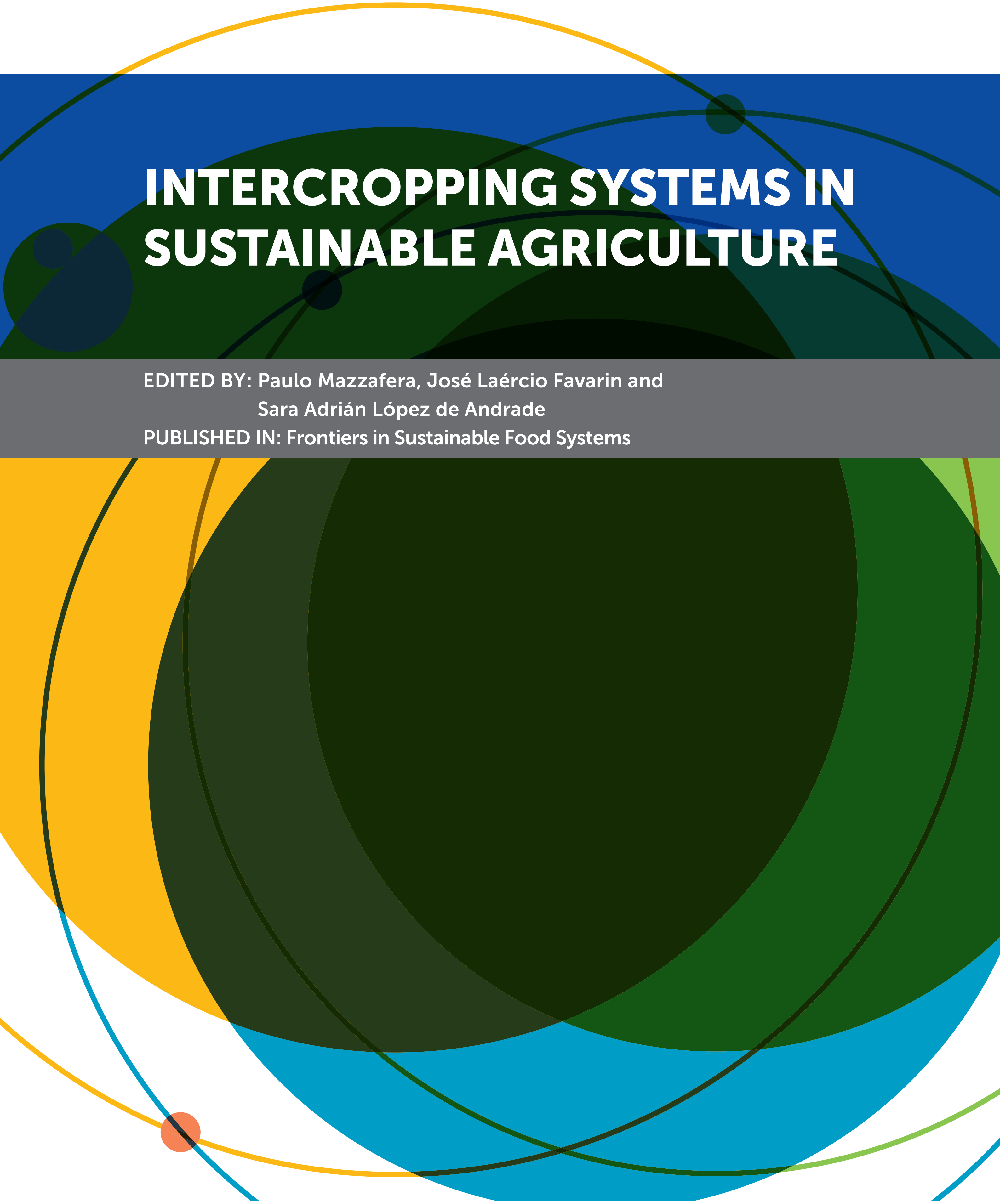

INTERCROPPING SYSTEMS IN SUSTAINABLE AGRICULTURE

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Sara Adrián López de Andrade

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INTERCROPPING SYSTEMS IN SUSTAINABLE AGRICULTURE

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EDITORIAL
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Editorial: Intercropping Systems in Sustainable Agriculture

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Editorial on the Research Topic

Intercropping Systems in Sustainable Agriculture

INTRODUCTION

Intercropping involves cultivating two or more crops in a field simultaneously, and is mainly practiced in regions where soils are relatively degraded. Intercropping is receiving increasing global interest as an agricultural practice as farmers strive to be more sustainable and maintain soil health (Glaze-Corcoran et al., 2020). Early research focused on supplying nitrogen by using legume species as intercropping plants (Stagnari et al., 2017). However, several non-cash plants are now used as cover crops to improve soil quality (Crusciol et al., 2012).

In this special issue, we received reports on recent research discussing the advantages and challenges of using intercropped systems in highly diverse world regions (sub-Saharan and Central Africa, North and South America, Asia, and Northern Europe).

TROPICAL GRASSES, NON-TILLAGE MANAGEMENT, AND NUTRIENT CYCLING

The use of tropical grasses as cover and companion crops is increasing in conservation agriculture. Herein, we review recent scientific publications on the use of *Urochloa* species in agroecosystems, and their contributions to more secure and sustainable agriculture (Baptistella et al.). *Urochloa* species employed as forage or intercropped with cash crops such as soybean, coffee, or citrus may protect soil, reduce soil erosion, suppress weeds, and mobilize nutrients. Many of the practical benefits are related to the extensive *Urochloa* root system that contributes to the cycling of nutrients from deep soil layers, and to soil carbon storage, thereby improving soil fertility and quality. Nevertheless, appropriate management when introducing these perennial grasses is essential to ensure diversity in native ecosystems.

Crusciol, Mateus et al. demonstrated the viability of maize intercropped with tropical grasses, palisadegrass (*Urochloa brizantha*), or guineagrass (*Megathyrus maximus*), and highlighted the requirement for nitrogen fertilization to guarantee yields and profitability. Meat production and land use were increased when nitrogen fertilizer was applied, and these intercropping systems involving maize and forage grasses were considered a good option for farm diversification. In another article, Crusciol, Portugal et al. reported that intercropping with palisadegrass or guineagrass led to a reduction in rice yield compared with monocropped rice. Nitrogen fertilization of intercropped rice increased the grain yield, grain protein, and milled productivity of rice. Despite the negative impact on yield, the authors concluded that intercropping rice with forage grasses was more favorable from both economic and environmental perspectives.

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Palisadegrass and guineagrass sowing time was evaluated by Costa et al. in terms of forage production and quality, soybean yield, and soil chemical properties when used as intercrops under a no-tillage system. In an experiment in Central-West Brazil, annual crops and semi-evergreen crops were grown for three growing seasons. The authors showed that sowing guineagrass after maize harvest increased soybean nutrients and yield, and improved soil fertility, by increasing soil organic matter, exchangeable Ca and Mg, and cation exchange capacity, while no-tillage reduced the pH value. Thus, this species can contribute to greater sustainability of tropical agriculture.

In a 5 year long-term study, de Azevedo et al. intercropped *Urochloa ruzizienzis* in high-density citrus (Tahiti acid lime) orchards, and compared four tillage systems: no-tillage, no tillage and herbicide, minimum tillage, and conventional tillage. Under non-irrigation conditions, incorporating *U. ruziziensis* in the no-tillage system increased fruit yield and improved water and nutrient-use efficiencies, as well as soil physical and chemical characteristics.

Pariz et al. studied a triple intercrop system with maize, pigeon pea (*Cajanus cajan*), and palisadegrass, combined with over-sown black oat. They concluded that the system improved nutrient cycling because the content of nutrients in standing plant residues and surface mulch was increased, resulting in better land- and nutrient-use efficiency.

In a soybean-wheat cropping system under no-tillage management, Moreira et al. showed that phosphate application as triple superphosphate efficiently supplied plants with phosphorus and increased grain yields, with stronger effects for fertilizer rates than application type on foliar phosphorus concentrations.

CROP RESILIENCE IN THE FACE OF CLIMATE CHANGE

Due to global warming, high temperatures and consequent drought have had a particularly detrimental impact in sub-Saharan Africa (Meehl et al., 2007; Spinoni et al., 2020). In this issue, Nyawade et al. and Chimonyo et al. present two examples of intercropped systems that contribute to increasing crop yield and improve water use efficiency (WUE). In the first paper, potato (*Solanum tuberosum*) intercropped with either dolichos (*Lablab purpureus*) or hairy vetch (*Vicia sativa*) and treated with granular silicon resulted in higher yield and better WUE under water deficit (Nyawade et al.). In the second, using an *ex-ante* approach, Chimonyo et al. used the Agricultural Production Systems sIMulator (APSIM) model to assess maize landraces intercropped with Bambara groundnut (*Vigna subterranean*). This intercropping system reduces land and water demand, and offers an inexpensive management strategy in environments projected to face water scarcity.

INTERCROPPING AND SOIL MICROORGANISMS AS BIOFERTILIZERS

Traditional nitrogen-fixing legumes have been used to improve soil fertility and staple crop performance for a long time, and a rich diversity of local species have been employed such as mung bean (*Vigna radiata*), Bambara groundnut, cowpea (*Vigna unguiculata*), black gram (*Vigna mungo*), and groundnut (*Arachis hypogaea*). Lengwati et al. evaluated the contribution of local food legumes as rotation crops for improving maize grain yield. They established a series of field experiments in South Africa, and used ^{15}N/^{14}N and ^{13}C/1^{2}C isotopic analyses to determine the percentage of nitrogen derived from symbiotic fixation. They also evaluated maize grain yield and nutrient concentrations. The results showed that the inclusion of nodulated legumes as biofertilizers enhanced the yield of the cereal crop and the nutritional quality of the grains.

Arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) have been used as biofertilizers due to their beneficial effects on plant performance, mainly by mobilizing soil nutrients and producing phytohormones that stimulate root growth (Akinola and Babalola, 2021). In a comprehensive field study in Southern India over three successive cropping seasons, Mathimaran et al. demonstrated the potential of AMF and PGPR application in intercropping of pigeon pea and finger millet (*Eleusine coracana*). AMF (*Ambispora leptoticha* and *Rhizophagus fasciculatus*) and PGPR (*Pseudomonas* sp. strain MSSRFD$_{41}$) systematically improved grain yield for both pigeon pea and finger millet, mainly when applied as a consortium in transplanted pigeon pea-finger millet systems.

FRUIT-BASED INTERCROPPING SYSTEMS

Adverse climatic events contribute to a decline in soil fertility and an increase in pests and disease incidence in agriculture, reducing crop yield. Smallholders adopt banana-based intercropping systems in East and Central Africa, where fruit production contributes to food security and economic (Abele et al., 2007). In this issue, Gambart et al. quantified the profitability, sustainability, and nutritional yield of current banana-based systems using a FarmDESIGN model (Ditzler et al., 2019). A total of 120 farm households from two districts in Uganda were interviewed, and qualitative and quantitative information was collected. The agroecological intensification levels of farms were clustered according to key variables such as farm size, number of crop and hedge species, number of agroforestry species, number of shade- and drought-tolerant species, and production constraints and orientation. The authors demonstrated the disparity in agroecological practices and socio-economic constraints between farmers, and the FarmDESIGN model was a valuable tool to assess farm performance, and may help to reduce costs and time-consuming trials.

In banana-based production systems, intercrops beneath the tree canopy are challenged by low light availability, and the balance between the spacing density of banana plants and the shade-tolerance of intercropped crops is crucial. In this issue, Blomme et al. revealed the need for careful selection of crops depending on canopy density and according to seasonally available water. Taro, soybean, mucuna, chili, eggplant, and *Crotalaria* sp. performed well in a low-density banana plantation. Cassava and soybean showed limited tolerance to shade. Chickpea and mucuna were suitable for the long dry season as they adapt to low water availability.

FORAGE DIVERSITY, SOIL FUNGAL PATHOTROPHS, AND WEED CONTROL

The concomitant growth of two or more crops in the same area without spatial rearrangement characterizes mixed intercropping (Glaze-Corcoran et al., 2020). In high latitudinal regions, the adoption of intercropped systems is challenging due to the short growing season and the wide temperature range through the year, especially extreme cold. In this issue, Lizarazo et al. reviewed sustainable mixed cropping systems in the Boreal-Nemoral region and explored possible combinations of crops in terms of their unique features, advantages, and limitations. The need for more sustainable options for crop production has fostered interest in traditional cropping systems with mixed plant species. However, despite being a sustainable option with clear environmental benefits, mixed cropping systems still face resistance from end-users and industries, and natural constraints related to harsh climate conditions.

Bainard L. D. et al. reported the effects of increasing plant species diversity on forage productivity and soil chemical and biological characteristics in Canadian prairies. While in this system, mixtures performed better than monoculture, producing more nutritious forage, the abundance of fungal pathotrophs in soils increased with plant diversity, highlighting the need to select forage species based on local growing conditions and plant–pathogen dynamics. However, in another study by this group reported in this issue, Bainard J. D. et al. showed that the diversification of forage species in the semiarid prairies of Canada is an effective way to control weeds, which may have particular relevance for reducing agrochemical inputs in these systems.

FINAL REMARKS

This Research Topic highlights the high diversity of intercropping systems around the world. The articles discuss the advantages of conservation agriculture practices, and the limitations and bottlenecks to be overcome. Overall, intercropping is a sustainable practice that can improve resource use efficiency for both nutrients and water, thereby facilitating low-input agricultural practices.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Can Palisade and Guinea Grass Sowing Time in Intercropping Systems Affect Soybean Yield and Soil Chemical Properties?

Nídia Raquel Costa [1,2]*, Marcelo Andreotti [3]*, Carlos Alexandre Costa Crusciol [1], Cristiano Magalhães Pariz [4], João William Bossolani [1,2,3], André Michel de Castilhos [4], Carlos Antonio Costa do Nascimento [1], César Gustavo da Rocha Lima [5], Carolina dos Santos Batista Bonini [6] and Eiko Eurya Kuramae [2,7]

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In tropical regions, intercropping systems under no-tillage improve biomass quantity, soil conservation, and cash crop productivity. However, the optimal sowing time for forage species in these cropping systems is unknown. The objective of this study was to evaluate the effects of two sowing times of palisade and guinea grass on forage production and quality, soybean yield and soil chemical properties. Palisade and guinea grasses were sown for intercropping with maize or after maize silage harvest (hereafter succession) in an experiment carried out over three crop seasons. We evaluated forage dry matter production, pasture nutritive values, straw nutrient content, soybean leaf nutrients, yield, and soil fertility. The highest dry matter production was 8.1 Mg ha^{-1} for guinea grass in the intercropping system (sum of 3 cuts). Sowing forage after maize silage harvest provided 4% more crude protein compared with intercropping, regardless of grass species. Soybean yield was over 1.0 Mg ha^{-1} higher when soybean was cropped in succession compared with intercropping; however, the effects of the two forage grasses on soybean production were similar. Soil pH, calcium and magnesium content, cation exchange capacity, and base saturation were higher in the intercropping systems than in the succession systems, particularly when guinea grass was cultivated. Sowing guinea grass after maize harvest provided better forage quality, nutrient cycling, soybean yields, and soil chemical properties in tropical conditions.

Keywords: intercropping crops, *Glycine max*, *Megathyrsus maximus*, *Urochloa brizantha*, sustainability, tropical agriculture

INTRODUCTION

Leaving the straw and roots of successive crops on agricultural fields, such as under no-tillage systems (NTS), improves the physical, chemical, and biological properties of soil (Castro et al., 2015; Calonego et al., 2017). In tropical regions, soil quality can be improved by adopting integrated crop-livestock systems, intercropping, and crop rotation management under

NTS (Costa et al., 2015; Moraes et al., 2019). These conservationist management practices preserve natural tropical resources (Crusciol et al., 2015), provide high yields of most grain crops (Pariz et al., 2017; Mateus et al., 2020) and are good options to increase food production during irregular periods of rain (Borghi et al., 2013). Worldwide, these crop systems can reduce poverty, allowing farmers to achieve better productivity and increase profits with minimum environmental impact (FAO – Food Agriculture Organization of the United Nations, 2017).

In tropical and sub-tropical regions, intercropping crops under NTS may be one of the best alternatives for farmers to gain income and simultaneously achieve sustainability and nutrient cycling (Carvalho et al., 2010; Mateus et al., 2020). Intercropping systems promote grain crop yields during the growing season, pasture production during the off-season, and remaining straw to maintain the NTS (Mateus et al., 2020). One of the key factors determining the adoption of these crop systems is their relative profitability compared with other land-use practices (Telles et al., 2018), as well as their perceived environmental benefits, which are recognized as a potential means of improving socioeconomic, biodiversity and environmental sustainability in many regions of the world (Franzluebbers and Stuedemann, 2014; FAO – Food Agriculture Organization of the United Nations, 2017). However, in regions with dry winters, high temperatures and water deficits, such as the Brazilian Cerrado and African Savannah, it is very difficult to establish and maintain effective NTS due to the high rate of straw decomposition on soil (Costa et al., 2014). In these environments, the selection of an appropriate plant species for use as cover crop is crucial to achieve the benefits of NTS.

For tropical soils, forage species such as palisade grass (*Urochloa brizantha* cv. Marandu; syn. *Brachiaria brizantha* cv. Marandu) and guinea grass (*Megathyrsus maximum* cv. Tanzania; syn. *Panicum maximum* cv. Tanzania) are recommended as cover crops (Crusciol et al., 2012; Pariz et al., 2017; Mateus et al., 2020). These grass species are drought tolerant, have deep root systems, produce high biomass, cycle nutrients, and maintain soil moisture through the cash crop cycle. In general, palisade and guinea grass provide adequate nutritional quality in intercropping systems and exhibit good potential for regrowth and pasture production during the off-season, thus increasing animal carrying capacity (Costa et al., 2016a; Pariz et al., 2017; Moraes et al., 2019). However, compared with grasses grown in succession, the physiological maturation of grasses intercropped with maize is more advanced, resulting in higher levels of cell wall components. The greater nutritional quality of intercropped forage grasses is of extreme importance for crop systems. Nutritional quality is measured by the balance between crude protein content and fiber digestibility, which affect the consumption of fodder by animals (van Soest et al., 1991).

Sowing these cover crop species in intercropping seems to be the best option for effective NTS establishment and maximum cover crop biomass production (Borghi et al., 2013; Mateus et al., 2020). Ceccon et al. (2013) reported higher soybean yield following maize intercropped with palisade and guinea grass compared with monocropped maize. In addition, Pacheco et al. (2017) observed that intercropping ruzigrass and maize improved nutrient cycling, and Pariz et al. (2016) found higher availability of P, K and Mg in the soil after intercropping of maize and palisade grass. Thus, intercropping grain crops with tropical perennial forage can increase food production (grain or silage + pasture) per unit area in tropical regions (Costa et al., 2016a).

Although the interspecific competition between intercropped plants is small (Borghi et al., 2013; Crusciol et al., 2015; Mateus et al., 2016), appropriate plant management and lower co-existence times can favor the development of both crops (cash crop and tropical perennial forage) by further reducing inter-specific competition (Crusciol et al., 2014). The time of grass sowing is an important factor for minimizing competition for water, light, and nutrients between the grass and cash crop (Crusciol et al., 2012). Environmental conditions also affect grass cover crop development; for example, the initial development of palisade and guinea grass is limited by low temperatures and insufficient water (Costa et al., 2005). Depending on the climatic conditions, sowing tropical fodder after harvesting commercial crops may not provide enough soil cover during the off-season (Costa et al., 2016a; Mateus et al., 2016). However, the best sowing period of these two forage species in tropical regions is not yet known.

The aim of this study was to evaluate the effects of two sowing times of palisade and guinea grass on forage production and quality, soybean yield, and soil chemical properties. We hypothesized that the sowing times (intercropped or in succession with maize silage) of palisade and guinea grass affect forage characteristics, remaining straw (mulch), soybean yield in succession, and soil fertility. To test this hypothesis, we evaluated the effects of different crop systems of maize silage with tropical perennial grasses (cultivated during the summer of the first two crop seasons) on (i) forage yield and chemical composition during winter, (ii) surface mulch (production, nutrient content, lignin/N ratio and C/N ratio) during spring, (iii) soybean cultivated during the summer of the third crop season (plant nutrition, production components and yield), and (iv) soil improvement in an experiment over three consecutive crop growing seasons in a tropical region with dry winters (Brazilian Cerrado).

MATERIALS AND METHODS

Site Description

This experiment was carried out in Central-West Brazil (20°18'S, 51°22'W, 370 m above sea level). The climate in this region is classified as Aw, characterized by a tropical and humid climate with a rainy summer season and a dry winter, according Köppen (Unicamp – Centro de Pesquisas Meteorológicas e Climáticas Aplicadas a Agricultura., 2016). The long-term (1956–2013) average annual maximum and minimum temperatures are 31.3 and 18.4°C, respectively The precipitation rate, and maximum and minimum temperatures of the area of this study were measured (**Table 1**).

The soil of the experimental area is a Ferralsol (FAO – Food Agriculture Organization of the United Nations., 2006), clayey,

TABLE 1 | Rainfall, maximum and minimum temperatures, and photoperiod at Selvíria, Mato Grosso do Sul State, Brazil, during the study period.

Climate characteristics	Month											
	Nov	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
						2010–2011						
Monthly rain, mm	214	288	202	230	356	157	9	41	0	0	3	137
Mean max. temp. (°C)	31.7	31.7	32.2	31.9	30.0	31.3	28.7	27.8	30.4	31.8	34.3	32.3
Mean min. temp. (°C)	19.5	21.4	21.3	21.2	20.7	18.7	15.8	14.1	16.0	16.7	18.5	20.8
Photoperiod, h day^{-1}	12.9	13.2	13.1	12.7	12.1	11.5	11.0	10.8	10.9	11.4	11.9	12.5
						2011–2012						
Monthly rain, mm	123	103	330	123	89	69	34	60	8	0	50	17
Mean max. temp. (°C)	32.0	33.6	31.2	33.0	33.1	32.0	28.0	27.8	28.2	30.9	33.6	34.9
Mean min. temp. (°C)	20.0	21.8	20.2	20.4	20.2	19.8	17.1	16.9	13.2	16.7	18.2	21.2
Photoperiod, h day^{-1}	12.9	13.2	13.1	12.7	12.1	11.5	11.0	10.8	10.9	11.4	11.9	12.5
						2012–2013						
Monthly rain, mm	138	83	110	265	166	60	-	-	-	-	-	-
Mean max. temp. (°C)	33.4	33.9	32.3	31.8	31.5	29.3	-	-	-	-	-	-
Mean min. temp. (°C)	21.4	22.5	21.8	20.9	20.3	18.5	-	-	-	-	-	-
Photoperiod, h day^{-1}	12.9	13.2	13.1	12.7	12.1	11.5	-	-	-	-	-	-
						Long-term (50-yr) average						
Monthly rain, mm	146	211	226	178	135	81	59	30	23	23	73	125
Mean max. temp. (°C)	33.0	33.0	32.0	32.1	32.0	31.1	29.3	28.4	28.8	31.6	31.0	33.0
Mean min. temp. (°C)	22.0	22.0	20.4	20.5	22.0	17.4	14.9	13.7	13.1	14.8	19.0	21.0
Photoperiod, h day^{-1}	12.9	13.2	13.1	12.7	12.1	11.5	11.0	10.8	10.9	11.4	11.9	12.5

with 482, 140, and 378 g kg^{-1} of clay, silt, and sand, respectively. Before October 2010, annual crops and semi-evergreen crops (maize, soybeans, sorghum, dwarf pigeon peas, palisade grass, beans, rice, and maize) were grown for 8 years in NTS. The chemical characteristics of the soil (0–0.20 m) were determined according to the methods described by van Raij et al. (2001). Before initiating the experiment, soil analyses indicated pH = 5.1, total soil organic matter = 25 g dm^{-3}, P (resin) = 33 mg kg^{-1}, exchangeable K, Ca, Mg, and total acidity at pH 7.0 (H + Al) = 4.1, 28, 16, and 29 mmol$_c$ kg^{-1}, respectively, and base saturation = 48.1%. The soil pH was determined in a 0.01-mol L^{-1} $CaCl_2$ suspension (1:2.5 soil/solution).

Experimental Design and Treatments

The experimental design was a randomized block, arranged in a 2 × 2 factorial scheme, with four replications. Treatments consisted of two different forages, palisade grass [*Urochloa brizantha* (A. Rich.) Stapf Marandu] and guinea grass [*Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs Tanzania] and two sowing times, i.e., the tropical grasses were sowing intercropped with maize at the same time, in alternative rows, according each treatment and sowed after maize silage harvest in the monocropped maize plots. The soybean (*Glycine max* (L.) Merrill) crop was sown in succession in crop rotation. The experiment was performed in the same location for three growing seasons. During the second growing season, the plots were established in the same location as the first growing season. The maize intercropped with palisade or guinea grass and forage in the off-season was carried out in 2010–2011, 2011–2012 and soybean in 2012–2013. The third growing season was a residual effect of intercropping systems from previous growing seasons with the same soybean crop system applied to all plots. Each plot consisted of four 20-m long maize rows that were spaced 0.90 m apart. The usable area was the two central rows; the 0.5 m at the end of each plot and the two external rows constituted the edge.

Tillage and Crop Management

Maize and Pasture

On November 22, 2010, the plants and weeds in the area were eliminated by applying glyphosate [isopropylamine salt of N-(phosphonomethyl) glycine] (1.44 g acid-equivalent ha^{-1}) at a spray volume of 200 L ha^{-1}. On November 26, 2010, the plants were cut using a plant residue crusher.

Triple hybrid BG 7049 maize [*Zea mays* (L.)] was sown on December 2, 2010–2011 for silage production. For the second growing season (2011–2012), simple hybrid AG 8088 YG was sown on December 10, 2011–2012. The same management plan was adopted in both growing seasons. The maize was sown at a depth of 0.05 m using a no-till drill, at a density of 60,000 seeds ha^{-1}. For all treatments and during both growing seasons, the basic fertilization in the sowing furrows consisted of 20 kg ha^{-1} of N, 70 kg ha^{-1} of P_2O_5, and 40 kg ha^{-1} of K_2O. Topdressing fertilization was conducted between maize rows without incorporation (90 kg ha^{-1} of N as urea) during both growing seasons when the maize plants had four expanded leaves. Subsequently, the experimental area was irrigated with 15 mm of water to minimize the N losses due of volatilization.

In the intercropping treatments, the grasses were sown at densities of 7 kg ha^{-1}. The forage (palisade grass or guinea grasses) seeds were sown simultaneously alternating rows with the maize, on the same day, at a depth of 0.08 m below the soil surface, using a no-till drill with a row spacing of 0.34 m. When the maize grains reached the ¼-milk grain stage (grains with 34–35% moisture), the crops in each plot were harvested using a mechanical silage forage harvester (Model JF-90 with 12 knives). The cutting height of the species for silage was ∼0.30 m above the soil surface.

On April 15, 2011 and April 12, 2012, the palisade grass and guinea grass were sown in the plots where the monocropped maize was cultivated. The sowing management of grasses in the off-season was the same as described previously in the crop season in intercropping systems.

Soybean in Crop Rotation

Soybean (*Glycine max* (L.) Merrill) "BRS Valiosa RR" was sown on October 30, 2012, at 4-cm depth, 0.45-m row spacing, and 260,000 seeds ha^{-1} density using a no-till seeding under palisade grass or guinea grass implanted during the intercropping or in succession after maize. Soybean seeds were inoculated with *Bradyrhizobium japonicum* (SEMIA 5079-CPAC 15 and SEMIA 5080-CPAC 7) at 5 g inoculant kg^{-1} seed. All soybean crop systems were fertilized in furrows with 90 kg ha^{-1} of P_2O_5 and 60 kg ha^{-1} of K_2O. The soybean plants were harvested 127 days after emergence (at grain physiological maturity).

Sampling and Analyses

Forage Dry Matter Production (FDMP) and Pasture Nutritive Values

Forage dry matter production (FDMP) was determined in both treatments at 50 (first cut), 100 (second cut), and 150 days (third cut) after sowing the forage species in the plots in monocropping systems during the off-season. All leaves (0.30 m from the soil surface) within a 2 m^2 area per plot were cut using a mechanical rotary mower. After cutting, all forage was removed from the plots, which was also performed using a mechanical rotary mower. This cutting height was used to provide faster forage regrowth. The collected material was dried by forced air circulation at 65°C for 72 h. The dry matter was weighed, and the data were extrapolated to kg ha^{-1} (FDMP). Samples were collected on June 4, 2011, July 24, 2011, and September 12, 2011 in the first growing season (2010–2011) and on June 1, 2012, July 21, 2012, and September 9, 2012 in the second growing season (2011–2012). In all cuts, the forage nutritional quality was determined. The crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) content was determined according to the methods described by Silva and Queiroz (2002) and Association of Official Analytical Chemists (2005).

Remaining Straw and Nutrient Content

On October 19, 2011, and October 14, 2012, following pasture and weed desiccation with herbicide, estimates of plant dessicated material (i.e., remaining straw) was evaluated. All leaves (0.05 m above the soil surface) within a 2-m^2 area per plot were cut using a mechanical rotary mower. After this management, the grasses in the plots were sprayed with glyphosate [isopropylamine salt of N-(phosphonomethyl) glycine] (1.44 g acid-equivalent ha^{-1}), using a spray volume of 200 L ha^{-1}, on October 25, 2011 and October 19, 2012.

Content of N, P, K, Ca, Mg and S were determined (Malavolta et al., 1997) in the accumulated straw. Lignin content was determined according to the method described by Silva and Queiroz (2002) and used to calculate the total lignin/N ratio. The C content (Tedesco et al., 1995) was determined to calculate the total C/N ratio, indicative of the durability of produced straw.

Soybean Agronomic Characteristics and Yield

Soybean leaf samples were collected from the upper third trifoliate at the R2 growth stage, full bloom (Fehr and Caviness, 1977). Petioles from 30 plants per plot were collected as proposed by Ambrosano et al. (1996). Leaf samples were washed with demi water and then dried under forced air circulation at 65°C for 72 h before grinding and analyzing for chemical composition. Contents of N, P, K, Ca, Mg, and S were determined using methods described by Malavolta et al. (1997).

The soybean plants were harvested 127 days after emergence, i.e., at physiological maturity. The plants contained in the four central rows were harvested to determine the soybean grain yield per plot, on March 12, 2013. The grains were weighed and corrected to a moisture content of 130 g kg^{-1}. The calculated agronomic characteristics were the final plant population (PP, calculated from the number of plants in the four central rows, excluding 1 m from the end of each side of the row in each plot), plant height (PH), height of the first pod insertion (HFPI), number of pods per plant (NPP), number of grains per pod (NGP), and 100-seed weight (W100, calculated from eight random samples per plot).

Soil Fertility

Soil chemical attributes were determined after soybean harvest, on April 03, 2013. Five single soil samples from 0.0 to 0.20-m depth were collected per plot in the soybean crop interlines and subjected to soil analysis according to methods described by van Raij et al. (2001).

Statistical Analyses

All data were normally distributed ($W > 0.90$) according the Shapiro-Wilk Test using UNIVARIATE procedure (version 9.3; SAS Inst. Inc., Cary, NC, USA) (SAS Institute, 2015), with the results indicating that all data were distributed normally (W C 0.90). The homogeneity of variances was tested by Levene's test for residual errors. The data for all variables were analyzed using the PROC MIXED procedure of SAS and the Satterthwaite approximation to determine the denominator's degrees of freedom for the tests of the fixed effects. The sowing times and forage species were considered fixed effect. Data were analyzed using the replication (block), year, and block (sowing times × forage species) as random variables. The growing seasons and their interactions between sowing times and forage species were not significant at $P < 0.05$ for any of the dependent variables. Thus, the data were combined for the growing seasons. The model statement that was employed to analyze the forage

TABLE 2 | Forage dry matter production (FDMP), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) as a function of sowing times and forage species.

Treatments	FDMP[§]	CP	NDF	ADF
	Mg ha^{-1}	g kg^{-1}		
Sowing times				
Intercropped	6.7 a	81.6 b	673 a	358 a
Succession	5.4 b	126.0 a	663 b	350 b
Forage species				
Palisade grass	5.4 b	97.4 b	667 a	343 b
Guinea grass	6.8 a	110.2 a	668 a	366 a
		ANOVA (F probability)		
Sowing (S)	0.015	<0.0001	0.031	0.093
Forage (F)	0.009	0.012	0.798	<0.0001
S × F	0.030	0.095	0.095	0.164
CV (%)	8.73	6.52	11.12	9.56
		Interactions		
FDMP (Mg ha^{-1})		Palisade grass		Guinea grass
Intercropped		5.5 aB		8.1 aA
Succession		5.3 aA		5.5 bA

[†] *Values followed by the same lowercase letter in a column (separated by sowing times and forage species) or the same uppercase letter in a row and their interactions are not significantly different at P < 0.05 according to the LSD test.*

[§] *FDMP results are presented as a sum of three cuts and average of two growing seasons. CP, NDF, and ADF results are presented as the average among three cuts of two growing seasons.*

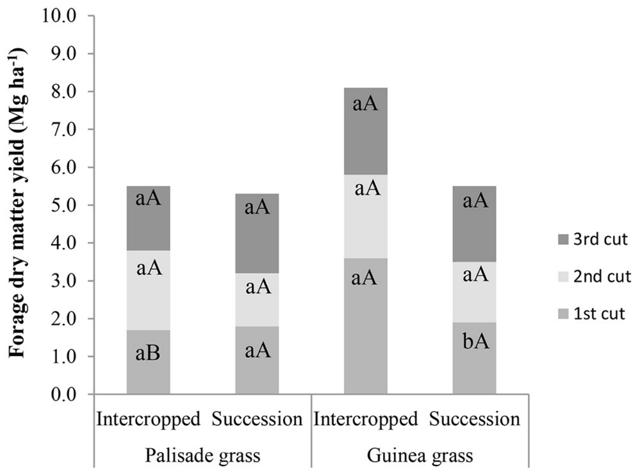

FIGURE 1 | Forage dry matter production (FDMP) of three cuts as a function of sowing times and forage species (average of two growing seasons). Values followed by the same lowercase letter (comparison of sowing times for the same forage specie) or the same uppercase letter (comparison of forage species for the same sowing time) for each cut are not significantly different at $P < 0.05$ according to the LSD test.

production encompassed the effects of the sowing times, forage species, cuts, and their resultant interactions. The specified term for the repeated statement was cuts, for the subject was replications (sowing times × forage species), and for the covariance structure utilized for all repeated statements was autoregressive, which provided the best fit for these analyses according to the Akaike information criterion (AIC). The results were reported as least square means (LSMEANS) and separated using the probability of differences (PDIFF) option. Mean separations were conducted using an LSD test. The effects were considered statistically significant at $p \leq 0.05$.

RESULTS

Forage Dry Matter Production (FDMP) and Pasture Nutritive Values

There was a significant effect of the sowing time × forage species interaction ($p < 0.030$) on forage dry matter production (FDMP) (sum of 3 cuts) (**Table 2**). The FDMP obtained during the off-season was highest when guinea grass was intercropped with maize compared to other treatments, reflection of this effect in the first cut (**Figure 1**).

Crude protein (CP) content was significantly influenced by sowing time ($p < 0.0001$) and forage species ($p = 0.012$) (**Table 2**). Guinea grass provided the highest CP content, and both species had the highest values when sown in succession to maize. Neutral detergent fiber (NDF) content differed based on sowing time ($p = 0.031$), and acid detergent fiber (ADF) differed ($p < 0.0001$) between forage species. For both species, NDF was lowest for sowing in succession, whereas ADF was lowest for palisade grass sown in succession to maize (**Table 2**).

Remaining Straw and Nutrient Content

There was a significant effect of the sowing time × forage species interaction on remaining straw and straw macronutrient accumulation (N, K, Ca, Mg, and S) (**Table 3**). Remaining straw production was highest for guinea grass sown in succession to maize (9.8 Mg ha^{-1}) and was similar in the other treatments (∼5.2–5.4 Mg ha^{-1}). Nutrient accumulation was greatest in guinea grass sown in succession to maize due to the highest remaining straw production of this species. The straw production and nutrient accumulation of palisade grass were similar at the two sowing times. P accumulation ($p < 0.001$; $p < 0.0001$) and the lignin/nitrogen (LIG/N) ($p = 0.032$; $p = 0.038$) and carbon/nitrogen (C/N) ($p = 0.009$; $p = 0.003$) ratios differed significantly as a function of sowing time and forage species (**Table 3**). P accumulation was highest for guinea grass, regardless of sowing time. The highest LIG/N and C/N ratios were obtained in the grasses intercropped with maize in the summer. Guinea grass provided the highest LIG/N ratio, whereas palisade grass resulted in the highest C/N ratio (**Table 3**).

Soybean Agronomic Characteristics and Yield

The soybean leaf contents of K, Ca and S were significantly influenced by the sowing time × forage species interaction (**Table 4**). In general, K and S content were higher when soybean was sown after guinea grass in succession to maize, whereas Ca content was higher when soybean was sown after palisade grass intercropped with maize. By contrast, soybean leaf N, P, and Mg content were influenced by forage species and sowing time (**Table 4**). N content differed significantly based on the sowing time ($p = 0.049$) and forage species ($p < 0.0001$). Leaf N and P content were highest in soybean sown under guinea grass straw or after maize harvest. On the other hand, leaf Mg content was higher ($p < 0.0001$) when soybean was sown after intercropped maize or under palisade grass straw, similar to leaf Ca content (**Table 4**).

With respect to agronomic parameters, none was influenced by the sowing time × forage species interaction (**Table 4**). The plant population (PP), height of the first pod insertion (HFPI), number of pods per plant (NPP), and number of grains per pod (NGP) were not significantly influenced by the sowing time or forage species. The plant height (PH), 100-seed weight (W100), were significantly influenced by the sowing time and forage species and grain yield (GY) by sowing time. Higher values were obtained when soybean was sown on straw produced by forage in succession to maize compared to the sown on straw produced by forage intercropped with maize (sowing times).

Soil Fertility

The soil chemical attributes were evaluated at the end of the experimental period. The changes in soil chemical properties (soil pH, exchangeable Ca and Mg, H + Al, CEC and BS) were greatest when the forage species were sown in succession to maize, particularly in the plots where guinea grass was cultivated (**Table 5**). These treatments provided soil with less acidity (pH $\sim$ 5.0), higher levels of exchangeable Ca (78–81 mmol$_c$ dm^{-3}) and Mg (13.6–14.6 mmol$_c$ dm^{-3}), and lower values of H + Al (34.7–36.0 mmol$_c$ dm^{-3}), resulting in higher CEC (78–81 mmol$_c$ dm^{-3}), and BS (42–46%).

TABLE 3 | Remaining straw production, nutrient content, lignin/N (LIG/N) and C/N ratio in forage species sown at different times over two growing seasons.

Treatment	P kg ha^{-1}	LIG/N	C/N
Sowing times			
Intercropped	10.42 b	2.30 a	38.38 a
Succession	16.78 a	1.62 b	27.19 b
Forage species			
Palisade grass	11.21 b	1.84 b	34.81 a
Guinea grass	15.98 a	2.08 a	30.77 b
		ANOVA (F probability)	
Sowing (S)	<.001	0.032	0.009
Forage (F)	<.0001	0.038	0.003
S × F	0.654	0.677	0.792
CV (%)	4.47	13.85	8.46
		Interactions	
	Palisade grass		Guinea grass
Remaining straw (Mg ha^{-1})			
Intercropped	5.2 aA†		5.4 bA
Succession	5.2 aB		9.8 aA
N (kg ha^{-1})			
Intercropped	56.3 4aA		61.81 bA
Succession	78.9 1aB		168.20 aA
K (kg ha^{-1})			
Intercropped	83.40 aA		99.68 bA
Succession	87.68 aB		186.01 aA
Ca (kg ha^{-1})			
Intercropped	15.87 aB		23.73 bA
Succession	15.43 aB		38.41 aA
Mg (kg ha^{-1})			
Intercropped	12.78 aA		13.01 bA
Succession	13.63 aB		26.26 aA
S (kg ha^{-1})			
Intercropped	5.74 aA		6.26 bA
Succession	6.29 aB		11.81 aA

†Values followed by the same lowercase letter in a column (separated by sowing times and forage species) or the same uppercase letter in a row and their interactions are not significantly different at $P < 0.05$ according to the LSD test.

DISCUSSION

Forage Dry Matter Production (FDMP) and Pasture Nutritive Values

In the present study, the different sowing times of forage grasses in this specific tropical production systems differently improves soil quality and the soybean yield response, consequently increasing the FDMP provided by forage grasses, an important objective. Here was showed just guinea grass intercropped with maize strongly influenced the FDMP in the first cut (3.6 Mg ha^{-1}) compared to other treatments (1.7–1.9 Mg ha^{-1}) (**Figure 1**), reflecting in the sum of three cuts (8.1 Mg ha^{-1}) compared to other treatments (5.3–5.5 Mg ha^{-1}) (**Table 2**). These positive results are attributable to the climatic conditions after maize harvest in both agricultural years (15.5 and 28°C) (**Table 1**), which were suitable for the development of forage (Costa et al., 2005). Additionally, the three-month precipitation totals of 200 and 160 mm (growing seasons 2012 and 2013, respectively), guaranteed plant establishment, even when sown in succession to maize. The combination of adequate luminosity incidence and photosynthetic rates during forage development promoted FDMP. Guinea grass is competitive and has a higher N demand than palisade grass; therefore, guinea grass has a higher potential for biomass production (Mateus et al., 2016). Moreover, the FDMP values obtained in the current study are superior to previously reported values for tropical soils under similar management systems (Mateus et al., 2020). FDMP is an important index for farmers who need to provide food to livestock through mechanical cutting or for grazing (Pariz et al., 2011).

Moreover, crude protein is an important parameter of forage quality, which decreased in the palisade grass and intercropped (**Table 2**). The nutritional quality of pasture is an extremely important factor for animal weight gain, economic viability

TABLE 4 | Leaf nutrient content, plant population (PP), plant height (PH), height of the first pod insertion (HFPI), number of pods per plant (NPP), number of grains per pod (NGP), 100-seed weight (W100), and grain yield (GY) for soybean cultivated in crop rotation systems under forage species sown at different times as a cover crop.

Treatments	**N**	**P**	**Mg**	**PP**	**PH**	**HFPI**	**NPP**	**NGP**	**W100**	**GY**
		g kg^{-1}		**n° × 1000**	**cm**				**g**	**Mg ha^{-1}**
Sowing times										
Intercropped	32.55 b	3.28 b	4.33 a	239.58 a	65.03 b	11.7 a	49.80 a	1.85 a	14.34 b	2.46 b
Succession	40.40 a	4.08 a	3.98 b	246.52 a	85.21 a	11.3 a	56.71 a	1.95 a	16.63 a	3.53 a
Forage species										
Palisade grass	36.15 b	3.53 b	4.20 a	234.72 a	74.64 b	11.7 a	56.31 a	1.85 a	15.24 b	2.97 a
Guinea grass	36.50 a	3.83 a	4.10 b	251.39 a	75.60 a	11.2 a	50.20 a	1.95 a	15.73 a	3.02 a
					ANOVA (*F probability*)					
Sowing (S)	0.049	0.006	0.223	0.366	0.001	0.414	0.358	0.562	0.043	0.003
Forage (F)	<0.0001	<0.0001	<0.001	0.702	0.036	0.588	0.301	0.887	0.050	0.059
S × F	0.105	0.589	0.078	0.406	0.104	0.634	0.913	0.291	0.246	0.479
CV (%)	7.12	6.25	9.62	16.23	11.45	11.73	9.66	17.87	5.21	8.14
					Interactions					
				Palisade Grass			Guinea grass			
K (g kg^{-1})										
Intercropped				17.00 bB[†]			19.00 bA			
Succession				20.00 aA			20.00 aA			
Ca (g kg^{-1})										
Intercropped				9.00 aA			7.10 aB			
Succession				6.80 bA			7.00 bA			
S (g kg^{-1})										
Intercropped				2.15 bB			2.60 aA			
Succession				2.65 aA			2.75 aA			

[†] *Values followed by the same lowercase letter in a column (separated by sowing times and forage species) or the same uppercase letter in a row and their interactions are not significantly different at $P < 0.05$ according to the LSD test.*
The results for the 2013 growing season are shown.

TABLE 5 | Soil chemical characteristics of the experimental site at 0–0.20 m depth after soybean harvest.

Treatments[†]	**pH**	**SOM**[‡]	**P$_{(resin)}$**	**K$_{ex}$**	**Ca$_{ex}$**	**Mg$_{ex}$**	**H+Al**	**CEC**[††]	**BS**[‡‡]
	(CaCl$_2$)	**(g dm^{-3})**	**(mg dm^{-3})**			**(mmol$_c$ dm^{-3})**			**%**
Sowing times									
Intercropped	4.7 b	22.0 a	26.5 a	3.8 a	16.63 b	10.0 b	40.1 a	70.5 b	30.4 b
Succession	5.1 a	23.3 a	20.6 a	3.3 a	28.13 a	14.6 a	34.7 b	80.8 a	46.0 a
Forage species									
Palisade grass	4.7 b	22.7 a	21.6 a	3.3 a	20.13 b	11.0 b	38.9 a	73.3 b	34.4 b
Guinea grass	5.0 a	22.5 a	25.5 a	3.8 a	24.63 a	13.6 a	36.0 b	78.1 a	42.1 a
					ANOVA (*F probability*)				
Sowing time (S)	0.050	0.815	0.610	0.226	0.050	0.032	0.020	0.050	0.029
Forage (F)	0.001	0.237	0.447	0.271	0.021	0.037	0.050	0.047	0.035
S × F	1.005	0.813	0.913	0.880	0.770	0.951	0.894	0.762	0.871
CV (%)	6.44	9.54	8.77	7.56	11.42	9.51	7.19	11.44	8.56

[†] *Values followed by the same lowercase letter in a column (separated by sowing times and forage species) are not significantly different at $P < 0.05$ according to the LSD test.*
[‡] *Soil organic matter.*
[††] *Cation exchange capacity.*
[‡‡] *Base saturation.*

and greater agricultural sustainability in tropical regions. In the present study, crude protein exceeded 70 g kg^{-1} in all treatments, and the NDF and ADF values were within the necessary ranges for maintaining the population of microorganisms in the animal rumen and for good digestibility (van Soest et al., 1991). Therefore, both forage grasses are great options for Cerrado regions since both provided adequate FDMP and nutritional composition in the off-season.

Remaining Straw and Nutrient Content

The remaining straw (mulch) in NTS contributes to soil quality and protection and to nutrient cycling (Crusciol et al., 2015; Costa et al., 2016b; Pariz et al., 2017). For successful implementation of NTS in the tropics, sufficient remaining straw production is key for maintaining favorable conditions for successive planting, as straw is a slow-release source of nutrients for cash crops (Costa et al., 2016b). In contrast to the FDMP results, the amount of remaining straw was highest for guinea grass sown in succession to maize (9.8 Mg ha^{-1}). In general, guinea grass has a higher biomass production capacity than palisade grass; however, this higher biomass productivity (the total for the three cuts) also increases the export of nutrients from the area, which can limit subsequent straw production. Guinea grass sown in succession to maize was best able to convert residual nutrients in the soil into biomass.

In the present study, forages sown in succession to maize showed lower lignin content and C/N ratios due to the younger age of the grasses, a strong indication that this plant material is capable of decomposing more quickly, cycling nutrients and improving soil quality (Costa et al., 2016b). The high production of remaining straw from guinea grass sown in succession to maize enabled greater accumulation of nutrients that can potentially return to the soil. Forage grasses with high remaining straw and nutrient accumulation (mainly N) provide a lower C/N straw ratio, accelerate the decomposition process and nutrient cycling, and increase the organic matter content of the soil (Mendonça et al., 2015), which are extremely important in tropical conditions.

Soybean Agronomic Characteristics and Yield

Soybean sown in the third agricultural year was positively influenced by sowing after forage species planted in succession to maize (regardless of forage species) or sowing after guinea grass (regardless of sowing time) (**Table 4**). Leaf N and P content and grain yield increased under these conditions. In addition, the leaf contents of K and S were highest when soybean was sown under the straw of both forage species planted in succession to maize. Ca content was highest when soybean was sown under palisade grass intercropped with maize. The grain yield of soybean was 1.07 Mg ha^{-1} higher when sown on straw produced by forage in succession to maize than that sown on straw produced by forage intercropped with maize (sowing times). The effects of the two forage grasses on soybean production were similar, only 0.05 Mg ha^{-1} higher when sown on straw produced by guinea grass than that sown on straw produced by palisade grass. Soybean benefits from the residual effect of nutrient cycling from predecessor crops, especially N and K (Crusciol et al., 2012; Pariz et al., 2016, 2017; Bossolani et al., 2018). The combination of both forage and maize residues provides soil cover for most of the soybean crop and results in lower soil temperature variation and higher moisture and greater soil decompaction and nutrient release as the plant and root residues decompose (Kliemann et al., 2006; Costa et al., 2014; Calonego et al., 2017). Furthermore, tropical grasses produce greater dry matter yield when following soybean in the rotation system because of the increase in N availability (Filizadeh et al., 2007; Pereira et al., 2016; Pariz et al., 2017). Thus, our results demonstrate positive effects of intercropping systems under NTS, as nutrient cycling due to straw decomposition and mineralization of the predecessor crops favors succeeding crops (Pereira et al., 2016; Pacheco et al., 2017; Pariz et al., 2017; Franzluebbers and Gastal, 2019).

Soil Fertility

Two years of maize cropping with forage species during or after maize cultivation and diversification with soybean altered soil chemical properties and macronutrient content. Specifically, the forage species in succession to maize (regardless of forage species) and guinea grass (regardless of sowing time) were associated with increased soil pH, exchangeable Ca and Mg, CEC and BS and lower H + Al content, probably due to the nutrient content in the crop residues (Brandan et al., 2017). The lower C/N ratio of remaining straw for forage sown in succession to maize (lower age) suggests that compared with intercropping, sowing forage in succession to maize could have greater potential to transform organic material into mineral nutrients, particularly in tropical regions with higher temperature and rainfall in the spring/summer season.

The results of this research will promote the sustainability of tropical soils because improving remaining straw (surface mulch) production and soil fertility in intercropping systems could enable a constant input of organic matter into the soil (Costa et al., 2015) to improve soil quality. Cropping systems that incorporate plant diversification through associations and rotations are sustainable and innovative (Moraes et al., 2019; Mateus et al., 2020). Species with high biomass production in the same area, such as maize, palisade grass, and guinea grass, result in a higher concentration of roots in the soil than under monocropping, and root exudates can reduce soil pH (Calonego et al., 2017). In the present study, the observed reduction in pH was not followed by a reduction in macronutrient content because NTS reduce pH values while increasing nutrient accumulation at the soil surface and soil organic matter (Castro et al., 2015).

CONCLUSIONS

Intercropping crops under NTS is very promising, but studies of the effectiveness of different evaluation parameters for achieving multiple objectives in tropical regions are lacking, particularly analyses of the maintenance of these crop systems for greater sustainability. In the current study, forage biomass production was highest for guinea grass intercropped with maize. Guinea grass had similar nutritive quality but higher crude protein levels than palisade grass. Sowing guinea grass after maize harvest increased soybean nutrient content and yield and improved soil properties after three consecutive growing seasons in tropical conditions. These results indicate that guinea grass can improve the productivity of crop systems and the long-term sustainability of tropical agriculture in the Brazilian Cerrado.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

NC and MA: Design the experiment. NC, MA, CC, CP, JB, CL, and CB: Obtain and process the data. NC, AC, and JB: Analyze the data. NC, JB, and EK: Wrote the paper with contribution of all co-authors. All authors confirm being contributor of this work and has approved it for publication.

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Impact and Opportunities of Agroecological Intensification Strategies on Farm Performance: A Case Study of Banana-Based Systems in Central and South-Western Uganda

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Agroecological intensification (AEI) practices relying on on-farm diversity tend to close nutrient cycles and reduce dependency on external inputs in agricultural systems. These practices improve the productivity of banana-based systems in Uganda, but their extent of implementation differs between and within regions. However, the impact of AEI practices on a broader range of objectives including environmental and nutritional objectives, is hardly quantified. Additionally, recommendations to improve the farm performance, given these options, are lacking. We, therefore, analyzed the current farm performance for these broad range of objectives and explored optimal farm reconfigurations in two Ugandan districts, one in Central Uganda and one in Southwestern (SW) Uganda. Given the heterogeneity of smallholder farms, a farm typology based on the applied AEI practices was developed. It classified the subsistence farms in Central Uganda into two extreme groups with an average of 11.0 and 16.4 AEI practices applied per farm. Farms in SW Uganda were moderately intensified (i.e., 13.0 practices). The FarmDESIGN model revealed a higher species diversity, relatively higher profitability (2,039 – 3,270 $/ha/year) and nutritional yield on farms in Central Uganda. However, relatively high soil erosion levels (0.243 – 0.240) and negative nitrogen (N) balances (−72 to −50 kg N/ha/year) were indicative of unsustainable practices. In contrast, farms in SW Uganda were less diverse and more market oriented. Their commercial orientation allowed investments in soil fertility management, resulting in more sustainable [low soil erosion level (0.172) and positive N balance (5 kg/ha/year)], but less profitable (506 $/ha/year) systems. To improve farm performance, bananas and other perennials played a key role. Explorations with *Calliandra calothyrsus* (Calliandra) hedgerow or *Mucuna pruriens* (Mucuna) cover crop increased on-farm mulch production, improved sustainability indicators and profitability. We conclude that AEI practices can improve farm performance, and a more intensive use would be beneficial. In addition, the FarmDESIGN model provides a useful tool for redesigning these farms, proposing different redesigns depending on farmers' objectives (profitability, productivity or sustainability), and for evaluating *ex ante* the impact of new agricultural measures on farm performance.

Keywords: agroecology, farm typology, FarmDESIGN, farm performance, farm optimization, nutritional yield, profitability, sustainability

INTRODUCTION

East African highland bananas and ABB type cooking/beer bananas (*Musa* spp.) are important components of the production landscape in Uganda (Gold et al., 2002; Němečková et al., 2018), especially in the country's Central and South-western region (UBOS and MAAIF, 2010). As a cheap all year-round energy source, rich in vitamin A, B6 and C, bananas contribute to small-scale farmers' food and income security (Frison and Sharrock, 1999; Abele et al., 2007). However, over the past 16 years, banana yield decreased with 1.5 tons/ha, resulting in an average yield of 4.3 tons/ha in 2017 (FAO, 2019)[1]. Increased land pressure due to rising population densities (Fermont et al., 2008), soil fertility decline (Stoorvogel and Smaling, 1990; Bazira et al., 1997; Bekunda and Manzi, 2003), drought (Wairegi et al., 2010; van Asten et al., 2011) and biotic stresses of pests, diseases and weeds (Tinzaara et al., 2009, 2018) are reported to contribute to this yield decline.

Sustainable intensification of the land currently under agricultural production is required to avoid further expansion into forest- or non-cultivated land (Wairegi and van Asten, 2010). Although fertilizer application is reported to have beneficial effects on banana production (Meya et al., 2020), adoption remains low due to high prices, low availability and poor market access. The practice is also only profitable in regions with good farm gate prices (e.g., close to Kampala) and good crop responses (e.g., Southern-Uganda) (Wairegi and van Asten, 2010). Therefore, agroecological intensification (AEI) practices that enhance the profitability, sustainability and nutritional yield on smallholder farms could offer opportunities for substantial improvement of the performance of these farms. As defined by Wezel et al. (2015), "AEI is a way of improving the performance of agriculture while minimizing environmental impacts and reducing dependency on external inputs through integration of ecological principles into farm- and system management." Besides increasing production and minimizing environmental impact, elements such as social and cultural perspectives are also included. Practices like mulching, intercropping, crop rotations, integrated soil- and nutrient management, soil- and water conservation, integrated pest management and biological control strategies and a balanced and more efficient use of fertilizers are mentioned as possible AEI strategies by different authors (Gliessman, 2000; Côte et al., 2010; Karamura et al., 2013; Vanlauwe et al., 2013; Wezel et al., 2015).

Several studies report the application of AEI practices on banana-based farms in Uganda and the Great Lakes region (Katungi et al., 2006; Jassogne et al., 2013; Mpiira et al., 2013; Ocimati et al., 2013; Ssebulime et al., 2017). Here, bananas are integrated in a wide range of cropping systems, ranging from monocultures to intercropping- and mixed farming systems (Karamura et al., 1998). While in Uganda's Southwestern region banana plots are more likely to be pure stands, crop diversity within the banana field increases toward the country's Central region. Beans (*Phaseolus vulgaris*) (Karamura et al., 1998), coffee (*Coffea* spp.) (Jassogne et al., 2013) or agroforestry species such as jackfruit (*Artocarpus heterophyllus*), natal fig (*Ficus natalensis*), albizia (*Albizia coriaria*) and mango (*Mangifera indica*) are often intercropped with banana (Mpiira et al., 2013). However, the system's performance (profitability, productivity and sustainability) in general and the effect of the different farm components on farm performance, more specifically, are not yet fully quantified. Moreover, recommendations on how to intensify banana-based systems with these AEI practices are lacking (Ssebulime et al., 2017).

Therefore, this study focuses on the quantification of current banana-based systems in terms of their profitability, sustainability (soil N balances and soil erosion levels) and nutritional yield (dietary energy, vitamin A and iron) at the farm system level. Additionally, windows of opportunities for the improvement of the systems, given the initial farm components, are explored. As smallholder farms are characterized by a large array of farm components, all interrelated, the FarmDESIGN model (Groot et al., 2012; Ditzler et al., 2019), was used to facilitate the redesign process. The different agroecological and socio-economic conditions farmers are faced with, also result in a large diversity between farms even within the same geographic region. In addition, production constraints faced by farmers determine their possibilities to adopt intensification practices. To overcome the potential challenges due to the heterogeneity among farmers, this study uses farm typologies to group farmers into more homogeneous groups on the basis of the currently applied AEI practices.

MATERIALS AND METHODS

Project, Site Selection, and Study Area

The fieldwork was conducted in two Ugandan districts: Kiboga district [0° 49′ 48.8″ N, 31° 51′ 36.8″ E; 1,210 meters above sea level (m.a.s.l.)] in Central Uganda and Isingiro district [0° 50′ 43.8″ S, 31° 0′ 30.1″ E; 1,510 m.a.s.l.] in SW Uganda.

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[1] http://www.fao.org/faostat/en/#data/CC

The two study sites are diverse so that most findings could become applicable to other banana production zones with similar production systems and constraints in the East and Central African region. Additionally, differences in AEI practices depending on the production orientation of the banana-based farming systems can be explored since production of bananas in Kiboga is mainly oriented toward home consumption, while farmers in Isingiro mainly produce for the market.

Both districts are characterized by a mean annual temperature of 21°C. Besides, a bimodal rainfall pattern is present in both regions, consisting of two rainy seasons, one from April until May and the other from September until December. The total annual precipitation in Kiboga is 1,200 mm, while in Isingiro it is 1,140 mm (FAO, 2005)[2].

Farm Typology

Dataset

In order to capture the diversity among farms considering AEI practices, 120 farm households were selected in a first round of interviews using the snowball and maximum variation sampling approach. Thus, farmers and/or key informants were interviewed to identify very and less diverse farms in their neighborhoods to capture the conventional as well as rarely adopted AEI practices. In total, 60 households each were visited in Lwamata sub-county in Kiboga district, and Rugaaga sub-county in Isingiro district. During the interviews, a preliminary questionnaire was used, to collect both qualitative and quantitative information on farm, farm location, and household and farm management, including the farm, household and livestock size, number of cultivated crop species, number and type of applied agro-ecological practices, and the production orientation, constraints and objectives.

Typology Construction

Sixteen potential key variables were selected to cluster the farms according to their AEI level (**Table 1**). As in Alvarez et al. (2018), multivariate statistics, executed in R (version 3.5.1), were used for the typology construction. By drawing pairs plot, boxplots and histograms, the data were checked for missing values and possible outliers, which were subsequently removed. In addition, key variables with a non-normal distribution were transformed to a more normal distribution. Out of the 120, 112 households were retained and the logarithm ($\log_{10}$) and square root function applied to the variables "farm size" and "tropical livestock units," respectively. Tropical livestock units (TLU) were calculated according to FAO guidelines (Otte and Chilonda, 2002), using the conversion factors : cattle = 0.70, pigs = 0.20, sheep and goats = 0.10, and chickens = 0.01. These factors were multiplied by the number of each animal species present on farm and summed up to come up with the final TLU value. The transformed variables are represented by the codes area2 and tlu2, respectively.

In order to reduce the number of key variables into non-correlated dimensions, the Factor Analysis of Mixed Data (FAMD) was executed with the packages "factoextra" (version 1.0.5) and "FactoMineR" (version 1.41). However, highly correlated key variables resulted in a poor outcome of the factor analysis. Therefore, key variables with the lowest contribution to the dimensions were omitted during several rounds of analysis, until only seven variables were kept for the final grouping. These variables included five quantitative (farm size, tropical livestock units, number of cultivated crop species, number of applied AEI practices, number of agroforestry species) and two qualitative variables (farm location and farmer's objectives). To determine the number of dimensions kept in the analysis the Kaiser criterion was used, to keep only the dimensions with an eigenvalue larger than one. The remaining dimensions were used for further analysis to classify the farms into different groups.

The clustering analysis was performed by the HCPC function (Hierarchical Clustering on Principal Components) from the "FactoMineR" package (version 1.41). This function starts with assigning every observation to its own cluster and subsequently merges the two most similar clusters until only one cluster is left (Crawley, 2013). The optimal number of clusters was found by using Ward's method, which maximizes both intra-cluster homogeneity and inter-cluster heterogeneity (Murtagh and Legendre, 2014). In order to identify the variables that characterized the different clusters, boxplots were drawn for the quantitative variables and statistical significances between the different clusters were detected with the pairwise Wilcoxon test. Moreover, a classification tree was built using the R package "rpart" (version 4.1–13). The Margalef index (M), representing species richness, and Shannon index (H), representing both species richness and evenness, were calculated for each identified cluster, based on the following formulas, respectively:

$$M = \frac{S-1}{ln(A)} \text{ and } H = -\sum_{i=1}^{S} \left(p_i * \ln\left(p_i\right)\right)$$

where S is the number of crop species present on farm, A the total farm area (m^2) and p_i the area proportion of crop i.

Farm Redesigns

Dataset

In order to calculate the performance of each farm type and reveal windows of opportunities for farm optimization, we used the FarmDESIGN model (Groot et al., 2012; Ditzler et al., 2019). Three farms from every cluster, identified by the farm typology, were selected for a second round of interviews. This selection was based on the willingness of the farmer to participate and on the quality of the information provided by the farmer during the previous interview. Additionally, farmers as close as possible to the cluster centers, as identified by the HCPC analysis, were selected. Then, a more detailed questionnaire was used, which captured necessary input variables for the FarmDESIGN model, including household and herd composition, and for each crop, crop yield, product destination, cultivation costs, labor requirements and market prices. Additional information such as, data on environmental and crop characteristics, like soil type and climatological conditions, N fixation and nutrient composition was obtained from secondary data available within the FarmDESIGN model or from literature (Gutteridge and Shelton, 1994; Groot et al., 2012; Kongkijthavorn, 2017; de

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[2] http://www.fao.org/land-water/land/land-governance/land-resources-planning-toolbox/category/details/en/c/1032167/

TABLE 1 | Main characteristics of the 16 key variables of the dataset.

Quantitative variable	Unit	Included in FAMD	Code	Mean	±Stdev	Min	Max
Household size	Capita		hhsize	7.29	3.33	1.00	18.00
Farm size	ha	√	area2	4.43	5.89	0.20	28.33
Tropical livestock units	TLU	√	tlu2	1.92	2.91	0.00	15.85
Number of cultivated crop species	Integer	√	nrcr	15.63	5.38	4.00	27.00
Number of applied AEI* practices	Integer	√	nraei	13.14	2.68	4.00	19.00
Number of hedge species	Integer		nrhed	0.33	0.82	0.00	5.00
Number of agroforestry species	Integer	√	nraf	8.59	8.65	0.00	29.00
Number of shade-tolerant species	Integer		nrsh	2.82	1.60	0.00	6.00
Number of drought-tolerant species	Integer		nrdr	2.35	1.16	0.00	5.00

Qualitative variable	Unit	Included in FAMD	Code	Levels	Min	Max
Farm location	District	√	distr	Isingiro, Kiboga	Isingiro	Kiboga
Education of the household head	Diploma		educ	Graduate, institution, no, primary, secondary	Graduate	Primary
Use of hedge species	Classes		usehed	No hedges, not used, used	Used	No hedges
Use of agroforestry species	Classes		useaf	No AF**, not used, used	No AF	Used
Production constraints	Classes		constr	Diseases, drought, erosion, fertility, land, pests	Pests	Diseases
Farmer's objectives	Classes	√	obj	Dietary diversity, income, productivity	Productivity	Dietary diversity
Production orientation	Classes		or	Commercial, subsistence	Commercial	Subsistence

*AEI, Agroecological intensification.

**AF, Agroforestry species.

The unit, code, mean value, standard deviation, minimum and maximum value for the quantitative variables are given in the upper part of the table. The lower part of the table shows the unit, code, different levels and the least (min) and most (max) observed levels of the qualitative variables. The column 'Included in FAMD' indicates whether the variable was used to execute the FAMD or not.

Jager, 2018; HarvestPlus, 2018; USDA, 2018; World Agroforestry Centre, 2018a,b,c).

FarmDESIGN Model

Besides being able to calculate the performance of the original farm, the FarmDESIGN model can explore redesigns that outperform the original farm by using a multi-objective Pareto-based Differential Evolution algorithm. Considering as well-objectives as constraints, the performance of Pareto-optimal redesigns cannot be improved without compromising one of the other objectives (Groot et al., 2007). For every run, the number of iterations was set at 1,000. We wanted to maximize operating profit, dietary energy yield, vitamin A yield, iron yield and to improve the farm sustainability by minimizing N losses and soil erosion (objectives). Constraints were set so that the total crop area could not increase and that each fruit species could occupy maximally 0.4 ha. Redesigns varied in the area allocated to each (mixture of) crop(s) and destination of the crop products and residues (decision variables). A summarizing table listing the objectives, decision variables and constraints used for the FarmDESIGN model is given in **Table S1**. In addition, two scenarios were created, in which *Calliandra calothyrsus* (as hedge row) and *Mucuna pruriens* (as cover crop) were each separately introduced on farm to evaluate the effect on farm performance.

Each time the FarmDESIGN model was run, it generated 800 Pareto-optimal redesigns. Those redesigns which outperformed the original farm in all objectives were selected. As trade-offs between objectives made it impossible to reach the best performance for each indicator simultaneously, objectives had to be ranked to select the most optimal redesign. In this study, profitability was prioritized, followed by soil quality and nutritional yield improvement. As postulated by Kansiime et al. (2018), it is believed that higher incomes allow on-farm investments, which in turn can lead to increased soil quality, if the right investments are made, and hence improved sustainability.

The operating profit was calculated by subtracting both variable (for labor, manure, fertilizer and other inputs) and fixed costs (for land) from the crop and animal product revenues. To calculate the nutritional yield, expressed in persons/ha/year, the nutrient supply of the products, annually produced on one ha of land, was compared with the recommended daily reference intake multiplied by 365 days. The N balance was computed by considering both N inputs (symbiotic and non-symbiotic N fixation, atmospheric N deposition, and the N content of green manure, animal manure and fertilizers) and outputs (N content of exported crop and animal products, soil erosion and N volatilization) (Groot et al., 2012). Erosion levels were determined for each crop by the soil erosion C factor, defined as the ratio between soil losses on bare soil and those on land cultivated by every specific crop (FAO, 2001). Therefore, soil erosion C factors are dimensionless, ranging from zero to one, whereby low values are assigned to crops which cause limited soil erosion.

Due to time constraints, the outcome of the model runs was discussed with the interviewed farmers in Kiboga only during two focus group discussions. The best redesigns and scenarios were presented to the farmers with slides each representing the composition of the modeled farm, after which farmers opinions were asked.

RESULTS

Farm Type Characterization

Rainfed cooking bananas (further referred to as bananas) were the major component in agricultural production systems in Kiboga and Isingiro. However, there are some considerable differences. In Kiboga, farms were smaller (on average 2.2 ha) compared to Isingiro, where the farm size was on average 6.8 ha. Crops like coffee, maize, beans and cassava were intensively intercropped with banana in Kiboga. Moreover, agroforestry species were present on all farms within the banana fields unlike hedges which were on 25% of the farms in that region. In Isingiro, maize, beans and cassava were also present, but farmers mainly focused on monocrops of each crop. Instead of plot diversity, these farmers invested more in soil and water-conservation practices. Here, mulch was applied on every farm, compared to 74% of the farms in Kiboga. Likewise, 80% of the farmers in Isingiro, compared to 58% in Kiboga, applied animal manure. The same trend could be noticed for the occurrence of water retention ditches: 78% in Isingiro compared to 39% in Kiboga.

To exploit the differences between farms in more depth, a multivariate analysis was executed. The first two dimensions of the Factor Analysis of Mixed Data explained together 60% of the total variability between farms, based on seven key variables. The first dimension explained 39% of the variability and was closely related to the farm location, farm size, number of agroforestry species and farmer's objectives, thus, expressing general farm information. The second dimension, explaining 21% of the variability, was mainly correlated to the number of applied AEI practices and seemed to explain farm management. The clustering analysis grouped the farms into four different farm types (**Figure 1**). However, since the third farm type (WC; wrongly classified) consisted of only two observations (**Figure 1**), these observations were classified according to the classification tree into the three remaining farm types: farm type LI (low intensified), MI (moderately intensified) and HI (highly intensified), ordered with increasing number of applied AEI practices (**Figure 2**).

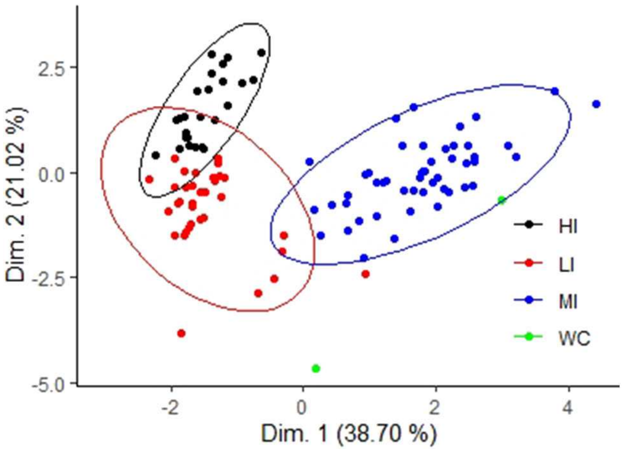

FIGURE 1 | Hierarchical clustering of identified clusters LI, MI, HI, and WC, referring to low intensified farms, moderately intensified farms, highly intensified farms and wrongly classified farms, respectively.

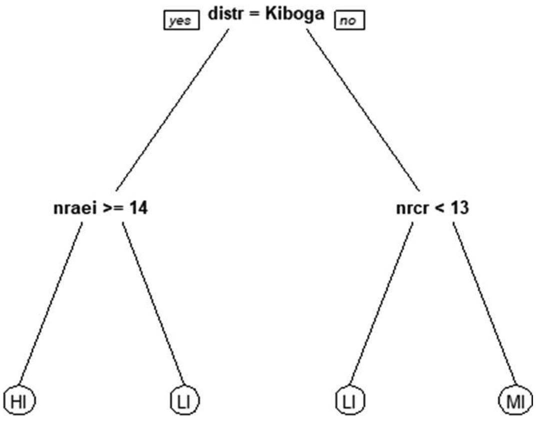

FIGURE 2 | Classification tree, with overall accuracy of 82%, to classify the two observations from cluster WC into the three remaining clusters (LI, MI, and HI, referring to low, moderately, and highly intensified farms). Distr represents the considered district, nraei the number of applied agroecological intensification practices and nrcr the number of cultivated crop species.

Farm Type LI: Low Intensified Farms (n = 36, 32% of the Sampled Farms)

LI farms were the least intensified of the three farm types. On average, 11.0 out of the 34 AEI practices (**Table S2**) such as intercropping, integration of agroforestry species, application of kitchen and household waste and manual control of pests and diseases were applied in these farms (**Figure 3**). Additionally, LI farms were the smallest (1.41 ha; 6.3 household members), with the smallest number of cultivated crop species (11.1) including banana, coffee, maize, beans, cassava and groundnuts. Annual species covered about 49% of the land. Perennials mainly comprised banana and coffee. However, 10% of the land was allocated to trees, i.e., 7% agroforestry species and 3% fruit species (**Table S3**). While the fruit species [i.e., *Artocarpus heterophyllus* (jackfruit), *Mangifera indica* (mango), *Persea americana* (avocado)] were mainly grown as an additional food source for the farm household, agroforestry species (i.e., *Eucalyptus grandis*, *Maesopsis eminii*, *Albizia coriaria*, *Erythrina abyssinica,* and *Syzigium cuminii*) were valued for shade, fodder, mulch, firewood and timber. Hedges, in contrast, were not abundant. If present, hedges were used as a mulch, fodder and/or firewood source. LI farms had a Margalef index, indicating species richness, of 2.32. An index of 0 represents monocultures, while larger indices indicate more species in a determined area or the same amount of species on a smaller area. The Shannon index, a measure of both species richness and evenness mainly ranging from 1.5 to 3.5, reached a level of 2.12, indicating that the relatively high number of species were more or less evenly

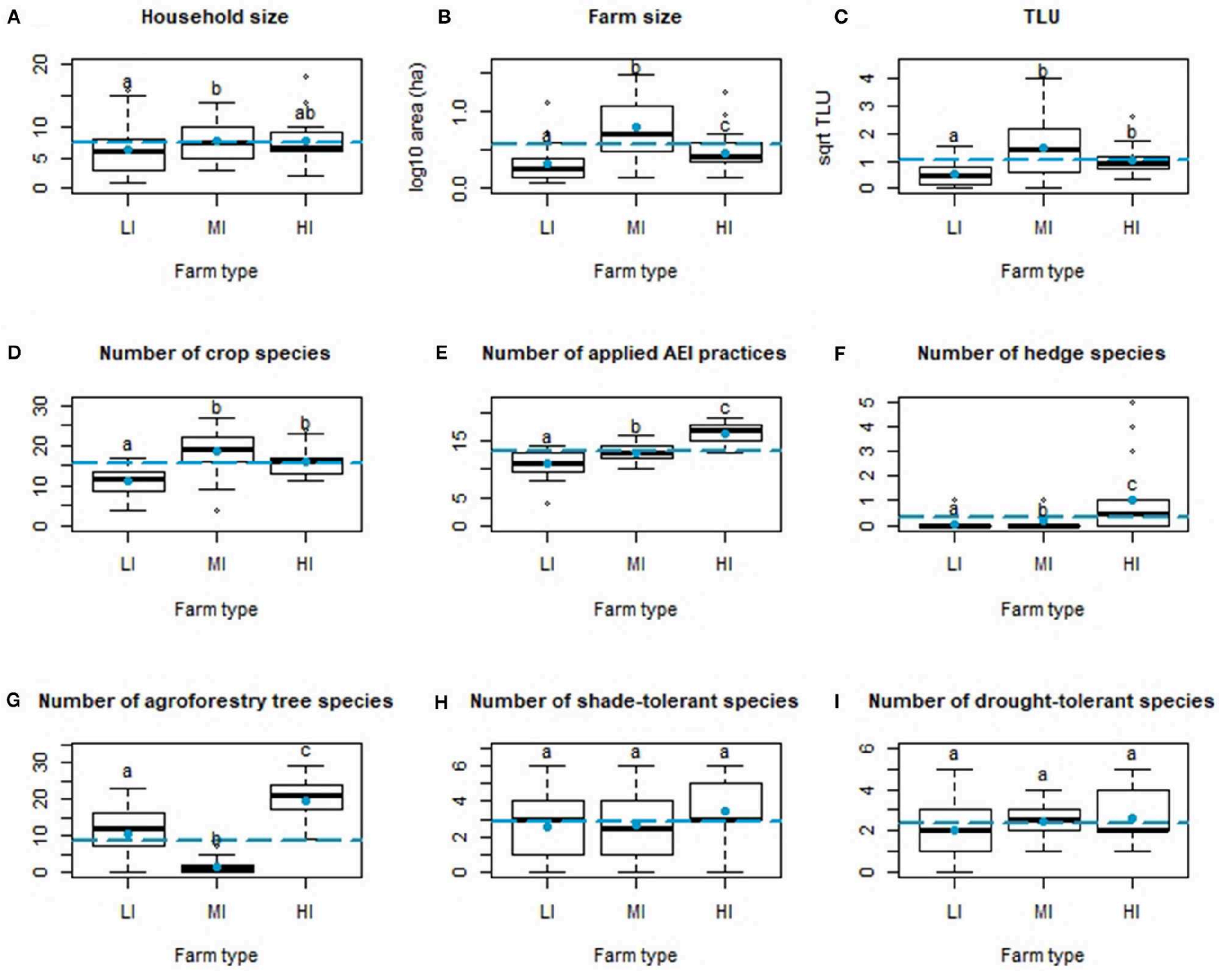

FIGURE 3 | Boxplots of the three farm types, ordered by increasing number of applied AEI practices (LI: low intensified farms, MI: moderately intensified farms and HI: highly intensified farms), for the variables household size **(A)**, farm size **(B)**, tropical livestock units **(C)**, number of cultivated crop species **(D)**, number of applied AEI practices **(E)**, number of hedge species **(F)**, number of agroforestry species **(G)**, number of shade- and drought-tolerant species **(H,I)**. Blue points represent the cluster means, thick solid horizontal lines the median values, box outlines the interquartile range containing the middle 50% of values, whiskers the 90th percentile values, gray points the outlier values and the dashed blue line the survey means of each variable. Small letters above the whiskers of the boxplots represent the output of the pairwise Wilcoxon test. Cluster means that are significantly different at the 0.05 significance level are indicated by a different letter. Only boxplots of the same quantitative variable can be compared.

distributed on farm. Few animals, like chickens and pigs were on farm, resulting in the lowest TLU of 0.45 amongst all farm types. While chickens were free ranging, pigs were tethered in the farmyard. These least intensified farms were mainly located in Kiboga (83.3%) and managed by subsistence farmers who mainly produced for home consumption.

Farm Type MI: Moderately Intensified Farms (n = 50; 45% of the Sampled Farms)

In MI farms, defined as the moderately intensified farms, 13.0 AEI practices were on average applied (**Figure 3**). Unlike farm type LI, MI farms were the largest (7.57 ha; 7.8 household members). Although about 80% of the total land area was covered by banana, many crop species (18.8), like beans, maize, groundnut and millet, were cultivated mainly as monocrops. Crops grown for home consumption only, such as eggplants, leafy vegetables and pumpkins, were found on a very small to negligible scale, consisting of one or two plants per species and this was reflected by the species indices. The Margalef index was the smallest for all farm types and reached a level of 1.32. Moreover, the relatively small number of species was not evenly distributed (Shannon index = 0.92). Perennials other than banana occupied 1% of the land and included *Carica papaya* (papaya), *Ricinus communis* and *Eucalyptus grandis*. While the former two species were grown within the banana fields, the latter was allocated to a separate field to avoid possible competition for water, soil nutrients and solar radiation with the cultivated crops. Cattle, goats and chickens were managed by the farm household (TLU = 3.32). While cattle and goats were on communal grazing areas during day time and kept in kraals overnight, chickens were found in the farmyard. This resulted in a large availability of animal excreta, which was applied on the banana field(s). In addition to application of farmyard manure on banana fields, kitchen and household waste, mulch and external cow dung were applied and soil and water retention ditches dug. These farms were mainly (98%) located in Isingiro, where farmers had commercial objectives.

Farm Type HI: Highly Intensified Farms (n = 26; 23% of the Sampled Farms)

Intercropping, integration of agroforestry- and hedge species, application of kitchen and household waste, mulch, manure and even mineral fertilizers were some of the 16.4 AEI practices applied on HI farms (**Figure 3**). These highly intensified farms had, on average, a size of 2.59 ha (7.6 household members), of which 45% was allocated to annual crop species, such as maize, sweet potato, cassava, beans and tomato. Coffee and banana occupied together about 50% of the total area. Half of the remaining land was covered by agroforestry/hedge species while the other half with fruit tree species (i.e., jackfruit, mango and avocado). Additionally, these farms were characterized by the highest diversity of agroforestry and hedge species, with on average 19.9 and 1.0 species, respectively. The five most occurring agroforestry species were *Markhamia lutea* (96%), *Albizia coriaria* (92%), *Maesopsis eminii* (92%), *Ficus natalensis* (85%), and *Vernonia amygdalina* (85% of HI farms). Hedges occurred on 50% of the farms and were used by 77% of these households as a fodder, mulch and/or firewood source. Especially *Calliandra calothyrsus* and *Morus* spp. were used for these production objectives. HI farms were highly diverse with a Margalef index of 3.12. Additionally, the relative high number of species were equally divided among the available land (Shannon index = 2.49). As for all farm types, animals were kept on farm. Here, livestock mainly consisted of goats, pigs and chickens, resulting in a TLU of 1.25. While the former two were mainly zero grazed, chickens were free ranging in the farmyard. This type of farmers were all located in Kiboga district, defining themselves as subsistence farmers.

Performance of the Farms

The different compositions of the farm types resulted in contrasting farm performances, as calculated by the FarmDESIGN model using the inputs of the second round of interviews (**Table 2**). LI (with a profit of 2,039 $/ha/year) and HI (with a profit of 3,270 $/ha/year) farms, predominantly located in Kiboga, were more profitable compared to MI farms (with a profit of 506 $/ha/year), mainly located in Isingiro. The species rich LI and HI farms were also able to meet the dietary requirements of a relatively larger number of people compared to the less diverse MI farms. However, the relatively high soil erosion levels and negative N balances indicated unsustainable farming practices for LI and HI farms. In contrast, MI farms in Isingiro were more sustainable given the positive N balance (+ 5 kg N/ha/year) and the relatively low soil erosion level (0.172).

TABLE 2 | Performance of the farm types and the contribution of banana to the performance parameters (%), as calculated by the FarmDESIGN model.

Performance	LI		MI		HI	
		%*		%**		%*
Operating profit/ha [USD/ha]	2,039	67	506	19	3,270	60
Soil erosion C factor	0.243	−5	0.172	−75	0.240	−7
N balance [kg/ha/year]	−72	−26	5	192	−50	−23
Dietary energy yield [persons/ha/year]	26	41	9	65	23	46
Vitamin A yield [persons/ha/year]	18	75	9	83	18	77
Iron yield [persons/ha/year]	21	43	16	56	31	25

*contribution of banana to the performance parameters.

**contribution of banana and its management practices (mulch and manure) to the performance parameters.

As the major farm component, bananas were paramount in those farms (**Table 2**). They were the major vitamin A supplier and provided 60 to 67% of the total profit in Kiboga, but only 19% in Isingiro. If income from sales was considered, bananas provided 31% of the total farm income in the latter region, although still reaching lower levels than expected. The sale of tomato and cassava contributed 24 and 25% to the total farm income in Isingiro, respectively (data not shown). However, bananas were responsible for the sustainability of MI farms by reducing soil erosion by 75% and improving the N balance by 192%, meaning that more nitrogen is added to the banana plot than is removed by its harvest.

Farm Redesigns

An Optimal Redesign

In order to improve the farm performances (profitability, productivity and/or sustainability), redesigns of each farm type were generated by multi-objective optimization by first varying the area allocated to every species, already present on the farm and, hence, without introducing new species. As an illustration, out of the clouds of redesigns, one Pareto-optimal redesign which outperformed the original farm in terms of all objectives was chosen for each farm type (**Table 3A**). Unlike for farm type MI, whereby all objectives could be enhanced, farm composition changes did not profoundly improve the N balance in farm type LI, while in farm type HI N balance improved but still remained negative. Nevertheless, profits could be doubled and even quadrupled. Also, the nutritional parameters were improved.

To obtain these performances, perennials seemed to be important, covering 54% to 88% of the total farm area (**Table 3B**).

TABLE 3 | Performance (**A**) and composition (**B**) of the best redesigns for each farm type, as calculated by the FarmDESIGN model, and the percentage change in objective (**A**) and farm components (**B**) relative to the original farm performance.

	LI		MI		HI	
		Δ%		Δ%		Δ%
(A) PERFORMANCE						
Operating profit/ha [USD/ha]	4,049	+99	2,593	+412	7,990	+144
Soil erosion C factor	0.183	−25	0.160	−7	0.218	−9
N balance [kg/ha/year]	−85	−18	4	+19	−35	+30
Dietary energy yield [persons/ha/year]	43	+65	12	+34	45	+97
Vitamin A yield [persons/ha/year]	50	+177	15	+64	38	+115
Iron yield [persons/ha/year]	22	+5	24	+48	42	+36
(B) COMPOSITION (%)						
Annuals	14	−71	12	−37	46	+2
Perennials (total)	86	+69	88	+9	54	−2
Banana	80	+122	85	+6	15	−57
Fruit spp.	4	+33	3	/	11	+175
Other agroforestry spp.	1	−86	0	−100	28	+833

While for farm type LI and MI, banana was the main perennial on farm, agroforestry tree species and other perennials comprised the largest area allocated to perennials on farm type HI. *Erythrina abyssinica*, *Pennisetum purpureum,* and *Maesopsis eminii* were the most prominent perennial species here, comprising each 86%, 4% and 3% of the total area under perennial species other than banana, respectively (data not shown). Considering annuals, cassava occupied the largest area in all redesigns (56, 22, and 43% of the total area under annuals on farm type LI, MI, and HI, respectively). The rest of the area was mainly allocated to maize, tomato, peas and cabbages. The detailed composition of the original farm types and the best redesigns can be found in **Tables S3, S4**, respectively.

For a given farm type, the model generated a wide range of redesigns (**Table 4A**), allowing farmers to choose a redesign in line with their own preferred ranking of objectives. However, trade-offs between objectives impeded the existence of compositions which performed better for all objectives simultaneously than the original farm composition. Increased nutritional yield, for instance, was related to a reduced N balance. Likewise, a positive N balance on farm type HI was associated with a lower operating profit. Moreover, the variation of the performance was not equally large for all farm types. For farm type LI, only three better performing but similar redesigns were identified by the model.

Similar to the best performing composition (**Table 3**) perennials accounted for more than 50% of the total farm area in these redesigns (**Table 4B**), with at least 80% of land allocated to banana on farm types LI and MI. In contrast, agroforestry tree species, specifically *E. abyssinica* accounted for the largest area under perennials on farm type HI. To increase the area under perennials, the area under annuals had to decrease in most cases. Major annual crops were cassava, maize and beans on farm type LI, cassava, tomato, groundnuts, beans and peas on farm type MI and cassava and cabbages on farm type HI (data not shown).

Explorations of New Species on Farm

Besides changing the compositions of the initial components, new species, which potentially reduce the dependency on external inputs, can be added to the model to investigate their effect on the farm performance. In a first scenario *Calliandra calothyrsus*, a N fixing species with high biomass production (8.5 tons DM/ha/year; Kongkijthavorn, 2017), was integrated as a hedge around farmers' fields in the model. Explorations by the FarmDESIGN model revealed that with exception of the N balance for farm type LI, all redesigns with *C. calothyrsus* outperformed the original farm type for all objectives considered (**Table 5A**). Again, perennials played a key role (**Table 5B**): i.e., banana on farm type LI and MI, occupying 80% and 87% of the total area, respectively, and other agroforestry species on farm type HI, covering 52% of the total area. Here, *E. abyssinica* was the most prominent, comprising 91% of the area allocated to agroforestry species. Important annuals were cassava on farm type LI, tomato and *Solanum* potato on farm type MI and cabbages and sweet potato on farm type HI, covering 69, 27, 13, 18, and 18% of the area allocated to annuals, respectively (data not shown).

In a second scenario, the N fixing cover crop *Mucuna pruriens* was integrated below the banana canopy on farm type MI. This introduction alone, without changing any of the current farm components, reduced soil erosion and farm profitability by 5% and tripled the N balance.

DISCUSSION

Farms differ in terms of land accessibility, resource endowment, production and consumption decisions, experience and management skills of the owners (Tittonell et al., 2010; Alvarez et al., 2014). These differences influence the interest and capacity of farmers to adopt AEI strategies that can potentially improve the farm performance (Kuivanen et al., 2016). Therefore, the classification of farmers in more homogenous groups facilitates tailor-made recommendations and allows to increase adoption rates of new technologies by farmers.

Farm Type Characterization

In this research, farm typology revealed different farm management strategies across both districts. These differences can be explained by the difference in farm size. With an average farm size of 7.57 ha, MI farmers cultivated their crops as pure stands to reach the household dietary requirements. Moreover, the practice of intercropping did not fit within their mindset,

TABLE 4 | Performance range (**A**) and composition range (**B**) of the redesigns outperforming the original farm, as calculated by the FarmDESIGN model, and the percentage change in objective (**A**) and farm components (**B**) relative to the original farm performance.

	LI		MI		HI	
		Δ%		Δ%		Δ%
(A) PERFORMANCE RANGE						
Operating profit/ha [USD/ha]	(3,902) – (4,049)	(+91) – (+99)	(622) – (2,593)	(+23) – (+412)	(3,527) – (7,990)	(+8) – (+144)
Soil erosion C factor	(0.183) – (0.189)	(−25) – (−22)	(0.153) – (0.171)	(−11) – (−1)	(0.163) – (0.218)	(−32) – (−9)
N balance [kg/ha/year]	(−85) – (−83)	(−18) – (−16)	(+ 1) – (+ 10)	(−80) – (+103)	(−48) – (+19)	(+4) – (+138)
Dietary energy yield [persons/ha/year]	(40) – (43)	(+53) – (+65)	(8) – (15)	(−11) – (+67)	(25) – (67)	(+9) – (+193)
Vitamin A yield [persons/ha/year]	(44) – (50)	(+144) – (+177)	(10) – (28)	(+10) – (+207)	(28) – (203)	(+59) – (+1049)
Iron yield [persons/ha/year]	(20) – (22)	(−5) – (+5)	(15) – (24)	(−8) – (+48)	(35) – (58)	(+14) – (+88)
(B) COMPOSITION RANGE (%)						
Annuals	(14) – (18)	(−71) – (−63)	(7) – (19)	(−63) – (0)	(14) – (48)	(−69) – (+7)
Perennials (total)	(82) – (86)	(+61) – (+69)	(81) – (93)	(0) – (+15)	(52) – (86)	(−5) – (+56)
Banana	(77) – (80)	(+114) – (+122)	(78) – (90)	(−3) – (+13)	(11) – (24)	(−69) – (−31)
Fruit spp.	(4)	(+33)	(1) – (4)	/	(7) – (17)	(+75) – (+325)
Other agroforestry spp.	(1)	(−86)	(0) – (1)	(−100) – (0)	(17) – (59)	(+467) – (+1867)

as, according to these farmers, species integrated within the banana plot compete for water, soil nutrients and solar radiation. Therefore, *E. grandis* was cultivated in a separate field, while beans were planted at the border of the banana plot and only specific species, like papaya and *R. communis*, were grown within the field. The latter two species are characterized by a limited above-ground branching habit resulting in limited competition for light (CABI, 2018)[3]. However, below-ground, papaya is characterized by an extensive root system in the first 30 cm of the soil layer, which can potentially result in competition with the banana plants, while *R. communis* has a tap root system with prominent lateral roots, therefore, avoiding competition with other crops (CABI, 2018)[3]. The limited available land in Kiboga (farm types LI and HI) forced farmers to increase the production intensity by intercropping, integrating fruit- and other agroforestry species to meet family dietary needs and keep their land productive. Integration of several crops on the same piece of land has been reported to be a smallholder strategy to increase land- and other resource-use efficiency (Sileshi et al., 2007) and minimize risks of crop failures (Dapaah et al., 2003).

In addition, farm size is reported to be linked to market participation, i.e., the larger the farm size the more these farms are involved in sales (Tittonell et al., 2010; Chapoto et al., 2013; Kuivanen et al., 2016), suggesting that MI farmers are more oriented toward the market than type LI and HI farmers. Moreover, profits from sales can, on the one hand, be invested in soil amendments, such as mulch and animal manure, to compensate for nutrient losses, and, on the other hand, in expansion of the herd (Kuivanen et al., 2016). Besides, the large farm size enables farmers to keep more livestock to provide manure and fertilize the fields. In addition, animals can be kept as a form of insurance. During periods of cash shortage, they might be sold to pay medical bills and school fees. Among all animal species, cattle are the most valuable. Indeed, MI farmers owned the largest cattle herds, while in Kiboga, where farms were smaller and market participation was less, livestock mainly consisted of small ruminants. These differences in management practices were reflected by the farm performances. While farmers in Isingiro invested in soil fertility by buying mulch and animal manure, farmers in Kiboga did not, resulting in less profitable but more sustainable farms in Isingiro, compared to profitable but unsustainable farms in Kiboga. These results are supported by findings of Kansiime et al. (2018), who showed that labor and fertilizers were more efficiently used on farms with a commercial orientation, suggesting that the expectation to generate farm income motivates farmers to produce more efficiently.

In Kiboga the number of applied AEI practices differed significantly between both farm types (cf. 11.0 practices on farm type LI and 16.4 on farm type HI). The fewer number of AEI practices on LI farms may be due to the small land LI farmers owned. Tittonell et al. (2010) showed that poor resource-endowed (land and labor) farmers face constraints, such as education and health for several generations. This limits their interest and possibilities to adopt new intensification strategies. Hence, lack of knowledge or adequate labor forces could be given as some of the reasons for the lower adoption of AEI practices on LI farms compared to HI farms.

Farm Redesigns

The current performance of the different farms urged for improvement measures in terms of all objectives considered, i.e., operating profit, sustainability in terms of soil erosion and N balance, and nutritional yield. Farmers' information, acquired during the interviews, revealed that income from sales of commonly cultivated crops, like banana, coffee, maize, beans and groundnuts, was relatively low. Additionally, commonly eaten food products by the Ugandan population are rich in carbohydrates, while vitamins and minerals are often lacking. Fruits and vegetables showed potential to increase farm profitability and nutritional yield.

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[3] https://www.cabi.org/isc/

TABLE 5 | Performance **(A)** and composition **(B)** of the best redesign with *C. calothyrsus* integrated on farm, as calculated by the FarmDESIGN model, and the percentage change in objectives **(A)** and farm components **(B)** relative to the original farm performance.

	LI		MI		HI	
		Δ%		Δ%		Δ%
(A) PERFORMANCE						
Operating profit/ha [USD/ha]	4,143	+103	1,132	+123	4,450	+36
Soil erosion C factor	0.175	−28	0.158	−8	0.178	−26
N balance [kg/ha/year]	− 81	−13	5	+3	5	+109
Dietary energy yield [persons/ha/year]	43	+63	20	+126	26	+14
Vitamin A yield [persons/ha/year]	48	+167	52	+468	31	+78
Iron yield [persons/ha/year]	63	+62	28	+74	42	+36
(B) COMPOSITION (%)						
Annuals	10	−80	11	−42	21	−53
Perennials (total)	90	+76	89	+10	79	+44
Banana	80	+122	87	+9	12	−66
Fruit spp.	7	+133	1	/	14	+250
Other agroforestry spp.	3	/	1	+0	52	+1,633

In order to improve the farm performance, the FarmDESIGN model was run for each farm type. Optimal redesigns, i.e., compositions which outperformed the original farm in almost all objectives, with positive N balances attributed more than 50% of the total farm area to agroforestry species. The limited available land on farm type LI seemed to be the biggest limitation for improving the N balance. Since a large area needed to be assigned to crops and fruits to improve both profitability and nutritional yield, not enough space was left for agroforestry species, necessary to maintain adequate soil fertility levels.

However, concerns of farmers in Isingiro may be appropriate, as some agroforestry species can exert above- and/or below-ground competition with intercropped species. Agroforestry species with complementary characteristics to banana, i.e., a limited above-ground branching habit or a canopy with sufficient light transmission and a low competitive or deep root system, offer opportunities to improve the system. Of the commonly integrated species in Kiboga, only *A. coriaria* and *F. natalensis* are suitable companion species to banana. As a N fixing species with a deep tap root and sparse canopy, *A. coriaria* produces good decomposable litter (Buyinza et al., 2019; Ssebulime et al., 2019). Also *F. natalensis* produces litter with a sufficient decomposition rate (Ssebulime et al., 2019). In contrast, while the dense canopy of *M. lutea* and fruit species, like jackfruit, mango and avocado, prevent sufficient solar radiation transmission to the crops underneath, the extensive and competitive root system of papaya and *M. eminii* compete for soil nutrients (Wajja-Musukwe et al., 2008; World Agroforestry Centre, 2018b)[4]. However, species unsuitable as upper canopy species, can also be planted in hedgerows to avoid above-ground competition through heavy foliage. Additionally, pruning can improve solar radiation transmission, although, as above- and below-ground biomass is balanced (Van Noordwijk and Purnomosidhi, 1995), it also changes the root system, leading to a reduced rooting depth and increased root branching in the topsoil (Schroth, 1995). Despite potential competition with crops, integrating agroforestry species can have many advantages. They might recycle nutrients leached to deeper soil layers, aggregate soil particles and, thus, increase the water holding capacity and water infiltration and reduce erosion (Giller and Wilson, 1991).

Besides the scientific prove that an agroecological intensification practice is effective, farmers' willingness to adopt the practice is also of utmost importance. Therefore, two feedback sessions with farmers in Kiboga were organized, one for each farm type. All farmers showed great interest in integrating *M. pruriens* as a cover crop underneath the banana canopy. Since weeds, lack of mulch and labor forces were a big concern, *M. pruriens* can offer a solution here. Some farmers, who wanted to keep small ruminants, were more interested in integrating *C. calothyrsus* as a hedge around their homestead and field. These findings suggest that future research should focus on a more in-depth exploration of these redesigns in close collaboration with the farmers (Le Gal et al., 2011).

Model Perspectives

Various tools for the analysis and exploration of strategic improvements in farming systems have been developed in the last decades, each using different programming techniques and algorithms (Dogliotti et al., 2005; Tittonell et al., 2007a,b; Groot et al., 2012). An advantage of the FarmDESIGN model is the generation of a large set of alternative farm designs, providing rapid insights into the consequences of a range of reconfigurations on farm performance (Groot et al., 2012). Moreover, synergies and trade-offs among different objectives are revealed by the Pareto-based multi-objective optimization algorithm (Groot and Rossing, 2011). This makes the model a useful tool to support farmers in their management-related decision making and facilitates the discussion on alternative production systems between researchers and farmers.

It should be noted that all calculations were purely based on farmers' estimations. Therefore, detailed measurement of essential model inputs and confirmation of outputs by on-farm trials will be necessary to validate the FarmDESIGN results. However, estimates as reported in this study, can be a good reflection of reality. Additionally, animal feed balance, nutrient cycles and efficiency calculations in the model allow to check the correctness of the estimated

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[4] http://www.worldagroforestry.org/treedb2/speciesprofile.php?Spid=17981

material flows at the core of the model. Hence, the FarmDESIGN model is a suitable tool to determine the overall performance given the entangled system variables and reduces the need for risky, costly and time-consuming trial and error experiments.

CONCLUSION

As the agroecological and socio-economic constraints faced by farmers differ a lot between and even within the same geographic locations, the adoption potential of new technologies depends on each individual. Farm size, location and orientation (market vs. home-consumption) were the major factors influencing the number and type of adopted AEI practices on farm. While in Kiboga (Central Uganda, 1,210 m.a.s.l.; 1,200 mm/year; small to medium sized farms; home-consumption) farms tended to be more diverse, farmers in Isingiro (SW Uganda; 1,510 m.a.s.l.; 1,140 mm/year; large sized farms; market oriented) mainly focused on banana monocultures. Unlike in Kiboga, farmers in Isingiro invested in mulch and animal manure and controlled soil erosion by digging water retention ditches, resulting in a more sustainable farming system. In contrast, farms in Kiboga were relatively profitable, but soil quality parameters forecast unsustainable farming practices. Banana, as the major cash crop, was of key importance for the overall farm performance, in particular for the operating profit, vitamin A yield and reduction of soil erosion. Agroforestry species were fundamental to improve the N balance, requiring a coverage of at least 50% to obtain positive N balances. Composition changes, suggested by the FarmDESIGN model, allowed to double or even quadruple the operating profit, increase the nutritional yield by 5% to 180% and improve the sustainability by 10% to 30%. We conclude that the FarmDESIGN model is a useful tool to evaluate *ex ante* the impact of integrating theoretical promising species on farm performance and shows potential to be used as a tool in support of agricultural policy measures.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

WO, RS, RR, and GB supervised the experiments. CG and WO performed the experiments and analyzed the data. CG, WO, RS, and JG wrote the manuscript. All authors reviewed and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2020.00087/full#supplementary-material

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Intercropping Transplanted Pigeon Pea With Finger Millet: Arbuscular Mycorrhizal Fungi and Plant Growth Promoting Rhizobacteria Boost Yield While Reducing Fertilizer Input

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Pigeon pea (*Cajanus cajan*) and finger millet (*Eleusine coracana*) are staple food crops for millions of the rural population in Asia and Africa. We tested, in field trials over three consecutive seasons at two sites in India, an intercropping and biofertilization scheme to boost their yields under low-input conditions. Pigeon pea seedlings were raised during the dry season and transplanted row-wise into fields of finger millet, and arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (*Pseudomonas*) were added alone or in combination to both pigeon pea and finger millet. Our major findings are (i) effects of the biofertilizers were particularly pronounced at the site of low fertility; (ii) dual inoculation of AMF+PGPR to finger millet and pigeon pea crops showed increased grain yields more effectively than single inoculation; (iii) the combined grain yields of finger millet and pigeon pea in intercropping increased up to +128% due to the biofertilizer application; (iv) compared to direct sowing, the transplanting system of pigeon pea increased their average grain yield up to 267% across site, and the yield gains due to biofertilization and the transplanting system were additive. These technologies thus offer a tool box for sustainable yield improvement of pigeon pea and finger millet.

Keywords: pigeon pea (*Cajanus cajan*), finger millet (*Eleusine coracana*), intercropping, arbuscular mycorrhizal fungi (AM fungi), plant growth promoting rhizobacteria (PGPR), biofertilizers

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) and plant growth promoting rhizobacteria (PGPR)—generally called "biofertilizers"—are two classes of microbes that are well-known for their broad spectrum of beneficial effects to plants, by mobilizing phosphate, producing plant growth hormones, alleviating drought by the production of ACC deaminase

(Aroca and Ruiz-Lozano, 2009; Qiao et al., 2011; Ortiz et al., 2015; Bender et al., 2016; Mathimaran et al., 2017; Rahimzadeh and Pirzad, 2017) which helps improving crop nutrient uptake (Bender et al., 2016; Igiehon and Babalola, 2017). Both AMFs and PGPRs have been successfully evaluated in many field crops, such as wheat and rice (Mäder et al., 2011). However, currently there is lack of comprehensive knowledge on whether application of AMF+PGPR could potentially reduce the mineral fertilizer inputs in finger millet and pigeon pea without compromising their yields.

Finger millet (*Eleusine coracana*) and pigeon pea (*Cajanus cajan*) are two important crops predominantly grown in marginal rainfed regions of Africa and Asia, particularly in India, and Latin America (Chandrashekar, 2010; Krishna, 2010; Rao et al., 2015; Gupta et al., 2017; Varshney et al., 2017). Both crops are rich in protein and minerals, and serve as staple food for millions of people (Chandrashekar, 2010; Gupta et al., 2017). In India, pigeon pea and finger millet are grown on about 3.38 and 2.5 million hectares, respectively. Their yields are reduced due to various biotic and abiotic stresses, and in particular to low soil fertility, which cannot be readily overcome by the application of mineral fertilizers because of their high cost (Varshney et al., 2012; Gupta et al., 2017).

Biofertilizers, such as AMF and PGPR, have been successfully applied to improve the grain yields and nutritional quality of food crops (Schutz et al., 2018), including pigeon pea and finger millet (Patro et al., 2008; Dutta et al., 2014; Sekar and Prabavathy, 2014; Gopalakrishnan et al., 2016). Biofertilizer can be complementary to other fertilization or plant nutrition strategies, they can help reduce chemical fertilizers and increase the efficiency of nutrient uptake and soil organic matter stabilization. Our study examines to what extent biofertilization is effective in mono- or mixed culture of the two crops in order to reduce the quantity of mineral fertilizers while safe-guarding farmers practice of cultivating pigeon pea and finger millets as intercrops. In particular, biofertilization with AMF in the intercropping system might lead to sustainable crop production through improved soil fertility via a common mycorrhizal network. Under rainfed conditions, a transplanting system of pigeon pea cultivation is gaining importance due to its yield advantage compared to direct sowing (Ashok et al., 2010; Murali et al., 2014). In the transplanting system, pigeon pea seedlings are pre-cultured in polybags, a type of pot alternative, and well-watered before the start of monsoon, a seasonal rainfall pattern found in tropics. They are transplanted in the field after about 6 to 7 weeks at the start of the monsoon season. Polybag transplanting systems in pigeon pea have been successfully tested both under mono- and intercropping systems (Ashok et al., 2010; Murali et al., 2014; Praharaj et al., 2015). Under such a scenario, the use of biofertilizers, particularly the AMF, may provide an additional benefit, by forming a common mycorrhizal network from the start. Inoculating pigeon peas during pre-culturing in polybags may allow to reduce the amount of AMF inoculum, which is often considered to limit the application of AMF in arable crops.

Our study was aimed to address following four hypotheses (i) compared to application of 50% recommended dose of fertilizer (RDF) and farm yard manure (FYM), application 50% RDF + FYM + biofertilizer has a potential to significantly improve the grain and straw yields of pigeon pea and finger millet; (ii) under pigeon pea-finger millet intercropping system, application 50% RDF + FYM + biofertilizer, has potential to improve the total grain and straw yields of pigeon pea and finger millet on par with the 100% RDF + FYM; similarly compared to application of FYM alone (0% RDF), application of FYM + biofertilizer has a potential to marginally improve the total grain and straw yields of pigeon pea and finger millet; (iii) under pigeon pea (transplanted system)-finger millet intercropping system, application (placement) of AMF biofertilizers to the seedlings of pigeon pea in polybags is sufficient to obtain the grain and straw yields on par with the yields obtained when AMFs applied to both the crops; (iv) under pigeon pea (transplanted system)-finger millet intercropping system, application (placement) of AMF biofertilizers to the seedlings of pigeon pea in polybags would result better grain and straw yields compared to application of biofertilizers under direct sown pigeon-finger millet intercropping system.

MATERIALS AND METHODS

Field Site, Rainfall Pattern, and Soil Properties

Main field trials were conducted at two field sites in South India at Bangalore (Karnataka) and Kolli Hills (Tamil Nadu) over two seasons (July 2014 to January 2015 and July 2015 to January 2016), with a third trial at both locations during July 2016 to January 2017 for selected treatments. The geographic location of the two sites, and their climatic conditions and soil properties for the three cropping seasons are given in **Supplementary Table 1**. In each season, trials were established on fields with a cropping history of either cassava or finger millet as pre-crops, or remained fallow in the season before the trials were established. While rainfall distribution was normal for the season 2014-15, extremely strong monsoon rainfall caused heavy flooding in the season 2015-16. In the season 2016-17, there were severe drought spells after the crop establishment.

Plants: Variety and Provider

At Bangalore, for all three seasons of the field trials, GPU-28 (finger millet—*Eleusine coracana*) and BRG-2 (pigeon pea—*Cajanus cajan*) varieties, were used. The seeds were obtained from National Seed Project at GKVK, Bangalore, and had a germination percentage of more than 95%. At Kolli Hills, Suratai Kelvaragu (finger millet) and SA-1 (pigeon pea) varieties were used, except for the season 2015/16, where Vamban-3 (seed provided by Tamil Nadu Agricultural University) was sown instead of SA1 with the objective to reduce the relatively long duration of SA-1 compared to BRG-2. However, due to uneven flowering of Vamban-3 (National Pulses Research Centre, Vamban) at Kolli Hills, we reverted to SA-1 variety for the third

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Abbreviations: DS, direct sowing system; TP, transplanting system; FM, finger millet; PP, pigeon pea; AMF, arbuscular mycorrhizal fungi; PGPR, plant growth promoting rhizobacteria; AMFfm, AMF applied to finger millet; AMFpp, AMF applied to pigeon pea.

season field trial. Germination percentage of the seeds ranged from 80 to 90% across sites and year.

Microbial Inoculants: Strains, Provider, and Multiplication

Two species of AMF inoculants *viz., Rhizophagus fasciculatus* (AMFfm) and *Ambispora leptoticha* (AMFpp) were selected from finger millet (Rao et al., 1983a) and pigeon pea (Reddy and Bagyaraj, 1991), respectively. PGPR strain (*Pseudomonas* sp. MSSRFD41 isolated from finger millet rhizosphere) selected for both finger millet and pigeon pea (Sekar and Prabavathy, 2014), and *Rhizobium* commercial product for pigeon pea alone were used for this study. The two AMFs were multiplied in a vermiculite based carrier material (substrate) using Rhodes grass (*Chloris gayana*) as host plant for 40 to 45 days. The inoculum was prepared by allowing the grass to dry after which the roots were chopped into pieces (ca., 0.5–1 cm), and mixing homogenously in the same substrate in which the grass was grown. Dried (ca. 5% moisture w/w) inoculum thus prepared was evaluated for the infective propagules through MPN method. The harvested AMFpp inoculum consisting of 24 spores per g substrate, was applied at the rate of 5 g inoculum per pigeon pea seedling in polybags and 278 kg inoculum per ha in field as a band application. AMFfm inoculum consisting of 15 spores per g substrate was applied at the rate of 444 kg inoculum per ha for finger millet as a band application. The PGPR strains were multiplied in King's B medium and a liquid culture consisting of 1 x 10^9 CFU per ml of *Pseudomonas* sp. The liquid culture was prepared by dissolving the pellet using sterile water and then mixing with PEG, glycerol and PVP (3% v/v). MSSRFD41 was applied as seed coating at the rate of 5 ml per kg seed. Additionally, a band application (along the planting rows) was applied at the rate of 49.5 l (consisting 1 x 10^9 CFU per ml). All control treatments for PGPR were treated with "cell free" broth with same volume as with cells (*Pseudomonas*) and all AMF controls were treated with vermiculite alone. Rhizobium (consisting of 1 x 10^9 CFU per ml) was applied as seed inoculation at a rate of 10 ml per kg of all treatments with pigeon pea.

Pre-culturing System of Pigeon Pea Seedlings

Pigeon pea seedlings were raised in polythene bags as described by Praharaj et al. (2015). In brief, polythene bags of 10 cm diameter x 15 cm height were used and filled with a mixture of field soil: FYM: sand mixture ratio of 15:1:1 (v:v:v). A seeding hole of about 5 cm was made using a stick, into which *Ambispora leptoticha* inoculum with vermiculite as carrier material was first added at the bottom of the seeding hole at 5 g per plant. Pigeon pea seeds treated with PGPR and rhizobium at a dose of 10 ml and at 5 ml per kg of seed, respectively, were added to two seeds per hole. For the no inoculation treatments, only the carrier material was added and *Rhizobium* (5 ml per kg of seed) treated pigeon pea seeds were sown in the hole. Seedling were thinned after 2 weeks to leave one seedling per polythene bag. The pigeon pea seedlings, thus raised, were watered based on need and were grown until 40 to 45 days before transplanting into the field at the time of sowing finger millet.

Establishment of Field Trials

Experimental Design in Seasons 2014-15 and 2015-16

The experiment was laid out with a plot size of 6.6 × 3.9 m in a randomized block design (RBD) with a total of 20 treatments: **T1**:Sole crop of finger millet at 100% RDF; **T2**:Sole crop of finger millet at 50% RDF; **T3**:T2 + AMF; T4:T2 +PGPR; **T5**:T2+ AMF+PGPR; **T6**:Sole crop of pigeon pea at 100% RDF; **T7**:Sole crop of pigeon pea at 50% RDF; **T8**:T7 + AMF; **T9**:T7 + PGPR; **T10**:T7+AMF+PGPR; **T11**:Finger millet + Pigeon pea (8:2) inter cropping +100% RDF; **T12**:Finger millet + pigeon pea (8:2) inter cropping + 50% RDF; **T13**:T12 + AMF; **T14**:T12 + PGPR; **T15**:T12+AMF+PGPR; **T16**:Finger millet + Pigeon pea (8:2) inter cropping + 50% RDF +AMF; **T17**:Finger millet + pigeon pea (8:2) + 50% RDF +AMF+PGPR; **T18**:Finger millet + Pigeon pea (8:2) + No fertilizer + No biofertilizer (absolute control); **T19**:Finger millet + pigeon pea (8:2) + No fertilizer + AMF; **T20**:T19 + PGPR; (T1 - T20) (see **Table 1** for further details). Each treatment was replicated four times (=80 plots) per site for seasons 2014-15 and 2015-16 laid out in randomized block design (**Supplementary Figure 1**). The experimental design included (i) three mineral fertilizer levels (100% RDF, 50% RDF, or 0% RDF)—to test the potential of biofertilizer reduce the mineral fertilizer and to compare with the yield obtained at 100% fertilizer dose recommended to farmers; (ii) three cropping systems (finger millet and pigeon pea mono and intercropping), and (iii) two to four levels of biofertilization. For two mineral fertilizer levels (50% RDF and 0% RDF), the combined microbial inoculants [AMF application to Finger millet (AMFfm) Pigeon pea (AMFpp) and PGPR], were tested against a treatment without biofertilizers. At 100% RDF we tested only the no biofertilizer treatments (T01, T06, and T11). In addition, at 50% RDF (T02, T03, T05, T05, T07, T08, T09, T10, T12, T13, T14, and T15), we tested the application of PGPRs and AMF separately and in combination. Additional treatments were T16 – 50% RDF + AMFpp; T17 – 50% RDF + AMFpp+PGPR were used to test the "placement effect" (effect of placing the AMFpp or AMFpp+PGPR at the time of raising pigeon pea seedling in polybag in comparison with applying AMFfm alone or AMFfm+PGPR additionally in field as band application); T18 – 0% RDF + no inoculation (absolute control); T19 – 0% RDF + AMFpp; and T20 – 0% RDF + AMFpp+AMFfm + PGPR.

We used following treatments for verifying each of the four hypotheses indicated above: hypothesis 1: T02, T05, T07, T10, T12, and T15; hypothesis 2: T11, T12, T15, T18, and T20; hypothesis 3: T13, T15, T16, and T17; hypothesis 4: T12, T15, T12d, and T15d.

Experimental Design in Season 2016-17

In the third cropping season (2016-17), the performance of biofertilizer (AMF + PGPR) application on the transplanted vs. direct-sown pigeon pea in the intercropping system was tested (**Table 1**), using six treatments (T11, T12, T15 with transplanting as in the previous years, and, T11d, T12d, T15d with direct-sown

TABLE 1 | An overview of the 20 treatments in cropping seasons 2014-15 and 2015-16 and the six treatments in season 2016-17.

Cropping season	Trt. Nr.	FM planting system	PP planting system	Crop	Min. Fert.	Bio. Fert. applied to PP	Bio. Fert. applied to FM
2014-15 & 2015-16	T01	DS		FM	100	NA	No
	T02	DS		FM	50	NA	No
	T03	DS		FM	50	NA	AMFfm
	T04	DS		FM	50	NA	PGPR
	T05	DS		FM	50	NA	AMFfm+PGPR
	T06		TP	PP	100	No	NA
	T07		TP	PP	50	No	NA
	T08		TP	PP	50	AMFpp	NA
	T09		TP	PP	50	PGPR	NA
	T10		TP	PP	50	AMFpp+PGPR	NA
	T11	DS	TP	FM+PP	100	No	No
	T12	DS	TP	FM+PP	50	No	No
	T13	DS	TP	FM+PP	50	AMFpp	AMFfm
	T14	DS	TP	FM+PP	50	PGPR	PGPR
	T15	DS	TP	FM+PP	50	AMFpp+PGPR	AMFfm+PGPR
	T16	DS	TP	FM+PP	50	AMFpp	No
	T17	DS	TP	FM+PP	50	AMFpp+PGPR	No
	T18	DS	TP	FM+PP	0	No	No
	T19	DS	TP	FM+PP	0	AMFpp	AMFfm
	T20	DS	TP	FM+PP	0	AMFpp+PGPR	AMFfm+PGPR
2016-17	T11	DS	TP	FM+PP	100	No	No
	T12	DS	TP	FM+PP	50	No	No
	T15	DS	TP	FM+PP	50	AMFpp+PGPR	AMFfm+PGPR
	T11d	DS	DS	FM+PP	100	No	No
	T12d	DS	DS	FM+PP	50	No	No
	T15d	DS	DS	FM+PP	50	AMFpp+PGPR	AMFfm+PGPR

pigeon pea) comprising two levels biofertilizers (No inoculation and AMFpp+PGPR) at 50% RDF and one level of biofertilizer (no inoculation) at 100% RDF under intercropping system. Pigeon peas were directly sown at the time of transplanting the polybag seedlings and sowing of the finger millet. Two pigeon pea seeds were sown per seeding hole and later thinned out to have one seedling per hole as before. Sowing in the field was done with the same spacing as in the main trials (see above). Both transplanted and direct sown system resulted in total 24 plots per site. All other operations, such as field preparation and plant protection measures, were the same as in the main trials in previous seasons.

All pigeon pea plants were inoculated with rhizobium. All control treatments for PGPR were treated with "cell free" broth with same volume as with cells (*Pseudomonas*). Similarly, all AMF control plots were amended with vermiculite alone, the carrier material for AMF propagation. A dose of 7.5 t per ha farmyard manure was applied to all plots.

Field Preparation and Inputs

The land was prepared by passing a disc plow followed by a cultivator twice to remove weeds and to crush the soil clods. All plots received a blanket application of FYM at a dose of 7.5 t per ha prior to sowing. The 100% RDF for finger millet and pigeon pea were applied at the rate of 50:40:25 and 25:50:25 NPK (Urea: Single Superphosphate; Muriate of Potassium) kg per ha, respectively. Using custom-made wooden markers, furrows or lines were opened at a row spacing of 30 cm for finger millet and 60 cm for pigeon pea in the main field and as per the plan of layout (**Figure 1**). In each plot, the crops were planted row-wise, with 22 rows of finger millet in monoculture (**Figure 1A**), 11 rows of pigeon pea in monoculture (**Figure 1B**) and 16 rows finger millet plus 4 rows pigeon pea in the intercropping system (**Figure 1C**). Finger millet and pigeon pea harvest area were marked in mono and intercropping system (**Figure 1**). After 20 days of finger millet sowing and transplanting of pigeon pea, thinning and gap filling of finger millet was done manually to maintain target plant density. Weeds were controlled by a manual hoeing 30 days after planting, followed by a hand weeding on 40 days to keep the plot weed free and for better soil aeration. At Bangalore, pigeon pea was protected against pod borer (*Helicoverpa armigera)* incidence through prophylactic measures twice at fortnightly intervals during flowering at pod development stage. At Kolli Hills, neem oil was sprayed against the blister beetles, *Mylabris* spp. (Meloidae: Coleoptera). The crops were grown for about 4 months (finger millet) and pigeon pea grown for 8 and 5 months for SA-1 and Vamban-3 varieties respectively.

