

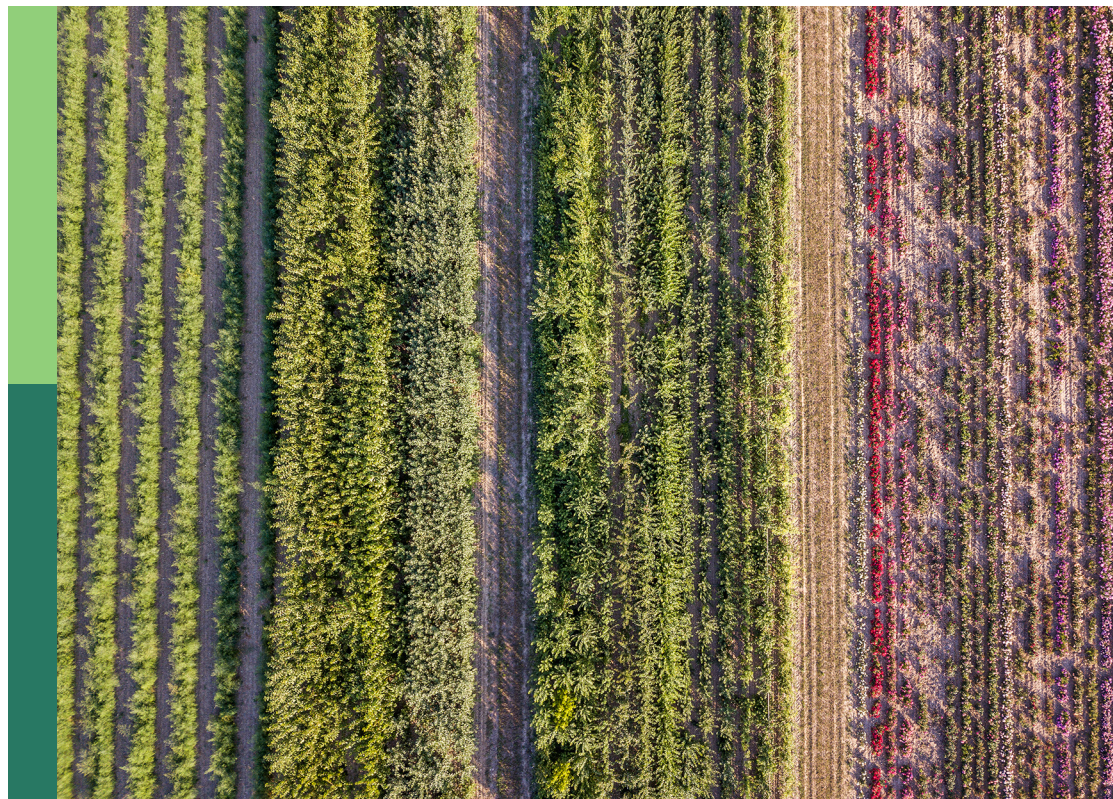
Nitrogen use to improve sustainable yields in agricultural systems

Edited by

Sudhakar Srivastava and Andrews Opoku

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Nitrogen use to improve sustainable yields in agricultural systems

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Editorial: Nitrogen use to improve sustainable yields in agricultural systems

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KEYWORDS

agricultural systems, biological nitrogen fixation, food security, nitrogen balance, nitrogen use efficiency

Editorial on the Research Topic

Nitrogen use to improve sustainable yields in agricultural systems

Nitrogen (N) plays a vital role in plant nutrition. It is required in larger amounts than other essential nutrients as N is an integral part of a plethora of plant metabolites such as amino acids, chlorophyll, nucleic acids, ATP and phytohormones. Thus, N acts as the backbone of optimum plant growth and development (Anas et al., 2020). The food demands of growing population in twentieth century could be met with prolific crop production; thanks to the discovery of urea, a nitrogenous fertilizer. The sufficient food availability in turn allowed population to further increase exponentially. The increasing trend of population and diminishing land resources over the years have compelled crop production systems to become more intensive and to use high unwarranted amounts of nitrogenous fertilizers for vigorous growth and yields. In the USA, N fertilizer use increased from $0.22 \text{ gN m}^{-2} \text{ y}^{-1}$ to $9.04 \text{ gN m}^{-2} \text{ y}^{-1}$ between 1940 to 2015; an about 40-fold increase (Cao et al., 2018). An increase of about 4-fold in cereal production has been monitored since 1960s to present times but it occurred with a 9-fold increase in the N fertilizer application (Ladha et al., 2022). However, a substantial amount of the N fertilizer applied, is lost through leaching, ammonia volatilization and denitrification. Málinas et al. (2022) highlighted the severity of N losses from agricultural fields by ascertaining that nearly 50% of N applied globally is lost, contributing to groundwater pollution, eutrophication, soil acidification and global warming. For sustainable crop production, agricultural practices must intensify productivity while maintaining environment quality simultaneously (Govindasamy et al., 2023). To this end, augmenting biological N fixation and reuse of organic wastes can play significant role in reducing N fertilizer applications, and in sustaining soil and environmental health.

This Research Topic presents original research and review articles on recent scientific advances in N use efficiency, N balance assessment, biological N fixation and integrated nutrient management. In all, ten papers are presented. Four articles by Castillo, Kirk, Rivero, Fabini et al., Chivenge et al., Winnie et al., and Ntinyari et al. explored the options for optimizing N use efficiency by moderating N losses. Chivenge et al. demonstrate that the combined use of site-specific nutrient management (SSNM) and digital decision support tools such as Rice Crop Manager, Nutrient Expert, and RiceAdvice improved rice yields, profit, and N use efficiency, and reduced N losses. They advocated

that the use of SSNM would improve farmers' profits too. Winnie et al., showed that the Abjua declaration of increasing fertilizer consumption in West Africa to 50 kg nutrients (i.e., N + P + K)/ha was too low to improve food security and optimize NUE. Castillo, Kirk, Rivero, Fabini et al. used N balance, N use efficiency, and N surplus (NSURP) to suggest that crop-livestock systems could be efficiently propagated and promoted to greater crop yields and higher livestock productivity without increasing N fertilizer user. Castillo, Kirk, Rivero, Haefele et al. demonstrated the application of a model, DNDC in optimizing N management in rice, rice-soybean and rice-pasture crop systems. Kebede presented a state-of-the-art review of legumes-driven biological nitrogen fixation. The author suggested that selection of appropriate legumes and their use at the maximum genetic potential, inoculation of legumes with compatible effective rhizobia, and the use of appropriate agronomic practices can sustainably utilize biological N fixation and increase crop yields. Paramesh et al. presented an excellent review of integrated nutrient management (INM) practices and opined that INM is vital for the revival of soil health along with achieving higher crop yields and decreasing environmental pollution and greenhouse gas emissions. INM approaches should indeed be practiced widely to sustainably utilize soil resources of the earth.

Ferdous et al. assessed the feasibility of reducing ammonia volatilization from irrigated rice system by applying lower rates of N fertilizers. They could achieve both aims by utilizing biochar and compost, either alone or in combination, as nutrient providers. The article by Thakur et al. investigated the effect of multitrait *Pseudomonas* sp as a growth-promoting bioinoculant on a medicinal plant, *Andrographis paniculata* (Kalmegh). The authors found the bioinoculant to be a potent plant growth-promoting agent and an environmentally friendly approach for improving crop performance. Lastly, Betts et al. explored the relationships among nitrogen balance, fertilizer application advice, and farm financial performance and found N balance to be a responsive indicator of farm financial performance.

Overall, these ten articles published in this Research Topic have clearly identified the "problems and issues" associated with N use

in crop production in diverse agricultural systems. The articles also showcased feasible sustainable strategies for improving N use efficiency, tracking N flows, reducing N losses while increasing crop yields and reducing environmental pollution. The nutrient management strategies opined in the presented articles shall pave way for improved N management in crop production while attaining the goals of food security.

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Improving Nitrogen Use Efficiency—A Key for Sustainable Rice Production Systems

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Fertilizer use and genetic improvement of cereal crops contributed to increased yields and greater food security in the last six decades. For rice, however, fertilizer use has outpaced improvement in yield. Excess application of nutrients beyond crop needs, especially nitrogen (N), is associated with losses to the environment. Environmental pollution can be mitigated by addressing fertilizer overuse, improving N use efficiency, while maintaining or improving rice productivity and farmers' income. A promising approach is the site-specific nutrient management (SSNM), developed in the 1990s to optimize supply to meet demand of nutrients, initially for rice, but now extended to other crops. The SSNM approach has been further refined with the development of digital decision support tools such as Rice Crop Manager, Nutrient Expert, and RiceAdvice. This enables more farmers to benefit from SSNM recommendations. In this mini-review, we show how SSNM can foster sustainability in rice production systems through improved rice yields, profit, and N use efficiency while reducing N losses. Farmer adoption of SSNM, however, remains low. National policies and incentives, financial investments, and strengthened extension systems are needed to enhance scaling of SSNM-based decision support tools.

Keywords: precision nutrient management, sustainability, rice agri-food systems, digital tools, profitability

INTRODUCTION

Optimal nutrient management in rice is important for food security, climate change mitigation, adaptation and transformation, and attainment of several sustainable development goals (Cakmak, 2002; Kanter et al., 2019; Lal et al., 2020). Fertilizer use has reduced agriculture expansion into natural ecosystems by increasing crop productivity on existing land. However, while yields increased with fertilizer use in the 1960s, they stagnated in intensive rice systems in the mid-1980s despite the development of varieties with greater yield potential (Dawe and Dobermann, 1999). This resulted in large yield gaps. This was largely due to excessive or imbalanced fertilizer use based on increased reliance on blanket fertilizer application, coupled with a rapid decline in the efficiency of fertilizer uptake by plants, indicating that increased fertilizer use outpaced yield improvements (Cassman and Pingali, 1995; Tilman et al., 2002). The orientation of producing more food, associated with fertilizer overuse, particularly nitrogen (N), has caused a deterioration in soil physical, chemical, and microbiological properties and functions and increased soil and water pollution (Pingali, 2012; Srivastava et al., 2020). With increasing pressure to meet global food demand while fostering environmental sustainability, a paradigm shift is needed to a more judicious use of N fertilizer.

About 50% of global N fertilizer is applied to major cereals: *Zea mays* (maize; 17%), *Triticum aestivum* (wheat; 18%), and *Oryza sativa* (rice; 16%) (Heffer et al., 2017). However, globally, N use efficiency, a measure of the short-term balance between N used for grain production and N lost to the environment, has remained below 40% (Omara et al., 2019), indicating that more than 60% of applied N remains unused or is lost from soil (Dobermann, 2000; Ladha et al., 2005). Increasing N use efficiency in rice agri-food systems becomes all the more important, given that the commodity is a staple food for more than half the global population (GRiSP, 2013). More than 90% of the rice is produced in Asia, mostly by smallholder farmers. Due to high subsidy on urea fertilizer, farmers tend to apply large quantities of N fertilizer in excess of plant requirements (Ladha et al., 2005). However, grain yield response diminishes as N fertilizer rate increases and may cause lodging and susceptibility to pest and disease damage when overapplied (Balasubramanian et al., 1998; Duy et al., 2004). Excess reactive N has detrimental effects in agroecosystems, such as nitrous oxide emissions, increased soil acidity, decreased biodiversity, and groundwater contamination (Galloway et al., 2003).

Nitrogen Use Efficiency Trends in Rice Production Systems

In rice production, farmers apply large amounts of N fertilizer to maximize yield, but only 20–50% of N is taken up by the crop. N use efficiency remains low with a global average partial factor of productivity N (PFP N) of about 40 kg grain kg⁻¹ N applied (Figure 1). This is largely due to farmers applying large quantities of N fertilizer at early growth stages when the rice plants have not fully developed the root system. The resulting loss of the applied N, which is a mobile nutrient leads to increased water and land pollution and greenhouse gas (GHG) emissions (Shaviv and Mikkelsen, 1993; Xu et al., 2012; Zhang et al., 2013). Dobermann (2007) reviewed the commonly used N use efficiency indices in agronomy research, which include agronomy efficiency, recovery efficiency, internal efficiency, physiological efficiency, and PFP N. PFP N is commonly used in agronomy and is useful when comparing across different management practices and where there are no N omission plots to enable calculation of other indices (Dobermann, 2007). PFP N is an aggregate index which integrates indigenous N supply from the soil and that applied from external sources. It generally declines with increasing N application rates.

A PFP N of 60 kg grain kg⁻¹ N applied or greater indicates well-managed systems (Dobermann, 2005). PFP N has remained well below this threshold in many rice growing Asian countries compared to developed countries (Heffer et al., 2017). While PFP N increased between 2006 and 2014 in China, Indonesia, and Vietnam, it stagnated in India at 33 kg grain kg⁻¹ N (Figure 1). In contrast, in the Philippines, Thailand, Iran, and Pakistan, PFP N declined during the same period. It should be noted, however, that available data on fertilizer use by crop and country are unreliable. The contrasting PFPs could be due to management, e.g., the reduction in N fertilizer rate associated with the controls introduced in China (Chen et al., 2014) could

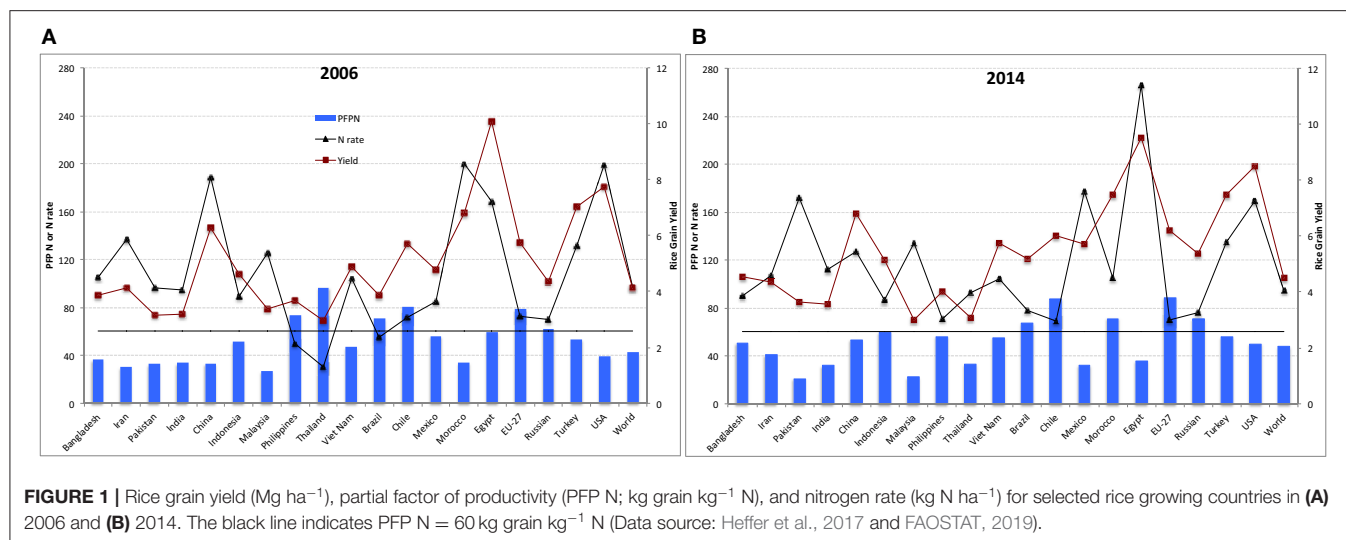
account for the increase in PFP N. The Rice Research Institute of the Guangdong Academy of Agricultural Sciences introduced the Three Controls Technology to control the amount on N fertilizer. This technology includes controls on the amount and timing of N fertilizer using site-specific nutrient management (SSNM) approach, controls on the number of tillers and controls on the use of pesticides and herbicides.

While increasing N use efficiency in rice systems is essential for sustainability, low input rice systems in Sub-Saharan Africa (SSA) are characterized by high N use efficiency, sometimes > 100 kg grain kg⁻¹ N applied. These systems are associated with mining of nutrients, resulting in land degradation (Dobermann, 2005, 2007; Edmonds et al., 2009). Africa contributes about 4.5% to global rice production (FAOSTAT, 2019). This is not enough to meet rice demand in Africa, which is increasing as dietary preferences shift from the traditional coarse grains owing to urbanization and changing family occupational structure. Rice production in SSA has increased in recent years, largely due to expansion of area than increased productivity, i.e., yield per unit area (FAOSTAT, 2019), but has been outpaced by consumption demand; much of which has been supported by imports, mostly from Asia. While global rice yields average at 4 t ha⁻¹, yields in SSA average 2 t ha⁻¹ (GRiSP, 2013); <50% of attainable yield. This is caused by a myriad of issues, among them; low soil fertility and limited fertilizer use, use of home retained seeds and traditional varieties, labor shortage, weak markets, and lack of infrastructure and equipment for irrigation. Rice productivity in SSA can be increased via the introduction of improved cultivar and agronomic management practices.

Exploitable rice yield gaps remain in Asia and SSA (Stuart et al., 2016). There is a need for tailored solutions that are sustainable and meet the increasing global demand for food, feed, and energy while protecting the environment. Rice production also needs to be profitable for farmers; this can be partly achieved by farmers applying appropriate types and amounts of fertilizers. Fertilizers typically constitute 20% of the input costs in rice production (Pampolino et al., 2007) and achieving efficient fertilizer management is challenging in smallholder farming systems where soils and crop management can vary even within short distances. SSNM enable farmers to apply adequate and appropriate amounts of nutrients to suit soil, crop variety, and climate, hence mitigating the potential trade-offs between productivity and environmental health.

The SSNM Approach

The SSNM approach was developed in the 1990s to calculate field-specific requirements for fertilizer N, P, and K for cereal crops, taking into consideration the indigenous nutrient supply and the target yield (Dobermann et al., 2002, 2004). SSNM was initially conceptualized for small holder rice producers in Asia, where fields tend to be small with large spatial variability in terms of nutrient status and management. SSNM is based on the principles of the Quantitative Evaluation of the Fertility of Tropical Soils (QUEFTS) model (Janssen et al., 1990) to estimate the requirement for a fertilizer nutrient from the gap between the total amount of nutrient required to achieve a specific target yield and the indigenous supply of



the nutrient (Witt and Dobermann, 2004). The approach allows balanced application of major nutrients. Timing of fertilizer application is adjusted to meet peak nutrient demand of the crop to enhance nutrient use efficiency and foster environmental sustainability. Using SSNM principles, field-, crop-, and season-specific requirements for N, P, and K are calculated at the beginning of the season (Dobermann et al., 2002; Buresh et al., 2010).

SSNM-Based Decision Support Tools

SSNM has evolved along a research to impact pathway, with refinement in the science and methods, expansion to new geographies, and the development of decision support tools for its dissemination. Leaf color charts, typically plastic strips containing four to six panels with colors ranging from yellowish green to dark green, were developed to monitor leaf greenness, which is related to N status and thus aid assessment and adjustment of N requirements during the season (Singh et al., 2002; Witt et al., 2005). An ICT decision support tool, the Nutrient Manager, was developed to give field-specific fertilizer recommendations for rice production, initially as Microsoft Access, but it evolved to be web-based (Buresh et al., 2014) and was eventually developed as Rice Crop Manager [RCM] (Buresh et al., 2019; Sharma et al., 2019a). RCM is a web-based and open access decision tool that provides farmers with simplified, easy to follow, and appropriate nutrient management recommendations (<https://phapps.irri.org/ph/rcm/>, <http://webapps.irri.org/in/od/rcm/>). Similar tools were developed for different regions and crops: Nutrient Expert (Pampolino et al., 2012; Xu et al., 2017a) and RiceAdvice in West Africa (Zossou et al., 2020; Arouna et al., 2021). These tools can be integrated with GIS and remote sensing for holistic and precise knowledge delivery to greater numbers of farmers. The tools provide information on yield predictions, which is useful for more accurate estimation of nutrient requirements, and allow for dynamic nutrient management with mid-season nutrient management adjustments to match crop condition and needs.

METHODS

Data from 46 published articles with studies conducted between 2001 and 2020 were compiled in a database (Table 1). These studies were conducted in 11 countries: eight in Asia and three in Africa. Using Web of Science, Science Direct, and Google Scholar, we used the following search terms: SSNM, SSNM rice, SSNM maize, SSNM wheat, SSNM cereals, and SSNM vs. farmers' fertilizer practice (FFP). The studies included peer reviewed journal publications, book chapters, and technical reports that show direct comparison between SSNM and FFP in the same fields. We excluded studies when SSNM was compared with other treatments such as the no input control, blanket fertilizer recommendation, government or institute recommendation, or soil test-based recommendations. We included only studies that followed the generic SSNM approach and excluded studies where factors other than fertilizer management differed between SSNM and FFP. When multiple publications reported the data from the same experiments, we used the paper with the most complete dataset. Studies were excluded if the experimental method was vague and when there are varied factors other than fertilizer management between SSNM and FFP treatments. Hence, agronomic practices except nutrient management were the same or similar in both treatments. Of the 46 studies, 23 of them conducted N omission treatment thus enabling them to calculate and report AEN (Equation 1). In some cases, AEN values were extracted from the papers. Partial factor of productivity of N (PFP N) was calculated for all studies (Equation 2).

$$\text{AEN} = [\text{GY}_N (\text{kg ha}^{-1}) - \text{GY}_0 (\text{kg ha}^{-1})] / \text{N rate} (\text{kg N ha}^{-1}) \quad (1)$$

$$\text{PFP N} = \text{GY}_N (\text{kg ha}^{-1}) / \text{N rate} (\text{kg N ha}^{-1}) \quad (2)$$

Where GY_N is the grain yield in a treatment with N application.
 GY_0 is the grain yield in a treatment without N application.

TABLE 1 | A synthesis of studies conducted in rice cropping systems on different sites in different countries in Asia and Africa under different agronomic management practices that evaluated the site-specific nutrient management (SSNM) compared to the farmer fertilizer practice (FFP) in terms of grain yield, agronomic efficiency of N (AEN), partial factor productivity of N (PFP N), and gross return above fertilizer cost (GRF).

References	Country (# of sites)	Crop. system β	Residue manag. α	Crop estab. ψ	Decision tool ξ	N rate (kg ha ⁻¹)		P rate (kg ha ⁻¹)		K rate (kg ha ⁻¹)		Grain yield (kg ha ⁻¹)		AEN (kg grain kg ⁻¹ N)		PFP (kg grain kg ⁻¹ N)		GRF (USD ha ⁻¹)	
						SSNM	FFP	SSNM	FFP	SSNM	FFP	SSNM	FFP	SSNM	FFP	SSNM	FFP	SSNM	FFP
Abdulrachman et al. (2004)	Indonesia (1)	R-R	Retained	TPR	SPAD	106	121	20	8	57	5	4,500	4,275	13	9	45	38	990	977
AfricaRice (2016)	Ghana (1)	R-R	Removed		RA	126	151	24	23	46	44	4,900	4,300			39	28	1,076	914
Alam et al. (2005)	Bangladesh (6)	R-R	Removed	TPR	LCC	130	150	26	26	38	38	5,206	4,688	19	14	40	32	1,153	1,011
Alam et al. (2006)	Bangladesh (2)	R-U	Removed	TPR	LCC	117	149	25	30	41	46	4,550	4,000	17	10	39	27	997	827
Banayo et al. (2018)	Philippines (4)	R-R	Removed	TPR	RCM	82	97	10	11	21	19	4,538	4,228			56	44	1,053	965
Singh (2014)	India (6)		Removed	TPR	LCC	99	132					6,469	6,384	23	18	67	49		
Biradar et al. (2006)	India (1)	R-U	Removed	TPR	Others	200	120	44	13	83	25	5,520	3,686			28	31	1,130	808
Budhathoki et al. (2018)	Nepal (1)	R-U	Removed	DSR	NE							5,140	4,020						
Gines et al. (2004)	Philippines (1)	R-R	Retained	TPR	SPAD	110	107	19	15	49	23	5,200	4,700	15	12	48	45	1,169	1,068
Gupta et al. (2016)	Nepal (1)	R-U	Removed	TPR	NE							5,460	4,430						
Hu et al. (2007)	China (1)	R-R	Removed	TPR	LCC	142	177					6,100	5,900			43	33		
Islam et al. (2007)	India (2)	R-R	Removed	DSR	LCC	104	129					3,908	3,848			37	30		
Khuong et al. (2007)	Vietnam (3)	R-R	Removed	DSR	Others	96	106	19	21		39	5,620	5,525	15	13	59	52		
Khurana et al. (2007)	India (6)	R-U	Removed	TPR	SPAD	136	148	11	3	30	0	6,000	5,117	17	9	44	35	1,376	1,180
Khurana et al. (2009)	India (1)	R-U	Removed	TPR	Others	137	148					6,000	5,050	16	10	44	34		
Mandal et al. (2015)	India (1)	R-U	Removed	TPR	NE	111	85	14	23	41	39	5,784	4,627			52	54	1,326	1,041
Marahatta (2017)	Nepal (1)	R-U	Removed	TPR	Others	96	53	19	11	60	7	6,350	4,620			66	88	1,459	1,099
					LCC	100	53	19	11	60	7	6,660	4,620			67	88	1,533	1,099
Nagarajan et al. (2004)	India (2)	R-R	Retained	TPR	SPAD	129	98	22	22	75	35	6,021	5,350	15	14	47	57	1,340	1,218
Pampolino et al. (2007)	India (2)	R-R	Removed	TPR	LCC	125	115	14	20	66	36	6,425	6,000			52	53	1,463	1,373
	Philippines (2)	R-R	Removed	TPR	LCC	113	125	12	17	48	31	5,200	4,850			46	42	1,178	1,087
	Vietnam (3)	R-R	Removed	DSR	LCC	92	106	20	20	36	41	4,917	4,583			54	44	1,116	1,021
Pampolino (2016)	India (1)	R-U	Removed	TPR	NE	111	85	15	17	41	39	5,780	4,630			52	54	1,325	1,051
	China	R-U	Removed	TPR	NE	156	170	31	26	72	71	8,000	7,800	15	12	51	46	1,806	1,756

(Continued)

TABLE 1 | Continued

References	Country (# of sites)	Crop. system β	Residue manag. α	Crop estab. ψ	Decision tool \S	N rate (kg ha ⁻¹)		P rate (kg ha ⁻¹)		K rate (kg ha ⁻¹)		Grain yield (kg ha ⁻¹)		AEN (kg grain kg ⁻¹ N)		PFP (kg grain kg ⁻¹ N)		GRF (USD ha ⁻¹)	
						SSNM	FFP	SSNM	FFP	SSNM	FFP	SSNM	FFP	SSNM	FFP	SSNM	FFP	SSNM	FFP
Peng et al. (2006)	China (4)	R-R	Removed	TPR	SPAD	87	205	40	40	100	100	7,544	7,163	13	4	98	35	1,704	1,532
	Philippines (1)	R-R	Removed	TPR	SPAD	133	90	30	30	40	40	6,650	6,150	20	23	51	68	1,505	1,407
Tan et al. (2004)	Vietnam (1)	R-R	Retained	DSR	SPAD	98	112	22	20	62	20	4,663	4,390	20	15	48	40	1,029	983
Qureshi et al. (2018)	India (1)	R-U	Removed	TPR	NE	118	130	12	17	43	17	6,531	6,064	25	19	55	47	1,511	1,394
Rajendran et al. (2010)	India (2)	R-R	Removed	TPR	LCC	121		15		50		6,363	5,825						
Saito et al. (2015)	Senegal (1)		Removed	DSR	RCM	133	153	17	18	26	0	7,467	5,967			57	39	1,738	1,365
Satawathananont et al. (2004)	Thailand (1)	R-R	Retained	DSR	SPAD	112	112	18	23	43	5	4,795	4,725	9	8	43	43	1,071	1,070
Segda et al. (2005)	Burkina Faso (1)	R-R	Removed	TPR	Others	116	79	21	16	20	15	6,440	5,203			56	66	1,490	1,215
Sharma et al. (2019a)	India (1)	R-R	Removed	TPR	NE	127	122	14	21	38	46	4,833	4,300			38	36	1,081	935
Sharma et al. (2019b)	India (4)	R-R	Removed	TPR	RCM	102	85	13	22	28	44	5,094	4,561			50	55	1,170	1,023
Singh et al. (2015)	India (1)	R-U	Removed	TPR	Others	180		26		75		9,110	6,800						
Son et al. (2004)	Vietnam (1)	R-U	Retained	TPR	SPAD	94	104	16	20	53	62	6,175	6,013	18	14	67	58	1,425	1,366
Van Hach and Tan (2007)	Vietnam (3)	R-R	Removed	DSR	LCC	99	113	17	21	40	40	5,807	5,447			59	49	1,335	1,231
Varinderpal et al. (2010)	Bangladesh (1)	R-R	Removed	TPR	SPAD							5,100	5,000	26	17				
	India (13)	R-R	Removed	TPR	LCC	68	92					5,656	5,540			99	76		
Wang et al. (2001)	China (1)	R-R	Removed	TPR	SPAD	133	171	21	22	80	75	6,350	5,900	11	6	48	34	1,419	1,283
Wang et al. (2004)	China (1)	R-R	Removed	TPR	SPAD	126	171	14	19	52	57	6,475	6,192	12	7	52	36	1,483	1,371
Wang et al. (2007)	China (2)	R-R	Removed	TPR	SPAD	136	236	14	19	52	58	8,240	7,770	16	9	61	35	1,743	1,599
Wang et al. (2020)	China (1)			DSR	NE	169	185	29	33	75	73	9,400	9,200	20	17	56	50	1,208	1,163
Xu et al. (2010)	China (1)	R-R	Retained	TPR	SPAD	102	150	39		62		6,538	6,550			64	44		
Xu et al. (2017a)	China (3)	R-R	Removed	TPR	NE	156	191	30	34	76	88	8,342	7,858	17	13	54	42	1,890	1,733
Yang et al. (2017)	China (7)	R-R	Removed	TPR	NE	156	169	30	26	66	70	8,429	7,957	17	14	54	48	1,917	1,795

Country (# of sites) is country where study was conducted and in parenthesis is the number of sites within the country.

Crop. system β is cropping system where R-R is rice-rice; R-U is rice-upland crop.

Residue manag. α is residue management.

Crop estab. ψ is crop establishment method; TPR is transplanted and DSR is direct seeded rice.

Decision tool \S : SPAD is SPAD chlorophyll meter; LCC is leaf color chart; NE is nutrient expert; RA is RiceAdvice; RCM is Rice Crop Manager.

Averages of yields and AEN of both SSNM and FFP were obtained from each study, while in some instances, manual estimation from the figures was performed when these data were only presented in figures. Individual studies have variable number of replicates with 323 as the highest number. Also, nutrient rates among the fields varied and were reported as a range because there is a high spatial variability even for neighboring fields. Thus, the average between the minimum and maximum values reported was determined across the replicates and was used as nutrient rate for the reported grain yield. The most common sources of fertilizers used in the studies were urea for nitrogen, DAP for P, and muriate of potash (KCl) for potassium.

The cost benefits were reported as gross return above fertilizer cost (GRF) in this paper, which was derived from the other two economic performance metrics: total fertilizer cost (TFC; Equation 3) and gross return (Equation 4). GRF was calculated as in Equation 5. Fertilizer prices of the most common sources: urea (46-0-0) for N, DAP (18-46-0) for N and P, and KCl (0-0-60) for K (urea, DAP, and KCl), were estimated from the 10-year average across countries listed in the database (Indexmundi, 2020; <https://www.indexmundi.com>). The prices were calculated as per unit of nutrient leading to US\$0.642 kg⁻¹ N, US\$2.151 kg⁻¹ P, and US\$0.633 kg⁻¹ K. Farm gate price of paddy rice was used at US\$0.25 kg⁻¹ paddy rice based on the trend of the market price for the past 25 years (Indexmundi, 2020; <https://www.indexmundi.com>).

$$\text{TFC (US\$ ha}^{-1}\text{)} = (\text{pN} \times \text{N}_{\text{rate}}) + (\text{pP} \times \text{P}_{\text{rate}}) + (\text{pK} \times \text{K}_{\text{rate}}) \quad (3)$$

$$\text{Gross return (US\$ ha}^{-1}\text{)} = \text{FGP} \times \text{GY} \quad (4)$$

$$\text{GRF (US\$ ha}^{-1}\text{)} = \text{Gross return} - \text{TFC} \quad (5)$$

Where pN, pP, pK = prices of N, P and K fertilizers, respectively (US\$ kg⁻¹).

N_{rate}, P_{rate}, K_{rate} = amount of N, P, and K applied (kg ha⁻¹).

FGP = farm gate price of paddy rice, maize, or wheat (US\$ kg⁻¹).

GY = grain yield of paddy rice, maize, and wheat (kg ha⁻¹).

Performance of SSNM

We conducted a mini-review using 46 studies (43 from Asia and three from SSA). This shows the paucity of research in SSA, despite the low rice productivity against an increasing rice demand in the region. Our analysis shows that on average, the implementation of SSNM recommendations resulted in 644 kg ha⁻¹ (11.4%) more rice yield compared to the farmer fertilizer practice (FFP; **Table 1**). This was associated with 38.2, 18.2, and 8.6% greater agronomic efficiency of N, PFP N, and gross return above fertilizer cost (GRF; a measure of economic performance) with SSNM compared to FFP, respectively. These benefits accrued while using 14% less N fertilizer than FFP (**Table 1**), similar to observations by Peng et al. (2010). The lower N fertilizer also resulted in an increased GRF by US\$178 ha⁻¹ and a higher agronomic N use efficiency (AEN) under SSNM (17 kg grain kg⁻¹ N) than FFP (12 kg grain kg⁻¹ N applied) and PFP N (58 vs. 47 kg grain kg⁻¹ N). The FFP is often based on blanket recommendations with unbalanced nutrient application in many

cases (Wang et al., 2001; Dobermann et al., 2002; Peng et al., 2010).

