SOIL FERTILITY MANAGEMENT FOR SUSTAINABLE FOOD PRODUCTION IN SUB-SAHARAN AFRICA

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SOIL FERTILITY MANAGEMENT FOR SUSTAINABLE FOOD PRODUCTION IN SUB-SAHARAN AFRICA

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EDITORIAL
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Editorial: Soil Fertility Management for Sustainable Food Production in sub-Saharan Africa

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Keywords: agri-food systems, integrated soil fertility management, sustainability, food production, farming system, crop modeling, climate change

Editorial on the Research Topic

Soil Fertility Management for Sustainable Food Production in sub-Saharan Africa

Agriculture is the mainstay of the economies of sub-Saharan Africa as it employs 60% of the active population and contributes up to 35% of the GDP (AGRA, 2014). Yet, agri-food systems are bedeviled with a lot of challenges regardless of the unrelenting efforts made by the scientific community and the flagship agricultural programs initiated by governments of the sub-region. Agriculture in SSA is predominately rainfed and is managed by smallholder farmers who are too cash-strapped to afford the recommended inputs. Cereal yields in SSA, for example, are extremely low and average about 1.6 tons ha^{-1} compared to the global average of 3.9 tons ha^{-1} (FAOSTATS, 2020). While a plethora of constraints may account for the fragile food systems, prolonged decline in soil fertility, and low and erratic rainfall have been cited as the key biophysical constraints (IFDC, 2007).

These precarious agri-food systems are worsened by the impact of climate change. According to IPCC estimates, climate change-induced drought may shorten growing periods in SSA by an average of 20% by 2050 and cause a decline of 40% in cereal yields (Lobell et al., 2011). While judicious use of fertilizers could improve soil fertility and increase crop yields, smallholder farmers in SSA only use limited amounts of fertilizers. The fertilizer use in SSA is constrained by several factors such as high cost, limited access, and poor producer price. Consequently, smallholder farming systems often rely on inherent soil organic matter (SOM) content to sustain crop production. While SOM can help to maintain soil fertility by enhancing the retention and release of soil nutrients as well as improving the water holding capacity of the soil, continuous cropping and soil erosion can reduce the native SOM content, resulting in a rapid decline in soil fertility.

Integrated soil fertility management is a credible pathway for building resilient agri-food systems to mitigate the adverse effect of climate change and boost agricultural productivity. This Research Topic presents original research articles on recent scientific advances in the detection of limiting nutrients, quantification of soil loss and integrated nutrient management, and its effects on crop quality. Integrated soil fertility management was studied in detail in six of the articles in this special issue. Of special interest, soil organic amendments such as bioplant, neem seed cake, biochar, and biosol were shown to be effective soil conditioners for optimizing mineral fertilizer use efficiency in cropping systems (Ebanyat et al., Nartey et al., Traore et al.). In these studies, the role of organic amendments in increasing soil buffering capacity and preventing soil

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acidification, which is a chronic problem in the humid zones of SSA was demonstrated. Two articles by Yaro et al. and Wilson et al. investigated the effectiveness of native rhizobia strains and rhizobium biofertilizers for improving the grain yield of groundnut and highlighted biological N fixation as an environmentally friendly approach to improving soil fertility. One study (Jaiswal et al.) also evaluated mineral nutrient concentrations, P-enzyme activity, and changes in microbial communities in the rhizosphere of sole and intra-hole planted cowpea. This study suggested that an intra-hole cropping system can provide protection against soil-borne diseases possibly through elimination by antibiotics and/or phytoalexins present in plant and microbial exudates in the rhizosphere.

The study by Baijukya et al. addresses the issues associated with "non-responsive" soils in sub-Saharan Africa. In a study to estimate soil loss for sustainable crop production in the semi-deciduous forest zone of Ghana, Sekyi-Annan et al. using modeling demonstrated how site-specific land cover management strategies such as tree-cover intercropping, and reduced tillage could have a huge potential in reducing soil loss and sustain soil fertility. In a related study, Baath et al. demonstrated the potential of using readily accessible satellite data to develop methods for improved crop management decisions in precision agriculture, related to finger millet, an important small grain crop in the savannas of West Africa. The paper by Nkansah et al. addresses challenges associated with tomato production under greenhouse conditions in tropical environments.

Overall, these ten contributions published in the special issue further strengthen the role of integrated soil fertility management and modeling in addressing widespread land degradation and food insecurity on the continent.

AUTHOR CONTRIBUTIONS

AO made the first draft which was edited by SA-N. ID and KF made additional contributions. All authors contributed to the article and approved the submitted version.

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ORIGINAL RESEARCH
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Detecting Biophysical Characteristics and Nitrogen Status of Finger Millet at Hyperspectral and Multispectral Resolutions

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Finger millet (*Eleusine coracana* Gaertn L.) is an important grain crop for small farmers in many countries. Reliable estimates of crop parameters, such as crop growth and nitrogen (N) content, through remote sensing techniques can improve in-season management of finger millet. This study investigated the relationships of hyperspectral reflectance with canopy height, green canopy cover, leaf area index (LAI), and N concentrations of finger millet using an optimal waveband selection procedure with partial least square regression (PLSR). Predictive performance of 13 vegetation indices (VIs) computed from the original hyperspectral data as well as synthesized Landsat-8 and Sentinel-2 data were evaluated and compared for estimating various crop parameters with simple linear regression (SLR) and multilinear regression (MLR) models. The optimal wavebands determined by PLSR were mostly concentrated within 1,000–1,100 nm for both LAI and dry biomass but were scattered for other canopy parameters. The SLR statistics resulted in the simple ratio pigment index (*SRPI*) and red/green index (*RGI*) performing best when predicting LAI (R^2_V = 0.53–0.59) and canopy cover (R^2_V = 0.72–0.76). The blue/green index (BGI_1) was strongly related to canopy height (R^2_V = 0.65–0.78), dry biomass (R^2_V = 0.42–0.49), and N concentration (R^2_V = 0.70–0.83) of finger millet, regardless of spectral resolutions. The MLR approach, using four maximum VIs as input variables, improved the prediction accuracy of N concentration by 14% compared to both SLR and waveband selection methods. VIs computed from synthesized Landsat-8 and Sentinel-2 satellite data resulted in similar or greater prediction accuracy than hyperspectral data for various canopy parameters of finger millet, indicating publicly accessible multispectral data could serve as alternative to hyperspectral data for improved crop management decisions via precision agriculture.

Keywords: waveband selection, vegetation indices, remote sensing, landsat- 8, sentinel-2, *Eleusine coracana*

INTRODUCTION

Finger millet is an annual grass that serves as an essential cereal crop in several drought-prone areas globally. It is extensively cultivated in Asia (India, Nepal, Myanmar, China, Sri Lanka, and Japan), and Africa (Kenya, Uganda, Ethiopia, Zaire, Tanzania, Somalia, and Rwanda) (Upadhyaya et al., 2010). The nutritious finger millet grain helps prevent malnutrition and is an important component of diets for breastfeeding mothers, growing children, and patients (Singh and Raghuvanshi, 2012). Furthermore, the biomass of finger millet is used as forage for livestock in many Asian and African countries (Sumathi et al., 2005).

Finger millet has been receiving increased attention as a forage resource in the southern United States (US) due to its high nutritive value. Research conducted in the Southern High Plains reported that the nutrient concentrations of its forage were higher than forage of corn and sorghum. Moreover, it can be mixed with corn and sorghum to improve the overall quality of silage for dairy cattle (Gowda et al., 2015). Finger millet could also serve as an alternative to perennial pastures of bermudagrass [*Cyanodon dactylon* (L.) Pers.] and old world bluestems (*Bothriochloa* spp.), to fill declines in forage quality during late-summers in the Southern Great Plains (Baath et al., 2018a,b). Extensive research has focused on developing strategies for agronomic management, including optimum rates of nitrogen (N) application to sustain forage production and quality of finger millet in the southern US.

Estimation and monitoring of biophysical and biochemical characteristics are essential for agronomic research and forage management of crops, including finger millet. Biophysical parameters such as plant height, leaf area index (LAI), green canopy cover, and biomass are important indicators of plant growth, foliage density, canopy interception, and crop productivity, respectively (Thenkabail et al., 2000). In contrast, N concentrations in plant biomass represent an estimate of forage quality and any stress associated with photosynthetic activity of the parent plant (Feng et al., 2008). Although the traditional approaches of measuring biophysical attributes or plant N are reliable, they are time-consuming, laborious, and do not provide real-time N status of crops (Foster et al., 2017). In comparison, remote sensing techniques have potential to provide real-time, non-destructive estimates of different biophysical or biochemical attributes in many crops (Thenkabail et al., 2000; Nguyen and Lee, 2006). Remote sensing can also capture seasonal variations, which are often missed in traditional techniques due to limitations associated with the time and human resources (Hatfield and Prueger, 2010). While many diverse applications of remote sensing have been developed for major crops (Bégué et al., 2018), there are very few reports of such studies for finger millet (Dayananda et al., 2019).

Hyperspectral reflectance has been established as a valuable alternative to traditional methods of remote sensing. Hyperspectral remote sensing provides many contiguous narrow bands (<10 nm) of information related to biophysical and biochemical characteristics, which are usually missed by broadband multispectral reflectance due to low spectral resolution (Sahoo et al., 2015). Past research showed the potential of hyperspectral reflectance in estimating many biophysical parameters, involving plant height (Yue et al., 2017), leaf area index (LAI; Zhao et al., 2007a), canopy cover (Muharam et al., 2015), biomass (Foster et al., 2017), and plant N (Zhao et al., 2005). Therefore, the technique could be useful in monitoring the growth parameters and plant N status of finger millet and defining responses to different application rates of fertilizers (Jain et al., 2007).

Though hyperspectral imaging can provide more comprehensive analyses of the attributes of the canopy of finger millet, the high-dimensional data captured by hyperspectral sensors are challenging for conventional analytical techniques. Such data are affected by relatively large volumes, require Big Data analytics, have storage problems, and include redundant data/wavelengths (Becker et al., 2005). One possible solution is to reduce the dimensions of captured hyperspectral data by extracting optimal bands of interest for specific crop parameters (Wang et al., 2008). The partial least square regression (PLSR), an extension of multiple linear regression, is one of the most efficient methods used to extract relevant information from the large dimensional hyperspectral data and generate reliable models for predicting crop characteristics (Nguyen and Lee, 2006; Li et al., 2014). As hyperspectral data often consist of highly collinear wavebands, the use of PLSR is assumed more appropriate than other statistical methods since it avoids model overfitting.

Hyperspectral analysis is not the only option for remote sensing of canopy characteristics. In contrast to the complicated selection procedure for wavebands in hyperspectral analyses, the approach of spectral vegetation indices (VIs) has been widely utilized for many crops due to its simplicity and straightforward analytics (Fang and Liang, 2008). VIs are commonly-used numerical computations derived from specific narrow bands of the electromagnetic spectrum, primarily the visible and near-infrared regions (Viña et al., 2011). Many VIs can be developed from hyperspectral data and correlated individually to different canopy parameters (Hatfield and Prueger, 2010). Simple ratio (*SR*) and Normalized Difference Vegetation Index (*NDVI*) are the most commonly used for estimating various canopy characteristics (Zhao et al., 2007b; Hatfield et al., 2019); however, other VIs such as Triangular Vegetation Index (*TVI*) performed better at predicting LAI for soybean (*Glycine max*), maize (*Zea mays*), and wheat (*Triticum aestivum*) (Jiang et al., 2008). Likewise, indices that involve red-edge wavelengths were found to be more robust at predicting canopy N in wheat than normalized or simple ratio indices (Cammarano et al., 2014). The suitability of VIs varies for specific applications, growth stages and crop species (Thenkabail et al., 2002), and hence some caution is needed when considering VIs for practical applications in finger millet. Also, a combination of different VIs could further enhance the prediction accuracy of crop parameters (Tong et al., 2019), but the approach is not yet well-established. As such, the multilinear regression (MLR) models could prove effective at selecting multiple non-correlated VIs to develop significant correlations with various canopy parameters.

Spectroradiometer equipment involves a high monetary cost, which limits its availability to small farmers and crop consultants. New multispectral sensors, including Sentinel-2 and Landsat-8, that accompanied improvements in spectral

and spatial resolution, could serve as useful platforms for precision agriculture-based applications (Flynn et al., 2020). Since the applicability of VIs could also differ depending on the spectral resolution and instrument used (Xue and Su, 2017), it is important to evaluate whether VIs developed from hyperspectral data can successfully translate to the multispectral bands captured by Sentinel-2 or Landsat-8 for the estimation of crop parameters of finger millet.

The main objectives of this study were to: (i) investigate the relationships of hyperspectral reflectance with biophysical characteristics and N status of finger millet using the PLSR optimal waveband selection procedure, and (ii) evaluate capabilities of VIs developed from the original hyperspectral data and those derived from synthesized Landsat-8 and Sentinel-2 data for estimating various crop parameters through simple linear regression (SLR) and multilinear regression (MLR) models. Finally, an overall comparison of prediction accuracies obtained using different methods was performed to define their capacity as a remote sensing tool in agronomic research and forage management of finger millet. We hypothesized that the optimal waveband selection procedure and VI based models developed using hyperspectral and multispectral band resolutions would result in similar prediction accuracy for various canopy parameters of finger millet.

MATERIALS AND METHODS

Experimental Setup

The experiment was conducted at the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) Grazinglands Research Laboratory (35°34′N, 98°02′ W, elevation 409 m above mean sea level), near El Reno, Oklahoma, US. The experimental field soil type was described as Brewer silty clay loams with a pH of 6.9, slope of 0–1%, water permeability ranging between 0.2 and 1.5 cm h^{-1}, and rarely flooded (USDA-NRCS, 1999). Finger millet cv. PI302662 was planted 2-cm deep at 38-cm row spacing using a Monosem planter (Monosem, Kansas City, KS) on 16 June 2018. Inter-plant spacing was adjusted to achieve 15 seeds m^{-1} row length. About 50 mm of irrigation water was applied with a sprinkler system at planting to assure good emergence. Five nitrogen treatments of 0, 30, 60, 90 and 120 Kg N ha^{-1} were arranged in a randomized complete block design with four replicated plots per treatment. Nitrogen treatments were top-dressed in two equal split doses at 10 and 50 days after planting (DAP), using dry urea (46-0-0) fertilizer. The site received a total of 235 mm of rainfall during the growing season of finger millet and encountered a mean air temperature of 19 °C during the experiment (**Figure 1**).

Crop Characteristics and Hyperspectral Reflectance

Biophysical and reflectance data were collected simultaneously at 38, 46, 52, and 76 days after planting (DAP). The spectral data were measured with a spectroradiometer [FieldSpec Pro FR: Analytical Spectral Devices (ASD), Boulder, CO, USA] by choosing a random location within each plot. The reflectance measurement of the spectroradiometer used ranges between 350 and 2500 nm, comprising a spectral interval of 1.4 and 2.0 nm from 350 to 1,000 and 1,000 to 2,500 nm, respectively. Spectral data were collected from 1.2 m above ground with a 25-degree cone of acceptance, generating a 0.53 m diameter footprint for each sample. The LAI values were collected using a plant canopy

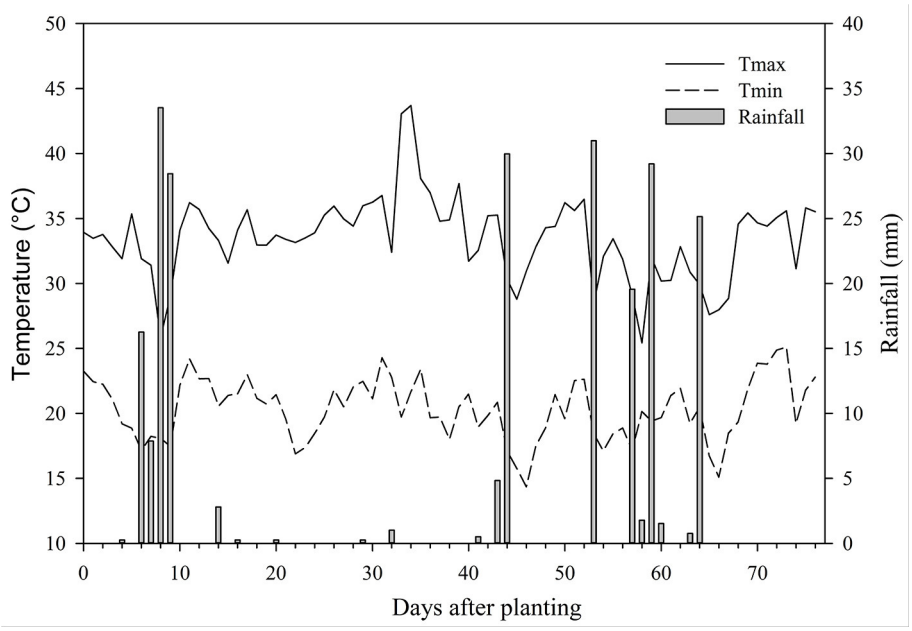

FIGURE 1 | Daily maximum (Tmax) and minimum (Tmin) temperatures and rainfall encountered during the summer growing season of 2018 at El Reno, Oklahoma, USA.

analyzer (LAI-2200C, LI-COR Inc., Lincoln, Nebraska, USA), canopy cover was collected using the Canopeo app (Patrignani and Ochsner, 2015), and canopy height were observed at the same location within the plot. Sampled areas were then harvested to 5.0 cm aboveground from 0.5 m row lengths. Biomass samples were oven-dried at 60°C to constant weight, and dry weights were determined to define aboveground biomass. Each biomass sample was ground to 2-mm in a Wiley mill, and total N content was determined using an auto-analyzer (Model Vario Macro, Elementar Americas, Inc., Mt. Laurel, NJ, USA). The measured crop characteristics are summarized in **Table 1**.

Spectral Preprocessing

Three spectral measurements were averaged to obtain a single spectral curve for each location/sample point before preprocessing. The resulting spectral curves were subjected to the Savitzky-Golay smoothing method to reduce spectral noise (Savitzky and Golay, 1964). Further, wavelength centered bands were created based on the average value derived from a set of 5 nm wavelengths (Kawamura et al., 2008, 2018); for instance, a band centered at 450 nm was averaged from wavelength reflectance values observed from 448 to 452 nm. The procedure for creating centered bands aids in reducing the noise of data and removes wavelengths considered to be similar. Bands ranging between 1,290 and 1,495, 1,705 and 2,045, and 2,355 and 2,500 nm were removed due to atmospheric moisture absorption noise, while 350–395 and 1005–1015 were removed to avoid overlapping noise within the two sensors of the spectroradiometer. The spectral preprocessing resulted in a total of 277 spectral wavebands ranging from 400 to 2,350 nm.

The 5 nm bands matching Landsat-8 (*L8*) and Sentinel-2 (*S2*) bands were synthesized using hyperspectral (*Hy*) data. Bandwidths used for both *L8* and *S2* are presented in **Table 2**. The reflectance values for *Hy* bands within a given bandwidth were added together and divided by number of wavebands to calculate each of *L8* and *S2* bands.

TABLE 1 | Descriptive statistics of the measured parameters for the finger millet.

Parameter	*n*	Minimum	Maximum	Mean	SD
Canopy height (cm)	80	15.24	111.76	38.70	23.41
Leaf area index (LAI)	56	0.43	4.26	2.11	0.94
Canopy cover (%)	79	7.60	84.40	47.3	20.53
Dry biomass (Mg ha^{-1})	80	0.57	16.77	3.70	3.65
N concentration (%)	80	1.27	4.53	3.32	0.79

n, number of samples; SD, standard deviation.

Data Analyses

The canopy reflectance data collected over four sensing dates for finger millet grown under five nitrogen treatments were analyzed as a randomized block design using PROC MIXED procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Nitrogen treatment and spectral wavelength served as fixed effects within the analysis of variance (ANOVA) model, while block was considered as a random element and individual plots were treated as a subject. Sensing dates were taken as repeated elements, and compound symmetry covariance structures were used to take covariance and auto-correlation into account. All possible three-way and two-way interactions among nitrogen treatment, sensing date and wavebands were accounted for within the final model.

Partial Least Square Regression (PLSR) and Optimal Waveband Selection

The PLSR technique projects both dependent and independent variables into a new higher dimensional space to develop a linear regression model. It generates latent variables, also known as score vectors, to capture the variability related to the dependent variable(s). Technically, PLSR develops a model by deriving *X*-scores from latent variables to predict *Y*-scores (Baath et al., 2020). A redundancy analysis on the *X*- and *Y*-scores brings directionality in the factor space to obtain the most accurate prediction (Wu and Yu, 2016). While applying PLSR on spectral data, the number of latent variables (*NLV*) should not exceed the number of independent variables as it can lead to overfitting (Kawamura et al., 2008).

The waveband selection is a modified PLSR method generally used for spectroscopic analyses. The selection process involves reducing the number of wavebands to the most relevant bands based on plant/crop characteristics before carrying out the regression. The method resembles step-wise regression as it eliminates the least important wavebands. PLSR assigns a weighed regression coefficient (βw) to each independent variable based on its contribution to the model. The waveband selection starts with all 277 wavebands and removes the waveband with the least contribution to the model. This removal process is repeated with 276 wavebands and continues until it is reduced to one waveband. After every iteration, the predictive ability is assessed for each set of wavebands by computing the root mean squared error (*RMSE*), and the PLSR model resulting in the lowest *RMSE*

TABLE 2 | Convolved hyperspectral wavelengths (nm) to match Landsat-8 OLI (L8) and Sentinel-2 MSI (S2) bands (B).

Satellite	B_1	B_2	B_3	B_4	B_5	B_6	B_7	B_8
Landsat-8	Ultra Blue	Blue	Green	Red	NIR	–	–	–
	435-451	452-512	533-590	636-673	851-879	–	–	–
Sentinel-2	Ultra Blue	Blue	Green	Red	Red-Edge 1	Red-Edge 2	Red-Edge 3	Narrow NIR
	430-457	447-546	538-583	646-684	695-713	731-749	770-797	848-882

determines the optimal wavebands and the number of wavebands to be used.

Spectral Indices and Linear Regression Models

Original *Hy*, and synthesized *L8* and *S2* bands were used to compute 13 vegetation indices (**Table 3**). The relationships between each index and measured parameters were best-fit with the simple linear regression (SLR) models. In addition, multilinear regression (MLR) models were developed for each of *Hy*, *L8* and *S2* datasets using the stepwise regression and Akaike information criterion (AIC) approach (Zhang, 2016). The MLR approach is based on a primary hypothesis that a subset of VIs is more predictive than others for tested canopy parameters. It starts with no VI in the regression model and then keeps on adding the most statistically significant VIs (with the lowest *p*-value) at every step. The process stops at the point when the regression model shows no improvement on adding more VIs (Fritz and Berger, 2015). A multicollinearity analysis of datasets was conducted based on the Variance Inflation Factor (VIF) calculated using the 'car' package in R (Fox et al., 2012), and predictors with a VIF > 5 were not included in the regression models. The maximum number of VIs (nvmax) selected by the MLR models were limited to four using the 'caret' package in R (Kuhn, 2008).

The predictive capabilities for the PLSR based waveband selection method, and SLR and MLR models developed using VIs were evaluated using a bootstrapping procedure. The procedure involves dividing the data into two random subsets: calibration (75%) and validation (25%), with 1,000 replacements (Efron, 1979). The prediction accuracy of models was compared using the resulting coefficient of determination (R^2_v) and root mean squared error ($RMSE_v$) values for the validation.

RESULTS

Significant three-way interaction ($P < 0.0001$) among nitrogen treatment, sensing date and waveband was noticed for canopy reflectance spectra. The responses of various spectrum regions changed across five nitrogen treatments on different sampling dates (**Figures 2A–D**). Although there were differences in intensity, reflectance showed a typical increase in the NIR region (>700 nm) with increasing nitrogen fertilizer level at all sampling dates, except some relatively mixed responses observed at 52 DAP.

Each of the crop parameters showed a wide range and high standard deviation among observed values (**Table 1**), which represents the variability caused by different N rates applied and age of plant materials at sampling dates during the

TABLE 3 | Formulas of vegetation indices computed for hyperspectral (Hy) data and synthesized Landsat-8 OLI (L8) and Sentinel-2 MSI (S2) data [derived from Zarco-Tejada et al. (2005) and le Maire et al. (2004)].

Type	Vegetation Index	Hyperspectral (Hy) Indices	Synthesized Landsat-8 OLI (L8) Indices	Synthesized Sentinel-2 MSI (S2) Indices	References
Band Ratios	Normalized Difference Vegetation Index (*NDVI*)	$Hy\_NDVI = \frac{(R_{900}-R_{685})}{(R_{900}+R_{685})}$	$L8\_NDVI = \frac{(B_5-B_4)}{(B_5+B_4)}$	$S2\_NDVI = \frac{(B_7-B_4)}{(B_7+B_4)}$	Rouse et al., 1974
	Simple Ratio (*SR*)	$Hy\_SR = \frac{R_{900}}{R_{685}}$	$L8\_SR = \frac{B_5}{B_4}$	$S2\_SR = \frac{B_7}{B_4}$	Jordan, 1969; Rouse et al., 1974
	Modified Simple Ratio (*MSR*)	$Hy\_MSR = \frac{\frac{R_{900}}{R_{685}}-1}{(\frac{R_{900}}{R_{685}})^{0.5}+1}$	$L8\_MSR = \frac{\frac{B_5}{B_4}-1}{(\frac{B_5}{B_4})^{0.5}+1}$	$S2\_MSR = \frac{\frac{B_7}{B_4}-1}{(\frac{B_7}{B_4})^{0.5}+1}$	Chen, 1996
Triangulated	Triangular Vegetation Index (*TVI*)	$Hy\_TVI = 0.5[120\,(R_{750}-R_{550}) - 200\,(R_{670}-R_{550})]$	$L8\_TVI = 0.5[120\,(B_5-B_3) - 200\,(B_4-B_3)]$	$S2\_TVI = 0.5[120\,(B_7-B_3) - 200\,(B_4-B_3)]$	Broge and Leblanc, 2001
Soil Adjusted	Improved SAVI with self-adjustment factor L (*MSAVI*)	$Hy\_MSAVI = \frac{1}{2}[2R_{800}+1-\sqrt{(2R_{800}+1)^2-8(R_{800}-R_{670})}]$	$L8\_MSAVI = \frac{1}{2}[2B_5+1-\sqrt{(2B_5+1)^2-8(B_5-B_4)}]$	$S2\_MSAVI = \frac{1}{2}[2B_7+1-\sqrt{(2B_7+1)^2-8(B_7-B_4)}]$	Qi et al., 1994
	Optimized Soil-Adjusted Vegetation Index (*OSAVI*)	$Hy\_OSAVI = \frac{(1+0.16)(R_{800}-R_{670})}{(R_{800}+R_{670}+0.16)}$	$L8\_OSAVI = \frac{(1+0.16)(B_5-B_4)}{(B_5+B_4+0.16)}$	$S2\_OSAVI = \frac{(1+0.16)(B_7-B_4)}{(B_7+B_4+0.16)}$	Rondeaux et al., 1996
Simple Pigment	Red/Green, Blue/Green, and Blue/Red Pigment indices (*RGI, BGI, BRI*)	$Hy\_RGI = R_{690}/R_{550}$ $Hy\_BGI_1 = R_{400}/R_{550}$ $Hy\_BGI_2 = R_{450}/R_{550}$ $Hy\_BRI_1 = R_{400}/R_{690}$ $Hy\_BRI_2 = R_{450}/R_{690}$	$L8\_RGI = B_4/B_3$ $L8\_BGI_1 = B_1/B_3$ $L8\_BGI_2 = B_2/B_3$ $L8\_BRI_1 = B_1/B_4$ $L8\_BRI_2 = B_2/B_4$	$S2\_RGI = B_4/B_3$ $S2\_BGI_1 = B_1/B_3$ $ST\_BGI_2 = B_2/B_3$ $S2\_BRI_1 = B_1/B_4$ $ST\_BRI_2 = B_2/B_4$	Zarco-Tejada et al., 2005
	Simple Ratio Pigment Index (*SRPI*)	$Hy\_SRPI = R_{430}/R_{680}$	$L8\_SRPI = B_1/B_4$	$S2\_SRPI = B_1/B_4$	Peñuelas et al., 1995
Red-Edge	Red-Edge Linear Extrapolation	*Inflection point:* $R_{re} = (R_{670}+R_{780})/2$ $Hy\_REP = 700 + 40\left(\frac{R_{re}-R_{700}}{R_{740}-R_{700}}\right)$	–	*Inflection point:* $R_{re} = (B_4+B_7)/2$ $S2\_REP = 700 + 40\left(\frac{R_{re}-B_5}{B_6-B_5}\right)$	Cho and Skidimore, 2006

*See **Table 2** for band convolutions for synthesized Landsat-8 OLI and Sentinel-2 MSI.*

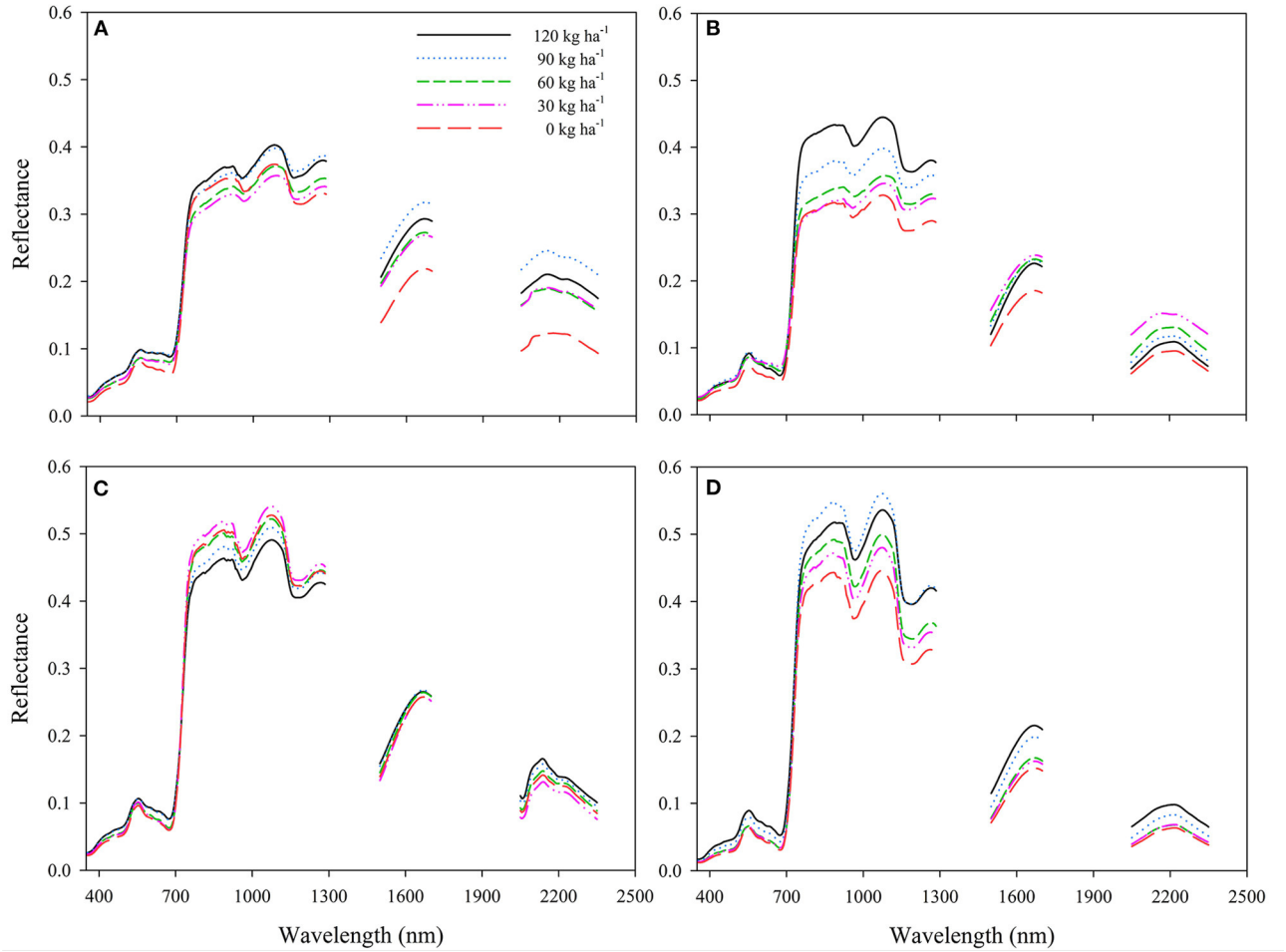

FIGURE 2 | Mean canopy reflectance of finger millet under five nitrogen fertilizer rates when sampled at **(A)** 38, **(B)** 46, **(C)** 52, and **(D)** 76 days after planting.

growing season. Datasets of canopy height, dry biomass, and N concentration include 80 values each, while there was one missing value for canopy cover data. Also, LAI measurements were not recorded at final sampling (76 DAP), and four values were identified as outliers; hence a total of 56 values were used for LAI analyses.

Waveband Selection Approach

Among parameters, the best prediction accuracy was observed for canopy height ($R^2_v = 0.86$), followed by canopy cover ($R^2_v = 0.81$), with the waveband selection approach (**Figures 3A,C**). In contrast, R^2_v for dry biomass and plant N concentration were 0.71 and 0.77, respectively (**Figures 3D,E**), and the lowest R^2_v (0.55) among measured canopy parameters of finger millet was noted for LAI (**Figure 3B**).

It should be noted that the results for dry biomass and LAI were based on only eight spectral bands, with the most optimal bands positioned within the electromagnetic wavelength range of 1,000–1,100 nm. In contrast, plant N concentration, canopy cover and canopy height used a relatively higher number of spectral wavebands (52–59), with ∼ 25, 29, and 44% of selected spectral bands, respectively, within the near-infrared region (NIR; 700–1000 nm).

Vegetation Indices (VIs)

VIs computed using Hyperspectral (*Hy*) data, synthesized Landsat-8 (*L8*), and Sentinel-2 (*S2*) data showed variable linear relationships with canopy height (**Table 4**). Within each of *Hy*, *L8*, and *S2* indices, the simple pigment blue/green index (BGI_1) performed best at predicting canopy height through SLR models, with an R^2_v of 0.78 for *L8_* BGI_1, and R^2_v of 0.65 and 0.66 obtained for *Hy_* BGI_1 and *L8_* BGI_1, respectively. In contrast, blue red index (BRI_1) was the least accurate at estimating canopy height of finger millet, irrespective of tested spectral resolutions.

The MLR model resulted in greater prediction accuracy of canopy height than SLR models in each of three categories of VIs (*Hy, L8,* and *S2*; **Table 5**). The MLR model, comprised of two VIs, resulted in comparable R^2_v of 0.83-0.84 for *Hy* and *L8* indices, while R^2_v of 0.86 was obtained with an additional VI used in the model for *S2* data (**Table 5**).

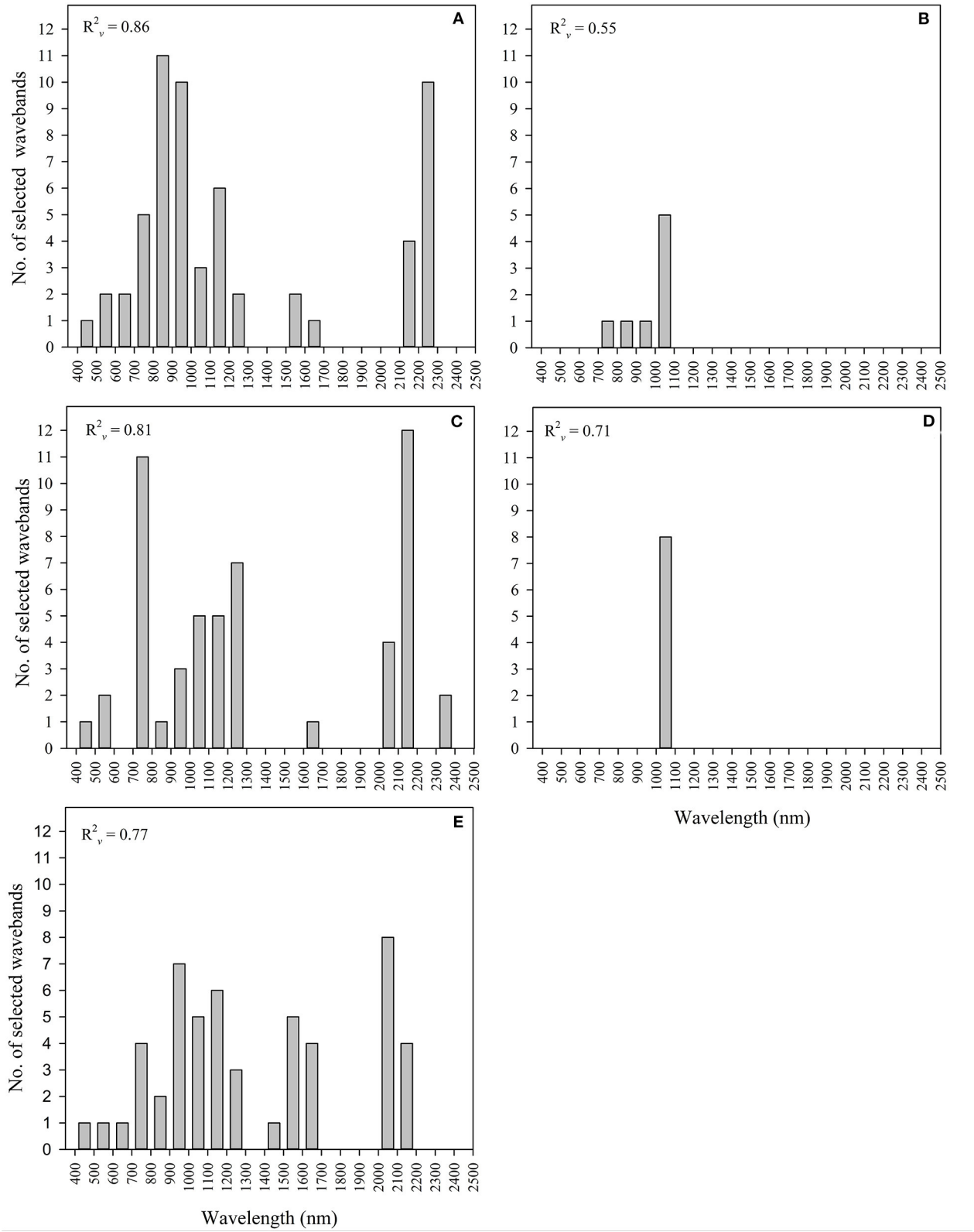

FIGURE 3 | Frequency of optimal wavebands selected across 350-2500 nm spectral wavelength for **(A)** canopy height, **(B)** leaf area index (LAI), **(C)** canopy cover, **(D)** dry biomass and **(E)** N concentration of finger millet, using the partial least square regression (PLSR) based procedure for waveband selection.

Red/Green index (*RGI*), another simple pigment index, outperformed the other indices for canopy cover estimations within each category (**Table 6**). *Hy*, *L8* and *S2* data resulted in a comparable range of R^2_v (0.72–0.76) values for the linear relationships between *RGI* and canopy cover. Besides *RGI*, all VIs calculated with *Hy* and *L8* data performed reasonably well at predicting canopy cover with $R^2_v \geq 0.50$ for SLR models, except $L8\_BGI_1$ (**Table 6**). For *S2* data, five out of 13 indices resulted in $R^2_v < 0.50$, with $S2\_BGI_1$ performing as the least accurate ($R^2_v = 0.30$).

Unlike canopy height, the resulting MLR models for canopy cover were consistently based on the same two VIs (*SR* and *RGI*; **Table 5**). Although MLR performed better ($R^2_v = 0.75$-0.78) than

TABLE 4 | Validation statistics ($RMSE_v$ and R^2_v) of simple linear regression models of Hyperspectral (*Hy*) indices, and synthesized Landsat-8 (*L8*) and Sentinel-2 (*S2*) indices for canopy height of finger millet.

Height	Hyperspectral (*Hy*) indices		Landsat-8 (*L8*) indices		Sentinel-2 (*S2*) indices	
	$RMSE_v$	R^2_v	$RMSE_v$	R^2_v	$RMSE_v$	R^2_v
NDVI	8.52	0.34	8.58	0.34	8.34	0.32
SR	7.03	0.58	5.88	0.68	6.15	0.64
MSR	7.46	0.51	6.65	0.60	6.77	0.55
TVI	10.13	0.06	9.84	0.16	9.07	0.21
MSAVI	9.26	0.21	9.00	0.28	8.44	0.33
OSAVI	9.03	0.25	8.94	0.29	8.50	0.30
RGI	9.20	0.22	9.10	0.28	8.65	0.26
BGI_1	**6.90**	**0.65**	**5.83**	**0.78**	**5.93**	**0.66**
BGI_2	7.21	0.56	6.37	0.71	6.50	0.58
BRI_1	NS	NS	NS	NS	9.86	0.04
BRI_2	NS	NS	NS	NS	8.90	0.22
SRPI	10.38	0.02	NS	NS	9.86	0.04
REP	7.34	0.51	NA	NA	6.89	0.61

NDVI, Normalized Difference Vegetation Index; SR, Simple Ratio; MSR, Modified Simple Ratio; TVI, Triangular Vegetation Index; MSAVI, Modified Soil Adjusted Vegetation Index; OSAVI, Optimized Soil Adjusted Vegetation Index; RGI, Red/Green Index; BG, Blue/Green Index; BR, Blue/Red Index; SRPI, Simple Ratio Pigment Index; REP, Red-Edge Linear Extrapolation; NS, non-significant; NA, not available. Bold values represent vegetation index with highest R^2_v.

TABLE 6 | Validation statistics ($RMSE_v$ and R^2_v) of simple linear regression models of Hyperspectral (*Hy*) indices, and synthesized Landsat-8 (*L8*) and Sentinel-2 (*S2*) indices obtained for canopy cover of finger millet.

	Hyperspectral (*Hy*) indices		Landsat-8 (*L8*) indices		Sentinel-2 (*S2*) indices	
	$RMSE_v$	R^2_v	$RMSE_v$	R^2_v	$RMSE_v$	R^2_v
NDVI	12.22	0.67	11.10	0.66	8.83	0.69
SR	11.60	0.71	11.58	0.63	9.98	0.61
MSR	11.07	0.73	11.07	0.66	9.44	0.64
TVI	13.74	0.62	11.68	0.61	14.19	0.44
MSAVI	12.13	0.71	11.20	0.64	13.04	0.49
OSAVI	11.96	0.70	10.97	0.67	11.58	0.56
RGI	**11.06**	**0.74**	**9.92**	**0.76**	**8.81**	**0.72**
BGI_1	15.18	0.50	15.42	0.36	13.60	0.30
BGI_2	13.71	0.59	13.48	0.50	12.47	0.39
BRI_1	15.79	0.54	11.17	0.70	11.97	0.59
BRI_2	13.63	0.62	10.19	0.74	9.46	0.69
SRPI	11.91	0.71	11.17	0.70	11.97	0.59
REP	14.22	0.56	NA	NA	13.29	0.34

NDVI, Normalized Difference Vegetation Index; SR, Simple Ratio; MSR, Modified Simple Ratio; TVI, Triangular Vegetation Index; MSAVI, Modified Soil Adjusted Vegetation Index; OSAVI, Optimized Soil Adjusted Vegetation Index; RGI, Red/Green Index; BG, Blue/Green Index; BR, Blue/Red Index; SRPI, Simple Ratio Pigment Index; REP, Red-Edge Linear Extrapolation; NS, non-significant; NA, not available. Bold values represent vegetation index with highest R^2_v.

TABLE 5 | Validation statistics ($RMSE_v$ and R^2_v) of multilinear regression models of Hyperspectral (*Hy*) indices, and synthesized Landsat-8 (*L8*) and Sentinel-2 (*S2*) indices obtained for canopy height, canopy cover, dry biomass and nitrogen (N) concentration of finger millet.

Parameter	Indices	Regression equation	$RMSE_v$	R^2_v
Canopy height	Hyperspectral (*Hy*)	$Y = 3.64\ Hy\_SR - 66.21\ Hy\_SRPI + 34.16$	3.20	0.84
	Landsat-8 (*L8*)	$Y = 3.75\ L8\_SR - 86.22\ L8\_BRI_2 - 55.04$	3.36	0.83
	Sentinel-2 (*S2*)	$Y = -56.55\ S2\_MSAVI - 176.47\ S2\_BGI_1 - 56.55\ S2\_REP - 2317$	3.79	0.86
Canopy cover	Hyperspectral (*Hy*)	$Y = 1.69\ Hy\_SR - 72.42\ Hy\_RGI + 93.29$	10.26	0.78
	Landsat-8 (*L8*)	$Y = 2.19\ L8\_SR - 82.74\ L8\_RGI + 96.92$	9.49	0.78
	Sentinel-2 (*S2*)	$Y = 1.95\ S2\_SR - 79.62\ S2\_RGI + 93.74$	9.81	0.75
Dry biomass	Hyperspectral (*Hy*)	$Y = 16.7\ Hy\_SR - 365.47\ Hy\_BRI_1 + 122.37$	59.89	0.50
	Landsat-8 (*L8*)	$Y = 20.21\ L8\_SR - 334.8\ L8\_BRI_1 - 153.53$	34.43	0.55
	Sentinel-2 (*S2*)	$Y = 20.27\ S2\_SR - 323.0\ S2\_BRI_1 + 150.27$	63.59	0.52
N concentration	Hyperspectral (*Hy*)	$Y = 9.26\ Hy\_BGI_2 - 0.016\ Hy\_REP + 10.28$	0.46	0.88
	Landsat-8 (*L8*)	$Y = 1.81\ L8\_OSAVI + 16.66\ L8\_BGI_1 - 6.54$	0.35	0.85
	Sentinel-2 (*S2*)	$Y = 1.18\ S2\_MSAVI + 16.35\ S2\_BGI_1 - 5.83$	0.33	0.81

SR, Simple Ratio; MSR, Modified Simple Ratio; MSAVI, Modified Soil Adjusted Vegetation Index; OSAVI, Optimized Soil Adjusted Vegetation Index; RGI, Red/Green Index; BG, Blue/Green Index; BR, Blue/Red Index; SRPI, Simple Ratio Pigment Index; REP, Red-Edge Linear Extrapolation.

SLR models at predicting canopy cover for each of three datasets, observed statistics (R^2_v and $RMSE_v$) were not largely different.

The SLR model of simple ratio pigment index (*SRPI*) performed consistently (R^2_v = 0.53–0.59) at estimating LAI, with *L8_SRPI* performing best (R^2_v = 0.59) among *Hy*, *L8* and *S2* indices (**Table 7**). Apart from *SRPI,* the predictive accuracy of BRI_2 was also relatively better (R^2_v = 0.52–0.57) than the remaining indices. Besides, *SR*, *MSR,* and *RGI* indices developed comparable linear relationships with LAI and their R^2_v ranged between 0.43 and 0.51, regardless of spectral resolutions. However, in contrast to canopy height and canopy cover, MLR models did not result in any significant combination of VIs that could outperform the SLR model for LAI prediction accuracies with either of three datasets.

Among the VIs, none of them resulted in a consistently best linear relationship with dry biomass for each of *Hy*, *L8,* and *S2* datasets (**Table 8**). Among *Hy* indices, the best linear relationship of dry biomass was observed with red-edge linear extrapolation (*REP*) index (R^2_v = 0.48), while BGI_1performed with an R^2_v of 0.46 and 0.49 among *L8* and *S2* indices, respectively (**Table 8**). Other than BGI_1, *SR* showed a consistent prediction accuracy, with R^2_v ranging between 0.40 and 0.46, compared to remaining VIs in all three categories. In contrast, linear relationships of dry biomass with BRI_1, BRI_2 and *SRPI* were least significant (R^2_{cv} = 0.02–0.20) among VIs.

The MLR model improved the prediction accuracy of dry biomass for all three datasets (**Table 5**). Among MLR models developed for dry biomass, best performance (R^2_v = 0.55) was observed with *L8* indices, accompanied by *S2* (R^2_v = 0.52) and *L8* (R^2_v = 0.50) indices.

Among VIs, the best predictive accuracy for N concentration was obtained with BGI_1within each of *Hy*, *L8,* and *S2* indices (**Table 9**). The greatest R^2_v of 0.83 was observed for Hy_BGI_1, followed by $S2\_BGI_1$ (R^2_v = 0.80). Whereas, $L8\_BGI_1$ had a relatively lower R^2_v of 0.70 for N concentration, which was otherwise higher than the remaining *L8* indices. As observed for canopy height and dry biomass, the least effective SLR models for estimating N concentration were observed with BRI_1, BRI_2, and *SRPI* indices.

MLR models, comprising two VIs, improved prediction accuracies of N concentration compared to SLR models within each category (**Table 5**), though little difference was observed for performance given by *Hy* indices. The best MLR model performance (R^2_v = 0.90) was noticed for *S2* data, followed by *Hy* and *S2* data (R^2_v = 0.83–0.88).

DISCUSSION

Though not generally grown in large-scale commercial settings, finger millet is still an essential cereal crop in many drought-prone areas around the world. Therefore, the ability to estimate different parameters of crop canopies of finger millet through remote sensing could be important for improving in-season management. Remote sensing applications have not been widely applied to finger millet, and this study showed that relationships between hyperspectral reflectance and different canopy parameters using the PLSR based procedure for

TABLE 7 | Validation statistics ($RMSE_v$ and R^2_v) of simple linear regression models of Hyperspectral (*Hy*) indices, and synthesized Landsat-8 (*L8*) and Sentinel-2 (*S2*) indices obtained for leaf area index (LAI) of finger millet.

LAI	Hyperspectral (*Hy*) indices		Landsat-8 (*L8*) indices		Sentinel-2 (*S2*) indices	
	$RMSE_v$	R^2_v	$RMSE_v$	R^2_v	$RMSE_v$	R^2_v
NDVI	0.631	0.42	0.751	0.41	0.727	0.38
SR	0.667	0.48	0.711	0.45	0.663	0.51
MSR	0.657	0.49	0.711	0.45	0.676	0.47
TVI	0.702	0.41	0.803	0.35	0.674	0.50
MSAVI	0.698	0.41	0.769	0.39	0.682	0.48
OSAVI	0.673	0.46	0.757	0.41	0.700	0.44
RGI	0.644	0.49	0.697	0.47	0.695	0.43
BGI_1	0.844	0.11	0.927	0.09	0.871	0.12
BGI_2	0.791	0.27	0.828	0.25	0.831	0.20
BRI_1	0.644	0.50	0.616	0.59	0.631	0.54
BRI_2	0.632	0.52	0.629	0.57	0.631	0.53
SRPI	**0.621**	**0.53**	**0.616**	**0.59**	**0.631**	**0.54**
REP	0.848	0.13	NA	NA	0.827	0.24

NDVI, Normalized Difference Vegetation Index; SR, Simple Ratio; MSR, Modified Simple Ratio; TVI, Triangular Vegetation Index; MSAVI, Modified Soil Adjusted Vegetation Index; OSAVI, Optimized Soil Adjusted Vegetation Index; RGI, Red/Green Index; BG, Blue/Green Index; BR, Blue/Red Index; SRPI, Simple Ratio Pigment Index; REP, Red-Edge Linear Extrapolation; NS, non-significant; NA, not available. Bold values represent vegetation index with highest R^2_v.

TABLE 8 | Validation statistics ($RMSE_v$ and R^2_v) of simple linear regression models of Hyperspectral (*Hy*) indices, and synthesized Landsat-8 (*L8*) and Sentinel-2 (*S2*) indices obtained for dry biomass of finger millet.

Biomass	Hyperspectral (*Hy*) indices		Landsat-8 (*L8*) indices		Sentinel-2 (*S2*) indices	
	$RMSE_v$	R^2_v	$RMSE_v$	R^2_v	$RMSE_v$	R^2_v
NDVI	71.89	0.29	63.24	0.31	77.14	0.30
SR	65.35	0.40	57.64	0.46	69.78	0.44
MSR	66.42	0.38	58.80	0.43	71.33	0.41
TVI	76.75	0.21	61.95	0.33	78.97	0.25
MSAVI	71.50	0.31	60.47	0.37	75.89	0.32
OSAVI	72.18	0.29	61.63	0.34	76.66	0.31
RGI	75.50	0.22	66.64	0.22	80.37	0.23
BGI_1	**65.82**	**0.42**	**59.40**	**0.46**	**70.30**	**0.49**
BGI_2	66.20	0.40	61.95	0.38	73.55	0.42
BRI_1	NS	NS	72.04	0.06	88.02	0.04
BRI_2	NS	NS	69.90	0.12	82.51	0.19
SRPI	83.48	0.04	72.04	0.06	88.02	0.04
REP	63.53	0.48	NA	NA	73.17	0.32

NDVI, Normalized Difference Vegetation Index; SR, Simple Ratio; MSR, Modified Simple Ratio; TVI, Triangular Vegetation Index; MSAVI, Modified Soil Adjusted Vegetation Index; OSAVI, Optimized Soil Adjusted Vegetation Index; RGI, Red/Green Index; BG, Blue/Green Index; BR, Blue/Red Index; SRPI, Simple Ratio Pigment Index; REP, Red-Edge Linear Extrapolation; NS, non-significant; NA, not available. Bold values represent vegetation index with highest R^2_v.

TABLE 9 | Validation statistics ($RMSE_v$ and R^2_v) of simple linear regression models of Hyperspectral (*Hy*) indices, and synthesized Landsat-8 (*L8*) and Sentinel-2 (*S2*) indices obtained for N concentration of finger millet.

N	Hyperspectral (*Hy*) indices		Landsat-8 (*L8*) indices		Sentinel-2 (*S2*) indices	
	$RMSE_v$	R^2_v	$RMSE_v$	R^2_v	$RMSE_v$	R^2_v
NDVI	0.625	0.39	0.574	0.36	0.632	0.36
SR	0.550	0.54	0.463	0.57	0.560	0.50
MSR	0.567	0.50	0.492	0.52	0.586	0.47
TVI	0.713	0.19	0.660	0.24	0.675	0.23
MSAVI	0.681	0.27	0.612	0.33	0.653	0.31
OSAVI	0.660	0.31	0.605	0.32	0.650	0.32
RGI	0.621	0.39	0.597	0.31	0.665	0.32
BGI_1	**0.368**	**0.83**	**0.397**	**0.70**	**0.362**	**0.80**
BGI_2	0.394	0.76	0.420	0.66	0.408	0.74
BRI_1	NS	NS	0.705	0.03	0.794	0.05
BRI_2	0.761	0.08	0.685	0.09	0.758	0.20
SRPI	0.729	0.15	0.705	0.03	0.794	0.05
REP	0.735	0.19	NA	NA	0.615	0.31

NDVI, Normalized Difference Vegetation Index; SR, Simple Ratio; MSR, Modified Simple Ratio; TVI, Triangular Vegetation Index; MSAVI, Modified Soil Adjusted Vegetation Index; OSAVI, Optimized Soil Adjusted Vegetation Index; RGI, Red/Green Index; BG, Blue/Green Index; BR, Blue/Red Index; SRPI, Simple Ratio Pigment Index; REP, Red-Edge Linear Extrapolation; NS, non-significant; NA, not available. Bold values represent vegetation index with highest R^2_v.

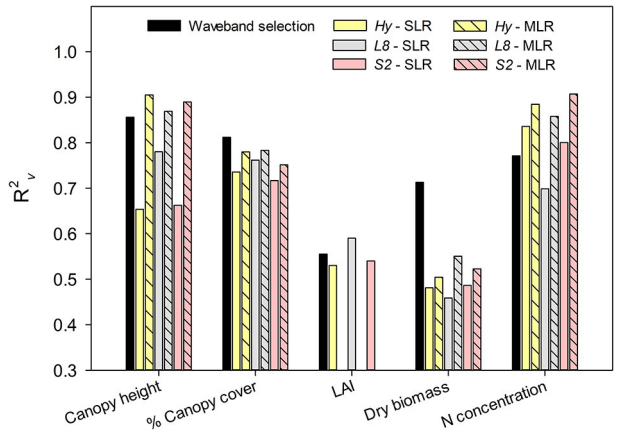

FIGURE 4 | Comparison of predictive performance (R^2_v) given by waveband selection procedure, and Hyperspectral (*Hy*) indices and synthesized Landsat-8 (*L8*) and Sentinel-2 (*S2*) indices with simple linear regression (SLR) and multilinear regression (MLR) models, for estimation of different canopy parameters of finger millet. Within each group, SLR models with the best statistics were selected for comparison.

selecting wavebands have value. Further, given the cost of spectroradiometers, the complexity of the approach used in waveband selection, and the production of finger millet by mostly small farmers in developing countries, indicates simpler methods based on VIs derived from *Hy*, *L8* and *S2* datasets may also have great value.

Canopy height is an important parameter characterizing plant growth, and it is often required as input for determining energy balance components using remote sensing data (Hunsaker et al., 2003). The predictive accuracy of canopy height by VIs remained lower through SLR approach in this study. Similarly, Payero et al. (2004) reported lower prediction accuracy for plant height of a grass (*Festuca arundinacea*) using the linear function of 11 similar VIs. Whereas, the predictive performance was significantly improved using MLR models based on VIs calculated from *Hy, L8,* and *S2* data, even when compared to the waveband selection procedure (**Figure 4**), which suggests a strong potential for height estimations of finger millet with this approach. The unavailable *REP* index for *L8* data could explain a slightly lower prediction accuracy of the *L8*-multilinear model compared to other MLR models developed for canopy height. Nonetheless, it appeared that MLR models allowed the use of effective information of different VIs, and hence improved the prediction accuracy of canopy height in finger millet.

The predictive performance of canopy cover estimations by MLR was comparable ($R^2_v = 0.75$–0.78) to the best SLR model obtained for the *Hy*, *L8* and *S2* datasets, though PLSR based waveband selection resulted in a slightly greater prediction accuracy (**Figure 4**). These findings suggest that estimations of green canopy cover could be directly achieved with sufficient accuracy in finger millet using *RGI*. Likewise, the green cover was reported as highly correlated with *RGI* in another grasslands study (Cundill et al., 2015). Canopy cover estimation involves sensing healthy green grass, dry grass (litter), and bare soil. If the proportion of litter and bare soil is greater, the reflectance in red region increases more than in the green region and vice versa (Asner, 1998). Therefore, the combination of green cover, litter and bare soil can be better assessed by VI_S such as *RGI* since it accounts for both red and green regions. It is important to emphasize that prediction accuracy for detecting green cover remained similar across VIs, regardless of bandwidths associated with different instruments. This is in agreement with previous studies (Broge and Leblanc, 2001; Zhao et al., 2007b), which suggested that narrow-band and broad-band indices could result in similar performance for some parameters, like canopy cover, depending on the optimum waveband positions.

The prediction accuracy of LAI obtained in this study was lower than the other canopy parameters, which could be due to fewer data points (56 vs. 80) available for calibration and validation of LAI models. It is generally argued that narrow-band indices result in better estimation of important canopy parameters such as LAI (Sahoo et al., 2015; Din et al., 2017). However, broad-band indices computed from both *L8* and *S2* outperformed the narrower bands of *Hy* indices in this study. The better performance of *S2* and *L8* indices could be explained by the reduction in surrounding environmental noise with aggregated bands, which otherwise exist within the original narrow-band hyperspectral data (Kawamura et al., 2005; Flynn et al., 2020). Further, the predictive performance of *SRPI* in estimating LAI exceeded the most commonly used VIs, involving *NDVI* (Tan et al., 2020), *REP* (Dong et al., 2019), *SR* (Nguy-Robertson et al., 2012), or modified soil-adjusted vegetation index (*MSAVI*; Din

et al., 2017). These findings supported that LAI-VI relationships are not universal but crop-specific (Kang et al., 2016; Dong et al., 2020).

Another interesting finding observed from PLSR waveband selection was that both LAI and dry biomass of finger millet were strongly related to the spectral bands within the electromagnetic range of 1,000–1,100 nm. Since all VIs were derived from spectral bands ranging between 400 and 900 nm, it explains the lower prediction performance observed with both SLR and MLR models of all VIs, especially in the case of dry biomass estimations, compared to the standard PLSR based waveband selection approach. The optimal wavebands determined for finger millet biomass were 686, 694, and 774–814 nm were reported in earlier research in India by Dayananda et al. (2019), using a hyperspectral sensor with the spectral range of 450–998 nm. However, our findings suggest it is essential to focus on the spectral range of 1,000–1,100 nm to improve the prediction accuracy of biomass in future precision agriculture research related to finger millet.

In agreement with results of this study, Tong et al. (2019) also observed an improvement in the predictive performance of aboveground biomass using MLR methods on VIs. Therefore, such VI-multilinear algorithms should also be encouraged for improved estimation of pasture biomass with hyperspectral or multispectral instruments in other crops.

The PLSR waveband selection revealed that several hyperspectral regions between 400 and 2,200 nm were important for predicting nitrogen content in finger millet. Similar findings were reported in other studies estimating nitrogen from hyperspectral data (Caporaso et al., 2018; Flynn et al., 2020). While it was also noted that equal or better predictive accuracy for N concentration in finger millet could be achieved using the SLR approach involving VIs, potentially avoiding the complexity related to PLSR waveband selection procedure. Based on R^2_v and $RMSE_v$ values obtained for simple linear relationships between VIs and N concentration, simple pigment indices (BGI_1) derived from blue and green spectral bands, irrespective of band widths, resulted in more accurate estimations of plant N concentration than other traditional VIs, such as *NDVI* and *SR* (Zhu et al., 2008; Zhao et al., 2018). Simple pigment indices were also good predictors of biochemical components in another cereal crop known as tef (*Eragrostis tef*; Flynn et al., 2020).

Although blue bands are less commonly adopted for detecting nutritional quality, the combination of blue and green bands through *BGI* have been found more useful than *BRI* in retrieving chlorophyll contents in barley (*Hordeum vulgare*) and vineyard (*Vitis vinifera*) (Zarco-Tejada et al., 2005; Aasen et al., 2015). Similarly, Hansen and Schjoerring (2003) found only visible wavelengths, particularly the combination of blue and green bands, as strongly correlated to N concentrations. Moreover, *BGI* was earlier reported best at predicting N concentration in wheat (Prey and Schmidhalter, 2019). Results also suggested that the application of MLR models could further enhance the N prediction accuracy for *Hy*, *L8* and *S2* datasets by 14%, and hence the approach has a major significance for precision agriculture.

CONCLUSION AND IMPLICATIONS

Hyperspectral reflectance data could be effectively utilized to estimate canopy parameters for rapid and improved in-season management decisions of finger millet. In the case of SLR models, the best prediction accuracies were observed with simple pigment indices for all of the tested crop parameters in this study. BGI_1 performed best for canopy height, dry biomass, and N concentration, while *RGI* and *SRPI* were found strongly related to canopy cover and LAI of finger millet, respectively. Also, results showed that the application of MLR approach on VIs could provide a predictive performance better than the methods, involving SLR models and PLSR waveband selection procedure, for the estimation of canopy height and N concentration in finger millet. Whereas, dry biomass of finger millet was estimated with greater accuracy using waveband selection procedure than approaches involving VIs, owing to optimal spectral bands positioned within the electromagnetic range of 1,000–1,100 nm.