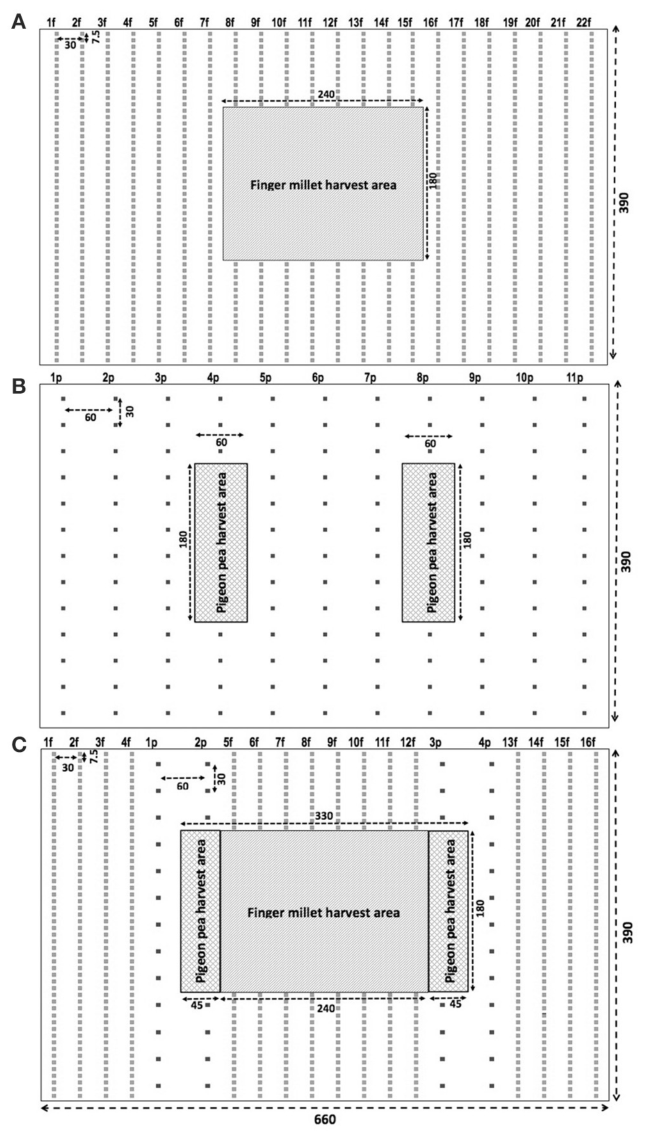

FIGURE 1 | Layout of a plot of **(A)** Finger millet monocropping **(B)** Pigeon pea monocropping **(C)** Finger millet + Pigeon pea intercropping. Plot dimensions are in centimeters. Finger millet harvest area and pigeon pea harvest area are shaded with their respective width and lengths. For clarity, finger millet rows are indicated in a series of 1f,2f,3f…and pigeon pea rows are indicated in a series of 1p,2p,3p.

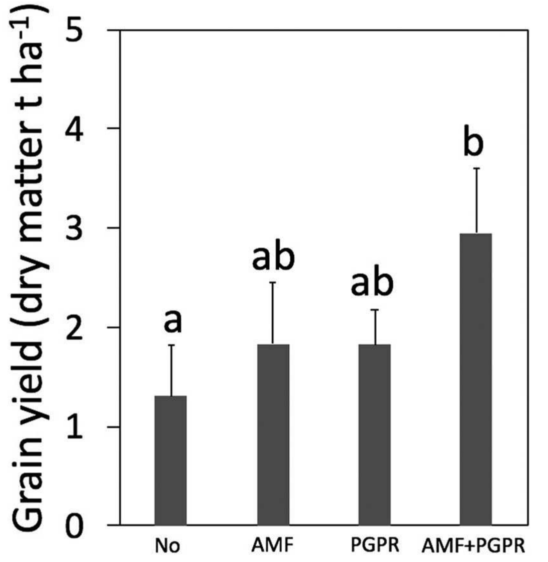

FIGURE 2 | Grain yield of finger millet as affected by single and combined microbial inoculants (AMF+PGPR) at 50% recommended dose of fertilizer (RDF) in Kolli Hills site during 2014-15. Each error bar represents +1 standard deviation of the mean. Bars not sharing the same letters are significantly different at $p < 0.05$.

Harvest, Analyses, and Report

After attaining maturity, finger millet and pigeon pea straw and grains were harvested from their respective harvest area marked in each plot (**Figure 1**). The harvested grains and straw were sun-dried, and their weights were recorded. A subsample of the sun-dried material was oven dried for 24 h at 80°C. Oven dried weight straw and grains were used for calculating the dry matter yield expressed in metric tons per ha. Number of tillers in finger millet were counted in 0.6 m row length (8 plants) and the average per plant was calculated. Seed weights of harvested crops were measured (in grams) in 1,000 and 100 randomly selected seeds of finger millet and pigeon pea, respectively.

Statistical Analyses

Statistical analysis was performed using JMP software v.11. Two-way ANOVA was performed to find if there was any interaction effect between crop and biofertilizer. We found no interaction effect, indicating that the effect of biofertilizers for pure crop (for both finger millet and pigeon pea) was similar. Therefore, we compared the means of the biofertilizer treatment (AMF+PGPR) to the treatments without biofertilizers (No) across each year and site. A *t*-test was performed to find whether the means differed significantly between the "No" and "AMF+PGPR" treatments. In the intercropping system an additional *t*-test for the total mean of both the crops was performed. A multi-axis panel figure was constructed using two or more individual graphs.

RESULTS

Effect of Biofertilizers at 50% RDFs

Across the two sites (Bangalore and Kolli Hills) and seasons (2014-15 and 2015-16), at 50% RDF, there was a general trend of improved grain yields of finger millet and pigeon pea when inoculated with both AMF+PGPR compared with inoculation with either AMF or PGPR alone. During 2014-15, under finger millet monocropping system at Kolli Hills site, the combined inoculation of AMF+PGPR increased the grain yield of finger millet by +126% as compared to uninoculated control (**Figure 2**).

At the Bangalore site, there was a trend of increased grain yields of finger millet and pigeon pea in the mono-

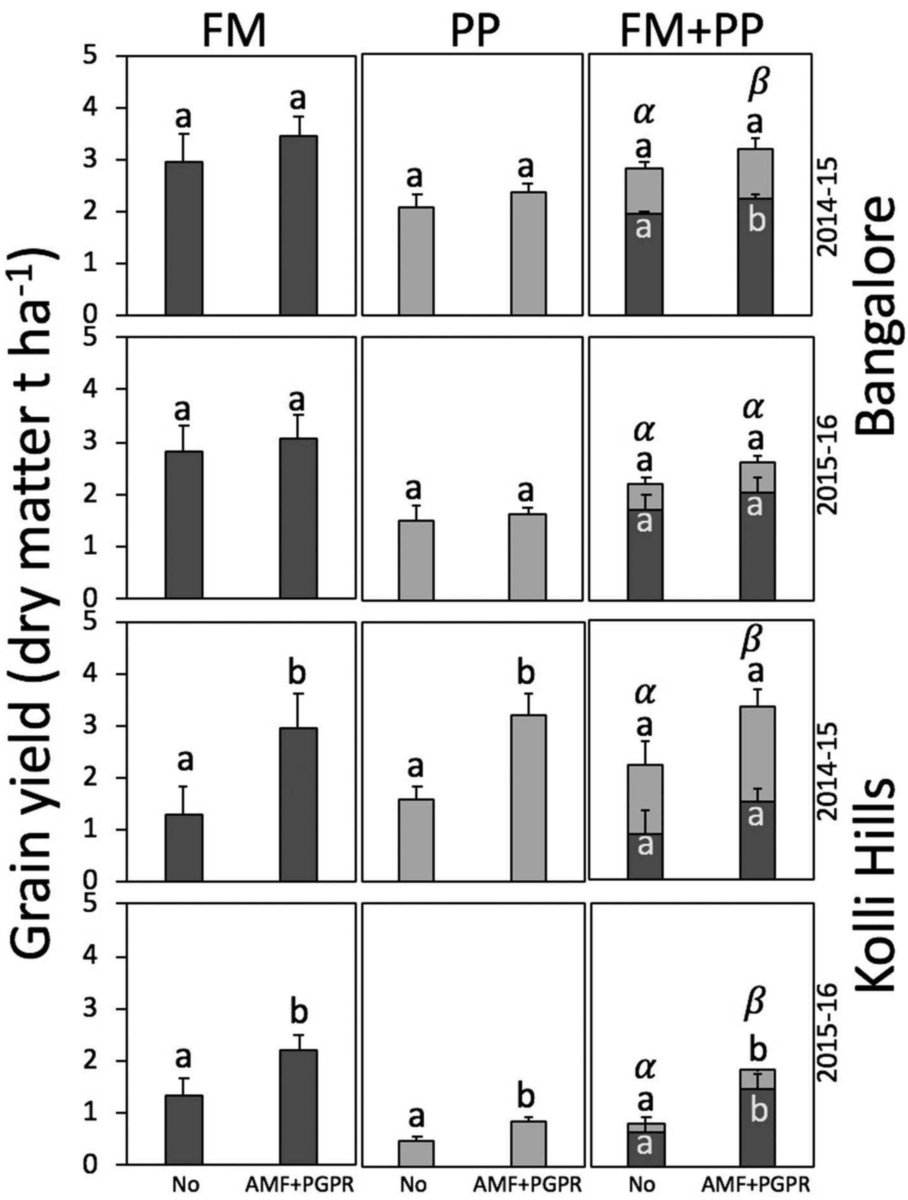

FIGURE 3 | Finger millet (dark shaded bars) and pigeon pea (light shaded bars) grain yield at 50% recommended dose of fertilizer (RDF) at Bangalore and Kolli Hills. Significant differences among means were calculated crop wise. Each error bar indicates +1 standard deviation of the mean. Alpha (α) and beta (β) above the bars indicate letters of significance for combined grain yields of finger millet and pigeon pea. Bars not sharing the same letters are significantly different at $p < 0.05$.

and intercropping systems in response to the biofertilizers, although there was significant effect only during 2014-15 under intercropping system (**Figure 3**). However, at the Kolli Hills site, as compared to the uninoculated control, inoculation of AMF+PGPR improved the grain yields of finger millet and pigeon pea both in monoculture and in the intercropping system. For intercropping yield increase due to inoculation was +126% and +128% during 2014-15 and 2015-16, respectively (**Figure 3**). Relatively lower grain yield of the pigeon pea during 2015-16 may be caused by a change in variety and flooding (see section Materials and Methods).

Effects of Biofertilizers at Three RDFs (0, 50, and 100%) Under Intercropping

At 0% RDFs and 50% RDFs, across the sites and seasons, there was a trend of improved grain yields of finger millet and pigeon pea, due to application of AMF+PGPR (**Figure 4**). At Bangalore, the inoculation of AMF+PGPR increased the combined grain yields of finger millet and pigeon pea by +12 and +13% at 0% RDFs and 50% RDF, respectively. At Kolli Hills during 2014-15 at 0% RDF, the grain yields of pigeon pea and finger millet were +69% higher when inoculated with AMF+PGPR as compared to uninoculated control. There was a general trend that grain yields

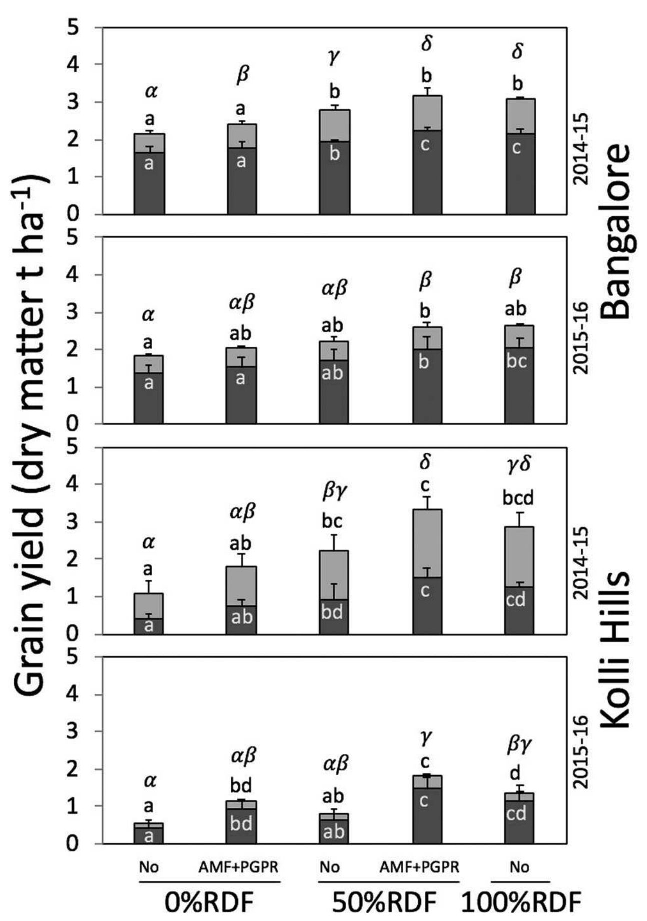

FIGURE 4 | Effect of combined biofertilizer inoculation on grain yield of finger millet (dark shaded bars) and pigeon pea (light shaded bars) in the intercropping system at the 0% recommended dose of fertilizer (RDF) and at 50% RDF as compared to no inoculation at 0, 50, and 100% RDF. Significant differences among means were calculated crop wise. Means were compared crop wise across fertilizer levels. Each error bar represents +1 standard deviation of the mean. Alpha (α) and beta (β) above the bars indicate letters of significance for combined grain yields of finger millet and pigeon pea. Bars not sharing the same letters are significantly different at $p < 0.05$.

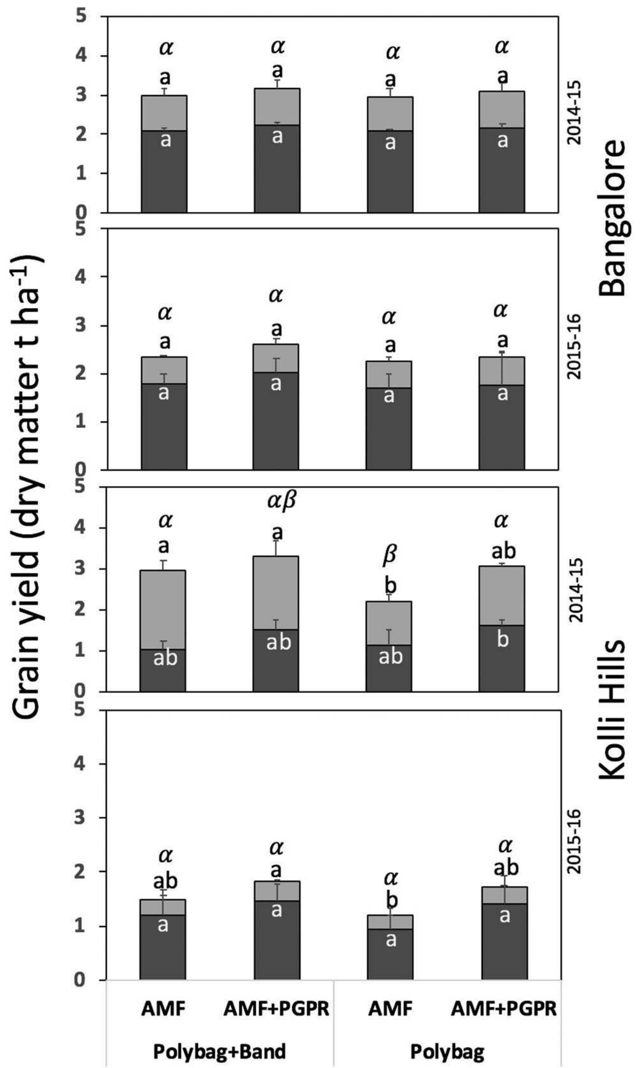

FIGURE 5 | Effect of single inoculation of AMF and combined application of AMF+PGPR in precultured pigeon pea in polybag vs. application in precultured pigeon pea alone and as band in the field on grain yield of finger millet (dark shaded bars) and pigeon pea (light shaded bars) in intercropping system at Bangalore and Kolli Hills during the seasons 2014-15 and 2015-16. Significant differences among means were calculated for total yield of finger millet and pigeon pea. Each error bar indicates +1 standard deviation of the mean. Alpha (α) and beta (β) above the bars indicate letters of significance for combined grain yields of finger millet and pigeon pea. Bars not sharing the same letters are significantly different from each other at $p < 0.05$.

of finger millet and pigeon pea at 50% RDF plus AMF+PGPR were on par (sometimes slightly higher) with the ones obtained at 100% RDF without inoculation. At the Bangalore site, during 2015-16, the combined grain yields of finger millet and pigeon pea at 100% RDF (without AMF+PGPR) was identical with the grain yield obtained at 50% RDF with AMF+PGPR. Similarly, during 2014-15, at Kolli Hills site, the combined grain yields of finger millet and pigeon pea at 50% RDF plus AMF+PGPR was +14% higher than the grain yields obtained at 100% RDF without AMF+PGPR.

"Placement Effect" of the Biofertilizers at 50% RDF

An overview of the "placement effect" (for definition see Materials and Methods section) is presented in **Figure 5**. In brief, the "placement effect," as we hypothesized in this study, is to find whether application (placement) of AMF biofertilizers to the seedlings of pigeon pea grown in the polybags would be sufficient to obtain the grain and straw yields of pigeon pea (transplanted) and finger millet on par with the yields obtained when AMFs applied to both the crops. There was a trend showing that grain yields of pigeon pea and finger millet due to application of AMFpp alone was on par with the grain yields obtained when both AMFpp+AMFfm were

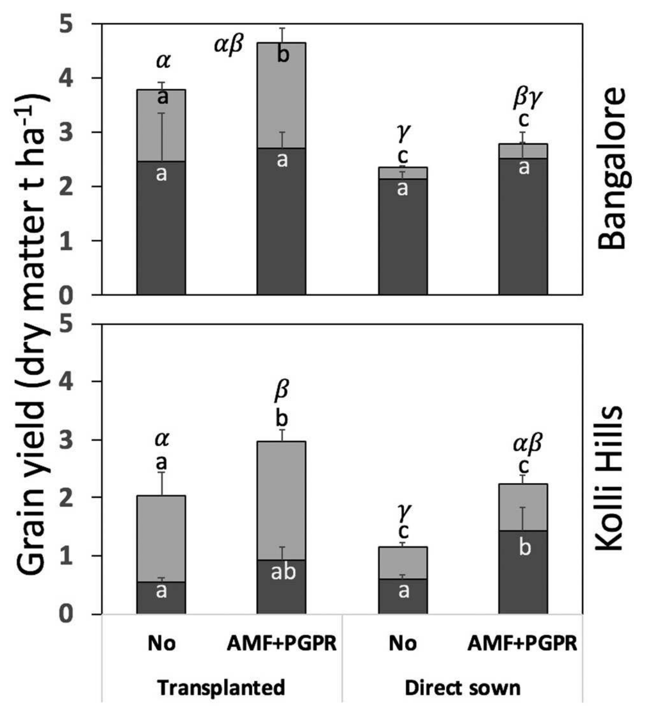

FIGURE 6 | Effect of no inoculation and combined application of AMF and PGPR on grain yields of finger millet (dark shaded bars) and pigeon pea (light shaded bars) in transplanted vs. direct sown intercropping system at Bangalore and Kolli Hills during the season 2016-17. Significant differences among means were calculated for total yield of finger millet and pigeon pea. Each error bar indicates +1 standard deviation of the mean. Alpha (α) and beta (β) above the bars indicate letters of significance for combined grain yields of finger millet and pigeon pea. Bars not sharing the same letters are significantly different from each other at $p < 0.05$.

applied (**Figure 5**). A similar trend was observed in the grain yields obtained when AMFpp was applied in combination with PGPR. At Bangalore site, during 2014-15, the grains yields of finger millet and pigeon pea obtained by applying AMFpp+PGPR during preculturing pigeon pea (polybag) and in addition applying the AMFfm as band to the finger millet did not significantly increase the grain yields of finger millet and pigeon pea compared to applying the AMFpp+PGPR to the pigeon pea during the polybag alone. Similarly, in the same year, at Bangalore site, applying AMFpp alone to the pigeon pea during the preculturing (polybag) stage had the same grain yields of finger millet and pigeon pea when AMFfm was added as band in addition to the AMFpp. In contrast to the Bangalore site, at Kolli Hills (with inherently poor soil), in the first year, application of AMFpp and AMFfm during preculturing and band application, respectively, showed higher values (+34%) than application of AMFpp alone during preculturing. A similar trend was also observed in the second year although the effect was not significant. At Kolli Hills site, there were no significant "placement effect."

Effect of Biofertilizers in Transplanted vs. Direct Sown Pigeon Pea Intercropping System

As expected, the transplanted system showed distinctly higher grains yields of pigeon pea than the direct-sown system at both sites, both under AMF+PGPR and no-inoculation treatments. Inoculation with AMF+PGPR increased pigeon pea yield in the transplantation system at both sites; the effect of transplanting system on finger millet was significant only in Kolli Hills in the direct-sown system (**Figure 6**).

Effect of Biofertilizers on the Straw Yields and Other Growth Parameters of Finger Millet and Pigeon Pea

In general, there were no significant changes in the straw yields of both the crops due to inoculation at the Bangalore site for both seasons (**Supplementary Figure 2**). At Kolli Hills, for both seasons, the straw yields of pigeon pea in monocropping were significantly improved due inoculation as compared to no inoculation. Similarly, during the season 2014-15, the combined straw yields of pigeon pea and finger millet in intercropping system were significantly higher in inoculated treatments as compared to non-inoculated treatments. At 50% RDF, across site and year, the combined straw yield of pigeon pea and finger millet in the inoculated treatments were on par with the yields obtained at 100% RDF without inoculation (**Supplementary Figure 3**) (for example, see data for Bangalore and Kolli Hills during 2014-15). On the other hand, the finger millet and pigeon pea combined straw yields did not differ significantly between the treatments, particularly during the season 2015-16 at Kolli Hills. In general, the straw yields were not affected by the placement of the biofertilizer (**Supplementary Figure 4**), i.e., either in polybag alone or application as both band as well as in polybags. Similarly, inoculation did not significantly improve the straw yields under transplanting system (**Supplementary Figure 5**). Improved crop yields, particularly the pigeon pea grains, may be due to relatively better establishment of pigeon pea seedling during the pre-culturing stage in polybags (**Supplementary Figure 6**), although the results were not consistent across sites and year. Similarly, the higher grain yields in finger millets and pigeon pea may be due to improved number of tillers and number of branches per plant respectively (**Supplementary Figure 7**). Inoculation with AMF+PGPR caused a slightly increase in seed weights in finger millet and pigeon pea (**Supplementary Figure 8**).

DISCUSSION

Here we show that biofertilizer application can be useful for improving grain yields under 0% RDF (organic farming) and 50% RDF (reduced fertilizer input) for monocultures and intercropping of pigeon pea and finger millet. For pigeon pea, this is true both when sown directly-a common practice adopted by marginal farmers-or when transplanted in polybags-a labor intensive improved system (Fehle, 2016) (**Figure 7**). Specifically,

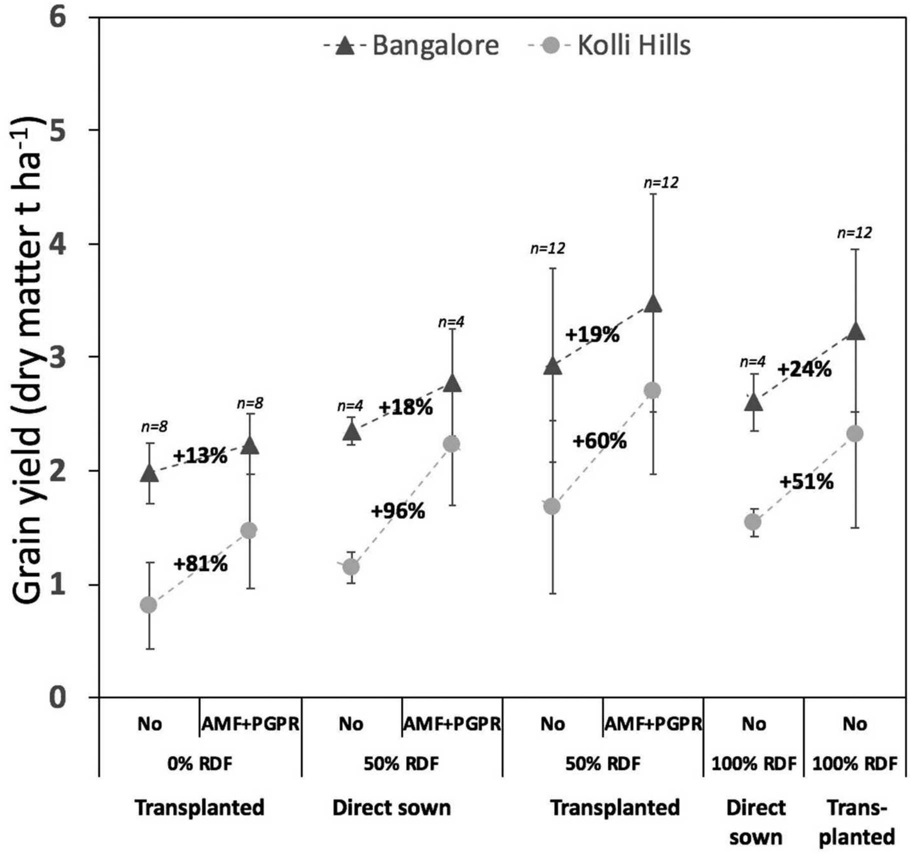

FIGURE 7 | Summary graph depicting potential of attaining higher grain yields of pigeon pea and finger millet in two sites under three mineral fertilizer levels and two different sowing systems through application of AMF+PGPR. Each error bar indicates ±1 standard deviation of the mean. Dotted lines connecting two points are used only for the purpose to indicate the trend for better visualization of the effects of inoculation. Bangalore, site with high inherent soil fertility; Kolli Hills, site with low inherent soil fertility. RDF, recommended dose of chemical fertilizers; no, no inoculation; AMF, arbuscular mycorrhizal fungi; PGPR, plant growth-promoting rhizobacteria; transplanted, transplant.

we show that total grain yield of pigeon pea and finger millet under intercropping system can be systematically improved by applying biofertilizers (AMF and PGPR) separately or together in pigeon pea-finger millet mono as well as in intercropping system. We found that application of biofertilizers + 50% RDF improved grain and straw yields. Reduced mineral fertilizer input through supplementing with biofertilizers could minimize certain detrimental effect, such as leaching of nitrogen to nearby water bodies, potential negative impact on microbial diversity, including mycorrhizal fungi. Furthermore, on economical view point, attaining grain yield equivalent to 100% RDF using 50% RDF plus biofertilizers shows potential savings for the farmers on the cost of mineral fertilizer input despite cost for purchasing biofertilizers need to be accounted. Improved yields obtained for pigeon pea and finger millet in this study indicates there is a great potential in systematically (through biofertilizers) reducing the existing large yield gap between potential yield (ca. 2.5 and 3.5 t per ha for pigeon pea and finger millet, respectively) and average yields (ca. 0.8 and 1.5 t per ha for pigeon pea and finger millet, respectively) obtained on farmer's fields in Asia (Ashok et al., 2010; Varshney et al., 2012). Below we discuss scenarios under which biofertilizers either in combination with transplanting or intercropping system affects grain yields of pigeon pea and finger millet.

Biofertilizer Effect on Grain Yield

Various studies have shown that application of single microbial species or consortia improve crop growth, including finger millet and pigeon pea (Mäder et al., 2011; Gupta et al., 2015; Schutz et al., 2018), although the outcome may also depend on the host genotype. Our results corroborates with Mäder et al. (2011) showing that combined application of AMF+PGPR improves grain yield than application of single microbial inoculant. Although it was beyond our scope to understand exact mechanism for improved grain yields in pigeon pea and finger millet due to application of biofertilizers, earlier studies have shown that better phosphorus uptake via AMF, crop tolerance to biotic and abiotic stresses via PGPRs, regulation of plant hormones are among the common mechanism through which biofertilizers help to increase crop growth (see reviews (Aroca and Ruiz-Lozano, 2009; Reddy, 2012; Mathimaran et al., 2017) in this regard). In our case the improved finger millet and pigeon pea growth may be primarily due to the choice of AMFpp and AMFfm which were originally isolated from the rhizosphere of pigeon pea and finger millet respectively via screening (Rao et al., 1983a,b; Reddy and Bagyaraj, 1991). Similarly the PGPR strain MSSRFD41 was isolated from finger millet and may have been co-evolved as better symbiont for the host (Sekar et al., 2010, 2018). The observed variation in the microbial performance across the sites and years could be attributed to the differences in the soil physio-chemical properties and climate (**Supplementary Table 1**).

Furthermore, improved crop yields, particularly the pigeon pea grains, may be caused by better establishment of pigeon pea seedling during the pre-culturing stage in polybags, although the results were not consistent across sites and season (**Supplementary Figure 6**). Similarly, the higher grain yields in finger millets and pigeon pea may can be explained by higher number of tillers and number of branches per plant, respectively (**Supplementary Figure 7**). Inoculation seems to have only a slight effect on the increase in seed weights in finger millet and pigeon pea (**Supplementary Figure 8**).

In general, we observed that the straw yields were only marginally improved through biofertilizer application as compared to grain yields (see **Supplementary Figures 2–5**). We are aware that crop residue (or straw) is major factor in the soil nutrient cycles (Correia et al., 2005) and for livestock (Chandrasekharaiah et al., 2003). Nevertheless, considering the significance of our work, especially from farmers view point, here we primarily report only the grain yields. Furthermore, reporting additional data such as root biomass/architectures/microbial diversity is beyond scope of this study, although this would have allowed us to better interpret our results.

Effect of Transplanting and Biofertilizer on Grain Yield

Transplanting system is common in several crops, including finger millet and pigeon pea (Ghosh et al., 2007; Praharaj et al., 2015; Thilakarathna and Raizada, 2015), primarily due to yield advantage associated with better establishment of root and resistance to pest and diseases (Ashok et al., 2010; Mallikarjun et al., 2015; Praharaj et al., 2015). However, benefits of transplanting method in combination with biofertilizers has not been established yet. Here we show that pre-colonized pigeon pea under transplanted system results in higher grain yields of pigeon pea and finger millet, particularly under monocropping system, which may be attributed to the better root-growth, nutrient uptake and improved soil structure via the AMFs (Cartmill et al., 2012). Due to less labor and input costs, direct sown crops is common farming practice among marginal farmers but in recent times transplanting system of pigeon pea is being advocated due to higher yields attainable particularly under delayed monsoon and avoiding pest infestation during early stages of direct sown crops (Praharaj et al., 2015). Our study shows that transplantation benefits in pigeon pea can be further improved by application of biofertilizers. Improved yield of pigeon pea and finger millet due to biofertilizers under transplanting system could serve as criteria for the farmers to consider adopting a labor intensive system (Fehle, 2016). Inoculation of pigeon pea during pre-culturing stage would reduce the labor cost and quantity of inoculum required of applying biofertilizer in standing crops. Furthermore, it would be practically easier to apply any bio-fertilizer in small polybags than applying large fields.

Effect of Intercropping and Biofertilizers on Grain Yield

Intercropping is considered a productive system through improved soil biodiversity (Li et al., 2007), nutrient acquisition (Brooker et al., 2015), particularly N and P. On the other hand, intercropping can be less productive due competition for resources such as light and nutrient. It is known that beneficial microbes play a key role in below-ground resource-sharing but their role in intercropping is less explored primarily due to technical challenges such as tools required to accurately measure the nutrient and water sharing between the plant and the symbionts. We observed, compared to no biofertilizer, application of AMF+PGPR improved the combined grain yields of finger millet and pigeon pea although the results were not always same across season and site, which may be due to "unequal return of investments" between pigeon pea and finger millet via the common mycorrhizal network (Walder et al., 2012), a possibility that needs to be investigated. Improved growth of finger millet under intercropping system may have been due to possible "bioirrigation" via the common mycorrhizal network (Saharan et al., 2018), although this needs to be further verified using stable isotopes and by measuring appropriate physiological parameters under field conditions.

CONCLUSIONS

Our comprehensive field study conducted for three successive cropping seasons and at two geographical locations clearly shows the potential of a combined application of AMF (*Ambispora leptoticha* and *Rhizophagus fasciculatus*) + PGPR (*Pseudomonas* sp. strain MSSRFD41) to considerably reduce the mineral fertilizer input without jeopardizing the yields in pigeon pea and finger millet. The use of biofertilizers turned out to be efficient not only in systems with reduced mineral fertilizer input, but also in systems with addition of only farmyard manure. Thus, biofertilization is a sustainable and viable technology both in low-input and organic farming systems, particularly in transplanted pigeon pea-finger millet systems. Biofertilizers in combination with transplanting may offer an efficient cropping system of pigeon pea and finger millet because yield increase was found to be additive. Our comprehensive analysis would form a basis to improve the yield and productivity of finger millet and pigeon pea, particularly for marginal farmers of Southern India. Nevertheless, our results obtained in experimental fields need to be interpreted with caution when recommending the biofertilizer application to marginal farmers, particularly with different soil and environmental conditions not tested in this work.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

PM, NM, TB, AK, SJ, VP, MT, and EA designed the study. SJ, VP, PY, BM, MT, EA, MS, and NM conducted the experiment. SJ, VP, PY, BM, MT, EA, and MS provided data. NM and PM analyzed the data. NM wrote the manuscript. DB provided AMF cultures. VP and SJ provided PGPR culture. All authors read and contributed in the revision of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2020.00088/full#supplementary-material

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Impact of Diverse Annual Forage Mixtures on Weed Control in a Semiarid Environment

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Intercropping with different crop species and different spatial patterns is suggested to lead to increased competition with weeds and reduced weed abundance and biomass. In this study, our objective was to explore the ability of multi-species annual forage crop mixtures to control weeds while providing productive forage. We utilized field and greenhouse trials to evaluate the impact of different crop mixtures and row spacing on weed control in the semi-arid Brown soil region of southwestern Saskatchewan, Canada. Seven different mixtures of up to eight annual forage crops were grown with row spacing of 15 or 30 cm in a replicated field trial. Weed abundance and biomass were significantly affected by crop species mixtures. Crop mixtures that contained radish and barley generally had higher weed suppression. Row spacing did not significantly impact weed abundance or biomass across the treatments. Results were similar over both years in spite of drastically different precipitation conditions. Forage production was significantly different between cropping mixtures in July in both years. The barley-radish mixture had the highest crop biomass in July, and this early crop production was linked to weed suppression because crop biomass had a significant effect on weed abundance and biomass in July, but not August. A greenhouse experiment was used to further evaluate the crops (i.e., barley and radish) that demonstrated the highest weed suppressive activity in the field trial. Crop identity and row spacing were both significant factors affecting weed and crop biomass production. Radish exhibited stronger control of common lamb's quarters (*Chenopodium album*) compared to barley, but the binary mixture of the two species produced the highest crop biomass and equivalent weed control compared to the radish monoculture. This research suggests that cropping with multiple species (particularly forage radish) may be an effective way to control weeds in semiarid environments.

Keywords: weed suppression, multi-species forage mixtures, polyculture, row spacing, intercropping

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INTRODUCTION

The shift toward more sustainable agricultural practices due to concerns over biodiversity loss, environmental pollution, and greenhouse gas emissions has promoted reduced reliance on agrochemicals (Gomiero et al., 2011). This is particularly relevant for weed control in annual cropping systems due to the extensive use of herbicides. Diversification of cropping patterns and crop species selection may provide one way to lower reliance on chemical inputs. Multiple species cropping (polyculture) is the spatial or temporal intermixing of growing multiple crop species selected based on their contributions to the system (e.g., legumes included for nitrogen fixation) or complementarity with other species [e.g., varying plant architecture and resource use Anil et al., 1998] on the same land base in the same year. Multiple cropping systems can increase productivity through resource partitioning and other ecosystem benefits may accompany these diverse systems including increased soil health and water use efficiency (Anil et al., 1998; Bedoussac and Justes, 2011). In many cases, polycultures have also been associated with weed suppression via increased competition or allelopathic potential (Liebman and Dyck, 1993; Mohler, 2001; Szumigalski and Van Acker, 2005).

Important factors that influence weed dynamics in multiple cropping or intercropping systems are planting density, row spacing, and selection of crop species. From an agronomic perspective, it has long been suggested that increased crop density can reduce the competitive ability of weeds, although this is not always correlated with narrower row spacing (Mohler, 2001). In general, narrower row spacing of crops should increase the interception of light by crop plants, and row intercropping with species of varying canopy structure can increase light use efficiency and increase the competitive pressure on weeds (Anil et al., 1998). Seeding functionally diverse crop mixtures in different densities and arrangements can increase the competitive ability and resource utilization of the crop, both of which can reduce weed abundance (Lowry and Smith, 2018). In a meta-analysis, Verret et al. (2017) found that intercropping with a companion crop (i.e., a crop not harvested as a cash crop) resulted in significantly lower weed biomass and often higher crop yield. For example, several studies have shown that intercropping mixtures had better weed suppression than either crop planted alone (Izaurralde et al., 1993; Hauggaard-Nelson et al., 2001; Nelson et al., 2012; Wang et al., 2012) or had comparable weed suppression to the better of the crops planted alone (Poggio, 2005; Deveikyte et al., 2009; Begna et al., 2011). However, the opposite has also been found, for example, intercropped mixtures of maize and climbing bean didn't show any improvement in weed control over the monoculture maize and in some cases decreased crop yield (Nurk et al., 2017). Most intercropping studies focus on binary mixtures, and little is known about weed dynamics in annual intercropping with three or more crops, particularly in temperate forage-based systems.

In this study, we explored the potential for weed control through the combination of two aspects of intercropping—crop diversity and variable row spacing—in annual forage crops in the semiarid prairie region of southwestern Saskatchewan, Canada. Annual forage crops are currently being evaluated for their potential as a low-input addition to standard crop rotations in Saskatchewan, and maximizing low-input weed control in these crops will enhance the benefit of this system for producers. Our hypotheses were: (1) higher crop species diversity will be better at suppressing weeds, and (2) narrower crop row spacing will reduce weed abundance and biomass. The hypotheses were tested in two field trials followed by two greenhouse trials.

MATERIALS AND METHODS

Site

The field trials were located on a Brown Chernozem soil at the Swift Current Research and Development Center, southeast of Swift Current, Saskatchewan (50°16′N lat., 107°43′W long., 824 m elev.). The land for the 2015 study was seeded to oats in 2013 and chem fallowed in 2014, and the land for the 2016 study was seeded to wheat in 2014 and chem fallowed in 2015. Both sites were tilled and harrow packed prior to seeding.

Field Trials

Field trials were carried out in the summers of 2015 and 2016. Eight annual forage crops were selected that are known to grow well in the local environment, and that were also being tested in long-term multi-species cropping trials. The species represent four functional groups: cool season grasses—barley (*Hordeum vulgare* "AC Metcalfe"), oats (*Avena sativa* "Common No. 1"), triticale (*Triticosecale* "Bunker"); a warm season grass—corn (*Zea mays* "Roundup Ready Corn 2"); legumes—forage pea (*Pisum sativum* "CDC Leroy"), hairy vetch (*Vicia villosa* "Common No. 1"); and brassicas—radish (*Raphanus sativus* "Common No. 1"), turnip (*Brassica rapa* subsp. *rapa* "Purple Top"). These crops were used in seven different mixtures (see **Table 1**) seeded in two different row spacings: species planted together in rows 30 cm apart, or planted in alternating rows 15 cm apart. Total crop densities were the same in all plots regardless of row spacing.

The experimental design was a full factorial randomized complete block with the seven cropping treatments, two row spacing treatments, and four blocks. Each block had 14 plots measuring 6 m long by 1.8 m wide. These proportions matched the dimensions of the self-propelled hydrostatic seeder used, which seeded six rows with 30 cm between rows. The 15 cm spaced treatments were seeded in a second pass (a second pass without seeding was included on the 30 cm plots). Crop density was 50 live seeds per linear meter at the 15 cm spacing and 100 live seeds per meter at 30 cm, resulting in the same amount of live seeds per unit area. In the mixtures, the component crops were seeded in equal proportions. A seeding depth of 2 cm was selected as an intermediate depth suitable for all species. Plots were seeded on June 4, 2015 and June 3, 2016. The later seeding dates are a reflection of dates selected for the provision of late-season grazing material as well as environmental conditions at the time of seeding (e.g., extremely wet spring in 2016). Data

TABLE 1 | List of crop mixtures in the 2015 and 2016 field trials.

Mixture	Crops
1	Barley
2	Oats, peas
3	Barley, radish
4	Triticale, corn, peas, radish
5	Barley, corn, peas, hairy vetch
6	Barley, oats, hairy vetch, turnip
7	Barley, triticale, oats, corn, peas, hairy vetch, radish, turnip

collection from each plot included crop biomass (clipped at 5 cm from a 0.25 m^2 quadrat, and dried at 60°C) and weed count and identification (within a 0.25 m^2 quadrat). Sampling dates were July 22 and August 20 in 2015, and July 19 and August 22 in 2016. In 2016 weed biomass was also assessed by clipping, drying and weighing all weeds in the 0.25 m^2 quadrat at the time of crop biomass sampling.

Greenhouse Trials

Following the 2015 field trial, the crop mixture with the best weed control (barley-radish mixture) was selected for further studies in the greenhouse. As radish was not grown as a monoculture in the field trial, we wanted to investigate the relative impact of both crops on weed control. The greenhouse trial was conducted twice, in 2016 and 2018. In the greenhouse, 24 plastic bins (53 cm long × 39 cm wide × 18 cm high) were filled with a soil mix of 1 part field soil, 1 part silica sand, and 1 part potting mix (Sunshine Mix #4, Sun Gro Horticulture). The cropping treatments were barley monoculture, radish monoculture, and barley-radish mixture (grown in the same rows). In each bin, two crop rows were seeded, with a spacing treatment of either 15 cm apart or 30 cm apart. Crops were seeded at the same rate as the field trials (100 live seeds per linear meter) resulting in two rows of 50 plants per bin. In the very middle of the bin between the cropping rows, *Chenopodium album* (common lamb's quarters) was seeded and thinned back to 20 plants after emergence. *C. album* was selected because it is a common agricultural weed in Western Canada that was frequently present in our field trials. An additional control treatment of *C. album* seeded alone was included. Three replicates of each treatment were seeded (barley at 15 cm, barley at 30 cm, radish at 15 cm, radish at 30 cm, barley and radish at 15 cm, barley and radish at 30 cm, and weeds alone) resulting in a total of 28 bins. The greenhouse conditions were set for a 14 h day length, with a day temperature of 20°C and a night temperature of 15°C. Plants were watered consistently as needed (based on measuring soil moisture levels), and fertilizer (20-20-20) was applied twice in the middle of each trial, when plants showed signs of nutrient stress. Pots were rotated on the bench every 2 weeks to reduce spatial effects. Plants were harvested 8 weeks after seeding. Measurements included crop biomass, weed height, weed shoot dry weight, and weed root dry weight.

Statistical Analysis

All statistical analyses (field and greenhouse) were carried out in R v. 3.3.1 (R Core Team, 2016), using the packages Lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2014). A mixed model fit via lmer was used with row spacing and crop mixture as fixed effects and block as a random effect. Multiple comparisons were made using the function difflsmeans. In both the field and greenhouse trials, preliminary analyses indicated that there were significant year by treatment effects, so in both studies the 2 years were analyzed independently. Generalized linear models (GLM) were used to determine if there was an effect of crop biomass production on weed biomass and abundance.

RESULTS AND DISCUSSION

Field Trials

Weed abundance was significantly affected by crop mixtures in both July and August in 2015 and in July 2016 (**Tables 2**, **3**). Total weed abundance was much lower in 2016 than 2015 and the barley-radish mixture had the lowest weed abundance overall. The four species mix (triticale-corn-peas-radish) also had relatively low weed populations over both site-years, and the eight species mix had low weed abundance in 2015. Treatments that included radish generally had fewer weeds than the other

TABLE 2 | Mixed model results of weed abundance (2015, 2016), weed biomass (2106), and crop biomass (2015, 2016) with crop spacing and crop mixture from the field trials.

	Factor	df (num, den)	Weed abundance		Weed biomass		Crop biomass	
			F	*p*	*F*	*P*	*F*	*p*
2015								
July	Spacing	1, 39	0.058	0.811	–	–	4.283	**0.045**
	Mixture	6, 39	5.807	**<0.001**	–	–	5.519	**<0.001**
	Spacing × mixture	6, 39	1.072	0.396	–	–	1.835	0.117
August	Spacing	1, 39	3.055	0.088	–	–	1.049	0.312
	Mixture	6, 39	4.321	**0.002**	–	–	2.620	**0.031**
	Spacing × mixture	6, 39	0.339	0.912	–	–	0.280	0.943
2016								
July	Spacing	1, 39	1.074	0.307	0.184	0.670	4.132	**0.049**
	Mixture	6, 39	2.693	**0.028**	4.458	**0.002**	4.728	**0.001**
	Spacing × mixture	6, 39	1.572	0.181	0.487	0.814	1.326	0.269
August	Spacing	1, 39	0.639	0.429	2.349	0.133	0.724	0.400
	Mixture	6, 39	1.172	0.341	0.768	0.600	1.507	0.201
	Spacing × mixture	6, 39	0.790	0.583	0.084	0.998	1.322	0.270

Bold values are significant at $p < 0.05$.

TABLE 3 | Mean weed abundance by crop mixture treatment and row spacing (see **Table 1** for list of cropping treatments) in July and August, from the 2015 and 2016 field trials.

		MEAN WEED ABUNDANCE (per 0.25 m^2)		
	Mixture	**30 cm spacing**	**15 cm spacing**	**Mean ± sem**
2015				
July	1	23.3 ± 9.6	16.8 ± 6.7	**20.0 ± 5.6** BC
	2	45.3 ± 12.6	39.5 ± 20.6	**42.4 ± 11.2** A
	3	8.5 ± 2.8	0.8 ± 0.8	**4.6 ± 2.0** C
	4	11.3 ± 5.1	8.5 ± 5.5	**9.9 ± 3.5** C
	5	41.8 ± 20.2	30.5 ± 10.3	**36.1 ± 10.7** AB
	6	8.3 ± 3.2	33.3 ± 15.2	**20.8 ± 8.6** BC
	7	9.0 ± 3.2	10.5 ± 7.3	**9.8 ± 3.7** C
	Mean	**21.0 ± 4.4**	**20.0 ± 4.6**	
August	1	16.3 ± 7.3	6.8 ± 2.0	**11.5 ± 3.9** C
	2	31.5 ± 11.1	28.8 ± 20.2	**30.1 ± 10.7** A
	3	8.0 ± 6.4	1.5 ± 0.9	**4.8 ± 3.2** C
	4	6.3 ± 2.7	7.8 ± 5.8	**7.0 ± 3.0** C
	5	32.0 ± 19.5	18.8 ± 12.8	**25.4 ± 11.1** AB
	6	20.3 ± 14.6	8.0 ± 4.7	**14.1 ± 7.5** BC
	7	6.5 ± 2.1	4.8 ± 3.1	**5.6 ± 1.8** C
	Mean	**17.3 ± 4.1**	**10.9 ± 3.6**	
2016				
July	1	12.0 ± 0.8	11.3 ± 0.3	**11.6 ± 0.4** A
	2	13.0 ± 2.7	10.8 ± 1.5	**11.9 ± 1.5** A
	3	8.3 ± 1.7	4.3 ± 1.0	**6.3 ± 1.2** B
	4	6.8 ± 1.3	10.8 ± 1.8	**8.8 ± 1.3** AB
	5	12.3 ± 1.1	9.5 ± 1.0	**10.9 ± 0.9** A
	6	12.5 ± 4.2	9.0 ± 0.0	**10.8 ± 2.0** A
	7	7.5 ± 1.4	10.0 ± 2.0	**8.8 ± 1.3** AB
	Mean	**10.3 ± 0.9**	**9.4 ± 0.6**	
August	1	4.8 ± 1.5	3.5 ± 1.5	**4.1 ± 1.0**
	2	8.3 ± 2.1	8.8 ± 3.0	**8.5 ± 1.7**
	3	7.3 ± 4.0	1.8 ± 0.3	**4.5 ± 2.1**
	4	5.8 ± 3.5	2.8 ± 1.2	**4.3 ± 1.8**
	5	7.3 ± 5.7	5.3 ± 1.8	**6.3 ± 2.8**
	6	4.5 ± 1.0	4.3 ± 1.3	**4.4 ± 0.8**
	7	5.8 ± 2.3	10.0 ± 3.9	**7.9 ± 2.2**
	Mean	**6.2 ± 1.1**	**5.2 ± 0.9**	

All values are means ± standard error. Row mean values that do not share the same capital letter (within a year and month) are significantly different from each other ($p < 0.05$). Column mean values that do not share the same lower case letter (within a month) are significantly different from each other ($p < 0.05$).

cropping combinations (**Table 3**). The forage radish used in this study has several factors that give it strong competitive ability: it is quick to germinate, grows rapidly, and has large aboveground biomass. Radish has the potential for allelopathy (e.g., Norsworthy, 2003), but it is likely the rapid canopy development of radish that plays the biggest role in weed suppression (Lawley et al., 2012). Barley has also been recognized as a good weed suppressant, both in monoculture and in mixture. In a study by Nelson et al. (2012), weed suppression was highest in treatments containing barley seeded alone and in intercrops. In 2016 we also measured weed biomass, which showed the same patterns as weed abundance (**Tables 2, 4**).

The effect of row spacing on weed abundance was not statistically significant (**Tables 2, 3**), although the lowest weed abundance overall was attributed to the 15 cm row spacing in the barley-radish intercrop. One factor that might lead to the high variability in results is the spatial heterogeneity of weed growth. Each field site had an uneven distribution of weed diversity likely related to weed dispersal and the existing seed bank. This resulted in certain site areas that had greater abundance of weeds, or the presence of large aggressive weeds which contributed considerably to the weed biomass measures in 2016, regardless of crop mixture and spacing. Other factors that may contribute to the lack of crop spacing effects include nutrient management,

TABLE 4 | Mean weed biomass by crop mixture treatment and row spacing (see **Table 1** for list of cropping treatments) in July and August, from the 2016 field trial.

		MEAN WEED BIOMASS (kg ha^{-1})		
	Mixture	**30 cm spacing**	**15 cm spacing**	**Mean ± sem**
2016				
July	1	625.5 ± 172.5	436.1 ± 132.6	**530.8 ± 106.9** BC
	2	1397.2 ± 375.4	1413.8 ± 351.4	**1405.5 ± 238.1** A
	3	309.7 ± 95.9	353.7 ± 72.9	**331.7 ± 56.4** C
	4	855.2 ± 303.3	604.8 ± 301.9	**730.0 ± 203.7** BC
	5	1210.2 ± 324.8	811.8 ± 487.5	**1011.0 ± 281.4** AB
	6	504.5 ± 295.0	615.4 ± 178.6	**560.0 ± 161.0** BC
	7	561.1 ± 119.6	845.2 ± 330.3	**703.2 ± 171.2** BC
	Mean	**780.5 ± 112.1**	**725.8 ± 117.1**	
August	1	899.3 ± 393.2	508.7 ± 274.6	**704.0 ± 234.0**
	2	1216 ± 347.8	1156.6 ± 441.7	**1186.3 ± 260.5**
	3	863.9 ± 467.7	132.0 ± 69.7	**498.0 ± 258.9**
	4	888.4 ± 661.5	447.2 ± 220.4	**667.8 ± 333.4**
	5	1466 ± 1059.0	1060.8 ± 907.4	**1263.4 ± 650.1**
	6	885.6 ± 287.1	481.2 ± 208.5	**683.4 ± 181.2**
	7	1222.1 ± 640.4	897.5 ± 461.9	**1059.8 ± 370.6**
	Mean	**1063.0 ± 205.5**	**669.1 ± 163.5**	

All values are means ± standard error. Row mean values that do not share the same capital letter (within a year and month) are significantly different from each other ($p < 0.05$). Column mean values that do not share the same lower case letter (within a month) are significantly different from each other ($p < 0.05$).

the relative heights of the crops, and the timing of weed and crop emergence (Mohler, 2001). Our seeding dates were later than those recommended for our region, and thus the emergence timing of the weeds and crops was similar. The close emergence timing meant that many weeds were established by the time the crop canopy was fully expanded. The different crop species also are likely to have variable rates of seedling recruitment (Szumigalski and Van Acker, 2005).

Crop biomass in 2015 significantly differed between species combinations in both July and August, but was only affected by row spacing in July (**Tables 2**, **5**). At the July sampling date in both 2015 and 2016, the barley-radish intercrop had the highest biomass production at both crop row spacings. This early strong biomass production is a good indicator of the success of this mixture at controlling weeds. The GLMs supported this, showing a significant effect ($p < 0.05$) of crop biomass production on weed abundance and weed biomass in July. Crop spacing was not significant in August of both years. The barley monoculture and oats-peas mixture had the highest late season (August) crop biomass in 2015, while no significant differences among the crop mixtures were detected in 2016 (**Table 5**). The oats-peas mixture had the highest weed abundance and biomass each year (only significant at $p < 0.05$ in 2015), suggesting that late season crop growth has little impact on overall weed suppression. This is also supported by the GLMs, which found no significant effect ($p > 0.05$) of August crop biomass production on weed abundance or biomass.

Increased crop diversity is suggested to lead to higher productivity and yield (e.g., intercropping vs. monocultures; Bedoussac et al., 2015). This trend was not observed in our study. In 2015, the monoculture and two species mixtures had higher biomass production than the four and eight species mixtures, and in 2016 there were no significant differences in biomass between any of the cropping treatments (**Table 5**). However, the productivity of the two species mixtures (barley-radish, oats-peas) were comparable to the barley monoculture. This shows that the inclusion of certain crops (i.e., radish and barley) in mixtures can improve weed control, but not at the expense of reduced forage production. Further, more diverse mixtures can provide improved forage quality (Mischkolz et al., 2013), enhancing the advantages of multi-species crops.

During the growing season, monthly total precipitation was considerably different in 2015 and 2016 (**Supplemental Figure 1**). The spring of 2015 was particularly dry in southwestern Saskatchewan, experiencing one of the lowest levels of precipitation on record from May to mid-July, while the same period in 2016 was one of the wettest on record (**Supplemental Figure 1**). We expected that these contrasting conditions would have considerably altered the results of the spacing component. In particular, the dry field conditions in 2015 may have impacted the establishment and growth of some crop species (e.g., legumes) which would have reduced canopy closure in the narrower row spacing. Additionally, water availability may have been a more limiting factor for weed growth than light, reducing the importance of row spacing in a dry year relative to a wet year. However, even under the heavy precipitation of spring 2016, the lack of relationship between weed abundance and crop spacing was the same. We did see much lower weed abundance in 2016 than 2015, but this could also be a result of the different site locations. Olsen et al. (2012) found that drought conditions decreased the effect of crop spacing pattern on weed biomass production relative to non-drought conditions. When

TABLE 5 | Mean crop biomass by crop mixture treatment and row spacing (see **Table 1** for list of cropping treatments) in July and August, from the 2015 and 2016 field trials.

		MEAN CROP BIOMASS (kg ha^{-1})		
	Mixture	**30 cm spacing**	**15 cm spacing**	**Mean ± sem**
2015				
July	1	2675.4 ± 180.5	2145.7 ± 189.5	**2410.6 ± 157.2** A
	2	1579.3 ± 178.9	1765.3 ± 258.7	**1672.3 ± 149.8** C
	3	3046.2 ± 422.3	2212.3 ± 158.0	**2629.3 ± 261.5** AB
	4	2451.2 ± 100.5	2032.6 ± 168.0	**2241.9 ± 120.3** AB
	5	1405.2 ± 59.7	1660.7 ± 232.4	**1533.0 ± 121.1** C
	6	2232.8 ± 363.1	1541.2 ± 267.8	**1887.0 ± 246.4** B
	7	2105.6 ± 417.1	2280.4 ± 263.8	**2193.0 ± 230.8** AB
	Mean	**2213.7 ± 140.3** a	**1948.3 ± 90.9** b	
August	1	7483.5 ± 165.8	7685.8 ± 270.3	**7584.7 ± 151.7** AB
	2	7906.2 ± 538.2	7519.3 ± 217.4	**7712.8 ± 278.5** A
	3	6743.0 ± 595.9	6437.8 ± 439.5	**6590.4 ± 347.6** BC
	4	6845.2 ± 1520.3	5988.2 ± 194.9	**6416.7 ± 727.8** C
	5	6599.9 ± 120.0	5918.0 ± 406.7	**6259.0 ± 234.8** C
	6	6424.4 ± 228.3	6527.9 ± 508.7	**6476.2 ± 258.8** C
	7	6484.7 ± 265.0	6399.7 ± 253.2	**6442.2 ± 170.4** C
	Mean	**6926.7 ± 243.7**	**6639.5 ± 169.7**	
2016				
July	1	5582.5 ± 587.7	4338.5 ± 713.8	**4960.5 ± 488.3** B
	2	3394.6 ± 914.5	2344.8 ± 116.1	**2869.7 ± 470.6** C
	3	8891 ± 2505.2	5558.5 ± 248.0	**7224.8 ± 1324.7** A
	4	4072.2 ± 500.8	4218.2 ± 550.6	**4145.2 ± 345.6** BC
	5	3607.8 ± 909.6	4299.3 ± 1172.4	**3953.6 ± 699.2** BC
	6	4455 ± 588.2	4619 ± 520.1	**4537.0 ± 364.8** BC
	7	5521.6 ± 744.4	3557.2 ± 328.1	**4539.4 ± 528.8** BC
		5075.0 ± 512.8 a	**4133.6 ± 270.2** b	
August	1	12132.6 ± 854.0	12053.4 ± 973.6	**12093.0 ± 599.7**
	2	10155.7 ± 1066.8	9322.2 ± 786.3	**9739.0 ± 633.4**
	3	10318.9 ± 941.0	10009.0 ± 1396.4	**10164.0 ± 781.7**
	4	11115.2 ± 1154.7	10618.0 ± 688.1	**10866.6 ± 629.3**
	5	9598.2 ± 1846.5	9857.6 ± 985.6	**9727.9 ± 970.1**
	6	13090.1 ± 1703.5	9499.9 ± 806.0	**11295.0 ± 1105.2**
	7	9391.2 ± 1030.0	11246.5 ± 816.3	**10318.9 ± 702.2**
	Mean	**10828.8 ± 491.0**	**10372.4 ± 361.8**	

All values are means ± standard error. Row mean values that do not share the same capital letter (within a year and month) are significantly different from each other ($p < 0.05$). Column mean values that do not share the same lower case letter (within a month) are significantly different from each other ($p < 0.05$).

we assessed weed biomass in 2016, we did observe consistently reduced weed biomass in the 15 cm row spacing treatments at the August sampling date, which was supported by visual observations of stunted and senescing weeds that did not have nearly the seed set of weeds found in the 30 cm row spacing. If annual weeds are present but out-competed to the point of reduced fitness, then there is still a long-term benefit to these seeding patterns.

Greenhouse Trials

To better understand what factors contributed to weed control in the best cropping mixture of barley and radish, we grew these crops in the greenhouse to isolate the effect of row spacing and crop combination on weed control from environmental variation. In the 2016 greenhouse trial, crop spacing and crop mixture significantly affected *C. album* shoot and root biomass, as well as height (**Table 6**). In 2018, there was a significant effect of crop spacing and mixture on weed shoot biomass, but not root biomass, and weed height was only affected by mixture (and not spacing). In both years, all cropping and spacing treatments significantly lowered the shoot weight, root weight, and height of *C. album* compared to the control treatment of weeds grown alone (**Tables 6**, **7**). In both trials, the radish monoculture and barley-radish mix reduced weed shoot biomass and height significantly more than barley alone. In all cropping treatments, the 15 cm row spacing reduced weed shoot biomass

TABLE 6 | Greenhouse experiments (2016 and 2018) mixed model results of crop biomass, weed shoot biomass, weed root biomass, and weed height with crop mixture and row spacing as fixed factors.

Factor	Df	Crop biomass		Weed shoot biomass		Weed root biomass		Weed height	
	(num, den)	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*P*
2016									
Spacing	1, 18	0.4	0.534	45.4	**<0.001**	12.7	**0.003**	15.7	**0.001**
Mixture	2, 18	9.2	**0.002**	58.2	**<0.001**	8.2	**0.004**	52.0	**<0.001**
Spacing × mixture	2, 18	2.8	0.091	0.4	0.685	0.8	0.472	2.6	0.110
Mixture (incl. control)*	3, 24	–	–	73.3	**<0.001**	31.4	**<0.001**	167.6	**<0.001**
2018									
Spacing	1, 18	0.4	0.541	21.9	**<0.001**	2.3	0.149	4.3	0.052
Mixture	2, 18	16.8	**<0.001**	141.4	**<0.001**	1.6	0.237	174.6	**<0.001**
Spacing × mixture	2, 18	0.9	0.417	6.0	**0.012**	0.3	0.713	1.6	0.234
Mixture (incl. control)*	3, 24	–	–	392.5	**<0.001**	7.0	**0.002**	359.0	**<0.001**

**Control treatment did not have any crop seeded (only Chenopodium). A separate mixed model was used to test the fixed factor crop mixture.*
Bold values are significant at $p < 0.05$.

TABLE 7 | Mean crop biomass, weed shoot biomass, weed root biomass, and weed height with crop mixture and row spacing for the 2016 and 2018 greenhouse experiments.

Mixture	Spacing	Crop biomass (g)	Weed shoot biomass (g)	Weed root biomass (g)	Weed height (cm)
2016					
Barley	15 cm	129.72 ± 3.45 **BC**	5.98 ± 1.41 **B**	0.39 ± 0.08 **AB**	27.24 ± 2.41 **B**
	30 cm	128.32 ± 4.43 **BC**	11.15 ± 2.56 **A**	0.67 ± 0.22 **A**	36.10 ± 3.44 **A**
Radish	15 cm	124.32 ± 12.41 **C**	1.37 ± 0.37 **D**	0.12 ± 0.04 **C**	16.90 ± 1.32 **C**
	30 cm	135.59 ± 7.83 **BC**	3.10 ± 0.57 **C**	0.26 ± 0.02 **B**	19.32 ± 1.52 **C**
Barley-Radish	15 cm	165.04 ± 2.23 **A**	1.63 ± 0.37 **D**	0.14 ± 0.04 **C**	16.60 ± 1.55 **C**
	30 cm	144.58 ± 5.14 **B**	3.44 ± 0.60 **C**	0.33 ± 0.04 **AB**	20.11 ± 1.34 **C**
Control (no crop)		–	113.93 ± 3.93*	5.33 ± 0.71*	75.39 ± 1.98*
2018					
Barley	15 cm	126.57 ± 7.02 **C**	15.34 ± 2.17 **B**	3.79 ± 0.90	42.22 ± 2.98 **A**
	30 cm	123.36 ± 6.16 **C**	23.99 ± 1.71 **A**	5.39 ± 1.45	46.73 ± 1.28 **A**
Radish	15 cm	134.38 ± 6.53 **C**	1.46 ± 0.37 **D**	1.33 ± 0.35	18.31 ± 1.00 **B**
	30 cm	139.25 ± 3.22 **BC**	5.15 ± 0.40 **C**	4.94 ± 0.85	22.05 ± 0.88 **B**
Barley-Radish	15 cm	161.71 ± 5.48 **A**	2.27 ± 0.39 **CD**	5.60 ± 2.77	19.75 ± 0.86 **B**
	30 cm	151.61 ± 7.09 **AB**	3.06 ± 0.39 **CD**	6.57 ± 2.29	19.25 ± 0.92 **B**
Control (no crop)		–	141.39 ± 3.93*	12.23 ± 1.73*	76.42 ± 1.11*

**Control treatment (no crop) is significantly different ($p < 0.05$) from all other mixtures.*
All values are means ± standard error. Column mean values that do not share the same capital letter within each year (excluding control treatment) are significantly different from each other ($p < 0.05$).

and height more than at 30 cm spacing, although the effect was not always significant. Finally, the barley-radish mixture had the highest crop biomass in both trials.

The greenhouse trials were different from the field trials in that row spacing had a significant effect on weed growth. This could be due to the growth of plants in a more restricted space, where competition for resources was accentuated by the limited space in a bin. Even though seeding density was the same in the greenhouse and the field, in the field there was a greater surrounding area for roots to grow into or draw resources from. In addition, the greenhouse trials only observed one weed species, *C. album*, which could be more susceptible to the increased competition for light at the 15 cm spacing than some of other weed species which we observed in the field. Other weed species in the field may have been more aggressive in their growth rate and form, which would have contributed more to weed biomass (e.g., large weeds such as redroot pigweed, *Amaranthus retroflexus*) or abundance (e.g., small prostrate weeds such as *Portulaca oleracea*).

The use of radish as a cover crop has previously been highlighted as a mechanism for weed control (e.g., Haramoto and Gallandt, 2004; Kruidhof et al., 2008; Lawley et al., 2011, 2012). In our greenhouse study, while the narrower row spacing had a significant effect on *C. album* shoot biomass, it is not clear which mechanism was most responsible for this—aboveground competition for light, or belowground competition for resources and/or the presence of allelopathic root compounds. Kruidhof et al. (2008) found that an autumn fodder radish cover crop severely decreased the abundance of *C. album* in the field, citing the main mechanism of control as light interception, although interestingly, seeding density did not impact weed suppression. Lawley et al. (2012) found that in-crop competition for light had the greatest impact on weed control, while the alellopathic potential of forage radish could not be documented in multiple field and controlled environment studies.

CONCLUSIONS

Weed abundance in the field was associated with the selection of different cropping mixtures, while cropping pattern with closer row spacing was less important in the field but was significant in the greenhouse trials. The selection of specific crops appears to provide significant weed control, in particular forage radish grown alone and in mixture with barley. The ability to maintain strong weed control when the two crops are grown together is important in a forage context, where having the two crops together would provide forage with a more balanced nutritional profile compared to radish alone. This study provides incentive for improving species selection in forage intercropping trials and testing spatial arrangements that might provide greater weed suppression.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

JB, EL, and MPS designed the field experiment. JB conducted the field work. JB, EL, and MS designed the greenhouse experiment. JB and MS conducted the greenhouse work. JB, LB, and EL conducted statistical analyses. JB and LB wrote the manuscript, with contributions and editing from all authors.

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SUPPLEMENTARY MATERIAL

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No-Tillage and High-Density Planting for Tahiti Acid Lime Grafted Onto Flying Dragon Trifoliate Orange

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The management of soil cover plants (intercropping) in orchards can contribute to increase productivity of citrus trees. Thus, the present research aimed to evaluate different planting systems for Tahiti acid lime grafted onto Flying Dragon trifoliate orange, a dwarfing rootstock, at high planting density (1,157 trees ha^{-1}). The study was set up in four tillage systems, using *Urochloa ruziziensis* as an intercrop species in the orchard, and conducted for 5 years: no-tillage (NT), no-tillage and no-herbicide (NT-NH), minimum tillage (MT) and conventional tillage (CT; without intercropping). Dry matter (DM) production of biomass in the row and interrow of the orchard was evaluated yearly, as well as weed density, soil physical and chemical characteristics, plant water and nutritional status, and fruit yield of trees. Greater deposition of DM of biomass was observed in the row of citrus planting for treatments NT and NT-NH compared to CT and MT treatments, which led to reduced undesirable weed populations. The NT treatment also provided increases of 79% in potassium (K) nutrient concentrations in the leaves of trees and 60% in exchangeable K in the soil surface layer, in the first 2 years evaluated. The maintenance of the *Urochloa ruziziensis* mulch in the NT system also provided higher soil volumetric moisture content and consequently lower soil resistance penetration and water stress on trees, evidenced by the predawn plant leaf water potential (<1 MPa). Moreover, the NT treatment provided an average increase of 56% of fruit yield from trees compared to the CT treatment during three harvests. This long-term study demonstrated the contribution of the no-tillage system using a favorable cover crop to increase the yield of Tahiti acid lime fruits and maintain the soil quality most required for the sustainability of citrus production.

Keywords: ***Citrus latifolia*, ecological mower, intercropping, soil protection, *Urochloa ruziziensis*, glyphosate management, yield**

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INTRODUCTION

The increasing change in the lifestyle habits of the world's population has led to changes in the food production situation. Concerns about CO_2 emissions have increased, calling for low-carbon cultivation practices (Gregory and George, 2011). With the heightened consumer demand, the link between healthy food and the production system (agroecosystem) has been tightened. Consequently, there is a search for less-impacting production systems, making more conservationist, organic and even so-called urban farming techniques gain space in the world's agricultural sphere (James and Friel, 2015).

Brazil stands out on the world stage as an agricultural producer, not only for being one of the largest producers, but also for the generation of new techniques and more-sustainable planting systems (Ribeiro et al., 2017). Among the foods most-produced in Brazil, citrus fruits stand out, the country being the world's largest producer of oranges, the fifth in the ranking of mandarins and the sixth in limes and lemons. However, large variation on average productivity has been observed (Fundo de Defesa da Citricultura [Fundecitrus], 2019), which results from significant differences in the adoption of technology (planting and cultural practices) among producers in the different Brazilian citrus fruit regions (Corá et al., 2019).

The state of São Paulo accounts for 79% of Brazil's production of limes and lemons, with a production of 1.3 million tons, with the highlight being Tahiti acid lime [*Citrus latifolia* (Yu Tanaka) Tanaka], with 30.4 thousand hectares planted and nine million productive trees (IBGE, 2018). It is important to highlight the socio-economic impact for the state, since this production area is concentrated in the hands of eight thousand small producers, who are the most in need of sustainable planting and cultural practices for remaining in business (Santos et al., 2016).

In this context, the implementation of the FAO's Conservation Agriculture (CA) concept, involving (i) minimum soil tillage, (ii) cover crop usage, and (iii) diversity of cultivated species, in sequence (crop rotation) or in association (intercropping) (Food and Agriculture Organization [FAO], 2017), is key to the success of the agricultural activity.

Earlier studies demonstrate the possibility of using minimum soil tillage for the implantation of orange orchards (Auler et al., 2008; Fidalski et al., 2010), keeping the inter-rows permanently planted with cover crops (*Urochloa* spp.) after the establishment of the orchard (Martinelli et al., 2017). This kind of management is not usual in areas of the conventional tillage system, where the soil is exposed to sunlight and raindrops, increasing erosion. Minimum tillage and no-tillage provide for the accumulation of organic material and carbon content in the soil, for stabilizing its structure, besides cycling nutrients and contributing to the control of weeds (Trumbore and Camargo, 2009).

Two techniques have been highlighted as alternatives for the conservationist production of citrus fruit, replacing the conventional system. The secondary cultivation of species of perennial brachiaria is highlighted, mainly of the Poaceae family [*Urochloa* (syn). *Brachiaria*] in the orchards (Matheis et al., 2006), and the handling of this vegetation with the use of the ecological mower. This mower is characterized by cutting the inter-row vegetation and distributing it simultaneously on the row of citrus planting, producing a mulching effect (Corá et al., 2019).

The production of biomass of covering plants in the inter-rows of citrus groves and the launch of it for citrus row planting was the target of a few short-term studies with citrus fruit (a few harvests), focusing on specific evaluations of different species of cover crop, water storage capacity and control of weeds (Bremer Neto et al., 2008; Fidalski et al., 2009; Azevedo et al., 2012; Martinelli et al., 2017). However, the no-tillage and mulching effects on the rows of citrus over a long period of time were not evaluated in these studies, not even the possible responses to new rootstocks, such as the dwarfing varieties, which may differ in the consumption and absorption of water in the soil.

The diversification of rootstock in citriculture has occurred with great speed, with one of the objectives being varieties that induce less vigor to the canopy, thus allowing for denser planting in citriculture (Azevedo et al., 2015). Rootstock and scion with low vigor are essential for achieving better results in high-density planting systems, and mechanical harvest, and one of the dwarfing rootstocks that allows for greater density of planting is the trifoliate orange Flying Dragon [*Poncirus trifoliata* var. monstrosa (T. Ito) Swingle] (Cantuarias-Avilés et al., 2012). However, this rootstock shows low tolerance for water deficit, which justifies studies of alternative planting and cultural practices to increase the quality of the soil, as regards the storage of water from in the soil and fertility characteristics.

On the basis of the above, this research had the objective of assessing different systems of planting and cultural practices for Tahiti acid lime grafted onto Flying Dragon as a high-density planting orchard, considering the use or non-use of *Urochloa ruziziensis* as intercropping, and the consequent production and transfer of biomass from the inter-rows to the row of citrus planting, on the physical and chemical characteristics of the soil, over a long time period, which may affect the fruit production of the orchard.

MATERIALS AND METHODS

Growing Conditions and Experimental Design

The experiment was installed in São Paulo State, Brazil (22° 18′ 26″ S; 47° 23′ 11″ W and 620 m altitude). The climate of the region, according to Köppen, is of the Cwa type, tropical altitude, rainy in summer and dry in winter, with annual rainfall of 1,384.5 mm (Alvares et al., 2013). The soil has been classified as a dystrophic Red Latosol, with clay texture, with a moderate A horizon (Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA], 2013). During the experimental period (2015–2019), maximum and minimum temperatures and the monthly rainfall accumulated were monitored, with data collected at a meteorological station located 1 km from the experimental area.

The initial chemical characteristics of the soil from the experimental area were evaluated (Raij et al., 2001) for a soil sample collected in July 2014 (0.0–0.20 m depth). The following average levels were found: P (phosphorus; 51.5 mg dm^{-3}), K (potassium; 3.7 $mmol_c$ dm^{-3}), Ca (calcium; 52 $mmol_c$ dm^{-3}), Mg (magnesium; 17 $mmol_c$ dm^{-3}), and base saturation (68%). These values are considered suitable for planting citrus fruit (Corá et al., 2019; Mattos-Junior et al., 2020). In November 2014, *Crotalaria juncea* and *Urochloa ruziziensis* were sown, 30 and 12 kg ha^{-1} of seeds, respectively, with the aim of increasing soil carbon and nitrogen, in total area.

In March 2015, when the cover crops exhibited maximum growth (**Figure 1A**), they were cut. The furrows were opened and 0.45 kg of monoammonium phosphate (MAP) was applied per linear meter of the furrow (**Figure 1B**). *U. ruziziensis* was kept as a permanent covering crop (**Figure 1C**) in the orchard inter-rows, in minimum tillage (MT), no-tillage (NT) and no-tillage + no-herbicide (NT-NH) treatments (**Table 1**).

The planting of Tahiti acid lime IAC-5, grafted onto Flying Dragon trifoliate orange, was carried out in March 2015, using a spacing of 4.8 m × 1.8 m (1,157 plants ha^1). The treatments were as follows: conventional tillage (CT), minimum tillage (MT), no-tillage (NT) and no-tillage + no-herbicide (NT-NH). Different planting operations and cultural practices were performed on each treatment (**Table 1**). The experiment was installed with four treatments in a randomized block design, with five replications. Each plot was composed of 18 trees, distributed in three rows with six trees each and the evaluations were concentrated on the four central trees of the central row.

The orchard was maintained without irrigation, and the fertilization followed the recommendations by Mattos-Júnior et al. (2009), which were carried out manually, subdivided into four applications from November to March of each harvest. In addition, 2 t ha^{-1} of limestone was applied in 2014 (before planting), 2016 and 2018, over the total area. The other routine treatments for culture (control of pests and diseases, pruning, etc.) were carried out on all the plots, annually.

Biomass Production

In all the treatments, three mowing of the cover crops were carried out per year, during 2016 to 2019. The inter-rows of the NT and NT-NH treatments were managed with an ecological mower (Kamaq® Ninja Eco 230 model, with six blades and a 2.60-m cutting width, that throws the mowed biomass into the row area), while the MT and CT treatments have been managed with a conventional mower (Kamaq® Ninja 230 model, with four blades and a 2.60-m cutting width, that leaves the mowed biomass in the inter-row area).

After the mowing (all treatments) and manual hoeing in the rows (NT-NH), biomass sampling was carried out on the orchard rows and inter-rows of the orchard, in all treatments, in four points per plot, using a gage area of 0.25 m^2. The samples were weighed and dried at 65 ± 2°C for 72 h.

Weed Density

Thirty days after each treatment management (mowing, herbicide and hoeing, according **Table 1**), the plant protection survey was carried out on all treatments in the four evaluation years (2016 to 2019). An area of 0.25 m^2 was randomly evaluating four times in the plot, totaling a sample area of 1 m^2 in the citrus planting rows, as described by Martinelli et al. (2017). Weeds (including *Urochloa ruziziensis*) were counted to obtain the number of individuals per square meter (density).

Soil Penetration Resistance and Soil Moisture

The soil penetration resistance (SPR) was assessed using the Stolf penetrometer in the citrus rows. The measurement was obtained by a calculation using the number of impacts of a known weight driving into the soil and the depth measurement achieved by each impact, according to the methodology described by Stolf et al. (2014). Four points per plot (citrus row) were analyzed in the layers of 0–20 and 20–40 cm in depth, in September of the second (2016) and fourth (2018) years of the experiment, a period of low rainfall (**Figure 2**).

On the same dates, the soil moisture was determined by the gravimetric method (Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA], 2018), by taking four samples from the row planting, at depths of 0–20 and 20–40 cm, with the aid of an auger. The soil samples collected were packed in aluminum capsules and weighed before and after drying at 105 ± 3°C for 24 h. By difference in mass, the percentage of soil moisture was calculated using the formula:

$$SM(\%) = \frac{100(WW - DW)}{DW}$$

Where SM is the soil moisture as a percentage of water, WW is the wet weight and DW is the dry weight of the sample.

Leaf Water Potential

Leaf water potential (Ψ_L) was measured on clear days during the dry season (August to September, **Figure 2**) annually from 2017 to 2019. Measurements were performed using a pressure chamber (Soil Moisture Equipment Corp., model 3000, Santa Barbara, CA, United States) according to Kauffmann (1968) and following the recommendations of Turner (1988). Sampling was performed at predawn (05:00 AM), collecting the third leaf in reproductive branches, four trees per plot. Leaves were collected and preserved in hermetic plastic bags on ice until measurement. At the same time soil samples (0–20 cm) were collected and soil moisture evaluated, according Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA] (2018).

Chemical Analysis of Soil and Leaves

Each year in April (2016–2019), with the use of "Dutch"-type auger, eight soil subsamples per plot, were collected, in the citrus rows, in the layer of 0–20 cm depth to determine the availability of phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg), using the methods described by Raij et al. (2001). On the same date, sample leaves were collected for the assessment of the nutritional status of the trees, with the determination of total levels of macronutrients according to Bataglia et al. (1983). The third leaf of fruit branches was collected, with four trees being sampled per plot and five leaves per plant.

Vegetative and Productive Development of Tahiti Acid Lime

The vegetative growth of the Tahiti acid lime plants was assessed annually by measuring the height and diameter

FIGURE 1 | *Crotalaria juncea* and *Urochloa ruziziensis* at the most vegetative development, before the cut, February 2015 **(A)**; opening of furrows for planting in no-tillage system, March 2015 **(B)**; and plants of Tahiti acid lime in no-tillage system, October 2015 **(C)**.

TABLE 1 | Treatments and description of the operations of planting and cultural practices.

Treatments		Planting and cultural practices
CT		*Planting:* disk plow and disk harrow; opening of the furrows and planting in the uncovered soil; inter-row soil also uncovered. *Cultural practices:* disk plow in the inter-rows once a year (May); application of glyphosate (1080 g acid equivalent ha^{-1}) to the row; and conventional mowing in the inter-rows, three times a year (October to March).
MT		*Planting:* disk plow and disk harrow; opening of the furrows and planting in the uncovered soil; keeping *Urochloa ruziziensis* as intercropping. *Cultural practices:* application of glyphosate (1080 g acid equivalent ha^{-1}) to the row; and conventional mowing in the inter-rows, three times a year (October to March).
NT-NH		*Planting:* without disk plow or disk harrow; opening of the furrows and mulch planting; keeping *U. ruziziensis* as intercropping. *Cultural practices:* ecological mowing in the inter-rows, and manual hoeing in the rows, three times a year (October to March).
NT		*Planting:* without disk plow or disk harrow; opening of the furrows and mulch planting; keeping *U. ruziziensis* as intercropping. *Cultural practices:* application of glyphosate (1080 g acid equivalent ha^{-1}) to the row; and ecological mowing in the inter-rows, three times a year (October to March).

CT, conventional tillage; MT, minimum tillage; NT, no-tillage; NT-NH, no-tillage + no-herbicide.

of the trees using a graduated ruler, for the subsequent calculation of the canopy volume by the equation proposed by Mendel (1956):

$$CV = 2/3 \times \pi \times r^2 \times H$$

Where CV is the canopy volume (m^3), r is the radius (m) and H is the height (m) of Tahiti acid lime plants. In each month of the period 2017–2019, all ripe fruit were harvested and weighed and then the cumulative productivity of each year was calculated, expressed in t ha^{-1}. All evaluation were performed in four central trees per plot and in five replications.

Statistical Analysis

For the statistical analysis, all data were analyzed separately for each growing season, subjected to ANOVA. When the effects were significant, differences among treatments were

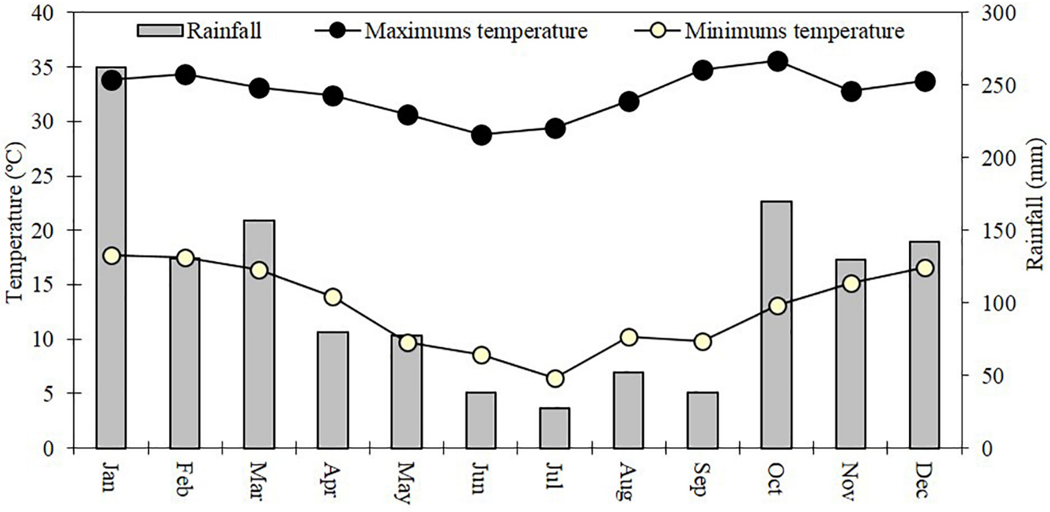

FIGURE 2 | Mean values of air temperature minimums and maximums and of rainfall across the five growing seasons (2015–2019) in which the experiment was conducted.

determined using Tukey's multiple comparison test at a significance level of 5%.

RESULTS

Biomass of Cover Crops and Weed Density

In the conventional (CT) and minimum-tillage (MT) treatments, where the planting was carried out without the presence of mulch in the citrus rows and the biomass of the inter-rows was managed with a conventional mower, a greater quantity of biomass of the covering plants was deposited in the inter-rows of the Tahiti acid lime orchard (**Table 2**).

On the other hand, in no-tillage systems, whether with or without herbicide, where the ecological mower was used on the inter-rows, higher biomass values were observed in the orchard row, with 10-fold increases in these treatments (NT and NT-NH) compared to CT and MT (**Table 2**); these results were similar over four consecutive years (2015–2019).

In NT treatment, the presence of mulch and the use of the herbicide glyphosate, two weed control methods (physical and chemical) in the citrus rows led to a significant reduction in the number of weeds in all years evaluated (**Table 2**). The average reduction in the number of weeds in the NT in relation to the CT treatment was 75%, reaching close to 80% in the first year evaluated, coming from the use of the herbicide and from the high values of biomass present in the row in this year (**Table 2**).

Resistance to Soil Penetration and Soil Moisture

With the newly planted experiment (2016), the penetration resistance values (0–20 cm depth) did not differ between treatments and remained below 2 MPa (**Figure 3A**). On the other hand, in the fourth year (2018), at the same depth, these values doubled in some treatments (NT and NT-NH) and quadrupled

TABLE 2 | Inter-row cover crop biomass yield, orchard row cover crop mowed biomass deposition and weed density, in four different planting systems of Tahiti acid lime, over four growing seasons (2015–2019).

Treatments	Inter-row cover crop biomass (t ha^{-1})	Orchard row cover crop mowed biomass deposition (t ha^{-1})	Weed density (weeds m^{-2})
		2016	
CT[a]	6.2 b[b]	0.5 b	26.9 a
MT	9.0 a	0.6 b	24.1 a
NT	1.8 c	6.5 a	5.9 b
NT-NH	2.1 c	6.4 a	22.9 a
		2017	
CT	5.5 a	0.6 c	24.3 a
MT	6.3 a	1.1 c	23.5 a
NT	0.8 b	4.5 b	6.7 c
NT-NH	0.6 b	7.4 a	19.0 b
		2018	
CT	6.6 a	0.4 b	18.8 a
MT	5.1 a	0.5 b	15.7 a
NT	0.8 b	5.3 a	5.2 b
NT-NH	1.0 b	4.2 a	14.3 a
		2019	
CT	7.6 a	0.3 b	78.3 a
MT	5.7 a	0.4 b	55.3 b
NT	0.6 b	3.9 a	19.5 c
NT-NH	1.8 b	4.7 a	47.0 b

[a] *CT = conventional tillage; MT = minimum tillage; NT = no-tillage; NT-NH = no-tillage + no-herbicide.* [b] *Means followed by the same letter, in the same column, for each parameter evaluated and each year separately, do not differ (n = 20; Tukey test at $p < 0.05$).*

in conventional tillage (>8 MPa) (**Figure 3A**). Value greater than 10 MPa (**Figure 3B**) of resistance to penetration in the soil was observed in the CT treatment at the deeper layer (20–40 cm depth).

The soil moisture, taken together with resistance to penetration, was greater in mulch treatments at a depth of 0–20 cm in the second and fourth years (**Figure 3C**), however, in the 20–40 cm layer, the CT treatment showed lower soil moisture than the NT and NT-NH treatments in the second year, and the NT and NT-NH treatments had the highest moisture values in the fourth year (**Figure 3D**).

Leaf Water Potential and Soil Moisture

The leaf water potential was higher in the no-tillage (NT) treatment, in the third- and fourth-year assessments (**Figure 4A**), demonstrating a higher degree of hydration in the plants, even in low-precipitation periods of the year (**Figure 2**) when such assessments were carried out (September 2017, 2018, and 2019). The soil moisture, determined in parallel with the leaf water potential, was greater in the NT and NT-NH treatments than the other treatments, in all the years of evaluation (2017–2019) (**Figure 4B**).

Soil Fertility and Nutritional Status of the Tahiti Acid Lime

Phosphorus (P) levels in the soil were adequate (13–30 mg dm^{-3}) (Mattos-Junior et al., 2020) for a young Tahiti acid lime orchard (<5 years), for all years evaluated, and there was no significant difference between treatments (data not shown). However, although the concentrations of potassium (K), calcium (Ca) and magnesium (Mg) in the soil did not differ between the treatments in the first year evaluated, they were found to have sufficient levels for the crop, resulted from liming and soil fertilization conducted before tree planting. In the fourth year, the K content in soil in treatment NT (6 $mmol_c dm^{-3}$) was higher than in the other treatments (**Figure 5A**). In the leaf analyses, the K content was higher in treatment NT in the second and third years than in conventional tillage without mulch (CT) (**Figure 5B**).

Calcium and magnesium had higher levels, in soil, in NT treatment in years 4 and 5 compared to CT and NT-NH treatments (**Figures 5C,E**). No differences in leaf Ca levels were observed between years 2 and 4, however, there is a higher Ca content in MT compared to NT and NT-NH in the fifth year (**Figure 5D**). The leaf Mg content in MT was higher than in the other treatments in the second year (**Figure 5F**).

In the second year of the orchard (2016), the leaf N levels were higher in mulch treatments (NT and NT-NH) (data not shown). In all evaluation years, the levels were adequate for Tahiti acid lime (>25 g kg^{-1}) in all treatments (Mattos-Junior et al., 2020). The soil organic matter content in treatment NT was higher than in the other treatments in years 4 and 5 (**Figure 6A**). Corroborating the K, Ca and Mg data in the soil, increased base saturation was attained in NT treatment (**Figure 6B**) in years 4 and 5 (75 and 58%, respectively). In year 1, high levels of base saturation (∼70%) were also observed, but in all the treatments in this case, as a result of the soil liming carried out before trees planting.

Vegetative and Productive Development of Tahiti Acid Lime

The NT-NH treatment led to lower vegetative development for Tahiti acid lime plants in the second year (2016). The canopy volume in the CT treatment was lower than in the other treatments in the fifth year of evaluation (**Figure 7A**). Although the orchard is in the process of being formed, the results showed an early production of the Tahiti acid lime plants in high-density planting grafted to rootstock of Flying Dragon trifoliata orange, since the plots in the no-tillage system with application of herbicide (NT) produced more than 22 t ha^{-1} in the third year (2017). From the third to the fifth year of evaluation, the NT treatment showed a greater production than the other treatments, reaching 30% greater than the CT system in the last harvest (**Figure 7B**). Although the NT-NH treatment showed the lowest productivity (9.5 t ha^{-1}) in the third year, this treatment did not differ from the CT and MT treatments in the fourth and fifth years (**Figure 7B**).

DISCUSSION

Biomass of Cover Crops and Density of Weeds

The largest quantity of biomass in the citrus row, in the NT and NT-NH treatments, was due from the planting carried out in the mulch (**Figure 1**). The subsequent maintenance of this mulch on the soil surface results from the management of the cover crops in the inter-rows (*U. ruziziensis*) with the use of an ecological mower. This agricultural equipment throws the mowed biomass into the row area; this is a sustainable management technique, providing greater soil protection and a reduction in the use of herbicides, as an aid in the control of weeds (Bremer Neto et al., 2008; Azevedo et al., 2012; Martinelli et al., 2017).

The presence of the *U. ruziziensis* as cover crop in the inter-rows of the NT, NT-NH and MT treatments produced quantities of approximately 7 t ha^{-1} biomass in these systems, on the rows or inter-rows of the orchard. These biomass values are similar to the results reported by other authors for the state of São Paulo and Mato Grosso, Brazil (Bauer et al., 2011; Torres et al., 2016). In spite of other species of *Urochloa* such as *U. decumbens* showing biomass production (>9 t ha^{-1}) higher than that of *U. ruziziensis*, there are reports of *U. decumbens.* allopathy in citrus trees (Souza et al., 2006). Thus, the use of *U. ruziziensis* in the inter-rows of Tahiti acid lime orchards has been recommended, because it exhibits the least competition with citrus fruit (Martinelli et al., 2017).

The use of two methods of control, the physical, with the planting and maintenance of the mulch of *U. ruziziensis*, and the chemical, applying the herbicide glyphosate in the NT treatment, led to an efficient control of weeds (**Table 2**). This underlines the importance of using more than one control method; a fundamental premise for the integrated management

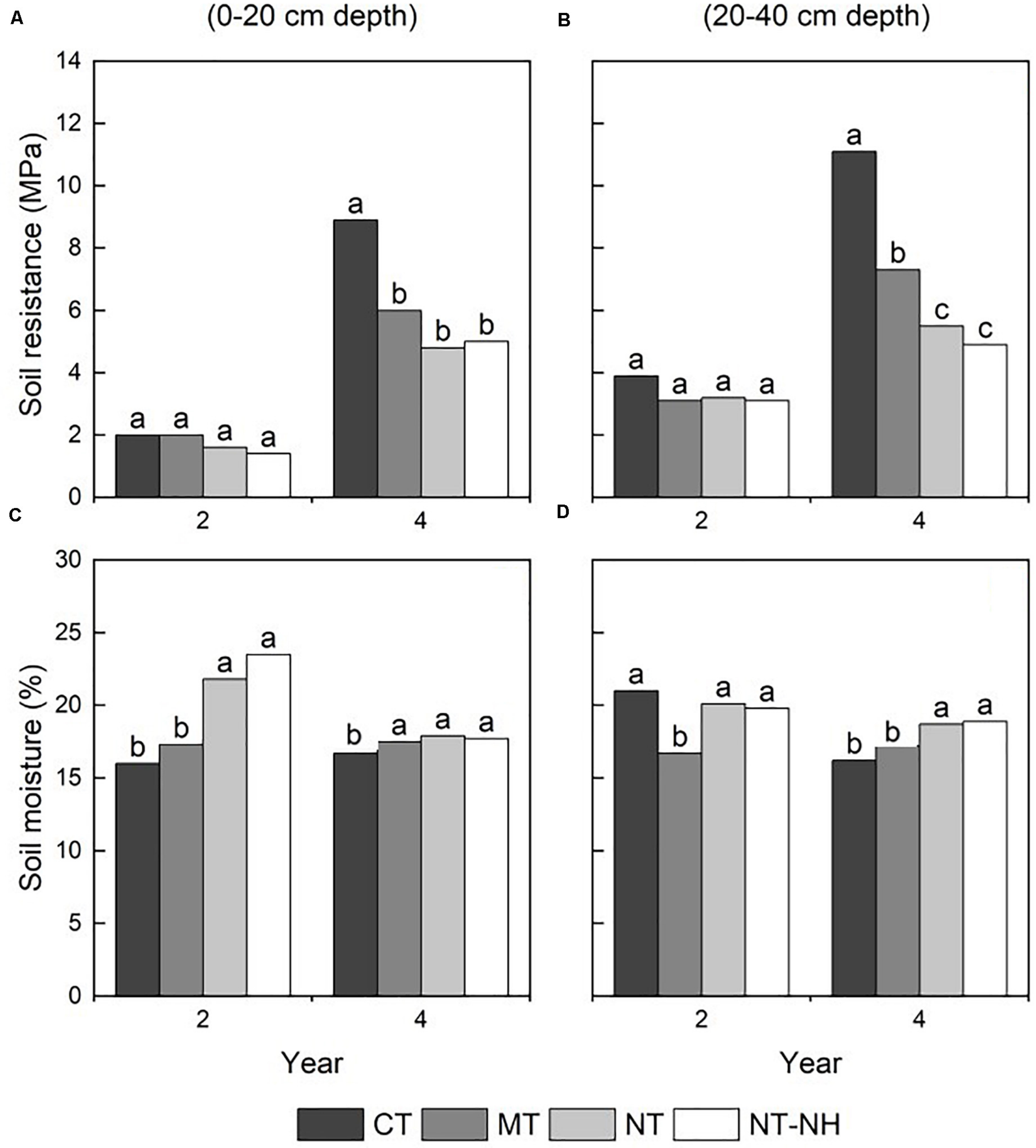

FIGURE 3 | Soil resistance penetration **(A)**, 0–20 cm depth and **(B)**, 20–40 cm depth) and soil moisture **(C)** 0–20 m depth and **(D)** 0–20 m depth) in four different planting systems of Tahiti acid lime, over two growing seasons (2016 and 2018). CT, conventional tillage; MT, minimum tillage; NT, no-tillage; NT-NH, no-tillage + no-herbicide; Years: 2 = 2016 and 4 = 2018. Means followed by the same letter, for each evaluating and year separately, do not differ (*n* = 20; Tukey test at $p < 0.05$).

of weeds for citrus fruit and other crops (Christoffoleti et al., 2007). On the other hand, the absence of herbicide use and the presence of mulch alone in the NT-NH treatment, and the use of glyphosate (chemical) alone in the MT and CT treatments, were not sufficient to maintain the density of weeds at low levels, as they use only one control method (Teasdale and Mohler, 2000).

The presence of the natural mulching of the soil in treatment NT likely influenced the density of weeds due to physical effects, as it limits the passage of light, hindering the germination of positive photoblastic seeds, creates a physical barrier for the emergence of such seeds and reduces the germination of seeds that need thermal amplitude to start the germination process (Noce et al., 2008).

The NT-NH treatment was inefficient in controlling weeds, which could lead to lower productivity in relation to treatments where weeds are controlled by the use of cover crop cuttings and

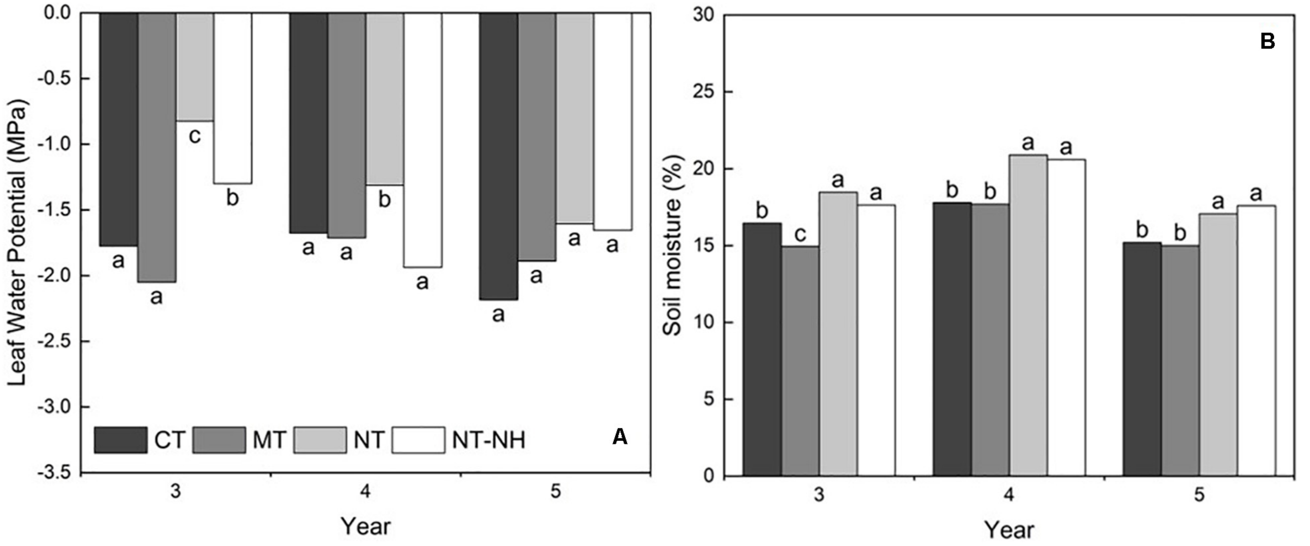

FIGURE 4 | Soil moisture **(A)** and leaf water potential **(B)** in four different planting systems of Tahiti acid lime, over three growing seasons (2017–2019). CT, conventional tillage; MT, minimum tillage; NT, no-tillage; NT-NH, no-tillage + no-herbicide. Years: 3 = 2017, 4 = 2018, and 5 = 2019. Means followed by the same letter, for each evaluating and year separately, do not differ (*n* = 20; Tukey test at $p < 0.05$).

herbicide (NT). However, good preparation of the soil and good handling of the soil with covering and mulching plants may be the most economical option for controlling weeds in comparison with the use of organic or chemical herbicides, electroshock or steam (Hiltbrunner et al., 2007).

Soil Penetration Resistance, Soil Moisture and Leaf Water Potential

The SPR in the second year evaluated (2 MPa) for all treatments resulted from good soil preparation for the planting of the orchard (**Figures 3A,B**). The conventional practices of grazing and gradient modify the structure of the soil, altering its properties, which reduces the resistance to penetration of the soil and improves the porosity of the soil and the capacity for water retention, but these benefits are temporary (Nkakini and Fubara-Manuel, 2012).

In the evaluations carried out in the fourth year, in both soil layers, there were lower resistance values to penetration of the soil in the NT and NT-NH management systems, in which the soil of the citrus row is covered by mulch, compared to those of CT (**Figures 3A,B**), with values close to 4 MPa. Values up to 4 MPa are considered adequate for root growth, demonstrating that did not adversely affect the physical structures of the soil (Hamza and Anderson, 2005). Lima et al. (2007) consider that SRP values between 2.6 and 5.0 MPa may indicate some problems in plant growth, mainly in the root system.

The two soil layers assessed in treatment CT yielded values greater than 8 MPa of SRP, therefore outside the appropriate range. Several studies showed that the plants tolerate higher resistance to penetration of the soil (above 4 MPa), but in areas with no-tillage systems. This is due to better structure of the soil and greater presence of continuous biopores in this system (Moraes et al., 2014), which is not the case in the CT treatment. The SRP depends on some specific factors, such as the handling of the area, texture, structure and mineralogy, depending highly on the soil moisture (Assis et al., 2009).

The greater moisture in the soil in the no-tillage systems compared to that in the CT, in the fourth year of evaluation, in the layer of 20–40 cm depth (**Figures 3C,D**), is associated with improvements in the soil structure, by the addition of organic material on the surface (Fidalski et al., 2010), resulting in lower resistance to penetration of the soil in these treatments (NT and NT-NH).

Proper management of soil cover crops is important in improving the soil structure and porosity, protecting it from erosion and increasing water infiltration, as well as improving the soil ability to sustain adequate moisture for plant growth (Melloni et al., 2008), which was observed in NT and NT-NH. Yoshida and Stolf (2019) report that when the soil is moister, its particles are further away from each other, which results in less difficulty in separating them, reducing the resistance to penetration of equipment into the soil.

In China, in apple orchard Liu et al. (2013) report that the covering of the soil with mulch has a positive effect on the conservation of the water content in the soil, besides the control of erosion and the loss of nutrients, such as P and K. Still in this context, according to Ahmad et al. (2020), the Chinese government has encouraged its citriculturists to recover degraded areas and to keep them productive by ways that prioritize the conservation of soil and water, including the use of organic mulching.

Although rainfall rates in August and September were low (**Figure 2**), treatment NT showed greater water potential of the leaf (about −1.1 MPa) compared to the other treatments (**Figure 4A**) in the third and

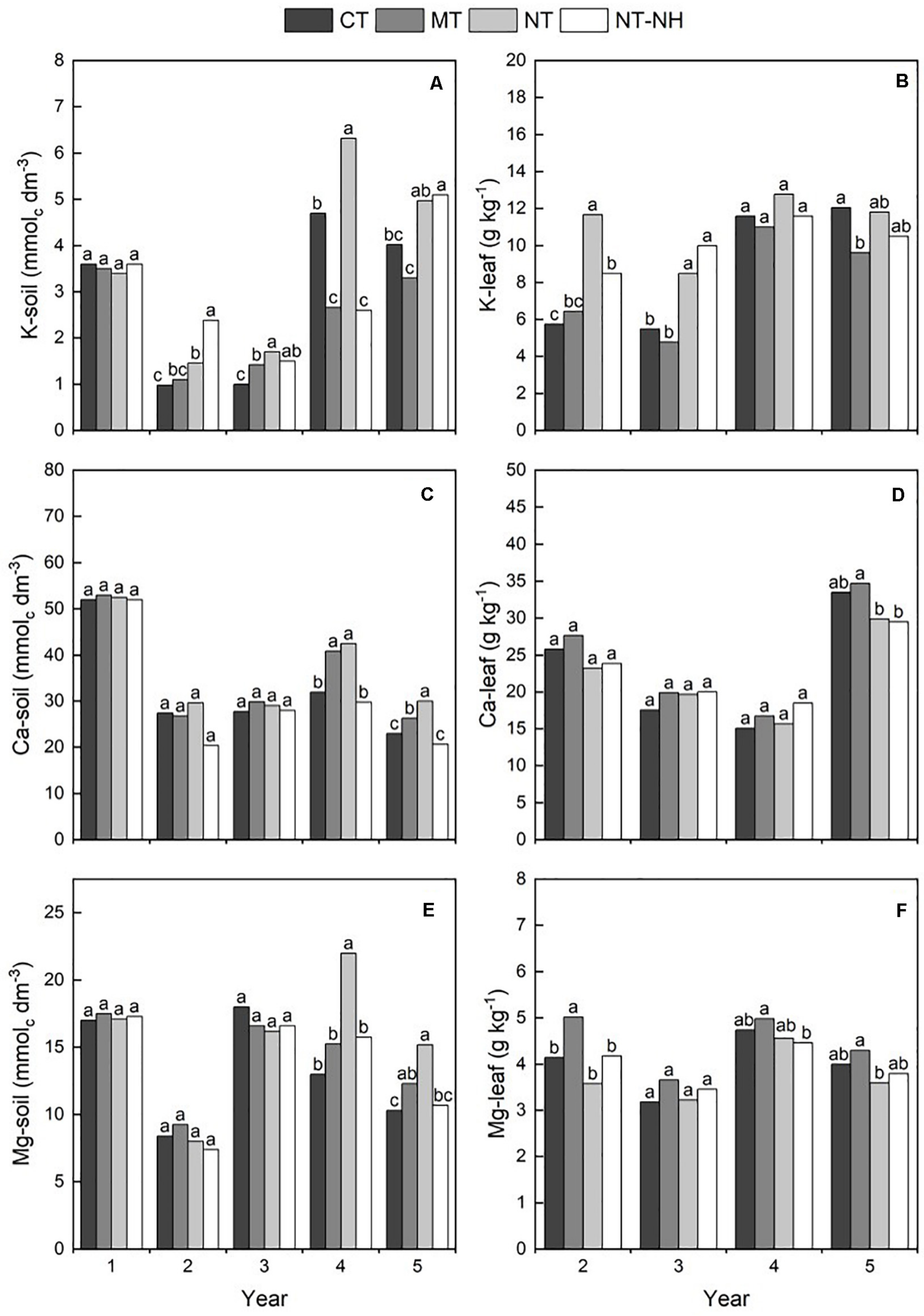

FIGURE 5 | Nutrient soil availability (0–20 cm depth) and leaf content: K – **(A,B)**, Ca – **(C,D)**, Mg – **(E,F)** in the tree planting row of different planting systems of Tahiti acid lime, over five growing seasons (2015–2019). CT, conventional tillage; MT, minimum tillage; NT, no-tillage; NT-NH, no-tillage + no-herbicide. Years: 1 = 2015, 2 = 2016, 3 = 2017, 4 = 2018 and 5 = 2019. Means followed by the same letter, for each evaluating and year separately, do not differ (n = 20; Tukey test at $p < 0.05$).

fourth years, even using a rootstock highly susceptible to drought such as the Flying Dragon trifoliata orange (Espinoza-Núñez et al., 2011). Values for the predawn water potential should be less than −1.3 MPa in order to avoid negative effects on the final production of citrus fruit (García-Tejero et al., 2010).

Conventional and minimum tillage do not show mulching in the citrus row, due to the use of the conventional mower, keeping the citrus row exposed to the greater incidence of sunrays. As a result, the evaporation of water occurs more quickly, bringing about more-negative values of water potential in the leaves (∼ −2 MPa). Reduced hydraulic conductance may be the main cause

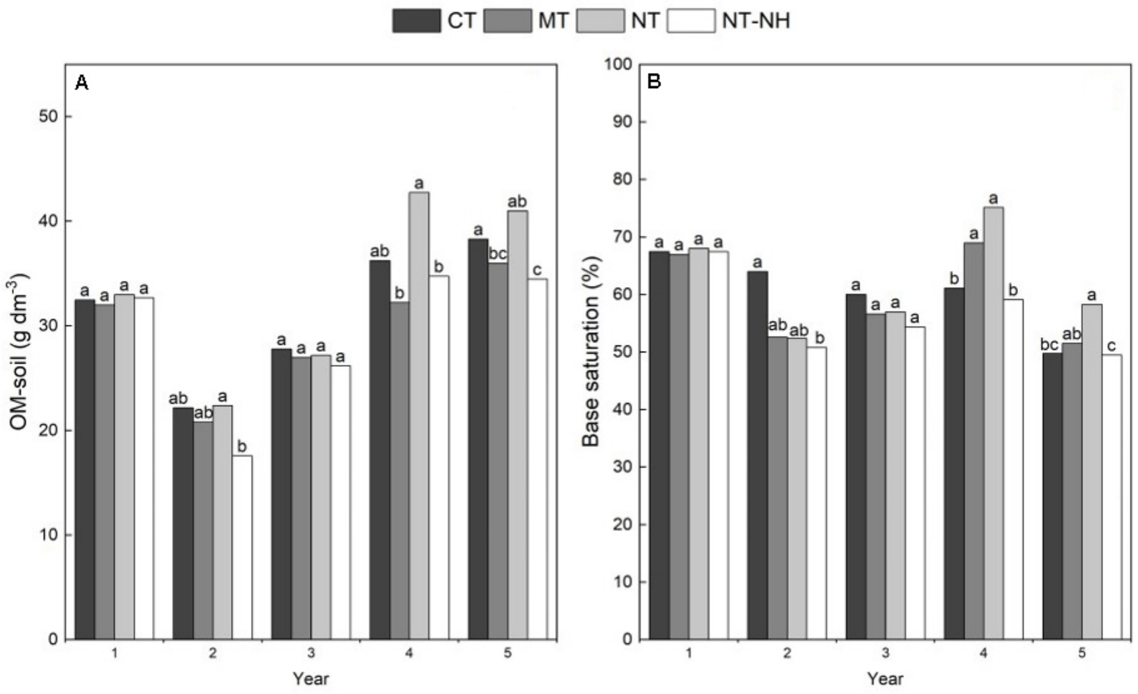

FIGURE 6 | Organic matter (OM) **(A)** and base saturation **(B)** of soil in four different planting systems of Tahiti acid lime, over three growing seasons (2015–2019). CONV, conventional tillage; MT, minimum tillage; NT, no-tillage; NT-NH, no-tillage + no-herbicide. Years: 1 = 2015, 2 = 2016, 3 = 2017, 4 = 2018, and 5 = 2019. Means followed by the same letter, for each evaluating and year separately, do not differ (n = 20; Tukey test at $p < 0.05$).

of the low vigor of some species used as anchoring rootstocks in citrus fruit, as is the case of the Flying Dragon. A lower amount of xylem of Flying dragon trifoliate orange, brings about a reduction in the capacity for transporting water from the roots to the leaves (Martínez-Alcántara et al., 2013).

The treatments that have covering of the soil by mulch (NT and NT-NH) allowed higher soil moisture values (**Figure 4B**). This occurs due to the protection that the cover crop provides, showing greater protection against the rays of the sun, besides reducing the effects of rain and water leaching, favoring the infiltration of water. Favarin et al. (2015) and Pedrosa et al. (2014) studying *Urochloa* spp. with intercropping to coffee, obtained a 49% increase in soil moisture resulting from the biomass left by cover crops. Furthermore, this biomass left by brachiaria can release high levels of N into the soil which can be used by the coffee crop.

Soil Fertility and Foliar Analysis

Higher levels of P are often reported in the surface layers of soil in the no-tillage system due to the application of surface fertilizers and non-revolving and the low mobility of P, in oxidic soil, to the deeper layers, in addition to the mineralization of the organic matter of the cover crop (Spera et al., 2011). However, the P levels in the soil and the leaves of Tahiti acid lime did not differ between the proposed planting systems (data not shown). Bremer Neto et al. (2008), working with management of a Pera sweet orange orchard [*Citrus sinensis* (L.) Osbeck], with and without mulch in the citrus row, also did not observe any differences in the levels of P in the soil. Ulén et al. (2010) and Raghothama and Karthikeyan (2005) emphasized the importance of maintaining vegetation cover on the planting row, since the availability of P is directly related to the aeration, moisture and temperature of the soil, factors influenced by the addition of phytobiomass in productive systems.

The highest concentration of K in the soil, found in the treatments with the presence of *U. ruziziensis* mulching on the citrus row, the NT and NT-NH (**Figure 5A**), is also reported for other fruit plants such as apple trees, using spontaneous vegetation as a cover crop (Oliveira et al., 2016), and in various no-tillage researches for maize cultivation, using a consortium of *Urochloa* spp. (Borghi et al., 2013). The use of *Urochloa* spp. in a system of agricultural and livestock integration makes it possible, for example, to absorb non-interchangeable forms of K and to subsequently liberate this nutrient in forms of greater availability, for the cultivation of corn (Garcia et al., 2008). Salton and Lamas (2011), assessing the quantity of macronutrients released by the decomposition of the *U. brizantha* mulch, observed the release of 100 kg ha^{-1} of K per year.

The release with K is greater in no-tillage systems because of characteristic mineral composition of mulch, enriched with K, as found in the present work (**Table 2**), since the mineralization of the K of the mulch can be relatively fast, and the nutrient remains almost entirely in the ionic form in the plant tissue and, consequently, the K is quickly released into the soil. This monovalent cation is not associated with any particular component structure in the plant tissue (Boer et al., 2008); the

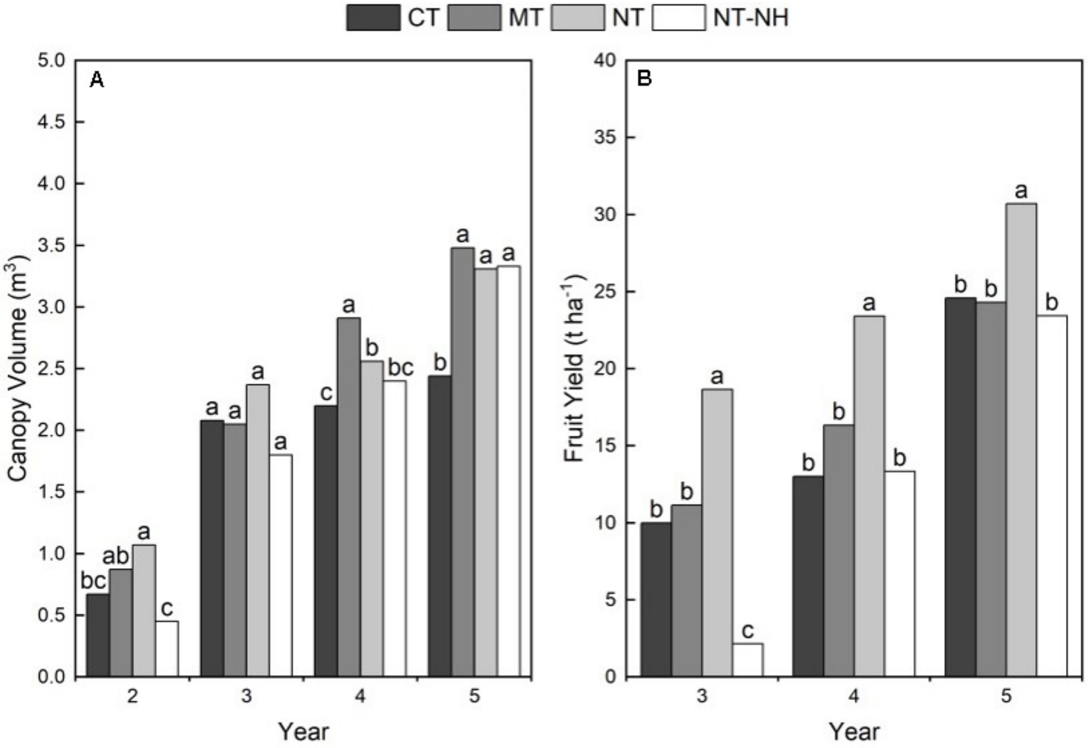

FIGURE 7 | Canopy volume **(A)** and fruit yield **(B)** in four different planting systems of Tahiti acid lime trees, over three growing seasons (2017–2019). CT, conventional tillage; MT, minimum tillage; NT, no-tillage; NT-NH, no-tillage + no-herbicide. Years: 1 = 2015, 2 = 2016, 3 = 2017, 4 = 2018, and 5 = 2019. Means followed by the same letter, for each evaluating and year separately, do not differ ($n = 20$; Tukey test at $p < 0.05$).

K released from mulch is in the soluble form, easily absorbed by the plants, resulting in rapid absorption (Zalamena et al., 2013), as observed for Tahiti acid lime in NT and NT-NH treatments (**Figure 5B**).

Calcium and Mg showed similar distribution to K in the soil (**Figures 5C,E**), result from the cycling of these nutrients in the treatment with mulching and herbicide (NT), leading to an increase in the soil levels in the fourth and fifth years after the planting of Tahiti acid lime, contributing to the increase in soil base saturation (**Figure 6B**). Oliveira et al. (2001) explained that Ca and Mg have a similar rate of release from the mulch, slower than that of K.

In the second year (2016), the levels of soil nutrients were low, probably because it was a less rainy year in Araras, São Paulo State, Brazil. In 2016 it rained 20% less (1,700 mm) than the average history for the region, which is a 1,500 mm. In February 2016, it rained only 50 mm, less than half the historical average. The low soil moisture, due to the scarcity of rain, negatively affects the availability of nutrients to plants and microorganisms in the soil (Cavagnaro, 2016).

Potassium was more concentrated in citrus plants in treatments with mulching than was Mg (**Figures 5B,D,F**). This is strong evidence that K is absorbed more rapidly, actively or by facilitated diffusion, and strongly competes with other cations such as those of Ca and Mg (Mattos-Junior et al., 2004; Quaggio et al., 2011; Medeiros et al., 2014).

Although soil Mg levels increased in the fourth and fifth years after planting in the treatments with mulching and herbicide (NT), this did not reflect the leaf levels of this nutrient. Cations such as Ca^{2+} and K^{+} compete and depress Mg absorption (Marschner, 2002); in the NT treatment high levels of K in the soil were observed, justifying this assumption of competition at the surface of the roots, and one evidence of competition between cations for absorption is that the sum total of cations in a plant is not very variable with an increase in the supply of a specific cation, since a reduction in the proportion of the other cations present generally occurs (Mengel and Kirkby, 2001).

The greater intake of N in the leaves of the Tahiti acid lime, observed in the NT treatment in the second year of evaluation (data not shown), was also observed by Oliveira et al. (2019) for apple trees, using biomass treatment of spontaneous plants covering the orchard planting row. Fidalski and Stenzel (2006) also observed an increase in the leaf levels of N in the Folha Murcha sweet orange grafted onto Rangpur lime, brought about by the covering of the inter-rows with the use of *Urochloa brizantha*. Liu et al. (2013) explain that, in addition to increasing the N levels, mulching also significantly reduces the loss of that nutrient by surface rainwater flow.

Vegetative and Productive Development

In the fifth year of evaluation, the canopy volume of the Tahiti acid lime was still small, due to the use of the Flying Dragon trifoliate orange, a dwarfing rootstock (**Figure 7A**), on the other hand, Martinelli et al. (2017) studying a vigorous rootstock, the Swingle citrumelo, reported values greater than 15 m^3 at the same age (5 years), confirming the dwarfing effect of the Flying Dragon. This characteristic of the Flying Dragon makes it possible to manage high-density planting, bringing good results in productivity, without altering the quality of the fruit. For the small citrus fruit growers, this is important because they can increase their income by making better use of the land area and by the precociousness of the fruit production, anticipating the financial return (Azevedo et al., 2015).

The productivity of Tahiti acid lime in the NT treatment reached a value close to 30 t ha^{-1} in the fifth year (**Figure 7** B), exceeding the Brazilian average of mature orchards, which is 25.6 t ha^{-1} (Food and Agriculture Organization [FAO], 2017). The lower fruit productivity in the CT system, in relation to NT, may be related to the field competition (**Figure 7B** and **Table 2**). The weed competition in the NT treatment was minimized by the presence of mulch and by the use of glyphosate, providing lower density of weeds in the citrus row, reducing competition for water and nutrients (Granatstein et al., 2014). Unfavorable productivity results are reported in no-conservative plantings, because of competition for water between citrus plants and the intermediate vegetation in periods of water stress, however, this is only observed when the handling of the covering plants is ineffective (Wright et al., 2003; Auler et al., 2008).

The differences in crop production reflect all the characteristics of the production systems used. Greater efficiency in productivity will depend on effective adaptation to the region's soil and climate characteristics. There are great prospects for reducing the use of herbicides, but for this to occur it is essential to apply the correct handling of the litter. This will not only benefit the control of weeds but also a series of factors involved in the conduct of the culture, mainly reflecting on productivity (Gomes and Christoffoleti, 2008). Thus, the NT-NH treatment is promising, since it shows similar productivity to CT and MT (**Figure 7B**), but with the advantage of eliminating a chemical input (herbicide), so more sustainable.

The economic situation of Brazilian citriculture, with the presence of *huanglongbing* (HLB), the most important citrus disease, is very critical and requires high productivity for the producer to maintain profitability. It is necessary to adopt economically viable and efficient techniques to guarantee the longevity of the orchards, ranging from a suitable planting system to the high-density planting (Azevedo et al., 2015). For dense systems, the use of combinations of scion and rootstock producing smaller mature plant size is fundamental (Moreira et al., 2019). Maintaining the mulch on the planting rows contributes toward the adaptation of dwarfing rootstocks under non-irrigation conditions, due to a more favorable environment for roots grow that increase water and nutrient use efficiency and decreased problems with weeds, which, makes the system more economically profitable and environmentally sustainable. Increased productivity highlights the importance of these systems as sustainable alternatives for medium and small citrus growers.

CONCLUSION

The reported no-tillage (NT) system, including the correct management of intercropping *Urochloa ruziziensis* with the use of an ecological mower, and glyphosate in rows, has improved the physical and chemical properties of the soil, in comparison with conventional tillage. Thus, results lead to the conclusion that the NT system tested for Tahiti acid lime grafted onto Flying Dragon trifoliate orange rootstock is effective, increasing crop productivity, in conditions without irrigation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

FA, PC, and DM designed the experiment. AA, AP, FA, RA, PC, RL, and RM performed, collected, and analyzed data. DM, RB, and VD supervised assessment of nutrition and plant physiological traits. AA, DM, FA, PC, RB, RM, and VD critically revised and edited the final manuscript version. All authors discussed the results and commented on the manuscript.