Increased N use efficiency in SSNM was attributed to the distribution of N fertilizer applications (i.e., timing, amount, and frequency), resulting in an optimized balance between N supply and crucial stages of crop growth and demand for N. On average, there were 3.5 N-fertilizer splits under SSNM compared to 3.0 under FFP. In addition to reduced N fertilizer, the SSNM approach ensures balanced N, P, and K application contributing to increased N use efficiency. In our review, the largest increases in N use efficiency with SSNM were observed in China, where farmers generally use excessive amounts of N fertilizer (Wang et al., 2001; Peng et al., 2010). Peng et al. (2010) reviewed the performance of SSNM across 107 sites in China conducted over 10 seasons and showed on average, higher N input in FFP (195 kg N ha⁻¹) compared to SSNM (133 kg N ha⁻¹). While that study showed a 5% yield advantage with SSNM, the greater benefits were observed with AEN, which was 61% higher compared to FFP. This suggests significant reduced N losses.

Although most of the reviewed studies were in irrigated lowland ecosystems, where the SSNM approach was developed, Biradar et al. (2006) and Banayo et al. (2018) in India and in the Philippines, respectively, conducted studies under rain-fed ecosystems and reported higher rice yield and GRF under SSNM compared to FFP. However, the higher yield for SSNM in India was achieved with about 1.7 times more N fertilizer than in FFP, resulting in a lower PFP N under SSNM. It is likely that the algorithms of SSNM evaluated a greater N requirement in rain-fed systems but that could be an overestimation since SSNM has not been optimized with limited trials under rain-fed systems. Overestimation could lead to lower PFPN, thus further evaluation and calibration of SSNM under rain-fed environments are needed.

Rice yield, N use efficiencies and GRF responses to SSNM recommendations varied depending on crop establishment methods. Greater benefits from SSNM compared to FFP were observed for transplanted than direct-seeded rice (DSR; **Table 1**). Faced with labor and water scarcity in transplanted systems (Pampolino et al., 2007), farmers are increasingly adopting DSR (Kumar et al., 2018). However, weeds are a major constraint in DSR systems, leading to reduced crop productivity. A key strategy to enhance rice yield under DSR is to apply N late in the season (Liu et al., 2019), this is in line with SSNM which emphasizes the need to time N supply with demand. There are opportunities for improving SSNM recommendations for DSR, encompassing local conditions and weed management.

Traditional tools based on leaf greenness to assess N status (leaf color charts and SPAD or chlorophyll meter) were used in 21 of the studies and increased rice yield, PFP N and GRF by 6.8, 18.9, and 9.1%, respectively, compared to FFP (**Table 1**). On the other hand, the use of SSNM-based digital decision-support tools (RCM, Nutrient Expert, RiceAdvice) increased rice yield by 11.7%, PFP N by 11.5%, and GRF by 11.8%. However, RiceAdvice was only used in one study (AfricaRice, 2016). Digital tools provide pre-season recommendations, and allow for in-season N fertilizer adjustments to improve the performance of the recommendations given by the tools. However, extensive

adoption of SSNM-based digital tools by farmers in the field has been limited by factors like poor access to the tools, non-availability of internet facilities, low penetration of digital devices in rural areas, non-availability of recommended fertilizers, limited credit for buying the fertilizers, labor shortage lack of concentrated efforts by the local extension agents (Florey et al., 2020). Integrating digital tools with geospatial approaches facilitates improved yield targets and in-season N adjustment based on crop performance and enhances scaling of SSNM recommendations (Xu et al., 2017b).

DISCUSSION

Our mini-review shows SSNM as an effective N management strategy for improving rice productivity and profit for farmers while increasing N use efficiency, thus attaining environmental benefits. On average, optimized nutrient management reduced N fertilizer inputs, improved yields, and hence, increased N use efficiency. While N use efficiency has been used to indicate the balance between N used for grain production and losses to the environment (Dobermann, 2007; Omara et al., 2019), only a few studies have quantified N losses under SSNM. For example, a recent study using Nutrient Expert showed both agronomic and environmental benefits of SSNM in a rice–maize cropping systems in China (Wang et al., 2020). In that study, N losses and GHG emissions with SSNM were 10.1 and 6.6% lower than FFP for rice and 46.9 and 37.2% for maize, respectively. The reduced losses arose from increased N use efficiency. Nutrient Expert was also used for winter wheat in North China where N fertilizer rates were 41.4% lower with SSNM than FFP, leading to a 70% increase in agronomic N use efficiency and 55% lower emissions of N_2O (Zhang et al., 2018).

Similarly, using Nutrient Expert in a wheat cropping system in India, GHG emissions were 16–42% lower under SSNM than FFP, both under conventional and no-till, but with greater benefits under no-till (Sapkota et al., 2014). Sapkota et al. (2021) observed a 2.5% reduction in global warming potential associated with reduced GHG emissions, increased rice yields and profit in India when Nutrient Expert was compared to FFP. Earlier studies in the Philippines and Vietnam (Pampolino et al., 2007) and in China (Wang et al., 2007) also showed increased N use efficiency with reduced N losses through leaching, runoff, and N_2O emissions with SSNM compared to FFP. SSNM, hence, provides a climate mitigation nutrient management option compared with the FFP. Considering the wide range of conditions where rice is grown, there is need to evaluate the benefits of SSNM in more locations and using different digital tools. However, adoption of SSNM recommendations has been low, highlighting the need to strengthen extension systems through public and private partnerships.

Currently, the SSNM-based digital tools, including RCM, provide pre-season nutrient management recommendations and focus on balanced application of N, P, and K with little emphasis on micronutrients. On the other hand, farmers generally lack awareness of the importance of micronutrients and there are less obvious and/or immediate yield gains and profit associated with

micronutrient fertilization. This has resulted in mining of these micronutrients from rice soils, while malnutrition, particularly due to zinc and iron deficiency, is common for communities relying on rice-based diets (Palanog et al., 2019). While much of the research on improving micronutrient nutrition in rice have been through breeding (Dixit et al., 2019), there is also need to optimize micronutrient management, along with major nutrients, for the production of healthier rice through agronomic bio-fortification as soil or foliar application (Hakoomat et al., 2014). Given that more iron and zinc is needed during the early growth stages of rice, application in the soil is more practical than foliar application, which requires the plant leaves to have been developed significantly in order to effectively take up the foliar applied nutrients. However, zinc has been shown to convert to unavailable forms immediately after the application of zinc sulfate in flooded soils (Bunquin et al., 2017). Thus, soil application of zinc is not highly effective in flooded rice fields. Although foliar application of zinc at later growth stages of rice does not result in yield gain, it has been shown to increase grain zinc content and is therefore important for the production of healthier high-zinc rice (Hakoomat et al., 2014; Rubianes et al., 2018).

While micronutrient fertilization improves the nutrition value of rice grain, farmers are often not compensated for the extra input costs. They are unwilling to invest in micronutrient management in the absence of other incentives. Policy shifts are needed to reward farmers for the production of more nutritious and healthier rice; some of them necessarily require active public–private partnerships. For example, the Sustainable Rice Platform, which is promoting a premium price for sustainably produced rice (SRP, 2019). Such efforts can foster environmental sustainability, ensuring that rice systems in the Global South deliver essential ecosystem services while also improving farmers' livelihoods. The changing climate and other driving forces like shortage of labor and water dictate shifts from continuous intensive rice systems to changes in agronomic management practices, such as direct seeded rice, non-puddled rice, and water saving technologies. Increasing rice productivity while minimizing adverse environmental consequences require the adoption of integrated nutrient and crop management practices that increase system-level efficiency.

CONCLUSION

Our mini-review clearly shows that SSNM in rice cropping systems increases rice yield, profit, and N use efficiency while reducing N losses and GHG emissions when compared with the farmer practice. AEN and PFP were 38.2 and 18.2% greater with SSNM than the farmer practice. This was achieved using 14% lower N fertilizer. The superior performance of SSNM compared to the farmer practice is mainly due to better distribution with more splits of N fertilizer during the growing season coupled with balanced fertilization. However, SSNM has mainly focused on the major nutrients, ignoring micronutrients, and thus, impacting human nutrition for those whose diets are rice-based, while potentially mining the soils of the micronutrients. SSNM-based

digital decision support tools enable dissemination of SSNM recommendations at scale, but this requires a pluralistic approach that fosters collaboration among multiple organizations and service providers with support from governments. Additionally, linking the digital tools with GIS and remote sensing tools allows fine-tuning of SSNM recommendations, addressing the huge spatial variability in smallholder farming systems. SSNM research and evaluation has focused on favorable environments in Asia, despite the increasing demand and production in Africa. More research is needed on SSNM under diverse management practices, such as direct seeding, and in marginal environments along with quantification of nutrient losses, along with inter-disciplinary approaches to enhance farmer uptake of SSNM.

AUTHOR CONTRIBUTIONS

PC, SS, and JH conceived the project. MB extracted data from publications. PC and MB conducted analyses. PC wrote

the manuscript draft. All authors contributed to the literature search, interpretation of the results, and writing of the final paper.

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Contribution, Utilization, and Improvement of Legumes-Driven Biological Nitrogen Fixation in Agricultural Systems

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Legumes improve soil fertility through the symbiotic association with microorganisms, such as rhizobia, which fix the atmospheric nitrogen and make nitrogen available to the host and other crops by a process known as biological nitrogen fixation (BNF). Legumes included in the cropping system improve the fertility of the soil and the yield of crops. The advantages of legumes in the cropping system are explained in terms of direct nitrogen transfer, residual fixed nitrogen, nutrient availability and uptake, effect on soil properties, breaking of pests' cycles, and enhancement of other soil microbial activity. The best benefits from the legumes and BNF system can be utilized by integrating them into cropping systems. The most common practices to integrate legumes and their associated BNF into agricultural systems are crop rotation, simultaneous intercropping, improved fallows, green manuring, and alley cropping. However, the level of utilizing nitrogen fixation requires improvement of the systems, such as selecting appropriate legume genotypes, inoculation with effective rhizobia, and the use of appropriate agronomic practices and cropping systems. Therefore, using legumes at their maximum genetic potential, inoculation of legumes with compatible rhizobia, and using appropriate agronomic practices and cropping systems are very important for increasing food production. Importantly, the utilization of legumes as an integral component of agricultural practice in promoting agricultural productivity has gained more traction in meeting the demand of food production of the world populace. Priority should, thus, be given to value the process of BNF through more sustainable technologies and expansion of knowledge to the system.

Keywords: biological nitrogen fixation, cropping system, inoculation, legume residue, nutrient transfer

INTRODUCTION

Most soils are facing a decline in soil nutrient status, which is a basic limitation to food production (Sanginga et al., 2003). It was projected, in Africa, that the yearly net nutrient depletion exceeds 30 kg/ha of N and 20 kg/ha of K for arable land in Ethiopia, Kenya, Malawi, Rwanda, and Zimbabwe (Smaling, 1993). The replenishment and enhancement of soil fertility are, thus, progressively regarded as serious to the practice of alleviating poverty. Additionally, developing countries experience the demand of more costs for synthetic fertilizer utilization although their use has adverse and unpredictable problems in the environment: mainly soil, water, and natural area contamination. Consequently, legumes increase soil fertility through the action

of microorganisms, which are imperative to affect the soil properties, including soil biological, chemical, and physical properties (Stagnari et al., 2017; Nanganoa et al., 2019; Vasconcelos et al., 2020). The increasing attention of low-input crop production and sustainable agricultural systems from an environmental, economic, and managerial standpoint opened the door for sustained adoption and inclusion of legumes in the agricultural systems (Ghosh et al., 2007; Stagnari et al., 2017; Kebede, 2020b). This is because of the contribution of legumes through their nitrogen (N_2)-fixing capabilities and ability to restore soil fertility and break the cycles of diseases and other pests attacking crops (Kebede, 2020a).

The total nitrogen fixation in the world is estimated to be about 1.75×10^{11} Kg, of which symbiotic nitrogen fixation in legumes accounts for about 8.0×10^{10} Kg by fixing, on average, 20–200 kg N fixed ha^{-1} year $^{-1}$, and the other near half is industrially fixed while producing N fertilizers (about 8.8×10^{10} Kg) (Shah et al., 2021). Biological nitrogen fixation (BNF) through rhizobia–legume symbiosis is, thus, the best alternative and a more sustainable process by a group of symbiotic bacteria, so-called rhizobia, which fix the atmospheric N_2 and make the fixed nutrient available to the host legume and other crops in the cropping system (Stagnari et al., 2017). Increments in crop yield following nitrogen-fixing legumes arises from the role of this system in modifying the activity of soil organisms; the chemical or physical characteristics of the soil; and/or through breaking the cycles of diseases, insects, and other pests (Wani et al., 1995). The use of legumes in agricultural systems and utilization of associated BNF systems provides economically feasible and environmentally sound ways of decreasing external inputs and improving the soil nutrient content and, hence, can be suggested for the nutrition of sustainable agriculture (Peoples et al., 1995; Postgate, 1998; Kebede, 2020a).

Full utilization of BNF and maximal benefit from BNF systems can be recognized through integrating legumes into agricultural systems in which the benefits of BNF can be extended to crops and cropping systems (Fujita et al., 1992; Stagnari et al., 2017; Kebede, 2020a). The well-known agricultural systems of integrating legumes into cropping systems include crop rotation, simultaneous intercropping, improved fallows, green manuring, and alley cropping (Ghosh et al., 2007; Meena et al., 2018; Nanganoa et al., 2019; Kebede, 2020a; Lengwati et al., 2020). Wider legume technology adoption and utilization, including methods that improve the BNF system and integrate it into agricultural cropping systems are imperative to enhance agricultural production. Consequently, an increment in the level of nitrogen fixed could be attained by adopting management practices that influence BNF in agricultural production systems, such as selecting legume genotypes, inoculating with effective rhizobia, and the use of good agronomic practices and cropping systems (Wani et al., 1995; Montañez, 2000; Vanlauwe et al., 2019). Therefore, improvement and utilization of BNF are very important, particularly in the developing world, where much of the increases in food production must come to accommodate the increasing world population. Hence, this paper aims to review the contribution, utilization, and improvement of legumes-based BNF in agricultural systems.

TABLE 1 | BNF capacity ($kg\ ha^{-1}$) of commonly cultivated legumes.

Common name	Scientific name	BNF ($kg\ ha^{-1}$)	Cultivation area
Fava bean	<i>Vicia faba</i> L.	118.6–311	Greece, Italy
Pea	<i>Pisum sativum</i> L.	36.6–125.3	Canada, Greece
Common vetch	<i>Vicia sativa</i> L.	107–131	Switzerland
Grass pea	<i>Lathyrus sativus</i> L.	101–149	Switzerland
White lupin	<i>Lupinus albus</i> L.	53.1–64.1	Italy
Chickpea	<i>Cicer arietinum</i> L.	21.0–103.6	Canada
Lentil	<i>Lens culinaris</i> Med.	23.0–86.8	Switzerland, Canada
Common bean	<i>Phaseolus vulgaris</i> L.	16.3–71.9	Canada
Cowpea	<i>Vigna unguiculata</i> (L.) Walp.	36–75	Brazil
Soybean	<i>Glycine max</i> (L.) Merr.	90–95	United States
Alfalfa	<i>Medicago sativa</i> L.	103–209	Canada, China
Egyptian clover	<i>Trifolium alexandrinum</i> L.	35–59	Switzerland
Red clover	<i>Trifolium pretense</i> L.	35.4–389	Denmark, United States

Adapted from Vasconcelos et al. (2020).

BNF IN CROPPING SYSTEMS

BNF enables legume crops to rely upon atmospheric nitrogen, which is essential in legume-based agricultural systems in which nitrogen fertilizers are limited as legumes integrated within different cropping systems increase the fertility of the soil (Ghosh et al., 2007; Meena et al., 2018; Lengwati et al., 2020). Legume crops, such as common bean, cowpea, soybean, lablab, and groundnut, are important hosts for rhizobia to perform BNF. In addition to providing the fixed nitrogen in the cropping system, legumes also aid in solubilizing unsolvable phosphorus (P) in the soil, increasing soil microbial activity, ameliorating the soil physical environment, restoring organic matter, and smothering weeds (Giller, 2001; Stagnari et al., 2017). BNF by legumes (e.g., fava bean, lentil, pea, chickpea, alfalfa, red clover, etc.) ranges from 21 to 389 $kg\ ha^{-1}$ (Vasconcelos et al., 2020). Stagnari et al. (2017) indicated that the magnitude of BNF and associated contribution varies across legume species, soil properties, climatic conditions, and cropping systems (i.e., monoculture, mixed culture, crop rotations, etc.) as well as soil management strategies. The BNF capability of commonly cultivated legumes across the world is shown in Table 1, and the tables indicated that BNF capability varied across legume species and locations.

Furthermore, soil fertility can be restored with nitrogen obtained from legume residue decomposition, which mainly depends on how their residues are exploited (whether incorporated, which is of more benefit; totally removed from the field; or burned) (Ghosh et al., 2007; Thilakarathna et al., 2016). Legume species commonly used for grain production and green manure can fix nitrogen ranging from 100 to 300 $kg\ ha^{-1}$ from the atmosphere (Fujita et al., 1992). In a normal ecosystem, legumes can fix nitrogen in the range of 11.34–34.02 kg of nitrogen per acre per year. In cropping systems, for example, perennial legume crops, such as alfalfa, sweet

clovers, true clovers, and vetch, can fix up to 250–500 lb of N per acre per year (Walley et al., 1996). Similarly, legumes such as cowpeas, peanuts, fava beans, and soybeans can fix up to 113.4 Kg nitrogen ha⁻¹. The use of these legumes in a cropping system, including rotation, intercropping, green manure, and legume-enriched pastures, has significant advantages over sole cropping systems in terms of fertilizer use and, hence, emissions of the greenhouse gases CO₂ and N₂O (Peoples et al., 1995; Kebede, 2020a). Stagnari et al. (2017) considered legumes to be competitive crops in terms of both environmental and socioeconomic benefits with the potential to be included in modern agricultural systems, which are characterized by a reducing crop diversity and excessive use of fertilizers and agrochemical inputs. Particularly, the positive contributions of legumes to the agricultural system mainly arise from legume-specific traits of fixing atmospheric nitrogen into nitrogen-rich organic compounds and indirectly from their reduced reliance on agronomic inputs (Vasconcelos et al., 2020). Besides this, the legume-based nitrogen fixation is of importance to the cropping systems as it is used by the N₂ fixing and nonfixing crops growing nearby as the benefits can be supplemented from the fixing plants (Vanlauwe et al., 2019; Kebede, 2020a,b; Shah et al., 2021).

MECHANISMS OF CONTRIBUTION OF LEGUMES AND BNF IN CROPPING SYSTEM

Direct Nutrients Transfer

Legumes in the mixed agricultural system can lead to more efficient use of soil nutrients and the significant release and transfer of fixed N during the cereal phase, which also results in an improved N yield of the mixed cereal and the overall mixture (Louarn et al., 2015). Direct nutrient transfer is the transfer of nutrients from a nitrogen-fixing legume to another crop during the growth of the crop in an intercrop association with a legume's component and/or as remaining nitrogen for the advantage of a subsequent crop. Thilakarathna et al. (2016) stated that nutrient transfer in a legume-based BNF system is the transfer of nutrients from a donor plant (legume) to receiver plants (cereals) either without undergoing mineralization or through mineralization followed by the uptake of nutrients by the receiver plants. In many instances, it is predictable that an amount of the fixed nitrogen by the intercropped legume crop is made accessible to the accompanying non-legume during the cropping season, and the direct transfer of fixed nitrogen from legumes to a companion crop happens in a mixed cropping system (Fujita et al., 1992). Confirmation of nutrient transfer from legumes to cereals is achieved in intercropping and rotation studies through the excretion of the root, nutrients leached from plant leaves, and leaf fall (Yusuf et al., 2009). The main ways of nutrient transfers can be grouped into above- and below-ground nutrient transfer. According to Thilakarathna et al. (2016), nitrogen is obtained from decayed legume nodules, roots, root border cells, root caps, sloughed cells, and the epidermis (water-insoluble resources), which more

importantly donate to the below-ground nutrient, primarily nitrogen, transfers.

When examining various mechanisms of nitrogen transfers, nodules, and root decomposition are more significant but can differ significantly by legume species. It is projected that nitrogen ranging from 3 to 102 kg ha⁻¹ yr⁻¹ can be transferred through the decomposition of nodules and roots in legumes, which is equal to 2–26% of the biologically fixed nitrogen in legumes (Thilakarathna et al., 2016). The below-ground nutrient transfer comprises three different pathways, i.e., decomposition, root exudation, and mycorrhizae-mediated nutrient transfer and is shown in **Figure 1**.

The transfer of biologically fixed nitrogen to neighboring and/or succeeding crop plants is highly variable and can range from as low as 0% to as high as 73%, depending on various factors (Islam and Adjesiwor, 2017), which can be the highest reported levels of 75–110 kg N ha⁻¹ yr⁻¹ (Louarn et al., 2015). A common practice in agricultural systems for utilizing nutrient transfer from BNF is intercropping N₂-fixing legumes with non-N₂-fixing crops. A study by Eaglesham et al. (1981) revealed that 24.9% of nitrogen fixed by cowpea can be transferred to intercropped maize. Up to 35% of N in maize grown after pigeon pea was revealed by isotope dilution to be from N fixation, and part of the fixed N was from below-ground parts. Similarly, Osunde et al. (2004) revealed that the quantity of nitrogen derived from N₂ fixation is 40% in the intercropped soybean and 30% in the sole crop without the addition of fertilizer. Furthermore, Mandimba (1995) reported that the N input of groundnut to the growth and yield of maize in an intercropping system is equal to the fertilization of 96 kg of N/ha at a proportion of plant population densities of individual maize plants to four groundnut plants. The estimates of the amount of plant phosphorus and nitrogen derived from symbiotic N₂ fixation for frequently produced legume crops in tropical and subtropical agricultural systems are shown in **Table 2**.

The degree of nitrogen transfer depends upon the quantity and concentration of legume N, microbial mineralization and immobilization in the rhizosphere, the availability of other N sources, and the degree of utilization by the associated crop (Peoples and Craswell, 1992; Thilakarathna et al., 2016; Islam and Adjesiwor, 2017). The amount of N available and the pathway of transfer are also seen to depend on the legume species (Wolfe and Cormack, 2002). For instance, Dubach and Russelle (1994) measured 13 kg/ha of symbiotically fixed N could be released from decomposing alfalfa roots but only 2 kg/ha of fixed N from decomposing three-leaved plant roots. By comparison, the three-leaved plant was seen to have further root nodules, estimated to provide 6 kg/ha N to the top 30 cm soil as compared with only 2 kg/ha from alfalfa nodules. The quantities of nitrogen fixed and the amount transferred to the soil and/or subsequent crops in different agricultural systems are indicated in **Table 3**.

Decomposition of nodules and roots of legumes are also believed to be significant in the transfer of nitrogen (Thilakarathna et al., 2016). Though these parts, generally, have only a portion of the entire plant's nutrients, the amount of the plant root system, which might be decaying during plant growth, has not been estimated yet. Moreover, the likelihood of nitrogen

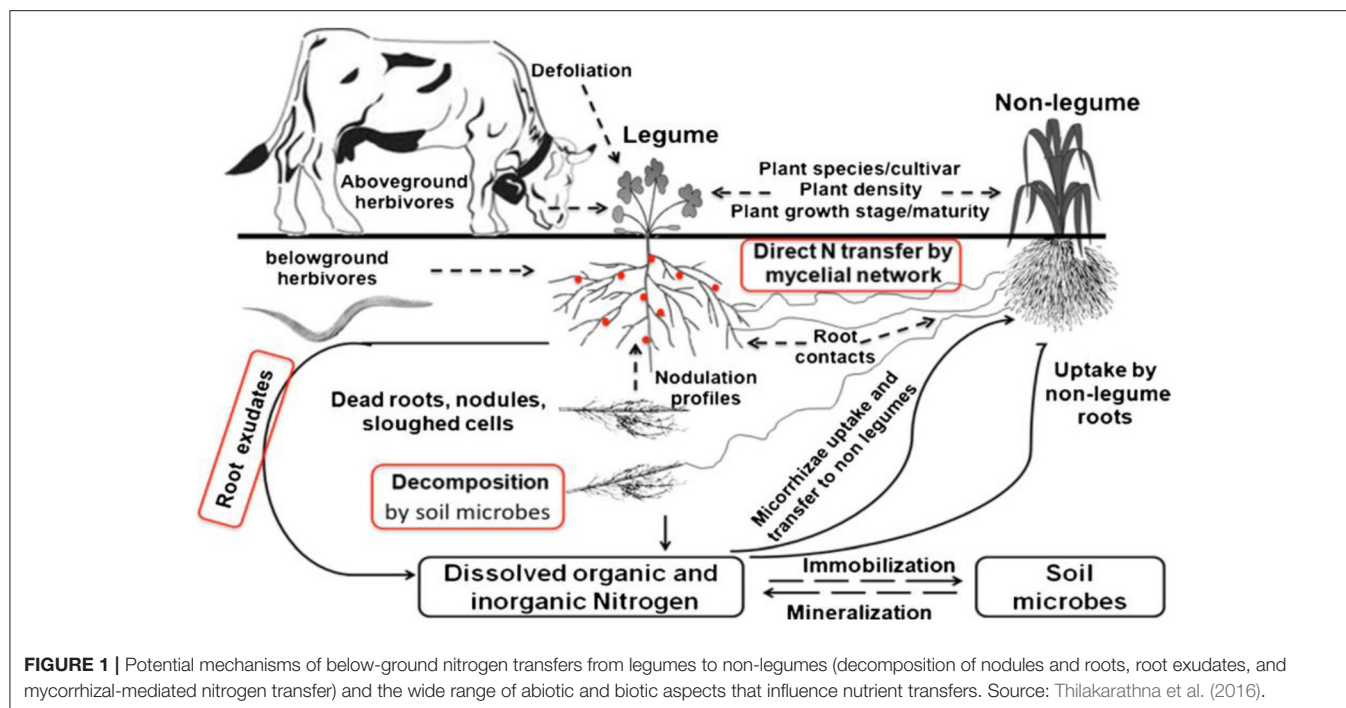


TABLE 2 | The proportion of phosphorus and amount of plant nitrogen attained from symbiotic N_2 fixation of grain legumes frequently produced in tropical and subtropical systems as adapted from Peoples and Craswell (1992).

Species	Location	Treatment variable	Total crop N (Kg N ha ⁻¹)	N ₂ fixed	
				P (kg ha ⁻¹ crop ⁻¹)	Amount (kg N ha ⁻¹ crop ⁻¹)
Groundnut	Australia	Water supply	171–248	0.22–0.53	37–131
		Cultivar	254–319	0.55–0.65	139–206
		Rotation	181–247	0.47–0.53	85–131
	Brazil	Inoculation	147–163	0.47–0.78	68–116
Pigeon pea	India	Cultivar	126–165	0.86–0.92	109–152
		Season	77–92	0.88	68–88
Soybean	Brazil	Site/season	112–206	0.70–0.80	85–154
	Hawaii	Temperature	120–295	0.97–0.80	117–237
	Indonesia	Rotation	79–100	0.33	26–33
		Cultivar	33–65	0.78–0.87	26–57
	Thailand	Cultivar	121–643	0.14–0.711	17–450
Common bean	Brazil	Water supply	157–251	0–0.45	0–113
		Cultivar	18–71	0.16–0.71	3–32
		Phosphorus	128–183	0.16–0.32	17–57
Cowpea	Brazil	Site/season	25–69	0.32–0.70	9–51
	Indonesia	Rotation	67–100	0.12–0.33	12–22
		Phosphorus	92–94	0.26–0.35	24–39
Green gram	Thailand	Cultivar	71–74	0.89–0.90	64–66
Black gram	Thailand	Cultivar	125–143	0.95–0.98	119–140

released from the living roots of legumes can also contribute to the transfer of nitrogen (Fujita et al., 1992). On the other hand, the transfer of nutrients to companion non-legume crops within the cropping system and growing season depends on the legume species, the amounts of the plant components in the

stand, the comparative maturities of the accompanying crops, and the vigor and period of plant growth (Thilakarathna et al., 2016). Soil-related factors also influence the efficiency of nutrient transfer. The estimates of probable N_2 fixation are also unlike in legumes and under various farming systems. The levels of

TABLE 3 | The quantities of nitrogen fixed and the amount transferred to soil or subsequent plants in different agricultural systems as taken from Islam and Adjesiwor (2017).

Crop(s)	Amount of N transferred	% of fixed nitrogen
Caragana (<i>Caragana arborescens</i> Lam.)–oat (<i>Avena sativa</i> L.)	38–45 kg N ha ⁻¹	60–70
Alfalfa-tall fescue (<i>Schedonorus arundinaceus</i> (Schreb.) Dumort.)	0–650 kg N ha ⁻¹	0–12
White clover-perennial ryegrass	0–340 kg N ha ⁻¹	0–47
Mung bean-oat	12.8 mg N plant ⁻¹	9.7
Soybean-maize	7.84 mg N pot ⁻¹	7.57
Soybean-maize	10.77–13.72 mg N pot ⁻¹	1.26–2.17
Faba bean-wheat	0.17 mg N plant shoot ⁻¹	14.9
Red clover-bluegrass (<i>Poa pratensis</i> L.)	35.85 mg N plant ⁻¹	1.5
Pigeon pea (<i>Cajanus cajan</i> (L.) Mill sp.–coffee (<i>Coffea arabica</i> L.)	21.8 g N kg ⁻¹	na
Crotalaria-coffee	13.5 g N kg ⁻¹	na
Velvet bean (<i>Mucuna pruriens</i> (L.) DC.)–coffee	19.7 g N kg ⁻¹	na
Red clover-perennial ryegrass and forbs	25–58 kg N ha ⁻¹	9.5–15

na, could not be estimated from data.

nitrogen fixation and amount of nutrient transfer are also reliant on soil moisture and water supply, rhizobia inoculation, cropping system, nitrogen fertilizer applications, and soil fertility status (Peoples and Craswell, 1992).

Contribution of Legume Residues

Part of the symbiotically fixed nitrogen in legumes is available to succeeding crops through the decomposition and mineralization of the legume residues (Thilakarathna et al., 2016; Islam and Adjesiwor, 2017). The residues of legumes can be a source of more mineral nitrogen to succeeding crops than the residues of cereal due to their relatively high nitrogen contents and relatively low C:N ratio in legume residue as compared with cereal residues. Nutrients derived from decomposed roots, nodules, root caps, root border cells, sloughed cells, and the epidermis (water-insoluble materials) significantly contribute to below-ground nutrient transfer (Louarn et al., 2015). Cereals cultivated in sequence with legume crops obtain nitrogen benefits as compared with cereal monoculture. The utilization of legume crops in various cropping systems can cause a significant and progressive yield increment on subsequent non-legume crops as compared with rotations with non-legume crops (Kebede, 2020a). Many factors are assumed to explain these results, including enriched nitrogen availability following the legume and other rotational effects, such as decrease of disease and other pests and higher mycorrhizal colonization rate and diversity

in the soils. Murphy-Bokern et al. (2017) stated that the nitrogen-rich root, shoot, and leaf biomass of legumes, which is enabled by BNF, improves the availability of N to neighboring or succeeding nonlegume crop plants as exudates, and living and senescent biomasses provide additional below-ground N-enriched input to the soil. However, reliable estimates of nitrogen fixation and residual soil nitrogen are required to determine nitrogen contribution of legume to subsequent or associated crops (Adeleke and Haruna, 2012).

Nutrients derived from decomposed roots, nodules, root caps, root border cells, sloughed cells, and the epidermis (water-insoluble materials) significantly contributes to below-ground nutrient transfer (Louarn et al., 2015). Cropping systems, such as crop rotation comprising legumes, can decrease the amount of nitrogen fertilizer applied in succeeding crops. According to Mayer et al. (2003), nitrogen derived from legume rhizodeposits contribute to an increase of 35–44% in residual nitrogen content in the soil and constituted 79–85% of the below-ground nitrogen of plants at the maturity stage. Besides this, Jensen (1996) revealed that 47% of the whole below-ground nitrogen derived from plants can be acquired from legume root depositions. Moreover, enhanced BNF through the use of rhizobia inoculants helps in improving available nitrogen and phosphorus in the soil once the crop is harvested, and it can be utilized by the next crop (Matse et al., 2020).

Several reports reveal that BNF can be improved by inoculating host legumes with compatible rhizobia (Wani et al., 1995; Zahran, 1999; Adeleke and Haruna, 2012; Bhowmik and Das, 2018). The improved nitrogen fixation can leave crop residual nitrogen in the soil, and it contributes to organic matter and can be a source of inexpensive nutrients for the next cropping season. In food legumes, the nitrogen is divided into either the harvested seed or the non-harvested vegetative parts of the crops, such as nodulated roots, stems, and leaves, which usually stay as crop residues. The leaf falls throughout legume crop growth in the nodulated roots are also testified to comprise up to 40 kg N ha⁻¹ (Buresh and De Datta, 1991). Ghosh et al. (2007) stated that legumes, such as soybean, pigeon pea, cowpea, and groundnut, cultivated as an intercrop in maize had a positive residual contribution on the yield of the following wheat crop. The use of legume crops in the cropping system and utilization of BNF is, therefore, considered to have soil fertility enhancement and economic benefits (Ndakidemi et al., 2006).