This study demonstrated that VIs derived from hyperspectral data could be translated to multispectral bands of Landsat-8 and Sentinel-2 satellite data with similar (or greater) prediction accuracy for canopy parameters of finger millet. Hence, there is a great potential of utilizing such readily accessible sources of satellite data for developing methods of precision agriculture related to finger millet, especially in the developing countries where the crop is mostly cultivated. Future research needs to be focused on evaluating the effectiveness of VIs developed from Landsat-8 and Sentinel-2 data at predicting nutritive value of forage, and grain yields, of finger millet across environmental gradients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

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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frontiers in Agronomy

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Native Rhizobia Strains Enhance Seed Yield of Groundnut Varieties in Northern Ghana

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Rhizobia inoculation with effective strains is an environmentally friendly approach for enhancing nodulation and yield of legumes. To obtain an ideal strain for inoculant production, the strain's performance must be matched to the environmental conditions. A 2 × 9 cross-factorial experiment laid in a randomized complete block design with three replications on farmers' fields in the northern part of Ghana was set up to evaluate the rhizobia's performance. The factors were groundnut varieties (Chinese and Samnut 22) and nine strains consisting of five native rhizobia (KNUST 1001, 1002, 1003, 1032, and 1031), two commercial strains (Biofix and BR3267), and a positive (N+) and absolute control (N–). In addition, the population of the strains was assessed after a cropping season. The strains' performance on groundnut was location and variety dependent. At Cheshegu, KNUST 1031 and 1002 significantly increased seed yield of the Chinese variety compared to other strains; however, KNUST 1031 elicited a 24% yield increase while KNUST 1002 caused a 16% yield increase over the control. Strain KNUST 1031 increased the seed yield of the Chinese variety by 24% while KNUST 1002 increased the seed yield of Samnut by 16%. Only KNUST 1002 elicited a significant seed yield increase in Samnut 22 at Cheshegu. At Binduri, strain KNUST 1003 significantly increased the seed yield of the Chinese variety by 35% relative to the control. Samnut 22 did not show a clear preference for any of the strains. The Chinese variety did not show a clear response to the strains at Tanina. However, Samnut 22 responded to KNUST 1002 as it increased seed yield by 45% relative to the control. On average, the seed yield at Tanina (846.15 kg ha^{-1}) was less than the seed yield recorded at Binduri (1,077.66 kg ha^{-1}) and Cheshegu (1,502.78 kg ha^{-1}). Inoculation with strains KNUST 1002, 1003, and 1031 was all profitable under the current experimental conditions as they recorded value cost ratios (VCRs) above the threshold of 3–4. The study has shown that strains KNUST 1002, 1003, and 1031 have the potential to be used in inoculant formulation to increase groundnut production and enhance the income of smallholder groundnut farmers in northern Ghana.

Keywords: native strains, value cost ratio, biological nitrogen fixation, grain yield, smallholder farmers

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INTRODUCTION

Groundnut is an important source of protein and forms part of many delicacies in Ghana. The crop provides income to smallholder farmers through the sale of its seed and the oil produced from it. Groundnut biomass and haulms are used to improve the fertility of soils. The annual production of groundnut in Ghana is 521,030 Mt from an area of 319,680 ha (Statistics, Research and Information Directorate (SRID), 2019). The northern part of Ghana contributes about 75.6% of the annual production (Breisinger et al., 2008).

Grain legumes such as groundnut (*Arachis hypogea* L.) require high amounts of nitrogen for optimum growth (Peoples and Herridge, 1990). The nitrogen demand of such crops can be met through mineral nitrogen fertilization or inoculation with highly competitive and effective rhizobia strains. Nitrogen fertilization is very effective under optimum field conditions; however, the high cost and accessibility limit its use by most smallholder farmers in sub-Saharan Africa (SSA) (Zengeni et al., 2006). Mineral nitrogen fertilizers are one of the main sources of nitrous oxide, which contributes to greenhouse gas emissions and ultimately climate change and its attendant effects (Erbas and Solakoglu, 2017). Also, mineral nitrogen fertilizers have been identified as a major source of water pollution (Erbas and Solakoglu, 2017). These negative effects on the environment threaten the sustainable use of mineral nitrogen fertilizers.

Rhizobia inoculation with highly effective strains is a feasible environmentally friendly approach for enhancing the grain yield of legumes. Several reports in SSA show the positive effect of inoculating groundnut with appropriate rhizobia strain (Yakubu et al., 2010; Kabir et al., 2013; Biswas and Gresshoff, 2014).

However, smallholder farmers have difficulty in accessing quality inoculant(s) partly because there are few inoculant-producing centers in SSA. The quality of inoculants is sometimes compromised before and/or after the farmer(s) receives them due to poor handling and storage practices as well as a lack of strong quality control measures. There has been contrasting reports on field evaluation of imported inoculants due to the degree of effectiveness of the strains and their adaptability to local conditions in SSA (Mathu et al., 2012; Thuita et al., 2012; Masso et al., 2016; Ulzen et al., 2016), creating doubt about the contribution of rhizobia inoculants to legume grain yields. In recent times, there has been a renewed interest in inoculant production in SSA by N2AFRICA (www.n2africa.org) and COMPRO II (www.compro2.org) projects. This has led to the establishment of an inoculant production facility in the northern part of Ghana, necessitating the need to bioprospect, from the indigenous rhizobia population, highly saprophytic and effective strains to feed this production center and to boost groundnut production in northern Ghana.

Several types of research aimed at selecting effective rhizobia for use as inoculants revealed that effective strains capable of inducing high N_2 fixation exist within the native rhizobia populations (Ampomah et al., 2008; Yakubu et al., 2010; Kawaka et al., 2014; Osei et al., 2018). Also, the native rhizobia are well-adapted to local conditions and are therefore presumed to have a highly competitive advantage. An equally important attribute is the high saprophytic competence relative to exotic strains, which allows researchers to make informed decisions about subsequent inoculation practices. Thus, bioprospecting for and characterizing native rhizobia to identify effective and persistent strains for use in prescribed doses as inoculants will increase the likelihood of successful inoculation responses. Although this is an entry point for successful inoculation, the success or otherwise of rhizobia inoculation is influenced by the specificity between rhizobia, legume genotype used, and environmental conditions as well as management practices (Woomer et al., 2014). Consequently, studies geared toward matching superior rhizobia strains with improved crop varieties under different environmental conditions are needed. The present study hypothesized that superior effective strains with saprophytic abilities that can meet the N requirement of groundnut exist in soils of Ghana and can effectively increase seed yields of groundnut. This study was, therefore, conducted to (i) determine the response of groundnut to inoculation with rhizobia originating from native populations in soils of northern Ghana and (ii) quantitively assess the population of the introduced strains after a cropping season. Identifying effective and saprophytic competent strains will not only be a major step toward increasing groundnut production and seed yield but will also reduce the frequency of reinoculation.

MATERIALS AND METHODS

Site Selection and Characteristics

The field trials were conducted at three different locations in the northern part of Ghana during the 2015 cropping season. The experimental sites were located at Cheshegu (northern region) (latitude N09°27′18.2″ to longitude W000°57′22.4″), Binduri (upper east region) (latitude N10°56′57.6″ to longitude W000°18′52.0″), and Tanina (upper west region) (latitude N09°53′13.0″ to longitude W002°27′48.5″). The study sites have a unimodal rainfall distribution with an annual rainfall amount of 1,000–1,200 mm and mean temperature between 26 and 30°C with little variation within the year. The rainfall distribution during the cropping season is presented as **Supplementary Figures 1A–C**. All the fields had no known history of rhizobia inoculation. Before planting, five soil core samples were taken from each plot and thoroughly mixed, and composite samples were taken into transparent polythene bags and kept in the refrigerator at 4°C before laboratory analysis. The soil parameters analyzed were particle size (hydrometer method), soil pH (1:2.5 soil to distilled water ratio), organic carbon (OC) (modified Walkley and Black procedure) as described by Nelson and Sommers (1996), total nitrogen (Kjeldahl method) as described by Bremner and Mulvaney (1982), available soil phosphorus (Bray No. 1 solution) as described by Olsen (1982), and exchangeable potassium [ammonium acetate (NH_4OAc) extract] as described by Helmke and Sparks (1996); details of the characteristics are given in **Table 1**. Calcium and magnesium were also determined in 1.0 M ammonium acetate (NH_4OAc) extract (Black, 1965). The study was conducted under rainfed conditions; hence, no manual irrigation was carried out.

TABLE 1 | Soil physicochemical characteristics and MPN count at the study sites.

Soil parameters	Cheshegu	Binduri	Tanina
Total N (%)	0.024 ± 0.001*	0.022 ± 0.0011	0.03 ± 0.001
Available P (mg kg^{-1})	17.61 ± 0.01	17.61 ± 0.011	20.54 ± 0.005
Exch. K ($cmol_{(+)}$ kg^{-1})	0.03 ± 0.004	0.016 ± 0.0003	0.015 ± 0.003
Organic carbon (%)	0.42 ± 0.006	0.96 ± 0.005	0.62 ± 0.006
pH (1:2.5, H_2O)	6.1 ± 0.01	6.24 ± 0.012	6.38 ± 0.001
Exch. Ca ($cmol_{(+)}$ kg^{-1})	3.64 ± 0.012	2.54 ± 0.015	2.6 ± 0.015
Exch. Mg ($cmol_{(+)}$ kg^{-1})	0.22 ± 0.002	0.28 ± 0.0006	0.18 ± 0.0001
Exch. Na ($cmol_{(+)}$ kg^{-1})	0.39 ± 0.026	0.28 ± 0.0005	0.35 ± 0.004
% Sand	56.4 ± 0.002	78.6 ± 0.005	84.56 ± 0.005
% Clay	5.88 ± 0.003	14.56 ± 0.002	5.88 ± 0.005
% Silt	37.72 ± 0.002	6.84 ± 0.006	9.56 ± 0.015
Texture	Sandy loam	Loamy sand	Loamy sand
MPN (rhizobia cell g^{-1} soil)	26.3	11.2	32.8

Standard deviation.

Estimation of Rhizobia Population

Enumeration of indigenous rhizobia population was done using the most probable number (MPN) of plant infection techniques (Vincent, 1970; Somasegaran and Hoben, 2012) before planting and after a cropping season. Soil samples were taken from a depth of 0–20 cm using the W design and bulked. A composite sample was taken and used for the MPN count. Seeds of groundnut varieties (Samnut 22 and Chinese) were used as a trap host for the estimation of the indigenous rhizobia population. The groundnut seeds were surface-sterilized with 95% ethanol and 3% (v/v) hydrogen peroxide (H_2O_2) solution. The seeds were rinsed in five successive changes of sterilized distilled water and pre-germinated on moistened filter paper in Petri dishes at a temperature of 30°C. After 2 days, well-grown healthy seedlings of similar radicle length were transferred aseptically into growth pouches containing 65 ml nitrogen-free mineral nutrient solution (Broughton and Dilworth, 1971). Twenty grams of the composite soil sample was serially diluted in distilled water using the fivefold, six-step serial dilution method (5^{-1}) and was mixed thoroughly on a vortex mixer. One milliliter of each dilution was used to inoculate seedlings. Four seedlings were inoculated per dilution level. Plants were watered as and when required. The setup was monitored for 28 days, after which scoring was done for the presence or absence of nodules. Population estimates were assigned using the MPNES software (Woomer et al., 1990).

Inoculant Preparation

The peat-based inoculant was prepared at the soil microbiology laboratory of Kwame Nkrumah University of Science and Technology, Kumasi, as previously described by Ulzen et al. (2016) following the method of Somasegaran and Hoben (2012).

Field Preparation, Experimental Design, and Treatments

The field at Cheshegu was planted on July 13, that at Binduri on July 18, and that at Tanina on July 28, 2015. The experimental fields were disc plowed and harrowed using a harrow to a depth of 15 cm. Each plot measured 4 × 4 m with an alley of 1 m within plots and 2 m between blocks. Each experiment was 9 × 2 cross factorial, laid out in randomized complete block design (RCBD) with three replications. The treatment factors were rhizobia strains and varieties. There were nine strains consisting of five native rhizobia strains (KNUST 1001, 1002, 1032, 1003, and 1031) (Osei et al., 2018), two reference strains (BR3267 and Biofix, USDA 110), a positive control with 100 kg N ha^{-1}, and an absolute control (uninoculated and without mineral N). The strain BR3267 is from Brazil and has originally been described by Leite et al. (2018). The two groundnut varieties were Chinese and Samnut 22. The seeds were inoculated under a shade with a peat-based inoculum of respective strains at a rate of 5 g peat kg^{-1} of seeds using 10% of gum Arabic solution as a sticker. Three seeds of the groundnut varieties, Samnut 22 and Chinese, were sown per hill at a spacing of 60 × 20 cm at each location. The seedlings were thinned to two, after 2 weeks to maintain optimum plant population. Other cultural practices such as weeding and earthing-up were carried out accordingly as and when needed. Samnut 22 is a dual-purpose groundnut cultivar with good oil content, high haulms, and high pod yield (Ajeigbe et al., 2015). It matures within 115–120 days with a potential yield of 2.5 t ha^{-1} (Ajeigbe et al., 2015). The Chinese variety is an early-maturing (100 days) cultivar with a potential yield of about 3.5 t ha^{-1}. A basal dose of 20 kg P ha^{-1} in the form of triple superphosphate (46% P_2O_5) was applied through band placement 2 weeks after planting. Nitrogen (urea) was split-applied at 20 kg N ha^{-1} 2 weeks after emergence and 80 kg N ha^{-1} at mid-flowering.

Measurement of N_2 Fixation

The amount of nitrogen derived from the atmosphere through biological nitrogen fixation (BNF) was estimated using the total nitrogen difference (TND) method in both field and greenhouse experiments. Guinea grass (*Panicum maximum*) was used as a reference plant (Ashworth et al., 2015). The total amounts of nitrogen in the shoot at mid-flowering and in the reference plant were assessed, and the amount of N_2 fixed was calculated using the modified equations of Viera-Vargas et al. (1995). To account

for the nitrogen that remained in the roots and/or was lost in the soil, the calculated value was then multiplied by a factor of 1.4 (Unkovich and Baldock, 2008).

$$\text{Total N in plant} = \frac{\text{Shoot dry weight x \% N in shoot}}{100} \quad (1)$$

$$\text{Amount of N fixed} = \text{Total N in legume} - \text{Total N in reference plant} \quad (2)$$

Harvesting for Nodulation and Grain Yield

At the R_1 stage (beginning bloom) (Fehr et al., 1971) of Samnut 22 and Chinese varieties in the field, 10 plants were randomly selected from the row directly adjacent to the border rows for each treatment plot. With the help of a spade, the selected plants were carefully uprooted by digging 15 cm around each plant, after which the shoots were separated from roots. The adhered soil on the roots was carefully washed in 1-mm sieve mesh after, which the nodules were detached and counted. The nodules and shoots were oven-dried separately at 60°C for 72 h, after which the dry weights were recorded. At the R_8 stage (Fehr et al., 1971), the plants were harvested for their pods and grains.

Economic Viability of Using Indigenous Rhizobia Isolates

The profitability of using indigenous rhizobia strains in inoculant formulation was determined through the value cost ratio (VCR) (Roy et al., 2006; Dittoh et al., 2012).

$$VCR = \frac{A(\$ha^{-1})}{B(\$ha^{-1})} \quad (3)$$

where A represents total benefits as a result of the applied treatment and B represents the total treatment cost. Rhizobia inoculant was estimated at a cost of US$6 ha^{-1}. The cost of 1 kg of groundnut from the open market was US$0.7. An exchange rate of US$1 to GH 3.60 was used in the conversion of the cost to US dollars. A VCR value greater than a threshold of 3–4 was considered profitable (Dittoh et al., 2012).

Statistical Analysis

The data from each location were checked for normality using Shapiro–Wilk's test. Analysis of variance (ANOVA) was applied to the data through Sisvar version 5.6 (Ferreira, 2008). The Scott–Knot test at 5% probability was used to compare treatment means.

RESULTS

Physical and Chemical Properties and the Most Probable Rhizobia Count

The physical and chemical soil properties of the study sites are shown in **Table 1**. The texture of the soils from Binduri and Tanina was loamy sand while that from Cheshegu was sandy loam. The soil pH levels (6.10–6.38) were considered to be in the medium range. The OC levels at all the study sites were very low: 0.42% at Cheshegu, 0.96% at Binduri, and 0.62% at Tanina. Total N at all the study sites was generally very low, ranging from 0.022 to 0.030%. Available phosphorus (P) ranged from low to medium (17.61–20.54 mg kg^{-1}). In general, the fertility status of the soil at the study sites was very low. The estimated population sizes of the indigenous rhizobia at the study sites were 26.3, 11.2, and 32.8 cells g^{-1} soil for Cheshegu, Binduri, and Tanina, respectively.

Nodulation, N_2 Fixation, Pod Formation, and Grain Yield

The results showed a significant interaction between the strains and groundnut variety for all the parameters measured at Cheshegu. The seed yield increases of the Chinese variety inoculated with strain KNUST 1031 was statistically higher than all the other treatments except strain KNUST 1002 and the mineral N-fertilized treatment. Except for strains KNUST 1002 and 1031, the seed yield of all other test strains was not significantly different from that of the control treatment. Strains KNUST 1002 and 1031 increased seed yield over the control by 16% and 24%, respectively. The Samnut 22 variety, however, responded better to the KNUST 1002 strain as it markedly increased seed yield over the control and the other strains. The seed yield increase by KNUST 1002 on Samnut 22 was comparable only to the yield of plants that received mineral N fertilizer (**Table 2**). Strain KNUST 1002 significantly increased seed yield over control by 30% and above 22% over the other strains. The results followed a definite pattern at Cheshegu and showed a clear response of the Chinese variety to KNUST 1031 and of Samnut 22 to KNUST 1002 in all the measured parameters. For instance, KNUST 1031 increased the seed yield of the Chinese variety by 24% compared to Samnut 22. Meanwhile, KNUST 1002 increased seed yield on Samnut 22 by 21% compared to the Chinese variety. Generally, seed yields observed for the control treatment on both varieties were at par with all the inoculated treatments (test strains and reference strains) except KNUST 1031 on the Chinese variety and KNUST 1002 on Samnut 22. The two strains KNUST 1031 and KNUST 1002 increased nodulation, shoot biomass, pod number, and BNF of Chinese and Samnut 22 varieties, respectively, relative to the control and the other strains (**Table 2**).

At Binduri, a significant interaction between the strains and groundnut variety was observed. Unlike Cheshegu, the Chinese variety showed a response to KNUST 1003. The isolate KNUST 1003 significantly increased shoot biomass, BNF, pod number, and seed yield of the Chinese variety relative to the other strains. KNUST 1003 increased the seed yield of the Chinese variety by 35% over the control treatment (**Table 3**). On the other hand, Samnut 22 did not show a clear response to one particular strain. Although KNUST 1003 recorded the highest grain yield, it was only significant compared to KNUST 1032, BR3267, and the control (**Table 3**). A seed yield increase of 21% over the control was recorded by strain KNUST 1003 on Samnut 22. The isolate KNUST 1003 recorded a significantly higher yield on Samnut 22 than on the Chinese variety.

Similar to the results at Cheshegu and Binduri, a significant interaction was observed between the strains and groundnut varieties at Tanina for all the parameters measured. On average, the seed yield recorded at Tanina was lower than that recorded at Cheshegu and Binduri (**Table 4**). The pattern of results was

TABLE 2 | The response of groundnut to rhizobia inoculation and mineral N application at Cheshegu.

Strains (S)	Variety (V)	Nodule number (10 plants^{-1})	Nodule dry weight (mg 10 plants^{-1})	Shoot dry weight (kg ha^{-1})	BNF (kg N ha^{-1})	Pod number (plant^{-1})	Seed yield (kg ha^{-1})
KNUST 1001	Chinese	103 ± 1.15^{Ab}	0.26 ± 0.0173^{Bb}	763.7 ± 28.9^{Bc}	26.48 ± 1.15^{Bc}	24 ± 1.73^{Ad}	$1{,}267.5 \pm 57.8^{Ab}$
KNUST 1002		119 ± 1.15^{Ba}	0.34 ± 0.0173^{Ba}	$1{,}186.8 \pm 28.9^{Ba}$	44.2 ± 1.15^{Ba}	51 ± 1.15^{Aa}	$1{,}594.7 \pm 55.8^{Ba}$
KNUST 1032		100 ± 1.15^{Bb}	0.24 ± 0.0173^{Bb}	697.8 ± 28.9^{Bc}	25.92 ± 1.15^{Bc}	27 ± 0.577^{Ad}	$1{,}393.3 \pm 57.4^{Ab}$
KNUST 1003		97 ± 1.15^{Ac}	0.22 ± 0.0173^{Bc}	741.6 ± 28.9^{Bc}	29.85 ± 1.15^{Bb}	32 ± 1.45^{Ac}	$1{,}264.4 \pm 58.7^{Bb}$
KNUST 1031		121 ± 1.15^{Aa}	0.38 ± 0.0173^{Aa}	$1{,}206.6 \pm 28.9^{Aa}$	46.78 ± 1.15^{Aa}	52 ± 1.73^{Aa}	$1{,}704.8 \pm 56.3^{Aa}$
Biofix		104 ± 1.15^{Ab}	0.26 ± 0.0173^{Bb}	716.8 ± 28.9^{Bc}	26.5 ± 1.15^{Bc}	26 ± 1.2^{Ad}	$1{,}304.6 \pm 57.4^{Bb}$
BR3267		101 ± 1.15^{Bb}	0.24 ± 0.0173^{Ab}	847.6 ± 28.9^{Bb}	29.99 ± 1.15^{Bb}	32 ± 1.45^{Ac}	$1{,}341.9 \pm 62^{Bb}$
N–		97 ± 1.15^{Bc}	0.19 ± 0.0173^{Bc}	531.8 ± 28.9^{Bd}	21.49 ± 1.15^{Bd}	31 ± 0.882^{Ac}	$1{,}372.7 \pm 54.5^{Ab}$
N+		94 ± 1.15^{Bc}	0.19 ± 0.0173^{Bc}	$1{,}124.8 \pm 28.9^{Ba}$	32.98 ± 1.15^{Bb}	44 ± 0.882^{Ab}	$1{,}685.6 \pm 60.9^{Aa}$
KNUST 1001	Samnut 22	106 ± 1.15^{Ac}	0.36 ± 0.0173^{Ab}	$1{,}214.1 \pm 28.9^{Ab}$	42.23 ± 1.15^{Ab}	24 ± 0.882^{Ac}	$1{,}365.2 \pm 60.8^{Ab}$
KNUST 1002		135 ± 1.15^{Aa}	0.49 ± 0.0173^{Aa}	$1{,}307 \pm 28.9^{Aa}$	49.63 ± 1.15^{Aa}	42 ± 1.76^{Ba}	$1{,}933.9 \pm 50.6^{Aa}$
KNUST 1032		111 ± 1.15^{Ab}	0.33 ± 0.0173^{Ac}	$1{,}070.4 \pm 28.9^{Ac}$	$37.76 \pm 1.1A^{5c}$	25 ± 0.882^{Ac}	$1{,}458.5 \pm 56.9^{Ab}$
KNUST 1003		99 ± 1.15^{Ad}	0.23 ± 0.0173^{Ae}	$1{,}050.3 \pm 28.9^{Ac}$	40.25 ± 1.15^{Ab}	27 ± 1.45^{Bc}	$1{,}520.7 \pm 54.1^{Ab}$
KNUST 1031		94 ± 1.15^{Be}	0.19 ± 0.0173^{Be}	932.7 ± 28.9^{Bd}	31.13 ± 1.15^{Bd}	21 ± 0.882^{Bd}	$1{,}371.7 \pm 59.2^{Bb}$
Biofix		101 ± 1.15^{Ad}	0.33 ± 0.0173^{Ac}	967.7 ± 28.9^{Ad}	36.05 ± 1.15^{Ac}	22 ± 1.15^{Bd}	$1{,}537.4 \pm 48.5^{Ab}$
BR3267		105 ± 1.15^{Ac}	0.28 ± 0.0173^{Ad}	$1{,}186.3 \pm 28.9^{Ab}$	43 ± 1.15^{Ab}	31 ± 1.15^{Ab}	$1{,}590.9 \pm 54.9^{Ab}$
N–		106 ± 1.15^{Ac}	0.38 ± 0.0173^{Ab}	$1{,}025.2 \pm 29^{Ac}$	41.93 ± 1.15^{Ab}	31 ± 0.882^{Ab}	$1{,}490.2 \pm 54.3^{Ab}$
N+		98 ± 1.15^{Ad}	0.32 ± 0.0173^{Ac}	$1{,}375.3 \pm 28.9^{Aa}$	41.02 ± 1.15^{Ab}	39 ± 0.882^{Ba}	$1{,}852.1 \pm 55^{Aa}$
P-values							
S		0.0005	0.00001	0.00001	0.00001	0.00001	0.00001
V		0.00001	0.00001	0.00001	0.00001	0.00001	<0.00001
S × *V*		0.00001	0.00001	0.00001	0.00001	0.00001	0.0001
CV (%)		1.95	9.45	4.95	5.3	6.22	6.59

Figures followed by the same small letters compare the same variety among different strains, while figures followed by capital letters compare the same strain between different varieties.

different from Cheshegu and Binduri. Between the varieties, the Chinese variety did not show a clear response to the strains; however, Samnut 22 responded to KNUST 1002 (**Table 4**). The strain BR3267 recorded the highest seed yield on the Chinese variety but was only significantly different from the yields recorded by the control, Biofix, KNUST 1001, and KNUST 1003 (**Table 4**). KNUST 1002 significantly increased the seed yield of Samnut 22 over all the other strains except the mineral N-fertilized treatment. KNUST 1002 increased the seed yield of Samnut 22 over the control by 45%. Strain KNUST 1002 performed better on Samnut 22 than on the Chinese variety as it significantly increased all parameter measures on Samnut 22 compared to the Chinese variety.

Economic Viability of Using Indigenous Rhizobia Isolates

About five of the strains (KNUST 1001, 1003, and 1032; Biofix; and BR3267) had VCRs below the threshold of 3–4 at Cheshegu (**Figure 1A**). Strains KNUST 1002 and 1031 had VCRs of 5.6 and 8.3 for the Chinese variety, which were above the threshold of 3–4. Only strain KNUST 1002 was profitable on Samnut 22 with a VCR of 11.

The VCR results at Binduri showed that strain KNUST 1003 recorded the highest VCR of 7.83 on the Chinese variety. The other strains had a VCR less than or within the range of 3–4. On the other hand, strains KNUST 1002, 1003, and 1031 recorded VCR values of 4.24, 5.87, and 4.09, respectively, on Samnut 22 (**Figure 1B**).

None of the tested strains were profitable on the Chinese variety at Tanina per the threshold of 3–4. Only the industrial strain BR3267 had a VCR of 4.17. However, strain KNUST 1002 had a VCR of 9.04 for Samnut 22 at Tanina. The other strains had VCR values that were less than or within the threshold of 3–4 (**Figure 1C**).

Rhizobia Population After a Cropping Season of Introduction Into the Soil

The rhizobia population count varied among the strains and across different locations; however, strain KNUST 1002 demonstrated consistency in persistence. The highest rhizobia population count (283.4 cells g^{-1} soil) after a cropping season was recorded at Binduri by strains KNUST 1002 and 1003. However, only strain KNUST 1002 persisted in high numbers at Cheshegu and Tanina (**Figure 2**). The population of KNUST 1002 in inoculated soils was more than double that of the uninoculated plots after a year of introduction at all three study locations.

DISCUSSION

Until recently, groundnut was regarded as a promiscuous grain legume that nodulated freely with indigenous rhizobia and therefore did not require inoculation during establishment.

TABLE 3 | The response of groundnut to rhizobia inoculation and mineral N application at Binduri.

Strains (S)	Variety (V)	Nodule number (plant^{-1})	Nodule dry weight (mg plant^{-1})	Shoot dry weight (kg ha^{-1})	BNF (kg N ha^{-1})	Pod number (plant^{-1})	Seed yield (kg ha^{-1})
KNUST 1001	Chinese	84 ± 1.15^{Bb}	0.14 ± 0.0115^{Bc}	524.3 ± 28.3^{Bd}	24.47 ± 1.13^{Bc}	20 ± 1.73^{Bb}	912.1 ± 29^{Bc}
KNUST 1002		92 ± 2.08^{Bb}	0.14 ± 0.0115^{Bc}	517.4 ± 29.8^{Bd}	24.48 ± 1.26^{Bc}	19 ± 1.73^{Bb}	893.3 ± 29^{Bc}
KNUST 1032		97 ± 2.89^{Bb}	0.1 ± 0.0115^{Bd}	531.2 ± 29.1^{Bd}	27.51 ± 1.26^{Bc}	21 ± 1.73^{Ab}	824.3 ± 31^{Bc}
KNUST 1003		105 ± 2.89^{Ba}	0.38 ± 0.0115^{Ba}	882.1 ± 28.6^{Aa}	36.41 ± 1.11^{Ba}	33 ± 1.73^{Aa}	1195.8 ± 29.4^{Ba}
KNUST 1031		93 ± 2.89^{Bb}	0.23 ± 0.0115^{Ab}	727.8 ± 29.9^{Ab}	31.64 ± 1.21^{Bb}	24 ± 1.73^{Bb}	985.7 ± 29^{Bc}
Biofix		106 ± 2.89^{Aa}	0.35 ± 0.0115^{Aa}	744.6 ± 28.2^{Ab}	31.47 ± 1.19^{Bb}	23 ± 1.73^{Ab}	892.9 ± 29.9^{Bc}
BR3267		88 ± 2.89^{Bb}	0.11 ± 0.0115^{Bd}	635.6 ± 29.3^{Ac}	29.54 ± 1.04^{Ab}	22 ± 1.73^{Ab}	857.2 ± 29.4^{Bc}
N–		92 ± 2.89^{Bb}	0.13 ± 0.0115^{Bc}	496.5 ± 27^{Bd}	23.82 ± 1.11^{Ac}	18 ± 1.73^{Ab}	882.8 ± 27.9^{Bc}
N+		90 ± 2.89^{Bb}	0.06 ± 0.0115^{Be}	845.4 ± 28.4^{Aa}	31.52 ± 1.12^{Ab}	32 ± 1.73^{Aa}	1076.9 ± 27.9^{Bb}
KNUST 1001	Samnut 22	127 ± 2.89^{Ab}	0.43 ± 0.0115^{Ab}	825.3 ± 28.6^{Aa}	39.41 ± 1.05^{Aa}	31 ± 1.73^{Aa}	1275.7 ± 26.5^{Aa}
KNUST 1002		128 ± 2.89^{Ab}	0.23 ± 0.0115^{Ad}	825.3 ± 28^{Aa}	41.66 ± 1.25^{Aa}	33 ± 1.73^{Aa}	$1,293.9 \pm 27^{Aa}$
KNUST 1032		117 ± 2.89^{Ac}	0.21 ± 0.0115^{Ae}	730.8 ± 29.2^{Ab}	35.69 ± 1.12^{Ab}	23 ± 1.73^{Ab}	973.4 ± 28.9^{Ac}
KNUST 1003		144 ± 2.89^{Aa}	0.47 ± 0.0115^{Aa}	885.3 ± 27.1^{Aa}	41.36 ± 1.22^{Aa}	37 ± 1.73^{Aa}	$1,359.1 \pm 29.4^{Aa}$
KNUST 1031		128 ± 2.89^{Ab}	0.24 ± 0.0115^{Ad}	794.9 ± 29.9^{Aa}	40.57 ± 1^{Aa}	32 ± 1.73^{Aa}	$1,287.9 \pm 28.2^{Aa}$
Biofix		105 ± 2.89^{Ad}	0.3 ± 0.0115^{Bc}	725.8 ± 28.5^{Ab}	35.34 ± 1.13^{Ab}	25 ± 1.73^{Ab}	$1,191.4 \pm 28.6^{Aa}$
BR3267		119 ± 2.89^{Ac}	0.18 ± 0.0115^{Ae}	717.3 ± 27.5^{Ab}	32.42 ± 1.2^{Ab}	19 ± 1.73^{Ab}	$1,112.9 \pm 29^{Ab}$
N–		128 ± 2.89^{Ab}	0.2 ± 0.0115^{Ae}	651.5 ± 29.1^{Ab}	34.73 ± 1.25^{Ab}	20 ± 1.73^{Ab}	$1,124.3 \pm 28.1^{Ab}$
N+		126 ± 2.89^{Ab}	0.19 ± 0.0115^{Ae}	821.6 ± 28.6^{Aa}	34.75 ± 0.974^{Ab}	30 ± 1.73^{Aa}	$1,258.2 \pm 29.7^{Aa}$
P-value							
S		0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
V		0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
S × *V*		0.00001	0.00001	0.00001	0.00001	0.0003	0.0009
CV (%)		4.28	8.54	7.03	6.23	11.97	4.74

Figures followed by the same small letters compare the same variety among different strains, while figures followed by capital letters compare the same strain between different varieties.

This skepticism has been challenged by the outcome of several inoculation studies (Ashraf et al., 2006; Sajid et al., 2010; Yakubu et al., 2010; Biswas and Gresshoff, 2014; Grönemeyer et al., 2016; Osei et al., 2020). Groundnut has been reported to respond positively to inoculation with effective rhizobia, leading to enhanced nodulation, BNF, and ultimately yields in Ghana and elsewhere (Ashraf et al., 2006; Sajid et al., 2010; Yakubu et al., 2010; Osei et al., 2020, Grönemeyer et al., 2016). The results of this study corroborate these reports as inoculation with effective native strains particularly KNUST 1002, 1031, and 1003 elicited seed yield increases of Samnut 22 and Chinese varieties at different locations, thus confirming the widely known assertion that most strains are site specific and variety specific (Date, 2000; Woomer et al., 2014). The significant interaction response indicates that the strain performance varied with location and variety. The positive response observed in this study may have been influenced by the superior symbiotic effectiveness of the introduced strains over the indigenous rhizobia population, the low population of native rhizobia before the establishment of the experiment, and the low fertility of soils at the study sites. Buri et al. (2010) indicated that soils in the Savanna zones of Ghana are predominantly low in organic matter, nitrogen, and available P. These low nutrient contents, particularly N (ranging between 0.022 and 0.03%), coupled with the rhizobia population, which was below the threshold reported by Thies et al. (1991) to obviate inoculation response, revealed a need for plant N that was impossible to obtain from the soil by groundnut plants. Hence, introducing effective native strains led to an efficient symbiosis, subsequently making N available for plant growth and increased yields. Mathenge (2017) reported that the potential of legumes to fix nitrogen is likely to be high when the mineral N of the soil is low compared to when soils have higher N levels. The response of groundnut varieties (Chinese and Samnut 22) to inoculated strains, which varied significantly, could be due to the specificity in the interaction between rhizobia and plant genotype (de Alcantara et al., 2014). For instance, inoculation with strain KNUST 1031 enhanced nodulation of the Chinese variety compared to Samnut 22 at Cheshegu. Furthermore, nodulation of the negative control was higher for Samnut 22 than for the Chinese variety. This shows that although the native rhizobia population was low, Samnut 22 was highly compatible with the native soil rhizobia, giving them a competitive advantage over some of the introduced strains. According to Sanginga et al. (2000), genotypes of the same legume may differ in terms of their nodulation and N_2 fixation capacities, hence influencing the performance of strain(s). Abi-Ghanem et al. (2011) also reported that crop genotype and its interaction with rhizobia significantly influence nitrogen fixation. This is due to the inherent genetic capacity of a strain. Perret et al. (2000) reported

TABLE 4 | The response of groundnut to rhizobia inoculation and mineral N application at Tanina.

Strains (S)	Variety (V)	Nodule number (plant^{-1})	Nodule dry weight (mg plant^{-1})	Shoot dry weight (kg ha^{-1})	BNF (kg N ha^{-1})	Pod number (plant^{-1})	Seed yield (kg ha^{-1})
KNUST 1001	Chinese	88 ± 1.73^{Ab}	0.11 ± 0.0115^{Ac}	646 ± 30.1^{Ac}	20.80 ± 1.1^{Ac}	22 ± 1.73^{Aa}	756.6 ± 27.9^{Ab}
KNUST 1002		85 ± 1.73^{Bb}	0.12 ± 0.0115^{Bc}	697.6 ± 28.9^{Bc}	21.37 ± 1.15^{Bc}	23 ± 1.73^{Ba}	826.3 ± 27.6^{Ba}
KNUST 1032		87 ± 1.73^{Ab}	0.12 ± 0.0115^{Ac}	561 ± 28.5^{Bd}	17.52 ± 1.01^{Bd}	25 ± 1.73^{Aa}	807.9 ± 28.9^{Aa}
KNUST 1003		85 ± 1.73^{Ab}	0.1 ± 0.0115^{Ba}	586.3 ± 31^{Bd}	17.47 ± 1.21^{Ad}	20 ± 1.73^{Ba}	744.8 ± 30.9^{Bb}
KNUST 1031		92 ± 1.73^{Aa}	0.18 ± 0.0115^{Ab}	963.9 ± 28.7^{Ba}	33.73 ± 1.44^{Aa}	23 ± 1.73^{Aa}	833.5 ± 30.3^{Aa}
Biofix		89 ± 1.73^{Ab}	0.15 ± 0.0115^{Ac}	694 ± 27.6^{Bc}	22.35 ± 1.2^{Bc}	19 ± 1.73^{Aa}	765.2 ± 27.7^{Bb}
BR3267		95 ± 1.73^{Aa}	0.2 ± 0.0115^{Aa}	975 ± 28.2^{Aa}	34.3 ± 1.18^{Aa}	27 ± 1.73^{Aa}	873.7 ± 28.7^{Aa}
N–		86 ± 1.73^{Ab}	0.15 ± 0.0115^{Bb}	835.3 ± 28.3^{Ab}	30.45 ± 1.09^{Ab}	20 ± 1.73^{Aa}	707.1 ± 29.1^{Bb}
N+		86 ± 1.73^{Ab}	0.14 ± 0.0115^{Ab}	974.5 ± 27.2^{Aa}	29.63 ± 0.93^{Bb}	23 ± 1.73^{Ba}	813.8 ± 29.6^{Ba}
KNUST 1001	Samnut 22	84 ± 1.73^{Ac}	0.14 ± 0.0115^{Ac}	705.1 ± 27^{Ad}	23.56 ± 1.37^{Ad}	24 ± 1.73^{Ab}	796.3 ± 28.6^{Ac}
KNUST 1002		108 ± 1.73^{Aa}	0.3 ± 0.0115^{Aa}	1074.9 ± 27.8^{Aa}	42.74 ± 1.21^{Aa}	34 ± 1.73^{Aa}	1185.5 ± 28.4^{Aa}
KNUST 1032		86 ± 1.73^{Ac}	0.12 ± 0.0115^{Ac}	763.8 ± 28.4^{Ad}	26.26 ± 1.16^{Ac}	19 ± 1.73^{Bb}	757.2 ± 29.9^{Ac}
KNUST 1003		87 ± 1.73^{Ac}	0.2 ± 0.0115^{Ab}	993.6 ± 26.9^{Ab}	33.59 ± 1.23^{Bb}	28 ± 1.73^{Aa}	975.1 ± 29^{Ac}
KNUST 1031		91 ± 1.73^{Ab}	0.16 ± 0.0115^{Ab}	838.2 ± 29.4^{Ad}	27.56 ± 0.96^{Bc}	22 ± 1.73^{Ab}	756.4 ± 27.7^{Ac}
Biofix		86 ± 1.73^{Ac}	0.13 ± 0.0115^{Ac}	876.5 ± 29.6^{Ac}	27.55 ± 1.25^{Ac}	24 ± 1.73^{Ab}	873.8 ± 28.9^{Ac}
BR3267		92 ± 1.73^{Ab}	0.18 ± 0.0145^{Ab}	975.6 ± 27^{Ab}	35.347 ± 1.14^{Ab}	23 ± 2.03^{Ab}	814.6 ± 59.8^{Ac}
N–		85 ± 1.73^{Ac}	0.11 ± 0.0115^{Ac}	914.9 ± 28.4^{Ab}	29.60 ± 1.18^{Ac}	21 ± 1.73^{Ab}	824.1 ± 30.1^{Ac}
N+		87 ± 1.73^{Ac}	0.13 ± 0.0115^{Ac}	1024.4 ± 28.9^{Aa}	33.36 ± 1.16^{Ab}	30 ± 1.73^{Aa}	1118.8 ± 24.1^{Aa}
P-value							
S		0.00001	0.00001	0.00001	0.00001	0.0035	<0.00001
V		0.00001	0.00001	0.00001	0.00001	0.0012	<0.00001
S × *V*		0.00001	0.00001	0.00001	0.00001	0.0003	<0.00001
CV (%)		3.44	12.65	5.86	7.32	12.79	6.48

Figures followed by the same small letters compare the same variety among different strains, while figures followed by capital letters compare the same strain between different varieties.

that the specificity between symbiotic partners minimizes the formation of non-fixing nodules by the host plant to enhance N_2 fixation.

The fact that introduced rhizobia strains increased nodulation over the native rhizobia population suggests that the introduced strains were probably competitive for nodulation sites. The performance of some of the introduced strains was comparable to the commercial strain, suggesting their potential for use in inoculant formulation. Even though nodulation was higher in this study, it was limited by moisture stress at Tanina as indicated in **Supplementary Figure 1C**. Moisture stress has also been reported to generally affect nodulation (Bordeleau and Prévost, 1994; Hungria and Vargas, 2000). Moisture stress compromises plant and rhizobia growth and is a major cause of nodulation failure due to its effect on rhizobia survival, growth, and population structure in soil; the formation and longevity of nodules; synthesis of leghemoglobin; and nodule function (O'Hara, 2001).

Our results suggest the potential saprophytic competence abilities of the introduced strain, especially KNUST 1002, as its population was higher compared to other strains after a cropping season of its introduction. Confirming the strain's ability to sustain effectiveness after a cropping season will be a major step toward reducing the number of successive inoculations. This has positive implications for groundnut farmers, as inoculants are rarely available in the remote areas and thus costs are cut down.

The VCR values showed that the strains varied in profitability between groundnut varieties and across locations. Using the threshold of 3–4 indicated by Dittoh et al. (2012), strains KNUST 1002 and 1031 were profitable on Samnut 22 at Cheshegu, while KNUST 1003 was profitable on the Chinese variety and KNUST 1002, 1003, and 1031 were profitable on Samnut 22 at Binduri. Per the threshold, only KNUST 1002 was profitable on Samnut 22 at Tanina. Ronner et al. (2016) and Ulzen et al. (2018) indicated that the use of rhizobia inoculant is financially rewarding for farmers in northern Ghana and northern Nigeria. Thus, this study has demonstrated that not only will the use of the test strains improve yields of groundnut for smallholder farmers under similar environmental conditions but that it will also increase their livelihoods.

CONCLUSION

The findings of this study support the hypothesis that superior effective strains with saprophytic abilities that can meet the N requirement of groundnut exist in soils of Ghana and can effectively increase seed yields of groundnut. The study

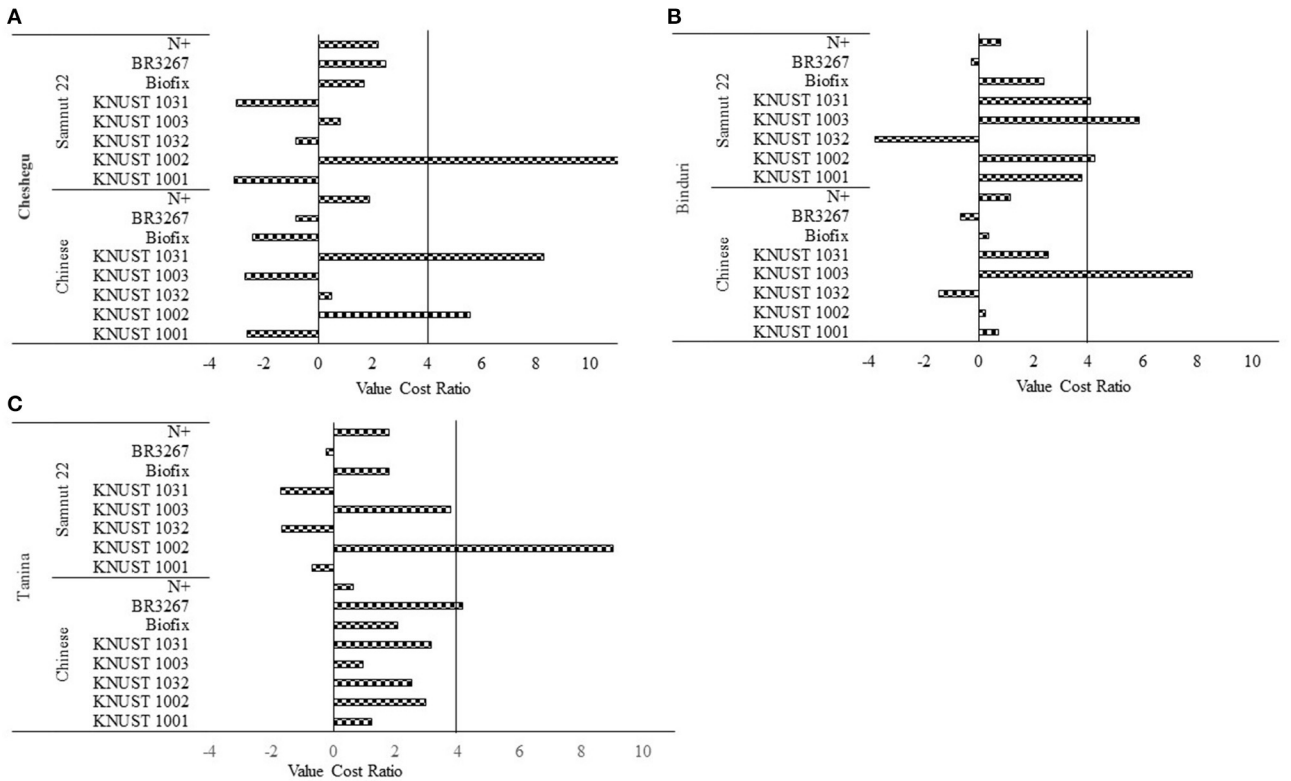

FIGURE 1 | Value cost ratio of strains on Samnut 22 and Chinese varieties Cheshegu **(A)**, Binduri **(B)** and Tanina **(C)**. The vertical line indicates the threshold beyond which a treatment is considered profitable.

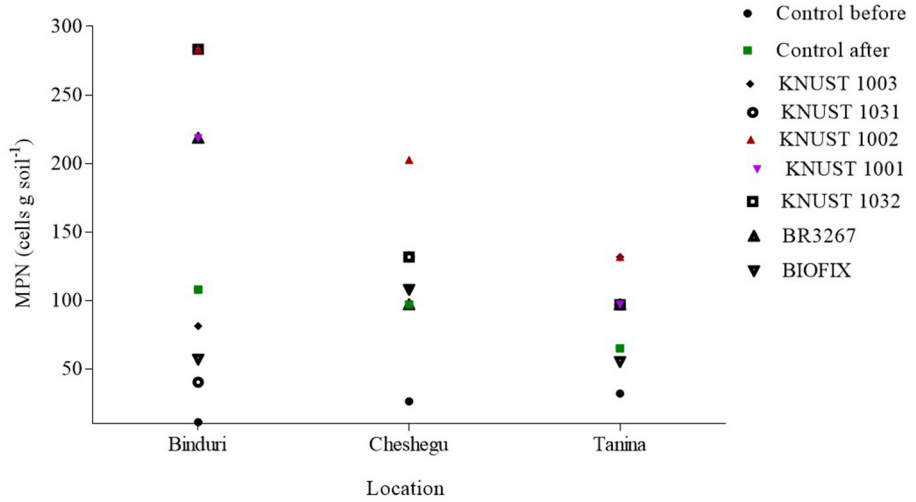

FIGURE 2 | Rhizobia population after one cropping season of introduction. The legends describe the rhizobia population in the control plots before and after the experiment as well as the plots that received the various strains.

has shown the potential of strains KNUST 1002, 1003, and 1031 as possible candidates for use in the commercial production of rhizobia inoculant for Samnut 22 and Chinese varieties in Cheshegu, Binduri, and Tanina as the grain yields produced by these strains were better or comparable to those produced by mineral N-fertilized treatment. Strain KNUST 1002 can persist in the soil for at least one cropping season irrespective of the location. The study also revealed that strains KNUST 1002, 1031, and 1003 were highly profitable under the current environmental conditions

of the study. Our results showed that with the same rhizobia strain, genotypes, and management practices, environmental conditions are the most important factors that limit rhizobia efficiency. Long-term studies are required to unravel the mechanisms underlying rhizobia survival under field conditions and the interaction between environmental conditions and rhizobia strains.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the Corresponding author.

AUTHOR CONTRIBUTIONS

GW, JU, RA, AO, OO, and SA-N designed the experiment. GW, JU, RA, AO, and OO planned the research activities. GW, JU, and OO conducted the field work, collected and analyzed the data, and drafted the manuscript. RA, AO, and SA-N fine-tuned 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/fagro.2021.653044/full#supplementary-material

Supplementary Figure 1 | Rainfall distribution during the cropping season [**(A)**: Cheshegu, **(B)**: Binduri, and **(C)**: Tanina].

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Response of Peanut Varieties to Phosphorus and Rhizobium Inoculant Rates on Haplic Lixisols of Guinea Savanna Zone of Ghana

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Peanut forms a major component of the predominantly cereal-based farming systems in Northern Ghana. However, yields are low, prompting the need to evaluate the effects of phosphorus (PR) and rhizobium inoculant (IR) rates on growth, nodulation, and yield of peanut varieties. On-station and on-farm experiments were conducted to determine the interaction effects of three P rates (0, 30, and 60 kg P_2O_5/ha), three IR rates (0, 3, and 6 g/kg seed), and two peanut varieties [Chinese and Nkatie Savannah Agricultural Research Institute (SARI)] on growth, nodulation, and yield of peanut on Haplic Lixisols of Northern Ghana. Both experiments were conducted using a split-split plot design replicated three times for the on-station experiment and on six farmer's fields (on-farm experiment). In both experiments, combined application of 60 kg P_2O_5/ha and IR at 6 g/kg seed increased pod number in the Nkatie SARI and Chinese varieties compared to their control counterparts. PR × V interaction influenced growth, effective nodule number, and podding capacity with 60 kg P_2O_5/ha combined with Nkatie SARI to produce significantly higher values. The interaction of IR × V improved pod number, nodule number, and harvest index, such that inoculant at 6 g/kg seed combined with Nkatie SARI gave the best performance. PR × IR also had a significant interactive influence on peanut grain yield. Higher grain yields were recorded from 60 kg P_2O_5/ha in combination with 6 g/kg seed of rhizobium inoculant. Therefore, these results suggest that the use of P fertilizer at 60 kg/ha and rhizobium inoculant at 6 g/kg seed increase peanut productivity on Haplic Lixisols in Northern Ghana. However, it was prudent not to suggest any recommendations from the P rates in interaction with IR, since the result between the on-station and on-farm experiments appeared not consistent.

Keywords: peanut varieties, phosphorus, inoculant, nitrogen fixation, grain yield

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INTRODUCTION

Peanut (*Arachis hypogea* L.) is among the three most important grain legumes in West Africa, which forms a major component of the predominantly cereal-based farming systems. It is primarily grown as food and cash income, but in the dry savannas of Western Africa, the haulms and husk after harvest are excellent sources of quality livestock feed (Adjei-Nsiah et al., 2018). According to

the Food and Agriculture Organization (FAO, 2018) estimate for 2018, 28.5 million hectares of peanut were harvested worldwide, of which the West African sub-region harvested 7.1 million hectares. In Ghana, 95% of the total national production of peanut occurs in Northern Ghana (Ministry of Food and Agriculture, 2012). Despite this, peanut yield on farmers' fields is low due to several biotic and abiotic factors, three of which are low and declining soil fertility, high cost and/or unavailability of inputs (seed, fertilizer, an inoculant), and use of low-yielding varieties (Kolawole, 2012).

Phosphorus (P) is an important mineral nutrient that promotes the growth of leguminous crops to produce their own N sources. Therefore, P deficiency can negatively affect access to atmospheric N by legumes (Adjei-Nsiah et al., 2018). In confirming this, Asante et al. (2020) indicated that rhizobium activity and N2 fixation are negatively affected when the system lacks phosphorus (P) since P serves as an energy source for the rhizobia. Similarly, the requirement of P in nodulating legumes is higher compared to non-nodulating crops as it contributes significantly to root development (Tang et al., 2001), root nodulation (Nwaga and Ngo Nkot, 1998), and shoot uptake of N, P, and K (Ramesh et al., 1997) in legumes. Due to the important role played by P in plant growth, its addition to P-deficient soils leads to an increase in grain yield (Uchida, 2000). However, there are conflicting results on the optimum application rate of P on yield of peanut as some authors have reported higher yields at 60 kg P_2O_5/ha (Panwar and Naidu, 2002) while no significant effect on yield was recorded by Taruvinga (2014), when P rate was increased beyond 45 kg P_2O_5/ha. This has therefore renewed interest for further research.

Leguminous crops are also able to meet a significant portion of their nitrogen requirement when they are cultivated in the presence of effective rhizobia (Bekere and Hailemariam, 2012). It has been reported that leguminous plants with symbiotic nitrogen-fixing bacteria are able to fix about 15–210 kg/ha of nitrogen per season (Dakora and Keya, 1997). However, the effective functioning of rhizobia can be influenced by the availability of other soil nutrients, especially P. In confirming this, Asante et al. (2020) stated that rhizobium activity is reduced when the soil lacks phosphorus (P). Thus, in the presence of P, the inoculation of peanut with the appropriate rate of rhizobium inoculant can be a beneficial strategy to improve productivity.

In addition to phosphorus and rhizobium inoculant application, the grain yield of peanut may be increased through the cultivation of improved peanut varieties. According to Zhou et al. (2016), the identification and use of P-efficient legume genotypes is a sustainable P management strategy for enhancing yield and P use efficiency. While studies have shown that the use of improved varieties (Konlan et al., 2013) and the application of the appropriate rate of P fertilizer (Kamara et al., 2011) and rhizobium inoculant (Catroux et al., 2001) have the potential to improve the productivity of peanut, there is limited information on the interaction effects of variety, phosphorus rate, and rate of rhizobium inoculation on the yield of peanut varieties under on-farm conditions. Similarly, there is a gap in knowledge on the effect of P fertilizer and rhizobium inoculant rates on productivity of peanut varieties in Ghana. The need therefore arises to undertake a study on the comparative and interactive effects of P fertilization and rhizobium inoculation on productivity of peanut varieties to help inform measures that aim to increase productivity of the legume.

In this paper, we study the comparative and interactive effects of P fertilization and rhizobium inoculation on growth, nodulation, yield, and nutrient uptake in two peanut varieties on Haplic Lixisols in the Guinea savanna zone of Ghana.

MATERIALS AND METHODS

The study was conducted under both on-station and on-farm conditions in the Guinea savanna agro-ecological zone of Ghana during the 2018 cropping season (July–October).

On-station Experiment

This was carried out at the experimental field of the Faculty of Agriculture, University for Development Studies (UDS), Nyankpala, in the northern region of Ghana. The site is located on latitude 9°25′N, longitude 0°59′W (**Figure 1**) and at an altitude of 183 m above sea level. The total annual rainfall received by the area ranges between 800 and 1,200 mm, which occurs from May to October, with a dry season characterized by harmattan winds occurring between October and April. The mean monthly temperatures range from 25°C in December to 38°C in April. The soil of the study area was Haplic Lixisols (International Union of Soil Sciences Working Group, 2014) with organic carbon of about 4.2 g/kg, total nitrogen of 4 g/kg, exchangeable potassium of 42.6 mg/kg, and available phosphorus of 6 mg/kg.

On-farm Experiment

The on-farm experiment was carried out in two communities in the Guinea savanna agro-ecological zone of Ghana. These were at Gurumanchenyili (9°24′N, 1°0′W) and Zangbalun Fandu (9°36′N, 0°58′W) (**Figure 1**). The climate is warm, semi-arid tropical and has a unimodal rainfall of 800–1,200 mm, which increases from March until a maximum is reached in August/September. There is considerable variation between years in the time of onset, duration, and amount of rainfall. The vegetation is basically Guinea savanna which is interspersed with trees and grassland that are drought resistant. Bush fire is an annual event, which usually destroys the vegetation (Amoako et al., 2018). The fires are noted to result in substantial losses of plant nutrients, which has a negative impact on the livelihood of the people.

Experimental Design and Agronomic Management

On-station Experiment

The experimental field was plowed and harrowed with a tractor, after which the field was laid out. The experimental design was a split-split plot arranged in a randomized complete block with three replications. The treatments were made up of three rhizobium inoculant rates (IR) (0, 3, and 6 g/kg seed) as the main plot factor, three P fertilizer rates (PR) (0, 30, and 60 kg P_2O_5/ha) as the sub-plot factor, and two peanut varieties (V)

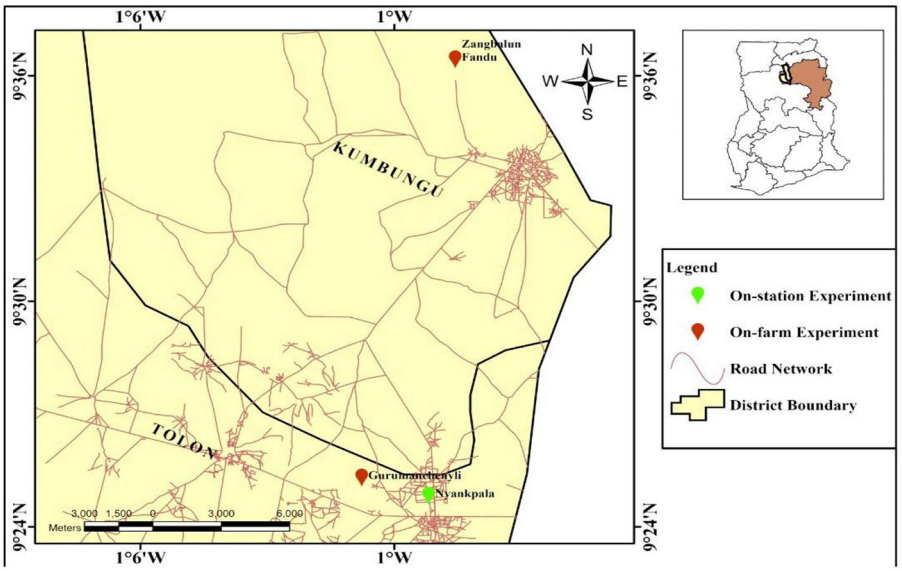

FIGURE 1 | Map of Ghana showing location of the experimental sites.

[Chinese and Nkatie Savannah Agricultural Research Institute (SARI)] as the sub-sub-plot factor. Variety was used as the sub-sub-plot, followed by P fertilization as the sub-plot and inoculant rate as the main plot to reduce potential error attributable to factor migration. Variety is the least migrating factor, followed by P, while the bacteria in the inoculant are the most mobile during the growing season. The inoculant rates were halved to understudy productivity of nodulation under a limited- or scarce-inoculant condition. The sub-plot size was 4 × 2.4 m, and an alley of 1 and 2 m separated sub-plots within and between replications, respectively. The sub-plot consisted of six rows, each 4 m long with an inter-row spacing of 40 cm and an intra-row spacing of 20 cm. The peanut was sown at two seeds per stand and thinned to one seedling per stand 2 weeks after planting.

On-farm Experiment

The same experimental design, planting distance, and treatment combinations as used in the on-station experiment were used in the on-farm experiment. These included three rates of rhizobium inoculant, three rates of P fertilizer, and the two peanut varieties. The treatments were replicated on six farmers' fields across the two communities, thus one replication per farmer. To ensure minimal soil variation, farmers' fields with similar soil type, slope, and minimal shading with no history of peanut cultivation in previous years were selected.

Agronomic Management

Two groundnut varieties—Chinese, a spreading type with 100 days of maturity duration, and Nkatie SARI, an erect bunch habit with 110 days of maturity duration released by the Council for Scientific and Industrial Research (CSIR)—were used in the experiment. A commercial *Bradyrhizobium* inoculant product, SARIFIX, containing strain BR 3267 was obtained from the Savanna Agricultural Research Institute, Tamale, Ghana, for the experiment.

Seed inoculation was performed just before planting by weighing 1 kg of seeds into a plastic container and adding 10 ml of dissolved gum Arabic solution as a sticker. The seeds and gum Arabic solution were mixed thoroughly, and the respective mass of SARIFIX inoculant (strain BR 3267) at 10 g/kg seeds of a 100-g pack was applied to the seed and mixed thoroughly to ensure that all the seeds were effectively covered with the inoculant before planting.

Data Collection

Soil Sampling and Analysis

Prior to treatment application and before planting, representative soil samples were collected from a depth of 0–20 cm using a soil auger. Similar sampling was done after harvest on each treatment plot. A composite was made from 30 samples (on-station experiment) and 10 samples (per farmer's field) collected randomly from different parts of the plot and thoroughly mixed, sub-sampled, air-dried, and then sieved with a 2-mm sieve. The sieved soil samples were analyzed at SARI for chemical and physical soil properties. The soil pH was determined according to the electrometric method. Organic carbon was analyzed by the Walkley–Black procedure as described by Nelson and Sommers (1982), total N by the Kjeldahl method as described by KIT (1984), P by the Bray-1 method as described by Bray and Kurtz (1945), and K by the flame photometer method as described by Hald (1947). Particle size distribution was carried out by the hydrometer method as described by Bouyoucos (1962).

Growth and Nodulation Assessment

At 8 weeks after sowing, data on growth (plant height) and nodulation assessment were collected for both on-station and

on-farm experiments. The plant height of five peanut plants was measured from the two central rows of each plot. The average height of the five plants were then calculated for each plot. For nodulation assessment, five plants outside the two central rows of each plot were carefully uprooted and placed in a plastic bowl containing water for 20 min to loosen adhering soil on the roots. The nodules were then removed, counted, and dissected to determine effective nodules using the procedure described by Ishizawa and Toyoda (1955).

Yield and Yield Components

At physiological maturity, plants from the two middle rows of each sub-sub-plot (3.2 m^2) were harvested, after which five plants were randomly selected from the harvested plants for each sub-sub-plot to determine their podding capacities (pod number and weight). The average number and weight of pods were then calculated for each sub-sub-plot, after which both plant and pods were added back to the harvested plants for each sub-sub-plot. The plants (whole plant with pod) from the harvested area (3.2 m^2) were weighed with an electronic scale, after which the pods were removed and shelled. The seed was then weighed and subtracted from the initial weight (whole plant with pod) to obtain the haulm weight per plot. It was then expressed in kg/ha. Grain yield per hectare was determined by shelling the pods from the harvested area (3.2 m^2) of each sub-sub-plot. The shelled seeds

TABLE 1 | Chemical and physical properties of soil at the experimental sites.

Properties	On-station experiment	On-farm experiment					
		Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
pH	4.93	5.13	5.12	5.14	5.12	5.13	5.13
†[a]OC (%)	1.05	1.09	1.09	1.09	1.09	1.09	1.09
[b]TN (%)	0.05	0.05	0.04	0.07	0.05	0.06	0.05
[c]P (%)	0.06	0.03	0.03	0.02	0.03	0.02	0.02
[d]K (%)	0.02	0.05	0.06	0.06	0.05	0.04	0.04
Sand (%)	72.6	54.9	60.0	56.2	59.9	58.9	56.2
Silt (%)	16.8	10.3	5.0	9.1	6.1	6.3	6.1
Clay (%)	12.2	34.8	35.0	34.7	34.0	34.8	37.7
Texture	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam

† [a] OC, organic carbon.
[b] TN, total nitrogen.
[c] P, available phosphorus.
[d] K, available potassium.

TABLE 2 | Summary of ANOVA for variety (V), phosphorus (PR), and rhizobium inoculant rate (IR) effects on plant height, nodulation, yield, and yield components of groundnut in the on-station and on-farm experiments.