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REVIEW
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Sustainable Mixed Cropping Systems for the Boreal-Nemoral Region

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Mixed cropping, including intercropping, is the oldest form of systemized agricultural production and involves the growing of two or more species or cultivars of the same species simultaneously in the same field. However, mixed cropping has been little by little replaced by sole crop systems, especially in developed countries. Some of the advantages of mixed cropping are, for example, resource use efficiency and yield stability, but there are also several challenges, such as weed management and competition. The boreal-nemoral region lies within the region 55 to 70°N. In this area, for example in Finland, the length of the thermal growing season varies from >105 to over 185 days. Typically, variation between locations and years is marked. However, during the year, there can be a wide range of temperature extremes between −70 and +30°C. The majority of cropping systems in this region are usually monocultures, except for forage grass mixtures. The possibility of having several crops in a mixture is very challenging in the region due to the short growing season and extreme cold temperatures, meaning that crop earliness and overwintering capacity are a considerable restriction for year-round mixed cropping. A further restriction is the quality requirements set by the industry. Our review will explore a range of mixed cropping possibilities for the boreal-nemoral region, including different possible combinations of spring, winter, perennial, biennial, catch, and cover crops. The reviewed mixed cropping systems could considerably improve the sustainability and efficiency of crop production.

Keywords: catch crops, cover crops, double cropping, intercropping, nitrogen management, relay cropping

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INTRODUCTION

The oldest form of systemized agricultural production was based on mixed cropping (Plucknett and Smith, 1986). In mixed cropping and intercropping, growing of two or more crop species or cultivars takes place simultaneously in the same field with the aim of improving the resource use efficiency and yield stability, and decreasing losses due to possible pathogen and pest infestation. The main difference is the definite pattern of the crops in intercropping. Relay cropping as well as catch and cover cropping can also be considered as mixed cropping. The specific feature of relay cropping is that the second crop is seeded after the first crop; thus the first crop is harvested well before the second crop, even in the following growing season. In catch cropping, nutrient scavenging crop species are used between the main crops cultivated for yield. Nutrients are fixed into living plant tissues, which minimizes nutrient leaching into the environment (Dabney, 1998; Dinnes et al., 2002). Catch cropping can also improve sustainability, since after incorporation into soil, the nutrients are available for the following main crop (Thorup-Kristensen and Nielsen, 1998).

Cover cropping is sometimes associated with catch cropping, even though the term refers mainly to a crop covering the soil and thus reducing water and wind erosion (Dabney, 1998).

The advantage of mixed cropping is the higher number of plants per unit area and differences in pest and pathogen resistance as well as stress tolerance of different plant species and cultivars. Due to a dense plant stand, the foliage and roots cover a larger area, thus increasing the radiation (Keating and Carberry, 1993), water (Morris and Garrity, 1993a), and nutrient (Midmore, 1993; Morris and Garrity, 1993b) capture. Mixed cropping could result also in further benefits, such as a lower number of weeds in dense plant stands and a lower number of pests and diseases due to difficulties in detecting hosts and an increased number of natural enemies (Altieri and Liebman, 1986; Trenbath, 1993). However, the plant stand architecture, growth, and dry matter partitioning of species in the mixture can vary due to competition for available resources and species interaction (Silvertown, 1982).

The boreal-nemoral region lies between 55 and 70°N. The land in this zone experienced heavy glaciation and, as a result, features such as moraines and eskers are common surface features in the region (Metzger et al., 2012). Arable land is limited and agricultural production challenging because of the short length of the growing season (few frost-free days and a small sum of growing degree days or heat units) and the striking changes in day length through the year. In summer, the sun does not set and day length may range from 17 to 19 h at 55 to 60°N, respectively [Baldocchi et al., 2000; Tveito et al., 2001; FMI (Finnish Meteorological Institute), 2020]. Conversely, in winter, above the arctic circle the sun does not rise above the horizon, causing a period known as "polar night" when very short days are common and can be as short as 7 h at 60°N, for example in Southern Finland [Baldocchi et al., 2000; Anonymous, 2020; FMI (Finnish Meteorological Institute), 2020]. Large variations in day length mean that the region also experiences large variations in temperature range during the year, and thus crop production is restricted to the southern edge of the region, while grasslands are cultivated further north (Heikkilä and Seppä, 2003; Metzger et al., 2012).

For the present review, we will define the boreal-nemoral region in Europe as including Denmark, Estonia, Finland, Latvia, Lithuania, Norway, and Sweden, all countries that lie above 55°N. Strict or constant limits for the southern fringe of the boreal-nemoral region are elusive; for example, in some reports the southern edge of Norway and the whole of Denmark are outside the nemoral zone (Metzger et al., 2012), while in others they are part of the boreal-nemoral region (Hagen et al., 2013). In addition to cases from the above countries, we also include examples from Canada, where the provinces of Alberta, Manitoba, Nova Scotia, and Saskatchewan are all above 49°N and where there are valuable examples of mixed cropping systems that, thanks to their similar climate and land features, could be implemented in the boreal-nemoral region in Europe.

In the boreal-nemoral region, most common mixtures include legumes because of their symbiosis with atmospheric nitrogen (N_2) fixing *Rhizobium* species, for which reason the requirement for fertilizer application is either decreased or excluded (e.g., Andersen et al., 2005). In mixtures, legumes are mainly grown with forage grasses but in some cases also with cereals and rapeseed. Typical examples are oat (*Avena sativa* L.)-vetches (Lauk and Lauk, 2009), ley mixtures, and small grain cereals-pea (*Pisum sativum* L.) (Harper, 1983) as well as leys undersown with small grain cereal (Känkänen et al., 2001; Känkänen and Eriksson, 2007).

In this review, we will discuss different mixed cropping possibilities, including also intercropping and relay, catch, and cover cropping, for the boreal-nemoral region. The main emphasis is on ways to improve the sustainability and efficiency of crop production.

MIXED CROPPING

In mixed cropping, two to several different plant species or cultivars are grown at the same time in a plant stand. Therefore, the individual plants have complex interactions with each other, which might result in an altered assimilate partitioning and thus growth and senescence both in above- and belowground plant parts (Silvertown, 1982). Furthermore, competition for resources also changes throughout the growing season. Early in the season, plants compete mainly for water and nutrients, whereas competition for light takes over later in the season as the foliage expands. At the end of the season, the original plant stand density affects the severity of competition and thus the final number of remaining plant individuals. Environmental conditions, such as temperature and precipitation, may be more favorable to one over another species. Finally, the properties of species in the mixture and their ability to utilize available resources will determine the dominance. For example, deep-rooted and tap-rooted species restrict the growth of shallow-rooted ones due to better access to deep soil moisture and nutrients (Harper, 1983; Vandermeer, 1989). A further challenge is caused by weeds (Vandermeer, 1981).

When planning mixed plant stands, it is important to take into account the different characteristics and features of the component species of the mixture and the mixture itself, especially the growth habit to avoid competition (Vandermeer, 1989); for example, root systems of different species often avoid each other (Silvertown, 1982), the nutritional requirement and timing of nutrients as well as other resources differ between species, not to mention the synthesis and tolerance of allelochemicals (Zimdahl, 2004). Solutions to alleviate the competition include adjusted seeding times and densities of the component species to maximize the plant stand productivity (Davies et al., 1986). Growth advantage for inferior species of the mixture can be achieved for example by seeding the dominant species later than the other species (Andersen et al., 2007).

Properly planned mixed cropping can improve the sustainability, productivity, as well as yield (Vandermeer, 1989; Fukai and Trenbath, 1993). Mixed stands remove higher amounts of nutrients with yield in comparison with sole crops, resulting in higher nutrient use efficiency (Midmore, 1993; Morris and Garrity, 1993a). This should be taken into account especially in environments with limited nutrient availability (Midmore, 1993). Radiation use efficiency is usually also improved in mixed crops due to increased leaf area index (Keating and Carberry, 1993), restricting also the existence of weeds. However, in a dense canopy, shading increases and can result in a situation where assimilation is exceeded by respiration (Black, 1963). Bigger leaf cover also leads to improved water use efficiency because of lower evaporation and soil temperature (Morris and Garrity, 1993b). Even though mixed crops usually consist of species reaching maturity at the same time, most yield advantage is obtained from crops reaching maturity at different times. As an extreme, in relay cropping, the second component of the two-crop mixture is seeded markedly later than the first one, although well before harvest of the first crop (Francis, 1986). This allows the later seeded crop to utilize the resources without marked competition (Fukai and Trenbath, 1993) as well as consecutive growing of two crops in a limited growing season (Tuulos et al., 2015b). Examples of relay cropping in the boreal-nemoral region include mixed spring and winter crops, such as oilseeds and cereals, which can potentially be used for forage in the vegetative stage (Davidson et al., 1990; Tuulos et al., 2015a) and harvested for seed yield in the later stage (Tuulos et al., 2015a).

Perennial Forage Mixtures

The best-known and most utilized crop mixtures around the world are most likely forage. Also in the boreal-nemoral region, forage is commonly grown in perennial mixtures, either in binary or more complex mixtures, typically including both grasses and legumes. For example, in Finland most of the forage produced for cattle, both for silage and grazing, consists at least of a few grass species and one forage legume species. The most commonly used species include timothy (*Phleum pretense* L.), meadow fescue (*Festuca pratensis* Huds.), tall fescue (*Festuca arundinacea* Schreb.), perennial ryegrass (*Lolium perenne* L.), × *Festulolium*, red clover (*Trifolium pratense* L.), and white clover (*Trifolium repens* L.). In pastures, Kentucky bluegrass (*Poa pratensis* L.) is also commonly used. The same species combinations are used at the same latitudes also, for example, in Sweden, Norway, and Canada, with some variation depending on the local climatic factors. Winter tolerance is one of the most important features when selecting perennial crops for forage production at high latitudes; thus species and cultivars must be carefully selected. However, recently more winter tolerant cultivars, for example for perennial ryegrass, have been introduced, and the cultivation area has expanded to more harsh winter climates in the continental regions (Helgadóttir et al., 2018a). Alfalfa (*Medicago sativa* L.) is not yet cultivated extensively, but there might be more possibility for this in the future due to the warming climate and breeding of new cultivars (Annicchiarico et al., 2019).

A higher yield in perennial forage mixtures is mainly due to higher biodiversity and different functional plant groups. In perennial forage mixtures, plants are typically divided into three groups: grasses, N fixing legumes, and other herbaceous species. Although species belonging to different functional groups are supposed to be more complementary, even mixtures containing only two different grass species have been shown to produce higher dry matter yield than sole one species stands (van Ruijven and Berendse, 2003; Ergon et al., 2016; Helgadóttir et al., 2018b). Moreover, a stable yield increase was seen when more grass species were added to a mixture of 2, 4, 6, or 8 species (van Ruijven and Berendse, 2003). Although van Ruijven and Berendse (2003) included also grass species with non-agronomic importance in their study, valuable information on the biodiversity effect of mixtures containing only grasses was gained. However, in a study where legumes, red and white clover, and alfalfa were grown in sole stands and mixtures without grasses, the over-yielding effect was not observed (Dhamala et al., 2017).

Different physiological characteristics and growth habits of grasses benefit biodiversity; for example, timothy has shallower (Bertrand et al., 2008) and *Festuca* deeper root systems (Humphreys et al., 2013; Mäkinen et al., 2018) and thus resources can be allocated more evenly among species. In the spring, timothy has quite fast growth but the regrowth ability in the following cuts is slower and dry matter yield lower (Seppänen et al., 2010; Virkajärvi et al., 2012) compared with perennial ryegrass and *Festuca*, which can produce higher dry matter yields in the following cuts (Frame and Laidlaw, 2011). These differences between species benefit forage growth and thus enable larger yields.

The main advantage of using legumes in the perennial grass mixtures is the improvement of N supply through biological N fixation for non-legume species (Dahlin and Stenberg, 2010). Legumes are able to fixate the atmospheric N into the soil, and grasses and other species growing in the same mixture can use N for their growth. Plant diversity in the perennial mixtures increases the N_2 fixation mainly as a result of the non-legume species competition for N from soil (Carlsson and Huss-Danell, 2003; Rasmussen et al., 2012). For example, Li et al. (2019) showed that an unfertilized timothy–red clover mixture had higher dry matter yield than N fertilized sole stands in Finland because through biological N fixation plants were able to utilize resources more efficiently. Dhamala et al. (2017) concluded that perennial mixtures need to have non-legume species to maximally benefit from the N_2 fixation. However, under northern growing conditions it should be taken into account that symbiotic N_2 fixation is very dependent on temperature and is possible only during the most favorable summer months.

Grass–legume mixed swards produce higher dry matter yields in comparison with grass swards, possibly due to biological N fixation. For example, dry matter yield was 33–65% higher in grass–clover mixtures compared with sole stands of perennial ryegrass, cocksfoot (*Dactylis glomerata* L.), and white or red clover canopies in a 3-year field trial in southern Sweden (Frankow-Lindberg et al., 2009) and 21–32% higher in grass–clover mixtures compared with sole stands of timothy, Kentucky bluegrass, and white or red clover canopies in a 3-year field trial in northern Europe and Canada (Sturludóttir et al., 2013). In Iceland, Helgadóttir et al. (2018b) reported as high as 71% yield advantage in mixtures compared with sole stands of timothy, meadow fescue, and red and white clover across a 5-year field trial. Large differences between separate studies are partly explained by the differences in the experimental locations as well as the species used and their ability to use resources efficiently. Research conducted at 31 different sites, including Finland, Sweden, Norway, and Iceland, reported that mixtures containing both grasses (timothy, perennial ryegrass, cocksfoot, Kentucky bluegrass) and legumes (red and white clover, alfalfa) produce on average 32% higher yield compared with monocultures (Finn et al., 2013). Interestingly, adding alfalfa to the mixture increased dry matter yield only by 7% (Finn et al., 2013), 8% (Bélanger et al., 2014), and 12% (Thompson, 2013) over the best monoculture.

Including several species in the perennial forage production generally also improves feed security. In the boreal-nemoral growing conditions, winter hardiness is still one of the key issues for forage production, and some species and cultivars are better adapted. Generally, grasses have better winter hardiness than legumes; thus the former must be included in the mixtures.

Legumes in perennial swards typically improve the nutritional quality of the yield. According to Mela (2003), grass and red clover mixtures produce higher protein and crude fiber content compared with sole grass swards in Finland. Sturludóttir et al. (2013) reported better digestibility and high crude protein content in grass–clover mixed swards compared with grass monocultures in northern Europe and Canada. Adding alfalfa to a grass mixture improved neutral detergent fiber (NDF) concentration and digestibility in a Canadian study (Bélanger et al., 2014). Moreover, adding red clover to a mixture has shown to increase the milk production and quality in dairy cows (Heikkilä et al., 1992). It can be concluded that adding legumes to mixtures has a positive effect on the nutritive value of the yield without a negative effect on the dry matter yield.

Mixtures also reduce the invasion of weeds and other unwanted species. In sole stands of grasses and legumes even 10–60% of the dry matter yield can be weeds, whereas in mixtures the proportion can be <2–5% of the dry matter yield as shown in several studies conducted in the boreal-nemoral region (Frankow-Lindberg et al., 2009; Finn et al., 2013; Sturludóttir et al., 2013; Bélanger et al., 2014; Helgadóttir et al., 2018b). Mixtures are able to use resources more efficiently compared with sole stands, and therefore weeds are not able to invade the plant stand. Furthermore, mixtures produce higher dry matter yields which partly suppress weeds and other unwanted species.

Perennial Forage Mixtures With Forage Herbs

Recently, even more exotic species, i.e., non-leguminous dicotyledon forage herbs (forbs), such as ribwort plantain (*Plantago lanceolata* L.), chicory (*Cichorium intybus* L.), and salad burnet (*Sanguisorba minor* L.) have been tested for perennial forage mixtures. Reasons to introduce these species include the potential to increase plant diversity, competitiveness, and tolerance to different weather conditions (Eriksen et al., 2012; Pirhofer-Walzl et al., 2012), and improvement in the nutritional quality (Pirhofer-Walzl et al., 2011).

In several Danish experiments, forage herbs, including ribwort plantain and chicory, have been studied to reveal the potential for forage production. Dhamala et al. (2018) showed that forbs can be used in perennial mixtures without a negative effect on the yield or amount of biological N_2 fixation, but the amount of forbs in the mixture needs to be low. Typically, forbs are rich in minerals and thus could possibly serve as balancing supplements in mixtures. Plantain and chicory had higher concentrations of some macro- and micronutrients, such as phosphorus, magnesium, potassium, boron, sulfur, and zinc, compared with grasses and legumes (Pirhofer-Walzl et al., 2011). Using a multispecies grass–forage–forb mixture as the main feed source for milking cows reduced the need for artificial mineral supplements and simultaneously increased some ecosystem services, for example, foraging sites for pollinators (Pirhofer-Walzl et al., 2011). In multispecies mixtures, grasses took a higher amount of N fixed by clover, whereas forbs used soil N for growth (Dhamala et al., 2017). It seems that forbs could balance perennial forage mixtures in a sustainable way and use resources differently compared with grasses and legumes.

Forbs are not commonly used in the boreal-nemoral region in grasslands and thus not many extensive field trials with forbs have been conducted. In Finnish advisory groups, chicory has been tested in multispecies mixtures for silage production. Preliminary results have indicated that chicory has potential also in Finnish growing conditions, especially in fields facing drought problems and for plots intended for fast rotation grazing (Proagria, 2017).

Mixed Cropping by Combining Spring Crops

Traditionally the most typical spring crop combinations in the boreal-nemoral region have been the grain legume–cereal mixtures grown for whole crop forage, mainly pea (*Pisum sativum* L.) mixed with oat (*Avena sativa* L.), barley (*Hordeum vulgare* L.), and wheat (*Triticum aestivum* L. em. Thell.). Due to their usage as forage, the majority of studies have focused on the nutritive value and ensiling of mixed spring crops. In grain legume–cereal mixed crops the forage quality is higher in comparison with sole cereal forage. Grain legume–cereal mixed crop forage has higher crude protein content, higher protein yield, and higher relative feed value, and it can provide alternatives to more traditional forage (Strydhorst et al., 2008). However, less focus has been paid to environmental, ecological, and physiological traits of the grain legume–cereal, cereal species, and cereal cultivar mixtures. The major obstacle for species and cultivar mixtures for grain has most likely been the problems in marketing the yield, since the industry has so far been interested in sole crop grains. Separating the seeds of different cultivars and species for industrial processes is time-consuming and expensive. A further challenge has been harvesting mixed grain crops, since the components have to reach maturity at the same time. However, in low-input cropping systems there can be both ecological and economic advantages of cultivating spring crop mixtures not only in tropical regions but also in the boreal-nemoral region.

Due to its suitability to boreal-nemoral growing conditions and the long tradition of including it in mixed crops, pea has been the most studied grain legume as a component crop. Pea–barley mixtures have been extensively studied for example in Denmark, and pea in combination with other spring cereals, for example triticale (× *Triticosecale* Wittm. ex A. Camus), wheat, and oat in Estonia, Finland, and Lithuania. Other grain legumes studied for mixed crops with cereals include narrow-leafed lupin (*Lupinus angustifolius* L.), faba bean (*Vicia faba* L.), and oilseed rape

(*Brassica napus* L. ssp. *oleifera* (Moench.) Metzg.). In Denmark and Canada, studies have also involved tri-crop combinations, i.e., oilseed rape and pea with either wheat or barley. A less traditional faba bean–maize (*Zea mays* L.) mixture for forage production has gained interest in Sweden (Stoltz et al., 2018).

In general, the pea–cereal mixtures (**Table 1**) have been higher yielding than the sole crops, especially when grown without N fertilizer on soils with low fertility (Ghaley et al., 2005; Lauk and Lauk, 2008). According to Knudsen et al. (2004), the dominant species in grain legume–barley mixtures were legumes on sandy loam and barley on sandy soil. In Estonia, pea–cereal mixtures with oat, wheat, and barley grown on sandy-clayey soil without fertilizers produced higher grain yield and protein yield than sole crops. The highest yielding combination was a pea–oat mixture, in which case the yield of oat was higher than the yield of the sole oat crop, especially when the seeding rate of pea ranged from 20 to 80 seeds m^{-2}, when the optimum for pea as a sole crop is from 100 to 120 seeds m^{-2} (Lauk and Lauk, 2008). Increased plant stand density favors pea over barley, which is seen in decreased yield and yield stability of the barley component and increased yield stability of the pea component (Jensen, 1996). Similarly, the lupin component is suppressed in a mixed crop with barley when the plant stand density increases (Hauggaard-Nielsen et al., 2008). Furthermore, competition by pea in mixed crops decreased both the grain size and the protein content of cereals (Lauk and Lauk, 2008), indicating that the increase in protein yield of the mixed crop was due to the pea component. In Denmark, a pea–wheat mixture grown on sandy loam soil was most productive without fertilizers (Ghaley et al., 2005). Increasing the rate of N fertilizer gave a competitive advantage to wheat, thus suppressing the growth of pea, although without a decrease in intercrop grain yield. Without N fertilizer, pea as a component of a pea–wheat mixed crop fixed more N than a sole crop (Ghaley et al., 2005), although faba bean and lupin are even more efficient in N fixation as component crops in grain legume–cereal mixtures (Hauggaard-Nielsen et al., 2008).

In pea–barley mixed crops, barley has proven to be a strong competitor for N and thus seems to have a negative effect on N fixation when dominant (Jensen, 1996; Hauggaard-Nielsen and Jensen, 2001). Hauggaard-Nielsen et al. (2001) observed that N fixation of pea decreased from 120 kg ha^{-1} in sole pea crops to 30 kg ha^{-1} in a pea–barley mixed crop. According to Jensen (1996), N fixation decreases with increased N fertilization, because it gives further competitive advantage to barley over the grain legume. Barley seedling emergence and the growth of its root system in time and space are faster than those of pea, thus allowing barley to explore a larger soil volume earlier in the growing season as well as shade the pea foliage (Hauggaard-Nielsen et al., 2006). However, the competition between the grain legume and the cereal depends on the cultivar traits of the component crops, such as the emergence and growth rates, determinate/indeterminate growth type, height, leaf area, and tiller formation. For example, Hauggaard-Nielsen et al. (2001) reported that unlike determinate pea, indeterminate pea in the mixture decreased both the N uptake and the grain yield of barley. For a list of the traits of an optimal pea cultivar for mixed crops with cereals, see the review by Hauggaard-Nielsen et al., 2003.

When pea–barley and pea–oilseed rape mixtures were compared with their sole crop counterparts in Saskatchewan, Canada, it was found that both oilseed rape and barley grown in a mixture together with pea yielded better than sole crops without N fertilizer, resulting in land equivalent ratio values up to 1.56 (Malhi, 2012). However, the yield of individual components in a mixture decreased. In mixtures, the protein content of both barley and oilseed rape was higher, but the oil content of oilseed rape was lower than in the sole crops (Malhi, 2012). Similarly, in Finland, a mixed crop of faba bean and oat produced higher grain yield than their sole components but only under poor growing conditions. This was related to better growth of faba bean in a mixture with wheat, mainly resulting in heavier grains of faba bean but also an increased number of panicles of oat (Helenius and Ronni, 1989). However, oat benefited more than faba bean from the mixture (Helenius, 1990).

Helenius and Ronni (1989) observed in Finland that mixed cropping of faba bean and oat increased the number of bird cherry-oat aphids (*Rhopalosiphum padi* L.) on the oat component but decreased the number of black bean aphids (*Aphis fabae* Scopoli) and weevils (*Sitona* spp.) on the faba bean component. In a faba bean–maize mixture, the severity of leaf spots on the faba bean decreased by up to 57% in comparison with a sole crop in Sweden (Stoltz et al., 2018) and by 20–40% in comparison with a grain legume–cereal mixed crop in Denmark (Hauggaard-Nielsen et al., 2008). Stoltz et al. (2018) concluded that the decrease could be related to a higher copper content of the plants in mixed crops. Mixed cropping increased the macro- and micronutrient uptake and thus nutrient use efficiency of component crops. Interestingly, in Finland, only the macronutrient content of oat increased in the faba bean–oat mixed crop (Helenius, 1990). A further advantage of the mixed crops was observed in Lithuania in the rate of weed infestation, which was up to 1.6-fold less in pea–cereal mixtures in comparison with a sole pea crop (Deveikyte et al., 2009). Similar marked decreases in weed infestation of grain legume–cereal mixed crops in comparison with sole grain legume crops have been reported also in other studies conducted within the boreal-nemoral region (e.g., Hauggaard-Nielsen et al., 2001, 2006, 2008; Sarunaite et al., 2010). In the majority of cases, mixed cropping tends to increase the reliability and stability of the grain legume component. Therefore, farmer interest toward the mixtures especially in the low N input systems, such as organic systems, and protein production is expected to increase as knowledge increases.

Adding the third crop component into the mixture pea–oilseed rape–wheat further increased the grain yield of the mixture in Canada (Szumigalski and Van Acker, 2006). Szumigalski and Van Acker (2006) explained the increased grain yield through N use complementarity of the three crops as well as increased light interception and spatial partitioning of water extraction between the crops (Szumigalski and Van Acker, 2008). Further advantages of three component mixtures

TABLE 1 | Land equivalent ratio (LER) values of different mixed spring crops grown in the boreal-nemoral region.

Harvest	Component crops	LER value	References
Biomass	Pea–barley	1.02	Hauggaard-Nielsen et al., 2006
Biomass	Pea–barley	1.25 (10 N kg ha^{-1})	Hauggaard-Nielsen et al., 2001
Biomass	Pea–barley	1.17 1.05 (50 N kg ha^{-1})	Hauggaard-Nielsen and Jensen, 2001
Biomass	Pea–wheat	∼1.34 ∼1.00 (40 N kg ha^{-1}) ∼0.85 (80 N kg ha^{-1})	Ghaley et al., 2005
Grain yield	Pea–barley	1.18 1.07 (50 N kg ha^{-1})	Hauggaard-Nielsen and Jensen, 2001
Grain yield	Pea–barley	1.54 1.29 (40 N kg ha^{-1})	Malhi, 2012
Grain yield	Pea–barley Faba bean–barley Lupin–barley	∼1.05 ∼1.10 <1.0	Knudsen et al., 2004
Grain yield	Pea–barley Faba bean–barley Lupin–barley	1.18 1.40 1.14	Hauggaard-Nielsen et al., 2008
Grain yield	Barley cultivars	0.99 (50 N kg ha^{-1}) 0.99 (100 N kg ha^{-1})	Jokinen, 1991b
Grain yield	Oilseed rape–wheat	1.00 (N unknown)	Hummel et al., 2009
Grain yield	Pea–wheat Pea–oilseed rape Wheat–oilseed rape Pea–wheat–oilseed rape	0.99 1.20 1.09 1.14	Szumigalski and Van Acker, 2005
Biomass	Pea–barley	1.15 (5 N kg ha^{-1}) 1.00 (40 N kg ha^{-1})	Andersen et al., 2005
	Pea–oilseed rape	1.32 (5 N kg ha^{-1}) 1.16 (40 N kg ha^{-1})	
	Oilseed rape–barley	1.33 (5 N kg ha^{-1}) 0.98 (40 N kg ha^{-1})	
	Pea–barley–oilseed rape	1.26 (5 N kg ha^{-1}) 1.16 (40 N kg ha^{-1})	

All crops received 0 N kg ha^{-1} fertilizer unless otherwise stated.

were achieved in the competitive ability and yield stability of the mixture. A three-crop mixture of pea, oilseed, and wheat increased the weed suppression ability of the plant stand in comparison with sole crop and two-crop mixtures. The weed biomass as well as relative weed density and biomass were lowest in the three-crop mixture. Competition ability and the ability to withstand competition were nearly the same in the three-crop mixture and wheat and wheat–oilseed rape mixtures. Even though a pea–oilseed rape mixture produced the highest grain yield, adding the third component crop, wheat, into the mixture increased the grain yield stability over years and locations (Szumigalski and Van Acker, 2005). However, in Denmark, when only the biomass production was evaluated in a similar three-crop mixture, the results indicated that two-crop mixtures had higher productivity in comparison with three-crop mixtures, both mixtures out yielding the sole crops. This was related to suppressed barley growth in three-crop mixtures (Andersen et al., 2007). Andersen et al. (2007) concluded that the most marked effect on productivity is between the sole crop and mixed crop systems, whereas adding further components into a mixture does not considerably affect the productivity of the mixture.

Less attention gained are spring cereal cultivar and species as well as oilseed rape–cereal (Hummel et al., 2009), cereal–flax (*Linum usitatissimum* L.), and cereal–oriental mustard (*Brassica juncea* L.) mixtures (Pridham and Entz, 2008). The oilseed rape–wheat mixture produced similar grain yields to sole crops, and the oil content of the oilseed rape as well as protein content of wheat increased in mixed crops. However, the leaf disease infestation of wheat increased in mixed crops, most likely due to higher humidity in the plant stand with increased foliage brought along by oilseed rape. Furthermore, flea beetle damage was similar in sole crops and mixed crops (Hummel et al., 2009). Although the wheat flag leaf disease level decreased in a wheat–flax mixture, flax outcompeted wheat, resulting in poor grain yield. A wheat–oriental mustard mixture was in general higher yielding than the sole crops, but suffered from flea beetle (*Phyllotreta cruciferae* Goeze), disease, and weed infestations (Pridham and Entz, 2008).

In Finnish and Canadian experiments conducted with cereal cultivar and species mixtures, and sole crops, only limited advantages have been observed in cereal cultivar and species mixtures over sole crops. According to Jokinen (1991a,b) growing barley in two to four cultivar mixtures did not increase the grain yield and the yield stability of the plant stand, contrary to the common argument of increased stability from diversity of genotypes [reviewed in Vandermeer (1989)]. Similar results were obtained by Pridham and Entz (2008) when wheat was grown in a mixture with oat, barley, and spring rye (*Secale cereale* L.). However, in Finland, a barley–oat mixture produced slightly higher total yield in comparison with sole crops (Jokinen, 1991d). In the mixed plant stands, species competition affected mostly the number of panicles, heads, and grains. However, the weight and protein content of the grains was not affected (Jokinen, 1991d). As part of a mixture, barley was more competitive than oat, especially with an increased rate of N fertilization. This could be explained by the ability of barley to respond through variation in yield components in response to changes in plant stand density and the availability of nutrients (Jokinen, 1991c). However, the cereal species mixtures could decrease the yield losses due to diseases. In spring, wheat cultivar mixtures based on glume blotch (*Septoria nodorum* Berk.) susceptible and resistant cultivars, Karjalainen (1986) observed that in the mixed crops the amount of disease was always less in comparison with sole crops regardless of whether the disease level was low or high. Although under the low disease level the advantage of a mixed crop was clear, under the high disease level only the progress of the disease was slowed down (Karjalainen, 1986). Based on these observations, cereal species and cultivar mixtures could be ecological options, since there is a possibility of minimizing the need for pesticides. The challenge so far with cereal species and cultivar mixtures has been marketing the yield for industrial purposes.

Mixed Cropping by Combining Spring and Winter Crops

The majority of investigations have focused so far on mixed spring crops in the boreal-nemoral region. A less well-known and less investigated type of mixed crop is the combination of a seed-producing winter crop established as a relay crop with a spring cereal. A few potential crop combinations have been studied and to some extent used in practical farming at least in Finland.

Tuulos et al. (2015a,b) studied the establishment of winter turnip rape (*Brassica rapa* L. ssp. *oleifera* (DC.) Metzg.) by undersowing with various spring cereals in Finland. Winter turnip rape is better suited to the cold temperatures and low-input cropping systems of the boreal-nemoral region than winter oilseed rape (Mäkelä et al., 2011). However, winter turnip rape needs to be sown by the end of July for successful overwintering under Finnish conditions, which is early compared with winter oilseed rape. Therefore, in a winter turnip rape sole crop during the early part of the growing season, the field needs to be an early-harvested grass–ley, fallow, or set-aside land, as none of the other crops cultivated in Finland generally reaches maturity by mid to late July. Establishing winter turnip rape simultaneously with a spring cereal allows harvesting of the cereal seed yield during the first growing season of the plant stand, while winter turnip rape remains in a vegetative growth stage (Tuulos et al., 2015b). Winter turnip rape enters the reproductive growth stage during the second growing season, after overwintering and vernalization (Tuulos et al., 2015a).

Undersowing winter turnip rape with spring cereals was attempted in the 1950s (Valle, 1951), but the method remained marginal. The main reason for abandoning the method after the early 1950s was the difficulty in cereal harvesting, especially in the case of the cereal stand lodging over the winter turnip rape stand, suppressing its growth. However, there are currently five different active ingredients and over 20 commercial plant growth regulator products available for the prevention of cereal lodging in Finland [TUKES (Finnish Safety and Chemicals Agency), 2020]. The use of plant growth regulators for cereals would decrease the risk of lodging and thus the risks related to cereal harvest and suppressed turnip rape growth.

In the work of Tuulos et al. (2015a), winter turnip rape was sown either with normal seeding density (150 viable seeds m^{-2}) or double seeding density (300 viable seeds m^{-2}) in Finland. The seeding densities of cereals were normal or reduced by 20%, respectively. The seeding time of winter turnip rape was either simultaneous with the spring cereal in May (mixed and sole stands) or as sole stands at the end of July. Two separate passes were required with a seeder; the seeding rows were parallel to each other. The establishment cost could be reduced if the crops were sown simultaneously. All tested spring cereals (oat, wheat, two-row barley, six-row barley) were suitable as nurse crops for undersown winter turnip rape (Tuulos et al., 2015b). Undersown winter turnip rape did not decrease cereal yields, even though wheat yield was affected by year. A cereal yield increase due to undersown winter turnip rape was observed with six-row barley and oat in some years. An explanation for the phenomenon was not identified by Tuulos et al. (2015b), but a similar increase in barley yield with undersown field cress (*Lepidium campestre* (L.) W.T. Aiton), a biennial crucifer, was observed by Merker et al. (2010) under Swedish conditions. Cereal yields tended to be slightly lower in the reduced cereal seeding density plant stands (Tuulos et al., 2015b), but with winter turnip rape, differences between different seeding methods and seeding densities were not evident (Tuulos et al., 2015a). Differences in winter turnip rape yields between years were attributed to overwintering conditions. In overwintering conditions similar to the long-term average in Finland, winter turnip rapeseed yields ranged from 1,800 to 2,300 kg ha^{-1} in stands established by undersowing or as the sole crop (Tuulos et al., 2015a).

Undersowing winter oilseed rape with a spring cereal does not necessarily create advantage in crop establishment, since winter oilseed rape can be sown later than winter turnip rape and after the harvest of an early spring cereal or winter cereal. Nordestgaard (1982), however, investigated the undersowing of winter oilseed rape with spring barley under Danish conditions. Nordestgaard (1982) concluded that the overwintering percentage of winter oilseed rape was decreased when the crop was established by undersowing, mostly due to hypocotyls growing too tall during the first growing period and thus being later in the winter exposed to freezing temperatures above the snow cover. Additionally, some of the winter oilseed rape hypocotyls were already cut off during the barley harvest, thus destroying the plants (Nordestgaard, 1982).

Kakko et al. (1997) studied in Finland the possibility of establishing winter rye and winter wheat by undersowing with spring barley in an attempt to decrease the need for soil tillage in cereal production. An additional benefit of the method was the avoidance of winter cereal seeding under poor seeding conditions with rains and excess soil moisture typical of late August and early September. Winter cereal seeding was done in a separate pass with a seeder before the emergence of spring barley. In order to minimize interrow competition and to establish as evenly distributed a mixed stand as possible, the second pass with seeder was performed crosswise to the direction of the first pass. Interestingly, winter rye (cv Ponsi) was not suitable for undersowing due to a large proportion of it entering the generative growth stage already during the first growing period. In contrast, winter wheat remained fully in the vegetative stage during the first growing period, while barley entered generative growth and produced harvestable seed (Kakko et al., 1997).

Overwintering performance of winter wheat was good, without winter damage observed. Growth of undersown winter wheat after overwintering was ∼9 days ahead of autumn-sown, sole crop winter wheat. However, both barley and winter wheat yield in the undersowing method was decreased compared with normally established sole crops, although not drastically. The yield of undersown winter wheat was on average 450 kg ha^{-1} smaller in comparison with the yield of autumn-sown sole crop. The yield decrease in barley in mixed cropping was 320–520 kg ha^{-1} in comparison with the sole crop. Additionally, the barley yield of undersown plots fulfilled the quality limitations set for malting due to decreased protein content, which was 0.5–1.0% points lower in the mixed crop compared with the sole crop barley (Kakko et al., 1997). The decrease in barley yield seemed to be dependent on the cultivar, as yield reduction was on average only 100 kg ha^{-1} with cv Kymppi, a late two-row cultivar, but 900 kg ha^{-1} with cv Arve, an early six-row cultivar. Despite the lower tillage cost, the economic profit of the undersowing was €53.71 ha^{-1} lower than in separate spring and autumn sowing due to lower yields of both barley and winter wheat. In 1997, the barley producer price was €157.43 t^{-1} and the price of wheat was €250.04 t^{-1} (Kakko et al., 1997). However, the average producer prices in 2009–2019 have been €155.69 t^{-1} for barley and €173.41 t^{-1} for milling wheat (LUKE (Natural Resources Institute Finland), 2019). Therefore, the difference in economic profit between undersowing and separate sowing methods would be nowadays smaller than 22 years ago, suggesting that undersowing winter wheat to barley could now be more attractive than in 1997.

There were, however, also additional challenges with undersowing winter wheat, namely increased occurrence of Hessian fly [*Mayetiola destructor* (Say)], which is difficult to control with the common pyrethroids available in Europe. The Hessian fly larvae are usually deep in the base of the plant and therefore cannot be reached with insecticide spray. Seed treatment with an insecticide reduced the number of Hessian flys in winter wheat plants in the experiments of Huusela-Veistola and Känkänen (2000). However, currently there are no insecticide seed treatments registered for use in cereals in Finland [TUKES (Finnish Safety and Chemicals Agency), 2020].

An advantage in relay cropping is the reduced leaching of mineral N from the agricultural environment. According to Tuulos et al. (2015c), winter turnip rape undersown with barley decreased the amount of soil mineral NO_3^--N more than 50% in the topsoil and more importantly 60–80% in the subsoil, when compared with NO_3^--N content in the topsoil and subsoil of plots with sole stand barley that was plowed after harvest. Tuulos et al. (2015c) discussed different explanations for the performance in NO_3^--N uptake by barley–winter turnip rape mixed stands and concluded that as crucifers tend to have deeper root systems than Graminae species they are commonly used as catch crops. A deep root system depletes subsoil NO_3^--N more efficiently than shallow root systems. Combining barley with winter turnip rape in a mixed stand results in a stand with densely distributed plant individuals and roots distributed to different depths in the soil. As a crop undersown already in spring, winter turnip rape has ∼10 more weeks to expand its root system than winter turnip rape sole crops, which are usually established in late summer. This was manifested as a higher amount of depleted subsoil N in the mixed stands of winter turnip rape and spring barley (Tuulos et al., 2015c).

Another benefit of relay cropping is the reduced need for soil tillage, as crops for two subsequent years are established simultaneously or almost simultaneously. Reduced tillage brings many benefits, including less use of fuel in agriculture and also less detrimental effects on the soil structure.

Spring and Winter Crop Mixtures for Silage

In cattle production, whole crop cereal silage is also used as a supplement to ordinary grass, legume, or maize based silage. Whole crop cereal silage feeding is common in areas where the cattle's indoor feeding season is long and outdoor feeding season, as well as the whole growing period, are short and variation in the produced amounts and availability of ordinary silage occurs. Usually, whole crop cereal silage is harvested from sole stands of spring or winter cereals, between the late milk and early dough stages (Jedel and Salmon, 1995). Jedel and Salmon (1995) studied spring barley and spring triticale grown as intercrop mixtures with winter triticale and winter rye for silage production in Alberta. As spring-sown winter cereals do not vernalize during the first growing season, the silage from winter–spring cereal mixtures consisted mostly of winter cereal leaves and spring cereal stems, leaves, and heads. Jedel and Salmon (1995) found out that the silage yield of winter–spring cereal mixtures was usually similar to or smaller than the silage yield of the sole crop of the higher yielding component of the mixtures, on average 8.46 t ha^{-1}. Similarly, the silage yield of cereal mixture tends to be equal or slightly lower than the silage yield of monocrops (Baron et al., 1992). However, the silage quality, measured as soluble fiber content, may slightly be improved due to the higher palatability of the vegetative parts of winter cereals in the silage made of mixed crops (Jedel and Salmon, 1995), even if the amount of winter cereal is modest (Baron et al., 1992). However, in a situation where the seeding ratio of winter cereal and spring cereal is 1:1, it is likely that the spring cereal dominates the winter cereal in a mixed stand and therefore contributes to the formation of total biomass more (Baron et al., 1992), indicating that adjusting the seeding ratio to the benefit of winter cereal could affect silage quality. An additional benefit of mixed stands is that as the silage quality is an intermediate of both components of sole stands, the appropriate time interval for harvesting could be wider than that of sole crops, without compromising yield quality. Regarding dry matter yield and silage quality, spring triticale seemed to be more suitable to mixtures than spring barley (Jedel and Salmon, 1995).

Juskiw et al. (2000) reported that mixtures of spring cultivars of oat, barley, and triticale as well as winter cultivars of rye and triticale had quality attributes intermediate of the sole component in the mixture and would therefore enable a wider harvest window for silage. As an opposite to the work of Jedel and Salmon (1995), increasing the sowing rate did not bring an advantage in the quantity of the silage yield (Juskiw et al., 2000). The most productive mixture was barley–oat intercrop giving slightly higher silage yields than either of the components as sole crops. Winter cereal mixtures tended to be lower yielding than sole stands of the components. Intraspecific variety mixtures, such as some combinations of barley varieties, could be more productive than sole variety stands (Juskiw et al., 2000).

Tuulos et al. (2015a) studied the possibility of harvesting the winter turnip rape leaves when grown mixed with spring cereals and as sole stands. Forage yields of winter turnip rape were in the range of 1,000–3,000 kg of dry matter ha^{-1}, sole stands always yielding the best. When the forage quality was evaluated, the mixed stand had a markedly lower D-value and crude protein and crude fat content but higher dry matter, crude fiber content, and NDF value. The differences were due to the cereal stubble in the mixed crops. The drawback was a weakened overwintering of the plant stands (Tuulos et al., 2015a).

Biennial Crops in Mixed Cropping Systems

There are few biennial crops of commercial importance in the boreal-nemoral region and few cases of their inclusion in mixed cropping systems. The most important biennial crop in the region is caraway (*Carum carvi* L.), which is rarely in mixed cropping systems because of the yield penalty, particularly on the second year yield. However, the possibility of using spring wheat, oat, flax, faba bean, and pea as intercrops for caraway has been studied in Finland. Mixed cropping with spring wheat and flax gave an extreme reduction in caraway yield; depending on the row system used, the yield in the second year was as low as under 100 kg ha^{-1} and the yield was ∼30% lower in the third year (Keskitalo, 2014). In contrast, mixed cropping with pea, faba bean, and barley, although reducing the second year caraway yield by 30–50%, resulted in, in the third year, up to a 3-fold higher caraway yield in comparison with a sole caraway crop. Specifically, when in mixed crop with barley or faba bean, caraway yielded ∼1,500 kg ha^{-1} and with pea ∼1,200 kg ha^{-1} (Keskitalo, 2014).

In vegetable cropping, there is evidence of some biennial legumes as good alternatives to be included in the mixed cropping systems. For example, yellow sweet clover (*Melilotus officinalis* L.), hairy vetch (*Vicia villosa* Roth.), and crimson clover (*Trifolium incarnatum* L.) were shown to be suitable alternatives in field trials in Norway (Brandsaeter and Netland, 1999; Brandsaeter et al., 2000). In addition, the biennial field cress was selected in Sweden as a potential new oilseed crop and catch crop. Field cress reduced N leaching remarkably well, leaving a significantly lower mean total N in soil than other catch crops, such as the mixture of hairy vetch and winter rye, although it had a negative effect on total phosphorus leaching (Ulén and Aronsson, 2018).

Two crops that have gained interest lately again are flax and Jerusalem artichoke (*Helianthus tuberosus* L.). Flax is an important fiber and oil crop in the region, particularly in Finland and Canada. In Canada, Halde and Entz (2014) grew barley and hairy vetch with and without tillage, and seeding flax the following year. It seems that including crimping of cover crops is effective at stopping the growth of barley but not hairy vetch. Moreover, the success of the system under no-till requires a well-established cover crop and an absence of excess soil moisture. Keeping the cover crop biomass until mid-summer harvest was a good strategy and did not cause a yield penalty in the flax crop subsequently harvested (Halde and Entz, 2014).

In Finland, Jerusalem artichoke obtained interest, since the aboveground biomass could be used as bioenergy feedstock. Vetch (*Vicia sativa* L.), sweet clover (*Melilotus albus* Medik.), goat's rue (*Galega orientalis* L.), and red clover were tested as intercrops. Although there were no significant differences between the effect of intercrops on Jerusalem artichoke yields and mineral element composition (compared with N fertilizer), the dense shade and soil disturbance during the harvest of tubers hindered the durability and ease of use of these intercrops (Epie et al., 2018).

Mixed Cropping Systems Including Catch Crops and Cover Crops

The use of cover and/or catch crops in mixed cropping systems is a key practice within conservation agriculture, which seeks to protect the soil cover and improve soil function, while preventing nutrient losses and erosion (Lahmar, 2010). Crops that have the ability or are chosen specifically to reduce N leaching are often referred to as "catch crops" (Valkama et al., 2015), while the umbrella term "cover crops" is used for cases when the crop provides other services, such as preventing phosphorus losses and soil erosion (Aronsson et al., 2016). The term "subsidiary crops," coined in recent years, includes crops that are mainly cultivated because of the range of agroecological benefits they provide rather than for economic profit (Reimer et al., 2019).

The climate in the boreal-nemoral region makes it prone to high N leaching during winter (Zhao et al., 2020). Thus, it is of utmost importance to reduce soil NO_3^--N levels left in autumn and to choose efficient catch crops (Wahlström et al., 2015). The role of cover crops in achieving more sustainable crop rotations has been well-studied, and current environmental policy in the region encourages farmers to often include them in the cropping systems. For example, in Denmark, farmers are required to grow cover crops in autumn on at least 10% of their farm area (Thorup-Kristensen and Kirkegaard, 2016) and in Finland there is an allowance of €100 ha^{-1} for cover crops (Salonen and Ketoja, 2019).

Advantages

Cover crops increase the vegetation cover in the off-season and have the potential to reduce NO_3^--N leaching by increasing the uptake of mineral N surplus (Känkänen et al., 2001; De Notaris et al., 2020; Zhao et al., 2020). Moreover, cover crops improve nutrient cycling as their life cycle terminates, since the following crops can reuse the N and other nutrients (e.g., sulfur, potassium, and phosphorus, among others) left in the surface soil layer and crop residues (Eriksen et al., 2004; Toom et al., 2019; Yang et al., 2019). Although catch crops may affect the cycling of several nutrients, when choosing catch crops the priority is mainly to maximize N efficiency in the cropping system, and the efficiency of other nutrients is assessed in that perspective (Eriksen et al., 2004; Løes et al., 2011).

Non-legume cover crops are the most effective option to reduce N leaching. Reducing up to 51–80% of N leaching, they are effective in a wide variety of soils and weather conditions (Knudsen et al., 2006; Sapkota et al., 2012; Jabloun et al., 2015; Valkama et al., 2015; Pugesgaard et al., 2017; Vogeler et al., 2019; Yang et al., 2019; De Notaris et al., 2020; Zhao et al., 2020). N leaching varies depending on soil type and rainfall (Askegaard et al., 2005, 2011; Hashemi et al., 2018; Pandey et al., 2018). For example, Askegaard et al. (2005, 2011) found that N leaching was higher in a coarse sandy soil with high rainfall than in a sandy loam with low rainfall.

Although legume cover crops are not as efficient in reducing N leaching, they are valuable as they fix atmospheric N and retain N, increasing N availability for the following crop, and thus reducing the need for external N fertilizer inputs (De Notaris et al., 2019, 2020; Zhao et al., 2020). Moreover, Pandey et al. (2018) pointed out that besides the effect of soil type and rainfall on N leaching, the response of N leaching to N input is critical, as N input varies as a consequence of different levels of biological N fixation by different cover crops and their successful establishment or not.

Mixtures of legumes and non-legumes as catch crops have been tested in order to enhance N supply for the next crop, and striving for minimum N leaching risk (Vogeler et al., 2019). It is estimated that while a legume catch crop can reduce N leaching by 28–55%, a mixture of a non-legume and legume catch crop can reduce leaching by 49–81%, which could outperform the reported values for sole stands of non-legume catch crops (Vogeler et al., 2019). In addition to reducing N leaching, legume and mixed catch crops are able to increase grain yield and grain N content by up to 6% (Valkama et al., 2015).

Several cover crops are credited with improving soil properties including bulk density, aggregate size distribution, water stability of aggregates, and soil organic matter (Breland, 1995; Foereid and Høgh-Jensen, 2004; Bronick and Lal, 2005). Deeper-rooted crops, such as fodder radish (*Raphanus raphanistrum* subsp. *sativus* (L.) Domin), have been found to increase gas diffusivity, lower pore tortuosity, and increase soil macroporosity, all of which could potentially ameliorate soil compaction (KadŽienŽ et al., 2011).

Other benefits of cover crops for the soil include a reduction in soil erosion (Dabney, 1998; Känkänen et al., 2001; Vico et al., 2014), reduction in phosphorus losses (Liu et al., 2015), an increase in soil total N and carbon content (Sapkota et al., 2012), and prevention of drain water acidification (Ulén et al., 2008). Some cover crops may increase the yield of the following crop (Bergkvist et al., 2011) and may help to control weeds in organic farming systems (Peigné et al., 2016; Masilionyte et al., 2017), although their effectiveness in weed control may depend on interactions between the chosen tillage system and choice of cover crops (Reimer et al., 2019).

The combined effect of these advantages often means that catch crops are widely accepted and a proven key management practice for climate and environmentally friendly agricultural policy schemes. Indeed, a survey among farmers in five countries—Denmark, Estonia, Finland, Poland, and Sweden—showed that a catch crop contract would be the most preferred strategy to reduce nutrient leaching and greenhouse gas emissions compared with other measures, such as set-aside and fertilizer technology contracts (Hasler et al., 2019).

Challenges

In the boreal-nemoral region, depending on the latitude, snow cover and temperatures below zero can last between 3 and 6 months, making cultivation during the off-season difficult. The risk of winter damage in the region is a big constraint for utilizing catch and cover crops in mixed cropping systems, and it is a constant risk due to prolonged snow cover, frequent, and erratic freeze-thaw cycles, and cold and frost spells (Hutchinson et al., 2007; Vico et al., 2014; Yang et al., 2019). In addition, the short and "unreliable" period without snow cover makes the establishment success of cover crops difficult, and nutrient retention in clay soils common in the region is challenging (Liu et al., 2015).

The main challenges in systems with cash or cover crops are to: (a) decide whether to undersow with the main cash crop or sow after the harvest of the cash crop, and (b) choose an appropriate and effective method to terminate the cash or cover crop so that its growth ends in time for the next cash crop to be sown. Undersown cover crops are usually paired with spring cereals (Aronsson et al., 2016). Some alternatives for terminating the cover crop growth are use of herbicides (e.g., glyphosate), plowing (Breland, 1995, 1996), synchronizing the end of the crop with a frost period, and use of roller crimping machinery (Kornecki et al., 2009). Although the latter technique has gained popularity, it does not always succeed in killing all cover crops (Halde and Entz, 2014).

A challenge specific to legume cover crops is that in order for them to be able to improve soil N availability, there needs to be sufficient biomass accumulation, so early establishment, for example performing undersowing, is of utmost importance (De Notaris et al., 2019). Although the timing of undersowing is critical to maximize biomass and plant N, the best timing for undersowing has been tested in very few studies. Early sowing is reported to achieve N fertilizer replacement values of ∼40 N kg ha^{-1} and biological N fixation rates of up to 85% of N accumulated in the cover crop biomass. In such a scenario, N supply and long-term soil fertility are improved and could result in a "yield stabilization effect" over time (De Notaris et al., 2019). However, mixtures of cover crops that include legumes need careful planning in aspects such as increased interrow spacing to reduce the competition between crops and minimize the impact of cover crops on the main crop yield (De Notaris et al., 2019). The impact of both legume and non-legume catch crops undersown in spring on grain yields has been reviewed by Valkama et al. (2015) who did a meta-analysis of 35 studies in the Nordic countries. According to them (Valkama et al., 2015), Italian ryegrass was the best catch crop, depleting up to 60% of soil N and being more effective than perennial ryegrass.

When using catch and cover crops, it is common that grain yields of the main cash crop in the first year are decreased (Breland, 1996; Cicek et al., 2015; Yang et al., 2019). This yield penalty is often equated to the density of the catch crop, meaning that a denser cover crop frequently leads to a bigger yield penalty of the main cash crop; thus choosing the suitable cover crop should go hand-in-hand with customizing the interrow spacing (Breland, 1996; De Notaris et al., 2019).

The use of and research into catch and cover crops has been widely explored in southern Sweden, Denmark, and Canada, while less so in Finland, Norway, Estonia, Latvia, and Lithuania. The most effective cover and catch crops in the region are perennial ryegrass, Italian ryegrass (*Lolium multiflorum* Lam.), white clover, red clover, hairy vetch, fodder radish, winter rye, winter oilseed rape, and white mustard (*Sinapis alba* L.). Further details about the main crops used in rotation and the benefit of cover crops for the mixed cropping system are listed in **Tables S1**, **S2**.

CONCLUSIONS

Crop mixtures have a long tradition in cropping systems in the boreal-nemoral region, although for decades these have been neglected in practical crop production. In the future challenges associated with climate change, both environmental and economic, crop mixtures could provide a sustainable option for increased resilience of crop production especially since crop mixtures can decrease nutrient leaching and pathogen and weed infestation, and thus the need for agrochemicals, as well as increase the availability of N among other nutrients, and yield stability. However, in order to reach wider acceptability in practice, also the end users, such as industry, should develop the means to utilize the raw materials resulting from mixed cropping systems. Although the advantages of mixed cropping are clear, challenges in the boreal-nemoral region are set by the climatic conditions restricting the seeding and harvesting times, the length of the growing season, and thus the limited availability of suitable crops. Therefore, further research is needed to find the most suitable species, cultivars, and management practices for crop mixtures for different purposes as well as to gather information regarding the ecological, economic, and environmental effects of these mixtures.

AUTHOR CONTRIBUTIONS

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SUPPLEMENTARY MATERIAL

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Urochloa in Tropical Agroecosystems

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Increasing biodiversity is an important issue in more secure and sustainable agriculture. Diversified systems are more resilient to climate change, environmental stresses and enhance soil health, nutrient cycling and nutrient use efficiency. In tropical agroecosystems, cover crops and intercrops are an alternative toward a more diverse and sustainable production. *Urochloa* spp. (syn. *Brachiaria* spp.) are perennial grasses, known for their high biomass production. They are commonly used as cover and companion crops in conservation agriculture in the tropics and the residues left in the field after cutting protect the soil and provide nutrient to the next crop cycle or intercropped culture. *Urochloa* species roots are vigorous, abundant and deep, as opposed to the more shallow and scarce roots of common crops. These traits contribute to carbon sequestration, soil organic matter stabilization and nutrient cycling. *Urochloa* roots also improve soil physical characteristics and influence soil nutrient dynamics, reducing nutrient losses and enhancing cycling, what is key to achieve greater nutrient use efficiency in agriculture. For instance, *Urochloa* root exudates can reduce nitrogen losses by denitrification and leaching through a process called biological nitrification inhibition; root exudates can mobilize recalcitrant phosphorus from soils and make it available for plant uptake; the deep roots of these grasses have the potential to recover nutrients that are virtually lost away from the root zone of other crops. This review compiles scientific progress regarding the introduction of *Urochloa* in agroecosystems, mainly on the aspects related to the contribution to more secure and sustainable agriculture.

Keywords: brachiaria, nutrient use efficiency, sustainability, crop production, nitrogen use efficiency, intercrop, no-till system, soil carbon stock

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INTRODUCTION

It is well-known that in natural systems where there is greater diversity in plant species there is also a tendency for an increase in the natural fertility of the soil (Dybzinski et al., 2008). Several factors may be involved in this process, from the incorporation of organic matter of different plant sources to the diversity of root structures. This contributes not only to explore a greater volume of soil, and to the formation of new pores in the soil, but also to diversifying the composition of root exudates and mucilage, which results in the recruitment of specific rhizosphere microbiota for each species, promoting the interaction of plants with microorganisms and with soil nutrients (Andreote and Pereira e Silva, 2017). Therefore, it is possible to extend this information to agroecosystems, where it becomes evident that the greater diversity of species results in benefits ranging from increased productivity, greater resilience, enhanced nutrient cycling and an altogether safer and more sustainable food production (Altieri, 1999; Frison et al., 2011).

There is a wide variety of conservation agriculture (CA) practices that are used in the tropics and subtropics. CA relies on conservation premises, such as minimum tillage, crop rotation/intercrop and permanent soil cover with crop or cover crop derived residues (Hobbs et al., 2008). CA can be applied to annual and/or perennial crop systems and their adoption depends on climate regional differences, crop management, cash crop and spatial/temporal disposition of plant species in the cultivated areas (Gil et al., 2015; Bieluczyk et al., 2020). Because CA practices have similarities to other conventional systems, the transition from conventional agriculture systems is relatively simple and it can be applied and/or adapted to large areas (Gil et al., 2015).

Most studied and common CA systems adopted in the tropics include no-tillage (NT), with grain production, cover crops and crop rotation; integrated crop-forestry system (ICFS), with simultaneous production of grains and trees; integrated crop-livestock system (ICLS), with the production of grains, forage and animals; integrated livestock-forestry system (ILFS), with the production of forage, animals and trees; and integrated crop-livestock-forestry system (ICLFS), with the production of grains, forage, animals, and trees (Carvalho et al., 2014; Gil et al., 2015; Bieluczyk et al., 2020). These systems can be seen as levels of complexity and intensification, NT being the less intensified, ICLFS the most and ICFS/ICLS/IFLS intermediates, which means that the more species (plant and/or animal) are introduced, the more the agroecosystem resembles a natural ecosystem, and interactions among species become more complex and more total biomass is produced (Bieluczyk et al., 2020).

In all CA systems described above, there is the opportunity to include forages to serve either as cover crops or pasture to animals. Introduction of forage species is justified when it provides services to the agroecosystem (Foley et al., 2005; Cherr et al., 2006). These services may be related to nutrient supply, increased nutrient use efficiency (NUE), soil protection and health, weed suppression and/or to the enhancement of crop or companion crop production and yields; also, they should be suited to local socioeconomic context (Cherr et al., 2006; Horrocks et al., 2019; Paul et al., 2020). Some plant traits are linked to the provision of these services, such as adaptation to tropical environmental conditions, low soil fertility requirements, high biomass production capacity, biomass recalcitrance, efficient root morphology and exudation activity, to name a few (Cherr et al., 2006; Horrocks et al., 2019).

Urochloa is a genus of perennial C4 grasses used as cover crops and as pastures. In Brazil and other South American countries, *Urochloa* species represent the forage with the largest pasture area (Rao et al., 1995; Dias-Filho, 2016) and their use in intercropping systems with annual and/or perennial cash crops has grown substantially in recent years, thanks to the adoption of CA practices in the tropics (Ragassi et al., 2013; Almeida et al., 2017a). There is evident scientific interest in the agronomic characteristics of *Urochloa* species. The International Center for Tropical Agriculture (CIAT) maintains a germplasm bank of more than 600 accessions of *Urochloa* spp., which can be used in breeding programs (Keller-Grein et al., 1996) or investigated for desirable traits for soil health improvement (Horrocks et al., 2019). The interest on this genus is justified for the genetic variability found for tolerance to drought, flooding, nutritional limitation, soil acidity, and against several diseases. The cultivation of *Urochloa* reveals positive characteristics related to soil quality and health in agroecosystems, such as greater NUE, less risk of erosion, better soil structure, higher levels of organic matter and biological activity (Boddey et al., 1996).

Because *Urochloa* species produce large amounts of biomass and have a vigorous, abundant and deep root system, these plants can explore a large volume of soil and absorb substantial amounts of nutrients available in soil regions that are away from the roots of the companion crop, which are generally more superficial and scarce (Rosolem et al., 2017). Additionally, by adding and stabilizing soil organic matter, studies have shown that *Urochloa* roots influence the mobility and availability of some nutrients in the soil, reducing losses and benefiting the cycling process (Almeida and Rosolem, 2016; Nuñez et al., 2018). For example, certain *Urochloa* radicular exudates can reduce nitrogen (N) losses, through leaching and denitrification, by preventing the nitrification process from occurring through biological nitrification inhibition (BNI) (Ishikawa et al., 2003; Subbarao et al., 2009; Byrnes et al., 2017; Nuñez et al., 2018) and in the case of phosphorus (P), exudates can mobilize recalcitrant forms of soil P, making it available for plant absorption (Janegitz et al., 2013; Almeida and Rosolem, 2016). *Urochloa* can also affect directly the NUE of agroecosystems, a key aspect for food production sustainability (Hobbs et al., 2008).

The aim of this review is to gather the current state of knowledge on *Urochloa* grasses use in agroecosystems, with main focus as intercrop in tropical regions. For a comprehensive review we searched the databases Web of Science, Scopus, Scielo, and Google Scholar, with special attention to the most recent articles. We also included references of our own reference libraries, which are not available in these databases. "Brachiaria," "*Urochloa*," "intercropping," and "agroecosystems" were among the key terms used in this search, resulting in 182 references.

UROCHLOA IN TROPICAL AND SUBTROPICAL CONSERVATION AGRICULTURE

Although *Urochloa* is increasingly being adopted as cover and companion crop in agricultural systems through the tropics, other grasses and leguminous have also been used as rotation or cover crops in CA, such as *Megathyrsus maximus* [syn *Panicum* spp.], *Stylosanthes* spp., and *Desmodium* spp., amongst other less explored species (Jank et al., 2017). This variety of species gives complexity to the matter and simplified approaches regarding forage species choice might not be adequate as it has to provide multiple benefits to the system (Cherr et al., 2006; Paul et al., 2020). Few studies are comparing *Urochloa* and other species.

M. maximus is the second largest utilized grass species in the tropics (Dias-Filho, 2016). It is the most productive tropical forage multiplicated by seed (Jank et al., 2011) and it has been used successfully in intercropping systems with corn (Almeida et al., 2017b). There are several commercial cultivars of *M.*

maximus, almost all with tufted growth habit (Jank et al., 2011). They need frequent management interventions (Jank et al., 2011; Santos et al., 2012) and demand high fertility environments, without N limitations, what may result in low BNI capacity (Subbarao et al., 2007, 2009; Simon et al., 2020) and subsequent higher N_2O emissions than *Urochloa* (Subbarao et al., 2007). Simon et al. (2020) found 20% greater N_2O emissions from cattle urine over *M. maximus* pastures, compared to that in pastures of *U. humidicola*.

However, *M. maximus* BNI capacity is still debatable. In a greenhouse experiment, Villegas et al. (2020) compared BNI capacity, N_2O emission, forage productivity and quality of 119 *M. maximus* germplasm accessions, including four commercial cultivars (Mombaza, Sabanera, Vencedor, and Massai) and *U. humidicola* as control. The authors reported that different levels of BNI are spread through the analyzed population of *M. maximus,* but none of the 4 commercial cultivars showed high BNI capacity. Some accessions showed reduced nitrification rates, similar to *U. humidicola,* specially cultivar Tobiata, which showed the lowest values among all accessions. High BNI accessions had equal cumulative N_2O emissions to *U. humidicola*, but low BNI accessions emitted twice as much N_2O. This is the first study showing high BNI capacity in *M. maximus*.

Horrocks et al. (2019) compared the influence of two genotypes of *U. humidicola*, two of *M. maximus* and *U.* hybrid cv. Mulato I on soil health. The soil organic carbon, aggregate stability and friability were highest with the *U. humidicola* genotypes. Less soil loss was also observed in the plots with these materials. *U.* hybrid cv. Mulato I had an intermediate effect but tended to be more alike *M. maximus.* The authors highlighted that these differences are related to one important, but less considered aspect, the forage growth habit. As *U.* hybrid cv. Mulato I and *M. maximus* grow in clumps, the benefits they add to soil are restricted to areas near the plant tussock as they leave more soil areas uncovered. As *U. humidicola* and other *Urochloa* species cover all soil, their benefits on soil traits reach the whole area. Forage growth habits and their relationship to the benefits they add to the system it is not well-understood and should be investigated further by research.

Relatively to grasses, forage legumes are less utilized in the tropics (Karia et al., 2011). But, their ability to biologically fix N is of particular interest, although they usually produce less biomass (Paul et al., 2020) and have lower NUE than grasses (Rao, 2001). Additionally, because they have low C:N ratio, the intense soil microbial activity in the tropics accelerates residue decomposition, as opposed to the more recalcitrant residues of grasses, which provides longer periods of soil cover (Gerlach et al., 2019; Soratto et al., 2019). However, it should be noted that intercropping legume with *Urochloa* species may increase its benefits to the system (Fisher et al., 1994; Costa et al., 2012).

Stylosanthes spp. are native of tropical Americas and used in Africa and Australia, being adapted to low rainfall and low soil fertility (Karia et al., 2011; Philp et al., 2019). The main uses of *Stylosanthes* are as green manure and in intercrops with perennial crops or grasses in pastures (Karia et al., 2011). Depending on the cultivar, *Stylosanthes* spp. can fix up to 200 kg N ha^{-1}, but is less productive and tolerant to grazing than *Urochloa* or *M. maximus* (Karia et al., 2011; Philp et al., 2019). Gerlach et al. (2019) studied the intercropping of the legumes *Sylosanthes capitate*, *Cajanus cajan*, and *Crotalaria spectabilis* with corn for three consecutive years in the Brazilian savanna (Cerrado). All species were monocropped or intercropped between the rows of corn either on the day of corn planting or after the corn had 4–5 fully expanded leaves. All corn-legume intercropping treatments produced more total biomass compared to corn monoculture, however, no increase on corn yield was obtained and *S. capitata* hardly passed 130 kg dry mass ha^{-1} when intercropped, what was low compared with the other species.

The genus *Desmodium* spp. comprises leguminous forages species that are native from tropical and subtropical regions and are suitable to be used in intercrops, as cover and forage crop (Paul et al., 2020). In low input agroecosystems *Desmodium* spp. have demonstrated great potential in improving corn yield and soil C stock (Chidowe et al., 2019), as well as suppressing aggressive weeds, like *Striga hermonthica,* when intercropped or in rotation with corn (Khan et al., 2006; Midega et al., 2013). Like many other leguminous in the tropics, *Desmodium* spp. residue showed low recalcitrance and half-life of residues covering the soil was only 29 days (Dubeux et al., 2007).

As the available literature is concerned, *Urochloa* can provide more services to tropical agroecosystems. In CA, *Urochloa* can be introduced as a sole crop, for covering the soil or combined with annual or perennial crops in intercrop systems in a variety of ways. But how should *Urochloa* be combined with crops or introduced in the system? To answer that, we took Brazil as an example of CA evolution and research.

Facing pasture and land degradation back in the 80s, Brazilian agriculture and livestock production had to evolve. First, empirically by farmers, and then supported by research institutions, the use of *Urochloa* in intercrop systems made the restoration of agricultural and pasture productivity feasible, leading to the creation of two successful systems: "Sistema Barreirão" in 1991 (Oliveira et al., 1996) and "Sistema Santa Fé," 10 years later (Kluthcouski et al., 2000) by EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária). Both systems rely on diluting costs of forage implantation/restoration by intercropping *Urochloa* with grain crops, where the development of the forage is improved by residual fertilizers from the cash crop after its harvest (Ceccon, 2013).

In the "Sistema Barreirão," annual crops like corn, sorghum (*Sorghum bicolor*), pearl millet (*Pennisetum glaucum*) and particularly upland rice (*Oryza sativa*), were intercropped with forages, such as *Urochloa* and *Andropogon* spp. to establish new pasture for cattle; on the other hand, in "Sistema Santa Fé" the intercrop is established every year and aims at producing forage for the off-season and residues in quantity and quality to cover the soil under CA (Torres et al., 2018). The research and promotion of these intercrop systems made possible the adoption and development of more integrative CA systems (ICLS, ICF, ILF, and ICLF) and Brazilian agriculture became more integrative and sustainable along the process. These two basic systems can be used as guidance to introduce *Urochloa* in tropical agroecosystems and be adapted to the local context.

With annual crops, most commonly corn, *Urochloa* can be sowed before, during or after corn sowing; mixed to corn fertilizers, in special seed boxes between the corn ones or broadcasted before or after corn emergence (Ceccon et al., 2013). Either way, both species will coexist through the corn cycle. To guarantee no corn yield loss, forage growth must be partially suppressed to reduce competition for water, light, nutrients and space with corn; consequently, corn gains a competitive advantage over the forage, which can lead to normal growth, development and production during the intercrop without compromising forage production afterwards (Ceccon et al., 2013; de Oliveira et al., 2019). Broadcasting *Urochloa* before/after corn can be a way to reduce its growth (Ceccon et al., 2013) and the use of lower herbicide rates is also recommended for this aim (Almeida et al., 2018d; de Oliveira et al., 2019). In this case, nicosulfuron (2-[[[[(4, 6-dimethoxy-2-pyrimidinyl) amino] carbonyl] amino] sulfonyl]-N, N-dimethyl-3-pyridinecarboxamide) is the most used herbicide to suppress *Urochloa* growth in intercropping systems (Almeida et al., 2018d; de Oliveira et al., 2019). According to Anésio et al. (2017), applying nicosulfuron to *Urochloa* at a lower rate affords the suppression needed, without killing it. After corn harvest, the forage can be used as a cover crop or for grazing. This process can be repeated every year in NT, to generate residues for the next crop; or when the pasture needs to be renewed in systems that include the animal component.

More recently, *Urochloa* has been intercropped with perennial crops, such as coffee (Franco Junior et al., 2019; Silva et al., 2019) and citrus (Auler et al., 2008; Martinelli et al., 2017). Cultivated as stripes between rows, *Urochloa* protects the soil, reduces erosion, increases infiltration, suppresses weeds and cycles nutrients (Auler et al., 2008; Ragassi et al., 2013; Favarin et al., 2018). The forage can be either sowed between the rows of producing crops or sowed in the total area before the perennial crop establishment. As an aggressive forage species, *Urochloa* must be controlled to maintain at least 0.5 m distance from the plants to avoid competition for resources and mainly to facilitate crop management. This is usually done by applying herbicides periodically and by ecological mowing of *Urochloa* shoot when flowering, directing its residues toward the crop row (Martinelli et al., 2017; Favarin et al., 2018). As *Urochloa* growth is reduced dramatically under low light availability, the establishment of the intercropped system must take in consideration the sun path, the row spacing and the height of the perennial crop. In drastic situations, pruning of the crop may be necessary to avoid forage re-sowing.

In intercrops, a larger volume of soil is explored, both between the rows and at depth, depending on the agronomic characteristics of the crops chosen for cultivation. In NT systems, *Urochloa* produces large amounts of residues, which contribute to the increase of the SOM and to reduce soil erosion, water and nutrients runoff and water evaporation (Tully and Ryals, 2017; Tanaka et al., 2019). Both crop rotation and intercrop favor the cycling of nutrients, as they increase the efficiency in the use of fertilizers and nutrients (Pedrosa, 2013), in addition to recovering elements virtually lost beyond the reach of the root system of the main crop (Maciel de Oliveira et al., 2020).

Urochloa As an Invasive Species

Every exotic introduced species may be a potential threat to ecosystem native biodiversity, especially if the species has aggressive growth and is adapted to local edaphoclimatic conditions (Pivello et al., 1999a,b; Foxcroft et al., 2010). This is the case of most of the native African grasses introduced in the American neotropical savannas and Australia, for example (Foxcroft et al., 2010).

Urochloa species can outcompete native forage species in savannas grasslands because they have efficient photosynthetic rates, high water and nutrient use efficiency, are relatively tolerant to abiotic stresses and produce significant amounts of above and belowground biomass (Pivello et al., 1999a,b; Foxcroft et al., 2010). But its invasive character is particularly linked to prior ecosystem disturbance, i.e., usually, *Urochloa* is not the agent of disturbance, but a consequence of it (Vitousek et al., 1996; Zenni and Ziller, 2011). Alien grasses, such as *Urochloa* that are nearby an area that has been cleared or set on fire, can invade the area after the disturbance and dominate. Once established, it changes the microclimate, the quantity and quality of residues and the propensity to new fires, creating new fire regimes (Gorgone-Barbosa et al., 2016). Additionally, *Urochloa* is well-adapted to fire in its original habitat meaning that it remains in the area even after new fire events, thus in a long term suppressing native species regeneration (Vitousek et al., 1996).

Nonetheless, because *U. decumbens* is highly colonized by AM, it has been used in initial stages of land restoration programs in disturbed areas, where other species do not grow well under harsh soil conditions (Leite et al., 2019). Also, *Urochloa* is highly responsive to the increased availability of nutrients after fires (Pereira-Silva et al., 2019).

Most of the risk posed by *Urochloa* species introduction into savanna biome is related to lack of good management practices. Thus, it is necessary to define practices to avoid dispersal and further negative effects on the native ecosystems in which the agroecosystems may be inserted. Also, pasture/cover crops should be managed and suppressed when needed, to avoid disturbances to nearby native grasslands. This may be done regularly when *Urochloa* is used as intercrop or as a cover crop by adopting desiccating management or mowing; when used in pastures, the grazing regime can prevent over-accumulation of residues, that are flammable, avoiding disturbances occurrence and the spread of fire and seeds to new native grassland areas (Sühs et al., 2020).

NUTRIENT CYCLING BY *UROCHLOA* IN CONSERVATION AGRICULTURE

Nutrient cycling comprises the many transformations nutrients undergo in the soil-plant-atmosphere system. In agroecosystems, nutrient cycling is influenced by soil and climate conditions, landscape disposition and agricultural management practices (Tully and Ryals, 2017). Thus, factors intrinsically related to the soil and landscape, such as mineralogy, texture, groundwater depth, and topography; together with climatic factors, such as rainfall and temperature; and plant diversity, govern nutrient flows in agroecosystems (Tully and Ryals, 2017).

The efficient use of nutrients in agriculture is vital for system sustainability and it is intimately linked to nutrient cycling, that can be optimized through the adoption of management practices to maintain soil fertility and biodiversity, increase soil C sequestration and minimize negative climatic effects (Power, 2010). In this context, nutrient cycling can be considered a process favored by CA systems.

Mainly because of its rapid growth and large amounts of biomass/residues produced in tropical conditions, *Urochloa* stand out among cover and intercrop species, such as crotalarias (*Crotalaria* spp.) and millet (*Pennisetum* spp.) (Wutke et al., 2014). Besides, *Urochloa* are low soil fertility demanding species (Rao et al., 1996; Miles et al., 2004). Even influenced by seasonal variations, perennial grasses, such as *Urochloa*, have a wider opportunity to grow and absorb nutrients throughout the year than annual crops. Therefore, they have a great influence on how and when released nutrients are available (Tully and Ryals, 2017). Senescence and pruning/cutting management or desiccation of aboveground parts of cover crops constitute the main route in which nutrients return to the soil after decomposition/mineralization of the residues, closing the cycle. Besides, perennial grasses can build up dense root systems over time, and also affect the dynamic of nutrient cycling in the soil and consequently soil fertility profile (Yé et al., 2017).

Shoot Biomass and Nutrient Accumulation in *Urochloa*

The cycling potential of any plant species depends on nutrient accumulation and its rate of residue decomposition. The plant cycle, that is, the time that it remains in the field, also influences this potential, as it can enable a greater accumulation of nutrients. The accumulation of nutrients is defined by the productivity of dry mass (kg of dry matter ha^{-1}) and the concentration of nutrients in the plant tissues (g kg^{-1}). The higher these two factors, the higher the accumulation of nutrients will be.

Urochloa can accumulate between 2 and 16 Mg of dry matter ha^{-1} in their aerial parts, per year, in crop rotation or intercropping (Macedo, 2009; Bernardes et al., 2010; Costa et al., 2016; São Miguel et al., 2018), with the amount varying according to the species and the system management practices adopted, such as fertilization and cutting timing/frequency.

The large production of biomass combined with the relatively high concentrations of nutrients in the tissues (**Table 1**) results in a significative accumulation of nutrients in the *Urochloa* shoot (**Table 2**). According to the species and system management, nutrient accumulation will differ. In general, *Urochloa* species accumulate about 100 kg ha^{-1} of N and 130 kg ha^{-1} of potassium (K), in addition to more than 15 kg ha^{-1} of P, 40 kg ha^{-1} of calcium (Ca) and about 25 kg ha^{-1} of magnesium (Mg).

It is of special relevance to know what part of the soil profile the accumulated nutrients originate from, that is, from what position in the soil they were absorbed (surface, subsurface, between cropping rows) by the roots. This information is not yet available and to obtain it, it is imperative to deepen our understanding of the growth dynamics of the *Urochloa* root system in different soils and through the profile.

TABLE 1 | Concentration of nutrients in the shoot of *Urochloa*.

N	P	K	Ca	Mg	References
g kg^{-1}					
11.7	2.2	24.8	2.2	2.4	de Magalhães et al., 2002
12.2	1.7	21.9	2.8	3.7	Cruz et al., 2008
24.3	1.8	18.0	3.9	2.9	Costa et al., 2017
16.1	**1.9**	**21.6**	**3.0**	**3.0**	**Mean values**

N, nitrogen; P, phosphorus; K, potassium; Ca, calcium; and Mg, magnesium.

TABLE 2 | Nutrient accumulation in the shoot of *Urochloa* species.

Urochloa	N	P	K	Ca	Mg	References
	kg ha^{-1}					
Urochloa brizantha	60	12	130	12	13	de Magalhães et al., 2002
	86	8	ni	14	14	Torres et al., 2008
	48	11	165	23	17	de Barcellos Ferreira et al., 2010
	135	13	118	87	45	Pacheco et al., 2013a
	165	45	246	77	31	
	106	21	154	ni	ni	Costa et al., 2014a
	87	24	101	15	13	Costa et al., 2014b
	57	11	103	13	11	
	120	12	164	12	18	Costa et al., 2015
	143	14	127	91	59	
	103	13	117	35	23	Costa et al., 2016
Mean values	**101**	**17**	**142**	**38**	**24**	
Urochloa ruziziensis	156	12	119	63	35	Pacheco et al., 2011
	144	12	119	63	37	Pacheco et al., 2013a
	162	44	211	111	28	
	121	35	79	24	22	Pacheco et al., 2013b
	138	20	203	ni	ni	Costa et al., 2014a
	74	19	83	15	12	
	57	11	101	16	11	Costa et al., 2014b
	44	16	104	32	23	São Miguel et al., 2018
	94	18	46	46	35	
Mean values	**110**	**21**	**118**	**46**	**25**	
Urochloa decumbens	31	4	12	16	8	Alcântara et al., 2000
	142	20	254	33	43	Cruz et al., 2008
Mean values	**87**	**12**	**133**	**24**	**26**	

ni, values not informed in the report. N, nitrogen; P, phosphorus; K, potassium; Ca, calcium; and Mg, magnesium.

Urochloa Root System and Soil C Stock

Although most studies focus on shoot biomass production, the great potential of *Urochloa* as a cover or intercropping culture resides in the roots. Like other tropical grasses, *Urochloa* produces more root mass compared to herbaceous legumes (Rao et al., 1996). Some reports show that the accumulation of root dry mass of *Urochloa* can range from 5.3 to 38 Mg ha^{-1} (Razuk, 2002; Volpe et al., 2006; Apolinário et al., 2013; Saraiva et al., 2014). Considering these values and the estimated root turnover for tropical grasslands of 0.85 year $^{-1}$ (Gill and Jackson, 2000), the results indicate that up to 32 Mg root dry mass ha^{-1} year $^{-1}$ might be produced, thus causing great impact on soil C stocks. Furthermore, the architecture, morphology, physiology and interaction of the roots with soil microbiota help the formation and stabilization of soil aggregates, increase soil porosity, C sequestration and can increase the availability of nutrients and reduce its losses, influencing the cycling of these elements (Bardgett et al., 2014).

The root system of some *Urochloa* species can easily reach three meters in depth (Rodrigues et al., 2011). However, root biomass tends to decrease significantly with depth (Guenni et al., 2004). Deep roots of several plants have functional specialization (da Silva et al., 2011; Wang et al., 2015). These roots are thinner and have a larger diameter of conductive vessels compared to superficial roots, in addition to the greater number of vessel elements and tracheids. These attributes increase hydraulic conductivity and water uptake efficiency (Wang et al., 2015). Thus, these roots can contribute to the maintenance of plant growth and nutrition, especially when the soil superficial layers are under water deficit (Bleby et al., 2010; da Silva et al., 2011). Additionally, the formation of aerenchyma as a "constitutive" characteristic of some species' roots, such as *U. humidicola*, gives this species high adaptability in situations of flooding and nutritional deficiencies, without impairing nutrient uptake and plant growth (de la Jiménez et al., 2019). It is known that under nutritional limitation, ethylene accumulates in the roots and induces the formation of aerenchyma and the development of new lateral roots, characteristics that are related to lower metabolic cost of the roots and lower cost of soil exploration (Postma and Lynch, 2011; de la Jiménez et al., 2019). A detailed study of *Urochloa* root systems could clarify whether this functional variation exists and what is the contribution to plant survival in times of drought and nutrient and water absorption at greater depths.

Generally, the amount of nutrients in the subsoil is lower than in the topsoil (Brady and Weil, 2013) but this varies with soil texture, rainfall distribution, management and especially according to the chemical transformations and interaction of each nutrient in the soil particles. However, this may not be the rule depending on the nutrient. For example, Mekonnen et al. (1997) quantified the NO_3^- in tropical soil and found between 40 and 199 kg ha^{-1} from the topsoil to the subsoil at 4 m. Therefore, the absorption of nutrients from deep layers would allow these nutrients to be used for plant growth and development. Thus, it is reasonable to admit that because the large and deep root system *Urochloa* can reduce the loss of nutrients by absorbing them from superficial and deep soil layers.

Urochloa root system growth is not strongly limited in compacted soils (Silva et al., 1992; Barreto et al., 2006; Stumpf et al., 2016). Stumpf et al. (2016) reported that among several perennial grasses growing in a constructed mine-soil recovery, *U. brizantha* roots had the highest potential to penetrate the compacted zone under the first 0.1 m, reconstituting aggregates and thus recovering soil physical properties. According to these authors, the high density, volume and length of *U. brizantha* roots make this species a good candidate for soil decompression. The formation of aggregates of larger diameters in soil cultivated with forage grass species, including *Urochloa*, is related to its abundant root systems and the constant renewal and decomposition of root biomass (root turnover) (Six et al., 2004). In Kenya, the cultivation of *Urochloa* species was compared to other local grass species (*Chloris gayana* and *Pennisetum purpureum*) in their capability to change the size and distribution of soil aggregates (Gichangi et al., 2017). *Urochloa* species significantly improved soil aggregation and enhanced soil microbial biomass carbon.

The *Urochloa* root system can substantially increase C storage in the soil by producing massive root biomass in comparison to other species (Gichangi et al., 2017). However, this depends on how *Urochloa* is managed (Carvalho et al., 2010). Agronomically well-managed *Urochloa* pastures in the Brazilian Amazonia, with adequate liming, fertilization and cutting/grazing regimes, had great potential to increase soil C stocks and reduce CO_2 emissions (Eri et al., 2020), because of greater biomass production and greater cattle yields per unit area (de Figueiredo et al., 2017). According to Carvalho et al. (2014), CA systems are a good way to mitigate greenhouse gas emissions on Brazilian Cerrado. On the other hand, degraded pastures are prone to C losses and higher CO_2 emissions (Carvalho et al., 2010).

A classic study on the impact of deep-rooted grasses on soil C stocks in American neotropical savannas was done by Fisher et al. (1994). The authors reported that under agronomically well-managed grass-based pastures (*U. humidicola* and *Andropogon gayanus*) soil stored significant amounts of C (4.3 Mg C year^{-1}) in deeper layers, which reached 1.0 m depth; significantly higher if compared to native savanna grasses. In the same study, *U. humidicola*/*Arachis pintoi* intercrop showed a greater increase, 11.7 Mg ha^{-1} year $^{-1}$, suggesting that the legume's ability to fix N improved C sequestration in the system.

Multivariate geostatistics were used by Tavanti et al. (2020) to estimate CO_2 emissions from soil under degraded pastures areas in Central Brazil, and it was verified that sorghum intercropped with *U. brizantha* promoted organic carbon storage in the mineral fraction of the soil, which consequently reduced soil CO_2 emissions. The proposed pasture management with intercropped systems deserves further studies in the actual climate change scenario considering other management practices as liming and fertilizers application or grazing intensities (Tavanti et al., 2020).

Nonetheless, some recent studies show less optimistic C storage capacities in *Urochloa*-based pastures. For example, in agronomically well-managed *Urochloa* pastures, C storage was around 0.44 Mg C ha^{-1} year $^{-1}$ (Carvalho et al., 2010), which can go up to 3.0 Mg ha^{-1} year $^{-1}$ (Bustamante et al., 2006); in the ICLS, carbon storage by pastures grasses vary from 1.0 to 2.5 Mg ha^{-1} year $^{-1}$, depending on the prevalent edaphoclimatic conditions and management practices (Carvalho et al., 2010). The inclusion of *U. ruziziensis* to a soybean-corn-cotton ICLS promoted soil C sequestration and the system became a significant C sink, with rates reaching 0.36 Mg C ha^{-1} year $^{-1}$ (Carvalho et al., 2014). Intensifying system productivity through ICLS showed to be the best alternative in increasing soil C storage in a six-year evaluation experiment (Bieluczyk et al., 2020), accumulating 0.28 Mg carbon ha^{-1} year $^{-1}$. These authors observed that corn-*U. brizantha* cv. Piatã intercrop promoted the increase of SOM, whereas further introduction of *Eucalyptus urograndis* stands in the system, thus becoming an ICLFS system, reduced soil C and N contents, as shade limited crop and pasture growth. On the other hand, Rice et al. (2020) verified that the inclusion of *Eucalyptus* spp. to an *Urochloa* pasture added more C below 0.6 m depth, while *U. brizantha* increased C in the first 0.2 m, showing complementarity between the two species, although forage shoot and root production was impaired compared to control. dos Santos et al. (2019) evaluated soil C stocks 16 years after the conversion of native vegetation to *U. brizantha* pastures (cv. Arapoti and cv. Xaraés) in Brazil and found 43.2 Mg C ha^{-1} in soil derived from the pastures, an accumulation rate of 2.7 Mg C ha^{-1} year $^{-1}$. Soil C storage did not differ among *Urochloa* cultivars, but it was higher than native vegetation, especially in the first 0.3 m of depth, due to higher root biomass; additionally, an increase in soil C storage at 1 m depth was also observed. ICLS that relied on intercrops to establish *Urochloa* pastures increased soil C stocks (Soares et al., 2020).

Root recalcitrance and decomposition, distribution in the soil profile, rhizodeposition, and the establishment of mycorrhizal associations are root traits that contribute to SOM stabilization (Poirier et al., 2018). Roots can also indirectly impact soil C cycling due to the influence of its activity on soil microbiota. Roots with higher levels of lignin and lower levels of N promote soil C sequestration, by stimulating fungi growth over bacteria, which respire more C-based substrates per unit of C incorporated in biomass (de Deyn et al., 2008; Bardgett et al., 2014). Thus, the abundance, depth, composition and rhizodeposition show the high potential of *Urochloa* species for stabilizing SOM.

Rhizodeposition

The compounds that plant roots exude during their growth—exudates, mucilage, border cells—constitute what is known as rhizodeposits, which may interact with minerals in the clay fraction of the soil as well as with microorganisms in the rhizosphere (Kuzyakov and Domanski, 2000). Root exudates greatly vary in chemical composition and function and can represent about 30–50% of all fixed C by pasture grasses (Kuzyakov and Domanski, 2000). These exuded compounds represent an excellent energy source for rhizosphere microorganisms, which can increase their activity and favor SOM stabilization, both in topsoil and in subsoil profile (Poirier et al., 2018). Recently, diazotrophic bacteria of the genera *Azospirillum* and *Nitrospirillum* were identified in the rhizosphere of more than 20 genotypes of *Urochloa* (da Silva Ribeiro et al., 2019). These endophytic bacteria can colonize root tissues and the rhizosphere promoting plant growth due to their ability to fix dinitrogen, produce auxins, and siderophores, and/or solubilize phosphorus (P) and zinc (Zn) from the soil particles (da Silva Ribeiro et al., 2019). Biological nitrogen fixation (BNF) is an important input in systems that use *Urochloa* and will be addressed later in the N cycling topic.

Rhizodeposits may also inhibit soil processes mediated by microorganisms, such as nitrification (Subbarao et al., 2007) or increase micronutrient availability and plant uptake by the release of siderophores (Dakora and Phillips, 2002), or the reduction of metal phytotoxicity, such as the case of Al and Mn (Wenzl et al., 2002). Therefore, compounds exuded by roots play an important role in nutrient cycling in the rhizosphere, improving NUE and reducing the effect of toxic elements, possibly contributing to better plant performance and thereby constituting a promising area of research to stimulate more sustainable agriculture.

N Cycling

Nitrogen (N) is generally the nutrient of highest demand by plants and a highly dynamic element with one of the most complex biogeochemical cycles, due to its various chemical forms in the soil-plant-atmosphere system (Brady and Weil, 2013). For agricultural production, some of the chemical transformations of N, such as nitrification and denitrification, may represent N losses from the soil system, as N may be lost both by nitrate (NO_3^-) leaching in the soil profile and by forming volatile compounds, such as N oxide forms (NO and N_2O) that are released into the atmosphere.

N inputs to the system derive from atmospheric deposition, fertilization and BNF processes. The amount of N from biological fixation associated with diazotrophic bacteria on *Urochloa* roots can be substantial. According to Boddey and Victoria (1986), BNF can account for 30 and 40% of N accumulation in *U. decumbens* and *U. humidicola*, respectively. BNF contribution on total N accumulation of four *Urochloa* species (*U. brizantha, U. decumbens, U. humidicola* and *U. ruziziensis)* can account for 3–26%, varying seasonally and between genotypes (Reis et al., 2001). Similar results were found by Silva et al. (2010), showing that BNF contributed with 10–42% of *U. decumbens* and 10–39% of *U. humidicol*a total N, depending on season and species. For example, *U. ruziziensis* increased the population of N-fixing microorganisms in the system compared to *U. brizantha* and *M. maximus* (Rocha et al., 2020b). Considering that the mean N accumulation among *Urochloa* species is about 100 kg N ha^{-1} (**Table 2**), up to 42 kg N ha^{-1} could be added into the soil from BNF. *Urochloa* and *Panicum* grasses used as cover crops in rotation under N-limited conditions help to prevent N leaching (Rocha et al., 2020a) and its associated BNF may substantially contributed to part of the N-demand of the subsequent maize culture (Rocha et al., 2020a,b).

The highest N inputs into the soil are via fertilization and can vary depending on management practices and the purpose of *Urochloa* cultivation, i.e., pasture, cover crop in rotation or intercropping system. In *Urochloa* pastures, N inputs through fertilization vary according to the intensification level (Pereira et al., 2018), for instance, in high input pasture systems, fertilization can reach up to 300 kg N ha^{-1} (Santos et al., 2010), but common fertilization recommendations are 50 kg N ha^{-1} for pasture maintenance and additional 50 kg N ha^{-1} after each grazing cycle (Werner et al., 1997). When *Urochloa* is cultivated as a cover crop, N fertilizers might be applied at sowing at a maximum rate of 40 kg N ha^{-1} to guarantee the establishment of the pasture (Werner et al., 1997). When *Urochloa* is intercropped with corn or coffee, fertilization is commonly applied uniquely to the cash crop, and in these systems, N inputs follow the N requirements of the cash-crops. In corn, 200 kg N ha^{-1} may be used (Cantarella, 2007); in coffee, N fertilization may reach up to 450 kg N ha^{-1}, at an average of 300 kg N ha^{-1} (Quaggio et al., 2018).

The main forms of N losses in agricultural systems are the volatilization of ammonia (NH_3), soil erosion, nitrate leaching and runoff and denitrification (Brady and Weil, 2013). Conservation practices consistently prevent N losses from soil erosion and runoff when compared to conventional systems (Wutke et al., 2014). The volatilization of NH_3 is a more challenging issue when N is supplied as urea-based fertilizers, while NO_3^- losses, by leaching and denitrification, are prone to occur in every form of N-fertilizer (Villalba et al., 2014). In Brazil, NO_3^- leaching can stand for the loss of up to 87 kg ha^{-1} of N under favorable conditions (Villalba et al., 2014). N loss can also occur by denitrification, which is the process in which NO_3^- is used as the final electron acceptor in anaerobic respiration and transformed into volatile forms (N_xO) by some groups of soil bacteria. Although denitrification N losses represent only about 1% of the total N applied (Villalba et al., 2014), they are very harmful as N_2O, a powerful greenhouse gas, is potentially more harmful to the environment than CO_2 (IPCC, 2019).

The high N use efficiency of *Urochloa* can contribute to the reduction of N losses when used in intercropped systems. In an experiment using ^{15}N-enriched fertilizer in a coffee-*Urochloa* intercrop system, the N recovery efficiency increased from 38 to 53% when the element was supplied in equal doses between *Urochloa* and coffee plants, compared to when supplied solely to coffee plants. This value was over 80% when it was supplied only for *Urochloa* (Pedrosa, 2013). In the corn-*Urochloa* intercrop, the recovery of N in fertilizer was higher than in corn monoculture (Almeida et al., 2018d) and the greater recovery in soil profiles between 0.6 and 1.0 m was probably related to *Urochloa* roots reaching deep layers, thus reducing N-losses by leaching. Reduced leaching might as well be attributed to the BNI promoted by *Urochloa* root exudation (Karwat et al., 2018). Also, Galdos et al. (2020) reported that the finer roots of *U. brizantha* promoted a more complex pore system in the soil, reducing solute flow and reducing N leaching when compared to corn or even *U. ruziziensis*.

In this context, *Urochloa* roots exudates can act directly on the N cycle, inhibiting undesirable processes in terms of production sustainability. This can contribute to the high N use efficiency of these tropical grasses and the decrease of N losses in agroecosystems.

Biological Nitrification Inhibition

The roots of certain plant species are capable of exuding substances that inhibit and/or reduce nitrification, a process carried out by bacteria of the genera *Nitrosomonas* and *Nitrobacter*, that transform ammonium (NH_4^+) to nitrite (NO_2^-) and NO_2^- to NO_3^-, respectively. This inhibitory action on this process is called biological nitrification inhibition—BNI (Ishikawa et al., 2003; Subbarao et al., 2007, 2009) and can contribute positively to the crop's N use efficiency (Sun et al., 2016). Several studies show the effectiveness of some compounds exuded by roots in reducing nitrification, as well as NO_3^- leaching and N_2O emissions (Subbarao et al., 2009; Byrnes et al., 2017). It is important, however, to note that the main N-form lost in soil systems is NO_3^-, due to the various chemical transformations it can undergo. For this reason, several studies addressing nitrification inhibition have been carried out.

Commercial products that inhibit nitrification exist and are used for said purpose. The most common are those based on nitrapyrin, DMPP (3,4-dimethylpyrazole phosphate) and DCD (dicyandiamide) (Trenkel, 2010). The efficiency of these compounds is questionable, and its use has been rarely translated into gains in productivity (Rosolem et al., 2017). This low efficiency may be due to environmental factors (Villalba et al., 2014) and to the fact that these inhibitors act in only one of the stages of the nitrification process (Subbarao et al., 2009), causing the inhibitory effect to last a few weeks at most (Villalba et al., 2014). The first stage of nitrification is carried out by the enzyme ammonia monooxygenase and the second by hydroxylamine oxidoreductase (Subbarao et al., 2013).

Urochloa species are capable of exuding nitrification inhibiting substances (NIS) by their roots, although their production varies according to species, cultivar and soil pH (Subbarao et al., 2007). Among the *Urochloa* species, *U. humidicola* and *U. decumbens* had greater inhibitory effect on nitrification compared to other grasses, cereals and legumes (Subbarao et al., 2007). Among nitrification inhibiting substances, brachialactone is responsible for more than 60% of BNI in soils under *U. humidicola* influence (Subbarao et al., 2009), which is considered the model plant species for BNI studies.

Brachialactone is a cyclic diterpene produced in the presence of the NH_4^+ ion and ensures that most of the inorganic N remains in this ionic form (Subbarao et al., 2009). This compound is exuded by *Urochloa* roots in response to the presence of NH_4^+ in the rhizosphere and affects the two stages of nitrification. Brachialactone is more effective in suppressing this process when compared to the synthetic inhibitor nitrapyrin, which acts only on the enzyme ammonia monooxygenase (Subbarao et al., 2009). Although this inhibitory effect is immediate, it takes a year of cultivation for the BNI to reach its maximum (Nuñez et al., 2018). Subbarao et al. (2009) observed that after three years of *Urochloa* pasture establishment, nitrification and denitrification processes were strongly suppressed in the soil. Some genotypes of *U. humidicola* possess high BNI capacity and can reduce the emission of N_2O (Subbarao et al., 2009). On this regard, it has been shown that *U. humidicola* significantly reduced N_2O emissions from cattle urine residues in soils (Byrnes et al., 2017; Simon et al., 2020).

Pastures of tropical grasses are commonly efficient in soil N use and demonstrate low losses of this element (Karwat et al., 2017), so gains of BNI promoted by *Urochloa* would be more evident for the next crop or in the companion crop, in the case of crop rotation and intercrops, respectively. Early stages of crop development are critical for N loss, as the roots are still underdeveloped and exploit small soil volumes. As a residual BNI effect of *Urochloa* can last up to 4 months after cutting or desiccation—due to the accumulation of NIS in the soil during its cultivation, or to the root biomass that remains in the field releasing these inhibitors during decomposition (Karwat et al., 2017)—these losses would be reduced, with benefits to the next sown crop (Karwat et al., 2017).

The cultivation of corn in an area previously cultivated with *U. humidicola* showed higher productivity, higher N absorption and use efficiency compared to corn monoculture and the use of synthetic nitrification inhibitor DCD (Karwat et al., 2017). However, NIS effect does not last for long periods in soil environment, since the result did not repeat in the second corn cultivation, indicating that the residual inhibitory effect of *Urochloa* had ended (Karwat et al., 2017). It is expected that in intercropping systems where *Urochloa* is used between the rows, the effect will be more evident because of the proximity and co-occurrence of the crops.

BNI would lead to greater NUE from the soil by *Urochloa* roots, allowing both NO_3^- and $NH_4{}^+$ uptake, as observed by Nakamura et al. (2005) in *U. humidicola*. Species, such as *U. humidicola* are naturally adapted to N-limited environments and can absorb both $NH_4{}^+$ and $NO_3{}^-$. Its performance under high levels of $NH_4{}^+$ is superior to that of other *Urochloa* species, although the growth under $NO_3{}^-$ is relatively higher (Rao et al., 1996; Rao, 2001). These differences to other *Urochloa* species seem to be linked to the characteristic high production of NIS of *U. humidicola*, among other factors (Rao, 2001). Therefore, it is plausible that genotypes from other *Urochloa* species with high BNI potential also behave similarly when subjected to high concentrations of $NH_4{}^+$. As urea is frequently the main form of N-fertilizer used in agriculture and, that when solubilized in acidic pH conditions it is transformed to $NH_4{}^+$, this *Urochloa* response would be of interest, as it could further reduce N-losses through the absorption of N-$NH_4{}^+$.

P Cycling

P is one of the less available plant nutrients in soils (Marschner, 2012). The concentration of P in the soil solution rarely exceeds 10^{-4} M (Johnston et al., 2014), with phosphate ion ($H_2PO_4{}^-$) diffusion being the main mechanism for this nutrient to reach the roots. Besides, inorganic P (Pi) can be retained/fixed to soil colloids, making it unavailable to plants in the short term, even though it is mobilized at small rates over the years (Syers et al., 2008; Johnston et al., 2014). Organic P (Po) can represent between 20 and 80% of the total P of the soil (Neumann, 2016) and the most representative organic forms of P in the soil are inositol phosphates or phytates, which can also be strongly fixed to the mineral surfaces of the soil, and phosphate monoesters and diesters, such as sugar-phosphates, phospholipids and nucleic acids (DNA and RNA), which have higher mobility in soil (Gerke, 2015).

Although over 50% of the P fixed in the soil is represented by orthophosphate (Pi), Po as inositol phosphate can represent up to 40% of the fixed P depending on the soil and the cropping system, being considered a highly recalcitrant form of P (Menezes-Blackburn et al., 2017). These fixed fractions could sustain agricultural production for hundreds of years if they were made available to plants (Menezes-Blackburn et al., 2017). The inclusion of tropical grasses, such as *Urochloa*, in agricultural systems can contribute to the use of these poorly available P fractions.

Plants have mechanisms to increase the acquisition of unavailable P forms in the soil, among them, the exudation of surplus protons, organic acids and enzymes by the roots (Lambers et al., 2006; Louw-Gaume et al., 2017; Wang and Lambers, 2020), the emission of root hairs in a greater number and length (Wang and Lambers, 2020), in addition to the association with arbuscular mycorrhizal fungi (AMF) (Lambers et al., 2006). Acid phosphatase enzymes released in the rhizosphere by the roots are also able to release Pi from mono and diester phosphates, making it available for plant absorption (Tian and Liao, 2015). In addition, Pi fixed on soil colloids and Po can be mobilized by the action of organic acids, such as citric, oxalic, and malic acids, among others (Lambers et al., 2015).

In response to P-limitation *Urochloa* increases acid phosphatase activity (Louw-Gaume et al., 2010) and exudes organic acids (Louw-Gaume et al., 2017). Even at high P availability, *U. humidicola* roots showed a large exudation of acid phosphatases to the rhizosphere (Teutscherova et al., 2019). A study on the influence of green manure species on soil P retention showed that soil under *U. ruziziensis* as the cover crop had the lowest P adsorption by the colloidal fraction amongst other leguminous covers, such as *Crotalaria juncea*, *C. cajan*, and *Mucuna* aterrima (Silva et al., 1997). The cultivation of these same species as cover crops also reduced the maximum P adsorption capacity in a Rhodic Hapludox soil, but in this soil peanut (*Arachis hypogaea*) and sorghum also used as cover crops were the most effective in this reduction (Janegitz et al., 2017). The lower P adsorption capacity of soils under *Urochloa* cover compared to soils under legumes-based cover has been related to the lower rate of decomposition of grasses residues relative to legumes (Silva et al., 1997; Janegitz et al., 2017).

When fertilized with P, the increase of *U. ruziziensis* exudation was related to the reduction of soil P recalcitrance, an effective indication of P cycling and mobilization in the soil-plant system (Almeida et al., 2018a). In rotation with soybean, *U. ruziziensis* increased the labile and moderately labile fractions of P, reducing the residual fraction of the nutrient in the soil (Almeida and Rosolem, 2016). However, this does not necessarily translate into higher P absorption or higher productivity of subsequent crops (Almeida et al., 2018b,c).

Po accumulation may occur in the soils covered with vegetation (Rodrigues et al., 2016), as a result of the decomposition of plant residues on the surface and roots belowground (Zaia et al., 2008). Po fractions may be the main source of P in deeper layers of the soil profile, mainly originated from dead roots decomposition or even from Po mobilized in the soil profile. Oehl et al. (2002) identified a significant increase of Po in subsoil layers in organic and conventional agroecosystems. This fraction may be important for plant nutrition after mobilization and Pi release, especially when the levels of P on the soil surface decrease. On the other hand, the binding of Pi with soil organic matter and formation of metallic phytates can occur after cultivation with *Urochloa*, which would potentially reduce in the short term the availability of P to plants (Almeida et al., 2018b). In contrast, the cultivation of *U. ruziziensis* as a cover crop reduced the concentration of myo-inositol and labile P degraded by inducing soil phytase activity (Almeida et al., 2018a), especially in those soils in which phosphate fertilizers were applied. This may be due to the direct exudation of phytases by *U. ruziziensis* roots or because its organic exudates favored the activity of microbial communities capable of degrading inositol. It should be noted that myo-inositol reduction was accompanied by an equivalent increase in decomposed fractions of inositol, which are easier to be mineralized and subsequently release Pi to plants.

Another plant response to P-limitation is establishing symbiotic AMF. AMF colonize roots and produce extra radicular hyphae that extend through the soil, functioning as an extension of the plant's root system, thus increasing soil volume exploitation and the acquisition of nutrients, among other non-nutritional benefits to the host (Clark and Zeto, 2000; Pérez-Tienda et al., 2012). In return, the plant provides carbohydrates and lipids as substrates for the fungus growth. The association with AMF has a lower energy cost for the plant than root hair production (Lambers et al., 2008).

Tropical C4 grasses, such as *Urochloa*, are highly mycotrophic and very responsive to mycorrhizal associations (Smith and Read, 2008). It has been found that in nutrient-poor pastures, *Urochloa* depends more on mycorrhizal fungi than C3 plants, especially in the initial seedling stages and during regrowth after periods of drought (Hetrick et al., 1990; Veenendaal et al., 1992). The use of *U. decumbens* in pastures in the Brazilian Cerrado has revealed high colonization by AMF, at a greater degree than native plants (Leite et al., 2019), which would partially explain the capacity of this species to develop in low fertility soils.

It has been suggested that in environments with low nitrification rates, where NH_4^+ predominates, arbuscular mycorrhizae may increase the efficiency of N and P utilization (Teutscherova et al., 2019). A short-term study sheds light on the relationship between mycorrhization, P and N uptake and soil phosphatase activity after ammonium-N fertilization of *Urochloa* genotypes with different BNI capacities. Mycorrhizal colonization of genotypes with high BNI capacities was positively correlated with acid phosphatase activity under N fertilization (Teutscherova et al., 2019). In this study, *U. humidicola* genotypes confirmed their high BNI capacity and maintained higher mycorrhizal colonization than low-BNI *Urochloa* hybrid cv. Mulato.

In a comprehensive review of P absorption in intercropping systems, Xue et al. (2016) reported numerous cases of mutual benefits in the absorption of P, whether due to greater AMF colonization, plant-microbe interactions, complementary niches or absorption facilitation via root exudates or increased microbial activity. Likewise, *Urochloa* has various mechanisms to increase the availability of P and other nutrients, and their inclusion in the productive system may provide greater absorption of these nutrients by the intercropped species.

K Cycling

A few studies were carried on K cycling and *Urochloa* in agroecosystems. According to Garcia et al. (2008), *U. brizantha* extracts substantial amounts of K from subsoil layers, increasing its availability on the surface soil. This is a piece of important information, since K leaching can represent up to 50% of the total nutrient applied as fertilizer in conventional systems (Rosolem and Steiner, 2017). Unfortunately, this study did not provide detailed information on the contribution of K cycling from the exploration of roots from deep soil layers.

On the other hand, using rubidium (Rb) as a marker for K, Maciel de Oliveira et al. (2020) compared K cycling and the vertical stratification of K uptake in soybean cultivation followed by ruzigrass (*U. ruziziensis*), corn or corn-ruzigrass intercrop during 2 years. In the first year, K accumulation was 358 kg ha^{-1} for ruzigrass monoculture, 56 kg ha^{-1} for corn monoculture and 184 kg ha^{-1} for corn-ruzigrass intercrop. In the second year, K accumulation was in general lower, but ruzigrass monoculture and corn-ruzigrass intercrop had higher K uptake, 209 and 157 kg ha^{-1}, respectively, compared to 106 kg ha^{-1} for corn as a sole crop. These authors also showed that soil deep layers had a higher contribution for K uptake by ruzigrass (monoculture and intercropped with corn) in both years, as 34% of total K was absorbed from 0.6 m depth and 40% from 0.3 m. Topsoil accounted for only 26%. These results show that ruzigrass can accumulate high quantities of K and that most of it may come from deep soil layer.

We do not know any similar research with other nutrients and the contribution of the deep layers is also unknown. Therefore, research on the vertical stratification of nutrients uptake by *Urochloa* deserves more attention.

Decomposition of *Urochloa* Residues and Nutrient Release

Urochloa cultivation in rotation systems or as intercrop generates considerable amounts of residues above and belowground that will be prone to decomposition and mineralization (Momesso et al., 2019; Tanaka et al., 2019). However, the total amount of the nutrients released in the soil do not become fully available for plant absorption. The composition and quantity of plant residues left by a crop, alter soil nutrient cycles in complex ways (Urquiaga et al., 1998). For instance, depending on the quality (chemical nature) of the residues and the prevalent environmental conditions, nutrients, such as N, can be either immobilized in soil/residues or mineralized and released into the soil solution, becoming available for plant uptake (Urquiaga et al., 1998; Marchezan et al., 2020). There are also nutrient losses due to volatilization and leaching during the mineralization process, and depending on residue quality, these elements may not be fully released during the next crop cycle, although they may become available in the system in the long term.

The decomposition process can be defined as the breaking of complex organic compounds into simpler and soluble compounds (Cardoso and Andreote, 2016). Soil micro and macrofauna regulate the process by reducing particle size which facilitates microorganism access to further chemical transformation of the organic matter. Mineralization is the process through which simpler organic molecules are degraded and transformed into inorganic forms by microorganisms, which obtain energy and nutrients necessary for its multiplication and development. Thus, part of the nutrients is immobilized in the microbial biomass and the surplus released to the environment, making it available for plant uptake (Cardoso and Andreote, 2016).

The factors that influence the activity of soil biota govern the decomposition/mineralization processes responsible for the release of nutrients. Among those factors, temperature, pH, water availability and the chemical composition of the residue play important roles. At this respect the C:N ratio and the content of lignin, waxes, polyphenols and tannins are especially relevant defining SOM recalcitrance (Cardoso and Andreote, 2016). The high C:N ratios and high lignin content of the residue contribute to the temporary immobilization of N in the soil (Horwath, 2017) and delay decomposition/mineralization process (Pacheco et al., 2011). Residues from *Urochloa* species, such as *U. ruziziensis*, for example, have a high C:N ratio in their biomass if compared to other Poaceae residues (Souza et al., 1999) or to legumes, which have low C:N ratio. Although it is beneficial for soil conservation to maintain soil coverage for longer periods in a tropical environment, this practice can slow down nutrients release (São Miguel et al., 2018; Momesso et al., 2019).

Residue degradation rates are established by half-life time, that is, the number of days required for 50% of residue biomass to be completely mineralized to CO_2, which varies between 70 and 120 days for *Urochloa* (Ceballos et al., 2018; Neves et al., 2018). As the residue is decomposed, mineral nutrients are released and made available to other organisms in the system.

According to Costa et al. (2014b), it takes 6 months for 90% of all P and K and 60% of all N to be released from the *Urochloa* residue. To release half of the N, P, K, Ca, and Mg contents of the *Urochloa* biomass, it takes 52, 20, 16, 61, and 47 days, respectively (Costa et al., 2016). In corn-*Urochloa* intercrop, up to 89% of *U. brizantha* and *U. ruziziensis* residues were already decomposed 3 months after corn harvest (Momesso et al., 2019). These relatively high decomposition rates were attributed to favorable rainfall and temperature conditions during the study, and consequently, a fast nutrient release was observed, as more than 80% of all the macronutrients contained in the residues were released.

Tanaka et al. (2019) reported that depending on the growing season, the cover crop biomass production, decomposition rates and nutrient release are affected by rainfall distribution. The homogeneous rainfall distribution was responsible for great biomass production, high decomposition rate and nutrient release after 90 days of *Urochloa* desiccation (Tanaka et al., 2019). After this period more than 90% of the residues were decomposed and nutrients released; on the other hand, although producing fewer residues, only 74% of it was decomposed and 30% of N was remaining when the condition was not favorable.

Because nutrients form an array of compounds of different complexity in the plant cells, their release rates may differ. Bernardes et al. (2010) found that nutrient release from *U. brizantha* residue was lower than *P. maximum* residue after 75 days of cutting. While the first released 38, 49, 59, 35, and 55% of the N, P, K, Ca, and Mg, the later species released 55, 61, 70, 40, and 53%, respectively. This difference probably reflects the chemical nature of each plant matrix (quality).

The nutrients from residues fulfill a large amount of the nutritional requirements of the companion crop or in succession, what otherwise would be supplied by the soil or by fertilization. Data in the literature support evidence that nutrients cycled via *Urochloa* increases use efficiency and the sustainability of the production system. On the other hand, there may be a lack of synchrony between the release of nutrients from the residues and the stage of highest demand by the crop during succession or intercropping. Regarding *Urochloa* intercropped with perennial crops, such as coffee and citrus, grass cutting can be programmed to coincide with the time the crop's demand is the highest. In any case, with or without synchrony, perennial crops absorb nutrients throughout the year. For annual crops in succession/intercrop, synchronization is not possible. Thus, residues release the most nutrients after cutting/degradation at the beginning of the crop cycle, when the root system is still underdeveloped and restricted to recover all the elements released in the total area, which initially, can lead to low utilization of this nutritional source.

Moreover, while residues on soil surface may not immobilize N, root residues can decrease N absorption of the next crop (Rosolem et al., 2017). Root turnover is a function of climatic conditions, root diameter and composition (Gill and Jackson, 2000) and can be slower than the shoot residues (da Silva et al., 2019). According to Urquiaga et al. (1998), more root biomass increased N immobilization and grasses had more non-decomposable C in the roots, reducing decomposition process and immobilizing N. *Urochloa* root biomass decay was enhanced with N fertilization and moderate grazing (da Silva et al., 2019), but after 512 days of incubation 30% of the root biomass was remaining. Based on this, root decomposition may be higher when *Urochloa* is mowed and N fertilization for the next crop is applied. Nevertheless, there is not enough information in the literature on root grasses decomposition and nutrient supply, indicating that further research is necessary to better understand this process and the consequences for plants nutrition.

In any case, the nutrients resulting from *Urochloa* residue decomposition represent a way to increase NUE in CA, which in the long run generates savings in the use of fertilizers, increases the useful life of reserves and mineral deposits used to manufacture them and enables safer and more sustainable production of food.

FINAL REMARKS

CA practices are a sustainable and secure way of producing food, providing the opportunity to introduce more species than conventional systems. Introduction of forage species can provide multiple services to agroecosystems and among these species, *Urochloa* stands out, as far as the literature is concerned. Introduction of this grass in CA systems can be done through intercrops and crop rotation, with both annual and perennial cash-crops. *Urochloa* accumulates large amounts of residues, which will not only protect the soil but also improve soil health and C storage. In addition to exploiting a large volume of soil and producing a large amount of biomass, *Urochloa* root system can recover elements from deep soil layers and directly influence N and P cycles, with reduced losses, greater recovery efficiency and better use of fertilizers by the plant. It is important to extend our knowledge to other nutrients as most of the available literature focus on N and P.

Urochloa can accumulate ~100 kg ha^{-1} of N, 130 kg ha^{-1} of K, 15 kg ha^{-1} of P, 40 kg ha^{-1} of Ca, and 25 kg ha^{-1} of Mg in its shoots. Depending on residue quality, system management and edaphoclimatic conditions, these accumulated nutrients can be made available by the decomposition/mineralization processes or immobilized in the soil. Although immobilization might not be a problem for shoot residues deposited over the soil surface, root decomposition potentially immobilizes N due to its higher recalcitrance and reduces the next crop or companion crop uptake. Consequently, understanding root decay dynamics and how it affects nutrient cycling is important and further research is encouraged on this issue. The amount of nutrients released by the *Urochloa* biomass is certainly not sufficient to supply for the total demand of the intercropped or succession crops, but it can still increase NUE in agroecosystems. This occurs especially in the case of perennial crops, since the cutting can be synchronized with the plant's stage of highest demand.

It should be restated that the benefits attributed to *Urochloa* introduction require proper management of this grass, i.e., *Urochloa* should be limed, fertilized, grazed, mowed or suppressed according to recommendation standards to provide such benefits. These good practices and native ecosystems protection ensure that the species does not invade native ecosystems, which can be a problem in neotropical savanna and Australia, concerning the reduction in native biodiversity.

For these reasons and others still not well-studied, including *Urochloa* in tropical agroecosystems present a feasible way to increase efficiency and making agricultural production more sustainable. We underscore the importance of future studies concerning the effects of the root system on the cycling of different nutrients and in different production systems, to enable incorporating this resource.

AUTHOR CONTRIBUTIONS

JB and PM designed the review and together with SA and JF wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Rotation Benefits From N_2-Fixing Grain Legumes to Cereals: From Increases in Seed Yield and Quality to Greater Household Cash-Income by a Following Maize Crop

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We investigated Bambara groundnut, groundnut, mung bean, cowpea, and black gram for use as biofertilizers in cropping systems. The ^{15}N natural abundance technique was used to measure N_2 fixation in this study. The percent of N derived from fixation by mung bean (*Vigna radiata* L. Wilczek), Bambara groundnut (*Vigna subterranea* L. Verdc.), cowpea (*Vigna unguiculata* L. Walp.), black gram (*Vigna mungo* L.), and groundnut (*Arachis hypogaea* L) was 98, 83, 79, 66, and 45% respectively. Nitrogen contribution from these legumes was 83, 67, 39, 36, and 32 kg.ha^{-1} respectively for Bambara groundnut, groundnut, mung bean, black gram, and cowpea. Maize grain yield without N fertilizer was 2,449, 2,291, 2,204, 2,046, and 1,671 kg.ha^{-1}, respectively, for maize following groundnut, Bambara groundnut, cowpea, mung bean, black gram, and maize. Grain yield increase of maize after legumes without N fertilizer was 47, 46, 37, 32, and 22%, respectively, for groundnut, Bambara groundnut, cowpea, mung bean, and black gram. Supplying 0 to 60 kg N ha^{-1} to maize plants increased shoot DM from 3,264 to 4,279 kg.ha^{-1}, grain yield from 2,184 to 3,586 kg.ha^{-1}, and whole-plant DM from 5,448 to 7,865 kg.ha^{-1}, which represented a 31, 64, and 44% increase with N fertilizer supply from 0 to 60 kg N ha^{-1}. Symbiotic N benefit of preceding legumes to maize without N fertilizer was 20–40 kg N. ha^{-1} in fertilizer equivalents. The preceding legumes increased maize grain concentrations of P, Ca, S, Fe, Mn, and Zn in zero-N plots relative to maize after maize. There was a 225, 222, 154, 149, and 108% increase in marginal returns of maize after groundnut, Bambara groundnut, cowpea, mung bean, and black gram, respectively, without N fertilizer.

Keywords: grain legumes, N_2 fixation, biofertilizers, crop rotation, grain nutritional quality, mineral nutrients, household cash income, marginal returns

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INTRODUCTION

Maize is a major staple food crop in Africa, especially in Southern Africa, and is the most important component of smallholder cropping systems in the continent. In Africa, maize is either grown as a monoculture or intercropped with cowpea, groundnut, or Bambara groundnut without any specific planting pattern or rotational system (Mathews and Beck, 1994). Though a staple, maize production in Africa is often constrained by drought and low rainfall, as observed during the 2018 drought in Southern Africa. But maize production can also be low on farmers' fields due to other abiotic factors.

Although commercial farmers in countries like South Africa can obtain maize yields of 4,210–6,470 kg ha^{-1}, grain yield is $<$2,000 kg ha^{-1} on smallholder fields due to inherently low soil fertility, severe soil degradation, intensive cereal monoculture, low inputs, and inappropriate land use (Von Loeper et al., 2016; DAFF, 2018). While the use of mineral fertilizers could improve maize yields in Africa, they are inaccessible to resource-poor farmers due to their high cost. The low or lack of fertilizer use is currently the major factor limiting increased crop yields (Sinclair and Vadez, 2012) as, on average, only about 8.8 kg NPK fertilizer is applied per hectare by smallholder farmers in Africa (Henao and Baanante, 2006). The inclusion of N_2-fixing legumes in traditional cropping systems can improve soil N fertility and increase crop yields for enhanced food/nutritional security (Walley et al., 2007; Lithourgidis et al., 2011; Ngwira et al., 2012).