During the harvest of legume seeds and residue incorporation in the soil, the entire nutrient found in the plant is unequally dispersed between the various vegetative parts (Louarn et al., 2015; Thilakarathna et al., 2016). These parts also vary from each other in the level to which they release mineral nutrients to the soil and, eventually, to the subsequent crops. The nutrient alterations happening during decomposition of crop residues are influenced by residue management, temperature, soil physical and chemical properties, and whether the soils are flooded and/or remain aerobic (Buresh and De Datta, 1991). However, decomposition of the residues may be improved by lignin and polyphenol content even though tissue nitrogen concentration, C:N ratio, and soil water status are the principal aspects affecting the rate of mineralization and obtainability of legume residues

to succeeding crops (Peoples and Herridge, 1990). When legume green manure is grown and intended to be used in the agricultural system, however, all plant parts are returned to the soil. In this case, the number of nutrients left in the soil and nutrient concentration in the legume's parts returned to the soil are expected to be greater than when the seed is harvested from food legumes. As a result, decomposition might also be anticipated to be quick, and the proportion of legume nitrogen mineralized is high (Peoples and Herridge, 1990). According to Beri et al. (1989), a considerable increment in yield of cereals following legume green manuring could range from 50 to 120 kg ha⁻¹ of nitrogen fertilizer. Therefore, legume green manuring can offer substantial quantities of nutrients to the soil that can significantly benefit succeeding crops.

Nutrient Availability and Uptake

Legumes-based cropping systems improve several aspects of soil fertility, including soil organic carbon and humus content, N and P availability and uptake, and organic C and N as well as releasing hydrogen gas as a by-product of BNF, which promotes bacterial legume nodules' development in the rhizosphere (Stagnari et al., 2017). Plant nutrient availability and uptake are dependent mainly on the amount, concentrations, and activities taking place in the root zone of the soils along with the ability of the soils to replace the limiting nutrients in the soil's solution (Makoi et al., 2013). Nitrogen and phosphorus are the principal elements that are abundantly available in the soil and atmosphere, respectively, but found in forms inaccessible to plants. These elemental nutrients are usually the limiting factors for the growth and development of plants (Mmbaga et al., 2014). Stagnari et al. (2017) divided the agricultural contribution of grain legumes in two as (1) the "nitrogen effect" component, which is due to the nutrient provision from BNF, which is highest in situations of low N fertilization to subsequent crop cycles, and (2) the "break crop effect" component, which includes nonlegume-specific benefits, such as improvements of soil organic matter and structure, phosphorus mobilization, soil water retention and availability, and reduced pressure from diseases and weeds. Hence, the contributions of legumes are highest in legumes included in diverse cropping systems.

Legumes-based BNF has a positive effect on the availability and real chemistry of soil nutrients, which, thus, stimulate the availability and uptake of plant nutrients. For instance, Fujita et al. (1992) showed that the maximum wheat nitrogen uptake was obtained when the crop is cropped following a maize-soybean or maize-groundnut intercrop system than after lone maize cropping. Besides this, microbial inoculants, especially rhizobia species, have come to be a promising solution to some of the problems associated with intensive agriculture by enhancing nutrient availability and uptake and eventually enhanced yield. Makoi et al. (2013) revealed improvement in the uptake of N and P following inoculation of different legumes with effective strains of rhizobia. Nyoki and Ndakidemi (2014) found that *Bradyrhizobium japonicum* inoculation complemented with phosphorus application in cowpea enhanced the uptake of nutrients such as N and P. Similarly, Desta et al. (2015) indicated that the inoculation of legume crops can improve nodulation

capacity and nitrogen fixation, which, in turn, enhance nutrient uptake of fava bean.

Legume species also have mechanisms to solubilize and recover phosphorus from unavailable forms in association with rhizobia. For instance, Stagnari et al. (2017) reported an increase in P availability at the rhizosphere level in the intercropping system than in sole cropping. One mechanism is the organic acid exudation from the root legumes, which reduces the pH in the soil neighboring the roots and solubilizes and discharges phosphorus. The second mechanism is the release of phosphatase enzymes into the soil, which can decompose organic material containing phosphorus. The third mechanism is an interaction reaction between the root surface of the legume and the insoluble phosphorus surrounding the plant roots (Ae and Shen, 2002). However, the degree of the contribution of legumes in phosphorus acquisition to the cropping system is greatly reliant on the type of soil and existing soil atmosphere. More broadly, Peoples and Craswell (1992) described the benefits of legumes in the agricultural system in the following ways:

- i. Improvements in soil structure following legumes or improvements in soil water-holding and buffering capacity and increased nutrient availability associated with incorporation of legume residues.
- ii. Breaking of cycles of crop pests and diseases and phytotoxic and allelopathic effects of different crop residues.
- iii. Improvement of soil microbial activity and probably heterotrophic nitrogen fixation following the addition of legume residues.
- iv. Additional residual nitrate found in the soil resulting from a legume rather than after a cereal or other non-legume, which might be associated with fewer nitrates being taken up by the legume or result from stimulation of mineralization rates under a legume cropping system.

METHODS OF BNF UTILIZATION IN CROPPING SYSTEM

Legumes are considered as competitive crops in terms of both environmental and socioeconomic benefits with the potential to be included in modern agricultural systems, which are characterized by a reduced crop diversity and excessive use of fertilizers and agrochemical inputs (Stagnari et al., 2017). They play fundamental roles at the production system level due to their ability to fix atmospheric nitrogen, making them potentially highly suitable for inclusion in low-input agricultural systems as well as at cropping-system levels as diversification crops in agroecosystems, breaking the cycles of pests and diseases, and balancing the soil nutrient deficit in the agricultural system. Full utilization of legumes-driven BNF and maximal benefit from these systems can be recognized through investigation and resolution of the most important limitations to the ideal performance in the field, their adoption, and practices by farmers (Fujita et al., 1992). There are practices utilized to integrate the BNF benefits into agricultural systems in which the benefits of BNF can be extended to crops and cropping systems. The most common agricultural systems of integrating legumes

into cropping systems include crop rotation, simultaneous intercropping, improved fallows, green manuring, and alley cropping. These cropping systems are used worldwide to exploit the nitrogen-fixing symbiotic relationship, and they can be applied in separation or hybrid mixtures. However, the selection of an appropriate cropping system is only one management step; i.e., pairing the system with a nitrogen-fixing symbiotic relationship adds dimension to the decision-making process.

Crop Rotations

Crop rotation is the oldest agricultural practice that comprises the long-term, everlasting farming of well-ordered sequences of crops on the same land. Crops are planted in accordance with their nutrient needs in which deep-rooted plants are followed by their shallow-rooted counterparts to allow nutrients to restore at various depths. Legumes are usually handled as components of crop rotations to optimize the management of pests, weeds, and diseases and to exploit nutrient availability through the soil profile (Murphy-Bokern et al., 2017). For example, deep-rooted legume crops, such as the common bean, cowpea, and pigeon pea can access nutrients below the cereal rhizosphere (1–3 m) rather than cereal crops. The incorporation of these legumes into crop rotations has proven to increase the yields of subsequent crops (Peoples et al., 2009). For example, Sanginga et al. (2003) described that the real proportions of nitrogen fixed by soybeans and the residual nitrogen contributions to the succeeding cereal crops in a crop rotation system range from 38 to 126 kg N ha⁻¹. According to Lengwati et al. (2020), nitrogen contribution obtained from Bambara groundnut, groundnut, mung bean, black gram, and cowpea is 83, 67, 39, 36, and 32 kg ha⁻¹, respectively. The fixed nitrogen benefit of these five legume crops to the subsequent maize crop, which is assessed using grain yield, shoot biomass, and total biomass as compared with monocropping and zero nitrogen plots is shown in **Figure 2**.

Legume rotations are widely recognized to improve soil structure, permeability, microbial activity, water storage capacity, organic matter content, and resistance to erosion, thereby increasing crop yields and sustainability of the agricultural systems (Murphy-Bokern et al., 2017). Therefore, the addition of legume crops in crop rotation systems by smallholder farmers is a profitable and ecologically sound way of improving the nutrition of both the legume and subsequent crops (Kebede, 2020a). Consequently, the net outcome is an increment in the yields of crops and soil nutrient uptake and availability, thus reducing the use of synthetic fertilizers. For instance, a comparison of the grain yields from zero nitrogen application in maize planted subsequently after different legumes with grain yields of maize under sole cropping receiving nitrogen fertilizer indicate that the symbiotic nitrogen contribution of the legumes to the maize was about 20 kg N ha⁻¹ for legumes, such as groundnut, black gram, cowpea, mung bean, and Bambara groundnut (Lengwati et al., 2020).

Simultaneous Cropping

Mixed and relay cropping are two practices that involve simultaneous cropping of two or more crops. Mixed cropping is the same as intercropping; however, it does not include a precise

geometric pattern, such as rows. Relay cropping is a cropping system that involves establishing the second crop directly onto the first crop before harvesting, thus, permitting the cultivation of two crops during the same year. In a mixed cropping system, the action of rhizodeposition advances the nitrogen uptake in the companion crop (Fustec et al., 2010). For instance, intercropping of legumes and cereals provides an opportunity to increase the input of fixed N into an agricultural system in both the short term through direct N transference, and the long term through mineralization of residues (Murphy-Bokern et al., 2017). Yield increment and other benefits from simultaneous cropping as compared with monocropping are usually accredited to joint balancing effects of the component crops, such as improved total utilization of existing nutrients and resources (Fujita et al., 1992). Ghosh et al. (2007) stated that the yield of sorghum in sorghum–legume intercropping is by far greater than that of the maximum yield of sorghum in sole cropping systems (**Figure 3**). The authors ascribed the possible reason to improved growth and enhanced uptake of N, P, and K by sorghum along with effective weed oppressing by the legume's intercrops. Murphy-Bokern et al. (2017) also revealed that intercropping has positive effects on phosphorus availability and uptake as phosphorus can be released into the soil solution in the form of phosphate ions and become available for plant uptake.

Improved Legume Fallows

Improved fallows involve planting a beneficial legume crop during the fallow period to restore soil fertility to enhance subsequent crop production. It is the planting of rapid-growing legume tree/shrub species as a replacement to normal fallow to attain the advantages of the latter in a smaller time. In legume tree fallows, the wood can be harvested, and N-rich components (leaves, pods, and green stems) tend to be incorporated into the soil before the rainy season (Sanchez, 1999). The nutrient supply, such as nitrogen, is greater in improved fallow than in cropped land as the plants store nutrients from the air and bottomless layers of the soil, and falls their leaf litter to improve the soil and preserve moisture (Lemage and Tsegaye, 2020). Nanganoa et al. (2019) indicated that different legume fallow systems had variable effects on the soil physicochemical properties of which the maximum distinguished effect is the total nitrogen content (ranging from 0.19 to 0.24%), organic carbon (SOC) content (ranging from 2.77 to 3.36%), and available phosphorus (ranging from 12.50 to 16.12 mg/kg) (**Table 4**).

In particular, the off-season farming of improved legume fallows and their succeeding incorporation as green manure is vital to improve soil production and productivity by adding nutrients such as N and organic C and by smothering the weeds (Lemage and Tsegaye, 2020). Nair et al. (1999) observed that 1-year *Sesbania sesban* fallow increased the yields of succeeding maize crops by 50–80%, and 2-year fallows showed yield increases of 150–270%. The residual benefits of different legume fallows were observed for 4 years after fallowing, and yields were three times greater than monocropped maize. Lemage and Tsegaye (2020) showed that abandoned agricultural land can be rehabilitated using improved legume fallow, which can enhance soil pH, organic carbon, available phosphorus, available

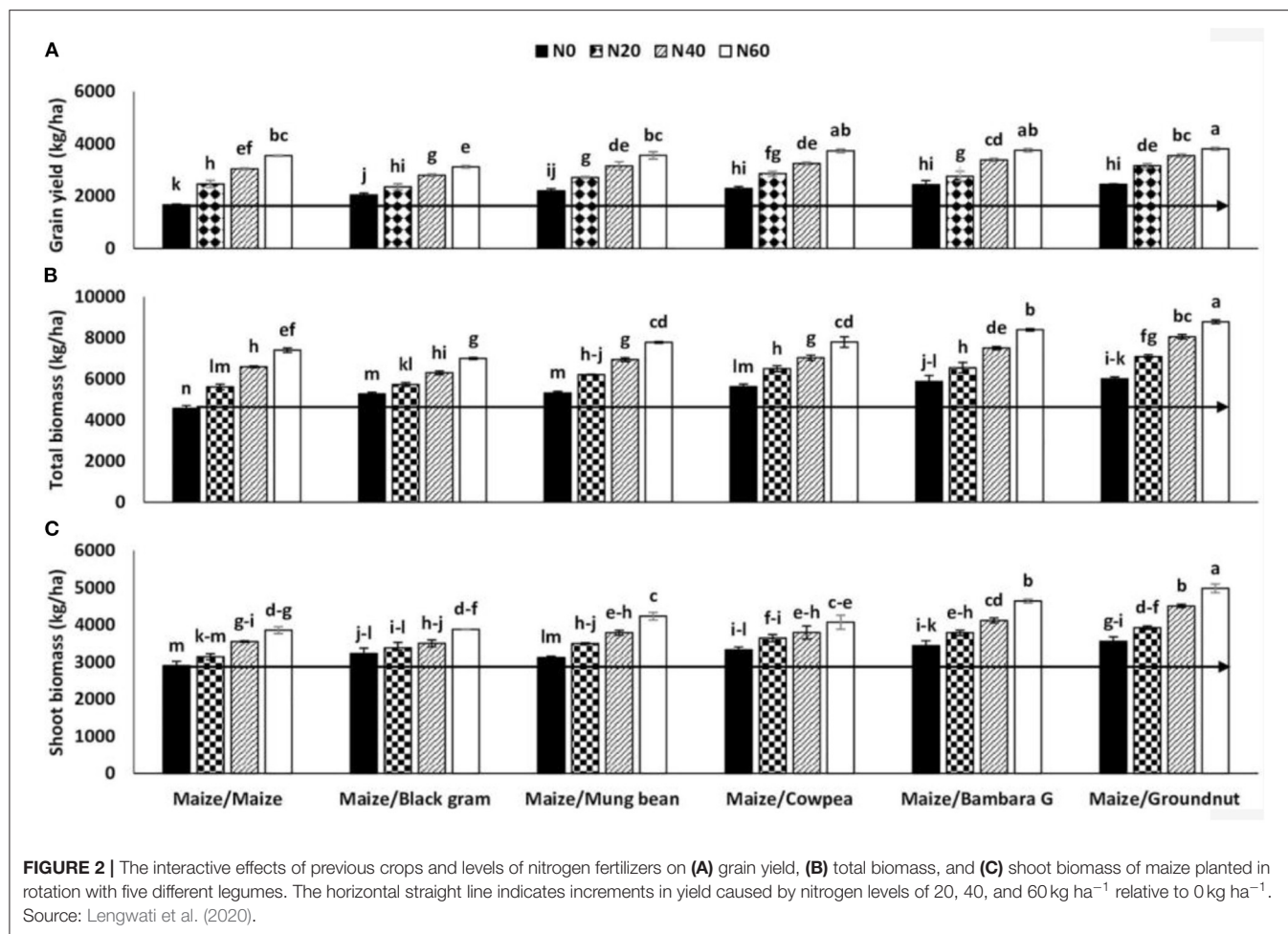


FIGURE 2 | The interactive effects of previous crops and levels of nitrogen fertilizers on (A) grain yield, (B) total biomass, and (C) shoot biomass of maize planted in rotation with five different legumes. The horizontal straight line indicates increments in yield caused by nitrogen levels of 20, 40, and 60 kg ha⁻¹ relative to 0 kg ha⁻¹. Source: Lengwati et al. (2020).

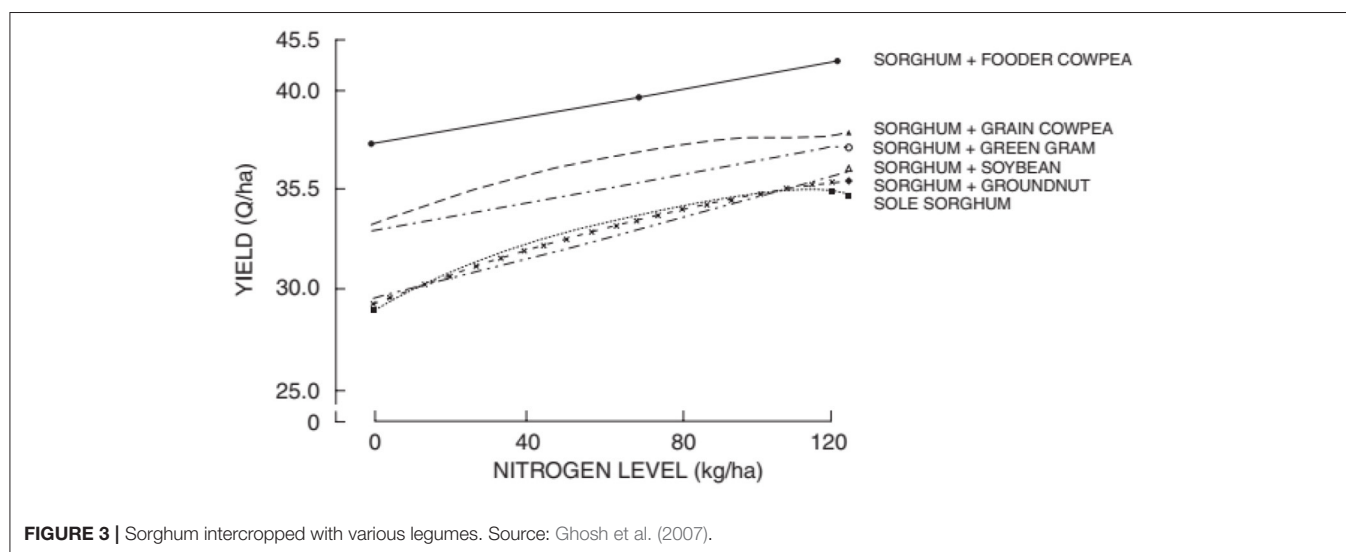


FIGURE 3 | Sorghum intercropped with various legumes. Source: Ghosh et al. (2007).

potassium, and total nitrogen content. Besides this, Nanganoa et al. (2019) indicated that different legume fallow systems had varying grain yield and economic benefits, ranging from

enhanced soil nutrient contents, increased grain yield, and increased economic value of the fallow land. The authors revealed that grain yield ranging from 1.0 to 1.9 t/ha can be obtained using

TABLE 4 | Effect of five different fallow systems (natural, soybean, groundnut, cowpea, and common bean) on soil physicochemical properties (Mean \pm SD) after 19 weeks of crop cultivation.

Soil physicochemical parameters	Fallow systems				
	Natural	Soybean	Groundnut	Cowpea	Common bean
pH	5.53 \pm 0.54 ^a	4.99 \pm 0.22 ^a	4.98 \pm 0.25 ^a	5.24 \pm 0.29 ^a	4.97 \pm 0.16 ^a
Organic carbon (%)	2.77 \pm 0.12 ^a	3.04 \pm 0.28 ^a	3.27 \pm 0.37 ^a	3.36 \pm 0.23 ^a	3.08 \pm 0.24 ^a
Carbon/Nitrogen	14.45 \pm 0.99 ^a	15.17 \pm 1.59 ^a	16.10 \pm 1.62 ^a	15.66 \pm 1.57 ^a	12.68 \pm 0.69 ^b
Phosphorus (mg/kg)	14.12 \pm 5.77 ^a	12.50 \pm 1.90 ^a	16.12 \pm 2.62 ^a	13.82 \pm 2.36 ^a	14.26 \pm 2.31 ^a
Sodium (cmol/kg)	0.21 \pm 0.00 ^a	0.21 \pm 0.01 ^{ab}	0.20 \pm 0.01 ^{ab}	0.20 \pm 0.01 ^{ab}	0.18 \pm 0.02 ^b
Potassium (cmol/kg)	0.94 \pm 0.04 ^a	0.69 \pm 0.24 ^a	0.77 \pm 0.14 ^a	0.75 \pm 0.22 ^a	0.70 \pm 0.05 ^a
Calcium (cmol/kg)	9.99 \pm 0.06 ^a	9.52 \pm 0.99 ^a	9.50 \pm 0.79 ^a	9.61 \pm 0.64 ^a	8.99 \pm 1.18 ^a
Magnesium (cmol/kg)	3.40 \pm 0.08 ^a	3.38 \pm 0.23 ^a	3.28 \pm 0.11 ^a	3.51 \pm 0.33 ^a	3.28 \pm 0.26 ^a
Acidity (cmol/kg)	0.06 \pm 0.02 ^a	0.05 \pm 0.01 ^a	0.05 \pm 0.01 ^a	0.06 \pm 0.01 ^a	0.07 \pm 0.02 ^a
ECEC (cmol/kg)	14.60 \pm 0.17 ^a	13.85 \pm 1.28 ^a	13.79 \pm 0.90 ^a	14.12 \pm 0.36 ^a	13.20 \pm 1.42 ^a

(Source: Nanganoa et al., 2019).

Values within columns with different letters are significantly different ($P < 0.05$).

different legume fallow systems with the highest in groundnut (1.9 t/ha), followed by soybean (1.6 t/ha), common bean (1.3 t/ha), and cowpea (1.0 t/ha) (Figure 4).

Legume Green Manuring

Green manures are locally produced and non-decomposed plant matter, especially legumes, which are applied to the soil surface (as in conservation agriculture) or tilled into the soil so that they help as a mulch and soil amendment. Green manures are cultivated for the specific purpose of providing nutrients to the agricultural system through biomass decomposition (Gangwar et al., 2004; Fageria, 2007; Ghosh et al., 2007). Legume-based green manures are grown with the specific aim of increasing N availability in a system by making use of the N fixed from the atmosphere by the legume (Murphy-Bokern et al., 2017). Green manuring is advantageous for improving the yields of crops and the fertility of the soil. Legume crops are higher-ranking green manure crops as compared with non-leguminous crops due to their ability to fix atmospheric nitrogen. Incorporation of legume green manures and their decomposition has a solubilizing consequence of macronutrients, such as N, P, and K, and micronutrients (Zn, Fe, Mn, and Cu) in the soil and can also alleviate deficiency of different nutrients by recycling nutrients through green manuring (Ghosh et al., 2007). Further, Meena et al. (2018) revealed that legume green manuring can increase the sustainability of agriculture by enhancing retention of different nutrients, improving soil fertility, and decreasing soil erosion and global warming. The BNF process and mineralization of legume green manure crops in soils are shown in Figure 5.

Gangwar et al. (2004) revealed that *Leucaena* incorporated into the soil as green manure at the rate of 6 t ha⁻¹ year⁻¹ before sowing of rice and wheat contributed about 80 kg organic N ha⁻¹ year⁻¹, which can substitute 25% mineral N fertilizer and substantiated to be more financially rewarding than monocropping of rice-wheat. The integration of legumes' green manure into the soil provides organic constituents, such as organic acid, amino acids, sugars, vitamins, and mucilage,

during crop growth and after decomposition. These materials are important for binding soil particles together and forming better soil aggregates, which result in increased hydraulic conductivity, water infiltration, water-holding capacity, and overall pore space of the soil, which are desirable characteristics to enhance soil fertility and crop yield (Meena et al., 2018). The green manuring legumes can also increase the uptake of phosphorus in subsequent crops by changing the unavailable natural and residual phosphorus to more available forms. Furthermore, legume green manure residue decomposition can produce bicarbonates (H₂CO₃), which can solubilize soil mineral phosphorus and, accordingly, result in higher phosphorus availabilities to plants (Fageria, 2007). The contents of different nutrients and C:N ratios found in the above-ground portions of various important legume green manure crops are shown in Table 5.

Alley Cropping

Alley cropping is a cropping practice in which cultivated crops are planted in alleys designed by trees or shrubs, well-known primarily to restore soil fertility and enhance productivity. It is the simultaneous growing of crops between hedgerows of trees that are nitrogen-fixing trees. Alley cropping with nitrogen-fixing trees appears to have a greater impact on long-term nutrient acquisition from deep soil strata. Besides this, the alley cropping approach guarantees the utilization of leaf manures as the trees/shrubs are trimmed during the cropping season (Ghosh et al., 2007). Green leaf manures falling from nitrogen-fixing trees/shrubs supplement the efficacy of applied fertilizers, sustain the soil health and, thus, improve the crop yields and returns. A report on the decomposition residues in alley cropping systems by Wilson et al. (1986) revealed that 50% of the supplemented legume nitrogen can be available to the crops within 1–9 weeks although it depends on the primary nitrogen contents and predominant ecological conditions.

According to Ghosh et al. (2007), the pruning of *Leucaena leucocephala* in an alley cropping system provides a considerable quantity of nutrients that aids the companion crop. In addition,

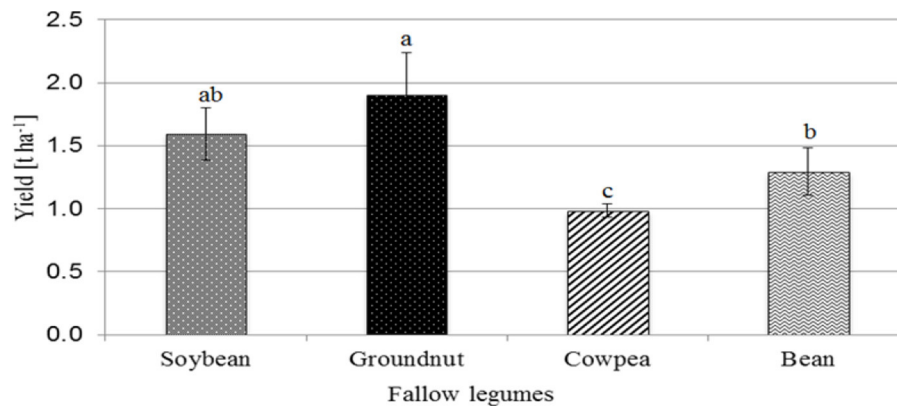


FIGURE 4 | Yield ($\text{t ha}^{-1} \pm \text{SD}$) of four different fallow grain legumes (soybean, groundnut, cowpea, and bean). Data with different letters are significantly different ($P < 0.05$). Source: Nanganoa et al. (2019).

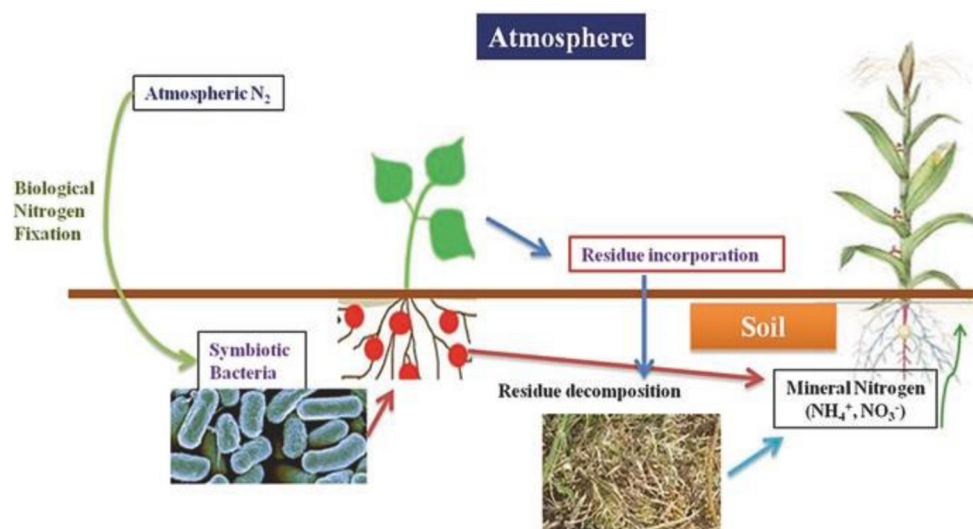


FIGURE 5 | Nitrogen fixation and mineralization of legumes green manure in the soil. Source: Meena et al. (2018).

TABLE 5 | The contents of nutrient and C:N ratios of above-ground portions of some important green manure crops (Source: Ghosh et al., 2007).

Green manure crops	Total nutrient concentration (% dry weight)						C:N ratio
	N	P	K	S	Ca	Mg	
Sesbania (<i>Sesbania aculeata</i>)	2.62	0.32	1.48	0.19	1.40	1.62	16.4
Sunnhemp (<i>Crotalaria juncea</i>)	2.86	0.34	–	–	–	–	16.1
Cowpea (<i>Vigna unguiculata</i>)	2.69	0.28	2.26	0.28	1.50	1.73	17.1
Cluster bean (<i>C. tetragonoloba</i>)	2.80	–	–	–	–	–	17.3
Mung bean (<i>Vigna radiata</i>)	2.21	–	–	–	–	–	16.1
Subabul (<i>L. leucocephala</i>)	3.15	0.20	1.73	–	1.88	0.41	12.7
Gliricidia (<i>Gliricidia maculata</i>)	3.49	0.22	2.44	–	1.89	0.43	10.42

the requirement for fertilizers is decreased, and the system offers an alternative system for achieving sustained yield with low agricultural inputs. Ghosh et al. (2007) also showed that there

is an average decrease of 38, 34, and 29% in the yield of maize, black gram, and cluster bean, respectively, as compared with pure crops when grown as an intercrop with *Leucaena leucocephala*.

TABLE 6 | Seed yields (kg ha⁻¹) of castor as influenced by sole cropping, alley cropping, green leaf manures, and nitrogen levels.

Treatment	N ₀	N40	N80	Mean
Sole cropping				
No green leaf manuring	263	470	570	434
<i>Leucaena</i> green leaf manuring	349	618	679	548
<i>Albizia</i> green leaf manuring	335	585	666	528
<i>Dalbergia</i> green leaf manuring	347	529	653	510
Mean	323	551	642	505
Alley cropping				
No green leaf manuring	348	672	745	588
<i>Leucaena</i> green leaf manuring	500	760	851	704
<i>Albizia</i> green leaf manuring	428	753	833	671
<i>Dalbergia</i> green leaf manuring	425	756	831	671
Mean	425	735	815	659
LSD (0.05)				
Cropping systems	8.2			
Nutrient	8.78			
Interaction	12.57			

Source: Ghosh et al. (2007).

The authors indicated that the highest earnings can be achieved when *Leucaena* is grown in alley cropping with cluster bean and black gram than growing of sole maize or *Leucaena*. Kang and Shannon (2001) also indicated that nitrogen yields are typically around 200–300 kg ha⁻¹ in alley cropping of which ~50% of the nitrogen is derived from the atmosphere (Giller, 2001). The continued presence of alley cropping, which utilizes nitrogen-fixing legume trees, can, thus, act as a way of utilizing the BNF system. The influence of sole cropping, alley cropping systems, green leaf manures, and nitrogen levels on seed yields of castor is shown in Table 6.

STRATEGIES FOR IMPROVING BNF OF LEGUMES AND ASSOCIATED CONTRIBUTIONS

The potential of nitrogen fixation in every legume system is dependent on the plant's reliance upon nitrogen fixation for growth and development and plant nitrogen yield. Improving the BMF for higher nitrogen productivity and gain will increase the sustainability of agricultural production systems. Improving the contribution of BNF by legumes to agricultural systems@ is currently central to different strategies to mitigate the environmental impacts of agriculture as it contributes to recoupling the carbon and nitrogen cycles in agro-ecosystems and enhancing soil nutrient pools while increasing nutrient use efficiency at the levels of diverse cropping systems (Louarn et al., 2015). Various strategies are used to improve the process in an existing symbiotic relationship and widen the scope to non-symbiotic commercial crops, and the success of these strategies depends on how well the process of BNF is understood (Goyal et al., 2021). As a whole, an increment in the level of

nitrogen fixed could be attained by exploiting the legumes yield within the constraints imposed by agronomic, nutritional, or ecological factors. In addition, several management practices influence BNF in agricultural production systems. The strategies widely recognized and adopted for improving BNF of legumes and their associated contributions in the agricultural systems include the selection of legume genotypes, inoculation of legumes with effective rhizobia and their improvements, and the use of appropriate agronomic practices and cropping systems.

Selection of Legume Genotypes

Enhancement in agricultural sustainability needs the utilization of BNF as a key source of nutrients for plant growth and development. Mixed cropping and crop rotations of a non-legumes with legumes have been utilized for centuries to exploit BNF from legumes. The selection of appropriate legume hosts plays a dominant role in interaction with rhizobia (Liu et al., 2020; Plett et al., 2021). The selection of improved host legumes must be developed if higher levels of production and sustainability are to be achieved. Best production from any environment can only be achieved by the use of the most appropriate biological material most matched for a particular environment and adopting management practices intended to avoid or minimize the particular ecological stress most likely to affect the symbiosis. According to Plett et al. (2021), understanding how a genotype supports nodulation and N-fixation in legumes is important to maximize the benefit of N-fixation and reduce reliance on nitrogenous fertilizers. Vanlauwe et al. (2019) stated that the primary methods to improve the potential of an effective BNF are (i) breeding of legume genotypes with increased BNF efficiency with elite strains; (ii) choosing of grain legumes (legume genotypes) that are satisfactorily promiscuous to nodulate successfully with the native rhizobia existing in the soils; and (iii) inoculating of legumes with effective and superior rhizobia strains.