Source of variation	On-station experiment						On-farm experiment						
	Degree of freedom	Plant height (cm) at 8 WAP	Pod no. 5 plants^{-1}	Grain yield (kg ha^{-1})	Haulm weight (kg ha^{-1})	Harvest index	Degree of freedom	Nodule no. 5 plants^{-1}	Effective nodules no. 5 plants^{-1}	Pod wt. (kg ha^{-1})	N uptake (%)	P uptake (%)	K uptake (%)
Rep	2						5						
IR	2	0.000	0.000	0.002	0.522	0.040	2	0.833	0.131	0.006	0.000	0.000	0.000
Error Rep × IR	4						10						
PR	2	0.121	0.000	0.031	0.012	0.086	2	0.285	0.522	0.000	0.000	0.004	0.000
IR × PR	4	0.080	0.000	0.011	0.068	0.061	4	0.193	0.771	0.886	0.004	0.041	0.048
Error Rep × IR × PR	12						30						
V	1	0.000	0.000	0.000	0.056	0.001	1	0.577	0.159	0.362	0.000	0.766	0.976
IR × V	2	0.987	0.026	0.401	0.000	0.024	2	0.009	0.197	0.946	0.038	0.919	0.007
PR × V	2	0.001	0.000	0.074	0.417	0.279	2	0.163	0.023	0.004	0.554	0.647	0.008
IR × PR × V	4	0.563	0.004	0.562	0.402	0.444	4	0.115	0.451	0.810	0.002	0.984	0.222
Error Rep × IR × PR × V	18						45						
Total	53						107						

were then oven-dried at 65°C to a moisture of 13% to measure grain yield.

Plant nutrient analysis was not performed for the on-station experiment. At physiological maturity, a quadrat (0.5 m^2) was placed outside the two central rows of each plot, and representative plants per each farmer's plot were harvested to determine the effects of PR, IR, and V on nutrient uptake. Plant shoots and roots from on-farm experiments were cut into pieces and then oven-dried at 70°C for 2 days to a constant weight. The oven-dried samples were then milled and analyzed chemically for N using the Kjeldahl method (Bremner and Muluvaney, 1982), P using the VV/VIS spectrophotometer through colorimetric determination, and K using the flame photometer (Myers et al., 1947). To determine biological nitrogen fixation (BNF), the reference crop used (maize) was sown on plots that received application rates of P and rhizobium inoculant as the peanut plots. Whole plants of both maize and peanut were sampled by treatment and prepared for the quantitative determination of total nitrogen, using the Kjeldahl method. The amount of nitrogen fixed (N%) in the plant was then calculated by using the nitrogen difference technique (Mweetwa et al., 2014; Anglade et al., 2015) as follows:

Q = N yield (groundnut) – N yield (maize) + [N soil (groundnut) – N soil (maize)]

where

Q = Quantity of legume N derived from N_2 fixation

N yield (groundnut) = Total N in groundnut plant

N yield (maize) = Total N in maize plant

N soil (groundnut) = Soil mineral N for groundnut plots after harvest

N soil (maize) = Soil mineral N for maize plots after harvest.

Statistical Analysis

The general linear model procedure of the Statistix 10 analytical package (Statistix, 2013) for Windows was used to analyse data from the two experiments. The Duncan multiple-range test was used to separate treatment means of significant difference at the 5% probability level.

RESULTS

Soil Properties at Study Sites

Table 1 shows the physicochemical properties of soil for both experiments (on-station and on-farm). Generally, the soil at the study sites was sandy loam with a slightly acidic pH (**Table 1**). The rating for the soil was done according to the classification by Sela (2020). The organic carbon level was very low (1.05–1.09%), while available phosphorus was low (0.02–0.03%); similarly, the exchangeable potassium range of 0.04–0.06% for the on-farm sites was low. The exchangeable potassium value of 0.02% recorded for the on-station site was low. The total nitrogen recorded before planting at the sites was generally very low.

Growth, Yield, and Yield Components

On-station Experiment

The IR × PR × V, likewise IR × PR and IR × V, was not significant on plant height. However, the PR × V and the effect of IR and V were significant on plant height (**Table 2**), such that PR at 60 kg P_2O_5/ha combined with the Chinese variety produced significantly higher values for plant height than did Nkatie SARI (**Figure 2**).

IR × PR × V and IR × PR, IR × V, and PR × V were not significant on nodulation parameters. However, significant differences in nodule number per plant and effective nodule number per plant occurred among IR, PR, and V treatments. Inoculation or P application increased nodule number per plant by an average of 26 or 50%, respectively, compared with their control, while the use of Nkatie SARI over the Chinese variety increased nodule number per plant by 7% (**Table 3**). Applying inoculant or P alone increased significantly the effective number of nodules per plant by an average of 46 or 25%, respectively, compared to their control, while sowing with Nkatie SARI produced 29% more effective nodule number per plant than did the Chinese variety (**Table 3**).

The effects of IR, PR, and V and their interactions on podding capacity were not consistent. For pod number per plant, the effects of IR × PR × V, IR × PR, IR × V, and PR × V were significant (**Table 2**). Inoculation and P application either alone or in combination with variety increased significantly the pod number per plant compared with the control. However, inoculation and P application appeared to favor higher pod number in Nkatie SARI than in the Chinese variety (**Figure 3**). Conversely, pod weight followed a different trend as that for the pod number. The IR × PR × V interaction was not significant on pod weight; however, the effects of IR and PR contributed significantly to pod weight such that IR at 6 g/kg seed and PR at 60 kg P_2O_5/ha produced the heaviest pod weight compared with un-inoculated and un-applied control plots, respectively.

IR × PR × V, IR × V, and PR × V were not significant on grain yield; however, significant differences in grain yield occurred among IR, PR, V, and IR × PR treatments (**Table 2**). Grain yield of IR at 3 and 6 g/kg seed combined with PR at 60 kg P_2O_5/ha increased significantly compared with the control (**Figure 4**). Grain yield was positively correlated with effective

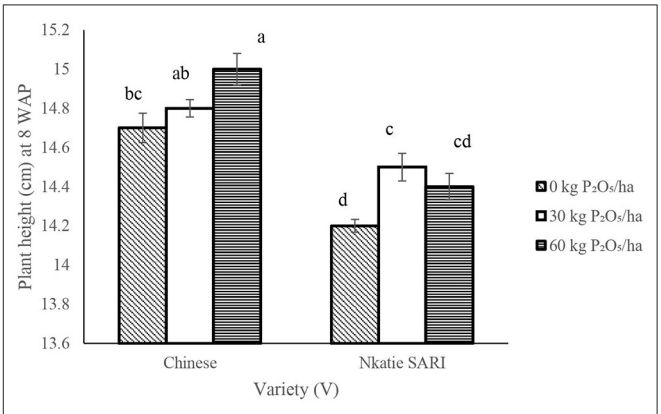

FIGURE 2 | Interactive effect of P-rate (PR) and variety on plant height (cm) of groundnut at 8 weeks after planting (8 WAP). Bars with different letters (in horizontal direction) show significantly different means. Error bars represent standard error of mean.

TABLE 3 | Plant height, nodulation, yield, and yield components of groundnut as affected by variety, phosphorus, and rhizobium inoculation rates in the on-station experiment.

Treatment	Plant height (cm) at 8 WAP	Nodule no. 5 plants^{-1}	Effective nodules no. 5 plants^{-1}	Pod no. 5 plants^{-1}	Pod wt. (kg ha^{-1})	Grain yield (kg ha^{-1})	Haulm weight (kg ha^{-1})	Harvest index
Rhizobium inoculation rate (IR) (kg/ha)								
0	14 ± 0.26^{c}	23 ± 7.75^{c}	15 ± 3.60^{c}	22 ± 7.04^{c}	5,538 ± 2,774.10^{c}	1,510 ± 581.90^{b}	10,677 ± 3,504.90^{a}	0.14 ± 0.03^{b}
3	15 ± 0.26^{b}	25 ± 7.85^{b}	20 ± 3.50^{b}	25 ± 7.32^{b}	6,493 ± 2,877.20^{b}	2,101 ± 565.21^{a}	9,479 ± 2,954.90^{a}	0.25 ± 0.10^{a}
6	16 ± 0.34^{a}	33 ± 8.00^{a}	25 ± 3.50^{a}	29 ± 8.43^{a}	7,638 ± 3,433.70^{a}	2,014 ± 688.19^{a}	9,913 ± 3,949.70^{a}	0.25 ± 0.13^{a}
Phosphorus rate (PR) (kg/ha)								
0	14 ± 0.27^{a}	20 ± 4.97^{c}	17 ± 4.79^{c}	20 ± 6.57^{c}	2,881 ± 1,353.90^{c}	1,441 ± 452.29^{b}	7,361 ± 3,462.00^{b}	0.26 ± 0.17^{a}
30	15 ± 0.22^{a}	23 ± 4.31^{b}	20 ± 4.75^{b}	26 ± 6.60^{b}	7,378 ± 1,109.50^{b}	1,962 ± 676.26^{a}	11,059 ± 2,807.70^{a}	0.18 ± 0.06^{b}
60	15 ± 0.38^{a}	37 ± 5.24^{a}	23 ± 4.66^{a}	30 ± 7.71^{a}	9,409 ± 1,846.90^{a}	2,222 ± 633.12^{a}	11,649 ± 2,467.20^{a}	0.20 ± 0.06^{ab}
Variety (V)								
Chinese	15 ± 0.25^{a}	26 ± 9.13^{b}	17 ± 4.45^{b}	19 ± 4.72^{b}	6,597 ± 2,968.90^{a}	1,516 ± 5,32.19^{b}	9,595 ± 3,599.60^{a}	0.18 ± 0.08^{b}
Nkatie SARI	14 ± 0.21^{b}	28 ± 8.36^{a}	22 ± 4.69^{a}	32 ± 5.33^{a}	6,516 ± 3,296.60^{b}	2,234 ± 418.00^{a}	10,451 ± 3,333.60^{a}	0.25 ± 0.14^{a}
***P*-value**								
PR × IR	0.080	0.672	0.110	0.000	0.308	0.011	0.068	0.027
PR × V	0.001	0.703	0.646	0.000	0.093	0.074	0.417	0.279
IR × V	0.987	0.670	0.286	0.026	0.360	0.401	0.000	0.024
PR × IR × V	0.563	0.553	0.304	0.004	0.245	0.562	0.402	0.444

[a,b,c]*Mean values followed by the same letters in each column are not significantly different from one another.*

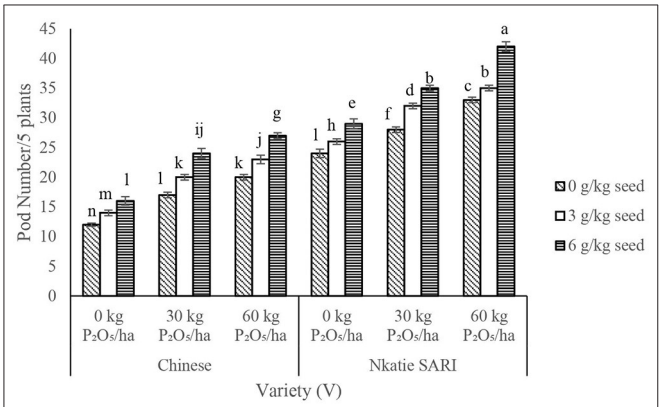

FIGURE 3 | Interactive effect of P-rate (PR), inoculant rate (IR), and variety (V) on pod number of groundnut grown in Haplic Lixisols of northern Ghana. Bars with different letters (in horizontal direction) show significantly different means. Error bars represent standard error of mean.

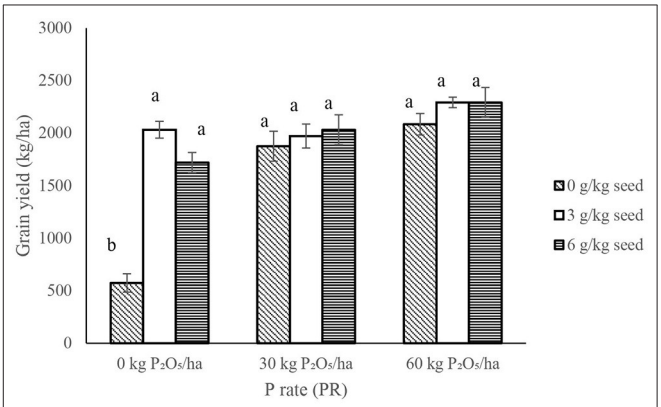

FIGURE 4 | Interactive effect of P-rate (PR) and inoculant rate (IR) on grain yield of groundnut grown in Haplic Lixisols of northern Ghana. Bars with different letters (in horizontal direction) show significantly different means. Error bars represent standard error of mean.

nodules and pod number (**Table 5**). These results indicate that when the number of effective nodules and pods increases, grain yield also increases.

IR × PR × V, IR × PR, and PR × V were not significant on haulm weight and harvest index (HI); however, significant differences occurred among IR × V treatments (**Table 2**). IR at 6 g/kg seed had higher ($P < 0.05$) haulm weight and HI at all levels of peanut variety compared with other IR at all levels of peanut variety (**Figure 5**).

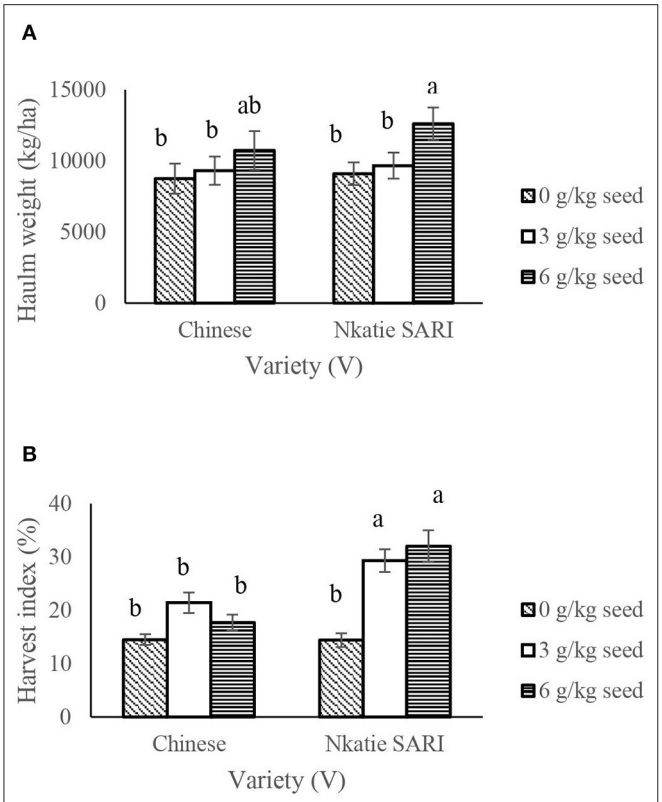

FIGURE 5 | Interactive effect of Innoculant rate (IR) and variety (V) on halum weight **(A)** and harvest index **(B)** of groundnut grown in Haplic Lixisols of northern Ghana. Bars with different letters (in horizontal direction) show significantly different means. Error bars represent standard error of mean.

On-farm Experiment

The IR × PR × V, IR × PR, PR × V, and IR × V as well as the effects of IR and PR did not affect ($P > 0.05$) plant height of peanut. However, the effect of V was significant on plant height, with Nkatie SARI recording higher ($P < 0.05$) plant height than the Chinese variety (**Table 4**).

For nodulation parameters, significant differences in nodule number per plant and effective nodule number per plant occurred among IR × V and PR × V treatments, respectively (**Table 2**). Applying the inoculant at 6 g/kg seed to Nkatie SARI increased significantly the nodule number per plant by 13% compared with that for the non-inoculated Nkatie SARI plots. However, applying the inoculant at 3 g/kg seed appeared to favor the Chinese variety, as it increased ($P < 0.05$) nodule number per plant in the Chinese variety by 8 and 7% compared to IR at 6 g/kg seed and the non-inoculated Chinese variety, respectively (**Figure 6A**). The application of 30 kg P_2O_5/ha combined with Nkatie SARI increased ($P < 0.05$) effective nodule number compared with the control (**Figure 6B**). Furthermore, the effective nodule number per plant observed from Nkatie SARI at each level of P was higher than that of the Chinese variety at each level of P.

The IR × PR × V, IR × PR, PR × V, and IR × V, likewise the effect of IR and PR, were not significant on pod number per plant; however, the effect of V significantly increased pod number per plant, with the Chinese variety recording higher ($P < 0.05$) pod number than did Nkatie SARI (**Table 4**). Pod weight was significantly influenced by PR × V (**Table 2**). PR at 60 kg P_2O_5/ha combined with the Chinese and Nkatie SARI varieties increased ($P < 0.05$) pod weight compared with the control (**Figure 7**).

IR × PR × V, IR × V, and PR × V, likewise the effect of V, were not significant on grain yield; however, significant differences occurred among IR and PR treatments. The grain yield of IR at 6 g/kg seed and PR at 60 kg P_2O_5/ha contributed to higher ($P < 0.05$) grain yields, respectively. Furthermore, the lowest grain yields from both IR and PR were recorded in their respective controls (**Table 4**). Grain yield was positively correlated with pod weight (**Table 5**). This indicates that an increase in pod weight results in a corresponding increase in grain yield.

IR × PR × V, IR × V, and PR × V, likewise the effect of V, were not significant on both haulm weight and HI; however, the effects of IR and PR were significant on both haulm weight and HI (**Table 4**). The haulm weight of IR at 0 g/kg seed increased significantly compared with that of the inoculated plots. Conversely, the HI of IR at 0 g/kg seed reduced significantly compared with that of the inoculated plots (**Table 4**). Similarly, the effect of PR on haulm weight and HI followed a similar trend as that for IR. The highest ($P < 0.05$) haulm weight and the lowest HI were observed in the control (0 g/kg seed) (**Table 4**).

Plant Nitrogen Fixation and Nutrient Uptake

On-farm Experiment

The IR, PR, and V interactions, likewise the effect of V, did not significantly contribute to N fixed in soil; conversely, the effects of IR and PR influenced N fixation in soil, with their respective controls performing poorly (**Table 6**).

The IR × PR × V, IR × PR, and IR × V were significant on N uptake in the shoot and root (**Table 2**). N uptake at the inoculant rate of 6 g/kg seed and P rate at 60 kg P_2O_5/ha combined with Nkatie SARI increased significantly compared to the control under both varieties (**Figure 8**). Furthermore, at all levels of IR × PR, uptake of N in the shoot and root appeared to be higher in Nkatie SARI than in the Chinese variety (**Figure 8**).

The IR × PR × V, PR × V, and IR × V were not significant on P uptake in the shoot and root; however, IR × PR interaction significantly improved P uptake (**Table 2**), such that the inoculant rate of 6 g/kg seed combined with P rate at 60 kg P_2O_5/ha gave the best performance (**Figure 9**). In contrast with the other levels of PR, 60 kg P_2O_5/ha at all levels of IR influenced higher ($P < 0.05$) uptake of P in the root and shoot (**Figure 9**).

The IR × PR × V was not significant on K uptake in the shoot and root; however, the IR × PR, PR × V, and IR × V were significant on K uptake in the shoot and root. K uptake in the shoot and root of IR at 6 g/kg seed combined with PR at 60 kg P_2O_5/ha increased significantly compared with the control (**Figure 10A**).

TABLE 4 | Plant height, nodulation, yield, and yield components of groundnut as affected by variety, phosphorus, and rhizobium inoculation rates in the on-farm experiment.

Treatment	Plant height (cm) at 8 WAP	Nodule no. 5 plants^{-1}	Effective nodules no. 5 plants^{-1}	Pod no. 5 plants^{-1}	Pod wt. (kg ha^{-1})	Grain yield (kg ha^{-1})	Haulm weight (kg ha^{-1})	Harvest index
Rhizobium inoculation rate (IR) (kg/ha)								
0	12 ± 4.50^{a}	40 ± 6.38^{a}	13 ± 1.85^{a}	15 ± 1.50^{b}	3,090 ± 1,580.70^{b}	1,380 ± 560.89^{b}	5,608 ± 1,993.20^{a}	0.31 ± 0.13^{b}
3	13 ± 4.41^{a}	39 ± 7.84^{a}	12 ± 1.91^{a}	16 ± 1.74^{ab}	3,064 ± 1,577.30^{b}	1,641 ± 777.67^{a}	4,748 ± 1,881.10^{b}	0.43 ± 0.12^{a}
6	13 ± 4.711^{a}	39 ± 6.25^{a}	13 ± 1.81^{a}	17 ± 1.81^{a}	3,550 ± 1,819.30^{a}	1,719 ± 806.29^{a}	5,238 ± 1,963.00^{a}	0.41 ± 0.12^{a}
Phosphorus rate (PR) (kg/ha)								
0	12 ± 4.95^{a}	41 ± 6.98^{a}	13 ± 1.59^{a}	17 ± 1.64^{a}	1,597 ± 857.54c	955 ± 325.14c	5,738 ± 1,936.70^{a}	0.19 ± 0.02^{b}
30	12 ± 5.08^{a}	38 ± 7.22^{a}	12 ± 1.88^{a}	17 ± 1.65^{a}	3,290 ± 1,057.70^{b}	1,641 ± 514.17^{b}	4,891 ± 2,049.60^{b}	0.44 ± 0.14^{a}
60	13 ± 3.31^{a}	39 ± 6.12^{a}	13 ± 2.12^{a}	17 ± 1.85^{a}	4,818 ± 1,121.50^{a}	2,144 ± 728.69^{a}	4,965 ± 1,835.60^{ab}	0.51 ± 0.18^{a}
Variety (V)								
Chinese	10 ± 3.97^{b}	39 ± 6.43^{a}	12 ± 1.88^{a}	17 ± 2.06^{a}	3,142 ± 1,689.30^{a}	1,580 ± 764.06^{a}	5,087 ± 2,405.70^{a}	0.43 ± 0.15^{a}
Nk^{a}tie SARI	15 ± 3.87^{a}	40 ± 7.22^{a}	13 ± 1.83^{a}	16 ± 1.14^{a}	3,328 ± 1,646.00^{a}	1,551 ± 703.81^{a}	5,309 ± 1,400.90^{a}	0.33 ± 0.12^{a}
***P*-value**								
PR × IR	0.750	0.193	0.771	0.249	0.886	0.228	0.321	0.922
PR × V	0.235	0.163	0.023	0.094	0.004	0.787	0.561	0.594
IR × V	0.641	0.009	0.197	0.683	0.946	0.752	0.978	0.939
PR × IR × V	0.654	0.115	0.451	0.405	0.810	0.667	0.689	0.857

[a,b,c]Mean values followed by the same letters in each column are not significantly different from one another.

TABLE 5 | Coefficient of correlation (*r*) among plant height, nodulation, yield, and yield components of groundnut in the on-station and on-farm experiments.

	†PH	NPP	ENP	PPP	PW	GY	HW	HI
On-station experiment								
PH	_							
NPP	0.22^{ns}	_						
ENP	0.03^{ns}	0.77***	_					
PPP	−0.29*	0.67***	0.82***	_				
PW	0.43***	0.80***	0.57***	0.55***	_			
GY	−0.34**	0.42***	0.55***	0.73***	0.36**	_		
HW	0.15^{ns}	0.34**	0.23^{ns}	0.40**	0.52***	0.27*	_	
HI	−0.38**	0.11^{ns}	0.31*	0.25^{ns}	−0.16^{ns}	0.51***	−0.61***	_
On-farm experiment								
PH	_							
NPP	−0.06^{ns}	_						
ENP	0.19*	0.08^{ns}	_					
PPP	−0.11^{ns}	0.46**	0.06^{ns}	_				
PW	0.16^{ns}	−0.07^{ns}	0.17^{ns}	0.05^{ns}	_			
GY	0.04^{ns}	−0.15^{ns}	0.10^{ns}	−0.04^{ns}	0.69***	_		
HW	0.02^{ns}	−0.04^{ns}	−0.09^{ns}	−0.09^{ns}	−0.07^{ns}	−0.19*	_	
HI	−0.05^{ns}	−0.12^{ns}	0.10^{ns}	−0.03^{ns}	0.49***	0.78***	−0.72***	_

†PH, plant height (cm) at 8 WAP; NPP, nodule no. 5 plants^{-1}; ENP, effective nodules no. 5 plants^{-1}; PPP, pod no. 5 plants^{-1}; PW, pod weight (kg ha^{-1}); GY, grain yield (kg ha^{-1}); HW, haulm weight (kg ha^{-1}); HI, harvest index.

*‡ns, $p > 0.05$; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.*

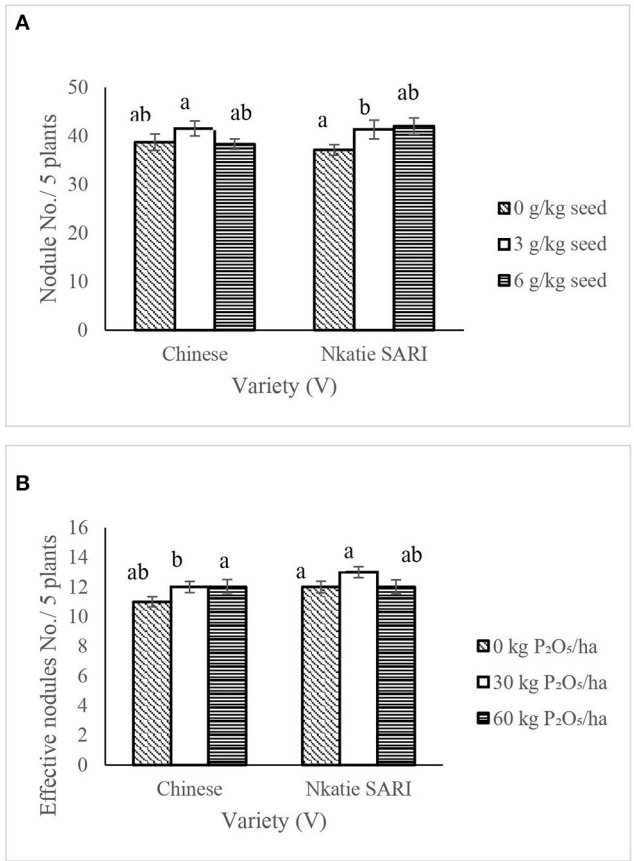

FIGURE 6 | Interactive effect of Innoclant rate (IR) and variety (V) on nodule number per five plants **(A)** and; P-rate and variety on effective nodule number per five plants of groundnut **(B)** grown in Haplic Lixisols of northern Ghana. Bars with different letters (in horizontal direction) show significantly different means. Error bars represent standard error of mean.

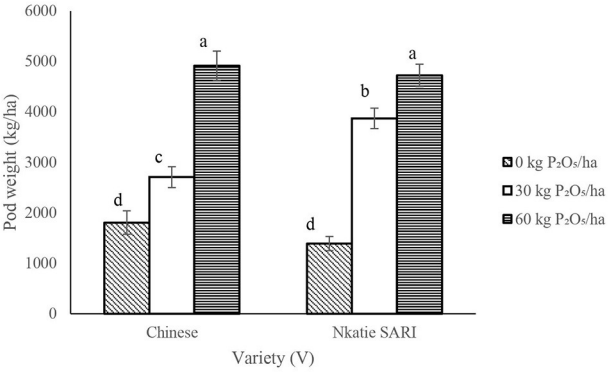

FIGURE 7 | Interactive effect of P-rate (PR) and inoculant rate (IR) on pod weight of groundnut grown in Haplic Lixisols of northern Ghana. Bars with different letters (in horizontal direction) show significantly different means. Error bars represent standard error of mean.

The PR at 60 kg P_2O_5/ha combined with the Chinese variety significantly increased K uptake in the shoot and root compared with the control (**Figure 10B**). Conversely, the IR at 6 g/kg seed combined with the Nkatie SARI variety increased K uptake in the shoot and root significantly compared to the control (**Figure 10C**).

DISCUSSION

Growth, Yield, and Yield Components

The significant interaction effect of PR × V on plant height as observed in the on-station experiment could possibly be due to the supply of plant nutrients to peanut varieties from P application. Several authors (Shiyam, 2010; Kabir et al., 2013) have reported on the positive influence of P on peanut growth and development. Similarly, Adjei-Nsiah et al. (2018) attributed rapid plant growth in soybean to P fertilization. Although the results of the on-farm experiment did not show any significant interaction effect on plant height, there was a significant difference in plant height among peanut varieties; the Nkatie SARI recorded higher plant height than the Chinese variety. The difference in plant height among the varieties could possibly be due to differences in genetic composition. In confirming this, Konlan (2010) observed significant differences in plant height among six peanut varieties (Adepa, Azivivi, Jenkaar, Kpanieli, Nkosour, and Manipintar) and attributed these differences to genetic factors. Similarly, Golakia et al. (2005) reported a significant difference in plant height between the Virginia runner type and the Spanish bunch type of peanut. However, the discrepancy in response of peanut plant height to inoculation and/or P application across experiments could be due to differences in prevailing soil conditions in the two experiments; although we did not assess soil temperature and moisture in our study, there is sufficient evidence that available soil moisture had a negative influence on peanut growth. In addition to soil nutrients and cultivar selection, germination, emergence, and plant vigor are primarily determined by the temperature and soil moisture in the seeding zone (Kumar et al., 2012).

The process of BNF heavily involves the formation of nodules. In this study, the nodule number and effective number of nodules were significantly influenced by IR × V and PR × V interactions, respectively, in the on-farm experiment. These results indicate that the ability to nodulate and further produce effective nodules is not only a genetic attribute but can also be influenced by the application of either P fertilizer or inoculant. In confirming this, several authors (Gentili et al., 2006; Lira et al., 2015) have reported on the significant influence of phosphorus and rhizobium inoculant on nodule and effective nodule formation in legumes. Similarly, Boddey et al. (2017) reported a higher nodule number in Northern Ghana due to the application of the *Bradyrhizobium* inoculant with P fertilizer. The study of Asante et al. (2020) on yield response of groundnut to inoculation and P application also supports this conclusion. In the on-station experiment, nodule and effective nodule formation did not differ significantly among IR, PR, and V interactions. This discrepancy in results between

TABLE 6 | N fixation and N, P, and K uptake by groundnut as affected by variety, phosphorus, and rhizobium inoculation rates in the on-farm experiment.

Treatment	N fixation (%)	Shoot and root uptake (%)		
	N	N	P	K
Rhizobium inoculation rate (IR) (kg/ha)				
0	0.40 ± 0.21^{b}	1.33 ± 0.65c	0.27 ± 0.13^{b}	0.52 ± 0.24^{b}
3	0.76 ± 0.45^{a}	1.68 ± 0.55^{b}	0.31 ± 0.17^{b}	0.64 ± 0.26^{b}
6	0.95 ± 0.51^{a}	2.22 ± 0.83^{a}	0.41 ± 0.18^{a}	0.98 ± 0.37^{a}
Phosphorus rate (PR) (kg/ha)				
0	0.49 ± 0.25^{b}	1.24 ± 0.56c	0.09 ± 0.05^{b}	0.36 ± 0.09c
30	0.61 ± 0.35^{b}	1.71 ± 0.57^{b}	0.29 ± 0.07^{ab}	0.74 ± 0.24^{b}
60	1.00 ± 0.60^{a}	2.28 ± 0.79^{a}	0.61 ± 0.22^{a}	1.05 ± 0.44^{a}
Variety (V)				
Chinese	0.65 ± 0.39^{a}	1.56 ± 0.70^{b}	0.33 ± 0.15^{a}	0.72 ± 0.43^{a}
Nk^{a}tie S^{a}RI	0.76 ± 0.50^{a}	1.93 ± 0.79^{a}	0.32 ± 0.25^{a}	0.72 ± 0.45^{a}
PR × IR	0.093	0.004	0.041	0.048
PR × V	0.225	0.554	0.647	0.008
IR × V	0.922	0.038	0.919	0.007
PR × IR × V	0.150	0.002	0.984	0.222

^{a,b,c}*Mean values followed by the same letters in each column are not significantly different from one another.*

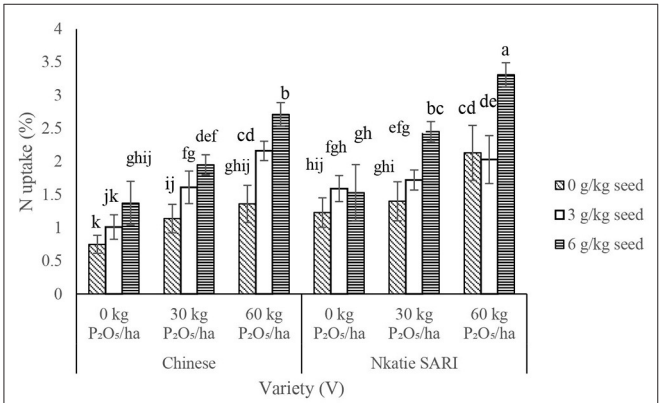

FIGURE 8 | Interactive effect of P-rate (PR), inoculant rate (IR), and variety (V) on N uptake of groundnut grown in Haplic Lixisols of northern Ghana. Bars with different letters (in horizontal direction) show significantly different means. Error bars represent standard error of mean.

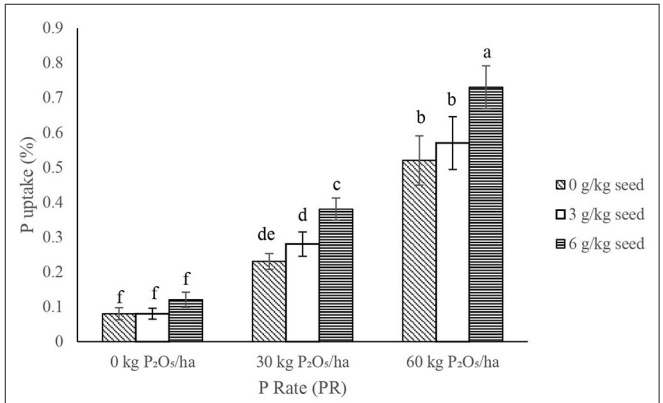

FIGURE 9 | Interactive effect of P-rate (PR) and inoculant rate (IR) on P uptake of groundnut grown in Haplic Lixisols of northern Ghana. Bars with different letters (in horizontal direction) show significantly different means. Error bars represent standard error of mean.

the on-farm and the on-station experiments suggests that, in the on-station experiment, the varieties responded similarly to inoculation and/or P application. This could be attributed to the low soil pH (4.9) at the on-station experiment (**Table 1**), which might have reduced phosphorus availability and impaired nitrogen fixation. This finding is affirmed by the report of Sela (2020) that the optimal pH range for enhanced phosphorus availability to crops is between 6.0 and 7.0.

The significant interaction effect of IR × PR × V on pod number per plant could possibly be due to the supply of nutrients by P and the introduction of effective rhizobia for peanut formation. The above interaction result indicated that peanut varieties require the application of both P and rhizobium inoculant to enhance its podding capacity. These results agree with earlier reports that inoculant combined with phosphorus fertilizer increased pod yield per plant more than the application of the inoculant or phosphorus fertilizer alone (Kyei-Boahen et al., 2017). Conversely, the interactive effect of IR, PR, and V did not affect ($P < 0.05$) pod weight. The failure of pod number to translate into pod weight could probably be due to drought conditions experienced during the pod filling period, which might have masked the positive influence of IR × PR on pod filling in the varieties. According to Martinson (2009), adequate soil moisture received during pod filling and seed formation periods results in rapid translocation of photosynthates to reproductive parts. PR × V influenced pod weight in the on-farm experiment. Statistically, Chinese and Nkatie SARI varieties

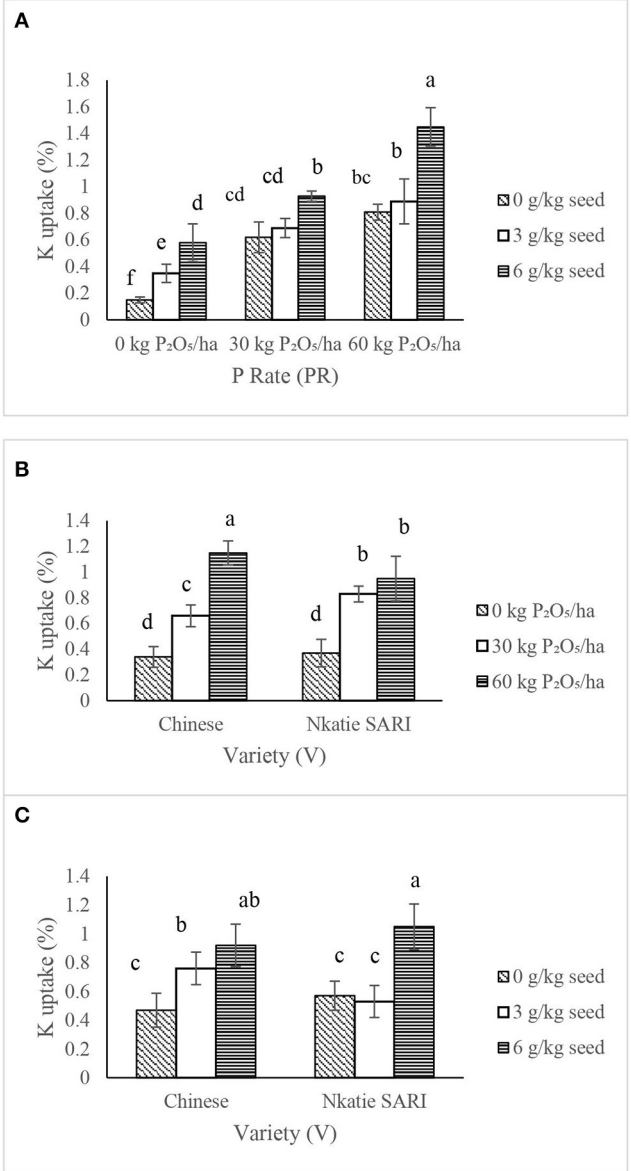

FIGURE 10 | Interactive effect of effects of P-rate (PR) and inoculant rate (IR) **(A)**; P-rate (PR) and variety (V) **(B)**; and inoculant rate (IR) and variety (V) **(C)** on K uptake of groundnut in Haplic Lixisols of northern Ghana. Bars with different letters (in horizontal direction) show significantly different means. Error bars represent standard error of mean.

combined with 60 kg P_2O_5/ha produced the highest pod weight. This increase in pod weight could possibly be due to enhanced stimulation of pod filling in such treatment combinations. In confirming this, Hernanadez and Cuevas (2003) recorded increased pod weight under 100 kg P_2O_5/ha application than the control (no P application).

The significant effect of IR × PR on grain yield in the on-station experiment could possibly be due to the positive interactive effect of the P fertilizer and rhizobium inoculant. The increase in grain yield with P fertilizer and rhizobium inoculant supports earlier findings that the combined application of P with rhizobium inoculant increased grain yield and nitrogenase activity as well as enhanced soil fertility (Fatima et al., 2007). Similarly, a study by Ulzen et al. (2018) reported significantly higher soybean grain yields from plots that received P and/or inoculation than those of the control plots. Accordingly, Ndakidemi and Semoka (2006) recommended that, for farmers who can afford P fertilization, its combined use with inoculants can further increase grain yield. In the on-farm experiment, grain yield did not differ significantly among IR, PR, and V interactions. It can be deduced that the IR and PR failed to play a complementary role with peanut varieties. This is corroborated by the insignificant response of P uptake (%) to IR × PR × V (**Table 2**). This observation contradicts research findings by Kyei-Boahen et al. (2017), where the application of P and inoculant affected ($P < 0.05$) grain yield of cowpea. Although there were no significant interaction effect on grain yield in the on-farm experiment, the effects IR and PR significantly affected grain yield. These results indicate that phosphorus and inoculant were the main deficient factors for peanut performance in the on-farm sites during this study, confirming earlier observations made by Stefanescu and Palanciuc (2000) that phosphorus and inoculation induced a pronounced effect on grain yield.

Haulm weight and HI were significantly affected by the interaction effect of IR × V in the on-station experiment. This could possibly be due to the supply of nutrients as enhanced by the application of rhizobium inoculant. The above interaction results indicated that higher dry matter accumulation in peanut requires the application of rhizobium inoculant. Amos et al. (2001) reported significantly higher dry matter accumulation, when rhizobium inoculation and P fertilizer were applied to common bean (*Phaseolus vulgaris*). In the on-farm experiment, haulm weight, and HI were significantly influenced by the effects of PR and IR. This result is in consonance with the findings by Emmanuel et al. (2021), who reported that the application of P and *Bradyrhizobium* inoculant increased dry matter of cowpea in savanna soils of Ghana. The application of either P fertilizer or inoculant most often influenced nitrogenase activity, and this nitrogen helps in vegetative plant growth, which might explain the significant effect of P and inoculant on haulm yield in this study.

N_2 Fixation and N, P, and K Nutrient Uptake

N_2 fixed in the soil was significantly enhanced by the effect of the phosphorus rate and rhizobium inoculant rate. This observation could be due to the availability of phosphorus needed by rhizobium for N_2 fixation, affirming the importance of both in peanut production. This result agrees with the report of Adjei-Nsiah et al. (2018) who stated that N fixation is strongly influenced by the availability of both P and effective rhizobium strain. However, in this study, the average nitrogen stored in plants was 148% higher in plants compared with that in the soil.

The significant effect of IR × PR × V interaction on N uptake in shoots and roots could possibly be attributed to the adequate plant nutrients supply by both P fertilizer and inoculant to peanut. P application influenced the growth of plant roots while the introduction of rhizobia ensured that there were enough

bacteria in the rhizosphere of the root to enhance formation of nodules for N fixation. Similar findings were reported by Rodelas et al. (1999) when they attributed N uptake in the shoots of fava beans to enhanced root development, which resulted in increased nutrient uptake.

The results from this study indicate that the combined application of phosphorus and inoculant affected P uptake in the shoots and roots, with the highest P rate and IR combining to influence larger uptake of P. The value recorded under the interaction of 6 g/kg seed and 60 kg P_2O_5/ha for P (0.73%) was higher than the sufficiency range for P (0.2–0.5%), as reported by Motsara and Roy (2008). This result suggests that a combined higher rate of P and inoculant application could lead to higher P uptake in the shoots and roots of peanuts. However, the results of our study contradict earlier reports by Taruvinga (2014) that higher P concentrations in the soil tend to "lock" and reduce the absorption and utilization of some soil nutrients.

The significant interaction effects of PR × V and IR × V on K uptake could possibly be due to the interactive effects of P and rhizobium to positively influence K uptake in the shoot and root. This concurs with earlier reports that P fertilizers and rhizobium inoculant may enhance root development and induce an increase in number of root hairs, thereby favoring nutrient uptake by exploring a larger volume of soils (Rodelas et al., 1999; Biswas et al., 2000). However, the observed values for K at all levels were not within the sufficiency or optimal range (1.0–5.0%) as described by Motsara and Roy (2008).

CONCLUSION

This study has revealed a variable response of peanut varieties to P fertilizer rates and rhizobium inoculant on Haplic Lixisols of the Guinea savanna zone of Ghana. P fertilizer rates combined with peanut varieties had a significant influence on growth, effective nodule number, and podding capacity. Nkatie SARI appears to have the potential to increase peanut productivity in P-deficient soils as it combined with 60 kg P_2O_5/ha to promote the best growth, nodulation, and podding capacity.

Peanut varieties treated with rhizobium inoculant improved pod number, nodule number, Haulm weight, and HI, with Nkatie SARI combined with inoculant at 6 g/kg seed giving the best performance. The interaction of IR × PR was significant on peanut grain yield. Higher grain yields were recorded in peanuts sown with P fertilizer rate at 60 kg P_2O_5/ha in combination with inoculant at 6 g/kg seed. This points to the fact that limited use of P fertilizers and inoculants by farmers within the study location is among the factors that have affected peanut productivity. Our results show that the use of P fertilizer and rhizobium inoculant has the potential to increase peanut productivity in areas with similar soil characteristics. However, since the result between the on-station and on-farm experiments appeared inconsistent, further studies are required to determine if the yield from this interaction is stable enough to form the basis of recommendation of this treatment combination to small-scale peanut farmers.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

AR and JK designed the experiment and protocol. RN, AR, JK, and AB performed the data collection. RN and AB analyzed the data and interpreted the results. RN did the literature searches. AB wrote the manuscript. All authors read and approved the submitted version.

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Rhizosphere P-Enzyme Activity, Mineral Nutrient Concentrations, and Microbial Community Structure Are Altered by Intra-Hole Cropping of Cowpea With Cereals

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In Africa, intercropping and intra-hole cropping systems are common practices used by smallholder farmers to optimize land use and tap the benefits of plant-to-plant interactions. The aim of this study was to evaluate mineral nutrient concentrations, P-enzyme activity and changes in microbial communities in the rhizospheres of sole cropped and intra-hole planted cowpea (cvs. TVu 546 and PAN 311), maize (cv. ZM 521), and sorghum (cv. M48). Cowpea cv. TVu546 intra-hole planted with sorghum (i.e., TVu546+M48) produced the highest rhizosphere acid phosphatase (APase) activity (230.0 μg p-nitrophenol.g^{-1} soil.h^{-1}). From 16S rRNA Miseq Illumina sequencing, the rhizosphere bacterial community structure was altered by intra-hole cropping, and was dominated by *Actinobacteria*, *Acidobacteria*, *Bacteroidetes*, *Firmicutes*, *Planctomycetes*, *Proteobacteria*, and *Verrucomicrobia*, which together accounted for about >95% of the total sequences. The *Sphingobacteria* phylum was the dominant microbial group in the rhizosphere soil of all the cropping systems. The *Proteobacter*ia phylum was the second most abundant in this study, which included the beneficial bacteria in all the rhizosphere soils studied. In contrast, typical pathogens like *Ralsotonia* and *Agrobacterium* were completely absent, indicating that the intra-hole cropping system can provide protection against soil-borne diseases possibly through elimination by antibiotics and/or phytoalexins present in plant and microbial exudates in the rhizosphere. *Mucilaginibacter* and *Flavobacterium* were however selectively present with intra-hole cropping.

Keywords: intra-hole and sole cropping, acid phosphatase activity, microbial community, 16S rRNA, Miseq Illumina sequencing

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INTRODUCTION

Intercropping, defined as the planting of two or more crops on the same piece of land within the same cropping season, is an ancient cultural practice common among smallholder farmers, especially in Africa. The intercropping system can vary from intra-row intercropping, inter-row intercropping to intra-hole cropping, which involves two or more different plant species. These intercropping systems are a common practice used by smallholder farmers in Africa to optimize

land use as well as tap the benefits of plant-to-plant interaction. This practice is regarded as low-input and self-sustaining, in that, it enhances the amount of biomass and yield that is harvested per unit land area (Matusso and Mucheru-Muna, 2014). With this practice, yield reliability and insurance against crop failure are key, with intercrops tending to have higher yield stability than sole crops especially in areas prone to extreme weather conditions (Papastylianou, 2004; Wang et al., 2014). Where intercropped plants have different root architecture, soil fertility is improved as roots of such crops explore a larger soil volume through increased root length and density, as well as root distribution (Li et al., 2013). Where a legume is intercropped with non-legume, direct transfer of fixed-N from the legume to the non-legume can occur, leading to enhanced N nutrition of the companion crop (Xiao et al., 2004).

Intra-hole cropping is a system where two different crop species (e.g., legume and cereal) are planted in the same hole (Dakora et al., 2008). The intra-hole legume/cereal pairing is often practiced where soils are low in N. As a result, the legume's N-rich compounds excreted by root nodules, released through rhizodeposition, leached from leaves, as well as N transferred through mycorrhizal fungal mycelia become available to the associated cereal plant belowground, in addition to products of organic matter decomposition becoming more available to succeeding crops (Hamel and Smith, 1991; Fustec et al., 2010).

Enhanced P nutrition of cereals from intercropping with legumes has been attributed to P-solubilization in the rhizosphere by symbiotic rhizobia and the secretion of P compounds in root exudates (Dakora and Phillips, 2002; Maseko and Dakora, 2013). However, the root exudates also contain phosphatases that can decouple unavailable P from complexes (Gunes et al., 2007), organic acids that can lower soil pH and make P more available (Li et al., 2007), and protons released during nitrogen fixation that can readily acidify the rhizosphere for increased P availability (Shen et al., 2011). Where roots of the intercropped species are intermingled and/or in close proximity, the solubilized P can benefit the component non-legume plant. In fact, Makoi et al. (2010) have shown that a sorghum/cowpea intercrop increased APase and AlkPase activity, leading to enhanced P nutrition, greater plant growth, and higher grain yield. A soybean/sugarcane intercrop in the glasshouse also resulted in increased total soil microbe number when compared to the monocultures (Li et al., 2013).

Being indigenous to the African continent, both cowpea and sorghum are widely adapted to different soil ecologies, including tolerance to drought, and are used as food and cash crops by most smallholder and commercial farmers in Africa together with maize. During intra-hole cropping, where the legume and cereal are planted in the same hole, the roots of the two species can become interwoven, leading to direct nutrient acquisition and increased mobilization (Li et al., 2010). There are also reports that an increase in rhizosphere microbial biomass and P-enzymes can lead to improved nutrient supply to both partners compared to either sole crop (Inal et al., 2007; Makoi et al., 2010). However, soil and plant management practices can have much greater influence on soil microbial mass (Gupta and Germida, 1988; Dick et al., 1994; Alvey et al., 2003), with monocultures tending to show reduced microbial biomass and activity in comparison with mixed cultures (Moore et al., 2000). Furthermore, legumes generally accumulate greater microbial biomass in the soil than cereals (Walker et al., 2003).

Although there is considerable information on the relationship between soil management and soil microbial activity, little is known about it under mixed cultures commonly practiced by smallholder farmers in Africa (Dick et al., 1988; Deng and Tabatabai, 1996; Yang et al., 2015). Although rhizosphere microbial composition used to be studied by culturing the bacteria for single species interactions, now however, root-microbe interactions and the composition of root-associated microbial community have become easier to assess, with the development of next generation sequencing (Mendes et al., 2013; Hacquard et al., 2015). Furthermore, while several studies have been conducted on intercropping (Wahua, 1984; Li and Wu, 2018; Dang et al., 2020; Li et al., 2020), to our knowledge little is known about intra-hole cropping of legumes and cereals. We hypothesized that intra-hole cropping of cowpea-maize, and cowpea-sorghum would influence soil P-enzyme activity, mineral nutrient accumulation and microbial communities based on the partners used in the intra-hole cropping system. Therefore, the aim of this study was to evaluate P-enzyme activity, mineral nutrient accumulation and microbial community structure in the rhizosphere of sole and intra-hole cropped cowpea with cereals (namely, sorghum and maize).

MATERIALS AND METHODS

Description of the Experimental Site and Experimental Design

The field experiment was carried out at the Marapyane Campus of the Lowveld College of Agriculture (24°57′53.777″S and 28°45′41.544″E), Mpumalanga Province of South Africa. The experimental field was grass fallow for over 30 years, but frequently grazed by livestock before the experiment was established in the 2013/2014 cropping season. The Mpumalanga Province is dominated by savanna vegetation and is semi-arid in climate with cold winter and warm summer seasons. The temperature and average rainfall can vary from 15.9 to 32.5°C and 0 to 34.4 mm, respectively, during planting summer season.

The experimental field layout consisted of plots measuring 4.2 m long and 2.4 m wide with an inter-row spacing of 80 cm, intra-row spacing of 30 cm and 90 cm between plots. The treatments included two cropping systems (monoculture and intra-hole cropping), two cowpea genotypes (PAN 311 and TVu 546), and two cereal species (maize cv. ZM521 and sorghum cv. M48). The experiment was laid out using a randomized complete block design with three replications. With sole cropping, two seeds were planted per hole for each crop species (cowpea, maize and sorghum), and later thinned to one plant per stand at 2 weeks after planting. With the intra-hole cropping system, four seeds (two of cereal and two of legume) were planted per hole, and later thinned to one plant of cowpea and one plant of cereal per stand at 2 weeks after sowing. The plants were naturally

rain-fed without irrigation. Weeds were controlled manually, when necessary.

Collection and Processing of Rhizosphere Soils

At 86 d after planting (DAP), samples of rhizosphere soil (defined here as soil adhering to the plant roots) were collected from each treatment, namely, sole cowpea, sole maize, sole sorghum, intra-hole cropped cowpea with maize and intra-hole cropped cowpea with sorghum. With the monocultures, soil adhering to the root system of each treatment was shaken off into a pre-labeled zipper plastic bag and sealed. With intra-hole planting, however, the soil around the intermingled roots of both cowpea and cereal was collected. The rhizosphere soil samples were immediately put into dry ice in a cooler box and transported to the laboratory where each sample was divided into three parts. One part was kept at −4°C for later use in APase and AlkPase assay, the second part stored at −80°C for later use in soil microbial DNA extraction, and the third part air-dried at room temperature, sieved (2 mm), and stored for mineral analysis.

Determination of pH and Chemical Properties of the Soil Samples

To measure pH, 50 mL 0.01 M $CaCl_2$ was added to 10 g of air-dried and sieved (2 mm) soil sample. The mixture was mixed thoroughly using an end-to-end shaker at 100 rpm for 3 h. After shaking, the suspension was allowed to settle for 30 min and the pH measured using a BT-675 pH benchtop meter [Boeckel + Co (GmbH + Co) KG, Hamburg, Germany].

Assay of Acid and Alkaline Phosphatase Activity in Rhizosphere Soils

APase and AlkPase activity in the rhizosphere soils was assayed as described by Tabatabai (1994). The solution was filtered through Whatman #2 filter paper and the absorbance of the supernatant was measured at 420 nm using a UV–VIS spectrophotometer (JENWAY 7300, Bibby Scientific Ltd, Stone, Staffs, UK). The absorbances of filtrates were compared with *p*-nitrophenol standards. For each assay, a control was included to account for non-enzymatic substrate hydrolysis, and the enzyme activity expressed as μg *p*-nitrophenol g^{-1} F wt soil h^{-1}.

Analysis of Mineral Nutrients in Rhizosphere Soils

The rhizosphere soil samples that were processed and kept for mineral analysis were used to analyze for P, K, Ca, Mg, Cu, Zn, and Fe according to the citric acid method developed by Dyer (Dyer, 1894) and modified by the Division of Chemical Services (1956), and later by Du Plessis and Burger (1965). The acid digestion method was employed to determine soil concentrations of P, K, Ca, and Mg and the EDTA method for Cu, Zn, and Fe using plasma-mass spectrometer (ICP-MS) (IRIS/AP HR DUO Thermo Electron Corporation, Franklin, Massachusetts, USA).

Extraction of Rhizosphere Soil Microbial DNA

The rhizosphere soils collected from each treatment plot were bulked together to obtain one composite sample. Genomic DNA was extracted from 0.5 g of the composite samples using PowerSoil^{TM} DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. The DNA concentrations were measured using PicoGreen (Invitrogen, cat. # P7589) method and Victor 3 fluorometry. Fluorescence was measured for three cycles of 30 s at 25°C in 96 well plates, and a standard curve generated by means of the fluorescence results, was used to determine the DNA concentration and adjusted to a final level of 3.5 ng/μl. The DNA samples with known concentrations were sent to Macrogen (The Netherlands) for sequencing.

Library Preparation and Cluster Generation

The sequencing library was prepared by random fragmentation of the DNA sample, followed by 5′ and 3′ adapter ligation. All samples of rhizosphere microbial DNA were subjected to PCR amplification in duplicates using primer pairs retaining the adapter-ligated fragments 5′TCGTCGGCAGCGT CAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG3′ and 5′GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GACTACHVGGGTATCTAATCC3′ targeting variable regions V3-V4 of the 16S rDNA gene (Klindworth et al., 2013). The polymerase chain reaction (PCR) was carried out with about 10–15 ng DNA in 25 μl reaction volume containing 12.5 μl 2× KAPA HiFi hotstart ready mix, and 5 μl of each primer (1 μM) with the following temperature profile: 95°C-30 s, 25×(95°C-30 s, 55°C-30 s, 72°C-30 s), 72°C-5 min. To verify the size of PCR enriched fragments, we checked the template size distribution by running it on an Agilent Technologies 2100 Bioanalyzer using a DNA 1000 chip. Subsequently, PCR amplified products were cleaned using AMPure XP beads. The sample libraries were quantified using qPCR according to the Illumina qPCR Quantification Protocol Guide. The PCR amplified products were indexed by ligation of indexing adapters to the DNA fragments, and amplified by PCR. The amplified products were again purified using AMPure XP beads.

For cluster generation, the library was loaded onto a flow cell where fragments were captured on a lawn of surface-bound oligos complementary to the library adapters. Each fragment was then amplified into distinct, clonal clusters through bridge amplification. After completion of clustering, the templates were sequenced.

Sequencing

Illumina SBS technology was used for paired-end-sequencing, a proprietary reversible terminator-based method that detects single bases as they get incorporated into DNA template strands. As all four reversible, terminator-bound dNTPs are present during each sequencing cycle, natural competition minimizes incorporation bias and greatly reduces raw error rates compared to other technologies. The results were a highly accurate base-by-base sequencing that virtually eliminated

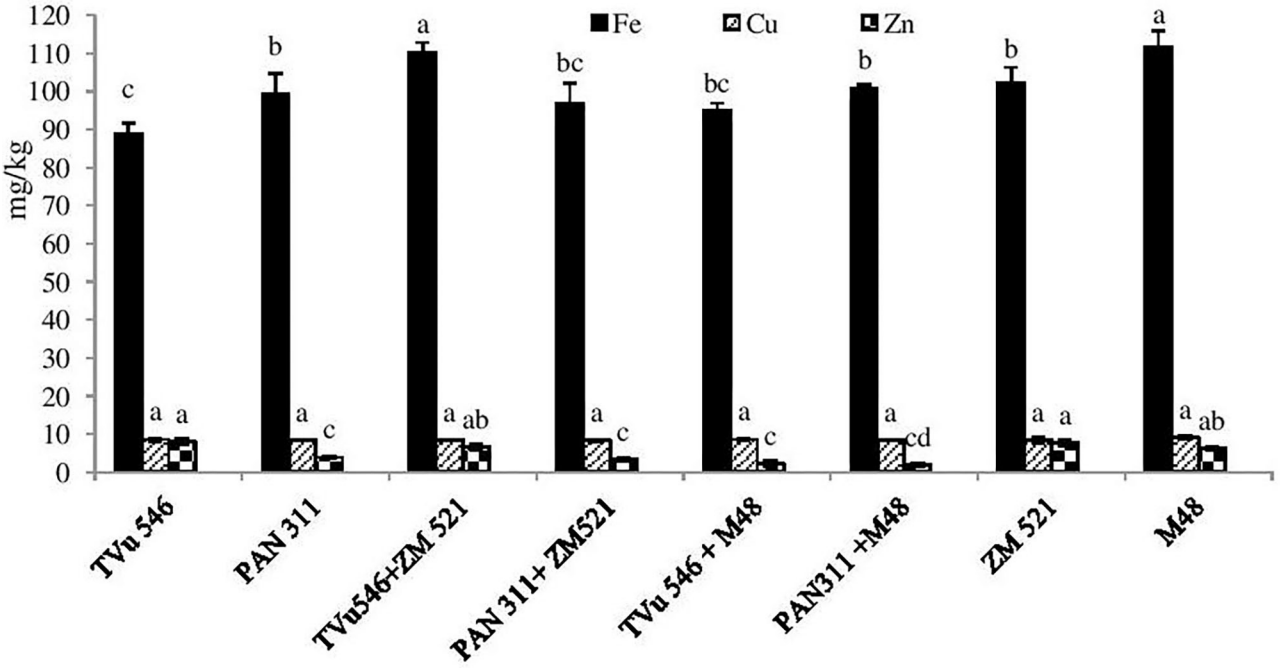

FIGURE 1 | Available micro-element in rhizosphere of solo and intra-hole planted cowpea, sorghum, and maize. Bars with dissimilar letters are significantly different ($P \leq 0.05$) for each element.

sequence-context-specific errors, even within repetitive sequence regions and homopolymers.

Data Assembling and Statistical Analysis

During data analysis and alignment, the newly identified sequence reads were aligned using FLASH to reference genome. The raw sequences were processed in CD-HIT-OTU for quality control (QC) assessment to remove noise data and to ultimately generate cluster files for each sample. All processed and QC passed cluster files were analyzed using Qunatitative Insights into Material Ecology (QIIME) pipeline (Caporaso et al., 2010). The representative reads from non-chimeric clusters were grouped using a greedy algorithm into OTUs at a user-specified OTU cutoff (e.g., 97% ID at species level). Genetic distance was calculated and sequences were clustered into operational taxonomic units (OTUs). The alpha diversity index (Chao1, Shanon, and Simpson) was calculated for each sample at both distances. The taxonomical abundance (%) of microbial communities of each rhizosphere soil sample was calculated using read files as queries against removed and de-replicated set of sequences from the small subunit (SSU) UCLUST (Edgar, 2010). The principal coordinate analysis (PCoA) was carried out using make_rd_plot.py of QIIME with output data of beta diversity (pairwise sample dissimilarity). Rarefaction analysis was performed at species level for each sample using alpha_rarefaction.py of QIIME. The data were submitted to NCBI Sequence Read Archive under the project Bioproject ID PRJNA397662. Soil AP*ase/AklPase* and nutrient data were statistically analyzed using STATISTICA software program version 10.1. The Duncan's multiple range test was used to separate means at $p \leq 0.05$.

RESULTS

Soil pH

Rhizosphere soil acidity ranged from pH 6.05–7.07 for the test species. Sole planted maize (cv. ZM 521) had soil pH 7.0, and sorghum (cv. M48) pH 6.61. In contrast, the two cowpea genotypes showed much lower soil pH levels, with cv. TuV 546 recording pH 6.34, and cv PAN 311 pH 6.28. When planted with sorghum in a single hole, cowpea cv. PAN311 lowered soil pH to 6.05, in contrast to cv. TVu546, which increased rhizosphere soil pH to 7.07 when planted in the same hole with sorghum. Planting cowpea cv. TVu 546 with maize (cv. ZM 521) in the same hole produced a slightly acidic rhizosphere soil reaction (pH 6.43).

Effect of Cropping System and Plant Species on Mineral Nutrient Concentrations in Rhizosphere Soils

Rhizosphere Levels of Fe, Cu, and Zn

Available Fe in the soil ranged from 88.75 to 111.70 mg/kg for all treatments (**Figure 1**). Fe concentration in the rhizosphere of maize and sorghum monocultures (102.36 and 111.70 mg/kg, respectively) was much higher than that of the sole-grown cowpea cultivars. Cowpea cv. PAN 311 also showed greater Fe level in its rhizosphere (99.28 mg/kg) than cv. TVu 546 (88.75 mg/kg).

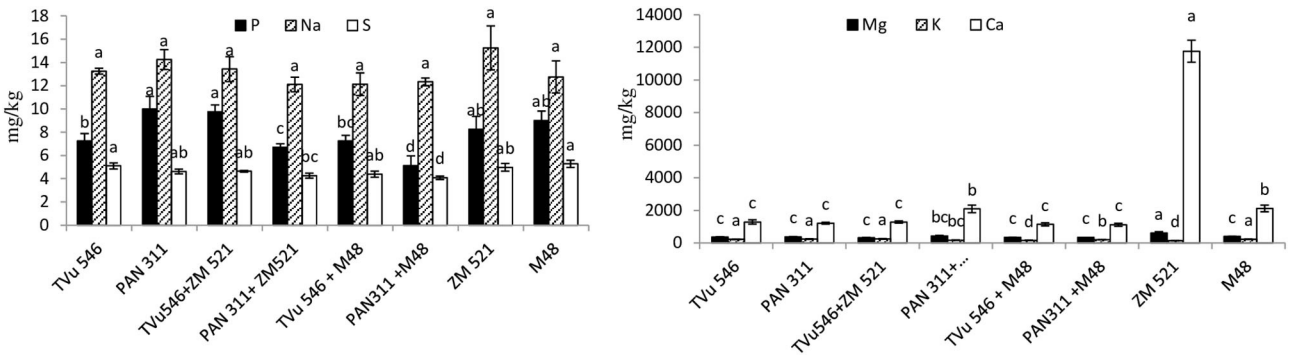

FIGURE 2 | Presence of exchangeable macro-element in rhizosphere of solo and intra-hole planted cowpea, sorghum, and maize. Bars with dissimilar letters are significantly different ($P \leq 0.05$) for each element.

Intra-hole planting of sorghum with the two cowpea genotypes increased Fe levels in the rhizosphere soil relative to sole cowpea, but decreased it compared to sole sorghum (**Figure 1**). Planting maize with cowpea cv. TVu 546 also increased rhizosphere Fe relative sole cv. TVu 546.