The main food legumes cultivated in Africa include cowpea, groundnut, Bambara groundnut, pigeon pea, common bean, and in recent times, soybean. Whether intercropped or cultivated as a monoculture, these legumes are often rotated with cereal crops. While crop rotation is an age-old practice that is not novel, the science behind it is still not properly understood. The work presented here is only a small part of a wider study to understand the changes in soil fertility, soil health, and soil microbial populations using metagenomics. Although all these factors influence yields of cereal crops rotated with legumes, the soil nutrient enrichment, increase in the population of beneficial microbes, and reduction in pathogenic microbes have not been quantified.

About 32 years ago, Dakora et al. (1987) showed that monocultured groundnut and cowpea, respectively, derived 79 and 89% of their N nutrition from symbiotic N_2 fixation, contributed 101 and 210 kg N ha^{-1}, and increased grain yield by 89 and 95% in zero-N plots when maize was rotated with groundnut and cowpea as preceding crops in Northern Ghana. The net N returns to soil in leguminous residues were 68 kg ha^{-1} for groundnut and 150 kg ha^{-1} for cowpea, while the N benefit of each legume to maize in the rotation was equivalent to 60 kg ha^{-1} of N fertilizer based on grain and dry matter yields (Dakora et al., 1987). Clearly, those findings have shown that crop rotation has the potential to improve soil health and increase plant productivity.

In Africa, the N_2-fixing ability and diversity of native soil rhizobia nodulating cowpea, groundnut, Bambara groundnut, common bean, soybean, and Kersting's bean have been established (Chibeba et al., 2017; Puozaa et al., 2017, 2019; Zinga et al., 2017; Chidebe et al., 2018; Gyogluu et al., 2018; Mohammed et al., 2018, 2019). However, their N contribution in cropping systems is still not properly understood. We also do not know the rotation effects of these legumes on the growth, grain yield, and quality of following cereal crops. The aim of this study was to evaluate N contribution by groundnut (*Arachis hypogaea* L.), Bambara groundnut (*Vigna subterranea* L. Verdc.), cowpea (*Vigna unguiculata* L.), black gram (*Vigna mungo* L.), and mung bean (*Vigna radiata* L. Wilczek), and to assess their rotation effect on grain yield, quality, and economics of a following maize crop.

MATERIALS AND METHODS

Description of Experimental Site and Type of Trials

Field experiments were conducted during 2011//2012 and 2012/2013 cropping seasons at Nelspruit (25°26′25′′ S, 30°58′57′′ E and 640 m above sea level), in the Mpumalanga Province of South Africa. The field trial in 2011/2012 evaluated five grain legumes for plant growth and N_2 contribution, while the field experiment in 2012/2013 measured the rotation effect of each legume on the grain yield and quality of a following maize crop. The rainfall received was 288 mm during the 2011/2012 planting season and 465 mm in the 2012/2013 cropping season. Classified as Avalon series, the soil at the study site is a deep and well-drained sandy-loam (8% clay) with pH 5.95, soil organic carbon (SOC) 0.18%, total nitrogen (N) 7.6 $mg.kg^{-1}$, plant-available phosphorus (P) 19.91 $mg.kg^{-1}$, and potassium (K) 38.86 $mg.kg^{-1}$ (SCWG, 1991).

Field Plot Techniques

The field experiment in 2011/2012 was laid using a randomized complete block design with four replications. A maize plot was included in each replication for assessing the residual effect of these legumes to a following maize crop planted in the 2012/2013 cropping season. Each plot measured 14.4 × 6.3 m in 2011/2012 and the legume species were planted to achieve the plant population shown in **Table 1**. Before planting, 10 soil samples were randomly cored at a depth of 0–30 cm across the experimental site, pooled, sieved, and analyzed for total N, extractable P, K, calcium (Ca), magnesium (Mg) copper, (Cu), zinc (Zn), manganese (Mn), and iron (Fe).

Measurement of Soil Properties

Soil pH was determined in 1 M KCl solution (Black et al., 1965) using a pH meter. The percent soil organic carbon (SOC) was determined as described by Walkley and Black (1934). Extractable P, K, Ca, Zn, and Mg in the soil were determined using the Ambic-1 method developed by Van der Merwe et al. (1984). Extractable P and K were measured on a continuous flow analyzer, and Ca, Mg, and Zn on an atomic-absorption spectrophotometer using an air-acetylene flame. Total N was determined by Kjeldahl digestion (AOAC, 1990).

TABLE 1 | Source and B-values of grain legumes used for estimating %Ndfa.

Grain legume	B-value (‰)	References	Plants.ha^{-1}
Groundnut	−2.700	Nyemba and Dakora, 2010	142,000
Bambara groundnut	−1.400	Nyemba and Dakora, 2010	71,000
Cowpea	−1.759	Belane and Dakora, 2010	200,000
Black gram	−2.200	Unkovich et al., 2008	71,000
Mung bean	−1.140	Unkovich et al., 2008	100,000
Maize	-		37,037

Plant Sampling and Processing

At flowering to early pod-filling stage, 10 plants were dug out from each plot, and separated into shoots, roots, and nodules. The shoot samples were oven-dried at 70°C for 48 h, weighed, and ground to a fine powder (0.85 mm) for analysis of ^{15}N and ^{13}C. The number of nodules per plant was recorded before oven-drying (70°C) to determine dry weight. Non-legume plants growing inside the plots were concurrently sampled and processed as was done for the legumes. At physiological maturity, 10 plants from the inner plots were harvested for yield determination.

^{15}N/^{14}N Isotopic Analysis of Cowpea Shoots

To determine the ^{15}N/^{14}N ratios of plant samples, about 2.0–2.5 mg of plant material was weighed in tin capsules, loaded onto the mass spectrometer, and analyzed using a Carlo Erba NA1500 elemental analyzer (Fisons Instruments SpA, Strada, Rivoltana, Italy) coupled to a Finan MAT252 mass spectrometer (Finnigan, MAT CombH, Bremen, Germany) via a Conflo II open-split device. An internal standard (*Nasturtium* spp.) was included in every five runs to correct for machine error during isotopic fractionation. The isotopic analysis was done for both legumes and reference plants. The combined average δ^{15}N signature of the non-legume reference plants (+2.01‰) was used to determine the %Ndfa of the test legumes (**Table 2**). The isotopic composition of ^{15}N was measured as (Junk and Svec, 1958; Mariotti, 1983):

$$\delta^{15}\mathrm{N}\,(‰) = \frac{\left(^{15}\mathrm{N}/^{14}\mathrm{N}\right)_{\mathrm{sample}} - \left(^{15}\mathrm{N}/^{14}\mathrm{N}\right)_{\mathrm{atm}}}{\left(^{15}\mathrm{N}/^{14}\mathrm{N}\right)_{\mathrm{atm}}} \times 1000$$

where $^{15}\mathrm{N}/^{14}\mathrm{N}_{\mathrm{sample}}$ was the abundance ratio of ^{15}N and ^{14}N in the plant sample and $^{15}\mathrm{N}/^{14}\mathrm{N}_{\mathrm{atm}}$ was the abundance ratio of ^{15}N and ^{14}N in the atmosphere.

Percent N Derived From Atmospheric N_2 Fixation and N-Fixed

The percent N derived from N_2 fixation (%Ndfa) by the selected legume species was determined as (Shearer and Kohl, 1986; Unkovich et al., 2008):

$$\%\mathrm{Ndfa} = \frac{\delta^{15}\mathrm{N}_{\mathrm{ref}} - \delta^{15}\mathrm{N}_{\mathrm{leg}}}{\delta^{15}\mathrm{N}_{\mathrm{ref}} - \mathrm{B}_{\mathrm{value}}} \times 100$$

TABLE 2 | Shoot δ^{15}N (‰) values of reference plants used for estimating %Ndfa in legumes.

Sample no.	Common name	Botanical name	Sample size (n)	δ^{15}N (‰)
1	Sickle thorn	*Asparagus falcatus*	5	+0.88
2	Starbur	*Acanthospermum hispidum*	2	+1.20
3	Khaki weed	*Alternanthera pungens*	5	+1.50
4	Pig weed	*Amaranthus spinosus*	6	+1.40
5	Wandering jew	*Commelina benghalensis*	8	+1.17
6	Milk weed	*Euphorbia hirta*	10	+0.48
7	Shoe black plant	*Hibuscus rosa-sinesis*	3	+2.64
8	Morning glory	*Ipomoea purpurea*	5	+0.28
9	Calabash	*Lagenaria siceraria*	3	+2.04
10	Brazil pusley	*Richardia brasiliensis*	3	+6.30
11	Castor	*Ricinus communis*	2	+0.27
12	Sesame	*Sesamum indicum*	3	+2.18
13	Devils thorn	*Tribulus terrestris*	3	+7.79
14	Coat buttons	*Tridax procumbens*	1	+1.24
15	Cocklebur	*Xathium strumarium*	2	+0.76
		Combined mean		+2.01

The number of plants sampled (n) per species were pooled, oven-dried, ground, and analyzed for ^{15}N/^{14}N ratio.

where $\delta^{15}\mathrm{N}_{\mathrm{ref}}$ is the combined mean ^{15}N natural abundance of the non-legume plant species sampled from the experimental plots and used as reference plants (**Table 2**), $\delta^{15}\mathrm{N}_{\mathrm{leg}}$ is the ^{15}N natural abundance of the legumes tested, and the B value is the ^{15}N natural abundance of the test legumes (Bambara groundnut, groundnut, cowpea, mung bean, and black gram) solely dependent on N_2 fixation for their N nutrition. The B values of the test legumes used in this study are shown in **Table 1**. The B value incorporates the isotopic fractionation associated with N_2 fixation and replaces the value of atmospheric N_2 (Shearer and Kohl, 1986). The amount of N-fixed was calculated as (Maskey et al., 2001):

$$\mathrm{N} - \mathrm{fixed} = \%\mathrm{Ndfa} \times \mathrm{legume\ biomass\ N}$$

The N content of plants was estimated as the product of %N and shoot biomass as (Pausch et al., 1996):

$$\mathrm{Shoot\ N} = \mathrm{dry\ matter\ of\ shoot} \times \%\mathrm{N\ of\ shoot}$$

The soil N uptake was calculated as the difference between total N in the shoots and N-fixed.

^{13}C/^{12}C Isotopic Analysis

The ^{13}C/^{12}C isotopic ratios in shoots of the test legumes were similarly analyzed as described for ^{15}N/^{14}N and reported in the standard notation relative to Pee Dee Belemnite standard as (Farquhar et al., 1989):

$$\delta^{13}\mathrm{C} = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} - 1\right) \times 1000$$

where $\delta^{13}C$ is the ^{13}C natural abundance of the plant shoot sample expressed in parts per million (‰), and R_{sample} and $R_{standard}$ the $^{13}C/^{12}C$ abundance ratios of the shoot sample (Bambara groundnut, groundnut, cowpea, mung bean, and black gram) and standard, respectively. The $^{13}C/^{12}C_{standard}$ used was the isotopic ratio of Belemnite Pee Dee limestone formation (Craig, 1957), a universally accepted standard. Shoot C content per plant was calculated as the product of %C and shoot dry matter weight.

Legume/Maize Rotation

After harvesting the grain, the biomass of each legume was incorporated back into its plots and planted with maize (cv ZM521) as a following crop in the next cropping season. Maize planted after maize was included as control. A spacing of 90 × 30 cm (plant population = 37 037 plants.ha^{-1}) was used. The plots (14.4 × 6.3 m) of preceding legume and maize crops planted in 2011/2012 were each divided into four sub-plots measuring 6.3 × 3.6 m with four rows per plot, and four N levels (N0, N20, N40, and N60 kg.ha^{-1}) super-imposed as sub-treatments using a factorial design with four replications. The five preceding grain legumes and maize planted in the year-1 trial represented the main treatment, and N levels (N0, N20, N40, and N60), the sub-treatments. The trial was planted on 05/10/2012; N was applied (N0, N20, N40, and N60 kg.ha^{-1}) as limestone ammonium nitrate (LAN 28% N) at planting and 9 weeks after planting prior to tasseling. At physiological maturity, shoot dry matter (DM) yield, grain yield, and harvest index were determined from 10 plants per plot. The harvest index was calculated as grain DM divided by the whole-plant oven-dried (60°C) weight times 100 as (Donald and Hamblin, 1976).

$$\text{Harvest index (HI)} = (\text{grain DM}/\text{whole-plant DM}) \times 100\%.$$

Determination of Mineral Nutrients in Maize Grain

The mineral nutrients in maize grain were analyzed at the Soil, Water, and Plant Laboratory, Western Cape Department of Agriculture, Elsenburg, South Africa. Briefly, to measure Fe, Zn, Cu, Mn, and B in cowpea leaves and grain, 1.0 g of ground plant sample was ashed in a porcelain crucible at 500°C overnight, followed by dissolving the ash in 5 ml of 6 M HCl (analytical grade) and placing it in an oven at 50°C for 30 min, after which 35 ml of de-ionized water was added. The mixture was filtered through Whatman No. 1 filter paper. Trace element concentration in plant extracts was determined from four replicate samples using inductively coupled plasma mass spectrometry (IRIS/AP HR DUO Themo Electron Corporation, Franklin, Massachusetts, USA) (Ataro et al., 2008).

Economic Analysis of Maize Grain Yield From Crop Rotation

Economic analyses involving monetary value, total variable costs, gross margin, percentage marginal returns, and cost/benefit ratio were done on maize grain yield produced by the subsequent crop treated to four N levels (i.e., N0, N20, N40, and N60). The economic parameters were estimated as (Gomez and Gomez, 1984):

$$\begin{aligned}
&\text{Totalvariable costs (TVC)} = \text{seed cost} + \text{land preparation cost}\\
&\qquad + \text{agrochemicals cost} + \text{labor cost} + \text{N fertilizer cost}\\
&\text{Monetary value (MV)} = \text{grain yield} \times \text{market price of grain.ton}^{-1}\\
&\text{Gross margin (GM)} = \text{monetary value} - \text{total variable costs (TVC)}\\
&\text{Percent marginal returns (\%MR)} = (\text{gross margin/monetary value}) \times 100\\
&\text{Cost/benefit ratio (C/B ratio)} = \text{monetary value/total variable costs.}
\end{aligned}$$

The maize market price (in South African Rands) of R2000 per ton (DAFF, 2018) and N fertilizer (LAN) cost of R4500 per ton (GRAIN, 2013) were used to estimate the total variable cost. The gross margin per hectare was based on the estimate of total variable costs per hectare for seed, land preparation, agrochemicals, labor, and N fertilizer, which amounted to R2650, R3238, R3575, and R3913 (in South African Rand currency), for the N0, N20, N40, and N60 treatments, respectively (i.e., the total variable cost was TVC_{N0} = R2650; TVC_{N20} = R3238; TVC_{N40} = R3575; TVC_{N60} = R3913).

Correlation Analysis

Correlation analysis was performed to assess if there was any relationship between fixed-N in the shoots of the preceding legume species and plant growth and/or grain yield of the following maize crop.

Statistical Analysis

The data were tested for normal distribution before being subjected to a 1-way or 2-way analysis of variance (ANOVA) using Statistica version 10.1 (Statsoft Inc., 2011). Where there were significant differences, the Dancan's multiple range test was used to separate the means at $p \leq 0.05$. Pearson's correlation was performed to determine the relationships between yield and symbiotic indices.

RESULTS

Soil Characteristics

The soil used for planting the five legumes in 2011/2012 had pH (KCl) 5.95 and contained 7.65 mg.kg^{-1} N, 19.91 mg.kg^{-1} P, 38.86 mg.kg^{-1} K, 143.50 mg.kg^{-1} Ca, 37.66 mg.kg^{-1} Mg, 3.19 mg.kg^{-1} Zn, 0.18% soil organic matter, and 8.0% clay.

$\delta^{15}N$ of Reference Plants

The $\delta^{15}N$ of non-legume plant species used as reference plants are shown in **Table 2**. Their values ranged from +0.27 to 7.79‰, and it is the combined mean value (+2.01‰) of the 15 plant species analyzed that was used to estimate the percent N derived from fixation by test legumes.

Plant Growth, N_2 Fixation, and N Contribution

There were significant differences in the shoot N concentration of the legume species studied, and these ranged from 2.19%

TABLE 3 | Plant growth, root nodulation, symbiotic performance, and soil N uptake of five grain legumes planted in the field at Nelspruit, South Africa, in the 2011/2012 cropping season.

Legume species	Nodule no. per plant	Nodule DM $g.plant^{-1}$	Shoot DM $g.plant^{-1}$	$\delta^{15}N$ ‰	N conc'n %	N content $g.plant^{-1}$	Ndfa %	N-fixed $kg.ha^{-1}$	Soil N uptake $kg.ha^{-1}$	$\delta^{13}C$ ‰	C/N ratio $g.g^{-1}$
Bambara	10.0 ± 1.7a	0.15 ± 0.01a	46 ± 2.1a	−0.83 ± 0.11b	2.99 ± 0.1a	1.39 ± 0.1a	83 ± 3.4a	83 ± 7.8a	16 ± 1.22b	−26.44 ± 0.04a	14.74 ± 0.21c
Black gram	22.0 ± 2.0a	0.25 ± 0.05a	22 ± 0.2c	−0.78 ± 0.08b	2.44 ± 0.7b	0.55 ± 0.0c	66 ± 1.9a	36 ± 1.2b	18 ± 1.2b	−27.31 ± 0.09b	17.63 ± 0.31b
Cowpea	15.0 ± 8.2a	0.15 ± 0.07a	24 ± 2.9c	−0.97 ± 0.10b	2.38 ± 0.0bc	0.57 ± 0.1c	79 ± 0.2.7a	32 ± 4.5b	8 ± 1.1c	−27.28 ± 0.10b	17.81 ± 0.08b
Groundnut	40.0 ± 11.6a	0.12 ± 0.04a	34 ± 2.8b	−0.10 ± 0.02a	3.12 ± 0.1a	1.05 ± 0.1b	45 ± 0.4b	67 ± 4.4a	83 ± 1.5a	−26.08 ± 0.14a	14.12 ± 0.42c
Mung bean	10.0 ± 0.9a	0.06 ± 0.01a	10 ± 1.0d	−0.91 ± 0.01b	2.19 ± 0.0c	0.21 ± 0.0d	93 ± 0.4a	39 ± 3.6b	3 ± 0.1d	−27.29 ± 0.21b	19.25 ± 0.11a
F-statistics	3.05 ns	2.80 ns	43.94***	20.23***	36.66***	59.97***	75.08***	21.58***	159.23***	19.7***	73.04***

*Values (Mean ± SE) followed by dissimilar letters in a column are significantly different at *$p \leq 0.05$, **$p \leq 0.01$, or ***$p \leq 0.001$, ns = not significant. The B-values used for estimating %Ndfa were −2.70‰ for groundnut, −2.70‰ for Bambara groundnut, and −1.40‰ for cowpea (Nyemba and Dakora, 2010) as well as −2.20‰ for black gram, and −1.14‰ for mung bean (Unkovich et al., 2008).*

in mung bean to 3.12% in groundnut (**Table 3**). Groundnut and Bambara groundnut showed significantly ($p \leq 0.05$) greater shoot N concentrations than the other legumes. But the shoot N concentration of black gram and cowpea were similar. Nitrogen content was significantly greater in Bambara groundnut than the other legumes (**Table 3**).

The results showed that shoot $\delta^{15}N$ was significantly greater ($p \leq 0.05$) in groundnut, followed by Bambara groundnut, black gram, cowpea, and mung bean (**Table 3**). The $\delta^{15}N$ in the shoots of the five test legumes ranged from −0.97‰ in mung bean to −0.10‰ in groundnut. As a result, percent N derived from atmospheric fixation (%Ndfa) was also in the range of 45% in groundnut to 93% for mung bean, which reflected the shoot $\delta^{15}N$ values of the legumes studied (**Table 3**). The amount of N-fixed was, however, significantly ($p \leq 0.05$) greater in groundnut and Bambara groundnut than the other legumes due to larger shoot biomass (**Table 3**). Legume N contribution by shoots ranged from 32 kg. ha^{-1} in cowpea to 83 kg. ha^{-1} in Bambara groundnut. Nitrogen uptake from the soil was significantly greater in groundnut (83 kg. ha^{-1}) and <20 kg. ha^{-1} in the other legumes which derived more N from fixation (**Table 3**).

Water-Use Efficiency and C/N Ratio

A one-way ANOVA of shoot $\delta^{13}C$ values showed significant variation between the test legumes (**Table 3**). The $\delta^{13}C$ discrimination was markedly greater (less negative) in groundnut (−26.1‰) and Bambara groundnut (−26.4‰) compared to the other legumes, which recorded −27.3‰. The shoot C/N ratios of the test legume species also revealed substantial differences, which ranged from 14.1 $g.g^{-1}$ in groundnut to 19.3 $g.g^{-1}$ for mung bean (**Table 3**). These differences in shoot C/N could imply potential variation in the decomposition of plant biomass when incorporated into soil.

CROP ROTATION STUDIES

Maize Plant Growth and Grain Yield

Shoot biomass of maize after groundnut was much greater, followed by maize after Bambara groundnut as the preceding crop (**Table 4**). In contrast, the shoot dry matter of maize after black gram was the lowest and similar to maize after maize. In the rotation, whole-plant maize DM (shoot + grain DM) was similar in trend to maize shoot DM, with whole-plant maize DM after groundnut being much higher than the others, followed by Bambara groundnut and cowpea (**Table 4**).

Grain yield also differed significantly between and among the rotation systems. Substantial differences were found in the grain yield of maize following food legumes and maize. This was evidenced by the greater grain yield of maize after legumes (except for black gram) than maize after maize (**Table 4**). More specifically, the grain yield of maize was 3,244 $kg.ha^{-1}$ after groundnut, 3,086 $kg.ha^{-1}$ after Bambara groundnut, 3,032 $kg.ha^{-1}$ after cowpea, 2,909 $kg.ha^{-1}$ after mung bean, 2,582 $kg.ha^{-1}$ after black gram, and 2,682 $kg.ha^{-1}$ after maize as preceding crops.

The supply of mineral N to maize plants in the rotation experiment resulted in significantly increased plant accumulation of shoot biomass and whole-plant dry matter (**Table 4**). Increasing N supply to maize grown after legumes markedly increased shoot biomass, total plant DM, and grain yield relative to the zero-N control (**Table 4**). In all instances, grain yield was much greater at N60 (3,586 $kg.ha^{-1}$), followed by N40 (3,197 $kg.ha^{-1}$) and N20 (2,722 $kg.ha^{-1}$), and lowest in control plants receiving N0 (2,184 $kg.ha^{-1}$). Shoot biomass and whole-plant DM followed the same pattern, and were similarly much higher at N60, followed by N40 and N20, and lowest in the control N0 plots (**Table 4**). In fact, supplying N0 up to N60 to maize plants increased shoot DM from 3,264 to 4,279 $kg.ha^{-1}$, yield grain from 2,184 to 3,586 $kg.ha^{-1}$, and whole-plant DM from 5,448 to 7,865 $kg.ha^{-1}$, which respectively represented a 31, 64, and 44% increase with N fertilizer supply at 0 compared with 60 kg N ha^{-1}.

Symbiotic N benefit in the rotation was assessed by measuring and comparing maize plant growth and yield from the N0 plots of each preceding legume. As shown in **Figure 1**, shoot biomass, grain yield, and whole-plant DM, respectively, recorded 2,905, 1,671, and 4,576 kg. ha^{-1} for maize after maize; 3,440, 2,440, and 5,879 kg. ha^{-1} for maize after Bambara groundnut; 3,558, 2,449, and 6,008 kg. ha^{-1} for maize after groundnut; 3,229, 2,046, and 5,275 kg. ha^{-1} for maize after black gram; 3,328, 2,294, and 5,622 kg. ha^{-1} for maize after cowpea; and 3,214, 2,204, and 5,328 kg. ha^{-1} for maize after mung bean. The increase

TABLE 4 | Two-way ANOVA of the effect of a preceding legume crop on growth, grain yield, and harvest index of maize planted after legumes in the field and super-imposed with four N levels at Nelspruit in the Mpumalanga Province during the 2012/2013 cropping season.

Preceding crop and N level	Shoot dry matter yield	Grain yield	Whole-plant dry matter	Harvest index
	kg ha^{-1}			%
Preceding crop (PC)				
Bambara groundnut	3,998 ± 139b	3,086 ± 158b	7,084 ± 292b	44 ± 0.63ab
Groundnut	4,245 ± 175a	3,244 ± 158a	7,489 ± 324a	43 ± 0.33bc
Black gram	3,497 ± 83d	2,582 ± 129d	6,079 ± 198d	43 ± 0.67bc
Cowpea	3,710 ± 85c	3,032 ± 166bc	6,741 ± 241c	44 ± 0.77ab
Mung bean	3,661 ± 128c	2,909 ± 154c	6,570 ± 277c	45 ± 0.42a
Maize	3,365 ± 117d	2,682 ± 218d	6,047 ± 331d	42 ± 1.66c
N level				
N0	3,264 ± 64d	2,184 ± 71d	5,448 ± 126d	41 ± 0.89b
N20	3,563 ± 67c	2,722 ± 76c	6,285 ± 132c	43 ± 0.48b
N40	3,878 ± 90b	3,197 ± 64b	7,075 ± 144b	45 ± 0.36a
N60	4,279 ± 106a	3,586 ± 61a	7,865 ± 150a	45 ± 0.42a
F-statistics				
PC	45.3***	29.8***	75.1***	5.2**
N-Level	122.0***	260.0***	381***	31.7***
PC × N level	2.1*	1.9*	2.4*	5.4***

Whole-plant dry matter = shoot biomass + grain dry matter.
*Values (Mean ± S.E.M) followed by dissimilar letters in a column are significantly different at *$p \leq 0.05$, **$p \leq 0.01$, or ***$p \leq 0.001$.*

in rotation benefit of maize after legume crops in the N0 plots ranged from 8 to 22% for shoot biomass, and 22–47% for grain yield (**Figures 1**, **2**).

The fixed-N benefit of the five preceding grain legumes to the following maize crop was estimated using maize grain yield, shoot biomass, and shoot + grain dry matter yield from zero-N plots (**Figures 1**, **2**). Comparing the grain yield from zero-N plots of maize planted after legumes with grain yield of maize after maize receiving N fertilizer showed that the symbiotic N benefit of preceding legumes to the following maize crop was about 20 kg.ha^{-1} in fertilizer-N equivalents for each of the test legumes (**Figure 2**). A similar comparison using whole-plant dry matter yield also showed that the fixed-N benefit was about 20 kg N.ha^{-1} for the five test legumes, except groundnut, which was 40 kg.ha^{-1} (**Figure 2**). Black gram as a preceding legume had little effect on shoot biomass when compared to maize after maize.

Plant harvest index (calculated as grain DM/whole-plant biomass × 100) was much higher for maize planted after mung bean as the preceding crop, followed by cowpea and Bambara groundnut, and lowest for maize after maize, maize after black gram, and maize after groundnut (**Table 4**). With N supply, however, the plant harvest index was significantly increased, and was greater at N60 and N40, followed by N20, and then N0 (**Table 4**).

Nutritional Quality of Maize Grain

Planting maize after legumes significantly increased maize grain quality relative to maize after maize. As shown in **Figure 3**, the grain of maize planted after groundnut recorded higher percentage increases in the concentrations of S, Mn, Fe, and Ca over maize after maize. The percentage increase in the grain levels of P, Zn, Mn, Fe, Cu, and Ca were also significant for Bambara groundnut as the preceding crop. Similar % increases in the concentrations of P, Ca, Zn, Mn, and Fe were found in maize grain after black gram as a preceding crop, just as maize after mung bean recorded a marked increase in the levels of P, Ca, Zn, S, Mn, Fe, and Cu in maize grain. Here, Fe and Cu were the only mineral nutrients whose concentrations were increased in the grain of maize grown after cowpea relative to maize after maize (**Figure 3**).

Economic Analysis of Maize Grain Yield From Rotation

A two-Way ANOVA of selected economic indicators revealed marked differences in profit margins and financial returns on maize grain yield from the positive effect of preceding legume crops. The significantly high maize grain yield after groundnut cultivation led to much greater monetary returns measured in South African currency (Rand) when compared to the other preceding legumes (**Table 5**). The grain yield was next highest in plots with Bambara groundnut and cowpea as preceding crops, and this also led to higher cash income from grain sale (**Table 5**). The gross margin, marginal returns, and the cost/benefit ratio of maize grain sale were similar in trend to the monetary value for each preceding crop (**Table 5**).

In this study, all the four economic parameters (i.e., monetary value, gross margin, marginal returns, and cost/benefit analysis) were expectedly and consistently higher with increasing N fertilizer application from N0 to N60 (**Table 5**). The monetary value of maize grain was thus generally greater with exogenous N supply due to the higher grain yields at increased N levels (**Table 5**). In contrast, the gross margin (GM) and percentage

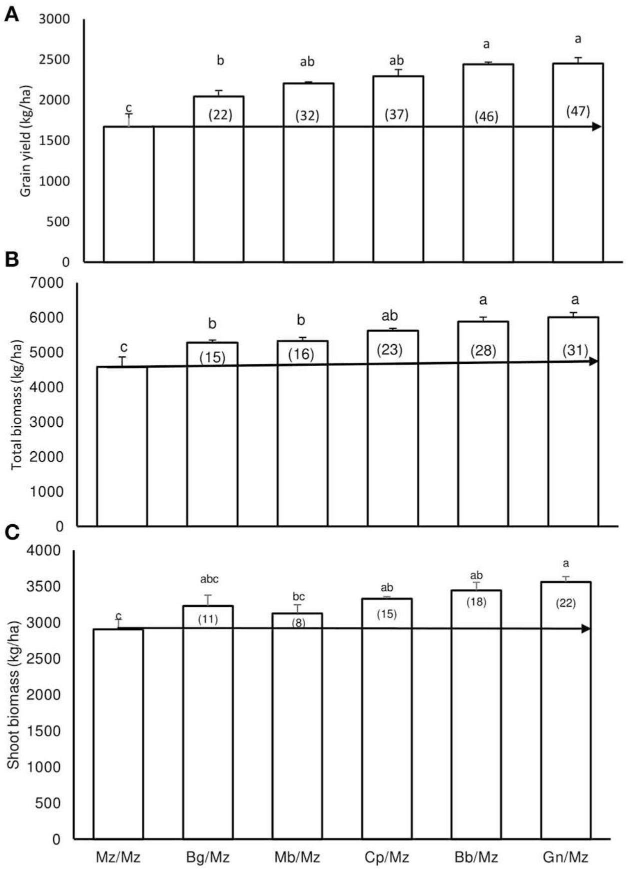

FIGURE 1 | A comparison of **(A)** grain yield, **(B)** whole-plant DM, and **(C)** shoot biomass of maize planted after maize with maize after legumes from zero-N plots at Nelspruit, Mpumalanga Province, in the 2012/2013 cropping season. Values in brackets represent % increases in yield of maize after legume relative to maize after maize as the preceding crop in zero-N plots. The horizontal line delineates increases in yield from zero-N plots caused by a preceding legume crop relative to a maize after maize monoculture. (Mz/Mz, maize after maize; Bg/Mz, maize after black gram; Mb/Mz, maize after mung bean; Cp/Mz, maize after cowpea; Bb/Mz, maize after Bambara bean; and Gn/Mz, maize after groundnut).

marginal returns (%MR) on the sale of maize grain was lower at the higher levels of N application due to the high cost of N fertilizers and the greater labor cost of N application when compared to N0 treatment (**Figure 4**). In essence, the monetary gain as cash income from N contribution by legumes in the cropping system was much higher after cost/benefit analysis than a cereal/cereal system. The increase in financial benefit with regards to percent marginal returns from these legumes over maize after maize at zero-N application was 225, 222, 154, 149, and 108% for groundnut, Bambara groundnut, cowpea, mung bean, and black gram, respectively (**Figure 5**).

Correlation Analysis

There was a strong and significant correlation between the amount of N-fixed by the preceding legumes and grain yield of the following crop, as well as N-fixed by preceding legumes and plant growth (shoot DM) of the following maize crop (**Figure 6**). Thus, symbiotic N in legume residues significantly promoted plant growth and grain yield of the subsequent maize crop in rotation.

DISCUSSION

Legume N Contribution and Water-Use Efficiency

Smallholder farmers account for over 70% of the crops produced in Africa and their grain yield is generally low due to both biotic and abiotic factors. Inherently high climate variability (Lal, 2004), low soil fertility (Dakora and Keya, 1997), high incidence of diseases, insect pests, and parasitic weeds are a major challenge to food production in Africa (Giles, 2007; Chianu et al., 2012). Nutrient depletion in soil is also a major problem affecting crop production by smallholder and resource-poor farmers in developing countries, where most of the grain legumes are produced. Although fertilizer use could increase crop yields in Africa, only a low 8.8 kg NPK fertilizer is applied per hectare per year due to the high cost and inaccessibility to resource-poor farmers (Henao and Baanante, 2006). Therefore, strategies are needed to develop sustainably green and affordable technologies for use by smallholder and resource-poor farmers in Africa in order to enhance soil productivity and ensure food/nutritional security.

In this study, the symbiotic performance of the five grain legumes studied (namely, Bambara groundnut, black gram, cowpea, groundnut, and mung bean) were evaluated in a field trial at Nelspruit in the Mpumalanga Lowveld of South Africa during the 2011/2012 cropping season as a first step in identifying effective technologies for small-scale farmers. The non-N_2-fixing reference plant species used to estimate soil N uptake by the test legumes showed greater $\delta^{15}N$ values (**Table 2**) than the grain legumes, which indicated that the %Ndfa of the legumes studied were reliably estimated using the ^{15}N natural abundance technique (Unkovich et al., 2008).

An assessment of legume performance in the field showed significant differences in plant growth of all the test species, with Bambara groundnut and groundnut exhibiting markedly greater shoot biomass production than the other legumes due to species differences and/or greater shoot N concentration and content. These results are consistent with those of Nyambati et al. (2011), who found a greater increased shoot biomass in Jack bean than hyacinth bean due to high N accumulation in the former.

Shoot $\delta^{15}N$ is a measure of symbiotic functioning in nodulated legumes, with low $\delta^{15}N$ values indicating high N_2 fixation, and greater $\delta^{15}N$ depicting low N_2 fixation. In this study, groundnut showed significantly higher $\delta^{15}N$ values, which resulted in less N derived from atmospheric N_2 fixation relative to the other legumes (**Table 3**). In fact, the %Ndfa was >66% in all the grain legumes, except groundnut which was 45%. As to be expected, this low %Ndfa of groundnut could not meet all of its N requirements. As a result, there was an increased soil N uptake by groundnut, up to 83 kg. ha^{-1} compared with 3–18 $kg.ha^{-1}$ for the other legumes that obtained over 65% of their N nutrition from symbiosis. But the 45% N derived from fixation by groundnut

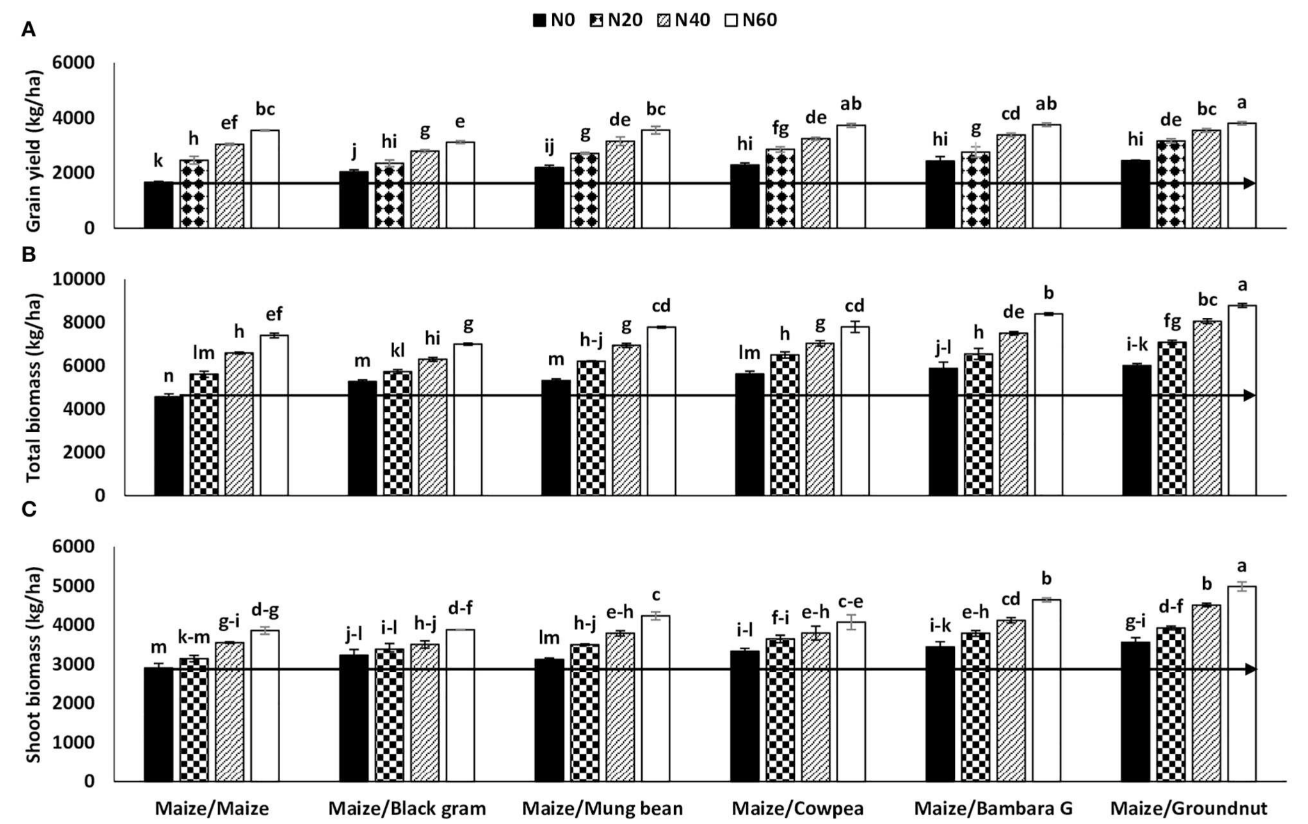

FIGURE 2 | Interactive effect of preceding crop and N fertilizer level on **(A)** grain yield, **(B)** total biomass, and **(C)** shoot biomass of maize planted in rotation at Nelspruit, Mpumalanga Province during the 2012/2013 cropping season. The horizontal line delineates increases in yield caused by N20, N40, and N60 relative to N0.

was nevertheless within the range reported for this legume in Ghana (32–57%), Zambia (27–70%), and South Africa (23–67%) (Nyemba and Dakora, 2010; Mokgehle et al., 2014; Oteng-frimpong and Dakora, 2018). Based on this study, the inclusion of food legumes in cropping systems of smallholder farmers can be a cost-effective and environmentally safe way of enhancing the N nutrition of both the legume and succeeding crops. The net result is sustainably increased yields with reduced soil N uptake, thus eliminating fertilizer N use, which can increase N_2O emission, and hence, global warming.

With climate change, there is a need to select crop species that are drought-tolerant for use in environments with low soil moisture, typically found under rain-fed, dry land conditions in Africa. In C3 plant species, which include members of the Leguminosae, long-term water-use efficiency (or drought tolerance) is commonly measured from analysis of tissue composition of ^{13}C and ^{12}C, the natural isotopes of carbon (Farquhar et al., 1989). In this study, shoot $\delta^{13}C$, which represents a measure of water-use efficiency, ranged from −27.31‰ in mung bean to −26.08‰ in groundnut, a clear indication of significant variations in the water relations of the five legumes tested. The $\delta^{13}C$ values obtained here were similar to those reported elsewhere for cowpea (Makoi et al., 2010) and Bambara groundnut (Mohale et al., 2014). Furthermore, Bambara groundnut and groundnut, which showed much greater $\delta^{13}C$ values (−26.44‰ and −26.08‰, respectively), contributed the highest N to the cropping system, while legume species with the least $\delta^{13}C$ (low water-use efficiency), made much smaller N contributions (**Table 3**). These findings suggest a functional relationship between symbiotic N nutrition and water-use efficiency in nodulated legumes. The significantly greater $\delta^{13}C$ values of Bambara groundnut and groundnut (i.e., better water-use efficiency) was strongly linked to the markedly high shoot N concentration and N content, as well as the increased amount of N-fixed by these legumes, which together supported greater plant growth and biomass accumulation (**Table 3**). These findings could prove useful when selecting legume species for enhanced N_2 fixation and tolerance to drought for use in a changing climate.

Maize Growth, Grain Yield, and Quality in a Legume/Cereal Rotation

Crop rotation is an ancient practice used for sustaining soil productivity, and involves planned successive cultivation of different crops in a specific order on the same field (Karlen et al., 1994). Legumes generally meet their N requirements from symbiotic N_2 fixation, while improving the N nutrition of associated non-legume crops either through N transfer

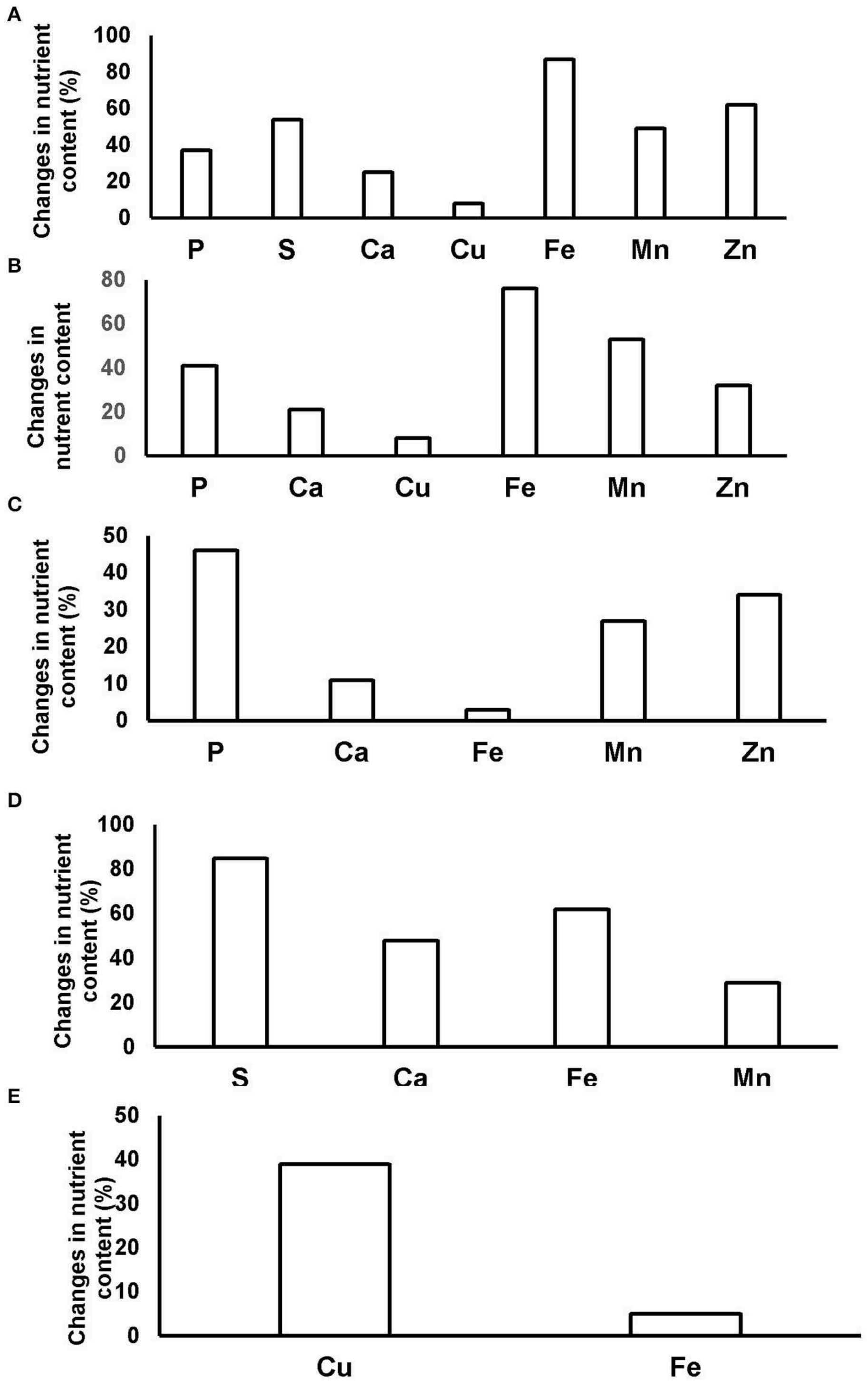

FIGURE 3 | Percentage increases in mineral nutrient composition of maize grain from zero-N plots of **(A)** mung bean, **(B)** Bambara groundnut, **(C)** black gram, **(D)** groundnut, and **(E)** cowpea over maize grain from zero-N plots of maize planted after maize. % mineral A = [(mineral A content of grain from zero-N plots of maize after legume–mineral A content of grain from maize planted after maize from zero-N plots)/(mineral A content of grain from maize grown after maize from zero-N plots)] × 100. Minerals not shown in **Figure 3** did not change significantly relative to maize after maize from zero-N plots.

(Eaglesham et al., 1981), enhancing the N-sparing effect of soil N by the legume, and/or increasing the release of symbiotic N by residues to following crops (Angus et al., 2006; Lithourgidis et al., 2011; Espinoza et al., 2012). Several studies have found substantial N contribution by legumes to subsequent crops, which led to increased plant growth

TABLE 5 | Two-Way ANOVA of the effect of the preceding crop on economic parameters of maize planted in rotation with four N-levels at Nelspruit in the Mpumalanga Province during the 2012/2013 cropping season.

Preceding crop and N-level	Monetary value	Gross margin	Marginal returns	Cost/Benefit ratio
	Currency (South African rand)		%	
Preceding crop (PC)				
Bambara	6,172 ± 317b	2,828 ± 188b	45 ± 1.03ab	1.84 ± 0.03b
Groundnut	6,489 ± 316a	3,145 ± 181a	48 ± 0.70a	1.93 ± 0.03a
Black gram	5,163 ± 257d	1,819 ± 132d	35 ± 1.18c	1.54 ± 0.03d
Cowpea	6,063 ± 231bc	2,719 ± 202bc	44 ± 1.01b	1.80 ± 0.03bc
Mung bean	5,818 ± 308c	2,474 ± 172c	42 ± 0.80b	1.73 ± 0.02c
Maize	5,364 ± 435d	2,020 ± 302d	35 ± 3.66c	1.58 ± 0.07d
N-Level				
N0	4,368 ± 143d	1,718 ± 143d	38 ± 2.57b	1.65 ± 0.05b
N20	5,444 ± 152c	2,206 ± 152c	40 ± 1.76b	1.68 ± 0.05b
N40	6,395 ± 129b	2,820 ± 129b	44 ± 1.15a	1.79 ± 0.04a
N60	7,172 ± 123a	3,259 ± 123a	45 ± 0.99a	1.83 ± 0.03a
F-statistics				
PC	259.7***	29.4***	19.8***	28.9***
N-Level	1.9*	81.1***	10.5***	13.8***
PC × N-level	1.9*	1.9*	2.9*	2.4*

*Means (±S.E.M) followed by dissimilar letters in a column are significantly different at *p ≤ 0.05, **p ≤ 0.01, and ***p ≤ 0.001.*

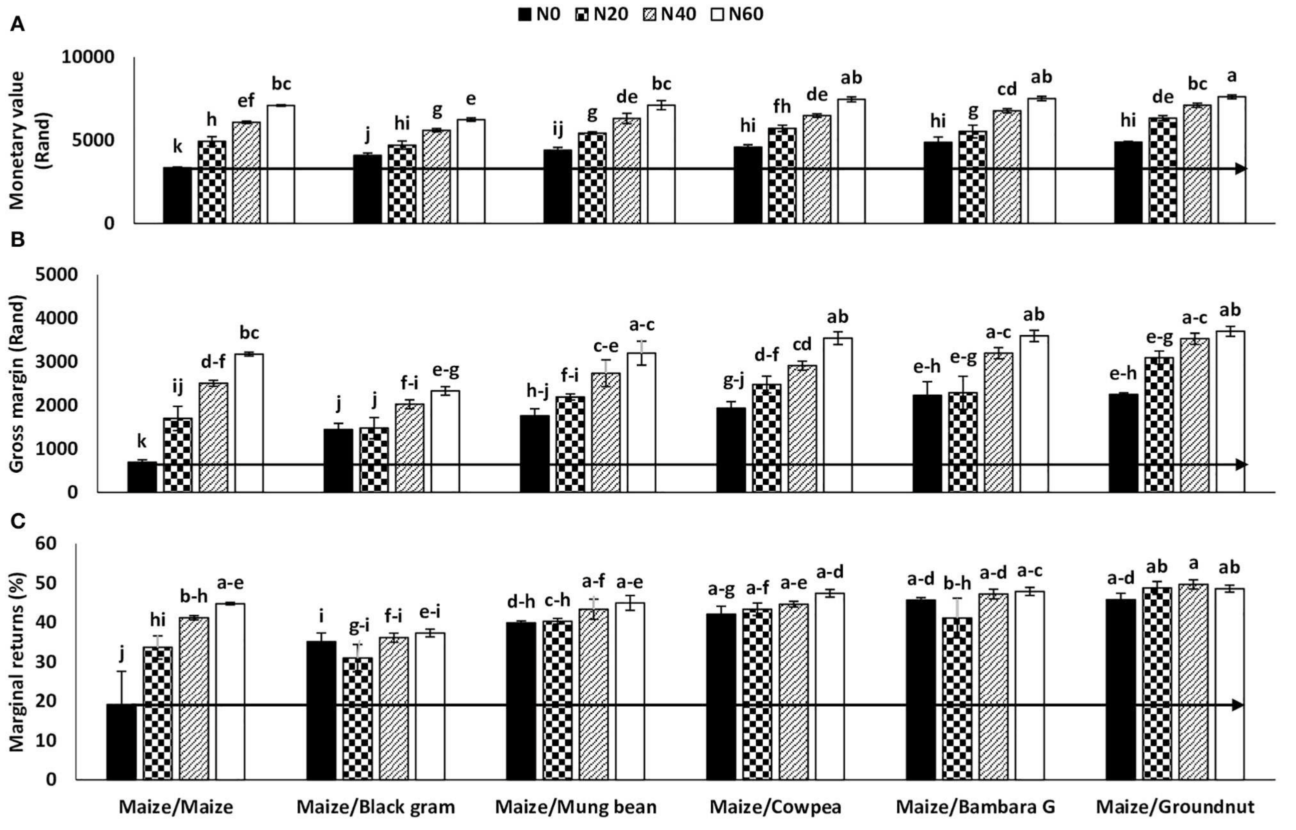

FIGURE 4 | Interaction effect of preceding crop and N fertilizer rate on the **(A)** monetary value, **(B)** gross margin, and **(C)** marginal returns of maize planted in rotation at Nelspruit, Mpumalanga Province during the 2012/2013 cropping season. The horizontal line delineates increases in yield caused by N20, N40, and N60 relative to N0.

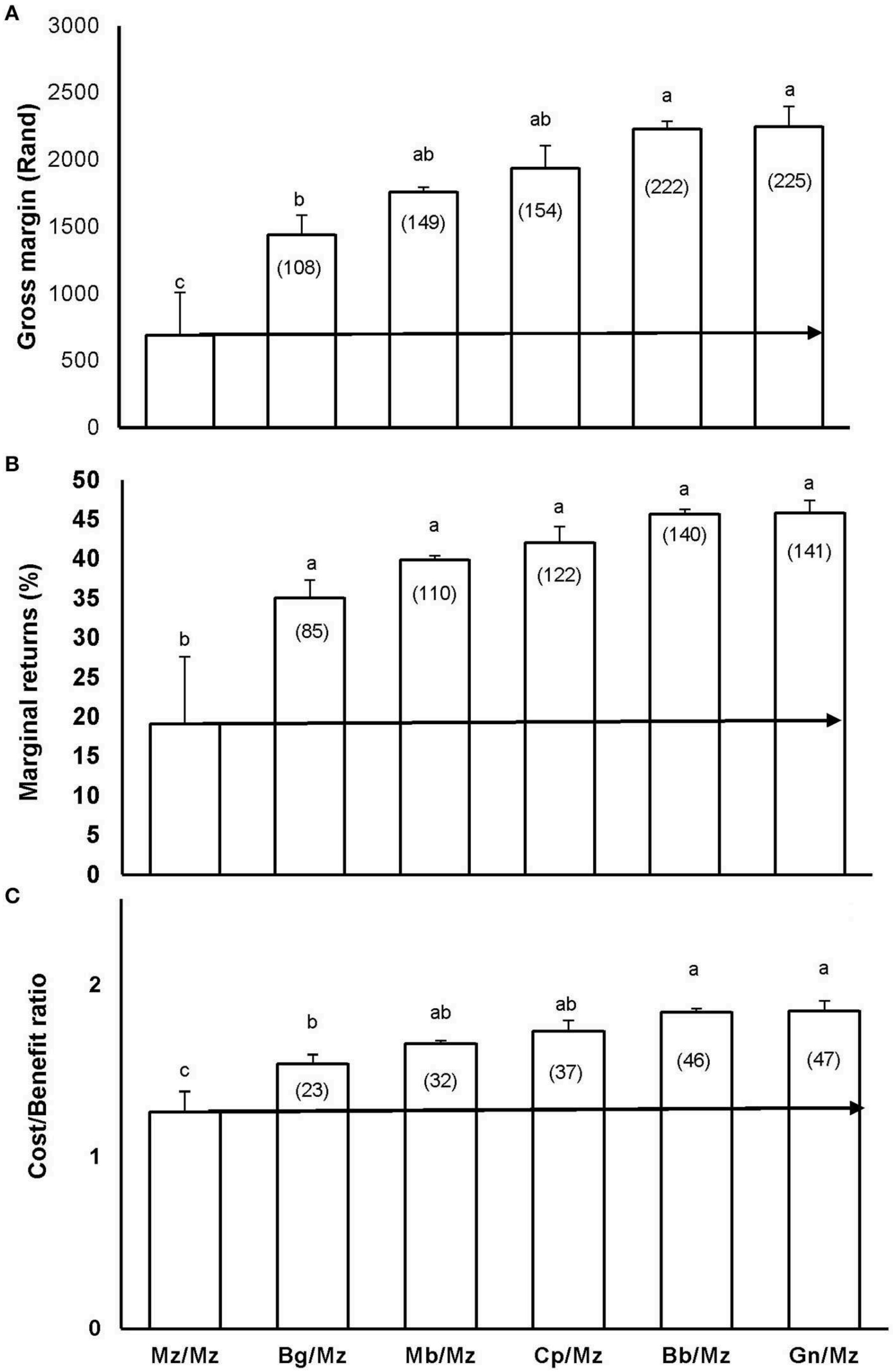

FIGURE 5 | A comparison of **(A)** gross margin, **(B)** marginal returns, and **(C)** cost/benefit ratio of maize after legumes with maize after maize at zero-N application at Nelspruit, Mpumalanga Province, in the 2012/2013 cropping season. Values in brackets represent % increases in yield of maize after legume over maize after maize in zero-N plots of the preceding crop. The horizontal line delineates increases in parameter caused by N20, N40, and N60 relative to N0. (Mz/Mz, maize after maize; Bg/Mz, maize after black gram; Mb/Mz, maize after mung bean; Cp/Mz, maize after cowpea; Bb/Mz, maize after Bambara bean; and Gn/Mz, maize after groundnut).

and grain yield of the following cereal crops (Dakora et al., 1987; Angus et al., 2014; Kirkegaard and Ryan, 2014; Abdel-Galil et al., 2016). The inclusion of symbiotic legumes in cropping systems can therefore help to reduce increased use of N fertilizers in global agriculture, and thus decrease N_2O gas emissions and global warming, thereby mitigating climate change (Dudeja and Duhan, 2005; Sengupta et al., 2015; Stewart, 2015).

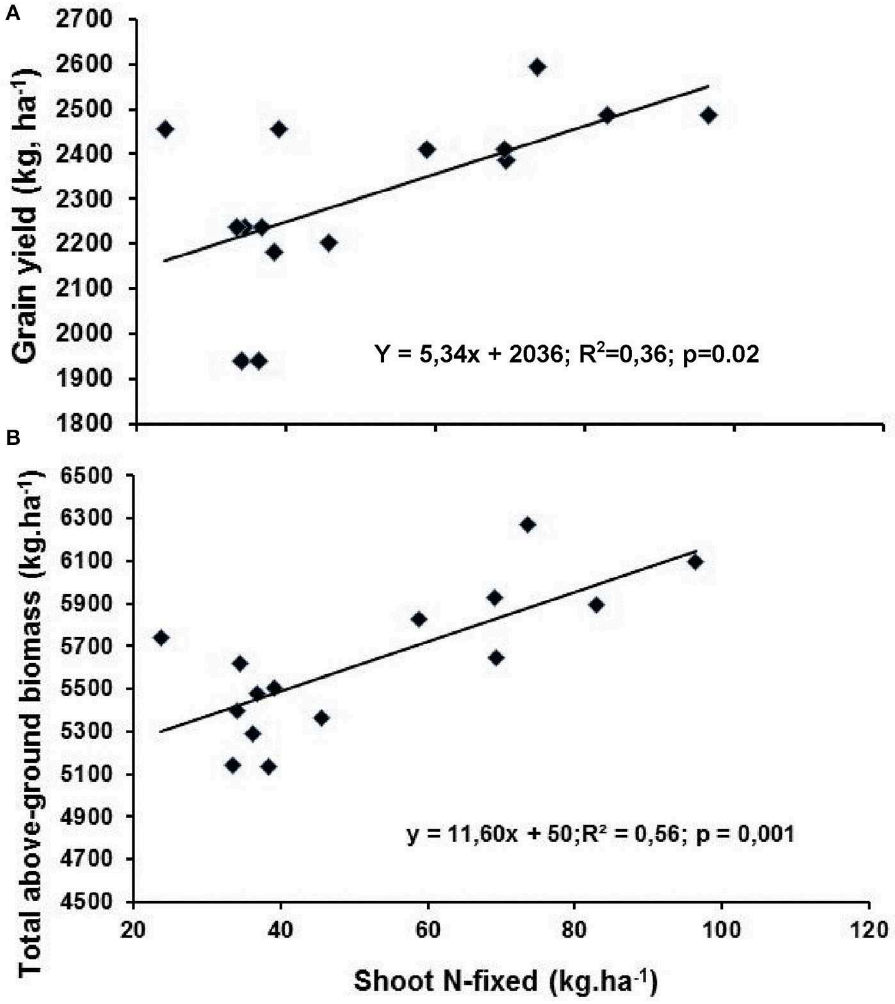

FIGURE 6 | Relationship between **(A)** average amount of N-fixed by all preceding legumes vs. maize grain yield from zero-N plots of the five grain legumes, and **(B)** average amount of N-fixed by all preceding legume and maize shoot DM from zero-N plots of the five grain legumes at Nelspruit, Mpumalanga Province, in the 2012/2013 season.

A comparison of the effect of symbiotic N contribution by the five food legumes to the following maize crop in this study revealed a significant increase in plant growth, shoot biomass, and grain yield of maize planted after legumes relative to maize after maize. The increase in grain yield of maize after legumes was 769, 777, 623, 533, and 375 kg.ha^{-1}, respectively, for Bambara groundnut, groundnut, cowpea, mung bean, and black gram from zero-N plots. The positive effect of legumes was also evident from the shoot biomass of maize planted after legumes. The increase in shoot dry matter yield was 535, 653, 423, 324, and 219 kg.ha^{-1}, respectively, for Bambara groundnut, groundnut, cowpea, mung bean, and black gram when the zero-N treatments were compared for all five legumes. These increases in maize biomass and grain yield could be attributed to the quantum of symbiotic N in legume residues. This was evidenced by the significant correlations found between the mean amount of N-fixed by all preceding legume vs. plant growth (shoot biomass) or grain yield of the following maize crop (**Figure 6**). Although the soil samples from the maize rotation were mixed up and therefore not analyzed in this study, reports from the same environment revealed soil N was 3.2–4.2% after pigeon pea and 1.0% after sole maize in 2009 (Mathew and Dakora, unpubl. data). These increases could, however, also be attributed to the inclusion of legumes in the cropping system which improved soil fertility and chemical characteristics (McCallum et al., 2004), and probably altered the populations of specific microbes in the rhizosphere to the benefit of the following crops (Osborne et al., 2000; Kirkegaard et al., 2008). As a result, cereal yields after legumes are often 40–80% greater than cereal after cereal without N fertilizer, and these increases can be as high as 450–1,000 kg of additional grain yield per hectare (Seymour et al., 2012). In fact, Dakora et al. (1987) found 89 and 95% increases in grain yield from the zero-N plots of maize after cowpea and groundnut as preceding crops when compared to a maize after maize monoculture. In this study, the increase in grain yield ranged from 375 to 777 kg.ha^{-1}, a finding consistent with the results of maize grain yield after groundnut and cowpea in Ghana (Dakora et al., 1987), or after mung bean, black gram, and soybean in Pakistan (Malik et al., 2006; Naveed et al., 2017).

Furthermore, this study revealed marked differences between and among the test legumes in their ability to promote yield increases of maize planted after legumes. As preceding crops, Bambara groundnut and groundnut, for example, caused greater increase in maize yield than the other legumes (Malik et al., 2006; Naveed et al., 2017). In contrast, there was a small increase in grain yield where maize was planted after black gram, a response similar to maize after maize. These differences in the effect of preceding legumes on maize plant growth and grain yield were likely due to the C/N ratios of the test legume species (**Table 3**). For example, even though Bambara groundnut and groundnut showed much greater shoot biomass, the two species also recorded significantly higher shoot N concentration, N content, and amounts of N-fixed (**Table 3**). As a result, Bambara groundnut and groundnut revealed the lowest C/N ratios among the five legumes tested (**Table 3**). It has, however, been known for a long time that plant residues with low C/N ratios tend to decompose faster under warm tropical conditions (Marschner, 1995; Nicolardot et al., 2001), leading to increased release of N and other mineral nutrients for uptake by subsequent crops. In this study, the low C/N ratio in shoots of Bambara groundnut and groundnut probably led to their increased decomposition and greater release of N and other nutrient elements for uptake by the maize crop following Bambara groundnut and groundnut. This would explain the better growth and increased grain yield of maize after Bambara groundnut and groundnut as preceding crops (**Table 4**). This argument is re-enforced by the results of mineral analysis of maize grain, which showed that maize after Bambara groundnut accumulated more mineral nutrients in grain (P, Ca, Fe, Mn, Zn, and Cu), in the same way that maize after groundnut showed greater mineral concentration (Ca, S, Fe, and Mn) in its grain (**Figure 3**).

The increase in maize grain yield caused by exogenous N supply relative to the zero-N treatment in this study was 355, 711, and 1,101 kg.ha^{-1} for 20, 40, and 60 kg N.ha^{-1}. Nitrogen fertilization thus resulted in 11, 22, and 34% increase in grain yield from supplying 20, 40, and 60 kg N ha^{-1}, respectively, when compared to the zero-N plots, which contained only symbiotic and endogenous soil N. The consistent increase in grain yield with increasing supplemental N suggests that N from the preceding legumes' residues was alone inadequate at meeting the N demand of the following maize crop to produce economic yields (**Supplementary Table 1**). The same could be said of the shoot + grain dry matter yield (**Supplementary Table 1**). The increase in plant growth with exogenous N supply was similar in pattern to grain yield, which confirmed the relationship between dry matter accumulation and grain production (Zhaosu, 1993; Zhang et al., 2008). Based on the grain yield, shoot biomass, and whole-plant dry matter, the symbiotic N benefit of legumes to the succeeding maize crop in rotation was estimated to be between 20 and 40 kg N.ha^{-1} fertilizer equivalents (**Supplementary Table 1** and **Figure 2**). These results are consistent with the findings of previous studies, which also found 20–60 kg N.ha^{-1} fertilizer equivalent when maize was planted as a following crop after grain legumes (Dakora et al., 1987; Myaka et al., 2006).

Economic Benefit of Legume Inclusion in Cropping Systems

For resource-poor farmers in Africa, 375–777 kg extra grain yield per hectare from the inclusion of grain legumes in the cropping system represents a substantial increase in household food security from BNF technology, which should be tapped for farmers' use, especially with the current high cost of N fertilizers. The monetary value of including legumes in the cropping system was also estimated, and found to be substantial, especially when measured against the commonly practiced cereal-after-cereal rotation of smallholder farmers in Africa. The increase in marginal returns recorded in this study was 222, 225, 154, 149, and 108% for Bambara groundnut, groundnut, cowpea, mung bean, and black gram, respectively, over a monoculture of maize after maize without N fertilizer (**Figure 5**). The monetary value of maize after legumes was significantly greater because of the higher maize grain yield produced in the zero-N plots of maize after legumes when compared to the zero-N treatment of maize after maize (**Table 5**). Although the maize grain yield was significantly higher with increased N supply, which resulted in a greater cash income for farmers, the percentage marginal returns from grain yield were markedly lower when the variable costs of fertilizer and labor were included (**Table 5** and **Figure 4**).

The legume/cereal rotation in this study also significantly improved the nutritional quality of maize planted after legumes. Whether produced as food for human consumption or feed for livestock, the grain from maize grown after legumes showed significant percentage increases in the concentrations of dietarily-important mineral nutrients relative to maize after maize (**Figure 3**). A number of studies have similarly found an increase in the levels of protein and nutritionally-important mineral nutrients in the grain of cereal crops planted after legumes (Hauggaard-Nielsen et al., 2006; Lithourgidis and Dordas, 2010). Taken together, this study has demonstrated that the inclusion of nodulated grain legumes as biofertilizers in cropping systems in Africa can significantly increase the grain yield of a following cereal crop, enhance the nutritional quality of maize grain, and overcome food insecurity of small-scale farmers, in addition to raising their household cash-income levels in a sustainable and environmentally-friendly manner.

CONCLUSION

Taken together, this study has demonstrated that the inclusion of nodulated grain legumes as biofertilizers in cropping systems in Africa can significantly increase the grain yield of a following cereal crop, enhance the nutritional quality of maize grain, and overcome food insecurity of small-scale farmers, in addition to raising the levels of their household cash-income in a sustainable and environmentally-friendly manner. The results of this study have provided impetus for conducting similar studies on traditionally neglected and under-researched African food legumes such as the Kersting's groundnut, African yam bean, and mucuna (velvet bean).

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

DL conducted the field experiments, collected and prepared plant samples for isotopes and nutrient analyses, and took part in drafting the manuscript. CM assisted in plant sampling, data analysis, and took part in drafting the manuscript. FD was the supervisor of DL, conceptualized the work, edited the final manuscript, and provided funding for the work. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2020.00094/full#supplementary-material

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Overcoming Competition From Intercropped Forages on Upland Rice With Optimized Nitrogen Input to Food Production in Tropical Region

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Intercropping forage grasses with upland rice is an alternative cropping system to improve agroecosystem diversification and could potentially enhance sustainability in tropical regions. However, nitrogen (N) immobilization and nutrient competition between rice and forage grasses could reduce rice grain yield and decrease overall productivity. Therefore, fertilizer N requirements of upland rice intercropped with forage grasses needs to be better defined. Field experiments were carried out during three growing seasons on a Typic Haplorthox soil in São Paulo state of Brazil. The experimental design was a randomized block design with a 3 × 4 factorial scheme with four replications. Treatments were cropping system [monocropped rice (*Oryza sativa L.*), rice intercropped with palisadegrass (*Urochloa brizantha*), and rice intercropped with guineagrass (*Megathyrsus maximus*) and sidedress N application rate (0, 40, 80, and 120 kg N ha^{-1}). Intercropped grasses were sown between upland rice rows 30 days after rice emergence. On average, intercropping of rice with palisadegrass or guineagrass decreased rice shoot dry matter and grain yield by 11% and milled rice productivity by 10% compared with monocropped rice. Grain yield, grain protein, and milled productivity of rice increased as N application rate increased. Forage dry matter production (first and second cut) and crude protein (second cut) were greatest in the rice + palisadegrass intercropping system. Production of both forage grasses increased with up to 80 kg N ha^{-1} in the first cut and increased linearly with N in the second cut. Intercropping of rice with palisadegrass or guineagrass with 80 kg N ha^{-1} application resulted in the greatest land equivalent ratio (1.96 and 1.55, respectively). Relative N yield was greatest at 120 kg N ha^{-1} (220 and 173%, respectively). Although rice monocropping had greatest grain yield, intercropping systems with forage grasses were more favorable from both economic and environmental perspectives by enhancing plant diversification, nutrient cycling with forage grasses, land use production per unit area, and profitability throughout the year.

Keywords: ***Oryza sativa L.*, *Urochloa brizantha*, *Megathyrsus maximus*, intercropping crops, sustainable agroecosystem**

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INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food consumed by more than half of the world's population (Emerick and Ronald, 2019) and one of the three most important cereal crops globally (Africa Rice Center - AfricaRice, 2019; Yadav et al., 2019). Rice cultivation is particularly prominent in Asia, Africa, and the Americas (CGIAR Science Council, 2006). The Cerrado of Brazil is the world's largest producer of upland rice (Silva et al., 2020), a region where soils are acidic and of low fertility (Allen et al., 2007). Low-level technology use by farmers and summer dry spells greatly restricts plant development and upland rice yields (Nascente et al., 2013).

A common agricultural practice in tropical regions is to cultivate upland rice in degraded pastures for two growing seasons before returning to pasture (Kluthcouski et al., 2000). Upland rice tolerates high soil acidity and exchangeable aluminum, which are frequent characteristics of degraded soils (Fageria, 1998). However, cropping systems based on conservation management with crop rotation, intercropping, integrated crop-livestock system (ICLS), and no-tillage system (NTS) are recommended to reduce soil degradation. These systems provide great efficiency in preserving natural resources and sustaining high agricultural production in tropical regions (Borghi et al., 2014; Crusciol et al., 2015; Moraes et al., 2019).

Intercropping upland rice with tropical forage grasses may be an excellent option to improve crop diversity and soil quality, particularly in tropical soils with low fertility and dry winters (Allen et al., 2007; Wood and Mendelsohn, 2014). In many regions of the world, including the tropics, ICLS may be a suitable alternative to improve food production and decrease poverty (Food and Agriculture Organization of the United Nations (FAO), 2017). Because ICLS reduces the need to cultivate new agricultural areas, these systems are considered more sustainable (Surve and Arvadia, 2011) and promote crop production diversity in the same area (Crusciol et al., 2014; Mateus et al., 2016).

Studies of intercropping systems have assessed yields of corn (Borghi et al., 2013a; Crusciol et al., 2013), sorghum (Crusciol et al., 2011; Borghi et al., 2013b), and soybean (Crusciol et al., 2012, 2014) in the summer season (Surve and Arvadia, 2011) and biomass production of tropical grass during the off-season (Pariz et al., 2017; Mateus et al., 2020). These studies aim to develop better management practices for increasing plant development and decreasing competition between intercropped species, thereby increasing yields (Crusciol et al., 2014; Pariz et al., 2016; Moraes et al., 2019; Mateus et al., 2020). However, information on N management of upland rice when intercropped with forage grasses in tropical regions is scarce.

Nitrogen is the most important nutrient impacting development and yield of rice, and its dynamics in the soil-plant system vary according to soil conditions and fertilizer management strategy (Fageria et al., 2011; Nascente et al., 2013). Increasing N use efficiency in agroecosystems is an ongoing goal to improve agricultural sustainability, promote high revenue per area, and increase upland rice yield (Nascente et al., 2013). In ICLS, cash crops and forage grasses are intercropped in the summer season, followed by forage grass production with animal grazing in the off-season (Crusciol et al., 2014, 2016; Franzluebbers and Stuedemann, 2014; Moraes et al., 2019). Successive grass-only cultivation can compromise the sustainability of ICLS due to soil N depletion through crop N removal (Garcia et al., 2016). Low N fertilizer recovery efficiency is common in grass crops. Optimal N fertilizer application rate varies according to soil conditions, crop technological level, and type of crop rotation with or without leguminous crops that fix atmospheric N (Borghi et al., 2014; Crusciol et al., 2016). Therefore, improved N fertilizer recommendations in intercropped systems, particularly those involving upland rice, are needed.

We hypothesized that intercropping systems can (i) increase biomass production of rice in the summer season and forage grasses in the off-season in tropical regions and, as a consequence, (ii) improve land-use efficiency compared with monocropped systems. Beyond that, (iii) better management of N fertilizer rate in intercropping systems could reduce the competition between intercropped forage grass and upland rice and improve the whole system of food production. Thus, the first objective was to compare the feasibility of production between monocropped rice and rice intercropped with the two most commonly used forage species (palisadegrass and guineagrass). Response variables included production and quality of rice, pasture production, land-use efficiency, and estimated meat production during three growing seasons. The second objective was to determine the most appropriate sidedress N rate for monocropped rice and rice intercropped with tropical grasses relative to food production, land use, competition factors, and economic aspects of the systems.

MATERIALS AND METHODS

Site Description

Field experiments were conducted during 2011–2012, 2012–2013, and 2014–2015 growing seasons in Botucatu, State of São Paulo, southeastern Brazil (48° 26′ W, 22° 51′ S; elevation of 740 m above sea level). The soil is classified as a clayey, kaolinitic, thermic Typic Haplothox (United States Department of Agriculture (USDA), 2014) with 630, 90, and 280 g kg^{-1} of clay, silt, and sand, respectively. At the beginning of the experiment, soil (0–0.2 m depth) was sampled to evaluate chemical characteristics (**Supplementary Table 1**) according to the methods of van Raij et al. (2001). The climate is Cwa, humid subtropical zone, with dry winters and hot summer, according to the Köppen climate classification system. Temperature and rainfall during the experimental period are reported in **Supplementary Figure 1**. Long-term (1956–2016) mean annual minimum and maximum temperatures are 15 and 26°C, respectively, with mean annual precipitation of 1,359 mm (Unicamp, 2016).

Each field experiment was performed in a new field previously cultivated with corn (*Zea mays* L.). Management history was NTS for 6 years. Prior to the 2011–2012 growing season, the field had previous crop history going back in time of corn, fallow/soybean [*Glycine max* (L.) Merr.], black oat (*Avena strigosa*)/common bean (*Phaseolus vulgaris* L.), black oat/soybean, palisadegrass

(*Urochloa brizantha*)/corn, palisadegrass/corn, and black oat/soybean. Prior to the 2012–2013 growing season, previous crop history was corn, castor bean (*Ricinus communis* L.)/common bean, forage grass/corn, forage grass/corn, forage grass/corn, black oat/soybean, and black oat/corn. Prior to the 2013–2014 growing season, previous crop history was corn, black oat/corn, black oat/soybean, black oat/corn, black oat/soybean, black oat/corn, and black oat/soybean.

Experimental Design and Treatments

The experimental design was a randomized complete block with a 3 × 4 factorial scheme and four replications implemented each of the three growing seasons. The three cropping systems were: monocropped rice, rice intercropped with palisadegrass, and rice intercropped with guineagrass (**Supplementary Figure 2**). Four sidedress N application rates were: 0, 40, 80, and 120 kg N ha^{-1}, applied as ammonium nitrate. Each plot was 3.4 × 20 m. Buffer area of 0.5–0.7 m along each plot edge was not sampled.

Crop Management

The upland rice cultivar BRS Monarca was sown on Nov. 21 (2011–12), Nov. 21 (2012–13), and Nov. 22 (2014–15) at a depth of 3 cm and row spacing of 0.34 m at a density of 200 viable seeds m^{-2} using no-till seeding (Semeato, Model Personale Drill 13, Passo Fundo, RS, Brazil). For all treatments, the basic fertilization in the sowing furrow consisted of 20 kg N ha^{-1} as urea, 50 kg P_2O_5 ha^{-1} as triple superphosphate, and 50 kg K_2O ha^{-1} as potassium chloride (Cantarella et al., 1997). Sidedress N fertilization rates were applied at tillering growth stage of upland rice (**Supplementary Figure 2**). Upland rice was cultivated according to crop needs.

At the time of sidedress N application, intercropping systems were sown with palisadegrass (*Urochloa brizantha* cv. Marandu) and guineagrass (*Megathyrsus maximum*) at densities of 15.3 and 15.9 kg ha^{-1} seed (34% viable seeds), respectively, with the same no-till drill in between all rows of rice. At the same time, replicated same-size plots of palisadegrass and guineagrass were sown solely as controls to calculate an intercropping competition factor.

Sampling and Analyses of Rice and Tropical Forage Grasses

Upland rice leaf samples were collected for nutrient concentration analysis when 50% of the panicles in each plot were at flowering stage. The selection was randomized by collecting 20 flag leaves of plants per plot (Cantarella et al., 1997). Leaf samples were dried by forced-air circulation at 65°C for 72 h and ground to pass a 0.85-mm stainless-steel sieve. Samples were digested with sulfuric acid for N determination and with a nitro-perchloric solution for P, K, Ca, Mg, and S determinations. Concentrations of N, P, and S were determined from digested solutions by semi-micro-Kjeldahl distillation, colorimetry, and turbidimetry methods, respectively. Concentrations of K, Ca, and Mg were determined by atomic absorption spectrophotometry (Malavolta et al., 1997).

At time of rice leaf sampling, shoots of plants from 1.0 m rows of the two central rows were cut at soil level for the determination of shoot dry matter (DM) of rice. Shoots were dried by forced-air circulation at 65°C for 72 h, weighed, and data extrapolated to Mg ha^{-1}. The following parameters were evaluated 85 days after rice emergence: number of panicles per m^2 (by counting the number of panicles in an area of 2.0 m in the two rows from the usable area of each plot), total number of spikelets per panicles (by counting the number of spikelets in 20 panicles in the useable area), spikelet fertility (calculated using the following function: number of grain-bearing spikelets/total number of spikelets per panicle × 100), and 1,000-grain weight (evaluated by randomly collecting and weighing four samples of 1,000 grains from each plot adjusted to a moisture content of 130 g kg^{-1}). Plants were manually harvested and panicles were dried in the sun for 2 days and later subjected to mechanical threshing using a research plot thresher. Rice grain yield was determined from unhulled grain weight, correcting moisture content to 130 g kg^{-1}, and converting to Mg ha^{-1}. Nitrogen-use efficiency (NUE) was defined as the increase in grain yield per unit of N applied (Fageria et al., 2005), which was determined by dividing the difference between the grain yield (kg ha^{-1}) in each N treatment and the grain yield of the control (no N application) by each N rate (kg ha^{-1}).

For industrial quality and milled rice productivity, 100-g sample of rice was collected from each plot and processed for 1 min in a proof mill for milling yield determination. Polished grains were weighed and calculated as percentage of total grain weight. Polished grains were placed in a grain sorting machine for 30 s to determine broken and unbroken kernels (i.e., milling fraction). Milling fraction was unbroken kernel yield divided by total grain weight. Milled rice productivity was calculated as: total grain yield × milling fraction (expressed as kg ha^{-1}). Grain protein (g kg^{-1}) was calculated from total N concentration of grain samples from Kjeldahl digestion multiplied by 5.95.

Palisadegrass and guineagrass DM production was evaluated at 60 days (first cut) and 150 days (second cut) after upland rice harvest. All forage from 2 m^2 per plot was cut with a mechanical rotary mower at 0.25 m from the soil surface. Forage was dried by forced air circulation at 65°C for 72 h, weighed, and data extrapolated to Mg ha^{-1}. Crude protein of first and second cut was calculated from total N concentration of forage samples from Kjeldahl digestion multiplied by 6.25 (Malavolta et al., 1997).

Statistical Analyses

All data were initially tested for normality using the Shapiro-Wilk test from the UNIVARIATE procedure of SAS v. 9.4 (SAS Inst. Inc., Cary, NC); all data were distributed normally ($W \geq 0.90$). Data were then analyzed using the MIXED procedure and the Satterthwaite approximation. Cropping system, sidedress N application rate, and resultant interactions were considered fixed effects. Block was a random variable. Growing season and its interaction with cropping system and sidedress N application rate were not significant at $P < 0.05$ for any of the dependent variables. Therefore, data were combined across growing seasons. Results were reported as least square means and separated using the probability of differences option (PDIFF).

Regressions of variables on the four rates of N fertilizer were tested across the replications of growing seasons. All data were fit to the non-linear models of quadratic function and, when the non-linear equation resulted an unrealistically outcome, a linear regression was fitted to data. Effects were considered significant at $P < 0.05$. Error bars are presented as standard error (SE) and the regressions were calculated using the SigmaPlot v. 14.0 (Systat Inc., San Jose, CA).

Economic Evaluation and Estimated Meat Production

Cost per hectare to produce each crop was calculated for each treatment (CONAB, 2018). The only differences among treatments were forage seed cost and sidedress N application cost. Return value of rice grain production was calculated using prices in US$.

Although we did not have livestock graze the palisadegrass and guineagrass after rice harvest, meat production was estimated using the Large Ruminant Nutrition System (LRNS; http://nutritionmodels.tamu.edu/lrns.html) model based on the Cornell Net Carbohydrate and Protein System (CNCPS) v. 5, according to Fox et al. (2004). To predict energy and protein requirements, performance and dry matter intake by 450 kg Nellore bull cattle in a continuous grazing system were used and assumed to produce 52% carcass yield with 22% Body Fat Grading System. Forage nutritive values of palisadegrass and guineagrass in the intercropping system with specific sidedress N application rate were used to predict performance of each treatment.

Dry matter intake was assumed as 9.9–10.0 kg DM day^{-1}. Due to the high forage crude protein (CP) concentration (9.5–14.4%), average daily gain (ADG) was based on metabolizable energy and protein to estimate meat production. Dry matter herbage allowance was double the amount of DM intake by individual cattle, considering a grazing efficiency of 60%, according to Braga (1983).

Animal grazing time was considered to be 150 days, assuming 112 days for rice production, 60 days forage accumulation period prior to stocking, and 43 days after animal grazing for biomass regrowth and desiccation to allow sufficient surface residue accumulation for effective ICLS management. Stocking rate was estimated from forage DM production, time available for grazing, DM intake by individual cattle, and grazing efficiency. Stocking rate was multiplied by ADG, time of animal grazing, and carcass yield (52%) to estimate total meat production per hectare averaged across the three growing seasons during off-season (150 days per year). Meat was produced in the fall-winter period, after the rice crop was harvested.

Gross revenue per hectare was calculate by the formula: (price per kg × rice yield) + (price per kg × estimated meat production). Net return per ha was calculated by the formula: (gross revenue ha^{-1} minus cost ha^{-1}). Unit values were from the Brazilian national average over the last 5 years and we converted these values to US dollars (Agrolink, 2020). Unit values were \$0.19 kg^{-1} for rice grain, \$3.00 kg^{-1} for meat, \$8.05 kg^{-1} for N fertilizer, and \$89.37 and \$90.40 kg^{-1} for palisadegrass and guineagrass seeds, respectively.

Intercropping Competition Factors

Competition effects between rice and forage crops were calculated by land equivalent ratio (LER), relative crowing (K), and aggressivity (A). The LER was calculated according Mead and Willey (1980) by the following formula:

$$\mathrm{LER} = \mathrm{Y}_{1,2}/\mathrm{Y}_{1,1} + \mathrm{Y}_{2,1}/\mathrm{Y}_{2,2}$$

where Y is aboveground biomass, and the suffixes 1 and 2 denote crop 1 (rice) and crop 2 (palisadegrass or guineagrass) and vice versa, respectively. The $\mathrm{Y}_{1,2}$ was the aboveground biomass of rice when grown in intercropping with grasses; $\mathrm{Y}_{1,1}$ was the yield of rice when grown monocropped; $\mathrm{Y}_{2,1}$ was the aboveground biomass of palisadegrass or guineagrass when grown in intercropping with rice; and $\mathrm{Y}_{2,2}$ was the aboveground biomass of palisadegrass or guineagrass when grown monocropped (Baumann et al., 2001; Biabani et al., 2008).

Aggressivity (A) is used to determine the competitive relationship between two crops in a mixture (Takim, 2012) and was calculated by the following formula (Agegnehu et al., 2006):

$$(A)_{\mathrm{rice}} = (\mathrm{Y}_{1,2}/\mathrm{Y}_{1,1} \times \mathrm{Y}_{1,2}) - (\mathrm{Y}_{2,1}/\mathrm{Y}_{2,2} \times \mathrm{Y}_{2,1}) \text{ or}$$
$$(A)_{\mathrm{forage}} = (\mathrm{Y}_{2,1}/\mathrm{Y}_{2,2} \times \mathrm{Y}_{2,1}) - (\mathrm{Y}_{1,2}/\mathrm{Y}_{1,1} \times \mathrm{Y}_{1,2})$$

The K is a measure of the competition experienced by crop 1 (rice) when grown in intercropping with crop 2 (palisadegrass or guineagrass) and vice versa. The calculation was according Agegnehu et al. (2006) as the following formula:

$$(K)_1 = \mathrm{Y}_{1,2} \times \mathrm{Z}_{2,1}/(\mathrm{Y}_{1,1} - \mathrm{Y}_{1,2}) \times \mathrm{Z}_{1,2} \text{ or}$$
$$(K)_2 = \mathrm{Y}_{2,1} \times \mathrm{Z}_{1,2}/(\mathrm{Y}_{2,2} - \mathrm{Y}_{2,1}) \times \mathrm{Z}_{2,1}$$

where $\mathrm{Z}_{1,2}$ is the sown proportion of rice, and $\mathrm{Z}_{2,1}$ is the sown proportion of the forage species. The plant density of each species used was from the day of rice harvest.

RESULTS

Leaf Nutrient Concentrations, Agronomic Characteristics, Yield, and Grain Quality of Upland Rice

Monocropped rice had similar N concentration as rice intercropped with palisadegrass and greater leaf P and K concentrations as with rice in the intercropping systems (**Supplementary Table 2**); N and P concentrations were lowest in rice intercropped with guineagrass. In the first year (2011–2012), greatest P and K concentrations were observed, while Ca and Mg concentrations were greatest in the third year (2014–2015). Across all cropping systems, sidedress N application rate significantly increased leaf N concentration, but with diminishing effect at higher N rates (**Figure 1A**).

Among agronomic characteristics, shoot dry matter (DM), panicles per m^2, spikelets per panicle, spikelet fertility, 1,000-grain weight, and grain yield of upland rice were influenced by treatment (**Table 1**). Agronomic characteristics were greatest in monocropped rice. Greater DM, panicles per m^2, spikelets

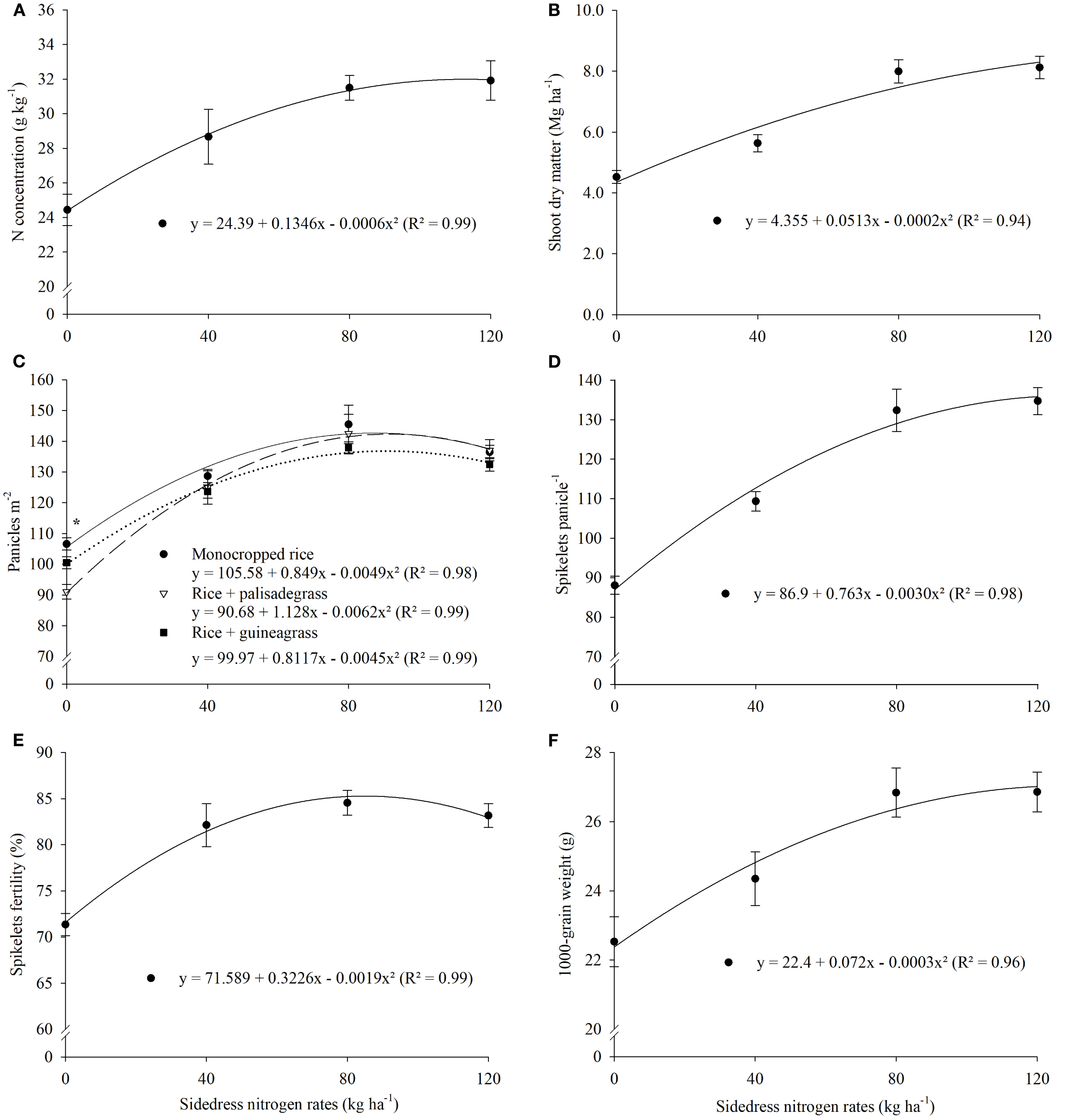

FIGURE 1 | Rice leaf nitrogen concentration **(A)**, shoot dry matter **(B)**, cropping system × sidedress nitrogen rates interaction effect on panicles m^{-2} **(C)**, spikelets panicle^{-1} **(D)**, spikelets fertility **(E)**, and 1,000-grain weight **(F)** as affected by sidedress nitrogen rate. Each data point is the mean of 36 replicates (three cropping systems, four blocks, and three growing seasons) and associated error bar is ± one SE. *$P \leq 0.05$.

per panicle, and grain yields with monocropped rice than rice intercropped with forage grasses was most dramatic in the first growing season. In addition, these agronomic characteristics responded positively to increasing N rate (**Table 1**).

A significant cropping system × N rate interaction occurred for panicles per m^2 (**Figure 1C**) and grain yield of upland rice (**Figure 2A**), with the highest values in the monocropped system. Shoot DM (7.6 Mg ha^{-1}) was greatest at 128 kg N ha^{-1} (**Figure 1B**), spikelets per panicle (135) at 127 kg N ha^{-1} (**Figure 1D**), spikelet fertility (85%) at 85 kg N ha^{-1} (**Figure 1E**), 1,000-grain weight (22.8 g) at 120 kg N ha^{-1} (**Figure 1F**), and NUE (32 kg kg^{-1}) at 68 kg N ha^{-1} (**Figure 2B**). The effects of

TABLE 1 | Shoot dry matter (SDM), panicles per m^2 (P), spikelets per panicle (SP), spikelet fertility (SF), 1,000-grain weight (W1000), grain yield (GY), and N-use efficiency (NUE) of upland rice as affected by cropping systems, sidedress nitrogen rates, and growing season.

Treatment	SDM Mg ha^{-1}	*P* n° m^{-2}	SP n°	SF %	W1000 g	GY Mg ha^{-1}	NUE kg kg^{-1}
Cropping systems							
Monocropped rice	7.1 a§	132 a	122 a	81.8 a	25.3 a	3.1 a	28.4 a
Rice + palisadegrass	6.4 b	123 b	111 b	79.6 b	25.2 a	2.8 b	27.2 a
Rice + guineagrass	6.2 b	123 b	112 b	79.5 b	24.4 b	2.7 b	27.1 a
Growing season							
2011–2012	6.8 a	127 a	116 a	80.6 a	25.2 a	3.0 a	29.3 a
2012–2013	6.7 a	125 a	116 a	80.4 a	24.9 a	2.9 a	26.8 a
2014-2015	6.3 b	125 a	113 a	79.9 a	24.8 a	2.8 a	26.7 a
ANOVA (F probability)							
Cropping systems (CS)	<0.0001	0.0004	0.0057	0.0021	0.0077	<0.0001	0.5920
Nitrogen rates (NR)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Growing season (GS)	0.0015	0.4516	0.3252	0.5880	0.3588	0.4259	0.1069
CS × NR	0.1526	0.0047	0.7925	0.9344	0.3204	0.0386	0.8578
CS × GS	0.1629	0.1944	0.9426	0.9913	0.7077	0.9485	0.9600
NR × GS	0.3710	0.5285	0.2933	0.1212	0.9066	0.6166	0.4126
CS × NR × GS	0.7807	0.7644	0.9291	0.6044	0.9529	1.0000	0.9992

§*Values followed by the same letter are not significantly different at P ≤ 0.05 according to the LSD test.*

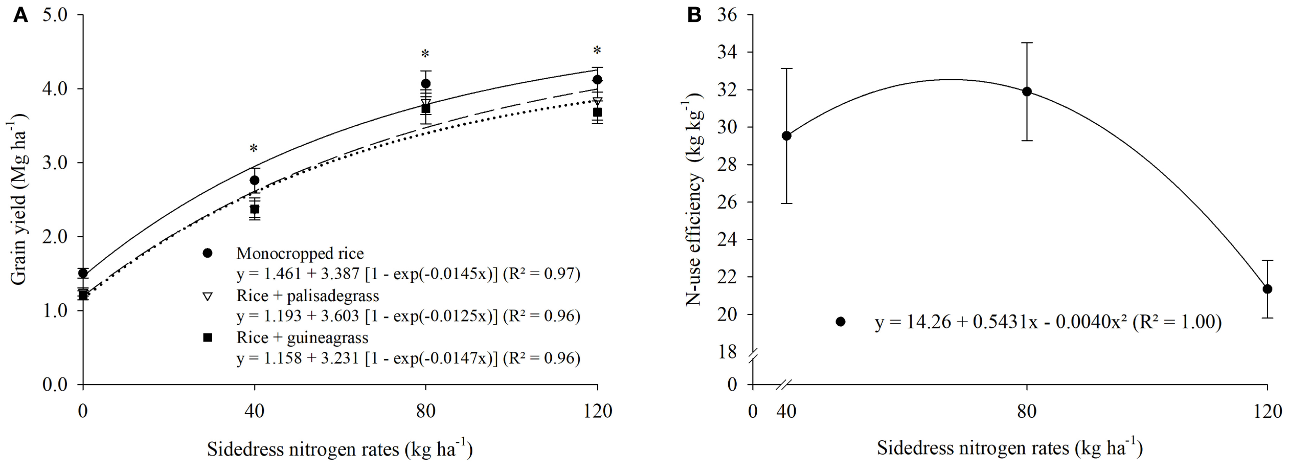

FIGURE 2 | Cropping system × sidedress nitrogen rates interaction effect on grain yield of upland rice **(A)**, and N-use efficiency as affected by sidedress nitrogen rate **(B)**. Each data point is the mean of 36 replicates (three cropping systems, four blocks, and three growing seasons) and associated error bar is ± one SE. *$P \leq 0.05$.

sidedress N rate on grain yield did not differ among cropping systems, with monocropped rice (4.0 Mg ha^{-1}) achieving a cost-to-value threshold of 15 kg gain per kg N at 86 kg N ha^{-1}, rice + palisadegrass (3.7 Mg ha^{-1}) at 87 kg N ha^{-1}, and rice + guineagrass (3.5 Mg ha^{-1}) at 83 kg N ha^{-1} (**Figure 2A**).

Milled rice productivity was influenced by treatment (**Table 2**), with greatest yields for the monocropped system. Milling yield, head rice yield, grain protein, and milled rice productivity were influenced by sidedress N application rate (**Table 2**). Industrial quality of upland rice increased with sidedress N rate; milling yield (72%) was greatest at 117 kg N ha^{-1}, head rice yield (62%) was greatest at 96 kg N ha^{-1} (**Figure 3A**), grain protein (11.6%) was greatest at 145 kg N ha^{-1} (**Figure 3B**), and milled rice productivity (3.5 Mg ha^{-1}) was greatest at 165 kg N ha^{-1} (**Figure 3C**).

Forage Dry Matter Production and Crude Protein and Economics

An advantage of intercropping systems compared with monocropping was the production of forage in the off-season. Palisadegrass intercropped with rice provided greater forage DM production, estimated animal stocking rate (SR), and estimated meat production in both cuts compared with guineagrass

TABLE 2 | Milling yield (MY), head rice yield (HRY), broken-grain yield (BGY), grain protein (GP), and milled rice productivity (MRP) of upland rice as affected by cropping systems, sidedress nitrogen rates, and growing season.

Treatment	MY	HRY	BGY	GP	MRP
		%		g kg^{-1}	Mg ha^{-1}
Cropping systems					
Monocropped rice	70.5 a[§]	60.8 a	9.6 a	108.8 a	2.2 a
Rice + palisadegrass	70.5 a	60.5 a	10.0 a	108.4 a	2.0 b
Rice + guineagrass	70.6 a	60.8 a	9.8 a	108.4 a	2.0 b
Growing season					
2011–2012	70.8 a	60.9 a	9.9 a	110.1 a	2.1 a
2012–2013	70.5 a	60.8 a	9.6 a	108.0 a	2.0 a
2014–2015	70.4 a	60.3 a	10.1 a	107.6 a	2.0 a
ANOVA (F probability)					
Cropping systems (CS)	0.8926	0.7972	0.8151	0.9608	<0.0001
Nitrogen rates (NR)	<0.0001	<0.0001	0.9747	<0.0001	<0.0001
Growing season (GS)	0.3696	0.3637	0.7051	0.2367	0.3321
CS × NR	0.9941	0.8355	0.9724	0.9931	0.9721
CS × GS	0.7153	0.9426	0.7720	0.5530	0.9610
NR × GS	0.9062	0.1015	0.1680	0.8012	0.6365
CS × NR × GS	0.9525	0.0624	0.1286	0.8316	1.0000

[§] *Values followed by the same letter are not significantly different at P ≤ 0.05 according to the LSD test.*

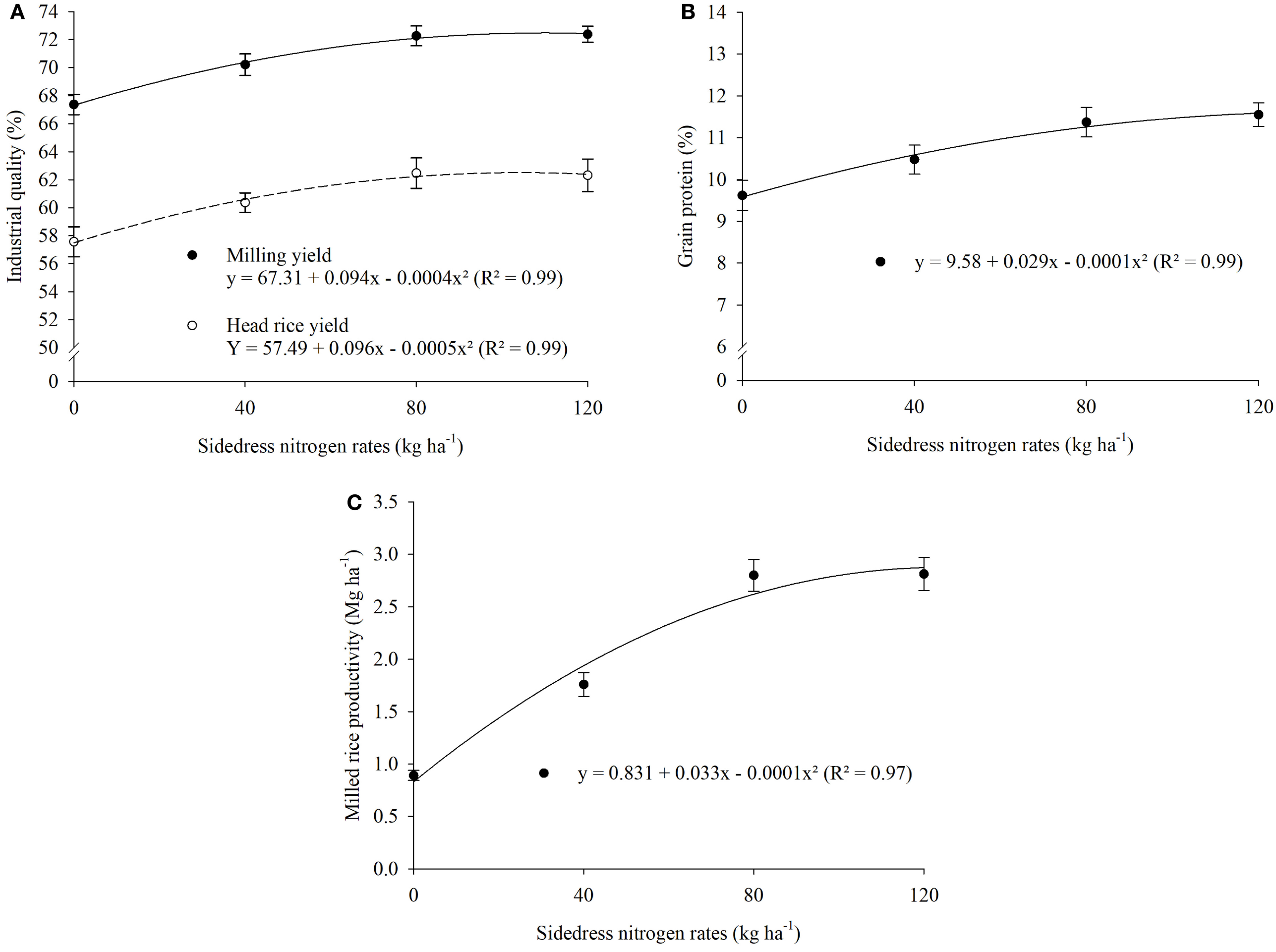

FIGURE 3 | Industrial quality **(A)**, grain protein **(B)**, and milled rice productivity **(C)** as affected by sidedress nitrogen rate. Each data point is the mean of 36 replicates (three cropping systems, four blocks, and three growing seasons) and associated error bar is ± one SE.

TABLE 3 | Forage dry matter production (FDMP), forage crude protein concentration (CP), estimated animal stocking rate (EASR), and estimated meat production (EMP) in the fall-winter as affected by cropping systems, sidedress nitrogen rates and growing season.

Treatment	FDMP		CP		EASR[¶]		EMP*	
	First cut[†]	Second cut[†]	First cut[†]	Second cut[†]	First cut[†]	Second cut[†]	First cut[†]	Second cut[†]
	Mg ha^{-1}		%		AU ha^{-1}		kg ha^{-1}	
Cropping systems								
Rice + palisadegrass	3.2 a[§]	6.5 a	11.7 a	11.9 b	3.2 a	6.5 a	69.7 a	145.1 a
Rice + guineagrass	2.9 b	5.9 b	12.0 a	12.5 a	2.9 b	5.9 b	64.7 b	139.9 b
Growing season								
2011–2012	3.0 a	6.1 a	11.8 a	12.1 a	3.0 a	6.1 a	67.1 a	139.9 a
2012–2013	3.1 a	6.4 a	11.7 a	11.9 a	3.1 a	6.4 a	68.4 a	144.2 a
2014–2015	3.0 a	6.0 a	12.0 a	12.6 a	3.0 a	6.0 a	66.3 a	143.2 a
ANOVA (F probability)								
Cropping systems (CS)	<0.0001	<0.0001	0.1036	<0.0001	<0.0001	<0.0001	<0.0001	0.0031
Nitrogen rates (NR)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Growing season (GS)	0.1521	<0.0701	0.2189	0.0654	0.4503	0.1287	0.1434	0.1058
CS × NR	0.0007	0.1226	0.3409	0.0693	0.0666	0.2514	0.0844	0.2474
CS × GS	0.9874	0.8567	0.1890	0.8895	0.9910	0.9005	0.9799	0.5345
NR × GS	0.6644	0.6375	0.4108	0.5208	0.8047	0.8045	0.0951	0.9260
CS × NR × GS	1.0000	1.0000	0.9854	1.0000	0.9999	0.9999	0.9981	0.9884

[†] *First and second cut in May and August, respectively.*

[§] *Values followed by the same letter are not significantly different at $P \leq 0.05$ (LSD test).*

[¶] *1 AU (animal unit) = 450 kg of body weight.*

**Estimated meat production = kg of body weight gain (cattle) per ha (estimated) × 52% of carcass yield.*

intercropped with rice (**Table 3**). However, guineagrass had greatest CP content of forage in the second cut.

Forage DM production, CP, SR, and meat production were positively influenced by increasing N rates (**Tables 3**, **4**, **Figures 4**, **5**). There was a significant cropping system × N rate interaction for forge DM production in the first cut (**Figure 4A**), with best results in the rice + palisadegrass intercropping system (5.15 Mg ha^{-1}) receiving 185 kg N ha^{-1}, followed by the rice + guineagrass intercropping system (4.6 Mg ha^{-1}) receiving 175 kg N ha^{-1}. In the second cut, forage DM production continued to increase with increasing N rates applied to the rice crop, demonstrating the positive carryover effect of N on forage production in the off-season (**Figure 4B**). Crude protein was greatest during both first and second cuts at 120 kg N ha^{-1} (**Figure 4C**).

The rice + palisadegrass and rice + guineagrass treatments with 120 kg ha^{-1} of applied sidedress N resulted in the highest net profits (US$ 1,189 and 1,149 ha^{-1}, respectively) (**Table 4**). Monocropped rice without sidedress N application rate resulted in the lowest net profit (US$ −158 ha^{-1}).

Intercropping Competition

Aboveground biomass of monocropped rice was greater than that of rice intercropped with palisade or guineagrass in all growing seasons (**Supplementary Table 3**). However, intercropping of rice with tropical forages increased total aboveground biomass in both intercropping systems. In general, the greater the N fertilizer rate, the greater the aboveground biomass. Total aboveground biomass was greater for rice intercropped with guineagrass than for rice intercropped with palisadegrass. The plant density of rice and forage increased with increasing N rate, demonstrating the efficiency of this nutrient in promoting plant development and crop establishment. Total N content of rice intercropped with palisadegrass or guineagrass was greater than that of monocropped rice (**Supplementary Table 3**). Among the N rates, 120 kg N ha^{-1} resulted in the greatest total N uptake.

Land equivalent ratio (LER) and relative N yield (RNY) of rice and forages were calculated from aboveground biomass and N content measured on the day of rice harvest (**Table 5**). All intercropping systems resulted in LER >1. Across growing seasons, LER and RNY were greater for rice intercropped with palisadegrass than for rice intercropped with guineagrass, and differences increased with increasing N rate. Compared with the control treatment (0 kg N ha^{-1}), sidedressing rice intercropped with palisadegrass increased total biomass by 31% at 80 kg N ha^{-1} and by 59% at 120 kg ha^{-1}.

The relative crowding coefficient (K) showed that intercropping competition between plants was similar in the two intercropping systems in the control treatment and at 40 kg N ha^{-1} (**Table 5**). In the treatments with 80 kg N ha^{-1}, the K value of rice intercropped with guineagrass was lower than that of rice intercropped with palisadegrass. Low values of aggressivity (A) were observed for forage crops independent of N rate, indicating low interspecific competition of either forage grasses (**Table 5**).

DISCUSSION

Upland Rice

Nutritional status of upland rice was influenced by cropping system and sidedress N rate. Availability of N for upland rice was lowest when intercropped with guineagrass (28 g kg^{-1}),

TABLE 4 | Economic evaluation of monocropped upland rice, upland rice intercropped with palisadegrass and upland rice intercropped with guineagrass as a function of sidedress nitrogen rates for upland rice (average of three growing seasons).

Treatment	Cost[Ω]	GY[¥]	Total rice[§]	Total EMP[α]	Total meat[¶]	Gross[†]	Net[‡]
	US$ ha^{-1}	Mg ha^{-1}	US$ ha^{-1}	kg ha^{-1}	US$ ha^{-1}		
Monocropped rice							
0 kg ha^{-1}	450	1.5	292	0	0	292	−158
40 kg ha^{-1}	466	2.8	545	0	0	545	79
80 kg ha^{-1}	482	4.1	798	0	0	798	316
120 kg ha^{-1}	498	4.1	798	0	0	798	300
Rice ± palisadegrass							
0 kg ha^{-1}	473	1.2	234	102	306	540	67
40 kg ha^{-1}	489	2.4	467	177	530	997	508
80 kg ha^{-1}	505	3.8	740	258	773	1,513	1,008
120 kg ha^{-1}	520	3.8	740	323	969	1,709	1,189
Rice ± guineagrass							
0 kg ha^{-1}	474	1.2	234	91	273	507	33
40 kg ha^{-1}	490	2.4	467	166	499	966	476
80 kg ha^{-1}	506	3.7	720	244	732	1,452	946
120 kg ha^{-1}	522	3.7	720	317	951	1,671	1,149

Meat production derived from pasture available in the fall-winter, after rice harvest.

[Ω] *Mean costs and production costs of monocropped upland rice and upland intercropped with palisadegrass or guineagrass; the only difference was the forage seeds cost and sidedress nitrogen rates used for the upland rice crop.*

[¥] *GY is the upland rice yield.*

[§] *Total = kg of rice ha^{-1} × US$ 0.19.*

[α] *Total estimated meat production (EMP) = kg of body weight gain (cattle) per ha (estimated) × 52% of carcass yield (sum of EMP First and Second cuts).*

[¶] *Total meat = meat production × US$ 3.00.*

[†] *Gross is the revenue per ha, which was calculated using the formula: total upland rice + total meat.*

[‡] *Net is the return per ha, which was calculated using the formula (gross ha^{-1}–cost ha^{-1}).*

indicating competition between rice and the forage for N. However, leaf N concentration was within the range considered ideal for rice (27–35 g kg^{-1}) according to Cantarella et al. (1997). Guineagrass has high N demand when intercropped with grain crops, because of high soil fertility requirement (Pires, 2006). Regardless of type of intercropping, leaf N concentration increased with sidedress N application up to 112 kg N ha^{-1}. Results of a previous study led to a sidedress recommendation of 40–60 kg N ha^{-1} (depending on the expected response to sidedress N fertilization) for monocropped upland rice (Cantarella et al., 1997). Our results suggest that N fertilizer recommendations might need to be greater than published in fertilization tables, but such changes in recommendation may need to be defined by soil N availability indices.

Across cropping systems, rice leaf P concentration varied from 2.3 to 2.6 g kg^{-1}, and rice leaf K concentration varied from 19 to 20 g kg^{-1}. These values were considered within the adequate range for rice, i.e., 1.8–3.0 g kg^{-1} for P and 13–30 g kg^{-1} for K according to Cantarella et al. (1997). Intercropping of palisadegrass and guineagrass with upland rice reduced rice leaf P and K concentrations due to competition for these nutrients between rice and the forage species.

Shoot DM, panicles per m^2, spikelets per panicle, spikelet fertility, 1,000-grain weight and grain yield of upland rice were lower in intercropped systems than in rice monoculture (**Table 1**). Rice grain yield varied from 2.7 to 3.1 Mg ha^{-1} among treatments. According to Crusciol et al. (2011) and Nascente et al. (2013), it is possible to achieve upland rice yields of 4.0–5.0 Mg ha^{-1} under well-distributed rainfall conditions using NTS for monocropped rice in tropical regions. Our results showed a small interspecific competition between intercropped plants. Therefore, forages reduced rice vegetative growth and grain yield in these intercropping systems.

Rice does not have a strong ability to compete with other plants, especially aggressive forage grasses (Fischer et al., 2001). Plant competition is one of the most yield-limiting constraints in upland rice production and can reduce rice yield by 50%. Tropical forage grasses can reduce plant development of cash crops, resulting in low crop yields. Intercropping may also lead to interspecific competition and may decrease crop yields when plants are not adequately managed (Baldé et al., 2011; Pariz et al., 2016, 2017). In addition, rice and forage grasses have different photosynthetic pathways, i.e., rice is C_3 (Karki et al., 2013) and these tropical grasses are C_4 (Silva et al., 2015). C_4 species are more efficient in converting energy intercepted by the canopy into biomass production (Zhu et al., 2010), resulting in greater competition compared with C_3 species under tropical conditions (Atkinson et al., 2016). Therefore, forage species likely reduced the availability of water, nutrients, and solar radiation for rice even when sown 30 days after rice emergence, resulting in reduced plant development and grain yield of upland rice. Studies have highlighted the challenges of food production in intercropping systems with grasses and the need to seek alternatives to reduce competition for resources among plants

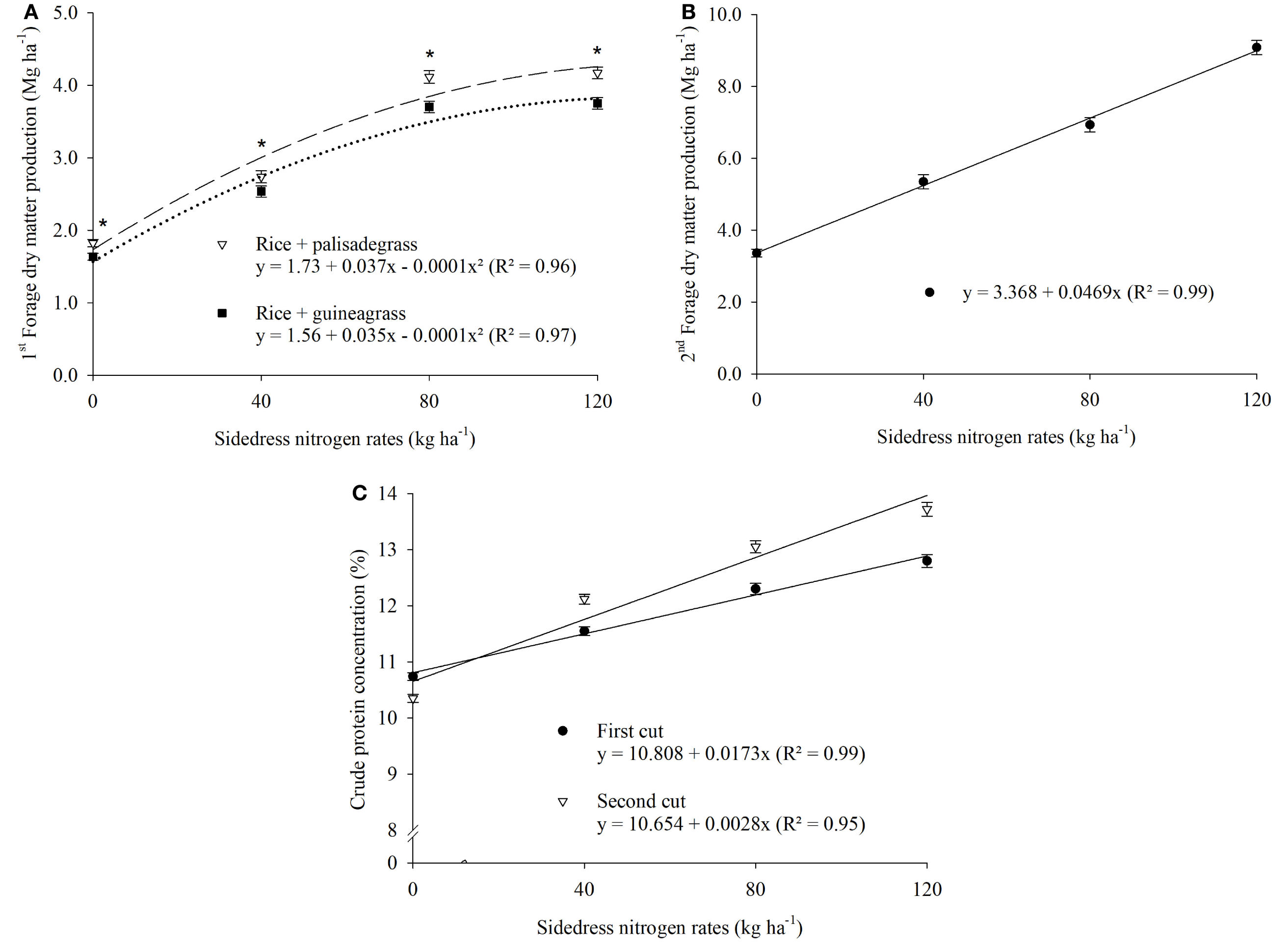

FIGURE 4 | Crop system × sidedress nitrogen rates interaction effect on forage dry matter production in the first cut **(A)**, forage dry matter production in second cut **(B)**, and crude protein concentration at second cut as affected by sidedress nitrogen rate **(C)**. Each data point is the mean of 24 replicates (two cropping systems, four blocks, and three growing seasons) and associated error bar is ± one SE. $^*P \leq 0.05$.

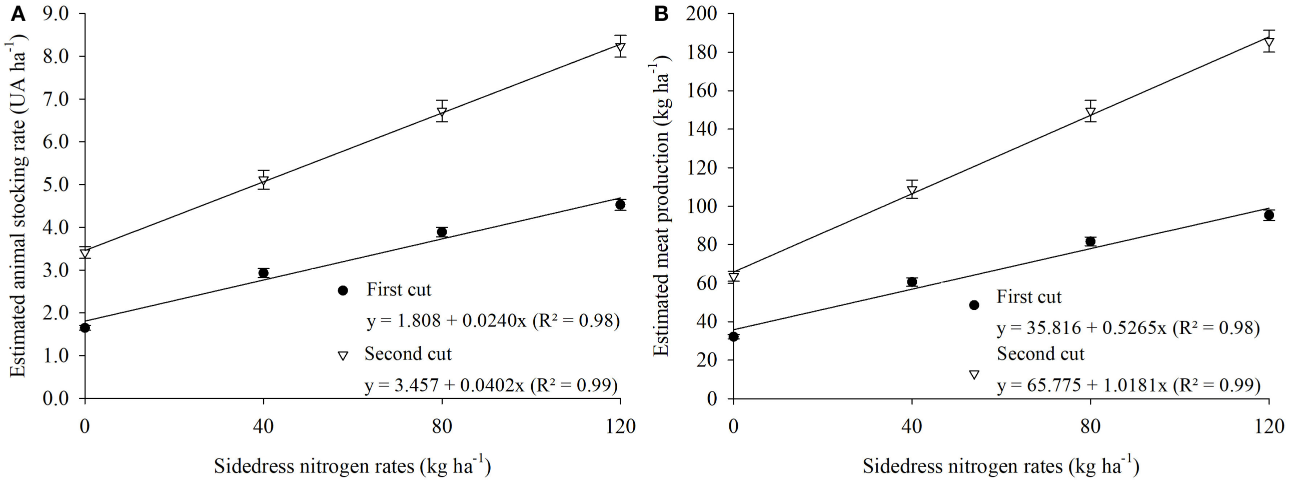

FIGURE 5 | Estimated animal stocking rate **(A)**, and estimated meat production as affected by sidedress nitrogen rate **(B)**. Average of three growing seasons. Stocking rate and meat production in the fall-winter period, after rice harvest. Each data point is the mean of 24 replicates (two cropping systems, four blocks, and three growing seasons) and associated error bar is ± one SE.

TABLE 5 | Land equivalent ratio (LER), relative nitrogen yield (RNY), relative crowding coefficient (*K*) and aggressivity (*A*) of upland rice, palisadegrass, and guineagrass intercropped as a function of sidedress nitrogen rates for upland rice crop.