Legume–rhizobial symbiosis is a highly specific interaction, such that particular legume genotypes form an efficient symbiosis with only a specific set of rhizobial strains (Liu et al., 2020). However, the importance of the legume genotypes in regulating nitrogen fixation has commonly been given negligible attention. Sinclair and Nogueira (2018) indicated that breeding programs ignore plant traits that might be related to improved nitrogen fixation potentials due to the constraints in tracking nodulation capacity and nitrogen fixation activity. In more recent research on legumes N₂ fixation, it is increasingly becoming clear that the host plant has a leading role in influencing N₂ fixation. The selection of legume genotypes now appears to be necessary to improve N₂ fixation potential and to have better growth and physiological capability, which can provide better nitrogen input to the plant. Therefore, host plant breeding is compulsory to increase BNF, particularly if inoculation with elite rhizobia strains is anticipated to improve crops yield. Efforts toward announcing BNF as a key property to be considered in plant breeding programs could have thoughtful influences on symbiotic potentials (Vanlauwe et al., 2019).

On the other hand, various stages of the plant–bacteria interaction could also be optimized to maximize the amount and

benefit of nitrogen fixation. Incompatibility occurring among host legumes and nodulating rhizobia at the initial stages of the interaction can block bacterial infection and nodule organogenesis, whereas incompatibility happening after nodule development can result in the development of infected nodules incapable of fixing nitrogen (Yang et al., 2017; Wang et al., 2018). Hence, it can be noted that nitrogen fixation is dependent on the capability of legumes to form an association with rhizobia in a given soil and the host's ability to selectively interact with the most mutualistic partners from a group of compatible indigenous strains. Therefore, it is important to select appropriate legumes and improve their ability to choose and cooperate with the best mutualistic rhizobial symbionts. For this purpose, Liu et al. (2020) suggested that genetic and genomic methods should be employed to identify genes and alleles through harnessing abundant genetic and phenotypic variation present in the legume varieties. According to Goyal et al. (2021), the chemical communication between the legume host and bacteria can be improved to enhance the number of nodules formed and favor occupation by desirable strains. Flavonoids produced by the plant are the earliest signals that promote the symbiotic interaction and induce the genes (nod genes) that are responsible for the synthesis of nod factors. Nod genes are lipochitooligosaccharides that allow the legume to initiate nodule formation, and hence, manipulation of these chemicals, particularly for their expression under stress and suboptimal environmental conditions can substantially improve BNF. Prithiviraj et al. (2003) indicated that increasing the availability of nod factors at low soil temperatures can improve nodulation and BNF. Modulation of stress response genes, phytohormone biosynthesis, phosphate solubilization, and antibiotic production in host plants are also important techniques for improving the symbiotic interaction and the effectiveness of BNF. Kebede (2021) also delineated that rhizosphere engineering can be an alternative approach through which plants are genetically modified to discharge compounds that boost the association and proliferation of beneficial microorganisms.

Inoculation of Legumes With Effective Rhizobia and Their Improvements

The most antique microbe used as inoculants is “rhizobia” bacteria that can colonize the rhizosphere and establish a symbiotic association with legumes, which are used as a plant growth promoter and protector through BNF, mobilization and solubilization of nutrients, production of siderophores, and discharge of phytohormones (Kebede, 2021). According to Murphy-Bokern et al. (2017), inoculation of the legume with the appropriate strain of rhizobia is necessary production technology if it is to be grown where it or a related species has not been produced within the previous 5 years, and this inoculation often results in improved BNF and, hence, soil fertility and crop yields. Hence, legume inoculation with rhizobia is a way of ensuring that the strain of rhizobia appropriate for the legume cultivar being grown exists in the soil at the proper period and in numbers adequate to make a rapid and effective infection and succeeding nitrogen fixation. The inventive objective of this practice is to

stimulate BNF to offer nitrogenous nutrients to a particular legume and other crops, and its return is an increment in plant growth, nutrient uptake, yield, seed protein, and other traits and a reduction in the use of chemical fertilizers with the subsequent decrease of ecological contamination (Wolde-meskel et al., 2018; Kebede, 2021).

Inoculation of legumes with rhizobia can be beneficial in providing a sufficient number of viable N-fixing rhizobia to offer early and effective symbiosis in legumes in the field. Moreover, inoculating the appropriate rhizobia results in the early formation of effective nodules for efficient nitrogen fixation. The utilization of rhizobial inoculants has also permitted the effective introduction of legumes to new agricultural systems in which compatible rhizobia were absent from the soils. A better knowledge of the questions of when, where, and how many inoculants to apply to the soil is the main factor to ensure effective inoculation and colonization of roots in competition with soil rhizobia (Wani et al., 1995). Vanlauwe et al. (2019) suggested future research consideration of an understanding of factors that regulate the persistence of inoculated rhizobia, and this may vary broadly among various rhizobial species and strains. Therefore, the use of appropriate inoculants in legumes offers an opportunity for improving the productivity of legumes and other crops grown in integrated cropping systems, such as crop rotation, intercropping, alley cropping, and green manuring. As a result, the phenomenon of rhizobial inoculation has gotten consideration due to its increasing contribution to agricultural productivity. Further, using rhizobia strains that nodulate well and are persistent in the soils when their host legumes are not cropped will be beneficial for farmers. Besides this, the necessity to inoculate should be well-understood along with the most suitable and effective rhizobium supply systems and improved supply chain conditions.

For effective nodulation and subsequent improvement in BNF, it is also imperative to improve the rhizosphere competence and survival of rhizobial inoculants and enhance their adaptation to diverse environments, which can be advantageous in producing a robust approach for usage by farmers (Kebede, 2021). It is reported that legume inoculation with effective rhizobia offers stimulation and accumulation of phenolic compounds, such as isoflavonoid phytoalexins, and triggering of enzymes such as L-phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), peroxidase (POX), and polyphenol oxidase (PPO), which are involved in phenylpropanoid and isoflavonoid pathways, a rich source of metabolites in plants (Das et al., 2017; Kebede, 2021). Furthermore, Das et al. (2017) recommended novel rhizobial formulation technologies, including polymer-based formulations, water-in-oil emulsion technology for producing liquid formulations, biofilm-based formulations, and application of nanotechnology for the manufacture of an effective inoculant that can ensure enhanced stability, survival, and competence as biofertilizers and biocontrol agents under adverse ecological conditions.

Symbiotic nitrogen fixation in rhizobia is mostly controlled by the *nod*, *nif*, and *fix* genes. Goyal et al. (2021) revealed that rhizobial strains could be improved at any stage beginning with their perception of flavonoids to initiate the nodulation process

through all the steps up to the delivery of fixed nitrogen to the host plant. Consequently, molecular studies and improvements and in-depth knowledge of factors responsible for rhizobial survival and persistence can play a vital role in improving the underlying mechanism of communication, occupation in the host, and nitrogen-fixing ability. For instance, Goyal et al. (2021) described that nitrogen reduction by overexpression of genes from *nif* and *fix* groups and modulation of stress response genes, phytohormone biosynthesis, phosphate solubilization, and antibiotic production are among technologies that are being explored to enhance rhizobial effectiveness. Tremendous efforts over a long period that have resulted in significant progress in different molecular modifications and improvements in rhizobial strains and their effect on host-bacteria symbiotic processes are presented in **Table 7**.

Overall, the use of rhizobial inoculants is a common agronomic practice to ensure adequate nitrogen availability as ~80% of biologically fixed N comes from this symbiosis, having the potential to fix up to 300 kg N/ha/year in different legume crops (Nosheen et al., 2021). In a mixed cropping system, these species improve the growth of nonlegumes by inducing changes in root morphology and growth physiology. One important approach to ensure the success of these species nowadays is to use their mixtures, which increase the competency of each component under field conditions. According to Vassilev et al. (2015), the interest in using mixed inoculants is based on the fact that species of microorganisms in the soil normally exist in communities in which they release diverse metabolites related to their association with plants as well as other microorganisms involved in naturally existing processes of defense and/or competition for space and nutrients. In this regard, Kebede (2021) delineated that the mixture of rhizobial inoculants allows persistence in the rhizosphere for an extended period even during the nonexistence of host legumes and exploitation of broader mechanisms of action that improve their efficiency and reliability. For instance, Kebede (2021) indicated that the co-inoculated mixture of rhizobia can produce signaling molecules, such as nodulation factors (nod factors) and polysaccharides that can stimulate root nodulation and improve the efficiency of biological nitrogen fixation. Hence, the use of mixtures of inoculants, especially various inoculants with diverse proven benefits to the plants, such as nitrogen fixing, phosphorus solubilizing, potassium mobilizing, and biocontrol of diseases, is essential.

The Use of Appropriate Agronomic Practices and Cropping Systems

Understanding the key agronomic constraints influencing legume yields is a prerequisite for improving the BNF in legumes. Stagnari et al. (2017) indicated that the magnitude of BNF and associated plant nutrient contributions varies across legume species, soil properties, climatic conditions, and cropping systems (i.e., monoculture, mixed culture, crop rotations, etc.) as well as soil management strategies. Inorganic nitrogen sources are reported to decrease the nodulation of legumes and reduce nitrogen fixation. The degree of inhibition achieved is reliant on numerous factors comprising the concentration and form,

the time of application, and also the host plant and bacterial strain. Sometimes a small amount of inorganic N, which is known to be starter nitrogen, stimulates early seedling growth and nodulation, leading to an increment in the quantity of nitrogen fixed per plant. In such cases, the nitrogen overcomes the period of nitrogen stress, which the legumes may experience before the commencement of vigorous N fixation. According to George and Singleton (1992), the amount of N gained from fixation is influenced strongly by the N level in the soil as well as nodule weight, nodule number, nodule size, and the amount of N fixed being inversely related to the increase in fertilizer N.

Phosphorus is also critical for both establishments of nodulation and N₂ fixation. Poor nodulation and poor plant vigor are observed in legumes grown in soils low in extractable phosphorus (Desta et al., 2015; Wolde-meskel et al., 2018). Uchida (2000) revealed that phosphorus is vital in several key plant functions, including photosynthesis, transfer of energy, conversion of sugars and starches, movement of nutrients within the plant, and transmission of genetic traits from one generation to the next. Rhizobia utilize phosphorus as a vital element in transforming atmospheric N₂ to nitrate or ammonium (NH₄), a form that is uptaken by plants (Dakora and Keya, 1997). Thus, nodulation, N fixation, and detailed nodule activity are directly correlated to the phosphorus supply in the soil. Under phosphorus stress, strains of rhizobia vary in their capability to extract and incorporate phosphorus from the external environment (Ahiabor et al., 2014; Desta et al., 2015; Wolde-meskel et al., 2018). Phosphorus is, thus, the basis for effective legume nodulation and efficiency of symbiotic nitrogen fixation. Under adequate conditions of P, potassium stimulates infection and N fixation, but high levels of K are inhibiting if P levels are low. Potassium is also well-known for stimulating nodule activity by improving carbohydrate supplies. Similarly, S deficiency affects nodulation by reducing nodule number, size, and N fixation. It is revealed that sulfur deficiency causes a failure of protein synthesis whether nitrogen is available to the plant symbiotically or in a combined form. Some disorders due to deficiency of trace elements are shown to affect the growth of legumes, including Ca, Mg, Mn, Al, etc. (Campo, 1995). Therefore, nutrient management is essential to maximize BNF and obtain the potential benefit from this system.

Crop agronomic practices, such as the frequency of tillage and cropping system practices, can change edaphic, chemical, and biophysical factors and also indirectly affect BNF. Therefore, agronomic management practices that enhance BNF are a promising avenue to increase yields of legumes and other crops in different cropping systems (Montañez, 2000). The potential of BNF and the amount of soil nutrients accessible to a non-legume crop can also be affected by soil and crop management and cropping systems. Legumes grown directly after a cereal crop can fix significantly higher nitrogen than when grown in formerly fallowed soil. With an appropriate selection of cropping systems and consideration of nutritional aspects, BNF can be managed for enhancing N₂ fixation. In areas in which water logging is a problem, the use of different drainage mechanisms, such as broad bed furrows, cumber plots, and furrows, improve the stress imposed by excess water on the growth of crops (Bergersen et al.,

TABLE 7 | Summarized molecular modifications of rhizobial strains and explored improvements in survival and nitrogen fixation characteristics.

Type of modification	Gene modified	Genotype improved	Phenotype improved
Adhesin biosynthesis	<i>rapA1</i>	Overexpression in <i>R. leguminosarum</i>	Increased competitiveness and nodule occupation in red clover.
Antagonism related	TFX (peptide antibiotic trifolitoxin)	Production in <i>Rhizobium etli</i>	Higher rhizosphere competitiveness and nodulation
Cellular replication	<i>parA</i>	Overexpression in <i>Azorhizobium caulinodans</i>	Single swollen bacteroid in one symbiosome, relatively narrow symbiosome space, and polyploid cells were observed when in symbiosis with <i>Sesbania rostrata</i>
EPS biosynthesis	<i>pssA</i> and <i>rosR</i>	Overexpression in <i>Sinorhizobium meliloti</i>	Increased competitiveness and induced more nodules in clover plants
	<i>exoY</i>	Extra copies in <i>M. mediterraneum</i>	Higher shoot fresh weight and shoot length in <i>Medicago truncatula</i>
Heat stress	<i>clpB</i>	Extra copies in <i>M. mediterraneum</i>	Improvement in symbiosis under normal and acidic conditions, and overexpression of <i>nodA</i> and <i>nodC</i> .
Hydrogen uptake	<i>groEL</i>	Overexpression <i>Mesorhizobium</i>	Improved symbiotic effectiveness in chickpea
	<i>Hup</i>	Gene from <i>R. leguminosarum</i> expressed in <i>R. tropici</i> and <i>R. freirei</i>	Increase in nodule efficiency and seed N content in <i>Phaseolus vulgaris</i>
Metal toxicity	<i>MTL4</i> and <i>AtPCS</i>	Genes from <i>Arabidopsis thaliana</i> expressed in <i>Mesorhizobium hawaii</i>	Increased Cd in nodules working on phytoremediation
	<i>MTL4</i> , <i>AtPCS</i> and <i>AtIRT1</i>	Genes from <i>Arabidopsis thaliana</i> expressed in <i>Mesorhizobium hawaii</i>	Higher sensitivity and higher accumulation of Cd and advantage in accumulation of Cu and As
	pSinA	Plasmid from <i>Sinirhizobium</i> inserted in several <i>Alphaproteobacteria</i>	Arsenic resistance and oxidation and heavy metal resistance
	<i>copAB</i>	Gene from <i>Pseudomonas fluorescens</i> expressed in <i>Sinorhizobium medicae</i>	Improved root Cu accumulation without altering metal loading to shoots in <i>M. truncatula</i> , and improved root Cu tolerance
	S-adenosyl-methionine methyltransferase	Gene from <i>Chlamydomonas reinhardtii</i> in <i>R. leguminosarum</i> bv <i>trifolii</i>	Methylation of arsenite
	<i>ropAe</i>	Deletion in <i>R. etli</i>	Cu tolerance enhanced
	<i>PsMT1</i> and <i>PsMT2</i>	Metallothionein genes from pea expressed in <i>R. leguminosarum</i>	Improved tolerance to Cd depicting normal development of nodules
Molecular transport <i>nif</i> genes	<i>dctA</i>	Overexpression of <i>Rhizobium meliloti</i>	Higher rate of nitrogen fixation in <i>Medicago sativa</i>
	<i>nifA</i>	Gene from <i>Klebsiella pneumoniae</i> overexpressed in <i>S. meliloti</i>	Increased nodulation competitiveness in alfalfa
		Extra copy in <i>S. meliloti</i>	Increased alfalfa biomass
		Overexpression in <i>S. meliloti</i>	Increased nodule formation efficiency and rhizopine synthesis
		Overexpression in <i>Bradyrhizobium japonicum</i>	Overexpression of <i>groESL3</i>
		Gene from <i>K. pneumoniae</i> overexpressed in <i>Sinorhizobium fredii</i>	Accelerated nodulation and increased competitiveness in soybean
		Overexpression in <i>S. meliloti</i>	Improved nitrogen fixing efficiency in <i>M. sativa</i>
	<i>nifHDK</i>	Overexpression in <i>R. etli</i>	Increased nitrogenase activity and increased weight and yield in <i>P. vulgaris</i>
<i>nod</i> genes	Random DNA fragment	Random DNA duplication in <i>R. tropici</i>	More competitive strains for nodule formation in <i>Macroptilium atropurpureum</i>
	<i>nodD1</i> , <i>nodABC</i> and <i>nifN</i>	Overexpression in <i>S. meliloti</i>	Increase in nodulation, nitrogen fixation (acetylene reduction activity) and growth of alfalfa
	<i>nod</i>	Overexpression in <i>R. leguminosarum</i>	Increased nitrogen fixation in <i>Vicia sativa</i> and <i>Trifolium repens</i>
	<i>nodD1</i> and <i>nodD2</i>	Overexpression in <i>R. leguminosarum</i>	Delayed nodulation and reduced number of nodules on <i>Vicia</i> plants
	<i>nolR</i>	Overexpression in <i>S. fredii</i>	Increased EPS production and fewer number of nodules on <i>Glycine max</i> and increased number of nodules on <i>Vigna unguiculata</i>
Oxidative stress	<i>Fld</i>	Gene from <i>Anabaena variabilis</i> and overexpressed in <i>S. meliloti</i>	Nodule senescence delayed in <i>M. sativa</i> ; reduced structural alterations in alfalfa nodules; less decline in nitrogenase activity under salinity conditions; improves tolerance to oxidative stress and the survival in the presence of the herbicides paraquat and atrazine

(Continued)

TABLE 7 | Continued

Type of modification	Gene modified	Genotype improved	Phenotype improved
Phosphate solubilization	<i>katB</i>	Overexpression in <i>S. melliloti</i>	Aberration infection thread formation and delayed nodulation on <i>M. sativa</i>
	<i>cbb3</i>	Overexpression in <i>B. japonicum</i>	Increase in the symbiotic effectiveness and in O ₂ consumption rate (free-living cultures) and enhancement in symbiotic nitrogen fixation
		Overexpression in <i>R. etli</i>	Reduced sensitivity of symbiosis with <i>P. vulgaris</i> in drought conditions
	<i>vktA</i> (catalase)	Gene from <i>Vibrio rumoiensis</i> expressed in <i>R. leguminosarum</i>	Increased N fixation activity into nodules, reduced H ₂ O ₂ production
	<i>ahpC</i>	Overexpression in <i>Anabaena</i>	Lowered the peroxide, superoxide and malondialdehyde contents in <i>Anabaena</i> strains
	<i>appA</i>	Gene from <i>Citrobacter braakii</i> overexpressed in rhizobia	Increased P content and shoot dry weight of <i>V. radiata</i>
Phytohormone modulation		Gene from <i>Escherichia coli</i> overexpressed in <i>S. melliloti</i>	Improvement of maize growth in low P soil
	<i>acdS</i> and <i>lrpL</i> (ACC deaminase)	Mutation in <i>R. leguminosarum</i>	Decreased nodulation in pea
		Genes from <i>R. leguminosarum</i> overexpressed in <i>S. melliloti</i>	Improved competitiveness, nodulation, and shoot dry weight in alfalfa
	<i>iaaM</i> and <i>tms2</i>	Overexpression in <i>S. melliloti</i>	Increased number of nodules in <i>M. truncatula</i> ; tolerance to UV, high salt, low pH, and phosphate starvation; improved nitrogenase activity and increased stem dry weight; lower expression of ethylene signaling genes, larger amounts of P-solubilizing organic acid and lower reduction in shoot dry-weight <i>M. truncatula</i> ; induction of transcriptional changes in free-living cells like those occur in nitrogen-fixing root nodule; increased expression of nitrogen fixation genes and stress response-related genes; higher tolerance of alfalfa in drought conditions and higher concentration of Rubisco and lower accumulation of ethylene in drought conditions
		Introduction of <i>iaaM</i> gene from <i>Pseudomonas savastanoi</i> and <i>tms2</i> from <i>Agrobacterium tumefaciens</i> in <i>R. leguminosarum</i>	Fewer number of nodules (but heavier) and increased nitrogenase activity in vetch
		Overexpression in <i>Mesorhizobium loti</i>	Higher nodulation in <i>Lotus japonicus</i> and <i>Lotus tenuis</i> , and improved competitiveness of the strain
	<i>acdS</i> (ACC deaminase)	Gene of <i>Pseudomonas putida</i> overexpressed in <i>Mesorhizobium ciceri</i>	Stimulated growth and increased nodulation on chickpea under normal and waterlogging stress conditions; increased nodulation, plant growth and biocontrol potential in chickpeas; improved growth of chickpea under saline conditions
		Gene of <i>P. putida</i> overexpressed in <i>S. melliloti</i>	Higher biomass of <i>Medicago lupulina</i> under copper stress and enhancement of antioxidant defense system
	<i>ipt</i> (cytokinin)	<i>ipt</i> gene from <i>Agrobacterium</i> overexpressed in <i>S. melliloti</i>	Increased survival of nodules and increased production of antioxidants under drought conditions in alfalfa
	<i>miaA</i> (cytokinin)	Mutation in <i>Bradyrhizobium</i>	Faster nodule formation and alteration of size and number of nodules in <i>Aeschynomene</i>
Polysaccharide biosynthesis	<i>celC</i>	Overexpression in <i>R. leguminosarum</i>	Reduction in biofilm formation, aberrant infection behavior, delay in nodulation and decreased root attachment in <i>T. repens</i>
		Gene from <i>R. leguminosarum</i> overexpressed in <i>S. melliloti</i>	Delay in nodulation in <i>M. truncatula</i>
Salinity and drought stress	<i>putA</i>	Overexpression in <i>S. melliloti</i>	Increased competitiveness in alfalfa plants under drought stress
	<i>betS</i>	Overexpression in <i>S. melliloti</i>	Rapid acquisition of betaines and better maintenance of nitrogen fixation in salinized alfalfa
	<i>otsA</i>	Overexpression in <i>R. etli</i>	Improved number of nodules, nitrogenase activity and biomass in <i>P. vulgaris</i> and plants recovered from drought stress

(Continued)

TABLE 7 | Continued

Type of modification	Gene modified	Genotype improved	Phenotype improved
Siderophore production	<i>fegA</i> <i>fhuA</i>	Gene from <i>S. melliloti</i> overexpressed in <i>M. ciceri</i>	Increased growth in saline media; improved nodules formation and shoot biomass accumulation in chickpea growing in presence of NaCl
		Gene from <i>B. japonicum</i> expressed in <i>Mesorhizobium</i> sp.	Increased growth and nodule occupancy in peanut plants
		Gene from <i>B. japonicum</i> expressed in <i>Rhizobium</i> sp.	Increased growth and nodule occupancy in pigeon pea
		Gene from <i>E. coli</i> overexpressed in <i>Rhizobium</i> ssp.	Increased nodulation and growth in pigeon pea

1989). Besides this, the most important agronomic practices for enhancing BNF are using improved pest management practices, enhancing soil structures, transforming from conventional tillage to minimal or zero tillage, enhancing the general fertility status of the soils, and maintaining the levels of existing soil nutrients (Montañez, 2000).

CONCLUSION

Legumes are essential sources of foods and feed proteins and are often integrated into agricultural systems for their beneficial effects on soil fertility through BNF, which makes a substantial contribution in cropping systems. Direct nitrogen transfer, decomposition, and mineralization of legume residues and mineral nutrient availability and uptake enhancement by crops are among the contribution of BNF in the cropping systems. To effectively utilize these returns in production systems, integrated cropping systems, such as crop rotation, intercropping, improved fallows, green manuring, and alley cropping, are commonly used. An increase in the amount

and utilization of the potential benefits in different cropping systems could be realized by exploiting legumes yield within the limitations imposed by different agronomic, nutritional, and ecological factors. Particularly, the BNF and its associated benefit in the agricultural system can be improved by selecting legume genotypes, inoculating with effective rhizobia, and using good crop and soil management practices. Generally, the use of legumes in the cropping system and adoption of BNF technology, such as an expansion of knowledge and development of economic applications and management systems should be given priority. Future research should engage different approaches to improving inoculant quality with special emphasis on the development of rhizobial strains, inoculant production and application methods, and utilization of legumes in different cropping systems.

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The author confirms being the sole contributor to this work and has approved it for publication.

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Assessment of the 2006 Abuja Fertilizer Declaration With Emphasis on Nitrogen Use Efficiency to Reduce Yield Gaps in Maize Production

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The Abuja Fertilizer Declaration in 2006 recommended the increase of fertilizer use from the current practice for Sub-Saharan Africa (SSA) to achieve food sufficiency and improve soil fertility status. However, the current recommended rates of fertilizer have not been evaluated for specific crops on their potential to reduce the yield gap and optimize nitrogen use efficiency (NUE). In this study, with nitrogen (N) being a significant yield-determinant nutrient, four N use scenarios were drawn from existing recommendations and were evaluated under field conditions for maize crops in two catchments of the Lake Victoria basin. The scenarios included Business as Usual (BAU, 0 kg N ha⁻¹), 25% of the Abuja declaration (ADS 12.5 kg N ha⁻¹), 50% of the Abuja declaration (ADS 25 kg N ha⁻¹), and Abuja declaration–Abuja scenario (ADS, 50 kg N ha⁻¹). The results revealed that increasing N input levels significantly influenced the growth and yield of maize crops. The ADS scenario recorded the highest grain yield increase (167.39%) in Nyando and 103.25% in Rangwe catchments compared to the BAU scenario. N deficits were observed in all the N use scenarios with a range of –66.6 to –125.7 kg N ha⁻¹ in Nyando and –62.5 to –105.4 kg N ha⁻¹ in Rangwe catchments with the 50% ADS scenario having the highest deficits. The deficits imply that the added N input is insufficient to create an N balance for optimal NUE with consequent high risks of soil N mining. In both catchments, all N use scenarios were within the recommended agro-physiological N efficiency (APE_N) level of between 40 and 60 kg kg⁻¹ N. The partial N balance obtained at Nyando (1.56–3.11) and Rangwe (1.10–4.64) was higher than the optimal values, a sign of insufficiency of N inputs and possible risk of soil N depletion in all the scenarios. Our findings conclude that the proposed N rates in the region are still very low for food sufficiency and optimized NUE. Therefore, there is a need to explore other sources of N such as biological N fixation and organic manure and inform policy- and decision-makers to recommend higher rates beyond the “Abuja declaration” with the prospect of reaching target yield and optimizing NUE values based on specific crop recommendations.

Keywords: nitrogen use efficiency, low yield, soil mining, nitrogen deficit, partial N balance, soil fertility, maize

INTRODUCTION

Low soil fertility in Sub-Saharan Africa (SSA) is a significant challenge limiting the realization of higher crop productivity among small-scale farmers (Vanlauwe and Giller, 2006; Ten Berge et al., 2019). In most farms, negative nutrient balances have been reported where the use of nutrients has been below 10 kg ha^{-1} , and in most cases, no mineral fertilizer is applied (Chianu et al., 2012; FAO, 2017; Gram et al., 2020) compared to over 100 kg ha^{-1} in Europe, North America, and China (Rurinda et al., 2020). Nitrogen (N) is a critical element for increased crop production and is a significant yield-determining nutrient in farming systems (Noor et al., 2020; Quemada et al., 2020). Ninety percent of fertilizers applied in Africa are used to supply N, although the current application rates are far below the recommended rates (Thar et al., 2021). Although N is required in adequate quantities to sustain yields, caution should be exercised to avoid excessive application. According to the African Union (2006), agricultural ministers pointed out that increasing fertilizer application rates to 50 kg ha^{-1} in SSA could be the main solution to lift the low productivity levels of maize. Against this background, with $\sim 90\%$ of the fertilizer used being of N form, assessing its efficiency on soil fertility is critical. According to Richards et al. (2016) and a report by the African Union (2014), the recommended rate of 50 kg ha^{-1} by the Abuja Declaration on Fertilizer for the African Green Revolution remains a nightmare for many farmers. The low inputs have contributed to the depletion of soil stocks, characterized as “soil mining,” leading to soil fertility losses (Ten Berge et al., 2019; Leitner et al., 2020), implying that the amount of nutrients absorbed/removed by crops is higher than fertilizers applied (Chianu et al., 2012). N depletion rates in SSA have been reported to be more than 100 kg N ha^{-1} (Akintoye et al., 1999; Nyamangara et al., 2003; Oikeh et al., 2003; Pasley et al., 2020).

Maize remains one of the most cultivated crops due to its essence in the food and livelihoods of the population in SSA (Badu-Apraku and Fakorede, 2017; Ten Berge et al., 2019). However, maize yields in this region are low due mainly to insufficient fertilizer inputs and accessibility of input (Jama et al., 2017; Beesigamukama et al., 2020; Gweyi-Onyango et al., 2021). Existing evidence shows that maize yield is as low as 1.4 t ha^{-1} against a potential of $4\text{--}13 \text{ t ha}^{-1}$ in SSA when proper nutrition and improved varieties are used (Mueller et al., 2012; Tamene et al., 2016). According to Mueller et al. (2012), closing yield gaps in SSA to $\sim 50\%$ of the attainable yields requires addressing the existing nutrient deficiencies, which remains a challenge for many smallholder farmers. As reported by Dzanku et al. (2015), smallholder agriculture in Africa experiences large food crop yield gaps under rainfed conditions. With the anticipated population increase in SSA, approaches are required to reduce the yield gap particularly for cereal crop that forms the most basic meal for every household (Van Ittersum et al., 2016). Small-scale agrarian livelihood is the most dominant production system, contributing to most national-level food production, with a significant farm size being $<2 \text{ ha}$ (Leitner et al., 2020). Low use N input and small crop land for most rural farmers contribute significantly to large yield gaps. However, increasing N input is

linked to increment of yield and minimizes the current gaps and food insecurities. This can be attested from Malawi's experience where maize yield doubled with fertilizers' subsidies that allowed farmers access to N fertilizers and use of improved maize varieties (Folberth et al., 2013; Masso et al., 2017; Katengeza, 2020).

Nitrogen use efficiency (NUE) is defined as a ratio between the amount of N removed with harvest and N applied in the cropping system. It is an established metric used to benchmark the management of N in defined systems (Congreves et al., 2021; Ntinyari et al., 2021). NUE provides information on the relative utilization of applied N to an agricultural production system to either specific plots or farms (Brentup and Pallière, 2010). The components of NUE that are critical in the analysis of N management are as follows: the partial N budget (PNB) that shows the nutrient recovery efficiency; the agronomic efficiency of N (AE_N) shows the measure of crop yield with the amount of N added (Dobermann, 2005); the agro-physiological N efficiency (APE_N) shows the economic yield per unit N accumulated from the N fertilizer applied (Dobermann, 2005); and N surplus/deficit that shows a balance between N input and output from the system with positive values indicating surplus and negative values showing N deficits.

Insufficient N inputs in most SSA countries have been linked to NUE values above 100% (Edmonds et al., 2009), compared to 70% in regions with good use of N in cropping systems (Sutton et al., 2013; Masso et al., 2020). The European Union Nitrogen Expert Panel (EUNEP, 2016) described the desired NUE to range between 50 and 90%. NUE levels higher than 90% represent chances of extreme risks of mining soil N stocks (Quemada et al., 2020). Comparatively, global NUE averages approximately 45–50%, indicating that a few countries have achieved a desirable NUE.

According to Elrys et al. (2020), the failure of African countries to achieve a six-fold increase in fertilizer input, as suggested by the Alliance for a Green Revolution in Africa (Trade and Africa, 2018), has led to poor NUE values posing a severe threat toward achieving food sufficiency and environmental sustainability. Therefore, there is a need to adopt better practices to optimize NUE and minimize the risks of excess soil nutrient mining in scenarios with low N inputs (Kuyah et al., 2021). According to Hirel et al. (2011), N fertilizer's in-season application is an essential facet toward improving NUE. Proper timing of N fertilizer application improves synchronization of available N to plants and maximizes uptake and utilization (Yadav et al., 2017; Ullah et al., 2019; Ishfaq et al., 2021). To optimize NUE, farmers are encouraged to apply the 4 R stewardship of nutrient management (i.e., right rate, right source, right timing, and right placement) to increase NUE while minimizing losses and environmental impacts (Davidson et al., 2016; Masso et al., 2017; Ladha et al., 2020; Ntinyari and Gweyi-Onyango, 2021).

Although recommendations by the Abuja declaration have been made to increase fertilizer inputs, their influence on NUE has not been evaluated at the plot level. Besides, most of the studies have relied on model projections in estimating the change in yield over time (Mueller et al., 2012; Leitner et al., 2020). Still, they have neglected key indicators for NUE for major crops within the region. Based on this, scenarios for N use were

simulated at field conditions to give insights into possibilities of reducing the yield gap, optimizing NUE, and contributing to farmers' knowledge on improving N management. The scenario's choices were based on the Comprehensive Africa Agriculture Development Programme (CAADP), Abuja declaration of 2006, and Malabo declaration of 2014 aligned with global agriculture and Sustainable Development Goals. The objective of this study was to evaluate the Abuja 2006 declaration by using gradual increases of N as a main inorganic fertilizer in SSA for maize for its effectiveness in reducing yield gaps and enhance achievement of optimal nitrogen use efficiencies. The scenarios described in this study show a projected transition of N inputs uses by the farmers from the current practices. This is one of the first studies to assess the effect of the Abuja fertilizer declaration (50 kg ha^{-1}) on optimization of NUE in yield, agronomic, and environmental sustainability, assuming that farmers will transit gradually from current practices. The results from this study can be a basis for the formulation of new policies and priorities for sustainable N management within the region.