Available Cu levels were generally similar in the rhizosphere soil of all the treatments, and ranged from 8.34 to 9.14 mg/kg. The concentration of Zn in the rhizosphere of all the treatments ranged from 1.96 to 8.06 mg/kg (**Figure 1**). The rhizosphere soil of cowpea cv. TVu 546 showed 2-fold higher Zn concentration (8.1 mg/kg) than cv. PAN 311 (3.9 mg/kg). However, the Zn level in the rhizosphere soil of the two cereals, sorghum (M 48) and maize (ZM 521), was much higher (6.30 and 7.74 mg/kg, respectively) relative to cowpea cv. PAN 311, but lower than cv. TVu 546. Intra-hole planting of sorghum or maize with the two cowpea cultivars decreased Zn levels relative to the monocultures of cowpea, sorghum, and maize (**Figure 1**).

Rhizosphere Levels of Ca, Mg, K, and P

The concentration of Ca in the rhizosphere soil of the test species ranged from 1012 to 11758 mg/kg (**Figure 2**), with the two cereals (maize and sorghum) registering the highest levels (11758 and 2118 mg/kg, respectively) relative to the legume. Cowpea cv. TVu 546 recorded 1287 mg/kg Ca and cv. PAN 311 1214 mg/kg. Intra-hole planting of maize with the two cowpea genotypes lowered Ca concentration in the rhizosphere relative to sole maize, but increased it when maize was planted in the same hole with cowpea cv. PAN 311 (**Figure 2**). Intra-hole planting of sorghum with each of the cowpea cultivars decreased rhizosphere Ca relative to monocultures of sorghum, cowpea cv. TVu 546 and cv. PAN 311.

The level of Mg in plant rhizospheres ranged from 324 to 605.10 mg/kg, with sole maize and sorghum exercising much greater concentration compared to sole cultures of the two cowpea genotypes (**Figure 2**). Planting maize with each of the two cowpea cultivars in same hole lowered Mg concentrations relative to monocultures of maize and cowpea cv. TVu 546, just as intra-hole planting of sorghum with the cowpea cultivars decreased the concentration of Mg in the rhizospheres of sorghum, cowpea cv. TVu 546 and cv. PAN 311 monocultures (**Figure 2**).

Potassium concentration ranged from 143.3 to 246.9 mg/kg in the rhizosphere soil of the test plants (**Figure 2**), with sole cowpea genotypes exhibiting greater K levels than the sole maize and sorghum. Intra-hole planting of maize with the cowpea genotypes increased rhizosphere K relative to sole-planted maize, as well as TVu 546 co-planted in one hole with maize. However, sowing sorghum seeds and cowpea seeds in one hole lowered rhizosphere K level when compared to sole-planted sorghum and cowpea (**Figure 2**).

Plant-available P in the rhizosphere soil of the test species ranged from 5.5 to 10.0 mg/kg, with cowpea cv. PAN 311 recording the highest P (10.00 mg/kg; **Figure 2**). Intra-hole planting of maize with cowpea increased the concentration of rhizosphere P relative to sole maize and cowpea cv. TVu 546, but decreased it when compared to sole maize and cowpea cv. PAN 311 (**Figure 2**). Similarly, planting sorghum and cowpea cv. PAN 311 in the same hole led to a decrease in P relative to monocultures of sorghum and cv. PAN 311, but had no effect with cv. TVu 546 (**Figure 2**).

Effect of Cropping System and Plant Species on APase and AlkPase Activity in Rhizosphere Soils

Relative to APase, AlkPase activity was generally higher in the rhizosphere of the test species. Furthermore, the APase and AlkPase activity of sole-planted cowpea cultivars was much greater than that of their cereal counterparts (**Figure 3**). Intra-hole planting of maize with each of the two cowpea cultivars resulted in an increase in rhizosphere AlkPase activity relative to sole-planted maize, and a decrease relative to monocultured cowpea genotypes. However, planting maize in the same hole with cowpea cv. TVu 546 increased rhizosphere APase activity relative to sole-planted TVu 546, while with PAN 311, it decreased APase activity when compared with sole stands of maize and cv. PAN 311. Intra-hole planting of sorghum with cowpea cv. TVu 546 increased APase activity relative to sole-planted sorghum and cowpea cv. TVu 546, while with cv. PAN 311, it decreased it relative to sole stands of sorghum and cv. PAN 311. Planting sorghum in the same hole with cowpea cv. TVu 546

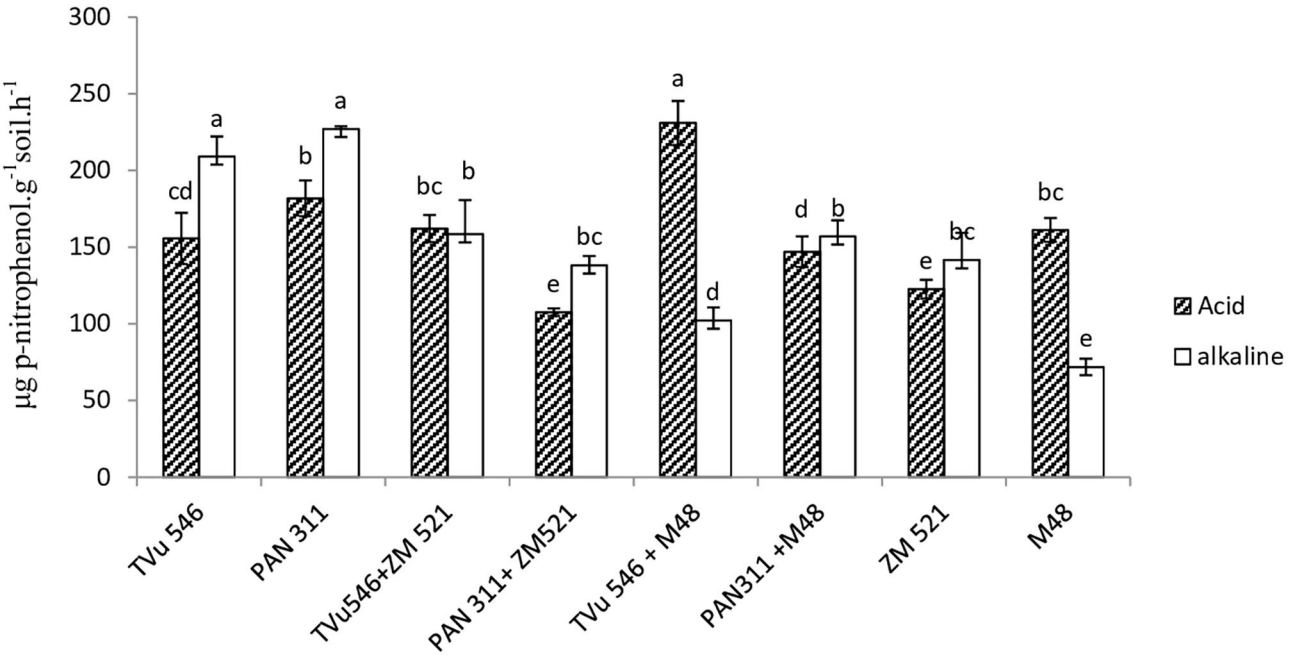

FIGURE 3 | Phosphatase activity in the rhizosphere of solo and intra-hole planted cowpea, sorghum, and maize. Bars with dissimilar letters are significantly different ($P \leq 0.05$) for each enzyme activity.

TABLE 1 | Information about number of generated raw sequences, quality sequences, chao1, and OTUs.

Sample name	Total bases	Raw read count	GC (%)	Q20 (%)	Q30 (%)	Chao1	Good coverage	OTUs
PAN311	53,424,001	118,025	55	96.99	87.46	806.09	0.98	642.0
TVu546	56,515,556	125,260	55.4	97.01	87.43	850.45	0.98	694.0
ZM 521	71,367,953	158,022	55.6	97.08	87.73	873.06	0.98	704.0
M48	54,653,244	120,691	55.4	97.1	87.73	847.43	0.98	694.0
PAN311+ZM521	77,105,901	170,531	55.2	96.99	87.43	923.34	0.98	780.0
TVu546+ZM521	76,938,622	169,559	54.1	96.99	87.48	910.79	0.99	739.0
PAN311+M48	63,795,130	141,302	55.5	97.0	87.44	832.36	0.98	721.0
TVu546+M48	78,365,292	173,180	54.8	97.11	87.78	906.85	0.99	778.0

or PAN 311 decreased AlkPase activity relative to sole-planted cowpea cvs. TVu 546 and PAN 311, but increased AlkPase activity when compared with sole-planted sorghum (**Figure 3**).

Analysis of Rhizosphere Microbial Community

The results of sequencing microbial DNA extracted from rhizosphere soils showed that the intra-hole planting system exhibited higher nucleotide sequences when compared to sole planting. Intra-hole planting of cowpea cv. TVu 546 with sorghum (cv. M 48) revealed the highest sequence bases (>78 million), while sole-planted cowpea cv. PAN311 recorded the lowest (<54 million). The highest GC (55.6%) was found in the rhizosphere metagenome sequences of maize (ZM 521), but Q20 and Q30 were highest in the rhizosphere metagenome of intra-hole planted cowpea (TVu 546) with sorghum (M 48) (**Table 1**).

A total of 1,176,570 raw reads were generated from analysis of the eight rhizosphere soil samples collected from sole and intra-hole planted maize, sorghum and cowpea. There were variations in read output across the analyzed samples, with >100,000 reads obtained per sample (**Table 1**). With subsequent analysis, however, 107,171 quality sequences were added which resulted in 11,677 as a median per sample with a range of 9523–22907. The read length was 300 bp. All quality sequences were aligned at >97% sequence identity using QIIME to determine operational taxonomic units (OTUs). The reads were all successfully classified as belonging to the domain bacteria. The coverage was between 98 and 99% for each of the eight test samples. The highest OTU (780) was found in the rhizosphere metagenome of cowpea (PAN 311) co-planted in the same hole with maize (ZM 521), and the

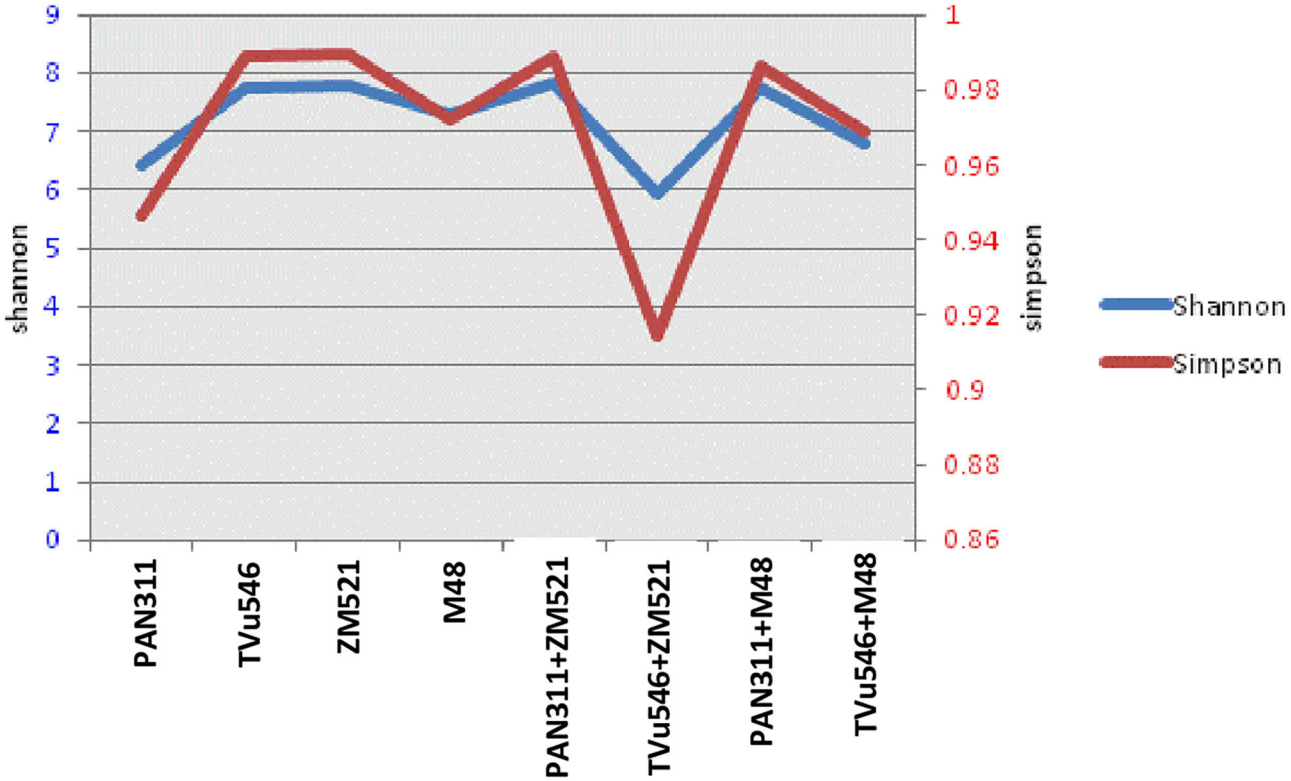

FIGURE 4 | Shannon/Simpson microbial community diversity index of test rhizosphere soil samples.

least (642) in sole-planted cowpea cv. PAN 311 (**Table 1**). Both the observed and estimated richness (Chao 1) were higher in the rhizosphere soil of cowpea (cv. PAN 311) planted in one hole with maize cv. ZM 521 (**Table 1**). The Shannon diversity index was 7.8 for rhizosphere soils from sole maize (cv. ZM 521) and intra-hole planted cowpea (cv. PAN 311) with maize, 7.7 for sole cowpea (cv. TVu 546) and cowpea (cv. PAN 311) co-planted with sorghum in one hole, 6.4 for rhizosphere soils of sole-planted cowpea (cv. PAN 311), 7.3 for sole sorghum, 5.9 for cowpea (cv. TVu 546) planted in the same whole with maize, and 6.8 for cowpea (cv. TVu 546) planted in one hole with sorghum (**Figure 4**).

The alpha rarefaction graph (**Figure 5**) for each soil sample did not reach the plateau phase, which indicates that more reads are required to capture all the diversity associated with the plateau part of the graph.

When the observed reads were assigned to high taxonomic ranks, the occurrence and relative abundance of different phyla suggested a strong dissimilarity among bacterial communities in all the rhizosphere soil samples tested. Except for cowpea (cv. PAN 311) co-planted with sorghum, the intra-hole planting system generally showed higher microbial phyla than sole cropping. Results showed that including undefined phylum the highest number of phyla (20) were found in the rhizosphere soil of intra-hole planted cowpea (cv. PAN 311) with maize, with the least number of phyla (16) detected in the rhizosphere soil of intra-hole planted cowpea (cv. PAN 311) with sorghum (**Figure 6**). The most dominant phyla in all the test samples were *Actinobacteria*, *Acidobacteria*, *Bacteroidetes*, *Firmicutes*, *Planctomycetes*, *Proteobacteria*, and *Verrucomicrobia,* as they accounted for >95% of the total bacteria observed. The microbial community profiles in the rhizosphere soils analyzed were not similar at the class, order and family levels due to little differences in the relative abundance of some bacteria. The rhizosphere soils of sole cowpea (cv. PAN 311), sole maize, and cowpea (cv. TVu 546) co-planted with maize showed 18 bacterial phyla, while sole cowpea (cv. TVu 546), sole sorghum and cowpea (cv. TuV546) intra-hole planted with sorghum revealed 17 bacterial phyla with some slight differences in relative presence and absence of certain bacterial phyla. For example, *Thermomicrobia* was absent in the rhizosphere soil of sole maize, as well as the two cowpea genotypes co-planted with sorghum (i.e., PAN311+M48 and TVu546+M48), but was present in the rhizosphere soils of sole-planted cowpea cultivars (i.e., cv. PAN 311 and TVu546), sole planted sorghum, and maize intra-hole planted with the two cowpea genotypes (i.e., PAN311+ZM521 and TuV546+ZM521). Similarly, *Fibrobacteres* was present in the rhizosphere soil of sole maize and maize intra-hole planted with the two cowpea cultivars (i.e., PAN311+ZM521, TVu546+M48), but absent in all other samples. Also, *Parcubacteria* was present in the rhizosphere soils of only sole sorghum and cowpea co-planted with maize. The phyla *Aerophobetes* and *Tenericutes* were present exclusively in the rhizosphere soils of intra-hole planted cowpea (cv. PAN 311) with maize, and sole-planted maize.

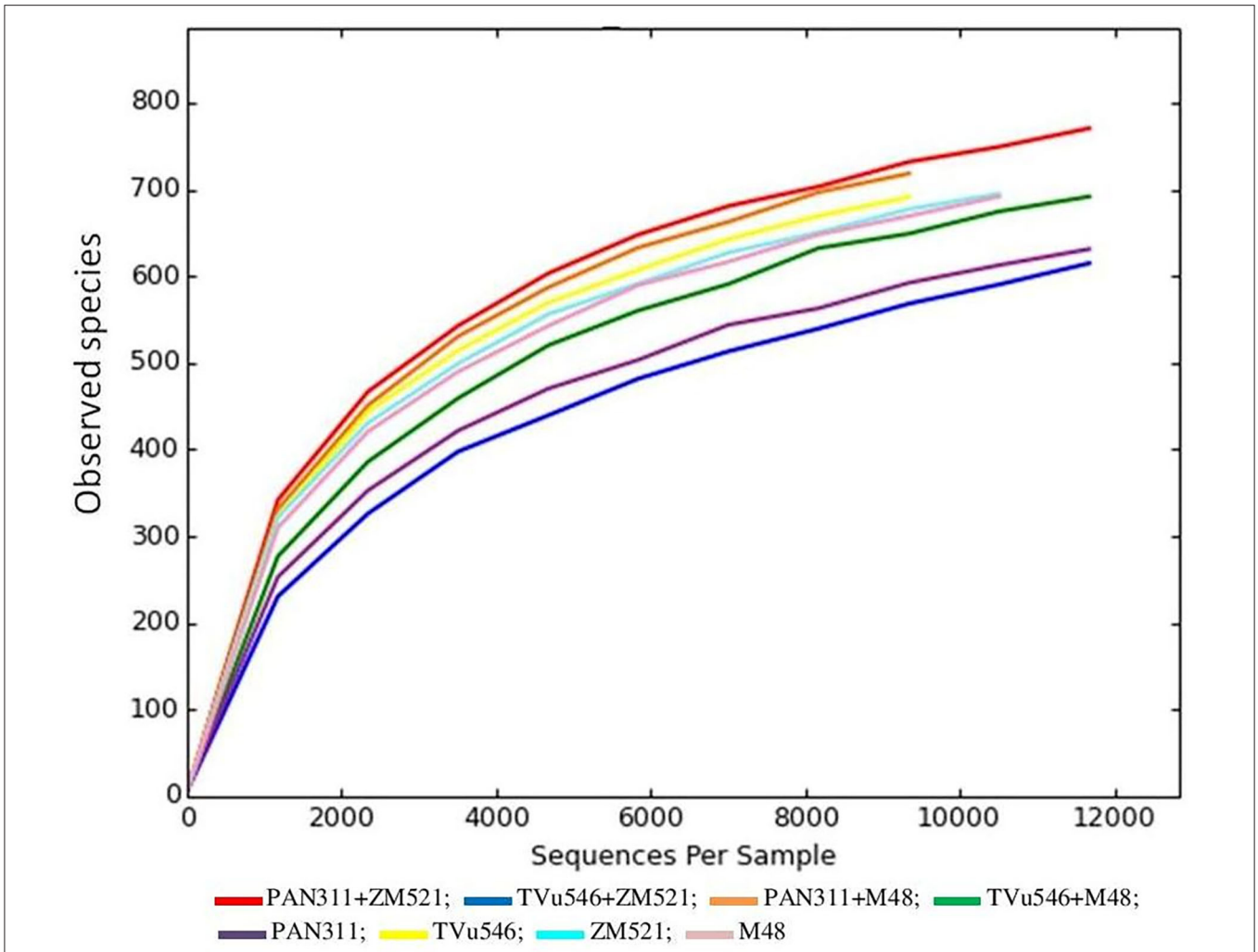

FIGURE 5 | Rarefaction curves of all samples were generated for observed microbial species which contained unique sequences and were defined at 97% sequence similarities.

At the class and order level, the *Sphingobacteria* was the most dominant microbial community present in all rhizosphere soils (except for the rhizosphere of sole cowpea (cv. PAN 311) which was dominated by Gammaproteobacteria). *Alphaproteobacteria* was the second dominant microbial community present in the rhizosphere soils of sole cowpea (cv. TVu 546) (16.44%), sole maize (17.03%), intra-hole planted cowpea (cv PAN 311) with maize (13.7%) and intra-hole planted cowpea (cv. PAN 311 with sorghum (14.7%). By proportions, *Sphingobacteria* represented 14.2% of the microbial community in the rhizosphere of sole cowpea (cv. PAN 311), Gammaproteobacteria 19.9% in the rhizosphere of sole-planted sorghum and *Flavobacteria* 14.5% in the rhizosphere of intra-hole planted cowpea (cv. TVu546) with maize. The phylum *Cyanobacteria* was present in the rhizosphere soils of all treatments, except cowpea (cv. TVu 546) intra-hole planted with maize.

At the genus level, *Lysobacter* was dominantly present in the rhizospheres of monocultured cowpea (cv. PAN 311) (20.9%) and sorghum (15.5%), as well as cowpea (cv. PAN311) co-planted in one hole with maize (7.4%), while *Sphingomonas* was dominant (8.1%) in the rhizosphere soil of sole-planted maize. In contrast, *Mucilaginibacter* was the most abundant genus in the rhizosphere soils of intra-hole planted cowpea (cv. TVu 546) with maize (27.7%) and cowpea (cv. TVu 546) co-planted in one hole with maize (11.9%). The genus *Flavobacterium* was dominant in the rhizosphere soils of sole cowpea (cv. PAN311) (8.9%), sole sorghum (1.2%), intra-hole planted cowpea (cv. PAN 311) with maize (4.6%), cowpea (cv. TVu546) co-planted with maize (10.0%), and intra-hole planted cowpea genotypes TVu 546 and PAN 311 with sorghum (7.2 and 8.4%, respectively). An undefined genus of the *Chitinophagaceae* family was the most abundant in rhizosphere soil of sole-planted cowpea cv. TVu 546. Despite the presence of diverse bacterial species in the test rhizosphere soils, most of them were classified as unculturable.

To compare microbial community variation between samples, principal coordinate analysis (PCoA) was done using weighted

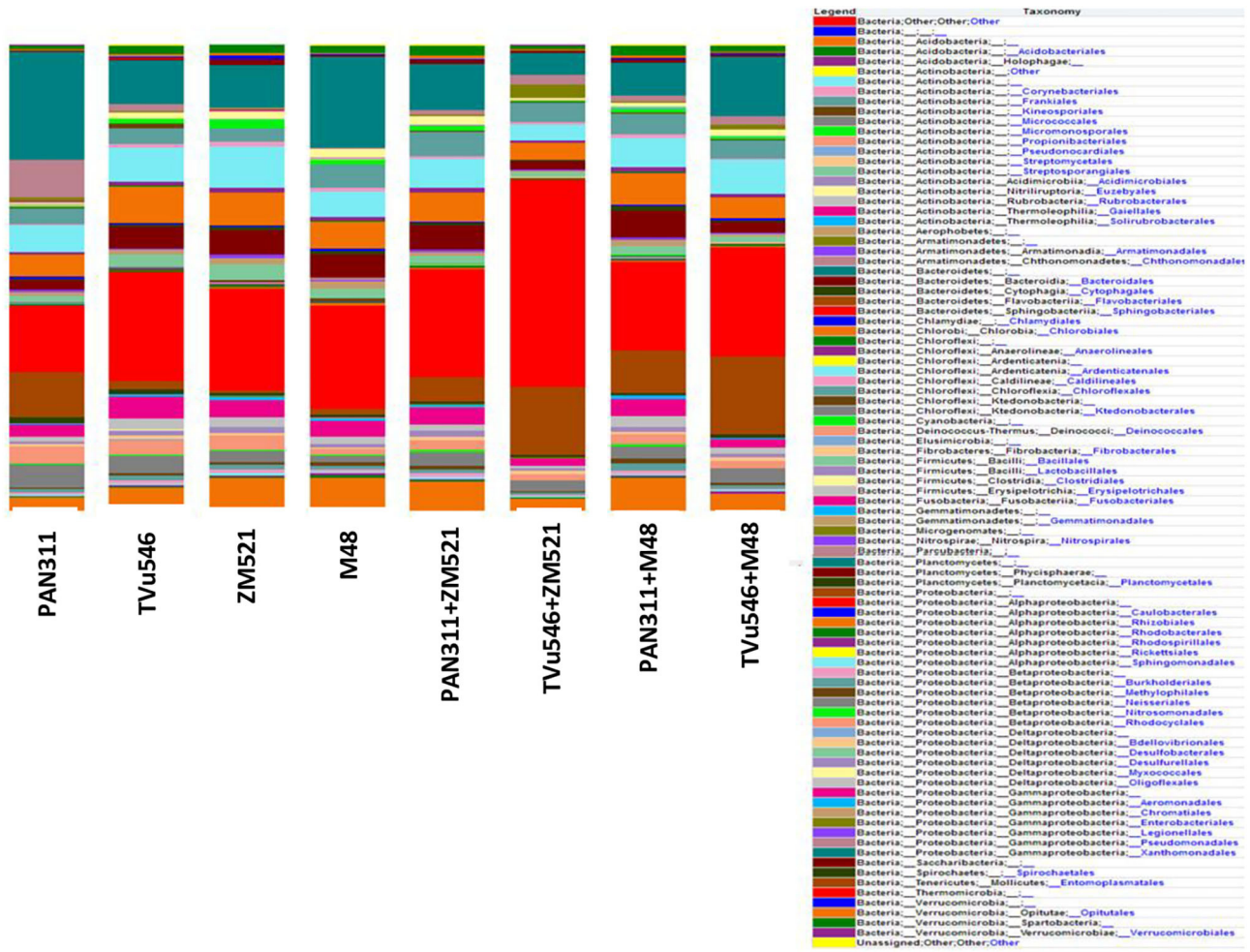

FIGURE 6 | Average distribution of 16S rRNA sequences classified at order level in rhizosphere of solo and intra-hole planted cowpea, sorghum, and maize.

UniFrac metric. The PCoA of the UniFrac metric illustrated clustering of soil microbial communities by number of sequences generated from each soil sample. Based on operational taxonomic units or OTUs (97% 16S rRNA identity), the results showed that the microbial communities were separated by rhizosphere soil of the test crop species and the cropping systems, indicating that there was a difference in the community composition (**Figure 7**). The two principal components (PC1 and PC2) accounted for 86.49% of the total microbial community variations in the rhizosphere soil samples tested. Despite this clear separation, the first axis in PC1 vs. PC2 explained 70.79% of the variance within the data, indicating that the relative abundance of the most OTUs was diverse between the rhizosphere soils of all the treatments.

The number of sequences attributed to each taxon was compared between and among the rhizospheres of the test treatments based on distance matrix estimated with UPGMA algorithm represented in the phylogenetic tree (**Figure 8**). The results yielded one major cluster with two subclusters (Subcluster Ia and Ib). The microbial communities from the rhizosphere of sole-planted sorghum, sole cowpea (cv. PAN 311), and intra-hole planted cowpea (cv. TVu 546) with maize and sorghum stood separately, which clearly indicated the presence of diverse microbial community structures in the rhizosphere soils analyzed. The results further showed that the microbial communities in the rhizosphere soils of cowpea (cv. PAN 311) co-planted with sorghum or maize in one hole (Subcluster 1a), or sole-planted cowpea (cv. TVu546) and maize (Subcluster 1b) were very close when compared to the other cropping systems. The results of this study therefore suggest that the changes in microbial community structure in the rhizosphere soil samples were most distinct in intra-hole cropping systems relative to monocultures.

DISCUSSION

Effect of Cropping System and Plant Species on P-Enzyme Activity in the Rhizosphere

Rhizosphere functioning is plant species-dependent, but can be altered by many biological processes, including soil enzyme activity, microbial numbers, root border cells, mineral nutrient

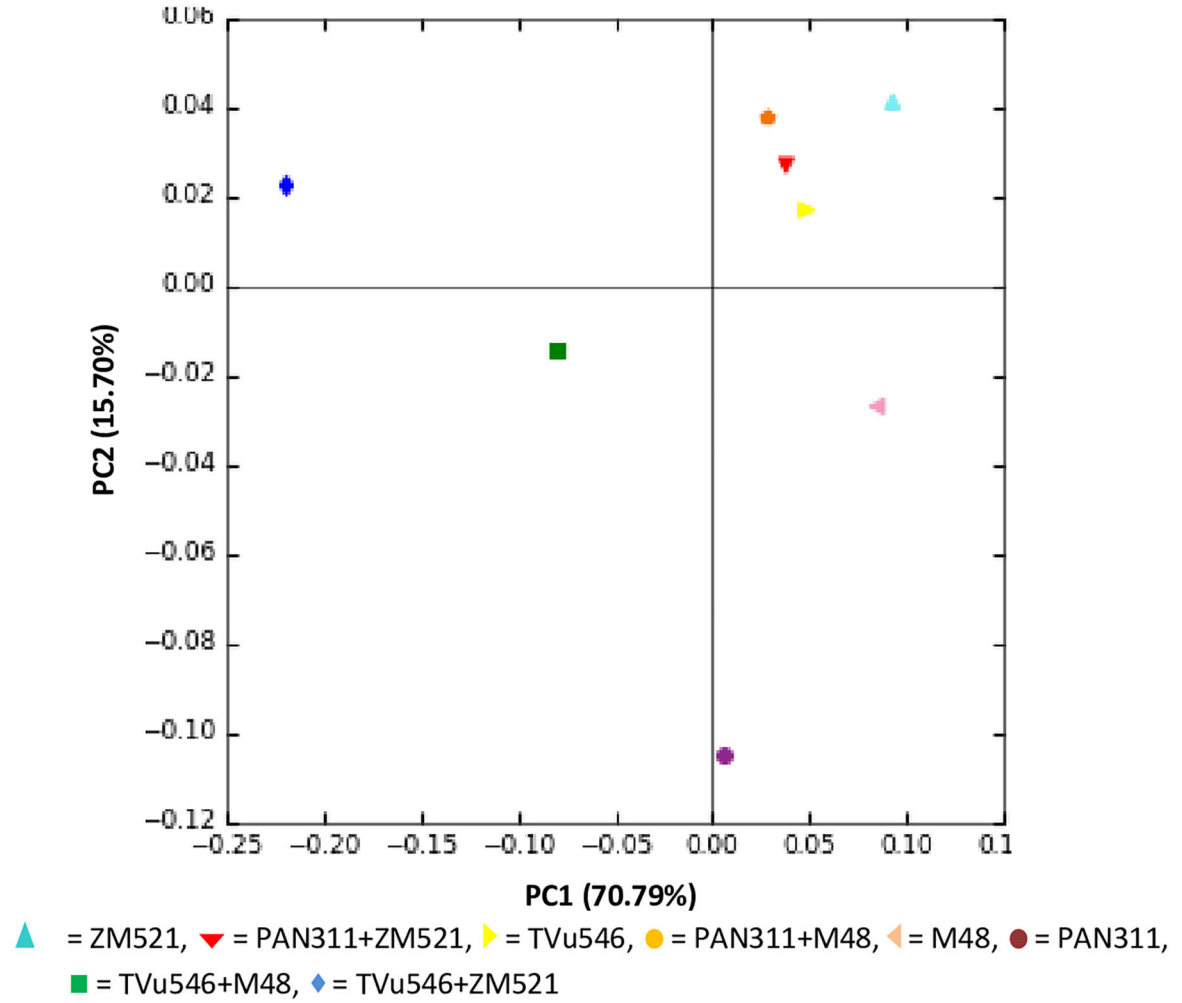

FIGURE 7 | Principal Co-ordinate analysis (PCoA) of microbial communities based on OTUs for all samples from rhizosphere of solo and intra-hole planted cowpea, sorghum, and maize.

concentrations, and root exudates (Dakora and Phillips, 2002; Jaiswal et al., 2019). Soil nutrient profiles are often affected not only by plant species but also by soil enzymes and root border cells, and could be bio-indicators for soil fertility (Dick and Tabatabai, 1993; Dick et al., 2000). For example, soil enzymes such as APase and AlkPase, whether in plants or soils, are biomarkers for P nutrition, especially in nodulated legumes (Maseko and Dakora, 2013).

In this study, APase and AlkPase activities in the rhizosphere of monocultures of the two cowpea genotypes were generally higher than those of the cereals, be it sorghum or maize (**Figure 3**). This is consistent with the findings by Li et al. (2004) which showed that chickpea secreted greater amount of APase than maize. Izaguirre-Mayoral et al. (2002) also found that phosphatase activity in plants can differ with crop species and varieties. The higher phosphatase activity in the rhizosphere of cowpea was not unexpected as nodulated legumes have a greater P requirement to support symbiotic functioning in root nodules when compared to non-legumes (Israel, 1987; Maseko and Dakora, 2013).

These enzyme activities were however altered by the cropping system. For example, co-planting of cowpea cv. TVu546 with sorghum in one hole significantly increased APase activity, whether compared with sole cowpea or sole sorghum, and is consistent with the results of other intercropping studies involving legumes and cereals (Li et al., 2004; Inal et al., 2007). It was further found that intra-hole cropping of cowpea cv. TVu546 with sorghum or maize showed greater enzyme activity relative to both sole legume and cereal. In contrast, co-planting cowpea cv. PAN 311 with any of the two cereals lowered APase activity relative to sole legume and cereal (**Figure 3**). Because APase activity of sole cowpea cv. TVU 546 was less than that of cv. PAN 311, the former would require higher APase activity to meet its P needs when compared to the latter. These results can therefore be interpreted to mean that intra-hole cropping of cowpea cv. TVU 546 with sorghum or maize optimizes P nutrition in both

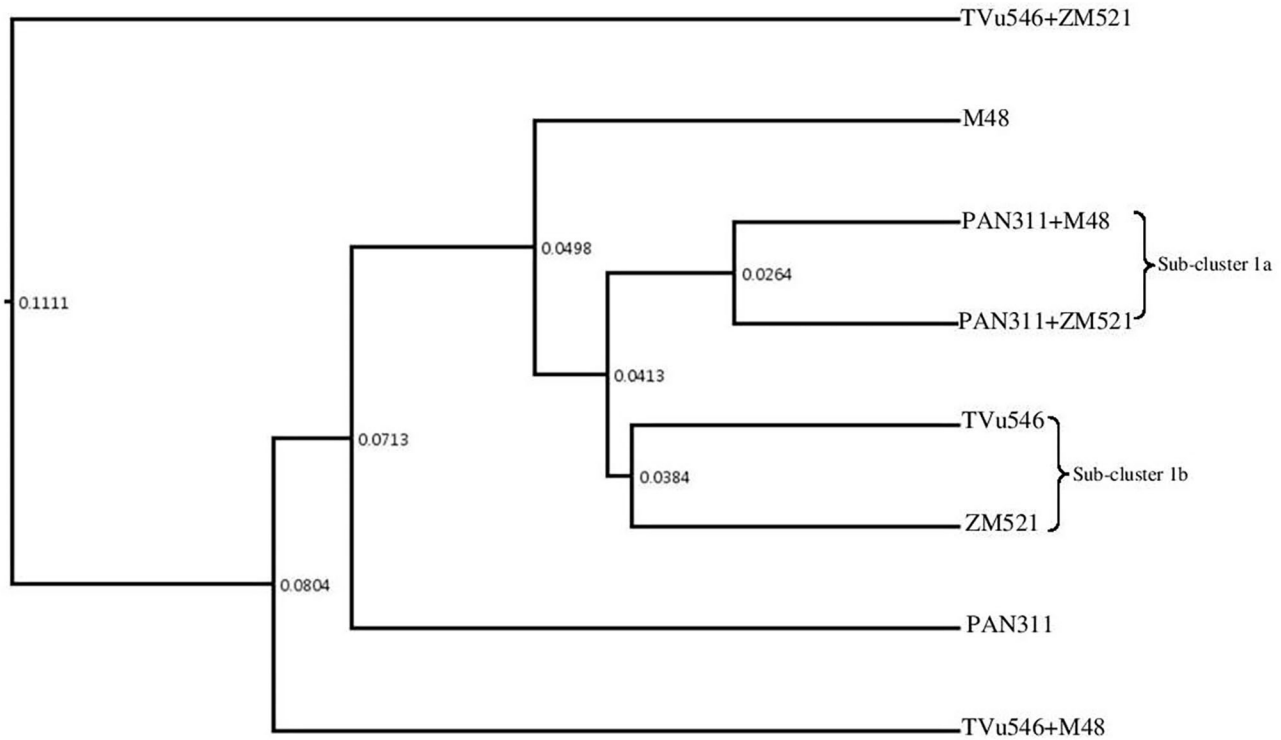

FIGURE 8 | Hierarchical cluster analysis based on distance matrix with the UPGMA algorithm for eight rhizosphere metagenomes of solo and intra-hole planted cowpea, sorghum, and maize.

the cereal and cowpea cv. TVU 546 via plant-plant interaction in the rhizosphere. Other studies have also suggested that the legume facilitates P availability for uptake by the cereal partner in the cropping system (El Dessougi et al., 2003; Li et al., 2004).

Rhizosphere AlkPase activity was much higher than APase activity in the rhizospheres of monocultured cowpea cv. TVu 546 and cv. PAN 311 (**Figure 3**), implying that microbial biota which are responsible for secreting AlkPases in the rhizosphere contributed significantly to P nutrition of the cowpea plants. The AlkPase activity in the rhizosphere soil of intra-hole planted cowpea cv. TVu 546 with maize or sorghum was always lower than the sole legume, but higher than sole cereals, a finding consistent with a report by Makoi et al. (2010). As a result, co-planting of cowpea cv. PAN 311 in one hole with sorghum increased AlkPase activity relative to sole cereal, but decreased it when compared to sole cv. PAN 411.

Effect of Cropping System and Plant Species on Mineral Accumulation in the Rhizosphere

In this study, there were strong species differences in mineral accumulation in the rhizosphere of cowpea, maize and sorghum. In general, the mineral concentrations (Na, Mg, Ca, S, Fe, and Cu) in the rhizosphere of sole cowpea were much lower than maize and sorghum. This is not unexpected as the symbiotic process in legumes imposes a greater demand on the host plant for enhanced mineral nutrition to support N_2 fixation (O'Hara, 2001). It has also been shown that high N_2 fixation in legumes generally results in greater mineral uptake from the soil compared to low-fixing symbiosis (Belane et al., 2014).

In this study, available P in the rhizosphere soil of the test species ranged from 5.5 to 10.0 mg/kg, with cowpea cv. PAN 311 recording the highest P (10.0 mg/kg; **Figure 2**). P supply in the rhizosphere is influenced by a number of factors, including the exudation of P-enzymes. As shown in **Figure 1**, cowpea cv. PAN 311 secreted more APases an AlkPases than all the test species, and this can explain the high P concentration in the rhizosphere of cv. PAN 311. However, the activity of P-solubilizing rhizobia has also been reported to increase P availability in rhizosphere soils of legumes (Rodríguez and Fraga, 1999). The fact that intra-hole planting of maize with cowpea increased P concentration in the rhizosphere relative to sole maize and sole cowpea cv. TVu 546, but decreased it when compared to sole maize and cowpea cv. PAN 311 (**Figure 2**) could suggest differences in microbial populations within the rhizospheres of the two systems. Any variation in the microbial community can affect P-solubilization and phosphatase secretion, and thus affect P availability.

The level of Ca and Mg were greater in the rhizospheres of sole maize and sorghum compared to monocultured cowpea cultivars TVu 546 and PAN 311. While this could be species-dependent, N_2-fixing legumes tend to accumulate more mineral nutrients than cereals (Gardner and Boundy, 1983). Intra-hole planting of sorghum or maize with each of the cowpea cultivars decreased

rhizosphere Ca relative to monocultures of sorghum, cowpea cv. TVu 546 and cv. PAN 311. This can be attributed to plant-plant competition by the species for nutrient uptake in the rhizosphere.

In this study, soil Cu levels were more than sufficient to support plant growth. Rhizosphere concentration of Fe and Zn varied among the treatments, but were generally higher in the rhizosphere of sole-planted maize and sorghum than the two cowpea genotypes, possibly due to greater uptake by the legume for symbiotic functioning. Intra-hole planting of sorghum or maize with cowpea cultivars TVu 546 and PAN 311 increased Fe levels in the rhizosphere relative to sole cowpea (**Figure 1**). While this could be attributed to increased secretion of phytosiderophores by the plant partners for Fe mobilization, the microsymbionts nodulating the legume probably also produced extra bacterial siderophores for increased Fe availability (Xue et al., 2016). Whatever the case, positive changes in mineral nutrient concentrations in the rhizosphere can be regarded as one of the benefits of legume/cereal intercropping systems.

Response of Rhizosphere Microbial Community to Cropping Systems

Plants and microorganisms are intricately inter-linked for growth and survival. Plant interactions with rhizosphere microbial community play a crucial role in agricultural productivity, in addition to contributing to the resilience of the ecosystem to climatic stress. Understanding plant/microbe interactions could reveal novel ways of using these microbes to promote plant health and productivity, and thus change ecosystem functioning. In this study, we performed sequence analysis of the bacterial communities in the rhizospheres of sole and intra-hole planted cowpea and cereals using the MiSeq Illumina method. The results revealed the presence of many unculturable bacterial populations in the plant rhizospheres, as earlier reported by Jaiswal et al. (2019). There were distinct bacterial community members across the rhizosphere environments of both sole and intra-hole cropped plants, which probably denoted differences in bacterial community function.

Although the reads were applied to species level, the sequences were summarized and classified according to bacterial phylum to ascertain the overall composition of the microbial community at a high phylogenetic resolution. The results showed differences in microbial communities between rhizosphere soils of sole and intra-hole planted cowpea with maize and sorghum. The differences in OTU richness, both within and among phyla, increased non-linearly with test plant species which suggested that selection of rhizosphere microflora was highly sole and intra-hole specific (Micallef et al., 2009; Turner et al., 2013). Although intra-hole planted cowpea cv. PAN 311 with maize recorded the highest number of phyla (20), cv. PAN 311 with sorghum showed only 16 microbial phyla. This suggests that specific plant combinations define the bacterial community composition in the rhizosphere, and this is probably influenced by plant-related changes in the soil such as root exudation (Marschner et al., 2001; Micallef et al., 2009; Jaiswal et al., 2019). In fact, root exudates are a crucial determinant of rhizosphere microbial diversity (Lynch and Whipps, 1990; Barea et al., 2005; Badri and Vivanco, 2009). The results also suggest that microbial biomass is more likely to increase where intra-hole cropped plant partners share close root proximity and/or exhibit intermingling of their roots.

Though present in the rhizosphere soil of sole-planted cowpea cv. PAN 311 and cowpea cv. TVu 546 co-planted with maize in the same hole, the phylum *Thermomicrobia* was absent in the rhizosphere soil of sole maize. Similarly, *Fibrobacteres* was present in the rhizosphere of sole maize, but absent in the rhizosphere soil of monocultured cowpea cv. PAN 311 and cowpea cv. TVu 546 co-planted with maize. The phylum *Parcubacteria* was also present in the rhizosphere of sole sorghum, but absent in sole cowpea cv. TuV 546 and cv. TVu 546 co-planted with sorghum. However, the phyla *Aerophobetes* and *Tenericutes* were exclusively present only in the rhizosphere soils of intra-hole planted cowpea cv. PAN 311 with maize, and sole-planted maize. The presence or absence of microbial phyla in some plant rhizospheres, but not in others, can be interpreted in number of ways. Components of plant root exudates generally serve as a source of carbon for many bacteria, and as chemoattractants for mutualistic microbes (Aguilar et al., 1988; Compton et al., 2020), often leading to the establishment of symbiotic relationships. In contrast, some components of plant root exudates also serve as defense molecules for warding off soil-borne pathogens (Dakora et al., 1993a,b). Various studies (Dakora et al., 1993a,b) have shown that both pathogens and rhizobial symbionts can elicit phytoalexin exudation in the rhizosphere of N_2-fixing legumes, and thus control populations of other microbes. Whether in this study any of these scenarios functioned to create the observed differences in rhizosphere microbial communities remains to be determined. Additionally, pioneer microbes in the rhizosphere can also produce antibiotics that reduce the populations of other microbial communities, but again, this was not assessed in this study. Similarly, the fact that the rhizospheres of the two test cowpea cultivars (cv. PAN 311 and cv. TVu 546) revealed different bacterial phyla can be attributed to differences in the profile of seed and root exudates produced (Tsamo et al., 2018, 2020). Root exudate composition can vary with plant taxa, and even within closely related plant species, as well as with genotypes and accessions of the same species (Czarnota et al., 2003; Warembourg et al., 2003; Micallef et al., 2009).

Cropping system *per se* has been reported to have an effect on rhizosphere microbial populations. For example, rhizosphere bacterial counts were found to increase with intercropping, and reportedly greater in intra-row than inter-row planting (Wahua, 1984). Because legumes and cereals release root exudates with different chemical profiles into their rhizospheres, intercropping and/or crop rotations are likely to significantly alter rhizosphere microbial communities (Alvey et al., 2003). Except for sole-planted cowpea cv. PAN 311, *Sphingobacteria* was the dominant microbial group in the rhizosphere soil of all the other cropping systems, followed by *Alphaproteobacteria*, which is home to the many rhizobial symbionts nodulating legumes. Similarly, all rhizosphere soils except that of cowpea cv. TVu 546 co-planted with maize showed high proportions of Cyanobacteria. These major groups were earlier identified in a PCR-based and metatranscriptomic study of both bulk and rhizosphere soils

(Urich et al., 2008; Inceoglu et al., 2011; Lundberg et al., 2012; Turner et al., 2013). Despite the fact that soil remains the richest microbial ecosystems on earth, our study has revealed that only a few bacterial phyla (namely, *Actinobacteria*, *Acidobacteria*, *Bacteriodetes*, *Firmicutes*, *Planctomycetes*, *Verrucomicrobia*, and *Proteobacteria*) actually colonize the rhizosphere, a finding similar to the results of earlier studies (Peiffer et al., 2013; Figuerola et al., 2015; Shi et al., 2015; Zarraonaindia et al., 2015; Coleman-Derr et al., 2016; Jaiswal et al., 2019). In this study, the major contributors to microbial community diversity in the rhizosphere included the *Flavobacteriales*, *Sphingobacteriales*, *Bacillales*, *Phycisphaerae*, *Rhizobiales*, *Sphingomonadales*, *Burkholderiales*, and *Xanthomonadales*, which are well-known for their mutualistic interactions with plant roots in the rhizosphere (Lu et al., 2006). Recently, Yang et al. (2016) found that intercropping of groundnut with sorghum was beneficial to the composition of bacterial communities in the rhizosphere.

In addition to identifying rhizobia and other beneficial bacterial taxa in this study, we also found the highest community of *Lysobacter* in the rhizosphere of sole-planted cowpea cv. PAN 311 (20.8%) and sorghum (15.5%), as well as in the rhizosphere of cowpea cv. TVu 546 co-planted with sorghum in one hole (10.8%) and cv. PAN 311 intra-hole planted with maize (7.4%). The genus *Lysobacter* is widely distributed in soil and has high P-solubilizing ability (Reichenbach, 2006). Its dominance in the rhizosphere of cv. PAN 311 probably explains the highest AlkPase activity observed in the rhizosphere soil of cv. PAN 311 (see **Figure 3**).

Furthermore, *Lysobacter* is reported to be an important source of new enzymes such as chitinases, glucanases, proteases, lipases, elastases, keratinases, phosphatases, endonucleases, endoamylases, and esterases (Zhang et al., 2001; Folman et al., 2003; Palumbo et al., 2005; Reichenbach, 2006; Stepnaya et al., 2008; Ko et al., 2009; Gökçen et al., 2014; Vasilyeva et al., 2014) that promote plant growth and enhance defense against pathogens (Gómez Expósito et al., 2015). Crop treatment with *Lysobacter* has been shown to enhance plant defense and decrease diseases in bean (Zhang et al., 2001), rice (Ji et al., 2008), pepper (Ko et al., 2009), cucumber (Folman et al., 2004; Postma et al., 2009) grapevine (Puopolo et al., 2014), and sugar beet, spinach (Islam et al., 2005).

The rhizosphere of sole-planted cowpea cv. PAN 311 was dominated by 8.9% *Flavobacterium,* and cv. PAN 311 co-planted with sorghum by 8.4%. The bacterium occurs in soil and other habitats (Bernardet and Bowman, 2006), and has extracellular macromolecular-degrading enzymes (amylase, cellulose, chitinase, peptidases and glycoside hydrolases) which enable it to digest degradable polymers like starch or chitin and therefore plays an important role in the degradation of bacteria, fungi, insects and nematodes (Peterson et al., 2006). Additionally, 70% of the rhizosphere carbon turnover is due to *Flavobacterium* (Johansen et al., 2009). As a result, *Flavobacterium* is a major member of the microbial community in the rhizosphere of pepper (Graber et al., 2010), lettuce (Cardinale et al., 2015), peanut (Haldar et al., 2011), and *Arabidopsis thaliana* (Lundberg et al., 2012; Bodenhausen et al., 2013). Recently, Qin et al. (2016) reported 0.9–8.5% *Flavobacterium* genera in wheat rhizosphere, and its presence correlated positively with plant biomass (Manter et al., 2010), plant disease resistance (Kolton et al., 2014), and bio-control against *Phytophthora capsici* in pepper (Sang et al., 2008). *Flavobacterium* also contains ACC deaminase activity for promoting plant growth (Maimaiti et al., 2007), in addition to the ability of some *Flavobacterium* isolates to elevate Rubisco gene expression and increase plant biomass in wheat and barley (Flynn et al., 2014).

The genus *Mucilaginibacter*, a member of the family *Sphingobacteriaceae* (Steyn et al., 1998; Pankratov et al., 2007) and phylum *Bacteroidetes*, was more abundant by 27.1 and 11.9%, respectively, in the rhizospheres of intra-hole planted cowpea cv. TVu546 with maize and sorghum. They are extracellular polysaccharide (EPS)-producing, plant-growth-promoting bacteria that were also isolated from cotton rhizosphere soil (Madhaiyan et al., 2010).

However, bacterial genera such as *Pedobacter*, *Pseudomonas*, *Flavisolibacter*, *Bacillus*, *Rhizobium*, *Sphingomonas*, and *Streptomyces,* which are beneficial microbes, were present in all the rhizosphere soils studied, thus indicating their general presence in the rhizosphere of crop species. A few studies have implicated *Bacillus* in the solubilization of Zn, Cu, and K in the rhizosphere (Pirhadi et al., 2016; Sunithakumari et al., 2016). Functionally, *Sphingomonas* is associated with IAA production in the rhizosphere of soybean for enhanced plant growth (Sugiyama et al., 2014), *Pseudomonas*, *Bacillus*, and *Rhizobium* with P solubilisation in the rhizosphere (Rodríguez and Fraga, 1999), and *Streptomyces*, *Bacillus*, and *Pseudomonas* with siderophore production for enhanced Fe mobilization (Matsuoka et al., 2013).

Although several studies have been undertaken on intercropping of legumes and cereals, few (if any) have addressed belowground processes involving critical analysis of the cowpea-sorghum intra-hole cropping system in terms of microbial community structure and mineral nutrition. This is possibly because data obtained from rhizosphere studies of mixed-cultured systems are often complex and difficult to interpret when compared to monocultures. The rhizosphere is home to millions of different microbial phyla, genera, families and species; and the complexity of this system increases with intercropping, especially with intra-hole planting where roots of legumes and cereals are closely interwoven and intermingled. In such an intimate system, there are plant-plant, plant-microbe and microbe-microbe interactions that are constantly occurring in the rhizosphere with different outcomes from mutualism and warfare. These interactions involve chemical products present in plant and microbial exudates. According to Dakora and Phillips (2002), plant root exudates consist of a complex mixture of enzymes, root border cells, phytosiderophores, phenolics, organic acids, gaseous molecules, organic and inorganic ions, as well as mineral nutrients, which are important for defense and promoting mineral uptake by roots. Soil microbes similarly secrete enzymes, organic acids and anions, siderophores and antibiotics for improving mineral nutrition and self-defense (Dakora and Phillips, 2002). For example, typical pathogens like *Ralsotonia* and *Agrobacterium* were completely absent in the rhizosphere of intra-hole cropping system possibly due to elimination by antibiotics and/or phytoalexins present in

plant and microbial exudates (Dakora and Phillips, 1996). It is also therefore likely that the *Pseudomonas* and *Xanthomonas* species found in the *Proteobacte*ria phylum in this study were the beneficial rather than pathogenic species, given the greater abundance of mutualistic microbes such as *Flavobacteriales*, *Sphingobacteriales*, *Bascillales*, *Phycisphaerae*, *Rhizobiales*, and *Sphingomonadales* found in this same study. Dang et al. (2020) found similar changes in some bacterial taxa, especially Proteobacteria, in a mung bean-millet intercropping system. Even with a tomato/potato-onion intercropping devoid of legumes, there was an alteration in soil microbial communities (Li et al., 2020), suggesting that changes in rhizosphere microflora is not unique to legume-cereal systems, but more likely an outcome of the composition of root exudates released by the intercropped partners.

In conclusion, intercropping of legumes with cereals is an old cultural practice perceived with the lens of "old science." With new tools and techniques, however, more data can be generated on belowground processes that would advance our understanding of both plant-plant and plant-microbe interactions in the rhizosphere with potential for increasing crop yields. In this study, APase and AlkPase enzyme activity, mineral nutrient accumulation and microbial community structure in the rhizosphere were influenced by crop species (legume and cereals), as well as cropping system. The cropping system promoted an increase in the numbers and diversity of some functional and unculturable rhizosphere microbes. Intra-hole cropping significantly altered rhizosphere microbial community structure when compared with monoculture.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/, PRJNA397662.

AUTHOR CONTRIBUTIONS

SJ executed molecular work and drafted the manuscript. MM did soil analysis. FD conceived the idea, edited and approved the final version of the paper. All authors contributed to the article and approved the submitted version.

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Estimating Soil Loss for Sustainable Crop Production in the Semi-deciduous Forest Zone of Ghana

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Quantitative information on soil loss is relevant for devising soil conservation and crop management strategies to ensure sustainable fertility management and crop production. Estimations from runoff/erosion plots are expensive and laborious and thus requiring the exploration of other less expensive but reliable methods such as modeling. This study aimed to estimate current and future rates of soil loss for conservation planning toward sustainable crop production in the semi-deciduous forest zone of Ghana. The Universal Soil Loss Equation (USLE), which took into consideration the rainfall characteristics of the study area, inherent soil physicochemical and hydraulic properties, variations in slopes and terrain differences, land cover types, and soil management practices, was employed to estimate the magnitude and rate of soil loss in the study area. Output from three regional climate models (RCMs) from Coordinated Regional Climate Downscaling Experiment including CCCma-CanESM2, GFDL-ESM2M, and HadGEM2-ES were used to estimate the impact of climate change on soil erosion in the study area. The results showed that soil loss estimated for bare soils was high ranging from 12.7 to 163.8 t ha^{-1} year^{-1} largely due to variation in slopes coupled with soil physicochemical and hydraulic properties. The simulated annual soil losses under various land cover options showed variable degrees of soil loss for maize cultivation under conventional tillage (8.2–106.5 t ha^{-1} year^{-1}), soya bean monocropping (4.4–57.3 t ha^{-1} year^{-1}), and low soil loss for oil palm plantation with grass or leguminous cover (2.5–32.8 t ha^{-1} year^{-1}). Evaluation of the RCMs showed excellent performance for CCCma-CanESM2 and GFDL-ESM2M. Predictions of climate change impact using outputs from CCCma-CanESM2 and GFDL-ESM2M indicated that 9–39% increase in soil loss is expected by 2070, and it will be more severe (16–42%) by 2100. The model predictions indicate that the adoption of site-specific land cover management strategies such as tree–cover crop intercropping and reduced tillage has a huge potential to reduce soil loss and sustain soil fertility. The model can be used as an advisory tool for mapping areas for appropriate cropping systems for a particular site.

Keywords: soil loss, universal soil loss equation, climate change, land use land cover, modeling, semi-deciduous forest of Ghana

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INTRODUCTION

Achieving a land degradation-free world by 2030 requires the implementation of urgent actions including the accurate estimation of the extent of soil erosion toward restoring degraded land and soils (Griggs et al., 2013; Keesstra et al., 2018a). These mostly short-term actions aimed at providing sustainable solutions should be embedded in long-term landscape planning, as the realization of the aforementioned United Nations' Sustainable Development Goal (SDG) of land degradation neutrality and restoration (i.e., SDG 15.3) requires a transition toward integrated nature-based solutions founded on an eco-socioeconomic system analysis (Keesstra et al., 2018a). Furthermore, a combination of initiatives, steering of knowledge flows, and regular assessment of the status of the transition process for planning further steps in the transition framework are crucial for achieving the SDGs by 2030 (Visser et al., 2019). Soil erosion, the most visible and widespread form of land degradation, coupled with low fertilizer application is the major cause of nutrient depletion in most sub-Saharan African countries resulting in low agricultural productivity and thereby a threat to food security (Obalum et al., 2012; Bashagaluke et al., 2018). The on-site effect of soil erosion on arable land is evidenced by the loss of the topsoil that supports crop productivity (Stocking, 2003). Apart from the reduction in soil depth, soil loss almost always occurs alongside depletion of soil organic matter and plant nutrients referred to as fertility erosion (Mesele, 2014).

In sub-Sahara Africa (SSA), soil degradation is driven by a myriad of interrelated natural and anthropogenic factors including, but not limited to, high population growth and unemployment rate, agricultural expansion and intensification, poor agronomic practices, land tenure insecurity, and climate change impact (Tully et al., 2015; Nkonya et al., 2016; Borrelli et al., 2020). Sand winning from inland valleys and lowlands, whose naturally nutrient-rich status supports livelihoods of smallholder farmers through year-round crop production and the provision of ecosystem services, is currently a major problem. Inland valleys (i.e., "flat-floored and shallow valleys that occur in the extensive plains and plateaus found across the African landscape") and lowlands cover an area of 1.9 M ha in Ghana and 190 M ha in SSA and support the production of food crops like rice, maize, sorghum, cowpea, soybean, vegetables, oil palm, cocoa, oranges, cassava, yam, plantain, and mangoes (Namara et al., 2012). Unfortunately, the aforementioned potential of inland valleys and lowlands is being threatened by indiscriminate sand winning across SSA (Aromolaran, 2012; Salifu, 2016). In Ghana, poor agronomic practices such as plowing along the slope and low fertilizer use by poor smallholder farmers result in increased soil erosion and nutrient mining, respectively (Buri et al., 2015). Excessive burning of vegetation (during land preparation) and biomass after harvesting is another farmer practice that degrade the soil by killing beneficial macroorganisms (e.g., mites, earthworms, millipedes, etc.) and microorganism (e.g., fungi) living in it (Tully et al., 2015) as well as oxidization of some important elements such as nitrogen (N), phosphorus (P), and potassium (K). The occurrence of bushfire is prevalent in northern Ghana, especially in the dry season when the vegetation is very dry. The region is consequently prone to wind erosion. Overgrazing also causes soil degradation in SSA, and especially in nomadic communities (Nkonya et al., 2016). Uncontrolled grazing by livestock renders the soil bare and prone to water and wind erosion (Tully et al., 2015; Zingore et al., 2015). According to the FAO and ITPS (2015) report, overgrazing accounts for 50% of physical soil degradation in Africa. Land tenure insecurity is a primary driver of soil degradation in Ghana and SSA at large. Smallholder tenant farmers are unwilling or averse to investing in good but expensive and/or laborious sustainable land management practices (Namara et al., 2012; Vlek et al., 2017), as they are not sure if they could keep their croplands in the next season. Land owners could easily abrogate a contract or amend it for their own interest engendering insecurity among migrant or tenant farmers.

Soil erosion (by water) is the largest threat to soil productivity in SSA, as it could occur gradually over a long period of time and elude detection until its adverse impacts are realized in low crop yields (Obalum et al., 2012; Desta et al., 2021). Soil erosion by water has a greater potential to limit soil productivity in most African regions characterized by humid tropical climate due to the torrential nature of rainfall in those regions (Obalum et al., 2012) as well as across the Mediterranean region (Rodrigo-Comino et al., 2020; Novara et al., 2021).

Although very few studies have estimated rates of soil loss from agricultural lands in Ghana (e.g., Owusu, 2012; Badmos et al., 2015; Bashagaluke et al., 2018, 2019), to the best of our knowledge, none has predicted the impact of climate change on soil erosion considering increasing rainfall variability in the region.

Methods commonly used for soil erosion estimation including runoff/erosion plots (Bashagaluke et al., 2018, 2019) and remote sensing and modeling (Owusu, 2012; Badmos et al., 2015; Gelagay and Minale, 2016; El Jazouli et al., 2017; Diwediga et al., 2018) are either laborious and expensive or qualitative (and therefore subjective). The Universal Soil Loss Equation (USLE) developed by Wischmeier and Smith (1978), which predicts long-term average annual soil loss comes in handy to provide easy but rigorous quantitative assessment of the magnitude of erosion, which is a prerequisite in designing an appropriate strategy for controlling soil erosion. According to Benavidez et al. (2018), USLE and related models including the Revised Universal Soil Loss Equation (RUSLE), the Revised Universal Soil Loss Equation version 2 (RUSLE2), and the Modified Universal Soil Loss Equation (MUSLE) have been applied globally to improve the estimation of soil loss. Furthermore, regional climate models (RCMs) from the Rossby Center Regional Atmospheric model (RCA4) and the Coordinated Regional Climate Downscaling Experiment (CORDEX) project at 44 km spatial resolution have been used in projecting climate change impact in many part of Africa (Vanvyve et al., 2008; Giorgi et al., 2009; Hewitson et al., 2012; Laprise et al., 2013; Dosio and Panitz, 2016; Nikiema et al., 2016; Sylla et al., 2016; Kisembe et al., 2018) and in Ghana (Owusu and Kluste, 2013; Bessah et al., 2018, 2020; Okafor et al., 2019).

From the foregoing context, this study aimed to estimate current and future rates of soil loss for soil conservation planning toward sustainable crop production in the Semi-deciduous Forest Zone of Ghana. The objectives of the study, therefore, include the following: (i) to quantitatively estimate soil loss under different land cover and soil management options using the USLE and (ii) to assess the impact of climate change and variability on soil erosion using outputs of applicable RCMs from CORDEX including CCCma-CanESM2, GFDL-ESM2M, and HadGEM2-ES.

MATERIALS AND METHODS

Study Area Description

The study was conducted in 2014 at Kwame Nkrumah University of Science and Technology (KNUST) research field at Anwomaso located in the Ejisu Municipality of the Ashanti region of Ghana (**Figure 1**). It lies within latitude 6° 40′57″N and 6° 42′30″N longitude 1° 30′2″W and 1° 31′44″W. The area covers ∼50 ha of semi-deciduous forest with some patches of cultivated oil palm, citrus, and cassava. The site is currently being encroached by urbanization (**Figure 1C**). The study area is characterized by a bimodal rainfall pattern occurring in March–July (major season) and September–November (minor season), which allows for two cropping seasons within a year. The total annual rainfall in the semi-deciduous forest agroecological zone of Ghana ranges between 1,300 and 1,500 mm (Fuji et al., 2009; Nkrumah and Adukpo, 2014). However, the total rainfall amount in a major and minor cropping season in the area is averagely 834 and 412 mm, respectively, for the period 1991–2014 (Bessah et al., 2021). Predominant crops cultivated by smallholder farmers in the area include oil palm, cassava, maize, cowpea, and soya bean. The soils of the site are mainly developed over deeply weathered granitic parent material consisting of heterogeneous soil series along the catena including Boamang, Bomso, Kotei, Akroso, and Nta (**Table 1**).