Treatment	**LER**			**RNY**			**K**		**A**	
	Rice	**Forage**	**Total**	**Rice**	**Forage**	**Total**	**Rice**	**Forage**	**Rice**	**Forage**
					%					
Rice + palisadegrass										
0 kg ha^{-1}	0.96	0.53	1.49	83	61	144	0.87	28.09	0.0010580	–0.0010580
40 kg ha^{-1}	0.83	0.63	1.46	86	87	173	0.18	44.54	0.0004831	–0.0004831
80 kg ha^{-1}	0.97	0.96	1.93	94	126	220	0.99	844.64	0.0001135	–0.0001135
120 kg ha^{-1}	0.88	0.95	1.83	93	136	230	0.25	588.09	0.0000152	–0.0000152
Rice + guineagrass										
0 kg ha^{-1}	0.96	0.34	1.30	80	41	121	0.85	13.75	0.0014596	–0.0014596
40 kg ha^{-1}	0.83	0.40	1.23	86	60	146	0.19	17.19	0.0008847	–0.0008847
80 kg ha^{-1}	0.94	0.59	1.52	92	82	173	0.51	41.25	0.0005151	–0.0005151
120 kg ha^{-1}	0.81	0.55	1.36	89	84	174	0.16	34.32	0.0004168	–0.0004168

grown simultaneously (Costa et al., 2012; Mateus et al., 2020). One alternative to reduce the negative effects of competition on yields of upland rice intercropped with palisadegrass is the application of a low rate of herbicide (cyhalofop-butyl), with the aim of reducing forage growth at sowing while simultaneously increasing food production in the system (Carvalho et al., 2010). Another alternative to reduce competition and increase rice yields is N application as fertilizer since N is essential for rice plant growth (Basuchaudhuri, 2016) and its cultivation in NTS usually develops slowly in the early stages as a result of N immobilized by microorganisms that decompose straw (Rosolem et al., 2017). Our study showed that a rate of ∼115 kg N ha^{-1} increased production components, and the highest NUE was obtained at a rate of 68 kg N ha^{-1}. NUE reduction in rates above 68 kg N ha^{-1} indicates that rice plants were not able to absorb the N applied in excess, because their absorption mechanisms could be saturated (Fageria, 2014).

There was an effect of the cropping system × N rate interaction on the number of panicles per m^2 such that in the absence of N application, the number of panicles per m^2 was greatest for monocropped rice. N application increases the number of rice tillers and ensures their survival to become producers of panicles (Gitti et al., 2012). With reduced N supply, intercropping of rice with palisadegrass or guineagrass intensified the competition for N between species, reducing the development of rice. For monocropped and intercropped rice, the number of panicles per m^2 was greatest at 89 kg N ha^{-1}.

At all N rates, grain yield of rice was greater with monocropping than with intercropping of forage grasses. This result shows the potential for interference from forages intercropped with rice due to competition for N. According to Atkinson et al. (2016), species with C_4 photosynthetic metabolism (palisadegrass and guineagrass) have faster growth than C_3 species (rice). For all cropping systems, rice yields were optimized with N rate of 85 kg N ha^{-1}. The rate of increase in all systems was 32 kg grain kg^{-1} N applied. With no N fertilizer application, rice yield was greater when monocropped than in the intercropping systems, and this difference increased as the rate of N fertilizer application increased. In the intercropping systems, forage grasses shaded rice plants due to vigorous growth of the grasses, and the consequent lower solar radiation on the rice leaves reduced plant development and photosynthetic processes during grain filling (Meirelles et al., 2019).

Milled rice productivity was 12% greater when monocropped than when intercropped with palisadegrass or guineagrass, reflecting this negative effect of competition. Industrial quality of rice grains, as assessed by milled and whole grain yields, achieved maximum production at an average of 106 kg N ha^{-1}. At this rate, milled rice productivity was 24 kg grain kg^{-1} N. Lower levels of N in the plant reduce the industrial quality of rice grains (Portugal et al., 2020). Low N availability causes chalkiness of grain (Zhou et al., 2015) due to grain opacity caused by the arrangement between starch and protein granules in the cells, resulting in a large percentage of broken grains (Marchezan et al., 1992). As N availability improves, starch and protein accumulation in rice grains increase. This accumulation results in densely compacted starch granules interspersed with protein bodies (Zhou et al., 2015), which increases grain resistance to breakage (Silva et al., 2013). Rice quality and actual productivity influence the market price of rice and consequently revenue in the growing area (Salassi et al., 2013).

Forage Characteristics and Estimated Meat Production

The optimal temperature range for palisadegrass and guineagrass development is 30–35°C. Temperatures of 10–15°C that occur during the winter greatly reduce the growth of these forage grasses (Costa et al., 2005). In addition, during the off-season in the Brazilian Cerrado (dry winters), rainfall is limited, further reducing the development of these forage grasses, mainly in June to July, the period corresponding to the 1st cut. Forage DM production of the 1st cut was greater for palisadegrass than for guineagrass, regardless of the N rate applied. For both forage grasses, greatest DM production was obtained with an application rate of 120 kg N ha^{-1} to rice, resulting in forage production of 4.7 and 4.3 Mg ha^{-1} for palisadegrass and guineagrass, respectively. Forage DM production of both grasses during the 2nd cut responded linearly to rice N fertilizer application. In general, greater stocking rate and meat production were obtained with greater forage DM production.

Tropical forage grasses have strong potential for regrowth and provide high availability of biomass (pasture) in the off-season (Costa et al., 2015), mainly in crop systems with N application. Forage DM production can be used as a parameter for hay production potential or as grazed pasture in the off-season (Pariz et al., 2009) to increase the sustainability of agricultural activities. In addition, rice monocropping in the summer season is usually followed by a fallow period in the dry season, which increases the number of weeds in agricultural areas (Nascente et al., 2013). In tropical regions, forage availability is usually lower in the off-season due to the dry winter weather conditions (Borghi et al., 2013a). Therefore, rice intercropped with palisade or guineagrass can be a good option for the diversification of farm activities year-round. Intercropping systems provide the possibility of grazing during the off-season period, when climatic conditions are unfavorable for the development of most crops. Sowing of tropical perennial grasses after rice harvest usually does not provide enough fodder in the off-season due to the short time period and slow plant development for the establishment of fodder under dry winter conditions, making it a risky option.

Intercropped forage crops accumulated significant DM, even after 6 months without rain, which is characteristic of these regions (Cerrado or African Savannas). Forage residue accumulation can favor the success of a NTS, leading to greater nutrient cycling from the large biomass production and establishment of deep root systems (Pacheco et al., 2011; Momesso et al., 2019). Forage roots can absorb nutrients otherwise lost by leaching and return them to the surface of the soil (Mateus et al., 2020). In addition, mineralization of plant and animal residues releases nutrients into the soil that can be absorbed by crops in succession (Carvalho et al., 2010; Pariz et al., 2016, 2017; Moraes et al., 2019).

Among cycled nutrients, N provides the greatest returns to the crop system and can be reused by crops in succession (Rosolem et al., 2017; Momesso et al., 2019). Thus, high mulch cover production by intercropped systems of grain crops with forage grasses is key for the successful maintenance of NTS in the tropics, as well as a very important strategy for enhancing the early establishment and successful production of forage for grazing by animals in the off-season (Barth Neto et al., 2014; Mateus et al., 2016; Pariz et al., 2017).

The benefits of intercropping and N fertilization for increased forage production and nutrient cycling are strongly reflected in the quality of the pasture established in succession. Crude protein was the same in the two forage grasses in the 1st cut, but greater in guineagrass than in palisadegrass in the 2nd cut. Crude protein was also greater in the 2nd cut than in the 1st cut. Both forages responded strongly to the previous application of N to rice. In general, CP exceeded the minimum of 70 g kg^{-1} (7%) considered necessary by van Soest (1994) to maintain the population of microorganisms in the rumen.

Palisadegrass growth was initially greater than guineagrass growth during the 1st cut (Pariz et al., 2017), but with greater N application guineagrass responded with greater growth during the 2nd cut (Mateus et al., 2016), resulting in equivalent overall DM production. The rate of N fertilizer needed to achieve optimum forage production potential is 80 kg N ha^{-1}. Recommended N fertilizer rates for monocropped upland rice have been established (Cantarella et al., 1997). However, there is no N fertilizer recommendation for intercropping systems, especially in NTS (Nascente et al., 2013; Arf et al., 2018). We can infer that the current recommended N fertilizer rate may be insufficient to optimize grain yield of rice and biomass and CP in intercropped forage grasses.

Greater forage production and quality can increase meat and milk production in the dry winter (Crusciol et al., 2012). The amount of N required by crops varies according to the environmental conditions and characteristics of the plants used in rotation, with greater needs for N in crop systems that include only grasses (Crusciol et al., 2012; Garcia et al., 2016; Mateus et al., 2020). Thus, our results provide a better understanding of the use of N in upland rice intercropping systems to support greater N fertilization efficiency and more sustainable agricultural systems.

Revenue

Intercropped systems can be considered a sustainable manner of food production to improve quality of pastures and animal carrying capacity. Our results demonstrated that intercropping of forages with rice using NTS is a feasible option for increasing sustainability in tropical areas and can result in higher revenues for farmers due to the productive, economic, and environmental benefits of these systems. Furthermore, these systems can increase global food production in the same area (Carvalho et al., 2010; Herrero et al., 2010; Franzluebbers and Stuedemann, 2014; Food and Agriculture Organization of the United Nations (FAO), 2017; Moraes et al., 2019). Therefore, our data indicated that rice intercropped with palisadegrass or guineagrass is a promising approach for farmers, especially in the tropical regions of South America, Africa, and parts of Asia, where additional opportunities to produce food are needed.

Except for monocropped rice without sidedress N rate, all treatments resulted in positive net profit, particularly the rice + palisadegrass and rice + guineagrass treatments, because in addition to rice yield in the summer/autumn, farmers could use forage DM production of palisadegrass and guineagrass for animal fodder in the winter/spring. In all treatments with low N sidedress rate (0 or 40 kg N ha^{-1}), net profits were negative when considering rice production only. However, when considering intercropping, one could produce 102–323 kg ha^{-1} meat during the fall-winter season, with net profits of US$ 33–1,196 ha^{-1}, depending on the rate of sidedress N application to rice. Greater N rates result in greater net profits as a function of greater forage DM production, which could effectively add an extra US$ 125–889 to the production system. In addition, the need for soil mulch would be satisfied in planning for the next crop.

Similar to those of rice yield, economic results highlight the need for sufficient sidedress N application rates for the rice + palisadegrass (80–120 kg ha^{-1} N) and rice + guineagrass (120 kg ha^{-1} N) treatments to achieve greatest net revenue (>US$ 1,000 ha^{-1}).

Intercropping Competition Factors

Intercropping changes the N dynamics and nutrient use in the system. Our study showed positive effects of N application in intercropping systems, resulting in greater yield per land area. Increases in total LER and RNY were observed when tropical forages were intercropped with rice, indicating that forage is a suitable option for agricultural systems. Crop performance in intercropping systems is measured by LER, which describes the relative land use per unit area compared with monocropping (Biabani et al., 2008). Values of LER <1 indicate that the species is disadvantageous in the system. RNY may have low nutrient cycling and imposes greater competition per area of crops. However, intercropping systems such as rice + tropical grasses are also beneficial due to straw production and soil coverage throughout the year in NTS. Palisadegrass exhibited greater growth rate than guineagrass when intercropped with rice, indicating that this grass is a viable option in intercropping systems. Systems that improve land use are important for the sustainability of the environment and food production, especially in regions with poor farmers (Costa et al., 2015; Mateus et al., 2020). In addition, the RNY values indicated a high percentage of N cycling in the intercropping systems with N fertilization. Palisadegrass and guineagrass increase nutrient cycling in agricultural systems (Pariz et al., 2017) due to their deep root systems and nutrient uptake in the soil profile (Rosolem et al., 2017). Our study highlights that, although monocropped rice produced the greatest grain yield, intercropping systems of rice, and forage increased total agricultural yields as well as enhanced land use and N cycling.

Competition between rice and forages was evident. Based on the high K values for the forage grasses, tropical forages are more competitive than upland rice due to the plant characteristics of these grasses (Zarochentseva, 2012; Rosolem et al., 2017). Pariz et al. (2017) and Mateus et al. (2016) reported similar high competition between forage grasses and maize and sorghum in intercropping systems, respectively. However, the aggressivity of upland rice was higher than that of the forage in our study, as the rice was sown before the forage (Namuco et al., 2009). In addition, aboveground biomass and plant density of crops were affected by environmental factors, such as climatic conditions and N fertilizer. A shorter time of co-existence in intercropping can favor the growth of crops by reducing interspecific competition (Crusciol et al., 2014). Although there was competition between grain and forage species, intercropping systems provided advantages, including soil coverage, high land use per area and food for cattle as pasture due to forage production. This study contributes to sustainable agricultural production and provides a foundation for subsequent research on the production of rice in intercropping with forage and the adequate rate of N fertilizer application in these tropical systems.

CONCLUSIONS

In this study, intercropping systems with upland rice and forage grasses showed considerable improvements in productivity, economic, and environmental outcomes over three growing seasons. Monocropping upland rice provided a superior rice performance, with high agronomic characteristics and yields; however, intercropping with forage grasses was superior in total food production due to increased diversification of production, land use per unit area, nutrient cycling, and profitability throughout the year. Both forages were viable for cultivation in intercropping system, production of forage dry matter, crude protein, and estimated meat. For upland rice monoculture or intercropped with perennial grasses, the recommendation of 85 kg N ha^{-1} was sufficient to optimize grain production. However, our study showed that with crop-livestock integration, food production (grains and meat), and economic return responded positively with the application of 120 kg N ha^{-1}. Additional studies with rates >120 kg N ha^{-1} and different management of N fertilizer in the intercropped rice should be considered, as well as long-term studies to understand the changes in soil nutrient cycling capacity that might develop in these systems. In addition, intercropping of upland rice and forage grasses enhanced land equivalency ratio at an application rate of 80 kg N ha^{-1} and relative N yield at an application rate of 120 kg N ha^{-1}. From an overall perspective considering grain yields, land use, nutrient cycling, and profitability, intercropping systems were effective to improve diversity and food production in the same area with a potential to decrease poverty in developing countries.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

CACC, CC, and HC: design of experiment. CASC, LM, and CHMC: obtain and process the data. CACC, JP, JB, CP, AC, and AF: analysis of data. CACC, JP, LM, JB, and NC: wrote the paper with the contribution of all co-authors. All authors confirm being contributors of this work and have approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2020.00129/full#supplementary-material

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Influence of Annual Plant Diversity on Forage Productivity and Nutrition, Soil Chemistry, and Soil Microbial Communities

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Forage cover crops are gaining in popularity on the Canadian prairies, where multi-species crop mixtures are grown for soil and ecosystem enhancing benefits, but also harvested for forage. As the use of these forage mixtures increases, more knowledge is needed to understand the impact these mixtures have on forage production systems. The objective of this study was to determine the effects of increasing plant species diversity on forage productivity, soil chemistry, and soil microbial communities. Field trials were conducted in 2016 and 2017 at two separate locations in the Canadian Prairie region that included four treatments: (1) oat monoculture, (2) three spp. mixture (one grass, one legume, one brassica), (3) six spp. mixture (two grasses, two legumes, two brassicas), and (4) nine spp. mixture (three grasses, three legumes, three brassicas). Soil and plant samples were collected at the mid and late growing season to assess soil chemistry, plant biomass and composition, forage nutrition and quality, and soil bacterial and fungal communities. Overall, the oat monoculture had the highest biomass productivity, while the nine spp. mixture produced the lowest biomass among the treatments. All three mixtures had a better nutritional profile [i.e., greater concentrations of Ca, Cu, Fe, total Kjeldahl nitrogen (TKN), total phosphorus (TP) and total potassium (TK), and lower concentrations of acid detergent fiber (ADF) and neutral detergent fiber (NDF)] compared to the monoculture. Differences in forage nutrition were particularly heightened at the end of the growing season. Soil chemical properties did not differ greatly among the treatments with the exception of higher levels of soil nitrate availability in the mixtures compared to the monoculture. Early indicators of a shift in soil microbial diversity and fungal community composition, and an increased abundance of fungal pathotrophs in the mixtures compared to the oat monoculture, was observed at one of the field sites. This study indicates that increasing plant species diversity does not always lead to an increase in biomass production or significant changes or improvements in soil microbial communities. However, the inclusion of multiple plant species can improve the quality and nutritive value of forages over a monoculture forage crop.

Keywords: forage cover crop, soil chemistry, bacteria, fungi, forage nutrition, polyculture, microbial community

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INTRODUCTION

Forage cover crops can offer nutritional resources to support grazing livestock while also leaving plant material behind to serve as a mulch or soil amendment. Forage cover crops have been utilized worldwide in multiple cropping systems, especially on organic farms to optimize environmental resource availability and enhance sustainable agricultural practices (Bergtold et al., 2017). Annual forage cover crops can be monocultures of grasses, legumes, or brassicas, or mixtures of these crops, and can be included in rotation with other cash crops. Currently, forage cover crop designs that include multi-species mixtures are becoming increasingly popular for use in forage production due to their perceived benefits to productivity and soil properties. There have been numerous studies showing that increasing cover crop diversity can lead to optimized environmental resources use, increased soil nutrients, restricted disease and pest pressure, enhanced weed suppression, reduced soil erosion and water runoff, all leading to yield increases (Anil et al., 1998; Tilman et al., 2001; Hartwig and Ammon, 2002; Lithourgidis et al., 2011; Bonin and Tracy, 2012). However, information regarding the selection and diversity of suitable crop mixtures is still very limited.

It is important for forage cover crops to contain sufficient amounts of nutrients to support livestock growth, which can lead to reduced grain feeding and purchased supplemental feeds, and result in economic benefits to farmers (Gardner et al., 1991). Mixed forage cover crops can yield better quality forage as the use of plant species from various functional groups can improve the nutritive value of forage and reduce the need for additional mineral supplementation. More specifically, the inclusion of legumes in cover crop mixtures has been shown to produce higher levels of important macronutrients and some micronutrients to support livestock (Pirhofer-Walzl et al., 2011). Choosing effective cover crop mixtures is also critical to maximize the ecological benefits that cover crops can bring into agricultural systems (Wittwer et al., 2017). For example, including legumes into crop mixtures has been shown to increase soil nutrient (nitrogen, phosphorus and potassium) budgets (Malézieux et al., 2009; Dahmardeh et al., 2010). Including other crops such as grasses or brassicas, which have a high capacity for N and C storage, can prevent nutrient loss, water pollution and greenhouse gas emissions from agro-ecosystems (Dabney et al., 2001; Blanco-Canqui et al., 2015). In addition, increasing species richness within plant functional groups has shown positive impacts on forage biomass, which demonstrates that species within the same functional group may not be redundant in their functions (Marquard et al., 2009). However, there may be a limit to the number of plant species in a mixture that are actually beneficial to forage productivity, and some high species mixtures may produce less biomass than a mixture with fewer species (Schellenberg et al., 2012). The positive effects of diverse cover crops may not always be realized by farmers as many factors can compromise these benefits, such as ineffective or incompatible crop selection, soil types, climate conditions, fertilization, and tillage strategies (Gabriel and Quemada, 2011; Dorn et al., 2015; Wittwer et al., 2017).

Despite a considerable amount of cover crop research, there is a limited understanding of forage cover crop impacts on soil microbial communities, which are integral components of functional cropping systems. Previous studies have shown that increasing plant species richness can support more diverse and functional soil microbial communities compared to monocultures in both annual and perennial systems (Zak et al., 2003; Qiao et al., 2012; Lange et al., 2015; Finney et al., 2017). In agroecosystems, the inclusion of specific crop species and plant functional groups in cover crops appear to be important regulators of microbial community composition and soil biological activity (Finney et al., 2017; Venter et al., 2017; Cloutier et al., 2020). Due to the critical role that soil microbial communities play in various agroecosystem services it is important to better understand the impacts of multi-species forage cover crops on soil microbial communities.

To address the uncertainties on how to optimize mixed forage cover crops and to systematically evaluate a selection of mixed forage cover crops and their benefits, we hypothesized that forage biomass productivity, forage nutrition, and soil nutrients would increase with increased cover crop diversity. We also hypothesized that increased cover crop diversity would restructure soil microbial communities and increase bacterial and fungal diversity. To examine these hypotheses, we tested the following objectives: (1) determine the effects of increased cover crop diversity on overall forage productivity and nutritive value; and (2) examine changes to soil chemistry and soil microbial communities due to increased cover crop diversity.

MATERIALS AND METHODS

Site Description and Experimental Design

Experimental field trials were conducted at two different sites in the Canadian prairie region. A small plot (0.004 ha plot size) field trial was located on a Brown Chernozem soil zone at the Swift Current Research and Development Center, southeast of Swift Current, Saskatchewan, Canada (50°16′N lat., 107°43′W long.). This field was seeded to wheat in 2014 and chemical fallowed in 2015. A larger plot (∼0.16 ha plot size) field trial was located on a loamy Black Chernozem soil zone at the Brookdale Research Farm (managed by Manitoba Beef and Forage Initiatives Inc.), which was located 18 km north of Brandon, Manitoba, Canada (50°05′N lat., 99°92′W long.). The field at the Brookdale research farm was seeded to an oats/peas green feed mixture in 2014, and left fallow in 2015. Both sites were tilled and harrow packed prior to seeding.

To test the effect of crop species diversity, the experimental design included four different cropping mixture treatments: (i) monoculture, (ii) three spp. mixture, (iii) six spp. mixture, and (iv) nine spp. mixture. The experiment was conducted for two consecutive growing seasons in 2016 and 2017, with the same cropping treatments grown on the same plots each year. The monoculture was seeded to oats (*Avena sativa* "Common No. 1"). The three spp. mixture consisted of one grass species (oats), one legume species (forage peas, *Pisum sativum* "CDC Leroy"), and one brassica species [Graza forage radish (*Raphanus x Brassica,* Stewart and Moorhead, 2004)]. The six spp. mixture consisted of two grasses (oats and Italian ryegrass, *Festuca perrenis*), two legumes [forage peas and hairy vetch (*Vicia Villosa,* "Common No. 1")], and two brassica species [Graza and Winfred forage brassica (*Brassica napus* ssp.)]. The nine spp. mixture consisted of these same six crops, with one additional representative from each functional group: sorghum (*Sorghum bicolor*), crimson clover (*Trifolium incarnatum*), Hunter forage brassica (*Brassica* sp.). In 2017, Graza forage radish was replaced by Groundhog forage radish (*Raphanus sativus* sp. "Common No. 1"). All treatments were replicated four times in a randomized complete block design for a total of 16 plots per site. Plots at the Swift Current site were 2 by 10 m with crop row spacing of 30 cm. Plots at the Brookdale site were 12 by 120 m with a crop row spacing of 15 cm. Seeding rates at each location were determined based on recommended plants m^{-2} for these regions (i.e., ∼240 grass and 150 brassica and legume plants m^{-2}). The number of live seeds per crop species in the mixtures was calculated as a proportion of the total mix (e.g., three spp. mixture: oats = 1/3*240 live seeds m^{-2}, forage peas = 1/3*150 live seeds m^{-2}, forage radish = 1/3*150 live seeds m^{-2}). A seeding depth of 3 cm was selected as an intermediate depth suitable for all species. Plots were seeded on May 19, 2016 and May 25, 2017 at the Swift Current site, and June 3, 2016 and June 10, 2017 at the Brookdale site.

Sampling

Soil samples were collected at two different dates during the growing seasons in 2016 and 2017: (i) mid growing season in late July and (ii) late growing season in late August or early September. Soil cores, 15 cm deep and 2.5 cm diameter, were collected from six random locations within each plot. Soil cores from each plot were bulked together and homogenized in the field immediately after sampling to form one composite soil sample per plot. A 10 g soil sub-sample was immediately flash frozen in a liquid nitrogen cryo-shipper for molecular analyses. The remaining soil was stored in a cooler for transportation and then stored at 4°C for up to 1 week until further processing. Soil samples were sieved through a 2 mm sieve, and a subsample was used to determine soil moisture content (gravimetric). The remaining soil was air-dried and ground for chemical analyses. Plant biomass and plant nutrient samples were collected at the same dates and sampling points as the soil cores. Six 1 m rows of crop were cut and bulked together within each plot. Plant tissues were oven-dried and weighed for plant biomass.

Soil and Plant Tissue Analyses

Soil organic carbon was determined using the dry combustion method (after acidification with HCl) using an Elementar vario MICRO cube elemental analyzer (Schumacher, 2002). Soil nitrate nitrogen (NO_3-N) and phosphate phosphorus (PO_4-P) were determined using sodium bicarbonate extractions followed by colorimetric analysis using a Technicon Autoanalyzer (Harm et al., 1973; Gentry and Willis, 1988). Soil sulfate sulfur (SO_4-S) was determined using calcium chloride extractions followed by colorimetric analysis using a Technicon Autoanalyzer (Harm et al., 1973). Soil pH was measured in water saturation paste (Hendershot et al., 2008) and paste extracts (Miller and Curtin, 2008).

Dried plant samples were ground using a Wiley laboratory mill with a 1 mm screen. Tissues were analyzed for Ca, Cu, and Fe content using the digestion method (U. S. Environmental Protection Agency, 1996) and analysis was performed by ICP-OES (inductive coupled plasma-optical emission spectroscopy) on a Fisher Scientific iCAP6300 Duo. Plant total Kjeldahl nitrogen, total phosphorus (TP), and total potassium (TK) were determined using the Kjeldahl digest method. Plant acid detergent fiber (ADF) was measured using the procedure of Goering and Van Soest (1970) and the neutral detergent fiber (NDF) was determined using the ANKOM^{200} fiber analyser (Model 200; ANKOM; Fairport, New York).

Amplicon Sequencing and Bioinformatics

DNA was extracted from 0.25 g of soil in duplicate for each sample using the DNeasy PowerSoil kit (Qiagen, Hilden, Germany) in batches of 12 samples using an automated system (QIAcube, Qiagen). Extracted DNA was quantified using a Qubit dsDNA BR Assay Kit (Thermo Fisher, Waltham, MA). For amplicon sequencing, DNA extracts were shipped on dry ice to the Genome Quebec Innovation Center (Montreal, Canada) for amplicon library preparation and Illumina MiSeq sequencing. The bacterial 16S rRNA genes were sequenced using primers 515-F and 806-R (Caporaso et al., 2012). The fungal ITS1 region was sequenced using primers 5.8S-Fun and ITS4-Fun (Taylor et al., 2016) for 2016 samples and ITS1F and 58A2R (Martin and Rygiewicz, 2005) for 2017 samples. For full description of amplicon library preparation and Illumina Miseq sequencing see Delavaux et al. (2020). The raw amplicon sequencing dataset is available in the NCBI Sequence Read Archive under BioProject ID: PRJNA656804.

Raw paired reads were processed using the UPARSE pipeline and USEARCH v.9 (Edgar, 2013). Paired reads were merged using the fastq_mergepairs command with a maximum of five (i.e., default) mismatches in the alignment. Merged reads were quality filtered using the command fastq_filter that discarded all reads that were <200 bp and those with expected errors > 1. Sequences were dereplicated and the command cluster_otus was used to perform operational taxonomic unit (OTU) clustering (based on 97% similarity) and chimera filtering. Taxonomic identity was assigned using the RDP classifier (Wang et al., 2007) and 16S rRNA training set (version 16) for bacteria/archaea and ITS UNITE database for fungi (Kõljalg et al., 2013) in R (v.3.6.1). Before all analyses, we filtered out all unmatching domains (including only archaea and bacteria in bacterial analyses; only fungi in fungal analyses). OTU tables for each analysis were filtered to include OTUs with a minimum of three sequences. Finally, OTU tables for each analysis were normalized to the lowest number of sequences in a sample (12,898 reads for bacteria, 7,035 reads for fungi in 2016, and 12,173 reads for fungi in 2017) using rrarefy function from the R package vegan (Oksanen et al., 2013).

Statistical Analyses

All statistical analyses were performed in R Studio using R 3.6.1 (The R Foundation for Statistical Computing). All univariate analyses (i.e., forage biomass, forage nutrition, soil

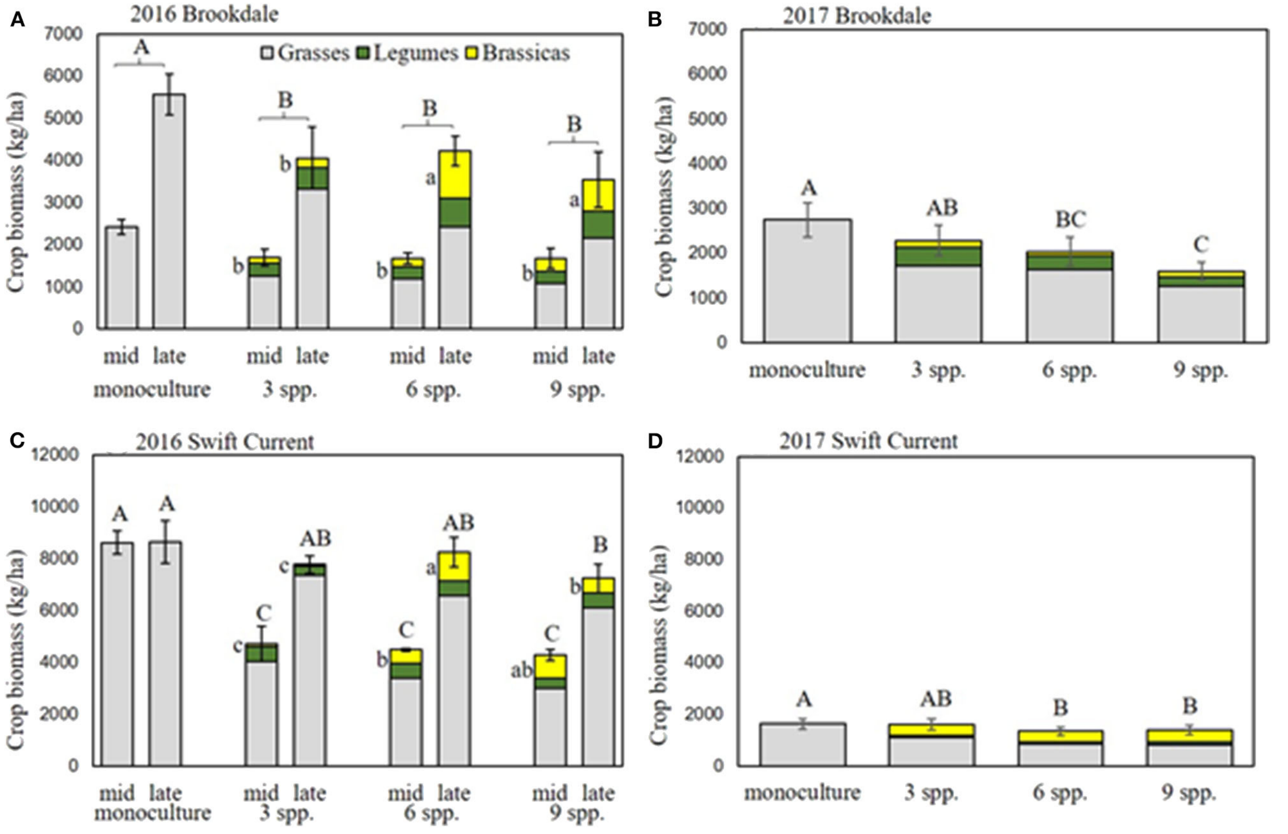

FIGURE 1 | Crop biomass production at Brookdale in **(A)** 2016 and **(B)** 2017 and at Swift Current in **(C)** 2016 and **(D)** 2017. Mid and late-season crop biomass are presented separately for eco-site years that had a significant mixture by date interaction (i.e., Brookdale 2016 for brassica biomass and Swift Current 2017 for total biomass). Bars with different capital letters are significantly different ($p < 0.05$) for total biomass, and lowercase letters are significantly different for brassica (yellow portion of bars) biomass.

chemistry, and microbial α-diversity and relative abundance) were conducted using a mixed model fit to test the fixed factors cropping mixture, sampling date and their interaction, and block as a random factor using the packages Lme4 (Bates et al., 2014) and lmerTest (Kuznetsova et al., 2017). Multiple means comparisons were made using the function difflsmeans. The effect of cropping mixture and sampling date on the bacterial and fungal community composition was tested using Permanova (adonis function in R using Bray-Curtis dissimilarity with 5,000 permutations) and visualized using principle coordinate analyses (PCoA). Each eco-site year (two sites × 2 years = four total eco-site years) was analyzed separately for statistical analyses.

RESULTS

Crop Biomass Production

The cropping mixtures had a significant effect on crop biomass production in 2016 and 2017 at the Brookdale field trial. The oat monoculture had the highest biomass production in both years and was significantly higher than all the mixtures in 2016 (**Figures 1A,B**, **Table 1**). There was no difference in the biomass production between the three mixtures, but in 2017 the three spp. mixture produced significantly higher biomass than the nine spp. mixture. In both years, the biomass significantly increased in all treatments from the mid-part of the growing season until the late growing season (**Table 1**). Brassicas were the only plant functional group that was affected by the cropping mixtures (**Table 1**). This was significant in 2016 where we observed higher brassica biomass production in the six spp. and nine spp. mixtures compared to the three spp. mixture at the end of the growing season (**Figure 1A**). This was primarily due to the poor establishment and production of the Graza forage radish in 2016, which was present in the three, six, and nine spp. mixtures. This variety was replaced with Groundhog forage radish in 2017 and there were no longer any differences in brassica biomass production between the mixtures.

At the Swift Current field trial, we observed a significant effect of the cropping mixtures and a cropping mixture by sampling date interaction in 2016 (**Figure 1C**, **Table 1**). The oat monoculture produced the highest biomass at the mid point of the growing season, but did not exhibit any further growth for the remainder of the season. In contrast, the mixtures increased their biomass by over 50% between the mid and late season sampling dates. As a result, there was no significant difference between the monoculture and the three and six spp. mixtures at the late season sampling date (**Figure 1C**). Similar to the Brookdale site, the brassica biomass production at Swift Current was significantly higher in the six and nine spp. mixtures compared to the three spp. mixture due to the poor growing Graza forage radish (**Figure 1C**). In 2017, all plots produced very low crop biomass at the Swift Current site, and the six and nine spp. mixtures produced significantly lower crop biomass compared to the oat monoculture (**Figure 1D**). The lower biomass production in Swift Current and in Brookdale to a lesser extent is likely due to the very low levels of precipitation and soil moisture content in 2017 compared to 2016 (**Supplementary Table 2**).

Forage Quality and Nutrition

The cropping mixtures had a significant effect on different measures of forage quality and nutrition at both field sites and years. Ca, Cu, TKN, and NDF were all significantly affected by the cropping mixtures in at least two of the four eco-site years (**Table 2**). Across all eco-site years the Ca, Cu and TKN were all higher and NDF lower in all three mixtures compared to the oat monoculture (**Supplementary Table 1**). Fe, TP, TK and ADF were significantly affected by the cropping mixtures at the Swift Current site in 2017. The three mixtures had higher concentrations of Fe, TP and TK and lower ADF compared to the oat monoculture. Sampling date was also a critical factor for forage quality and nutrition as TKN, TP, TK, ADF and NDF were all significantly affected and nutritional quality deteriorated at the later sampling date at all eco-site years (**Table 2**, **Supplementary Table 1**).

TABLE 1 | ANOVA results of the effect of cropping mixtures (monoculture, three, six, and nine spp.) and date of sampling (mid or late growing season) on total aboveground crop biomass production and of each functional group (grasses, legumes, brassicas) at the Swift Current and Brookdale research sites in 2016 and 2017.

Location	Year	Factors	Total Biomass	Grasses	Legumes	Brassicas
Brookdale	2016	Mixture	*	ns	ns	**
		Date	***	***	***	**
		Mixture: Date	ns	ns	ns	*
	2017	Mixture	**	ns	ns	ns
		Date	***	***	*	ns
		Mixture: Date	ns	ns	ns	ns
Swift Current	2016	Mixture	***	*	ns	**
		Date	***	***	ns	ns
		Mixture: Date	**	ns	ns	*
	2017	Mixture	*	**	ns	ns
		Date	***	***	ns	***
		Mixture: Date	ns	ns	ns	ns

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns, not significant ($P > 0.05$).

Soil Chemical Properties

The cropping mixtures had a minor impact on the soil chemical properties at both sites. Nitrate (NO_3-N) levels were significantly affected by the cropping mixtures in 2017 at the Brookdale site and in both years at the Swift Current site (**Table 3**). The only difference at the Brookdale site was an increase in soil nitrate levels under the six spp. mixture at the late sampling date compared to the other treatments (**Supplementary Table 2**). Nitrate levels were also strongly affected by sampling date at all four eco-site years. At the Swift Current site we observed higher levels of nitrate in the three spp. mixture in 2016 and in the nine spp. mixture in 2017. Phosphate (PO_4-P) and organic carbon were unaffected by the cropping mixtures, but did significantly change between the sampling dates in 2017 at both sites. Soil pH and soil moisture were only significantly affected by the cropping mixtures at one of the four eco-site years (pH: Brookdale in 2017, soil moisture: Swift Current in 2017). For both years in Swift Current, soil moisture significantly decreased from the mid to late sampling period.

Microbial Community

The cropping mixtures and sampling date had no significant effect on bacterial or fungal alpha-diversity (richness and inverse Simpson's index) at the Brookdale site in either 2016 or 2017,

TABLE 2 | ANOVA results of the effect of cropping mixtures (monoculture, three, six, and nine spp.) and date of sampling (mid or late growing season) on forage quality and nutrition at the Swift Current and Brookdale research sites in 2016 and 2017.

Location	Year	Factors	Ca	Cu	Fe	TKN	TP	TK	ADF	NDF
Brookdale	2016	Mixture	*	*	ns	ns	ns	ns	ns	**
		Date	*	*	ns	***	***	***	***	***
		Mixture: Date	ns	ns	ns	ns	ns	ns	ns	ns
	2017	Mixture	***	ns	ns	*	ns	ns	ns	ns
		Date	***	ns	*	***	***	***	***	***
		Mixture: Date	**	ns	ns	ns	ns	ns	ns	ns
Swift Current	2016	Mixture	ns	ns	ns	*	ns	ns	ns	ns
	2017	Mixture	***	**	*	***	*	**	**	***
		Date	ns	ns	***	**	***	***	**	**
		Mixture: Date	ns	ns	ns	ns	ns	***	ns	ns

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns, not significant ($P > 0.05$).

TKN, total Kjedahl nitrogen; TP, total phosphorus; TK, total potassium; ADF, acid detergent fiber; NDF, neutral detergent fiber.

TABLE 3 | ANOVA results of the effect of cropping mixtures (monoculture, three, six, and nine spp.) and date of sampling (mid or late growing season) on soil properties at the Swift Current and Brookdale research sites in 2016 and 2017.

Location	Year	Factors	NO_3-N	PO_4-P	Organic C	pH	Soil moisture
Brookdale	2016	Mixture	ns	ns	ns	ns	ns
		Date	***	ns	ns	*	ns
		Mixture: Date	ns	ns	ns	ns	ns
	2017	Mixture	ns	ns	ns	***	ns
		Date	***	*	*	ns	ns
		Mixture: Date	*	ns	ns	ns	ns
Swift Current	2016	Mixture	*	ns	ns	ns	ns
		Date	***	ns	ns	***	***
		Mixture: Date	ns	ns	ns	ns	ns
	2017	Mixture	*	ns	ns	ns	*
		Date	**	***	**	ns	***
		Mixture: Date	*	ns	ns	ns	ns

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns, not significant ($P > 0.05$).
SO_4-S, sulfate sulfur; NO_3-N, nitrate nitrogen; PO_4-P, phosphate phosphorus.

TABLE 4 | ANOVA results of the effect of cropping mixtures (monoculture, three, six, and nine spp.) and date of sampling (mid or late growing season) on bacterial and fungal α-diversity at the Swift Current and Brookdale research sites in 2016 and 2017.

Location	Year	Factors	Bacteria			Fungi		
			Observed	Chao	1/D	Observed	Chao	1/D
Brookdale	2016	Mixture	ns	ns	ns	ns	ns	ns
		Date	ns	ns	ns	ns	ns	**
		Mixture: Date	ns	ns	ns	ns	ns	ns
	2017	Mixture	ns	ns	ns	ns	ns	ns
		Date	ns	ns	ns	ns	ns	ns
		Mixture: Date	ns	ns	ns	ns	ns	ns
Swift Current	2016	Mixture	ns	ns	ns	ns	ns	*
		Date	ns	ns	ns	ns	ns	ns
		Mixture: Date	ns	ns	ns	ns	ns	ns
	2017	Mixture	*	ns	ns	ns	ns	ns
		Date	**	**	**	***	***	ns
		Mixture:Date	ns	ns	ns	ns	ns	**

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns, not significant ($P > 0.05$).

except for a decrease in fungal diversity (inverse Simpson's index) in 2016 (**Table 4**, **Figure 2**). At the Swift Current site, fungal alpha-diversity was affected by the cropping mixtures in 2016, with the oat monoculture exhibiting a higher inverse Simpson's index compared to the three mixtures (**Table 4**, **Figure 2**). In 2017, the bacterial and fungal diversity were both significantly affected by the cropping mixtures and sampling date (**Table 4**). Bacterial richness was significantly higher in the three and six spp. mixtures compared to the oat monoculture in 2017 (**Figure 2**). We detected a significant cropping mixture by sampling date interaction for fungal diversity, which is explained by the higher inverse Simpson's index at the late sampling date compared to the mixtures, but no difference at the mid sampling point (**Figure 2**). The sampling date effect was due to a decrease in bacterial diversity (richness and inverse Simpson's index) and increase in fungal diversity (inverse Simpson's index) between the mid to late sampling dates (**Figure 2**).

The cropping mixtures had very little impact on the composition and structure of the soil bacterial community at either field site. There was no significant effect on the composition of the total bacterial community (**Table 5**) or the composition of the top eight most abundant bacterial phyla (**Supplementary Table 3**) at either the Brookdale or Swift Current sites. In addition, the cropping mixtures had no effect on the relative abundance of these bacterial phyla except the Gemmatimonadetes and Verrucomicrobia at one of the eco-site years (i.e., Swift Current 2016; **Supplementary Table 5**). Sampling date was a significant factor explaining variation in the shift in bacterial communities between the mid to late sampling periods at all eco-site years except Brookdale in 2016 (**Table 5**). This effect is primarily explained by a shift in composition of taxa belonging to the Proteobacteria because it was the only phyla that had a significant sampling date effect at all sites (**Supplementary Table 3**) and it was one of the two most abundant phyla at all eco-site years (**Supplementary Table 5**).

The fungal community was not significantly affected by the cropping mixtures at three of the eco-site years (**Table 5**). However, the cropping mixtures did explain a significant proportion of the variation in the fungal community at the Swift Current site in 2016. The principle coordinate analysis (PCoA) revealed that the cropping mixture effect was the most evident at the late sampling date with the oat monoculture and nine spp. mixture being the most dissimilar (**Figure 3A**). Further analysis revealed that the variation between cropping mixtures at this sampling date was due to a shift in taxa belonging to the Ascomycota (**Figure 3B**; **Supplementary Table 4**). Although the total fungal community was not affected by the cropping mixtures at the Swift Current site in 2017, the composition of the Mortierellomycota were significantly affected (**Supplementary Table 4**). The cropping mixture effect was most evident at the late sampling date with the six spp. mixture being the most dissimilar from the other treatments (**Figure 3C**). Furthermore, when assessing the relative abundance of fungal taxa based on functional guilds, we found that the oat monoculture had a significantly lower abundance of pathotrophs compared to at least two of the mixtures at the Brookdale site in 2017 and Swift Current site in 2016 (**Figure 3**; **Supplementary Table 7**). Sampling date was a significant factor explaining a shift in the fungal community at three of the four eco-sites years (**Table 5**). This effect is due to a shift in the composition and relative abundance of taxa belonging to the

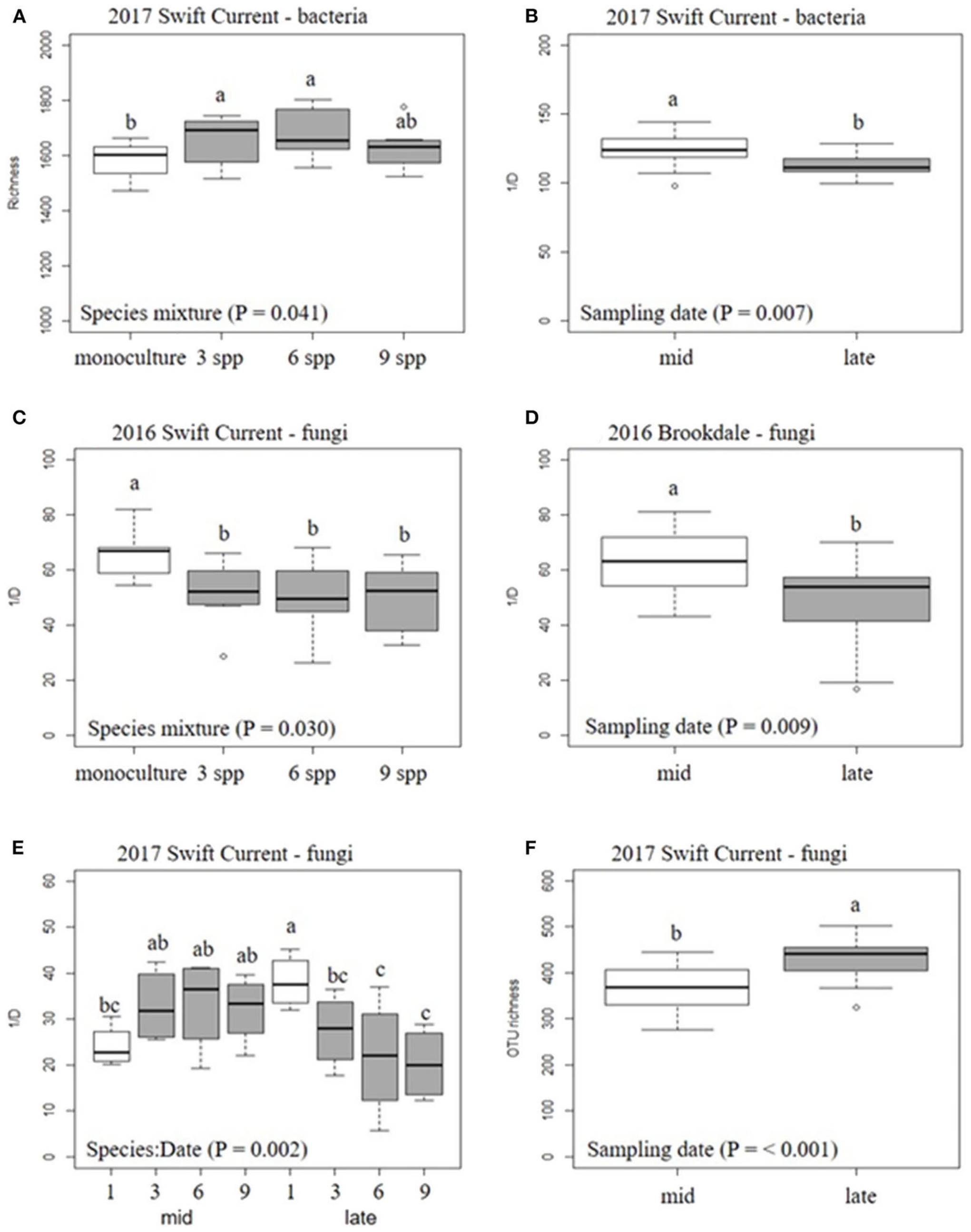

FIGURE 2 | Boxplots of the effect of cropping mixtures (monoculture, three, six, and nine spp.) and date of sampling (mid or late growing season) on bacterial **(A,B)** and fungal **(C–F)** α-diversity at the Swift Current and Brookdale research sites. Bars with different letters are significantly different ($p < 0.05$).

Ascomycota and Basidiomycota to a lesser extent, between the mid to late sampling dates (**Supplementary Tables 4**, **6**).

DISCUSSION

High Cover Crop Diversity in Mixtures Did Not Increase Forage Biomass

We found that the oat monoculture had significantly higher crop biomass than the diverse annual forage cover crops we tested at both experimental sites in 2016 and 2017, indicating that an increase in diversity does not necessarily lead to an increase in annual crop biomass. Similar studies have found that crop biomass was higher in monocultures and pure stands of a forage crop than in polycultures and cropping mixtures, and is suggested to be a result of plant competitiveness (Assefa and Ledin, 2001; Griffith et al., 2011; Sadeghpour et al., 2013). As oats are tall plants with rapid early-season growth, it could have caused interspecific competition for light and supressed the growth of the slower growing and shorter species of legumes and brassicas in the mixtures. Likewise, the growth of oats in the mixtures may have also been affected by competition from the other crops in the mixtures. In our study, the nine species cropping mixtures produced the lowest crop biomass at both experimental sites in 2016 and 2017, which could have been due to high interspecific competition in the mixtures that limited plant growth and thus reduced total forage biomass. Lithourgidis et al. (2011) also reported a reduced growth rate and lower dry biomass of cereals when intercropped with peas compared to cereals in monoculture due to interspecific competition. In addition, some of the individual crops had relatively low productivity in the mixtures (data not shown) which indicates that of the total amount of crop that was seeded, not all crops grew equally well. Therefore, selecting proper cover crop species to reduce interspecific competition in mixtures could be beneficial in increasing overall forage crop biomass.

Diversified Cover Crop Mixtures Increased Forage Quality and Nutrition

Although the total biomass of annual forage cover crops did not increase with increasing crop diversity in this study, we did find that mixtures can have beneficial effects for improving forage nutrition. In general, significantly higher levels of Ca, Cu, Fe, TP, TK, and TKN in the mixtures were found compared to the oat monoculture, especially at the Swift Current site in 2017. These results indicate that using forage cover crop mixtures of grasses, legumes and brassicas can significantly increase the nutritive value of forage compared to a grass monoculture. There are several reasons why mixtures can have better nutritive values than monocultures, related to the specific functional groups and species selected. For example, legumes are known to be high in nitrogen, which is a direct measure of crude protein and a key factor in forage nutrition (e.g., Schultz and Stubbendieck, 1983). Brassicas are also high in nitrogen and various micronutrients such as calcium (de Ruiter et al., 2009). Other studies have indicated that crop mixtures are beneficial for increasing forage nutrition such as P, Mg, K, S, and Zn (Assefa and Ledin, 2001; Pirhofer-Walzl et al., 2011), which can be attributed to the ability of different plant functional groups to uptake different minerals (Zhang et al., 2017). Including legumes in forage crop mixtures can boost biological nitrogen fixation in the system, which can further increase other soil nutrient availability such as available P (Dahmardeh et al., 2010). Brassica crops are good soil N and C sinks which can also increase soil nutrient use efficiency, while reducing the runoff and soil water loss when applied in forage cover crop mixtures (Blanco-Canqui et al., 2015). In this study, the selection of cover crops from three different functional groups (grasses, legumes, and brassicas) allowed diversified plants species to uptake nutrients from the soil both temporally and at different rates.

In regards to forage digestibility, NDF content was much lower in mixed forage cover crops than the oat monoculture, with two of the four eco-site years having significantly lower NDF. Lower levels of NDF are generally preferred, as NDF will impact the animal's intake of dry matter and the time of rumination, thus the concentration of NDF is usually negatively related to energy uptake. It is well-known that the inclusion of legumes at higher ratios in grass-legume mixtures will exhibit lower NDF values compared to cereal monocultures (Caballero et al., 1995; Ghanbari-Bonjar and Lee, 2003). Similarly, lower levels of ADF are generally indicators of higher quality forage, and lower levels of ADF were found in the mixtures at the Swift Current site in 2017. As NDF and ADF are widely used as good indicators of forage quality, our results demonstrated that mixing legume and brassica cover crops with grasses can be a good way to increase forage digestibility compared to an oat monoculture.

TABLE 5 | Permanova results of the effect of crop species mixture (monoculture, three, six, and nine spp.) and sampling date (mid or late growing season) on the bacterial and fungal community composition at the Brookdale and Swift Current research sites in 2016 and 2017.

Location	Year	Factors	Bacteria		Fungi	
			R^2	Pr(<F)	R^2	Pr(<F)
Brookdale	2016	Mixture	0.062	ns	0.090	ns
		Date	0.099	ns	0.185	***
		Mixture:Date	0.024	ns	0.050	ns
	2017	Mixture	0.012	ns	0.093	ns
		Date	0.186	***	0.025	ns
		Mixture:Date	0.049	ns	0.096	ns
Swift Current	2016	Mixture	0.067	ns	0.156	*
		Date	0.152	***	0.204	***
		Mixture:Date	0.061	ns	0.108	ns
	2017	Mixture	0.095	ns	0.083	ns
		Date	0.214	***	0.069	**
		Mixture:Date	0.061	ns	0.113	ns

$^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns, not significant ($P > 0.05$).

Significant differences were detected in forage quality and nutrition between sampling dates, as we found forage nutrition decreased in the late-season samples at both experimental sites in 2016 and 2017. The significant increase in NDF content in late-season samples is attributed to changes in the morphological growth of plants as they mature. For example, Assefa and Ledin (2001) observed that as the leaf : stem ratio of oats decreased during maturation, the NDF content of forage mixtures increased. The overall improvement of forage quality in mixtures vs. the cereal monoculture was particularly evident in Swift Current during the very dry conditions of 2017, which might indicate that annual forage mixtures might be a useful crop management option to provide higher quality forage under stressful growing conditions. In addition, the improved forage quality in mixtures could also lead to greater potential market value and improve animal performance in beef, dairy, and sheep production systems (Buza et al., 2014; McKenzie et al., 2017; Fruet et al., 2019; Tufail et al., 2020).

Variation of Soil Properties Under Mixed Cover Crops

Soil nitrate levels were significantly higher in the cropping mixtures than in the oat monoculture in 2017 at the Brookdale

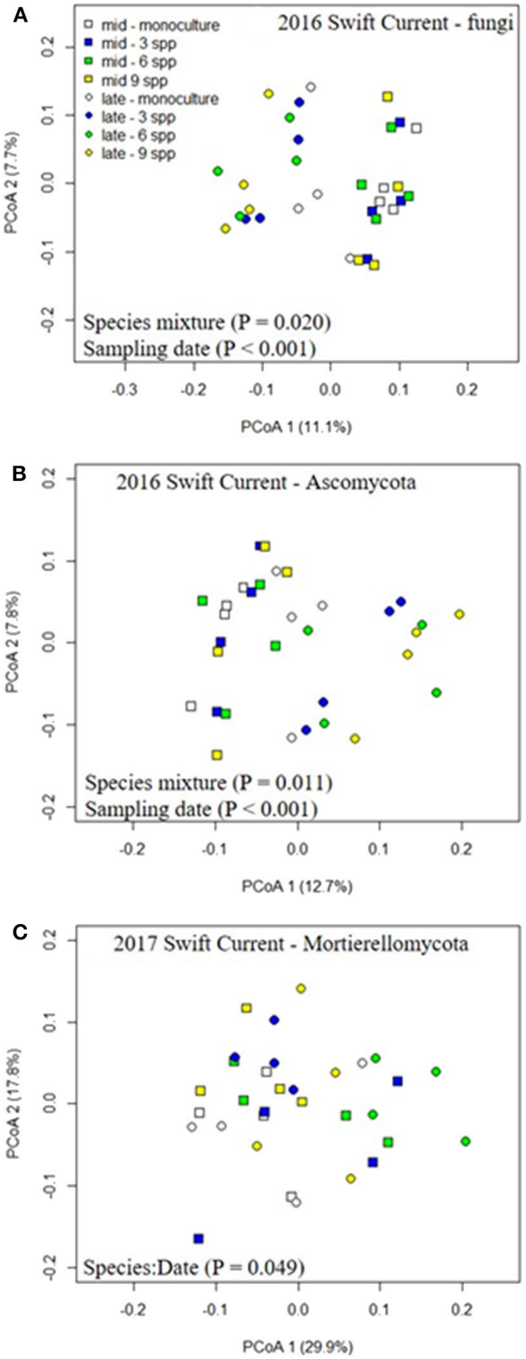

FIGURE 3 | Principle coordinate analysis (PCoA) demonstrating the effects of cropping mixtures (monoculture, three, six, and nine spp.) and date of sampling (mid or late growing season) on the community composition of the **(A)** fungi, **(B)** ascomycota, and **(C)** mortierellomycota at the Swift Current research site in 2016 **(A,B)** and 2017 **(C)**.

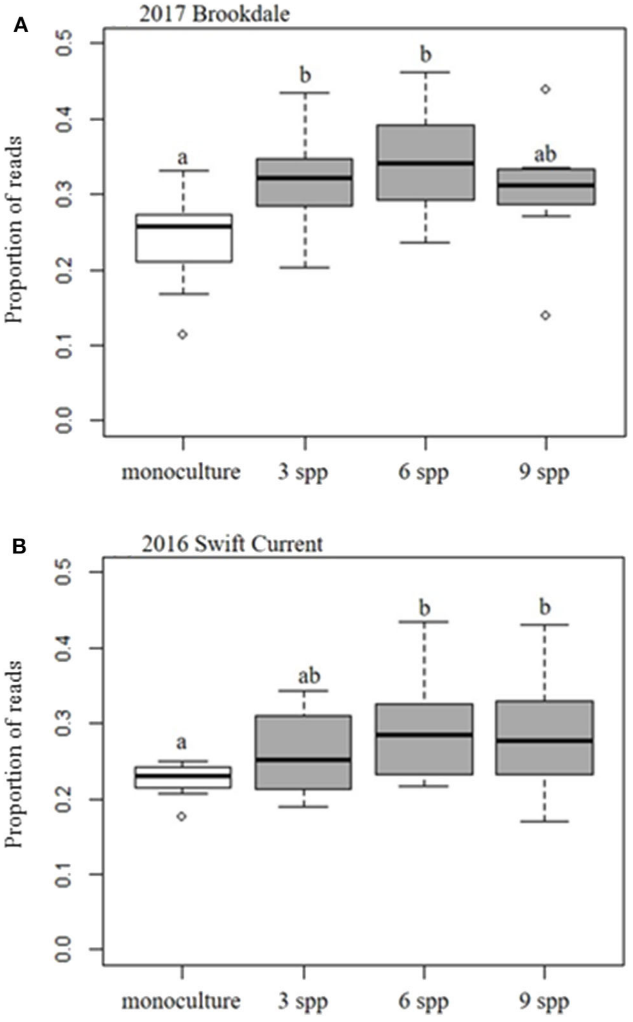

FIGURE 4 | Boxplots of the effect of cropping mixtures (monoculture, three, six, and nine spp.) on the relative abundance (proportion) of fungal pathotrophs at the **(A)** Brookdale site in 2017 and **(B)** Swift Current site in 2016. Bars with different letters are significantly different ($p < 0.05$).

site and in both years at the Swift Current site. Adding nitrogen-fixing legume species to a cropping mixture can affect total N levels within the soil during the growing season, increasing the amount of available N in soil, which is beneficial for other non-legume crops applied in mixtures (Dybzinski et al., 2008; Malézieux et al., 2009; Dahmardeh et al., 2010). Previous studies have shown changes in soil chemical properties with an increase in plant species (Dahmardeh et al., 2010; Zhang et al., 2016), although it may take several years to show effects (van Ruijven and Berendse, 2005; Dybzinski et al., 2008). This is especially true for changes in soil C in the Canadian prairies, due to the cool climate and decomposition rates (VandenBygaart et al., 2008). For example, Blanco-Canqui et al. (2013) found that including triticale and legume cover crops in cereal based monocultures increased soil N, P, and organic C content after 5 years of cropping. Thus, significant changes in soil properties other than N levels, such as soil C and available P, may not have been observed in our study due to the shorter study period.

We detected significant changes in soil N and P between sampling dates, indicating that soil properties can change significantly within a growing season. Many N-based soil nutrients, such as ammonium, nitrate, potentially mineralizable N, and urease activity, show seasonal changes due to changes in soil water content (López-Poma et al., 2020) and crop uptake, suggesting seasonal changes of soil chemical properties may be related to the variation in soil moisture and crop phenology. Soil moisture is the driver of many soil functions especially in arid and semi-arid regions, such as earthworm activity and the soil functional microbial community, all of which relates to soil nutrient cycles (He et al., 1997; Cavagnaro, 2016; Kooch et al., 2020). In our study, soil moisture at both the Brookdale site and Swift Current site decreased significantly in 2016 and 2017 from the mid to late sampling dates, therefore the observed change in soil nutrients may be linked to variation in soil moisture.

Response of Soil Microbial Communities to Cover Crop Mixtures

We identified minor shifts in the composition of the soil microbial communities among the forage cover crop mixtures and oat monoculture, and early indicators of changes to the microbial diversity. Previous research has shown that increasing plant diversity in polycultures can increase microbial diversity (Qiao et al., 2012; LeBlanc et al., 2015). In this study we found mixed results. At the Brookdale site we found no effect of increasing cover crop diversity on microbial diversity or community composition. This is similar to a study by Dassen et al. (2017), who found that the inclusion of specific plant functional groups and soil abiotic properties were more important than plant species richness. We included equal proportions of each plant functional group (e.g., grass, legume, brassica) in all of our mixtures and that may explain why we didn't observe differences in soil microbial diversity among the forage cover crop mixtures. This doesn't explain the lack of differences between the mixtures and the oat monoculture, which may instead be linked to the minimal changes in soil abiotic properties at Brookdale. Malý et al. (2000) also observed no significant effects of plant species composition or diversity on soil microbial properties and speculated that the length of their study (2 years) was not long enough to detect differences. A grassland plant diversity study supports this hypothesis as they were only able to detect significant effects of plant diversity on soil microbial communities after 4 years, suggesting a lag time for soil microbial responses to changes in aboveground vegetation (Habekost et al., 2008). The short time frame of our study (2 years) may partially explain the lack of changes we observed in the microbial community at Brookdale.

In contrast to the Brookdale site, at the Swift Current site we observed differences in the soil microbial diversity and composition among the crop treatments in both years. This included an increase in bacterial diversity in the mixtures (three and six spp.) compared to the oat monoculture in 2017, and decrease in fungal diversity in all three mixtures compared to the oat monoculture in both years. The decrease in fungal diversity in the mixtures could be linked to differences in plant biomass production and soil nitrate levels compared to the oat monoculture. A grassland biodiversity experiment revealed a strong link between plant productivity and subsequent resource availability and fungal diversity (Zak et al., 2003; Waldrop et al., 2006). In our study, the oat monoculture had higher aboveground plant biomass production than the mixtures (although not significant at all sampling dates) and likely had higher belowground production as well due to the larger rooting capacity of grasses compared to leguminous and some brassica crops (Liu et al., 2011), which may have provided greater resource availability for the fungal community. Swift Current was also the site where we observed the most differences in soil properties among the cropping treatments, including increased soil nitrate levels in the mixtures during both years. Detheridge et al. (2016) found that soil nitrate levels were one of the key factors driving shifts in soil fungal diversity in forage cropping systems. An alternate explanation for the decreased fungal diversity in the mixtures may be linked to the inclusion of leguminous forage crop species. For example, Bainard et al. (2017) found that increasing the frequency of leguminous crops in annual crop rotations led to a decrease in fungal diversity, particularly in comparison to wheat monocultures.

We also observed temporal shifts in the composition of the bacterial and fungal communities between the mid and late season sampling dates for three of the four eco-site years. Temporal shifts in bacterial and fungal communities within an agricultural growing season has been consistently reported (Gomes et al., 2001; Smalla et al., 2001; Habekost et al., 2008). Within a regional context, soil microbial community composition and activity in the Canadian prairies has been correlated with seasonal shifts in soil moisture and nutrients (Hamel et al., 2006; Bainard et al., 2016). This was particularly evident at the Swift Current site, where we detected a significant drop in soil moisture and nitrate levels between the sampling dates in both years, and corresponding shifts in the bacterial and fungal communities.

Interestingly, we found that the oat monoculture had a significantly lower abundance of fungal pathotrophs compared to the cover crop mixtures at the Brookdale site in 2017 and the Swift Current site in 2016. This contradicts previous studies that have reported higher disease suppression in intercropping systems (Boudreau, 2013). This may be due to the selection of crop species or functional groups that were included in our mixtures. Although we did not specifically assess the crops for disease symptoms, the relative abundance of fungal pathotrophs provides a potential indicator that these type of forage mixtures may not necessarily provide a break in the disease cycle for susceptible crops that follow in the rotation. This highlights the need for producers to focus on crop selection rather than the level of plant diversity in forage cover crop mixtures in order to reduce the potential carry-over or build-up of soil pathogens (Panth et al., 2020).

CONCLUSION

Given the many suggested benefits of diverse annual forage cover crops, it is important to quantitatively evaluate the impact of these cropping mixtures. Over 2 years and two research sites on the Canadian prairies, we found minimal impact of increased forage crop diversity on forage productivity, soil nutrients and soil microbial communities. However, diverse mixtures did have better forage nutrition compared to an oats monoculture, indicating that it is possible to improve forage quality of a crop, while not sacrificing productivity or negatively impacting the soil community. While the mixtures performed better than the monoculture, there wasn't any strong evidence to show that increasing species diversity improved forage nutrition, suggesting that the inclusion of different functional groups is the most important consideration. Within the functional groups tested here, producers should select specific species based on local growing conditions and plant-pathogen dynamics.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

LB and JB contributed to all aspects of the work. BE contributed to the field and laboratory work and writing of the manuscript. EM and TY contributed to the writing of the manuscript. All authors approved the manuscript for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2020.560479/full#supplementary-material

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Phosphate Fertilizer in Soybean-Wheat Cropping System Under No-Till Management

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Improvements in soybean (*Glycine max* [L.] Merrill) and wheat (*Triticum aestivum* L.) yields in cropping systems under no-till management (NTM) in the subtropics have been obtained through advances in phosphorus (P) utilization, cultivar selection, and planting and harvesting strategies. This fact, along with the P application in band application in consolidated planting, has resulted in adequate P utilization efficiency and small depleted soil P levels. The aim of this study was to evaluate the efficiency of P_2O_5 rates and application methods in soybean-wheat cropping under NTM during two growing seasons (2014–2016) in a Typical Eutrorthox. Four P_2O_5 rates (0, 30, 60, and 120 kg ha^{-1}) of triple superphosphate (45% P_2O_5) were applied using two application methods (broadcast and band application at sowing) before the soybean and wheat crops were cultivated. Both P application methods resulted in a significant yield response to P_2O_5 rates for the soybean and wheat crops. Under NTM, broadcast P_2O_5 application was more effective than band application in soybean, and maximum grain yields were obtained with 129.3 and 88.1 kg P_2O_5 ha^{-1}, respectively. Maximum wheat grain yields were obtained with 91.8 and 99.7 kg P_2O_5 ha^{-1} for broadcast and band application, respectively. Except for total P in leaves, nutrient uptake and yield components were not affected by the P application methods and rates. The results suggested that in soils with adequate available P levels of >15 mg kg^{-1} (Mehlich 1 extractant), the grain yield is equivalent or superior with broadcast P application as compared with band application for both soybean and wheat crops when cultivated in a Typical Eutrorthox under subtropical conditions. However, broadcast application is considered more effective for large areas of grain cultivation, where it is necessary to sow crops uniformly within the shortest possible time.

Keywords: ***Glycine max*, *Triticum aestivum*, broadcast application, band application, nutritional state of plants, subtropical conditions, Typical Eutrorthox**

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HIGHLIGHTS

- Phosphorus (P) application increased soybean and wheat grain yield independently of application method.
- Broadcast P_2O_5 application in a consolidated no-till management was efficient in making P available to plants.

- The P_2O_5 application methods (broadcast and band application) did not influence the nutritional status of soybean and wheat plants.
- Our study provides a comprehensive survey of the response of application type (broadcast and band application) and P_2O_5 rates in soybean-wheat cropping system in no-till management and provides novel insights to improve the sustainability and quality of P (P_2O_5) fertilization under subtropical conditions.

INTRODUCTION

Most soils in Brazil are deficient in phosphorus (P). Associated crop plant P deficiencies are more evident in grain crops, and result in reduced plant size, low pod insertion height, and small ears (Sfredo, 2008). In no-till management (NTM), crop rotation and/or cropping system in tropical and subtropical climates became viable only with the development of technologies to increase the use efficiency of fertilizers and correctives in different soil types in these regions. In these places, the use of large amounts of fertilizer, combined with the absence of abundant reserves of phosphate rocks of sedimentary origin and the need to maximize the time between one cultivation and the next, justifies studies to investigate optimization of phosphate fertilizer use efficiency in agriculture.

Crop rotation is feasible for several crops as it provides continuous soil protection, a good amount of soil organic matter (SOM), and nutrient accumulation which are of fundamental importance for maintaining high yields over the years. No-till management involves the implantation of crops in unturned soil and their protection by cover with straw residues. In NTM, the soil is not de-structured; however, this approach is restricted to band application (Fidelis et al., 2003).

The management of phosphate fertilizer influences the availability of nutrients in the soil and in the plants (Hansel, 2019). The application method can change the speed and capacity of the fertilizer to react in the soil, which in turn can change the solubilization and P availability for the plant, influencing the use efficiency (Lacerda et al., 2015). Fertilizer application must be planned from a long-term perspective, since the cost of fertilization and the responses in productivity are subject to many uncertainties and may vary from year to year (Fixen and Halvorson, 1991; Resende et al., 2006). Among the systems adopted, there are controversies about the ways of supplying the soil with adequate P levels since soluble phosphates, while promptly making P available, have shown good results in different application forms (Borges and Mallarino, 2003).

The use of soybean (*Glycine max* [L.] Merril) and wheat (*Triticum aestivum* L.) in a single cropping system is common in southern Brazil. Both crops benefit from this management system: the higher soil fertility requirement for wheat cultivation results in greater soil nutrient uptake in the straw, mainly P, and contributes to a reduction of fertilization needed for soybean crops, while wheat and other cereals benefit from the provision of significant amounts of N, P, and K left by soybean crop residues (Stainer et al., 2000; Rossi et al., 2013).

There are controversies about the efficiency of P_2O_5 application in relation to the method used (broadcast or band application) and the appropriate rate. Therefore, the objective of this study was to determine if the method and rate of P_2O_5 application has an influence on soil fertility, grain yield (GY), yield components, and nutritional status of soybean and wheat crops grown under subtropical conditions in a Kaolinitic Typical Eutrorthox.

MATERIALS AND METHODS

Site and Experimental Design

The experiment was carried out in the field under rainfed conditions in an NTM (operational for 10 years). The study was conducted across two growing seasons (2014–2015 and 2015–2016) in the same field rotation located in Londrina, Paraná State, in southern Brazil (23° 23′ 30″ LS and 51° 11′ 05″ LW) (**Figure 1**). The soil, classified as a loamy (710 g kg^{-1} clay) Kaolinitic Typical Eutrorthox (USDA, soil taxonomy classification), had the following chemical properties (determined at a depth of 0–0.1 m) prior to soybean cultivation: pH ($CaCl_2$) = 4.9, soil organic matter (SOM) = 31.3 g kg^{-1}, P (Mehlich 1 extractant) = 3.7 mg kg^{-1}, P (Resin extractant) = 9.1 mg kg^{-1}, potassium (K^+) (Mehlich 1) = 0.7 $cmol_c$ kg^{-1}, calcium (Ca^{2+}) (KCl, 1.0 mol L^{-1}) = 5.1 $cmol_c$ kg^{-1}, magnesium (Mg^{2+}) (KCl, 1.0 mol L^{-1}) = 1.9 $cmol_c$ kg^{-1}, sulfur (S-SO_4^{2-}) = 79.1 mg kg^{-1}, aluminum (Al^{3+}) = 0.1 $cmol_c$ ‘kg^{-1}, potential acidity (H+Al) = 3.8 $cmol_c$ kg^{-1}, cation exchange capacity (CEC) = 11.5 $cmol_c$ kg^{-1}, base saturation (V) = 67%, boron (B) available = 0.6 mg kg^{-1}, copper (Cu) available (DTPA-TEA) = 18.3 mg kg^{-1}, iron (Fe) available (DTPA-TEA) = 101.6 mg kg^{-1}, manganese (Mn) available (DTPA-TEA) = 173.8 mg kg^{-1}, and zinc (Zn) available (DTPA-TEA) = 7.9 mg kg^{-1}. The region has a humid subtropical climate (Köppen classification: Cfa) and sequential water balance and temperature (**Figure 2**) in the soybean and wheat crop seasons (2014–2016) according to Thornthwaite and Mather (1955).

The experiment was set up as a randomized block design in a 2 × 4 factorial scheme [two application methods (broadcast, total area of plot, and band application together with the seed) and four P_2O_5 rates (0, 30, 60, and 120 kg ha^{-1}, equivalent to 0, 13.1, 26.2, and 52.4 kg ha^{-1} of P)] in a split-plot arrangement (growing season), with four replicates. For the first cultivation, the soybean cv. ‘M5947 IPRO’ crop was grown in plots of 4.0 × 8.0 m with a row spacing of 0.5 m. After each soybean crop, wheat cv. ‘Pardela’ was cultivated in succession with a spacing of 0.175 m to quantify the residual effects of phosphate application to the soybean crop. Phytosanitary treatments and weed control of the soybean and wheat crops were carried out according to the recommendations described in TPS (2013) and ITTT (2011).

The micronutrients (B, Cu, Fe, Mn, and Zn) were applied in the form of salts mixed with 2.0 Mg ha^{-1} of gypsum ($CaSO_4 \cdot 2H_2O$). In the second cultivation, fertilization of the soybean crop was based on soil chemical analysis sampled after the first wheat crop and carried out according to

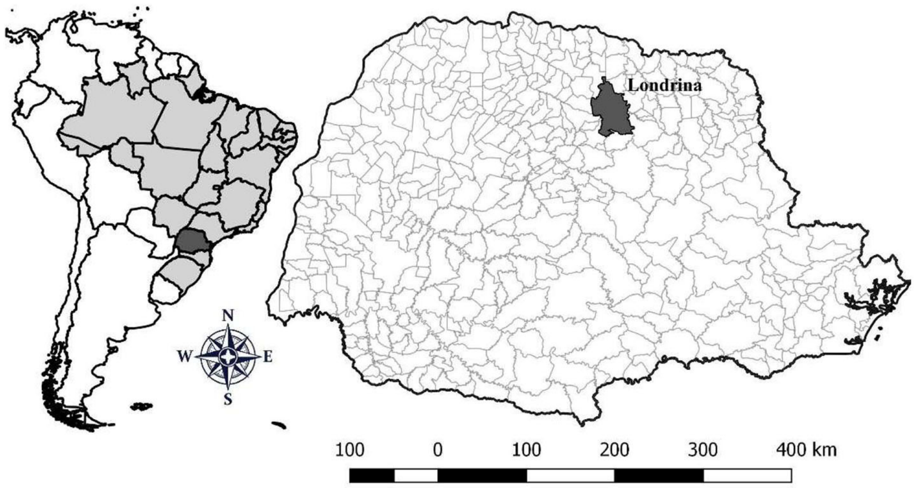

FIGURE 1 | Overview of the experimental site in Londrina County, Paraná State, Brazil.

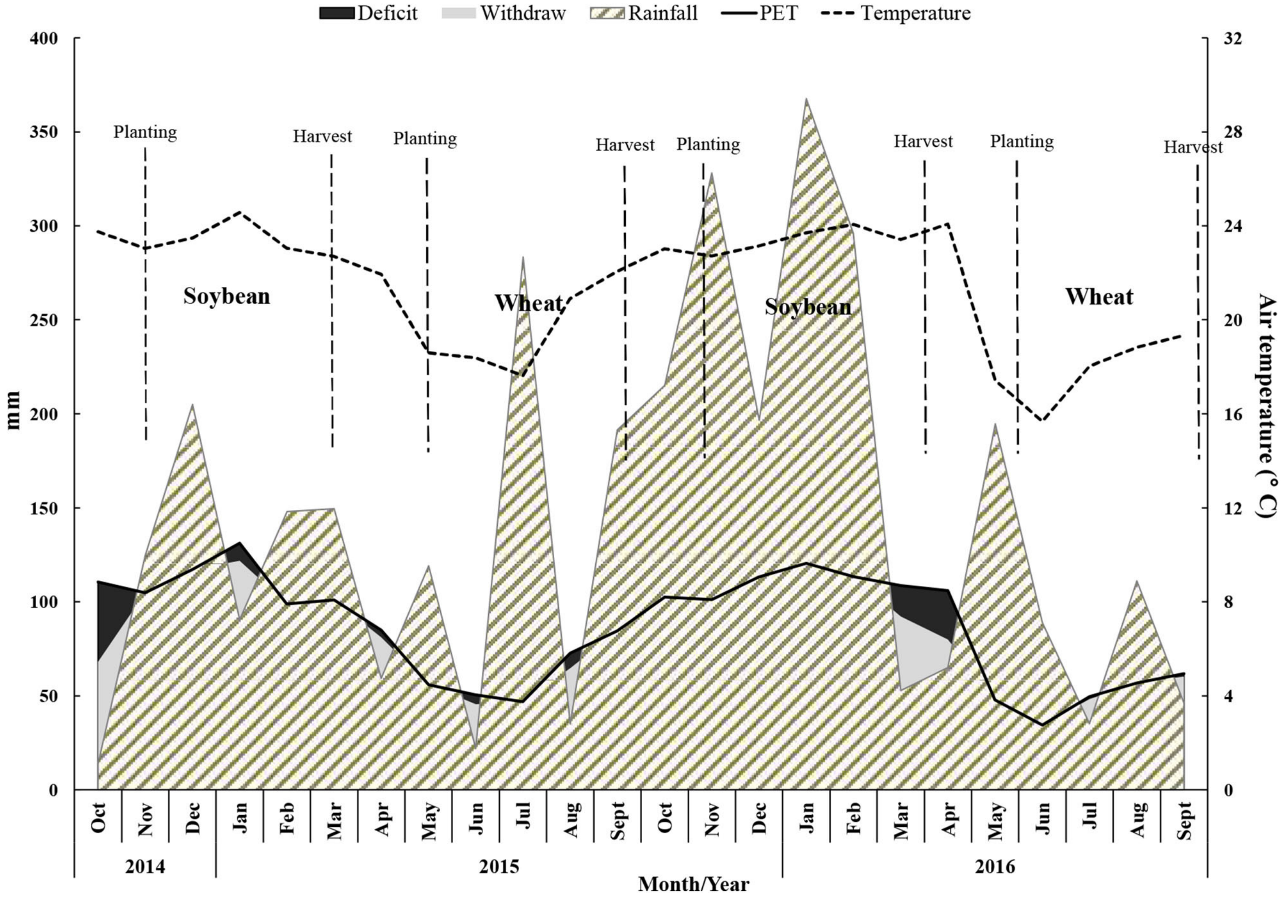

FIGURE 2 | Sequential water balance in the soybean and wheat crop seasons (2014–2016) according to Thornthwaite and Mather (1955). PET, potential evapotranspiration withdraw, H_2O removal system, and air temperature. Londrina, Paraná State, Brazil.

Moreira et al. (2019b). In both soybean cultivations, seeds were inoculated with *Bradyrhizobium elkanii* SEMIA 587 and SEMIA 5019 (4.0×10^9 viable cells g^{-1}) and treated with a solution containing 20 g ha^{-1} of molybdenum (Mo), 2.0 g ha^{-1} of cobalt (Co), and 10 g ha^{-1} of nickel (Ni). For the second soybean crop, in addition to the P_2O_5 treatments, the experimental area received 183 kg ha^{-1} of potassium chloride [KCl (60% K_2O)] (Moreira et al., 2019b). In both cultivations, the wheat crop received 180 kg ha^{-1} of urea (45% N) in sowing.

Evaluation of the Experiments

At the R2 reproductive stage, leaves 3 and 4 were collected at random from the apex of 30 plants to determine the nutritional status and chlorophyll level of the plants with hand-held chlorophyll meters (SPAD-502, Minolta, Japan) of the plants, afterwards, the data were transformed in chlorophyll contents (mg cm^{-2}) using the equation $y = 16.033 + (7.5774 \times \text{SPAD})$ using the equation $y = 16.033 + (7.5774 \times \text{SPAD})$ (Moreira et al., 2020). The leaves were collected and dried under forced ventilation oven at 65 ± 5 °C until constant weight. The total N was extracted by sulfuric digestion and determined by the micro-Kjeldahl method (Nelson and Sommers, 1972). The total P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn concentrations were extracted by digestion in a nitric-perchloric solution. The total P concentration was determined by spectrophotometry with molybdenum blue and the S concentration by turbidimetry. The total K, Ca, Mg, Cu, Fe, Mn, and Zn concentrations were analyzed by atomic absorption spectrophotometry, according to the methods described by Malavolta et al. (1997). Soil chemical analyses (pH, C, P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn) were carried out according to the methodologies described in Teixeira et al. (2017).

At the R6 growth stage (Fehr et al., 1971), 30 soybean plants were collected from the four central rows in each plot to determine the number of pods (NP), number of grains per pod (NGP), and plant height (PH). Soybean grain yield (GY) and 100 grain mass (R8 stage) were determined from an area of 3.0 × 7.0 m, leaving a 0.5-m border on all sides of each plot.

For the wheat crop, at the 10.1 stage, leaves were collected from 30 plants at random within each treatment to determine the nutritional status (N, P, K, Ca, Mg, S, Cu, Fe, Mn, and Zn—as per Malavolta et al., 1997), and chlorophyll level (SPAD-502, Minolta, Japan), and transformed with the equation described by Uddling et al. (2007). The GY, PH, hectoliter volume (HV), number of spikelet (NS), number of spikelets per clump (NSC), and 1000 grain mass were quantified. At the end of the crop cycle (harvest), soils were sampled from each treatment at a depth of 0–0.1 m to quantify available P with Mehlich 1 and resin extractants as described by Teixeira et al. (2017).

Statistical Analysis

Normality of the distribution of the traits was tested via Shapiro–Wilk's normality test (Shapiro and Wilk, 1965), testing the null hypothesis that the sample belongs to a population with normal distribution with the statistic X ($0 < X < 1$); if X is equal to 1, the data perfectly fit normal distribution, whereas small values of X are evidence of deviations from normality. The value of the statistic and the associated p-value were obtained; if this p-value was less than the nominal value of significance $p \leq 0.05$, the null hypothesis of normality was rejected. Normality of residuals was examined using the UNIVARIATE procedure ($p \leq 0.05$). Squared and absolute values of residuals were examined with Levene's Test to confirm homogeneity of variances ($p \leq 0.05$). After the normality test, the treatment variance data (soybean and wheat) were subjected to analysis of variance (ANOVA) and F-test using the standard least squares procedure of JMP by SAS. Regression and Pearson product-moment correlations were derived using

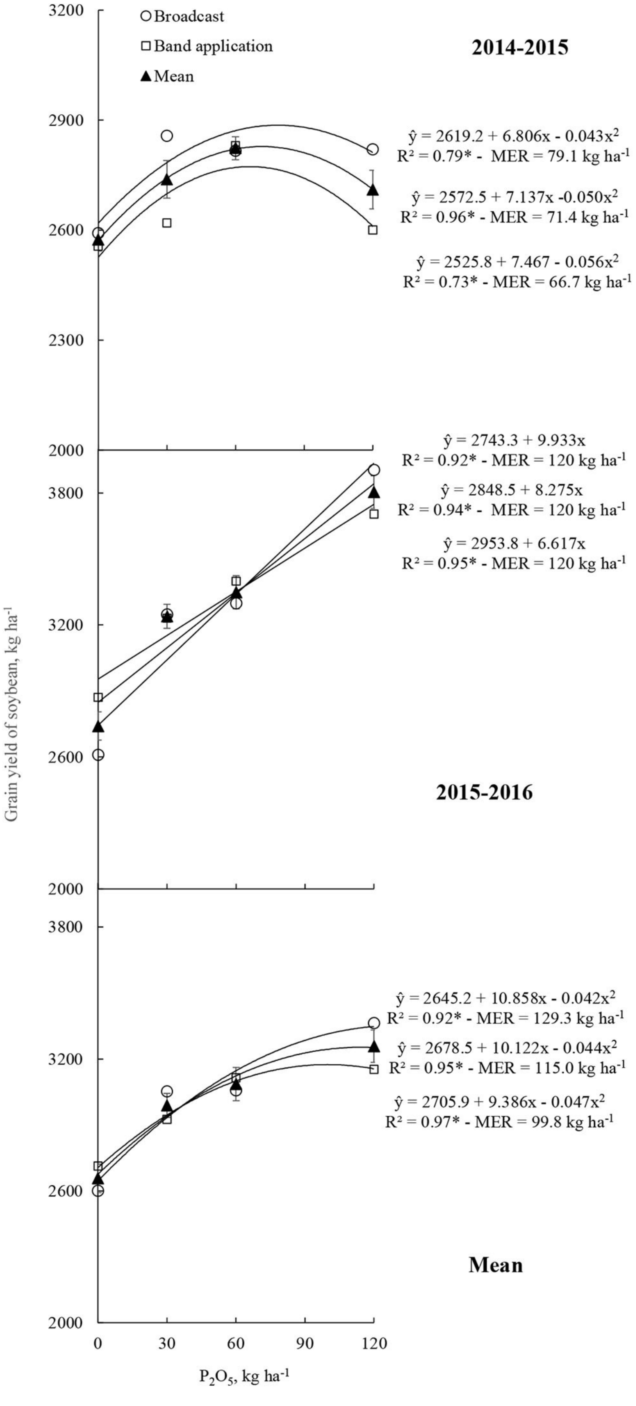

FIGURE 3 | Relationship of the effect of P_2O_5 rates applied through broadcast or band application in soybean crop, and soybean grain yield in two growing season. MER, maximum estimated rate. *Significant (F-test, $p \leq 0.05$). Londrina, Paraná State, Brazil.

the REG procedure of SAS to investigate the relation between P_2O_5 rates with grain yield (GY), yield components, nutrient concentrations, and chlorophyll level of soybean and wheat, and the regression equations for GY were chosen according to the best fit of the points within the curve. Where there was interaction of treatments × growing seasons ($p \leq 0.05$), the data were separated for each growing season.

RESULTS AND DISCUSSION

There was an interaction of P_2O_5 rate and application method on GY for both the soybean (**Figure 3**) and wheat (**Figure 4**) crops. In the two growing seasons, the yield of soybeans responded to the P_2O_5 application with a quadratic effect in the first and a linear effect in the second cultivation cycle. In addition, the results from the GY regression analysis under the effect of P_2O_5 rates indicated that, when grown in a Typic Eutrorthox, the broadcast application for the soybean crop showed an average value for the two harvests that was 5.4% higher for the maximum estimated rate (MER) of 99.8 kg ha^{-1} of P_2O_5 for the band application at sowing method and 129.3 kg ha^{-1} for the broadcast method with a MER of 115.0 kg ha^{-1} for the mean of the two application methods (**Figure 2**). These values are beyond the official recommendation for yield 3.0 to 4.0 Mg ha^{-1} (Moreira et al., 2019b). The wheat crop GY also increased with increasing P_2O_5 rates, but there was no effect of the application method (**Figure 4**). However, there was less yield in the second crop, probably due to less rain at the beginning of flowering (**Figure 2**). The mean GY of the two crops varied from 2,975.5 kg ha^{-1} for the MER of 91.8 kg ha^{-1} of P_2O_5 applied using the band application method to 2,977.7 kg ha^{-1} for the MER of 99.7 kg ha^{-1} of P_2O_5 applied using the broadcast approach.

Similar results reflecting the positive effects of P_2O_5 rates on GY of soybeans and wheat have been reported by Moreira et al. (2014) and Blue et al. (1990). Regarding the P_2O_5 rates and the two application methods, Heckman and Kamprath (1992) and Borges and Mallarino (2000) obtained positive responses in soybean GY with an increase in P rates application in the soil with both application methods. The band application or broadcast method showed no difference, even with available P levels in the soil within ranges considered low and very low (Moreira et al., 2019b). A low soil P level was a characteristic of the present study, where the average P content available in the soil before planting was 3.7 mg kg^{-1} (determined using the Mehlich 1 extractant). In a cultivation study carried out under "Cerrado" conditions, in a Typical Oxisol with a clayey texture and low P concentration, Broch and Chueiri (2005) obtained similar soybean yields when applying P fertilizer using the broadcast or the band application method. Regarding yield components, only the chlorophyll levels and number of grains per plant (NGP) in soybean crop and the number of spikelets (NS), and chlorophyll levels in wheat were significantly influenced by the P_2O_5 rates (**Table 1**). Fageria et al. (2011, 2013) reported that in phosphate fertilization studies, the NGP and NS have a greater influence in increasing GY as compared with other yield components, while P in the plants acts directly as an energy donor in the process of photosynthesis (Malavolta et al., 1997).

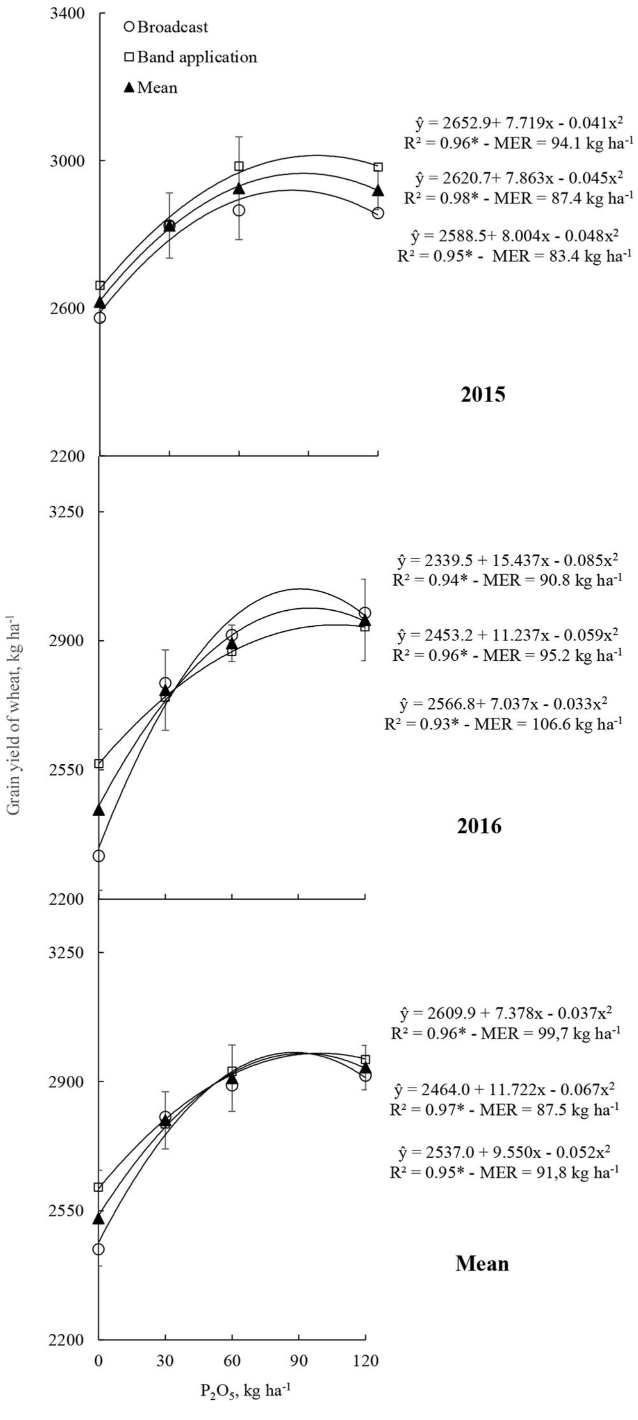

FIGURE 4 | Relationship of the residual effect of P_2O_5 rates applied through broadcast or band application in soybean crop and wheat grain yield in two growing seasons. MER, maximum estimated rate. *Significant (*F*-test, $p \leq$ 0.05). Londrina, Paraná State, Brazil.

Studies by Anghinoni (1992) and Sousa et al. (2002) have shown that rates higher than 100 kg ha^{-1} of P_2O_5 can be applied

TABLE 1 | Yield components and chlorophyll level as influenced by P_2O_5 rates, under broadcast and band application, on the average of two growing seasons of a soybean–wheat cropping system, Londrina, Paraná State, Brazil.

P_2O_5 rates ($kg\ ha^{-1}$)	**Soybean**						**Wheat**					
	Chlorophyll	**100 grains**	**Height**	**NPP**	**NGP**	**NGP/NPP**	**Chlorophyll**	**1000 grains**	**Height**	**Spikelet**	**SC**	**HV**
	$mg\ m^{-2}$	**G**	**Cm**	***n***	***n***		**$mg\ m^{-2}$**	**g**	**cm**	***n***	***n***	**kg**
BROADCAST												
0	246.1 ± 5.5	11.2 ± 0.9	56.4 ± 13.7	41.5 ± 15.2	103.5 ± 38.3	2.5 ± 0.2	340.3 ± 28.5	29.8 ± 0.7	81.2 ± 4.8	44.5 ± 1.7	34.8 ± 3.1	78.2 ± 0.8
30	248.5 ± 11.6	12.4 ± 0.7	60.4 ± 5.3	52.1 ± 10.8	129.3 ± 25.1	2.5 ± 0.1	346.5 ± 11.4	30.0 ± 0.6	78.8 ± 2.9	46.3 ± 3.6	36.0 ± 2.7	79.0 ± 0.5
60	248.7 ± 8.1	11.8 ± 0.5	62.7 ± 6.4	54.3 ± 9.0	137.9 ± 16.8	2.5 ± 0.2	349.5 ± 17.8	32.4 ± 0.9	80.6 ± 7.7	47.8 ± 3.0	30.0 ± 2.2	78.4 ± 0.7
120	253.8 ± 9.6	12.5 ± 1.1	65.0 ± 16.4	47.9 ± 12.5	121.2 ± 34.0	2.5 ± 0.2	361.9 ± 17.7	29.6 ± 0.6	79.9 ± 3.0	48.0 ± 2.6	31.5 ± 3.3	78.1 ± 0.3
Mean	249.3	12.0	61.1	49.0	123.0	2.5	349.6	30.5	80.1	46.7	33.1	78.4
BAND APPLICATION												
0	240.9 ± 13.0	12.0 ± 0.6	52.3 ± 4.1	46.5 ± 5.1	113.7 ± 13.3	2.4 ± 0.2	341.3 ± 29.8	30.3 ± 0.4	76.7 ± 6.1	42.5 ± 3.0	29.3 ± 2.9	78.8 ± 0.7
30	246.2 ± 10.8	11.8 ± 0.2	60.8 ± 4.6	49.7 ± 3.8	117.3 ± 11.7	2.4 ± 0.2	347.1 ± 25.0	29.8 ± 1.1	80.0 ± 3.0	46.3 ± 3.6	38.0 ± 3.4	78.5 ± 1.2
60	247.7 ± 5.1	12.3 ± 0.6	61.7 ± 4.6	53.2 ± 8.0	125.7 ± 17.2	2.4 ± 0.1	358.9 ± 19.5	29.8 ± 0.4	79.7 ± 4.9	46.8 ± 3.5	30.5 ± 1.8	78.4 ± 2.3
120	248.3 ± 13.8	12.1 ± 0.8	67.5 ± 4.3	51.4 ± 9.8	122.5 ± 22.2	2.4 ± 0.2	364.1 ± 27.3	29.8 ± 0.8	78.7 ± 1.3	47.0 ± 5.4	34.0 ± 2.6	78.7 ± 5.3
Mean	245.8	12.1	60.6	50.2	119.8	2.4	352.9	29.9	78.8	45.7	33.0	78.6
MEAN												
0	243.5 ± 9.3	11.6 ± 0.8	54.4 ± 8.9	44.0 ± 10.2	110.4 ± 25.0	2.5 ± 0.2	340.8 ± 27.8	30.1 ± 0.6	79.0 ± 5.5	43.5 ± 2.4	32.1 ± 3.0	78.5 ± 0.8
30	247.4 ± 11.2	12.1 ± 0.5	60.6 ± 5.0	50.9 ± 7.3	123.3 ± 18.4	2.5 ± 0.2	346.8 ± 18.2	29.9 ± 0.9	79.4 ± 3.0	46.3 ± 3.6	37.0 ± 3.1	78.8 ± 0.9
60	248.2 ± 6.6	12.1 ± 0.6	62.2 ± 5.5	53.8 ± 8.5	131.8 ± 17.0	2.5 ± 0.2	354.2 ± 18.7	31.1 ± 0.7	80.2 ± 6.3	47.3 ± 3.3	30.3 ± 2.0	78.4 ± 1.5
120	251.1 ± 11.7	12.3 ± 1.0	66.3 ± 10.4	50.0 ± 11.2	121.8 ± 28.1	2.5 ± 0.2	363.0 ± 22.5	29.7 ± 0.7	79.3 ± 2.2	47.5 ± 3.4	32.8 ± 2.5	78.4 ± 2.8
Mean	247.6	12.0	60.9	49.6	122.6	2.5	351.2	30.2	79.5	46.2	33.1	78.5
***F*-TEST**												
AM	ns	Ns	Ns	ns	ns	Ns	ns	ns	ns	ns	ns	ns
P_2O_5 rates	*	Ns	Ns	ns	*	Ns	*	ns	ns	*	ns	ns
AM × P_2O_5 rates	ns	Ns	Ns	ns	ns	Ns	ns	ns	ns	ns	ns	ns
CV (%)	3.9	6.0	12.2	18.8	18.0	8.0	6.2	2.4	5.3	6.9	8.0	1.9

**Significant (F-test, $p \leq 0.05$); ns, non-significant (F-test, $p > 0.05$); AM, application method; CV, coefficient of variation; SD, standard deviation; SC, spikelets per clump; NPP, number of pods per plant; NGP, number of grains per plant; HV, hectoliter volume.*

using the broadcast method without influencing the GY. Resende et al. (2006) and Borges and Mallarino (2003) investigated P sources, P rates, and application methods in corn (*Zea mays* L.) and soybean crop and observed that fertilization by broadcast and/or band application with soluble P sources yielded similar productivity. Prado et al. (2001) point out that in soils with a low P concentration, the adsorption of this nutrient is maximized when the phosphate fertilizer is applied as a broadcast with incorporation. This application methods lead to a greater contact of the P with the solid phase of the soil and, at the same time, the P contact with the plant root system is reduced. According to Lana et al. (2003), where there is non-incorporation of fertilizer in the soil, P fertilization can reduce losses due to fixation, being provisionally adsorbed on SOM, which becomes available to plants after mineralization (Anderson, 1980) and provides greater root development in the topsoil (Barber, 1995). Another factor to be highlighted is that in NTM, characterized by greater SOM accumulation in the upper layers of the soil, the restrictive effect described by Novais and Smyth (1999) was not observed. This restrictive effect is that more weathered tropical soils with high fixation power represent a drain preferential of P, and the broadcast application of P_2O_5 proves inadequate to meet plant demands. Although the results of the present study indicate better plant responses with broadcast application of P_2O_5, it is worth mentioning that due to the slope of the land and excessive rain (runoff effect), the band application at sowing method can reduce fertilizer losses by laminar leaching (Borges and Mallarino, 2003).

In this study, the available P levels in the soil under soybean and wheat crops showed linear increases in Mehlich 1 (P-M1) and ion exchange resin (P-RE) in relation to P_2O_5 rates (**Table 2**). In Brazil, these two methods are considered official; however, the interpretations must be in accordance with the corresponding calibrations made in each region of the country. The P-M1 extractant available *P*-values were lower than the P-RE values extracted until 5.7 times as much available P as that of P-M1, a result similar to that observed by Moreira and Malavolta (2001). In addition, both had a high correlation coefficient (soybean: y (P-RE) $= 6.628 + 3.202$ (P-M1), $r = 0.90$, and wheat: y (P-RE) $= 1.127 + 4.264$ (P-M1), $r = 0.86$, $p \leq 0.05$). These results confirm the findings of Moreira et al. (1997) and Moreira and Malavolta (2001) for these two extractants, indicating that both

TABLE 2 | Phosphorus availability in the soil with Mehlich 1 and resin extractants after a soybean-wheat cropping system due to the P_2O_5 rates and application method (AM) (broadcast and band application), Londrina, Paraná State, Brazil.

P_2O_5 rates (kg ha^{-1})	**Soybean**				**Wheat**			
	2014–2015		**2015–2016**		**2015**		**2016**	
	Mehlich 1 mg kg^{-1}	**Resin mg kg^{-1}**	**Mehlich 1 mg kg^{-1}**	**Resin mg kg^{-1}**	**Mehlich 1 mg kg^{-1}**	**Resin mg kg^{-1}**	**Mehlich 1 mg kg^{-1}**	**Resin mg kg^{-1}**
BROADCAST								
0	4.4 ± 0.4	9.1 ± 0.7	4.2 ± 0.3	8.8 ± 0.5	4.4 ± 0.8	17.7 ± 1.8	4.1 ± 0.5	13.5 ± 1.5
30	5.1 ± 0.4	12.5 ± 1.7	4.7 ± 0.4	13.3 ± 2.3	4.5 ± 0.3	19.0 ± 0.4	4.7 ± 0.6	19.7 ± 1.0
60	6.2 ± 0.6	14.0 ± 2.2	5.8 ± 0.5	13.3 ± 1.0	5.3 ± 0.2	21.7 ± 0.7	6.2 ± 0.3	23.9 ± 1.7
120	6.9 ± 0.4	23.4 ± 2.1	6.8 ± 0.4	23.8 ± 0.8	6.7 ± 1.3	25.4 ± 3.4	7.1 ± 0.9	27.4 ± 2.5
Mean	5.7	14.8	5.4	14.8	5.2	21.0	5.5	21.1
BAND APPLICATION								
0	3.5 ± 0.3	8.4 ± 1.4	3.6 ± 0.2	8.8 ± 1.0	4.3 ± 0.3	18.0 ± 0.5	3.9 ± 0.4	15.8 ± 1.1
30	4.1 ± 0.5	11.0 ± 3.5	4.2 ± 0.4	11.3 ± 3.6	5.1 ± 0.2	20.8 ± 0.4	5.3 ± 0.5	17.6 ± 1.9
60	5.5 ± 0.9	18.9 ± 4.2	5.4 ± 0.8	18.5 ± 3.4	6.0 ± 0.3	23.9 ± 0.8	6.1 ± 0.4	21.4 ± 2.4
120	6.0 ± 1.0	19.1 ± 5.3	5.8 ± 0.9	17.8 ± 6.2	7.7 ± 1.2	28.9 ± 2.7	7.6 ± 0.7	24.7 ± 1.9
Mean	4.8	14.3	4.8	14.1	5.8	22.9	5.7	19.9
MEAN								
0	4.0 ± 0.4	8.8 ± 1.1	3.9 ± 0.3	8.8 ± 0.8	4.4 ± 0.6	17.9 ± 1.2	4.0 ± 0.5	14.7 ± 1.3
30	4.6 ± 0.5	11.8 ± 2.6	4.5 ± 0.4	12.3 ± 3.0	4.8 ± 0.3	19.9 ± 0.4	5.0 ± 0.6	18.7 ± 1.5
60	5.9 ± 0.8	16.5 ± 3.2	5.6 ± 0.7	15.9 ± 2.2	5.7 ± 0.3	22.8 ± 0.8	6.2 ± 0.4	22.7 ± 2.1
120	6.5 ± 0.7	21.2 ± 3.7	6.3 ± 0.7	20.8 ± 3.5	7.2 ± 1.3	27.2 ± 3.1	7.4 ± 0.8	26.1 ± 2.2
Mean	5.3	14.6	5.1	14.5	5.5	23.0	5.7	21.0
***F*-TEST**								
AM	*	ns	ns	Ns	ns	ns	ns	Ns
Rates (*R*)	*	*	*	*	*	*	*	*
AM × *R*	ns	ns	ns	Ns	ns	ns	ns	Ns
CV%	13.9	19.5	12.8	17.9	13.9	6.5	12.2	8.9

**Significant (F-test, $p \leq 0.05$); ns, non-significant (F-test, $p > 0.05$); AM, application method; Rates, P_2O_5 rates; CV, coefficient of variation; SD, standard deviation.*

TABLE 3 | Leaf N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn concentrations as influenced by P_2O_5 rates, with two application methods (AM) (broadcast or band application), on the average of two growing seasons of soybean crops, Londrina, Paraná State, Brazil.

P_2O_5 rates (kg ha^{-1})	**N g kg^{-1}**	**P g kg^{-1}**	**K g kg^{-1}**	**Ca g kg^{-1}**	**Mg g kg^{-1}**	**S g kg^{-1}**	**B Mg kg^{-1}**	**Cu Mg kg^{-1}**	**Fe mg kg^{-1}**	**Mn Mg kg^{-1}**	**Zn mg kg^{-1}**
BROADCAST											
0	36.4 ± 3.8	2.6 ± 0.3	20.8 ± 2.7	8.5 ± 0.4	2.8 ± 0.2	1.7 ± 0.3	54.4 ± 5.7	8.2 ± 0.7	103.8 ± 14.2	133.5 ± 23.2	56.3 ± 7.3
30	37.1 ± 2.0	2.8 ± 0.3	21.2 ± 2.1	8.2 ± 0.3	2.8 ± 0.2	1.8 ± 0.2	48.7 ± 7.5	8.1 ± 0.8	118.4 ± 25.8	126.3 ± 16.8	49.2 ± 2.8
60	35.8 ± 1.8	3.0 ± 0.3	20.7 ± 1.0	8.0 ± 0.4	2.8 ± 0.2	1.9 ± 0.2	42.5 ± 6.8	7.9 ± 0.6	124.1 ± 37.9	132.8 ± 22.4	52.3 ± 4.4
120	34.3 ± 4.6	3.7 ± 0.4	21.3 ± 3.0	8.2 ± 0.2	2.7 ± 0.1	1.8 ± 0.1	45.9 ± 11.0	8.1 ± 0.9	128.8 ± 43.4	128.4 ± 27.3	49.0 ± 3.1
Mean	35.9	3.0	21.0	8.2	2.8	1.8	47.9	8.1	118.8	130.3	51.7
T8BAND APPLICATION											
0	34.7 ± 2.8	2.9 ± 0.1	21.2 ± 2.2	7.7 ± 0.5	2.8 ± 0.2	1.9 ± 0.2	44.5 ± 4.9	8.1 ± 0.1	109.8 ± 12.4	121.0 ± 8.8	50.4 ± 3.3
30	35.0 ± 2.9	3.1 ± 0.2	21.3 ± 2.4	8.1 ± 0.5	2.9 ± 0.3	1.9 ± 0.2	59.9 ± 9.8	7.7 ± 0.3	98.0 ± 19.0	124.7 ± 9.8	51.5 ± 1.9
60	35.8 ± 2.8	3.2 ± 0.4	21.8 ± 1.4	7.9 ± 0.4	2.9 ± 0.2	2.0 ± ± 0.2	53.4 ± 7.2	7.7 ± 0.6	104.3 ± 9.4	124.1 ± 12.3	49.3 ± 1.8
120	37.4 ± 2.8	3.4 ± 0.3	21.8 ± 2.4	8.2 ± 0.6	3.0 ± 0.3	2.5 ± 0.2	49.6 ± 5.0	8.3 ± 0.4	127.8 ± 42.3	128.2 ± 8.7	54.4 ± 5.4
Mean	35.7	3.2	21.5	8.0	2.9	2.1	51.9	8.0	110.0	124.5	51.4
MEAN											
0	35.5 ± 3.3	2.7 ± 0.2	21.0 ± 2.5	8.1 ± 0.5	2.8 ± 0.2	1.8 ± 0.3	49.5 ± 5.3	8.1 ± 0.4	106.8 ± 13.3	127.3 ± 16.0	53.4 ± 5.3
30	36.1 ± 2.5	3.0 ± 0.3	21.2 ± 2.3	8.1 ± 0.4	2.9 ± 0.3	1.8 ± 0.2	54.3 ± 8.7	7.9 ± 0.6	108.2 ± 22.4	125.5 ± 13.3	50.3 ± 2.4
60	35.8 ± 2.3	3.1 ± 0.4	21.3 ± 1.2	8.0 ± 0.4	2.8 ± 0.2	1.9 ± 0.2	47.9 ± 7.0	7.8 ± 0.6	114.2 ± 23.7	128.4 ± 17.4	50.8 ± 3.1
120	35.8 ± 3.4	3.5 ± 0.4	21.6 ± 2.7	8.2 ± 0.4	2.8 ± 0.2	2.1 ± 0.2	47.8 ± 8.0	8.2 ± 0.7	128.3 ± 42.9	128.3 ± 18.0	51.7 ± 3.7
Mean	35.8	3.1	21.3	8.1	2.8	1.9	50.0	8.2	114.4	127.4	51.6
***F*-TEST**											
AM	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
P_2O_5 rates	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
AM × P_2O_5 rates	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	7.9	12.5	9.3	5.4	7.4	14.9	17.0	7.0	23.5	12.0	8.5

**Significant (F-test, $p \leq 0.05$); ns, non-significant (F-test, $p > 0.05$); AM, application method; CV, coefficient of variation; SD, standard deviation.*

TABLE 4 | Leaf N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn concentrations as influenced by the P_2O_5 rates, with two application methods (AM) (broadcast or band application), on the average of two growing seasons of wheat crops, Londrina, Paraná State, Brazil.

P_2O_5 rates (kg ha^{-1})	**N g kg^{-1}**	**P g kg^{-1}**	**K g kg^{-1}**	**Ca g kg^{-1}**	**Mg g kg^{-1}**	**S g kg^{-1}**	**B mg kg^{-1}**	**Cu mg kg^{-1}**	**Fe mg kg^{-1}**	**Mn mg kg^{-1}**	**Zn mg kg^{-1}**
BROADCAST											
T2 0	29.7 ± 6.4	1.9 ± 0.2	23.5 ± 2.7	3.5 ± 1.2	2.1 ± 0.4	3.5 ± 0.4	25.6 ± 2.1	8.7 ± 1.1	119.6 ± 23.3	142.0 ± 35.4	35.5 ± 3.7
30	30.9 ± 6.3	2.3 ± 0.2	23.5 ± 1.1	5.1 ± 1.2	2.0 ± 0.2	3.6 ± 0.4	37.6 ± 2.7	8.2 ± 0.8	125.8 ± 47.5	151.8 ± 23.8	34.4 ± 2.1
60	29.9 ± 8.3	2.4 ± 0.1	22.5 ± 1.5	5.9 ± 0.7	1.8 ± 0.2	3.8 ± 0.3	27.3 ± 3.2	8.0 ± 0.9	123.1 ± 18.5	165.3 ± 22.6	31.8 ± 4.8
120	31.1 ± 5.8	2.7 ± 0.1	23.9 ± 1.5	4.4 ± 1.1	2.1 ± 0.3	3.9 ± 0.1	35.8 ± 2.8	8.0 ± 1.2	136.3 ± 27.5	176.2 ± 29.2	31.4 ± 1.3
Mean	30.4	2.3	23.4	4.7	2.0	3.7	31.6	8.2	126.2	158.8	33.3
BAND APPLICATION											
0	31.2 ± 2.2	2.1 ± 0.1	21.4 ± 1.6	7.4 ± 2.5	2.1 ± 0.3	3.6 ± 0.2	33.8 ± 2.5	8.8 ± 0.5	147.2 ± 49.7	171.6 ± 18.0	33.4 ± 4.1
30	30.3 ± 5.5	2.4 ± 0.1	18.8 ± 2.2	6.1 ± 0.9	2.0 ± 0.3	4.0 ± 0.5	37.5 ± 1.9	8.1 ± 0.8	137.3 ± 40.4	161.4 ± 13.0	32.9 ± 3.1
60	30.6 ± 6.6	2.5 ± 0.1	21.6 ± 2.2	5.6 ± 2.6	2.1 ± 0.2	3.6 ± 0.4	28.5 ± 2.4	8.7 ± 0.7	165.9 ± 41.6	157.7 ± 12.1	32.7 ± 1.4
120	24.1 ± 3.4	2.7 ± 0.1	20.6 ± 4.3	4.9 ± 1.4	2.0 ± 0.2	4.0 ± 0.1	38.3 ± 2.2	8.6 ± 0.6	161.3 ± 43.6	174.6 ± 20.8	32.6 ± 3.7
Mean	29.1	2.4	20.6	6.0	2.1	3.8	34.5	8.6	152.9	166.3	32.9
MEAN											
0	30.5 ± 4.3	2.0 ± 0.2	22.5 ± 2.2	5.5 ± 1.9	2.1 ± 0.4	3.5 ± 0.3	29.7 ± 2.3	8.7 ± 0.8	133.4 ± 36.5	156.8 ± 26.7	34.5 ± 3.9
30	30.6 ± 5.9	2.3 ± 0.2	21.1 ± 1.7	5.6 ± 1.1	2.0 ± 0.3	3.8 ± 0.5	37.6 ± 2.3	8.1 ± 0.8	131.6 ± 44.0	156.6 ± 18.4	33.7 ± 2.6
60	30.3 ± 7.5	2.5 ± 0.1	22.0 ± 1.9	5.7 ± 1.7	1.9 ± 0.2	3.7 ± 0.3	27.9 ± 2.8	8.3 ± 0.8	144.5 ± 30.1	161.5 ± 17.4	33.3 ± 3.1
120	27.6 ± 4.6	2.7 ± 0.1	22.2 ± 2.9	4.7 ± 1.3	2.1 ± 0.3	3.9 ± 0.1	37.0 ± 2.5	8.3 ± 0.9	148.8 ± 35.6	175.4 ± 25.0	32.0 ± 2.5
Mean	30.0	2.4	22.0	5.4	2.1	3.8	33.1	8.4	139.6	162.6	33.1
***F*-TEST**											
AM	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
P_2O_5 rates	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
AM × P_2O_5 rates	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	18.6	8.3	9.9	27.8	14.3	7.9	7.5	10.7	26.2	13.4	9.1

**Significant (F-test, $p \leq 0.05$); ns, non-significant (F-test, $p > 0.05$); AM, application method; CV, coefficient of variation; SD, standard deviation.*

are efficient ways of quantifying P availability in the soil. The P available with the Resin method increased from the first to second crop in wheat (**Table 2**), possibly due to the positive effect of the applied gypsum recovering the P in depth, and the greater sensitivity of extractant in quantifying the availability of nutrient in the soil.

There was a significant relationship between application methods and soybean leaf P, Ca, and S concentrations. Band application at a rate of 120 kg ha^{-1} of P_2O_5 resulted in a higher foliar P concentration than for the other rate treatments. However, it did not differ significantly from the broadcast application at the same rate (**Table 3**). The both methods types, the P-M1 and P-RE were well correlated with foliar P concentration for both species ($r = 0.65^*$ and $r = 0.56^*$, for soybean; $r = 0.58^*$ and $r = 0.56^*$, for wheat, respectively, $p \leq 0.05$), that is, the relationship between P-M1 and foliar P concentration was better than that of P-RE and foliar P concentration. Similar results were obtained by Antonangelo et al. (2019) with P rates in the same edaphoclimatic conditions. For K, there was no statistical difference; however, it was observed that P_2O_5 application using the band application method at a rate of 120 kg ha^{-1} resulted in a 7.0% higher K concentration in soybean leaves in comparison with application using the broadcast method. Probably, there must have been an increase in root volume in depth with the P application in the band application with a consequent increase in the K concentration in the plant. The foliar Ca concentration showed a linear effect ($y = 8.120 + 0.004x$, $R^2 = 0.68$, $p \leq 0.05$) only in the application to the broadcast, possibly due to the P source used that contained 12–14% Ca (Ribeiro et al., 1999). For leaf Mg, B, Cu, Fe, Mn, and Zn concentrations, there were no significant differences between treatments (**Table 3**). Rosa et al. (2015), in studies on P_2O_5 rates and application methods in soybean crop, observed that the broadcast approach was more effective at increasing the P, K, Ca, S, Mn, and Zn concentrations in the leaves than the band application method. Despite this variability, the foliar nutrient levels found in this study are within the ranges considered by Urano et al. (2006) as adequate in soybean crop. In this study, the negative P × Zn interaction was not observed in the uptake of these nutrients by plants as described by Malavolta et al. (1997) and verified by Moreira et al. (2006) in alfalfa (*Medicago sativa* L.) crop. Regarding the foliar nutrient concentrations of the wheat crop (**Table 4**), the only significant effect was of residual P_2O_5 rates on the P concentration ($y = 2.080 + 0.006x$, $R^2 = 0.77$, $p \leq 0.05$). The leaf N, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn concentrations in wheat were not influenced by growing season and treatments, and were close to the concentrations according with Malavolta et al. (1997) and obtained by Moreira et al. (2019a) in a study on the soybean-wheat intercropping system with Cu rates cultivated under the same soil and climate conditions.

CONCLUSIONS

Fertilization with a soluble phosphate source (triple superphosphate) in a soybean-wheat cropping system under NTM and subtropical conditions increased the productivity of soybeans and wheat over two growing seasons, regardless of the application method used (broadcast or band application). Given the similarity in soybean and wheat yield responses observed in this study, it is advised that the cost-benefit ratio for selecting the best method of P_2O_5 application should largely be determined by the difference in application costs and the relief of the area to be cultivated. The MER obtained for the mean of the two application methods are beyond the official recommendation for yield 3.0–4.0 Mg ha^{-1}. The soybean and wheat foliar P concentrations and the soil available P at a 0–0.1 m depth (determined using P-M1 and P-RE) were, for the most part, influenced by the P_2O_5 rates and not by the P application methods. Available P in the soil extracted by P-M1 and P-RE was highly correlated, with P-RE extracting until 5.7 times as much available P as P-M1.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because no requests to access the datasets should be directed to Adônis Moreira, adonis.moreira@embrapa.br.

AUTHOR CONTRIBUTIONS

AM: conducting the experiment, data tabulation, statistical analysis, and text writing. LM: conducting the experiment, and text writing. RP: conducting the experiment, and data tabulation. All authors contributed to the article and approved the submitted version.

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Optimizing Traditional Cropping Systems Under Climate Change: A Case of Maize Landraces and Bambara Groundnut

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Traditional crop species are reported to be drought-tolerant and nutrient-dense with potential to contribute to sustainable food and nutrition security within marginal production systems under climate change. We hypothesized that intercropping maize landraces (*Zea mays* L.) with bambara groundnut (*Vigna subterranea* (L.) Verdc.), together with optimum management strategies, can improve productivity and water use efficiency (WUE) under climate change. Using an ex-ante approach, we assessed climate change impacts and agronomic management options, such as plant ratios, and plant sequences, on yield and WUE of intercropped maize landrace and bambara groundnut. The Agricultural Production Systems sIMulator (APSIM) model was applied over four time periods; namely past (1961–1991), present (1995–2025), mid-century (2030–2060) and late-century (2065–2095), obtained from six GCMs. Across timescales, there were no significant differences with mean annual rainfall, but late century projections of mean annual temperature and reference crop evaporation (ET_0) showed average increases of 3.5°C and 155 mm, respectively. By late century and relative to the present, the projected changes in yield and WUE were −10 and −15% and 5 and 7% for intercropped bambara groundnut and maize landrace, respectively. Regardless of timescale, increasing plant population improved yield and WUE of intercropped bambara groundnut. Asynchronous planting increased yield and WUE for both maize landrace (5 and 14%) and bambara groundnut (35 and 47%, respectively). Most significant improvements were observed when either crop was planted 2–3 months apart. To reduce yield gaps in intercrop systems, low-cost management options like changing plant populations and sequential cropping can increase yield and WUE under projected climate change. To further increase sustainability, there is a need to expand the research to consider other management strategies such as use of other traditional crop species, fertilization, rainwater harvesting and soil conservation techniques.

Keywords: climate change adaptation, climate change impacts, food and nutrition security, multicropping, neglected and underutilized crops, resilience, water use

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INTRODUCTION

Sub-Saharan Africa has a dualistic food system with the formal system taking a more national focus, and also focused on a few strategic crops while the informal system supports local food systems, which support household food and nutrition security (Tcoli, 2016; Mabhaudhi et al., 2019a). While several nations are food secure at a national level, household food insecurity remains problematic with an estimated 821 million people currently food insecure and malnourished (Abegaz, 2018; Gashu et al., 2019; Xie et al., 2019). Most of these people rely on agriculture as their mainstay; thus, the importance of agriculture within these communities provides an opportunity to improve food and nutrition security, reduce poverty, and enhance rural economic development [New Partnership for Africa's Development (NEPAD), 2014]. However, current crop yields are low and challenged by worsening land degradation, especially declining soil fertility (Ukeje, 2010; Rippke et al., 2016; Badu-Apraku and Fakorede, 2017), and low water use efficiency (WUE) (Mabhaudhi et al., 2018b; O'Leary et al., 2018; Nouri et al., 2019). Furthermore, climate variability and change are adversely affecting productivity through increased incidences and intensity of droughts (Mpandeli et al., 2018; O'Leary et al., 2018; Nhamo et al., 2019). There is consensus that rural agricultural systems must increase resource use efficiencies and adopt strategies to adapt to climate risk (Isaacs et al., 2016; Matthews and McCartney, 2018).