MATERIALS AND METHODS

Study Site Characteristics

Two field experiments were carried out in two distinct catchments of the Lake Victoria basin, namely, Nyando and Rangwe. Nyando is located in Kisumu County 34.912190°S , -0.148550°E at an elevation of 1,154 m above sea level. The average monthly temperatures are between 24.0 and 25°C . The soils in the study site are Vertisols black cotton soils with shallow depths, high organic matter, and moderate pH levels (Gachene and Kimaru, 2003). The catchment receives cumulative rainfall of 1,350 mm annually. Rangwe is located in Homabay county at 34.573104°S , 0.623583°E with an elevation of 1,166 m above sea level. The average monthly temperatures range between 22.1 and 23.9°C . The soils are Eutric Fluvisol with low organic matter and moderate pH levels. The cumulative rainfall for the catchment is 1,646 mm annually. The analysis of the selected physical-chemical characteristics were done according to Okalebo et al. (2002) (Table 1).

Nitrogen Fertilizer Scenarios Applied

The change in N fertilizer use was assumed to be influenced by implementing various recommendations and policy interventions set aside for the Africa's Green revolution and livelihood transformation through Agriculture (Trade and Africa, 2018).

Scenario 1: Business as Usual (BAU)

The first scenario evaluated maize simulation (BAU) representing zero N input (0 kg N ha^{-1}), reflecting the actual farmers' practices in the two catchments. This scenario was guided by the fact that 60% of the farmers in the region do not use N fertilizer input in their maize fields. Therefore, this scenario assumes that the farmers in this category would continue to grow crops without any N inputs over time.

TABLE 1 | Selected chemical and physical characteristics of the experimental soils.

Parameter	Nyando		Rangwe	
	0–20 cm	20–40 cm	0–20 cm	20–40 cm
Total N%	0.07	0.06	0.08	0.07
TOC (%)	1.35	1.16	0.56	0.82
NO_3^- (mg kg^{-1})	18.90	10.6	16.20	12.00
NH_4^+ (mg kg^{-1})	22.20	36.5	45.20	32.60
pH (1:2.5 water)	5.70	5.9	6.13	6.23
EC (ms cm^{-1})	0.13	0.14	0.07	0.04
Available P (ppm)	28.8	62.3	7.20	9.70
Ca (cmol kg^{-1})	7.50	6.2	2.85	3.10
K (cmol kg^{-1})	1.76	1.14	1.18	0.48
Bulk density (g cm^{-3})	1.30	1.32	1.40	1.43

Case 2: 25% of the Abuja Declaration (25% ADS)

The second scenario represented 25% of the recommended rate by the Abuja declaration of 2006 on the set target of 50 kg ha^{-1} and recommended by Africa.fertilizer.org. In this scenario, N input was set to $12.5 \text{ kg N ha}^{-1}$, which was the average nutrient application rate by 2015 from Abuja fertilizer declaration in 2006.

Case 3: 50% of the Abuja Declaration (50% ADS)

Scenario 3 represented 50% of the recommended rate by the Abuja declaration of 2006 on the set target of 50 kg ha^{-1} of nutrients. The N input for this scenario was 25 kg N ha^{-1} and represented a double increment and a transition from the 25% ADS scenario that was the base value in the fertilizer declaration summit. This scenario also reflects the current N input rates by some farmers in selected countries in SSA (Sheahan et al., 2014).

Case 4: Abuja Declaration Scenario (ADS)

This scenario used 50 kg N ha^{-1} , which was adopted as the set target for Abuja fertilizer declaration. Nevertheless, many farmers are still far short of the Abuja fertilizer summit. The scenario represented 100% transition by the farmers to the "summit adopted" rate of N application. This scenario assumes that there will be changes in favor of fertilizer accessibility; hence, farmers will purchase/access and apply the recommended N inputs in maize cropping systems.

Experimental Design and Data Collection

Maize (*Zea mays* L.) seeds of Sc Duma-43 from the Seed Co. (hybrid variety and recommended for the Lake Victoria catchment) were sown in the fields during the cropping season of September 2020–January 2021. A total of 16 plots measuring $5 \times 5 \text{ m}$ with border widths of 0.5 and 1 m for plots and blocks, respectively, were adopted and arranged in a randomized complete block design (RCBD) with four replications for each scenario. The spacing for the maize plants was $75 \times 25 \text{ cm}$. Three plants per hill were planted and thinning was done to 2 seedlings per hill after 2 weeks of germination. Urea, a commonly available source of nitrogen fertilizer in the region, was applied into fields except for control (BAU) and other treats

as 12.5 kg N ha⁻¹, 25 kg N ha⁻¹, and 50 kg N ha⁻¹. The N fertilizer was applied in two splits; the first split was at planting, while the second application was during the vegetative stage, which corresponded to 30 days after planting (DAP). During the experiment, the standard agronomic practices of maize crop production, including weeding and pest control, were carried out.

Data on biomass and other growth parameters were collected at three critical stages of maize production: vegetative, which was 30 DAP, tasseling (60 DAP), and physiological maturity (90 DAP). Five plant samples were collected from the experimental plots at vegetative (V6), reproductive (R1), and physiological maturity (R6) harvesting stages. The samples were thoroughly washed in running water to free them from soil and any other surface impurities. The samples were separated into leaves, stems, roots, and grain at harvesting and taken to the laboratory for drying at a temperature of 70°C for 48 h (after achieving constant weight). The dried samples were then ground using a mechanical grinder.

At maturity, yield data were collected from each plot after all the ears had reached physiological maturity. Plants were harvested by cutting at ground level, and ears were threshed. Both grain and stover were air-dried and then oven-dried in the laboratory until a constant moisture content of 12.5% was reached. The yield obtained from the net plot of each N use scenario was determined and extrapolated into tons per hectare (t ha⁻¹).

Laboratory Analysis Methods

Soil samples were collected from the two experimental fields at the start of the experiment to analyze the selected chemical and physical compositions of the soils. The soils were sampled from depths (0–20 and 20–40 cm) for analysis of total organic N, available N (NO₃⁻), and NH₄⁺, organic carbon, available P, pH, bulk density, electrical conductivity (EC), soil texture, and exchangeable cation (K and Ca). Electrical conductivity (EC) and pH were determined using extracts 1:2.5 [weight/volume (w/v)] for soil to distilled water. The pH and EC were then read directly using a pH (AD1000, Adwa, Romania) and EC meter (AVI, Labtech, India), respectively (Okalebo et al., 2002). The available N (NH₄-N and NO₃-N) was extracted from soil using 0.5 M potassium sulfate at a ratio of 1:10 (w/v). The potassium sulfate mixture was shaken for 1 h using an orbital and linear shaker (KOS-3333/KCS-3333, MRC, UK). Filtration of the solution was done using Whatman No. 1 filter paper, and the filtrate was used for further analysis using the colorimetric method at 655 and 419 nm as described by Okalebo et al. (2002). Total N in soil was determined using the Kjeldahl digestion and distillation method. Exchangeable Ca and Mg were determined using Atomic Absorption Spectrometry at 422.7 and 285.2 nm, respectively (iCE 3300 AA system, Thermo Scientific, Shanghai, China), and K was determined using flame photometry. Available phosphorus was analyzed using Bray 2 method as described by Okalebo et al. (2002). Air-dried samples were ground using an analytical mill for N concentration in grain and plant tissues per N use scenario. The N content in the plant tissue (grain and stover) will be determined by Kjeldahl digestion procedure (Baker and Thompson, 1992). A sample of 0.3 g of milled plant

material was put in a digestion tube and digestion mixture, 1% NaOH was added, and total N was determined through distillation. To determine nitrogen partitioned to roots, stem, leaves, and grain, the N content obtained was divided by the total amount of N in the whole plant and later converted to a percentage by multiplying by 100.

Nitrogen Use Efficiency

The calculated N use efficiency indicators, according to Fixen et al. (2015), were as follows:

- Partial nutrient balance (PNB) was determined to show nutrient recovery efficiency, usually expressed as nutrient output per unit of nutrient input (a ratio of “removal to use”) (Equation 1).

$$PNB = \frac{N_{\text{content of the harvested (edible portion)}} (kg N ha^{-1})}{Rate of N fertilization (kg N ha^{-1})} \quad (1)$$

- Agro-physiological N efficiency (APE_N kg grain kg⁻¹) was calculated to determine the economic yield per unit N accumulated from each fertilizer treatment (Equation 2).

$$APE_N = \frac{yield \text{ with } N (kg ha^{-1}) - yield \text{ without } N (kg ha^{-1})}{biomass \text{ uptake with } N (kg N ha^{-1}) - biomass \text{ uptake without } N (kg N ha^{-1})} \quad (2)$$

- Agro-physiological N efficiency (APE_N kg grain (kg N)⁻¹) was calculated to show the increase in yield per the unit of N increase applied (Equation 3).

$$AE_N = \frac{Yield \text{ N } (kg N ha^{-1}) - Yield \text{ without } N (kg N ha^{-1})}{Rate of N application (kg N ha^{-1})} \quad (3)$$

- N surplus/deficit was calculated to show the balance between the applied N and the crop N removal (Equation 4).

$$N \text{ surplus/deficits} = N_{\text{inputs}} (kg N ha^{-1}) - N_{\text{outputs}} (kg N ha^{-1}) \quad (4)$$

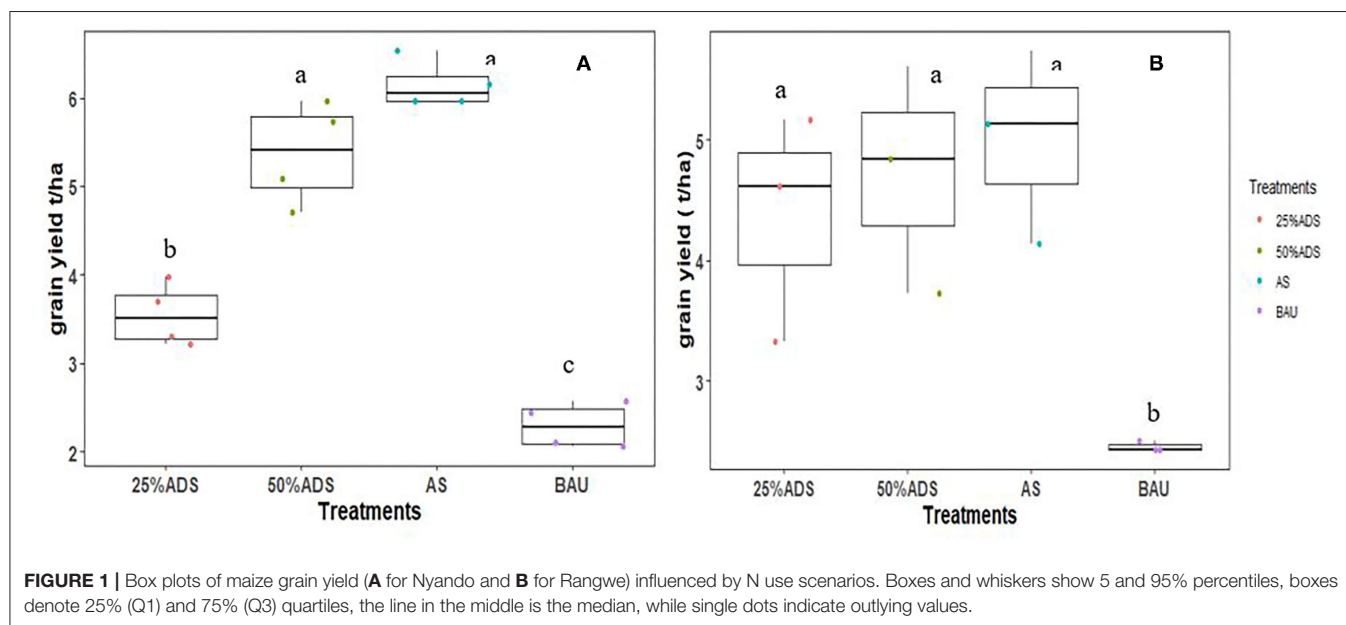
Statistical Analysis

The collected data were checked for normality using the Shapiro–Wilk test. Plant biomass, N grain yield, NUE, and total nitrogen crop production were analyzed using a one-way analysis of variance test using R software version 4.1.0. Computation of least squares means was done using “lsmeans” package,

TABLE 2 | Biomass accumulation of maize as influenced by various N use scenarios during maize phenological stages.

N scenarios	kg N ha ⁻¹	Biomass accumulation (t ha ⁻¹)					
		Nyando			Rangwe		
		Vegetative (V6)	Reproductive (R1)	Harvesting (R6)	Vegetative (V6)	Reproductive (R1)	Harvesting (R6)
BAU	0	0.17 ^c	2.45 ^b	5.47 ^b	0.13 ^c	2.68 ^b	4.44 ^b
25% ADS	12.5	0.39 ^b	3.25 ^b	8.91 ^a	0.35 ^{ab}	3.10 ^b	4.66 ^b
50% ADS	25	0.76 ^a	4.65 ^{ab}	10.17 ^a	0.37 ^{ab}	4.86 ^{ab}	6.62 ^{ab}
ADS	50	0.72 ^a	7.00 ^a	10.42 ^a	0.42 ^a	6.27 ^a	8.62 ^a
<i>p</i> -value		0.002	0.002	0.004	0.04	0.03	0.004

BAU, Business as Usual; 25% ADS, Abuja declaration scenario; AS, Abuja scenario; vegetative, reproductive, and harvesting = 30, 60, and 90 days after planting, respectively. In the same column, means with the same letter superscripts are not significantly different.



followed by mean separation using adjusted Tukey's method implemented using "cld" function from the "multicompView" package. Distribution of means for grain and stover yield was done using the ggplot command from the ggpubr package (R Core Team, 2019).

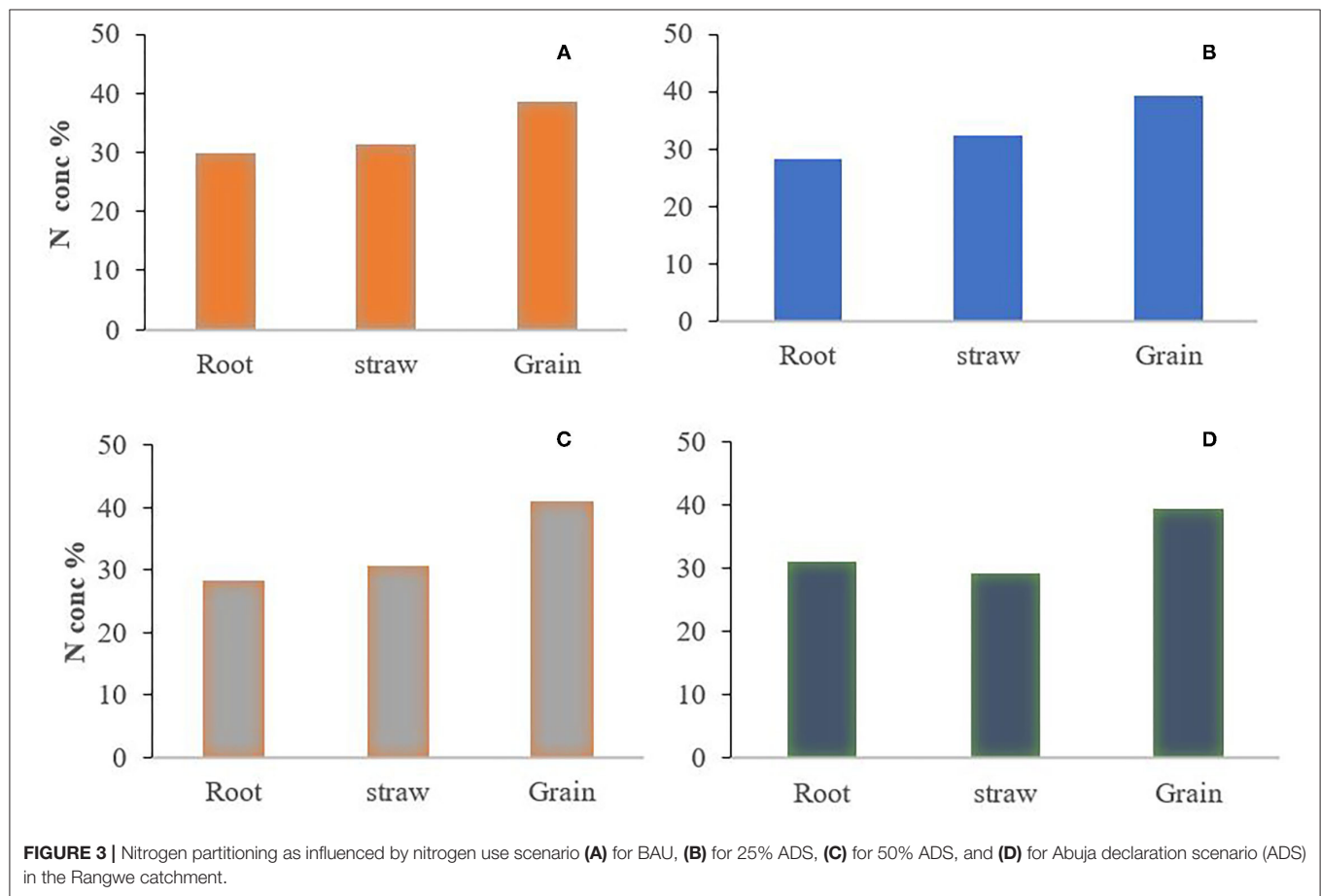
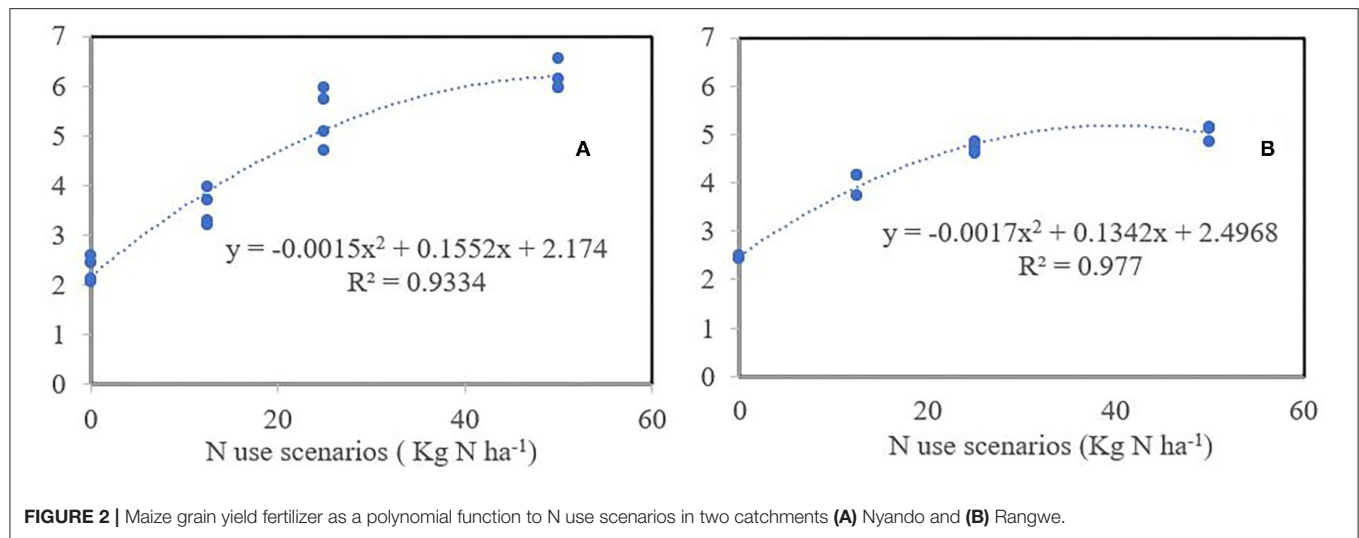
RESULTS

Biomass Accumulation, Grain Yield, and N Partitioning

Biomass accumulation varied significantly ($p \leq 0.05$) among the N use scenarios during vegetative (V6), reproductive (R1), and physiological maturity (R6) stages of maize production (Table 2). There was a significant increase in biomass accumulation from vegetative (V6), reproductive (R1), and maturity (R6) in the two catchment areas (Table 2), with ADS (50 kg N ha⁻¹) having the highest biomass accumulation of 0.72–10.42 t ha⁻¹ in Nyando and 0.42–8.62 t ha⁻¹ in Rangwe across all the three phenological stages.

The different N use scenarios showed significant differences ($p < 0.001$) in maize grain yield in the two catchments (Figure 1). A positive trend on grain yield increase with increasing N application rates was observed. In Nyando, a significant ($p < 0.001$) difference was observed across the scenarios, with ADS 50 kg N ha⁻¹ having the highest yield of 6.15 t ha⁻¹, which was 167.39% higher compared with the BAU (0 kg N ha⁻¹) (Figure 1A).

In Rangwe, a similar trend of maize yield was observed with ADS at 50 kg N ha⁻¹, recording the yield of 5.00 t ha⁻¹, which was 103.25% higher than the BAU (0 kg N ha⁻¹). However, the yield differences were not significantly different between AS and the other two N scenarios (i.e., 25 and 50% ADS) in Rangwe. The regression analyses (Figure 2A) showed that grain yield increased with N rates, and the response assumed a polynomial function, with R^2 values of 0.93 and 0.98 for Nyando and Rangwe, respectively. However, in Rangwe (Figure 2B), the curve seems to plateau, which could result from other factors, including calcium and potassium, which were relatively low in the site, hence affecting N



in the test variety. In all the N use scenarios, in both catchments, the highest N concentration was partitioned to the grain, representing 39.01–42.07 and 38.62–41.09% of the entire plant uptake in both Nyando and Rangwe catchment areas (**Figures 3, 4**).

The results indicate that ~40% of N uptake was removed from the field with the grain harvested for all nitrogen use scenarios. The lowest partitioning of N concentration was observed in the roots with mean percentage ranges of 28.19–31.19 and 28.41–29.85% in Rangwe and Nyando, respectively (**Figure 4**).

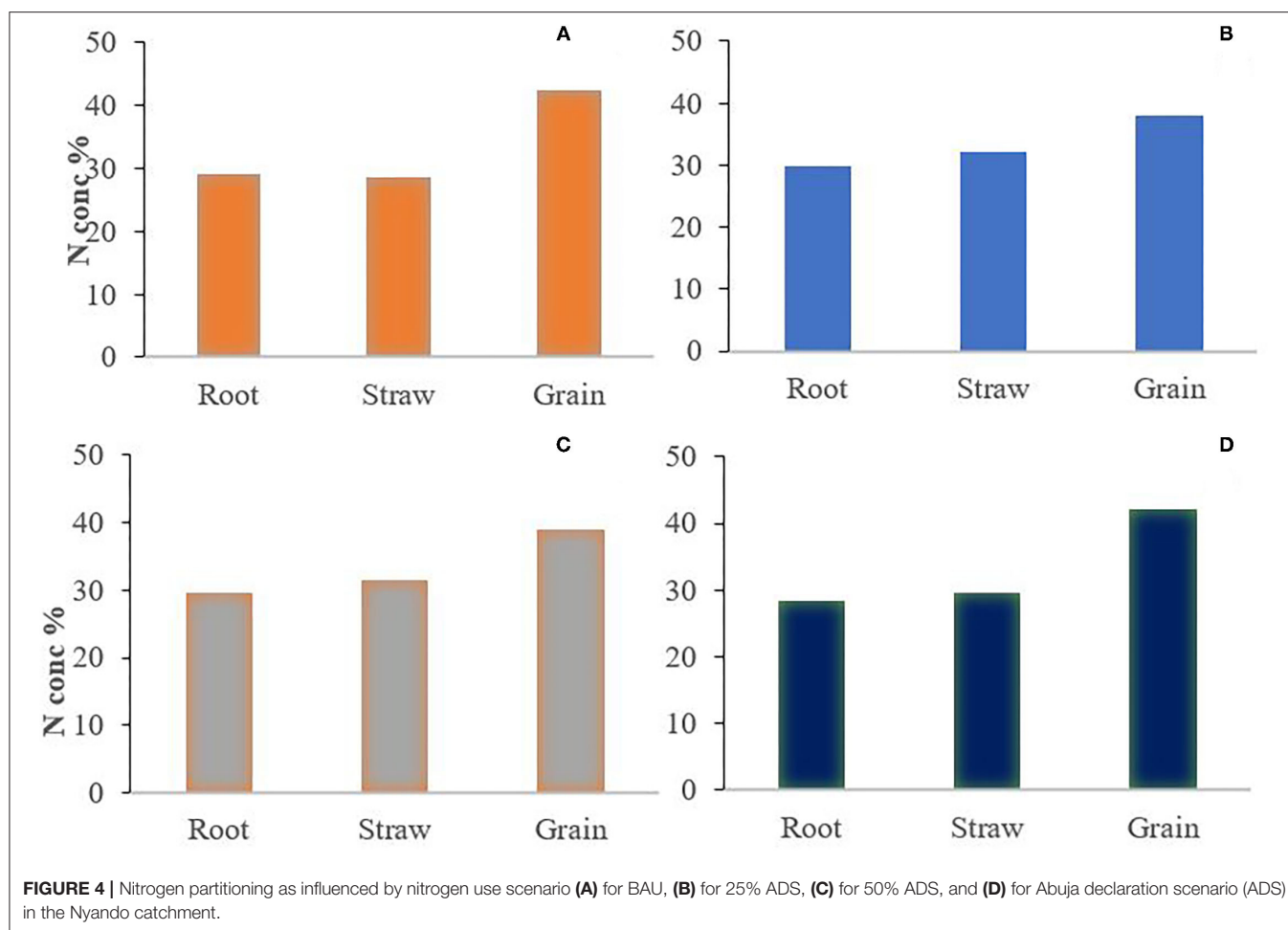


TABLE 3 | Effects of N use scenario fertilizers on nitrogen use efficiencies and partial N balance maize.

N use scenarios	Nitrogen use efficiency						
	kg N ha ⁻¹	Nyando			Rangwe		
		APE _N	PNB	N surplus/deficit	APE _N	PNB	N surplus/deficit
BAU	0	n.a.	n.a.	−66.6 ^a	n.a.	n.a.	−62.5 ^a
25% ADS	12.5	47.4 ^a	3.11 ^a	−109.1 ^b	52.4 ^a	4.64 ^a	−82.4 ^{ab}
50% ADS	25	48.9 ^a	2.39 ^b	−125.7 ^b	45.4 ^a	2.99 ^{ab}	−105.4 ^b
ADS	50	51.7 ^a	1.56 ^c	−119.6 ^b	57.6 ^a	1.10 ^b	−63.30 ^a
p-value		0.030	0.001	0.001	0.75	0.013	0.001

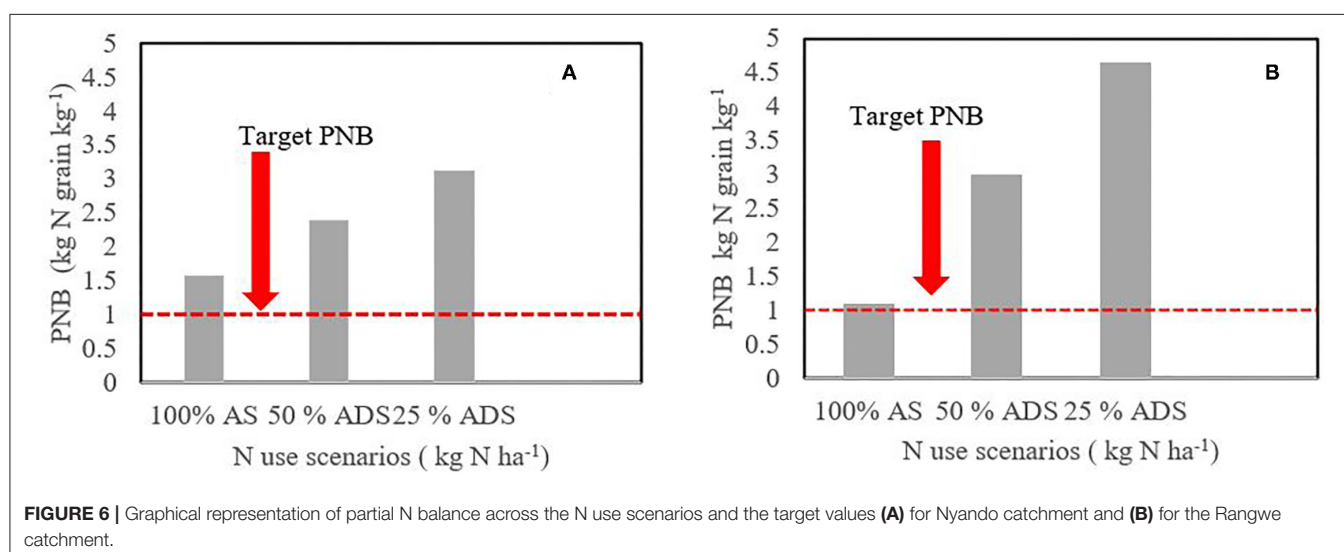
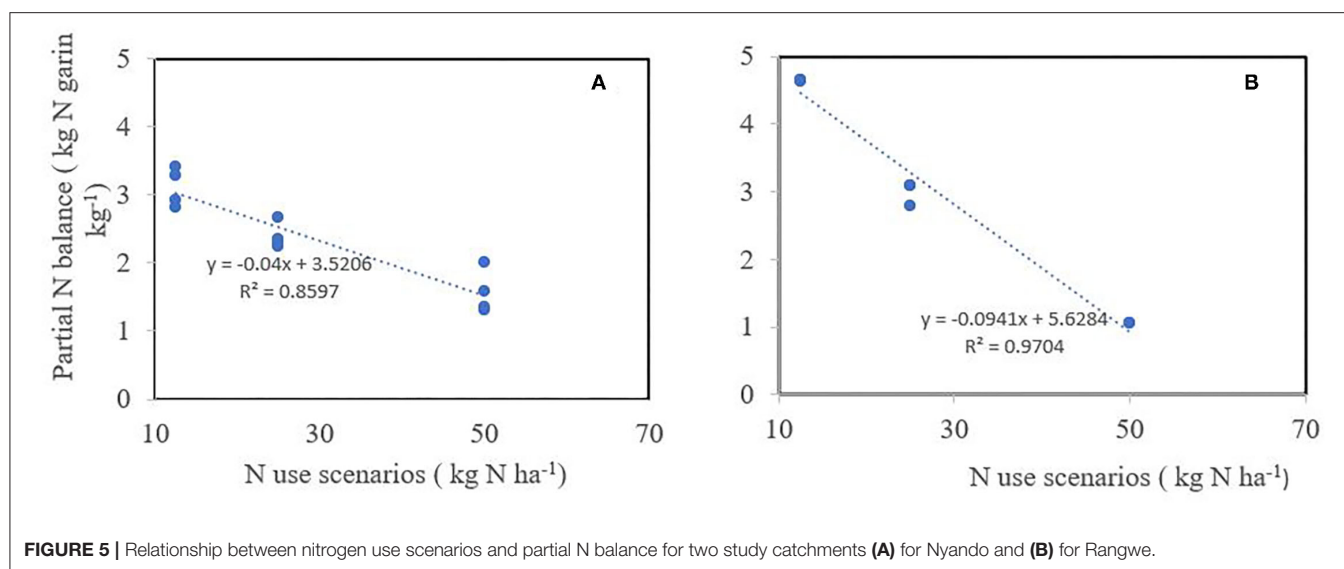
BAU, Business as Usual; 25% ADS, Abuja declaration scenario; ADS, Abuja scenario; APE_N, Agro Physiological Nitrogen Efficiency; PNB, Partial N Budget; n.a., not applicable. In the same column, means with the same letter are not significantly different.

Nitrogen Use Efficiency

Agro-physiological nitrogen efficiency (APE_N) was not significantly ($p < 0.05$) different among the N use scenarios in the two catchments. However, there was a clear trend with the highest N inputs (ADS 50 kg N ha⁻¹) having higher APE_N values. The lack of differences could be influenced by the N scenario adopted in this study being too low to affect maize grain significantly. Moreover, degraded soils could have contributed

to the lack of differences and N inputs being too low. The ADS scenario at 50 kg N ha⁻¹ recorded the highest APE_N, with a mean value of 57.60 kg grain kg⁻¹ N in Rangwe and 51.7 kg grain kg⁻¹ N in Nyando catchment. In both catchments, all N use scenarios achieved the recommended APE_N level of between 40 and 60 kg grain kg⁻¹ N (Table 3).

N surplus/deficit of maize varied significantly ($p < 0.001$) across all the scenarios in the two catchments evaluated (Table 3).



The 50% ADS (25 kg N ha⁻¹) had the largest N deficit of -125 and -105 kg N ha⁻¹ in Nyando and Rangwe catchments. This indicates that the addition of more N input could optimize N and reduce the negative imbalances in the soils; however, the 50 kg N ha⁻¹ suggested at the Abuja fertilizer summit is still very low for maize crop to contribute to the optimization of overall NUE.

The PNB of maize varied significantly ($p < 0.001$) across all the N use scenarios evaluated in the two catchments (Table 3). The 25% ADS (12.5 kg N ha⁻¹) had the highest values of PNB of 3.11 kg grain N (kg N)⁻¹ in Nyando, while 50% ADS (25 kg N ha⁻¹) and AS (50 kg N ha⁻¹) had 2.39 and 1.56 kg grain N (kg N)⁻¹ PNB, respectively.