Model Description

The USLE (**Equation 1**) is an empirical erosion model that estimates the long-term mean annual rate of soil loss on a specific field slope based on salient biophysical variables including rainfall, topography, soil type, and cropping system, as well as soil management practices (Wischmeier and Smith, 1978; Renard et al., 1997). Although USLE has been developed for application in cropping and soil management systems and rangeland, it is applicable to non-agricultural conditions such as construction sites (Renard et al., 1997). Furthermore, USLE only predicts the amount of soil erosion resulting from sheet or rill erosion on a specific slope but does not account for additional soil losses that might occur from gully, wind, or tillage erosion (Wischmeier and Smith, 1978). Input parameters for simulations in USLE are as follows: (i) rainfall–runoff erosivity factor (R)—a function of rainfall amount and its maximum intensity in 30 min—measures the erosive force and intensity of rain in a normal year and represents a geographical location within an agroecological zone; (ii) soil erodibility factor (K), measures the susceptibility of soil particles to detachment and transport by rainfall and runoff and affected by soil structure, organic matter, and permeability; (iii) slope length and steepness factor (LS), quantifies the combined effect of slope length and slope steepness and represents a ratio of soil loss per unit area on a specific site corresponding to soil loss from a 22.13-m long experimental plot with a slope steepness of 9%; (iv) land cover management factor (C), the ratio of soil loss from land under specified crop or mulch conditions to the corresponding soil loss from tilled, bare soil and determines the relative effectiveness of soil and crop management systems for preventing soil loss; and (v) support practice factor (P), the ratio of soil loss with a given surface condition to soil loss with up-and-down hill plowing and reflects the effectiveness of the support practices that will reduce the amount and rate of runoff, and thus reduce the amount of erosion; the most commonly used supporting cropland practices are cross-slope cultivation, contour farming, and strip cropping. Each of the aforementioned factors is the numerical estimate of a specific condition that affects the severity of erosion at a specific location.

$$A = R \times K \times LS \times C \times P \quad (1)$$

where A is the annual amount of soil loss (t ha^{-1} year^{-1}); R is the rainfall erosivity (MJ mm ha^{-1} h^{-1} year^{-1}); K is the soil erodibility (t ha h ha^{-1} MJ^{-1} mm^{-1}); LS is the slope length and steepness factor (dimensionless); C is the land cover management factor (dimensionless); and P is the support practice factor (dimensionless).

Data Collection and Analysis

Landscape and Vegetation Survey

A baseline and traverses were demarcated throughout the field at predetermined distances (100 m) using compasses, ranging poles, and a 100-m tape measure. Chisel and auger holes were dug along the grid points of 50 m on the traverses to examine, identify, and describe the soils at specific recording points. Sampling points were also recorded on the base map, and boundaries of soil units were drawn by interpolation of the points identified to create a rigid grid on the base map. The line level method was used to measure the length and steepness of slope of the identified soils in the study area. Records of observed vegetation types were taken.

Soil Profile Sampling and Analysis

Five soil profile pits were dug on identified soil series after the soil survey for a detailed soil profile description. A double ring infiltrometer was installed *in situ* for the measurement of the soil–water infiltration rate of the identified soil types (Touma and Albergel, 1992). The soil physical (texture, bulk density), chemical (organic carbon), and hydraulic (moisture content) properties were determined using standard procedures as described by Allison (1960) and Ibitoye (2006) at the analytical laboratory of the CSIR-Soil Research Institute in Ghana.

Rainfall Data

Rainfall data for the period 1981–2005 were accessed from the Kumasi synoptic station of Ghana Meteorological Agency (GMet) as well as simulated historical data (1981–2005) and future rainfall data (2011–2100) from CORDEX via Climate for Impact platform of the EU (climate4impact.eu). Climate

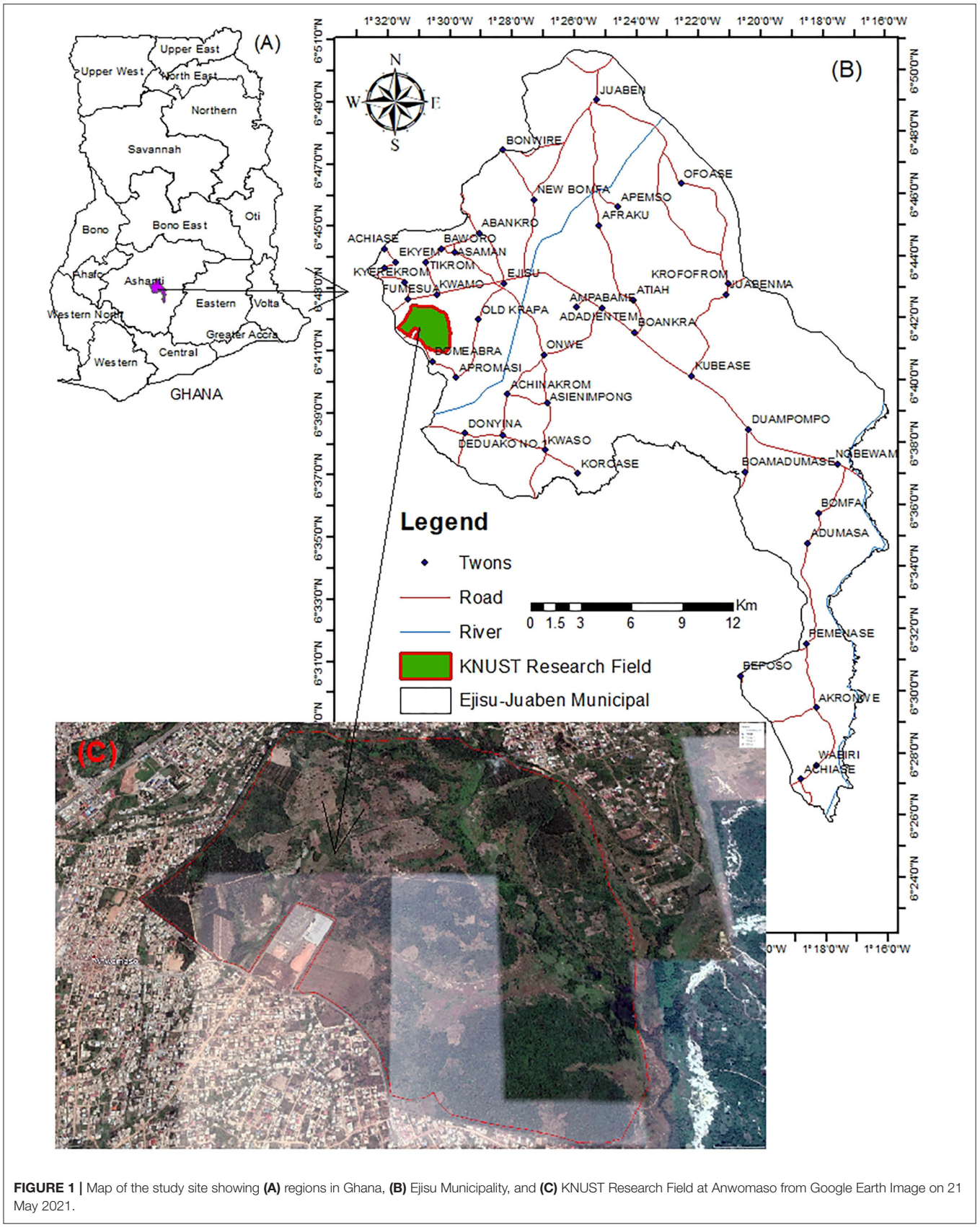

FIGURE 1 | Map of the study site showing **(A)** regions in Ghana, **(B)** Ejisu Municipality, and **(C)** KNUST Research Field at Anwomaso from Google Earth Image on 21 May 2021.

TABLE 1 | Characteristics of identified soil types in the study area.

Soil series	Soil type (FAO, 2014)	Section along the catena	Characteristics
Boamang	Orthic–Ferric Acrisol	Upper slope	• Consists of a red well-drained soil developed over deeply weathered granite. • The soil occurs on nearly level summit and on gentle to moderate steep upper slopes. • The topsoil is reddish-brown, loamy sand; the subsoil is red and has a gritty texture. • The soil is free from concretions and gravels.
Bomso	Plinthic–Ferric Acrisol	Middle slope	• It is a reddish gravelly, well-drained soil developed over sedentary granite parent material. • The topsoil is sandy loam with granular structure; the subsoil is yellowish to dark red with clay. • There were quartz and ironstone gravel in the subsoil and the occurrence of white mica flakes on the soil surface.
Kotei	Albic Arenosol	Middle slope	• Consists of red and moderately well-drained and gravelly soils developed over granite parent rock • The topsoil is dark gray and dark yellowish-brown clay loam; the subsoil is strong brown to yellowish red and gravelly
Akroso	Gleyic Lixisol	Lower slope	• It is brown to yellowish-brown, a moderately well-drained soil developed in colluviums of gentle slopes. • The soils are characteristically free from gravels up to underlying subsoil concretions. • The topsoil is dark brown sandy loam with fine granular structure; the subsoil is a strong brown to yellowish brown with gritty sandy loam to clay loam texture.
Nta	Gleyic Arenosol	Lower slope	• It is light yellowish brown, pale brown to brown, imperfectly drained sand and loamy sand developed in colluviums of gentle lower slopes. • The soils are free from gravel; the topsoil has a texture of fine sand, sandy loam or loam, and the subsoil sand and sandy loam

model outputs were extracted in R software and bias corrected with the quantile–quantile mapping method in CMhyd software (Teutschbein and Seibert, 2012).

Estimation of Soil Loss for Different Land Cover Options

The USLE was used to predict the amount of soil loss from the identified soil series in the study area under different land cover/crop management options including oil palm with cover crops, soya bean monocropping, and maize under conservation tillage. To this end, the input parameters of USLE for the different land cover options based on the field survey and land information were determined as follows.

Rainfall–Runoff Erosivity Factor

The modified Fournier index (MFI) method, which has proven to be suitable for the tropical region, was used to determine R by adopting the interpolation table of Elbasit et al. (2013). Based on the rainfall and erosivity interpolation table, 150 mm rainfall corresponds to 400 MJ mm ha^{-1} h^{-1} $year^{-1}$ erosivity (Elbasit et al., 2013).

Soil Erodibility Factor

K was read from a soil erodibility nomograph for the identified soil types using site-specific physical and hydraulic soil characteristics including soil structure (very fine granular–blocky or massive), soil permeability (very slow–rapid), soil texture (% sand, % silt + very fine sand), and soil organic matter (low) (Wischmeier et al., 1971).

Slope Length and Steepness Factor

The slope steepness determined for the identified soil types from the field survey and the predetermined slope length of 100 m were used to calculate the LS values using Equation (2) (Stone and Hilborn, 2012). LS values for the different soil types include 0.26 (Boamang), 0.59 (Bomso), 2.10 (Kotei), 1.93 (Akroso), and 1.84 (Nta).

$$LS = [(0.065 + (0.0456 \times slope) + (0.006541 \times slope^2)] \times (slope\ length \div 22.1)^m \quad (2)$$

where $m = 0.5$ if the percentage slope is ≥ 5, 0.4 on slopes in the range of 3.5–4.5%, 0.3 on slopes of 1–3%, and on 0.2 on uniform gradients of $<1\%$.

Land Cover Management Factor

C was adopted from Roose (1975) and Stone and Hilborn (2012) for the land cover options under study including bare soil (1.0), sole maize (0.65), oil palm with cover crop (0.2), and sole soya bean (0.35).

Support Practice Factor

P was assigned 1.0 for the entire study area, as farmers applied no soil conservation measure there (Shiono et al., 2002; Stone and Hilborn, 2012).

Climate Change Impact Assessment

Outputs from three RCMs from CORDEX were used to estimate the impact of climate change on soil erosion for all the different land cover types under study. The RCMs applied include the second-generation Canadian Earth System Model (CCCma-CanESM2) from the Canadian Center for Climate Modeling and Analysis, the General Fluid Dynamics Laboratory Earth System Model (GFDL-ESM2M), and the Hadley Global Environment Model (HadGEM2-ES). CCCma-CanESM2 and GFDL-ESM2M have been found to be good models for projecting temperature and rainfall over the Pra River Basin where the current study

is located (Bessah et al., 2018, 2020). HadGEM2-ES has been used by several studies over Ghana (Okafor et al., 2019). The emission scenario selected for this study was the Representative Concentration Pathways (RCP) 4.5, as tropical West Africa is reported to be a hotspot of climate change under RCP4.5 pathway projected to occur by late 2030s to early 2040s (Diffenbaugh and Giorgi, 2012; Mora et al., 2013).

Statistical Analysis

The data were subjected to analysis of variance (ANOVA) using Genstat package. Significant influence of soil properties on soil loss were separated using least significant difference (LSD) at 5% probability.

Evaluating the Performance of the Regional Climate Models

The performance of the RCMs was assessed with multiple statistical indicators using estimated erosivity based on observed rainfall data for the period 1981–2005 and those estimated with simulated historical rainfall data for the same period (i.e., 1981–2005). The statistical indicators include (i) the coefficient of determination (R^2), indicating the proportion of the variance in observed data explained by the model and ranges between 0 (no agreement) and 1 (perfect agreement); typically, $R^2 > 0.5$ is acceptable for watershed simulations (Moriasi et al., 2007); (ii) Nash–Sutcliffe model efficiency coefficient (EF), quantifying the relative magnitude of the residual variance in comparison to the variance of the observed data, and ranges from 1 (perfect match) to $-\infty$ (poor predictability); (iii) Willmott's index of agreement (d), quantifying the extent to which the observed data correlates with the simulated data and ranges between 0 (no agreement) and 1 (perfect agreement); and (iv) normalized root mean square error (NRMSE), signifying the relative difference between the simulated results and the measured data, with NRMSE <10% showing excellent model performance, 10–20% (good), 20–30% (fair), and >30% (poor performance) (Loague and Green, 1991).

RESULTS AND DISCUSSION

Identified Soil Types in the Study Area

The identified soil types include Orthic–Ferric Acrisol, Plinthic–Ferric Acrisol, Albic Arenosol, Gleyic Lixisol, and Gleyic Arenosol (**Table 1**). The soils are well-drained to imperfectly drain from the uplands to the bottom soils. The texture was sandy loam to loamy sand, with soil depth above 1.8 m with no gravels except in parts of the middle slope (Kotei series) (**Table 2**). The soils studied have a detrimental tendency of forming a crystallized, cemented, and hardened irreversible ferric oxide (Petro-plinthite) beneath the surface that could restrict infiltration and aid excessive runoff during rainfall (Adu, 1969).

Rainfall–Runoff Erosivity and Soil Erodibility Factors

The mean observed (1981–2005) R applied in the study was 287.1547 MJ mm ha^{-1} h^{-1} year^{-1}, whereas the mean simulated historical (1981–2005) R values were 245.5028, 261.7396, and 112.4751 MJ mm ha^{-1} h^{-1} year^{-1} for CCCma-CanESM2, GFDL-ESM2M, and HadGEM2-ES, respectively (**Table 3**). Furthermore, mean R for the future periods (i.e., 2011–2040, 2041–2070, 2071–2100) estimated based on simulated rainfall data from the three RCMs using the aforementioned procedure have been presented in **Table 4**. The R values estimated from both observed and simulated rainfall data were found to be very high. According to Balogun et al. (2012), *R* values >160 MJ mm ha^{-1} h^{-1} year^{-1} are considered very high and characteristic of high erosion risk zones. Essel et al. (2016) reported R values in the range of 73.5–200.4 MJ mm ha^{-1} h^{-1} year^{-1} for a location within the coastal savanna agro-ecological

TABLE 3 | Estimated erosivity based on observed (1981–2005) rainfall data from the Kumasi synoptic station and simulated observed rainfall data from three regional climate models for the period 1981–2005.

Month	R (MJ mm ha^{-1} h^{-1} year^{-1})			
	Observed	CCCma-CanESM2	GFDL-ESM2M	HadGEM2-ES
January	50.9547	6.4779	0.0000	54.6475
February	114.1867	60.6933	23.1147	102.3531
March	312.9813	324.5536	279.5285	158.3413
April	407.2427	425.4037	422.5291	113.3237
May	423.2960	445.3451	450.0000	40.6891
June	538.2080	528.2763	565.2171	57.6981
July	365.4293	307.2981	409.7291	137.2277
August	219.1573	217.0389	239.4155	111.6939
September	420.5227	420.2059	378.3552	233.6149
October	394.1333	176.4821	338.7829	180.3243
November	123.8933	34.2581	34.2037	77.8080
December	75.8507	0.0000	0.0000	81.9797
Annual mean	287.1547	245.5028	261.7396	112.4751

TABLE 2 | Rated soil physical characteristic of soils of the study site.

Soil series	Slope (%)	Drainage	Texture	Effective soil depth (mm)	Gravel and concretions
Boamang	1.4–2.1	Well-drained	Sandy loam	>200	No gravels
Bomso	3.3–4.1	Well-drained	Sandy loam	>200	No gravels
Kotei	8.2–9.6	Moderately well-drained		>200	Gravels
Akroso	6.2–10.5	Imperfectly drained	Loamy sand	>180	No gravels
Nta	6.9–9.3	Imperfectly drained	Loamy sand	>180	No gravels

zone of Ghana. Similarly, Roose (1976) found high R values ranging between 200 and 650 MJ mm ha^{-1} h^{-1} year^{-1} in Burkina Faso (formerly Upper Volta). However, very high R values in the range of 414.9–701.1 MJ mm ha^{-1} h^{-1} year^{-1} have been estimated in the Guinea-sudano savanna zone of Ghana (Badmos et al., 2015).

Estimated K values for this study ranged between 0.17 and 0.31 (**Table 5**). These K values were considered moderate to high on tropical soils (Roose, 1976). K values in the range of 0.02–0.32 have been recorded across West Africa (Roose, 1976).

Effect of Soil Physicochemical Properties on Erosion

The results from the soil survey conducted showed variations in the indices related to the estimated erosion. There were significant differences between soil properties of the different soil series studied along the catena, namely, Boamang, Bomso, Kotei, Akroso, and Nta, and subsequent estimated soil losses (**Tables 2**, **5**). A surface (0–20 cm) bulk density in the range of 1.3–1.5 g cm^{-3} (**Table 5**) suggests that soils were loose and showed less sign of compaction and thus will enhance infiltration during rainfall. The generally low soil organic matter content (1.01–2.12%) coupled with a compacted subsoil (i.e., bulk density $\geq$1.8 g m^{-3}) will most likely restrict water infiltration into the soil and thus facilitate excess runoff causing detachment of loose soil particles. Increased soil erosion could result from the combined effect of low organic matter content, high bulk density, and low soil–water infiltration (Charman and Roper, 2007; Novara et al., 2021).

Effect of Slope Steepness on Soil Erosion

The results show a positive linear relationship ($R^2 = 0.78$) between slope steepness and soil loss from bare soil resulting from a respective increase in the velocity and volume of surface runoff. The steepness of the slopes (1.8–8.9%) coupled with the soil physicochemical and hydraulic properties contributed to the varying amounts of eroded soil ranging from 12.7 to 163.8 t ha^{-1} year^{-1} estimated for the various soil series (**Figure 2**). Boamang series found on the summit with a gentle slope (1.4–2.1%) experience less runoff that culminated in lower soil loss (12.7 t ha^{-1} year^{-1}) compared with the other soil series along the middle slope to the lower slope including Bomso, Kotei, Akroso, and Nta (37.3–163.8 t ha^{-1} year^{-1}). There is, thus, the need to reduce the effect of the slope characteristics on soil erosion within the catchment area by planting strip crops (e.g., *Vertiver* spp.) or terracing to break the slopes and reduce the volume and speed of runoff (Stone and Hilborn, 2012; Cerdà and Rodrigo-Comino, 2019). However, Cerdà and Rodrigo-Comino (2019) found that runoff and soil erosion were evenly distributed along the top, middle, and lower slopes in a Mediterranean vineyard, which could be due to a homogenization effect of a millennia-old tillage practice in the study area.

TABLE 4 | Estimated erosivity (MJ mm ha^{-1} h^{-1} year^{-1}) for future periods between 2011 and 2100.

Period	CCCma-CanESM2	GFDL-ESM2M	HadGEM2-ES
2011–2040	268.8213	421.2404	159.3960
2041–2070	268.8240	429.8901	169.6079
2071–2100	292.6910	450.5689	216.1072

Effect of Vegetation Cover Type on Soil Erosion Control

Under oil palm with cover crop, quantity of eroded soil was similar for all the soil series (ranging from 2.5 to 32.8 t ha^{-1} year^{-1}) (**Figure 3**). This shows that growing oil palm with cover crops on any soil of similar characteristics will not result into excessive soil erosion even at 10% slope. Erosion is reduced when raindrop impact energy is absorbed on non-erodible surfaces like vegetation and mulches, and thus, initial detachment and subsequent transport of particles is reduced (Keesstra et al., 2018b).

Soil erosion under sole soya bean crop was similar for Boamang (1.8% slope) and Bomso (3.7% slope) soil series ranging from 4.4 to 13.0 t ha^{-1} year^{-1} (**Figure 3**). Growing soya bean on these soils is recommended since soil erosion is not severe. Under the same crop management (i.e., sole soya bean), soil erosion for Kotei (8.9% slope), Akroso (8.4% slope), and Nta (8.1%) were similar and higher than Boamang and Bomso soil series ranging from 33.0 to 57.3 t ha^{-1} year^{-1}. Sole cropping of soya bean or any crop of similar structure on slopes of above 5% will most likely result in excessive soil erosion of above 33 t ha^{-1} year^{-1}. Maize cultivation under conventional tillage is most appropriate in Boamang (8.2 t ha^{-1} year^{-1} soil loss) followed

TABLE 5 | Erosion related properties of soils of the study site.

Soil series	Soil organic matter (%)	Bulk density (g cm^{-3})		Mean infiltration (mm h^{-1})	K (t ha h ha^{-1} MJ^{-1} mm^{-1})
		0–20 cm	20–40 cm		
Boamang	1.43bc	1.5	1.7	30.0bc	0.17b
Bomso	2.12a	1.3	1.8	46.7a	0.22b
Kotei	1.69ab	1.5	1.7	32.5bc	0.23b
Akroso	1.58b	1.4	1.8	31.7bc	0.17b
Nta	1.01c	1.5	1.8	43.3ab	0.31a

Values with the same letters for the different soil types are not statistically different at P < 0.05.

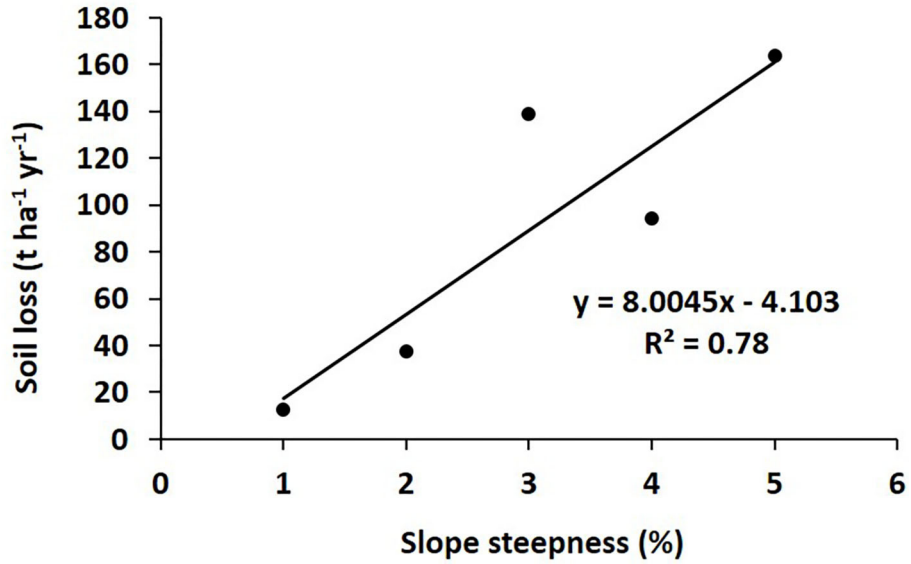

FIGURE 2 | Effect of slope steepness on soil loss.

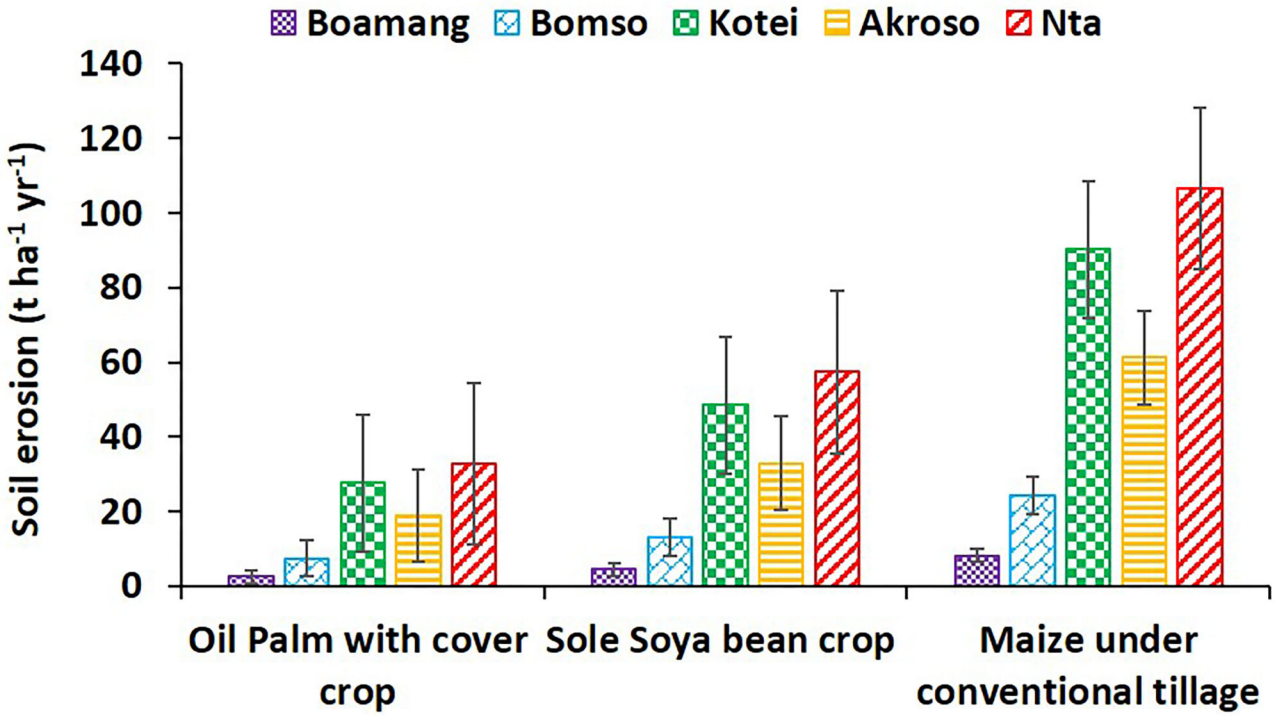

FIGURE 3 | Estimated soil losses under different land cover options.

by Bomso (24.2 t ha^{-1} year^{-1} soil loss) soil series (**Figure 3**). However, soil erosion for Kotei, Akroso, and Nta were similar and significantly higher than Boamang and Bomso. Soil erosion ranged from 61.2 to 106.5 t ha^{-1} year^{-1} for these soil series and highly inappropriate for maize cultivation under conventionally tilled conditions. Issaka et al. (2015) reported soil erosion of 8.2–10.7 t ha^{-1} year^{-1} when maize was cultivated on a 5° (8.8%) slope under tilled conditions as against relatively low eroded soil (1.5–3.2 t ha^{-1} year^{-1}) under no-tilled conditions. With increasing slope, tillage should be reduced, and the use of cover crops

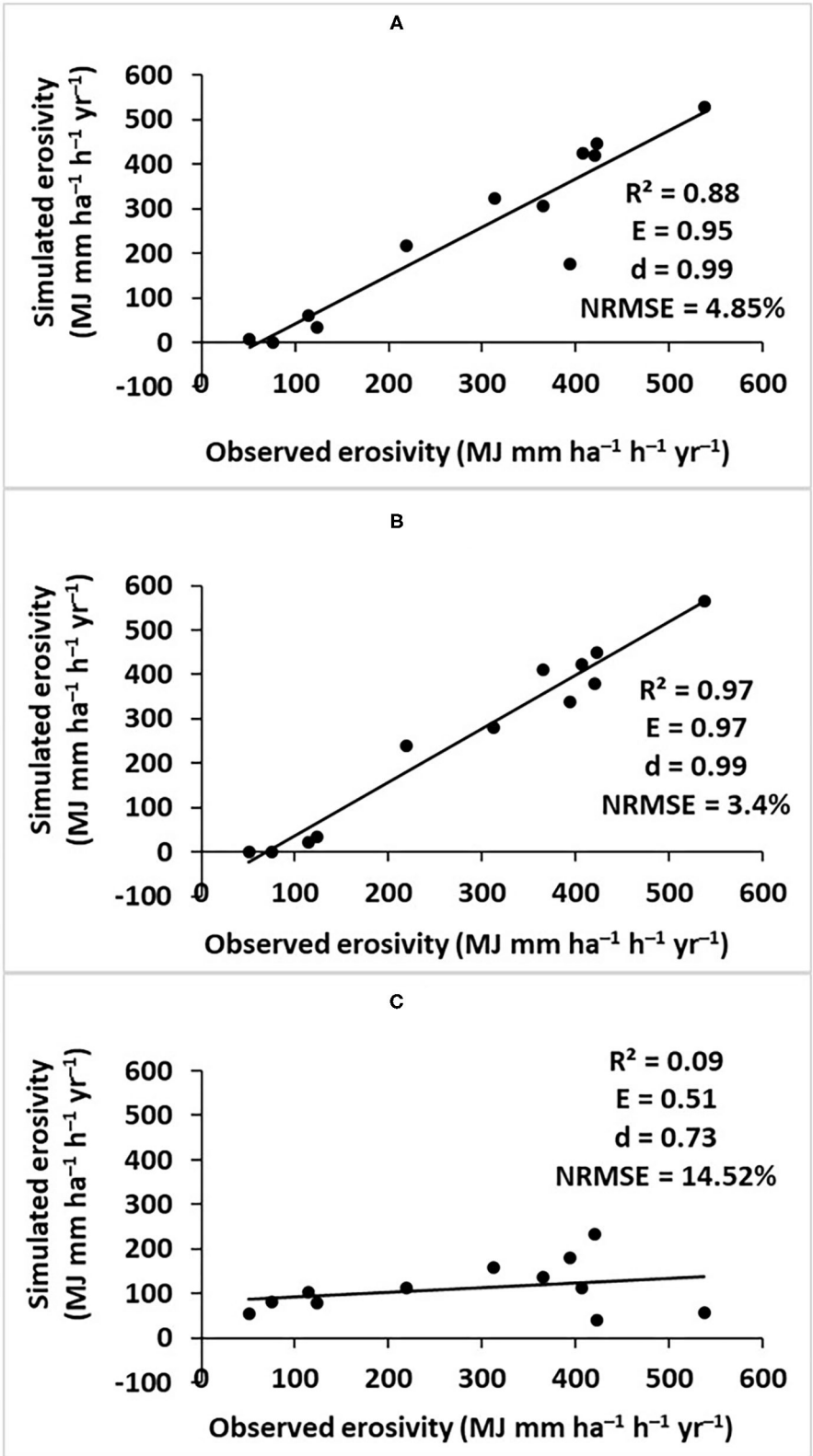

FIGURE 4 | Performance evaluation of regional climate models including **(A)** CCCma-CanESM2, **(B)** GFDL-ESM2M, and **(C)** HadGEM2-ES.

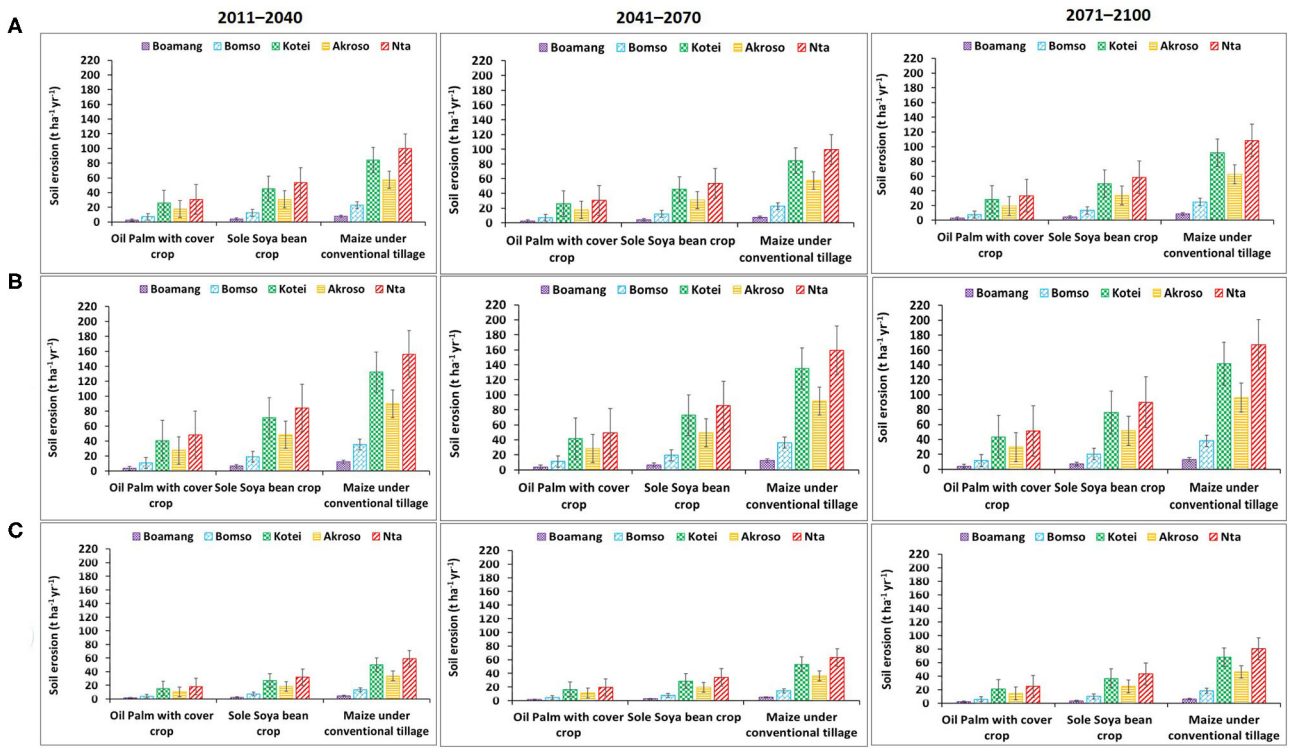

FIGURE 5 | Predicted soil loss under climate change impact using **(A)** CCCma-CanESM2, **(B)** GFDL-ESM2M, and **(C)** HadGEM2-ES model outputs.

or mulch should be considered (Cerdà and Rodrigo-Comino, 2019).

The rates and quantities of soil loss depend on the cultural practices adopted by farmers. Estimates of soil loss for maize with no-till and oil palm with cover crop gave lower soil losses as compared with maize under conventional tillage on the same stretch of land. Capello et al. (2019) reported a reduction in runoff by 76% and soil loss by 83% under soil management with grass cover compared with tillage in northwest Italy. Soil is likely to be affected by the disturbance of the soil surface that affects soil aggregates especially during tillage; the soil is easily pulverized by the plow. The use of tractors for land preparation and other farm practices affect the structure and hydraulic properties of the soil (Krebstein et al., 2014; García-Tomillo et al., 2018; Novara et al., 2021), and thus, periodic subsoiling to reduce the compaction of the lower depths could be helpful. The soil with low clay and low organic matter contents lends its particles easily to detachment when pulverized, and they are splashed and carried away by runoff (Krebstein et al., 2014; García-Tomillo et al., 2018).

Estimated losses from the various crop factors used recorded a comparatively higher rate apart from the no-till maize and oil palm with cover crop. The values could as well be lowered when conservation practices were put in place. Farmers should engage in contour farming practices that would slow down runoff and thereby enhance water infiltration with a resultant reduction in soil loss (Stone and Hilborn, 2012; Desta et al., 2021). For example, planting of strips of *Vertiver* spp. at 30-m intervals on moderate slopes could also be a useful strategy to reduce surface runoff. Planting *Vicia sativa* is also recommended for controlling soil and water losses at the early stages of vineyard plantation (Rodrigo-Comino et al., 2020).

Performance Evaluation of Climate Models Applied

Evaluation of the performance of the RCMs for simulation showed excellent performance (i.e., R^2, E, and d approximately = 1, and NRMSE < 10%) for CCCma-CanESM2 and GFDL-ESM2M (**Figures 4A,B**). However, the performance of HadGEM2-ES was only moderate, as R^2 < 0.5 and NRMSE was in the range of 10–20% (**Figure 4C**). Hence, model outputs from CCCma-CanESM2 and GFDL-ESM2M were more reliable. The excellent performance of these two RCMs confirms similar reports by Bessah et al. (2020) and Bessah et al. (2018). Sekyi-Annan et al. (2018) reported EF and d in the range of 0.65–0.83 and 0.87–0.96, respectively, and NRMSE between 17.7 and 42%, indicating good performance of an agro-hydrological model (i.e., AquaCrop) for simulating dry aboveground biomass of tomato in the Guinea-sudano-savanna agroecological zone of Ghana.

Impact of Climate Change on Soil Loss

Simulation of the impact of climate change on soil loss across all land cover types in the study area varied based on RCMs applied (**Figure 5**). For CCCma-CanESM2, the rate of soil loss is expected to increase by 9% during 2011–2040 and 2041–2070

and 16% during 2071–2100. For GFDL-ESM2M, increasing rate of soil loss by 38, 39, and 42% are expected during the periods 2011–2040, 2041–2070, and 2071–2100, respectively. HadGEM2-ES predicted 29, 34, and 48% increase in soil loss for the same periods.

Overall, predictions of climate change impact based on outputs from CCCma-CanESM2 and GFDL-ESM2M indicated that 9–39% increase in soil loss is expected in the study area by 2070, and it will be more severe (16–42%) by 2100. The USLE's estimated high annual soil erosion rates in the study area is predicted to be exacerbated by the impact of climate change requiring urgent soil management interventions such as tree–cover crop intercropping and reduced tillage practices. Similarly, Routschek et al. (2014) predicted significant increase in soil erosion by 2050 in Germany resulting from climate change, and thus, a refusal to adapt soil management and land use would aggravate soil erosion rates. Furthermore, Borrelli et al. (2020) forecast 30–66% increase in global soil erosion due to climate change by 2070. However, Routschek et al. (2014) predicted a partial decrease in soil loss rates by 2100 in Germany probably due to differences in climate (i.e., temperate vs. tropical) and soil properties.

CONCLUSIONS

Estimation of erosion loss and the effectiveness of erosion control measures are crucial in sustainable agricultural land management considering the increasing rate of land degradation and the impact of climate change across SSA. The USLE as a predictive tool provided a relatively easier but rigorous approach to estimating the rate and quantity of soil erosion, which serves as early warning signals for land users in order to implement appropriate erosion control measures. The extent of compaction indicated by high bulk density in the subsoil is useful information for agricultural extension staff in the planning and management of agricultural mechanization services. The simulated annual soil losses under various land cover options showed variable degrees of soil losses for maize cultivation under conventional tillage (8.2–106.5 t ha^{-1} $year^{-1}$) and for soya bean monocropping (4.4–57.3 t ha^{-1} $year^{-1}$) and low soil loss for oil palm plantation with grass or leguminous cover (2.5–32.8 t ha^{-1} $year^{-1}$). There existed a strong correlation between observed and simulated soil loss for all three climate models applied including CCCma-CanESM2, GFDL-ESM2M, and HadGEM2-ES. Evaluation of the RCMs showed excellent performance for CCCma-CanESM2 and GFDL-ESM2M. Predictions of climate change impact based on outputs from CCCma-CanESM2 and GFDL-ESM2M indicated that soil loss in the study area will increase by 9–39% during 2011–2040 and 2041–2070, while it will be more severe (16–42%) during 2071–2100 owing to the impact of climate change and variability. Hence, the adoption of cropping systems and cultural practices that maintain a thick layer of mulch or surface contact vegetation to prevent exposure of the soil surface to the adverse effect of the weather is crucial. The model predictions indicate that the adoption of site-specific crop management strategies such as tree–cover crop intercropping and reduced tillage has a huge potential to reduce soil loss and sustain soil fertility. Additionally, the model can be used as an advisory tool for mapping areas for appropriate cropping system suitable for a particular site.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

ES-A: conceptualization, data analysis, and writing original draft of manuscript. EG: conceptualization, data collection and analysis, and review and editing of manuscript. RI: data analysis and review and editing of manuscript. GQ: data collection and review and editing of manuscript. SA: review and editing of manuscript. EB: data collection and analysis and review and editing of manuscript. All authors contributed to the article and approved the submitted version.

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Efficacy of Nutrient Management Options for Finger Millet Production on Degraded Smallholder Farms in Eastern Uganda

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Poor soil fertility is a major problem constraining crop productivity in smallholder farms of sub-Saharan Africa due to inadequate nutrient replenishment. Differential management of nutrients creates areas of accumulation and depletion of nutrients within farms with the latter increasing in spatial coverage. Nutrient additions are required to increase crop production in such degraded areas. We used experimental data to evaluate the potential of inorganic fertilizers and organic manures to offset finger millet yield differences or gap between degraded fields and former kraals, which are recognized as niches for obtaining the best yields within the Teso farming system in eastern Uganda. Nitrogen (N) and phosphorus (P) fertilizers were sole applied at 0, 30, 60, and 90 kg ha^{-1} and in combination (N+P) at equal rates of sole application, and manure (3 t ha^{-1}) supplemented with N (0, 30, 60, and 90 kg ha^{-1}) to degraded fields located in upper and middle landscape positions in Chelekura and Onamudian villages. A second control treatment of finger millet grown on soils of former kraal sites (high fertility niches) was included as a benchmark to evaluate the efficacy of nutrient management options on degraded field. Average grain yield ranged from 404 to 2,026 kg ha^{-1} and differed significantly ($p < 0.001$) between villages and seasons. Significant effects ($p < 0.05$) of landscape position on grain yield were observed only in Onamudian village. Although the treatments significantly increased millet yields on degraded fields above the control, they could not eliminate the yield differences between degraded fields and former kraals. The largest average grain yields on degraded fields were obtained from combined application of N+P resulting in average grain yields of 800 and 1,171 kg ha^{-1} in Chelekura village and Onamudian village, respectively. These yield responses resulted in only 24 and 43% of yields obtained on former kraal fields in Chelekura and Onamudian, respectively. The physiological efficiencies, agronomic efficiencies, and apparent recoveries of N and P were low; often <25%. Pot experiments conducted in a greenhouse showed that Sulphur (S) and potassium (K) were additional limiting nutrients to N and P for finger

millet production in Chelekura and Onamudian and may partly explain the large yield differences of finger millet between fertilized fields and former kraals in the smallholder farming systems. Nutrient management strategies for sustainable millet production in these farming systems need consideration of site-specific nutrient limitations.

Keywords: integrated soil fertility management, nutrient use efficiencies, limiting nutrients, finger millet (*Eleusine coracana*), sub-Sahara Africa (SSA)

INTRODUCTION

The livelihood of smallholder farmers in sub-Saharan Africa (SSA) is hinged on agriculture to combat food insecurity and poverty through direct production of food and cash crops and sale of excess production to generate incomes. This is, however, threatened by poor soil fertility, now a major constraint to crop productivity in smallholder farms resulting from inadequate replenishment of nutrients removed (Holden, 2018). The magnitude of nutrient depletion varies across the different spatial scales from continent to farm (Buresh et al., 1997). Uganda is among the countries in the continent with the largest depletion rates: 20–40, 3.5–6.6, and 17–33 kg ha^{-1} $year^{-1}$ for N, P, and K, respectively (Smaling et al., 1997). Although negative nutrient balances are prevalent throughout SSA, areas of nutrient accumulation are created through management reinforcing heterogeneity in soil fertility and variability in crop productivity within farms (e.g., Rowe et al., 2006; Tittonell et al., 2007; Zingore et al., 2007). Continued depletion of nutrients results in poor fertility or degraded patches within farms.

The area covered by poor fertility fields in smallholder African farms is substantial and will increase if no action is taken to replenish and sustain soil fertility. Tittonell (2008) found the proportion of poor fertility fields according to farmers' categorization to account for ∼30% of total farm fields in six farming systems in western and central Kenya and eastern Uganda. In two villages in Pallisa district, the degraded fields covered 13–29% of the land area (Ebanyat, 2009). Together with reducing farm size, this threatens the food security of smallholders who rely on farming for their livelihood.

Finger millet (*Eleusine corocana* (L.) Gaertn) is an important food and nutrition crop as well as an income crop for smallholder farmers in the Teso farming system in eastern Uganda (Esele, 1989; Wanyera, 2007; Ndungu-Magiroi et al., 2017). It is rated third after maize and sorghum in order of production among smallholder farmers, and 15% of the national production is from this region [Uganda Bureau of Statistics (UBOS), 2020]. However, soil fertility depletion and labor constraints have affected its productivity (<1 t ha^{-1}) despite the availability of improved varieties (Tenywa et al., 1999; Kidoido et al., 2002; Owere et al., 2014). The farming system used to be a strongly integrated crop–livestock system and sustained finger millet production until depletion of livestock through rustling, and due to decline in soil fertility and other factors has changed from a millet-based to a cassava-based farming system (Ebanyat et al., 2010a). The United Nations Convention to combat Desertification and Drought land degradation neutrality initiative proposes reversal of past degradation to improve productivity and enhance food security (Orr et al., 2017). Therefore, measures for improving/restoring fertility in degraded fields are needed for smallholders to sustain crop production and close yield differences within farms (Mueller et al., 2012).

Potential options to restore soil fertility include the use of inorganic fertilizers to optimize locally available organic inputs and addressing site-specific constraints to restore and improve soil fertility as guided in integrated soil fertility management paradigm (Vanlauwe et al., 2010). The use of inorganic fertilizers is constrained by high costs and inaccessibility, and a lack of economic returns (Morris et al., 2007; Barungi, 2012; Chianu et al., 2012; Bonilla Cedrez et al., 2020) as well as low soil organic matter (Barret et al., 2017). At the same time, amounts of organic resources available on smallholder farms are limited and their poor nutrient quality constrains their use and effectiveness in soil fertility management (Ridder et al., 2004). Combined use of organic and inorganic fertilizers is a potential approach to ameliorate soil fertility because of their complementary benefits (Vanlauwe et al., 2002; Chivenge et al., 2011; Stewart et al., 2020).

Strategies for fertility regeneration in smallholder farming systems can best be designed with the knowledge of field responsiveness to nutrient management interventions. Vanlauwe et al. (2010) propose a stepwise approach in targeting and adapting nutrient management interventions and germplasm to local variations as a way to moving toward integrated soil fertility management. The stepwise approach requires recognizable benchmarks to step up to and against which to evaluate efficacy of the intervention strategies. In the Teso farming system in eastern Uganda where this study was conducted, areas where manure accumulated over years of night corralling (former kraals) are fertile, give good yields of finger millet, and are readily observed by smallholders. We used these former kraal sites as benchmarks to assess within-farm differences in finger millet yield and to evaluate responsiveness of degraded fields to nutrient interventions in two study sites in Pallisa district. Nitrogen and phosphorus are the major nutrients limiting millet production in the low input farming systems in eastern Uganda (Tenywa et al., 1999). Research on nutrient limitations and management practices has concentrated more on major nutrients than on secondary nutrients and micronutrients (Kihara et al., 2016, 2017; Wortmann et al., 2019). A complete understanding of limiting nutrients is important for making appropriate soil and nutrient management recommendations for soil fertility remediation and needed to be done for degraded fields in the smallholder systems of eastern Uganda. We tested the hypothesis that differences in finger millet yield between the former

kraals and degraded fields can be eliminated by application of N and P from organic and inorganic sources on degraded fields and results in improved nutrient use efficiencies by finger miller. The specific objectives of the study were (1) to determine finger millet yield response to applied nutrients of organic and inorganic origin on degraded fields and assess the extent to which yield differences between benchmark sites and degraded fields amended with nutrient inputs are reduced, (2) to determine nutrient use efficiencies by finger millet, and (3) to identify other nutrients limiting finger millet production in degraded fields.

MATERIALS AND METHODS

The Study Sites

Field experiments were conducted in two villages: Chelekura in Chelekura parish (1°24′N; 33°30′E) and Onamudian in Akadot parish (1°11′N; 33°43′E) in Pallisa district (1°09′N, 33°48′E) representative of the Teso farming system in eastern Uganda. Soils of Chelekura are formed from lake deposits and those of Onamudian from basement complex rocks (granitic gneisses) (Harrop, 1970). The landscape is characterized by wide gently convex interfluves separated by wide swampy valleys (Ollier et al., 1969). The toposequence can be divided into three sub-zones: the upland zone at the summits (upper landscape positions), the midland zones located on pediments (middle landscape positions), and the valleys that may be seasonally or permanently wet (lower landscape positions). In both villages, soils are Ferralsols and Dystric Fluvisols in the uplands and valley bottoms, respectively (Ebanyat et al., 2010a).

Mean annual rainfall ranges from 900 to 1,200 mm and is distributed in a bimodal pattern. The first rains are from March to June and the second rains from August to October or November. A dry spell stretches from November to March. Both study sites were within the same rainfall zone of 900 mm year^{-1}. Monthly temperature ranges from 15 to 36°C, with an annual mean of 25°C (Yost and Eswaran, 1990). Cumulative total rainfall during the growing period of short rainy season in 2005 (2005B) was low (≈400 mm), and above normal (≈615 mm) in both short rain season of 2006 (2006B) and long rain (≈580 mm) season of 2006 (2006A) (**Figure 1**).

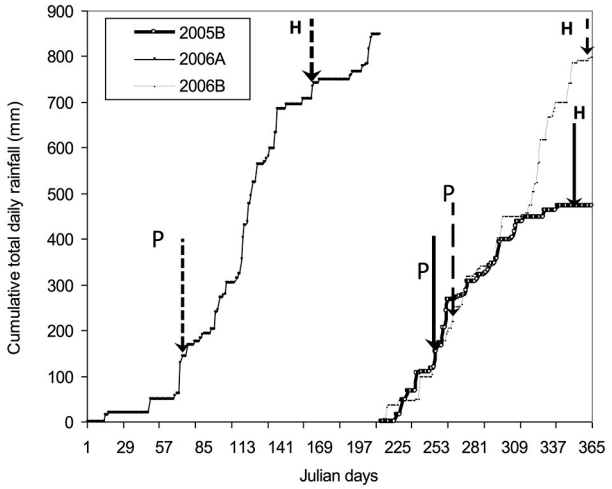

FIGURE 1 | Cumulative daily rainfall received in the study area during field experimentation for three subsequent seasons 2005B, 2006A, and 2006B. P and H denote planting and harvesting, respectively.

Field Experiments

Field Selection and Soil and Manure Characterization

Ten degraded fields (five on upper and five on middle landscape positions) based on farmers' perceptions of fertility status and five former kraal sites last used for night corralling five years before this study were selected for experimentation in each of two study villages of Chelekura and Onamudian. Five soil subsamples were taken from each field from 0 to 20 cm, thoroughly mixed, and by quarter sampling composite samples were obtained. Manure was collected from two (Chelekura) and three (Onamudian) former kraals and bulked together, and after thorough mixing, subsamples were taken for oven drying at 65°C for 48 h to obtain the average moisture content. Air-dried composite samples of soil (<2 mm) and manure samples were subjected to physico-chemical analysis at the World Agroforestry Center (ICRAF) following spectral and standard wet chemistry analysis procedures (Shepherd and Walsh, 2002). Extractable P was determined at Kawanda National Agricultural Laboratories Research Institute in Uganda using the modified Olsen method (Anderson and Ingram, 1993).

Field Preparation and Experiment Establishment

The fields were ox-plowed twice and plots of 3 × 3 m demarcated. Experimental design was a randomized complete block experiment with 10 replicates (farms) per village. Kraal manure, N (urea), and P as triple super phosphate (TSP) were applied as single replicates per farm and landscape position as follows: control (with no nutrient inputs), N and P alone at rates of 30, 60, and 90 kg ha^{-1} and combinations of N and P each at equal rates (i.e., 30N+30P, 60N+60P, and 90N+90P); kraal manure at 3 t ha^{-1} and kraal manure 3 t ha^{-1} with N at 30, 60, and 90 kg ha^{-1} (i.e., M+30N, M+60N, and M+90N). Manure and TSP fertilizer were basal applied by spreading and worked into soil with a hand hoe. Improved finger millet variety SEREMI 2 with yield potential of 2.5 t ha^{-1} and maturity period of 110 days was planted during the short rains of 2005 (2005B) at a spacing of 0.3 m between rows and thinned to 0.05 m within rows at 2 weeks after planting (WAP). Nitrogen was applied in two equal splits at first weeding (2 WAP) and second weeding (4 WAP). In the subsequent seasons of 2006A and 2006B, land preparation was done on a plot basis using a hand hoe and planting. Plots of similar size were established on former kraal sites as a second control but without addition of any nutrient inputs. The experiments were replanted in the same plots without any nutrient input additions in the long rainy season (2006A), but with nutrient inputs at the same rates during the short rainy season (2006B). All other agronomic operations were carried in the same way across the seasons.

Millet Sample Collection, Preparation, and Analysis

In 2005B, only straw was harvested because of poorly distributed rains (**Figure 1**) that did not allow panicle filling. In 2006, millet panicles were harvested by cutting with thumb knives (farmers' practice) and straw cut at 0.05 m above the ground surface from two quadrats of 1 m^2 along the three middle rows of each plot. The panicles and straw samples were oven dried at Makerere University's Soil and Plant analytical laboratories at 65°C for 72 h. Panicles were threshed and weights of grains and husks, and straw were obtained before they were ground to pass through a 1-mm sieve. The samples were analyzed at the ICRAF, Nairobi, Kenya for pH and total (Tot.) nutrients—N (nitrogen), K (potassium), Ca (calcium), and Mg (magnesium)—using NIR spectroscopy as detailed in Shepherd et al. (2003). Total P was determined using the wet chemistry procedure as detailed by Anderson and Ingram (1993) for plant materials at Kawanda National Agricultural Laboratories Research Institute in Uganda.

Greenhouse Pot Experiment

Bulk soils were collected from five locations within the degraded fields used for experiments at 0–20 cm; three each from upper and middle landscape positions of each village. The samples were bulked and mixed using a manually rotated drum. The mixed soils of each village were weighed into 5-kg pots to provide a rooting volume of 2,000 cm^3, i.e., 3.48 and 3.10 kg for degraded soils, and 2.84 and 2.78 kg for former kraal soils from Chelekura and Onamudian villages, respectively. Treatments applied constituted macro- and micronutrients N, N+P, N+P+K, N+P+K+S, N+P+K+S+Ca, N+P+K+S+Ca+Mg, N+P+K+S+ micronutrients, and N+P+K+S+Ca+Mg+ micronutrients. The source and amount of each nutrient applied (g pot^{-1}) to soils from Chelekura and Onamudian village were N (NH_4NO_3; 0.2429, 0.2214), P (NaH_2PO_4; 0.1974, 0.1800), K (K_2O; 0.0410, 0.0374), S [$(NH_4)_2SO_4$; 0.2104, 0.1918], Ca (CaO; 0.2380, 0.2170), Mg (MgO; 0.1133, 0.1033), Mo (Na_2MoO_4; 0.0010, 0.0009), Mn ($MnSO_4$; 0.0200, 0.0183), Cu ($CuSO_4$; 0.0150, 0.0137), Zn ($ZnSO_4$; 0.0150, 0.0137), Bo ($Na_2B_4O_7$; 0.0010, 0.0009), and Co ($CoCl_2$; 0.0025, 0.0023). The nutrients were dissolved in the amounts of distilled, water required to bring the soils in pots to field capacity. The pots were left to stand for 2 days and then planted with 0.5 g of finger millet seed of improved variety, SEREMI 2, with a potential yield of 2.5 t ha^{-1} and maturity period of 110 days. The experimental design was a complete randomized block with three replicates. At two weeks after emergence, only 20 plants were maintained per pot. Water was added after every 2 days to maintain moisture content of the pots at 70% of field capacity during the experimental period. Millet shoots were cut at 0.05 m from the soil surface at 8 weeks after planting (WAP) and oven dried at 65°C for 48 h to obtain shoot dry weights. Roots were recovered by washing soil from each pot through a 2-mm sieve. The roots were then oven dried to obtain root dry weights. Total biomass was a total of recovered roots and shoot biomass.

Data Calculations and Analysis

Total nutrient uptake in straw and grain was determined as a product of straw or grain yield with mass respective percentage total N or total P and the nutrient physiological or internal nutrient efficiencies for N and P computed using the equation of Witt et al. (1999):

$$PhE = \frac{GYT}{UNT} \tag{1}$$

where *PhE* is physiological nutrient efficiency (kg kg^{-1}), *GYT* is grain yield for treatment (kg ha^{-1}), and *UNT* is the total uptake of nutrient (kg ha^{-1}).

Agronomic efficiency and apparent nutrient recovery fractions of nutrients applied to degraded fields were computed from the following equations:

$$AE = \frac{GYT - GYC}{RN} \tag{2}$$

where *AE* is agronomic efficiency (kg kg^{-1}), *GYT* is grain yield of treatment (kg ha^{-1}), *GYC* is grain yield of control treatment (kg ha^{-1}), *RN* is rate of applied nutrient (kg ha^{-1}), and

$$ARN = \frac{UT - UC}{RN} \tag{3}$$

where *ARN* is apparent recovery of nutrient (kg kg^{-1}), *UT* is total uptake of nutrient in straw and grain (kg ha^{-1}), *UC* is total uptake in straw and grain in the control treatment (kg ha^{-1}), and *RN* is the rate of applied nutrient (kg ha^{-1}).

Statistical analysis was performed using the linear mixed-effects models of the Genstat 11.1 statistical package for field experiments with the fixed model term: Constant+Landscape position+Treatment+Season+Landscape position × Treatment+Landscape position × Season+Treatment × Season+Landscape position × Treatment × Season, and the random term: Farm+Farm × Plot. Analysis was only conducted on data from 16 of the 20 farms because some plots were destroyed by livestock. Only data for the two seasons of 2006 are used in the analysis as the crop of 2005B season failed. For the greenhouse limiting-nutrient pot experiment, a two-way ANOVA was conducted on millet biomass and the factors compared were sites (villages) and nutrient application.

RESULTS

Soil and Manure Quality

Initial soil quality of degraded fields selected by farmers differed significantly in pH, SOC, total N, exchangeable Mg, CEC, and total P between sites but not between landscape positions (**Table 1**). In both sites, the fields were moderately acidic and poor in extractable P (<10 mg kg^{-1}). The former kraals differed significantly between sites in pH, SOC, total N, exchangeable Mg, CEC, and total P, but were richer reflecting niches of good soil fertility. Manure quality varied between sites and

TABLE 1 | Initial soil properties of degraded fields compared with poor fields and former kraals used for experimentation by landscape positions in the study villages.

Village/ landscape position	pH (H_2O)	SOC	Tot N	Extr. P	Exc. K	Exc. Ca	Exc. Mg	CEC	Total P	Sand	Clay	Silt
		(%)	(%)	(mg kg^{-1})	($cmol_c$ kg^{-1})					(%)		
Chelekura												
					Degraded fields ($n = 10$)							
Upper	6.2	0.59	0.06	4.7	0.30	2.5	0.7	3.2	0.01	69	20	11
Middle	6.1	0.55	0.06	6.2	0.26	2.4	0.6	3.9	0.01	70	18	12
SED	0.29	0.08	0.01	1.7	0.07	0.7	0.1	0.9	0.002	3	3	1
					Degraded fields ($n = 10$)							
Onamudian												
Upper	5.3	0.90	0.11	4.56	0.30	2.04	0.86	8.6	0.03	60	27	13
Middle	6.3	0.81	0.09	7.13	0.41	2.74	1.19	6.6	0.02	69	20	11
SED	0.3	0.21	0.02	1.01	0.15	0.64	0.32	1.6	0.01	5	4	2
SED (village)	0.21*	0.11*	0.01*	1.00	0.08	0.48	0.17***	0.98**	0.003*	3	3	1
					Former kraals ($n = 10$)							
Chelekura												
Upper	7.2	1.7	0.15	14	0.8	4.5	1.2	7.5	0.03	68	22	10
Middle	7.3	1.6	0.16	18	1.1	5.2	1.3	8.4	0.04	66	23	11
SED	0.32	0.39	0.03	3.9	0.2	0.9	0.11	1.3	0.01	3.8	3.1	1
					Former kraals ($n = 10$)							
Onamudian												
Upper	6.6	2.1	0.20	24	0.7	5.4	2.0	12.0	0.06	59	26	15
Middle	6.7	2.4	0.23	21	0.8	5.1	1.70	11.2	0.05	66	25	9
SED	0.4	0.34	0.04	6.2	0.16	1.6	0.22	1.40	0.02	2.6*	1.95	1.5
SED (village)	0.24*	0.25*	0.03*	3.5	0.13	0.88	0.13***	0.89**	0.01*	2.5	1.71	1.4

*Significance: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.*
SOC, soil organic carbon; Tot N, total nitrogen; Extr. P, extractable P (Olsen); Exc. K, exchangeable K; Exc. Ca, exchangeable Ca; Exc. Mg, exchangeable Mg; CEC, cation exchange capacity; SED, standard error of difference.

TABLE 2 | Chemical properties of cattle manure used in the experiments.

Site/season	pH (H_2O, 1:2.5)	Total C (%)	Total N (%)	Total P (%)	Total K (%)	Total Ca (%)	Total Mg (%)	C/N ratio
Chelekura								
2005B	8.0	5.24	0.55	0.15	0.57	0.56	0.14	10
2006B	9.5	7.99	0.71	0.26	0.94	0.62	0.19	11
Onamudian								
2005B	7.5	8.31	1.09	0.39	0.85	1.24	0.49	8
2006B	7.0	5.61	0.70	0.28	0.52	0.60	0.17	8

seasons, and was poor in carbon (**Table 2**). The narrow C:N ratio (8–11) implies that manure used in both study sites was well decomposed.

Finger Millet Yield, Nutrient Uptake, and Nutrient Use Efficiencies

Analysis of yield, nutrient uptake, and physiological efficiencies data showed significant ($p < 0.001$) differences between sites; thus, further analysis was conducted by site to assess landscape position, treatment, season, and their interaction effects. Landscape position was significant ($p < 0.05$) for only N uptake in Chelekura village and for grain yield and physiological P efficiency in Onamudian village. Treatments were highly significantly different ($p < 0.001$) for almost all of the variables in both locations with the exceptions of PhEP and ANR in Chelekura. Landscape × treatment interactions were rare and only for PhEP and AEP in Chelekura and Onamudian villages, respectively.