A considerable amount of literature depicts the adoption of improved technologies such as the use of high yielding, improved crop varieties (Hammer et al., 2014; Ran et al., 2017; Mabhaudhi et al., 2019a). However, marginalized farmers have experienced several challenges when trying to adopt conventional farming practices. Chief among these include inadequate access to agrochemicals, loss in agro-biodiversity and an increase in the vulnerability of the system to climate risk (Mabhaudhi et al., 2019b; Malik and Chaudhary, 2019). The low adoption and consequent challenges have partly contributed to the widening gaps in food and nutrition security (Midega et al., 2015; Mrema et al., 2018). Within the context of marginal systems, agriculture needs to sustainably contribute to food and nutrition security and rural economic development, while reducing negative impacts on the environment or improving the environment (van Ittersum et al., 2016). Demand for more sustainable agriculture, which is less dependent on external inputs and better suited to marginal environments, has revived interest in traditional systems (Keatinge et al., 2015; Govender et al., 2016; Saharan et al., 2018). In line with this, there is a renewed focus on the inclusion of neglected and underutilized crops (NUS) as alternative crop choices in marginal cropping systems (Mabhaudhi et al., 2019a).

Neglected and underutilized crops, also referred to as underutilized indigenous and traditional crops, are defined as "plant species that are part of more substantial biodiversity, were once popular (in and out of their centers of diversity), and are neglected by users and research but remain relevant in the regions of their diversity" (Dansi et al., 2012). They are associated with high nutritional value, adaptation to marginal soils, and tolerance to drought and heat stresses (Slabbert et al., 2004; Chibarabada et al., 2015; Chimonyo et al., 2016a; Hadebe et al., 2017; Mabhaudhi et al., 2017). They often require fewer inputs such as fertilizer and agrochemicals, as they are also tolerant of several pests and diseases (Mabhaudhi et al., 2019a). Their nutritional attributes and adaptability make them suitable crops for promotion in marginal areas where poverty and food and nutrition insecurity remain high; however, their contribution to mainstay agriculture remains low (Massawe et al., 2016). As is reflected by their name, the potential of underutilized crops has not yet been fully harnessed, but most of them contribute to diversification and resilience of agroecosystems. Therefore, they have the potential for future agriculture under adverse agro-climatic conditions (Padulosi et al., 2011). Many proponents of modern agriculture and the Green Revolution have discouraged their continued production, highlighting low productivity and resource use efficiencies (Tokatlidis and Vlachostergios, 2016; Missio et al., 2018). For example, water use efficiency of bambara groundnut was reported to be 0.45 kg ha^{-1} mm^{-1} compared to 0.89 kg ha^{-1} mm^{-1} for groundnut (Chibarabada et al., 2017), while landrace sorghum varieties had 20% less WUE relative to hybrid varieties (Hadebe et al., 2019). However, the argument is to not promote them as replacement crops for high yielding major crops, but as complementary crops (Mabhaudhi et al., 2019a), especially in marginal areas where the major crops may not perform well (Massawe et al., 2016). Within these areas, NUS have potential to contribute to improving rural livelihoods and may be "better bet" technologies; however, this potential remains largely untapped due to limited information detailing their genetic, eco-physiological and agronomic performance (Chivenge et al., 2015). It is against this backdrop we hypothesize that, by optimizing resource use, yields of NUS can be sustainably increased. Intercropping involves growing of two or more crops simultaneously or overlapped on the same piece of land, which can sustainably increase WUE (Martin-Guay et al., 2018).

In this study, we hypothesize that intercropping a maize landrace (*Zea mays* L.) with bambara groundnut (*Vigna subterranea* (L.) Verdc.) is beneficial because the latter's smaller canopy offers little competition to the cereal crop (Saxena et al., 2018). As a legume, bambara groundnut also fixes atmospheric nitrogen. It contributes to soil fertility (Sprent et al., 2010), and the low cost of bambara groundnut seed makes it an exemplar crop for enhancing food and nutrition security within cereal producing households (Muhammad et al., 2016; Mayes et al., 2019). While traditional cropping systems featured multicrops (Muzari et al., 2012), intercropping maize with bambara groundnut is no longer a common practice. Little information is known about crop interaction and the impacts of climate variability and change on productivity and water productivity. While intercropping, in general, could be considered positive in terms of yield (Martin-Guay et al., 2018), the performance of each crop in an intercrop system is determined by the interaction between different crops and the availability of resources. With the impacts of climate variability and change, adapting agronomic management in response to changing resources can allow for sustainable intensification of the traditional cropping systems through improved resource use efficiency. Using an ex-ante approach in APSIM, the current study assessed the productivity and water use of a maize landrace - bambara groundnut intercrop under changing climate and in response to different management options. APSIM has been used widely to study impacts of climate change on crop growth and productivity across Africa (Beveridge et al., 2018; Duku et al., 2018; Xiao et al., 2020). However, its application for studying intercrop systems remains scanty, with no known research on its application for climate change studies.

MATERIALS AND METHODS

Study Area

The study area was the University of KwaZulu–Natal's Ukulinga Research Farm (29° 40′S; 30° 24′E; 809 m a.s.l.). Ukulinga Research Farm is classified as semi-arid with 77% of the mean annual rainfall of 750 mm received mostly between October and April. The summer months are warm to hot, with an average temperature of 26.5°C (Kunz et al., 2015). Soil textures are characterized as predominantly clay to clay loam and are moderately shallow, ranging from 0.6 to 0.8 m (Chimonyo et al., 2016a).

APSIM Maize – Bambara Groundnut Intercrop Model

Brief Description of the APSIM Model

The APSIM version 7.10 is a daily time step, field-scale multi-year, a multi-crop model that provides an analytical tool for assessing the impacts of climate, soil factors and farming management on cropping system production (Holzworth et al., 2014). The model is driven by daily temperature, precipitation, and solar radiation and is capable of simulating soil carbon (C), soil water, phosphorus (P), and nitrogen (N) dynamics and their interaction (Keating et al., 2003). Management practices include sowing date, variety selection, irrigation water management, fertilizer application, crop residue management, crop rotations and conservation tillage; this makes the model ideal for assessing the impacts of various management options on resource use. APSIM also allows users to set up atmospheric CO_2 concentration (Jones et al., 2001), which is ideal for assessing climate change impacts. Furthermore, through the CANOPY module, the model can simulate resource use within intercrop systems. For detailed information on the technical workings of the APSIM model, refer to McCown et al. (1996), Dimes and Revanuru (2004), and Holzworth et al. (2006; 2014).

The CANOPY module determines resources intercepted by each component of the intercrop using leaf area index (LAI), extinction coefficient and height for each crop. Arbitration for water and nitrogen uptake is done based on the module changing the order each day (on a rotational basis) in which the competing species are allowed to capture soil resources. Through the CANOPY module, the model accounts for the vertical profiles of LAI in different species in a mixture (Keating et al., 2003), and assumes a horizontally homogeneous canopy for each species (Gou et al., 2017). The CANOPY module has been published and successfully applied by Smith et al. (2016) and Snapp et al. (2018) for maize and pigeon pea; Carberry et al. (1996) for maize and bean; Chimonyo et al. (2016a) for sorghum and cowpea; and Hoffmann et al. (2020) for various maize intercrop systems. Although Nelson et al. (1998a,b) used APSIM to simulate a maize and *Desmanthus virgatus* intercrop system, the two crops were grown as monocultures and did use the CANOPY module. It was not clear whether Amarasingha et al. (2017) used the CANOPY module when maize and mung bean intercrop systems were simulated in APSIM. In contrast, Knörzer et al. (2011) found that APSIM was unable to simulate wheat-pea and maize-pea intercropping systems in Germany because it strongly underestimates the competitive ability of the species that was planted the first relative to the one that was planted last. In this study, we used the CANOPY module to simulate the effects of climate change on a maize landrace and bambara groundnut intercrop system. The current study, therefore, adds to the existing body of knowledge on the use of ASIM in simulating intercrop systems. It goes further to simulate different management options under different climate change impacts on the intercrop system.

Model Calibration, Testing and Application

The calibration and testing of the APSIM were carried out using observed data obtained from field experiments conducted during the 2015/16 growing season for a maize landrace–bambara groundnut intercrop established at the University of KwaZulu-Natal's Ukulinga Research Farm. Sub-plots comprised intercrop combinations, that is, sole maize landrace, sole bambara groundnut and maize landrace–bambara groundnut intercrop. The irrigated treatments were used for calibration, while the rainfed treatments were used to validate the model. For a detailed description of the experiment, refer to **Supplementary Information 1**. The simulation files were, therefore, created using observed data collected from the rainfed and irrigated treatment.

Met File

For model calibration and testing, a 10-year (2009–2019) weather data file that contained daily estimates of rainfall, minimum and maximum temperatures, solar radiation and reference evapotranspiration was sourced from SASRI weather site (http://sasex.sasa.org.za/irricane/tables/Ash_tables_AR.pl) using the nearest station to the location except for Ukulinga where there is a weather station on-site. With the 5-year climate file, we were able to back-calculate and estimate the initial soil water and initial soil nitrogen at planting. APSIM require an average ambient temperature (TAV) and the annual amplitude in monthly temperature (AMP). These values are calculated using long-term daily minimum and maximum temperatures by software program named "tav_amp."

Soil File

The soil file was generated using soil details at Ukulinga Rresearch Farm. Soils at the research farm have been described as being shallow clayey to clayey loam with medium fertility (Mabhaudhi et al., 2013). The soil file selected to represent this description best was Clay_Shallow_MF_101 mm. The soil module was created using information obtained from

Chimonyo et al. (2016b) (**Table 1**), and this was matched to a pre-existing soil file available in APSIM soil module – Africa (Generic).

Crop Files

Within maize APSIM crop file, we used the maize cultivar "mwi_local" as it best described the maize landrace used in terms of days to maturity and yield potential of 3 t ha^{-1}. However, slight iterations to genetic coefficients were done using an iterative approach until simulated values were within 9–20% of observed values (**Table 2**). Since APSIM does not have a bambara groundnut crop file, the groundnut cultivar "kangwana" was modified as it closely resembled bambara groundnut in terms of physiology, growth habit and phenology (**Table 2**). The groundnut crop module was iterated by first adjusting the reproductive parameters within the crop life cycle (phenology, e.g., time to emergence, first leaf, reproductive stages, and maturity) to resemble what was observed from the monocropped treatment during the field experiment. After that, where simulations disagreed with observations, parameters in the groundnut module were modified in a sequential approach following the order proposed by Boote et al. (2002). The steps were: (1) leaf appearance rate, canopy height, and width, (2) specific leaf area, leaf area index, and partitioning among vegetative organs, including the rate of total biomass accumulation and lastly, (3) onset, rate, and duration of pod addition and seed growth. Besides modifications based on comparisons with the observed data, some parameter modifications were made based on a literature review.

TABLE 1 | Soil water properties at different depths for soil at the experimental site.

Texture	BD[a]	HC[b]	PWP[c]	FC[d]	TAW[e]	SAT[f]	K_{SAT}^{g}
	gm^{-3}	mm m^{-1}					mm h^{-1}
Clay	1.35	0.33	294	416	152	489	19,70

[a] *Bulk density;* [b] *Hygroscopic moisture content;* [c] *Permanent wilting point;* [d] *Field capacity;* [e] *Total available water;* [f] *Saturation;* [g] *Hydraulic conductivity.*

Management File

The management file considered planting date, plant densities, fertilizer rate, irrigation and harvest rules. The plant populations used to calibrate and test the model were 2 0 and 2.2 (plants m^{-2}) of the maize landrace and bambara groundnut, respectively. The plant population used represented the densities observed in the field experiment and were less than the recommended densities for dryland maize (2.6 plants m^{-2}) and bambara

TABLE 2 | Modification of groundnut crop coefficients based on experimental data and data obtained from the literature.

Parameter	Description	Default peanut crop file (*cv* kangwana)	New bambara groundnut crop file
Temp units	Temperature table for thermal time	9.0 29.0 39.0	8.5 28.0 38.0
leaf_dm_init	Initial leaf dry matter	0.045	0.035[b]
ratio_root_shoot	ratio_root_shoot	0 0 1.0 1.0 0.33 0.33 0.087 0 0 0 0	0 0 1.0 0.67[a] 0.33 0.33 0.087 0 0 0 0
frac_leaf units	Fraction of remaining dry matter allocated to leaves	0 0 0.58 0.58 0.58 0.45 0.45 0 0 0 0	0 0 0.60[b] 0.60[b] 0.60[b] 0.55[b] 0.55[b] 0 0 0 0
frac_pod units	Fraction of dry matter allocated to pod or multiplier of grain dry matter to account for pod dry matter	0 0 0 0 0 0.18 0.25 0 0 0 0	0 0 0 0 0 0.30[b] 0.45[b] 0 0 0 0
leaf_size	leaf_size	2,000 4,000 4,000 4,000 4,000	4,800 4,800 4,800 4,800 4,800[b]
sla_max description	Maximum specific leaf area for delta LAI	35,000 30,000 25,000 20,000 20,000 20,000 20,000	45,000 45,000 40,000 40,000 38,000 34,000 30,000
hi_incr	Rate of HI increase	0.0056	0.0024
hi_max_pot	Maximum harvest index potential	0.45	0.35[a,c]
Floral initiation (°Cd)		680	220[a]
Flowering (°Cd)	Time from flowering to start grain fill	300	340[a]
Start_grain_fill	Duration of grain filling	440	550[a]
End_grain_fill	Duration of seed maturation	10	85[a]
Height (mm)	Plant height	700	400[a]
		Default maize crop file (*cv* mwi_local)	**Iterated maize crop file**
tt_flower_to_maturity description (°Cd)	Time from flowering to maturity	780	750
PotKernelWt (g 100 kernels^{-1})	Potential kernel weight	260	160

[a] *Field observation;* [b] *model iteration;* [c] *Karunaratne et al. (2010).*

groundnut (4.4 plants m^{-2}) production (Jensen et al., 2003). Since the field experiment used to calibrate and test the model was conducted in one season, we used the irrigated treatments for calibration and the rainfed treatments to test model. The module "irrigate on the date" was used to apply irrigation on dates corresponding to actual irrigation dates. Observed irrigation applied per event for the field experiment was calculated to be, on average, 15 mm, which was applied thrice during the experiment. Nitrogen fertilizer was applied automatically within 50 cm depth in the soil at a rate of 50 kg ha^{-1} to avoid any nitrogen stress.

Climate Scenarios

Ukulinga Research Farm is located within quinary sub–catchment 4,697 of quaternary catchment U30J (Schulze et al., 2011). In addition to historical data, the study also used downscaled future climate projections for the Ukulinga quinary. The climate projections were developed by the Council for Scientific and Industrial Research (CSIR) (**Table 3**) using output from six global climate models (GCMs) from the CMIP5 archive that was forced by Representative Concentration Pathway 8.5 (RCP 8.5). The climates produced under RCP 8.5 were used as they represent the most extreme scenarios. The selection of these six GCMs was based on their ability to provide a reasonable representation of the El Nino-Southern Oscillation (ENSO) phenomenon for the region.

The climate projections were dynamically downscaled to improve spatial resolution to 0.5° (∼50 km) using the CCAM regional climate model developed by the Commonwealth Scientific and Industrial Research Organization, CSIRO (McGregor and Dix, 2001, 2008; McGregor, 2005). After that, a multiple-nudging strategy was followed to obtain a downscaling to 0.1° (∼10 km) resolution using CCAM in stretched-grid mode over South Africa (see Mabhaudhi et al., 2018a). Climate scenarios were then extracted for the gridded pixel that overlapped quinary sub–catchment 4,697. For application in crop modeling at a local scale, it is necessary to correct for systematic and localized biases in rainfall and temperature projections produced by the climate models. When compared to observed rainfall data from the historical quinary climate database for sub-catchment 4,697, the downscaled climate projections were found to have a substantially larger number of rain days, with many rain days having minimal rainfall depths (i.e., < 0.1 mm). Therefore, a quantile delta mapping method, as described and assessed by Cannon et al. (2015), was applied to bias correct the climate scenarios using a multiplicative factor for rainfall and an additive factor for temperature.

The bias-corrected climate data provide daily rainfall and temperature scenarios for a continuous period from 1961 to 2100. Daily reference crop evaporation (ETo) estimates were then computed as described for the historical data set (see Schulze et al., 2011). Solar radiation for each GCM for Ukulinga was then calculated as described by Schulze and Chapman (2007). The climate database, therefore, satisfied APSIM's climate file input requirements and was used to develop projections for the past (1961–1991), present (1995–2025), mid-century (2030–2060) and late-century (2065–2095) periods. Throughout the analysis, the "present" timescale was regarded as the baseline.

TABLE 3 | Global climate models used in this study.

Abbreviation	Model name	Model center	Horizontal resolution
ACC	ACCESS1-0	Commonwealth Scientific and Industrial Research Organization, Australia (CSIRO), and Bureau of Meteorology, Australia (BOM)	1.250 × 1.875°
CCS	CCSM4	National Center for Atmospheric Research (NCAR), USA	0.9424 × 1.250°
CNR	CNRM-CM5	Center National de Recherches Meteorologiques, Meteo-France, France	1.4005 × 1.4065°
NOR	NorESM1-M	NorESM (Norwegian Earth System)	1.250 × 0.940°
GFD	GFDL-CM3	Geophysical Fluid Dynamics Laboratory, USA	2.000 × 2.500°
MPI	MPI-ESM-LR	Max Planck Institute for Meteorology, Hamburg Germany	1.8653 × 1.875°

Management and Agronomic Scenarios

Two management scenarios were used to develop recommendations for best management practices. The scenarios were as follows.

Scenario 1: Planting Dates

Maize production guidelines published by the Department of Agriculture, Forestry and Fisheries suggest that maize should be planted between October 1 and mid-December throughout South Africa [Department of Agriculture Forestry Fisheries (DAFF), 2003]. As it is, South Africa exhibits a wide variation of agro-ecologies, both at the micro and macro level. Due to climate variability and change, this variation has increased, and there is an observed increase in the land area occupied by semi-arid arid agro-ecologies since 2000 (Cairns et al., 2013). Conversely, there is a continual need to redefine planting dates. In this study, we adopted five fixed dates between September 15 to January 15 as this approach is much easier for farmers to use. These dates were assumed to represent early to late planting. However, a significant weakness of this approach is the need to redefine the dates because of continuous shifting in agro-ecologies.

Scenario 2: Plant Populations

Model simulations were performed using plant populations that were 50% less to 50% more than the recommended values. Simulations were carried out by maintaining the recommended plant population of one component and changing the other. The total number of simulations was a 3 by 3 factorial with maize populations of 13,000, 26,000, and 39,000 plants ha^{-1} and bambara groundnut populations of 6,500, 13,000, and 19,500 plants ha^{-1}. The lower populations would reduce resource competition and improve productivity for either component crop, while higher populations assumed that there was a need to minimize unproductive resource use from the system and improve their productive use. From this, optimum plant populations were determined for both landraces.

Model Runs

For model calibration and testing, the APSIM intercrop model was run for 10 consecutive years from 2009 to 2019. The 10-year run allowed for soil conditions to stabilize around what was observed in the actual experiment. During the scenario analyses management options were run independently from each other across the six climate projections to minimize the interactive effects of the scenarios. The RCPs were run continuously from 1961–2095 periods.

Data Analyses

Since APSIM does not calculate WUE directly, simulated outputs of water use (WU in mm) and yield (Y in kg ha^{-1}) or biomass (B in kg ha^{-1}) were used to determine water use efficiency (WUE in kg mm^{-1} ha^{-1}) over the growing season (sowing to harvest) as follows:

$$WUE = \frac{Y/B}{WU} \tag{1}$$

Within the model, WU was determined as crop water uptake from the soil profile by either maize landrace or bambara groundnut crop, i.e., maize Ep and bambara Ep and soil evaporation Es [Ep (for either maize landrace or bambara groundnut +Es)].

For model calibration and validation, model performance was evaluated by comparing simulated (S) vs. observed (O) values for phenology, leaf area index, WU, WUE^B, grain yield and biomass. Model performance was evaluated using the coefficient of determination (R^2), root mean square error (RMSE) and normalized RMSE (nRMSE). Values of R^2 range between 0 and 1 with high values indicating less error variance. Since the interpretation of R^2 is independent, low values are only acceptable if n is large. However, R^2 values are sensitive to outliers and insensitive to additive, and proportional differences between S and O. The simulation was considered excellent when nRMSE $< 10\%$, good if 10–20%, acceptable or fair if 20–30%, and poor if $>30\%$ of the observed mean (Jamieson et al., 1991; Granderson and Price, 2014).

Simulation outputs for yield and water use were subjected to descriptive statistics, *t*-test analyses and generalized linear mixed analysis (GLMM) using R statistical software (version 3.6.0). Descriptive statistics such as means, standard deviations, bubble charts and box and whisker plots were used to analyse outputs. Box and whisker plot can show stability and general distribution of data sets. The GLMM was used to identify significant factors influencing maize landrace and bambara groundnut yield.

Developing Guidelines

The Food and Agriculture Organization (FAO) suggested a list of guiding questions to review transformative elements within an intervention (Carter et al., 2018). These questions are meant to provide clarity on the adaptation planning process. In this study, we adopted selected questions to assess the implications of the research, provide actionable recommendations and provide a way forward. Key findings were summarized in **Table 5** and implications outlined.

RESULTS AND DISCUSSION

Model Performance

Comparisons of simulated and observed values for maize landrace and bambara phenology and LAI, and biomass, yield and water use (WU) and water use efficiency (WUE) are given in **Figure 1** and **Table 4**, respectively. For phenology, the close alignment of the points to the 1:1 line indicates that the model was able to simulate the maize landrace and bambara phenology correctly. The model could explain more than 90% of the variation of either crop in phenological stages (**Figure 1**). During the calibration, the nRMSE for the system LAI was $< 10\%$ of the observed LAI for the maize landrace and bambara groundnut intercrop system. The nRMSE for the system LAI during model validation increased slightly to 14%; this implied good simulation for the intercrop system grown under rainfed conditions. Reasonable simulations of crop water use (WU) by the model during calibration, were also observed (RMSE $= 41$ mm); however, during model validation, WU was over-estimated by 48%. The output suggests that the APSIM model might not be sensitive to water. A closer look at model outputs for maize landrace and bambara groundnut simulated under irrigated (used for calibration) and rainfed (used for validation) conditions showed that transpiration was mostly unaffected by the reduction in water availability. In nature, the low availability of water results in a reduction in transpiration due to the reduction in stomatal conductivity. Field results showed no significant differences between the irrigated and rainfed treatments (see **Supplementary Information 1**). In this case, the model appropriately captured maize landrace and bambara groundnut physiology.

For biomass and grain yield, the model tended to overestimate the outputs for maize landrace and bambara groundnut. During model calibration, simulated yield and biomass for maize landrace as 11 and 16% higher than observed, and this implied reasonable simulation. Model simulation of maize landrace yield and biomass under rainfed conditions were satisfactory (RSME $= 49$ and 267 kg ha^{-1}) However, simulated yield and biomass for bambara groundnut were 32 and 55% higher than those for observed yields. The performance of APSIM would suggest that, for improved model simulations, additional parameterisation may be required to simulate bambara groundnut adequately. The WUE calculated based on model simulated biomass (WUE^B) of both the maize landrace and bambara groundnut showed a good fit (1 and 4 kg mm^{-1} ha^{-1}, respectively), for simulated and observed results (**Table 4**). Then again, the bambara cultivar used to calibrate the crop file was a landrace selection. It could be that performance under low water availability had adverse effects on its productivity, and model did not capture this response. Considering that the model was still able to simulate low yields

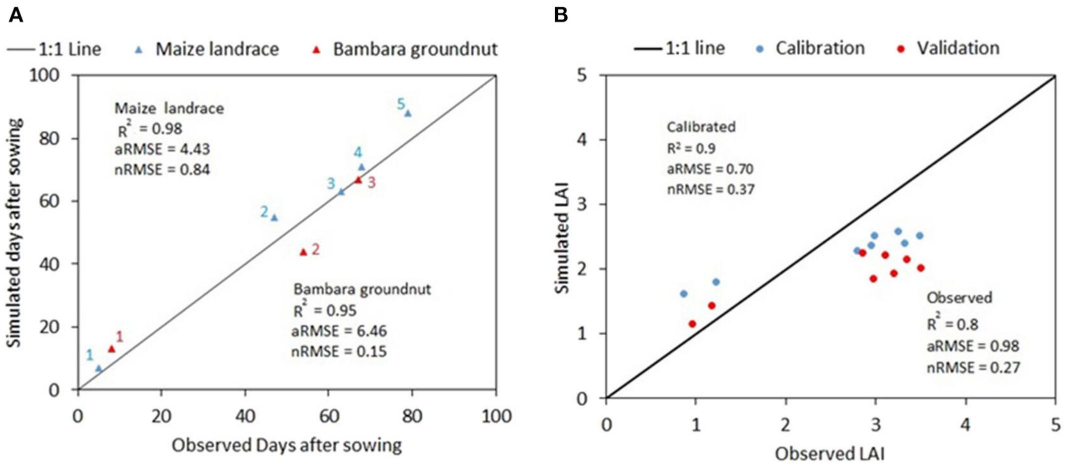

FIGURE 1 | Comparison of observed and simulated **(A)** phenology (days after sowing) and **(B)** leaf area index for maize landrace and bambara groundnut during model calibration and validation. Red triangles represent bambara groundnut, and associated numbers 1, 2, and 3 represent phenological stages; emergence, the onset of flowering and start of grain filling respectively. Blue triangles represent maize landrace, and associated numbers 1, 2, 3, 4, and 5 represent phenological stages as emergence, floral initiation, flag leaf formation, the onset of flowering, and start of grain filling respectively.

TABLE 4 | Calibration and validation results for observed and simulated outputs for maize landrace and bambara groundnut intercrop system for final biomass (kg ha^{-1}), yield (kg ha^{-1}) and intercrop system water use (mm).

		Observed	Simulated	RSME
Model calibration (Irrigated treatment)				
Maize landrace (kg ha^{-1})	Yield	820	918	98
	Biomass	2,370	2,741	371
Bambara groundnut (kg ha^{-1})	Yield	230	244	14
	Biomass	1,060	1,375	315
Intercrop WU (mm)		291	332	41
Intercrop WUE^b (kg mm^{-1} ha^{-1})		11	12	1
Model validation (rainfed treatment)				
Maize landrace (kg ha^{-1})	Yield	870	919	49
	Biomass	2,470	2,737	267
Bambara groundnut (kg ha^{-1})	Yield	150	213	63
	Biomass	950	1,248	398
Intercrop WU (mm)		179	266	87
Intercrop WUE^b (kg mm^{-1} ha^{-1})		19	15	4

for the maize landrace and bambara groundnut and possible errors in the observation data (e.g., iterated cultivar parameters), the APSIM model performance was considered to be acceptable for the simulation of the intercrop system.

Change in Climate During the Growing Season

Dynamically downscaled and bias-corrected climate projections for six GCMs forced by RCP 8.5, together with an impact model APSIM 7.7, were used to simulate bambara groundnut and maize landrace yields for past, present, mid-, and late-centuries. The primary aim was to assess how climate change impacts on yield, WU and WUE of the maize landrace and bambara groundnut intercrop. Secondary to that, we assessed the impacts of various management options on mitigating the impacts of climate change. The median value of climate change projections for minimum and maximum temperatures for Ukulinga showed a consistent warming trend across all months from past to late century. **Figure 2** indicates a warmer future (mid- and late-century) with mean maximum temperature increasing by 4.5°C relative to the baseline maximum temperature of 24°C. This suggests an increased probability of heat stress, especially for the maize landrace. This warming trend across the selected timescales is consistent with projected trends for South Africa (Mangani et al., 2018).

The six GCMs project an increase in mean minimum temperatures in the future (mid- and late-century) that ranges from 2.0–4.8°C from a minimum baseline temperature of 13°C. The projected increases suggest an increased probability of hot nights and longer and more frequent heatwaves. The warmer temperatures may result in a faster accumulation of heat units and a reduction in growth duration and accumulation of photosynthesis and increase in night-time respiration, all resulting in reduced crop yield (Schlenker and Roberts, 2009). Unlike bambara groundnut (C3 plant), maize (C4 plant) generally originates from warmer climates (Leff et al., 2004; Jia et al., 2016) and thus, may be more resilient to projected increases of temperature (Choudhary et al., 2019). Then again, for bambara groundnut, optimum temperatures range between 28 and 35°C and the lethal temperature has been reported to be 50°C (Soni et al., 2015). The wide temperature adaptation makes the crop ideally suited for building resilience to cropping systems located in areas where temperature increases have been projected.

Results across the GCMs show that mean annual rainfall (MAP) for the future is projected to remain somewhat unchanged (**Figure 3**). for the late-century period, data showed that ACC and CCS predict a 10.6 and 8.3% increase in MAP, respectively, while slight reductions of 3.5 and 2.5% are predicted by CNR and NOR respectively. However, the more extended box and whisper plots for ACC predict an increase in the inter-annual variability

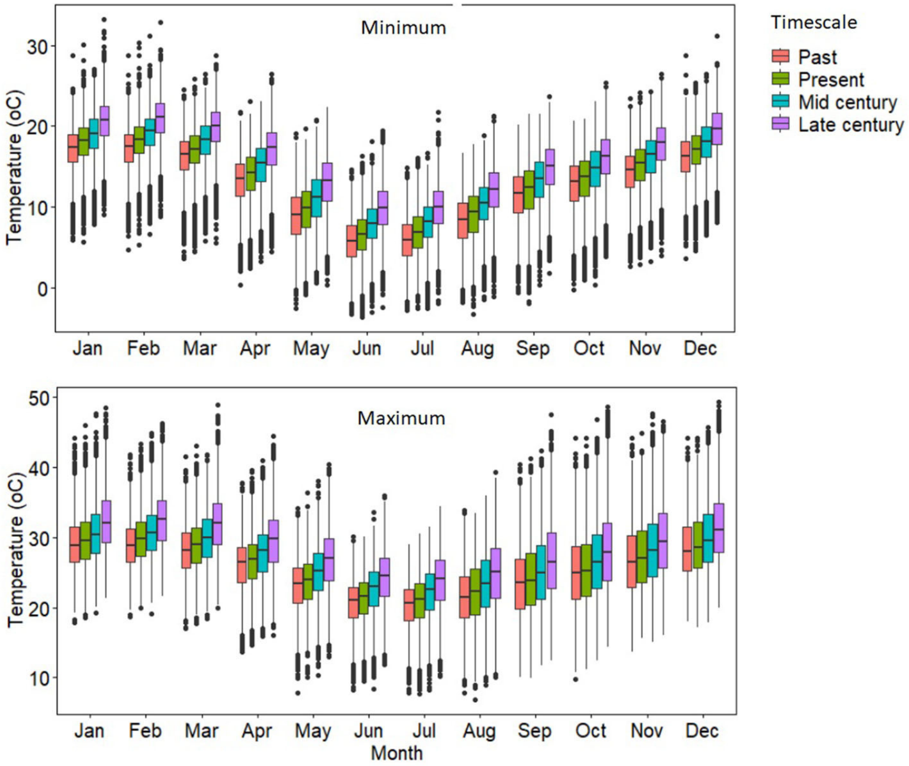

FIGURE 2 | Distribution of average monthly minimum **(A)** and maximum **(B)** temperature data for the different timescales (past, present, mid-, and late-century) as simulated by the six GCMs (ACC, CSS, CNR, GFD, NOR, and MPI).

of mean rainfall (750 mm) (**Figure 3**). This suggests an increase in the probability of extreme weather events such as drought and floods. In all instances, projected ET_o was observed to be higher (35%) than projected rainfall and is set to increase in the future (mid and late century) (**Figure 3**). In this regard, the rainfall: ET_o ratio is projected to decrease in the near future. The increase in ET_o is consistent with the projected increase in minimum and maximum temperature and suggests an increase in crop water stress (Zhao et al., 2017). Then again, intercrop systems with cereals and legumes are advantageous as the cereal over-story can lower canopy temperature and minimize evaporative losses (Eskandari, 2011; Chimonyo et al., 2016b). The modification of microclimate within intercrop systems makes it an ideal system to mitigate against projected temperature and ET_0 increases. Our results suggest that, while the tolerances of traditional crops to high temperatures may vary, intercropping crop species with different physiological and morphological traits can be a strategy to increase the resilience of marginalized production systems to projected temperature ET_0 increases.

Yield, Water Use and Water Use Efficiency

Across the GCMs, yield trends for intercropped bambara groundnut showed a gradual reduction toward the late century by 24% when compared to the baseline yield of 365 kg ha^{-1}. The observed trend for simulated bambara groundnut yield was late-century (285 ± 57) < mid-century (323 ± 62) < present (365 ± 67) < past (450 ± 65 kg ha^{-1}) (**Figure 4**). Across the GCMs, the magnitude of change in simulated bambara groundnut yield during the mid- and late-century periods was consistent with corresponding projected increases in ET_o and temperature. On the other hand, the mean yield trends for intercropped maize landrace across the GCMs and time scales were inconsistent with projected increases in ET_o and temperature. The observed trend for simulated maize landrace yield was past (845) < late-century (855) < present (923) < mid-century (967 kg ha^{-1}). For mid-century, although the model predicted a slight increase in maize landrace yield, results also showed larger yield variations relative to past and present. Standard deviations for the intercropped maize landrace yield were past (288) < present (363) < mid-century (351) < late-century (436 kg ha^{-1}) (**Figure 4**). These results are in line with the increased probability of extreme weather events such as drought and floods (Schulze, 2011). Although bambara groundnut yield decreased across the time scales, the magnitude of yield variations within each timescale and GCM was somewhat consistent with an average standard deviation of 63 kg ha^{-1}. Within each timescale, our results would suggest that yields of bambara groundnut are more stable to climate fluctuation; however, it could be more sensitive to significant climate changes.

The trend for water use in the intercropped maize landrace and bambara groundnut was inconsistent across the GCM and

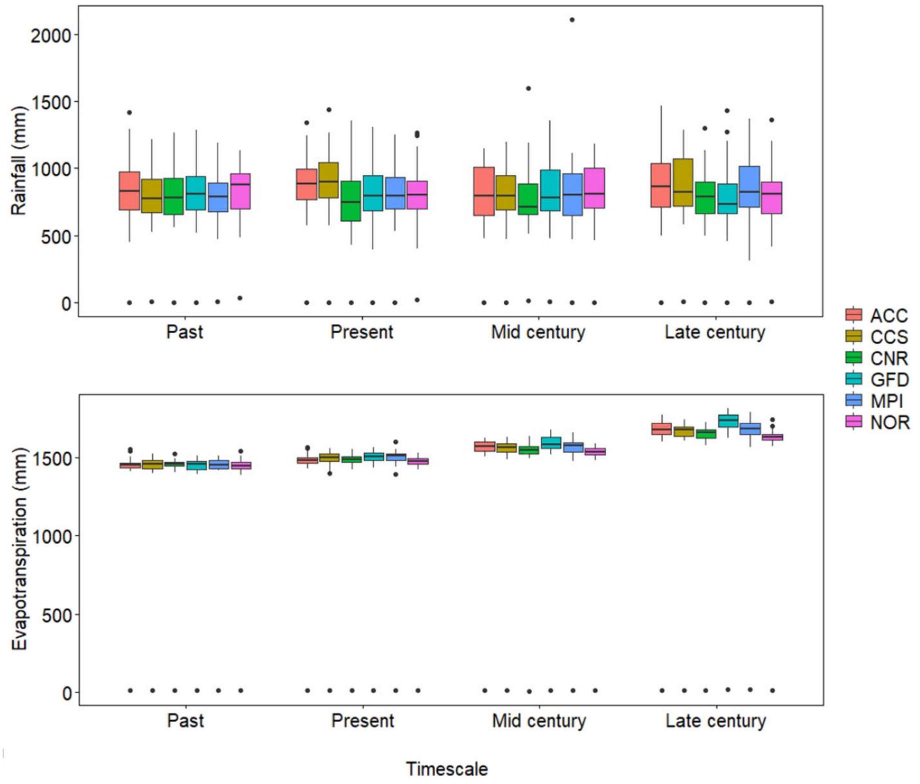

FIGURE 3 | Rainfall data representative of four different timescales (past, present, mid-, and late-century) as simulated by the six GCMs (ACC, CSS, CNR, GFD, NOR, and MPI). The average yearly rainfall calculated from observed rainfall data received between 2004 and 2019 was used as the mean annual rainfall.

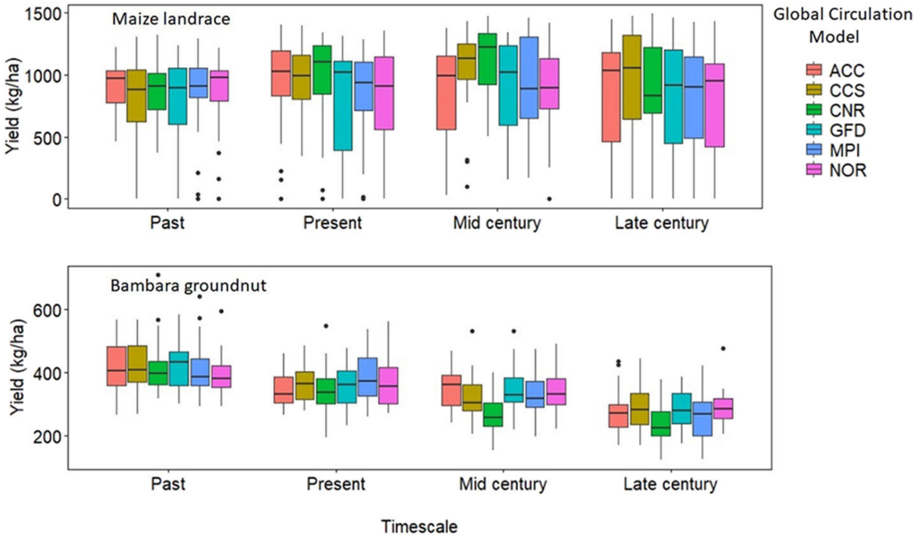

FIGURE 4 | Simulated yield (kg ha^{-1}) for maize landrace and bambara groundnut during four different timescales (Past, Present, Mid-, and Late-century) under rainfed conditions obtained from the six GCMs (ACC, CSS, CNR, GFD, NOR, and MPI). The effect of timescale on maize landrace grain yield - *F*-statistic: 3.492 on 3 DF, $P = 0.01$. The effect of the interaction between GCM and timescale on bambara groundnut grain yield - *F*-statistic: 1.953 on 15 DF, $P = 0.01$.

timescales. Overall, CCS predicted the highest water use (265 and 253 mm), while the lowest was under NOR (242 and 235 mm) for maize landrace and bambara groundnut, respectively (**Figure 5**). Differences in simulated WU across the GCMs could be that each climate model has been developed based on its assumptions and unique mathematical representations of physical climate system processes, providing different climate projections (Confalonieri et al., 2016). There were slight reductions in WU across the

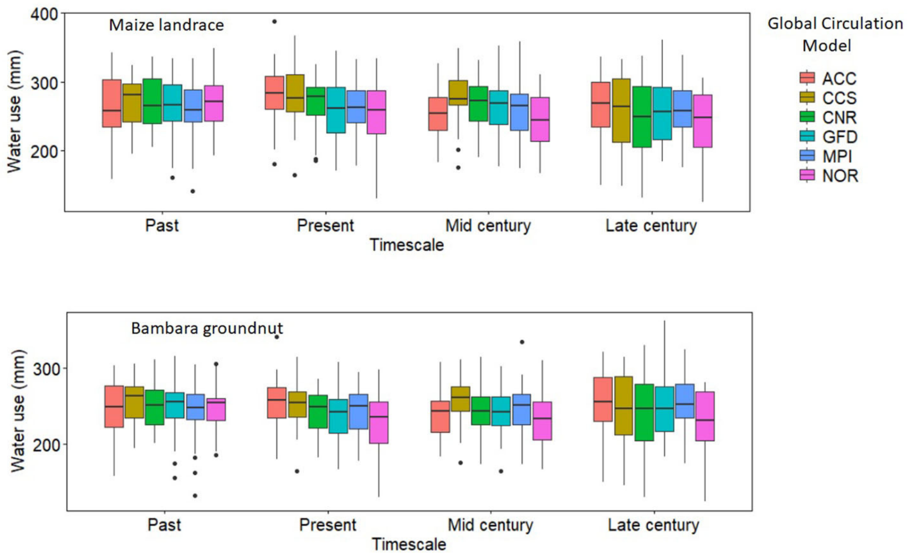

FIGURE 5 | Calculated water use (mm) of maize landrace and bambara groundnuts from soil evaporation (Es), crop water use (Ep) as simulated by APSIM across the six GCMs (ACC, CSS, CNR, GFD, NOR, and MPI) for each timescale (past, present, mid-century, and late-century). The effect of timescale on maize landrace water use - *F*-statistic: 2.989 on 3 DF, $P = 0.03$. The effect of GCM on maize landrace water use - *F*-statistic: 2.6392 on 5 DF, $P = 0.02$. The effect of GCM on bambara groundnut water use - *F*-statistic: 3.315 on 5 DF, $P = 0.005$.

timescale; however, based on the pairwise *t*-test analysis, the reductions were not significant ($P > 0.05$). On the other hand, simulated results for crop water use efficiency (WUE) for intercropped bambara groundnut showed a reduction across time scales. The trend was such that past (1.78 ± 0.45) > present (1.52 ± 0.41) > mid-century (1.37 ± 0.38) > late century (1.16 ± 0.42 kg ha^{-1} mm^{1}) (**Figure 6**). The observed trend was consistent with the observed reduction in future yield. Large inconsistencies were observed for maize landrace WUE across GCMs and time scales. For example, CNR predicted the highest water use (4.01 ± 1.98 kg ha^{-1} mm^{-1}), while the lowest was under NOR (3.25 ± 1.05 kg ha^{-1} mm^{-1}). The trend for maize landrace WUE across the timescale was such that present (3.58 ± 1.25) < past (3.62 ± 0.81) < late century (4.37 ± 1.38) < mid-century (4.56 ± 1.82 kg ha^{-1} mm^{-1}) (**Figure 6**). The observed trend was consistent with the simulated improvements of maize yield.

Optimizing the Performance of Bambara Groundnut in Intercrop Systems

Impacts of Planting Density on Yield and Water Use Efficiency of a Maize Landrace and Bambara Groundnut Intercrop System

Simulation results of yield and WU for intercropped maize landrace and bambara groundnut across the six GCM were not significantly ($P > 0.05$) different; therefore, the results presented in this section are average values across the six GCMs. Across timescales, the trend for maize landrace yield was past (850 ± 288) < late-century (853 ± 443) < present (893 ± 359) < mid-century (959 ± 362 kg ha^{-1}) (**Figure 7**). Increasing maize landrace plant population resulted in a significant increase in mean yield but did not affect WUE (**Figure 8**). Regardless of bambara groundnut plant population, increasing maize landrace plant population resulted in a 12% reduction in its mean yield while reducing the population resulted in an 8% improvement in its mean yield (**Figure 8**). On the other hand, simulated yield and WUE for intercropped bambara groundnut was significantly ($P < 0.05$) affected by timescales and by the interaction between maize landrace and bambara groundnut planting date.

Across timescales, the trend for bambara groundnut yield was past (806 ± 406) > present (760 ± 404) > mid-century (717 ± 359) > late-century (674 ± 332 kg ha^{-1}) (**Figure 7**). Likewise, the trend for bambara groundnut WUE was past (2.54 ±1.10) > present (2.37 ± 1.23) > mid-century (2.33 ± 0.98) > late-century (2.17 ± 0.89 kg ha^{-1} mm^{-1}) (**Figure 8**). Although the observed WUE for bambara groundnut in the late century represented an 87% improvement relative to the baseline (1.16 ± 0.42 kg ha^{-1} mm^{-1}) for the same period, there was a 52% increase in its variability. Increasing bambara groundnut plant population increased simulated yield by 43% at the highest plant population, but also increased yield variability (standard deviation). The simulated mean yields (in kg ha^{-1}) and corresponding standard deviations were 520 ± 247 < 777 ± 353 < 921 ± 406 for intercropped bambara groundnut simulated at 2.2, 4.4 and 6.6 plants m^{-2}, respectively (**Figure 9**). A similar trend was observed for the calculated WUE (in kg ha^{-1} mm^{-1}), which was 1.61 ± 0.60 < 2.41 ± 0.94 < 2.98 ± 1.07 for intercropped bambara groundnut at 2.2, 4.4 and 6.6 plants m^{-2} (**Figure 9**). This would suggest that the currently recommended plant populations of 4.4 plants m^{2} might be low for optimum use of resources such as water.

There was a reduction in simulated mean yield for bambara groundnut with the increase in maize landrace plant population.

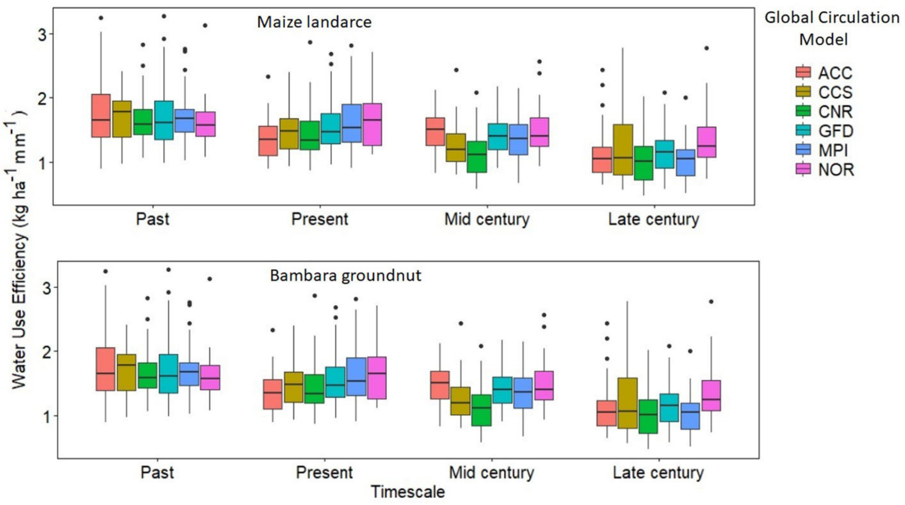

FIGURE 6 | Calculated water use efficiency (kg ha^{-1} mm^{-1}) for maize landrace and bambara groundnut across the six GCMs (ACC, CSS, CNR, GFD, NOR, and MPI) for each timescale (Past, Present, Mid-, and Late-century). Bambara groundnut *F*-statistic: 2.122 on 3 DF, *P*-value: 0.1 and bambara groundnut *F*-statistic: 4.543 on 3, $P = 0.003$.

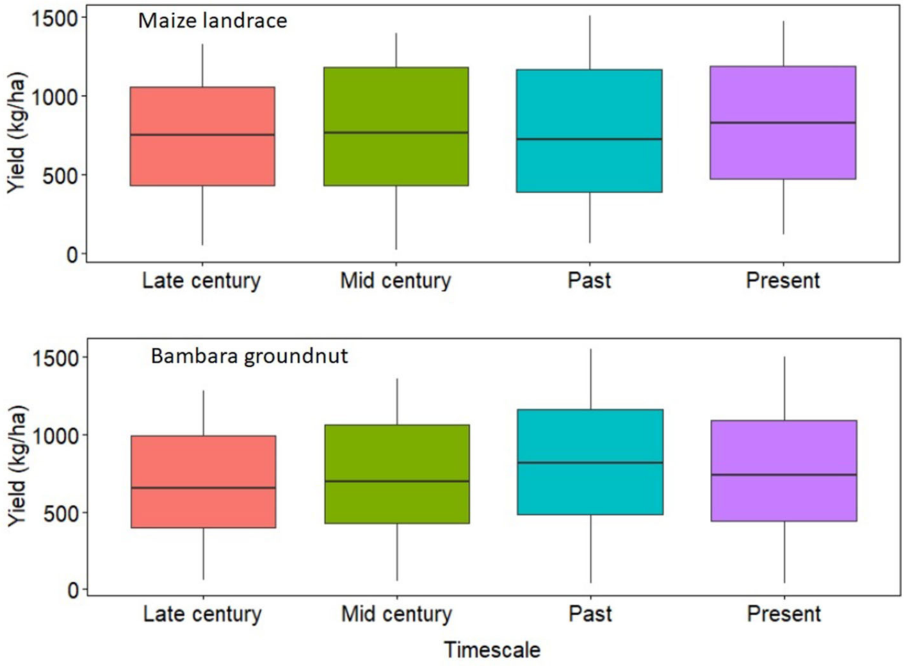

FIGURE 7 | Simulated maize landrace and bambara groundnut yields (kg ha^{-1}) across different timescales from six GCM (ACC, CSS, CNR, GFD, NOR, and MPI). The effect of timescale on bambara groundnut yield *F*-statistic: 5.031 on 3 DF, $P = 0.001$.

The trend for bambara groundnut yield was 841 ± 305 > 720 ± 379 > 657 ± 422 (kg ha^{-1}) when intercropped with maize landrace at plant populations of 1.3, 2.6 and 3.9 plants m^{-2}, respectively (**Figure 7**). Similar to the simulated yield trend of intercropped bambara, increasing maize plant population resulted in a reduction of calculated bambara WUE and an increase in its variability (standard deviation) (**Figure 7**). The reduction of simulated yield and WUE maxima and minima for bambara groundnut and the increase in yield variability under high maize landrace plant populations could be attributed to increased competition for resources with the maize landrace. Peake et al. (2008) observed that increasing maize plant populations beyond a specific limit could increase the risk of crop failure due to an increase in competition for water and

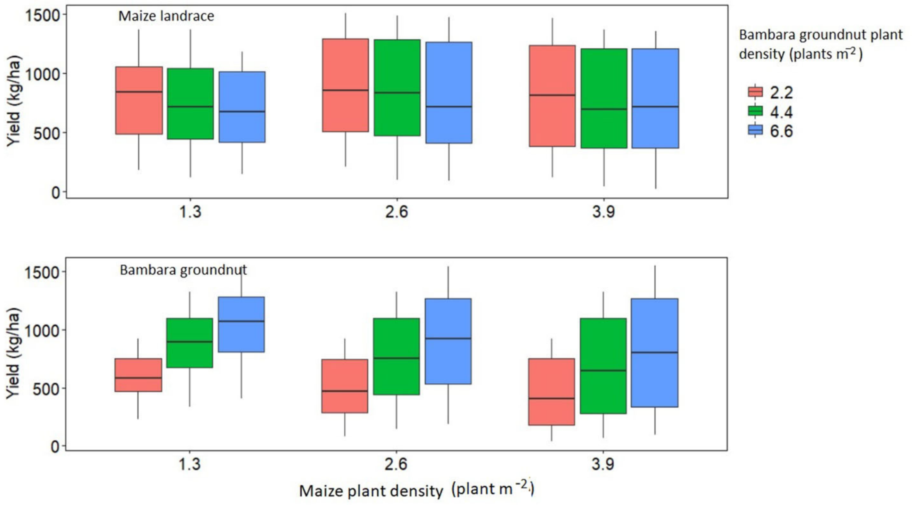

FIGURE 8 | Simulated yield response of maize landrace and bambara groundnut to plant population (plant m^{-2}) for climate scenarios obtained from six GCM (ACC, CSS, CNR, GFD, NOR, and MPI). Different colors of boxplots represent the bambara groundnut plant density (plant m^{-2}). The effect of the interaction between maize landrace plant density and bambara groundnut plant density on maize landrace grain yield - *F*-statistic: 62.47 on 8 and 891 DF, $P = 0.000$. The effect of the interaction between maize landrace plant density and bambara groundnut plant density on bambara groundnut grain yield - *F*-statistic: 38.93 on 24 and 875 DF, $P = 0.000$.

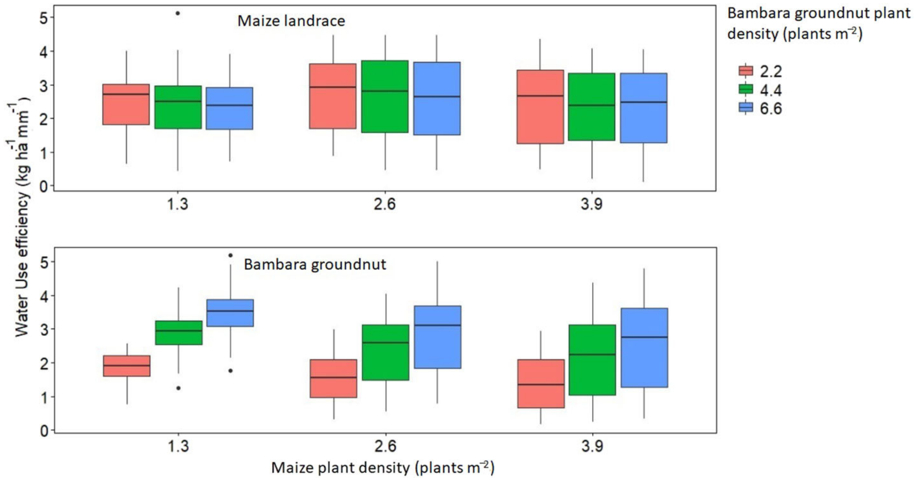

FIGURE 9 | Calculated water use efficiency maize landrace and bambara groundnut plant population (plant m^{-2}) for climate scenarios obtained from six GCM (ACC, CSS, CNR, GFD, NOR, and MPI). The colored boxplots represent the bambara groundnut plant density (plant m^{-2}). The effect of maize landrace plant density on maize landrace WUE - *F*-statistic: 6.78 on 2 and 891 DF, $P = 0.001$. The effect of the interaction between maize landrace plant density and bambara groundnut plant density on bambara groundnut WUE - *F*-statistic: 38.93 on 24 and 875 DF, $P = 0.000$.

solar radiation. In cases were both yields of the maize landrace and bambara groundnut are desired by a farmer, it might be worthwhile to reduce maize landrace plant populations to maximize yield for bambara groundnut. Alternatively, there is a need to improve water availability through rainwater harvesting and conservation techniques to reduce competition for water within the intercrop.

Impacts of Planting Dates on Yield and Water Use Efficiency of Maize Landrace and Bambara Groundnut Intercrop System

Simulation results for yield and WU for maize landrace and bambara groundnut across the six GCM were also not significantly different ($P > 0.05$); therefore, the results presented in this section were average values across the six GCMs. Simulated yield for maize landrace and bambara groundnut was significantly ($P < 0.05$) affected by the interaction of their planting dates (**Figures 10**, **11**). Overall, early planting (September) of maize landrace or bambara groundnut resulted in higher simulated yields relative to late planting (January). Across the planting dates, mean yield trends for intercropped bambara groundnut was September (992 ± 296) > October (889 ± 357) > November (681 ± 383) > December (548 ± 301) >January (486 ± 283 kg ha^{-1}). On the other hand, calculated WUE trend for intercropped bambara groundnut was September (2.81 ± 0.78) > October (2.53 ± 0.96) > November (2.46 ± 1.15) > January

(1.99 ± 1.12) > December (1.94 ± 1.01 kg mm^{-1}). For maize landrace, mean yield trends was September (1,052 ± 116) > October (902 ± 197) > November (835 ± 213) > December (648 ± 261) >January (596 ± 283 kg ha^{-1}). On the other hand, calculated WUE trends for intercropped bambara groundnut was September (3.01 ± 0.88) > October (2.73 ± 0.76) > November (2.33 ± 0.95) > January (2.09 ± 1.00) > December (1.84 ± 0.66 kg mm^{-1}). The calculated WUE was lower (24–107%) than the calculated WUE baseline of 4.12 kg mm ^{1}. This could be attributed to a reduction in the lower quartile values (**Figure 10**), which would suggest an increase in maize landrace yield gap with later planting. According to several research outputs, climate change is expected to reduce the length of the growing season and increase the occurrence of dry spells (Mitchell et al., 2015; Ajetomobi, 2016; Paff and Asseng, 2018). Despite the loss of growing days, our result suggests that, when planting on the same day, early planting (September) may ensure stable yields and WUE are obtained. Rezvani Moghaddam et al. (2014) found that early planting could be used as an adaptation strategy for maize under future climate in arid regions of Iran. Hussain et al. (2018) also highlight that, regardless of planting date, yield responses are highly dependent on resource availability and distribution, in this case, rainfall.

Intercropping bambara ground at different planting dates to maize landrace improved the mean yield (**Figure 10**), and WUE (**Figure 11**) provided it was done before November 15. Planting bambara groundnut a month earlier than maize landrace - for instance, planting in the former September and maize landrace in October, resulted in a 176 and 57% increase in its mean yield and WUE, respectively, relative to the baselines. Planting bambara groundnut 2 and 3 months earlier than maize landrace resulted in a 184% increase in yield (**Figure 10**) and improved WUE by 61% increase in WUE (**Figure 11**). Planting maize landrace a month earlier than bambara groundnut - for instance, planting in September and bambara groundnut in October, resulted in the most significant mean yield increase (56%) relative to the baseline (**Figure 11**). The asynchronous or sequential planting did not result in the overlap in critical phenological stages for both the maize landrace and bambara groundnut. This minimized the competition for water and other resources and maximized resource use through extending canopy duration, therefore improving yield and WUE for maize landrace and bambara groundnut within the intercrop. When critical periods overlap, Yu et al. (2016)suggested that the competitive balance in cereal-legume intercrops can be maintained by planting the legumes earlier than the cereals. This can be viewed as a strategy to minimize the risk of yield loss in the event of intermittent dry spells within the season. However, sequential cropping in rainfed systems is constrained by the length of growing period (Inthavong et al., 2011; Kotir, 2011; Vadez et al., 2012; Duku et al., 2018; Minda et al., 2018). In this study, we did not assess the impacts of climate change on changes in the length and shifts of the growing season, nor the probability of dry spell occurrence and duration.

WAY FORWARD AND RECOMMENDATIONS

Overall, crop simulation models (CSM) and climate scenarios provided a monitoring and surveillance system to identify climate trends and associated impacts on intercropped maize landrace and bambara groundnut yield and WUE. In this regard, the use of a CSM driven by climate projections from six GCMs provided an opportunity to assess the suitability and sustainability of intercropping traditional crops as a potential climate adaptation strategy under low input–low output production systems. Our study demonstrated that the availability of a range of GCM outputs provided useful indications of the potential magnitude of yield and WUE changes and the temporal variation that could occur for the intercrop system. This type of analysis was, therefore, helpful in improving our understanding of the type of climate risk on the maize landrace and bambara groundnut intercrop system (**Table 5**). We recommend that the use of a CSM with GCM output should be considered when assessing the applicability of agricultural adaptation strategy.

Our results further showed that, at present, functional crop diversity could enhance crop productivity, stability, and thus food security, through efficient water utilization. Also, the adoption of asynchronous or sequential planting and moderating plant populations of either maize landrace or bambara groundnut can be viewed as a low-cost option to improve productivity and WUE under increasing temperature. This allows for the identification of short, medium and long-term strategies to aid in mitigating the impacts of climate change on the productivity and WUE of maize landrace and bambara groundnut intercrop system (**Table 5**). However, these approaches do not represent the diversity and breadth of adaptation strategies that can be adopted by marginal farmers.

To better represent adaptation, there is a need to expand the research to consider other management strategies (e.g., other traditional crop species, different cropping sequences, fertilization, rainwater harvesting and soil conservation techniques) (Seyoum et al., 2017). In addition, more system (agroecosystem) and place-based approaches that can represent local context, knowledge and aspects of food and nutrition security other than availability (e.g., nutrition, access, utilization and stability) may be required (Beveridge et al., 2018). To increase the contribution of agriculture to improving food and nutrition security, poverty reduction, and enhance rural economic development, climate impact modeling studies should be coupled with social, economic and environmental system models. This will ensure that traditional crops and associated cropping systems are assessed in a holistic manner that informs their sustainable integration into existing cropping systems. However, the adoption of traditional crops and intercropping should not be viewed as a panacea to solve all climate adaptation challenges, nor is it the only adaptation strategy. The inclusion of traditional crops into cropping systems should be considered as a complementary strategy to increasing climate resilience in marginal cropping systems.

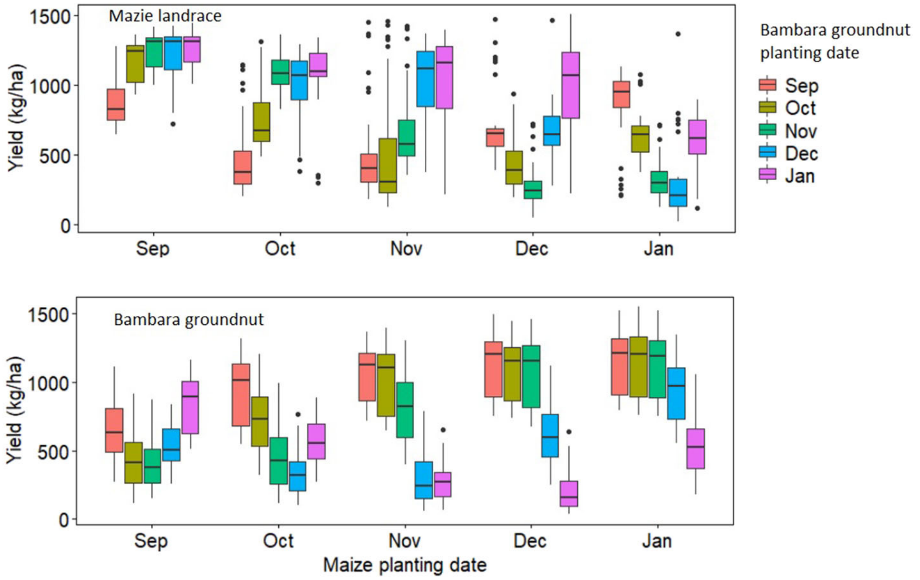

FIGURE 10 | Simulated yield for maize landrace and bambara groundnut across different planting date combinations under rainfed conditions obtained from six GCMs (ACC, CSS, CNR, GFD, NOR, and MPI). The x-axis represents the maize landrace planting dates and the colored boxplots represent the planting date for bambara groundnut. The effect of the interaction between maize landrace planting date and bambara groundnut planting date on maize landrace grain yield - *F*-statistic: 49.93 on 24 and 875 DF, $P = 0.000$; The effect of the interaction between maize landrace planting date and bambara groundnut planting date on bambara groundnut grain yield - *F*-statistic: 75.37 on 24 and 875 DF, $P = 0.000$.

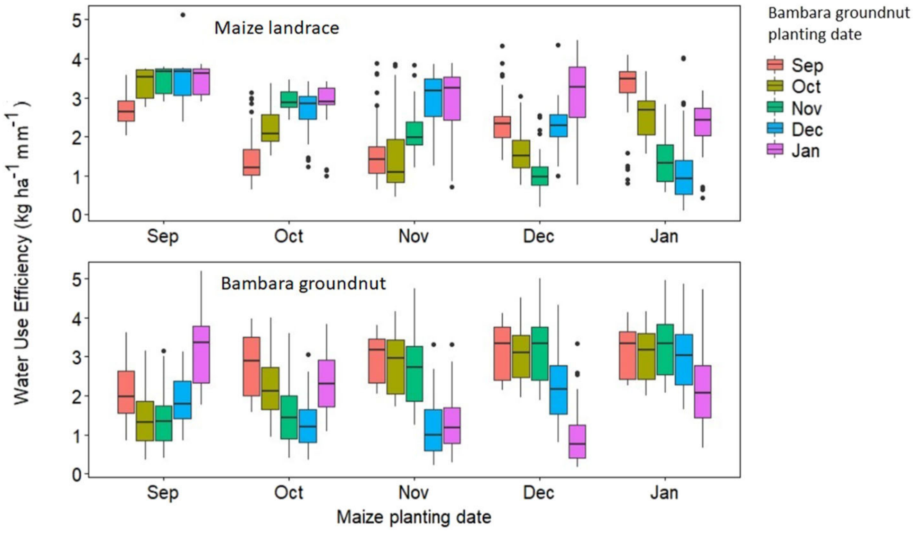

FIGURE 11 | Calculated water use efficiency (kg ha^{-1} mm^{-1}) for maize and bambara groundnut across different planting date combinations under rainfed conditions obtained from six GCMs (ACC, CSS, CNR, GFD, NOR, and MPI). The x-axis represents the maize landrace planting dates and the colored boxplots represent the planting date for bambara groundnut. The effect of the interaction between maize landrace planting date and bambara groundnut planting date on maize landrace WUE - *F*-statistic: 38.93 on 24 and 875 DF, $P = 0.000$ and The effect of the interaction between maize landrace planting date and bambara groundnut planting date on maize landrace WUE - *F*-statistic: 35.16 on 24 and 875 DF, $P = 0.000$.

A gap between the potential and practical realization of adaptation exists, and the evidence from our study supports the view that adaptation strategies need to be both climate-informed and context-specific to be viable (Beveridge et al., 2018; Carter et al., 2018). The cultivation of traditional crops has been done for millennia; however, to our knowledge, no study has quantified the yield and WUE responses in an intercrop system and under the impacts of climate change. Further to this, the FAO guidelines and key questions provided a useful framework to contextualize the observed results in an

TABLE 5 | FAO guidelines and key questions for assessing the impacts of adaptation strategy.

FAO Guideline Question	Key Findings	Comments	Implication
How can CSMs and climate scenarios assist in articulating decision windows?	• They provided a monitoring and surveillance system to identify short-, medium- and long-term climate trends and associated impacts on intercropped maize landrace and bambara groundnut yield and WUE • Data and trends on climate indicators allowed for the identification of possible responses to increasing system resilience	• By late century, there will be an increase in temperature and ET_0, while rainfall remains somewhat unchanged • Maize landrace yield responses are in line with rainfall trends • Bambara groundnut yield and WUE will be negatively impacted by increasing temperature • Adopting "better bet" management options in bambara can mitigate the projected impacts of climate change and improve the overall performance of the intercrop system	• Useful for improving understanding of climate risk and impacts • Useful in building the resilience of smallholder farming systems to possible impacts of climate change • For low input–low output systems, the adoption of traditional crops has the potential to support positive transformative adaptation to climate change
What are the likely short-, medium-, and long-term climate change impacts and risks for agriculture? How does risk shift further into the future?	• *Short-term:* an increase in yield variability resulting in increases in yield gaps • *Medium-term:* increases in climate risk will increase competition for resources within the intercrop system • *Long-term*: reduction in water availability through increases in temperature and evaporative demand	• *Short-term:* Use of adaptable crop species and cropping systems can reduce yield minima in marginal systems • *Medium-term:* reducing competition of resources within intercrop through enhanced niche differentiation • *Long-term*: There is a need to reduce the unproductive loss of water	• *Short-term*: intercropping maize landrace and bambara groundnut under recommended guidelines will improve overall system productivity and WUE relative to corresponding monocrop systems • *Medium-term:* adopt asynchronous or sequential planting to reduce competition within the intercrop systems • *Long-term:* adopt rainwater harvesting and soil water conservation strategies to enhance soil water capture, storage and minimize unproductive loss of soil water
Which of these interventions are likely to stand the test of time rather than becoming obsolete?	• Intercropping maize landrace at low plant population and bambara groundnut at high population can sustainably improve yield and WUE of the system under projected climate change • Early planting improves yield and WUE of maize landrace and bambara groundnut intercrop system under projected climate change • Planting bambara groundnut 2 months earlier than maize landrace can minimize resource competition and enhance productivity	• Manipulating planting densities and dates can aid in maintaining the competitive balance within an intercrop system	• Sequential cropping in rainfed systems may be constrained by the length of the growing period • Good agronomy can result in high yield and WUE

informative manner (**Table 5**) and less prescriptive. With the impacts of climate variability and change, our results provide evidence that adapting agronomic management could allow for sustainable intensification of the traditional systems through improved resource use efficiencies. However, we acknowledge that this type of study should be repeated across other agro-ecologies different from that of Ukulinga, allowing for more robust crop management practices and adaptation strategies to be identified.

The calibration and validation process concerning the APSIM maize landrace and bambara groundnut intercrop study was the first attempt to evaluate the impacts of climate change on growth and water use. In this study, data to calibrate and validate the model were obtained from irrigated and rainfed experiments, respectively, in the same growing season. This may not fully meet strict requirements for using an independent data set for model validation. Therefore, future studies should repeat the experiment across various agro-ecologies and time scales different from that of the calibration data set; this will allow for better validation of model performance and robustness.

CONCLUSIONS

There is a high probability that yield and WUE for intercropped bambara groundnut will decrease in the near to distant future if current management options are maintained. Assuming future rainfall remains mostly unchanged, the primary limitations to intercropped bambara groundnut yield and WUE will be temperature and ET_o under minimal rainfall changes. However, projected changes in temperature and ET_o will increase yield and WUE variability for a maize landrace and bambara groundnut intercrop system. Improving WU, through increased plant density or asynchronous planting of the maize landrace and bambara groundnut mitigated the negative impacts of climate change on yield and WUE. In this regard, optimum plant management can optimize traditional production systems. Thus, intercrop systems of maize landrace and bambara groundnut should be promoted as a potential future system for climate change adaptation in rainfed production systems.

While the results of these simulations are limited to one agro-ecology and a single intercrop system, the findings confirm the views that several traditional crops are drought tolerant and thus, are suitable for cultivation in marginal agricultural production areas. Furthermore, intercropping them can increase system resilience under climate change. The concept of WUE, among other parameters, has been suggested in selecting management options that can sustainably increase productivity under climate changes, heat and water stress, and interactions among them.

Intercropping maize landraces and bambara groundnut with the appropriate place-based management practices can be used as an adaptation strategy in environments that are projected to face increasing water scarcity. Reduced land and water demand from intercropping maize landraces and bambara groundnut and improved water use efficiencies mitigate the risks associated with increasing climate variability and extreme events such as drought. For resource-poor farmers that are inherently risk-averse, the production of traditional crops such as maize landraces and bambara groundnut, and their optimisation through inexpensive management strategies present an opportunity to build resilient cropping systems. Our results have important implications on how traditional crops and cropping systems should be viewed, in that their incorporation into marginal production systems can be an alternative adaptation strategy that may lead to sustainable intensification outcomes under increasing climate risk.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

VC and TM: conceptualization, methodology, and writing—draft preparation. VC: investigation and data analysis. TM: resources and project administration. VC, EW, and RK: data curation. VC, EW, TM, and RK: writing—review and editing. TM and AM: funding acquisition. All authors: contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2020.562568/full#supplementary-material

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Nitrogen-Fertilized Systems of Maize Intercropped With Tropical Grasses for Enhanced Yields and Estimated Land Use and Meat Production

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Intercropping grain with forage crops bridges the gap between agriculture and sustainability. In tropical regions, forage grasses are increasingly being adopted as winter pasture intercropped and in rotation with maize to maximize food production. However, current recommendations for nitrogen (N) fertilizer application are based on monocropped maize (*Zea mays*), and the best N management approach for intercropping systems remains unclear. A field experiment was carried out in three growing seasons with three intercropping systems [monoculture maize, intercropped with palisadegrass (*Urochloa brizantha*), and intercropped with guineagrass (*Megathyrus maximus*)] combined with six different split applications of N to maize (0–0, 100–0, 70–30, 50–50, 30–70, and 0–100 kg N ha^{-1} at seeding-sidedressing) with four replicates. We measured dry matter (DM) and accumulated N in maize and forage grasses, as well as maize production components and yields. Additionally, land equivalent ratio, relative crowding coefficient, aggressivity of maize with forage grasses, forage crude protein (CP) concentration, estimated animal stocking rate, and estimated meat production and economic outcomes. Greatest maize yield was 8.7 Mg ha^{-1} for monocropped maize. However, favorable maize yield was also obtained in intercropping systems. Although no difference was observed between intercropping systems, applying all N at sidedressing of maize negatively affected maize and forage yields and, consequently, land use and economic evaluation. For both intercropping systems, estimated meat and land use were 114 and 10% higher when N fertilizer was applied than the control (0–0 kg N ha^{-1}), on average. Maize-forage grass intercropping is a viable alternative production system for improving yields and land use. In addition, estimated meat production and revenue can be enhanced with palisadegrass or guineagrass. At least half of the N fertilizer must be applied early in the growing season of maize to maximize production of the entire system.

Keywords: *Brachiaria brizantha*, *Megathyrsus maximus*, *Zea mays* L., tropical agriculture, intercropping grasses, no-tillage system

INTRODUCTION

Intensive use of agricultural land is a global concern. The challenge of agricultural systems is to increase crop and food production, while reducing land use. A new commercial practice of intercropping grain and forage crops bridges the gap between agriculture and environmental sustainability (Mateus et al., 2016; Martin-Guay et al., 2018). Additionally, fertilizer management, such as N application, benefits these intercropping systems by enhancing yields and minimizing plant competition. However, the appropriate time for N management in intercropping systems remains incompletely studied. A new approach of maize-grass intercropping systems for crop and meat production has been suggested since intercropping with forages results in yield improvements and in satisfactory socioeconomic outcomes for integrated crop-livestock systems (ICLS) with a no-tillage system (NTS) (Derpsch and Friedrich, 2009; Himmelstein et al., 2017; Pariz et al., 2017a).

Intercropping tropical forages and cash crops is an alternative for farmers to develop temporary pasture using ICLS combined with NTS (Pariz et al., 2017b). With ICLS, food production potential (meat and grains) can be enhanced on the same land area and thus limit deforestation of new agricultural areas (Moraes et al., 2019). In the tropical region, forage grasses are being increasingly adopted in ICLS under NTS for winter pasture to maximize system production (Crusciol et al., 2015; Pariz et al., 2016, 2017a,b). Palisadegrass [*Urochloa brizantha* (=syn. *Brachiaria*)] and guineagrass [*Megathyrsus maximus* (=syn. *Panicum maximum*)] has been suitable species for intercropping with cash crops (Costa et al., 2015; Mateus et al., 2016; Pariz et al., 2016). Importantly, this strategy of intercropping forages with grain crops enhances the success of forage production in the dry winter season with low and irregular rainfall (Borghi et al., 2013a). Therefore, more reliable forage biomass production raises the protein concentration and potential meat production by animals grazing fodder in ICLS (Crusciol et al., 2012, 2014; Moraes et al., 2019).

Maize-forage grass intercropping has increased as cultivation practice (Sulc and Tracy, 2007; Tracy and Zhang, 2008; Moraes et al., 2019). Intercropping grasses with maize improves soil quality and increases soil organic C and N stocks by promoting deep root systems and better nutrient retention compared with monocrops (Costa et al., 2012, 2015; Cong et al., 2015). Because of the potential of cycling N from soil by plant N uptake and consequent high straw decomposition, providing diversity of residues, and nutrient back to the soil (Pariz et al., 2017b; Martin-Guay et al., 2018). However, maize and grass may compete for N sources at the vegetative growth stages in intercropping systems, since grasses can immobilize N by microbial processes and increase the dependence on N fertilizer for crop yields (Pariz et al., 2011; Mateus et al., 2016), especially during the first several years of cultivation in NTS with accumulation of soil organic matter. In addition, N demand by maize is high during early- to mid-season growth (Anghinoni, 2007; Borghi et al., 2014; Garcia et al., 2016).

Current recommendations for N application are based on maize monocropping (Cantarella et al., 1997), however, the N fertilizer recommendations for intercropping systems with maize-grasses have not been adequately studied for crop yields and meat production. Although agricultural models of possible N fertilizer application in ICLS based on N rates have been documented (Borghi et al., 2014; Mateus et al., 2020), there is a lack of information on how to achieve maximum potential of intercropping systems through fertilizer management. Nitrogen recommendations need to be tested based on suitable application timing. Managing N fertilizer in intercropping systems by dividing the rate into two application timings may promote greater N uptake and yield of maize, as has been shown for intercropped sorghum and forage grasses (Mateus et al., 2016). In addition, split N application may provide sufficient N for the high N demand of maize and forage, thus tightening the N cycle and minimizing environmental pollution.

While studies have shown reduction of soil erosion and degradation, stimulation of root growth and increase of forage yields in intercropping systems in relation to sole-cropping systems (Pariz et al., 2017b; Moraes et al., 2019), the potential food supply for livestock and farmers' profitability do not appear to have been investigated in maize-grasses intercropping systems with proper N management. The aim of this study was to evaluate the effects of split N application to maize-forage intercropping systems on crop yield, land equivalent ratio (LER), crop competition, estimated meat, and overall system revenue. We hypothesized that N management applied at maize seeding and at sidedressing of maize at V_5 growth stage, i.e., the initiation of maize ear development, would (i) increase yields of maize and forage grasses, (ii) increase efficiency land use and estimated meat production, (iii) decrease competition between intercropped crops, and (iv) provide high revenue. To test these hypotheses, we used the same N rate of 100 kg ha^{-1} divided into two applications (seeding + sidedressing) at different ratios for maize intercropped with palisadegrass and guineagrass.

MATERIALS AND METHODS

Site Description and Experimental Design

A field experiment was carried out during three growing seasons (2004–2005, 2005–2006, 2006–2007) in Botucatu, São Paulo, Brazil (48° 26′W, 22° 51′S, 740 m above sea level). The climate is Cwa, i.e., tropical with dry winter and warm, rainy summers, according to the Köppen classification. Mean annual precipitation is 1,358 mm and mean annual temperature is 20.7°C. Precipitation and temperature during the experiment are shown in **Figure 1**. The soil type was a clayey, kaolinitic, thermic Typic Haplorthox [United States Department of Agriculture (USDA), 2014] with 630, 90, and 280 g kg^{-1} of clay, silt, and sand, respectively. At the beginning of the experiment, selected chemical properties were determined according to methodology proposed by van Raij et al. (2001) and are shown in **Table 1**. The soil pH was determined in a 0.01 mol L^{-1} $CaCl_2$ suspension (1:2:5 soil:solution). Soil organic matter was determined by chromic acid digestion (Heanes, 1984). The total acidity at pH 7.0 (H+Al) was extracted by calcium acetate (0.5 mol L^{-1} at pH 7.0) and evaluated by titration with 0.025 mol L^{-1} NaOH solution. The available P and exchangeable basic cations (K^+, Ca^{2+}, and Mg^{2+})

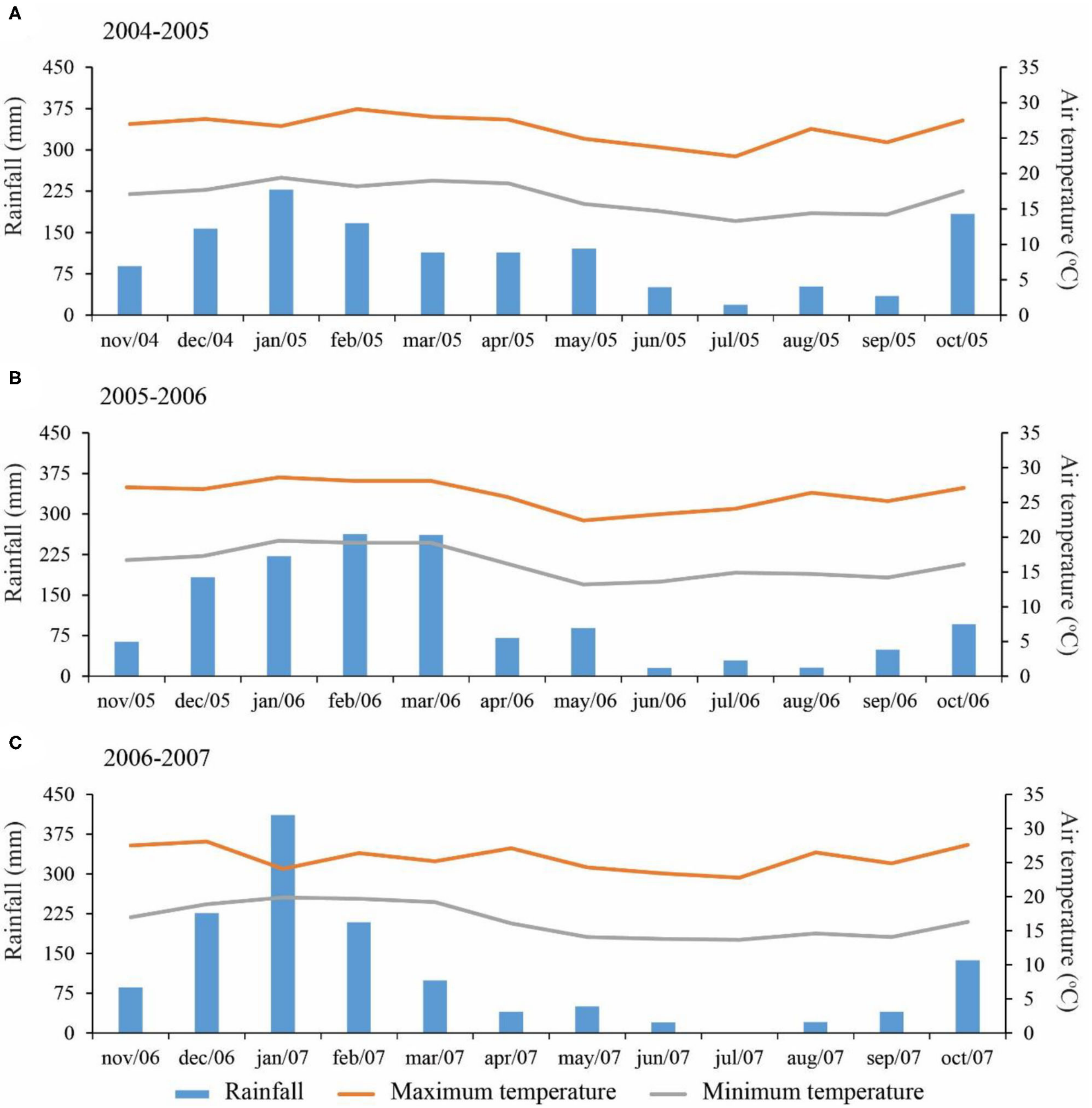

FIGURE 1 | Monthly rainfall, maximum and minimum temperatures during 2004–2005 **(A)**, 2005–2006 **(B)**, and 2006–2007 **(C)** growing seasons.

were extracted using an ion resin. The P_{resin} concentration was determined colorimetrically (Murphy and Riley, 1962) with a FEMTO 600S spectrophotometer. Exchangeable K^+, Ca^{2+}, and Mg^{2+} in the extracts were determined by an atomic absorption/flame-emission spectrophotometer (Shimadzu AA-6300). The cation exchange capacity (CEC) was obtained by summing the individual cations (H, Al, K, Ca, and Mg). The base saturation (BS) values were calculated using equivalents exchangeable bases and total acidity results (van Raij et al., 2001). The experimental area had been cropped under NTS since 1999 and the historical crop rotation is presented in **Supplementary Table 1**.

The experimental design was a randomized complete block with four replicates on different parcels of the same field each year. Treatments consisted of monocropped maize, maize intercropped with palisadegrass [*Urochloa brizantha* (Hochst. Ex A. Rich) R. Webster “Marandu”], and maize intercropped with guineagrass [*Megathyrsus maximus* (Jacq.) B. K. Simon and S.

TABLE 1 | Soil chemical characteristics at two depths in the experimental areas before initiating the experiment ($n = 8$).

Growing season	Depth	pH ($CaCl_2$)	SOM[†] (g dm^{-3})	$P_{(resin)}$ (mg dm^{-3})	H+Al ($mmol_c$ dm^{-3})	K^+ ($mmol_c$ dm^{-3})	Ca^{2+} ($mmol_c$ dm^{-3})	SO_4^{2-} ($mmol_c$ dm^{-3})	Mg^{2+} ($mmol_c$ dm^{-3})	CEC[‡] ($mmol_c$ dm^{-3})	BS[§] (%)
2004/2005	0.00–0.20 m	4.7	25	14	52	1.3	20	4.7	10	83	39
	0.20–0.40 m	4.4	22	8	76	0.7	15	9.8	8	99	24
2005/2006	0.00–0.20 m	4.5	24	14	49	1.7	19	5.1	9	82	39
	0.20–0.40 m	4.3	21	7	73	0.7	11	10.3	8	92	23
2006/2007	0.00–0.20 m	4.8	26	15	47	1.6	18	4.4	12	78	41
	0.20–0.40 m	4.6	24	9	66	1.0	14	9.5	9	90	27

[†] *Soil organic matter.*
[‡] *Cation exchange capacity.*
[§] *Base saturation.*

W. L. Jacobs "Mombaça"] factorially arranged with N applied at seeding and sidedressing of maize: (i) 0–0 (control), (ii) 100–0, (iii) 70–30, (iv) 50–50, (v) 30–70, and (vi) 0–100 kg N ha^{-1}, respectively (**Figures 2A,B**). The rate of 100 kg N ha^{-1} was based on current recommendation and studies in intercropping systems (Cantarella et al., 1997; Mateus et al., 2020). The relatively low rate aimed to reduce environmental impacts from N loss; however, there is currently no specific recommendation of N fertilizer application for intercropping systems.

Crop Management

Soil acidity was ameliorated with dolomite lime application over the soil surface, without soil incorporation. Lime rate was calculated to increase soil base saturation of the surface 0.20 m of soil to 70% (Cantarella et al., 1997) and was applied at concentrations of 3.05, 2.95, and 2.66 Mg ha^{-1} in August 2004, August 2005 and August 2006, respectively. Dolomitic lime consisted of 400 kg CaO ha^{-1} and 120 kg MgO ha^{-1}, with 85% effective calcium carbonate equivalence.

Pearl millet (*Pennisetum glaucum*) was sown on 2 Oct. 2004, 5 Oct. 2005, and 3 Oct. 2006 at 0.3-m depth using a no-till drill at a seed density of 20 kg ha^{-1} to produce crop residues for the ICLS prior to maize in a short-term cultivation. Pearl millet was terminated with glyphosate (1.8 kg ha^{-1} acid-equivalent), using a spray volume of 250 L ha^{-1} 20 days before maize sowing. Maize (hybrid 30F90) was sown on 15 Dec. 2004, 18 Dec. 2005 and 20 Dec. 2006 at a depth of 0.3 m and a density of 60,000 seeds ha^{-1} using a no-till drill. Each plot consisted of ten 20-m-long rows of maize and row spacing of 0.45 m. Sampling area was considered within a buffer zone of 0.45 m from the perimeter of each plot.

Baseline fertilization of maize in the sowing furrows consisted of 84 kg ha^{-1} P_2O_5 as triple superphosphate and 48 kg ha^{-1} K_2O as potassium chloride in a 08–28–16 NPK formula for all treatments. At seeding, N application treatments were applied as urea and distributed between 0.5 and 0.10 m next to the seed row by superficial broadcasting. For treatments with intercropping, palisadegrass and guineagrass were simultaneously sown with maize at densities of 15.3 and 15.9 kg ha^{-1} seed (34% viable seeds), respectively. Palisadegrass and guineagrass were mixed with fertilizer and sown at depths of 0.08 and 0.06 m below soil surface, respectively. Monocropped maize was sown at the same time using the same practices. In addition, monocropped palisade grass and guinea grass were seeded at the same time as the forages in intercropping systems using the same practices. The monocropped forages plots were the same size and were only used to calculate the intercropping competition factors.

Maize seedlings emerged 5 days after sowing (20 Dec. 2004, 23 Dec. 2005 and 25 Dec. 2006) and forage seedlings emerged 15 days after sowing, on average, for each growing season. Maize and forage were cultivated according to crop needs; atrazine [6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine] (1.0 kg ha^{-1} acid-equivalent) using a spray volume of 200 L ha^{-1} was applied to control emergence of annual broadleaf weeds, deltamethrin [(*S*)-cyano-(3-phenoxyphenyl)-methyl] (1*R*,3*R*)-3-(2,2-dibromoethenyl)-2,2-dimethyl-cyclopropane-1-carboxylate (5 g ha^{-1} active ingredient) was used against fall armyworm (*Spodoptera frugiperda*). Sidedress N fertilization was applied according to the treatments at V_5 maize growth stage (five expanded leaves). Physiological maturity averaged 128, 132, and 130 days after emergence in the 2004–2005, 2005–2006, and 2006–2007, respectively. Maize harvest was 7 days after physiological maturity using a mechanical harvester. Maize, palisadegrass and guineagrass were harvested separately from eight central rows.

Sampling and Analyses

When 50% of maize plants were in full flowering stage, 20 random leaf samples per plot were collected from the fourth leaf with visible sheath from the apex for nutrition diagnoses (Cantarella et al., 1997). Leaves were washed, dried in forced air circulation at 65°C for 72 h, ground, and N, P, K, Ca, Mg, and S concentrations in leaves were determined according to Malavolta et al. (1997). The samples were digested with sulfuric acid for N determination and with a nitro-perchloric solution for the other nutrients. The leaf N, P, S concentrations were determined by semi-micro-Kjeldahl distillation, colorimetry, and turbidmetry methods, respectively. The leaf K, Ca, and Mg concentrations were determined by atomic absorption spectrophotometry.

Kernel weight was determined and transformed to maize yield ha^{-1} by correcting to 13% grain moisture. Plant population was determined by counting the number of plants in the four central 5-m rows per plot at harvest. Number of ears per plant, number of

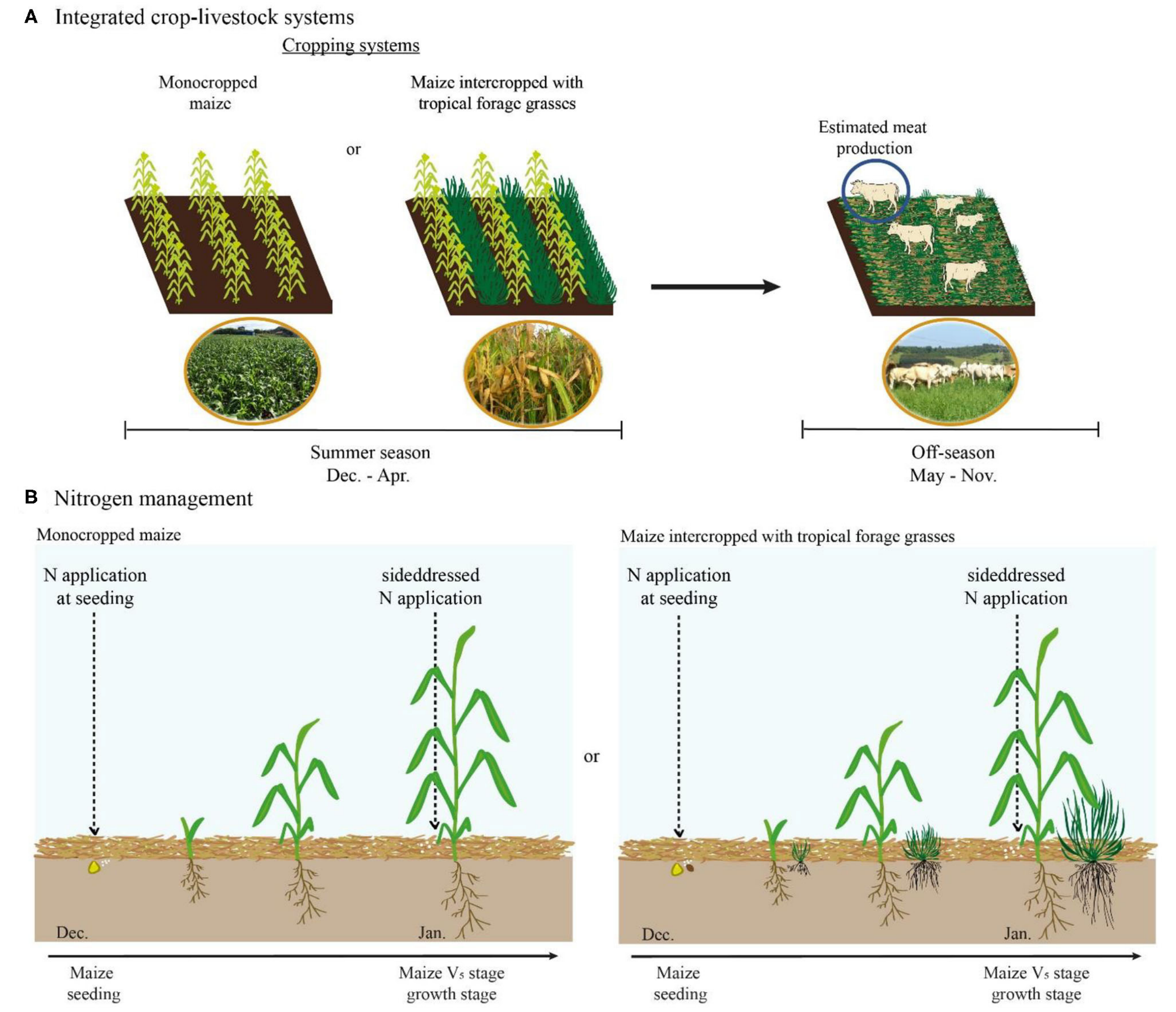

FIGURE 2 | (A) Scheme of cropping systems arrange in the summer season. Grazing by animals was not performed after maize harvest in off season and meat production was estimated using Large Ruminant Nutrition System model. **(B)** Timing of N application (seeding + sidedressing) in the monocrop and intercrop system in the summer season.

kernels per ear, and 100-kernel weight were determined at harvest and evaluated from 10 plants per plot chosen at random.

From the time of maize harvest, forage dry matter of palisadegrass and guineagrass were evaluated at 55 days (first cut) and 145 days (second cut), in June and September, respectively. Forages were cut at 0.25 m from the soil surface (2 m^2 area each area and row spacing = 0.45 m) and removed from the plots. The remainder of plots were cut using a manual mechanical rotary mower to provide faster forage regrowth. Forage dry matter was dried by forced-air circulation at 65°C for 72 h until constant weight, and weighed. Data were extrapolated to Mg ha^{-1}. A sub-sample of forage dry matter was used to determine total N concentration for crude protein (CP). CP was calculated by formula: CP (%) = total N (%) × 6.25 (Horwitz, 1980).

Intercropping Competition Factors

To study the competition effects between crops and to evaluate intercrop performance, different competition functions were calculated: land equivalent ratio (LER), relative crowding (K), and aggressivity index (A). The LER was used to evaluate the land use advantage provided by intercropping (Mead and Willey, 1980):

$$\text{LER} = \text{Y}1,2/\text{Y}1,1 + \text{Y}2,1/\text{Y}2,2$$

where Y is the aboveground biomass of crops, and suffixes 1 and 2 denote the crops: (1) maize and (2) palisadegrass or guineagrass. Therefore, $\text{Y}_{1,2}$ is the aboveground biomass of maize when grown in a mixture with grasses, $\text{Y}_{1,1}$ is the yield of maize when grown in a monoculture, $Y_{2,1}$ is the aboveground biomass of the forage (palisadegrass or guineagrass) when grown in a mixture with maize, and $Y_{2,2}$ is the aboveground biomass of the forage (palisadegrass or guineagrass produced 839 and 1,327 Mg ha^{-1}, respectively) when grown in a monoculture.

Relative crowding coefficient (*K* values) is a measure of plant competition theory as an index of the relative competitive abilities between plants in an intercropping system to evaluate and compare the competitive ability of one species to another in a mixture (Zhang et al., 2011). *K* was calculated according the method of Agegnehu et al. (2006) as follows:

$$(\mathrm{K})\mathrm{maize} = \mathrm{Y1,2} \times \mathrm{Z2,1} / (\mathrm{Y1,1} - \mathrm{Y1,2}) \times \mathrm{Z1,2} \text{ or}$$
$$(\mathrm{K})\mathrm{forage} = \mathrm{Y2,1} \times \mathrm{Z1,2} / (\mathrm{Y2,2} - \mathrm{Y2,1}) \times \mathrm{Z2,1}$$

where Y and suffixes 1 and 2 denote as described for LER, $Z_{1,2}$ is the sown proportion of maize, and $Z_{2,1}$ is the sown proportion of the forage species. For this calculation, the plant density of each species was evaluated on the day of maize harvest. Greater *K* value of one species indicates it is more competitive and dominant than another species in the intercropping system (Li et al., 1999; Wahla et al., 2009).

Aggressivity index (*A*) was calculated to determine relative yield of crop 1 with crop 2 in intercropping (Takim, 2012):

$$(\mathrm{A})\mathrm{maize} = (\mathrm{Y1,2/Y1,1Y1,2}) - (\mathrm{Y2,1/Y2,2Y2,1}) \text{ or}$$
$$(\mathrm{A})\mathrm{forage} = (\mathrm{Y2,1/Y2,2Y2,1}) - (\mathrm{Y1,2/Y1,1Y1,2})$$

where Y and the suffixes 1 and 2 denote the same as used in LER and (K). If $(\mathrm{A})_{\mathrm{maize}} = 0$, crops were equally competitive, if $(\mathrm{A})_{\mathrm{forage}}$ was negative, then maize dominated, if $(\mathrm{A})_{\mathrm{forage}}$ was positive, then forage dominated.

Economic Valuation and Estimated Meat Production

Production costs per hectare of monocropped maize and maize intercropped with forages were estimated (CONAB, 2018). Differences in input costs were forage seed and N fertilizer, as sowing maize monocrop and intercropped forage seeds were the same process. Maize grain yield (kg ha^{-1}) was calculated and multiplied by the value per kg.

Although grazing by animals was not carried out for the palisadegrass and guineagrass after maize grain harvest, meat production was calculated using Large Ruminant Nutrition System (LRNS; http://nutritionmodels.tamu.edu/lrns.html) model to estimate grazing performance by animals on tropical perennial grasses. The LRNS model is based on the Net Carbohydrate and Protein System (CNCPS), version 5 (Fox et al., 2004). Energy and protein requirements, performance and dry matter intake by each individual cattle fed in a group were predicted for continuously grazed 450 kg Nellore bulls with 52% carcass yield and 22% Body Fat Grading System. Performance values were predicted from the nutritional composition of palisadegrass and guineagrass and N fertilizer applied.

Dry matter intake by each individual cattle fed in a group was 9.9–10.0 kg of dry matter day^{-1}. Average daily gain (ADG) was used to estimate meat production based on the allowable metabolizable energy and protein gain, since CP of forage was 9.3–14.6%. A animal grazing time was calculated using a method similar to Crusciol et al. (2012), in which a 55 d forage accumulation period occurred after maize harvest followed by two 60-day grazing periods with a 30-day rest period in between grazing periods. Stocking rate was estimated from forage dry matter production, time of animal grazing (days per cut), dry matter intake, and grazing efficiency. Total cattle meat produced per hectare was calculated from stocking rate multiplied by the components of ADG, time of animal grazing, and carcass yield (52%).

Gross revenue ha^{-1} was calculated by the formula: (price per kg × maize yield) + (price per kg × estimated meat production). Net return per ha was calculated by the formula: (gross revenue – cost ha^{-1}). The Brazilian national average price used was from the last 5 years and values were converted to US$ (Agrolink, 2018).

Statistical Analyses

All data were initially tested for normality using the Shapiro-Wilk test from the UNIVARIATE procedure using the statistical software R (version 3.5.2) with the package "agricolae" (Mendiburu, 2015). All data were distributed normally ($W \geq 0.90$). Cropping systems, N management treatments, and their interactions were considered fixed effects. Growing season and its interaction with cropping systems and N managements were not significant at $P < 0.05$ for any of the dependent variables. Thus, data were combined across growing seasons. Block was considered a random variable. Analysis of variance (ANOVA) was performed and if the null hypothesis was rejected, means were compared using LSD teste ($P \leq 0.05$).

RESULTS

Plant Nutrition, Agronomic Characteristics, Kernels and Production Attributes of Maize

Monocropped maize and maize intercropped with palisadegrass had greater leaf N concentrations than maize intercropped with guineagrass (**Supplementary Table 2**). Maize intercropped with palisadegrass had greater leaf P, K, and S concentrations than monocropped maize and maize intercropped with guineagrass. Although the interaction of intercropping system and N management was not statistically significant for nutrient concentration, all intercropping systems that received N application had greater leaf N, P, and S concentrations than the control without N fertilizer (**Supplementary Table 2**).

Intercropping system did not influence plant population, ears per plant, kernels per ear, and 100-kernel weight (**Table 2**). However, monocropped maize had greater shoot dry matter and grain yield compared with intercropping systems of maize with palisadegrass and guineagrass. Time of N application did not influence plant population, but all treatments with some N application led to greater number of ears per plant, number of kernels per ear, 100-kernel weight, shoot dry matter, and grain yield of maize compared to the control without N application (**Table 2**). Shoot dry matter and grain yield of maize were greater in all N management systems with some N applied at seeding

TABLE 2 | Agronomic characteristics (plant population and number of ears per plant), kernels attributes (number of kernels per ear and 100-kernel weight) and production attributes (shoot dry matter and grain yield) of maize as affected by intercropping system, N management in the three growing seasons.

	Agronomic characteristics		Kernel attributes		Production attributes	
Treatment	**Plant population**	**Ears per plant**	**Kernels per ear**	**100-kernel weight**	**Shoot dry matter**	**Grain Yield**
	Thousand plants ha^{-1}	**no.**	**no.**	**g**	**Mg ha^{-1}**	**Mg ha^{-1}**
Intercropping system (IC)						
Monocropped maize	60.1 a[§]	1.13 a	403 a	32 a	17.6 a	8.7 a
Maize + palisadegrass	59.7 a	1.14 a	380 a	32 a	16.0 b	8.2 b
Maize + guineagrass	59.9 a	1.13 a	370 a	31 a	15.1 b	7.9 b
N management (NM)[‡]						
0–0	59.2 a	0.89 c	333 b	29 b	9.6 c	4.9 c
100–0	60.0 a	1.22 a	381 a	33 a	17.7 a	9.1 a
70–30	60.5 a	1.19 a	399 a	32 a	18.2 a	9.3 a
50–50	59.7 a	1.20 a	404 a	33 a	18.4 a	9.4 a
30–70	60.2 a	1.20 a	396 a	32 a	17.7 a	9.1 a
0–100	59.7 a	1.10 b	391 a	32 a	15.7 b	8.0 b
F *probability*						
IC	0.313	0.109	0.074	0.563	<0.001	<0.001
NM	0.549	<0.001	<0.001	<0.001	<0.001	<0.001
IC x NM	1.000	0.083	0.656	0.724	0.532	0.963

[§] *Values followed by the same letter are not significantly different at $P \leq 0.05$ (LSD test).*

[‡] *A rate of 100 kg N ha^{-1} applied in two-split management at maize seeding and V_5 growth stage sidedressing.*

(i.e., 100–0, 70–30, 50–50, and 30–70 kg N ha^{-1}) than with no N applied at seeding (i.e., 0–0 and 0–100 kg N ha^{-1}).

Forage Characteristics and Estimated Meat Production

Forage dry matter production, estimated animal stocking rate and estimated meat production were influenced by intercropping system in the second cut but not in the first cut (**Table 3**). Forage dry matter production in the second cut was 23% greater when maize was intercropped with palisadegrass than with guineagrass, although CP in the second cut was similar in the two intercropping systems. For both intercropping systems, forage dry matter production, CP, estimated animal stocking rate, and estimated meat production were greater when N fertilizer was applied than in the control (0–0 kg N ha-^{1}) in the first and second cuts.

Land Equivalent Ratio and Intercropping Competition Factors

All LER values of maize and forages were lower under intercropping compared with the respective monoculture (**Table 4**). When combined, LER of maize intercropped with palisadegrass (1.06 average) was more productive than each component separately when receiving N, independent of the split-N ratio. In contrast, LER of maize intercropped with guineagrass (0.95 average) was less productive than individual components grown separately for all N application conditions.

The intercropping competition factor (K) values shown in **Table 4** are the interspecific competitive abilities. Compared with the unfertilized control, K_{maize} and K_{forage} were greater for maize intercropped with both palisadegrass and guineagrass. The aggressivity index (A) showed that maize was less competitive than palisadegrass and guineagrass in all treatments. Maize was more competitive with guineagrass without N fertilizer application.

Revenue

Not supplying N fertilizer to cropping systems resulted in the lowest estimated net profit (**Table 5**). When supplying 100 kg N ha^{-1}, net profit was similar among the different split N applications, except when no N fertilizer was applied at seeding (0–100 kg N ha^{-1}), which had lower net profit compared to other treatments with N application. Intercropping maize with either forage grass had greater net profit compared to monocropped maize when supplied with N, because of significant meat production during the winter/spring.

DISCUSSION

Maize and Tropical Forage Grass Responses

Our study provides a novel alternative identifying potential agricultural systems to improve food production by intercropping maize with forage grasses and selecting the proper N management. Currently, recommendations for N fertilizer application consider only monocropping (maize or forage grass) in the summer/fall or fodder in the winter/spring for grain production (Cantarella et al., 1997). Our study shows that split N application timing can increase responses of both maize and forage grasses, while meeting grain crop requirements and high

TABLE 3 | Forage dry matter (DM) production and crude protein (CP) concentration, estimated animal stocking rate, and estimated meat production as affected by intercropping systems and N management in three growing seasons and ANOVA significance.

Treatment	Forage DM (Mg ha^{-1})		Crude protein (%)		Stocking rate (AU ha^{-1})[¶]		Meat production (kg ha^{-1})[Ψ]	
	First cut[†]	Second cut[†]	First cut	Second cut	First cut	Second cut	First cut	Second cut
Intercropping system (IC)								
Maize + palisadegrass	2.2 a[§]	5.8 a	12.5 a	12.8 a	2.2 a	5.8 a	53.1 a	140.5 a
Maize + guineagrass	2.1 a	4.7 b	12.6 a	13.4 a	2.1 a	4.7 b	49.5 a	118.4 b
N management (NM)[‡]								
0–0	1.4 b	3.1 b	11.2 b	10.1 b	1.4 b	5.7 a	30.0 b	59.0 b
100–0	2.3 a	5.7 a	12.8 a	13.8 a	2.3 a	5.8 a	55.7 a	145.2 a
70–30	2.4 a	5.8 a	12.9 a	13.9 a	2.4 a	5.7 a	57.0 a	147.6 a
50–50	2.3 a	5.7 a	12.5 a	13.7 a	2.3 a	5.7 a	54.5 a	140.0 a
30–70	2.3 a	5.7 a	12.8 a	13.8 a	2.3 a	5.6 a	56.3 a	144.5 a
0–100	2.3 a	5.6 a	12.7 a	13.7 a	2.3 a	5.7 a	54.3 a	140.5 a
F *probability*								
IC	0.148	<0.001	0.127	0.081	0.198	<0.001	0.102	<0.001
NM	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IC x NM	0.263	0.222	0.387	0.155	0.265	0.175	0.168	0.365

[†] *First and second cut in June and September, respectively.*

[§] *Values followed by the same letter are not significantly different at $P \leq 0.05$ (LSD test).*

[‡] *A rate of 100 kg N ha^{-1} applied in two-split management at maize seeding and V_5 growth stage sidedressing.*

[¶] *1 AU (animal unit) = 450 kg of body weight.*

[Ψ] *Estimated meat production = kg of body weight gain (cattle) per ha (estimated) × 52% of carcass yield.*

TABLE 4 | Land equivalent ratio (LER), relative crowding coefficient (*K*), and aggressivity (*A*) of maize, palisadegrass, and guineagrass intercropped as a function of N fertilizer applied for maize crop.

Treatment	LER			*K*		*A*	
	Maize[†]	Forage[‡]	Total[‡]	Maize	Forage	Maize	Forage
Maize ± palisadegrass							
0–0[Ψ]	0.86[§]	0.09	0.95	4.00	0.15	−0.0002994	0.0002994
100–0	0.91	0.16	1.06	6.93	0.27	−0.0007091	0.0007091
70–30	0.91	0.16	1.07	7.27	0.27	−0.0007196	0.0007196
50–50	0.91	0.15	1.06	7.30	0.26	−0.0007247	0.0007247
30–70	0.91	0.16	1.07	7.34	0.26	−0.0007070	0.0007070
100–0	0.91	0.14	1.05	7.24	0.24	−0.0006394	0.0006394
Maize ± guineagrass							
0 kg N ha^{-1}	0.82	0.06	0.88	3.19	0.09	−0.0001518	0.0001518
100–0	0.86	0.09	0.95	4.13	0.15	−0.0002579	0.0002579
70–30	0.86	0.09	0.95	4.39	0.14	−0.0002684	0.0002684
50–50	0.86	0.09	0.95	4.51	0.14	−0.0002735	0.0002735
30–70	0.86	0.09	0.95	4.24	0.15	−0.0002558	0.0002558
100–0	0.86	0.10	0.96	4.23	0.15	−0.0001883	0.0001883

[Ψ] *First value means the kg N ha^{-1} applied at seeding and the second value means the kg ha^{-1} applied sidedressing at maize V_6 growth.*

[†] *Relative to respective monoculture.*

[‡] *Relative to respective intercropping system.*

[§] *Value above 1 means positive impact.*

N demand of both crops. Furthermore, this research provides as a novel outcome that intercropping systems combined with fertilizer N management showed effectiveness in improving the overall productivity of the whole system, especially for enhancing meat production and revenue for farmers.

Competition between forage and maize may have been reduced in this study due to the relatively long growing season with the 130-day maturity maize hybrid (Crusciol et al., 2013). Sowing plants with earlier relative maturity may benefit an intercropping system and decrease the competition between

TABLE 5 | Economic evaluation of monocropped maize, maize intercropped with palisadegrass and maize intercropped with guineagrass as a function of N management for maize (average of three growing seasons).

Treatment	Cost[Ω]	CY[¥]	Total maize[§]	Meat production[α]	Total meat[¶]	Gross[†]	Net[‡]
	US$ ha^{-1}	Mg ha^{-1}	US$ ha^{-1}	kg ha^{-1}	US$ ha^{-1}	US$ ha^{-1}	US$ ha^{-1}
Monocropped maize							
0–0	604	5.3	1,081	0	0	1,081	477
100–0	643	9.5	1,937	0	0	1,937	1,294
70-30	643	9.7	1,978	0	0	1,978	1,335
50-50	643	9.8	1,998	0	0	1,998	1,355
30–70	643	9.5	1,937	0	0	1,937	1,294
0–100	643	8.4	1,713	0	0	1,713	1,070
Maize ± palisadegrass							
0–0	626	4.7	958	91	272	1,230	604
100–0	666	9.1	1,856	214	643	2,499	1,833
70–30	666	9.3	1,896	221	664	2,560	1,894
50–50	666	9.4	1,917	206	618	2,535	1,869
30–70	666	9.1	1,856	218	654	2,510	1,844
0–100	666	8.0	1,631	211	634	2,265	1,599
Maize ± guineagrass							
0–0	628	4.7	958	87	262	1,220	592
100–0	667	8.7	1,774	187	562	2,336	1,669
70–30	667	8.9	1,815	188	563	2,378	1,711
50–50	667	9.0	1,835	183	548	2,383	1,716
30–70	667	8.7	1,774	184	551	2,325	1,658
0–100	667	7.7	1,570	178	534	2,104	1,437

[Ω] *Mean costs and production costs of monocropped maize and maize intercropped with palisadegrass or guineagrass; the only difference was the forage seeds cost and sidedress nitrogen used for the maize crop.*

[¥] *CY is the maize yield.*

[§] *Total = kg of maize ha^{-1} × US$ 0.20.*

[α] *Meat production = kg of body weight gain (cattle) ha^{-1} (estimate) × 52% of carcass yield (sum of EMP First and Second cuts).*

[¶] *Total meat = meat production × US$ 3.00.*

[†] *Gross is the revenue per ha, which was calculated using the formula: total maize + total meat.*

[‡] *Net is the return per ha, which was calculated using the formula (gross ha^{-1}-cost ha^{-1}).*

species (Pariz et al., 2009; Crusciol et al., 2013). Although we observed high N uptake by monocropped maize, other studies have shown that intercropping systems with forage grasses do not impair N uptake by crops (Crusciol et al., 2011; Mateus et al., 2012; Borghi et al., 2013b). Another important finding of our study is evidence of high N demand, as the leaf N concentration was below the appropriate range for maize (27–35 g N kg^{-1}) (Cantarella et al., 1997) when there was no N application (control) for all intercropping systems and even for monocropped maize. Despite differences between intercropping systems, maize was adequately nourished in all treatments. Maize leaf concentrations of P, K, Ca, Mg, and S were within ranges considered adequate (Cantarella et al., 1997), and no nutrition problems were observed.

Although no differences in agronomic characteristics and kernel attributes were observed between monoculture and intercropping systems, greater shoot dry matter and grain yield of maize were observed in monocropped maize compared with the other treatments. The lack of competition with tropical forage grasses positively affected maize development and did not appear to limit dry matter in the early growth stages, which were characterized by high N uptake, implying high efficiency in intercepting photosynthetically active radiation (Amaral Filho et al., 2005; Sawyer et al., 2010). Grain yield is positively linked to dry matter accumulation and the supply of N and C to kernels (Kowles and Phillips, 1988). Previous studies have shown that intercropping maize/sorghum with palisadegrass/guineagrass did not affect grain yield or create better conditions for improving sorghum yield (Barducci et al., 2009; Borghi et al., 2013b), which may have been related to lower nutrient demand and difference in crop hybrid.

Shoot dry matter and grain yield of maize were strongly related to timing of N fertilizer in the cropping system. Lowest maize yield was a result of insufficient N supply to maize. Grass-grass rotation without N fertilizer addition can result in significant N immobilization via competition between plants and microorganisms (Schimel and Bennett, 2004; Kuzyakov and Xu, 2013). Introduction of legumes in the crop rotation can enhance soil N availability with NTS (Boddey et al., 2010). However, cultivation of forage grasses is well-established among farmers

(Moraes et al., 2019), and C4 grass residues are more favorable in long-term protection and coverage of the soil under tropical drought conditions than C3 residues due to slower residue decomposition rate (Mateus et al., 2016; Rosolem et al., 2017).

Nitrogen fertilizer application provided an average maize yield of 8.3 Mg ha^{-1}. While N addition increased maize yield, applying no N fertilizer at seeding (0–100 kg N ha^{-1} applied at maize seeding and sidedressing, respectively) resulted in the lowest ears per plant, shoot dry matter, and grain yield among cropping systems receiving N fertilizer. Similar reductions in maize yield under delayed application of N fertilizer or 100% application from maize growth stages V_6-V_{11} under monocropping have been reported previously (Scharf et al., 2002; Walsh et al., 2012; Muller et al., 2017). Applying the total N rate (100 kg N ha^{-1}) at sidedressing did not match optimum N uptake capabilities of maize, because significant N supply is needed during early growth stages. Adequate maize development and N accumulation in the plant are closely associated with metabolism of soluble protein and sugar utilization (Faleiros et al., 1996). Thus, a portion of the N fertilizer must be applied at maize seeding in this NTS with grass cover crop to achieve high yield potential. There was no difference in maize yield or forage characteristics whether application of N was all at maize seeding or split between seeding + sidedressing.

No difference in forage dry matter production between palisadegrass and guineagrass in the first cut may have been related to climate conditions. Low forage growth (2.1 Mg ha^{-1}) occurred with low rainfall and temperatures between 10 and 15°C in early winter (Mateus et al., 2016). For the second cut, climate conditions could also explain the 23% increase in dry matter for palisadegrass compared with guineagrass. Temperature increased and stimulated the production of forage biomass, apparently with a greater effect on palisadegrass. In general, greater values of estimated animal stocking rate and estimated meat production were obtained with greater forage dry matter production.

Production of dry matter for forage of up to 4 Mg ha^{-1} is considered good (Borghi et al., 2013a) and was achieved in the second cut, even though air temperature was not ideal for forage development (i.e., 30–35°C) (Costa et al., 2005). Furthermore, there was no effect of forage species in the intercropping system on CP in the first and second cuts. CP is an important parameter of nutritive value. Forage CP averaged 125 g kg^{-1}, which was more than adequate of the 70 g kg^{-1} minimum required for maintaining rumen microbial efficiency in cattle (van Soest, 1994).

Forage dry matter and CP were at highest levels as long as N was applied, irrespective of timing and split N ratio. As expected, grasses responded to N fertilizer because of high N demand (Boddey et al., 1996; Mateus et al., 2016). The N fertilizer rate of 100 kg ha^{-1} was considered relatively low for complex intercropping systems, but was compatible with our study's focus on finding an efficient N management strategy for enhancing productivity in a sustainable manner. Indeed, we observed greater maize yield and forage dry matter production compared to other studies with application rates of <100 kg N ha^{-1} (Mateus et al., 2016; Rosolem et al., 2017).

Land Use Efficiency, Intercropping Competition Factors and Economics

Based on observed yields, LER of maize intercropped with palisadegrass was 1.06. The LER indicates the productivity of land with intercropping relative to sole cropping on separate parcels of land. The value of 1.06 indicated that 6% less land would be needed to achieve the same yield as monocropped maize and palisadegrass separately. These results are in line with those of Meixiua et al. (2020), who found that the average LER in grass/grass (maize/wheat) intercropping was 1.59. Likewise, Pariz et al. (2017b) found that the average LER of maize/palisadegrass intercropping was 1.10. However, maize intercropping with palisadegrass without N application or any intercropping of maize with guineagrass resulted in LER <1, reflecting lower productivity of land use. Our study suggests that land saving potential for food production systems in tropical soil can only be obtained in maize-palisadegrass intercropping systems with N management, independent of the type of split-N application.

Maize was more competitive (K values) than the forage species; however, the dominant species in the system were forage grasses due to their aggressiveness. The K values of maize were greater than those of the forage species, in agreement with Zarochentseva (2012) and possibly due to the shading effect of maize on forage grass during maize development. Our results showed that maize was able to acquire more resources in the intercropping systems even though the forage grasses were the dominant species.

For LER and K values of intercropping competition, addition of N fertilizer was necessary to enhance competitiveness of maize. Nitrogen management promoted the competitiveness of maize and forage grasses by increasing vegetative growth and providing greater capacity for utilizing limited availability of water (Marschner, 2012; Yang and Udvardi, 2018). Previous studies have shown significant differences among crops in grass/grass intercropping systems, but not among different types of N addition (O'Leary and Smith, 1999; Baxevanos et al., 2017). When a species has high competitiveness, the plant acquires more resources and occupies a superior ecological niche (Grace and Tilman, 1990). In addition, the A index values were extremely low for all treatments, indicating a minimum dominance by forage grasses. These findings highlight the necessity of choosing suitable species for intercropping in maize-forage grass systems to enhance the interspecific complementarity and reduce interspecies competition (Davis and Woolley, 1993).

Intercropping is a sustainable practice of food production to improve quality of pastures and animal carrying capacity. Our results demonstrated that intercropping of tropical forage grasses with maize using NTS is a feasible option for increasing sustainability in tropical areas and can result in higher revenues for farmers due to the productive, economic, and environmental benefits of these systems. Furthermore, these systems can increase global food production from the same land area (Carvalho et al., 2010; FAO—Food and Agriculture Organization of the United Nations, 2010, 2017; Herrero et al., 2010; Franzluebbers and Stuedemann, 2014; Moraes et al., 2019). Therefore, our data indicated that maize intercropped with palisade or guineagrass is a promising approach for farmers, especially in the tropical regions of South America, Africa, and parts of Asia, where individuals need additional opportunities to produce food.

All treatments resulted in net profit, particularly the maize + palisadegrass and maize + guineagrass treatments with N management, because in addition to maize yield in the summer/autumn, farmers can use the forage dry matter production of palisade and guineagrass (**Table 3**) for animal fodder in the winter/spring. Thus, with maize intercropping, farmers could produce 87–218 kg ha^{-1} meat, with net profits up to US$ 1,600–1,800 ha^{-1}, depending of N management, which could add an extra US$ 500–600 than monocropped maize. In addition, the need for soil mulch would be satisfied in planning for the next crop.

Overall, our data suggest that intercropping systems are a great option for the diversification on farm and the increase of grain and forage yields. However, these agricultural systems deserve further investigations to assess the disadvantages and impacts of N fertilizer. Our study raises relevant questions about which changes occur in root systems and soil fertility and microbiology in deeper soil layers, as well as the reduction in diseases and pesticide applications, in maize-forage systems receiving N fertilizer, and the effects of fertilizer on N losses to environment and N recovery by plants in short- and long-term of intercropping systems.

CONCLUSION

Intercropping maize with forage grasses is a promising practice to meet the dual challenges of food production and sustainable development. Since agricultural systems are region- and soil-specific, variations of intercropping systems may require different N fertilization recommendations. Although monocropped maize produced greatest grain yield, intercropping systems were viable in terms of balanced grain and forage yields, land use, and profitability. Estimated meat production and revenue were enhanced with intercropping of palisadegrass or guineagrass with maize. Combining animal production with crop production in an intercropping system can be advantageous not only for farmers, but also for environmental quality and biological diversity of plants and soil microorganisms. However, N fertilizer application in these systems is still necessary for maize yields and profitability. At least a portion of total N input should be applied at seeding and the remainder at sidedressing of maize. Application of all N fertilizer at sidedressing was not a productive practice since in this study with pearl millet as previous cover crop under NTS, as it reduced maize yield and revenue. Future studies should examine biodiversity improvements in the soil-plant-microorganism interactions and the negative impacts of N losses and nitrous oxide gasses release to the environment in the short- and long-term intercropping systems.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

CACC, GM, CC, and HC designed the experiment. CACC, GM, EB, and JC obtained and processed the data. CACC, LM, CP, AC, and AF analyzed the data. CACC, LM, and CP wrote the paper, with contribution of all co-authors. All authors confirms being contributor of this work and has approved it for publication.