A similar reducing trend of PNB with an increased N was also observed at Rangwe. The declining trend of PNB with N was linear (Figure 5, Table 3). Moreover, ADS (50 kg N ha⁻¹) fertilizer application resulted in a near-optimal PNB of 1.10 kg grain N (kg N)⁻¹ (Figure 5). The higher N application rate

resulted in values closer to optimal PNB, while the low N rates had higher values, far from the optimal range. Figure 6 confirmed a strong association between nitrogen scenarios and partial N balance for both Nyando and Rangwe study sites. The increase of N application rates as defined by the N use scenarios resulted in lowering partial N balance with $R^2 = 0.86$ and 0.97 for Nyando and Rangwe, respectively (Figure 5).

Agronomic Performance

AE_N had significant differences across the N use scenarios in the two catchments. AE_N depicts the efficiency of N recovery from the applied fertilizers in grain yield. In all the N use scenarios, the AE_N obtained was greater than the common values of 10–30 kg grain (kg N)⁻¹ (Table 4).

The values obtained were between 81.7 and 118.7 kg grain (kg N)⁻¹ in Nyando and between 45.4 and 154.3 kg grain (kg N)⁻¹ in the Rangwe catchment, which are higher values than 30 kg grain

TABLE 4 | Effects of N use scenario fertilizers on Agronomic Nitrogen Efficiency (AE_N) on maize.

	Nyando AE_N	Rangwe AE_N
BAU	–	–
25% ADS	118.7 ^{ab}	154.3 ^a
50% ADS	132.1 ^a	103.0 ^a
ADS	81.7 ^b	45.4 ^b
p-value	0.02	0.01

BAU, Business as Usual; ADS, Abuja declaration scenario; AS, Abuja scenario. In the same column, means with the same letter are not significantly different. AE_N , Agronomic efficiency of nitrogen (dash means parameter could not be estimated).

(kg N)⁻¹, implying that the amount of N fertilizer supplied was very low to optimize AE_N .

DISCUSSION

Effects of N Use Scenarios on Nitrogen Use Efficiency Indicators

PNB that expressed the nutrient output per unit of nutrient input varied significantly across the scenarios. The values observed in this study were above the typically optimal range that indicates insufficient or low N level of the applied fertilizer. The PNB of 1.56–3.11 and 1.10–4.64 kg grain (kg N)⁻¹ (Table 3) showed that the amount of N used was not sufficient to supply the required quantity of N to optimize NUE, hence a need for an increased application. Increasing the fertilizer rate from the current ADS scenario could lead to more sustainable PNB in the region, a fact that is supported by the positive regression in Figure 5. With no plateaus observed in the regression lines, it may be an indication that increasing more N for the two regions would result in desirable PNB values (lower than <1) (Figure 6). Besides, the high PNB values >1 imply that more nutrients were being removed from the plot than what was applied; a situation of soil N mining. Therefore, there is lack of a mass balance between N used and N removed in the crop, with precise/optimal ranges shown in Figure 6.

Negative values of N balance observed in all the scenarios in the two catchments (Table 3) implied higher crop removal of N compared to N input, a process that is likely to contribute to extreme depletion of the soil N status. The N deficits indicate that even with the implementation of the Abuja recommendation, the soil fertility will still decline due to continuous mining. The current findings agree with those of Snyder and Bruulsema (2007), who suggested that a PNB value close to 1 is an indicator of the nutrient's mass balance and higher than 1 shows extreme deficits in the systems. However, this was not achieved in our case, and this could probably mean more N input needs to be added to correct the existing deficiencies of mineral N inputs. Similar results of higher PNB >1 were also reported by Fixen et al. (2015) in the global PNB analysis, with SSA having the highest values. APE_N of maize across the scenarios varied in the two catchments expressing the plants' ability to transform

nutrients applied into economic yield (Table 3). The increasing trend in APE_N values obtained in this study could suggest that an additional supply of more N inputs into the soils would lead to a positive contribution of the maize crop to transform the acquired N into economic yield. Therefore, achieving an optimal NUE for maize crops requires increment of N inputs as confirmed in this study and also agrees with the projection's analysis for Elrys et al. (2020) on Africa achieving food sufficiency in 2050. The APE_N values for ADS (50 kg N ha⁻¹) results in the Rangwe catchment agreed with Fixen et al. (2015), who reported APE_N optimal values of 40–60 kg grain kg⁻¹. The results are also supported by the findings of Snyder and Bruulsema (2007), who suggested that APE_N values >50 kg/kg could be obtained in low N use in some of the properly managed systems.

Effects of N Use Scenarios on Agronomic Performance of Maize

Our results on AE_N showed that by increasing the fertilization rate to 50 kg ha⁻¹ as the recommendation in the Abuja Fertilizer summit, there is a possibility of optimizing AE_N (Table 4), particularly for the Rangwe site though the optimal range was not achieved, and there was a significant milestone toward enhanced insights into the sustainability of the agricultural system. The decreasing trend of AE_N with increase in N fertilizer application rates indicates that with more N, there are possibilities of achieving an optimal value of AE_N for cereal crops between 10 and 30 kg grain (kg N)⁻¹. The AE_N values >30 kg grain (kg N)⁻¹ as reported by Vanlauwe et al. (2011) demonstrated that lower N rates resulted in higher AE_N in the African context, which strongly agrees with the findings of our current study. In addition to increasing N fertilizer input, hybrid maize varieties are also recommended to optimize AE_N for the region. Narrowing the agronomic use efficiency gap for the region can be achieved by increasing addition of N input coupled with improved management practices (Kuyah et al., 2021). According to Ahrens et al. (2010), higher values of AE_N result from fields with depleted soil N pools and are due to less fertilization, which is an implication that the current study did not achieve adequate N input to obtain optimized AE_N values. In addition, there is a need to understand other factors contributing to high AE_N beyond the optimal values at the farm level as a way of addressing the existing gap for example by including improving fertilization (Ahrens et al., 2010).

Influence of N Use Scenarios on Growth, Yield, and N Partitioning

The results indicate that more biomass could be achieved at higher N application. The findings also imply that increasing N fertilization reflected in the scenarios adopted in this study could result in more grain yield. Thus, this confirms that farmers need to change their current practices of little (or lack of) fertilizer application to more improved practices for higher yields to achieve food sufficiency and reduce the yield gap. Although the ADS of 50 kg ha⁻¹ results in incremental grain yield, exploring more alternative sources like animal manure and biological nitrogen fixation may be critical for the region for more

sustainable production. These results are in concurrence with findings of others (Abbasi et al., 2013; Chen et al., 2017; Hammad et al., 2017; Srivastava et al., 2018) who reported an increasing trend on yield and growth under influence of N application. The lower biomass accumulation at the vegetative stage reported in our study agrees with Mueller and Vyn (2018), who associated the low dry weight to reduced N uptake at vegetative as compared to silking and tasseling stages. Sen et al. (2016) also reported reduced biomass accumulation in maize crops grown in low N status compared to those supplied with N fertilizers. The higher biomass accumulation at the tasseling stage could be due to an increase in soil N status upon the second split application of the slow-release urea at the vegetative stage and the increased demand of N by the plants stage. Moreover, Wang et al. (2017) and Nasielski and Deen (2019) also reported a higher biomass accumulation of maize at reproductive stages under different N applications.

The increase in yield without any plateaus in the regression lines signifies the need for more N to achieve food sufficiency. However, in Rangwe (**Figure 2B**), the curve seems to plateau, which could result from other factors, including calcium (Ca) and potassium (K) that were relatively low in the site, hence affecting N in the test variety. The significant increase in grain yield with N rates confirmed the potential of meeting the food needs and reducing the yield gap if more N fertilizers were used (Pasley et al., 2019). In addition, the lack of a plateau in the yield response model indicates that increasing N fertilizers to 50 kg N ha⁻¹ could not be optimum, specifically for soils reported to have negative N balances like the case of SSA (**Figure 2A**). The variety (Sc Duma-43) used could also be more effective in scavenging for the available nutrients to promote growth and consequent positive increment in yield. Besides, the variety could be more effectively utilizing N use effectively although the rates used are lower to match the need for the crop. This may be a pointer that the rates used by the farmers are well below the plants' demands, which partially agrees with the low NUE in these sites (**Table 3**).

With optimal N management through proper application timing, specifically for slow-release fertilizers, there is a higher chance of obtaining a significant increase in yield (Grant et al., 2012; Davies et al., 2020). Although increasing application of N fertilizers may have a positive increase in both grain and stover yields, it may be difficult for some farmers in SSA due to financial constraints and therefore need for exploring alternative sources of N (IFDC, 2003; Pasley et al., 2019; Elrys et al., 2020). However, this can be made possible by increasing availability and subsidizing the cost of fertilizers, and exploring more organic sources of N. Therefore, policy instruments, including extension services, are critical for the region to offer technical efficiency in reducing the yield gap. This can be achieved by addressing N inputs management, including the timing for application for maximum as knowledge is limited (van Dijk et al., 2020).

The partitioning of about 50% of nitrogen into the root and the straw is an indicator practice of N recycling specifically for farms where plowing is done to incorporate both the straw and the roots in the soils after harvest (**Figures 3, 4**). These findings can be used to guide policy and decision-making on

straw management as a way of improving N management sustainability. Enlightening farmers through local extension services on the benefits of incorporating straws into the soils other than burning or feeding to livestock would be a milestone toward increasing nitrogen recycling into the cropping systems for the region.

The higher concentration of N in the grain is an indicator of N recovery from the cropping systems and the proportion of N that is exported from the field. The findings agree with Ning et al. (2017), who reported a significant decrease in N concentration in the stover and a higher increase of N concentration in the grain of maize both under low and high nitrogen inputs.

CONCLUSIONS

This is the first study to assess the Abuja 2006 fertilizer summit declaration from an African perspective on increasing fertilizer input, NUE optimization, and agronomic performance at a plot level and for specific crops. We conclude that the suggested fertilizer increment to 50 kg ha⁻¹ as spelt out in the Abuja declaration will slightly improve the growth and yield of maize, but is not sufficient to overcome the soil fertility decline, compared to other regions with plausible nitrogen management strategies coupled with strong policies. Negative N balances were also evident from this analysis, an indicator of higher N removal than the N input, indicating the presence of low N status that leads to soil N mining and degradation of the overall fertility and quality of the soil. In addition, the PNB showed increased soil N deficits in systems with values >1, an implication that more balanced N inputs are essential. Hence, policies should target higher N fertilizer levels to reduce the yield gap, optimizing the current NUE to a sustainable range. With the unlikelihood of most farmers achieving the Abuja recommendation rate, we recommend to explore other complementary sources of N such as animal manure and biological nitrogen fixation to improve the present scenario of low inputs into cropping systems within the region. In addition, we also recommended development of specific crop optimum rates as a basket of options and also take into consideration the socio-economic context of the smallholder farmers. Therefore, the findings of this study can be helpful in decision-making and policymaking to formulate new targets for fertilizers, particularly N input above 50 kg N ha⁻¹, to optimize NUE and reduce the yield gap for sustainability, and also focus more on integrated soil fertility management as a package for nutrient management in systems.

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

CM was the source of idea. MG, JG-O, JM, and GN reviewed the methodologies. NW collected the data, performed

statistical analysis, and wrote up the first manuscript. All authors reviewed the manuscript, provided input and suggestions in the text, and approved the submitted version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2021.758724/full#supplementary-material>

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Nitrogen budgets and nitrogen use efficiency as agricultural performance indicators in Lake Victoria basin

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Too little nitrogen (N) is a threat to crop productivity and soil fertility in sub-Saharan Africa (SSA). Nitrogen budgets (NB) and nitrogen use efficiency (NUE) are critical tools for assessing N dynamics in agriculture and have received little or no attention in the region. Data were collected from smallholder farmers clustered into two categories, farmers applying and farmers not applying N fertilizers. NB were calculated using the Coupled Human and Natural Systems (CHANS) model approach for field and farm spatial scales. The results showed spatial variabilities in NB and NUE at the field level (maize and rice) across all the catchments. At the field level, N balances were negative for the two crops in all the catchments. Similarly, at the farm gate, a deficit of $-78.37 \text{ kg N ha}^{-1}$ was observed, an indicator of soil N mining. NUE values at the field scale varied across the catchments for both crops, with values for maize grown without N ranging from 25.76 to 140.18%. Even with the application of mineral N at higher levels in rice fields compared to maize fields, NUE values ranged between 81.92 and 224.6%. Our study revealed that the Lake Victoria region suffers from inefficient N cycling due to depleted soil N pools and low synchrony between N input and N removal. Therefore, a challenge lies in exploiting more sustainable N sources for farmers in the region for sustainable farming systems. The NB and NUE provide critical information to agriculture stakeholders to develop environmental, agronomic, and economically viable N management solutions.

KEYWORDS

cropping system, mineral fertilizer, N losses, N management, soil depletion

Introduction

In recent decades, researchers have developed an increasing interest in understanding the role of nitrogen (N) cycling in agricultural systems, which involves mineral N inputs for improved crop production (Atieno et al., 2020). In sub-Saharan Africa (SSA), N constitutes 90% of the applied fertilizer (Sutton et al., 2013) and is sometimes accompanied with a little phosphorus (P) and potassium (K) but rarely with

secondary or micronutrients (Swarbreck et al., 2019). In SSA, soil fertility is declining due to several years of crop nutrient mining and limited replenishment of nutrients either taken up or lost from the soil (Jones et al., 2013). This imbalance between input and output from the soil system has resulted in extreme nutrient deficits in croplands, threatening agricultural production and associated ecosystem functions (Reynolds et al., 2015). According to Jayne and Sanchez (2021), although the current average use of N fertilizers has increased over the past decades the amount is still very low at $17.9 \text{ kg N ha}^{-1}$ and not enough to compensate for the harvested/exported nutrients. Therefore, an initial challenge in SSA is to provide more nutrients into their farms and building the soil health through soil organic matter (Vitousek et al., 2009).

Monitoring N fluxes in ecosystems is crucial for improved N management (Nimmo et al., 2013). Quantifying nitrogen budgets (NB) is the first step toward improved N management as it helps identify the significant N sources and sinks and inform sound N management practices and policies (Zhang et al., 2020). It is becoming succinctly clear that assessing the fate of applied N is crucial for effectively constructing partial and complete NB at both field and farm gate scales (Quemada et al., 2020). Further, understanding N balance is crucial for evaluating N performance and developing strategies for reducing its losses to the environment (McLellan et al., 2018).

NUE is used as a performance indicator to show the relationship between N inputs and agricultural products obtained from the system. It also indicates potential losses of reactive N (N_r) to the environment as farmers strive to improve crop yields to meet the increasing demand for food, feed, fiber, and fuel (Fixen et al., 2015). The concept of NUE is critical in evaluating crop production systems and is impacted by soil, plant, irrigation, and fertilizer/nutrient management (EUNEP, 2015). Poor N management practices contribute to un-optimal NUE due to poor synchrony between applied N and the crop demand (Sharma and Bali, 2017). According to Ntinyari et al. (2022a), NUE can be used as a reasonable indicator to show the target threshold with good management and close simulation of farmer practices for improved N performance. Presentation of NUE as a percentage or mass fraction of N input, output, surplus, and possible changes in the N stocks in the soil system would provide a specific indicator for improved N management (EUNEP, 2015).

In East Africa, N is the most limiting nutrient due to farmers' inability to afford and apply the recommended N fertilizer rates in the cropping systems (Chianu et al., 2012; Masso et al., 2017). The low N fertilizer application to crops in the region, the export of N with harvested crop product, and losses of N through different pathways such as volatilization, runoff, and leaching contribute to soil nutrient depletion and mining of nutrient stock (Zingore et al., 2015; Masso et al., 2017). Maize and rice rank second and third, respectively, following wheat in terms of their contribution to per capita calories consumed, food

supply, harvest area, total production, quantity imported, and Africa's share of the global import (Sileshi and Gebeyehu, 2021). Thus, the two crops are critical for meeting the United Nations Sustainable Development Goals (SDGs) on food security and poverty reduction in East Africa and across Africa (Majiwa, 2017; Sileshi and Gebeyehu, 2021).

Further, management of N input, outputs, and use efficiency in the Lake Victoria basin is crucial because the basin carries more than 20% of East Africa's population, and the Lake Victoria freshwater supports more than 4 million people through annual fishery production of about 1 million tons in East Africa in addition to being a source of river Nile. N loading is associated with declining lake water quality and the growth of water hyacinth (*Eichhornia crassipes*). Improving understanding of N dynamics from the Lake Victoria basin for significant cropping systems would be a crucial step toward reducing N accumulation and loading into Lake Victoria, thus managing potential negative impacts. Therefore, there are profound food security, economic and environmental impacts of improved N management in the Lake Victoria basin, for large populations in East Africa, and other areas where Lake Victoria is used as a source of water for humans, animals, and irrigation.

There are concerted efforts by the African governments and development partners to promote the increased use of fertilizers to close the yield gaps of most essential crops like maize and rice (AGRA, 2019). Consequently, fertilizer use has increased from 8 kg nutrient/ha in 2006 to slightly above 20 kg nutrient/ha in 2019 (AFDB, 2020; Ntinyari et al., 2022a). There is an overdue need to understand the sources, fate, and efficiency of N use in densely populated African agricultural regions to support future planning and investments. To our knowledge, there is no current information on NB and NUE for the smallholder, specifically maize and rice in East Africa. Therefore, the findings of this study will provide information to various stakeholders to facilitate the development of nutrient management strategies and policies that increase N management in cropland while reducing N losses to the environment. Based on this background, we hypothesized that estimation of N budgets at field and farm gate would give an explanation and interpretations on N cycling and management, reflecting smallholder farmer's scenarios in the Lake Victoria basin, which could trigger better adoption of measures for sustainable N management at farm level.

Materials and methods

Data sources and study site description

Data used in this study were obtained from participatory interviews using open-ended semi-structured questionnaires in an open data kit (ODK) as described by Ntinyari et al.

(2022b). A total number of 447 observations comprising of 154 farms, 135 rice fields and 158 maize fields were compiled from four catchments of the Lake Victoria basin, namely: Nyando, Sondu, Yala, and Nzoia. Each farm selected represented a farmer per household and the selected sites represented 50 % of the entire mass land area around the lake region on the Kenyan side. The Lake Victoria basin extends to five Eastern African countries (Burundi, Kenya, Rwanda, Uganda, and Tanzania), covering an approximate area of 194,000 km² (Kayombo and Jorgensen, 2006). The basin is inhabited by some of the most resource-constrained Eastern African rural populations, with an approximate population of 30 million and a projected annual increase of 6% (Kayombo and Jorgensen, 2006; Zhou et al., 2014). More than 70% of the population in the basin is involved in agricultural production activities. The main staple food crops within the catchment are maize and rice growing in predominant soil types, including Ferralsols, Nitisols, Vertisol, Cambisols, and Acrisols (Nkonya et al., 2015). The climate is equatorial, with temperatures modified by the high elevation of lakes and mountains like Mt. Elgon. However, since the temperatures are lower than the typical tropical conditions, it is classified as humid, with temperatures ranging from 20°C to over 35°C. The region experiences bimodal rainfall, with short rains occurring between mid-March and the end of May and long rains occurring from mid-October to the end of December. Annual rainfall quantities range from 1,000 to 1,500 mm in a year and lie at an elevation of 1,500 m (4,921 ft) (Okungu et al., 2005).

Farm and field sample selection

The farmers interviewed at field level that was defined as a crop, i.e., maize and rice as monocrop was drawn from the population given by the extension officers within the local region. The sample was selected using a purposive sampling technique, a non-probability sampling method that is selective to identify and choose information-rich participants. The purposive sampling procedure was followed by random engagement of the farmers for subsequent in-person interviews.

A similar selection approach defined at the field level was used at the farm level. Here a mixed farming system was considered, including all kinds of crops a farmer grows, mostly cereal-legume system and livestock and manure use practices. Data collected from fields and farms were categorized into two: i.e., Farmers applying mineral N fertilizers and farmers not applying mineral N fertilizers in their fields or farms. All collected data for N inputs and outputs at the field and farm level was reported as kg N ha⁻¹. The collected data included: land size, N inputs from fertilizers, the quantity of planted seeds, crop growth seasons, yield, and various planted crops.

Data collection field scale for maize and rice

At the field level, soil surface N budget (NB) and soil system NB approaches were used to quantify NB for maize and rice, as reported by Oenema et al. (2003). In each category of the N budget, there was the characterization of farmers applying and not applying mineral N. The soil surface NB considers all the significant N fluxes entering the soil *via* surface and only leaves the soil through crop uptake (Eq. 1). The soil system NB includes all N inflows and outflows, including N uptake, exported N harvested with the crop, and losses within and from the soil surface, as reported by EUNEP (2016) and shown in Eq. 2.

$$\text{Soil surface NB} = N_{\text{inputs}} (\text{SNF} + \text{ADN} + \text{NPM}) - N_{\text{outputs}} (\text{CNR}) \quad (1)$$

$$\text{Soil system NB} = N_{\text{inputs}} (\text{SNF} + \text{ADN} + \text{NPM}) - N_{\text{outputs}} (\text{CNR} + \text{Lch} + \text{RF} + \text{DNT} + \text{NH}_3 + \text{N}_2\text{O}) \quad (2)$$

Where; NB, nitrogen budgets; SNF, synthetic N fertilizer; AND, atmospheric N deposition; NPM, nitrogen in planting materials; CNR, crop N removal; Lch, leached N; RF, runoff; DNT, denitrification.

Data collected through field surveys included land size, N inputs as mineral fertilizers, the quantity of planted materials, yield, seasons, legume crops grown, and any other crops grown by the farmers in the fields. The actual rate of N applied from different N fertilizers sources was calculated by dividing the fertilizer application rate per ha by the proportion of N in the fertilizer used. The proportion of N in the fertilizer used is indicated in the local fertilizer grades, as shown in [Supplementary Table S1](#). The N in straw recycling was not considered in this budget because it is common for farmers around the catchment to burn crop residues. The N in irrigation water was not accounted for due to a lack of information on actual or estimated water supplied per hectare per growing rice season. Livestock manure as an N input source was not included in this budget because the majority of the farmers in the catchment area were practicing communal grazing. In many cases, the livestock excreta on overnight grazing are used as fuel, and others burn the droppings. BNF fixation was excluded because leguminous crops were not integrated into the target maize and rice plots as the focus was mainly on maize and rice monocrops.

Wet and dry atmospheric deposition of N was obtained from direct measurements from Lake Victoria (Kayombo and Jorgensen, 2006; Bakayoko et al., 2021). To calculate N input in planting material, the actual seeding rate in kg ha⁻¹ was multiplied by the N content for maize and rice seeds, while harvested N was determined by multiplying the respective N content for the specific crop and the amount of yield obtained from the fields as shown in [Supplementary Table S2](#). This was

achieved using a tiered approach to estimate various N inputs from the planting materials and harvested products.

For soil system budget, N loss in soils from applied mineral N fertilizer through ammonia (NH_3) volatilization and nitrous oxide (N_2O) emissions were calculated using country-specific emission factors according to Intergovernmental Panel on Climate Change (IPCC) (FAO, 2001; Bouwman et al., 2002) (Supplementary Table S3). To estimate gaseous emission, net N inputs were multiplied by the emission factors according to Eq. 3 (Eggleston et al., 2006). Soil denitrification was estimated by the N balance method using global estimates for upland and wetland crops (Supplementary Table S4) (Hofstra and Bouwman, 2005), and leaching and runoff losses of applied N were estimated using the IPCC factor of 0.3 kg N ha^{-1} of mineral N (FAO, 2001; Wang et al., 2019).

$$\text{N}_2\text{O} / \text{NH}_3 \text{ emission from applied SNF} = \text{Net N input applies} \\ \text{ha} - 1 \times \text{EF} \quad (3)$$

Where; SNF, synthetic N fertilizer; EF, emission factors.

Farm-gate spatial scale data collection

The farm-gate N budget was determined considering all the N sources flowing into the farm-gate as N inputs and leaving the farm-gate as N output (Eq. 4). For farm-gate, all crops grown by the farmer were considered in budgeting. The parameters collected and estimated for this scale included applied mineral N fertilizers, the quantity of planted seeds, seasons for specific crops, the yield for all crops, and the total land under agricultural production. At farm-gate, BNF was determined by multiplying the crop area under legume production by the global mean rate of N_2 fixation for each legume type as described by Smil (1999) (Supplementary Table S5). All N inputs and outputs were converted to kg N ha^{-1} . In this study, vegetables and fruits were excluded due to a lack of data on actual yield in kgs and analyzed N contents to estimate N removal by crop. N loss via gaseous NH_3 and N_2O emission was calculated using the same Emission Factors (EF) used for field level and denitrification rates for upland and wetland crops. There was no consideration of imported and exported feeds N because livestock were openly grazed, and feed trade was rare.

$$\text{FNB} = \text{N inputs (including all sources of N entering a farm)} \\ - \text{N (N sources leaving the farm and associated} \\ \text{N Losses)} \quad (4)$$

Where; FNB, Farm N balance.

Field nitrogen use efficiency

NUE in the field was determined using European Union Nitrogen Expert (EUNEP) methodology. EUNEP denotes that NUE is a ratio between harvested N in crops divided by the total sum of N inputs, including N from fertilizer, atmospheric N deposition, and N in the planted seeds (Eq. 5) (EUNEP, 2016).

$$\text{NUE} = \frac{\text{N output}}{\text{N input}} * 100 \quad (5)$$

Where; NUE, nitrogen use efficiency.

Data analysis and nitrogen budget estimations

All statistical analyses were performed with R Software (R Core Team R, 2020). The Least Square Means were computed using “lsmmeans” packages. Means were separated by adjusted “Tukey’s method using “cld” function from “multicompView” package for N inputs, N outputs, NUE, and N balance ($p < 0.001$). Mean distribution for N inputs, outputs, NUE, and N surplus/deficit were analyzed using the ggplot command from the ggpubr package. The Coupled Human and Natural Systems (CHANS) model version 1.3 (Gu et al., 2015) for the cropland subsystem was applied to estimate N budgets at different spatial scales. The choice of this model was based on its fundamental principle of mass balance of N fluxes in a whole system and each subsystem. CHANS model also accommodates data fluxes that are missing or could not be estimated, more so in Lake Victoria, where data on N flows is limited. In this study, the CHANS model was divided into two functional groups; N inputs and N outputs in the cropland subsystem.

Results

Maize fields N balances

For the soil surface, the maize farmers who do not apply mineral N fertilizers, the atmospheric deposition represented the primary flow with an average N input of $15.0 \text{ kg N ha}^{-1}$ (Table 1). In this type of N budget, farmers without application of mineral N recorded negative balances in Nyando and Sondu with average values of -3.45 and $-4.29 \text{ kg N ha}^{-1}$, respectively.

Similarly, farmers applying mineral N recorded negative balances for soil surface N budget in Nyando catchment. Despite the addition of N, the negative N budget in Nyando implies that more N was removed from the system than that added with fertilizers, an indicator of soil N mining. Among farmers who apply N fertilizers, positive balances were reported in three catchments, Nzoia, Yala, and Sondu, with average values of 13.27 , 18.04 , and $19.84 \text{ kg N ha}^{-1}$, respectively (Table 1).

TABLE 1 Soil system N budget for maize with and without mineral N fertilizer applications in four Lake Victoria catchment.

N inputs (Kg N ha ⁻¹)	Without mineral N				With mineral N			
	Nyando	Nzoia	Yala	Sondu	Nyando	Nzoia	Yala	Sondu
SNF	0	0	0	0	10.42	22.99	17.33	17.31
NPM	0.12	0.09	0.17	0.14	0.13	0.07	0.19	0.14
AND	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Sum _{Ninputs}	15.12	15.09	15.17	15.14	25.55	38.0	32.52	32.45
N outputs (Kg N ha⁻¹)								
CNR	18.57	9.56	3.57	19.43	33.38	24.79	14.48	12.61
NH ₃ emissions SNF	–	–	–	–	10.04	4.37	3.29	3.29
N ₂ O emissions SNF	–	–	–	–	0.69	0.30	0.22	0.22
Denitrification	15.0	15.0	15.0	15.0	17.0	17.0	17.0	17.0
Leached N	–	–	–	–	15.84	6.89	5.20	5.19
Runoff N	–	–	–	–	15.84	6.89	5.20	5.19
Sum _{Noutputs}	33.57	24.56	18.57	34.43	92.79	60.24	45.39	44.21
N balance (soil surface)	–3.45	+5.53	+11.6	–4.29	–7.83	+13.27	+18.04	+19.84
N balance (all)	–18.45	–9.47	–3.43	–19.29	–67.24	–22.24	–12.87	–11.76

Dash (–) means parameter could not be estimated due to data unavailability on fertilizer application. SNF, synthetic nitrogen fertilizer; NPM, nitrogen planting materials; AND, atmospheric deposition nitrogen; CNR, crop N removal; emission factors (EFs) applied for gaseous losses are only limited to applied SNF.

Both farmer categories were associated with negative N balances in soil system NB. In farmers not applying mineral N fertilizers, N balances ranged between -3.43 to -19.29 kg N ha⁻¹ across the four catchments areas, as shown in Table 1. More pronounced N losses were recorded to fertilizers associated outflows in the farmers applying mineral N categories. In Nyando balance of -67.24 kg N ha⁻¹ was recorded, indicating severe soil N depletion and mining in the farms. Soil system had higher N losses to significant outflows from denitrification, leaching, runoff, and N₂O and NH₃ emissions, pointing to the importance of appropriate soil management.

Rice fields N balances

In rice fields, soil surface NB showed variabilities across the catchments (Table 2). N balance was more pronounced in Sondu, with a value of -35 kg N ha⁻¹, while Nzoia showed a positive balance ($+31.94$ kg N ha⁻¹). The positive N balance in Nzoia could indicate N sufficiency Nyando and Nzoia had the highest mineral N application rates of 102.19 and 77.78 kg N ha⁻¹, respectively. The N in crop removal formed the largest N outflow across the three catchments in the two budget systems (Table 2). At soil system balance, all catchments recorded negative balances of -148.42 , -77.31 , and -51.88 kg N ha⁻¹ in Nyando, Nzoia, and Sondu, respectively (Table 2).

Farm-gate N balances

Farms without mineral N application, had the main N input from atmospheric deposition (15.0 kg N ha⁻¹) and biological N fixation with 6.99, 14.76, and 16.7 kg N ha⁻¹ for Nyando, Nzoia, and Sondu, respectively. At soil surface N budget, negative balances were observed in Nzoia for farms with no N fertilizer applications with value of -0.53 kg N ha⁻¹. In contrast, in Nyando and Sondu, the N budget was $+6.35$ and $+6.63$ kg N ha⁻¹, respectively. Conversely, in farms with mineral N application, positive balances of $+26.59$ and $+41.98$ kg N ha⁻¹ were observed with Nzoia and Sondu, respectively. In comparison, Nyando had a negative balance of -12.92 kg N ha⁻¹ (Table 3) due to increased N losses.

In Nyando, Nzoia and Sondu for the category of farmer using fertilizers, mineral N fertilizer was the dominant source of N input with 60.34, 46.07, and 49.42 kg N ha⁻¹, respectively. Farm-gate N balances were characterized by negative budgets for farm categories with and without mineral N fertilizer application. For farms without N fertilizers, the highest N balance was recorded in Nzoia, while the lowest value was Sondu (Table 4).

For farms where farmers applied N fertilizers, more losses and the largest negative balances were observed in Nyando with a mean value of -78.37 and the lowest in Yala with a mean value of -13.69 kg N ha⁻¹. In the two farm categories, the largest N outflow was recorded in crop removal (Table 4).

TABLE 2 Rice soil surface and soil system N budget in main rice-growing catchments in Lake Victoria basin.

Rice N inputs (Kg N ha ⁻¹)	Soil surface N budget			Soil system N budget		
	Nyando	Nzoia	Sondu	Nyando	Nzoia	Sondu
SNF	102.19	77.78	17.44	102.19	77.78	17.44
PNM	1.1	0.95	0.39	1.1	0.95	0.39
AND	15.0	15.0	15.0	15.0	15.0	15.0
Sum _{Ninputs}	118.29	93.73	32.83	118.29	93.73	32.83
N outputs (Kg N ha⁻¹)						
CNR	129.93	61.79	68.29	129.93	61.79	68.29
NH ₃ emissions SNF	–	–	–	21.46	16.33	3.66
N ₂ O SNF	–	–	–	13.49	10.26	2.30
Leached N	–	–	–	30.66	23.33	5.23
Runoff N	–	–	–	30.66	23.33	5.23
Denitrification	–	–	–	54	36	36
Sum _{Noutputs}	129.93	61.79	68.29	266.71	171.04	84.71
N Balance	–11.64	+31.94	–35.46	–148.42	–77.31	–51.88

SNF, synthetic nitrogen fertilizer; NPM, nitrogen planting materials; AND, atmospheric deposition nitrogen; CNR, crop N removal; no category for farmers without mineral N use for rice farmers. Emission factors (EFs) applied for gaseous losses are only limited to applied SNF.

TABLE 3 Soil surface N budget for farms with and without mineral N applications.