Seasonal Variations

The relationship between inherent soil fertility, rates of fertilizer, and yield was investigated with the aid of three-quadrant diagrams (Wit, 1992). With this procedure, fertilizer application and yield responses (quadrant i) are split into the relationships between total nutrient uptake and yield (quadrant ii) and between fertilizer rates and total nutrient

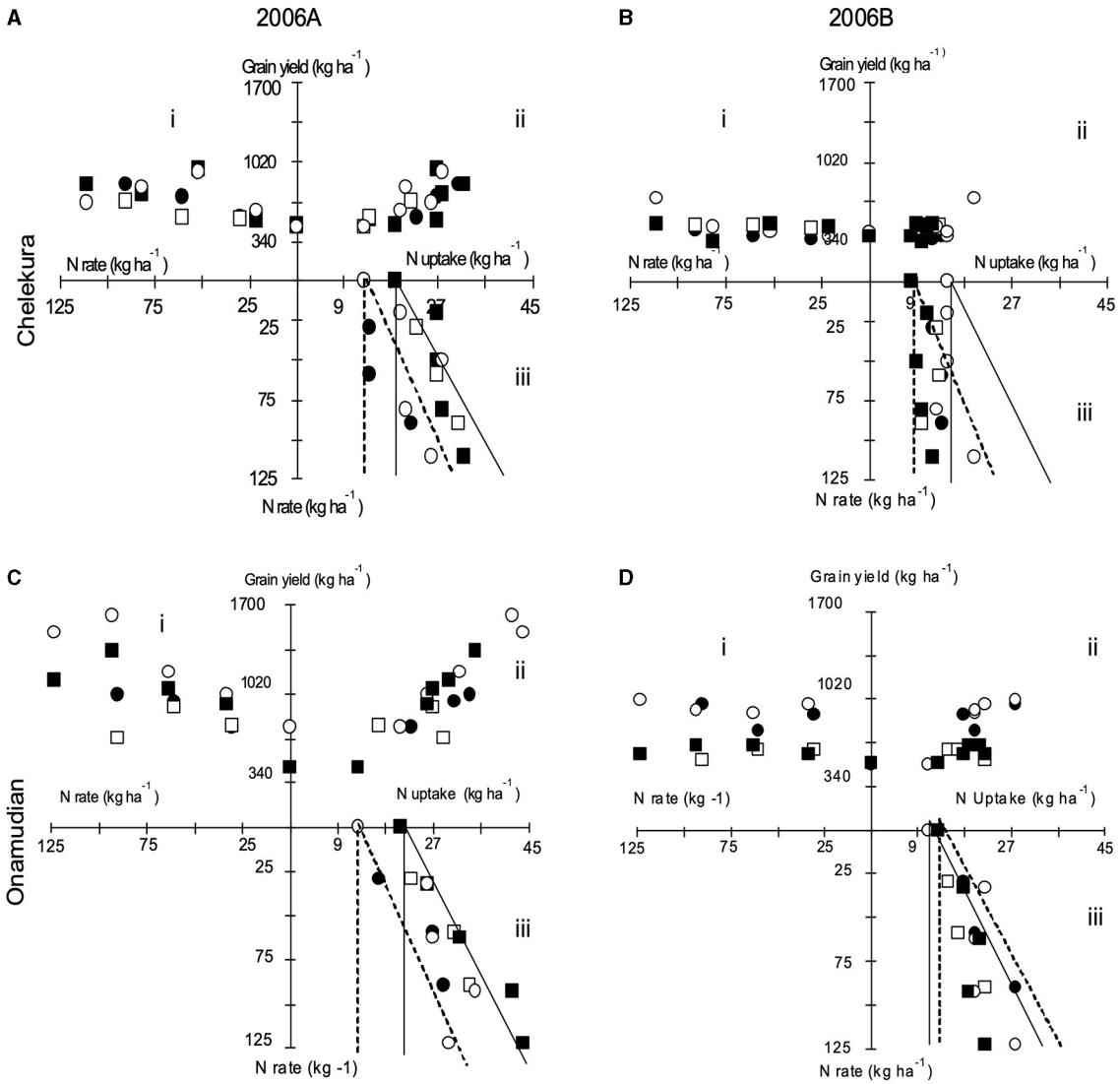

FIGURE 2 | Three-quadrant diagrams showing relationships between N application, N uptake, and grain yield of finger millet. (i) Yield against fertilizer rate (fertilizer use efficiency), (ii) yield against N uptake (physiological N use efficiency), (iii) N uptake against fertilizer application rate (fertilizer recovery) in degraded fields located on the upper and middle landscape positions in Chelekura **(A,B)** and Onamudian **(C,D)** village during seasons 2006A **(A,C)** and 2006B **(B,D)**. Open square, N alone, upper landscape position; full square, manure (3 t ha^{-1}) + N, upper landscape position; (full circle), nitrogen alone, middle landscape position; open circle, manure (3 t ha^{-1})+N, middle landscape position. Areas under bold line and dotted lines respectively represent ANR under 25% in the upper and middle landscape positions, respectively.

uptake (quadrant iii), for N (**Figure 2**) and P (**Figure 3**). These relationships were plotted for the grain yield of finger millet for treatments applied to degraded fields during seasons 2006A and 2006B by landscape position. Yield responses are related to nutrient uptake, which was influenced by the apparent nutrient recovery. Apparent nutrient recoveries were also determined by the indigenous nutrient supply by the soils and varied between seasons.

The indigenous supply of N was larger in both seasons in the upper than middle position with 19 kg N ha^{-1} and 13 kg N ha^{-1} (2006A) and 15 kg N ha^{-1} and 8 kg N ha^{-1} (2006B) in Chelekura village. In Onamudian, the indigenous supply of N was larger in the upper landscape position (21 kg N ha^{-1}) than in the middle position (13 kg N ha^{-1}) in 2006A. Indigenous supply of N, however, declined in the upper landscape position (11 kg N ha^{-1}) but remained the same in the middle landscape position (13 kg N ha^{-1}) in the 2006B season. Apparent N recoveries (ANR) were <25% for both the N-only treatments and manure+N treatments on both landscape positions in Chelekura village in each of the seasons. In Onamudian, only a few cases did it approach 40%. These low recoveries contributed to small total N uptake, which in turn determined the generally rather flat yield response curves in both villages (quadrant i). The responses in 2006A are due to nutrient application in 2005B and those

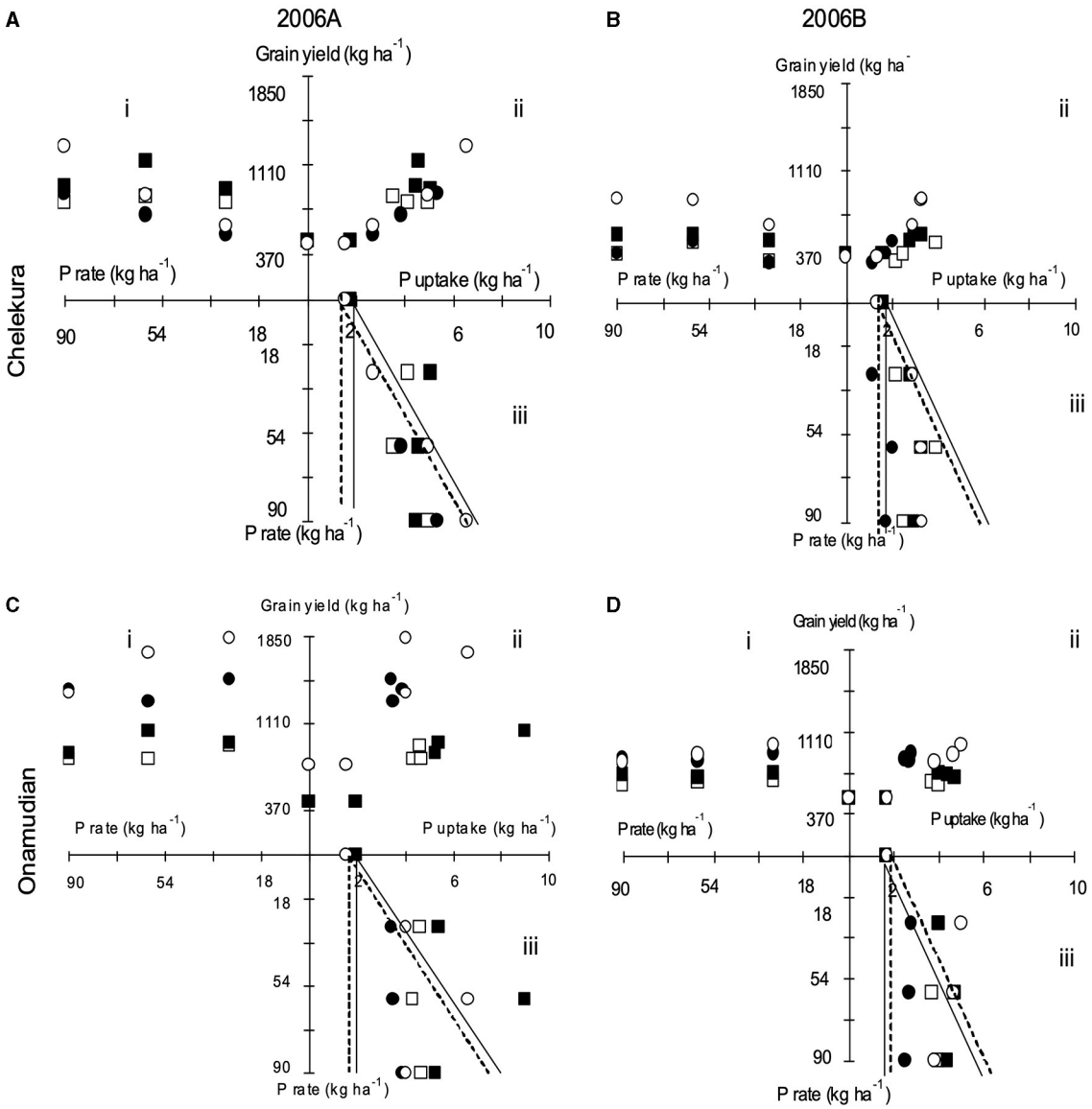

FIGURE 3 | Three-quadrant diagrams showing relationships between P application, P uptake, and grain yield of finger millet. (i) Yield against fertilizer P rate (fertilizer use efficiency), (ii) yield against P uptake (physiological P use efficiency), (iii) P uptake against fertilizer application rate (fertilizer recovery) in degraded fields located on the upper and middle landscape positions in Chelekura **(A,B)** and Onamudian **(C,D)** village during seasons 2006A **(A,C)** and 2006B **(B,D)**. Open square, P alone, upper landscape position; full square, N+P, upper landscape position; full circle, nitrogen alone, middle landscape position; open circle, N+P, middle landscape position. Areas under bold line and dotted lines respectively represent ANR under 25% in the upper and middle landscape positions, respectively.

for 2006B are due to nutrient application that season, plus any residual effect of P that was applied earlier. Application of manure with N occasionally gave slight increases in ANR, which was reflected in responses in N uptake and yield signifying an additive benefit from manure application, especially in the 2006A season.

The indigenous P supply was higher in 2006A than 2006B season for both landscape positions (**Figure 3**). In Chelekura village, indigenous P supply in the upper position (1.79 kg P ha^{-1}) was larger than in the middle position (1.59 kg P ha^{-1}) in 2006A and 1.61 kg P ha^{-1} in the upper position and 1.34 kg P ha^{-1} in the middle position in 2006B. Because of larger total P reserves, indigenous P supply in Onamudian village was larger than in Chelekura village: 1.93 kg P ha^{-1} (upper position) and 1.58 kg P ha^{-1} (middle position) in 2006A and 1.67 kg P ha^{-1} (upper position) and 1.70 kg P ha^{-1} (middle position) in 2006B. Apparent P recoveries (APR) were also small and usually <25% for both the P only and N+P treatments. P uptake was higher in N+P treatments than sole application of P in both study villages, implying that N and P were limiting in both sites. Yield responses were higher in 2006A than 2006B.

TABLE 3 | Average straw yield, grain yield, total nutrient uptake and internal nutrient use efficiencies, agronomic efficiencies, and recovery efficiencies of finger millet as affected by application of nutrient inputs to degraded fields on the upper and middle landscape positions in Chelekura village, 2 seasons of 2006.

Landscape position/ Treatment	Straw yield	Grain yield	RYI	N uptake	P uptake	Ph*EN*	Ph*EP*	AEN	AEP	ARN	ARP
	kg ha^{-1}		%	kg ha^{-1}		kg kg^{-1}					
Upper											
Control	1,043	443	0	17	1.7	28	276	–	–	–	–
30N	1,305	441	−0.5	18	2.6	26	194	−0.1	–	0.06	–
60N	1,341	550	24	21	2.4	28	251	−0.1	–	0.08	–
90N	1,649	625	41	23	3.0	28	228	1.9	–	0.05	–
30P	1,212	571	29	16	3.2	33	205	–	0.6	–	0.09
60P	1,268	668	51	15	3.4	52	223	–	6.0	–	0.05
90P	1,251	598	35	18	3.6	33	192	–	1.7	–	0.03
Manure*	1,255	552	25	21	3.5	36	251	10.3	43.3	0.38	0.51
M+30N	1,510	685	55	21	3.4	33	235	0.5	5.6	0.01	0.21
M+60N	1,650	783	77	23	2.6	28	237	2.6	44.0	0.02	0.28
M+90N	2,012	766	73	26	4.1	33	240	2.8	54.3	0.03	0.28
30N+30P	1,516	715	61	21	3.9	34	211	9.0	9.1	0.16	0.09
60N+60P	2,109	854	93	27	4.0	33	252	6.8	6.0	0.20	0.05
90N+90P	2,322	749	69	25	3.7	34	228	3.4	3.4	0.12	0.04
Middle											
Control	1,024	418	0	10	1.5	43	285	–	–	–	–
30N	1,147	493	18	13	2.1	37	254	2.5	–	0.12	–
60N	1,193	404	−3	14	1.5	35	263	1.8	–	0.11	–
90N	1,172	576	38	16	2.4	42	265	1.7	–	0.07	–
30P	1,425	679	62	18	3.5	42	234	–	−1.1	–	0.06
60P	1,185	600	44	17	2.9	37	217	–	3.6	–	0.04
90P	1,408	646	55	18	3.5	35	202	–	2.8	–	0.02
Manure*	1,065	462	11	15	2.8	30	239	11.5	46.8	0.45	0.43
M+30N	1,175	709	70	19	3.5	45	223	7.5	76.9	0.26	0.74
M+60N	1,536	628	50	17	2.1	37	229	12.6	29.3	0.64	0.24
M+90N	1,850	569	36	23	2.2	24	256	2.3	52.8	0.11	0.26
30N+30P	1,240	625	50	16	3.6	40	181	6.6	5.2	0.37	0.06
60N+60P	1,633	858	105	23	4.1	38	217	7.7	11.5	0.22	0.07
90N+90P	1,846	1,069	156	26	4.9	40	227	6.9	6.3	0.24	0.05
SED											
LP	330ns	156ns		3.2*	0.8ns	7ns	6ns	4.8ns	27ns	0.21ns	0.19ns
TRT	427***	201**		3.3***	1.1***	9ns	29***	5.2*	26.8*	0.22ns	0.21***
LP × TRT	380ns	180ns		3.2ns	0.9ns	8ns	18***	5.7ns	28.5ns	0.22ns	0.20ns

PhEN, physiological efficiency of nitrogen; PhEP, physiological efficiency of phosphorus; AEN, agronomic efficiency of nitrogen; AEP, agronomic efficiency of phosphorus; ANR, apparent nitrogen recovery; % RYI, relative grain yield increase calculated as (grain yield treatment−control yield)/control yield × 100; SED, standard error of difference; LP, landscape position; TRT, treatment.

Amount of N and P in manure is computed based on % N and P in manure; see **Table 2.*

*Significance: ns, not significant; *p<0.05; **p<0.01; ***p<0.001.*

Across-Season Analysis

Average straw and grain yield significantly differed between treatments ($p < 0.01$) but not between landscape positions nor treatment × landscape position interaction in Chelekura village (**Table 3**). Yield increased with increasing levels of nutrients and combinations in each landscape position. Grain yield ranged from 443 to 854 kg ha^{-1} and 418 to 1,069 kg ha^{-1} in the upper and middle landscape positions, respectively. The range of yield increase above the control or percent relative yield increase (RYI) was from −0.1% (30N) to 93% (60N+60P) on the upper landscape position and from −3% (60N) to 156% (90N+90P) in the middle landscape position and was generally better on combination of N+P and manure+N. Uptake of N was significantly ($p<0.05$) higher on the upper than middle landscape position. It also differed significantly ($p<0.001$) between treatments with the highest uptake usually obtained from the N+P treatments. The average N uptake ranged from 17 (control) to 27 kg ha^{-1} (60N+60P) on the upper landscape

TABLE 4 | Average straw yield, grain yield, total nutrient uptake and internal nutrient use efficiencies, agronomic efficiencies, and recovery efficiencies of finger millet as affected by application of nutrient inputs to degraded fields on the upper and middle landscape positions in Onamudian village, 2 seasons of 2006.

Landscape position / Treatment	Straw yield	Grain yield	GY increase	N uptake	P uptake	PhE*N*	PhE*P*	AEN	AEP	ARN	ARP
	kg ha^{-1}		%	kg ha^{-1}		kg kg^{-1}					
Upper											
Control	1,055	620	0	15	1.8	44	262	–	–	–	–
30N	1,381	826	33	20	3	41	217	7.0	–	0.15	–
60N	1,639	867	40	26	3.2	34	198	4.1	–	0.16	–
90N	1,863	987	59	31	3.6	32	200	4.2	–	0.18	–
30P	1,658	1,202	94	23	4.4	53	235	–	19.6	–	0.09
60P	1,938	1,077	74	23	4	50	200	–	7.7	–	0.05
90P	2,053	1,131	82	27	4.3	44	190	–	5.7	–	0.04
Manure	1,598	993	60	24	3.7	42	230	13.9	67.1	0.34	0.22
M+30N	1,564	1,037	67	26	3.7	41	233	6.6	37.2	0.15	0.19
M+60N	2,107	1,293	109	31	5.4	45	198	9.5	83.5	0.21	0.25
M+90N	2,374	1,241	100	36	5.8	35	154	5.2	67.1	0.16	0.62
30N+30P	2,154	1,405	127	27	4.7	57	231	25.7	19.6	0.53	0.12
60N+60P	2,527	1,889	205	37	8.2	56	157	12.0	12.2	0.31	0.10
90N+90P	2,803	1,102	78	32	4.4	39	151	4.8	5.0	0.20	0.05
Middle											
Control	823	482	0	13	1.6	39	250	–	–	–	–
30N	889	695	44	16	2.9	44	211	7.1	–	0.09	–
60N	1,524	766	59	22	3.5	37	156	4.7	–	0.15	–
90N	2,044	605	26	26	2.5	24	118	1.4	–	0.14	–
30P	1,174	798	66	19	3.1	45	214	–	10.5	–	0.05
60P	2,044	734	52	23	3.1	34	154	–	4.2	–	0.06
90P	1,387	717	49	20	3.2	38	167	–	2.6	–	0.02
Manure	1,417	790	64	24	3.7	34	159	12.5	31.5	0.40	0.29
M+30N	1,778	820	70	26	4.1	39	188	9.7	51.1	0.27	0.29
M+60N	1,413	858	78	22	4.1	36	150	3.7	22.2	0.10	0.52
M+90N	1,776	1,001	108	26	4.4	32	121	4.0	35.3	0.14	0.47
30N+30P	1,544	839	74	17	4.7	57	128	11.5	10.5	0.31	0.19
60N+60P	2,023	1,051	118	21	8.2	56	124	10.0	10.0	0.28	0.11
90N+90P	1,776	793	65	21	4.4	46	122	4.2	4.2	0.22	0.05
SED											
LP	347ns	153*		3.8 ns	0.59ns	4.0ns	25**	4.03ns	12.4ns	0.09ns	0.08ns
TRT	453***	195***		4.5***	0.76***	4.3***	26***	4.65***	13.3***	0.11***	0.09***
LP × TRT	401ns	175ns		4.2ns	0.68*	4.29ns	26ns	4.36ns	12.9*	0.10ns	0.09ns

PhEN, physiological efficiency of nitrogen; PhEP, physiological efficiency of phosphorus; AEN, agronomic efficiency of nitrogen; AEP, agronomic efficiency of phosphorus; ANR, apparent nitrogen recovery; % RYI, relative grain yield increase calculated as (grain yield treatment−control yield)/control yield × 100; SED, standard error of difference; LP, landscape position; TRT, treatment.

Amount of N and P in manure is computed based on % N and P in manure; see **Table 2.*

*Significance: ns, not significant; *p < 0.05; **p < 0.01; ***p < 0.*

position and from 10 (control) to 26 kg ha^{-1} (90N+90P) in the middle landscape position. Average P uptake also differed significantly ($p<0.001$) between treatments. Treatments that received P fertilizer generally resulted in significantly larger P uptake compared with the control. Only physiological P efficiencies differed significantly ($p<0.001$) between treatments and by treatment × landscape position and ranged from 192 to 276 kg kg^{-1} and 181 to 285 kg kg^{-1} in the upper landscape and middle landscape positions, respectively.

Agronomic efficiencies of N (AEN) were higher for manure+N and N+P treatments in both the upper and middle landscape positions. Manure treatments resulted in the highest agronomic efficiencies of P (AEP) ranging from 5.6 to 54 kg grain kg^{-1} P and from 29 to 77 kg kg^{-1} in the upper and middle landscape positions, respectively. Apparent N recovery (ARN) was <1 kg kg^{-1} for all the treatments although it tended to be better for manure-based and N+P treatments. Apparent recoveries of P were also small although again they tended to be

higher between 0.2 and 0.74 kg kg^{-1} for manure treatments in the upper and middle landscape positions.

Trends in average responses of yield, uptake, and physiological efficiencies in Onamudian village (**Table 4**) were similar to those in Chelekura with some minor differences. Grain yield was significantly ($p < 0.05$) larger on the upper than the middle landscape position. The RYI ranged from 33 to 205% in the upper landscape position and 26 to 108% in the middle landscape position. The yields in Onamudian village were higher than those in Chelekura village. N uptake was not significantly different between landscape positions, but physiological N efficiencies were significantly higher ($p < 0.001$) between treatments in the upper than in the middle landscape positions. They ranged from 32 to 57 kg ha^{-1} and from 24 to 57 kg kg^{-1} on the upper and middle landscape positions, respectively. Physiological P efficiencies were significantly higher in the upper (151 to 262 kg kg^{-1}) compared with the middle landscape position (118 to 250 kg kg^{-1}).

Agronomic efficiencies (AE), apparent N recovery fractions (ANR), and apparent P recovery (APR) from N, P, and manure+N applied to degraded fields were generally low. The AEN ranged from 3.7 to 26 kg grain yield per kilogram of N with usually better AEN recorded with manure N+P at equal rates of 30 or 60 kg ha^{-1} implying a complementarity role of P in agronomic use of N. The AEP were higher where manure and N were applied in both the upper and middle landscape positions reaching a highest level of 83.5 kg grain kg^{-1} of P with M+60N in the upper landscape position. The range for ANR was 0.10–0.53 kg kg^{-1} and for APR from 0.02 to 0.62 kg kg^{-1}.

Within-Farm Yield Differences

Across application rates, grain yield responses to application of N, P, manure, manure+N, and N+P treatments on degraded fields were variable, but yields were increased above the control treatment (**Figure 4**). Responses were larger in Onamudian than Chelekura village although in both villages yields obtained with fertilizers were always less than those on the former kraal sites. In Chelekura village, all treatments produced yields <1,000 kg ha^{-1}, and in Onamudian village, only manure+N (1,036 kg ha^{-1}) and N+P (1,171 kg ha^{-1}) produced yields >1,000 kg ha^{-1}. The trend in yield responses relative to the control were manure (0.21) < P (0.45) < N (0.47) < manure+N (0.62) < N+P (0.88) in Chelekura and (0.43) < N (0.64) < P (0.70) < manure+N (0.87) < N+P (1.11) in Onamudian village. Treatment application to degraded fields resulted in closing of gaps in grain yields between former kraals and the control treatment (1,532 kg ha^{-1}) by 6% (manure) to 24% (N+P) in Chelekura village and in Onamudian village (1,442 kg ha^{-1}) by 16% (manure) to 43% (N+P). Overall, grain yield responded more strongly to N+P application than to sole applications of either N or P, implying that both nutrients are limiting on both sites.

Other Limiting Nutrients

In the pot experiment conducted to explore whether nutrients other than N and P were limiting crop response in the field experiments (**Figure 5**), main effects of village and treatments were highly significant ($p < 0.001$) and village × treatment were significant ($p < 0.05$) on millet biomass. N alone significantly increased biomass yields of finger millet in degraded soils from Onamudian but not in the soils from Chelekura. When N and P were applied together, shoot growth increased much more strongly in soils from Chelekura and the increase in growth was doubled on Onamudian soils compared with the sole nutrients. Addition of K, together with N and P, significantly increased growth above the N+P treatment only in the soil from Onamudian. Adding sulphur increased plant growth only in the soil from Chelekura. Based on total biomass production, it appears that multiple nutrients limit productivity of millet on the degraded fields but that plant growth response depended on the interactions of N × P × S in Chelekura and N × P × K in Onamudian village. These combinations respectively resulted in 63 and 74% of biomass yield on former kraals. Adding other cations (Ca and Mg) or other micronutrients did not result in significant increases in biomass; to the contrary, they tended to give slightly depressed biomass yields.

DISCUSSION

Reversing nutrient depletion in smallholder farming systems is needed to assure food security for smallholders. Optimizing organic resources with inorganic nutrient sources together with improved germplasm and local adaptions can lead to restorative improvements of soil productivity (Vanlauwe et al., 2010). Knowledge on how to target nutrient resources is critical and using benchmarks within farming systems could be more realistic to evaluate the potential of nutrient interventions. In the Teso farming system, former kraal sites are the best for high productivity of finger millet and we used them as a benchmark

Application of N and P is recommended for improved millet productivity in the Teso farming system (Wortmann and Eledu, 1999). In this study, biomass production and yield of finger millet responded strongly when both N and P were supplied to the degraded fields, but in no case did yields match those found when finger millet was grown on former kraal sites. These former kraal sites are areas where manure has been accumulated over long periods of time through night corralling of cattle, although none of the sites where the experiments were situated had been used by cattle in the previous 5 years before this study. Persistence of good fertility in soils for at least four decades where kraals were formerly located has been reported from East Africa (Augustine, 2003).

The seasonal differences in millet yield response were strongly influenced by rainfall. No yield was obtained in the first season (2005B) due to drought. Yield and growth response in the subsequent season 2006A was dependent on nutrients applied as fertilizer and manure in the poor season (2005B), and thus observed variability in responses in 2006A may have been due to variability in nutrient losses. Although fertilizers and manure were applied again in the 2006B season, excessive rainfall (**Figure 1**) is likely to have caused substantial losses of N. Despite the excessive rainfall, strong responses in growth and yield of finger millet to combined applications of N and P were observed

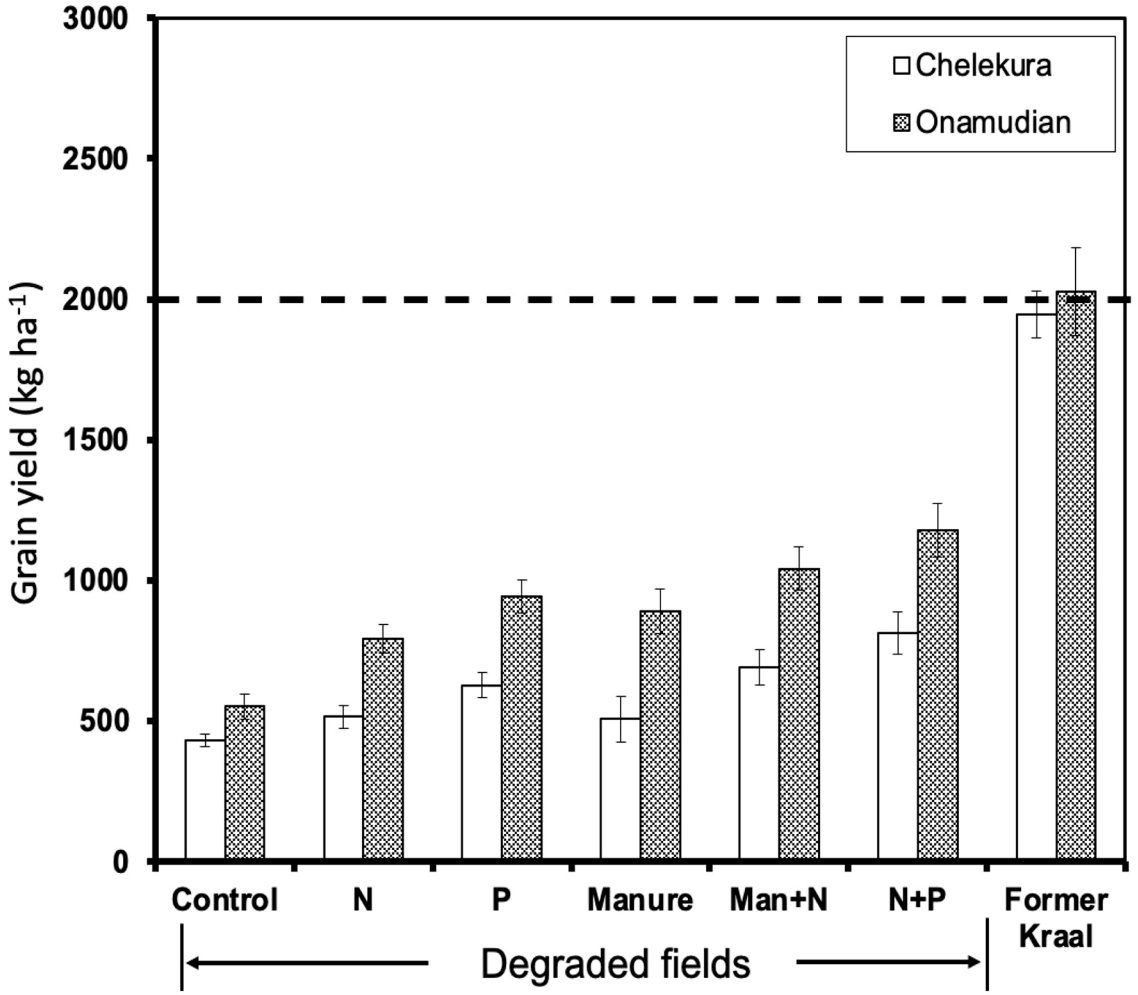

FIGURE 4 | Average responses of finger millet grain yields per applied treatment on degraded fields in comparison with the former kraal sites in the study villages, 2006. Bars are SEM.

in both 2006 seasons, although yields obtained were often only half those observed in the former kraal sites.

Soils differed between the two villages: the soils in Onamudian had greater silt+clay content (36%; sandy loam) than for those in Chelekura (31%; sandy clay loam). Silt + clay determine organic carbon storage through influencing physical protection of soil organic matter (Feller and Beare, 1997). In turn, these properties determine the capacity of soils to retain and supply cations. The soils in the former kraal sites had twice to three times as much SOC compared with the degraded fields in each of the landscape positions of each village. Variations in soil quality of former kraals between villages were also equally influenced by the differences in percent silt + clay of the soils, but could also vary due to different amounts of manure previously accumulated in those sites.

The effect of landscape position on millet response to fertilizer application was negligible and has been reported before in the area for legume-finger millet rotations (Ebanyat et al., 2010b). It implies that there is no strong soil fertility gradient in the villages but rather within locality or farm variability is critical for targeting and design of intervention options. The heterogeneity has been reinforced by lack of use of nutrient inputs affecting nutrient use efficiencies.

Internal, agronomic, and recovery efficiencies were generally low and explain the low millet yield response (**Figures 2**, **3**). Better responses have been associated with higher indigenous supply of nutrients (Xu et al., 2015), through increased availability of nutrients. Further, manure treatments often resulted in higher agronomic and recovery efficiencies of N and P through increased total amounts of nutrients and probably improved moisture availability. It should, however, be noted that the high PhEP for manure treatments is because of the small amounts of P supplied under conditions of P deficiency. Combining N and P improved agronomic and internal efficiencies of N indicating co-limitations of the two nutrients (**Figure 5**). Phosphorus provides energy for nitrate reduction promoting N uptake and utilization in plants (Gupta et al., 2014), hence a better agronomic N efficiency when N is combined with P. Agronomic P efficiencies were higher

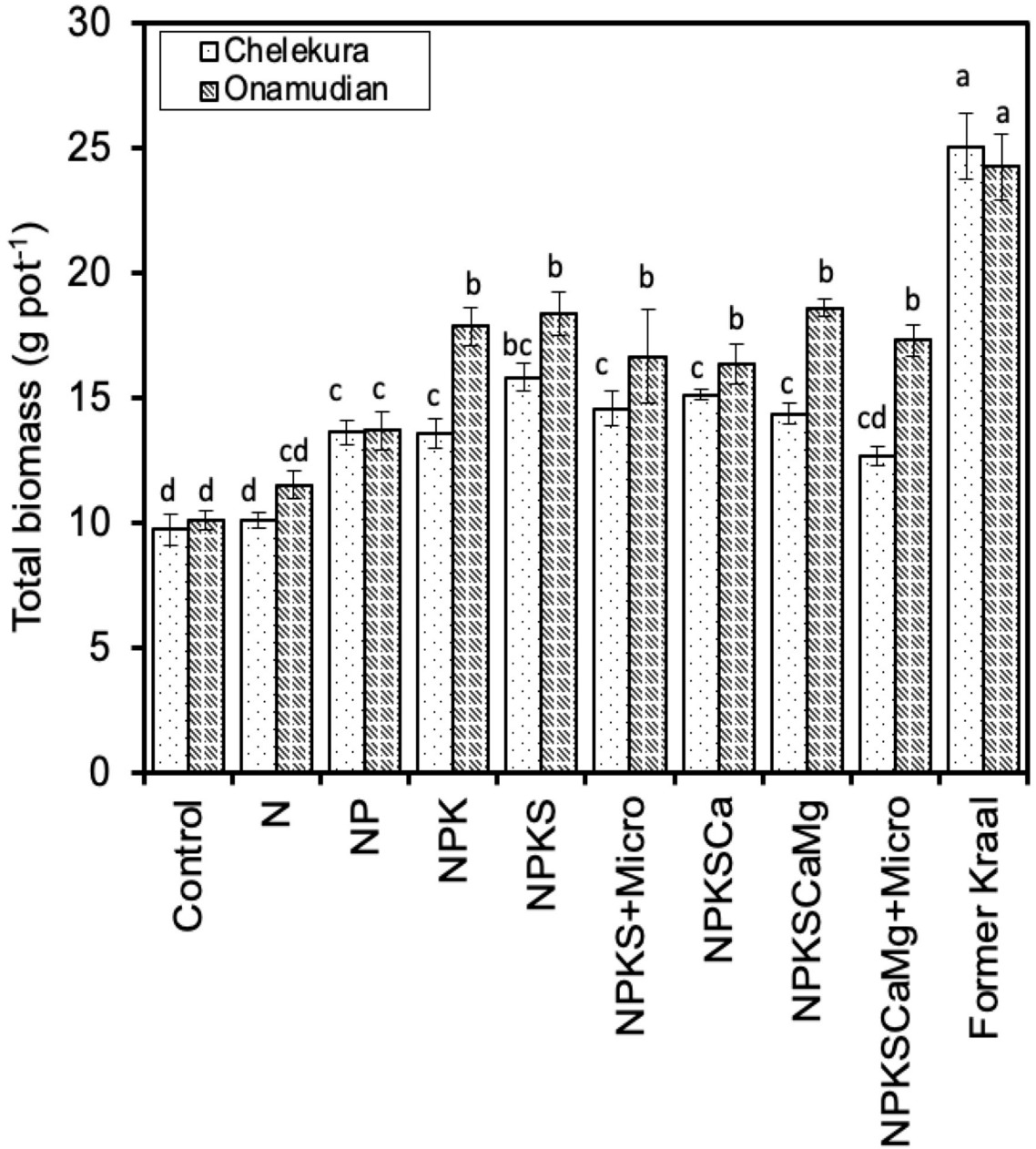

FIGURE 5 | Response of millet total biomass on soils from degraded fields from study villages amended with macro- and micronutrients and on former kraal soils in a greenhouse pot experiment over 8 weeks of growth. Bars are SEM. SE bars with same letters are not different.

in manure treatments probably because manure enhanced P availability as reflected in the higher ARP from the same treatments (**Tables 3**, **4**). This highlights an important role for manure in improving nutrient availability in the degraded fields.

The two nutrients alone, however, resulted in <45% of yields on former kraals (**Figure 4**) due to other constraints. Multiple nutrient deficiencies of N, P, Ca, and Zn were reported to constrain rehabilitation of productivity on degraded sandy soils in Zimbabwe (Zingore et al., 2008). The limiting nutrient experiment that we conducted in pots showed that S and K were additionally limiting millet growth in soils from Pallisa (**Figure 5**). We had expected that treatments where manure was added would have supplied other nutrients such as K, S, and micronutrients, but it seems that manure was unable to provide sufficient quantities of these nutrients in the short term. In field experiments in Zimbabwe, responses in growth and yield of maize to old kraal manure were seen only in the third year after application (Nyamangara et al., 2005; Zingore et al., 2007).

We further observed yield declines when P was applied at rates above 60 kg P ha^{-1} that may be associated with Zn–P antagonism arising from precipitation of zinc phosphate (Marschner, 1995). Soil organic matter also determines the physical properties of soils. The soils in the area are prone to surface sealing and often crusts are observed following rain events. Enhanced soil organic matter contents can improve the water balance in the degraded fields by reducing the susceptibility to crusting and enhancing infiltration. The good productivity of finger millet on the former kraal sites could be attributed to the beneficial effects of manure on many aspects of soil fertility: improving structure, moisture availability, nutrient availability including micronutrient supply, and biological activity, which can enhance nutrient cycling.

It is noteworthy that the conditions created in soils at former kraals arise from long-term accumulation of manure. Improving the conditions in the degraded fields will therefore require substantial time and large applications of manure. The quantities of manure available in this region are limited and difficult to increase—the smallholder farmers lack grazing land to feed cattle producing manure (Ebanyat, 2009), which means that more cattle cannot be supported in the area. Fertilizer use and recovery efficiencies were low probably because of the low SOC in the degraded fields and other losses. Rehabilitation of the degraded fields will require building up of organic matter to thresholds that can enhance fertilizer use efficiencies (Tittonell et al., 2007; Musinguzi et al., 2016), but it is unclear how the required amounts of organic matter can be sourced or created.

Attention to balanced crop nutrition, ensuring that fertilizers supply all of the necessary nutrients for crop growth, may give sufficient crop residues, which, if returned to the soil, may contribute to increase soil organic matter contents. Our results indicate that the declaration of the African Fertilizer Summit made in Abuja 2006 to aim for farmers to use 50 kg of fertilizer per hectare needs careful consideration because it will not yield much unless degraded fields are first rehabilitated. Responses of finger millet differed between sites with fields in the Chelekura site being less responsive compared with Onamudian because of the initial soil quality. The degraded fields in Onamudian had higher amounts of SOC compared with Chelekura (**Table 1**). Different amounts of inputs are required to raise productivity of fields in these two different villages reiterating the need for site-specific nutrient management and that such blanket fertilizer recommendations are inappropriate. Our experiments over three seasons yielded reasonable responses to fertilizers and manure, and from the knowledge that the process of rehabilitation takes time, dynamic modeling may help in designing strategies for intensification (e.g., Tittonell et al., 2008). Further experimentation is required to determine the quantities of organic manure and nutrients needed and the period it may take to restore fertility. Further field experiments are needed to assess the effects of application of all the limiting nutrients, including S, K, and micronutrients, on millet yield in the degraded fields.

CONCLUSION

Although growth and grain yield of finger millet in degraded soils were increased strongly by application of fertilizer and manure, none of the treatments could completely close the difference in yields obtained on sites of former kraals. The short-term nature of the experimentation, covering only three seasons, was insufficient to restore fertility of these degraded soils, even where cattle manure was applied in farmer's fields. The amounts of manure accumulated in former kraal sites were very large compared with the amounts added in the experiments, and probably insufficient to address the multiple nutrient limitations of S and K. Combined application of N+P fertilizer gave the strongest yield response compared with other options, but the strength of the crop response was variable with season, soil type, and, to a lesser extent, landscape position. Management aimed at increasing nutrient recovery efficiencies will need to accompany technological interventions to enhance sustainability. Thus, combining organic and inorganic resources (integrated nutrient management) because of their complementary benefits could lead to improved productivity of the degraded fields. Repeated applications of manure would be required to increase soil organic matter contents sufficiently to assist in improving capture (infiltration) and storage of water. The scarcity of manure in the area, due to the small number of cattle and the lack of grazing land, means that other means to restore soil organic matter contents of the soils and supply of other limiting nutrients must be sought.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

PE: field experimentation, data collection, analysis, and drafting of manuscript. NR, MB, KG, and RD: conceptualization and design experiments and editing of article. All authors contributed to the article and approved the submitted version.

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Integrated Bioplant and Groundnut Husk Biochar Compost Application on Yield of Lettuce in a Rhodic Kandiustalf

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Bioplant is a liquid soil conditioner that contains a consortium of beneficial fungi and bacteria manufactured by Artemis and Angel Company Limited in Bangkok. Bioplant is purported to stimulate beneficial microbial activity in soil and hence increase crop yield. However, the efficacy of Bioplant has not been evaluated on Ghanaian soils. A screen house trial was consequently conducted to evaluate the efficacy or otherwise of Bioplant on the yield of lettuce in a Rhodic Kandiustalf amended with or without compost. The soil was mixed with compost at 20 parts soil to 80 parts compost and 60 parts soil to 40 parts compost (v/v) and potted in 1.7-L pots. There was another potted soil with no compost amendment. To each of these potted soils, Bioplant was applied at four rates, viz., zero, half the manufacturer's recommended rate, the manufacturer's recommended rate, and twice the manufacturer's recommended rate, and allowed to equilibrate for 2 weeks. Seedlings of lettuce of the variety Eden were transplanted into the pots, and the treatments kept at 80% field capacity. The treatments were replicated four times in a completely randomized design. At physiological maturity, the lettuce was harvested, and fresh and dry matter yields were taken. The C and N contents and N uptake in the harvested plants were also determined. Results indicate that conditioning the soil with Bioplant at half and the manufacturer's recommended rates increased N uptake, resulting in higher carbon accumulation with concomitant increases in both fresh and dry matter yields. The results also show that amending the Rhodic Kandiustalf with Bioplant at twice the manufacturer's rate suppressed yield. Application of Bioplant at the manufacturer's recommended rate in combination with compost amended at 40 parts to 60 parts soil (v/v) saw a 47 and 90% respective significant yield increases in fresh weight and dry matter when only Bioplant was applied at the manufacturer's recommended rate. It is therefore recommended for Bioplant to be applied at the manufacturer's recommended rate of 825 mL/ha in combination with 40 parts of compost to 60 parts of soil (v/v).

Keywords: biochar, compost, nitrogen uptake, carbon, soil conditioner

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INTRODUCTION

Soils of Ghana, except for the few variants of Vertisols in the Accra plains, are generally low in fertility. Their organic carbon contents are <1%, and the levels of N and P are very low (SRID MoFA, 2007; Obiri-Nyarko, 2012). The soils are also dominated by low-activity clays, mainly kaolinites and sesquioxides, with a cation exchange capacity (CEC) usually below 15 cmol(+)/kg.

To obtain appreciable crop yields, the application of nutrient from external sources has become imperative. Application of inorganic fertilizers through the soil for plant uptake has been the commonest way by which nutrients are made available to crops in Ghana. Thus, there is application of mainly inorganic fertilizers, of which the ammonium-based ones (urea and sulfate of ammonia) are the most popular. Application of these fertilizers has also aggravated the acidity problem as a result of nitrification under aerobic conditions (Chang et al., 2007; Gong et al., 2009). Thus, about 85% of soils have become acidic (Buri et al., 2005), making them prone to P fixation. Consequently, the soils are low in productivity. Fertilizers are also expensive and generally beyond the reach of small-scale farmers, who are in the majority. The government subsidy policy on fertilizer implemented in 2008 has also not helped much because of cross-border smuggling (Yawson et al., 2010). Additionally, the inorganic fertilizers are usually not in close proximity to the farmers. Consequently, fertilizer use in Ghana as of 2019 stood at a paltry 20 kg/ha (SRID MoFA, 2019).

Nutrient use efficiency of the already low rate of fertilizers applied to soils in Ghana is low because of the poor nutrient holding capacities of the soils as a result of the low organic matter content and the presence of the low-activity clays. Returns on fertilizer application are thus low, negatively affecting soil and crop productivity, and consequently food security. Improving nutrient use efficiency would demand increasing the organic matter content of soils in Ghana to improve on their nutrient holding capacity (Tandy et al., 2009), while making the nutrient more readily available. An intervention to improve nutrient storage and increase nutrient use efficiency is to apply compost. Compost, while supplying nutrients in the readily available form, increases the soil humus content to improve on the nutrient holding capacity (Dutta et al., 2003). Compost, being a buffer and alkaline can offset pH decreases arising from nitrification under aerobic soil conditions (Akumah et al., 2021; Sulemana et al., 2021). The amendment also improves on the general health or quality of the soil (Meena et al., 2016), by boosting the micro and macro flora and fauna population and activity (Ros et al., 2003) and organic carbon content (Sulemana et al., 2021) in the soil. Composting could also be a way of closing the nutrient loop in circular photosynthesis by returning nutrient to the soil, thus minimizing nutrient mining.

A drawback of the use of compost, especially, in short duration crops is the slow release of nutrients (Zhang et al., 2009; Chen et al., 2013), which is not in synchrony with plant uptake. Should the availability of nutrients from compost be improved, it could be the panacea to the low fertility and productivity problem of Ghanaian soils. Any addition to compost that would accelerate the release of nutrients or make nutrients more readily available would be an impetus to improving the productivity of Ghanaian soils.

Bioplant is a soil conditioner manufactured by Artemis and Angel Company Limited in Bangkok, Thailand. Bioplant is a microbial liquid conditioner that contains bacteria and fungi and 7.00 g N/L, 4.00 g P_2O_5/L, and 5.10 g K_2O/L among other nutrients. It is purported to stimulate the activity of beneficial microbes in soil to improve nutrient uptake and hence increase crop yield. For Bioplant to be very effective, the soil must have high content of organic matter which would boost the proliferation of beneficial microbes and hence improve nutrient release[1] (Dutta et al., 2003).

Most soils in sub-Saharan Africa and particularly Ghana are low in organic carbon contents with levels lower than 10 g/kg (Jones et al., 2006). Bioplant may, therefore, not be very effective in such soils. As a means of increasing organic matter application to soils and managing organic waste, composting is being deployed in Ghana (Akumah et al., 2021). Many composting companies have thus sprung up in Ghana, and compost as a nutrient source has been included in the Ghanaian government's fertilizer subsidy program (SRID MoFA, 2019). Compost use, especially in northern Ghana where organic matter levels are exceptionally low, is expected to increase. Bioplant would be important to the Ghanaian farmer if it is applied in combination with compost to the soil. The efficacy of Bioplant would be ascertained better if it is applied with compost or decomposed organic matter under controlled conditions.

As a soil conditioner, Bioplant would be deemed efficacious if it aids in the release of nutrients rapidly to meet the demand of crops. This attribute could be evaluated best if Bioplant is assessed on short-duration crops. It is in the light of this that a screen house experiment was conducted using lettuce (*Lactuca sativa*), a leafy vegetable of short duration with a high N requirement as a test crop. Evaluation of the efficacy or otherwise of the conditioner was carried out on the Toje Series, a Rhodic Kandiustalf and one of the most widely cultivated soils of the Coastal Savanna Zone of Ghana but of poor fertility. Should Bioplant promote N uptake and increase yield of lettuce from the Toje Series amended with both the conditioner and compost over the crop grown only on a compost amended soil, then the conditioner would be deemed to be efficacious enough in enhancing the availability of nutrient from soils amended with organic fertilizers.

A screen house experiment was conducted using Bioplant as a booster for N availability in a compost-amended Toje Series with the hypothesis that the biofertilizer would enhance N availability and uptake and, consequently, increase the yield of lettuce.

The objectives of the study were two-fold:

i. to verify if the manufacturer's recommended application rate of the conditioner is effective in increasing N uptake and

[1]http://artemisthai.com/wp-content/uploads/2014/07/Regenerative-Agriculture (accessed March 02, 2021).

hence yield of lettuce in a highly weathered Ghanaian Rhodic Kandiustalf of low fertility and

ii. to ascertain the efficacy of Bioplant when applied in combination with compost on the yield of lettuce.

MATERIALS AND METHODS

Soil Sampling and Preparation

The Toje Series, a Rhodic Kandiustalf, was used as the soil for the study. This soil is widely cultivated in the Coastal Savanna Zone of Ghana but is inherently low in fertility. The plow layer of the soil was sampled from an area that had had no known history of fertilizer and any soil conditioner application. The soil was air-dried and passed through a 2-mm sieve to obtain the fine earth fraction for some physicochemical analyses. Undisturbed soil samples were taken for the determination of bulk density and water holding capacity (WHC). The particle size distribution of the fine earth fraction was determined using the Bouyoucos hydrometer method (Day, 1965). Moisture content of the soil at maximum WHC was determined by covering the water-saturated sample of the undisturbed soil with polythene sheets and determining the moisture content after allowing gravitational water to drain for 48 h. Bulk density was determined using the core method by Blake and Hartge (1986). The pH and electrical conductivity (EC) of the fine earth were determined electrometrically in water using a 1:1 soil-water ratio (w/v) on an Oakton PC 2700 pH and EC meters. Organic carbon content of the fine earth was determined on a Leco TruMac CNS analyzer after destruction of carbonates in the soil with 6 M HCl. Total N in the fine earth fraction was analyzed using the Leco TruMac CNS analyzer with total P being extracted by wet digestion using perchloric and nitric acids. Available P in the fine earth fraction was extracted according to the method of Bray and Kurtz (1945). Total and available P concentrations in the extracts were determined on a UV spectrophotometer after color development using the Murphy and Riley (1962) method. The CEC of the soil was determined by the ammonium acetate method.

Screen House Experiment

Maize stubble was co-composted with groundnut husk biochar in the ratio of eight parts of the maize stubble to two parts of groundnut husk biochar on volume-to-volume basis.

An unprocessed Toje Series (unprocessed soil) was packed into 1.7-L plastic pots with drainage holes to attain the field bulk density of the soil. Subsequently, the soil was mixed with the compost in two soil-to-compost ratios of 20:80 (C80) and 60:40 (C40) on a volume basis and packed. To another pot, only soil was packed (C0), and this served as the control. Bioplant (BP) as a conditioner was applied to the potted soils at four rates of zero (BP0), half the manufacturer's rate (BP1), the manufacturer's recommended rate (825 mL/ha) (BP2), and twice the manufacturer's rate (BP3). All the soils were allowed to equilibrate for 2 weeks amidst watering to attain 80% WHC. While allowing the soils to equilibrate, lettuce seeds of the variety Eden were nursed. At the end of the 2 weeks, the seedlings were transplanted at two per pot. The moisture content of the media for growth (soil ± compost ± Bioplant) was maintained at 80% WHC to avoid leaching. These were replicated four times. Thus, with three compost application rates of 0, 40, and 80 and four Bioplant application rates and four replications arranged in a completely randomized design, there were a total of 48 experimental units.

Agronomic Evaluation

At maturity, the lettuce was harvested, and the fresh weight determined. The lettuce was then dried in an oven at 65°C until a constant weight was attained and the dry matter content taken. The dried lettuce leaves were then milled, and the C and N contents determined on a TruMac C–N analyzer. The N uptake in the leaves per pot for each treatment was estimated by multiplying the leaf N content with the dry weight.

Residual Soils

After harvest, the soils from the various pots were poured out into plastic bowls, homogenized, and subsampled for air-drying. These were then sieved to obtain the fine earth fraction and analyzed for pH, organic carbon, total N, and available P as described earlier.

Data Analysis

The aforementioned measured parameters were subjected to analysis of variance (ANOVA) using Genstat 12th edition to establish, if any, significant treatment effects at $p < 0.05$. Mean separations were done using Tukey's least significant difference (LSD) (0.05).

RESULTS

Characterization of Soil

Some physical and chemical properties of the soil used for the study are presented in **Table 1**. The sand fraction of the soil at 74.4% was 12 times more than the clay fraction of 6.2%. The silt content was 19.4%, giving the soil a loamy sand texture. The bulk density of the soil was 1.3 Mg/m^3. The soil had a moderately acidic pH of 5.7 with a low EC of 0.35 dS/m. Typical of Ghanaian soils, the organic carbon content (8.0 g/kg) was below 10 g/kg. Consequently, total N and available P were also low with values of 0.7 g/kg and 5.96 mg/kg, respectively. The total P concentration of 108.2 mg/kg was also low. The CEC of 5.62 cmol(+)/kg was low and characteristic of highly weathered tropical soil with low-activity clay.

Some chemical properties of the compost used in the study are presented in **Table 2**. The compost had an ideal neutral pH of 7.4 with an EC of 4.67 dS/m. The total carbon content was 326.0 g/kg with a total N content of 13.2 g/kg and a low available N concentration of 0.9 g/kg. Consequently, the compost had a C:N ratio of approximately 24.7, which was slightly above the critical value of 20. The compost had a very high total P content of 7,366.7 mg/kg, of which about 5% was in the available form (370.0 mg/kg). The total calcium content of the compost was 6.05 g/kg, with the magnesium content of about 5.41 g/kg being 10% lower. The compost had a low sodium content of 2.16 g/kg, with the total potassium content of 50.03 g/kg accounting for almost 79% of the total bases.

TABLE 1 | Some physicochemical properties of Toje Series*.

Sand	Silt	Clay	Bulk density	pH	EC	OC	TN	Av. P	CEC
	%		Mg/m^3	(H_2O)	dS/m	g/kg		(mg/kg)	cmol(+)/kg
74.4	19.4	6.2	1.3	5.7	0.35	8.0	0.7	5.96	5.62

EC, electrical conductivity; OC, organic carbon; TN, total nitrogen; Av. P, available phosphorus; CEC, cation exchange capacity.

TABLE 2 | Some chemical properties of the compost used.

Parameters	Results
pH $(1:1)_{water}$	7.4
EC (dS/m)	4.67
Total carbon (g/kg)	326.4
Total nitrogen (g/kg)	13.2
Available nitrogen (g/kg)	0.9
Total P (mg/kg)	7,366.7
Available P (mg/kg)	370.0
Total Ca (g/kg)	6.05
Total Mg (g/kg)	5.41
Total Na (g/kg)	2.16
Total K (g/kg)	50.03

TABLE 3 | Effect of compost on yield, nutrient composition and N uptake in lettuce*.

Treatment	Fresh weight	Dry weight	C	N	N uptake
	(g)		(g/kg)		(g/pot)
C0	104.27a	3.98a	410.8a	36.7ab	0.15a
C40	220.7b	12.01b	473.2c	39.1b	0.49b
C80	107.3a	3.58a	432.8b	34.9a	0.13a
Cv (%)	18	17.8	4.5	8.8	18.5

*Means with the same alphabet are not significantly ($p < 0.05$) different.
C0, no application of compost; C40, compost-to-soil application ratio of 40:60; C80, compost-to-soil application ratio of 80:20; Cv, coefficient of variation.*

Compost Effect on Fresh and Dry Matter Yields and Nutrient Content of Lettuce

The effect of compost on lettuce yield, nutrient content, and uptake is presented in **Table 3**. The un-amended soil produced lettuce with fresh and dry matter weights of 104.3 g and almost 4 g, respectively. On amending the soil with 40 parts of compost (C40), the fresh and dry matter yields of the crop saw 2.1-fold and 3-fold significant increases, respectively ($p < 0.05$), to 220.7 and 12.01 g.

Increasing the volume of compost applied to 80 parts decreased yield by almost 51% from 220.7 g in the C40 amended soil to 107.3 g fresh weight ($p < 0.05$), statistically similar to the fresh weight of lettuce from the un-amended soil. The dry matter, which was 12 g in the C40 treatment, also decreased to 3.58 g, statistically similar ($p < 0.05$) to the dry matter yield of lettuce in the un-amended soil.

TABLE 4 | Effect of Bioplant on fresh and dry matter yields and N uptake of lettuce.

Treatment	Fresh weight	Dry weight	C	N	N uptake
	(g)		(g/kg)		(g/pot)
BP0	94.73a	3.33a	397.0a	32.3a	0.11a
BP1	146.52b	7.09c	452.2b	38.9b	0.29c
BP2	200.28c	9.9d	458.9b	37.9b	0.41d
BP3	134.82b	5.77b	447.8b	38.5b	0.22b
Cv (%)	18	17.8	4.5		8.8

*Means with the same alphabet are not significantly ($p < 0.05$) different.
BP0, no Bioplant application; BP1, Bioplant at half the manufacturer's rate; BP2, Bioplant at the manufacturer's recommended rate; BP3, Bioplant at twice the manufacturer's rate.
Cv, coefficient of variation.*

The carbon content of the lettuce in the un-amended soil was 410.8 g/kg. On application of 40 parts of the growing medium with compost (C40), the carbon content of the lettuce increased ($p < 0.05$) by 62.4 g/kg to 473.2 g/kg. A further 40-part increase in volume of compost added to the soil (C80), however, decreased carbon content in the lettuce to 432.8 g/kg, albeit 22 g/kg significantly higher ($p < 0.05$) than the carbon content in lettuce grown in the un-amended soil. The nitrogen content of lettuce from the un-amended soil was 36.7 g/kg, which was statistically similar ($p < 0.05$) to the 39.1 g/kg in lettuce grown in the soil amended with 40 parts of compost (C40). However, when 80 parts of compost were applied to the soil, the N content in the lettuce decreased significantly by 4.2 g N/kg from that in the C40 soils to 34.9 g/kg, albeit similar ($p < 0.05$) to the N contents in lettuce grown in the un-amended soil. Uptake of N by lettuce was in the order ($p < 0.05$) C40 > C0 = C80. It is worth noting that lettuce uptake of N in the soil amended with 40 parts of compost was over three times higher than that from the un-amended and amended soils with 80 parts of compost.

Effect of Bioplant on Fresh and Dry Matter Yields and Nutrient Content of Lettuce

The effect of Bioplant on fresh and dry matter yields of lettuce is presented in **Table 4**. The fresh weight of lettuce grown in the non-conditioned soil (BP0) was 94.73 g. On addition of the Bioplant at half the manufacturer's rate (BP1), there was an almost 55% increase ($p < 0.05$) in the fresh weight of lettuce to 146.52 g. Dry matter yield, which was 3.33 g in the non-conditioned soil, increased ($p < 0.05$), 2.13-fold to 7.09 g on addition of Bioplant.

Increasing further the Bioplant application rate to that of the manufacturer recommendation (BP2) saw a 2.1-fold and almost

1.4-fold increases in fresh weight of lettuce over those grown in BP0 and BP1 soils, respectively.

Carbon accumulations were generally statistically similar ($p < 0.05$) in lettuce grown in the Bioplant-amended soils, irrespective of application rate as the carbon contents were approximately between 450 and 460 g/kg.

Nitrogen levels in the lettuce followed a similar pattern as carbon, with the lettuce from the Bioplant-amended soils being similar in concentrations of the primary nutrient accumulated, albeit with significantly higher ($p < 0.05$) values than those accumulated in plants grown in the un-amended soils. The positive attribute of Bioplant was also evident in the 2.64-fold increase in N uptake of lettuce grown in soil conditioned with Bioplant at half the manufacturer's rate (BP1) over lettuce plants grown in the unconditioned soil (BP0). At the manufacturer's recommended rate (BP2), N uptake increased ($p < 0.05$) approximately 41% over uptake in lettuce grown in the soils amended with Bioplant at half the manufacturer's recommended rate (BP1).

Increasing the application rate to twice the manufacturer's recommended rate (BP3) saw a 33% reduction in fresh matter yield from that of lettuce plants grown in soils conditioned with Bioplant at the manufacturer's recommended rate. Uptake of N consequently decreased to almost half that in lettuce at the recommended rate. Uptake of N in lettuce induced by Bioplant application was in the order ($p < 0.05$) BP2 > BP1 > BP3 > BP0.

Interactive Effect of Bioplant and Compost on Yield and Nutrient Content

The interactive effect of both compost and Bioplant application on yield of lettuce is presented in **Table 5**. From the table, when the compost at 40 parts was applied in combination with Bioplant at half the manufacturer's recommended rate, the fresh and dry matter of lettuce harvested were almost 240 and 14.38 g, respectively. Carbon content in lettuce was 486.2 g/kg, much higher ($p < 0.05$) than when either compost or Bioplant only was applied at any of the rates. Nitrogen accumulation in the lettuce was 42.20 g/kg, which is 3.1 g N/kg higher ($p < 0.05$) than the highest accumulation in lettuce when either compost or Bioplant alone was amended to the soil. Uptake of N in lettuce, which was 0.61 g/pot, was far higher than when either of the two amendments only was applied to the soil.

On doubling the rate of Bioplant to the full recommended rate while maintaining the compost rate at 40 parts (C40), the fresh weight of the lettuce did not increase significantly from that at the half rate of Bioplant. However, the dry matter yield increased almost by 31% from 14.38 to 18.85 g. Carbon and N accumulations were similar to those in lettuce when the half Bioplant rate and C40 compost were applied to the soil. Nitrogen uptake increased significantly ($p < 0.05$) from that at the half Bioplant rate and C40 from 0.61 to 0.85 g/pot.

At twice the manufacturer's recommended rate of Bioplant application in combination with C40, both fresh and dry matter yields of lettuce declined significantly from those at the manufacturer's rate, corroborating the decline in yield when twice the recommended rate was applied with no compost amendment. Combining Bioplant at the recommended rate with 40 parts of compost produced lettuce that is almost 95 g heavier in fresh weight and 9 g heavier in dry matter than the corresponding lettuce grown in only Bioplant-conditioned soil at the manufacturer's recommended rate (BP2). These increases represented 47 and 90% significant yield increases ($p < 0.05$) in fresh weight and dry matter, respectively, in the combined application over the application of only Bioplant. Similarly, application of Bioplant at the recommended rate in combination with 40 parts of compost gave lettuce that is almost 74 g heavier in fresh weight and 6.84 g heavier in dry matter weight than lettuce counterparts grown in soil amended with only 40 parts of compost. These significant increases in yield represented 34 and 57% increment over yield of lettuce grown in compost only at 40 parts application rate to soil.

It is noteworthy that combining C40 and Bioplant at half the recommended rate produced lettuce with fresh weight that was statistically similar to the fresh weight obtained when C40 was combined with Bioplant at the manufacturer's recommended application rate. However, when the former (C40 + BP1) is compared with only Bioplant at half the manufacturer's recommended rate (BP1), there was a 64% increase in fresh weight of lettuce compared with the C40 + BP1-amended soil.

Combining 40 parts of the compost with all the three rates of Bioplant did not show any changes in C and N accumulation in the lettuce leaves, with the levels of carbon ranging between 471.2 and 504.7 g/kg and those of N between 35 and 45 g/kg. Nitrogen uptake among the treatments, however, was significantly different. Amendment of Bioplant and 40 parts of compost to the Toje Series saw N uptake in the following order: manufacturer's recommended rate > half the manufacturer's rate > double the manufacturer's rate (**Table 5**). Combination of 40 parts of compost with Bioplant at both half and full manufacturer's recommended rates gave more than 1.5-fold increases in N uptake in lettuce over lettuce grown in Bioplant-amended soils only (**Tables 3**, **5**). Nitrogen uptake of lettuce from the soil amended with C40 and twice the recommended Bioplant application rate (0.28 g/pot) was, however, similar to the uptake when lettuce was grown in the soil amended with only Bioplant at half and twice the manufacturer's recommended application rates.

When the compost was applied at 80 parts on a volume basis to 20 parts of the soil in combination with the three different rates of Bioplant, the yield of lettuce decreased with respect to those at similar rates of Bioplant and 40 parts compost amendment. At half the Bioplant recommended application rate with C40 compost amendment, fresh lettuce weight was 239.9 g, more than twice the weight when the same rate of Bioplant was amended with soils to which 80 parts of compost had been applied (**Table 5**). Similarly, when the full recommended rate of Bioplant was applied to C40 compost-amended soils, fresh lettuce weight was 1.88 times higher than the lettuce counterparts from the same rate of Bioplant application to a C80-amended soil. It is thus clear that compost application at 80 parts to 20 parts of soil is not conducive for cultivation of lettuce.

There was no difference in carbon contents of the leaves of lettuce among the three rates of Bioplant applied to 80 parts of

TABLE 5 | Interactive effect of compost and Bioplant on yield, nutrient composition, and N uptake of lettuce.