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SUPPLEMENTARY MATERIAL

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Enhancing Climate Resilience of Rain-Fed Potato Through Legume Intercropping and Silicon Application

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A large portion of sub-Saharan Africa is situated in belts of uncertain rainfall and are characterized by low soil fertility with limited capacity to adapt to and mitigate the impacts of climate change. A field study was conducted in the semi-humid potato-growing belt of Kenya to test the effect of legume intercropping and water soluble silicon (orthocilicic acid) on soil erosion, and use efficiency of light and water. Potato (*Solanum tuberosum* L.) was grown singly and intercropped with dolichos (*Lablab purpureus* L.) or hairy vetch (*Vicia sativa* L.). Each cropping system was subjected to granular water-soluble silicon (Si) amendment at two rates [2.5 kg Si ha^{-1} (+Si) vs. 0 kg Si ha^{-1} (–Si)]. Plants receiving Si maintained significantly higher ($p < 0.05$) percent relative leaf water content (62–89% vs. 52–72% in controls) and exhibited higher concentrations of proline (1.99–2.91 vs. 1–1.19 umol g^{-1}), soluble carbohydrates (28–59 vs. 10–28 umol g^{-1}) and electrolyte conductance (1,409–3,903 vs. 746–2,307 mS cm^{-1}). Legume intercropping enhanced groundcover establishment and reduced soil and nutrient losses by 45–80% compared with sole potato. Crop yields were 2–3-fold greater in intercropping relative to sole potato and were significantly greater in treatments subjected to Si application. Land equivalent ratios were above unity in intercropping but less than unity in sole potato, and were 8–20% increased by Si application. Use efficiency of water (5.99–9.09 Kg ha^{-1} m^{-3}) and light (1.98–2.98 g MJ^{-1}) were significantly greater under legume intercropping compared with sole potato (1.13–3.23 Kg ha^{-1} m^{-3} and 0.77–0.98 g MJ^{-1}, respectively) and increased with Si application. Integrative use of Si and legume intercropping presents the smallholder farmers an opportunity to increase productivity of potato while enhancing resource use efficiency and soil fertility in the semi-humid tropics.

Keywords: climate change adaptation, crop water productivity, legume intercropping, silicon, smallholder potato farmers, soil conservation

INTRODUCTION

Declining soil productivity and potato yield in sub-Saharan Africa (SSA) presents the need to develop more sustainable production systems. The average potato yield in this region is only 13 t ha^{-1} against potential yield of 40 t ha^{-1} (FAOSTAT, 2017; Gitari et al., 2018a). This situation is expected to deteriorate as climatic change is projected to lead to warmer and drier conditions with more variable and extreme weather events (Meehl et al., 2007). Declining rainfall and rising temperatures, which are associated with this change will have a direct negative effect on vegetation cover, which in turn will contribute to soil degradation because of the exposure of the soil surface to wind and water erosion. Consequently, SSA is projected to lose about 14% of cultivable land and about 20% of its pasture production potential by 2080 (Shah et al., 2008).

Potato is vulnerable to water deficit because of its fibrous root systems, which are concentrated in the upper (0–0.3 m) soil profile (Gitari et al., 2018a,b; Nyawade et al., 2018; Gitari et al., 2020b). Appreciable decrease in tuber yield occurs if a balance between soil temperature, soil moisture content, and crop nutrient uptake is not attained (Ferreira and Gonçalves, 2007; Nyawade et al., 2019b, 2020). High temperatures within the ridges at tuber initiation cause fluctuations in soil moisture, resulting in tuber malformation and tuber growth cracks (Polgar et al., 2017). Below soil water content of 20%, the matric potential of the ridges and furrows decline rapidly, slowing the redistribution of water within the soil profile (Nyawade et al., 2018). This impairs the nutrient uptake potential of potato leading to irregular plant growth.

There is therefore a growing need to provide farmers with practical and sustainable solutions to maintain potato yields under more challenging conditions while using water, nutrients, and soil resources more efficiently. Legume intercropping is one of such climate-smart cropping practices suitable to smallholder farmers. This is due to its potential to achieve multiple benefits that relate to climate mitigation and adaptation and general risk aversion via diversification (Gitari et al., 2019a,b; Nyawade and Gitari, 2020). Intercropping that includes forage legumes improves quality of forage while improving soil structure (Nyawade et al., 2018). This in turn increases water infiltration and air circulation, thus improving soil water-holding capacity.

The effectiveness of intercropping technology may be enhanced by use of silicon (Crusciol et al., 2009; Farhad et al., 2011; Pilon et al., 2014). Silicon is absorbed by plants mainly as orthosilicic acid (H_4SiO_4) also known as monosilicic or silicic acid (Mitani and Ma, 2005). At pH > 9, orthosilicic acid occurs in ionized form (H_3SiO_4/H_2SiO_4) (Knight and Kinrade, 2001). Most soils with pH < 9 thus contain Si as undissociated silicic acid over concentrations range of 0.1–0.6 mM and is in equilibrium with soil SiO_2 at pH 3 (Tubaña and Heckman, 2015). At low soil pH, Si may reduce soil phosphorus sorption by increasing the soil pH or by competing for Mn, Fe, and Al ions for the active sites thereby enhancing P availability for plant uptake (Pilon et al., 2014).

Nevertheless, little research has been conducted to assess the effect of Si application on nutrient availability and crop water productivity. This information is needed for identification of climate-smart management practices that would sustainably increase the productivity of soil in the current and future climatic scenario. Therefore, the present work was designed to test the hypothesis that potato-legume intercropping interacts with silicon application to increase light and water productivity, and minimize the high soil erosion rates in the semi-humid sloppy terrains of Kenya.

MATERIALS AND METHODS

Study Area

This study was conducted at Upper Kabete Research Farm of University of Nairobi, Kenya during the 5 rainy seasons commencing in 2016 long rains and running to 2018 short rains. The farm is located at latitude 1°14′45.00″S and longitude 36°44′19.51″E (**Figure 1**). The region is characterized by semi-humid climatic conditions and lies in agro-ecological region II, which receives an average rainfall amount of 1,000 mm per annum. These rains come in two seasons with the wet season occurring between March and August, and dry season between September and December. The area has an annual mean temperature of 20.4°C and is dominated by semi-intensive farming system where potato production accounts for more than half of the total cropped area (Jaetzold et al., 2006; Kibunja et al., 2018). The soils in Kabete are dark red friable clay classified as Humic Nitisol (Jaetzold et al., 2006). Details of the measured soil properties (0–0.3 m depth) before and after the experiment are provided in **Table 1**.

Experimental Design and Crop Husbandry

A split-plot randomized complete block study design was implemented in runoff plots measuring 5.8 m long by 2.4 m wide and were separated from each other by 1 m path with four replicates (blocks). Cropping system was considered a whole-plot factor and Si application as subplot factor. Each cropping system was subjected to granular water-soluble silicon (Si) amendment at rates of 2.5 kg ha^{-1} (+Si) and 0 kg ha^{-1} Si (–Si, the controls). The water-soluble Si was in the form of orthocilicic acid (OSA) with 0.4% Si and 5% fulvic acid (Privi Nutrifight-Privi Life Sciences PVT.LTD).

The cropping system consisted of potato (*Solanum tuberosum* L.) grown singly and intercropped with hairy vetch (*Vicia sativa* L.) or dolichos (*Lablab purpureus* L.), and the respective single crop of the legumes. The "Unica" (CIP 392797.22) potato cultivar used in this study is locally used for chips, crisp and boiling. This cultivar is heat and water stress tolerant making it well-adapted to wide range of agro-ecological conditions [National Potato Council of Kenya (NPCK), 2017; Rukundo et al., 2019]. The Rongai cultivar of dolichos (KT 003) used has a high biomass production with high crude protein content. This cultivar is used both as forage and as pulse and is a popular choice in the midlands and lowlands as a cover crop on infertile, acidic soils, and because of its high tolerance to drought. Vetch is not commonly grown by the farmers in this study area, but has great capacity to supplement the pasture and conserve the soil against erosion.

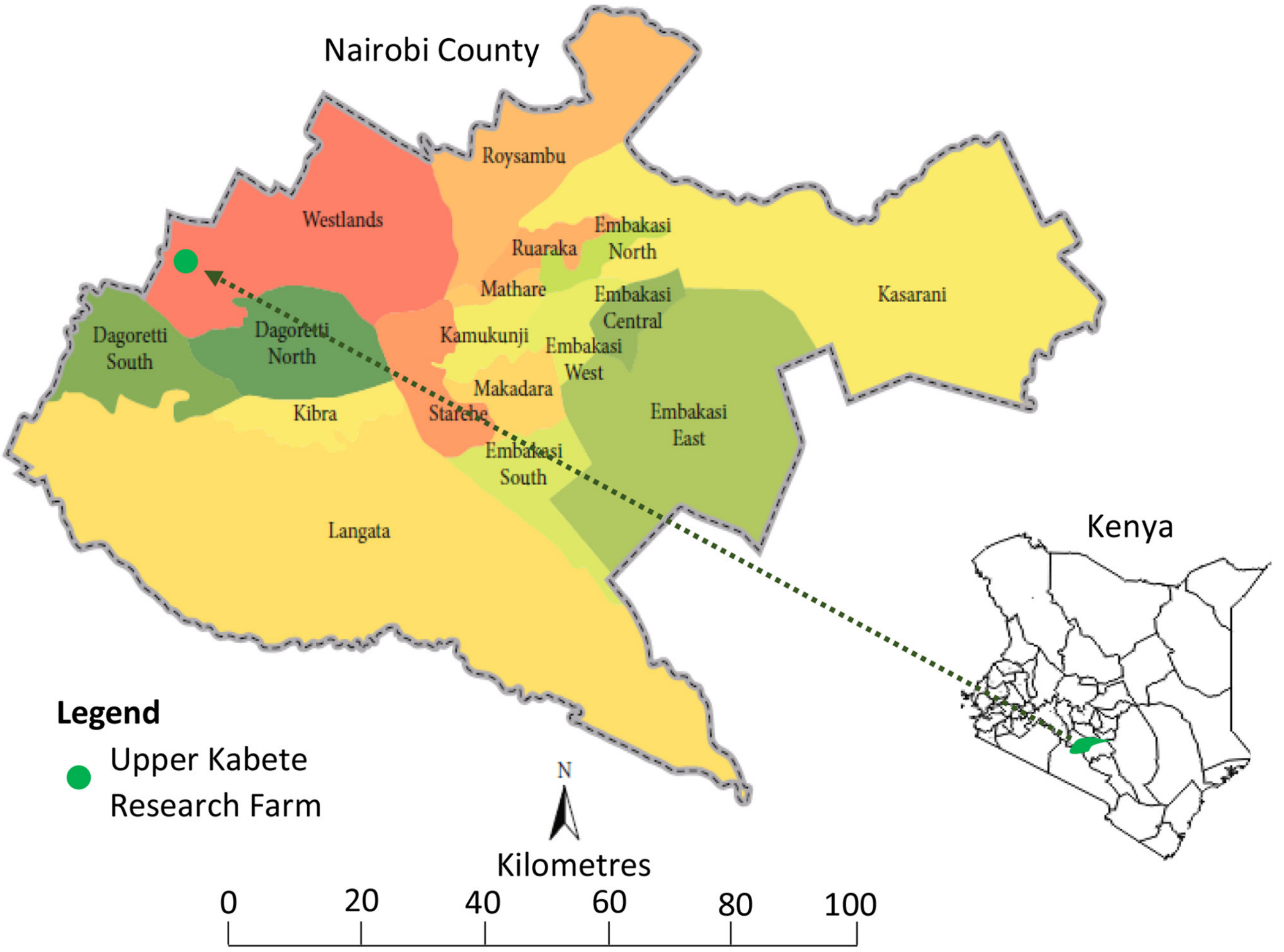

FIGURE 1 | Site map.

TABLE 1 | Soil properties measured before and after the experiment.

	Soil depth	Clay	Silt	Sand	Texture	pb	θfc	Si	Fe	Mn	pH	SOC	N	P	K
	m	%				gcm^{-3}	$cm\ cm^{-3}$		$mg\ dm^{-3}$			%	%	ppm	Cmol (+) kg^{-1}
Before	0–0.15	24.5	33.3	42.2	Clay loam	1.19	0.11	16.8	34.9	9.1	5.19	2.12	0.23	33.30	1.23
	0.15–0.3	24.2	36.9	38.9	Clay loam	1.24	0.09	17.7	31.9	8.2	5.19	1.34	0.23	23.40	1.33
	0.3–0.6	28.9	29.8	41.3	Clay loam	1.34	0.15	12.7	30.1	7.4	5.13	1.08	0.19	24.40	1.19
	0.6–0.9	23.8	32.4	43.8	Clay loam	1.35	0.18	18.9	28.5	9.1	5.12	0.83	0.10	20.20	1.09
After	0–0.15	25.2	32.2	42.6	Clay loam	0.99	0.28	37.1	33.8	10.2	5.22	2.45	0.25	44.40	1.33
	0.15–0.3	25.7	38.9	38.4	Clay loam	1.04	0.27	32.3	24.8	6.9	5.34	1.56	0.26	48.20	1.36
	0.3–0.6	27.9	27.8	44.3	Clay loam	1.14	0.26	33.2	23.8	6.7	5.36	1.57	0.25	43.70	1.31
	0.6–0.9	28.5	31.4	40.1	Clay loam	1.19	0.28	18.9	28.9	7.6	5.30	1.09	0.19	35.60	1.30

pb is soil bulk density, θwp, θfc, θs indicate soil water content at permanent wilting point, field capacity and saturation, respectively, Ks is saturated hydraulic conductivity.

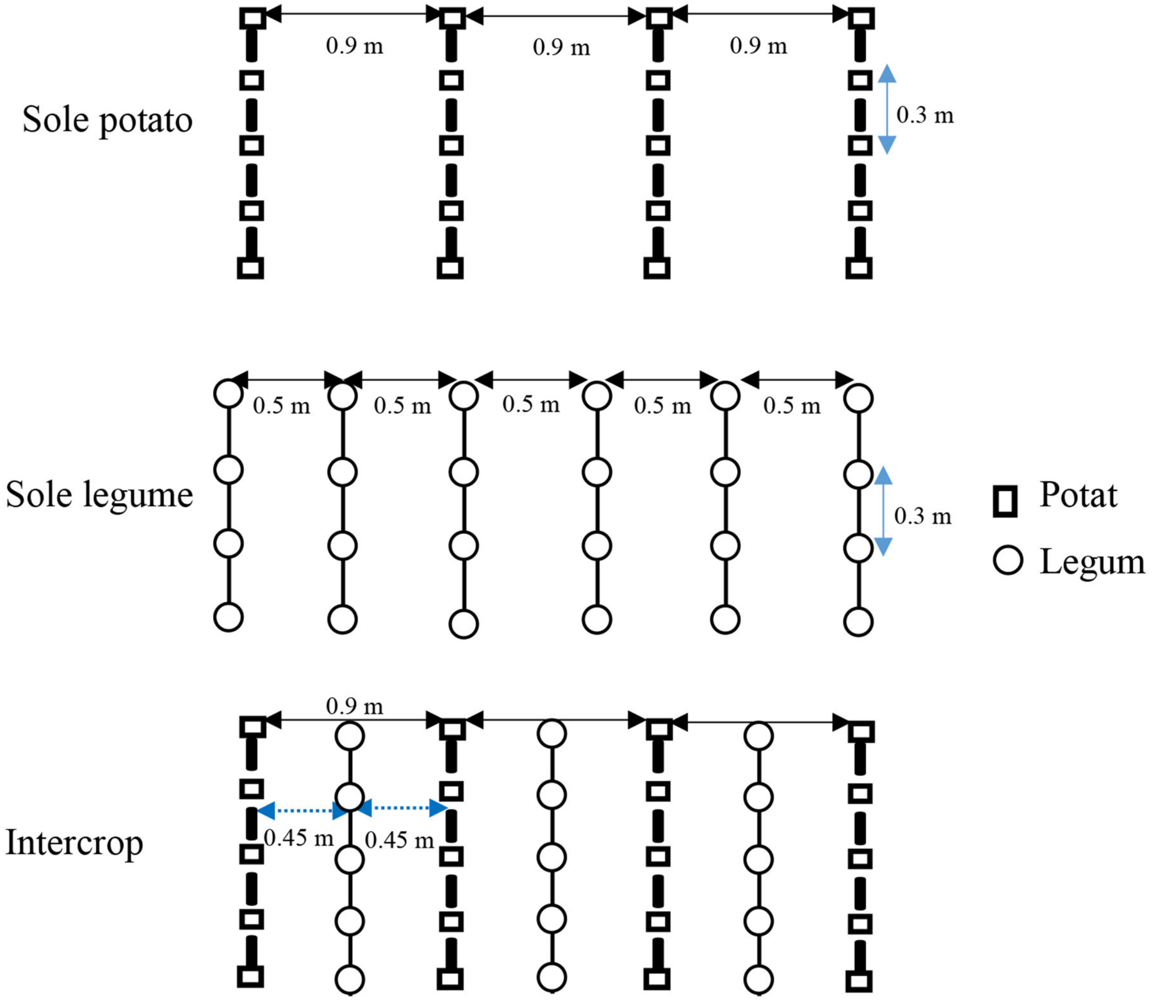

FIGURE 2 | Spacing used in treatments with potato and legumes grown singly or intercropped.

Sole potato rows were spaced 0.9 m with an interplant spacing of 0.3 m giving a plant population of 37,037 per ha. Sole legumes were spaced 0.5 m between rows and 0.3 m within rows giving plant population of 66,667 per ha. Intercropping was done in the inter-rows of potato spaced at 0.9 m, at ∼0.45 m from potato to the legume with interplant spacing of 0.3 m (**Figure 2**). This gave a plant population of 101,074 plants per ha. All the potato treatments received basal fertilization at a rate of 50 kg N ha^{-1}, 90 kg ha^{-1} of P_2O_5, 100 kg ha^{-1} of K_2O and topdress of 40 kg N ha^{-1}. Legumes received only basal phosphorus (46% triple super phosphate) applications at a rate of 20 kg ha^{-1}. Weeding and hilling were performed at 14–21 days after crop emergence by hand hoeing. The legumes were sprayed with Duduthrin 1.7 EC (Lambda-cyhalothrin 17.5 g L^{-1}) alternating with Bestox 100 EC (Alpha-cypermethrin 50 g L^{-1}) to control aphids while potatoes were sprayed alternately with Ridomil Gold MZ 68WG (Mefenoxam 40 g kg^{-1} + Mancozeb 640 g kg^{-1}) and Agriphite-600 (phosphonate) to control potato blight disease. Potatoes were harvested at maturity by digging out the tubers using hand hoes while dolichos were harvested by plucking out the pods and retaining the residues, which together with hairy vetch biomass were incorporated back into the soil.

Soil Sampling and Soil Physico-Chemical Analyses

Soil samples were taken from each plot at 0–0.3 m depths just before planting and at the end of each season using soil auger. For each plot, the soil samples were drawn from the inner rows and bulked to give one composite sample. The soils were passed through 2 mm sieve, analyzed for gravimetric moisture content, and stored at 4°C. Soil pH was measured in 1:2.5 soil to water suspensions, soil texture by the hydrometer method (Gee and Bauder, 1986), total N by modified Kjeldahl method (Bremner and Mulvaney, 1982) and organic carbon by modified Walkley and Black method (Nelson and Sommers, 1996). Extraction of soil samples for analysis of available P and extractable K was done using Mehlich 1 procedures (Mylavarapu et al., 2002) and determined using UV–vis spectrophotometer

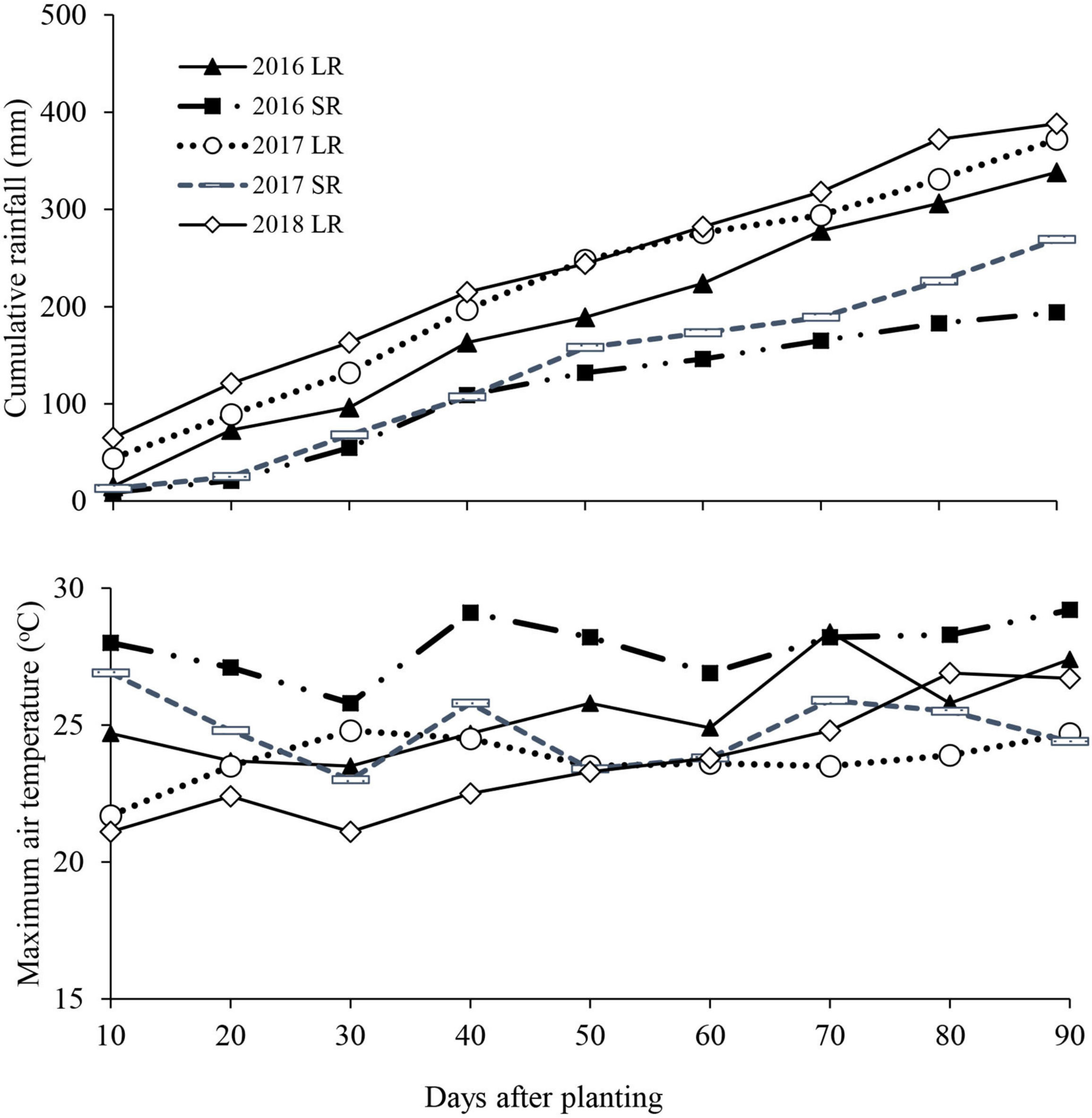

FIGURE 3 | Rainfall and temperature for the period between potato planting and harvesting. LR and SR denote long and short rains, respectively.

(Murphy et al., 1998) and flame photometry (Jackson, 1967) methods, respectively.

Climatic Data

Rainfall amount was recorded after every rainfall event using an onsite rain gauge. Air temperature were obtained from the onsite HOBO temperature sensors. Micro-lysimeters installed to a depth of 0.3 m were used to estimate the soil evaporation. The cumulative seasonal rainfall amounts received during the potato-growing period of 2016 long rains, 2016 short rains, 2017 long rains, 2017 short rains and 2018 long rains were 338, 194, 269, 372, and 388 mm, respectively (**Figure 3**). These rains occurred mainly at potato sprout development and at tuber initiation regardless of the season. The corresponding mean maximum air temperatures were 26.3, 21.2, 22.9, 22.3, and 21.2°C, respectively.

Soil Temperature, Soil Water Content, and Soil Water Retention

Tensiometers (0–100 kPa) installed at 0.3 m were used to measure the soil water contents and soil water potential. The tensiometers located at radial distances of 0.15 and 0.3 m represented ridge and furrow positions in the potato plots. Soil temperature were recorded by automatic Onset HOBO USeries, UX120-006 M data-logging equipment at 1 h common step.

Estimation of Root Length Density

Root samples were taken separately at 0–0.3 and 0.3–0.9 m soil depths to establish the spatial complementarity exerted by legume intercropping. The roots were extracted using 0.15 m × 0.1 m × 0.1 m metal cores directly driven under the plant rhizosphere (Bohm, 1979; Nyawade et al., 2018). Soil cores containing the roots were placed in a bucket of water and gently agitated to break down larger soil particles, and to remove the debris and dead roots. This mixture was sieved through 2 mm mesh placed in a shallow tub of water to wash away fine soil particles attached to the roots. The roots were floated on shallow water placed in a tray and scanned using Epson Expression 1680 Scanner (Seiko Epson Corp., Tokyo, Japan). Root length density analysis was done using WinRHIZO Root Analyzer System (Regent Instruments Inc., Quebec, Canada) (Equation 1).

$$\begin{aligned}&Root\ length\ density\ \left(cm\ cm^{-3}\right)\\&\quad=\frac{Root\ length\ (cm)}{Soil\ volume\ of\ corresponding\ depth\ (cm^{3})}\end{aligned}\qquad(1)$$

Assessment of Leaf Water Content

Four plants (potato and legumes) were harvested at 40 days after emergence for determination of leaf water content (LWC). Five leaves sampled from the fifth fully expanded leaf (from the plant apex) of the four plants were used (Barr and Weatherley, 1962). Sampling was done from the center of the leaves avoiding the veins and immediately weighed to prevent water loss, and to obtain the fresh weight (FW). The leaves were placed in petri dishes and soaked in deionized water for 12 h in the dark to attain saturation. The leaf samples were wiped with paper towels to remove the excess water and weighed again to get the turgid weight (TW). The samples were oven dried at 65°C for 24 h and placed in a desiccator for 30 min to reach ambient temperature. The leaves were weighed to obtain dry weight (DW) and the LWC computed using Equation (2).

$$LWC=\left[\frac{FW-DW}{TW-DW}\right]*100\qquad(2)$$

Physiological and Biochemical Processes

Canopy temperature and stomatal conductance were measured by a portable photosynthesis system (LI-6400, LI-COR, Lincoln, NE, USA) at vegetative phase of potato growth. Nine plants from each experimental plot were randomly selected and the measurements were conducted on the fourth petiole and averaged. All the measurements were done on a sunny day.

Computations of proline contents was done at 40 days after emergence (beginning of tuber bulking stage) (Pilon et al., 2014). The leaf samples used were obtained from the fourth leaf of the plant apex, which were wrapped in aluminum foil, immersed in liquid N and stored in a freezer (−80°C) for subsequent biochemical analysis. The leaf samples (1 g) were extracted with 3% sulphosalicylic acid and 2 mL of the extract added to 1 mL acid ninhydrin and 1 mL glacial acetic acid (Bates et al., 1973). The mixture was held in a water bath for 1 h before a 2 mL toluene was added. The proline concentrations were measured with a spectrophotometer (Shimadzu UVmini-1240, Shimadzu Corp., Kyoto, Japan) at a wavelength of 520 nm and calculated against a standard proline (Pilon et al., 2014).

For total soluble carbohydrates, leaves from 4 randomly sampled plants (both potato and the legumes) were placed in oven with forced air circulation at 70°C for 72 h. Upon drying, the leaves were ground to powdery and the powder stored in a glass container in the dark at 15°C until biochemical analyses. About 20 mg of the leaf powder was incubated with 2.0 μL of 80% ethanol at 95°C for 20 min and centrifuged for 5 min at 20°C. A 100 μL supernatant sample was carefully decanted and added to a solution containing 1.25 μL of 100% H_2SO_4, 70 μL of 15% phenol, and 580 μL H_2O before the soluble carbohydrate contents were measured at 490 nm using glucose as a standard (Dubois et al., 1956).

Leaf electrolyte conductance was measured at stolon and tuber formation stage of potato growth using procedure adapted from Hirashima et al. (2009). The uppermost fully expanded leaf trifoliate, both for potato and legumes was selected. The three leaflets, excluding the petioles were cut, placed in 50 ml eppendorf plastic tubes and covered to minimize water loss. About 20 mL of deionized water was added to 50 ml eppendorf plastic tubes, covered with lids and shaken for 2 h on an orbital shaker at 144 revolutions per minute (rpm). Initial conductance was measured using a conductivity meter (Model WTWCond3110 fitted with a tetracon 325 electrode; WTW 82362 Wellheim, Germany). This measurement indicated free leaf electrolytes outside the leaf cell membrane (apoplast). The leaf segments were boiled by placing the tubes on a 100°C water bath for 15 min followed by centrifuging at 144 rpm for another 2 h. The conductance of the boiled leaf tissues was measured after cooling down to a room temperature. This conductance indicated electrolyte status of electrolytes in the apoplast and electrolytes, which were previously bound within the leaf cell membrane (symplast) before being released because of aggravated membrane damage arising from exposure of the leaf segments to the hot water bath. To allow for direct comparison of apoplast, symplast and total leaf electrolyte status of the potato and legume species evaluated, the leaf segments were oven-dried to a constant weight at 85°C for 24 h.

Chlorophyll Content, Light Interception, Crop Yield and Radiation Use Efficiency

At 60 days after potato emergence (middle period of the tuber bulking stage), two leaf samples from the third leaf were collected for determination of chlorophyll content (Moran and Porath, 1980). The leaf samples were placed in 1.5 mL of dimethylformamide for 48 h and then incubated. The chlorophyll readings were taken using a spectrophotometer at wavelengths of 480, 646.8, and 663.8 nm.

Radiation interception of photosynthetically active radiation (PAR) and leaf area index (LAI) were measured from 14 days after potato emergence and progressively at 14–16 days interval until physiological maturity using a Sunfleck Ceptometer-LP-80 (Decagon Devices, Pullman, WA, USA). Measurements were taken only under blue-sky conditions with no or minimum clouds between 1130 and 0130 h (local time), and during a period of constant incident solar radiation. For each measurement, nine above and below canopy readings were taken perpendicularly to the crop rows to ensure that more leaf area was exposed to the light sensors. Corresponding LAI values were read upon averaging the above and below canopy readings. Plot values were computed from the average of four successive middle row readings. The PAR intercepted was calculated using Equations (3–5) (Koocheki et al., 2016).

$$\begin{aligned}\mathrm{PAR_{intercrop}} &= PAR_o\left[1-\exp\left(\left(-\lambda_{potato}{*}LAI_{potato}\right) + \left(-\lambda_{legume}{*}LAI_{legume}\right)\right)\right] && (3)\\ \mathrm{PAR_{potato}} &= \frac{\lambda_{potato}{*}LAI_{potato}}{(\lambda_{potato}{*}LAI_{potato})+(\lambda_{legume}{*}LAI_{legume})} && (4)\\ \mathrm{PAR_{legume}} &= PAR_{intercrop}-PAR_{potato} && (5)\end{aligned}$$

Where PAR = photosynthetically active radiation (400–700 nm); LAI = leaf area index, λ = light extinction coefficient; $\mathrm{PAR_o}$ = PAR incident equal to half the daily global radiation (Monteith and Unsworth, 1990). Daily global radiation was estimated from the daily sunny hours recorded from the adjacent meteorological station. The light extinction coefficient (λ) was determined from the slope of the linear regression between the natural logarithm of radiation transmission and leaf area index (Monteith, 1965).

The tuber and legume yields were estimated from the central 1.2 $\mathrm{m^2}$ area of each plot. About 500 g of tuber harvested from each plot were sliced and dried in an oven at 65°C for 72 h and reweighed to determine the tuber dry weight. For the legumes, the shoot biomass estimations was done by cutting the plants at the soil line using machetes. The dry mass was determined by oven-drying about 500 g samples at 65°C to a constant mass. The yields were converted into potato equivalent (PEY) using Equation (6) (Gitari et al., 2018a, 2020a). For dolichos, the estimations considered grain and shoot biomass separately as this legume is used as both pulse and forage.

$$PEY\left(t\ ha^{-1}\right) = PY\left(kg\ ha^{-1}\right) + \frac{LY\left(kg\ ha^{-1}\right) * LP\left(US\$\ kg^{-1}\right)}{PP\left(US\$\ kg^{-1}\right)} \tag{6}$$

Where PEY = potato equivalent yield, PY = potato yield, LY = legume yield, PP = market price of potato (0.38 US\$ $\mathrm{kg^{-1}}$) and LP = market price of the legume (0.51, 0.28, and 1.15 US\$ $\mathrm{kg^{-1}}$ for vetch forage, dolichos forage, and dolichos grain, respectively).

Radiation use efficiency (RUE) (g $\mathrm{MJ^{-1}}$) was estimated by fitting a linear regression (least square) to the cumulative amount of radiation absorption (MJ $\mathrm{m^{-2}}$) and dry matter accumulation from successive harvests (g $\mathrm{m^{-2}}$) (Monteith, 1994). The slope of each regression was taken as the RUE for each treatment.

Estimation of Soil Water Balance and Crop Water Productivity

The topsoil (0–0.3 m) water balance was estimated at the end of each season according to procedures outlined by Allen et al. (1998) (Equation 7).

$$D_{r,i} = D_{r,i-1} - (P - RO)_i - I - CR_i + ET_i + DP_i + \Delta SWC_i \tag{7}$$

where $\mathrm{D}_{r,i}$ and $\mathrm{D}_{r,i-1}$ are the cumulative depth of evaporation (root-zone moisture depletion) following complete wetting from the exposed and wetted fraction of the topsoil at the end of time i and time i-1, respectively, P is precipitation, RO is runoff from the soil surface, I is net irrigation depth that infiltrates into the soil, CR is capillary rise from the groundwater table, ET is the actual crop evapotranspiration, and Dp is deep percolation beyond the root-zone, ΔSW = change in soil water storage in root zone between planting and harvesting (ΔSW). All terms are expressed in mm relative to time i, that is, start of season and harvest. Capillary rise was assumed negligible because the groundwater table was more than 25 m below the soil surface (Karuku et al., 2014). The total amount of surface runoff was quantified from the runoff plots, soil evaporation by micro-lysimeters installed in each plot. Deep percolation (Dp) was estimated as residual term of potato root-zone water balance (Bethune et al., 2008). No irrigation water was applied throughout the growing period, similar to what the farmers in this area do.

Crop water productivity (CWP) was computed using Equation (8) (Pereira et al., 2012).

$$CWP = \frac{PEY}{P + CR + \Delta SW + I - RO} \tag{8}$$

Where PEY = potato equivalent yield; P = precipitation; CR = capillary rise of water; ΔSW = change in soil water storage in root zone between planting and harvesting period (ΔSW) and I = irrigation (I); R = runoff.

Quantification of Soil Loss and Runoff

Eroded sediment was quantified following procedures described by Wendelaar and Purkins (1979). The runoff-sludge mixture was thoroughly stirred, allowing the resultant suspension to settle for 30 min. The runoff water overlying the settled sludge was decanted and measured using a graduated bucket. A 100 ml suspension sample was oven-dried at 105°C for 48 h and expressed as dry soil mass in grams per liter. Total soil loss was computed using Equation (9).

$$\text{Soil loss }\left(\mathrm{g}\right) = \text{total runoff }\left(\mathrm{l}\right) * \text{sediment conc. }\left(\mathrm{g\,l^{-1}}\right) \tag{9}$$

Runoff from each plot was converted into mm depth using Equation (10).

$$Runoff\ (mm) = \frac{\text{Total runoff volume } (\mathrm{m^3})}{\text{Plot area}} \tag{10}$$

Nutrient loss was expressed as enrichment ratio, the ratio of nutrient element in the eroded sediment to that in the source soil (Polyakov and Lal, 2008).

Assessment of Intercrop Productivity

The land equivalent ratio (LER) (Mead and Willey, 1980) was used to evaluate the productivity of intercrops over sole cropping (Equation 11) while the system productivity index (SPI) (Odo,

TABLE 2 | Effect of silicon application and legume intercropping on soil, soil temperature, soil evaporation, and soil water balance.

	Cropping system	Root zone volumetric water	Matric potential	Soil temperature	Soil evaporation	Soil water balance
		$cm^3\ cm^{-3}$	cmH_2O	°C	mm	
+Si	Sole potato	0.15 ± 0.02bc	−85 ± 3.12b	23.1 ± 1.12d	92.2 ± 4.21g	5.1 ± 0.11b
	Sole dolichos	0.30 ± 0.04g	−31 ± 2.05f	18.8 ± 2.05a	42.2 ± 3.32a	14.2 ± 1.21g
	Sole vetch	0.27 ± 0.02f	−48 ± 3.12de	20.8 ± 2.12bc	53.3 ± 3.12b	9.3 ± 1.17de
	Potato + dolichos	0.24 ± 0.01e	−44 ± 3.94e	20.9 ± 1.94bc	61.1 ± 3.43bc	11.1 ± 1.41f
	Potato + vetch	0.20 ± 0.01d	−51 ± 3.01cd	21.4 ± 2.11b	70.9 ± 3.94de	9.9 ± 0.51def
–Si	Sole potato	0.08 ± 0.01a	−99 ± 4.12a	27.1 ± 1.12	101.3 ± 3.87h	3.3 ± 0.07a
	Sole dolichos	0.24 ± 0.02e	−42 ± 2.05e	20.6 ± 2.05b	53.3 ± 3.27b	10.1 ± 1.07e
	Sole vetch	0.21 ± 0.04d	−54 ± 2.34cd	22.9 ± 2.34d	64.5 ± 4.42c	6.5 ± 1.01bc
	Potato + dolichos	0.17 ± 0.03c	−56 ± 4.03cd	22.5 ± 2.03cd	76.9 ± 3.54e	7.7 ± 1.09c
	Potato + Vetch	0.14 ± 0.01b	−59 ± 3.06c	23.1 ± 2.06d	81.1 ± 3.64f	5.2 ± 0.31b
Silicon		*	ns	ns	ns	*
Cropping system		*	*	**	**	*
Silicon*cropping system		**	*	*	*	**
Season		**	**	**	**	**

*Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ns, not significant at the 0.05 probability level; +Si, silicon application; –Si, zero silicon application. Different letters indicate significant differences for means between the cropping systems and Si applications at $p \leq 0.05$ by Tukey's HSD test. Values (means ± standard error) are 4 replicates expressed as averages over five seasons.*

1991) was used to assess the stability of the intercropping systems (Equation 12).

$$LER = \frac{Y_{il}}{Y_{sl}} + \frac{Y_{ip}}{Y_{sp}} \tag{11}$$

Where Yil and Yip are intercrop yields of legume and potato, respectively, while Ysl and Ysp are the sole yields of legume and potato. LER > 1 indicates a production advantage; LER = 1 indicates no production advantage; and LER < 1 indicates a production disadvantage of the crop system.

$$\mathrm{SPI} = \mathrm{Y_{pi}} + \left(\frac{\mathrm{Y_{ps}}}{\mathrm{Y_{ls}}}\right) * \mathrm{Y_{li}} \tag{12}$$

Where $\mathrm{Y_{pi}}$ and $\mathrm{Y_{li}}$ depict the economic yield of potato and legume under intercropping, respectively, whereas $\mathrm{Y_{ps}}$ and $\mathrm{Y_{ls}}$ represents the respective yields under pure stands.

Data Analysis

The statistical analyses were performed in R software, version 3.4.2 (R Core Team, 2017) using linear mixed model analysis of variance (ANOVA). The datasets explored were soil temperature, soil moisture, canopy temperature, leaf water content, stomatal conductance, proline contents, total soluble carbohydrates, leaf electrolyte conductance, root length density, chlorophyll content, light interception, crop yield, radiation use efficiency, crop water productivity, soil loss, and runoff. The mixed-models were defined with the R-package lme4 (Bates et al., 2015) using likelihood based inference (Demidenko, 2013). Treatments and their interactions were considered as fixed factors while season and blocks (replications) were regarded as random factors because the study objective was to compute the variability of treatment differences across different seasons. Significant interactions between fixed factors were detected using likelihood ratio tests for generalized linear mixed models. Whenever treatment effects were significant, the response variable was subjected to Tukey's Honest Significant Difference (HSD) test ($p \leq 0.05$) for multiple mean comparisons with the agricolae package. Differences between treatments are indicated by different lower-case letters in figures and tables. Mean values are reported with standard errors, except for soil loss, runoff, and enrichment ratios where estimates are reported with 95% confidence intervals. Whenever necessary, data were either log transformed or an unequal variance model used to meet assumptions of normality and equal variance. Substitutive design was used to hold the total density of the intercrop constant and vary the ratio among the intercrop species.

RESULTS

Changes in Soil Moisture and Soil Temperature

There were significant differences ($p \leq 0.05$) in the mean volumetric soil water contents (SWCs) measured between the different cropping systems and Si applications (**Table 2**). Compared with the sole potato stands, which recorded the lowest SWC (0.08–0.15 $cm^3\ cm^{-3}$), the highest SWC in the surface soil layer (0–0.3 m) was measured under potato-dolichos intercropping (0.17–0.24 $cm^3\ cm^{-3}$) irrespective of Si application. The soil moisture content were influenced by Si application irrespective of the cropping system. The corresponding matric potential values varied among the treatments and were significantly higher ($p \leq 0.05$) in

TABLE 3 | Effect of silicon application and legume intercropping on leaf are index, leaf orientation, leaf nitrogen, stomatal conductance, and chlorophyll content.

	Cropping system	Leaf area index	Extinction coefficient	Specific leaf nitrogen	Stomatal conductance	Chlorophyll content
				$g\ N\ m^{-2}$ leaf	$molm^2\ s^{-2}$	$ug\ m^{-2}$
+Si	Sole potato	2.8 ± 0.21bc	0.53 ± 0.02e	1.63 ± 0.11b	0.36 ± 0.04b	4.93 ± 0.33b
	Sole dolichos	4.1 ± 0.02fg	0.38 ± 0.01a	2.13 ± 0.05h	0.69 ± 0.04g	8.45 ± 0.47e
	Sole vetch	3.4 ± 0.02cd	0.41 ± 0.04b	2.02 ± 0.12g	0.55 ± 0.06ef	7.56 ± 0.12e
	Potato + dolichos	4.5 ± 0.15g	0.41 ± 0.02b	2.84 ± 0.94j	0.55 ± 0.08ef	8.30 ± 0.28e
	Potato + vetch	3.9 ± 0.05ef	0.45 ± 0.01c	2.43 ± 0.14i	0.51 ± 0.07de	7.61 ± 1.04e
–Si	Sole potato	2.1 ± 0.04a	0.59 ± 0.02d	1.13 ± 0.08a	0.21 ± 0.01a	3.12 ± 0.11a
	Sole dolichos	3.4 ± 0.07c	0.40 ± 0.05b	1.97 ± 0.05fg	0.56 ± 0.03f	5.34 ± 0.27cd
	Sole vetch	2.9 ± 0.12bc	0.43 ± 0.02bc	1.76 ± 0.33c	0.43 ± 0.03c	4.78 ± 0.03b
	Potato + dolichos	3.7 ± 0.13def	0.42 ± 0.03b	1.94 ± 0.12efg	0.50 ± 0.05d	5.82 ± 0.49cd
	Potato + vetch	3.3 ± 0.04cde	0.45 ± 0.06c	1.88 ± 0.07d	0.48 ± 0.02cd	5.52 ± 1.01d
F statistics						
Silicon		*	*	*	ns	ns
Cropping system		*	*	Ns	*	*
Silicon*cropping system		*	*	*	**	*
Season		*	*	Ns	**	ns

**Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ns, not significant at the 0.05 probability level, +Si, silicon application; –Si, zero silicon application. Different letters indicate significant differences for means between the cropping systems and Si applications at $p \leq 0.05$ by Tukey's HSD test. Values (means ± standard error) are 4 replicates expressed as averages over five seasons.*

FIGURE 4 | Effect of Si application and legume intercropping on crop growth. **(a,b)** sole potato, **(c,d)** sole dolichos, **(e,f)** potato-dolichos intercrop, **(g,h)** sole vetch, **(i,j)** potato-vetch intercrop, with Si (+Si) and without Si (–Si) applications, respectively.

intercrops (−44–59 cmH_2O) relative to sole potato (−85–99 cmH_2O) and increased with Si application. Mean soil temperatures (23.1–27.1°C) were higher in sole potato plots than in potato-legume intercropping (20.9–23.1°C). Similarly, soil evaporation was significantly greater under sole potato (92.2–101.3 mm) than under intercrops (61.1–81.1 mm) and were significantly influenced by Si application ($p \leq 0.01$). The soil water balance was greatest under sole legumes (6.5–14.2 mm) followed by intercrops (5.2–11.1 mm) and lowest in sole potato (3.3–5.1 mm). Dolichos recorded higher soil water balance compared with vetch. Seasons, and interaction of Si and cropping system had significant effect on soil water balance ($p \leq 0.05$).

Intercropping and Silicon Application Effects on Crop Growth

Intercropping and Si interaction exhibited significant effect on crop growth and leaf nitrogen content (**Table 3**; **Figure 4**). Leaf area index was significantly greater in intercrops (3.3–4.5) followed by sole stands of legumes (3.3–4.1) and was lowest in

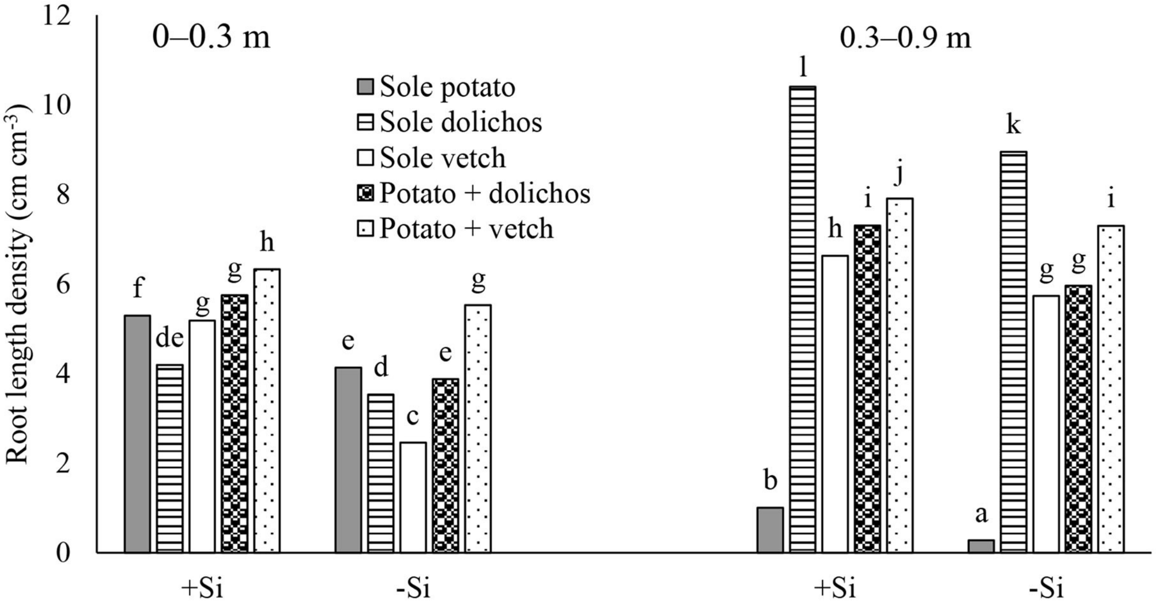

FIGURE 5 | Root length density as affected by intercropping and silicon application. +Si, silicon application; –Si, zero silicon application. Different letters indicate significant differences for means between the cropping systems and Si applications at $p \leq 0.05$ by Tukey's HSD test.

pure potato stands (2.1–2.8). Leaf area index increased by 18–33% with Si application. Intercropping significantly ($p \leq 0.05$) lowered the leaf extinction coefficient of both legumes and potato with mean value of 0.56 in sole potato compared with 0.41 in intercrops. Specific leaf nitrogen ranged from 1.13–1.63 g N m^{-2} leaf in sole potato compared with 1.88–2.84 g N m^{-2} leaf in intercrops and were up to 51% increased by Si application. Stomatal conductance ranged from 0.21–0.36 molm^{2} s^{-2} in sole potato compared with 0.48–0.55 molm^{2} s^{-2} in intercropping, and significantly increased with Si application. Chlorophyll contents were similarly higher ($p < 0.01$) in intercrops (5.52–8.30 ug m^{-2}) relative to sole potato treatments (3.12–4.93 ug m^{-2}) and increased with Si application. The LAI, chlorophyll content, stomatal conductance, and specific leaf nitrogen content responded significantly to Si application and to Si by cropping system interaction ($p \leq 0.05$). Season had significant effect on LAI, leaf extinction coefficient, and stomatal conductance.

Root length density (RLD) increased from 4.13–5.29 cm cm^{-3} in sole potato to 5.52–6.32 cm cm^{-3} in intercrops. The potato plants subjected to Si application exhibited significantly higher RLD both in the 0–0.3 and 0.3–0.9 m depths (**Figure 5**). In the 0.3–0.9 m depth, sole legumes exhibited significantly higher RLD (8.94–10.40 cm cm^{-3}) followed by intercrops (5.96–7.90 cm cm^{-3}) and lowest in sole potato (0.28–1.01 cm cm^{-3}). Dolichos generally showed significant higher RLD density ($p \leq 0.05$) in relation to vetch irrespective of cropping system and Si application.

Canopy Temperature and Biochemical Processes

Intercropping significantly ($p \leq 0.05$) lowered the canopy temperature from 25.6 to 27.8°C in sole potato to 18.7–21.8°C, the effect of which increased with Si application (**Table 4**). The percent leaf water content was significantly higher ($p \leq 0.05$) in the plants subjected to combined application of Si and intercropping (75.2–78.5%) compared with the –Si treatments (62.9–63.3%). Leaf electrolyte conductance ranged from 746–1,409 mS cm^{-1} in sole potato compared with 1,423–3,107 mS cm^{-1} in intercropping and were increased by 36–111% with Si application. Silicon application increased the contents of total soluble carbohydrates from 28 to 59 umol g^{-1} (110%). Similarly, Si application increased the contents of proline from 1.0 to 2.9 umol g^{-1} in sole potato (191%), 1.12–2.3 umol g^{-1} (104%) in potato-dolichos intercropping and 1.08 to 2.32 umol g^{-1} (115%) in potato-vetch intercropping. A significant effect of Si cropping system, season and Si by cropping system interaction was found on canopy temperature, leaf water content, proline content, total soluble carbohydrates and leaf electrolyte conductance.

Soil Erosion and Nutrient Loss

Cumulative soil loss and runoff differed significantly among the treatments ($p \leq 0.05$) and were consistently highest in sole potato plots (198–221 t ha^{-1} and 88–108 mm) and lowest in potato-dolichos intercrop (79–99 t ha^{-1} and 42–52 mm) plots, respectively (**Table 5**). Enrichment ratios of the measured parameters were above unity irrespective of treatments and differed significantly among the treatments ($p \leq 0.05$) except for the sand fractions. The highest values of enrichment ratios for NPK were recorded in sole potato plots (1.87–3.09) and lowest in sole dolichos plots (1.21–1.39). Even though an observable reduction in the enrichment ratio was observed due to Si application, these values were not significantly different from the controls (–Si treatments).

TABLE 4 | Silicon and intercropping effects on canopy temperature, leaf water content, and crop biochemical processes.

		Canopy temperature	Leaf water content	Leaf electrolyte conductance	Total soluble carbohydrates	Proline content
	Cropping system	**°C**	**%**	**mS cm^{-1}**	**umol g^{-1}**	
+Si	Sole potato	25.6 ± 2.12de	62.4 ± 3.17b	1,409 ± 23b	59.3 ± 2.32e	2.91 ± 0.33d
	Sole dolichos	20.7 ± 1.87bc	89.1 ± 3.34e	3,903 ± 29e	31.3 ± 2.21c	2.45 ± 0.41c
	Sole vetch	19.9 ± 1.78ab	71.7 ± 3.65d	2,401 ± 19c	28.9 ± 2.12c	1.99 ± 0.12b
	Potato + dolichos	18.9 ± 1.43a	78.7 ± 3.22cd	3,107 ± 31d	42.2 ± 2.76d	2.30 ± 0.28c
	Potato + vetch	18.7 ± 1.45a	75.2 ± 3.05cd	3,003 ± 27d	38.9 ± 2.88d	2.32 ± 0.17c
–Si	Sole potato	27.8 ± 1.54f	52.2 ± 3.14a	746 ± 17a	28.2 ± 2.12c	1.00 ± 0.01a
	Sole dolichos	23.6 ± 1.45d	72.3 ± 3.04c	2,307 ± 23c	18.1 ± 1.09b	1.19 ± 0.02a
	Sole vetch	24.4 ± 1.76e	60.3 ± 3.87b	1,764 ± 19b	10.3 ± 2.24a	1.14 ± 0.07a
	Potato + dolichos	21.8 ± 1.67c	62.9 ± 3.18b	1,654 ± 28b	18.1 ± 1.83b	1.12 ± 0.09a
	Potato + vetch	21.6 ± 1.89c	63.3 ± 3.76b	1,423 ± 24b	16.2 ± 1.32b	1.08 ± 0.03a
F statistics						
Silicon		*	ns	**	**	**
Cropping system		*	*	*	ns	ns
Silicon*cropping system		**	*	*	*	**
Season		**	*	ns	ns	**

**Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ns, not significant at the 0.05 probability level; +Si, silicon application, –Si, zero silicon application. Different letters indicate significant differences for means between the cropping systems and Si applications at p ≤ 0.05 by Tukey's HSD test. Values (means ± standard error) are 4 replicates expressed as averages over five seasons.*

TABLE 5 | Intercropping and silicon effects on soil erosion and soil nutrient loss.

	Cropping system	**Cumulative soil loss**	**Cumulative runoff**	**Enrichment ratio**								
		t ha^{-1}	**mm**	**pH**	**SOC**	**N**	**P**	**K**	**CEC**	**Sand**	**Silt**	**Clay**
+Si	Sole potato	198f	88g	1.08a	1.98h	1.87h	2.78h	1.89c	1.19b	0.98a	1.15a	1.17a
	Sole dolichos	48a	31a	1.03a	1.03a	1.11ab	1.23a	1..04a	1.07a	0.87a	1.13a	1.15a
	Sole vetch	72b	33a	1.01a	1.22d	1.32ef	1.43de	1.06a	1.08a	0.88a	1.14a	1.15a
	Potato + dolichos	79b	42bc	1.01a	1.45f	1.21cd	1.31b	1.34b	1.08a	0.89a	1.15a	1.16a
	Potato + vetch	98d	55ef	1.03a	1.54g	1.43g	1.46ef	1.38b	1.09a	0.87a	1.14a	1.16a
–Si	Sole potato	221g	108g	1.09a	2.09	1.92h	3.09i	1.92c	1.21b	0.99a	1.19a	1.19a
	Sole dolichos	78bc	35a	1.05a	1.13b	1.17bc	1.34bc	1.05a	1.09a	0.87a	1.17a	1.16a
	Sole vetch	92cd	48cd	1.04a	1.32e	1.38f	1.49fg	1.09a	1.08a	0.89a	1.15a	1.15a
	Potato + dolichos	99d	52de	1.03a	1.51cd	1.25de	1.39cd	1.36b	1.09a	0.88a	1.16a	1.17a
	Potato + vetch	123e	59f	1.04a	1.61c	1.46g	1.51g	1.38b	1.10a	0.89a	1.17a	1.18a
F statistics												
Silicon		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cropping system		**	*	ns	**	**	*	ns	*	ns	ns	ns
Silicon*cropping system		*	*	*	*	ns	**	ns	*	ns	ns	ns
Season		*	*	ns	ns	ns	ns	ns	*	ns	ns	ns

**Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ns, not significant at the 0.05 probability level; +Si, silicon application; –Si, zero silicon application. Different letters indicate significant differences for means between the cropping systems and Si applications at p ≤ 0.05 by Tukey's HSD test. Values are 4 replicates expressed as averages over five seasons.*

Intercropping Productivity

Cumulative biomass was significantly greater in intercrops (8.8–15.8 t ha^{-1}) than in sole potato stands (1.9–2.1 t ha^{-1}) and increased significantly with Si application (**Table 6**). Highest potato equivalent yields were recorded by intercrops relative to sole crop of potato, vetch, or dolichos. Potato equivalent yields for potato-dolichos system subjected to Si application was 14.5 t ha^{-1} greater than that of sole dolichos, and 23.3 t ha^{-1} than

TABLE 6 | Silicon and legume intercropping effect on biomass yield, potato yield, and resource use efficiency.

		Cumulative biomass	PEY	fPAR	RUE	CWP	LER	SPI
	Cropping system	t ha^{-1}	t ha^{-1}		g MJ^{-1}	Kg ha^{-1} m^{-3}		Kg ha^{-1}
+Si	Sole potato	2.1 ± 0.12a	13.9 ± 1.09b	0.43 ± 0.03b	0.98 ± 0.09b	3.23 ± 0.12b	0.89 ± 0.01b	2923 ± 21a
	Sole dolichos	18.8 ± 2.05e	25.2 ± 3.01e	0.76 ± 0.06f	2.03 ± 0.05ef	6.78 ± 1.03e	0.98 ± 0.04c	3223 ± 28a
	Sole vetch	10.2 ± 1.12bc	20.9 ± 2.09d	0.67 ± 0.07de	1.94 ± 0.13d	5.98 ± 1.12cd	0.97 ± 0.03c	3098 ± 34a
	Potato + dolichos	15.8 ± 1.34d	32.2 ± 3.00f	0.87 ± 0.05g	2.98 ± 0.08h	9.09 ± 1.04g	1.29 ± 0.08f	3987 ± 32b
	Potato + vetch	8.9 ± 1.01b	24.9 ± 2.12e	0.72 ± 0.01ef	2.56 ± 0.02g	7.38 ± 1.01ef	1.21 ± 0.07e	3765 ± 29b
–Si	Sole potato	1.9 ± 0.01a	8.9 ± 0.76a	0.31 ± 0012a	0.77 ± 0.11a	1.13 ± 0.12a	0.74 ± 0.03a	2421 ± 19a
	Sole dolichos	14.9 ± 1.03d	17.7 ± 2.01c	0.65 ± 0.06d	1.89 ± 0.05d	4.78 ± 0.08c	0.91 ± 0.07b	3018 ± 23b
	Sole vetch	8.8 ± 1.04b	13.3 ± 0.09bc	0.54 ± 0.12c	1.78 ± 0.10c	4.34 ± 0.34c	0.89 ± 0.03b	2967 ± 28b
	Potato + dolichos	11.8 ± 1.13c	25.1 ± 2.03e	0.77 ± 0.03f	2.09 ± 0.08f	6.89 ± 0.03e	1.12 ± 0.09d	3661 ± 27c
	Potato + vetch	8.8 ± 0.04b	17.3 ± 1.64c	0.65 ± 0.06d	1.98 ± 0.02de	5.99 ± 0.06cd	1.09 ± 0.05d	3587 ± 20c
Silicon (Si)		ns	**	*	*	ns	ns	*
Cropping system (CS)		*	*	*	**	*	**	**
Si* CS		**	**	**	**	*	*	*
Season		**	**	*	**	*	ns	ns

**Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ns, not significant at the 0.05 probability level; +Si, silicon application; –Si, zero silicon application; fPAR, fraction of photosynthetically active radiation intercepted; PEY, potato equivalent yield; CWP, crop water productivity; RUE, radiation use efficiency; LER, land equivalent ratio; SPI, System productivity index. Different letters indicate significant differences for means between the cropping systems and Si applications at p ≤ 0.05 by Tukey's HSD test. Values (means ± standard error) are 4 replicates expressed as averages over five seasons.*

that of sole potato. Yield increase due to vetch intercropping was 11.6 and 16 t ha^{-1} greater than that of sole vetch and sole potato respectively. Irrespective of the Si application, crop water productivity was 3–8-fold greater in intercrop relative to sole potato. Fractions of light intercepted were consistently greatest in intercrops (0.67–0.87) than in sole legumes (0.54–0.76) and lowest in the sole potato stand (0.31–0.43) ($p \leq 0.05$). Intercrop of potato and dolichos subjected to Si application recorded the highest significant RUE, which was 3-fold greater than that of sole potato.

Land equivalent ratios were greater than unity in intercrops compared with sole cropping system of potato, which recorded LER values below unity. Regardless of the cropping system, application of Si significantly increased the crop water productivity, radiation use efficiency, and light interception (PAR). Silicon and intercropping interaction had significant effect on land productivity as measured by LER. System productivity index (SPI) was significantly ($p \leq 0.05$) influenced by potato-legume intercropping systems. The index increased with Si application.

DISCUSSIONS

Intercropping and Silicon Effect on Soil Moisture and Soil Temperature

The significantly higher soil water content within the potato root-zone in intercropped plots indicated long-term moisture accumulation. The greater rooting system by potato grown in intercropping increased the capacity of crops to extract water by increasing the size of the water reservoir. While potato crop was characterized by roots that rarely exceeded a vertical depth of 0.3 m, dolichos and vetch roots colonized the 0.3–0.9 m depth. Legumes could therefore access soil water to deeper subsurface layers than the potato crop, allowing potato to draw the soil moisture in the 0–0.3 m depth. Giller (2015) highlighted that legumes have prolonged water uptake from deep soil layers. This helps the plants to improve their leaf water status and to maintain transpiration rates and dry matter production. As legume intercropping enhanced canopy formation and overlap, this meant a high groundcover that considerably reduced soil evaporation and increased soil water storage.

Soil temperatures were lower in intercropping due to the shade created by the canopy overlap between the intercrop components. The higher soil water content under intercropping further enhanced the soil cooling. Generally, a dry soil has lower heat capacity than a wet soil (Li et al., 2017). For the sole potato plots, heat from the topsoil was easily lost to the atmosphere through the intervening bare soil. Silicon application enhanced soil cooling due to its indirect effect on soil temperature as this amendment facilitated potato and legume growth thus enhancing canopy formation and subsequent shading of the soil (see the photo on **Figure 4**).

Effect of Silicon Application on Potato Growth, Physiology and Biochemical Composition

The greater LAI due to intercropping could be explained by the differences that existed in the vertical foliage arrangement and canopy architecture of the different crop varieties. Potato canopy was generally characterized by bare surfaces between the crop rows. While vetch provided low-density canopy, which closed the bare spaces between potato rows, the effective canopy overlap by dolichos bridged the inter-row spacing thus enhancing the LAI development. Nyawade et al. (2019a) observed that the larger and nearly vertical leaves exhibited by dolichos provided complementarity to the slender and more bent leaves of potato thus increasing the LAI. Even though the full canopy closure of potato occurred only after 40–45 days followed by rapid decline after physiological maturity, the legume components conferred a complimentary canopy that kept LAI relatively high during these periods. The higher leaf extinction coefficient (above 0.5) observed in pure potato stand was an indication of sagged leaves with increased propensity to converge solar radiation.

Plant growth and photosynthesis capacity were influenced by Si application. Silicon strengthened the plant tissues, an observation reflected in the relatively lower leaf extinction coefficient. This observation was supported by the measured high leaf water content in plants receiving Si relative to the controls. These plants were thus able to provide more leaf area toward light, thereby enhancing light interception. In addition, silicon increased the specific leaf nitrogen content, which is a major component of plant chlorophyll. Similar results have been found in a related study (Albiski et al., 2012). The increase in root length density due to Si application implies that Si promoted maintenance of root growth at the expense of shoot growth, a factor which has been shown to contribute to drought tolerance (Jefferies, 1993).

Both Si and legume intercropping significantly lowered the canopy temperatures and thus conferred an effective cooling mechanism to potato plants. This improved the plant tolerance to water deficit and to the high heat stress. Meunier et al. (2017) reported that Si stimulates the formation of plant-silicified structures, which offset leaf heat-load in plants. As was reflected in its additive effect on proline accumulations, Si altered the biochemical composition of potato tissues making the plant more resistant to heat stress under water deficit conditions. This is in agreement with the previous work, which demonstrated the role of proline in offsetting the adverse effects of water deficit thereby conferring stress tolerance (Sapre and Vakharia, 2016). Other studies have verified that proline concentrations in potato leaves increase with increase in Si concentrations under water-deficit conditions, which indicates that silicon may be associated with plant osmotic adjustment (Crusciol et al., 2009; Farhad et al., 2011; Pilon et al., 2014). This argument is supported by the fact that proline represents a water loss regulatory mechanism that reduces cell water potential (Fumis and Pedras, 2002), and is a biochemical marker of metabolic alterations generated by different types of stress (Lima et al., 2004). High proline levels enable potato plants to maintain cell turgidity, a fact attributed to the more negative osmotic potential of the cells and consequently alleviating the effects of water deficit (Moussa and Abdel-Aziz, 2008).

The increase in total leaf electrolyte conductance by treatments subjected to Si was possibly a consequence of Si-induced osmotic adjustment process that according to previous studies triggers accumulation of compatible solutes including sugars, ions, and amino acids in response to water deficit and heat stress (Crusciol et al., 2009; Farhad et al., 2011; Pilon et al., 2014). Accordingly, these plants had significantly higher total soluble carbohydrates content compared with the plants not subjected to Si application. The increased accumulation of solutes was a strategy meant to lower leaf osmotic potential and allow movement of water into the leaf cells, thereby maintaining turgor potential (Midega et al., 2017). This consequently increased plant tissue tolerance to low soil water conditions. According to Chaves et al. (2003), the accumulated solutes sequester water molecules and protect the cell membranes and protein complexes, thus allowing continued cell metabolic functioning. Leaf electrolyte conductance and total carbohydrate content of potato grown in intercrop without Si application were similar to those of sole potato receiving Si. This suggests that intercropping caused the potato plants to maintain their leaf cell membrane stability, which not only enhanced retention of water but also enabled accumulation of electrolytes within the symplast during exposure to heat and water deficit stress. This observation was attributed to the enhanced soil water content caused by decreased soil evaporation under higher ground cover contributed by legume intercrops.

Intercropping Effect on Soil Erosion

Compared with pure potato plots, soil loss was significantly reduced by intercropping relative to sole potato cropping. These losses were generally higher in the wet seasons, at emergence and after crop senesces when canopy cover was below 40%. This time constituted 60% of the cumulative soil loss in pure potato plots indicating that vegetal cover had effect on soil detachment. Seasonal effect was due mainly to the rainfall amounts, which were higher in the wet seasons. Potato took about 2 weeks to sprout and up to 45 days to close the canopy while dolichos emerged after 7 days and closed the canopy in only 21 days. The canopy closure with vetch occurred at 28 days after planting and was characterized by low dense cover with slender leaves and high propensity to control soil erosion. The canopy closure with dolichos was extended up to 1 month after potato harvest thus minimizing the offseason soil losses. The higher kinetic energy of rainfall that occurred early in the season sagged the weak leaf petioles, increasing the amount of bare soil between the potato rows and weakening the dissipating effect of the canopy on energy. The canopy heterogeneity under intercropping contributed by differences in plant heights, generally intercepted and dispersed the raindrops at different levels. The first raindrops that hit the canopy were intercepted and dispersed by the intercrops, thus gradually weakening their erosion potential. Ma et al. (2015) noted that the degree of leaf bending under splash effect differs with the type of plants with some leguminous crops being more prone to bending under high kinetic energy of rainfall due to their wider and softer leaves.

The highest values of enrichment ratio for total nitrogen, available phosphorus, extractable potassium, magnesium, and calcium were recorded in pure potato plots pointing to the high nutrient losses under pure potato stands. Potato delayed to establish protective cover and left the soil highly exposed to erosion, thus causing a substantial loss to the applied fertilizer. These losses occurred mainly in the first few weeks after planting when heavy rainstorms caused substantial soil erosion. Compared with vetch, growth of dolichos was more rapid and provided protective soil cover, which significantly minimized the nutrient losses. The enrichment ratio was particularly high for phosphorus because this element is usually adsorbed and fixed as iron phosphates in acidic soils (Quinton et al., 2001) and is therefore mobilized with the eroded sediment. The result thus implies that a slight soil loss through erosion may lead to a greater loss of phosphorus. The enrichment ratio for exchangeable potassium was lower than that of total nitrogen and phosphorus because phosphorus is uniformly distributed within the soil profile (Nyawade, 2015).

Across the treatments, the enrichment ratio of total soil organic carbon was above unity indicating that most of the eroded sediment was enriched in soil organic matter. This suggests the preferential transport of soil organic matter in sediments probably due to its low density. Rainfall splash effect may have peeled the soil aggregates exposing their outer layers, which have higher SOC concentration compared with the inner core resulting into SOC ratios above unity (Ghadiri and Rose, 1991). The soil pH enrichment ratio was above unity suggesting that the eroded soil material was enriched in bases relative to the source soil and may lead to Ca, Mg, K, and Na deficiency. This was asserted by the enrichment ratio of CEC, which was above unity in all the plots indicating that the eroded soil materials had higher positive charges relative to the source soil material. The enrichment ratio for clay and silt were greater than one, but that for sand was less than unity, indicating that the erosion process was selective, carrying with it the lighter material (clay and silt) and leaving the heavier material in the plots. This is because the energy required to entrain and transport silt and clay particles is comparatively lower than that of the coarser sand-sized aggregates (Boix-Fayos et al., 2009).

Intercropping and Silicon Effect on Crop Yield and Resource Productivity

Intercropping increased biomass yield, an observation that was associated with either increase in numbers of tubers per hill or increase in individual tuber weight. The yields were greater in the wet seasons due to the greater rainfall amounts. The intercropping effect on soil temperature was of great importance in explaining the yield variability between the treatments. In a study conducted by Radeni and Caesar (1986), heating the soil to 28°C reduced flow of assimilates to tubers. Similarly, Krauss and Marschner (1984) observed cessation of starch accumulation when developing tubers were subjected to soil temperature of 30°C. It could also be possible that allocation of assimilated carbon into non-structural and structural carbon was altered by the high soil temperature (Arai-Sanoh et al., 2010). For the legumes, the effects of high soil temperature were mediated in part by the deep roots coupled with their good adaptations to high temperatures.

Synergistic interaction between silicon application and intercropping on crop biomass accumulation under water deficit conditions was evident. Silicon enhanced the production of photoassimilates as was shown by the greater accumulation of total soluble carbohydrates. Further, the Si-mediated increase of water uptake under water deficit conditions enabled the potato plants to tolerate short periods of water- stress, minimizing its detrimental effects on tuber yield. For the sole potato plots not applied with Si, water stress not only restrained the potato foliage and plant development but also limited the number of leaves. Potato plants with few leaves are unable to produce large number of tubers and therefore exhibit lower yields (Obidiegwu et al., 2015).

The potato plants supplied with Si in intercropping recorded higher LAI and number of leaves, which enhanced light interception and biomass accumulation. In addition, potato intercrop exhibited greater root length density, which enhanced the crop's capacity to absorb soil water. The adverse effect of water shortage altered chlorophyll contents of the potato plants, decreased the leaf stomatal conductance and inhibited their net photosynthesis. These plants had limited capacity to mobilize the photosynthates to tubers leading to less dry matter formed per amount of water used.

The increased solar radiation interception and use efficiency by intercropping relative to monocropping was asserted to the increased canopy size and duration. The intercrops generally attained maximum canopy above 3, a value that corresponds to full groundcover by a typical potato cropping system (Burke, 2017). This was partly attributed to the increased number of leaves forming on lateral branches of potato grown in intercrops. Plants in intercropping systems were thus able to occupy all the empty niches thus contributing strongly to canopy size and radiation interception. Vetch put short, dense canopy with many slender leaves relative to dolichos, which established tall, broad dense crown with fewer interior leaves, which allowed more light to pass directly through the canopy. Unlike legumes, which indicated progressive growth with little response to prevailing heat stress, potato crop responded by developing leaves showing downward curvatures. This mechanism greatly reduced leaf area exposure to solar radiation and thus reduced radiation interception. When the crops suffered longer heat stress, potato leaves drooped followed by wilting that started from the lower strata leaves. Only leaves that exhibited some level of greenness recovered turgor and finalized their production cycle. These leaves however had limited capacity to absorb solar radiation, an observation affirmed by the proportional decrease of LAI with increasing soil and ambient temperatures.

The land equivalent ratios recorded under intercropping systems were greater than one, an indication that integration of legumes into potato-based cropping systems favored growth and yield of the companion crops grown in mixtures. The system productivity index of intercrops were greater than that of the sole potato, which was an indication that interspecific facilitation was greater than interspecific competition (Gitari et al., 2020a). This implies that intercropping resulted in greater land-use efficiency and resource productivity (Wahla et al., 2009; Machiani et al., 2018). This ability was enhanced by Si application as was indicated by the higher LER and SPI values. Seasonal effects on LER and SPI were due to fluctuating rainfall amounts, which were generally greater in the wet seasons.

CONCLUSION

This study demonstrated the beneficial effect of legume intercropping and silicon application on resource productivity, potato yield, and risk aversion in the semi-humid tropics.

For silicon to be fully integrated into potato cropping, the element should be considered essential for higher plants and should form part of soil amendment. Silicate fertilizer should be processed from the complex silicate minerals dominating in the tropics and be availed in the local markets. As this study did not integrate an open participatory learning approach, it lacked the farmer-researcher interactions necessary for enhanced adoption. To bridge this gap, close connections with the farming sector, including the farmers, extension services, and fertilizer blending companies is necessary during scaling up. Where possible, crowdsourcing applications can be adopted to provide inputs that meet the researchers' needs and help in closing the knowledge dissemination loop between researchers and practitioners and foster farmer-to-farmer interactions.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

SN conceptualized the idea, took part in the design of study, and drafted and proofread the manuscript. HG, NK, CG, KS, ES-G, and MP took part in synthesis of idea, design of the manuscript, and manuscript review and proofreading. All authors contributed to the article and approved the submitted version.

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An Innovative Corn to Silage-Grass-Legume Intercropping System With Oversown Black Oat and Soybean to Silage in Succession for the Improvement of Nutrient Cycling

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In the context of sustainable tropical agriculture, an innovative corn (*Zea mays* L.) to silage-grass-legume intercropping system can promotes plant diversity, improves agronomic performance and land-use efficiency, and increases the yield of oversown black oat (*Avena strigosa* Schreb) and soybean [*Glycine max* (L.) Merr.] to silage in succession. Thus, during three growing seasons on a Typic Haplorthox in Botucatu, São Paulo State, Brazil, four treatments of a corn to silage production system were implemented in summer/autumn with black oat oversown in winter/spring: (1) corn intercropped with palisade grass (*Urochloa brizantha* "Marandu") and black oat overseeded in lines; (2) corn intercropped with palisade grass and black oat overseeded in a broadcast system with superficial incorporation; (3) corn intercropped with palisade grass + pigeon pea [*Cajanus cajan* (L.) Millsp.] and black oat overseeded in lines; and (4) corn intercropped with palisade grass + pigeon pea and black oat overseeded in a broadcast system with superficial incorporation. During winter/spring, the black oat pastures were grazed by lambs, but results on forage allowance and nutritive value for animal grazing and on animal performance are not reported in the present manuscript. In the fourth growing season, the effect of soybean to silage intercropped with guinea grass (*Panicum maximum* "Aruana"), with only a residual effect of the four production systems from the previous three growing seasons, was evaluated. Despite greater interspecific competition of palisade grass and pigeon pea intercropped with corn, this more complex system produced better results. Thus, when analyzing this system as a whole, the triple intercrop (corn + pigeon pea + palisade grass) combined with oversown black oat in lines was the most effective option for silage production and for the improvement of other elements of system productivity, such higher surface mulch quantity, leaf nutrient concentrations, and yield of soybean to silage intercropped with guinea grass. This intercrop also generated better nutrient cycling because an increased quantity of nutrients was retained in standing plant residue and surface mulch, which resulted in better land- and nutrient-use efficiency, with an emphasis on nitrogen and potassium.

Keywords: *Cajanus cajan*, integrated crop-livestock systems, *Urochloa brizantha*, *Zea mays*, land use efficiency

HIGHLIGHTS

- Corn to silage is not affected by intercropping with palisade grass and pigeon pea.
- Pigeon pea increased the surface mulch quantity and soybean to silage in succession.
- The triple intercrop generated better nutrient cycling.
- The land equivalent ratio exceeded 1, showing the advantage of double and triple intercropping.

INTRODUCTION

The adoption of the no-till system (NTS) is growing in different edaphoclimatic conditions around of the world. However, this system is highly dependent of crops for the production and maintenance of straw on the soil surface (Borghi et al., 2013a). Several cover crops have been researched and used in tropical conditions, being that forage grasses, intercropped or not, especially of the genus *Urochloa* (syn. *Brachiaria*) are standing out. In addition, the use of integrated crop-livestock system (ICLS) can provide grain production in the summer, pasture in the autumn/winter and straw in the spring for continuity of the NTS in tropical regions, such as in the Brazilian "Cerrado" and African "Savannah" (Mateus et al., 2012). It is also noteworthy that in Brazil, the problem of degraded pasture cause low forage yield and a low animal stocking rate (Pariz et al., 2011a). Thus, the ICLS is an option to recover these degraded areas, increasing animal production. Furthermore, ICLS can contribute to increased global food production in the future (Wirsenius et al., 2010; Franzluebbers and Stuedemann, 2014).

In this context, the intercropping of tropical forage grasses with grain crops could be a key strategy for enhancing the early establishment and successful production of a winter season (with low and irregular rainfall) forage for grazing (Costa et al., 2016; Crusciol et al., 2016; Pariz et al., 2016). Nevertheless, many of these studies did not utilize animal grazing. According Moraes et al. (2014a), approximately only 5% of studies about ICLS directly utilized animal grazing, alternating with cash crops. These authors also highlighted that a diversity of field studies is needed with ruminant livestock, including sheep (*Ovis aries*), to adequately characterize the impacts of ICLS on animal performance and crop yield because stocking rates and management approaches can alter crop residue and forage quantity and quality.

Furthermore, over the years, grass-only intercropping can compromise the sustainability of ICLS due to soil nitrogen deficiency (Costa et al., 2012; Garcia et al., 2016). Therefore, according Moraes et al. (2019), the "sustainable no-tillage silage production systems are needed, especially those combined with promising ICLS production strategies, to improve overall agricultural functionality." Thus, corn intercropped with legumes (i.e., pigeon pea) is an alternative to improve the diversification of agricultural activities in tropical regions (Baldé et al., 2011). Pigeon pea is grown by smallholder farmers as a sole crop or intercropped with corn, sorghum [*Sorghum bicolor* (L.) Moench] or other crops, with cereal being the main crop that is common in Africa and Asia (Senkoro et al., 2017). When a tropical perennial grass is included in corn or sorghum-legume intercropping, there is the advantage of later pasture formation, and this intercropping system with legumes represents an alternative for the grower to implement nitrogen biological fixation in the soil because pigeon pea can fix up to 235 kg ha^{-1} of atmospheric N and can reduce the N fertilizer needed following cereal (Myaka et al., 2006) or pasture crops (Oliveira et al., 2011).

In general, results of Borghi et al. (2013a), Crusciol et al. (2014), Mateus et al. (2016), and Pariz et al. (2017c) demonstrated that in intercropping systems, the land equivalent ratio exceeded 1, showing the advantage in biomass production compared to sole crop systems. These results can be explained because companion crop stimulating the root growth of the grain crop (Hauggaard-Nielsen and Jensen, 2005). In addition, according Xiao et al. (2004), with the inclusion of legume intercropped with cereal crop (corn) and/or tropical forage grasses, it is also possible that the direct transfer of nitrogen from, e.g., a legume to a corn crop and grass. Results of Baldé et al. (2011) demonstrated that "high land equivalent ratio values provide evidence for the complementary and the high efficiency of use of available resources by the intercropped plants and thus the advantage of such systems to produce both corn grain and cover crop forage (pigeon pea and *Urochloa*)." The results presented by Ndungu-Magiroi et al. (2017) demonstrated that corn-bean (*Phaseolus vulgaris*) intercropping is more productive on a corn yield equivalent basis than monocropped corn, and the intercrop value is enhanced as bean to crop value ratios increase. Thus, despite several advances in research on intercropping systems, it is important to study the intensity of how each grain crop affects the growth of tropical perennial grasses and legumes in intercropping systems compared to monocropping systems (Brooker et al., 2015). The competition of crops in intercropping can be better explained by a land equivalent ratio (LER) (Mead and Willey, 1980), relative crowding coefficient (K) (Agegnehu et al., 2006), and aggressivity (A) (Takim, 2012).

Another good option in ICLS is the winter annual grazing grasses such as oat (*Avena*) (Moraes et al., 2014b). Results of Lopes et al. (2008) and Pariz et al. (2017b), demonstrated that in southern and central-southern Brazil, oversowing corn with oats

TABLE 1 | Rainfall, maximum and minimum temperatures, and radiation received at Botucatu, São Paulo, Brazil, during the study period and long-term averages.

Climate characteristics	Month											
	November	December	January	February	March	April	May	June	July	August	September	October
	2013–2014											
Monthly rain, mm	45	65	74	116	104	99	72	1	26	19	96	37
Mean max. temp., °C	28.1	30.0	30.7	31.3	28.8	27.3	24.0	24.5	23.7	26.8	28.0	30.2
Mean min. temp., °C	17.3	19.0	19.7	20.4	18.8	16.7	13.9	13.5	11.7	11.5	12.5	13.4
Radiation received, MJ m^{-2}	600	696	735	541	432	497	424	399	407	531	551	717
	2014–2015											
Monthly rain, mm	144	265	256	252	265	46	99	23	93	54	219	60
Mean max. temp., °C	28.1	28.6	31.7	28.4	27.1	27.0	23.4	23.5	22.6	26.7	27.3	28.7
Mean min. temp., °C	13.9	15.5	19.1	18.1	17.2	16.1	13.4	12.9	12.6	13.5	15.2	15.8
Radiation received, MJ m^{-2}	636	650	756	518	508	538	400	403	341	539	511	622
	2015–2016											
Monthly rain, mm	186	299	492	367	134	29	146	127	0	86	0	160
Mean max. temp., °C	27.2	28.4	28.1	29.6	28.4	29.6	22.9	20.5	24.1	25.3	26.0	27.2
Mean min. temp., °C	15.7	18.3	18.2	18.4	17.9	16.0	13.1	11.8	12.8	12.7	14.5	14.4
Radiation received, MJ m^{-2}	516	545	630	567	573	512	369	369	455	491	558	616
	2016–2017											
Monthly rain, mm	134	185	339	141	141	–	–	–	–	–	–	–
Mean max. temp., °C	27.9	27.9	26.4	29.8	28.0	–	–	–	–	–	–	–
Mean min. temp., °C	16.2	17.7	18.6	19.9	18.7	–	–	–	–	–	–	–
Radiation received, MJ m^{-2}	630	659	544	621	591	–	–	–	–	–	–	–
	Long-term (60 year) avg											
Monthly rain, mm	185	224	203	141	67	76	56	38	39	71	127	133
Mean max. temp., °C	27.2	28.1	28.0	28.0	27.0	24.0	23.0	23.0	25.0	26.2	26.7	27.2
Mean min. temp., °C	16.4	17.1	17.4	19.0	17.0	15.0	13.0	13.0	14.0	12.4	14.2	15.1
Radiation received, MJ m^{-2}	603	636	663	548	517	500	378	362	405	502	524	605

is a viable alternative to increasing forage and meat production in winter/spring. However, the oversown of oat in the winter/spring with seeds planted in lines or seeds broadcast with superficial incorporation may alter the forage mass of pasture as well as nutrient cycling.

Thus, our objective was evaluated the effect of inclusion of pigeon pea in the intercrop of corn with palisade grass, as well as, the modalities of oversown of black oat on: corn and soybean leaf nutrient concentrations, agronomic characteristics and yields, intercropping competition factors, land-use efficiency, relative nutrient yields, mulching, straw decomposition, and nutrient release rates in an ICLS during four growing seasons in the Brazilian "Cerrado."

MATERIALS AND METHODS

Site Description

The experiment was conducted in Botucatu in São Paulo, Brazil (48° 25′ 28′′ W, 22° 51′ 01′′ S; 777 m above sea level) over four consecutive growing seasons: 2013–2014, 2014–2015, 2015–2016, and 2016–2017. The soil was a clayey, kaolinitic, thermic Typic Haplorthox (FAO–Food Agriculture Organization of the United Nations, 2006) with 630, 90, and 280 g kg^{-1} of clay, silt, and sand, respectively. For 4 years, until October 2010, the field was fallow, with predominantly signal grass (*Urochloa decumbens* "Basilisk") and annual broadleaf weeds (*Bidens* spp., *Sonchus oleraceus*, *Raphanus raphanistrum*, *Commelina benghalensis*, *Ipomoea grandifolia*, *Chamaesyce hirta*, *Euphorbia heterophylla*, *Desmodium tortuosum*, *Leonotis nepetifolia,* and *Sida rhombifolia*). In the growing seasons of 2010–2011 and 2011–2012, corn to silage was intercropped with palisade grass in the summer/autumn, and yellow oat (*Avena byzantina* "São Carlos") was oversown, with grazing by lambs in the winter/spring (Pariz et al., 2017b). In the growing season of 2012–2013, soybean to silage was intercropped with guinea grass in the summer/autumn, and the pasture was cut in the winter/spring (Pariz et al., 2016, 2017a).

The climate of this area is Cwa, according to the Köppen climate classification system (Alvares et al., 2013). The long-term (1955–2015) mean annual maximum and minimum temperatures are 26.1 and 15.3°C, respectively, with a mean annual precipitation of 1,359 mm. The precipitation, temperature and incoming radiation were measured from 2013 to 2017 (**Table 1**).

The initial chemical characteristics of the soil [pH, organic matter, total acidity at pH 7.0 (H+, Al), exchangeable Al, available P and exchangeable Ca, Mg, and K] at depths of 0–0.20 and 0.20–0.40 m were determined (**Table 2**). The cation exchange capacity

TABLE 2 | Soil chemical characteristics at two depths in the experimental area before the initiation of the experiment.

Depth	pH	SOM[a]	P (resin)	H + Al	K_{ex}	Ca_{ex}	Mg_{ex}	CEC[b]	BS[c]
	$CaCl_2$	g dm^{-3}	mg dm^{-3}			mmol$_c$ dm^{-3}			%
0–0.20 m	5.1	38.1	12.2	41.4	1.0	31.5	15.8	89.7	53.8
0.20–0.40 m	4.8	38.0	5.5	59.6	0.7	21.9	11.4	93.6	36.3

[a] *Soil organic matter.*

[b] *Cation exchange capacity.*

[c] *Base saturation.*

(CEC) was calculated based on the sum of the concentrations of H+, Al, K, Ca, and Mg cations. The base saturation (BS) was calculated by dividing the sum of K, Mg, and Ca (the bases) by the CEC and multiplying the result by 100% (van Raij et al., 2001).

Experimental Design

The experiment had a completely randomized design as a function of soil fertility homogeneity, consisting of four treatments of corn to silage production system in summer/autumn with black oat oversown in winter/spring and twelve replications: (1) corn intercropped with palisade grass and black oat overseeded in lines; (2) corn intercropped with palisade grass and black oat overseeded in a broadcast system with superficial incorporation; (3) corn intercropped with palisade grass + pigeon pea and black oat overseeded in lines; and (4) corn intercropped with palisade grass + pigeon pea and black oat overseeded in a broadcast system with superficial incorporation. The cultivars used were: Marandu, Embrapa 29 and BRS Mandarim (palisade grass, black oat, and pigeon pea, respectively). During winter/spring, the black oat pastures were grazed by lambs, but results regarding forage allowance and nutritive value for animal grazing, as well as results regarding animal performance, are not reported in the present manuscript. The experiment was repeated in the same location for four growing seasons (2013–2014, 2014–2015, 2015–2016, and 2016–2017). Corn was ensiled in 2013–2014, 2014–2015, and 2015–2016, and soybean was ensiled in 2016–2017. The fourth growing season accounted for the residual effect of the crop systems from the previous growing seasons, as the same soybean crop system was applied to all plots. Each plot consisted of twenty 25-m-long rows spaced 0.45 m apart, thus providing a total area of 225 m^2. Monoculture plots of corn, palisade grass, or pigeon pea had the same size and number of replications as the other treatments. However, the corn, palisade grass, and pigeon pea monoculture plots were used only for determining the intercropping competition factors and land-use efficiency calculations.

Tillage, Crop Management, and Lamb Grazing Management

On 20 November 2013, plants comprising the remaining forage grasses and weeds were sprayed with glyphosate (1.44 kg acid-equivalent ha^{-1}) and 2,4-D amine (0.67 kg active ingredient ha^{-1}). On 09 December 2013, any weed regrowth was sprayed with glyphosate (1.08 kg acid-equivalent ha^{-1}). Both herbicide applications used a spray volume of 200 L ha^{-1}.

Corn hybrid 2B587 PowerCore (Dow AgroSciences, Indianapolis, Indiana, USA) with early relative maturity was sown in all crop systems on 16 December 2013, 16 December 2014, and 14 December 2015 at a 4-cm depth, with a row spacing of 0.45 m and a density of 80,000 seeds ha^{-1}, using no-till seeding (Semeato, model Personale Drill 13, Passo Fundo, Rio Grande do Sul, Brazil). When intercropped with corn, palisade grass was simultaneously sown at 12.0 kg ha^{-1} (pure live seed = 50%). Forage seeds were mixed with basic fertilizer (Pariz et al., 2016, 2017a) and sown at depths of 0.08 m below the soil surface, as described by Crusciol et al. (2012). Pigeon pea was first sown to a depth of 0.04 m using the same no-till seeding method (15 seeds per m) (~35 kg of seeds ha^{-1}), as recommended by Oliveira et al. (2011); then, the corn + palisade grass combination was sown. Therefore, pigeon pea emerged between the rows of corn + palisade grass. For all crop systems and in both growing seasons, basic fertilizer applied in the sowing furrows was 36 kg ha^{-1} of N as urea, 126 kg ha^{-1} of P_2O_5 as triple superphosphate, and 72 kg ha^{-1} of K_2O as potassium chloride, following the recommendation of Cantarella et al. (1997).

Corn seedling emergence occurred 11, 7, and 4 days after sowing (27 December 2013, 23 December 2014, and 18 December 2015, respectively). Differences were due to the absence of rain after sowing in the growing seasons (**Table 1**). Pigeon pea seedlings emerged 17, 12, and 9 days after sowing (02 January 2014, 28 December 2014, and 23 December 2015, respectively). Forage grass seedlings emerged 25, 22, and 21 days after sowing (10 January 2014, 07 January 2015, and 04 January 2016, respectively). In all growing seasons, due to the large amount of straw on the soil surface, there was no emergence of annual broadleaf weeds, and herbicide application during post-emergence of the corn crop was not necessary. Furthermore, it was not necessary to decrease the initial growth of palisade grass with a herbicide subdose due to the different emergence times of corn and grass seedlings.

On 15 January 2014, 13 January 2015, and 13 January 2016, when corn had four expanded leaves (V4 stage), mineral fertilizer was broadcast with no incorporation at 150 kg ha^{-1} of N as urea, 38 kg ha^{-1} of P_2O_5 as triple superphosphate, and 150 kg ha^{-1} of K_2O as potassium chloride, following the recommendation of Cantarella et al. (1997).

The average corn growing season length from emergence to grain production under 35% moisture was 104, 106, and 108 days

(10 April 2014, 08 April 2015, and 04 April 2016, respectively). Whole corn plants were harvested in each plot with a mechanical forage harvester (JF, model C-120, Itapira, SP, Brazil) at 0.45 m above the soil surface. Crops were chopped into particles with an average size of 1.0 cm, and knives were used to shred the corn silage grain. A two-row platform with reduced spacing (0.45–0.55 m between rows) was used to harvest the crops.

On 05 May 2014, 22 April 2015, and 13 April 2016, black oat was oversown in two modalities: (a) seeds were planted in lines to a depth of 3 cm at 65 kg ha^{-1} pure live seed density with 0.17 m row spacing, using no-till seeding (Semeato, model Personale Drill 13, Passo Fundo, Rio Grande do Sul, Brazil); (b) seeds were broadcast (manually) at 120 kg ha^{-1} pure live seed density, with superficial incorporation using a disk harrow (fully closed disks for minimum ground disturbance). Both oversown modalities followed the recommendation of Adami and Pitta (2012). In both oversown modalities, black oat seedlings emerged 24, 13, and 20 days after sowing (29 May 2014, 05 May 2015, and 03 May 2016, respectively). Differences in emergence were due to the absence of rain after oversowing during the growing seasons (**Table 1**).

In the first growing season, 48 (four crop systems × twelve lambs/crop system) uncastrated male crossbred Dorper, Texel and Ile de France lambs, with a mean age of 3 months and an initial body live weight of 27.0 ± 3.2 kg, were used. In the second growing season, 48 (four crop systems × 12 lamb/crop systems) uncastrated male crossbred Poll Dorset and Corriedale lambs with a mean age of 3 months and an initial body live weight of 24.4 ± 3.4 kg were used; 16 (four crop systems × four lambs/crop system) additional lambs were used to adjust the animal stocking rate. In the third growing season, 48 (four crop systems × 12 lambs/crop system) uncastrated male crossbred Dorper and Texel lambs with a mean age of 3 months and an initial body live weight of 26.4 ± 3.5 kg were used. The lambs were blocked based on weight variation and were randomly allocated to the crop systems. The management of lambs was conducted in accordance with the Ethics Committee on Animal Use (CEUA) of São Paulo State University (UNESP) at the College of Veterinary Medicine and Animal Science in Botucatu, São Paulo, Brazil, under protocol number 31/2014-CEUA.

The grazing method was rotational, with stocking at a fixed rate of 44, 55, and 44 lambs ha^{-1} in the first, second and third growing seasons, respectively, in a semi-feedlot scheme. The initial stocking rates were 1.2, 1.5, and 1.2 Mg ha^{-1} of body live weight, and the final stocking rates were 1.9, 2.2, and 1.9 Mg ha^{-1} of body live weight in the first, second, and third growing seasons, respectively. The grazing period in each paddock was 3 days, and the rest period was 33 days, totaling 12 paddocks per treatment. Two cycles of grazing were carried out, 36 and 33 days (first and second cycles, respectively), totaling 69 days. Lambs were on the pasture throughout the day (from 6:00 h. to 18:00 h.).

On 17 September 2016, 2.0 Mg ha^{-1} of dolomitic lime ($CaCO_3$. $MgCO_3$) with 28% CaO and 20% MgO was broadcast onto the soil surface, following the recommendation of Crusciol et al. (2016). On 02 December 2014, 16 November 2015, and 10 November 2016, plants composed of the remaining forage grasses and weeds were sprayed with glyphosate (1.44 kg acid-equivalent ha^{-1}) and 2,4-D amine (1.34 kg active ingredient ha^{-1}). On 17 December 2014, 04 December 2015, and 25 November 2016, any weed regrowth was sprayed with glyphosate (1.08 kg acid-equivalent ha^{-1}) and on 02 December 2016, mulch was sprayed with paraquat (0.4 kg active ingredient ha^{-1}). All herbicide applications used a spray volume of 200 L ha^{-1}.

The soybean cultivar "AS 3610 IPRO—INTACTA RR2 PRO" (super early cycle, maturity group 6.1, and indeterminate growth rate) was sown on 06 December 2016 at a 4-cm depth at a density of 350,000 seeds ha^{-1} and a row spacing of 0.45 m using no-till seeding (Semeato, model Personale Drill 13, Passo Fundo, Rio Grande do Sul, Brazil). The fungicide carboxin + thiram and the insecticide thiamethoxam were applied to soybean seeds at doses of 60 and 120 g of active ingredient (a.i.) to 100 kg of seeds, respectively. The soybean seeds were inoculated with *Bradyrhizobium japonicum* (SEMIA 5079—CPAC 15 and SEMIA 5080—CPAC 7) at 1,200 cells $seed^{-1}$. All soybean crop systems were fertilized in furrows, with 7 kg ha^{-1} of N as urea, 70 kg ha^{-1} of P_2O_5 as triple superphosphate and 70 kg ha^{-1} of K_2O as potassium chloride. In all crop systems, guinea grass cv. Aruana was intercropped with soybean and planted at 15 kg ha^{-1} (pure live seed = 32%). Forage seeds were mixed with basic fertilizer (Pariz et al., 2016, 2017a) and sown at depths of 0.06 m below the soil surface, as described by Crusciol et al. (2012). Soybean and guinea grass seedlings emerged 6 and 15 days after sowing, respectively (12 December 2017 and 21 December 2017, respectively). The herbicide glyphosate (0.54 kg acid-equivalent ha^{-1}) was applied 7 days after the emergence of the soybean seedlings. All crop systems were side-dressed 23 days after soybean emergence with 90 kg ha^{-1} K_2O as potassium chloride, with incorporation using a row crop cultivator for NTS (Tatu Marchesan, model CPD, Matão, São Paulo, Brazil).

Soybeans were cultivated according to crop needs (Embrapa—Empresa Brasileira de Pesquisa Agropecuária, 2006). The application of phytosanitary products was as follows: insecticide thiamethoxam + lambda-cyhalothrin (21 and 16 g of a.i. ha^{-1}, respectively) during the V4 stage; the fungicide trifloxystrobin + prothioconazole (30 and 35 g of a.i. ha^{-1}, respectively) and insecticide thiamethoxam + lambda-cyhalothrin (28 and 21 g of a.i. ha^{-1}, respectively) during the R1 stage; the fungicide trifloxystrobin + prothioconazole (38 and 44 g of a.i. ha^{-1}, respectively) and insecticide acephate (600 g of a.i. ha^{-1}) during the R4 stage; and the fungicide azoxystrobin + benzovindiflupyr (60 and 30 g of a.i. ha^{-1}, respectively) and insecticide thiamethoxam + lambda-cyhalothrin (28 and 21 g of a.i. ha^{-1}, respectively) during the R6 stage. All fungicide and insecticide applications used a spray volume of 200 L ha^{-1} and adjuvant mineral oil (65 g ha^{-1}).

The length of the soybean season (from emergence to growth stage R7—beginning of bean maturity and 50% yellow leaves), according to Fehr and Caviness (1977), was 95 days (16 March 2017). In this stage, whole soybeans and guinea grass plants were harvested according to the recommendation of Leonel et al. (2008) with a mechanical forage harvester (Model JF C-120 with 12 knives and total platform area of 1.30 m, Itapira, São Paulo, Brazil). The crops (soybean and guinea grass) were harvested at 0.15 m above the soil surface.

Sampling and Analyses

Corn and Soybean Leaf Nutrient Concentrations, Agronomic Characteristics, and Yield

Corn leaf samples were collected for nutrient analysis when 50% of corn plants were in the full flowering stage. Selection was randomized, with 30 plants chosen per plot. The fourth leaf with a visible sheath from the apex was collected following the methods of Cantarella et al. (1997). Soybean leaf samples were collected, i.e., the upper third trifoliate leaf from top to bottom at the R2 growth stage during full bloom (Fehr and Caviness, 1977). Petioles from 30 plants per plot were collected as proposed by Ambrosano et al. (1997). Leaf samples were washed, dried by forced-air circulation at 65°C for 72 h, grinding was carried out in a Willey mill (0.85 mm sieve) and analysis were carried out to determine the chemical composition. The concentrations of N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn were determined using the methods described by Malavolta et al. (1997). Nitrogen was extracted with H_2SO_4, and the other nutrients were extracted with a nitro-perchloric solution. The nitrogen concentration in the digested solution was determined by Kjeldahl analysis. The concentrations of the other nutrients were determined using atomic absorption spectrophotometry.

The corn plant population and number of ears (NE) (i.e., number of plants and ears in the four central rows, excluding 1 m from the end of each side of the row of each plot, extrapolated to plants, and ears per hectare) were evaluated. The plant height (PH), main ear insertion height (MEIH), and basal stalk diameter (BSD) were also evaluated. From harvested whole corn to silage, palisade grass, and pigeon pea plants, a representative sample was dried by forced-air circulation at 65°C for 72 h to determine the forage mass (Mg ha^{-1}) and the proportions (%) of corn grains, palisade grass, and pigeon pea in the forage mass. Using the same plant evaluation methodology, current-year corn, palisade grass, and pigeon pea mulch were collected from the soil surface to determine the remaining straw (Mg ha^{-1}). Concentrations of macro and micronutrients were determined (same methods described for corn leaf nutrient). Nutrient concentrations were multiplied by the quantity of forage mass and mulch to determine the nutrient contents (kg ha^{-1}).

The soybean plant population (SPP) (calculated from the number of plants in the four central rows, excluding 1 m from the end of each side of the row of each plot, extrapolated to plants per hectare) was evaluated. The PH, height of the first pod insertion (HFPI), number of pods per plant (NPP), number of seeds per pod (NSP), dry weight of 100 seeds (W100), and dry weight of seeds per hectare were also evaluated. Soybean and guinea grass were harvested at 0.15 m above the soil surface. A representative sample was dried by forced-air circulation at 65°C for 72 h to determine the forage mass (Mg ha^{-1}). Using the same plant evaluation methodology, current-year soybean, and guinea grass mulch were collected from the soil surface to determine the remaining straw (Mg ha^{-1}).

Intercropping Competition Factors and Land-Use Efficiency

The relative nutrient yield was calculated as the N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn yield of the species in the mixture divided by the nutrient yield of the species in the monoculture based on the harvested nutrient yields (crop yield multiplied by the % nutrient concentration of the aboveground biomass) according to Lüscher and Aeschlimann (2006). The land equivalent ratio (LER) was calculated according to Mead and Willey (1980). The relative crowding coefficient (K) and aggressivity (A) were calculated according Agegnehu et al. (2006).

Surface Mulch Quantity, Decomposition, Nutrient Content, and Release Rates

After grazing by lambs, following pasture and weed desiccation with glyphosate herbicide, estimates were obtained for the plant material killed (i.e., mulch quantity). Along two diagonal transects in each plot, three evaluators placed metal grid squares (1 m^2) on the ground in three areas per plot and cut all plant material to the ground level. The collected material was dried by forced-air circulation at 65°C for 72 h, weighed, ground, and reported in Mg ha^{-1}. Concentrations of macro and micronutrients were determined (same methods described for corn leaf nutrient). Nutrient concentrations were multiplied by the quantity of mulch to determine the nutrient contents (kg ha^{-1}). The lignin content was also determined according to the method described by Silva and Queiroz (2002) and was used to calculate the total lignin/N ratio.

To determine the rate of decomposition, fresh forage from each plot was placed in nylon bags (litter bags of 0.06 m^2, 0.3 × 0.2 m), proportionate to the standing mass (Pariz et al., 2011b). Litter bags were distributed and left on the soil for 15, 30, 60, 90, and 120 days. One litter bag per plot was removed at each sampling time as a function of days after desiccation (DAD) of pasture and weeds due to glyphosate herbicide following grazing. The contents of each litter bag were collected, purified by sieving, and rinsing with distilled water, and dried at 65°C to a constant weight to determine the dry weight. Subsequently, the concentrations of N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn of the remaining forage residue per litter bag were determined according to the method proposed by Malavolta et al. (1997). The nutrient concentrations were multiplied by the amount of remaining residues, calculating the respective release rates for a period of 120 days (Pereira et al., 2016).

Statistical Analyses

All data were initially tested for normality with the Shapiro and Wilk (1965) test using the UNIVARIATE procedure of SAS Institute (2015, SAS Institute Inc., Cary, NC). All data were distributed normally ($W \geq 0.90$). The data were analyzed using the PROC MIXED procedure of SAS and Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. The crop systems were considered fixed effects. A repeated statement was used with the growing season specified as the repeated variable. The covariance structure used in the analyses was autoregressive, which provided the best fit according to the Akaike information criterion. The results were reported as least square means and were separated by preplanned pairwise comparisons (PDIFF). The mean separations were conducted using an LSD test. The effects were considered statistically significant at $p \leq 0.05$. Straw

TABLE 3 | Leaf nutrient concentrations at the full flowering stage in corn to silage intercropped with palisade grass (C + PG) and with palisade grass and pigeon pea (C + PG + PP) with black oat (BO) oversown in lines and broadcast in three growing seasons.

Treatments	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
	g kg^{-1}						mg kg^{-1}				
Crop systems											
C + PG + BO line	29.0^{c§}	2.7^{a}	20.2^{a}	2.6^{a}	2.0^{a}	1.7^{a}	14.0^{a}	9.9^{b}	122.5^{a}	43.2^{b}	22.4^{a}
C + PG + BO broadcast	28.0^{d}	2.7^{a}	20.1^{a}	2.6^{a}	2.0^{a}	1.7^{a}	15.3^{a}	9.6^{b}	117.7^{a}	41.5^{b}	22.6^{a}
C + PG + PP + BO line	31.6^{a}	2.7^{a}	19.7^{a}	2.6^{a}	2.0^{a}	1.7^{a}	15.1^{a}	11.5^{a}	122.6^{a}	58.0^{a}	23.1^{a}
C + PG + PP + BO broadcast	30.6^{b}	2.7^{a}	20.3^{a}	2.7^{a}	2.1^{a}	1.7^{a}	14.8^{a}	11.7^{a}	125.8^{a}	57.7^{a}	22.6^{a}
Growing seasons											
First (2013–2014)	29.8^{a§}	2.8^{a}	19.9^{a}	3.3^{a}	2.4^{a}	1.7^{a}	17.9^{a}	10.3^{a}	122.0^{a}	53.5^{a}	22.5^{a}
Second (2014–2015)	29.6^{a}	2.6^{a}	20.5^{a}	2.5^{b}	2.1^{b}	1.7^{a}	18.6^{a}	11.0^{a}	122.5^{a}	47.9^{a}	22.8^{a}
Third (2015–2016)	30.0^{a}	2.7^{a}	19.9^{a}	2.2^{c}	1.5^{c}	1.8^{a}	7.9^{b}	10.7^{a}	122.0^{a}	48.9^{a}	22.8^{a}

§*Means within a column of the same category (i.e., crop systems and growing seasons) followed by the same letter are not significantly different at $P \leq 0.05$.*

decomposition and nutrient release rates were analyzed, as suggested by Wider and Lang (1982), with the litter bag method, using the PROC REG procedure of SAS, and the best adjustments chosen had the highest coefficients of determination (r^2) at $p \leq 0.05$. Error bars represent standard errors (SEs), and the means were determined using the PROC MEANS procedure of SAS.

RESULTS

Weather Conditions

The temperatures during the second (2014–2015) and third (2015–2016) growing seasons were relatively similar (**Table 1**) and appropriate for corn cultivation (Borghi et al., 2013b; Crusciol et al., 2013). According to Bergamaschi et al. (2004) and Araujo et al. (20111), the amount of precipitation would have allowed corn and intercropped forages to develop without water stress only during the second and third growing seasons (between 500 and 800 mm). In the fourth growing season (2016–2017), the weather conditions were also appropriate for soybean development (Crusciol et al., 2012, 2014). According to Embrapa—Empresa Brasileira de Pesquisa Agropecuária (2006), the amount of precipitation would have caused minimal water stress of soybean and intercropped guinea grass (between 450 and 850 mm).

In the first growing season (2013–2014), the rainfall (754 mm) was 45% lower than the historical average (1,360 mm), with low rainfall occurring in the summer (mainly from 17 January 2014 to 15 February 2014), which was associated with a greater mean maximum temperature than the historical average and caused severe water stress mainly during corn vegetative development, with precipitation around 300 mm between corn seedlings emergence until harvest (December 2013 to March 2014; **Table 1**). In the second and third growing seasons (2014–2015 and 2015–2016, respectively), rainfall amounts of 1,776 and 2,026 mm were 23 and 49% higher, respectively, than the historical averages, and the mean maximum and minimum temperatures were similar to the historical average. In the fourth growing season, the rainfall amount of 940 mm from November 2016 to March 2017 was 15% higher than the historical average during this period (820 mm), and the mean maximum and minimum temperatures were similar to the historical averages. The radiation received by corn between seedling emergence and harvest was similar in all growing seasons (1,986, 2,142, and 2,065 MJ m^{-2} in the first, second and third growing seasons, respectively), and in all months, the values were similar to the historical average. The radiation received by soybean between seedling emergence and harvest was 1,919 MJ m^{-2} in the fourth growing season.

Between the harvesting of corn and stocking of lambs on pastures, rainfall amounts of 151, 232, and 302 mm occurred in the first, second and third growing seasons, respectively, and after the oversowing of black oat, rainfall amounts of 85, 189, and 302 mm occurred in the first, second and third growing seasons, respectively. The radiation received by pasture between the corn harvest and stocking of lambs was similar for all growing seasons (1,243, 1,231, and 1,050 MJ m^{-2} in the first, second and third growing seasons, respectively).

Corn Leaf Nutrient Concentrations, Agronomic Characteristics, and Yield

The crop system affected the N, Cu, and Mn concentrations and the growing season affected Ca, Mg, and B concentrations of corn leaf tissue (**Table 3**). The crop system affected the standing plant residues, forage mass, and concentration of corn grains, and the growing season affected the corn plant population, NE per hectare, PH (corn, palisade grass, and pigeon pea), MEIH, bulk stalk diameter, standing plant residues, forage mass, proportions of corn grains, palisade grass, and pigeon pea (**Table 4**). Forage mass was also affected by the interaction between crop system × growing season.

Corn intercropped with palisade grass and pigeon pea had higher N, K, and Fe contents in the forage mass, and growing season affected only the N content in the forage mass (**Table 6**). Corn intercropped with palisade grass and pigeon pea had higher nutrient contents in standing residue than other crop systems, and growing season also affected all nutrient contents in standing residue.

TABLE 4 | Corn plant population (CPP), number of ears per hectare (NE), plant height (PH), main ear insertion height (MEIH), basal stalk diameter (BSD), standing plant residue (SPR), forage mass (FM), and proportions of corn grains (CG), palisade grass and pigeon pea in corn to silage intercropped with palisade grass (C + PG) and with palisade grass and pigeon pea (C + PG + PP) with black oat (BO) oversown in lines and broadcast in three growing seasons.

Treatments			PH							Concentration		
	CPP	NE	C	PG	PP	MEIH	BSD	SPR	FM	CG	PG	PP
	n° × 1,000		m				cm	Mg ha^{-1}		%		
Crop systems												
C + PG + BO line	$75.0^{a\S}$	82.3^{a}	2.0^{a}	1.0^{a}	–	0.9^{a}	21.7^{a}	2.2^{b}	14.1^{b}	50.0^{a}	1.2^{a}	–
C + PG + BO broadcast	74.7^{a}	81.2^{a}	2.0^{a}	1.1^{a}	–	0.9^{a}	22.3^{a}	2.4^{b}	14.0^{b}	50.5^{a}	1.3^{a}	–
C + PG + PP + BO line	75.9^{a}	81.8^{a}	2.1^{a}	1.1^{a}	1.6^{a}	1.0^{a}	21.4^{a}	3.1^{a}	15.0^{a}	47.5^{b}	1.1^{a}	6.4^{a}
C + PG + PP + BO broadcast	76.1^{a}	82.4^{a}	2.0^{a}	1.1^{a}	1.7^{a}	0.9^{a}	21.6^{a}	3.1^{a}	14.9^{a}	46.7^{b}	1.2^{a}	5.8^{a}
Growing seasons												
First (2013–2014)	$70.1^{b\S}$	71.4^{b}	1.5^{b}	0.8^{b}	2.1^{a}	0.7^{b}	18.6^{b}	2.2^{b}	13.9^{b}	50.2^{a}	1.5^{a}	9.4^{a}
Second (2014–2015)	77.6^{a}	87.0^{a}	2.3^{a}	1.2^{a}	1.4^{b}	1.0^{a}	23.8^{a}	2.9^{a}	14.8^{a}	47.4^{b}	1.0^{b}	4.6^{b}
Third (2015–2016)	78.6^{a}	87.5^{a}	2.2^{a}	1.2^{a}	1.4^{b}	1.0^{a}	22.9^{a}	3.0^{a}	14.8^{a}	48.5^{b}	1.1^{b}	4.4^{b}

[§] *Means within a column of the same category (i.e., crop systems and growing seasons) followed by the same letter are not significantly different at $P \leq 0.05$.*

Intercropping Competition Factors and Land-Use Efficiency

Intercropping corn with palisade grass and pigeon pea increased the total LER in the three growing seasons compared to intercropping corn with palisade grass alone (**Table 7**). Intercropping corn with palisade grass and pigeon pea affected the *K*-value of corn and palisade grass in the three growing seasons compared to intercropping corn only with palisade grass and affected the *A*-value of corn in the three growing seasons as well as the *A* value of palisade grass only in the first growing season compared to intercropping corn only with palisade grass.

Surface Mulch Quantity, Decomposition, Nutrient Content and Release Rates

The inclusion of pigeon pea in the production system with intercropped corn and palisade grass provided an increase in the surface mulch, mulch cover and nutrient contents and caused a decrease in the lignin/N ratio, mainly with black oat oversown in lines (**Table 9**). The growing season also affected all these attributes except the lignin/N ratio.

With a lower lignin/N ratio than other treatments, surface mulch in the palisade grass plots generated during triple intercropping had greater initial decomposition [logarithmic (log 10) decomposition]; furthermore, with a higher lignin/N ratio than other treatments, surface mulch in the palisade grass plots generated during double intercropping had lower initial decomposition (**Figure 1**). The release rate of N was similar to that of surface mulch decomposition in all crop systems. The rate of release of P, K, Mg, and B contained in the plant residues was logarithmic over 120 DAD; however, there was no crop system effect (**Figures 1–3**). The release of Ca, S, Cu, Fe, Mn, and Zn from the plant residues was exponential at 120 DAD; however, without a crop system effect, and in the first 30 days, the release of these nutrients was slower than that of the other nutrients (**Figures 2–4**).

Soybean Leaf Nutrient Concentrations, Agronomic Characteristics, and Yield

The residual effect of corn intercropped with palisade grass and pigeon pea, mainly with black oat oversown in lines, resulted in higher N and K concentrations in soybean leaves (**Table 10**). Residual crop system effects were significant for the SPP, PH, HFPI, NPP, dry weight of 100 seeds, dry weight of seeds per hectare, forage mass, and standing plant residue, with higher values after triple intercropping than after double intercropping (**Table 11**).

DISCUSSION

Corn Leaf Nutrient Concentrations, Agronomic Characteristics, and Yield

In all crop systems, corn leaf nutrient concentrations were optimal under sufficient ranges (Cantarella et al., 1997), despite following a recommended seeding and side-dressing fertilization programme (**Table 3**). Previous studies have shown higher cash-crop leaf nutrient concentrations when these crops are intercropped with palisade grass (Crusciol et al., 2012; Borghi et al., 2013b). However, the inclusion of pigeon pea in intercropping increased corn leaf N, Cu, and Mn concentrations (increase of 9, 18, and 36%, respectively), demonstrating a direct transfer of these nutrients to the cereal crop (Xiao et al., 2004).

A reduction in corn leaf Ca and Mg concentrations over the three growing seasons suggested the absorption of these nutrients that depended on the effectiveness of the root systems and nutrient availability (**Table 3**). Monteiro et al. (1995) found that "the omission of Mg in nutrient solutions for cultivating palisade grass reduced the production of root dry matter by 70% compared to a treatment with adequate concentrations of Mg." Although the levels of Ca and Mg in the soil were adequate before implementation of the experiment (**Table 2**), a short- to medium-term ICLS began in the growing season of 2010–2011 with dolomitic lime and agricultural gypsum application (Pariz et al.,

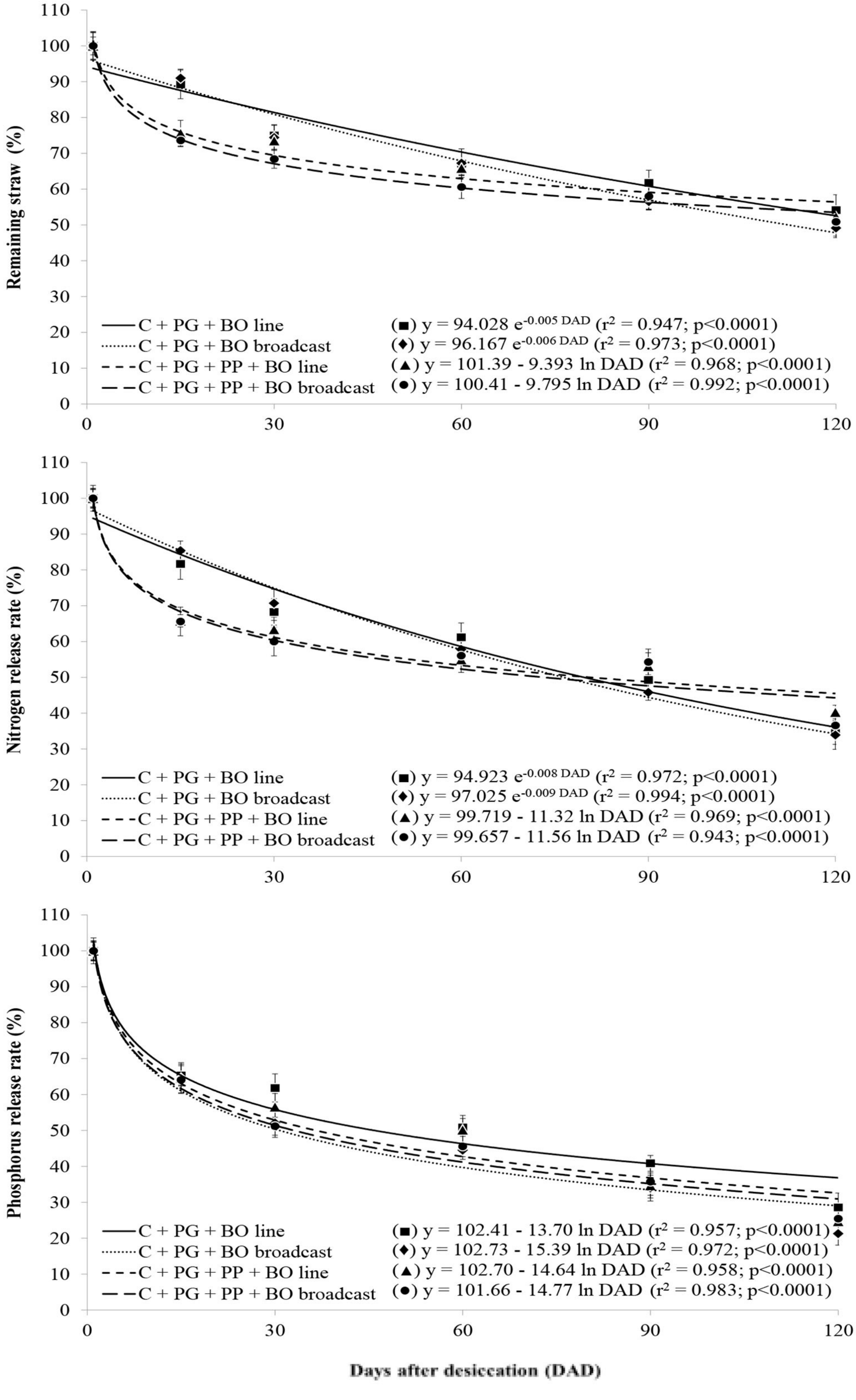

FIGURE 1 | Release rate of remaining straw, nitrogen, and phosphorus in palisade grass (PG) intercropped with corn (C) and with corn and pigeon pea (PP) to silage with black oat (BO) oversown in lines and broadcast after grazing by lambs as a function of days after desiccation (mean of three growing seasons). Values are the mean of 12 replicates, and the associated error bar is ± 1 SE. Days after desiccation (DAD): days after pasture and weed desiccation with glyphosate herbicide, after grazing by lambs.