N inputs (Kg N ha ⁻¹)	Without mineral N			With mineral N		
	Nyando	Nzoia	Sondu	Nyando	Nzoia	Sondu
NPM	0.38	0.64	0.50	1.52	0.49	1.49
SNF	0	0	0	60.34	46.07	49.42
BNF	6.99	14.76	16.7	7.5	22.07	18.88
AND	15.0	15.0	15.0	15.0	15.0	15.0
Sum _{Ninputs}	22.37	30.40	32.20	84.36	83.63	84.79
N outputs (Kg N ha⁻¹)						
CNR	16.02	30.93	25.57	97.28	57.04	42.81
Sum _{Noutputs}	16.02	30.93	25.57	97.28	57.04	42.81
N balance	6.35	–0.53	6.63	–12.92	+26.59	+41.98

SNF, synthetic nitrogen fertilizer; NPM, nitrogen planting materials; AND, atmospheric deposition nitrogen; CNR, crop N removal; BNF, biological N fixation.

Total N inputs, outputs, and NUE in maize and rice fields

In maize fields without application of mineral N fertilizer, analysis of variance for the catchments did not show significant effects of non-application of N fertilizers on total N inputs, outputs, and NUE ($p < 0.001$). Mean N inputs for the catchments ranged between 15.79 and 15.87 kg N ha⁻¹ (Figure 1). Similarly, the mean NUE for maize farms without N fertilizers in Nyando and Sondu exceeded 100% with 132.22 and 140.18%, respectively. In Yala, a lower mean NUE was recorded at 25.76%, while Nzoia had a close to optimal NUE of 69.21%. The NUEs also varied significantly across all the catchments for maize farmers with N fertilizer application. The NUE for

N fertilizer applied farms were a lesser variable as they ranged between 34.6 and 76.3 %.

Significant differences ($p < 0.001$) were observed in the data set for total N inputs, outputs, and NUE in fertilized rice fields. The highest mean total N inputs were observed in Nyando at 117.0 kg N ha⁻¹, while the least was in Sondu with 31.53 kg N ha⁻¹ (Figure 3). A similar trend was observed in the total N outputs data set, where Nyando had the highest mean value of 279.2 kg N ha⁻¹, whereas Sondu had the least mean of 120.7 kg N ha⁻¹.

In Nyando and Sondu, the mean NUE exceeded 100%, with Sondu having an extreme value of 224.6% (Figure 3C). Low N inputs could explain the extremely high NUE values in Sondu catchment in rice systems. Severe N deficits were observed in all

TABLE 4 Nitrogen budget for two categories of farms with and without mineral N applications.

N inputs (kg N ha ⁻¹)	Without mineral N			With mineral N			
	Nyando	Nzoia	Sondu	Nyando	Nzoia	Yala	Sondu
SNF	0	0	0	60.34	46.07	15.87	49.42
NPM	0.38	0.64	0.51	1.52	0.49	1.05	1.49
AND	15.0	15.0	15.0	15.0	15.0	15.0	15.0
BNF	6.99	14.76	16.7	7.5	22.07	23.22	18.88
Sum N_{inputs}	22.37	30.40	32.20	84.36	83.63	55.14	84.79
N output (kg N ha⁻¹)							
CNR	16.02	30.93	25.57	97.28	57.04	37.81	42.81
NH ₃ emissions SNF	–	–	–	11.46	8.75	3.01	9.39
N ₂ O emission SNF	–	–	–	0.79	0.61	0.21	0.65
Leached N	–	–	–	18.1	13.82	4.75	14.82
Runoff N	–	–	–	18.1	13.82	4.75	14.82
Denitrification	15	15	15	17.0	17.0	17.0	17.0
Sum $N_{outputs}$	31.02	45.93	40.57	162.73	111.04	67.53	99.49
N balance	–8.65	–15.53	–8.37	–78.37	–27.41	–12.39	–14.70

Dash (–) means parameter could not be estimated due to data unavailability on fertilizer application. SNF, synthetic nitrogen fertilizer; NPM, nitrogen planting materials; AND, atmospheric deposition nitrogen; CNR, crop N removal; BNF, biological N fixation emission factors (EFs) applied for gaseous losses are only limited to applied SNF.

catchments for rice fields, with Nyando having a twofold highest negative balance of $-162.2 \text{ kg N ha}^{-1}$, relative to Sondu with $-89.2 \text{ kg N ha}^{-1}$ and Nzoia with -78 kg N ha^{-1} (Figure 3D).

Discussion

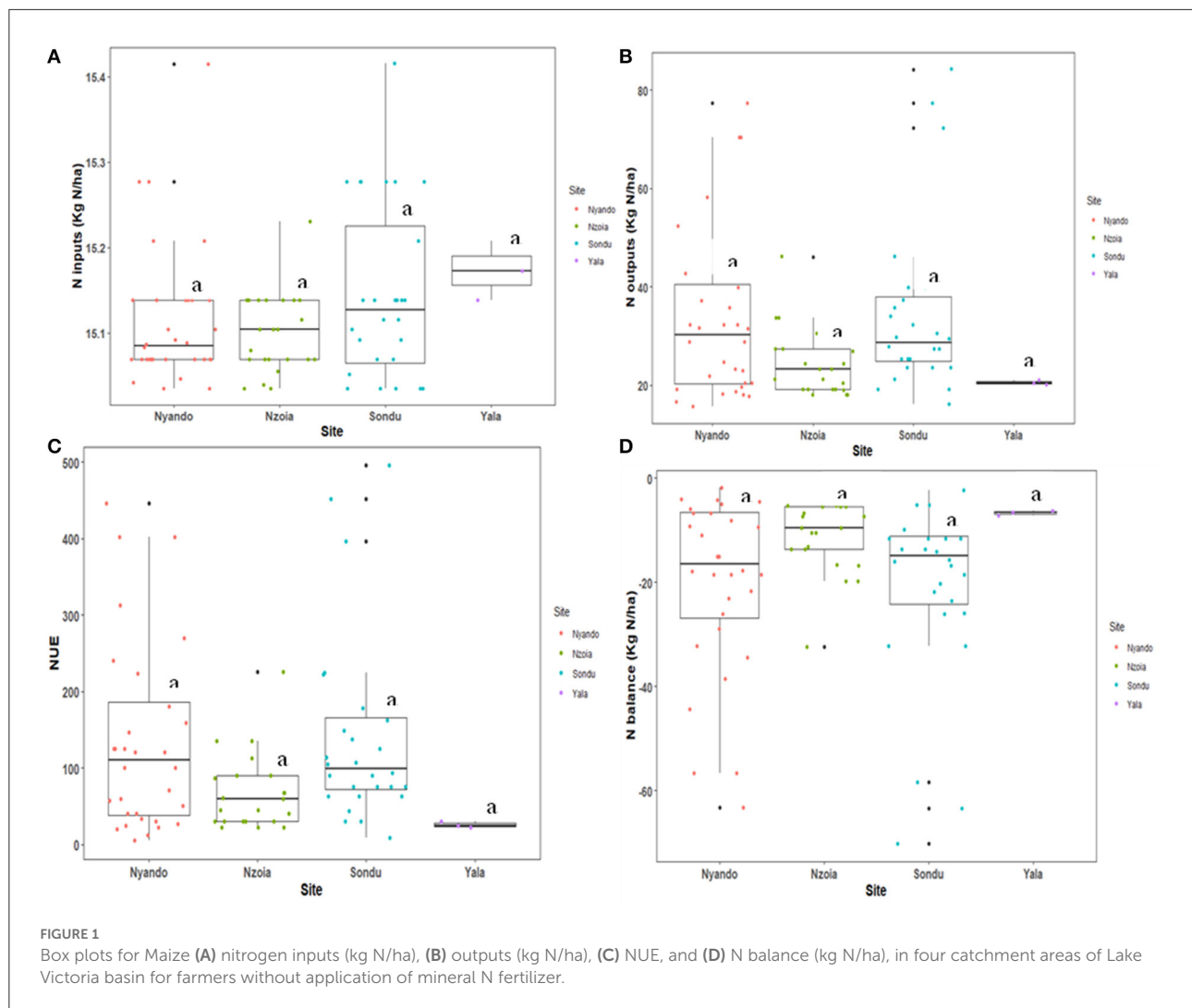
At the field level, estimated N balances in the two categories of farmers showed variability across the study catchments. The soil surface, that represented sources of N input and considered crop removal as the only output, NB ranged from -3.45 and $-4.29 \text{ kg N ha}^{-1}$ for maize farmers without mineral N application (Table 1). The negative values from our study are an indication of an insufficient amount of N in the systems contributing to further N depletion (Nkonya et al., 2005). The imbalances across farms can be attributed to poor management practices within the region, low availability, and low potential to purchase adequate N inputs. The maize fields with mineral N fertilizers application had a positive N balance except for Nyando with $-7.84 \text{ kg N ha}^{-1}$. The positive balances are due to more N applications than in Nyando.

Extreme N losses and variations at soil system NB between maize fields with and without were observed. The fields without mineral N application category had lesser negative ranging from -3.43 to $-18.45 \text{ kg N ha}^{-1}$ (Table 1), compared with the fields with mineral N application, more N losses were anticipated due to estimated losses from fertilizer applications (-11.76 to $-67.24 \text{ kg N ha}^{-1}$). Specifically, in Sondu catchment, the higher crop export from the category of farmers without mineral N could be due to more mining of nutrients from the soil by the

crops. Also, there could be more losses of applied N into the cropping system leading to less N available to be utilized by the crop and also depending on the time of application by the farmers as a management practice. Chianu et al. (2012) reported that exporting more nutrients than those applied is one of the most important causes of negative nutrient balances and soil N depletion in African agriculture.

In rice systems, the soil surface N budget, there was a positive balance of $+13.94 \text{ kg N ha}^{-1}$ (Table 3), in Nzoia catchment. In this site, the average N input from mineral fertilizers was $77.78 \text{ kg N ha}^{-1}$, this is an indicator that this range could be optimal to correct the negative balances in the soils but has to be coupled with the proper management practices, including the 4 R (right rate, right source, right placement, and right timing) stewardship. The high use of N fertilizer in both Nyando and Nzoia catchments on rice fields is closely associated with the influence of the National Irrigation Board (NIB), which supports farmers with farm inputs on credit and at subsidized rates, compared to Sondu, where rice fields are under individual farmer management.

More significant negative balances were observed in the rice soil system N budget (Table 2). The negative balances could result from various mechanisms of N losses: first, as these catchments are characterized by regular cycles of flooding and drying/wetting cycle; the wetness and flooding, cycles could elicit nitrification and denitrification processes due to changing soil gas diffusivity and water-filled pore spaces leading to enhanced losses of N as N₂O (Tan et al., 2005). High positive balances are not always desirable as they indicate excess N left in the system, susceptible to losses into surface/groundwater as NO₃–, or



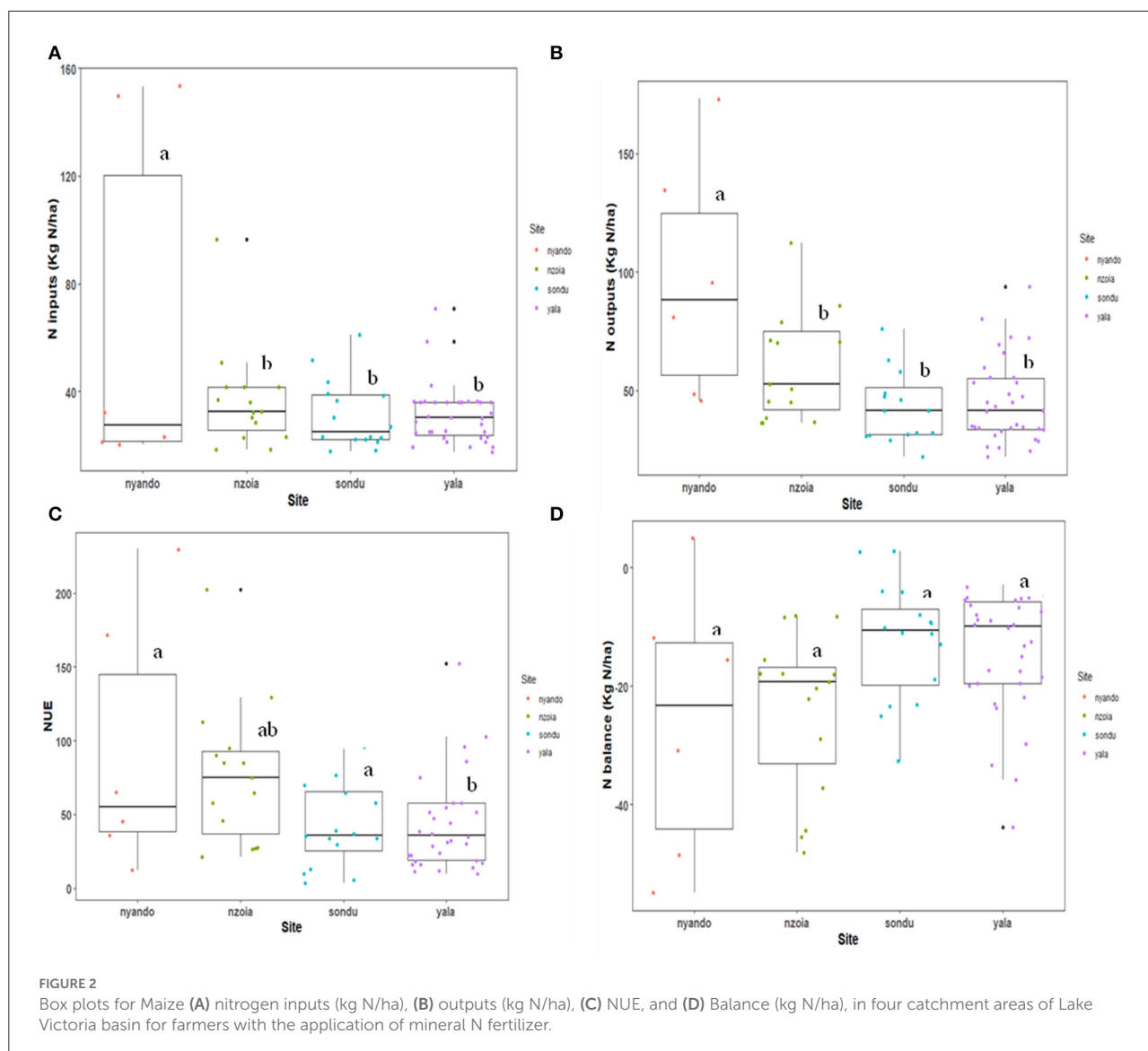
atmosphere as N_2O or NO_x emission, leading to environmental degradation (Dalggaard et al., 2017). All rice farmers in our case study had access to mineral N fertilizers due to the regulation, and the farmers could access more inputs in a coordinated manner. The regulation of rice farming by the National Irrigation Board (NIB) is one of the strategies that could be embraced in other cropping systems to guide management.

Without considering soil N transformations, the soil surface NB at farm scale shows an improved N content in the cropping systems since a positive surplus remains in the soil (Table 3). Considering the soil surface, NB positive balances were recorded at the farm level in farms with and without mineral N application). In farms without mineral N application, the primary source of N inflow was from atmospheric deposition of N estimated to be 15 kg N ha^{-1} . In addition, BNF estimated was also very low, ranging from 6.99 to $16.7 \text{ kg N ha}^{-1}$. Therefore, this category of farmers need to explore other sources of the organic source to replenish their fields and increase

N availability in the soils for higher crop yield and balanced nutrition because this particular inflow is too low and because of the high cost of fertilizers.

The soil system NB for farms recorded a deficit of $<50 \text{ kg N ha}^{-1}$ in the category of farmers not applying mineral N, while the farmer using mineral none catchment exceeded a deficit of above -50 kg N ha^{-1} (Table 4). The negative values indicated the extent of N depletion due to inadequate N supply, while positive values showed a loss of N that was taken up by the intended crop. Low or no N inputs among farmers could be attributed to the lack of policies by national and local governments to enhance affordability, accessibility, and use of mineral fertilizer in various cropping systems (Ciceri and Allanore, 2019).

NUE represented the efficiency of N in production process and showed in different categories of fields (maize and rice; either applying or not applying fertilizers) indicated poor efficiency of the available N resource in the cropping systems



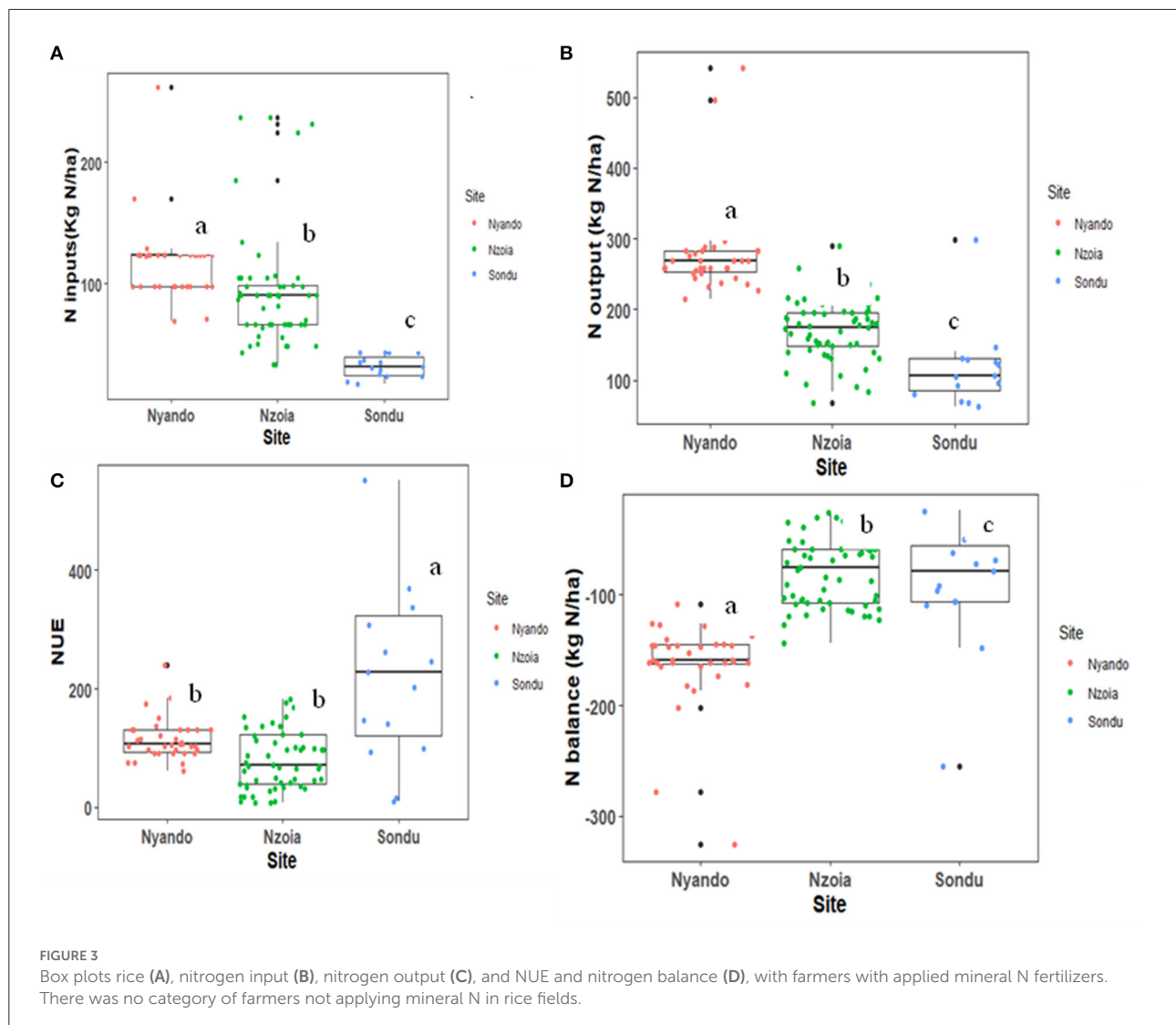
(Figures 2, 3). NUEs exceeding 100% in Nyando and Sondu for maize farmers (Figure 1C) not applying mineral N represented excessive N mining in the fields.

On the contrary, in rice fields where farmers had access to mineral N, there was higher NUE (224.6 %) in Sondu (Figure 3C). The higher NUE value is due to low N input and higher N output in crop harvest. This demonstrates that, despite the addition of mineral N, there must be a balance to match available soil N and crop demand. Higher soil N mining in fertilized rice fields may be due to higher biomass accumulation with more available N in the soil influencing crop N uptake, whereas this is not the case in non-N applied fields.

According to EUNEP, a threshold for assessing the efficiency of N in cropping systems is between 50 and 90%, which most scenarios in our studies exceeded, and thus this shows the need

for better strategies to optimize the NUE. These findings agree with Edmonds et al. (2009), on, estimated values of NUE are more than 100% in cereal production systems, especially in rice, due to low average application rates of mineral N that leads to a decline in soil fertility. Omara et al. (2019) revealed that high fertilizer applications in cereals result in lower NUE, while low or zero N inputs lead to extremely high depletion of the N pool in the soils. According to Bruulsema et al. (2009), it is also possible to apply sufficient N and boost N use efficiency through split application and adherence to 4R nutrient stewardship, which is crucial for closing the crop yield gaps without significant high losses of N to the environment.

Overall, our findings exhibited spatial variability on the indicators based on the four catchments under study. In Nyando, all the cases showed an extreme deficit for NUE in



both fields with and without the application of mineral N fertilizers. This could be due to different soil properties in the regions characterized and defined by Ntinyari et al. (2022a). Vertisol in Nyando, Fluvisols in Sondu, humic gleysols in Nzoia, and Ferralsols in Yala. In Nyando, the Vertisol having high N requirements resulting in more N mining in both fields with mineral N and without for both crops. Besides, The soils in Nyando have a higher clay percentage with swelling, shrinking, low soil organic carbon and deep cracking property causing deterioration in soil health; hence, more improved and sustainable practices for improved N cycling within the region. Therefore, these differences in soil properties could contribute to the variabilities in the two indicators assessed for the various catchments.

The relevance of NB in assessment of N management at the field and farm level as per the analysis of the current study is

to help understand N cycling and assess the efficacy of applied measurements through identifying deficits and surplus in the systems. This is achieved through quantifying the main fluxes in an input-output model as defined by either field or farm boundaries (Cameira et al., 2019). Moreover, it provides linkages between agricultural N uses and the losses to the environment. Furthermore, NB provides policymakers with a tool to monitor environmental impacts resulting from agricultural production and make informed choices. On the other hand, NUE, as determined in this study, is useful in evaluating the efficiency of applied N in the systems by defining the regions of soil N mining, N inefficiency, and N neutrality. As revealed from this analysis, NUE represented high values showing inefficiency for both fields with mineral N application and without. More pronounced values above the threshold were evident in rice farmers where mineral N was highly applied.

Implications and practical recommendations for N use and management

The results of this study show atmospheric deposition as the primary source of N in the Lake Victoria region in the majority of farms and fields where farmers do not use any mineral N. Managing N sources from atmospheric deposition could be challenging since a significant proportion may end up in unintended areas, for instance, water bodies or uncultivated land where runoff is more likely causing more pollution to the environment. Given that the N use of fertilizer in sub-Saharan Africa is low with many cases of no use, these results indicate a need for improved use of fertilizers to change farm N balances to +ve. Across the two cropping systems, the soil system registered higher N losses than the soil surface, implying significant outflows from denitrification, leaching, runoff, and N₂O and NH₃ emissions and pointing to a need for improved N management. These results affect environmental management, policy-making, and optimal agricultural resource management. These results could potentially be applied in projecting N dynamics for over 3 million hectares where maize and rice are grown in this region.

Several cases presented herein have indicated significantly higher negative balances in the N applied farms relative to non-N applied plots. The results indicate significant losses of applied N to the environment with a potential to pollute water bodies and contribute to increased N₂O emission (Tables 1–3). Furthermore, negative balances imply poor management, which results in the extraction of available N resources. This implies that farmers will require widespread capacity building in order to implement NUE optimizing practices. Furthermore, managing N losses could be accomplished through good agronomic practices such as integrated soil fertility management to improve nutrient balance.

An integrated approach is relevant toward reducing N losses in cropping systems to ensure a reduced negative impact on the environment soil degradation. These include promoting balanced nutrient management, the 4R nutrient stewardship, and integrated soil fertility management. As in recent studies by IFDC (2018), over 50% of fertilizers used in East Africa supply only N and P. Such nutrient imbalance alters crop uptake of N, contributing to losses and optimal NUE. A better-balanced application of fertilizers; ensuring availability of all essential nutrients is crucial for optimal N uptake, leading to minimizing losses. Additionally, whereas the majority of farmers apply most N fertilizers at planting, the 4R nutrient stewardship framework recommends splitting N application to 2- or 3-times during crop growth to match N supply with N demand by the crop, thus reducing the accumulation of N in soil and reducing such losses.

Our results revealed relatively low to no N use, particularly in maize fields, while in rice fields, most farmers had access

to fertilizers. However, both categories did not imply proper management due to higher NUE than optimal values and negative N balance. Therefore, decision support tools that guide farmers on the rate and time of application of nutrients to reduce excess N in the soil and match crop requirements with available N quantities, thus reducing the outflow of N from the agricultural system, should be given priority in this region. Some of the practical examples of decision support tools like the Nutrient Expert (NE) and Nutrient Manager for Rice (NMR) support the implementation of site-specific fertilizer management, leading to improved NUE (Sharma et al., 2019; Rurinda et al., 2020). Decision support tools make it possible to significantly manage N fertilizer application rates through improved NUE while sustaining or increasing crop yield levels (Wang et al., 2019).

Limitations and study assumptions

Calculating agricultural N budgets include generalizations and assumptions Lassaletta et al. (2014). Particularly in Africa, with limited data on quantification of N flows at field and farm levels due to a lack of specialized systems for monitoring N fluxes. We relied on specific conversion factors to calculate crop N removal and BNF, which could produce some uncertainties due to crop variations and adaptations to local environmental conditions. For instance, BNF is determined based on the crop area of the legume using global N₂ fixation rates. The approach for N budgeting does not also consider the available N in the soil but rather what goes in and comes out of the system.

We used the territorial emission factors as proposed by FAO (2001) and Bouwman et al. (2002) for different cropping systems (lowland or upland). In addition we applied, unified coefficient for all regions to estimate losses on leaching and runoff as proposed by IPCC (2006). These N-loss pathways could vary even on the same farm and could produce intermediate values and therefore future studies should avoid this kind of uncertainty. Similarly, losses via denitrification were determined on fixed factors based on the amount of N fertilizer application. Similarly, losses in gaseous losses, runoff, and leaching were limited to farms or fields with synthetic N fertilizer application due to the lack of emission factors for other sources of N, including BNF and atmospheric deposition.

Livestock manure was excluded from the N budget estimations due to the farmer's practices of burning manure or grazing the animals along the road. Of overnight grazing incidences, farmers use the dropping as a source of fuel/firewood. Due to the very low quality of the feed components, primarily grasses and crop residues, it is assumed that the excreta or the quality of the manure is very low and does not represent a significant N inflow into the farms. Burning and random grazing overall affect N cycling due to the associated

losses of NH_3 and N_2O that could be very high without proper manure management.

At the farm level, imported feeds were also omitted from the N budgeting method due to the farmers' economic limitations in buying feeds for their livestock. Also, there is scanty information on the availability of the N co-efficient for manure. More public knowledge and policy advocacy on manure management and its implications for N cycling is crucial. In addition, the role of straw management in N cycling should be emphasized to encourage farmers to leave crop residues in the field other than burning to enhance the sustainability of the system regarding nutrient cycling. N in irrigation water, particularly in rice systems, was omitted due to budgeting lack of data on N contents concentration and the amount of water supplied in the growing season. This could vary due to limitations in irrigation water. Leguminous trees in the farms were also not accounted for in the estimation due to the absence of N fixation rates to make the estimation.

The method used to determine field level NUE does not account for indigenous soil N or N mineralization during the cropping season's growth. It was challenging to determine NUE at the farm level due to the complexity in defining the farm boundary and the products needed to adopt the unified methodologies for NUE. At the farm level, there are complexities in nutrient flows. However, future studies should focus on defining the boundaries to enhance a more straightforward determination of N efficiency and overall farm management. Despite these limitations, the current study data provides a comprehensive scenario of the N budgets status at field and farm levels. Future studies on N budgeting within the Lake Victoria basin should focus on establishing long-term experiments at the field and farm level for accurate measurements for the transformation of N in soil gradients under specific rates of N fertilization to reduce existing uncertainties in such studies (Elrys et al., 2019).

Conclusions

Our analysis of nitrogen budget and use efficiencies offers a better interpretation and explanation on N management at field and farm levels. We report for the first time N budgets and NUE characterization for smallholder farms within Lake Victoria, highlighting excessive soil N mining and above the safe operating boundary for N in production systems, although with uncertainties due to limited data sources and lack of specialized systems to accurately monitor N flows at smaller spatial scale. The N mining reported is due to low input of N fertilizers and poor management practices in scenarios where farmers apply N in their field. Maize fields have relatively low N input with an average range of 10.42–22.99 kg N ha⁻¹. The insufficient use of N input also contributes to un-optimal NUE, with values surpassing NUE operational threshold for cropping systems. In

rice fields, particularly in Nyando and Nzoia, with a higher rate of N application 102.19 and 77.78 kg N ha⁻¹, better agronomic practices should be implemented to improve the NUE. The knowledge gap still exists due to difficulty quantifying actual N flows, hence the need for improved and better quantification of N fluxes into the cropping systems. Informing the strategic policy-making process will entail closing all the uncertainties in determining N budgets. In addition, effective policies should target improving the current scenario of low to zero N input through increasing the availability of fertilizers and affordable prices and encouraging the use of organic sources of N to increase sustainability in farms.

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

CM formulated the idea for this article. WN, MG, JG-O, JM, and GN reviewed the methodologies. WN collected the data, performed the statistical analysis, and wrote the first manuscript. WN and CM revised the manuscript. All authors reviewed the manuscript, provided input and suggestions in the text, and approved the submitted version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2022.1023579/full#supplementary-material>

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Co-application of biochar and compost with decreased N fertilizer reduced annual ammonia emissions in wetland rice

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Ammonia (NH₃) emission from rice fields is a dominant nitrogen (N) loss pathway causing negative impacts on farm profitability and the environment. Reducing N fertilizer application to compensate for N inputs in organic amendments was evaluated for effects on N loss via volatilization, rice yields and post-harvest soil properties in an annual irrigated rice (Boro) – pre-monsoon rice (Aus) – monsoon (Aman) rice sequence. That experiment was conducted using the integrated plant nutrition system (IPNS; nutrient contents in organic amendments were subtracted from the full recommended fertilizer dose i.e., RD of chemical fertilizers) where six treatments with four replications were applied in each season: (T₁) no fertilizer (control), (T₂) RD, (T₃) poultry manure biochar (3 t ha⁻¹; pyrolyzed at 450°C) + decreased dose of recommended fertilizer (DRD), (T₄) rice husk ash (3 t ha⁻¹) + DRD, (T₅) compost (3 t ha⁻¹) + DRD, and (T₆) compost (1.5 t ha⁻¹) + biochar (1.5 t ha⁻¹) + DRD. The N loss via volatilization varied twofold among seasons being 16% in irrigated rice and 29% in the pre-monsoon rice crop. In irrigated rice, T₆ had significantly lower NH₃ emissions than all other treatments, except the control while in pre-monsoon and monsoon seasons, T₆ and T₃ were alike. Pooling the three seasons together, biochar (T₃) or biochar plus compost (T₆) reduced NH₃ loss via volatilization by 36–37% while compost alone (T₅) reduced NH₃ loss by 23% relative to RD. Biochar (T₃) and biochar plus compost mixture (T₆) reduced yield-scaled NH₃ emissions by 40 and 47% relative to the RD of chemical fertilizer (T₂). The organic amendments with IPNS reduced the quantity of N fertilizer application by 65, 7, 24, and 45% in T₃, T₄, T₅, and T₆ treatments, respectively, while rice yields and soil chemical properties in all seasons were similar to the RD. This study suggests that incorporation of biochar alone or co-applied with compost and decrease of N fertilizer on an

IPNS basis in rice-based cropping systems can reduce N application rates and NH₃ emissions without harming yield or soil quality.

KEYWORDS

emission factor, NH₃ emissions, yield- rice yield, soil quality, scaled NH₃ emissions, ammonia emissions

1. Introduction

More than 90% of rice (*Oryza sativa*) in the world is grown in Asia, feeding more than 60% of the global population and it supports the livelihood of millions of small and marginal farm families in south Asia (Brolley, 2015). Bangladesh is the third largest rice producing country in the world where rice contributes about 4.5% to the country's gross domestic product. In this country, rice is grown in three seasons i.e., irrigated winter rice called Boro, pre-monsoon rice called Aus and monsoon rice called Aman. In 2020–21, gross annual production of 3-seasons' rice was 36.61 Mt (BBS, 2021). Despite large quantities of nitrogenous fertilizer being applied to maintain rice yields, there is low use efficiency (30–35%) of this fertilizer, and significant gaseous nitrogen (N) loss (Xia and Yan, 2012). In Bangladesh, N is applied at around 150 kg N ha⁻¹ season⁻¹, which is almost double the rate of Japan (80 kg N ha⁻¹ season⁻¹) and a little higher than in the United States (140 kg N ha⁻¹ season⁻¹) (Linguist et al., 2015; Xia et al., 2016). Ammonia (NH₃) is one of the most important by-products of applied N in rice field and volatilization of NH₃ is the primary source of soil nitrogen loss (Pan et al., 2016; Xu et al., 2019; Kuttippurath et al., 2020; Wang et al., 2021).