	Conditioner	Fresh weight	Dry matter	C	N	N uptake
		(g)		(g/kg)		(g/pot)
C40	No application	172.53e	6.59de	430.6bc	34.6abc	0.23cd
	Half rate	239.99f	14.38f	486.2de	42.2cd	0.61e
	Full rate	295.33f	18.85g	504.7e	45.1cd	0.85f
	Double rate	174.96e	8.23e	471.2.cde	34.6abc	0.28d
C80	No application	39.36a	1.00a	380.8a	29.4a	0.03a
	Half rate	107.53bcd	3.27abc	436.7bc	35.7abc	0.12abc
	Full rate	156.74de	5.79cde	448.8bcd	32.1ab	0.19bcd
	Double rate	125.57bcde	4.25bcd	464.8cde	42.4d	0.18bcd
Cv (%)	18	17.8	4.5	8.8	18.5	

Means with the same alphabet are not significantly ($p < 0.05$) different. C40, compost-to-soil application ratio of 40:60; C80, compost-to-soil application ratio of 80:20; Cv, coefficient of variation.

TABLE 6 | Effect of compost application on some residual soil properties*.

Treatment	pH (H_2O)	EC (ds/m)	OC (g/kg)	TN (g/kg)	Avail. P (mg/kg)
C0	5.8	0.37	7.8a	0.528a	3.20a
C40	6.5	1.45	11.8b	0.757b	11.45b
C80	7.1	2.34	14.5c	0.905b	18.10c

Means with the same alphabet are not significantly ($p < 0.05$) different.
**EC, electrical conductivity; TC, total carbon; TN, total nitrogen; Avail. P, available phosphorus; C0, no application of compost; C40, compost-to-soil application ratio of 40:60; C80, compost-to-soil application ratio of 80:20.*

compost-amended soil. However, doubling the manufacturer's recommended rate of Bioplant application reflected in the superior N accumulation to the other rates at 80 parts of compost amendment to the soil. This superior accumulation of N in lettuce grown in pots with double the manufacturer's recommended rate did not affect uptake of N as all the Bioplant treatments had statistically similar levels of N uptake under 80 parts of compost amended to the soil.

Effect of Compost on Residual Soil Characteristics

Some chemical properties of the residual soil after harvest are presented in **Table 6**. The pH of the residual soil which had had no amendment was 5.8, similar to that of the original soil before cultivation (5.7). There were, however, respective 0.7 and 1.3 pH increases to 6.5 and 7.1 in the residual soils which had been amended with compost at 40 and 80 parts compared with the soil before cultivation. It is worthy of note that the EC of the original soil, which was 0.35 dS/m, did not change in the residual non-conditioned soil after harvest. However, the EC increased over four-fold and six-fold in the residual C40 and C80 soils, respectively, after harvest. Organic carbon contents of the residual soil with no amendment (7.8 g/kg) was also similar to that of the original soil (7.8 g/kg). Total nitrogen and available P of the residual un-amended soil decreased marginally from the levels before planting with respective concentrations of 0.53 g/kg and 3.2 mg/kg. Organic carbon and available P levels of the residual soil seemed to increase with increasing application of the compost. There were almost 1.5-fold and 1.8-fold respective increases in organic carbon contents of the C40 and C80 residual soils and corresponding two-fold and three-fold increases in available P contents. Total N of the compost-amended residual soils was statistically similar (0.76–0.91 g/kg). It is, however, noteworthy that the nitrogen levels in the residual soils were between 0.23 and 0.38 g/kg higher than the contents in the residual un-emended soil.

DISCUSSION

Soil Characteristics

The loamy sand texture of the soil coupled with the bulk density of 1.3 Mg/m^3 gave a good indication of the soil's physical suitability for root growth and permeability, which are very important for the soil–plant–water relationship (McKenzie et al., 2004). The low organic carbon, total N, and available P contents are indications that the Rhodic Kandiustalf (Toje Series), chosen for the study, is indeed inherently low in fertility. Additionally, the loamy sand texture, coupled with the low organic carbon content, is likely to contribute to a low WHC of the soil and promote leaching of nutrients. These soil types should therefore not be irrigated above field capacity. They should rather be irrigated to a fraction of moisture content at field capacity and more regularly for optimum moisture availability to crops. The low clay content of the soil, which has been found to be dominated by low-activity clays like kaolinite (Eze, 2008), may, in part, explain the low CEC of 5.62 cmol(+)/kg. The moderately acidic pH of the soil in water coupled with the low organic carbon contents and the kaolinitic nature of the clay would promote low P availability (Nartey et al., 1997; Sulemana et al., 2021) as corroborated by the 5.96 mg/kg concentration. The poor fertility status of the soil justifies its choice and use as a medium for testing the efficacy of Bioplant. Lettuce grown on this Toje Series should respond positively to external inputs of fertilization and conditioning to improve soil and lettuce productivity.

Effect of Soil Amendments on Fresh and Dry Matter Yields and Nutrient Content of Lettuce

The addition of compost to soil generally helps to improve the structure and fertility status of the soil. Additionally, amendment of the soil with the biochar-compost should increase the pH of the moderately acidic soils to near neutral to enhance P availability and dry matter yield (Latifah et al., 2018; Sulemana et al., 2021) as evident in the pH and available P content, especially of the residual C40 soils. The respective 2.1-fold and 3-fold increases in fresh and dry matter yields over the lettuce grown in the un-amended soil following the application of 40 parts of compost (C40) may be due to higher availability of N and P from the compost. The increase in lettuce biomass indicates an improvement in nutrient availability in the soils after the amendment of (C40) as found elsewhere by Sulemana et al. (2021). This assertion is corroborated by the neutral pH, elevated total N, and available P contents in the residual levels of C40 treatments (**Table 6**). Increasing the volume of compost to 80 parts (C80), however, led to a 51% yield decline in fresh weight compared to amending the soil with only 40 parts of compost. This decline in yield was due to the 73% decrease in N uptake in the plants grown on soil with C80 amendments compared to those that had application of 40 parts compost as indicated in **Table 3**. The biochar compost used was in a ratio of two-part groundnut husk biochar to eight parts maize stover. The high Ca and K in the biochar certainly elevated the level of the two basic cations in the compost product. Addition of 80 parts of the biochar-compost to 20 parts of the soil must have elevated the Ca and K levels in the growing medium. This must have induced an osmotic stress on the lettuce roots, thus limiting N uptake with a concomitant lower leaf expansion, carbon accumulation, and hence fresh and dry matter yields. The EC of the residual C40 soil being lower than that of the C80 (**Table 6**) gives credence to the fact that absorption of nitrogen by lettuce would be hindered more from the C80 pots. The higher total N and available P levels in the residual C80 soil compared to the residual C40, albeit with a lower uptake in lettuce grown in the former, are an indication of the fact that even though the nutrients may be available, uptake was hindered.

As indicated in **Table 4**, addition of the soil conditioner (Bioplant) at half the recommended rate and the manufacturer's recommended rate resulted in 1.55-fold and 2.11-fold increases, respectively, in fresh weight of lettuce compared to those grown in the soil without any conditioner. These yield increases also translated into increases in dry matter yield of lettuce. The significant increases in both fresh and dry matter yields following the application of the soil conditioner could be a reflection of increased availability and higher uptake of N by the lettuce as a result of higher mineralization of native organic matter in the Toje Series used for the work. This assertion is corroborated by the 41.4% significant increase in N uptake from the lettuce grown in the soil amended with Bioplant at the manufacturer's recommended rate (BP2) over those grown in soils amended with half the manufacturer's rate (BP1) (**Table 4**).

The higher nitrogen uptake of lettuce in the BP1 and BP2 soils than their BP0 counterparts also reflected in their respective significantly superior carbon contents of about 450 g/kg relative to the 400 g/kg of BP0. A higher N uptake would promote better leaf expansion as a result of higher chlorophyll formation and hence higher assimilation of carbon (Gastal and Saugier, 2006). It is clear from the results that conditioning the soil with Bioplant at both manufacturer's recommended rate and half the rate significantly increased both fresh and dry matter yields. Considering the fact that lettuce is eaten fresh and the increase in fresh weight of lettuce is almost 37% higher at the manufacturer's recommended application rate relative to half the application rate, the former rate should be the preferred choice. However, for resource-poor farmers and clients interested in N content at cheaper cost, half the recommended rate could be used as there is no significant difference in the N composition of lettuce grown at the two rates.

A reduction in fresh matter yield following a 100% increase in the manufacturer's recommended Bioplant application rate to BP3 may have resulted in a lower N uptake, culminating in a lower dry matter yield of lettuce. It is evident that doubling the manufacturer's recommended application rate of Bioplant to the soil reduced the dry matter yield of lettuce significantly by 19 and 42% from the BP1- and BP2-conditioned soils, respectively. It thus appears that doubling the rate suppressed nutrient availability, leading to a decrease in leaf expansion and hence fresh and dry matter yields.

Interactive Effect of Bioplant and Compost on Yield and Nutrient Content

As indicated in **Table 5**, the superior interactive effect of compost and the soil conditioner was evident in the fresh and dry matter yields of lettuce when compared to those of either compost or the Bioplant alone. It is noteworthy that when C40 was combined with Bioplant application at half the recommended rate, lettuce fresh weight was statistically similar to that of C40 in combination with Bioplant at the manufacturer's recommended rate. However, C40 interacting with Bioplant at half the manufacturer's recommended rate yielded lettuce with a fresh weight that is 64% heavier than lettuce grown in soil amended with only Bioplant at half the manufacturer's recommended rate (BP1). The corresponding increase in dry matter of lettuce was two-fold. This increase in dry matter may be attributed to an increase in uptake of N over lettuce grown in either the BP1-conditioned soils or the C40 alone as seen in **Table 5** (Glaser et al., 2015; Kammann et al., 2015). The fact that combining compost at 40 parts to the soil and further amending it with Bioplant at the manufacturer's recommended rate produced lettuce that is 55.34 and 4.47 g, respectively, heavier in fresh and dry matter than their counterparts grown in C40 soils amended at half the manufacturer's recommended rate of Bioplant, and the former combination gave the highest N uptake of 0.85 g

N/pot, which shows that C40 and Bioplant at the manufacturer's recommended rate are a suitable mixture for growth of lettuce in the Toje Series. Thus, the combination of the conditioner at the manufacturer's recommended rate with 40 parts of compost to 60 parts of soil on (v/v) seems to be the ideal condition for lettuce production.

CONCLUSIONS

Results from the work have shown that Bioplant as a conditioner when applied to the Rhodic Kandiustalf at the manufacturer's recommended rate during land preparation boosts N uptake and increases yield of lettuce. For better and more efficient utilization of Bioplant, it is ideal to apply it in combination with some amount of compost.

It is recommended for Bioplant to be used in combination with compost or decomposed organic manure for better yield if the organic matter content of the soil is low. Under conditions of high organic matter contents of soils, Bioplant could be used without compost. Bioplant should be applied to the soil well-ahead of seeding or planting to boost microbial activity and mineralization to synchronize nutrient availability with uptake.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

All authors listed have made substantial intellectual contributions to the work and approved it for publication.

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Nutrient Deficiencies Are Key Constraints to Grain Legume Productivity on "Non-responsive" Soils in Sub-Saharan Africa

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Leguminous plants are known to require phosphorus fertilizers and inoculation with nitrogen fixing rhizobia for optimum yield but other nutrients may also be lacking. In this study, the most limiting nutrients for legume growth were determined in soils where the crops had not responded to P and rhizobial inoculation in field trials, using the double pot technique. Soils were collected from 17 farmers' fields in West Kenya, Northern Nigeria, Eastern and Southern Rwanda, South-west and North-west Sierra Leone. Plant growth and mean biomass were measured on soils to which a full nutrient solution, containing phosphorus (P), potassium (K), magnesium (Mg), sulfur (S) and micronutrients (MN) were added, and which were compared to a control (no nutrient added), and individual omissions of each nutrient. The relationship between soil properties and nutrient deficiencies was explored. Nutrient limitations were found to differ between soils, both within and across countries. Generally, each soil was potentially deficient in at least one nutrient, with K, P, Mg, MN and S emerging as most limiting in 88, 65, 59, 18, and 12% of tested soils, respectively. While K was the most limiting nutrient in soils from Kenya and Rwanda, P was most limiting in soils from Nigeria. P and K were equally limiting in soils from Sierra Leone. Mg was found limiting in two soils from Kenya and three soils from Rwanda and one soil each in Nigeria and Sierra Leone. Micronutrients were found to be limiting in one soil from Nigeria and one soil from Rwanda. Estimates of nutrient deficiency using growth and mean biomass were found to be correlated with each other although the latter proved to be a more sensitive measure of deficiency. With few exceptions, the relation between soil parameters and nutrient deficiencies was weak and there were no significant relations between deficiency of specific nutrients and the soil content of these elements. Although our results cannot be translated directly to the field, they confirm that individual and multiple nutrient deficiencies were common in these "non-responsive" soils and may have contributed to reported low yields. This highlights the need for balanced nutrition in legume production in SSA.

Keywords: missing nutrients, sustainability, double-pot technique, balanced nutrition, enhanced productivity, smallholder farms

INTRODUCTION

Grain legumes, particularly cowpea (Vigna *unguiculata* (L.) Walp), groundnut (*Arachis hypogaea* L.), soybean (*Glycine max* L. Merril) and common bean (*Phaseolus vulgaris* L.), play important roles in the livelihoods of smallholder farmers in sub-Saharan Africa (SSA) (Nedumaran et al., 2015; Vanlauwe et al., 2019). Household surveys generally identify grain legumes as an affordable source of dietary protein in the diets of the poor (Latham, 1997) and as a source of cash income, especially for women (Odendo et al., 2011). Across the savannah regions of West Africa cowpea and groundnut residues are fed to livestock as protein supplements when animal feeds are in short supply (Tarawali and Mohamed–Saleem, 1995; Savadogo et al., 2000) while rotation of grain legumes with cereals has been reported to enhance cereal yields on smallholder farms (Franke et al., 2017). Further, legumes are also known to be a critical source of folic acid, a necessary nutrient for prenatal and early childhood health, thus a potent tool to fight childhood stunting (Smith and Haddad, 2014; Bevis, 2015).

Despite their importance, legume yields on smallholder farms remain far below their potential, largely because the crops are grown on infertile soils without adding fertilizer (Sanchez et al., 1997). Integrated soil fertility management (ISFM) interventions, including use of P fertilizers, animal manure, composts, inoculants and seeds of improved varieties can increase productivity of grain legumes (Giller et al., 2013) but yield responses measured in field experiments are known to vary considerably (Ronner et al., 2016; Van Heerwaarden et al., 2018). Such variation may partly reflect the effect of additional nutrient constraints, something which has been observed explicitly in crops such as maize (Franke et al., 2008). The fact that local deficiencies of specific soil nutrients can limit crop yields and fertilizer responses would argue for more frequent field evaluations to detect such problems, in order to set priorities for future soil fertility management in the areas under study. Unfortunately, such *in situ* studies are typically expensive and hard to implement due to logistical, material and environmental constraints (Gibson et al., 1999). As a complement to such studies therefore, nutrient omission experiments performed in pots under controlled greenhouse conditions have been proposed as a powerful and cost-effective tool to identify key nutrient deficiencies in soils (Janssen, 1990). Although the transferability of results from such studies to natural conditions may be limited, because the sampled soil may not fully represent the field conditions, the technique has the advantage of being relatively quick and inexpensive, allowing soil limiting nutrients to be examined individually, with sufficient replication and in absence of complicating factors such as water stress and the occurrence of pests and diseases (Gibson et al., 1999).

This paper analyses a set of soils from selected sites in western Kenya, Nigeria, Sierra Leone, and Rwanda where grain legumes had failed to respond to added P fertilizer or rhizobial inoculation—so-called "non-responsive" soils (Vanlauwe et al., 2015). Our aim was to identify specific nutrient deficiencies that may constrain legume growth and to provide information that may help design future field-based studies into appropriate soil fertility amendments in the region.

MATERIALS AND METHODS

Data Sources

A database containing 672 data points (soil-nutrient-growth period combinations) was compiled from five greenhouse-nutrient omission experiments conducted at the International Institute of Tropical Agriculture (IITA)-Kano station in Nigeria (in 2012), the Rwandan Agriculture Board Rubona Research Station (2013), the Sierra Leone Agricultural Research Institute (SLARI) in Sierra Leone (2013) and the University of Eldoret in Kenya (2015), all employing a double pot technique. Results from the experiment in Kenya were reported by Keino et al. (2015), and are included in the present analysis for cross country comparison.

The Double-Pot Method

The double pot method is a rapid procedure used in diagnosing deficient plant nutrients in soils (Janssen, 1990). The set-up of the pots is as presented in **Figure 1**. Seedlings of test plants are sown in restricted quantity of soil placed in the upper pot (Pot 1), which has a gauze at the bottom. The growing roots pass through the gauze and reach the nutrient solution (in Pot 2) in which the desired test nutrient is omitted. The soil in Pot 1 is kept moist by irrigating with distilled water through a pipe filled with quartz. In this set up, two sources of nutrients are provided, which the plant can access simultaneously. The first is the test-soil itself, and the second the defined nutrient solution. By omitting one selected nutrient in the solution, the plant is forced to draw this nutrient from the soil. If the soil does not supply sufficient of this omitted nutrient, the plant will suffer from deficiency symptoms, such as limited growth and leaf deficiency symptoms. Such symptoms are visible in early growth stages, so that conclusions about further development and yield can be inferred after a few weeks.

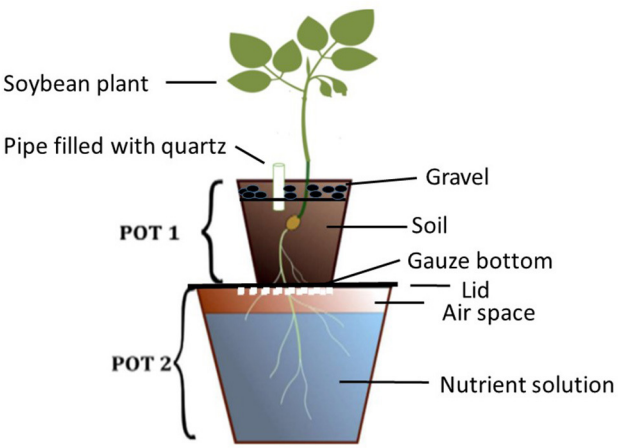

FIGURE 1 | Illustration of the setup of the double pot technique.

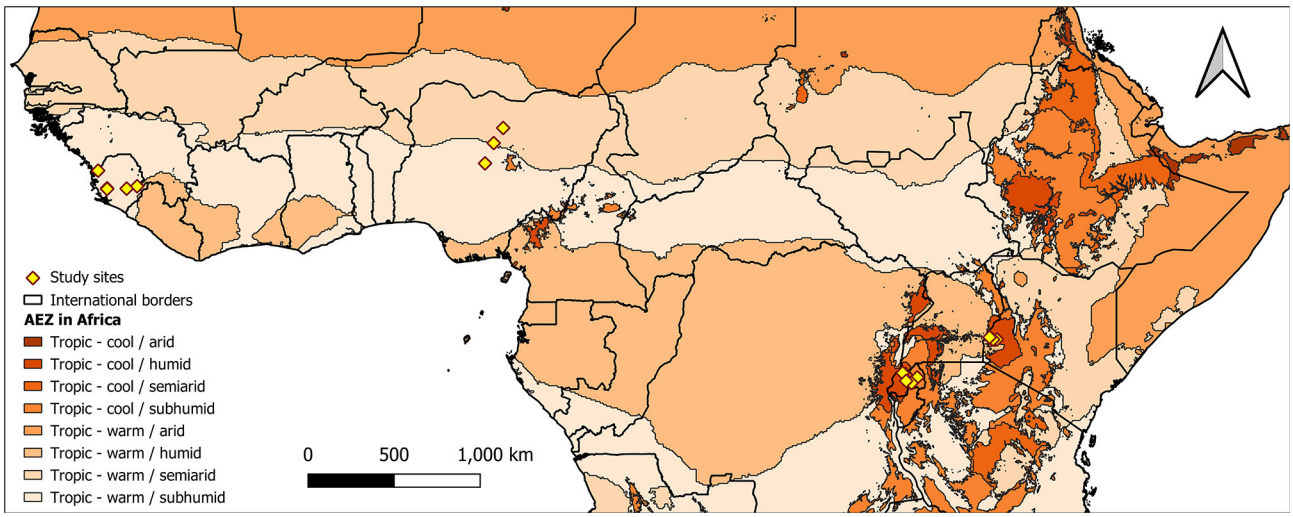

FIGURE 2 | Map showing location of farms (yellow diamond shape) where soils used in this study were collected from. Colored shading marks different agro-ecological zones.

Experimental Soils, Sampling, and Analysis

Soils were collected from farmers' fields in Western Kenya (5 soils); Northern Nigeria (3 soils); the Northern (1 soil), Southern (2 soils) and Eastern (1 soil) provinces of Rwanda; South-west (2 soils), and North-west (3 soils) Sierra Leone (**Figure 2**). Areas chosen for soil sampling were among the impact zones in which the N2Africa Project (www.n2africa.com) operated. In Kenya, Nigeria and Rwanda, previous agronomic experiments conducted in these soils (between 2009 and 2013) indicated poor yields of grain legumes with insignificant response to P fertilization and rhizobia inoculation (Van Heerwaarden et al., 2018). In Sierra Leone, soils were collected from fields where researchers suspected that nutrient deficiencies were limiting cowpea and soybean. The names of locations, GPS readings of fields and types of legumes grown prior to soil sampling are summarized in **Table 1**.

From each field ~60–70 kg of top soil (0–20 cm) was collected at 15–20 points in a zig-zag pattern across the field, using a spade or a hand hoe. The soil portions from each field were mixed to form a composite representative sample, air-dried, sieved to pass a 5 mm mesh then put in pots, each carrying 250 g. Roughly 200 g of soil per site was taken for chemical and physical analysis at Crop Nutrition Laboratory Services (CROPNUTS) in Nairobi, Kenya (for soils collected in Kenya and Rwanda); or the IITA-analytical laboratory at Ibadan, Nigeria (for soils collected in Nigeria and Sierra Leone). Soils were analyzed for particle sizes (sand, silt, and clay) using the hydrometer method (Gee and Or, 2002), pH in a 1:2 soil water volume ratio, total N using Kjeldahl method, soil organic carbon (SOC) by Walkley-Black dichromate oxidation method Nelson and Sommers (1982) and available P by Olsen method (Nelson and Sommers, 1982). Exchangeable cations (Ca, Mg, and K) were determined after Mehlich 3 extraction (Mehlich, 1984) at CROPNUTS, whereas the 0.1 M NH_4OACc extraction method (Thomas, 1982) was used at IITA-Ibadan.

TABLE 1 | Names of locations of farms where experimental soils were collected and corresponding legume grown on field before soil sampling.

Country	Site	GPS reading		Legume planted
		Latitude	Longitude	
Nigeria	Kachia	9.52083°	7.57065°	Cowpea
	Soba	10.59316°	8.03064°	Cowpea
	Garko	11.39364°	8.53008°	Soybean
Kenya	Masaba	0.19997°	34.46061°	Soybean
	Kakamega1	0.20722°	34.67233°	Common bean
	Kakamega 2	0.20406°	34.66817°	Common bean
	Butere	0.19759°	34.46581°	Soybean
	Butula	0.31994°	34.28025°	Soybean
Rwanda	Cyabingo	1.56732°	29.67895°	Common bean
	Kawangire	1.80811°	30.45027°	Common bean
	Nyarubaka	2.10722°	30.14798°	Soybean
	Musambira	1.99203°	29.86221°	Soybean
Sierra Leone	Gbombtrait	8.20059°	−12.43631°	Soybean
	Bondajuma	8.31088°	−10.84820°	-
	Kodenbotihun	8.16726°	−12.43614°	Cowpea
	Foya Junction	8.183589°	−11.40030°	-
	MeriCurve	9.13493°	−12.90843°	Cowpea

Most soils were acidic (pH 4.2 to 5.5), except soil Kawangile and soil Nyarubaka from Rwanda and soil Garko from Nigeria, had a pH between 6.0 and 6.1 (**Table 2**). It is difficult to assign minimum or maximum threshold values for soil organic carbon (SOC) as the values depend very much on texture. However, adapting criteria from Landon (1991) to indicate low, medium and high levels of SOC for top soils of different texture in the tropics, it was low for soils in Kenya (except soil Kakamega 2) and soils in Nigeria, but was high for soils in Rwanda (except soil

TABLE 2 | Top (0–20 cm) soil chemical and physical properties of the soils used in the experiments.

Country of origin	Site	pH	Total N (%)	OC (%)	Avail. P (mg kg^{-1})	Exchangeable cations (cmol$_c$ kg^{-1})			Particle size (g kg^{-1})			Textural class*
						K	Ca	Mg	Clay	Sand	Silt	
Nigeria	Kachia	5.1	0.09	0.93	1.0	0.16	1.93	0.95	330	470	200	SCL
	Soba	5.5	0.07	0.71	2.3	0.10	1.13	0.65	170	470	360	L
	Garko	6.0	0.05	0.55	trace	0.12	1.45	0.78	110	690	200	SL
Kenya	Masaba	4.7	0.13	1.50	4.4	0.16	1.10	0.51	300	540	160	SCL
	Kakamega 1	5.1	0.24	3.13	7.3	0.18	4.34	1.70	320	480	200	SCL
	Kakamega 2	5.0	0.20	2.59	4.6	0.17	3.22	0.93	320	520	160	SCL
	Butere	4.9	0.13	1.25	2.1	0.10	1.03	0.39	180	600	220	SL
	Butula	5.0	0.15	1.58	6.2	0.16	2.49	0.98	240	580	140	SCL
Rwanda	Cyabingo	5.0	0.12	2.33	8.4	0.12	1.30	0.22	329	451	220	SCL
	Kawangire	6.0	0.21	3.48	83.0	0.12	2.62	0.51	489	371	140	C
	Nyarubaka	6.1	0.20	2.27	61.1	0.06	0.51	0.14	209	672	120	SCL
	Musambira	4.6	0.09	1.42	13.2	0.09	0.08	0.02	409	551	40	SC
Sierra Leone	Gbombtrait	4.9	0.31	3.00	3.4	0.31	3.30	0.71	220	600	180	SCL
	Bondajuma	5.2	0.19	2.60	3.6	0.30	3.90	0.58	200	680	120	SCL
	Kodenbotihun	4.5	0.18	2.40	3.3	0.17	1.60	0.38	340	480	180	SCL
	Foya Junction	4.5	0.37	2.80	10.0	0.43	3.70	0.72	240	600	160	SCL
	MeriCurve	4.2	0.30	2.80	3.2	0.33	2.50	0.53	260	600	140	SCL

Textural class; C, Clay, SC, Sandy-Clay; SCL, Sandy-Clay-Loam; SL, Sandy-Loam; L, Loam; S, Sandy.*

Musambira) and soils in Sierra Leone. All soils were low in N, K, Mg and Ca applications as these nutrients were available in low levels. In most soils, except soils Kawangire and Nyarubaka from Rwanda, available P was below 10 mg kg^{-1} indicating that crops would respond to P fertilizer application (Landon, 1991).

Nutrient Treatments

Seven N-free nutrient treatments were considered across countries. These included two treatments aimed at establishing the response of soybean to the application of all nutrients minus N: 1. Control (no nutrients added), 2. Complete (P, K, Mg, Ca, S, and a combination of micronutrients (MN) Mo, B, Zn, Cu, Mo and Fe) added. The remaining five treatments evaluated omission of a single macronutrient and a combination of micronutrients in turn: 3.-P; 4. -K; 5. -Mg; 6. -S; 7. -MN. A -Ca treatment was not included because plant roots cannot not grow in Ca free nutrient solution (Janssen, 1974).

Salts, concentrations, chemical forms and rates of nutrients applied in different experiments are shown in **Table 3**. In Nigeria the concentration of nutrients was derived from a standard Hoagland's No. 2 solution (Hewitt, 1966). In Kenya, Rwanda and Sierra Leone the concentration of nutrients was derived from a standard Hoagland No. 2 solution in a half dilution and modified for the specific use with soybeans (Paradiso et al., 2012); the ion concentration in the Complete treatment was (in mM): P 0.5, K 3.0, Ca 2.5, Mg 1.0, S 1.0; (in μM): Fe 60.0, Mn 7.4, Zn 0.96, Cu 1.04, B 7.13, Mo 0.01.

Experimental Procedures

Soybean was used as the test plant. Test varieties were TGx1740-2F for the experiments in Kenya and Rwanda; TGx1448-2E in Nigeria and TGx1951-4F in Sierra Leone. Before sowing, soybean seeds were inoculated with rhizobia inoculant Legumefix (supplied by Legume Technology UK) for experiments in Nigeria, Rwanda and Sierra Leone, and with Biofix (supplied by MEA Kenya ltd) in Kenya. Inoculation followed a two-step method described in Somasegaran and Hoben, 1994. Three to four soybean seeds per pot were sown which were thinned to single uniform plants, five days after emergence (DAE). The top pots were watered daily with distilled water to keep the soils at field capacity. The nutrient solutions (with pH adjusted between 6.0 and 6.5 using NaOH) were added in Pot 2, 5 DAE and renewed every 8–10 days. The experiments were laid out in a completely randomized design (CRD) with four replications, except in Sierra Leone where three replications were used. Experimental factors were soil and nutrient solution. To allow for destructive sampling at 3–4 intervals, an extra 3–4 pots per treatment per replication were included.

Observations, Harvesting, and Measurements

From 10 days after emergence (DAE) onwards, regular observations were made on the experiments to detect visual nutrient deficiency symptoms in the leaves. Plant growth was determined at three growth periods (**Table 4**) through destructive sampling by cutting the plants at soil level followed by measuring and comparing shoot dry weight. Fresh weight (FW) was

TABLE 3 | Salt form and concentrations used to prepare the nitrogen-free nutrient solutions before application to the plants.

Nutrient	Compound	Concentration (mg /l)		
		Nigeria	Kenya and Rwanda	Sierra Leone
P	K_2HPO_4	200	–	102.1
	H_3PO_4	–	49.0	–
	$H_2NaO_4P.\ 2H_2O$	–	–	58.6
K	K_2CO_3	–	207.3	–
	KCl	750	–	447.6
Mg	$MgSO4{\cdot}7H_2O$	200	246.4	493.0
	$MgCl_2{\cdot}6H_2O$ (only in–S)	950	203.3	–
	$MgCO_3$ (only in–S)	–	–	42.2
S	$MgSO_4{\cdot}7H_2O$	–	–	–
	K_2SO_4 (only in -Mg)	174	174.3	–
Ca	$CaCl_2{\cdot}\ 2H_2O$	1100	368.0	596.6
	$CaSO_4$		–	435.0
	$CaHPO_4.\ 2H_2O$	1000	–	–
Mn	$MnCl_2\ {\cdot}4H_2O$	1.970	1.465	–
	$MnSO_4.\ H_2O$	–	–	3.170
B	H_3BO_3	2.860	0.441	1.100
Cu	$CuSO_4\ {\cdot}5H_2O$	0.080	0.260	0.650
Zn	$ZnSO_4{\cdot}7H_2O$	0.220	0.276	0.690
Mo	$Na_2MoO_4\ {\cdot}2H_2O$	0.140	0.002	–
	$(NH4)_6Mo_7O_{24}$	–	–	0.080
Fe	$FeCl_3.\ 6H_2O$	0.100	–	0.100

TABLE 4 | Harvesting periods (days after emergence for Nigeria, Kenya and Rwanda; days after planting for Sierra Leone) followed at each intermediate dry matter determination in different experiments.

Experiment	First harvest	Second harvest	Third harvest
Nigeria	21	26	31
Kenya	14	21	28
Rwanda	14	26	34
Sierra Leone	15	20	25

recorded, followed by oven drying of plant shoots at 60°C to constant weight.

Calculations

Biomass accumulation in treatments where a single nutrient had been omitted was compared with the biomass in the treatment with all nutrients applied, which is expected to have the largest biomass accumulation due to optimal conditions for growth. The concept of sufficiency quotient (Janssen, 1974) was used to measure availability of nutrients in a specific soil. Nutrient sufficiency quotient (SQ) is an index of the difference in growth between plants on a deficient and on a complete solution, because of the difference in nutrient availability. It may be estimated by determining the relative increase in plant weight (Rs) and the mean growth rate of plants at given time *t* as follows:

$$Rs = (1/S)\ (dS/dt) \tag{1}$$

where Rs is the relative growth rate; S = shoot dry weight in g and t = time in days.

Because of exponential growth, the mean growth at any given time is then estimated using the relation;

$$Rs = (\ln S2 - \ln S1)/(t2 - t1) \tag{2}$$

The SQ of respective nutrient elements are estimated as;

$$SQ_x = (Rs) - x/(Rs)\ C \tag{3}$$

Where; SQ_x = sufficiency quotient for nutrient element in question, (Rs)-x = Relative growth rate of plants growing in nutrient solutions with x (nutrient element) omitted and (Rs) C = Relative growth rate of plants growing on complete nutrient solution. Since the exact variance of a ratio of Rs-x and Rs-C is undefined, we express the relative deficiency as a difference, rather than a ratio, so that the standard error of the estimate can be calculated exactly using the statistical procedure described below.

We thereby define the sufficiency difference SD_x, as:

$$SD_x = (Rs) - x - (Rs)\ C \tag{4}$$

Values of SD_x significantly less than 0 provide evidence for deficiency of that particular nutrient.

Statistical Analysis

Statistical analysis was done using the programming language R (version 2.15.1). Data consisted of shoot dry weights (dwt_shoot) measured at three time points (time) for three to four replicates per soil and omitted nutrient (omitted_nutrient).

To evaluate evidence for deficiency of individual nutrients in the soil, the following linear mixed model was used to estimate the relative growth of each omitted nutrient treatment:

$$ln(dwt\_shoot) \sim soil + soil : omitted\_nutrient + soil : time + soil : omitted\_nutrient : time$$

A soil and replication specific time term was added as a random effect to account for repeated measurement in time by modeling the average growth per soil per replicate. The fixed terms *soil* and *soil:time* represent the average intercept and growth rate per soil. The complete nutrient treatment was defined as the reference level such that the coefficients for the interaction term interaction *soil:omitted_nutrient* corresponds to the difference in *ln(dwt_shoot)* of each omission treatment for a particular soil with respect to the complete treatment at t=0 and the coefficient for the interaction term: *soil:omitted_nutrient:time* represents the soil-specific regression slope of *ln(dwt_shoot)* against time, relative to the complete nutrient solution. As such, the latter provides a direct estimate of the sufficiency difference, SD_x for the different omitted nutrients, with a significantly negative t-statistic indicating deficiency.

Similarly, the model: *ln(dwt_shoot)*∼ *soil*+*soil:omitted_nutrient* was used to evaluate the effects of specific nutrient deficiencies in individual soils on average

shoot biomass accumulation. Data points with residuals larger or smaller than 4 standard deviations were removed before fitting final models for estimation.

Soil parameters were summarized per country and their pairwise correlations were calculated and visualized with bi-plots based on principal component analysis of the scaled parameters. The relationship between values of SD_x and average biomass for different soils and individual soil parameters was evaluated by linear regression with a correction for country.

RESULTS

Visual Deficiency Symptoms

In most cases, multiple deficiency symptoms occurred on the same plant simultaneously (**Table 5**). Deficiencies manifested by necrosis and yellowing of older leaves were common in all experimental soils and treatments, with severe symptoms recorded on -P and -K treatments in Kenya and Rwanda. P deficient plants were observed across soils in Kenya and Sierra Leone with Control and -P treatments. K deficiency symptoms were evident at an early growth stage (10 to 12 DAE) in the Control in Rwanda and across experimental soils in Kenya, Rwanda and Sierra Leone with the -K treatments. Mg deficiency symptoms were observed across soils and treatments in Rwanda, and in all soils in Kenya and Nigeria under -Mg treatment. Symptoms of Mo deficiency were observed on soil Masaba from Kenya with -MN treatment, and across soils from Nigeria under the Control and -MN treatments.

Sufficiency Difference for Omitted Nutrients

One soil from Soba, Nigeria had significantly negative sufficiency differences for all tested nutrient treatments (**Table 6**). Of the 16 remaining soils, 10 soils showed growth reduction due to deficiencies in one or more nutrients, with -K (9 soils), -P (4 soils), -Mg (4 soils), -S (two soils) and -MN (2 soils) treatments having sufficiency differences significantly below 0 (**Table 5**). Potassium (K) deficiency was observed in Butula and Masaba soils in Kenya, Garko and Soba soils in Nigeria, Cyabingo, Musambira, and Nyarubaka soils in Rwanda, as well as Bondajuma and Kodenbotihun soils in Sierra Leone. Significant P deficiency was observed in Bondajuma, Foya Junction and Kodenbotihun soils in Sierra Leone and Soba soil in Nigeria. Magnesium (Mg) deficiency was found to reduce growth in soil Kakamega 2 in Kenya and soils Cyabingo and Musambira in Rwanda. Poor growth due to micronutrient deficiency was only detected on the Rwandan Musambira soil. Interestingly, three of the soils with evidence for nutrient deficiencies, had the controls not showing significant reduction in growth rate.

Relative Shoot Biomass

Results for relative shoot biomass (**Table 7**), revealed more evidence for nutrient deficiency than growth rate. In addition to the Nigerian Soba soil, which again had significantly reduced biomass for all nutrient treatments except S, 16 soils were deficient for one or more nutrients. Potassium deficiency was again the most common (14

TABLE 5 | Symptoms of deficiency of nutrients (in bracket) and period when first observed on plants growing on a particular experimental soils for different treatments.

Treatment	Kenya	Nigeria	Rwanda	Sierra Leone
Control	From 10 DAE, in all soils; stunted plants with dark green leaves (P)	From 17 DAE, in all soils; reddish spots, interveinal chlorosis and yellowing of older leaves (N, Mg, Mo)	From 12 DAE, in all soils; yellowing and necrosis of margins of older leaves, early leaf drop (N, K and Mg)	From 12 DAE, in all soils stunted plants with dark green leaves (P)
Complete	From 20 DAE, in all soils; yellowing of older leaves (N)	No observed deficiency symptoms	From 17 DAE, in all soils; older leaves yellow (N, Mg)	From 20 DAE, in all soils; older leaves deep yellow (N)
-P	From 15 DAE, in all soils; stunted plants with dark green younger leaves, older leaves deep yellow (N, P)	From 12 DAE, in all soils; yellowing of older leaves (N)	From 17 DAE; in all soils, yellowing of older leaves with severe interveinal chlorosis (N, Mg)	From 12 DAE, in all soils stunted plants with dark green young leaves (P), old leaves yellow (N)
-K	From 12 DAE, in Masaba soils; older leaves pale yellow, necrotic, leaves dropping early (N, K, Mg)	No observed deficiency symptoms	From 12 DAE, in all soils; older leaves pale yellow, strongly necrotic and dropping. Severe in soil Cyabingo and soil Musambira (N, K, Mg).	From 12 DAE, in all soils; yellowing of older leaves, grey-brown spot progressive from older to younger leaves (N, K)
-Mg	From 10 DAE, in all soils; interveinal chlorosis yellowing of older leaves, early leaf drop (Mg, K)	From 11 DAE, in all soils; interveinal chlorosis on older leaves, severe on plant growing in soil Garko (Mg)	From 10 DAE, in all soils; older leaves chlorotic and necrotic, severe in soil Cyabingo and soil Musambira (K, Mg)	No observed deficiency symptoms
–S	From 20 DAE, in Masaba and Butere soils; younger and older leaves pale (S)	No observed deficiency symptoms	No observed deficiency symptoms	No observed deficiency symptoms
-MN	From 20 ADE, across soils; Irregular leaves, thick and brittle, dark brown with irregular lesions progressing to necrosis (Mo, Bo)	From 17 DAE, in all soils; thick pale leaves, scorched and rolled younger leaves (Mo, N)	From 12 DAE, older leaves yellow with interveinal chlorosis (N, Mg)	No observed deficiency symptoms

TABLE 6 | Averages of the sufficiency difference of control and omitted nutrients (relative to complete) ordered per experimental soil and per *P* values.

Country	Soil	Treatment					
		Control	-P	-K	-Mg	-S	MN
Kenya	Butula	−0.008	ns	−0.049	−0.061	ns	ns
	Kakamega2	−0.006	ns	ns	ns	ns	ns
	Masaba	−0.011	ns	−0.071	ns	ns	ns
Nigeria	Garko	−0.073	ns	−0.062	ns	ns	ns
	Kachia	−0.037	ns	ns	ns	ns	Ns
	Soba	−0.121	−0.087	−0.106	−0.073	−0.105	−0.097
Rwanda	Cyabingo	−0011	ns	−0.050	−0.049	ns	ns
	Kawangile	−0.042	ns	−0.023	Ns	ns	ns
	Musambila	−0.011	ns	ns	−0.047	ns	ns
	Nyarubaka	−0.002	ns	−0.065	ns	ns	ns
Sierra-Leone	Bondajuma	−0.049	−0.069	−0.071	ns	−0.009	ns
	Foya Junction	−0.059	−0.135	ns	ns	ns	−0.035
	Gbombtrait	−0.096	ns	ns	ns	ns	ns
	Kodenbotihun	−0.122	−0.120	−0.098	ns	ns	ns
	MeriCurve	−0.041	ns	ns	ns	ns	ns

Except for the Control, results are for treatments where values for sufficiency differences were significantly below zero (0), ns = not significant.

TABLE 7 | Averages of log biomass of control and omitted nutrients (relative to Complete) that were significantly below zero (0) ordered per experiment soil, ns = not significant.

Experiment	Soil	Treatments					
		Control	-P	-K	-Mg	-S	MN
Kenya	Butula	−0.471	−0.310*	−0.678	−0.361	ns	ns
	Butere	−0.521	−0.325*	−0.649	−0.358	ns	ns
	Kakamega 1	−0.519	−0.317	−0.307	ns	ns	ns
	Kakamega 2	−0.418	ns	−0.638	ns	ns	ns
	Masaba	−0.541	ns	−0.571	ns	ns	ns
Nigeria	Garko	−0.486	−0.444	ns	ns	−0.351	ns
	Kachia	−0.887	−0.763	−0.623	ns	ns	ns
	Soba	−1.057	−0.873	−0.929	−0.516	ns	−0.516
Rwanda	Cyabingo	−0.507	ns	−0.891	−0.381	ns	ns
	Kawangire	−0.119	ns	−0.765	−0.414	ns	ns
	Musambira	−0.232	ns	−0.724	ns	ns	ns
	Nyarubaka	−0.204	ns	−0.817	−0.364	ns	Ns
Sierra Leone	Bondajuma	−0.616	−0.347	−0.371	Ns	−0.417	ns
	Gbombrait	−0.603	−0.639	−0.451	Ns	ns	ns
	Kondenbothium	−0.722	−0.385	−0.527	Ns	ns	ns
	MeriCurve	−0.827	−0.595	ns	ns	ns	ns
	Foya Junction	−1.088	ns	−0.654	−0.358	ns	ns

soils), followed by phosphorus (10 soils), magnesium (8 soils), sulfur (2 soils) and micronutrients (1 soil). With the exception of soil Musambira in Rwanda, relative shoot biomass was significantly reduced in more than one nutrient treatment, and out of seventeen deficient soils, the relative biomass in the control treatment (no nutrient added) was significantly lower than in the complete nutrient solution in fourteen soils.

Relationships Between Sufficiency Difference and Relative Shoot Biomass

Overall, the correlation between the sufficiency difference and relative shoot biomass was moderate though highly significant ($R^2 = 0.30$, $p < 0.0001$) (**Figure 3**). Out of 101 soil/nutrient combinations only 22 were significant for both measures. Significant results for growth were basically a subset of those for biomass, with only three instances where a significant

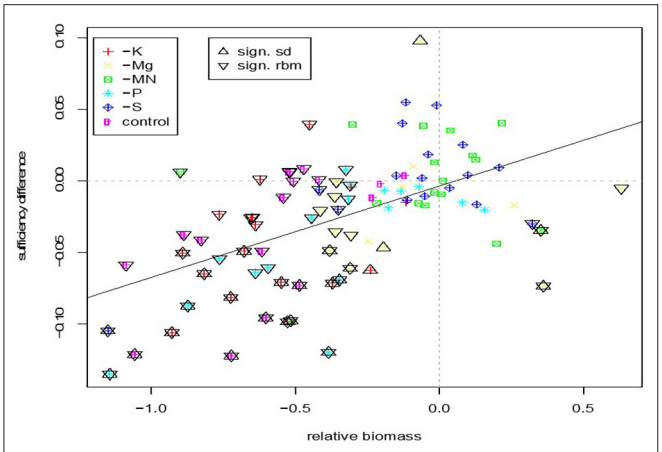

FIGURE 3 | Relationship between sufficiency difference and relative biomass for the different nutrients in specific soils. Significant values are marked by up and down facing triangles for sufficiency difference and relative biomass, respectively. Stars indicate simultaneous significance for relative biomass and sufficiency difference.

soil/nutrient combination was not significant for biomass. In contrast, 34 soil/nutrient combinations with significant reduction in biomass were not significant for growth. Considering significant results over both methods suggests that deficiencies of K, P, Mg, S, and MN occured in 82, 59, 47, 12, and 6% of tested soils, respectively.

Relationship Between Soil Properties and Nutrient Deficiencies

Overall, weak relationships between specific nutrient deficiencies and individual soil properties were observed (**Figure 4**). Of all the relations tested, only soil N and K concentration had a significant relationship with -K for growth rate and with -Mg and -P for relative shoot biomass. Nutrient deficiency was never significantly correlated with the soil concentration of the missing nutrient.

DISCUSSION

This study was the first to evaluate deficiencies of individual nutrients constraining legume growth in a diverse sample of "non-responsive" soils from SSA, providing evidence on the missing elements that could contribute to poor yields and nutrient responses in the region. Visual deficiency symptoms and decreased growth, as measured by sufficiency difference and mean shoot biomass, revealed that K, P, Mg and to lesser extent S, and MN were limiting legume production in the tested soils. Overall, all the tested soils lacked at least one nutrient, with the occurrence and extent of deficiency varying between soils within and across countries. This variability confirms that soil nutrient limitations are spatially heterogeneous, supporting the notion that soil fertility management amendments on smallholder farms in SSA could be improved by tailoring to local conditions (Giller et al., 2011; Vanlauwe et al., 2015). Our results confirm other studies reporting wide-spread deficiencies of P and, to a lesser extent S, on maize in soils of West African savanna (Vanlauwe et al., 2002; Franke et al., 2008; Nziguheba et al., 2009, 2016) and in soils of eastern and southern Africa, including Western Kenya (Kihara et al., 2016), although evidence for deficiencies of K, Mg, and micronutrients in the literature is relatively rare (Van der Zaag, 2010).

It is noteworthy therefore that K and Mg deficiencies were frequently detected in the present study, suggesting that these nutrients are perhaps a frequent cause of the lack of response to P and rhizobial inoculation. Although further confirmation is needed through field trials it is likely that including K and Mg in legume-specific fertilizers could improve legume yields where non-responsive soils are frequent. Similarly, the limitations found for S and micronutrients (usually in association with P or K deficiency) are an indication that limitations of secondary and micronutrients are locally important but less frequent. With many countries in SSA becoming progressively committed to a policy of agricultural intensification, adequate availability of these nutrients can no longer be taken for granted since the use of improved crop varieties with only N and P fertilization will in the long-term invariably lead to greater crop removal and deficiencies of other nutrients. Application of secondary and MN on soils revealing secondary nutrient limitations is one of the effective ways to enhance fertilizer use efficiency, and this can be done efficiently by blending commonly available NPK fertilizers with secondary and micronutrients (Vanlauwe et al., 2015). Legume-specific fertilizers with a wider blend of nutrients have been developed and marketed in several countries of sub-Saharan Africa based on this work (Giller and Ronner, 2019).

Two other notable results in our study were the multiple deficiencies observed for the soil from Soba in Nigeria and the absence of P deficiency in Rwandan soils. We have no clear explanation for the former observation but absence of P limitation in Rwanda soils was possibly a result of extensive use of P-fertilizers stimulated by the government-supported program on crop intensification (Ndushabandi et al., 2018). The crop intensification programme in Rwanda provides subsidies on fertilizers and seeds and support farmers to market their crops.

Janssen (1990) quantified nutrient deficiency by the reduction in growth rate. Here we applied a statistical model to get accurate estimates of this reduction and corresponding mean relative biomass in a replicated experiment. Although correlation between both measures of nutrient deficiency was moderate, relative biomass estimation yielded more significant deficiencies than the sufficiency difference (**Figure 2**), suggesting superior sensitivity. It is possible that estimates of growth are sensitive to deviations from linearity in the measured time period. The fact that our growth measurements started relatively late (10–15 DAE), to elimate effects of seed nutrients, might have caused measures of slope (i.e., sufficiency difference) to be estimated less reliably. Nevertheless, our results suggest that relying on growth estimates alone may not be the best approach and that average relative biomass offers an appropriate measure of deficiency.

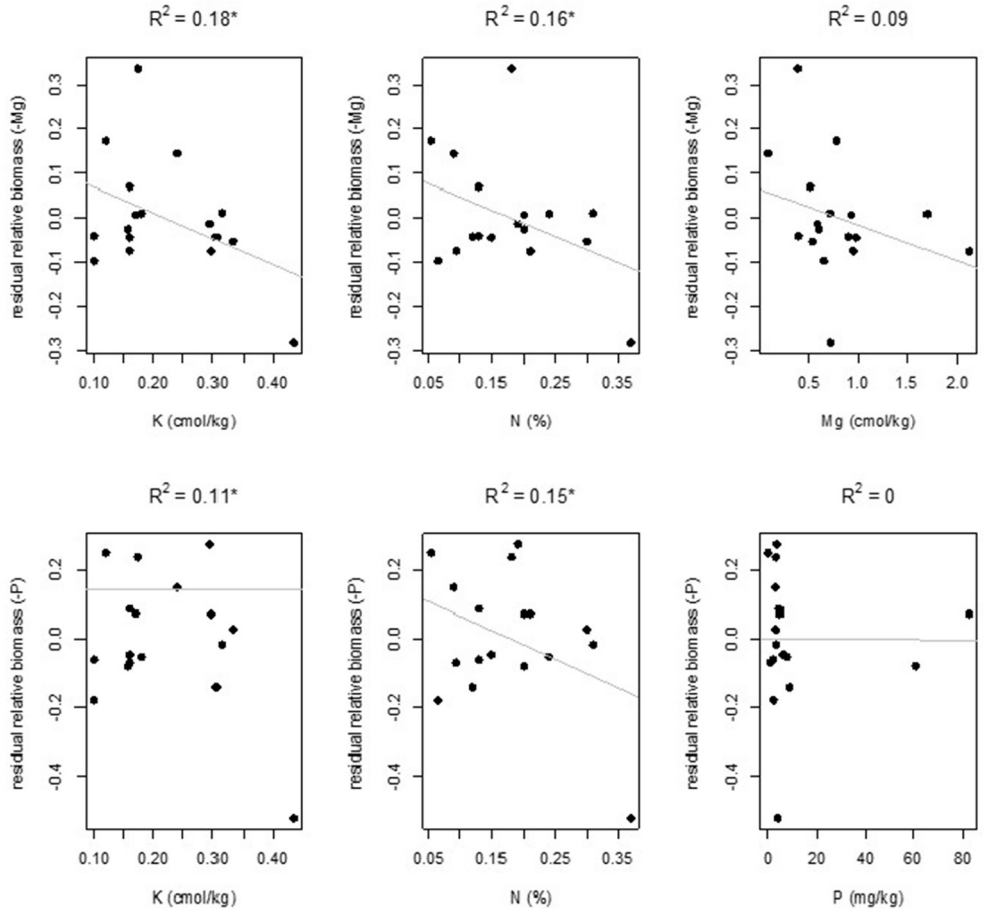

FIGURE 4 | Plots showing the relation between sufficiency difference (upper row) and relative shoot biomass (lower two rows) and soil parameters for the cases where a significant relationship was found (indicated by an asterisk) and for soil content of the missing nutrient. Top row from left to right: growth reduction for -K, against N (%) and K (cmol/kg). Second row left to right: biomass reduction for -Mg, against K (ppm) N (%) and Mg (ppm). Bottom row left to right: biomass reduction for -P, against K (cmol/kg) N (%) and P (mg/kg). Results are shown for the model residuals after correcting for country.

CONCLUSIONS

The present study examined a small range of soils from selected farmers' fields in four countries in SSA, identified nutrients limiting legume growth and provided data relevant for developing strategies and identified possible solutions to improve legume productivity. The double pot technique used here may not be as reliable as field experiments for soil nutrient diagnosis but the results nonetheless shed light on nutrients which could raise yields of legumes grown in soils with similar characteristics to those tested here. Based on our sample, and assuming representability, deficiencies of K, P, Mg, S and micronutrients seem to be wide-spread in non-responsive soils and were detected in 88, 65, 59, 18, and 18% of the soils, respectively. If these deficiencies indeed translate to reduced yields under field conditions, ignoring them will harm prospects for sustainable agricultural intensification in smallholder production. In that case, strategies for improvement of legume productivity should consider among others, blending of commonly available NPK fertilizers with secondary nutrients like Mg and S, and the MN, and organic resources amendments including animal manure (where available) to achieve a balanced crop nutrition. Application of these should take into account a targeted approach to address soil-specific deficient nutrients for a more efficient use of fertilizers and other inputs. Research is needed to verify the current results under field conditions and to define the extent of secondary and micronutrients limitation to crop growth in SSA.

DATA AVAILABILITY STATEMENT

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

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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Influence of Topping and Spacing on Growth, Yield, and Fruit Quality of Tomato (*Solanum lycopersicum* L.) Under Greenhouse Condition

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Tomato is an important vegetable in Ghanaian diet and contributes enormously in livelihood improvement. Tomato production is threatened by a high prevalence of biotic and abiotic stresses as well as increased postharvest losses and poor agronomic practices, thereby resulting in massive importation of tomato and its products to meet the local demands. The recent introduction of greenhouse vegetable cultivation technology in Ghana is a sustainable attempt in addressing and ensuring year-round production of vegetables including tomato. However, research on agronomic practices targeted to improving yield and fruit quality under greenhouse conditions in Ghana is scarcely available. Therefore, this study seeks to evaluate the effect of plant spacing and topping on tomato yield and fruit quality under greenhouse conditions. A 3×3 factorial treatment arranged in a completely randomized design (CRD) with three replications was used. Two factors, plant spacing and topping with each having three levels, were used. Thus, the levels for plant spacing were 0.15 m × 1.3 m, 0.2 m × 1.3 m, and 0.3 m × 1.3 m while topping treatments at trusses 2, 3, and 4 (control) were done. The results showed that yield was significantly influenced by plant spacing in both experiments. The interaction effect of 0.2 m × 1.3 m plant spacing and topping at truss 2 showed significantly higher yields. Furthermore, juice volume was significantly increased by plant spacing. Again, 0.2 m × 1.3 m plant spacing by truss 2 topping interaction produced the highest juice volume. Therefore, these agronomic practices could be an essential and effective approach in achieving higher tomato production with improved fruit quality under greenhouse cultivation to ensure sustainable food security.

Keywords: tomato, topping, plant spacing, fruit quality, yield, greenhouse

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), belonging to the Solanaceae family, is among the most widely cultivated vegetables in the world. Its fruits are consumed fresh or cooked and processed into paste, juice, puree, and sauce. Due to its increasing dietary importance, the production of tomatoes worldwide has increased significantly. World tomato production is around 134 million tons, with

Africa contributing about 11.9% [Food Agriculture Organization of the United Nations-Statistic Division (Food Agriculture Organization of the United Nations-Statistic Division (FAOSTAT)., 2019)]. In Ghana, tomato is one of the most important vegetable crops and essential ingredients for the daily food preparation in both urban and rural areas, which has contributed significantly to improving livelihoods. About 380,000 tons of tomatoes are grown annually [Food Agriculture Organization of the United Nations-Statistic Division (Food Agriculture Organization of the United Nations-Statistic Division (FAOSTAT)., 2019)], which is approximately USD 140 million in Ghana, of which 90% is consumed domestically (Osei et al., 2018). The demand for fresh tomato and its products in Ghana exceeds local production, leading to massive imports from other countries such as Burkina Faso, China, Italy, USA, and Spain (Awo, 2012). Ghana consumes more than 78,000 tons of tomato paste annually through imports, at a cost of about USD 100 million (Osei et al., 2018). The major contributors to the reduction in its production are attributed to the high prevalence of biotic (pest and diseases) and abiotic stresses (such as drought, heat stress) as well as increased postharvest losses and poor agronomic practices, posing a serious threat to food security. Also, due to population growth, lifestyle changes, and consumption, tomato production in Ghana is expected to increase in order to meet the rapid increase in its demand. Hence, the recent introduction of greenhouse vegetable cultivation technology in Ghana is a sustainable approach in addressing and ensuring year-round production of vegetables such as tomato, pepper, and cucumber. Likewise, this technology leads to an increase in productivity per unit area. The most commonly cultivated crop under greenhouse conditions in Ghana is tomato. To ensure sustainable tomato production, identifying adaptable and appropriate agronomic practices is highly recommended to improve yield and fruit quality, which could lead to increase in profit margin as well as meet consumer preferences (Maboko et al., 2017). Such agronomic practices include plant spacing, choice of cultivar (Maboko et al., 2017), and topping (Robinson and Kolavalli, 2010).

In Ghana, although both determinate and indeterminate tomato cultivars are grown under greenhouse conditions, the indeterminate type is commonly cultivated. Determinate cultivars have definite growth and as such are considered as self-topping. Thus, they grow, flower, and produce fruit at a specific stage. On the other hand, indeterminate cultivars grow indefinitely and are not self-topping, thereby producing fruits throughout the life of the plant (Rutledge et al., 1998; Panthee and Chen, 2010). Moreover, in self-topping or determinate cultivars, translocation of assimilates is targeted to the developing fruits at the reproductive stage, causing a decrease in harvest length compared to indeterminate types (Rutledge et al., 1998).

Topping is an agronomic practice that involves mechanical damage/wounding of the terminal bud to break the apical dominance of vegetable crops (Mohammed and Saeid, 2017). The technique of topping has been shown to offer advantages to yield and yield components increase in different crops (Tripathi et al., 2013) such as eggplant (Buczkowska, 2010), pepper (Adenle-Saheed et al., 2016), okra (Mohammed and Saeid, 2017), cotton (Aydin and Arslan, 2018), and jute (Das et al., 2014). The increase in yield and yield attributes promoted by the topping technique could be attributed to improved growth characteristics such as dry matter accumulation. For instance, in tobacco, at the maturity stage, topping caused a reduction in senescence-related metabolites and increased accumulation of secondary metabolites in mature leaves (Zhao et al., 2018). Hence, topping of indeterminate tomato cultivars could be a promising and effective agronomic technique in promoting fruit quality and yield through induced redistribution of sink-source assimilates (Kinet and Peet, 1997; Robinson and Kolavalli, 2010).

Furthermore, plant spacing is one of the agronomic techniques in achieving optimum yield. A recommended number of plants per unit area contributes to adequate utilization of the available planting space, thus ensuring an even distribution of water, nutrient, light, and air. However, an unregulated plant spacing may result in a relatively lower yield and poor fruit quality (Maboko et al., 2017). Tomato yield and yield components have been reported to be greatly affected by plant spacing (Balemi, 2008; Castoldi et al., 2010; Maboko et al., 2017). In Ghana, to the best of our knowledge, there is no report on planting spacing as well as topping for determinate tomato cultivars under greenhouse tomato cultivation. Hence, this study seeks to evaluate the effect of plant spacing and topping on tomato yield and fruit quality under greenhouse conditions.

MATERIALS AND METHODS

Experimental Site and Condition

In this study, two experiments were carried out with the second one being a repetition of the first using tomato variety, Anna F_1. The experiments were conducted under a greenhouse condition of a size of 270 m^2 at the research farm of the University of Ghana Forest and Horticultural Crops Research Center (FOHCREC), Okumaning-Kade, in the Eastern Region of Ghana from September 2019 to May 2020. The research area lies on latitude 6°0854′N and longitude 0°5400′W, with an elevation of 114 m above sea level (Ofosu-Budu, 2003). The climatic conditions in the greenhouse are shown in **Table 1**. The temperature and relative humidity in the greenhouse ranged between 24 and 32°C and 63 and 80%, respectively, during the experimental period.

Planting Material and Establishment

Seeds of tomato variety Anna F_1 were obtained from Macrofertil Ghana Limited (MGL, Tema, Ghana). Seedlings were raised in plastic trays filled with buffered cocopeat obtained from Hortirite Ventures (Tema, Ghana). Recommended nursery practices were observed to ensure that vigorous and uniform seedlings were obtained. Before transplanting, chlorpyrifos-ethyl at a rate of 30 ml/15l was applied to sanitize both the interior and exterior of the greenhouse. Seedlings were transplanted 4 weeks after sowing. Seedlings with an average height of 18 cm with 4–6 matured leaves were selected

TABLE 1 | Mean values of temperature and relative humidity in the greenhouse during the experimental period.

	Mean minimum temperature (°C)	Mean maximum temperature (°C)	Minimum relative humidity (%)	Maximum relative humidity (%)
September 2019	23.59	29.39	62.9	79.3
October 2019	24.71	31.54	61.4	78.9
November 2019	25.02	31.70	65.2	79.9
December 2019	24.89	32.03	66.4	78.9
February 2020	26.64	32.46	66.8	79.4
March 2020	30.44	32.63	69.1	79.7
April 2020	26.55	32.22	70.2	80.2
May 2020	21.94	32.54	74.1	80.4

Source: FOHCREC Greenhouse thermos-hydrometer, Kade.

TABLE 2 | Effect of spacing and topping on plant height of *Solanum lycopersicum* under greenhouse conditions.

Treatment	Plant height (cm)					
	Experiment 1			Experiment 2		
	2 WAT	4 WAT	6 WAT	2 WAT	4 WAT	6 WAT
Spacing (S)						
0.15 m × 1.3 m (S1)	21.6	45.5	99.6	21.1	45.3	105.5 b
0.20 m × 1.3 m (S2)	19.8	43.8	97.5	20.2	44.3	97.2 a
0.30 m × 1.3m (S3)	19.8	44.51	95.1	19.9	43.7	91.1 a
(*p*-value)	0.3	0.7	0.8	0.5	0.8	0.001
Topping (T)						
2 Truss (T1)	20.3	43.5	96.0	20.9	43.5	96.2
3 Truss (T2)	19.8	45.6	94.7	19.4	44.9	96.7
4 Truss (T3)	21.1	44.7	101.6	20.81	44.9	100.9
(*p*-value)	0.6	0.6	0.6	0.3	0.7	0.3
S × T	ns	ns	ns	ns	ns	ns

In a column, means followed by the same letters are not significantly ($p > 0.05$) different according to Fishers' protected F-test, ns, not significant; WAT, weeks after transplanting.

TABLE 3 | Effect of spacing and topping on plant stem girth of *Solanum lycopersicum* under greenhouse conditions.

Treatment	Stem girth (mm)					
	Experiment 1			Experiment 2		
	2 WAT	4 WAT	6 WAT	2 WAT	4 WAT	6 WAT
Spacing (S)						
0.15 m × 1.3 m (S1)	0.23	0.45	0.77	0.25 a	0.53	0.75
0.20 m × 1.3 m (S2)	0.27	0.46	0.74	0.30 b	0.54	0.71
0.30 m × 1.3 m (S3)	0.26	0.48	0.76	0.28 ab	0.55	0.77
(*p*-value)	0.06	0.64	0.81	0.04	0.86	0.41
Topping (T)						
2 Truss (T1)	0.26	0.48	0.78	0.27	0.55	0.76
3 Truss (T2)	0.23	0.45	0.72	0.30	0.54	0.74
4 Truss (T3)	0.27	0.46	0.78	0.27	0.53	0.73
(*p*-value)	0.11	0.42	0.35	0.15	0.82	0.80
S × T	ns	ns	ns	ns	ns	ns

In a column, means followed by the same letters are not significantly ($p > 0.05$) different according to Fishers' protected F-test, ns, not significant; WAT, weeks after transplanting.