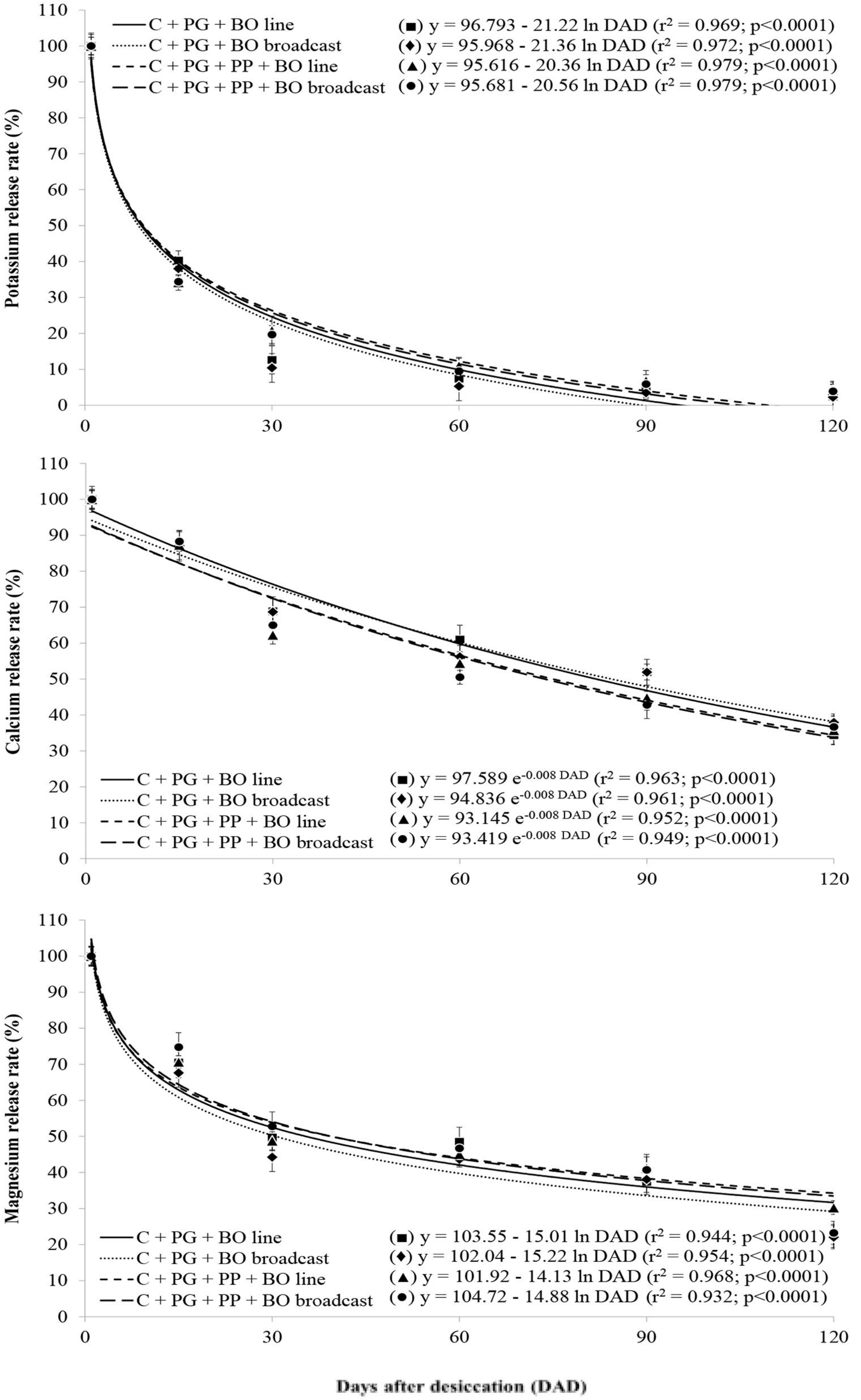

FIGURE 2 | Release rate of potassium, calcium, and magnesium in palisade grass (PG) intercropped with corn (C) and with corn and pigeon pea (PP) to silage with black oat (BO) oversown in lines and broadcast after grazing by lambs as a function of days after desiccation (mean of three growing seasons). Values are the mean of 12 replicates, and the associated error bar is ± 1 SE. Days after desiccation (DAD): days after pasture and weed desiccation with glyphosate herbicide, after grazing by lambs.

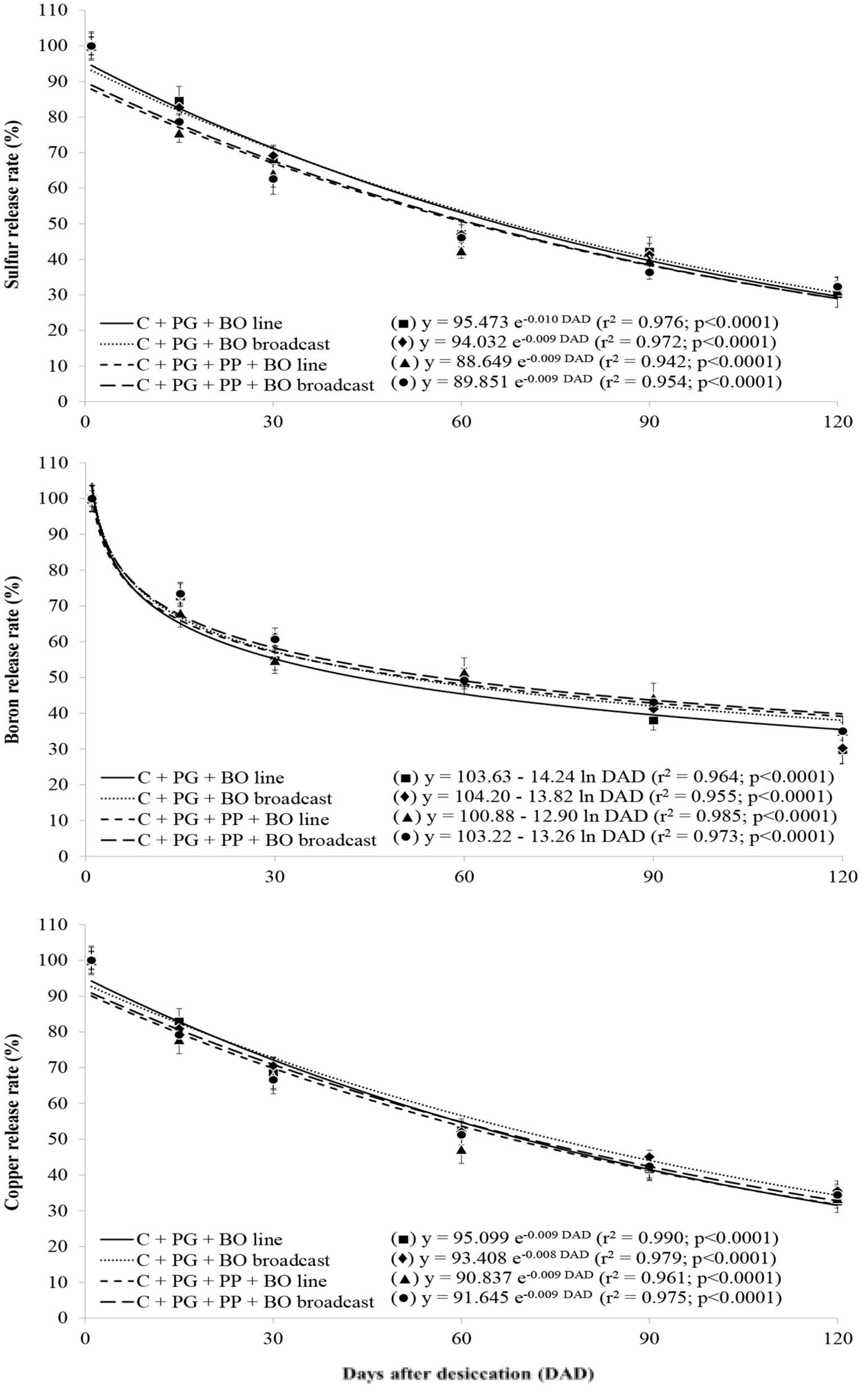

FIGURE 3 | Release rates of sulfur, boron, and copper in palisade grass (PG) intercropped with corn (C) and with corn and pigeon pea (PP) to silage with black oat (BO) oversown in lines and broadcast after grazing by lambs as a function of days after desiccation (mean of three growing seasons). Values are the mean of 12 replicates, and the associated error bar is ± 1 SE. Days after desiccation (DAD): days after pasture and weed desiccation with glyphosate herbicide, after grazing by lambs.

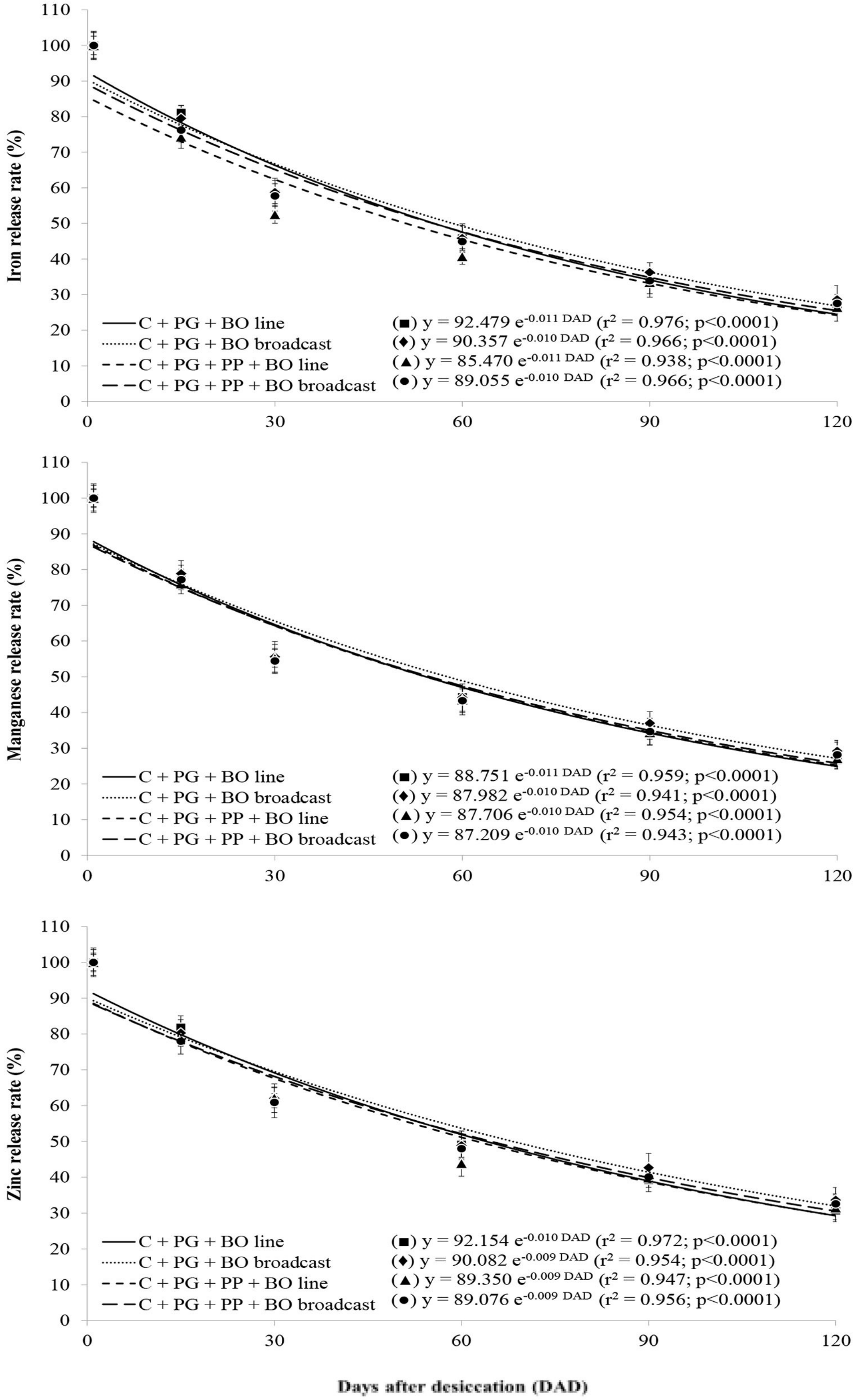

FIGURE 4 | Release rates of iron, manganese, and zinc in palisade grass (PG) intercropped with corn (C) and with corn and pigeon pea (PP) to silage with black oat (BO) oversown in lines and broadcast after grazing by lambs as a function of days after desiccation (mean of three growing seasons). Values are the mean of 12 replicates, and the associated error bar is ± 1 SE. Days after desiccation (DAD): days after pasture and weed desiccation with glyphosate herbicide, after grazing by lambs.

TABLE 5 | Forage mass of corn to silage intercropped with palisade grass (C + PG) and with palisade grass and pigeon pea (C + PG + PP) with black oat (BO) oversown in lines and broadcast in three growing seasons.

Treatments	Growing seasons		
	First (2013–2014)	Second (2014–2015)	Third (2015–2016)
		Mg ha^{-1}	
Crop systems			
C + PG + BO line	$12.9^{bB\S}$	14.6^{aA}	14.7^{aA}
C + PG + BO broadcast	12.8^{bB}	14.5^{aA}	14.7^{aA}
C + PG + PP + BO line	15.0^{aA}	15.0^{aA}	14.9^{aA}
C + PG + PP + BO broadcast	14.9^{aA}	15.0^{aA}	14.9^{aA}

§Means within a column followed by a different lowercase letter and within a line followed by a different uppercase letter are significantly different at $P \leq 0.05$.

2016, 2017a,b). In this context, the results of Crusciol et al. (2016) indicated that reapplication of lime to the surface of no-till soil can improve plant nutrition, dry matter production, crop yield, revenue and the long-term sustainability of tropical agriculture in the Brazilian "Cerrado." Therefore, since this agricultural system has high nutrient exports, the availability of Ca and Mg in the soil should be monitored annually, with surface lime reapplications when necessary to prevent deficiency in plants. Accordingly, prior to soybean sowing, dolomitic lime was broadcast onto the soil surface. Another nutrient that supported this necessary annual monitoring is B because the concentration of this nutrient in corn leaves was lower in the third growing season than in the first and second growing seasons.

Greater standing plant residue with the incorporation of pigeon pea as an intercrop was due to the stems of this crop growing closer to the ground (**Table 4**). Corn intercropped with palisade grass had a lower forage mass than that of other crop systems during the first growing season (**Table 5**). This was a function of low rainfall in the summer, which was associated with higher mean maximum temperature than the historical average and caused severe water stress during corn vegetative development. Thus, the inclusion of pigeon pea in the production system intercropped with corn and palisade grass generated an increase of 16% in the forage mass during the first growing season, probably due to the development of the pivotal root system of pigeon pea (up to 1 m deep in relation to the soil surface), which resulted in greater water absorption, causing the crop to be more resistant to the water deficit. Therefore, the mass that was produced by the pigeon pea intercropped with corn and palisade grass allowed for the forage mass (∼15 Mg ha^{-1}) to be similar to that produced during the other growing seasons without water deficiency. The inclusion of pigeon pea accounted for ∼5.8–6.4% in the forage mass (**Table 4**), reducing the proportion of corn grains in the forage mass from 50.0–50.5% to 47.5–46.7%.

The severe water stress during corn vegetative development during the first growing season also reduced the corn plant population, NE, PH (corn and palisade grass), MEIH, BSD, standing plant residue, and forage mass compared to the two other growing seasons (**Table 4**). However, as a function of the best resistance to the water deficit of pigeon pea previously discussed, associated with lower corn PH (greater incidence of light between the crop rows), the PH and proportion of pigeon pea in the forage mass were highest in the first growing season. Furthermore, it is noteworthy that after the flowering and pollination of the corn crop, the rainfall normalized, promoting adequate grain filling. Thus, as the corn plants had lower mass of leaves and stems, higher proportions of grains, and palisade grass were verified in the forage mass.

Considering that the proportion of pigeon pea in the forage mass was 9.4% in the first growing season and ∼4.5% in the second and third growing seasons (**Table 4**) and that this legume had a higher N concentration than corn and palisade grass, this result demonstrates how the inclusion of legumes in crop systems promotes the accumulation of plant nitrogen via the biological fixation of atmospheric nitrogen (N_2) (Oliveira et al., 2011), which increases the content of this nutrient in the forage mass and in standing residue (**Table 6**).

Furthermore, silage with more protein, as is the case in a corn-legume intercrop system, can reduce the use of protein concentrates, which increase the cost of an animal's diet. Also over the growing seasons [first (2013–2014) to third (2015–2016)], a greater accumulation of N occurred in the forage mass and in standing residue (**Table 6**), which can be considered an effect of the transition phase of NTS (between 5 and 10 years, considering that the experimental area has been managed since 2010). At this stage, the accumulation of straw on the soil surface begins, and N immobilization is similar to mineralization (Sá et al., 2009). A carryover effect of legumes may also occur in the crop system over the three growing seasons. The same carryover effect was also verified for all nutrients in standing residue, with higher contents in the second and third growing seasons than in the first growing season.

The higher N content in the forage mass and in standing residue from crop systems with oversown black oat in lines during the winter/spring compared to broadcast oversown black oat (**Table 6**) was due to the lower loss of soil nitrogen (emission of N_2O) in the line treatment because of the minimum ground disturbance under no-till seeding. In broadcast treatments, the superficial incorporation of black oat seeds using a disk harrow increased the pore space. High N_2O emission rates occur when the soil has a large proportion of water-filled pores, above 60%, which makes it difficult for O_2 to diffuse into the soil and favors the formation of anaerobic environments (Bateman and Baggs, 2005).

The higher K content in standing residue in the corn to silage systems likely provided significant K recycling and contributed to exchangeable forms in the soil (Garcia et al., 2008). Corn intercropped with palisade grass and pigeon pea also had a higher content of all nutrients in standing residue than other crop systems (**Table 6**). Thus, as a function mainly of stems that remained on the soil surface after harvest, pigeon pea had ∼0.7–0.9 Mg ha^{-1} more standing plant residue than that measured when there was no inclusion of this species in the cropping system (**Table 4**), which contributed to the greater accumulation

TABLE 6 | Nutrient content in forage mass and in standing plant residue of corn to silage intercropped with palisade grass (C + PG) and with palisade grass and pigeon pea (C + PG + PP) with black oat (BO) oversown in lines and broadcast compared to corn, palisade grass, and pigeon pea monoculture in three growing seasons.

Treatments	Nutrient content in forage mass										
	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
	kg ha^{-1}						mg ha^{-1}				
Crop systems											
C + PG + BO line	$153.2^{c\S}$	24.0^{a}	113.4^{b}	18.0^{a}	24.3^{a}	18.8^{a}	121.9^{a}	68.8^{a}	630.0^{b}	448.9^{a}	251.7^{a}
C + PG + BO broadcast	143.5^{d}	23.9^{a}	112.5^{b}	17.9^{a}	23.5^{a}	16.9^{a}	112.4^{a}	62.4^{a}	596.8^{b}	432.5^{a}	237.2^{a}
C + PG + PP + BO line	173.7^{a}	25.6^{a}	125.8^{a}	18.8^{a}	25.4^{a}	18.1^{a}	135.2^{a}	66.7^{a}	1476.7^{a}	540.8^{a}	266.5^{a}
C + PG + PP + BO broadcast	163.5^{b}	22.6^{a}	124.8^{a}	18.5^{a}	27.0^{a}	17.7^{a}	135.8^{a}	62.2^{a}	1455.1^{a}	537.0^{a}	239.4^{a}
Growing seasons											
First (2013–2014)	$148.2^{c\S}$	24.4^{a}	116.9^{a}	17.8^{a}	26.1^{a}	17.0^{a}	128.2^{a}	65.1^{a}	1046.8^{a}	506.8^{a}	266.6^{a}
Second (2014–2015)	158.7^{b}	23.6^{a}	120.1^{a}	18.1^{a}	23.5^{a}	18.6^{a}	122.7^{a}	64.2^{a}	1039.8^{a}	459.5^{a}	225.0^{a}
Third (2015–2016)	168.5^{a}	24.1^{a}	120.4^{a}	19.0^{a}	25.6^{a}	18.1^{a}	128.1^{a}	65.9^{a}	1032.4^{a}	503.1^{a}	254.5^{a}

Treatments	Nutrient content in standing residue										
	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
	kg ha^{-1}						mg ha^{-1}				
Crop systems											
C + PG + BO line	$9.8^{c\S}$	1.2^{b}	52.3^{b}	5.4^{b}	7.8^{b}	2.5^{b}	37.8^{b}	4.8^{b}	762.6^{b}	156.2^{b}	24.7^{b}
C + PG + BO broadcast	8.4^{d}	1.2^{b}	50.7^{b}	4.9^{b}	7.0^{b}	2.4^{b}	34.1^{b}	4.6^{b}	818.3^{b}	149.5^{b}	22.2^{b}
C + PG + PP + BO line	14.6^{a}	2.2^{a}	63.1^{a}	8.8^{a}	9.6^{a}	3.4^{a}	48.6^{a}	7.8^{a}	1032.8^{a}	205.1^{a}	41.9^{a}
C + PG + PP + BO broadcast	12.3^{b}	2.2^{a}	61.7^{a}	8.6^{a}	9.3^{a}	3.5^{a}	52.1^{a}	7.6^{a}	1088.5^{a}	203.9^{a}	40.4^{a}
Growing seasons											
First (2013–2014)	$8.7^{c\S}$	1.3^{b}	51.2^{b}	4.0^{b}	4.5^{b}	2.5^{b}	37.2^{b}	4.8^{b}	340.0^{b}	116.3^{b}	26.3^{b}
Second (2014–2015)	11.0^{b}	1.8^{a}	59.3^{a}	8.3^{a}	10.7^{a}	3.1^{a}	45.8^{a}	6.9^{a}	1335.5^{a}	218.3^{a}	35.5^{a}
Third (2015–2016)	14.0^{a}	1.8^{a}	60.3^{a}	7.0^{a}	10.0^{a}	3.2^{a}	46.4^{a}	6.8^{a}	1101.2^{a}	201.4^{a}	35.2^{a}

[§] *Means within a column of the same category (i.e., crop systems and growing seasons) followed by the same letter are not significantly different at $P \leq 0.05$.*

of all nutrients in standing residue. Furthermore, considering that the soil of the experimental area is a Typic Haplorthox rich in magnetite (iron oxide), pigeon pea exhibited a strong capacity for Fe absorption and accumulation.

Intercropping Competition Factors and Land-Use Efficiency

As previously reported, the low rainfall in the summer of the first growing season (**Table 1**) caused severe water stress during corn vegetative development. Thus, in the first growing season, as a function of the best resistance to water deficit and of increased competition with corn and palisade grass, pigeon pea resulted in higher individual (0.40–0.44 × 0.19–0.23) and total (1.63–1.59 × 1.28–1.31) LER than in the two other growing seasons (**Table 7**). During the second and third growing seasons, corn may have competed with pigeon pea, slightly reducing the individual LER (∼0.10). However, the LER of pigeon pea was ∼0.20, offsetting this reduction and resulting in a greater total LER.

In all growing seasons, palisade grass, and pigeon pea exhibited weak interspecific competition, but corn was strongly competitive in interspecific interactions (**Table 7**) because of the corn value (–K) and the palisade grass and pigeon pea value (+K) (Zarochentseva, 2012). However, the values of palisade grass and pigeon pea were extremely low, and the value of corn was closer to zero when intercropped only with palisade grass. In the triple intercrop (corn + palisade grass + pigeon pea), the K-value of corn and palisade grass was far from zero, demonstrating that these crops had to be much more competitive in the presence of pigeon pea.

Over three growing seasons, all of the crop system A-values for corn were negative, whereas such values for palisade grass and pigeon pea were always positive (**Table 7**), indicating that these crops presented higher aggression to compete with corn as a function of the high rates of corn dry matter accumulation (Pariz et al., 2017c). In the three growing seasons, the intercropping of pigeon pea reduced the aggressivity of corn, and in the first growing season, as a function of the less favorable weather conditions already discussed, the aggressivity of palisade grass was also lower. However, in all cases, the A-values were extremely low, indicating that this aggressivity was minimal.

Concerning the individual relative nutrient yields, only the N of corn was affected by the cropping system (**Table 8**). In the same way as N in corn leaves (**Table 3**) and the N content in the forage mass and in standing residue (**Table 6**), the relative N yield was higher in crop systems with triple intercropping (corn + palisade grass + pigeon pea) than in systems with

TABLE 7 | Land equivalent ratio (LER) of corn, palisade grass, and pigeon pea monoculture, relative crowding coefficient (*K*) and aggressivity (*A*) of corn to silage intercropped with palisade grass (C + PG) and with palisade grass and pigeon pea (C + PG + PP) with black oat (BO) oversown in lines and broadcast in three growing seasons.

Crop systems	LER				*K*			*A*		
	Corn	Palisade grass	Pigeon pea	Total	Corn	Palisade grass	Pigeon pea	Corn	Palisade grass	Pigeon pea
First growing season										
C + PG + BO line	1.09a§	0.07^{a}	–	1.16^{b}	−5.55^{b}	0.16^{b}	–	−0.0013238^{b}	0.0013238^{a}	–
C + PG + BO broadcast	1.07^{a}	0.12^{a}	–	1.19^{b}	−8.87^{b}	0.24^{b}	–	−0.0013238^{b}	0.0013238^{a}	–
C + PG + PP + BO line	1.10^{a}	0.10^{a}	0.44^{a}	1.63^{a}	−25.61^{a}	0.64^{a}	0.60^{a}	−0.0036451^{a}	0.0003264^{b}	0.0033187^{a}
C + PG + PP + BO broadcast	1.11^{a}	0.08^{a}	0.40^{a}	1.59^{a}	−23.25^{a}	0.52^{a}	0.54^{a}	−0.0036451^{a}	0.0003264^{b}	0.0033187^{a}
Second growing season										
C + PG + BO line	1.14^{a§}	0.02^{a}	–	1.17^{b}	−5.00^{b}	0.05^{b}	–	−0.0006853^{b}	0.0006853^{a}	–
C + PG + BO broadcast	1.17^{a}	0.03^{a}	–	1.19^{b}	−4.32^{b}	0.05^{b}	–	−0.0006853^{b}	0.0006853^{a}	–
C + PG + PP + BO line	1.06^{b}	0.02^{a}	0.20^{a}	1.28^{a}	−36.81^{a}	0.14^{a}	0.20^{a}	−0.0014979^{a}	0.0005580^{a}	0.0009399^{a}
C + PG + PP + BO broadcast	1.06^{b}	0.02^{a}	0.23^{a}	1.31^{a}	−36.48^{a}	0.12^{a}	0.24^{a}	−0.0014979^{a}	0.0005580^{a}	0.0009399^{a}
Third growing season										
C + PG + BO line	1.15^{a§}	0.04^{a}	–	1.19^{b}	−4.37^{b}	0.08^{b}	–	−0.0003647^{b}	0.0003647^{a}	–
C + PG + BO broadcast	1.17^{a}	0.04^{a}	–	1.21^{b}	−3.85^{b}	0.07^{b}	–	−0.0003647^{b}	0.0003647^{a}	–
C + PG + PP + BO line	1.04^{b}	0.04^{a}	0.20^{a}	1.28^{a}	−80.53^{a}	0.25^{a}	0.17^{a}	−0.0007567^{a}	0.0003375^{a}	0.0004193^{a}
C + PG + PP + BO broadcast	1.03^{b}	0.06^{a}	0.19^{a}	1.28^{a}	−97.01^{a}	0.35^{a}	0.17^{a}	−0.0007567^{a}	0.0003375^{a}	0.0004193^{a}

[§] *Means within a column of the same category in each growing season followed by the same letter are not significantly different at $P \le 0.05$.*

TABLE 8 | Relative nutrient yield (%) of corn to silage intercropped with palisade grass (C + PG) and with palisade grass and pigeon pea (C + PG + PP) with black oat (BO) oversown in lines and broadcast compared to corn, palisade grass, and pigeon pea monoculture (mean of three growing seasons).

Crop systems	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
	Corn										
C + PG + BO line	130.1^{c§}	124.3^{a}	61.1^{a}	46.0^{a}	64.1^{a}	95.1^{a}	59.2^{a}	91.7^{a}	51.3^{a}	34.5^{a}	117.8^{a}
C + PG + BO broadcast	122.2^{d}	122.8^{a}	61.6^{a}	38.4^{a}	60.1^{a}	95.0^{a}	54.2^{a}	90.1^{a}	56.3^{a}	30.2^{a}	115.0^{a}
C + PG + PP + BO line	149.6^{a}	122.4^{a}	59.9^{a}	40.2^{a}	60.3^{a}	95.9^{a}	56.5^{a}	91.0^{a}	65.0^{a}	35.7^{a}	120.3^{a}
C + PG + PP + BO broadcast	141.6^{b}	119.0^{a}	59.6^{a}	38.4^{a}	62.8^{a}	95.0^{a}	55.0^{a}	90.4^{a}	69.6^{a}	35.4^{a}	117.9^{a}
	Palisade grass										
C + PG + BO line	4.9^{a§}	5.9^{a}	6.6^{a}	5.4^{a}	4.6^{a}	5.2^{a}	9.7^{a}	3.8^{a}	9.3^{a}	4.5^{a}	4.4^{a}
C + PG + BO broadcast	4.9^{a}	7.5^{a}	6.4^{a}	7.3^{a}	5.6^{a}	6.4^{a}	9.9^{a}	5.4^{a}	9.7^{a}	6.5^{a}	4.2^{a}
C + PG + PP + BO line	4.9^{a}	6.4^{a}	6.4^{a}	6.1^{a}	4.6^{a}	5.4^{a}	9.2^{a}	4.1^{a}	8.7^{a}	4.7^{a}	3.7^{a}
C + PG + PP + BO broadcast	5.3^{a}	6.7^{a}	6.2^{a}	5.9^{a}	5.0^{a}	5.3^{a}	10.2^{a}	3.9^{a}	12.2^{a}	5.8^{a}	3.6^{a}
	Pigeon pea										
C + PG + BO line	–	–	–	–	–	–	–	–	–	–	–
C + PG + BO broadcast	–	–	–	–	–	–	–	–	–	–	–
C + PG + PP + BO line	27.0^{a§}	24.4^{a}	26.6^{a}	30.5^{a}	27.6^{a}	26.5^{a}	28.0^{a}	15.1^{a}	51.9^{a}	23.3^{a}	25.7^{a}
C + PG + PP + BO broadcast	23.4^{a}	20.6^{a}	25.9^{a}	26.0^{a}	25.6^{a}	24.4^{a}	24.8^{a}	13.2^{a}	40.3^{a}	15.7^{a}	18.6^{a}
	Total										
C + PG + BO line	134.9^{c§}	130.1^{b}	67.5^{b}	51.5^{b}	68.7^{b}	100.3^{b}	68.9^{b}	95.5^{b}	60.6^{b}	39.0^{b}	122.1^{b}
C + PG + BO broadcast	125.1^{d}	130.2^{b}	67.7^{b}	45.8^{b}	65.7^{b}	101.4^{b}	64.1^{b}	95.6^{b}	66.0^{b}	36.7^{b}	119.2^{b}
C + PG + PP + BO line	181.5^{a}	153.1^{a}	92.9^{a}	76.8^{a}	92.5^{a}	127.9^{a}	93.7^{a}	110.2^{a}	125.6^{a}	63.6^{a}	149.7^{a}
C + PG + PP + BO broadcast	170.3^{b}	146.3^{a}	91.7^{a}	70.3^{a}	93.4^{a}	124.7^{a}	90.1^{a}	107.4^{a}	122.1^{a}	56.9^{a}	140.1^{a}

[§] *Means within a column of the same category followed by the same letter are not significantly different at $P \le 0.05$.*

double intercropping (corn + palisade grass), mainly with black oat oversown in lines in winter/spring compared to oversown black oat that was broadcast, also reflecting a higher total relative N yield.

It should be noted that of all the nutrients, other than N, only P and Zn presented relative yields in corn plants >100% in all cropping systems (**Table 8**). Therefore, the results show that intercropping with palisade grass resulted

TABLE 9 | Surface mulch quantity, mulch cover, nutrient contents, and lignin/N ratio (Lig/N) in surface mulch of palisade grass intercropped with corn (PG + C) and with corn and pigeon pea (PG + C + PP) to silage with black oat (BO) oversown in lines and broadcast in three growing seasons after grazing by lambs.

Treatments	Surface mulch	Mulch cover	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn	Lig/N
	Mg ha^{-1}	%	kg ha^{-1}						mg ha^{-1}					
Crop systems														
PG + C + BO line	$5.2^{c\S}$	74^{c}	44^{c}	8^{b}	111^{c}	18^{b}	14^{b}	10^{b}	266^{b}	23^{b}	$7{,}123^{b}$	862^{b}	129^{b}	8.9^{a}
PG + C + BO broadcast	4.5^{d}	66^{d}	43^{c}	8^{b}	114^{c}	16^{b}	14^{b}	10^{b}	313^{b}	24^{b}	$7{,}458^{b}$	873^{b}	130^{b}	8.6^{a}
PG + C + PP + BO line	7.9^{a}	99^{a}	74^{a}	12^{a}	195^{a}	28^{a}	19^{a}	16^{a}	402^{a}	33^{a}	$9{,}106^{a}$	$1{,}220^{a}$	210^{a}	6.8^{b}
PG + C + PP + BO broadcast	6.1^{b}	86^{b}	60^{b}	11^{a}	141^{b}	28^{a}	18^{a}	14^{a}	382^{a}	30^{a}	$9{,}365^{a}$	$1{,}308^{a}$	190^{a}	6.4^{b}
Growing seasons														
First (2013–2014)	$3.9^{c\S}$	59^{c}	38^{c}	7^{c}	93^{c}	15^{c}	11^{c}	8^{c}	227^{c}	18^{c}	$5{,}578^{c}$	709^{c}	109^{c}	7.5^{a}
Second (2014–2015)	6.1^{b}	86^{b}	59^{b}	10^{b}	145^{b}	23^{b}	17^{b}	13^{b}	350^{b}	28^{b}	$8{,}309^{b}$	$1{,}088^{b}$	169^{b}	7.8^{a}
Third (2015–2016)	7.7^{a}	97^{a}	69^{a}	13^{a}	183^{a}	30^{a}	22^{a}	16^{a}	445^{a}	36^{a}	$11{,}002^{a}$	$1{,}400^{a}$	216^{a}	7.7^{a}

[§] *Means within a column of the same category (i.e., crop systems and growing seasons) followed by the same letter are not significantly different at $P \leq 0.05$.*

in an ∼20% higher accumulation of these nutrients in the corn plants than monocropping. Tropical perennial grasses introduced into a cropping system resulted in a higher soil labile P content than fallow systems, probably because tropical perennial grasses can take up moderately labile soil P fractions that are recycled in the system, regardless of the P fertilization strategy (Almeida and Rosolem, 2016).

The relative yield of the other nutrients (K, Ca, Mg, S, B, Cu, Fe, and Mn) in the corn plants was <100% in all crop systems (**Table 8**). Among these nutrients, what surprised us was K, of which the accumulation reached approximately only 60% in all crop systems compared to monocropped corn. This is very good from the standpoint of extracting this nutrient in corn to silage production areas because the replacement management of K in these areas is a problem since the harvesting of corn to silage plants extracts large amounts of K from the soil (Pariz et al., 2016, 2017a). Thus, assuming that plants in all crop systems were adequately supplied with K (**Table 3**), we can hypothesize that monocropped corn presented extensive absorption of this nutrient, mainly from exchangeable forms in soil, which may exhaust this more available form of K in the soil over the medium to long term.

According to Rosolem and Steiner (2017) "regarding recommendations on sustainable fertilizer practices, sampling the 0–0.2-m layer seems to be insufficient, and increased rates of fertilizer K intensify K leaching below 1 m in tropical sandy clay loam soil." In this context, the results of Garcia et al. (2008) demonstrated that "K recycling in intercropping systems is very important for plant growth to avoid losses in the soil profile. However, it must be emphasized that if K rates are underestimated in a fertilization programme, mainly corn to silage, soil K reserves may be depleted, as corn intercropped with palisade grass is very effective in recycling K, leading to increased exchangeable K contents in the surface soil layers; further, palisade grass is able to take up non-exchangeable forms of K in the soil. Ultimately, this K, after cover crop desiccation, is washed out of plant residues, and eventually, more K is available for crops in succession." Furthermore, corn to silage intercropped with palisade grass at greater heights, as performed in the present study (0.45 m above the soil surface), can contribute to K cycling from K contained in the lower internodes of plants (Pariz et al., 2016, 2017a). Therefore, the intercropping of palisade grass with corn becomes an excellent alternative in the cycling and maintenance of exchangeable K content in the soil, not allowing the extensive absorption of this nutrient by corn plants. The same analogy can be considered true for the other nutrients (Ca, Mg, S, B, Cu, Fe, and Mn) because the relative yield in corn plants was <100%, and the corn plants were well-fed (**Table 8**). Thus, we can also hypothesize that the contact of different intercropped crop roots can inhibit this expansive absorption of some nutrients by corn plants (Hauggaard-Nielsen and Jensen, 2005).

The relative yield of all nutrients in palisade grass and pigeon pea plants was <100% and was not influenced by crop system (**Table 8**). However, the incorporation of pigeon pea in corn intercropped with palisade grass increased the total relative yield of all nutrients. Therefore, considering that the presence of pigeon pea in the triple intercrop did not interfere with the relative nutrient yields of corn and palisade grass, it can be inferred that this legume possibly absorbed and accumulated nutrients that might not be absorbed by corn and palisade grass plants, especially in deeper soil depths, as a function of the pivotal root system of pigeon pea (up to 1 m deep in relation to the soil surface).

Surface Mulch Quantity, Decomposition, Nutrient Content, and Release Rates

The higher surface mulch quantity, mulch cover, and N and K contents, mainly in the triple intercrop with black oat oversown in lines compared to double intercrop with black oat oversown in broadcast (increase of 75, 50, 72, and 71%, respectively), despite subsequent grazing by lambs (**Table 9**), was probably due to the nitrogen dynamics and soil decompaction under the development of the pivotal root system of pigeon pea. In addition, results of Costa et al. (2021) demonstrated that the inclusion of pigeon pea in the intercrop of corn with palisade grass efficiently

TABLE 10 | Soybean leaf nutrient concentrations at the R2 growth stage intercropped with guinea grass to silage in the fourth growing season in succession with corn to silage intercropped with palisade grass (C + PG) and with palisade grass and pigeon pea (C + PG + PP) with black oat (BO) oversown in lines and broadcast in the first three growing seasons.

Crop systems	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
2016-2017 growing season	g kg^{-1}						mg kg^{-1}				
C + PG + BO line	36.8^{c§}	3.1^{a}	26.9^{c}	7.1^{a}	3.4^{a}	2.1^{a}	31.6^{a}	10.5^{a}	81.5^{a}	54.2^{a}	38.4^{a}
C + PG + BO broadcast	36.5^{c}	3.0^{a}	26.5^{c}	7.0^{a}	3.3^{a}	2.0^{a}	31.4^{a}	10.6^{a}	81.7^{a}	53.7^{a}	38.0^{a}
C + PG + PP + BO line	45.1^{a}	3.3^{a}	30.1^{a}	7.6^{a}	3.6^{a}	2.2^{a}	32.3^{a}	10.5^{a}	85.8^{a}	55.8^{a}	39.8^{a}
C + PG + PP + BO broadcast	41.8^{b}	3.3^{a}	28.3^{b}	7.6^{a}	3.5^{a}	2.1^{a}	32.0^{a}	10.7^{a}	83.8^{a}	54.7^{a}	38.6^{a}

§ *Means within a column followed by the same letter are not significantly different at $P \leq 0.05$.*

increased the use of N fertilizer by corn, promoted greater N recovery in the soil, and increased total N in the crop system, thus decreasing unrecovered N from fertilizer in the plant-soil system, what according to Sprent (2007) can be explained because occurs symbiosis between plants and bacteria found in root nodules. In the case of oversown oats, the in-row modality provides better distribution, seed burial, and uniformity of the plant population than the broadcast modality. Furthermore, superficial incorporation into the soil using a disk harrow (fully closed disks for minimal ground disturbance) is necessary for better germination and to reduce the number of seeds vulnerable to consumption by birds. However, this mechanical operation can reduce the soil nitrogen concentration because it increases the porous space and breaks the stability of the soil organic matter, favoring greater N_2O emission.

However, in all crop systems, the mulch remaining 120 DAD was ∼50–60% (**Figure 1**). Other nutrient (P, Ca, Mg, S, B, Cu, Fe, Mn, and Zn) contents were also higher when palisade grass was intercropped with corn and pigeon pea than when intercropped with other treatments; however, there was no effect of oversown black oat. The surface mulch quantity, mulch cover, and all nutrient contents increased in the third growing season compared with the second and first growing seasons, possibly due to a carryover effect and more time between the end of grazing by lambs and desiccation of the remaining plants.

The crop system affected the lignin/N ratio of the surface mulch of palisade grass (**Table 9**), which was lower in crop systems with triple intercropping than in those with double intercropping, because of the highest N content in plant surface mulch. Potassium was the nutrient with the highest accumulation in the mulch of palisade grass and exceeded 100 kg ha^{-1} in all crop systems and nitrogen was the nutrient with the second highest accumulation in the mulch of palisade grass (**Table 9**); in general, the residues released ∼50–60% of this nutrient at 120 DAD. The peak release of P, K, Mg, and B occurred in the first 30 days and in general, the residues released more than 60% of this nutrient prior to 120 DAD and at ∼90 DAD, K had been completely released from the palisade grass residues (**Figure 2**). The remaining Mg (30–40%) was subsequently and gradually released as part of the structural plant compounds. In this way, at 30 DAD, ∼50% of the Mg contained in the plant residues had been released.

Soybean Leaf Nutrient Concentrations, Agronomic Characteristics, and Yield

Concentrations of N in soybean leaves (**Table 10**) were slightly below a sufficiency level when previous crop systems were corn intercropped only with palisade grass (Ambrosano et al., 1997; Embrapa—Empresa Brasileira de Pesquisa Agropecuária, 2006). This result demonstrated that crop systems without the inclusion of legumes in intercropping or crop rotation over time may reduce the availability of N and consequently uptake by crops. The triple intercrop with black oat oversown in lines increased 23% the N content in soybean leaves compared to double intercrop with both black oat oversown systems. The differences in K concentrations in soybean leaves as a function of previous crop systems were possibly a function of the K content in the surface mulch (**Table 9**), demonstrating good nutrient cycling and a synchronized K release rate from mulch with absorption by soybeans during their growth cycle. The triple intercrop with black oat oversown in lines increased 13% the K content in soybean leaves compared to double intercrop with both black oat oversown systems. Despite no differences among crop systems with respect to other nutrients (P, Ca, Mg, S, B, Cu, Fe, Mn, and Zn), soybean leaf concentrations were within a range considered appropriate for soybean crops (Ambrosano et al., 1997; Embrapa—Empresa Brasileira de Pesquisa Agropecuária, 2006).

The higher values of SPP after triple intercropping (corn + palisade grass + pigeon pea) (**Table 11**) were a function of a higher quantity of surface mulch and the best mulch cover (**Table 9**), which reduced the bird attacks (mainly doves) on soybean seedlings. According to Yokomizo et al. (2000), the HFPI should be >0.12 m to enable proper mechanical harvesting of soybean. In our study, the HFPI of soybean was 0.13–0.15 m; therefore, mechanized harvesting was not a problem. The higher values of NPP, dry weight of 100 seeds (W100), dry weight of seeds per hectare, forage mass (FM), and standing plant residue (SPR) after triple intercropping can be explained by better mulch cover, N and K plant nutrition (**Table 10**) and possibly soil physical characteristics as a function of the root system of the pigeon pea and palisade grass discussed above, which favored the vegetative and reproductive growth of soybean. Thus, the combination of higher SPP, NPP, and W100 was reflected in ∼1.0, 1.5, and 0.4 Mg ha^{-1} more seeds, FM and SPR, respectively, than in crop systems with double intercropping (corn + palisade

TABLE 11 | Soybean plant population (SPP), plant height (PH), height of the first pod insertion (HFPI), number of pods per plant (NPP), number of seeds per pod (NSP), dry weight of 100 seeds (W100), dry weight of seeds per hectare, forage mass (FM), and standing plant residue (SPR) on the soil surface of soybean to silage intercropped with guinea grass in the fourth growing season in succession with corn to silage intercropped with palisade grass (C + PG) and with palisade grass and pigeon pea (C + PG + PP) with black oat (BO) oversown in lines and broadcast in the first three growing seasons.

Crop systems	SPP	PH	HFPI	NPP	NSP	W100	Seeds	FMS	SPR
2016–2017 growing season	***n°* × 1,000**	**m**		**n°**		**g**	**Mg ha^{-1}**		
C + PG + BO line	317^{b§}	73.5^{b}	13.2^{b}	42^{b}	2.5^{a}	13.1^{b}	4.4^{b}	9.4^{b}	1.1^{b}
C + PG + BO broadcast	319^{b}	73.0^{b}	13.0^{b}	41^{b}	2.5^{a}	13.1^{b}	4.3^{b}	9.2^{b}	1.0^{b}
C + PG + PP + BO line	337^{a}	79.2^{a}	15.3^{a}	45^{a}	2.6^{a}	13.4^{a}	5.5^{a}	11.0^{a}	1.5^{a}
C + PG + PP + BO broadcast	335^{a}	80.1^{a}	15.1^{a}	45^{a}	2.6^{a}	13.4^{a}	5.3^{a}	10.6^{a}	1.4^{a}

§*Means within a column followed by the same letter are not significantly different at $P \leq 0.05$.*

grass). Despite a lack of data on the N derived from the soil, pigeon pea may have provided some N (Baldé et al., 2011) to soybeans and guinea grass to enhance the seeds and FM (**Table 11**). The triple intercrop with both black oat oversown increased 24 and 16% the seeds and FM of soybean compared to double intercrop with both black oat oversown systems.

CONCLUSIONS

Our study revealed the potential for intercropping palisade grass and pigeon pea with corn in summer/autumn in the Brazilian "Cerrado" for produce forage mass and, subsequently, mulching in NTS. In addition, oversown black oat with palisade grass as a cover crop is also an good option for mulch production in the spring. Thus, with the inclusion of pigeon pea, maintaining long-term productivity would require fewer external N inputs. However, more constant monitoring of soil fertility, with an emphasis on the application of limestone, gypsum, phosphorus, and micronutrients, is necessary, especially because a majority of the biomass produced in these ICLS throughout the year is exported via the cutting of corn plants for ensilage.

Despite greater interspecific competition of palisade grass and pigeon pea intercropped with corn, this more complex system had better results. Thus, when analyzing this ICLS as a whole during the four growing seasons, the triple intercrop (corn + pigeon pea + palisade grass) combined with oversown black oats (mainly in lines) in the first three growing seasons was the most effective option for silage production and for the improvement of other elements of system productivity, such as higher surface mulch quantity (75%), leaf nutrient concentrations, mainly nitrogen and potassium (23 and 13%, respectively), and yield of soybean forage mass (16%) to silage intercropped with guinea grass in the fourth growing season. This type of intercropping also produced better nutrient cycling because it increased the quantity of nutrients retained in SPRs and surface mulch and provided better land- and nutrient-use efficiency, with an emphasis on nitrogen and potassium.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by this study was conducted in accordance with the Ethics Committee on Animal Use (CEUA) of São Paulo State University (UNESP) at the College of Veterinary Medicine and Animal Science in Botucatu, São Paulo, Brazil, under protocol number 31/2014-CEUA.

AUTHOR CONTRIBUTIONS

CP, NC, and FA: experimental work, data analysis, results interpretation, and writing. CC, CACC, PM, JC, MA, and AF: results interpretation and writing. AC: data analysis, results interpretation, and writing. DS, IC, and VL: experimental work and writing. VP, JS, MP, VM, RS, DF, and LS: experimental work. All authors contributed to the article and approved the submitted version.

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Sensitivity and Tolerance of Different Annual Crops to Different Levels of Banana Shade and Dry Season Weather

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Intercropping in small-holder production systems in East and Central Africa is very common and offers potential for significant yield and environmental benefits. However, the reduced light availability under banana canopies constrains the success of the intercrop in banana systems. Determining a balance between the optimal spacing/densities of banana plants with optimized intercrop selection based on their sensitivity and tolerance to shade is imperative. This study, through extensive field experiments performed in South Kivu, DR Congo investigated the resilience of a wide range of food and forage crops to varying banana shade levels. The same crop species grown as monocrops served as controls. Quantitative yield assessments showed yam, sweet potato, ginger and forage grasses to have a good potential to grow under moderately dense to dense banana fields. Taro, soybean, mucuna, chili, eggplant, and *Crotalaria* sp. performed well in sparsely spaced banana fields with moderate shading. Cassava and soybean showed limited tolerance to shade. Intercropping in banana systems is also generally confined to the rainy seasons due to the high sensitivity of most annual intercrops to long dry weather in the dry season months. We also thus assessed the sensitivity of chickpea and mucuna to the long dry weather of the dry seasons and found them to have great potential for extending farming production into the dry season. Overall, we show that careful selection and allocation of crops with varying sensitivity to various banana shade levels and dry season weather can potentially increase whole field productivity.

Keywords: banana, biomass, cover crop, Democratic Republic of Congo, intensification, mucuna

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INTRODUCTION

Banana (*Musa* sp.) producing landscapes in the East and Central African region are characterized by small (<2 ha) and highly fragmented farms (van Asten et al., 2004; Niroula and Thapa, 2005; UBOS, 2010; Wairegi et al., 2010; Tinzaara et al., 2018). Fragmentation of farms is hastened by the high population densities and land tenure system. Population densities of up to 470 people/km^2 have been reported in parts of the region (The World Bank Group, 2016). The land tenure system involves parents dividing their land between children, resulting over generations into smaller fragmented land sizes thus making it hard to consolidate land for agricultural production. The smallness and continuous fragmentation of farms hastens land degradation and limits the financial returns farmers can obtain from their land (Niroula and Thapa, 2005; UBOS, 2010). To use the available land optimally, farmers therefore often diversify crop types within fields through intercropping to augment yield without the additional need for more farmland (Ocimati et al., 2013, 2019; Tittonell and Giller, 2013; Ntamwira et al., 2014). Intercropping can provide potential co-benefits which can help maintain or even increase the sustainability of the farming systems. Firstly, soil degradation being a major issue in banana production systems (Wairegi et al., 2010; van Asten et al., 2011) can be mitigated through the intercrop. The increased soil coverage by plant canopy and an overall increased root network in the field improves conservation of soil moisture through reduced soil water evaporation and reduces the risk of erosion by stabilizing the soil surface layers (Lithourgidis et al., 2011). Additionally, intercropping can improve soil fertility of the field specifically when using nitrogen fixing crops (e.g., legumes), as will decomposed residue and mulch from parts of the intercrop not harvested (Lithourgidis et al., 2011; Gebru, 2015). This is particularly important for small-holder farmers in Central and East Africa where the use of fertilizers is minimal (Blomme et al., 2018). A second co-benefit from intercropping is a reduced incidence of pests and diseases often attributed to the improved biodiversity allowing for an increase in populations of natural enemies of the pests (Lithourgidis et al., 2011; Mulumba et al., 2012; Gebru, 2015; Ocimati et al., 2018). Intercropping has also been shown to effectively suppress weeds (Jensen et al., 2005; Gebru, 2015; Ocimati et al., 2019), leaving more resources for the crops that provide actual yield. The minimized effort in weed management and the reduced need for both herbicides and pesticides increase the cost-effectiveness of the intercropping system. Finally, the diversification of crops reduces the risk of total crop failure (Lithourgidis et al., 2011) providing greater financial stability for farmers.

In Central and East Africa, small-holder farms predominantly intercrop banana with short maturing crops in, respectively, open or young banana fields (Ntamwira et al., 2013). Intercropping in newly established banana fields e.g., works well with beans, coffee, maize and sweet potatoes (Ouma, 2009). Intensification of intercropping in more mature banana fields is however highly constrained by the reduced light availability. The light-demanding nature of most short-stature crop species therefore restricts their intercropping to the most sparsely spaced banana fields. In dense banana fields e.g., in eastern Democratic Republic of Congo (DR Congo), it's also common for farmers to cut banana leaves, at the onset of an annual cropping season, to increase the amount of light reaching the shorter intercrops, a practice that however reduces the economic efficiency of the system (Ocimati et al., 2019). Several more shade-tolerant crops, e.g., tuber crops *Colocasia* sp. (taro) and *Xanthosoma* sp. (cocoyam), have been shown to perform well under mature banana plantations (Blomme et al., 2018). Nevertheless, limited information is available on the shade-tolerance or sensitivity of a wider variety of crops for use in higher shade levels of denser banana fields. Given the small land sizes, exploration of species that can thrive under shade offers a good alternative for increasing yield and/or nutritional diversity of these farms.

Additionally, intercropping in banana systems in Central Africa is mostly confined to the rainy seasons with only few plant species [e.g., multipurpose trees, banana and coffee (*Coffea* spp.) allowing for a year-round production (Blomme et al., 2018)]. Farmers also often leave root and tuber crops [mainly taro, sweet potato (*Ipomoea batatas*) and cassava (*Manihot esculenta*) planted during the rainy season on the field during the dry season and harvest them based on household needs (Blomme et al., 2018)]. Most short-stature banana intercrops however cannot thrive in drier conditions and small-holder farmers cannot irrigate to extend their production to the dry seasons. Integration of crop species that are less sensitive to long dry weather (i.e., less sensitive to heat stress) conditions and can thrive when soil moisture content is low would enable farmers to extend their production into drier months thus offering an opportunity for higher total biomass yields. Soil coverage during the dry season would also be specifically advantageous for reducing the pressures of heat stress and moisture loss on the field (Blomme et al., 2018). Integration of crop species that are less sensitive to long periods of dry weather would thus allow for a more optimal use of available land as additional yield could be obtained.

This study therefore assessed the tolerance or sensitivity of a wide range of food and forage crop species to varying shade levels of open to dense banana fields. We assessed the performance of the known shade-tolerant crops taro and bird's eye chili (*Capsicum annum*) and the semi shade-tolerant crops cassava, yam (*Dioscorea* sp.) and ginger (*Zingiber officinale*) (Johnston and Onwueme, 1998, Sreelathakumary and Rajamony, 2002, Okwuowulu, 2005), and of crops for which less (or contrasting) information on shade-tolerance is available, including mucuna (*Mucuna pruriens*), chickpea (*Cicer arietinum*), soybean (*Glycine max*), sweet potato, and eggplant (*Solanum melongena*) (reviewed in Blomme et al., 2018). Two crops mucuna and chickpea shown to have a great potential for withstanding drier conditions (Johansen et al., 1994; Berger et al., 2004; Chiu, 2004), were also tested for their performance during the dry season. Finally, the integration of forage species (including grasses and shrubs) as hedges and borders as an additional means to augment land utilization within these banana systems is investigated.

MATERIALS AND METHODS

Study Area

This study was conducted through extensive field experiments set-up in the South Kivu province of the DR Congo. The experiments were carried out between March 2015 and July 2019 across 3 locations, namely, the Katana center (02°13.427′ S, 028°49.674′ E; 1,647 masl), the INERA-Mulungu research station (02°20.042′ S, 028°47.311′ E; 1,707 masl) and Kavumu (02°17.4′ S, 028°48.24′ E; 1,744.3 masl). The three sites are located within the same administrative boundary and within 20 km from each other. This region receives an annual precipitation of 1,656 ± 235 mm (2015–2018) with one long dry season from May till

September (monthly rainfall ≤ 150 mm) (**Figure 1**). The second short dry season (January–February), with an annual monthly rainfall of about 150 mm separates the two annual cropping seasons (**Figure 1**). For the first cropping cycle (season A), crops are planted in September and harvested between December and January, while for the second cropping cycle (season B) crops are planted from mid-February to March and harvested in May. Cumulative rainfall is higher in season A compared to season B (**Figure 1**).

The Katana site has highly fertile non-acidic volcanic granitic clay soils composed of a thick humus layer (Lunze, 1988, 2000). At INERA-Mulungu, soils are volcanic-derived and reasonably fertile (Kempers and Zweers, 1986). The Kavumu site has heavy clay soils with a pronounced A horizon, slightly acidic, with low organic matter, nitrogen and phosphorus levels, and altogether rather poor. Detailed characteristics of the soils at the three sites are provided in **Table 1**.

Experimental Design

Various crop species (roots and tubers, vegetables, forages, and cover crops) were planted within mature banana fields with different shading intensities. Across the various banana cultivars grown in the test plots, banana canopy size varied according to planting density and banana mat structure. The resulting shade level differences, which influence intercrop growth, were captured as photosynthetically active radiation (PAR) using a photometer as a basis for comparison across treatments. An ACCUPAR photometer probe (Model LP-80, Decagon Devices, Pullman, WA, USA; Decagon Devices, 2004) was used to measure the PAR (μmol/m^2/s) received under the leaf canopy for the different banana shade treatments and above the monocrops. At least 4 PAR measurements were taken in each intercropped treatment replicate (giving a total of at least 12 measurements per treatment). Measurements were taken at 50 cm from a banana plant at a height of 30 cm above the intercrops and between 11.00 a.m. and 3.00 p.m. In addition, four PAR measurements at a height of 30 cm above the sole annual crops were also taken at the center of each mono-cropped plot/ replicate. For all experiments across the three sites, a randomized complete block design was used. A total of 16 crop species were evaluated across sites, with only mucuna, taro, *Setaria sp.*, elephant grass (*Pennisetum* sp.), *Calliandra calothyrsus,* and *Leucaena leucocephala* evaluated at multiple sites.

Katana Experiments

At Katana, two mature banana fields, one comprising of the East African highland beer banana cultivar "Nshika" (AAA-EAH) and the other of a mixture of high yielding banana cultivars were used for the experiments. The mixed cultivar field had cultivars "T6" (plantain), "NSH20" (cooking), "NSH42" (beer) and "FHIA03" (dessert). The "Nshika" field was comprised of three banana plant densities (2 × 2 m, 2 × 3 m, and 3 × 3 m spacings) while the mixed cultivar field had a spacing of 3 × 4 m. The 3 × 3 m spacing is the most recommended spacing for banana in the study region, while lower spacings of 2 × 2 m and 2 × 3 m are common for banana mono-cropping and 3 × 4 m for banana intercrops. Prior to the establishment of crops to assess for shade-tolerance or sensitivity and/ or sensitivity to long dry weather, these fields had been intercropped with beans and amaranth until canopy shade level no longer allowed for these intercrops to grow. During this period, de-suckering to retain only 3 plants per mat (mother plant and two suckers) was carried out routinely at planting of the annual crops in the "Nshika" plots, while irregular de-suckering was carried out in the mixed banana cultivar plot. The crops assessed for sensitivity to shade or dry weather were established in February 2016 and September 2016 for the "Nshika" and mixed cultivar plot, respectively.

The crop species assessed for their sensitivity to shade were first introduced in February 2016 under the four banana planting densities. The crop species included the tuber crops cassava, a sweet variety named "Sawasawa," taro (*Colocasia* sp.; a shade-tolerant and prolific variety "Ishikazi") and yam (a pro-vitamin A rich variety "Nyaluliga"), the spice crop "red bird's eye chili' (variety "Lushendo") and the cover crop mucuna (*Mucuna pruriens*).

The tuber crops and chili were harvested after 12 months while mucuna was harvested after 4 months. Mucuna was grown across the various banana planting densities during the rainy cropping season B (planted in March and harvested in June) and in a separate trial also during the subsequent dry season (planted in May and harvested in August) (cf. **Figure 1**). The May to August mucuna crop was established to test for both shade and dry weather sensitivity or tolerance. Specifically, for cassava and taro, harvesting of young leaves as a vegetable prior to the final tuber harvest was carried out. To test the impact of this practice on the final tuber yield, young leaf harvesting treatments with intensities of a third or half of all leaves removed were performed. These tests were done across both the intercrops and the monocrops and compared to the yield when no young leaves were harvested.

INERA-Mulungu Experiments

At INERA-Mulungu, a mature cooking banana field (cultivar: AAA-EAH, "Barhabesha") at two spacing densities of 2 × 2 m and 4 × 4 m and a mature beer banana field (cultivar: AAA-EAH, "Nshika") with a 3 × 4 m spacing were available and used. Here the "Barhabesha" (2 × 2 and 4 × 4 m) banana fields were intercropped in the wet seasons A and/or B with chickpeas, ginger, eggplant, the forage crop C*rotalaria juncea,* four varieties of sweet potatoes and mucuna in subplots (**Supplementary Table 2**). Ginger and eggplant were harvested after 9 and 12 months, respectively. *Crotalaria* sp. was planted only during cropping season B and harvested after 4 months. All sweet potato varieties were planted during cropping season B and harvested after 5 months. For the chickpea crop, in addition to shade tolerance (assessed during cropping season A), the sensitivity of chickpea to long dry weather conditions of the dry seasons was assessed over the months of May to August and in the drier months in between the two cropping seasons (December till March) and chickpea yield during these seasons was compared with that from the cropping season A planting. The dry season chickpea was planted ∼1 month to the end of season B (early May) to ensure the crop benefitted from the residual water in the soil. In the third trial, chickpea was planted

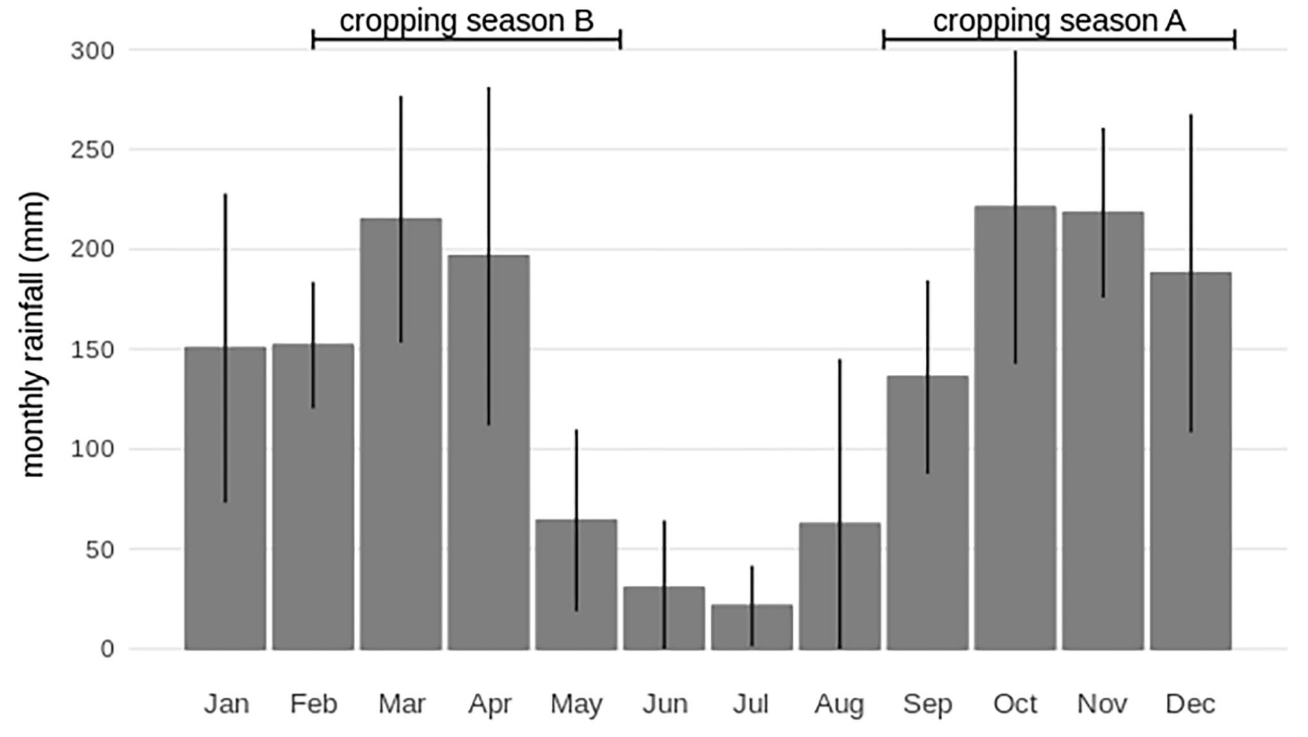

FIGURE 1 | Annual rainfall seasonality at the study site in South Kivu (DR Congo). Average monthly rainfall for the period 2015–2018 is provided; standard deviations are indicated. The timing of the two annual cropping seasons is shown, with cropping season A from September to December and cropping season B from mid-February–March to May.

TABLE 1 | Soil characteristics at Katana, Kavumu and INERA-Mulungu.

Sites	pH	OM (%)	N (%)	P (ppm)	Ca (ppm)	Mg (ppm)	K (ppm)
Katana	6.8a (±0.2)	7.2a (±0.6)	0.31a (±0.03)	100b (±18)	5569b (±861)	1480a (±121)	692a (±113)
Kavumu	6.1b (±0.2)	5.3b (±0.3)	0.26b (±0.06)	46c (±11)	4230c (±738)	928b (±80)	179c (±47)
INERA-Mulungu	6.8a (±0.5)	6.8a (±0.6)	0.30a (±0.02)	112a (±10)	6264a (±852)	1429a (±213)	371b (±99)
LSD	0.21	0.4	0.01	9.6	574.7	102.7	64.1
Fpr	0.001	0.001	0.001	0.001	0.001	0.001	0.001

For each site soil pH, organic matter (OM), nitrogen content (N), phosphorus content (P) and calcium (Ca), magnesium (Mg) and potassium (K) are provided. At Katana and Kavumu, averaged values are given of samples collected at the onset of the trials in 2014 and at the end of the trials in 2017. At INERA-Mulungu, soil characteristics were only determined at the end of the trials in 2017. Means in a column followed by the same letter are not significantly different from each other according to Tukey's HSD test ($p < 0.05$). Standard deviations are provided between brackets.

in December, i.e., during the last rains before the beginning of the drier period in between the two annual cropping seasons. The chickpea crop grew over a duration of 4 months in all three trails. The mucuna crop established at the beginning of the regular cropping seasons A and B, was allowed to grow into the drier months as cover crops. Thus, crops planted in September (season A) were harvested at the end of February, covering the periods in between the regular cropping seasons, and crops planted in March (cropping season B) were harvested in August, covering the dry period. The performance of mucuna as a cover crop was also assessed in the dry seasons between the two cropping seasons (September to December and March to May) in subplots under banana of different spacings (**Supplementary Table 2**). Mucuna planted in the dry season were planted 1 month early (May or December) to benefit from the residual soil moisture.

In the "Nshika" 3 × 4 m banana field only mucuna was grown. Mucuna was assessed in the drier periods in between the two cropping seasons as described for the 2 × 4 m "Barhabesha" fields above. For all crop and fodder species evaluated under different shade intensities, mono-cropped fields with equal replications as for the intercrops were established as controls.

Kavumu Experiment

At Kavumu a new banana field (beer cultivar; AAA-EAH, "Nshika") was established in March 2015 with a spacing of 3 × 4 m. Here, the performance of the tuber crop taro (*Colocasia* *esculenta,* variety "Astrida"), the legume crop soybean and of the forage grasses *Brachiaria ruziziensis* and *Setaria sphacelata* were assessed in intercrop and monocrop situations. The durations from planting (i.e., beginning of the rainy season) to harvest was 4 and 8 months for soybean and taro, respectively. The forage crops, planted at the onset of cropping season B, were first harvested at 6 months and thereafter monthly till the 23rd month of experimentation. The total cumulative biomass across harvests was used for the analysis. Additional trials on mucuna as a cover crop in both intercrop and monocrop formation were also performed, with performance tested through various planting and harvesting periods, including regular cropping seasons extended to the dry season months, year-round cropping and cropping during the dry season months in between the regular cropping seasons. Thus, the duration of the mucuna cropping cycle ranged from 4 to 12 months. At all locations the test crops were planted 30 cm from the banana mats to minimize damage to banana roots, competition and shading effects.

Forage grasses (elephant grass and *Setaria* sp.) and shrubs (*Calliandra* sp. and *Leucaena* sp.) were planted at all three sites as hedges at the boundary of fields for additional biomass production. These hedge crops were planted during cropping season A. These hedge crops were first harvested after 5 months and thereafter monthly, resulting in a total of 4 harvests. The total cumulative biomass across harvests was used for the analysis. Using forage crops as borders delineating property lines of adjacent farms allows for potential additional yields and optimal use of space. With the exception of taro, ginger, sweet potato for leaves and chili that had been observed to be shade-tolerant, and chickpea that had been reported to be drought-tolerant, the sensitivity of the other crop varieties evaluated to shade or long dry weather conditions was not known at the onset of the trials.

At all three sites above, each crop and treatment combination were replicated thrice while the same crop species grown as monocrops were assessed as controls in subplots separated by 0.5 m. Details on planting material, origin of the intercrops and spacing can be found in **Supplementary Table 1**, while size of the full subplots, the net plots assessed and/or corresponding number of plants assessed per growth and yield trait and intercrop are provided in the **Supplementary Table 2**.

Minimal tillage was also performed at planting while hand weeding was used as needed until the soil was covered by the respective test intercrop. In this region, smallholders hardly use inputs such as herbicides for weed control, fertilizers, pesticides for pest control or fungicides on their farms. Where these are used, they are only applied on high value crops. To mimic farmers' behavior, none of these inputs were applied in the various experiments across the three sites.

Data Collection and Analysis

Data collected in the various experiments/treatments included PAR values, growth and yield attributes. PAR values were measured during the cropping seasons as described in the section on "Experimental design" above. The growth and yield data depended on the crop characteristics and thus varied from one crop species to another. For all experiments and intercrops, fresh and dry biomass yields (kg) were measured at the end of the cropping cycle. According to the interest in yield for each crop, distinctions were made between the yield attributes leaf biomass, total aboveground biomass (stems and leaves), tubers, roots, grain and fruits yields. Specifically, data were collected on tuber yields for the tuber crops; rhizome yield for ginger; fruit yield for eggplants; grain yields for legumes; and vegetative biomass yield for legumes, chili, cover crops, the forage grasses, and the hedges crops. For the grasses and hedges, yields of multiple harvests were added up to obtain the total harvested biomass. For the root and tuber crops, cassava, taro and sweet potato, the weights of harvested leaves were also measured, while plant heights were measured for cassava, taro (at Katana) and the grasses *Setaria* sp. and *Brachiaria* sp. To obtain the dry biomass the fresh yields/biomass from the crops were dried in open air for 72 h and subsequently in an oven at 90°C for 48 h.

An analysis of variance using the GenStat v. 11 statistical software (VSN International Ltd, 2009) was conducted to determine the mean yields and effects of the different treatments. The Least Significant Difference (LSD) test at 5% probability level was used for means separation. Due to the inherent differences between the seasons, mean yields were compared between seasons and treatments. Treatments and crops that appeared across sites were also separately treated due to the inherent differences between the sites.

RESULTS

Light Reaching Shorter Crops

Light reaching the crops under the banana canopies varied with banana spacing/ plant densities and banana cultivar types (**Table 2**). At Katana, light availability to the short intercrops in the beer banana cultivar "Nshika" declined significantly ($p \leq 0.05$) with increasing plant density and was also significantly lower than in the open field. PAR declined by 73–89%, dropping from 1,835 ± 71 μmol/m^2/s in the open field to 209 ± 204 μmol/m^2/s in the 2 × 2 m plots (**Table 2**). The more sparsely spaced (3 × 4 m) mixed banana cultivars at Katana that were minimally de-suckered generally had a more robust canopy structure and had little light coming through (PAR of 288 ± 231 μmol/m^2/s) compared with the 2 × 3 m (475 ± 321 μmol/m^2/s) and the 3 × 3 m (501 ± 268 μmol/m^2/s) "Nshika" banana plots (**Table 2**).

At INERA-Mulungu, although field trials were performed in mature "Barhabesha" plots, relatively high light was recorded under both the open (4 × 4 m) and dense (2 × 2 m) banana canopies, with respective PAR values of 1,322–1,485 μmol/m^2/s and 603–742 μmol/m^2/s (**Table 3**). PAR at this site declined by 16%-27% in the 4 × 4 m and by 59–66% in the 2 × 2 m fields, respectively. The relatively high light under these fields was most likely due to the regular de-suckering that was practiced in these plots and the less robust structure of "Barhabesha" plants and mats. Here, banana canopy cover did not significantly differ in the period in between the two regular cropping seasons (PAR of 1,116 ± 299 μmol/m^2/s; short dry season from December–February), although light availability did reduce during the long

TABLE 2 | Yield and growth attributes of crops grown at the Katana center: tuber crops taro (variety "Ishikazi"), cassava and yam and spice crop chili.

Treatments	**% prior leaf harvesting**	**Taro**			**Cassava**			**Yams**	**Chili**	**PAR (μmol/m²/s)**
		Leaf yield (t/ha)	**Tuber yield (t/ha)**	**Height (cm)**	**Leaf yield (t/ha)**	**Tuber yield (t/ha)**	**Height (cm)**	**Tuber yield (t/ha)**	**Biomass yield (t/ha)**	
"Nshika" banana fields*										
2 × 2 m	0	0.2b	1.7cde (±0.8)	109abc (±62)	0.2c	0c (±0)	159bc (±94)	3.74b (±1.39)	0.13a (±0.09)	209c (±204)
	33	0.2b	1.4e (±0.5)	89bc (±18)	0.1c	0c (±0)	123de (±35)			
	50	0.4b	1.7cde (±0.8)	83c (±10)	0.1c	0c (±0)	122de (±54)			
2 × 3 m	0	0.3b	3.1ce (±0.6)	117abc (±9.5)	0.2c	0.3c (±0.5)	179bc (±84)	4.17b (±0.35)	0.15a (±0.07)	475b (±321)
	33	0.2b	2.4cde (±1.4)	93bc (±14)	0.1c	0c (±0)	102e (±47)			
	50	0.4b	1.6de (±0.6)	131abc (±71)	0.1c	0c (±0)	103e (±49)			
3 × 3 m	0	0.7b	7.6c (±1.6)	136abc (±36)	0.1c	0c (±0)	102e	5.66ab (±1.36)	0.18a (±0.01)	501b (±268)
	33	0.5b	5.7cde (±1.6)	90bc (±37)	0.1c	0c (±0)	157bc (±38)			
	50	0.6b	5.1cde (±2.9)	114abc (±45)	0.2c	0.04c (±0.1)	151bcd (±45)			
Mixed banana cultivar fields										
3 × 4 m	0	0.3b	7.5cd (±1.3)	93bc (±14)	0.4c	3.2c (±2.2)	149cd (±95)	3.45b (±3.61)	0.06a (±0.03)	288c (±231)
	33	0.4b	6.5cde (±1.4)	92bc (±28)	0.3c	0.6c (±0.8)	106e (±56)			
	50	0.6b	5.5cde (±2.7)	90bc (±31)	0.3c	0.5c (±0.4)	108e (±54)			
Monocrop										
	0	1.4a	40.2a (±9.9)	139ab (±10)	9.4a	27.7a (±5.0)	245a (±58)	9.82a (±4.98)	5.22b (±2.03)	1835a (±71)
	33	2.4a	30.8b (±3.6)	129abc (±13)	3.6b	8c (±3)	167b (±35)			
	50	1.9a	25.2b (±6.4)	160a (±13)	5.0b	13.1b (±6.5)	183b (±23)			
LSD		1.7	5.9	53.7	3.6	3.9	31.7	4.95	1.73	105
Fpr		0.854	0.049	0.793	0.029	0.001	0.001	0.086	0.001	0.001

The mixed cultivar types included: - "T6" (plantain), "NSH20" (cooking), "NSH42" (beer), and "FHIA03" (dessert).

Cumulative harvested yield of young leaves, tuber yield and total plant height at harvest are reported for tuber crops. For chili the total aboveground biomass was assessed. Yields are compared between intercrops in banana fields with various spacing (2 × 2 m, 2 × 3 m, 3 × 3 m, 3 × 4 m) and monocrops. For taro and cassava, the impact of young leaf harvesting (33% or 50% leaf removal) on yield is assessed. Leaf yield represents cumulative harvested leaves during the cropping period. Photosynthetic active radiation (PAR) was measured under the respective banana canopies or in the open field for the monocrops. Means in a column followed by the same letter are not significantly different from each other according to Tukey's HSD test ($p < 0.05$). Standard deviations are provided between brackets.

TABLE 3 | Yield and growth attributes of crops grown at INERA-Mulungu: chickpeas, eggplant, ginger, and the forage crop *Crotalaria* sp.

Planting time	Treatment (Banana spacing or monocrop)	Chickpea		Eggplant	Ginger	Crotalaria	PAR (μmol/m^2/s)
		Grain yield (t/ha)	Biomass (t/ha)	Yield (t/ha)	Yield (t/ha)	Yield (t/ha)	
At onset of cropping season A	2 × 2 m	0.00d (±0.00)	0.02d (±0.00)	1.51cd (±0.49)	1.19b (±0.21)	NA	NA
	4 × 4 m	0.01d (±0.00)	0.06d (±0.00)	2.70c (±0.57)	1.65b (±0.26	NA	NA
	Monocrop	0.02d (±0.00)	0.17d (±0.01)	6.37a (±1.60)	2.86a (±0.40	NA	NA
At onset of cropping season B	2 × 2 m	NA	NA	1.33d (±0.34)	1.02b (±0.69)	2.9a (±0.3)	742c
	4 × 4 m	NA	NA	2.29cd (±0.29)	1.13b (±0.70)	4.1a (±1.0)	1322b
	Monocrop	NA	NA	4.30b (±0.59)	1.46b (±0.83)	5.1a (±1.0)	1817a
Dry season	2 × 2 m	0.03c (±0.00)	0.11c (±0.01)	NA	NA	NA	603c (±10)
	4 × 4 m	0.11b (±0.05)	0.70b (±0.31)	NA	NA	NA	1485a (±12)
	Monocrop	0.27a (±0.03)	1.65a (±0.01)	NA	NA	NA	1767a (±40)
LSD		0.03	0.50	1.31	0.88	2.2	535
Fpr		0.001	0.004	0.091	0.13	0.112	0.001

Reported yields include grain and total aboveground biomass yields for chickpea, fruit yield for eggplant, root yield for ginger and aboveground biomass yield for Crotalaria sp. Yields are compared between the two annual cropping seasons for intercrops in banana fields with various spacing (2 × 2 m, 4 × 4 m) and monocrops, including an additional comparison in the dry season for chickpea. Photosynthetic active radiation (PAR) was measured under the respective banana canopies or in the open field for the monocrops. Means in a column followed by the same letter are not significantly different from each other according to Tukey's HSD test ($p < 0.05$). Standard deviations are provided between brackets. NA, not assessed.

dry season (May- August/September) to 865 ± 469 μmol/m^2/s (**Table 4**).

At Kavumu, PAR stayed relatively high, with PAR ranging between 587 and 903 μmol/m^2/s under the open spaced (3 × 4 m) and generally less robust-structured beer banana cultivar "Nshika", accounting for a 52–67% decline in light intensity relative to the light reaching the monocrops (**Table 5**).

Sensitivity of the Crops to Shade

An overview of the performance of all crops intercropped in the various banana fields compared to the monocrops is provided in **Figure 2**. Details on the yields attained in each cropping formation for each crop type can be found across **Tables 2**–**7**.

Taro

Two taro varieties, "Ishikazi" and "Astrida," were, respectively, assessed at the Katana and Kavumu sites. High tuber yields were achieved for both taro varieties in monocrop at either site (40.2 ± 9.9 t/ha and 37.9 ± 20.4 t/ha at Katana and Kavumu, respectively (**Tables 2**, **5**). At Katana however, significant reductions in tuber yield were recorded for intercropped plants, declining from 7.5 ± 1.3 t/ha in the 3 × 4 m plot to 1.7 ± 0.8 t/ha in the 2 × 2 m plot (**Table 2**). At Katana, total plant height did not significantly differ between the mono-cropped and intercropped taro plants at plant spacings of 2 × 2, 2 × 3, and 3 × 3 m. At Kavumu, tuber yields were high in both cropping seasons with an equally good performance in the intercrop (an average high yield of 44 ± 8 t/ha) compared with the monocrop (**Table 5**).

Taro leaf yields at Katana were higher (1.4 ± 0.3 t/ha) in the monocrop compared with yields of 0.2–0.7 t/ha under the intercropped "Nshika" fields (2 × 2, 2 × 3, 3 × 3 m) and 0.3 ± 0.1 t/ha in the 3 × 4 m mixed banana cultivar field (**Table 2**). There was a general reduction in taro tuber yields with increasing leaf pruning for both the mono-cropped and intercrops plants, though no significant differences were observed between the leaf cutting treatments within each planting density. For example, compared to 40.2 ± 9.9 t/ha in the treatment with no leaf cutting, yield of tubers was reduced to 30.8 ± 3.6 t/ha and 25.2 ± 6.4 t/ha when 33% and 50% of leaves were harvested, respectively, in the monocrop (**Table 2**).

Cassava

Cassava tuber and fresh leaf yield, only assessed at Katana (**Table 2**), was highest in the monocrop and when no young leaves were harvested, with mean yields of 27.7 ± 5.0 t/ha and 9.4 t/ha, respectively. Cassava tuber and leaf yield were significantly lower in the intercrop treatment than in the monocrop, with no significant differences observed between the intercrop treatments. Tuber yield under the 2 × 2 m to 3 × 4 m spaced banana fields varied between 0 and 3.2 t/ha while yield of fresh cassava leaves varied between 0.05 and 0.35 t/ha. Both cassava tuber and leaf yields decreased significantly in the monocrop when young leaves were harvested during the growing season, with an average retained yield (standing plant) of 8.0–13.1 t/ha and 3.6–5.0 t/ha for tubers and leaves, respectively. Similar trends to that of cassava tuber and leaf yield were observed for cassava plant height (**Table 2**).

Other cassava plant characteristics (including total stem weight and total root weight (combined weight of edible and non-edible roots; **Supplementary Table 3**) were also larger in the monocrop. In general, the reduction in the total root and stem weight also increased with increasing planting density.

Yam

Yams were only assessed at Katana. No significant differences ($p \leq 0.05$) in yam tuber yields were observed in between the treatments though a higher yield of 9.82 ± 4.98 t/ha was attained in the monocrop field (**Table 2**). In the "Nshika" banana-yam intercrop, yam yields declined with increasing shade intensity, varying between 5.66 ± 1.36 t/ha in the 3 ×

TABLE 4 | Mucuna biomass yields across the sites INERA-Mulungu, Kavumu, and Katana for various cropping periods.

Site	Cropping period	Cycle duration (months)	Banana spacing	Biomass yield (t/ha)	PAR (μmol/m^2/s)
Katana	Cropping season B	3	2 × 2 m	2.38ab (±2.27)	41b (±22)
			2 × 3 m	4.54a (±0.93)	192b (±151)
			3 × 3 m	4.95a (±0.31)	170b (±137)
	Long dry season (May–August)	4	3 × 4 m	0.8b (±0.88)	143b (±135)
			Monocrop	3.74ab (±3.08)	1700a (±0)
			LSD	3.51	211.5
			Fpr	0.011	0.001
INERA-Mulungu	Cropping season B, Extended over dry season	5.5	2 × 2 m	2.76d (±1.04)	407c (±295)
			4 × 4 m	4.53bc (±0.00)	720bc (±238)
			Monocrop	5.84a (±1.61)	1783a (±20)
	Cropping season A, Extended over period in between cropping seasons	7	2 × 2 m	1.54e (±0.54)	NA
			4 × 4 m	4.56bc (±0.57)	NA
			Monocrop	4.96b (±1.14)	NA
	Short dry season in between cropping seasons (December to February)	3	2 × 2 m	2.75d (±0.35)	NA
			4 × 4 m	3.78c (±0.33)	NA
			Monocrop	4.89b (±0.09)	NA
		3	3 × 4 m	1.18e (±0.30)	1116b (±299)
	Long dry season (May to August)	4	2 × 2 m	0.20g (±0.05)	NA
			4 × 4 m	0.73eg (±0.18)	NA
			Monocrop	1.10e (±0.22)	1828a (±189)
		4	3 × 4 m	0.55c (±0.09)	865b (±469)
			LSD	0.83	338.4
			Fpr	0.006	0.001
Kavumu	Cropping season B, Extended over dry season	6	3 × 4 m	0.91cd (±0.59)	543b (±197)
			Monocrop	6.54a (±2.85)	1756a (±0)
	Cropping season A, Extended over cropping season B	9	3 × 4 m	1.12c (±0.55)	639b (±410)
			Monocrop	4.13b (±0.79)	1896a (±34)
	Cropping season A, Extended year-round	12	3 × 4 m	1.14c (±0.53)	762b (±312)
			Monocrop	1.39c (±0.94)	1898a (±0)
	In between cropping seasons, extended over cropping season B	6	3 × 4 m	1.09c (±0.57)	543b (±197)
			Monocrop	4.12b (±0.79)	1756a (±0)
	Long dry season (May- August)	4	3 × 4 m	0.08f (±0.06)	639b (±410)
			Monocrop	1.04c (±0.79)	1896a (±34)
			LSD	0.69	568.0
			Fpr	0.001	0.924

*Cropping periods include the regular annual cropping seasons A and B, cropping periods in the dry season (May till September) and in the months in between the two annual cropping seasons (December – February.), and extended cropping seasons A and B. Yields are compared between intercrops in banana fields with various spacing (2 × 2 m, 2 × 3 m, 3 × 3 m, 3 × 4 m, 4 × 4 m) and monocrops. Photosynthetic active radiation (PAR) was measured under the respective banana canopies or in the open field for the monocrops. Means in a column followed by the same letter are not significantly different from each other according to Tukey's HSD test ($p < 0.05$). Standard deviations are provided between brackets. NA, not assessed (**Figure 3A–E**).*

3 m spaced banana fields to 3.74 ± 1.39 t/ha in the denser 2 × 2 m banana fields. In the 3 × 4 m mixed cultivar field that was heavily shaded, yam tuber yield was also relatively low (3.45 ± 3.61).

Chili

Red bird's eye (chili) was only assessed at Katana. Only aboveground biomass yield was assessed for chili (**Table 2**). A significantly higher ($p \leq 0.001$) chili biomass yield

TABLE 5 | Yield and growth attributes of crops grown at Kavumu: tuber crop taro (variety "Astrida"), legume crop soybean and forage grasses *Brachiaria* sp. and *Setaria* sp.

Planting period	**Banana spacing**	**Taro**	**Soybean**		***Brachiaria* sp.**		***Setaria* sp.**		**PAR (μmol/m^2/s)**
		Tuber yield (t/ha)	**Grain yield (t/ha)**	**Biomass (t/ha)**	**Height (cm)**	**Yield (t/ha)**	**Height (cm)**	**Yield (t/ha)**	
Cropping season A	3 × 4 m	49.7a (±18.2)	1.6ab (±0.1)	3.2a (±0.6)	56a (±43)	3.6a (±6)	46a (±38)	5.7a (±6.7)	903b (±267)
	Monocrop	41.7a (±19.4)	1.9ab (±0.5)	4.1a (±0.1)	48b (±41)	3.7a (±5.7)	45a (±38)	6.0a (±5.7)	1896a (±0)
Cropping season B	3 × 4 m	38.3a (±16.1)	0.2c (±0.17)	4.0a	NA	NA	NA	NA	587c (±185)
	Monocrop	34.1a (±21.3)	1.2b (±0.47)	5.3a	NA	NA	NA	NA	1756a (±0)
LSD		19.4	0.7	2.3	4.7	1.7	4.28	1.85	254.3
Fpr		0.78	0.2	0.8	0.002	0.89	0.65	0.66	0.8

Respective tuber yield, grain yield and total aboveground biomass yield are reported for taro and soybean, and plant height and aboveground biomass are provided for the forage grasses Brachiaria sp. and Setaria sp. Yields are compared between the two annual cropping seasons for intercrops in a banana field with a 3 × 4 m spacing and monocrops. Photosynthetic active radiation (PAR) was measured under the respective banana canopies or in the open field for the monocrops. Means in a column followed by the same letter are not significantly different from each other according to Tukey's HSD test ($p < 0.05$). Standard deviations are provided between brackets. NA, not assessed.

(5.22 ± 2.03) was observed in the mono-cropped field compared with 0.06 ± 0.03 to 0.18 ± 0.01 in the intercrops. Biomass yields under banana declined significantly with increasing shade intensity (i.e., reducing PAR) with an average of only 3% biomass retained across banana plant densities (**Table 2**).

Crotalaria sp.: Green Manure and Fodder Crop

Crotalaria sp. was only assessed at INERA-Mulungu. *Crotalaria* sp. yields increased with increasing PAR values, varying between 2.9 t/ha in the 2 × 2 m plots and 5.1 t/ha in the monocrop plots. No significant differences were observed between yields in the different shade/PAR levels despite a 20% and 43% yield decline in the 4 × 4 m and 2 × 2 m banana-crotalaria intercrop treatments, respectively, compared to the *Crotalaria* sp. monocrop (**Table 3**).

Chickpeas

Chickpeas were only assessed at the INERA-Mulungu site. Chickpea biomass and grain yield increased with declining shade intensity (**Table 3**). Significantly higher chickpea biomass and grain yields were attained when planted at the onset of the dry season (May) compared to planting at the onset of the wet season (September). The dry season planting resulted in 0.03 t/ha (in the 2 × 2 m plots) to 0.27 t/ha in the monocrop compared with yields of 0.00 ± 0.00 to 0.02 ± 0.00, respectively in the wet season (**Table 3**).

Eggplant

Eggplants were assessed at INERA-Mulungu only. Eggplant fruit yield was highest (6.4 t/ha) in the monocrop and when planted at the onset of cropping season A (**Table 3**). Eggplant yields declined with declining PAR (i.e., increasing shade level) from 6.4 t/ha in the monocrop to 1.5 t/ha in the 2 × 2 m intercrop in season A and from 4.3 t/ha in the monocrop to 1.3 t/ha in the 2 × 2 m intercrop in season B.

Ginger

Ginger was only assessed at INERA-Mulungu. Ginger had a higher yield for the crop planted under mono-cropped conditions compared to the intercrops, with a higher yield during cropping season A compared to cropping season B (2.9 ± 0.4 t/ha and 1.5 ± 0.8 t/ha, respectively) (**Table 3**). Under the intercrops, ginger yield declined with increasing banana plant density, though no significant differences between the 2 × 2 m and 4 × 4 m banana spacings were observed. No significant differences also occurred between the intercrop and the monocrop in season B.

Mucuna

The potential of mucuna as a cover plant under varying banana shade intensities and during both the wet season and dry season was assessed by planting it at the onset of a wet or dry season, and in separate trials allowing plants established in the wet season to continue growing during the subsequent dry season months (**Figures 3A–E**).

At Katana, the wet season mucuna yields increased with increasing PAR or plant spacing, varying between 2.4 t/ha in the 2 × 2 m "Nshika" banana spacing and 5.0 t/ha in the 3 × 3 m spaced banana plots. The dry season crop was only assessed under the 3 × 4 m spacing and under monocrop, with higher yields observed under monocropping.

At Kavumu, mucuna yields varied between 0.1 and 6.5 t/ha. The highest mucuna yields were observed in the monocrop established at the beginning of growing season B with extended growth during the subsequent dry season (6.5 ± 2.9 t/ha). High yields were also attained in monocrops in the extended season A and in monocrops planted in between the two growing seasons (early December) and extended toward the end of growing season B (end of May) (4.1± 0.8 t/ha). Mucuna yields were consistently higher in the monocrop treatments compared with the 3 × 4 m intercrop, though the yield of the monocrop planted in September 2015 (cropping season A), and harvested 12 months later, was unexpectedly low (1.4 ± 0.1 t/ha) compared to shorter crop cycles (**Table 4**). This was attributed to the fact that the harvest was delayed and all plants had already shed most of their leaves at time of harvest. Mucuna crop grown solely during the dry season (May–August) had low yields in both the monocrop

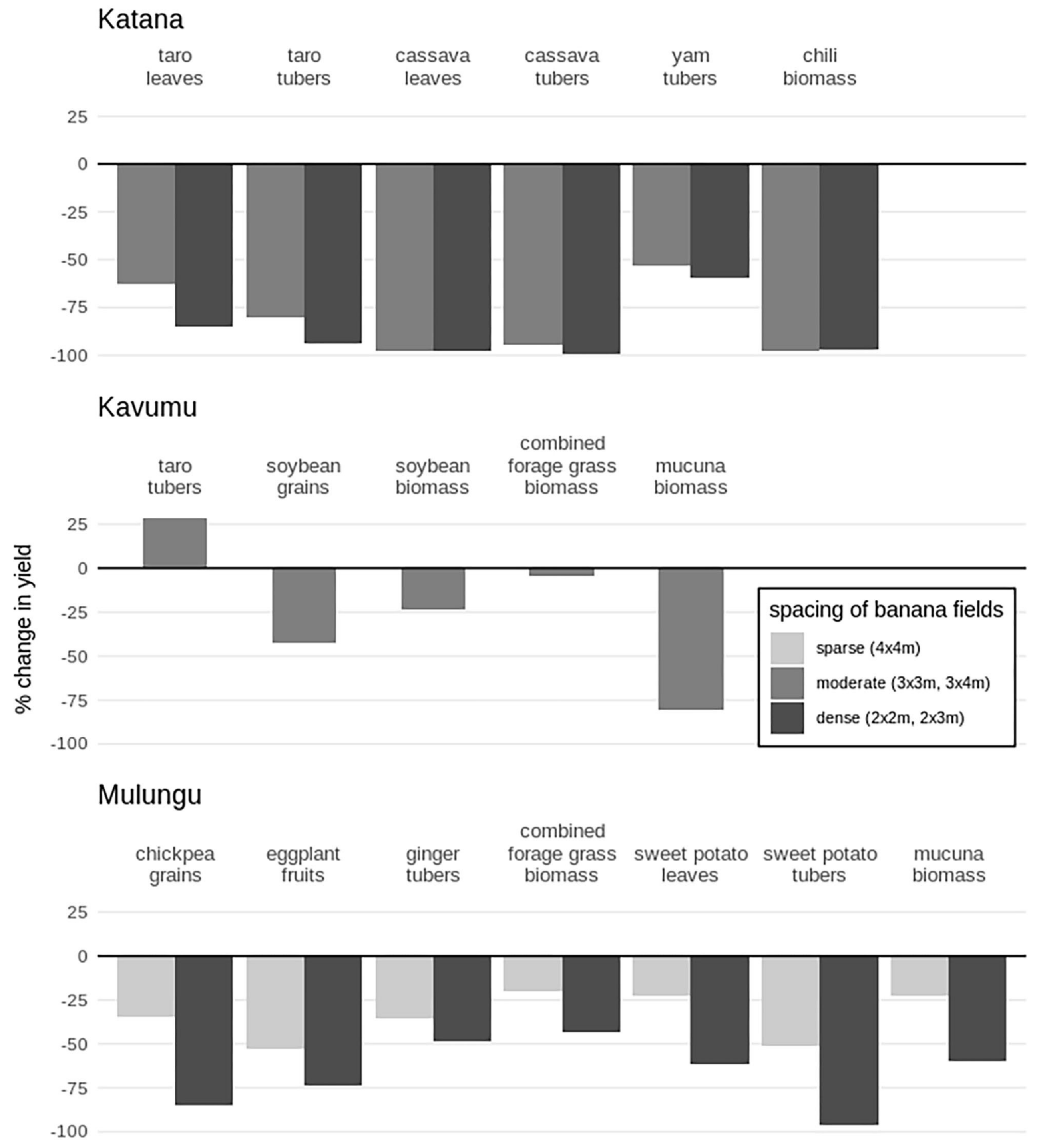

FIGURE 2 | Overview of percentage change in yield for various crops grown as intercrops in banana fields vs. as monocrop. Negative values represent a reduction in yield compared to the monocrop, positive values show an increase. Intercrops are grouped according to the spacing density of the banana field, with 4 × 4 m as sparsely spaced banana fields, 3 × 3 m and 3 × 4 m as moderate, and 2 × 3 m and 2 × 2 m as densely spaced banana fields.

(mean of 0.7 ± 0.5 t/ha) and the 3 × 4 m banana intercropped (mean of 0.08 ± 0.01 t/ha) fields.

At INERA-Mulungu, mucuna yields were also higher in the monocrop fields, with yields declining with decreasing PAR values (**Table 4**). A high mucuna yield of 5.8 ± 1.6 t/ha was obtained for mucuna monocrop fields (cf. **Figure 3A**) in the cropping season B (March–May) that was extended toward the end of the subsequent dry season (end of August) to provide soil cover. Similarly, high yields of 4.9 ± 0.1 and 5.0 ± 1.1 t/ha were attained for monocrops grown between the two cropping seasons (December–February) and during cropping season A (September–December) extended to February to provide soil cover in between the two cropping seasons, respectively (**Table 4**). Relatively high mucuna yields (3.8 ± 0.3–4.5 ± 0.0 t/ha; cf. **Figure 3B**) were also realized in the sparsely spaced banana fields (4 × 4 m) cropped between December and February, during the extended season A, and during the extended season B. Mucuna solely grown during the dry season (May–August) similar to other sites performed poorly with a yield of 1.1 ± 0.2 t/ha in monocrop. Mucuna yields were significantly decreased under the dense 2 × 2 m banana fields, varying between 0.2 ± 0.1 t/ha in the long dry season (May–August) and 2.8 ± 1.0 t/ha in season B extended over the long dry season.

Soybean

Soybean were only evaluated at Kavumu. Soybean were more vegetative, resulting in a higher biomass yield in season B compared to season A. In contrast, higher soybean grain yields were observed in the cropping season A (**Table 5**). Grain yields of the intercrop were only significantly lower than the monocropped fields during cropping season B, while no significant reduction occurred during cropping season A. Biomass yields were similar in intercrop (3 × 4 m spacing) and monocrop during both cropping seasons.

Forage Grasses

The forage grasses *Brachiaria* sp. and *Setaria* sp. were only assessed at Kavumu. Higher but non-significantly different yield ($p > 0.05$) was observed for the biomass yield of the forages under monocrops than in the intercrop (**Table 5**). *Brachiaria* sp. biomass yields varied between 3.6 and 3.7 t/ha while for *Setaria* sp. biomass varied between 5.7 and 6.0 t/ha. Despite the non-significant differences in biomass yield of the forages in the intercrop and monocrop, shade was observed to result in significantly taller plants ($p \leq 0.05$) for *Brachiaria* sp.

Sweet Potato

The aboveground (edible) biomass and tuber yields of multiple varieties of sweet potato was assessed at INERA-Mulungu (**Table 6**). For both the aboveground biomass and tuber yield, the highest yields across all varieties were observed in the monocropped field. "Mugande" and "Elendgi" with aboveground biomass yield varying from 4.5 to 5.8 t/ha and tuber yield of 14.4 to 15.8 t/ha in the monocrop, performed better than "M'Nzama" and "Vander Wall" with aboveground biomass of 3.0 to 3.2 t/ha and tuber yields of 6.9 to 7.5 t/ha. Similar trends in the performance of the sweet potato varieties was observed in the shade treatments as for the monocrop. For a given sweet potato variety, aboveground biomass and tuber yield declined with a declining level of PAR. Apart from aboveground biomass yields of "Mugande" and "Elendgi" that did not significantly differ under the monocrop and the 4 × 4 m treatment, significant differences were visible between the three treatments for both aboveground biomass and tuber yield.

TABLE 6 | Aboveground biomass yield for various sweet potato varieties at INERA-Mulungu.

Treatment	Sweet potato varieties	Biomass (t/ha)	Tuber yield (t/ha)
2 × 2 m banana spacing	Mugande	1.7def (±0.3)	0.3c (±0.2)
	Elendgi	1.7def (±0.3)	1.0c (±0.7)
	M'Nzama	1.3ef (±0.3)	0.2c (±0.2)
	Vander wall	1.0f (±0.0)	0.3c (±0.2)
4 × 4 m banana spacing	Mugande	4.0abc (±1.0)	6.1b (±2.6)
	Elendgi	4.8ab (±0.8)	7.8b (±4.3)
	M'Nzama	2.5cdef (±0.5)	4.7bc (±3.2)
	Vander wall	2.3cdef (±0.6)	3.3bc (±0.8)
Sweet potato monocrop	Mugande	5.8a (±2.3)	14.4a (±4.6)
	Elendgi	4.5ab (±1.0)	15.8a (±2.2)
	M'Nzama	3.2bcd (±2.0)	7.5b (±4.4)
	Vander wall	3.0bcde (±1.0)	6.9b (±3.4)
LSD		1.8	4.56
Fpr		0.3	0.099

Yields are compared between intercrops in banana fields with various spacing (2 × 2 m, 4 × 4 m) and monocrops. Means in a column followed by the same letter are not significantly different from each other according to Tukey's HSD test ($p < 0.05$). Standard deviations are provided between brackets.

Hedge Species

Forage grasses and shrubs planted along the borders of the banana plots had a similar performance in INERA-Mulungu and Kavumu (**Table 7**). The grasses of the Pennisetum species ("*Fraishe camerounaise*" and *Pennisetum purpureum*) obtained a higher yield than *Setaria sphacelata*. Of the two forage shrubs, *Calliandra calothyrsus* showed higher yields than *Leucaena leucocephala*. At Katana, lower yields were attained for forage grasses along the borders of the monocrop and the "Nshika" banana fields (3 × 3, 2 × 3, 2 × 2 m). This is potentially caused by higher local shading conditions from the dense "Nshika" fields and surrounding banana plots. Forage shrubs were not as severely affected by these high shading conditions, with similar yield attained as in Mulungu and Kavumu. Results at Katana show *Leucaena* sp. to be performing better under full light whereas *Calliandra* sp. performed better under shaded conditions.

DISCUSSION

Access to land is a major production constraint in the banana producing landscapes of the East and Central African region. Strategies are as such needed for the optimal use of the available land, especially niches/spaces under the perennial banana crop. Potential options to enable banana intercropping with other annual crops include (i) pruning banana leaves to allow for more light to the shorter stature crops, (ii) use of a wider spacing to minimize shading of the shorter crops, and (iii) integrating moderate to high shade-tolerant intercrops under the banana crop. The option of banana leaf pruning has been shown to be detrimental to the banana crop yield and to perpetuate the spread of the Xanthomonas wilt disease of banana through garden tool use (Ocimati et al., 2019). The efficacy of leaf pruning

TABLE 7 | Biomass yields of forage grasses and shrubs included as border species across the sites INERA-Mulungu, Kavumu, and Katana center.

Site		Forage grasses	Yield (kg/2m)	Forage shrubs	Yield (kg/2m)
Katana					
	Monocrop	*Fraishe camerounaise*	4.3abc (±6.3)	Calliandra	2.2ab (±1.8)
		Pennisetum sp.	3.0bc (±4.8)	Leucaena	1.1bc (±0.0)
		Setaria sp.	1.2c (±1.1)		
	Mixed cultivar field (3 × 4 m)	*Fraishe camerounaise*	8.9ab (±17.4)	Calliandra	3.3a (±2.9)
		Pennisetum sp.	10.4a (±26.6)	Leucaena	0.3c (±0.1)
		Setaria sp.	9.2ab (±23.4)		
	Nshika fields (3 × 3, 2 × 3, 3 × 3 m)	*Fraishe camerounaise*	0.6c (±0.9)	Calliandra	2.8a (±2.8)
		Pennisetum sp.	1.8c (±2.2)	Leucaena	0.5a (±2.8)
		Setaria sp.	0.8c (±0.7)		
		LSD	6.2		1.3
		Fpr	0.920		0.098
INERA-Mulungu		*Fraishe camerounaise*	14.0a (±13.8)	Calliandra	2.3a (±2.4)
		Pennisetum sp.	13.0a (±16.3)	Leucaena	0.5b (±1.4)
		Setaria sp.	5.3b (±5.5)		
		LSD	5.4		1.3
		Fpr	0.003		0.01
Kavumu		*Fraishe camerounaise*	12.2a (±10.6)	Calliandra	3.6a (±2.2)
		Pennisetum sp.	12.0a (±10.6)	Leucaena	1.2b (±0.9)
		Setaria sp.	5.3b (±3.5)		
		LSD	3.5		1.3
		Fpr	0.001		0.001

The forage grasses include "Fraishe camerounaise," Pennisetum sp. and Setaria sp. and the forage shrubs include Calliandra sp. and Leucaena sp. Yields are reported in kg for each 2m length of hedge. Means in a column followed by the same letter are not significantly different from each other according to Tukey's HSD test ($p < 0.05$). Standard deviations are provided between brackets.

will also depend on the level of leaves pruned and the spacing and size of banana mats. The extensive field trials performed in the South Kivu province of eastern DR Congo allowed for the identification of a variety of potentially favorable intercrops for the intensification of banana production systems. Extending production into the dry season months also offers an opportunity to optimally use the available land. Albeit only to a limited extent and for a few crops, this study also gives evidence that additional biomass for food or forage could be attained through the integration of crop spp. that are less sensitive to the long dry weather conditions of the dry seasons.

Sensitivity/Tolerance of Intercrops to Banana Shade

Although the investigated intercrops attained the highest yields when grown in open fields, a wide range of yield performance was observed under shaded conditions, with no significant differences observed between the intercrops and monocrops in a few cases (overview in **Figure 2**). Yam, sweet potato, ginger and forage grasses showed good potential for use in the high and moderate banana shade levels, while taro, soybean, mucuna, chili, eggplant, and *Crotalaria* sp. showed good yield in sparsely spaced banana fields with moderate shading. The cassava variety used in the study was highly sensitive to shade. Investigating variation in yield performance in contrasting cropping seasons revealed a differential performance of the intercrops between the two seasons. Several crops, including soybean, eggplant, and ginger performed better in cropping season A (September-December) which is characterized by a high rainfall over a longer rainfall duration.

Inter-cropping With Various Crop Types Under Banana Canopies

Of all investigated tuber crops, yam showed the best potential for intercropping in both sparsely spaced and dense banana fields. In sparsely spaced fields (3 × 3 m), yam achieved equally high yields as in an open field, while in the densest banana fields (2 × 2 m and 2 × 3 m) a reasonable reduction in tuber yield of 60% was attained (cf. **Figure 2**). At our trials, yam tuber yields were also least affected by shade (with yield reduction of 42–65%) compared to the other assessed crops (**Figure 2**), suggesting that yam could potentially contribute to increasing plot level biomass in banana fields. Reports on yam have mostly shown a moderate shade tolerance, with larger leaves and smaller tubers produced in shaded conditions (Onwueme and Charles, 1994; Johnston and Onwueme, 1998). In the current study only one yam variety was however explored. Studies to evaluate a wider set of yam cultivars for shade-tolerance is hence recommended.

Taro, while attaining high yields in the sparsely spaced banana field at Kavumu (3 × 4 m), showed a 88% drop in tuber yields under denser banana canopies at Katana. This contrasts other studies that have indicated that taro and other aroids show a high level of shade-tolerance related to a greater proportional increased leaf size combined with higher stomatal, chlorophyll

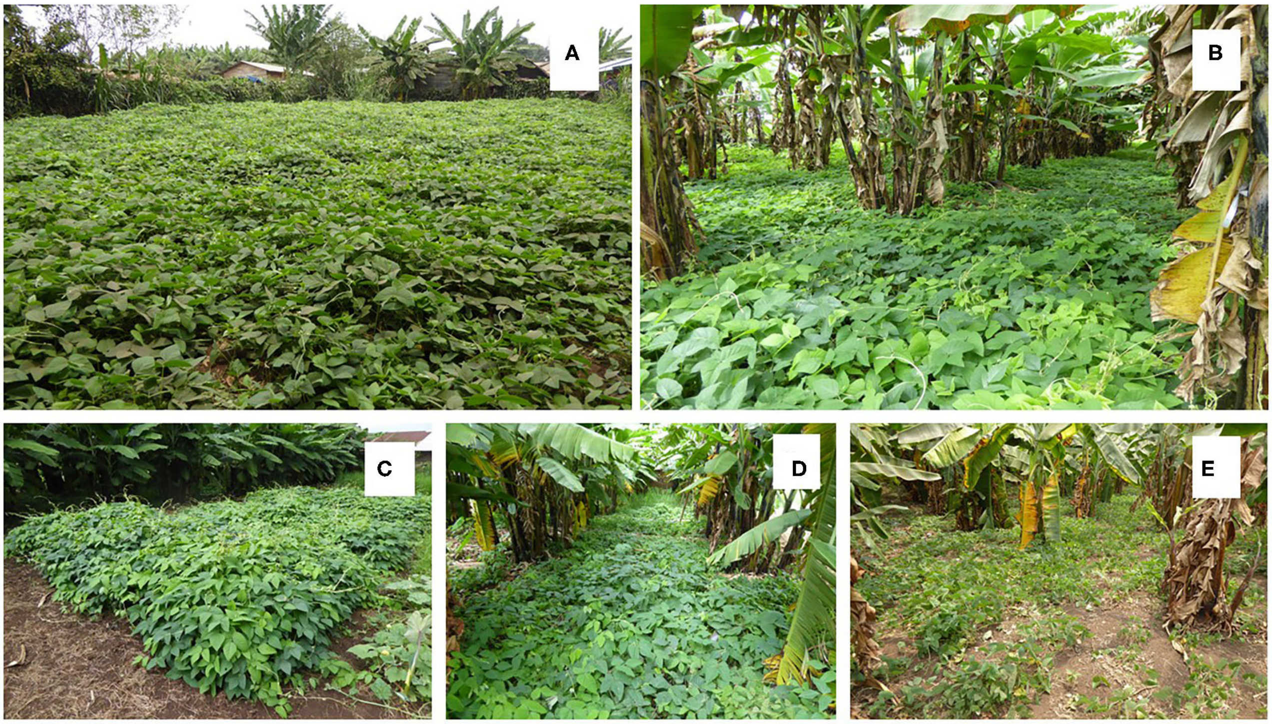

FIGURE 3 | Mucuna grown as a monocrop **(A,C)** and as an intercrop under 3 × 4 m spaced banana fields **(B,D,E)**. Mucuna monocrop **(A)** was planted in May 2016 (at the end of season B and start of the long dry season) (photo was taken in the dry season, in August 2016) while monocrop **(C)** was planted in December 2016 at the onset of the short dry season and extended over cropping season B (photo was taken in the rainy season, in April 2017). Mucuna intercrop in **(B)** was planted in early March 2016 at the onset of the rainy season B (photo taken in the rainy season in April); intercrop in **(D)** was planted at the start of the short dry season (December 2016) and extended over cropping season B (photo was taken in the rainy season, in April 2017); and **(E)** a mucuna intercrop planted at the onset of the long dry season in May 2016 (photo was taken at the end of the long dry season in August 2016). Rainy season B stretches from start of March to end of May, the long dry season from end of May/ start of June to end of August and the short dry season from end of December to end of February. Photos **(A,E)** were taken at the Katana site, **(C,D)** at the Kavumu site, while **(B)** was taken at the INERA-Mulungu site.

and carotenoids density compared to other tuber crops (Rogers and Iosefa, 1993; Johnston and Onwueme, 1998; Onwueme and Johnston, 2000). Rogers and Iosefa (1993) however reported a significant shade by cultivar interaction, and the taro variety "Ishikazi" used at Katana might not be suited for intercropping under the shade of banana fields. The taro variety "Astrida," although only tested in sparsely spaced banana fields, could be of a greater potential. Thus, the need to take stock and evaluate a larger number of taro and other aroids under banana shade.

The tuber crops cassava and sweet potato are reported to have a low tolerance to shade as compared to other tropical tuber crops (Johnston and Onwueme, 1998). Indeed, the tuber yield of cassava was particularly poor under heavily shaded conditions at the trial in Katana, with a yield reduction of 89% in the most sparsely spaced banana fields (i.e., 3 × 3 m and 3 × 4 m), and 100% tuber yield reduction in the denser plots. Cassava grown in shaded conditions show a large delay in their root bulking process, while the number of roots per plant is reduced (Okoli and Wilson, 1986). Dry matter (DM) allocation plasticity has been shown to lead to more DM allocation to the shoots when carbon dioxide or light is limiting and to the roots when nutrients or water are limiting (Bloom et al., 1985; McCarthy and Enquist, 2007). The production of cassava in shaded conditions is sometimes done specifically to obtain a harvest of edible leaves (Latif and Müller, 2015; Munyahali et al., 2017). Nevertheless, the cassava crop at Katana also showed a significant reduction in leaf yield in both sparsely spaced and dense banana fields, with a limited retained yield of 4% to 1% compared to the monocrop. Sweet potato performed better in this respect. Exploring a wider range of cassava varieties, especially for leaf production under banana shade is thus recommended. Tuber yields of the four investigated sweet potato varieties remained relatively high in sparsely spaced banana fields (4 × 4 m) with a yield retention of 49%, although retained yield dropped to 4% in the densest banana fields (2 × 2 m). Several varieties of sweet potato showed a good potential for leaf yield in shaded conditions. Although the varieties "Mugande" and "Elendgi" (of which the leaves are used as food and forage) attained higher leaf yields in monocrop than the varieties "M'Nzama" and "Vander Wall' (of which the leaves are consumed as a vegetable), all varieties maintained their leaf yields in the shading of sparsely spaced banana fields and retained ∼35% of yield in the densest fields.

The root crop ginger showed a great potential as an intercrop in banana fields, with a high retained yield even within the densest banana spacing (2 × 2 m). Ginger has been reported as a shade-tolerant crop (Okwuowulu, 2005; Lyocks et al., 2013).

Vanlalhluna et al. (2014) even reported increased rhizome yield from intercropped plots than monocrops, although this is not corroborated with our trial. Ginger additionally produced a higher yield when grown during cropping season A, which is characterized by heavier rainfall. Okwuowulu (2005) reported ginger to be sensitive to the variability in rainfall. Ginger planted during cropping season B might have been affected by its exposure to long dry conditions in the following dry season, which has been reported to affect rhizome yield (Okwuowulu, 2005). Ginger crop performance might be further optimized through trials with varied planting and harvesting times at the study area. It must also be noted that both ginger and *Musa* spp. are susceptible to the burrowing nematode (*Radopholus similis*) (Orton Williams and Siddiqi, 1973; Sipes et al., 2001), and close follow-up of the crops might be advisable.

The eggplant also showed reasonable fruit yields in shaded conditions, with a 46% yield retained in sparsely spaced banana fields and a further reduction to 27% in the denser fields. An experimental study indeed showed some degree of shade-tolerance of the eggplant with acclimation to low light conditions found for artificially shaded plants (Rosati et al., 2001), although decreased eggplant yields in shaded conditions have also been reported (Uzun, 2007; Pouliot et al., 2012).

The biomass yield of chili under shaded conditions during our trial was surprisingly low, with only ∼3% retained biomass yield across all banana fields (2 × 2 m to 3 × 4 m) in Katana. Inversely, Pouliot et al. (2012) have reported increased chili pepper yields under tree canopies of up to 150%. Shade tolerance in chili is however dependent on the variety used (Sreelathakumary and Rajamony, 2002), with an increase in chlorophyll density under shaded conditions, potentially increasing photosynthetic efficiency, in several but not all genotypes. Improved genotype selection could therefore potentially improve the performance of chili as an intercrop in banana fields. It is also important to note that in the current study the chili fruit yields were not assessed and there is a high chance that the abundant vegetative biomass in the monocrops could have resulted in lower fruit yields. Repeat experiments assessing fruit yields for multiple chili varieties are thus recommended.

Soybean, although often used as an intercrop in Central Africa, is susceptible to reduced light conditions (Ntamwira et al., 2013). In our experimental trial, soybean performed well in sparse shading of the 3 × 4 m banana field at Kavumu, although only during cropping season A characterized by a longer rainfall duration. In cropping season B and for the same intercropping formation, a lower grain yield of about 17% of monocrop yield was attained. This could possibly be attributed to the shorter rainfall duration. Other legume crops (e.g., bush bean and climbing bean), although also sensitive to reduced light (Blomme et al., 2018), cope better in low levels of shade than soybean (Ntamwira et al., 2013) and might be more suitable for intercropping in sparsely spaced banana fields.

The incorporation of forage grasses, legume mucuna and shrubs, either as an intercrop or as border delineation showed good potential to offer additional biomass on farm. Although grasses were not assessed for heavily shaded conditions, our experimental trials showed high yields in both sparsely spaced banana fields and along the border of the plots. An in-depth survey conducted by Mpiira et al. (2013) however showed that farmers generally don't plan for managed forage shrubs (and in extension grasses) which are rather present as spontaneous vegetation. Management of forage crops would however improve access to forage for livestock, improve nutrient recycling through mulch and manure and control soil erosion, thus benefiting the banana production system.

Crop Sensitivity to the Long Dry Weather During the Dry Seasons

The mucuna crop showed variable results when grown solely during the dry season. At Katana, relatively high yields were attained during the dry season while this was not the case at INERA-Mulungu. The high soil fertility at Katana could potentially explain this difference. Another option is to establish mucuna during the annual cropping season and extend its growth into the dry season. Results from this study have shown that mucuna is resilient to exposure to drier conditions after the crop has been established, and high yields can be retained. The ability of the crop to produce all year round enables for its differential promotion, targeting different farm types and priorities. For example, smallholder farms predominantly intercrop banana with other short-stature food crops during the rainy seasons, hence, the integration of mucuna as a cover crop with establishment in the rainy season and extension over the dry season could be hampered. Integration of mucuna toward the end of the rainy season (when annual intercrops are getting mature/ready to be harvested) could be promoted for this category of farmers. For medium to large scale farmers who often grow banana as a monocrop, mucuna could be planted in the rainy season, allowing it to establish all year round. This could enable farmers to cut on labor costs for weeding, support soil and water conservation and improve nutrient recycling in the system.

Chickpea showed great potential for intercropping in banana fields during the dry season. Although the grain yield does reduce with increased shading, an 8-fold yield increase was attained for the crop during the dry season relative to the wet season crop in both sparsely spaced and dense banana fields. Chickpea is indeed often grown in drier conditions through establishment on residual moisture from the rainy season (Varshney et al., 2014). While shading constrains chickpea yield production (Saxena and Sheldrake, 1980), implementation during the dry season not only provide yields outside of the usual cropping season but provides important soil cover protecting against soil degradation.

Challenges and Gaps in the Study

The current study did not establish the effects of the intercrops on the performance of the banana crop, whole field productivity level or system yield advantage calculations are hence not presented. Intercropping banana with legumes has been observed to reduce the performance of the banana crop though non-significantly (Ocimati et al., 2019). However, these authors observed an agronomic advantage of intercropping with these legumes but not an economic advantage (due to the higher value of the banana crop in the market), concluding that the choice of farmers could be influenced by their production objectives that could include nutrition, profitability and environment sustainability. It is thus recommended to also explore the agronomic and economic advantages associated with these crop species. Banana cultivars used in the study varied from one site or experiment to the other and the levels of shade did not only vary with the banana spacing/ plant densities but also with the banana cultivar type. To overcome this, the effect of level of shade measured as PAR was used as a uniform parameter to assess growth and yield of intercrops. Nevertheless, future studies could assess a wide range of *Musa* cultivars and their leaf structure or orientation, in relation to resulting shade levels. Such a study could pinpoint banana cultivars with e.g., more erect leaves and could lead to *Musa* cultivar specific recommendations for intercropping or a range of intercropping scenarios for a cultivar. These studies were also conducted at the high-altitude sites of eastern DR Congo, that receive a relatively high amount of rainfall. Replicating similar trials at sites with low rainfall might give different results.

Final Remarks

Shade in dense banana fields forms an important constraint for the integration of most short-stature crops. However, even though significant reductions in yield have been recorded, several of the investigated crops show a reasonable yield retention under moderate to high shade levels including taro, soybean, mucuna, chili, eggplant, *Crotalaria* sp., yam, sweet potato for leaves, ginger, and forage grasses and shrubs. Introducing these crops/species in denser banana fields can be highly beneficial for small-scale farmers, since additional yield is produced without the use of additional farming land and without excessive field management needs. Reduced soil degradation through intercropping with these crops is an important co-benefit of this farming system, although additional research is needed to quantify which crops perform best in this regard. Chickpea and mucuna showed great potential for extending farming production to the dry season, although both crops would probably benefit from establishment at the end of the rainy season, so that a well-established root system can develop before the rains stop. All in all, year-round biomass production and ground cover highly contribute to whole field productivity, and the overall health of the system.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

GB and WO conceived and developed the research concept, contributed to data analysis, and contributed to writing and editing the manuscript. JN contributed to the development of the research concept, conducted the field experiments, collected and analyzed the data, and contributed to manuscript writing. LB, DA, and NS contributed to shaping the research concept, supported the establishment of experiments, data collection, and data interpretation. EK supported data analysis and interpretation, and the writing and editing of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2020.545926/full#supplementary-material

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