TABLE 4 | Effect of spacing and topping on plant shoot dry weight of *Solanum lycopersicum* under greenhouse conditions.

Treatment	Shoot dry weight (g)					
	Experiment 1			Experiment 2		
	2 WAT	4 WAT	6 WAT	2 WAT	4 WAT	6 WAT
Spacing (S)						
0.15 m × 1.3 m (S1)	2.5a	13.1a	54.6a	2.4a	5.3a	32.8a
0.20 m × 1.3 m (S2)	2.8b	18.4b	68.4b	2.5a	7.1c	44.2b
0.30 m × 1.3 m (S3)	2.5a	19.1b	76.8b	3.2b	6.0b	50.9c
(*p*-value)	0.016	<0.001	<0.001	<0.001	<0.001	<0.001
Topping (T)						
2 Truss (T1)	2.0a	14.5a	58.6a	2.3a	6.0	43.6b
3 Truss (T2)	3.1c	17.6b	68.2b	3.1c	6.0	44.9b
4 Truss (T3)	2.7b	18.6b	73.1b	2.7b	6.5	39.4a
(*p*-value)	<0.001	0.003	0.013	<0.001	0.205	<0.001
S × T						
(0.15 m × 1.3 m) × 2 Truss	2.6b	8.6a	37.3a	2.6bc	5.0a	34.5b
(0.20 m × 1.3 m) × 2 Truss	1.8a	18.2cd	63.2b	2.8cd	7.6c	42.0c
(0.30 m × 1.3 m) × 2 Truss	1.6a	16.7bc	75.3bc	1.6a	5.4ab	54.3f
(0.15 m × 1.3 m) × 3 Truss	2.2b	14.0b	60.1b	2.2b	4.6a	34.7b
(0.20 m × 1.3 m) × 3 Truss	3.5c	17.3bc	62.5b	3.5e	5.3ab	48.6de
(0.30 m × 1.3 m) × 3 Truss	3.5c	21.5d	81.9c	3.5e	7.9c	51.4ef
(0.15 m × 1.3 m) × 4 Truss	2.6b	16.9bc	66.4bc	2.5bc	6.4b	29.1a
(0.20 m × 1.3 m) × 4 Truss	3.2c	19.7cd	79.5c	3.2de	8.3c	42.1c
(0.30 m × 1.3 m) × 4 Truss	2.3b	19.1cd	73.4bc	2.3bc	4.6a	46.9d
(*p*-value)	<0.001	0.026	0.040	<0.001	<0.001	0.024

In a column, means followed by the same letters are not significantly ($p > 0.05$) different according to Fishers' protected F-test, ns, not significant; WAT, weeks after transplanting.

and transplanted into black polythene bags of size 9 × 14 inches which were half-filled with buffered cocopeat. A 3×3 factorial treatment arranged in a completely randomized design (CRD) with three replications was used. Two factors, plant spacing and topping with each having three levels, were used. Thus, the levels for plant intra–interspacing were 0.15 m × 1.3 m, 0.2 m × 1.3 m, and 0.3 m × 1.3 m while topping treatments at trusses 2, 3, and 4 (control) were done. Topping of plants was carried out when three leaves above the preceding truss were fully developed to ensure that the required numbers of trusses (i.e., two, three, and four trusses) were kept in each treatment. The topping was done using sterilized secateurs.

Agronomic Practices

Greenhouse cultural practices such as trellising and pruning were done when necessary. At the early vegetative stage, through drip irrigation, a phosphate fertilizer at 10 g per plant was supplied to ensure proper root and shoot development. Three weeks after transplanting (WAT), compound fertilizers N:P:K (10:5:20) at 12 g per plant at the reproductive stage was applied. Twenty-five milliliters per 15 l of emamectin benzoate 1.9 EC insecticide was applied at 2 weeks after transplanting through to harvesting while 10 ml/16 l of tribasic copper sulfate 8.4% fungicide was also applied immediately after transplanting to prevent pest and pathogen infestation. A mist foliar application of B-naphthoxyacetic acid at 3 ml/l was applied twice a week between 4 and 9 weeks after transplanting to enhance fruit set. At maturity, harvesting was done by handpicking.

Data Collection and Analysis

For each replication, data were taken on five record plants in both experiments. Plant height was recorded at 2, 4, and 6 weeks after transplanting using a meter rule. A measure from the substrate level to the shoot tip was considered as the plant height. The stem girth was taken 5 cm above the substrate level using Vernier calipers. A leaf area meter (Model CI-202, Germany) was used to determine the leaf area by measuring the area of five randomly detached leaves collected from the middle part of the plant. Shoot and root samples were kept in an oven at 70°C for 72 h prior to measurements using a digital scale. The shoot-to-root ratio was computed using Microsoft Excel. Measurements of relative growth rate (RGR) and net assimilation rate (NAR) were carried out by destructive sampling at 2, 4, and 6 WAT.

RGR and NAR were calculated using the formulae below as reported by Sunaryanti et al. (2018).

$$\text{Relative growth rate (RGR)} = \frac{(\ln W2 - \ln W1)}{T2 - T1}$$

Where ln, natural log; W_1, dry weight of plant/m^2 recorded at time T_1; W_2, dry weight of plant/m^2 recorded at time T_2; T_1 and T_2, time interval.

$$\text{Net assimilation rate (NAR)} = \frac{(W2 - W1)(\ln A2 - \ln A1)}{(T2 - T1)(A2 - A1)}$$

TABLE 5 | Effect of spacing and topping on plant root dry weight of *Solanum lycopersicum* under greenhouse conditions.

Treatment	Root dry weight (g)					
	Experiment 1			Experiment 2		
	2 WAT	4 WAT	6 WAT	2 WAT	4 WAT	6 WAT
Spacing (S)						
0.15 m × 1.3 m (S1)	0.4a	4.3a	14.4a	0.5b	6.0	15.2a
0.20 m × 1.3 m (S2)	0.6b	5.9b	18.1b	0.4a	6.3	18.6b
0.30 m × 1.3 m (S3)	0.4a	5.8b	22.5c	0.4a	6.6	20.0b
(*p*-value)	<0.001	<0.001	<0.001	0.041	0.133	<0.001
Topping (T)						
2 Truss (T1)	0.4a	5.1a	23.1c	0.3a	6.9b	18.2ab
3 Truss (T2)	0.4a	5.2a	16.5b	0.4a	5.3a	19.1b
4 Truss (T3)	0.6b	5.7b	15.4a	0.5b	6.7b	16.5a
(*p*-value)	<0.001	0.010	<0.001	0.014	<0.001	0.025
S × T						
(0.15 m × 1.3 m) × 2 Truss	0.5de	0.8a	13.2a	0.5de	8.6e	14.1ab
(0.20 m × 1.3 m) × 2 Truss	0.3abc	9.3f	24.4d	0.3bc	6.8d	13.0a
(0.30 m × 1.3 m) × 2 Truss	0.3ab	5.3c	31.9e	0.2a	5.2ab	27.5f
(0.15 m × 1.3 m) × 3 Truss	0.2a	5.8cd	15.8b	0.2ab	4.1a	16.4bcd
(0.20 m × 1.3 m) × 3 Truss	0.6e	4.2b	15.8b	0.4cd	5.7bc	23.5e
(0.30 m × 1.3 m) × 3 Truss	0.5cde	5.5cd	17.8c	0.5cde	6.1bcd	17.5cd
(0.15 m × 1.3 m) × 4 Truss	0.4abcd	6.2de	14.3ab	0.6e	5.3bc	15.2abc
(0.20 m × 1.3 m) × 4 Truss	1.0f	4.3b	14.2ab	0.3bc	6.3cd	19.4d
(0.30 m × 1.3 m) × 4 Truss	0.4bcde	6.6e	17.7c	0.5cde	8.5e	14.9abc
(*p*-value)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means in a column followed by the same letters are not significantly (p > 0.05) different according to Fishers' protected F-test, ns, not significant; WAT, weeks after transplanting.

Where A1 and A2 are total leaf area at T1 and T2, respectively. W1 and W2 are the total dry weight of plant/m^2 recorded at time T1 and T2, respectively.

The numbers of flowers and fruits per plant were counted and recorded. The number of days to 50% flowering was recorded as the mean number of days from transplanting to 50% anthesis. At harvesting, fruit weight was measured using a weighing scale. Yield was calculated by multiplying the fruit weight (kg) by the total plant population per acre. Total soluble solids (TSS)/Brix and pH were measured using a refractometer (BEXCO Brix Handheld product) and pH meter, respectively. The juice volume of ground tomato fruits was measured using a graduated cylinder.

Statistical Analysis

Data were analyzed using GenStat statistical software version 19.0, and the least significant difference (LSD) at 5% was used in separating means.

RESULTS AND DISCUSSION

Results

Plant height was significantly affected by spacing at 6 WAT in experiment 2 (**Table 2**). Plants grown at spacing of 0.15m × 1.3m were also significantly taller than plants planted at spacings of 0.2 m × 1.3 m, and 0.3 m × 1.3 m. However, plant height was not significantly influenced by topping in 2, 4, and 6 WAT in both experiments. The interaction effects of spacing and topping (S × T) on plant height were not significant in both experiments (**Table 2**).

Stem girth was significantly influenced by spacing at 2 WAT in experiment 2 (**Table 3**). Stems of plants planted at a spacing of 0.20m 7 1.3m were significantly bigger (0.3mm) than stems collected from a plant spacing of 0.15m 7 1.3m. In both experiments, however, stem girth was not significantly influenced by topping at 2, 4, and 6 WAT. Also, the interaction effects of spacing and topping on stem girth showed no significant influence in both experiments (**Table 3**).

At 2, 4, and 6 WAT, shoot dry weight was significantly affected by spacing, topping, and its interaction in both experiments (**Table 4**). The interaction effects of 0.2 m × 1.3 m and 0.3 m × 1.3 m plant spacings and topping at the third truss showed a significantly higher shoot dry weight at 2 WAT in both experiments than the 0.3 m × 1.3 m plant spacing and topping at the second truss (**Table 4**). At 4 WAT, the interaction effects of the 0.3 m × 1.3 m plant spacing and topping at the third truss recorded a significantly higher shoot dry weight while the least was recorded at 0.15 m × 1.3 m plant spacing and topping at the second truss in experiment 1. However, at 6 WAT, the interaction effects of the 0.3 m × 1.3 m planting spacing and topping at the second and third trusses showed a significantly higher shoot dry weight in both experiments. On the other hand, the 0.15 m

TABLE 6 | Effect of spacing and topping on plant shoot-to-root ratio of *Solanum lycopersicum* under greenhouse conditions.

Treatment	Shoot-to-root ratio (g)					
	Experiment 1			Experiment 2		
	2 WAT	4 WAT	6 WAT	2 WAT	4 WAT	6 WAT
Spacing (S)						
0.15 m × 1.3 m (S1)	7.4b	5.4b	3.8	6.5a	1.0a	2.2a
0.20 m × 1.3 m (S2)	5.1a	3.5a	4.1	10.3b	1.1b	2.5b
0.30 m × 1.3 m (S3)	6.6ab	3.3a	3.7	6.7a	1.0a	2.7b
(*p*-value)	0.024	<0.001	0.473	<0.001	0.010	0.003
Topping (T)						
2 Truss (T1)	5.9a	5.4b	2.6a	7.1a	0.9a	2.6
3 Truss (T2)	7.8b	3.5a	4.2b	8.9b	1.1b	2.4
4 Truss (T3)	5.4a	3.4a	4.8c	7.6a	1.0ab	2.4
(*p*-value)	0.014	<0.001	<0.001	0.006	0.003	0.370
S × T						
(0.15 m × 1.3 m) × 2 Truss	4.9ab	11.1e	2.9	4.9a	0.6a	2.5b
(0.20 m × 1.3 m) × 2 Truss	6.2b	2.0a	2.6	8.4b	1.1bc	3.2c
(0.30 m × 1.3 m) × 2 Truss	6.7b	3.2abcd	2.4	8.0b	1.0bc	2.0a
(0.15 m × 1.3 m) × 3 Truss	9.9c	2.4a	3.8	10.6c	1.1bcd	2.1ab
(0.20 m × 1.3 m) × 3 Truss	5.9ab	4.1cd	4.0	8.5b	0.9b	2.1ab
(0.30 m × 1.3 m) × 3 Truss	7.6bc	3.9bcd	4.6	7.5b	1.3d	2.9c
(0.15 m × 1.3 m) × 4 Truss	7.5bc	2.7ab	4.6	4.1a	1.2cd	1.9a
(0.20 m × 1.3 m) × 4 Truss	3.3a	4.6d	5.7	13.9d	1.3d	2.2ab
(0.30 m × 1.3 m) × 4 Truss	5.4ab	2.9abc	4.1	4.6a	0.5a	3.1c
(*p*-value)	0.046	<0.001	0.083	<0.001	<0.001	<0.001

Means in a column followed by the same letters are not significantly ($p > 0.05$) different according to Fishers' protected F-test, ns, not significant; WAT, weeks after transplanting.

× 1.3 m plant spacing and topping at the second and fourth trusses recorded a smaller shoot dry weight in both experiments (**Table 4**). In experiment 2, the 0.20 m × 1.3 m plant spacing and topping at the fourth truss showed significantly higher shoot dry weight as compared with the 0.30 m × 1.3 m plant spacing and topping at the fourth truss (**Table 4**).

Similarly, at 2, 4, and 6 WAT plant spacing, topping and its interaction caused significant differences in root dry weight in both experiments (**Table 5**). At 2 WAT, the interaction effects of 0.2 m × 1.3 m and 0.15 m × 1.3 m plant spacings and topping at the fourth truss recorded significantly higher root dry weight in experiments 1 and 2, respectively. In addition, 0.15 m × 1.3 m plant spacing and topping at the third truss recorded small shoot dry weight in experiment 1. Plant spacings of 0.30 m × 1.3 m and 0.15 m × 1.3 m with topping at the second and third trusses, respectively, also showed smaller shoot dry weight in experiment 2 (**Table 5**). At 4 WAT, the interaction effects of 0.2 m × 1.3 m and 0.15 m × 1.3 m plant spacings and topping at the second truss recorded significantly higher root dry weight in experiments 1 and 2, respectively. However, plant spacing of 0.15 m × 1.3 m and topping at the second and third trusses showed relatively smaller root dry weights in experiments 1 and 2, respectively (**Table 5**). At 6 WAT, interaction effects of 0.3 m × 1.3 m plant spacing and topping at the second truss showed significantly higher root dry weight in both experiments while smaller root dry weights were recorded for plant spacings of 0.15 m × 1.3 m and 0.2 m × 1.3 m and topping at the second truss in experiments 1 and 2, respectively (**Table 5**).

The shoot-to-root ratio was significantly affected by topping in almost all the vegetative growth stages (**Table 6**) in both experiments. The interaction between plant spacing and topping significantly influenced the shoot-to-root ratio in both experiments except at 6 WAT in experiment 1. At 2 WAT, significantly higher shoot-to-root ratios were recorded for the interaction between plant spacing 0.15 m × 1.3 m with topping at truss 3 and 0.2 m × 1.3 m with topping at truss 4 in experiments 1 and 2, respectively (**Table 6**). At 4 WAT, the interaction effect of 0.15 m × 1.3m plant spacing with topping at truss 2 was significantly higher in experiment 1. At 6 WAT, the plant spacing of 0.2 m × 1.3 m with topping at the second truss showed a significantly higher shoot-to-root ratio while 0.15 m × 1.3 m plant spacing and topping at truss 4 showed a smaller shoot-to-root ratio in experiment 2 (**Table 6**).

Plant spacing and topping significantly affected the leaf area in both experiments. At 2 WAT, the interaction effects of 0.2 m × 1.3 m plant spacing and topping at truss 3 as well as 0.3 m × 1.3 m spacing and topping at truss 2 showed significantly higher leaf area in experiments 1 and 2, respectively (**Table 7**). At 4 WAT, the interaction effect of plant spacing of 0.3 m × 1.3 m with topping at the third truss resulted in a significant increase in leaf area while 0.15 m × 1.3 m plant spacing with topping at truss 2 recorded a smaller leaf area in experiment 2

TABLE 7 | Effect of spacing and topping on leaf area of *Solanum lycopersicum* under greenhouse conditions.

Treatment	Leaf area (cm^2)					
	Experiment 1			Experiment 2		
	2 WAT	4 WAT	6 WAT	2 WAT	4 WAT	6 WAT
Spacing (S)						
0.15 m × 1.3 m (S1)	0.5b	5.7	14.0b	0.6b	7.4a	13.1a
0.20 m × 1.3 m (S2)	0.5a	5.9	14.7b	0.5a	7.5a	15.1b
0.30 m × 1.3 m (S3)	0.7c	5.2	12.7a	0.7c	8.8b	12.7a
(*p*-value)	<0.001	0.062	0.001	<0.001	<0.001	<0.001
Topping (T)						
2 Truss (T1)	0.6b	5.3a	12.8a	0.6b	7.4a	14.1
3 Truss (T2)	0.7c	6.6b	12.4a	0.7c	7.8ab	13.8
4 Truss (T3)	0.4a	4.8a	16.2b	0.5a	8.4b	13.0
(*p*-value)	<0.001	<0.001	<0.001	<0.001	0.042	0.139
S × T						
(0.15 m × 1.3 m) × 2 Truss	0.6cd	4.9	9.0a	0.6cd	5.4a	10.1ab
(0.20 m × 1.3 m) × 2 Truss	0.5bc	6.0	16.1c	0.4b	8.0b	14.8c
(0.30 m × 1.3 m) × 2 Truss	0.7ef	5.1	13.b	0.8f	8.9b	17.3d
(0.15 m × 1.3 m) × 3 Truss	0.6de	7.1	16.3c	0.7ef	8.2b	17.7d
(0.20 m × 1.3 m) × 3 Truss	0.8f	6.5	12.6b	0.6de	6.2a	14.5c
(0.30 m × 1.3 m) × 3 Truss	0.6de	6.3	8.2a	0.6de	9.1b	9.2a
(0.15 m × 1.3 m) × 4 Truss	0.4b	5.1	16.6c	0.5bc	8.5b	11.5b
(0.20 m × 1.3 m) × 4 Truss	0.1a	5.1	15.4c	0.3a	8.2b	15.9cd
(0.30 m × 1.3 m) × 4 Truss	0.7ef	4.3	16.5c	0.7f	8.5b	11.6b
(*p*-value)	<0.001	0.162	<0.001	<0.001	<0.001	<0.001

Means in a column followed by the same letters are not significantly ($p > 0.05$) different according to Fishers' protected F-test, ns, not significant; WAT, weeks after transplanting.

(**Table 7**). At 6 WAT, interaction effects of 0.15 m × 1.3 m plant spacing and toppings at trusses 4 and 3 recorded a significantly higher leaf area in experiments 1 and 2, respectively. However, the interaction effect of 0.3 m × 1.3 m plant spacing with topping at the third truss recorded the smallest leaf area in experiment 1 at 6 WAT (**Table 7**).

RGR was significantly influenced by spacing at 4 WAT in both experiments but 6 WAT in experiment 2 (**Table 8**). NAR was significantly influenced by both spacing and topping at 4 and 6 WAT in both experiments. The interaction effects of plant spacing and topping on RGR at 4 WAT and NAR at 4 and 6 WAT in experiment 1 showed significant influence. Also, the interaction effects of plant spacing and topping (S × T) on RGR and NAR at 4 and 6 WAT showed a significant influence in experiment 2 (**Table 8**). The interaction effects of 0.2 m × 1.3 m plant spacing and topping at the fourth truss showed significantly higher RGR in experiments 1 and 2 at 4 WAT as compared with the 0.15 m × 1.3 m plant spacing and toppings at the second and fourth trusses with smaller RGR in both experiments at 4 WAT (**Table 8**). At 4 WAT, the interaction effects of 0.3 m × 1.3 m spacing and topping at the third truss showed significantly higher NAR in both experiments. However, the interaction effects of 0.15 m × 1.3 m spacing and topping at the third truss recorded smaller NAR both experiments at 4 WAT.

At 6 WAT, the interaction effect of 0.3 m × 1.3 m plant spacing and topping at the second truss showed significantly higher RGR as compared with 0.15 m × 1.3 m plant spacing and topping at the fourth truss with smaller RGR in experiment 2 (**Table 8**). However, the interaction effects of 0.3 m × 1.3 m plant spacing and topping at the third truss showed significantly higher NAR in both experiments. The interaction effects of 0.2 m × 1.3 m plant spacing and topping at the second truss showed significantly smaller NAR in experiment 1, and the interaction effects of 0.15 m × 1.3 m plant spacings and topping at the third truss also recorded smaller NAR in experiment 2 at 6 WAT (**Table 8**).

Days to 50% flowering and yield was significantly influenced by plant spacing in both experiments. However, fruits per plant and total fruit weight were significantly influenced by plant spacing in experiment 2 (**Table 9**). Again, number of flowers and days to 50% flowering were significantly affected by topping in both experiments; however, fruits per plant and weight per fruit were significantly affected by topping (**Table 9**). Days to 50% flowering, total fruit weight, and yield were significantly affected by the interaction between plant spacing and topping in experiment 1; however, weight per fruit, total fruit weight, and yield were significantly affected by plant spacing and topping interactions in experiment 2 (**Table 9**). The interaction effects of 0.2 m × 1.3 m and 0.3 m × 1.3 m plant spacings and topping at the second truss showed significantly more days to 50% flowering as compared with the 0.15 m × 1.3 m plant spacing and topping at the second truss with earlier days to 50%

TABLE 8 | Effect of spacing and topping on relative growth rate (RGR) and net assimilation rates (NAR) of *Solanum lycopersicum* under greenhouse conditions.

Treatment	Experiment 1				Experiment 2			
	RGR (g/day)		NAR (g cm^{-2}/day)		RGR (g/day)		NAR (g cm^{-2}/day)	
	4 WAT	6 WAT	4 WAT	6 WAT	4 WAT	6 WAT	4 WAT	6 WAT
Spacing (S)								
0.15 m × 1.3 m (S1)	0.181a	0.247	0.727a	1.003a	0.211a	0.289a	1.352a	1.853a
0.20 m × 1.3 m (S2)	0.217b	0.262	1.175b	1.197b	0.229c	0.306b	1.539b	2.059b
0.30 m × 1.3 m (S3)	0.222b	0.273	1.442c	1.687c	0.216b	0.314c	1.911c	2.757c
(*p*-value)	<0.001	0.247	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Topping (T)								
2 Truss (T1)	0.188a	0.260	0.905a	1.124a	0.218	0.305b	1.526a	2.135a
3 Truss (T2)	0.213b	0.265	1.509b	1.724b	0.219	0.305b	1.718b	2.401b
4 Truss (T3)	0.220b	0.258	0.930a	1.039a	0.219	0.299a	1.559a	2.132a
(*p*-value)	0.010	0.898	<0.001	<0.001	0.666	<0.001	0.023	0.011
S × T								
(0.15 m × 1.3 m) × 2 Truss	0.119a	0.255	0.764ab	1.278b	0.212ab	0.293b	1.752d	2.415de
(0.20 m × 1.3 m) × 2 Truss	0.229b	0.246	0.808b	0.824a	0.228d	0.299c	1.430bc	1.881b
(0.30 m × 1.3 m) × 2 Truss	0.216b	0.281	1.144c	1.272b	0.213b	0.321f	1.396bc	2.108bcd
(0.15 m × 1.3 m) × 3 Truss	0.205b	0.254	0.664a	0.866a	0.216b	0.290b	0.996a	1.337a
(0.20 m × 1.3 m) × 3 Truss	0.208b	0.263	1.470d	1.384b	0.217bc	0.314g	1.573cd	2.272cde
(0.30 m × 1.3 m) × 3 Truss	0.226b	0.276	2.393e	2.921c	0.225cd	0.312ef	2.585e	3.594f
(0.15 m × 1.3 m) × 4 Truss	0.221b	0.233	0.753ab	0.866a	0.205a	0.283 a	1.309b	1.807b
(0.20 m × 1.3 m) × 4 Truss	0.224b	0.277	1.247c	1.383b	0.243e	0.305cd	1.613cd	2.022bc
(0.30 m × 1.3 m) × 4 Truss	0.214b	0.263	0.789ab	0.869a	0.210ab	0.308de	1.753d	2.567e
(*p*-value)	<0.001	0.630	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means in a column followed by the same letters are not significantly ($p > 0.05$) different according to Fishers' protected F-test, ns, not significant; WAT, weeks after transplanting. Relative growth rate (RGR) (g/day) and net assimilation rates (NAR) (g cm^{-2}/day).

flowering in experiment 1 (**Table 9**). However, in experiment 2, the interaction effect of 0.2 m × 1.3 m plant spacing and topping at the second truss showed significantly higher weight per fruit as compared with the 0.2 m × 1.3 m plant spacing and topping at the fourth truss with smaller weight per fruit (**Table 9**). Also, the 0.2 m × 1.3 m plant spacing and topping at the second truss recorded significantly higher total fruit weight as compared with 0.15 m × 1.3 m plant spacing and topping at the second truss which recorded the smallest total fruit weight in experiments 1 and 2 (**Table 9**). The interaction effects of 0.2 m × 1.3 m plant spacing and topping at the second truss recorded significantly higher yields in both experiments. However, 0.3 m × 1.3 m plant spacing and topping at the fourth truss recorded a relatively lower yield in experiment 1. Likewise, the interaction effects of 0.3 m × 1.3 m plant spacings and topping at the third truss also recorded a lower yield in experiment 2 (**Table 9**).

Furthermore, juice volume was significantly influenced by spacing in experiment 2 (**Table 10**). The juice volume of fruits from plants planted at a spacing of 0.20 m × 1.3 m was significantly higher than that of plants planted at 0.15 m × 1.3 m spacing. Topping did not significantly influence Brix, pH, and juice volume in both experiments (**Table 10**). However, in experiment 2, the interaction effects of spacing and topping significantly influence pH and juice volume (**Table 10**). Thus, interaction effects of 0.15 m × 1.3 m and 0.2 m × 1.3 m spacings and topping at the second truss showed significantly higher pH and juice volume as compared with the 0.2 m × 1.3 m and 0.15 m × 1.3 m spacings and topping at the second truss with lower pH and juice volume in experiment 2 (**Table 10**).

Discussion

Improvement in the vegetative characters interacting with the genetic and optimum environmental conditions ensures the achievement of maximum yield of crop plants such as tomato. This study recorded significant differences among plant spacing and topping effects on the vegetative characters of tomato which include plant height, stem girth, and leaf area under greenhouse conditions. Plant height is an important vegetative character that enables the plant leaves to gain adequate access to full light when stems of plants are supported and maintained (Xu et al., 2020). Results from the study revealed that plant height increased with decreasing plant spacing. This could be as a result of increased plant competition for soil nutrients and sunlight between plants within the row and reduced competition between rows. The current result was in accord with the results of Ogundare et al. (2015) in tomatoes as well as in

TABLE 9 | Effect of spacing and topping on yield and yield components of *Solanum lycopersicum* under greenhouse conditions.

Treatment	Experiment 1						Experiment 2					
	No. flowers	Days to 50% flowering	Fruits/plant	Weight/fruit (g)	Total fruit weight (kg)	Yield (kg/acre)	No. flowers	Days to 50% flowering	Fruits /plant	Weight/fruit	Total fruit weight (kg)	Yield (kg/acre)
Spacing (S)												
0.15 m × 1.3 m (S1)	54	52a	7	65.3	2.4	9,904b	51	52a	8a	55.0	2.3 a	9,499b
0.20 m × 1.3 m (S2)	58	53b	8	82.0	3.0	9,357b	57	53b	10b	64.1	3.0 b	9,188b
0.30 m × 1.3 m (S3)	57	53b	8	71.1	2.7	5,659a	58	53b	9ab	64.5	3.0 b	6,148a
(*p*-value)	0.699	0.003	0.836	0.203	0.150	<0.001	0.132	0.017	0.019	0.337	0.005	<0.001
Topping (T)												
2 Truss (T1)	46a	54c	8	83.1	3.1	9,301	43a	54c	8a	78.6b	3.0	8.865
3 Truss (T2)	60b	53b	8	69.8	2.7	8,335	54b	53b	9b	59.2a	2.6	8.128
4 Truss (T3)	64b	52a	7	65.4	2.4	7,284	70c	52a	11c	45.9a	2.6	7.842
(*p*-value)	0.012	<0.001	0.882	0.156	0.065	0.158	<0.001	<0.001	<0.001	<0.001	0.151	0.347
S × T												
(0.15 m × 1.3 m) × 2 Truss	39	52ab	7	60.6	2.0a	8,374abc	40	53	7	53.6abc	1.9a	7,690ab
(0.20 m × 1.3 m) × 2 Truss	49	54c	8	107.9	4.2c	13,030d	44	54	8	103.4d	4.1d	12,735d
(0.30 m × 1.3 m) × 2 Truss	49	54c	8	80.9	3.1bc	6,500ab	43	54	8	78.9cd	3.0bc	6,171a
(0.15 m × 1.3 m) × 3 Truss	53	53ab	9	61.6	2.8ab	11,612cd	50	53	8	64.4bc	2.6bc	10,856cd
(0.20 m × 1.3 m) × 3 Truss	57	53b	7	74.5	2.6ab	8,039abc	58	53	10	52.1ab	2.5abc	7,689ab
(0.30 m × 1.3 m) × 3 Truss	60	53b	7	73.2	2.6ab	5,354a	55	53	9	61.1abc	2.8bc	5,840a
(0.15 m × 1.3 m) × 4 Truss	60	52a	6	73.7	2.3ab	9,726bcd	63	52	10	47.2ab	2.4abc	9,951bc
(0.20 m × 1.3 m) × 4 Truss	69	53ab	7	63.5	2.2ab	7,001ab	70	53	13	36.9a	2.3ab	7,141a
(0.30 m × 1.3 m) × 4 Truss	62	52a	9	59.1	2.5ab	5,124a	77	52	12	54abc	3.1c	6,433a
(*p*-value)	0.690	0.024	0.359	0.165	0.032	0.027	0.686	0.151	0.896	0.013	<0.001	<0.001

Means in a column followed by the same letters are not significantly ($p > 0.05$) different according to Fishers' protected F-test, ns, not significant; WAT, weeks after transplanting.

TABLE 10 | Effect of spacing and topping on fruit quality of *Solanum lycopersicum* under greenhouse conditions.

Treatment	Experiment 1			Experiment 2		
	Brix (%)	pH	Juice volume (ml)	Brix (%)	pH	Juice volume (ml)
Spacing (S)						
0.15 m × 1.3 m (S1)	3.9	4.3	2,359.0	3.8	4.3	2,159a
0.20 m × 1.3 m (S2)	3.9	4.2	2,866.0	3.9	4.3	2,976b
0.30 m × 1.3 m (S3)	4.4	4.3	2,642.0	4.4	4.3	2,785b
(*p*-value)	0.290	0.578	0.266	0.087	0.676	0.003
Topping (T)						
2 Truss (T1)	4.3	4.2	2,669.0	4.1	4.3	2,544
3 Truss (T2)	3.6	4.3	2,721.0	3.8	4.3	2,648
4 Truss (T3)	4.2	4.3	2,476.0	4.1	4.3	2,727
(*p*-value)	0.114	0.191	0.697	0.424	0.629	0.689
S × T						
(0.15 m × 1.3 m) × 2 Truss	4.0	4.3	1,742.0	3.9	4.4b	1,586a
(0.20 m × 1.3 m) × 2 Truss	4.3	3.9	3,583.0	4.0	4.1a	3,502d
(0.30 m × 1.3 m) × 2 Truss	4.6	4.3	2,681.0	4.5	4.2ab	2,545bc
(0.15 m × 1.3 m) × 3 Truss	3.0	4.3	2,869.0	3.4	4.2ab	2,683bc
(0.20 m × 1.3 m) × 3 Truss	3.5	4.4	2,649.0	3.7	4.3ab	2,534bc
(0.30 m × 1.3 m) × 3 Truss	4.3	4.3	2,646.0	4.4	4.3ab	2,729bc
(0.15 m × 1.3 m) × 4 Truss	4.7	4.3	2,465.0	4.2	4.2ab	2,208ab
(0.20 m × 1.3 m) × 4 Truss	3.9	4.3	2,366.0	3.8	4.3ab	2,893bcd
(0.30 m × 1.3 m) × 4 Truss	4.1	4.4	2,597.0	4.3	4.4b	3,081cd
(*p*-value)	0.263	0.234	0.077	0.619	0.039	0.011

Means in a column followed by the same letters are not significantly ($p > 0.05$) different according to Fishers' protected F-test, ns, not significant; WAT, weeks after transplanting.

Crotalaria juncea L. (Tripathi et al., 2013). On the contrary, other researchers reported higher plant height in wider plant spacing than closer spacing (Ara et al., 2007 and Chernet et al., 2017). The highest truss number induced an increase in plant height which corroborates with studies by Ayarna et al. (2019) probably due to the increase in the length of the internode.

Stem girth increased with increasing plant spacing where the thickest stems recorded in plants spaced 0.2 m × 1.3 m could be due to greater biomass allocation to the stem which is in accordance with Olaniyi et al. (2010), Ogundare et al. (2015), and Ayarna et al. (2019). Plants grown under narrow spacing seemed to be crowded which resulted in some degree of etiolation. This could be as a result of such plants utilizing the photosynthates produced in increasing their height to compete for maximum light at the expense of stem growth (Maurya et al., 2013).

The highest plant shoot dry weight was recorded in the wider plant spacing which is in line with a report by Naik et al. (2018). The presence of sufficient space, concomitant with reduced interplant competition, as well as the availability of sufficient moisture, nutrients, and light enhanced the growth and development of the plants. Thus, increased light interception by widely spaced plants with increased carbon assimilates for photosynthesis and efficient use of resources resulted in increased dry matter accumulation. Ibeawuchi et al. (2008) also reported similar results. On the contrary, a study by Makinde and Alabi (2002) reported higher shoot dry weight in closely spaced plants. The high dry matter accumulation at the third and fourth trusses could be a result of increased carbon and nitrogen metabolism, photosynthesis, and secondary metabolism (Zhao et al., 2018). Wider plant spacing promoted root dry weight accumulation. This finding is in agreement with reports by Jimba and Adedeji (2003), Moosavi et al. (2012), and Legwaila et al. (2014). On the other hand, narrower spacing contributed to an increase in root dry matter accumulation which was reported in sorghum (Snider et al., 2012) and soybean (De Bruin and Pedersen, 2008). The higher shoot-to-root ratio observed in narrow spacing may be attributed to plants channeling more nutrients and resources into the shoot development, thereby enhancing the manufacturing of photosynthates (Ayarna et al., 2019).

Wider spacing contributed to an increase in RGR and NAR, which may be as a result of less competition for nutrients and light. This finding is in contrast with a report by Law-Ogbomo and Egharevba (2008). Topping influenced the number of flowers in truss 4 which may be attributed to translocation of assimilates for flower development (Pék and Helyes, 2004) and may result in an increase in fruit number. In addition, moderate plant spacing of 0.20 m × 1.3 m influenced increased fruit number per plant as a similar pattern was observed by Jovicich et al. (2004) and Adenle-Saheed et al. (2016). In contrast, Ara et al. (2007) reported that narrow

spacing gave the maximum marketable number of fruits per plant. This could be caused by differences in crop varieties as early- to late-maturing crop cultivars do well in different plant spacing.

Moreover, topping at truss 2 also produced the maximum number of fruits per plant. This might be due to an increase in the accumulation of photosynthates for fruit development which is in line with a study by Ayarna et al. (2019). Wider plant spacing had a positive effect on fruit weight due to less competition for water, nutrients, and light as well as maximum photosynthetic area, thereby enhancing high assimilation and accumulation of photosynthates in tomato fruits as observed in study by Ayarna et al. (2019). This finding also corroborates with reports by Kultur et al. (2001) in muskmelon, Ibeawuchi et al. (2008) in maize, Naik et al. (2018) in clusterbean, and Aminifard et al. (2012) in sweet pepper. The higher yields observed in the narrow (0.15 × 1.3 m) and moderate (0.2 m × 1.3 m) spacing confirms maximum/efficient utilization of space under greenhouse cultivation of vegetables, which ensures an even supply and distribution of water and nutrients for efficient plant growth and development. Thus, the higher number of plants per unit area resulted in higher fruit number, thereby recompensing for the slight reduction of fruit weight as seen in wider spacing (Law-Ogbomo and Egharevba, 2008). Additionally, topping at truss 2 boosted yield, which could be attributable to the allocation of photosynthates and metabolites produced by leaves to strong carbohydrate sinks, such as fruit.

Moreover, spacing at 0.20 m × 1.3 m showed a positive effect on juice volume. This might be due to adequate reception of solar radiation, resulting in high leaf assimilates concomitant with increase in metabolite accumulation in the fruits. Furthermore, the less competition for light, space, and nutrient contributed to larger-sized fruits potentially enhancing more juice accumulation (Kirimi et al., 2011; Assefa et al., 2015).

CONCLUSION

Plant spacing and topping are useful agronomic practices that can be adopted to improve tomato yield and fruit quality under greenhouse conditions. In both experiments, the plant spacing 0.2 m × 1.3 m with topping at truss 2 produced higher yields as well as fruit juice volume. These agronomic practices could be an essential and effective approach in achieving multiple cultivations accompanied with higher tomato productivity and improved fruit quality under greenhouse cultivation to ensure sustainable food security.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

GN contributed to the funding acquisition, conceptualization of project, supervision, and manuscript preparation. CA contributed to the experimental design and supervision. MZ conducted the experiment and data collection. SO-N contributed to the writing, reviewing, and editing of the manuscript. PO contributed to the writing and reviewing of the original draft of the manuscript. FO-A contributed in data analyses and writing of the manuscript. All authors contributed to the article and approved the submitted version.

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Optimizing Tomato (*Solanum lycopersicum* L.) Growth With Different Combinations of Organo-Mineral Fertilizers

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With the aim of promoting sustainable agriculture that respects the environment and human health, a study was carried out to evaluate the impact of organic and mineral fertilizers on tomato plant cultivation. The study was carried out at the Research Station of Farako-Bâ in Burkina Faso. A complete randomized block of Fisher design with four replications was used to carry out the experiment. The treatments were as follows: T0: control (compost 15 t/ha); T1: compost (15 t/ha) + biosol (160 kg/ha) + urea (35 kg/ha) + NPK (87.5 kg/ha) + KCl (52.5 kg/ha); T2: compost (15 t/ha) + neem seed cake (10 t/ha) + urea (100 kg/ha) + NPK (250 kg/ha) + KCl (150 kg/ha); T3: compost (15 t/ha) + biochar (10 t/ha) + urea (100 kg/ha) + NPK (250 kg/ha) + KCl (150 kg/ha). The data collected were collar diameter, height of plants, number of fruits, fruit diameter, and tomato fruit yield. The results showed that the combination organo-mineral fertilizers had a significant effect on tomato plant productivity. Application of neem seed cake and mineral fertilizers was the most efficient treatment with a yield increase of 53% and 40% in 2019, respectively. In 2020, the yield increase was 32 and 85% for biochar and biosol, respectively. Incorporation of organo-mineral fertilizers has improved soil organic and nutrient status, which ultimately promotes crop growth of tomato plant. Neem cake can be effectively used to increase tomato plant productivity and farmer's income and also maintain soil fertility.

Keywords: organic amendment, mineral fertilizer, tomato productivity, yield, growth

INTRODUCTION

Agriculture remains a vital sector for sub-Saharan African countries. Burkina Faso's economy is based on agriculture, which employs more than 80% of the population and contributes about 31% to the gross domestic product (PNDES, 2016). Among the agricultural sectors, vegetable farming occupies a prominent place (MAHRH, 2011). The sector is an important source of foreign exchange for the national economy (CILSS, 2017). Tomato cultivation takes second place after onion in terms of cultivated area and volume of production. In 2018, tomato production has reached 167,400 tons, about 0.8% of African production, and generated more than 78 billion FCFA in Burkina Faso (MAAHA, 2019). However, the yield of tomatoes declined from 12.5 tons (t)/ha in 2012 to 10.9 t/ha in 2018 (Faostat, 2020). This decline in yield is linked to sub-optimal crop management, which

could include poor fertilization and irrigation, and to abiotic constraints (pedoclimatic variations, land degradation, etc.) (Ferrante and Mariani, 2018; Ullah et al., 2021). The fundamental cause is the degradation of land followed by their loss of fertility (physical, chemical, and biological fertility). In Burkina Faso, land degradation now affects 24% of arable land and threatens to undermine medium- and long-term food security. Tomato is grown by using conventional as well as organic fertilizers. The use of organic manure has been reported to improve biological, chemical, and physical properties of the soil and invariably increase plant growth and yield because of its high organic matter content due to high microbial activity (Mitran et al., 2017). Organic amendments such as such biochar, compost, and neem seed cake are compounds rich in organic matter that can be used for tomato production. Biochar is widely used in soil amendment for improving physicochemical properties and biological activities of agricultural soils. Biochars can be used as soil amendments for improving soil properties and crop yield, and secondly, storing biochars in soils is regarded as a means for permanently sequestering carbon (Glaser et al., 2002; Lehmann et al., 2006). Neem seed cake is rich in plant nutrients (crude protein 13–18%; carbohydrate 24–50%; crude fiber 8–26%; fat 2–13%; ash 5–18%; and acid-insoluble ash 1–17%, with nitrogen, phosphorus, calcium, and magnesium) and is used as manure for soil amendment and for urea coating (Puri, 1999). However, fertilizer sources can have a significant effect on tomato quality (Toor et al., 2006). Extensive use of inorganic fertilizer has a depressing effect on yield, by causing a reduction in number of fruits, and delays and reduces fruit setting, which consequently delays ripening and leads to heavy vegetative growth (Aliyu et al., 2003). Many other authors (Ayeni and Ezeh, 2017; Islam et al., 2017; Wu et al., 2020) mentioned the positive effect of the combination of organo-mineral fertilizers on tomato productivity. Also, the beneficial effect of combined organic manure with bio-fertilizer on availability of nutrients was reported by Fernandes and Bhalerao (2015) and Youssef and Eissa (2017). The objective of this study is to propose alternative solutions for maintaining or improving soil fertility and increasing tomato productivity by combining different sources of organic amendments and inorganic fertilizers.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted in 2019 and 2020 in Farako-Bâ Research Station (4° 20 W Longitude, 11° 06 N Latitude, 405 m above sea level). The climate is south-Sudanese type, with a rainy season ranging between May and October, and a dry season, from November to April. The annual average rainfall in the area is between 900 and 1,100 mm. The temperature varies between 17 and 37°C during the dry season and during the rainy season between 10 and 32°C. During the experiment period from 2019 to 2020, the mean annual rainfall was between 1,248 and 1,282 mm, received on 73 to 63 days, respectively. The rainfall distribution was characterized by frequent dry spells. The experiment was implemented on a tropical ferruginous soil (lixisol).

Soil Sampling

Diagonal soil sampling method was used to collect soil samples in each elementary plot in five points and soil composite sample was made per treatment for soil analysis before and after the experimentation. Soil samples were taken on 0–10 cm, 10–20 cm, and 20–30 cm depth in 2019 to determine soil chemical properties. Cation-exchange capacities (CEC) were determined, as well as total N, total P, total K, available P, available K, soil organic matter (OM), organic carbon (OC), and pH. Soil pH was measured using an electronic pH meter according to the international standard ISO 10390, carbon according to Walkley and Black (1934), total nitrogen and total phosphorus by mineralization according to the Kjeldahl method, and available phosphorus according to Bray 1 method (Bray and Kurtz, 1945), and available potassium was determined with a flame photometer. The Metson method (Metson, 1956) was used to determine the CEC. Calcium (Ca) of organic amendments was analyzed with the flame photometer method, and other parameters were analyzed by using the same method for soil analysis.

Plant Material

Hybrid tomato varieties Lindo F1 and Mongal F1 with a cycle of 65 days were used on January to March in 2019 and on May to September in 2020, respectively. The fruit weight of Lindo F1 and Mongal F1 are 90 g and 100–120 g, respectively. These varieties were chosen according to their adaptability to all the season and market demand.

Organic Amendments

Compost was obtained by the process of composting in pile of sorghum straw and cow dung during 3 months. For the composting, an area of 2 × 1.5 m was delimited to build a pile. The surface was filled with straw (30 cm of height) and cow dung (5 cm of height), and 1 cm of ash was added to complete one layer. Tree layer of the mixture was done to build one pile. Biochar was obtained by the pyrolyzing of cotton biomass, and neem seed cake was obtained after neem oil processing and biosol (bio-organic fertilizer) were used for the experiment. Their characteristics are presented in **Table 1**. Neem seed cake and biosol are compounds rich in organic matter, total nitrogen, total K, Ca, and acidic pH compared to compost and biochar, which are alkaline (9.09 to 10.16 pH value).

Mineral Fertilizers

Mineral fertilization of tomatoes was ensured by single urea [$CO(NH_2)_2$] with 46% nitrogen content, potassium chloride with 60% K_2O content, and NPK (14-23-14).

Experimental Design

This study was carried out in a complete randomized Fisher block design, with four treatments and four replications. Plants of 1 month in nursery were transplanted to their respective treatment plots at a spacing of 80 × 40 cm. A surface of 2.4 m^2 was assigned to each treatment. The treatments consisted of T0 (Control): compost (15 t/ha); T1: compost (15 t/ha) + biosol (160 kg/ha) + urea (35 kg/ha) + NPK (87.5 kg/ha) + KCl (52.5 kg/ha); T2:

TABLE 1 | Characteristic of organic amendments.

Organic amendments	pHw	OM (%)	N (%)	C/N	Total P (mg/kg)	Total K (mg/kg)	Total Ca (mg/kg)
Compost	9.09	41.06	1.20	19.90	1,528.43	13,667.24	21,631.99
Biochar	10.16	72.64	0.88	47.96	3,441.46	17,151.93	29,192.73
Neem seed cake	5.81	82.28	6.32	7.55	3,098.17	30,521.58	36,919.91
Biosol	7.00	77.23	15.30	2.93	1,118.15	40,490.82	76,217.47

compost (15 t/ha) + neem seed cake (10 t/ha) + urea (100 kg/ha) + NPK (250 kg/ha) + KCl (150 kg/ha); and T3: compost (15 t/ha) + biochar (10 t/ha) + urea (100 kg/ha) + NPK (250 kg/ha) + KCl (150 kg/ha). Fertilizer application was done according to the recommendation. Urea was applied at transplanting (50%) and at the flowering stage (50%), and the other mineral fertilizers were applied at transplanting (15 days after transplanting). Organic manure (compost) was applied at the rate of 5 t/ha during land preparation by broadcasting, and the other organic manures were locally applied 5 days after transplanting near the plant. Tomato protection was ensured by usual insecticides chlorpyriphos-ethyl 480 g/L and active Acetamiprid 16 g/L + indoxacarb 30 g/L. Irrigation was done when needed. The agronomic parameters were determined as follows: Plant height was determined by measuring the tomato stand from the base to the tip, plant diameter was determined by measuring the tomato stem from the base, the number of fruits were counted per treatment, and the fruits were hand-picked weekly and weighed.

Data Analysis

After verifying the normality of the data by Shapiro-Wilk test, the means of different treatments were subjected to Fisher's analysis of variance (ANOVA), with XL STAT 2016. Means were separated using LSD at 5% probability level.

RESULTS

Soil Characteristics With Organo-Mineral Fertilizers

The incorporation of soil organic amendments showed significant improvement on soil parameters (**Table 2**). In soil depth 0–10 cm, the addition of soil amendments led to enhance soil organic carbon contents and organic matter. The significant improvement in soil carbon was recorded by 64.1, 42.8, and 35.92%, when compost and neem cake, compost and biosol, and compost only were incorporated, respectively, over the initial soil. Similarly, total nitrogen improved with the association of organo-mineral fertilizers and the highest value (0.09%) was obtained with the treatment T1 [compost (15 t/ha) + biosol (160 kg/ha) + urea (35 kg/ha) + NPK (87.5 kg/ha) + KCl (52.5 kg/ha)]. Likewise, the application of both organic and mineral fertilizers significantly increased soil total P, total K, available P, available K, and CEC by 67.55, 45.03, 248.48, 13.63, and 30.61%, with the treatment T2 [(compost (15 t/ha) + neem seed cake (10 t/ha) + urea (100 kg/ha) + NPK (250 kg/ha) + KCl (150 kg/ha)] respectively, over initial soil.

Soil pH increases with the use of organic amendments and mineral fertilizers in both depths, generally. The increase was 6.09 and 12.42% in soil depths 0–10 and 10–20 cm with the treatment T0 [compost (t/ha)] respectively, over initial soil.

The use of biochar, compost, and mineral fertilizers (T3) efficiently ($p < 0.05$) increased soil organic matter, organic carbon, total N, available P, available K, and CEC in soil depth 10–20 cm by 65.38, 66.67, 71.43, 116, 11.64, and 29.89% respectively, over the initial soil.

Plant Growth With Organo-Mineral Fertilizers

Plant diameter was significantly ($p < 0.01$) higher with T3 [compost (15 t/ha) + biochar (10 t/ha) + urea (100 kg/ha) + NPK (250 kg/ha) + KCl (150 kg/ha)] 30 days after transplanting and lower plant diameter was obtained with T0 (compost only) in 2019 (**Figure 1**). At the end of the experiment, maximum plant diameter with T3 (1.11 cm) was 7% and 10% higher than T2 and T1, respectively. In 2020, higher plant diameter was observed with treatment T2 [compost (15 t/ha) + neem seed cake (10 t/ha) + urea (100 kg/ha) + NPK (250 kg/ha) + KCl (150 kg/ha)] and lower plant diameter was obtained with T0 (compost) during all the experiment time. Similarly, 27, 26, and 11% plant diameter increases were recorded with T2, T3, and T1 applications compared to T0 (0.80 cm). Significant difference was observed with plant height only in the later stage of growth between all the treatments in 2019 and higher plant height was recorded with T3 (53.33 cm). In 2020, all the treatments were significantly different in all stages. In general, lower plant growth rate was observed with T0 (55.17 cm) and higher growth rate was obtained with T2 (66.92 cm) (**Figure 2**).

Yield Component and Yield of Tomato Plant With Organo-Mineral Fertilizers

The number of tomato fruits and fruit diameter were significantly ($p < 0.001$) increased with the different combinations of organo-mineral fertilizers in 2020 (**Table 3**). The highest number of fruit per plant was obtained with T2 (15.39), and the increase over T3, T1, and T0 was 29, 77, and 171%, respectively. Fruit diameter was higher with T2 (6.16 cm) and lower fruit diameter was obtained with T0 (5.10 cm). Data presented in **Table 4** show a significant difference ($p < 0.001$) between treatment and fruit yield, and the highest yield in 2019 and 2020 was recorded with treatment T2 (19.17 and 49.82 t/ha, respectively). T2, T3, and T1 increased fruit yield of tomato by about 91, 53, and 41% compared to T0 (10.04 t/ha) in 2019. Similarly, 351, 84, and 32% fruit yield increases

TABLE 2 | Soil chemical characteristics at different depths.

Treatment	pHw	OM (%)	OC (%)	Total *N* (%)	C/N	Total P (mg/kg)	Av-P (mg/kg)	Total K (mg/kg)	Av-K (mg/kg)	CEC (cmol/kg)
Depth 0–10 cm										
IS	6.40b	1.03c	0.60b	0.053c	11.31a	109.7c	5.92e	1,375.4e	84.4b	3.43b
T0	6.79a	1.40b	0.81ab	0.083b	9.79a	160.5b	15.33c	1,613.0d	63.1c	5.08a
T1	6.66a	1.47b	0.86a	0.090a	9.50a	156.7b	11.06d	1,805.8c	56.8c	4.29ab
T2	6.48b	1.69a	0.98a	0.090a	10.88a	183.8a	20.63a	1,994.7a	95.9a	4.58a
T3	6.76a	1.43b	0.83ab	0.080b	10.36a	162.4b	16.94b	1,859.3b	88.3b	4.63a
Depth 10–20 cm										
IS	6.20b	0.78bc	0.45bc	0.042c	10.96a	94.1b	2.86d	1,424c	82.5b	3.78c
T0	6.97a	1.06b	0.62b	0.058b	10.58a	106.4a	4.40c	1,907a	45.4c	5.38a
T1	6.67a	0.97b	0.56b	0.058b	9.89a	106.4a	4.47c	1,705b	44.2c	5.21a
T2	6.68a	1.03b	0.60b	0.060b	9.94a	94.2b	5.64b	1,907a	75.7b	4.69ab
T3	6.68a	1.29a	0.75a	0.072a	10.37a	109.3a	6.88a	1,657b	92.1a	4.91a

IS, initial soil; T0, I (compost 15 t/ha); T1, compost (15 t/ha) + biosol (160 kg/ha) + urea (35 kg/ha) + NPK (87.5 kg/ha) + KCl (52.5 kg/ha); T2, compost (15 t/ha) + Neem seed cake (10 t/ha) + urea (100 kg/ha) + NPK (250 kg/ha) + KCl (150 kg/ha); T3, compost (15 t/ha) + biochar (10 t/ha) + urea (100 kg/ha) + NPK (250 kg/ha) + KCl (150 kg/ha). Means with the same letter in a column are not significantly different according to Fisher test ($p < 0.05$). OM, organic matter; OC, organic carbon. Means with the same letter in a column are not significantly different according to Fisher test ($p < 0.05$).

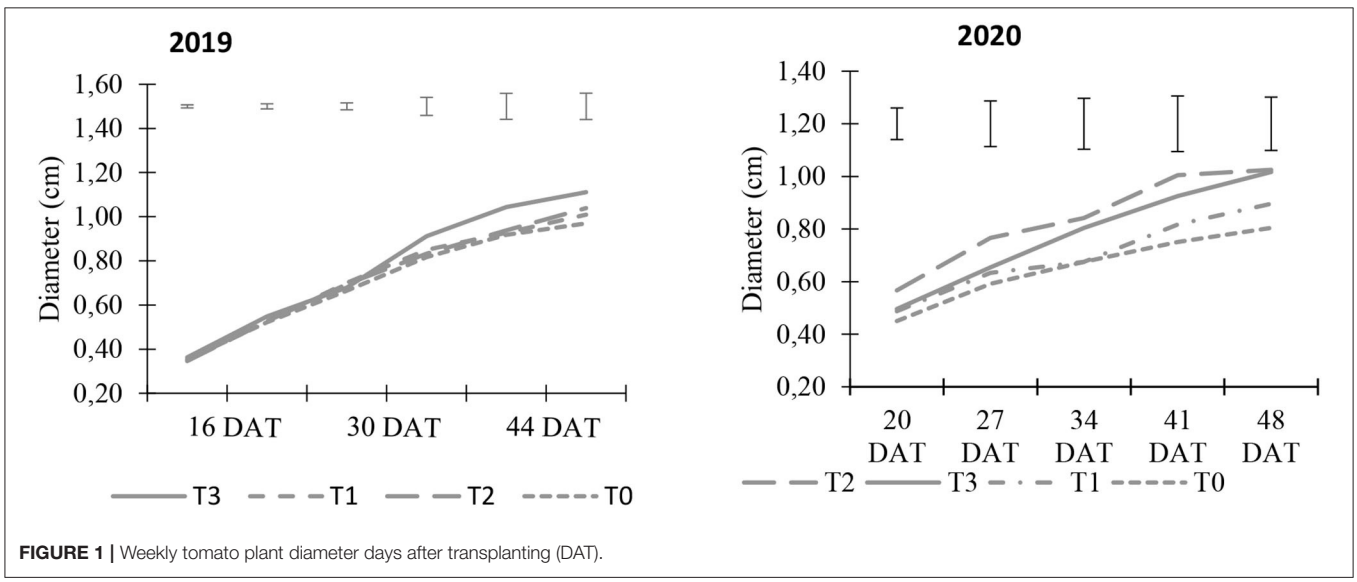

FIGURE 1 | Weekly tomato plant diameter days after transplanting (DAT).

were observed with T2, T3, and T1 compared to T0 (11.05 t/ha) in 2020.

DISCUSSION

The use organo-mineral fertilizers is required to supplement readily available nutrients and good growing environment (biological, chemical, and physical condition) for plant growth. Incorporation of organic amendments, especially addition of compost and neem cake or biochar, has efficiently increased soil organic status and soil nutrient level. The improvement of soil chemical properties and organic status is probably linked to the quality of the organic amendments that have been used for the study (**Table 1**). These results indicate that organic amendments are compounds rich in carbon and organic resources that provide an energy source for soil microorganisms that drive the various soil biological processes that enhance nutrient transformation and other quality parameters of soil (Fairhurst, 2012). The long-term use of organic amendments increases soil fertility by improving the structural and hydrological properties of soil and increasing soil organic matter and other macronutrients (Ramzan et al., 2021). According to Mulugeta and Getahun (2020), organic amendments play a positive role in chemical characteristics of the soil including increase in organic carbon (up to 58% with 120 t/ha vs. unfertilized soil) and organic nitrogen up to 90% depending on the type and the level applied. From the results, after 2 years of trials, soil fertility has increased with the combination of inorganic fertilizer and neem cake (30.61% carbon and 64.1% CEC), or biochar (42.8% CEC) and compost over the control. The finding of Ayamba et al. (2021), Bashir et al. (2021), Bergstrand et al. (2020), and Gorovtsov et al.

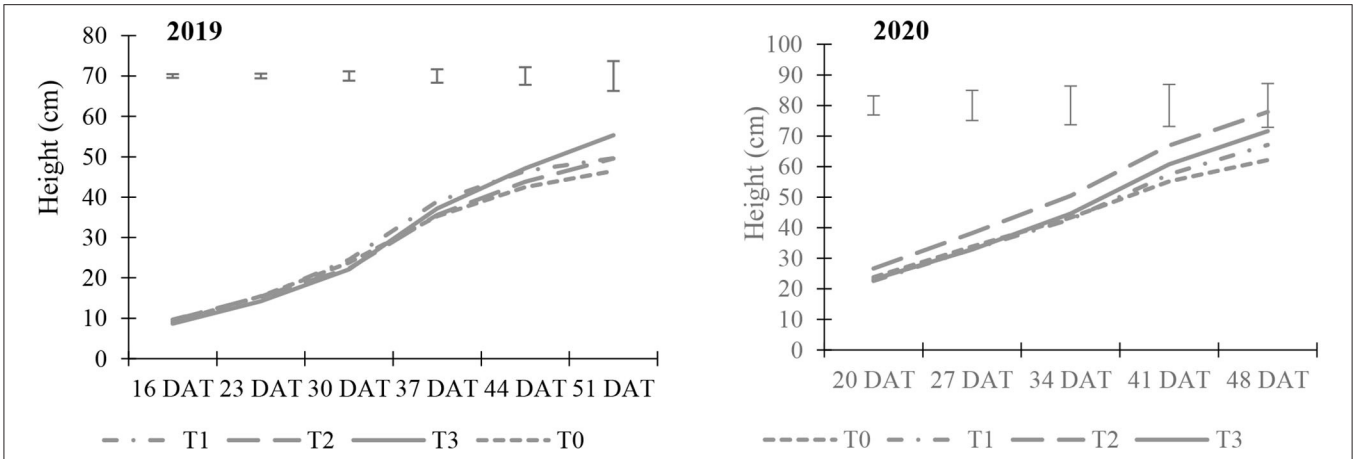

FIGURE 2 | Weekly tomato plant height days after transplanting (DAT).

TABLE 3 | Number of fruits and fruit diameter with organic and organo-mineral fertilizer application.

Treatments	Number of fruits	Fruit diameter (cm)
T0	5.66b	5.10d
T1	8.66b	5.36c
T2	15.39a	6.16a
T3	12.43a	5.88b

T0, I (compost 15 t/ha); T1, compost (15 t/ha) + biosol (160 kg/ha) + urea (35 kg/ha) + NPK (87.5 kg/ha) + KCl (52.5 kg/ha); T2, compost (15 t/ha) + Neem seed cake (10 t/ha) + urea (100 kg/ha) + NPK (250 kg/ha) + KCl (150 kg/ha); T3, compost (15 t/ha) + biochar (10 t/ha) + urea (100 kg/ha) + NPK (250 kg/ha) + KCl (150 kg/ha). Means with the same letter in a column are not significantly different according to Fisher test ($p < 0.05$).

TABLE 4 | Tomato yield with organic and organo-mineral fertilizer application.

Treatments	Tomato yield (t/ha)	
	2019	2020
T0	10.04c	11.05d
T1	13.63b	26.98c
T2	19.17a	49.82a
T3	12.55b	37.87b

T0, I (compost 15 t/ha); T1, compost (15 t/ha) + biosol (160 kg/ha) + urea (35 kg/ha) + NPK (87.5 kg/ha) + KCl (52.5 kg/ha); T2, compost (15 t/ha) + Neem seed cake (10 t/ha) + urea (100 kg/ha) + NPK (250 kg/ha) + KCl (150 kg/ha); T3, compost (15 t/ha) + biochar (10 t/ha) + urea (100 kg/ha) + NPK (250 kg/ha) + KCl (150 kg/ha). Means with the same letter in a column are not significantly different according to Fisher test ($p < 0.05$).

(2019) showed that organic manure and their combination with mineral fertilizers can be efficient to improve soil fertility and crop productivity.

Application of organo-mineral fertilizers increased the height and diameter of the tomato plant in this experiment. In general, the highest values were obtained with the association of compost and biochar with mineral fertilizers in 2019 and lower plant diameter was obtained with the application of compost only. This result can be attributed to the fact that biochar can increase soil cation exchange capacity and soil nutrient availability. High nutrient content and nutrient retention capacity lead to improved nutrient supply for plants and reduced nutrient losses by leaching (Glaser et al., 2002). Additionally, applying biochar to acidic soil improve soil pH and nutrient availability such as nitrogen and phosphorus (Van Zwieten et al., 2009; Blanco-Canqui, 2017; Fidel et al., 2018). These nutrients play an important role in plant growth by stimulating photosynthesis and biomass production. According to Hamer et al. (2004), biochar from maize residues in soils can promote mineralization of carbon compounds by enhancing the growth and dynamics (metabolism) of microorganisms. The finding of Nguyen et al. (2017) on short-term effects of organo-mineral biochar showed the positive effect of this combination over inorganic and organic fertilizer application separately. Also, Mustafa et al. (2010) showed that the application of biochar and mineral fertilizers increased plant height compared to the application of biochar or fertilizer alone. In 2020, growth parameters were positively influenced by mineral fertilizers and neem seed cake. Plant diameter and plant height increased about 27 and 21%, respectively, compared to the application of compost only. Similarly, application of neem seed cake with inorganic fertilizer improved the number of fruits by 171% and fruit diameter by 21% compared to application of compost only. The highest fruit yield of tomato in the current work was also recorded from the combined application of neem seed cake + compost with inorganic fertilizers in both years (91 to 35%). These results affirmed the finding of Wu et al. (2020) and Ayeni and Ezeh (2017) that the combination of organic and inorganic fertilizers has a positive effect on tomato productivity. The combination not only improves the organic status of soil but also increases inorganic fertilizer use efficiency. Also, neem seed cake amendment provides nutritional requirements, suppresses plant pest populations, and increases the yield and quality of agricultural crops. Koul and Shankar (1995) demonstrated that the major agricultural pests are sensitive to neem. The neem seed cake is also demonstrated to be rich in plant nutrients and is a

good manure for soil amendment (Puri, 1999). The results are in conformity with the finding of Oyinlola et al. (2017) who find an improvement of soil properties and tomato yield with the used of neem seed cake. Each organic amendment has a specific importance on the improvement of soil characteristics. However, farmers need fertilizers that have a high impact in the short term. Soil improvement must also be followed in the long term to sustain soil productivity.

CONCLUSION

Tomato cultivation required important demand of nutrient supply. Organo-mineral fertilizers are a combined source of nutrient that can be effectively used to increase the long-term productivity of tomato plant. This study clearly showed that the application of organic amendments and mineral fertilizers had more advantages than the application of organic amendment alone. The combined application of neem seed cake and mineral fertilizers was more effective on tomato productivity in this study.

DATA AVAILABILITY STATEMENT

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

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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