In 2021, Bangladesh ranked first globally in air pollution due to elevated concentrations of CH₄ and NH₃ in the air (IQAir, 2021). Even though NH₃ is not a potential greenhouse gas (GHS), its emissions and re-deposition can have negative impacts on the environment (Zhang et al., 2020). Volatilized NH₃ is a secondary source of N₂O and NO (Mosier et al., 1998), and NH₃ volatilization is responsible for around 30% of N deposition (Wolfe and Patz, 2002). Ammonia has a negative impact on regional air quality and human health generating aerosols in the atmosphere, influencing the radiation balance by scattering light and changing the earth's reflectivity (Xu and Penner, 2012; Stokstad, 2014). In Asia, agricultural gaseous N losses including NH₃ volatilization may reach 18.8 Tg N yr⁻¹ in 2030 (Zheng et al., 2002; Liu et al., 2021). The global estimate of NH₃ emissions from urea-fertilized soils ranges from 10 to 20%, although in warmer zones, it is substantially higher (Cantarella et al., 2018). Because of extensive rice cultivation, the Indo Gangetic Plain has been identified as a hotspot for NH₃ fluxes but estimates of the rates of loss are limited (Kuttippurath et al., 2020; Uddin et al., 2021; Jahangir et al., 2022).

Mitigating NH₃ emissions from agriculture will not only cut the cost of fertilizer N, but it will also improve air and water quality (Zhao et al., 2017). To limit N losses, various practices are proposed such as use of nitrification inhibitors, urease inhibitors (UI), elemental S, and polymers (He et al., 2018), crop residue removal management (Battaglia et al., 2018, 2021), and organic amendments (Saarnio et al., 2013; Malińska et al., 2014). The role of organic amendments like poultry manure, biochar, compost, etc. in mitigating NH₃ fluxes from wetland rice fields is unresolved since some researchers have reported positive effects (Saarnio et al., 2013; Malińska et al., 2014; Ali et al., 2019), while others reported negative effects (Feng et al., 2017; Chu et al., 2019; Rahaman et al., 2020). Ammonia emissions increase with the N fertilizer rate (Uddin et al., 2021; Jahangir et al., 2022) which suggests that with organic amendments the rate of N fertilizer application could be decreased to reduce both economic and environmental costs while maintaining soil quality. Biochar is a carbon-rich substance made from the pyrolysis of organic matter (Lehmann and Joseph, 2009). It has been reported to prevent NH₃ loss and improve soil health, crop output, and soil carbon sequestration, while also recycling organic waste (Diatto et al., 2020). Biochar and compost mixture can be utilized as fertilizer sources to increase soil nutrients and reduce nutrient losses (Banik et al., 2021).

Ammonia emissions are estimated by the IPCC Tier 1 method but only a single emission factor is scheduled (Bouwman, 1996). While a large amount of N loss as NH₃ can occur, the exact quantity is not known for accurate N balance calculations for many managed agricultural systems including the rice-based cropping patterns of South Asia. Previously, Uddin et al. (2021) evaluated the impact of Conservation Agriculture along with different N fertilization rates on NH₃ volatilization in winter rice (Boro rice). They reported that NH₃ volatilization accounted for 16–21% of the applied N. However, there is no baseline data of NH₃ volatilization in the other two rice growing seasons when temperature is higher (Aus and Aman rice), nor on the impacts of reduced N fertilizer application when co-applied with organic amendments (i.e., integrated plant nutrition system (IPNS) approach) on NH₃ volatilization. We hypothesize that co-application of organic fertilizer such as biochar, rice mill ash (RMA) and compost together with inorganic N fertilizers, which together provide the same amount of N as chemical fertilizer alone, would reduce

NH₃ volatilization loss without changing the soil N status. Thus, the study was conducted to evaluate the effects of rice husk ash, biochar alone or with compost (IPNS basis) on seasonal and annual NH₃ emissions, rice yields and soil quality.

2. Materials and methods

2.1. Experimental site description

The study was carried out on the Soil Science Field Laboratory (24° 71.59' N, 90° 42.50' E) of Bangladesh Agricultural University (BAU) in Mymensingh, Bangladesh. The experiment was done with an annual irrigated rice– pre-monsoon rice – monsoon rice cropping sequence, which is a common cropping sequence followed by the farmers of this country. The irrigated rice season, pre- monsoon rice season and monsoon rice season were occupied by Boro, Transplanted Aus (T. Aus), and Transplanted Aman (T. Aman) rice growing seasons, respectively. The field site was characterized as a Non-calcareous Dark Gray Floodplain soil (Aeric Haplaquept in US Soil Taxonomy), and belongs to agro-ecological zone-9, Old Brahmaputra Floodplain soil (FAO/UNDP, 1988). The soil is moderately drained with a silt loam texture and near neutral pH (6.5). The region has a sub-tropical monsoon climate with a mean annual temperature of 26°C, average annual rainfall of 1,800 mm, and relative humidity of 85% (Uddin et al., 2021, Supplementary Data 1).

2.2. Experimental design and crop management

The experiment was conducted with the same treatment combinations for Boro – T. Aus – T. Aman rice crops in sequence, but with different levels of a nutrient based on the requirements of individual crop and their target yields. That experiment was conducted under integrated plant nutrition system (IPNS; nutrient contents in organic amendments were subtracted from the full recommended fertilizer dose, i.e., RD of chemical fertilizers) where six treatments with four replications were applied in each season. The treatments were: (T₁) no fertilizer (control), (T₂) RD, (T₃) poultry manure biochar + decreased dose of recommended fertilizer (DRD), (T₄) rice husk ash + DRD, (T₅) compost + DRD, and (T₆) compost + biochar + DRD, laid out in a randomized complete block design (RCBD) with four replications. Total plot number was twenty-four for each season and the same plots were used for consecutive rice growing seasons and the unit plot size was 5 m × 4 m, with a 0.75 m inter-plot space, and 1 m inter-block space. The varieties were BRRI dhan28 for Boro, BINA dhan19 for T. Aus and BRRI dhan49 for T. Aman rice, respectively. Boro rice was grown during January–April (winter season), followed by T. Aus rice as a rainfed crop from May to August (pre-monsoon), and then T. Aman rice from August to November (monsoon).

The rate of chemical fertilizer application was based on Fertilizer Recommendation Guide (FRG, 2018) for the test crops. The nutrient contents of used organic amendments are presented in Table 1 while the recommended doses of nutrients for three seasons were presented in Table 2. Urea, triple super phosphate, muriate of potash, gypsum and zinc sulfate were used for N, phosphorus (P), potassium (K), sulfur (S) and zinc (Zn) sources, respectively. Except urea all the nutrients were applied during land preparation. For both Boro and T. Aman rice nitrogenous fertilizer (urea) was applied in equal three splits, followed interval for Boro rice was at 10, 31, and 53 Days After Transplanting (DAT) and for T. Aman that interval was at 9, 24, and 39 DAT. In T. Aus rice two splits of urea application were followed, at 11 and 29 DAT. Compost was collected from Mazim Agro Industries Ltd and rice husk ash from a local rice mill. Biochar was produced using poultry manure by an anaerobic pyrolysis process at 450°C for 4 hr. The organic materials were air dried to 15% moisture content, pulverized and sieved with a 2 mm mesh. In T₃, T₄, and T₅ treatments organic materials were applied at the rate of 3 t ha⁻¹ where T₆ was balanced by applying 1.5 t ha⁻¹ compost and 1.5 t ha⁻¹ biochar, the remaining nutrients were applied from chemical fertilizer based on FRG under IPNS approach. Glyphosate (Round up[®]) was sprayed over the field at a rate of 1.85 kg ha⁻¹ 3 days before final land preparation. The field was irrigated to maintain 3 cm standing water throughout rice growing seasons.

2.3. NH₃ gas sampling and analysis

Field measurements of NH₃ were conducted during January 2020–November 2021 in the rice field. A low-cost chamber was deployed in field conditions for NH₃ volatilization measurements (Nichols et al., 2018) and used for monitoring NH₃ fluxes in crop fields (Martins et al., 2021a,b; Zaman et al., 2021). The open chamber method was used to measure NH₃ fluxes in the field site on a daily basis. In the laboratory, the amount of NH₃ trapped in acid solution was estimated using the Kjeldahl principle (Keeney and Nelson, 1982). Measurements were done on the soil shortly after urea application and it was carried out until the fluxes were below the detection limit in each case.

2.4. NH₃ fluxes and emission factor calculation

The NH₃ fluxes were calculated following Equation 1.

$$\text{NH}_3 \text{ fluxes (mg N m}^{-2} \text{ d}^{-1}) = \frac{(\text{FBR} - \text{IBR}) \times 14.01 \times 0.01 \times 1000}{\text{Surface Area (m}^2\text{)} \times 1000} \quad (1)$$

TABLE 1 Chemical properties of organic amendments (poultry manure biochar, cattle compost, rice husk ash) used in three rice growing seasons.

Organic amendments	Soil organic carbon (%)	Total N (%)	Total P (mg kg ⁻¹)	Total S (mg kg ⁻¹)
Biochar	33.1	2.66	54.9	1990
Compost	25.3	0.98	14.4	770
Rice husk ash	3.10%	0.14%	3.9	126

TABLE 2 Amounts of nutrients added from each source of organic amendments used in three rice growing seasons.

	Treatment	N (kg ha ⁻¹)	P (kg ha ⁻¹)	K (kg ha ⁻¹)	S (kg ha ⁻¹)
Boro rice	Control	0	0	0	0
	Chemical fertilizer	144	21	60	8
	Biochar	66.5	3.43	58	4.97
	Rice husk ash	7	0.49	94	0.63
	Compost	24.5	0.9	23	1.93
	Compost + Biochar	45.5	2.17	40	3.45
T. Aus rice	Control	0	0	0	0
	Chemical fertilizer	72	7	40	3
	Biochar	66.5	3.43	58	4.97
	Rice husk ash	7	0.49	94	0.63
	Compost	24.5	0.9	23	1.93
	Compost + Biochar	45.5	2.17	40	3.45
T. Aman rice	Control	0	0	0	0
	Chemical fertilizer	90	8.5	50	4
	Biochar	66.5	3.43	58	4.97
	Rice husk ash	7	0.49	94	0.63
	Compost	24.5	0.9	23	1.93
	Compost + Biochar	45.5	2.17	40	3.45

Where, NH₃ flux was measured as mg N m⁻² d⁻¹; FBR, Final Burette Reading (ml); IBR, Initial Burette Reading (ml); molecular weight of N = 14.01 g; normality of H₂SO₄ = 0.01 N; and 1000 = unit conversion factor. The sum of NH₃ fluxes on sampling days across the whole sampling period was used to estimate cumulative NH₃ fluxes.

We derived EF (%) according to Equation 2 (Mazzetto et al., 2020).

$$EF(\%) = \frac{\text{Fluxes FT} - \text{Fluxes C}}{\text{Applied Fert}} \times 100 \quad (2)$$

Where EF (%) = Emission Factor, in%; Fluxes FT, Fluxes from fertilizer treatment (in kg N ha⁻¹); Fluxes C, Fluxes from control treatment (in kg N ha⁻¹); Applied Fert, Amount of fertilizer applied (in kg N ha⁻¹).

Yield-scaled NH₃ fluxes were determined following the Equation 3.

$$\begin{aligned} &\text{Yield-scaled NH}_3\text{fluxes}(\text{kg N t grain}^{-1}) \\ &= \frac{\text{Total fluxes from a plot (kg)}}{\text{Yield obtained from the plot (t)}} \quad (3) \end{aligned}$$

2.5. Measurement of grain yield

Before the final harvest of each rice growing season, plants from 1 m² area were collected from each plot, weighed and then oven dried to determine yield and system productivity i.e., pooling together the grain yields of the three rice seasons. For oven drying, 1000 grain samples of each plot were placed in an oven at 65°C until it reached constant weight to determine moisture content. After drying, rice grain samples were weighed and yields were estimated as tonne per hectare.

2.6. Soil sample collection and laboratory analysis

Composite soil samples were collected with an auger at 0–15 cm soil depth from the sites next to each NH_3 gas sampling chamber and preserved in sealable plastic bags in a cooler box. The field-moist soil was air-dried for 2 weeks in the shade at room temperature (25°C) and processed (2 mm sieved) for analysis of major soil physico-chemical parameters. During the NH_3 loss measurement, the pH of the soil was monitored in the field every seven days using a portable pH meter (HI12923; Hanna Instruments). The Kjeldahl method was used to determine total nitrogen (TN) content in the soil (Fawcett, 1954) and the wet oxidation method (Walkley and Black, 1934) was used for soil organic carbon (SOC) determination. Soil samples were extracted with 2 M KCl (1: 2.5; w/w) and NH_4^+ and NO_3^- contents were measured using the method described by Keeney and Nelson (1982).

2.7. Statistical analysis

One-way ANOVA was performed using treatments as fixed factors. The normality test on the NH_3 data was checked before analysis. *Post-hoc* tests were performed to separate differences among the treatments using the Tukey-Kramer multiple comparison Test. All statistical analyses were considered significant at $p \leq 0.05$, unless otherwise mentioned. All the statistical analyses were performed on Statistics 10 and Jamovi1.0.0.0 (R Package). Correlation among the parameters studied was tested by using Pearson's correlation coefficient comparison test.

3. Results

3.1. Time course of NH_3 fluxes after urea application

Ammonia fluxes reached their peaks at 2–3 days after each split of urea application in all seasons. The highest NH_3 fluxes were recorded during the second split application of urea in boro season but that was higher from first split application in both T. Aus and T. Aman seasons (Figure 1). In T. Aman rice, the NH_3 fluxes were 1.5–2.0 times higher in the first split compared to the second and third splits, while the latter two results were almost the same. The NH_3 flux peaks returned to background level at 7–10 days after each split urea application (Figure 1). The highest peak in all splits at each season ranked in the order of chemical fertilizer > RMA > compost > compost + biochar > biochar > control (Figure 1B). On the peak period at 1st, 2nd, and 3rd splits of urea fertilization the NH_3 fluxes from RD treatment were 117, 305, 160 $\text{mg N m}^{-2}\text{d}^{-1}$ in Boro season and that were

193, 165 $\text{mg N m}^{-2}\text{d}^{-1}$ in T. Aus and 289, 138 and 136 $\text{mg N m}^{-2}\text{d}^{-1}$ in T. Aman season, respectively.

3.2. Effects on mean and cumulative ammonia fluxes

The effect of organic and inorganic fertilizers on mean and cumulative ammonia fluxes of all three rice crops was significant. In Boro rice, the highest mean and cumulative NH_3 fluxes were observed in chemical fertilizer treated plot, which was statistically similar to RMA, and the lowest emission was observed in control (Table 3). Integrated use of organic and inorganic fertilizers reduced NH_3 emissions by 6–23% compared to the RD treatment. Either biochar or biochar plus compost reduced N loss via volatilization by 16–23%, while compost alone reduced it by 13%. Likewise, organic and inorganic fertilization also significantly influenced mean and cumulative NH_3 fluxes in T. Aus rice. Mean and cumulative NH_3 fluxes were higher in RD than in other treatments. Reduction in NH_3 fluxes ranged from 10% in rice husk ash to 52% in biochar. Disregarding the control, the highest mean and cumulative NH_3 fluxes were measured in chemical fertilizer treated plots, whereas the lowest emissions were measured in compost plus biochar treated plots. Combined application of organic and inorganic fertilizers reduced NH_3 volatilization by 20–45% compared to the RD application. Pooling the three rice growing seasons together, treatments comprised of biochar or biochar plus compost under IPNS basis reduced N loss via volatilization by 36–37% while biochar alone reduced it by 23% over sole application of full dose of recommended fertilizer as a treatment.

3.3. Effects on NH_3 emission factor and yield scaled NH_3 emissions

The NH_3 emission factor (EF) was significantly influenced by the application of organic and inorganic fertilizers. The NH_3 EF ranged from 12% in compost + biochar to 16% in chemical fertilizer-treated plots in Boro rice, from 21% in biochar to 29% in sole chemical fertilizer treated plots in T. Aus rice, and from 22% in biochar to 28% in chemical fertilizer-treated plots in T. Aman rice (Table 4). Yield-scaled NH_3 emissions in Boro rice varied from 0.17 kg t^{-1} in control to 2.88 kg t^{-1} in chemical fertilizer treated plots (Table 4). Except for the control treatment, yield scaled NH_3 emissions were similar among treatments in Boro rice. Mixture of biochar and compost reduced the NH_3 EF and yield-scaled NH_3 emission in all rice fields. Similarly, yield-scaled NH_3 emissions in T. Aus rice varied between 1.04 kg t^{-1} in control and 5.83 kg t^{-1} in chemical fertilizer treated

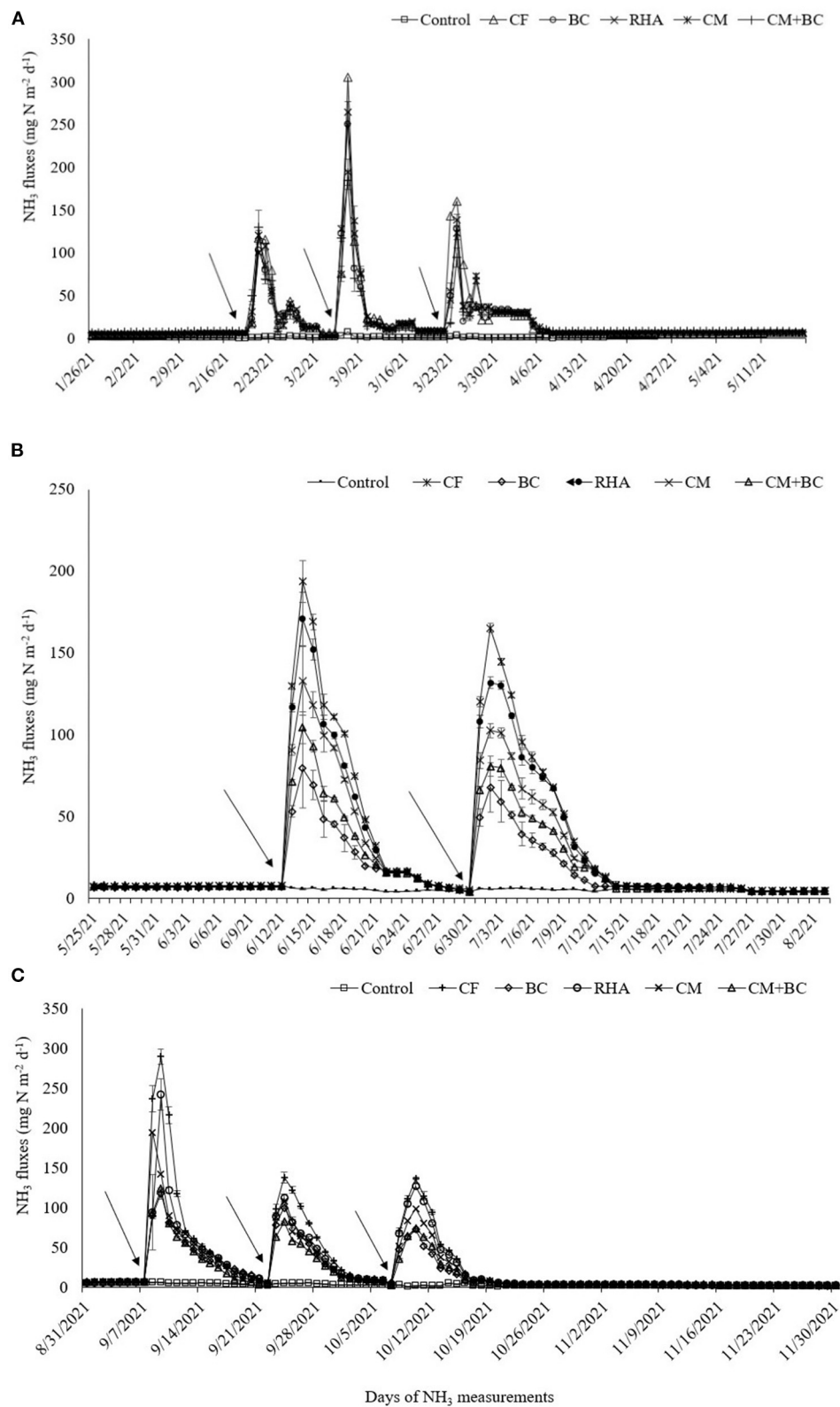


FIGURE 1

Daily NH₃ fluxes (mean ± SE; $R = 4$) from different plots treated with organic and inorganic fertilizers throughout the year [(A) Boro, (B) T. Aus, (C) T. Aman]. Arrows indicate the day of split urea application.

TABLE 3 Effects of organic and inorganic fertilizers on mean and cumulative ammonia fluxes in the Boro - T. Aus - T. Aman rice cropping pattern.

Treatment	Boro rice		Aus rice		Aman rice		Year round	
	Mean NH ₃ fluxes (mg N m ⁻² d ⁻¹)	Cumulative NH ₃ fluxes (mg N m ⁻²)	Mean NH ₃ fluxes (mg N m ⁻² d ⁻¹)	Cumulative NH ₃ fluxes (mg N m ⁻²)	Mean NH ₃ fluxes (mg N m ⁻² d ⁻¹)	Cumulative NH ₃ fluxes (mg N m ⁻²)	Mean NH ₃ fluxes (mg N m ⁻² d ⁻¹)	Cumulative NH ₃ fluxes (mg N m ⁻²)
Control	1.4 ± 0.4e	69 ± 1.7e	4.8 ± 0.1f	292 ± 3.0f	3.5 ± 0.1e	164 ± 3.3e	2.9 ± 0.1e	1055 ± 14.0e
Chemical fertilizer	41.0 ± 1.0a	1966 ± 47.5a	37.1 ± 0.9a	2262 ± 56.4a	56.8 ± 0.6a	2670 ± 29.3a	21.4 ± 0.4a	7822 ± 128.2a
Biochar	34.6 ± 0.5c	1660 ± 25.1c	17.7 ± 0.2e	1082 ± 14.2e	33.5 ± 0.4d	1575 ± 19.2d	14.4 ± 0.1d	5238 ± 33.4d
Rice husk ash	38.4 ± 0.7ab	1844 ± 32.0ab	33.5 ± 0.5b	2045 ± 27.4b	45.4 ± 1.0b	2133 ± 45.5b	19.3 ± 0.3b	7057 ± 93.6b
Compost	35.5 ± 1.0bc	1703 ± 47.1bc	27.9 ± 0.3c	1703 ± 18.4c	40.6 ± 1.2c	1909 ± 57.3c	17.2 ± 0.2c	6281 ± 82.7c
Compost + Biochar	31.4 ± 0.3d	1506 ± 12.8d	22.2 ± 0.3d	1357 ± 18.4d	31.2 ± 0.5d	1465 ± 25.2d	15.2 ± 0.4d	5530 ± 136.9d
CV (%)	4.43	4.43	2.67	2.67	3.50	3.50	3.23	3.23
Level of significance	***	***	***	***	***	***	***	***

*** $p < 0.001$. Columns (Mean ± SE) with different letters vary significantly.

plots (Table 4). Ignoring the control treatment, the highest yield-scaled NH₃ emissions were noted in chemical fertilizer, which was similar to RMA, and the lowest value was in biochar with or without compost treatment. Yield-scaled NH₃ emissions in T. Aus rice were 1 to 6 times higher than that in Boro rice. Similarly, yield-scaled NH₃ emissions in T. Aman rice ranged from 0.47 kg t⁻¹ in control to 4.43 kg t⁻¹ in chemical fertilizer-treated plots (Table 4). Discounting the control treatment, the highest yield-scaled NH₃ emission was recorded in chemical fertilizer, and the lowest value was in biochar with or without compost. Yield-scaled NH₃ emissions in T. Aman rice were 1.0 to 2.7 times higher than that in Boro rice, and 0.5 to 1.0 times that of T. Aman rice (Table 4).

3.4. Effects on crop yields and system productivity

Organic and inorganic fertilizers influenced the grain yield of Boro, T. Aus, and T. Aman rice ($p < 0.05$, Table 5), and system productivity of Boro - T. Aus - T. Aman rice cropping pattern ($p < 0.01$, Table 5). All the treatments were similar to each other in term of crop yield except T₁. Treatments under RD or IPNS had no statistical variation for crop yield and system production. In Boro and T. Aman rice, grain yields were the highest for application of compost and likely the system productivity was the highest for compost application and the lowest for rice husk ash, excluding the control treatment.

3.5. Effects on soil properties

Organic and inorganic fertilizers had a significant impact on soil organic carbon (SOC) during rice cultivation ($p < 0.05$, Table 6). Soil organic carbon increased by 6–14% over the control in plots treated with different amendments (Table 6). Biochar and RMA significantly increased soil total nitrogen (TN) content compared to the other treatments including control except in T. Aus season (Table 6). Likewise, organic and inorganic fertilizers significantly influenced soil C:N ratio only in Boro season but not in T. Aus and T. Aman seasons ($p < 0.05$, Table 6). The highest soil pH was measured in biochar-treated plots, which was similar to other treatments except for compost with biochar and control. Likewise, NH₄⁺ concentrations in soil were significantly influenced by different organic and inorganic fertilizers ($p < 0.001$, Table 6). The highest NH₄⁺ concentrations were found in plots treated with only chemical fertilizer and were lowest in control.

The relationship between the NH₃ fluxes and soil pH was positive and significant in all rice seasons (Figure 2). NH₃ fluxes had a strong correlation with soil pH in Boro rice ($R^2 = 0.79$; $p < 0.01$). Likewise, NH₃ fluxes had a moderate correlation with soil pH in T. Aus rice ($R^2 = 0.36$; $p < 0.05$) and in T. Aman rice ($R^2 = 0.50$; $p < 0.05$) (Figure 2). Like soil pH, the relationship between NH₃ fluxes and soil NH₄⁺ content was also positive and significant in all rice seasons (Figure 2). Ammonia fluxes had a strong correlation with soil NH₄⁺ content in Boro rice ($R^2 = 0.68$; $p < 0.01$), T. Aus rice ($R^2 = 0.86$; $p < 0.001$), and T. Aman rice ($R^2 = 0.91$; $p < 0.001$) (Figure 2).

TABLE 4 Effects of organic and inorganic fertilizers on emission factor and yield-scaled ammonia emissions in rice crops.

Treatment	Emission factor (%)			Yield scaled ammonia emission (kg t ⁻¹)		
	Boro rice	Aus rice	Aman rice	Boro rice	Aus rice	Aman rice
Control				0.17 ± 0.01b	1.04 ± 0.04d	0.47 ± 0.03e
Chemical fertilizer	15.8 ± 0.40a	29.2 ± 0.38a	27.9 ± 0.33a	2.88 ± 0.17a	5.83 ± 0.24a	4.43 ± 0.09a
Biochar	13.3 ± 0.21c	20.8 ± 0.37e	22.0 ± 0.30b	2.61 ± 0.24a	2.65 ± 0.18c	2.67 ± 0.11cd
Rice husk ash	14.8 ± 0.27ab	26.4 ± 0.41c	27.5 ± 0.53a	2.77 ± 0.12a	5.47 ± 0.19ab	3.51 ± 0.11b
Compost	13.6 ± 0.39bc	27.4 ± 0.78b	26.6 ± 0.87a	2.42 ± 0.04a	4.54 ± 0.37b	3.02 ± 0.08c
Compost + Biochar	12.0 ± 0.11d	24.3 ± 0.42d	23.7 ± 0.55b	2.34 ± 0.09a	3.28 ± 0.28c	2.36 ± 0.13d
CV (%)	4.43	2.67	3.47	10.94	14.14	9.38
Level of significance	*	*	**	***	***	***

p* < 0.05, *p* < 0.01, ****p* < 0.001, respectively. Columns (Mean ± SE) with different letters vary significantly.

TABLE 5 Effects of organic and inorganic fertilizers on the grain yield of crops and system productivity in the Boro - T. Aus - T. Aman cropping pattern.

Treatment	Grain yield (t ha ⁻¹)			System productivity (t ha ⁻¹)
	Boro rice	Aus rice	Aman rice	
Control	4.12 ± 0.25b	2.81 ± 0.14b	3.49 ± 0.15b	10.4 ± 0.20b
Chemical fertilizer	6.89 ± 0.31a	3.91 ± 0.27a	6.04 ± 0.14a	16.8 ± 0.31a
Biochar	6.55 ± 0.71a	4.13 ± 0.22a	5.92 ± 0.18a	16.6 ± 0.68a
Rice husk ash	6.70 ± 0.37a	3.75 ± 0.14a	6.09 ± 0.23a	16.6 ± 0.59a
Compost	7.06 ± 0.29a	3.83 ± 0.33a	6.32 ± 0.05a	17.2 ± 0.45a
Compost + Biochar	6.45 ± 0.21a	4.21 ± 0.33a	6.26 ± 0.29a	16.9 ± 0.51a
CV (%)	12.1	13.7	7.8	5.8
Level of significance	*	*	*	**

p* < 0.05, *p* < 0.01, respectively. Columns (Mean ± SE) with different letters vary significantly.

4. Discussion

In accord with our hypothesis, the co-application of biochar, RMA and compost together with N fertilizer, while supplying the same amount of N as N fertilizer alone, decreased NH₃ volatilization loss by 16–28% without changing the soil N status. In the following discussion, we first examine the dynamics of NH₃ fluxes, the NH₃ emission factors for treatments and the IPNS treatment co-benefits for soil properties and crop yield.

4.1. Peak of NH₃ fluxes

The NH₃ flux peak was within 2–3 days after urea application indicating that NH₃ volatilization was a rapid progress that was almost completed within 1 week after each split fertilizer application. The NH₃ volatilization flux patterns were consistent among treatments, suggesting that they were primarily driven by the urea applied. The NH₃ emission patterns were consistent with previous studies in the same (Uddin et al.,

2021) and dissimilar geographical areas (Fan et al., 2006) as our experiment. The NH₃ flux from urea hydrolysis usually peaks at 3–7 days after application (Rochette et al., 2009) which is in line with our results but not to Drury et al. (2017) who stated that the peak emissions can take up to 9–15 days if rain occurs after N application. That fluxes were highest from T₂ may be attributed to the highest rate of urea applied which rapidly converted into NH₄⁺ through the ammonification process, which was the first step of ammonia volatilization (Frimpong et al., 2016; Uddin et al., 2021). As NH₃ is in a dynamic equilibrium with NH₄⁺ and H⁺, urea treatment elevates soil pH through urease hydrolysis (Sommer et al., 2004). Following the peak on day 2–4 after urea application, the NH₃ fluxes rapidly declined. While organic amendments did not alter the timing of the peak of NH₃ fluxes, they decreased the magnitude of the peak which could be attributed to the lower rate of chemical N-fertilizer based on the IPNS approach. The decrease in soil NH₄⁺ content and a drop in pH with the organic amendments helps explain the decrease in NH₃ volatilization (Adviento-Borbe et al., 2010). Other processes leading to a decrease in NH₃ volatilization could

TABLE 6 Effects of organic and inorganic fertilizer on soil properties after urea application in Boro, T. Aus and T. Aman rice.

	Treatment	SOC (%)	STN (%)	Soil C:N ratio	Soil pH	Soil NH ₄ ⁺ content (mg N kg ⁻¹)	Soil NO ₃ ⁻ content (mg N kg ⁻¹)	Soil mineral N content (mg N kg ⁻¹)
Boro	Control	1.71 ± 0.05b	0.11 ± 0.01b	16.0 ± 0.27bc	6.55 ± 0.01c	14.3 ± 0.94c	3.7 ± 1.85b	18.0 ± 2.24c
	Chemical fertilizer	1.82 ± 0.05ab	0.10 ± 0.01b	18.7 ± 0.81a	7.56 ± 0.10ab	30.2 ± 1.28a	3.8 ± 1.56ab	34.0 ± 1.28a
	Biochar	1.98 ± 0.02a	0.13 ± 0.01a	15.1 ± 0.35c	7.78 ± 0.13a	20.1 ± 0.96b	4.8 ± 0.96ab	24.9 ± 1.91bc
	Rice husk ash	1.92 ± 0.04ab	0.13 ± 0.01a	15.2 ± 0.30c	7.55 ± 0.02ab	24.9 ± 1.10b	6.7 ± 0.96ab	31.6 ± 1.83ab
	Compost	1.95 ± 0.09ab	0.11 ± 0.01b	18.3 ± 0.97ab	7.64 ± 0.06ab	24.9 ± 1.10b	10.5 ± 1.83a	35.4 ± 1.83a
	Compost + Biochar	1.93 ± 0.06ab	0.10 ± 0.01b	18.8 ± 0.65a	7.36 ± 0.09b	23.0 ± 0.41b	6.7 ± 0.96ab	29.7 ± 1.29ab
	CV (%)	5.67	5.74	6.37	2.20	9.29	45.91	7.53
	Level of significance	*	*	*	**	***	*	**
Aus	Control	1.58 ± 0.03b	0.15 ± 0.01	10.26 ± 0.39	6.48 ± 0.06b	16.9 ± 0.45d	2.05 ± 0.68	18.9 ± 0.68d
	Chemical fertilizer	1.75 ± 0.04a	0.15 ± 0.01	11.57 ± 0.42	7.01 ± 0.17a	40.3 ± 0.68a	2.73 ± 0.00	43.0 ± 0.68a
	Biochar	1.67 ± 0.03ab	0.16 ± 0.01	10.42 ± 0.37	7.14 ± 0.06a	26.2 ± 0.68c	3.41 ± 1.72	29.6 ± 1.37c
	Rice husk ash	1.71 ± 0.04ab	0.15 ± 0.01	11.27 ± 0.22	7.11 ± 0.06a	35.5 ± 0.00b	1.37 ± 0.79	36.9 ± 0.79b
	Compost	1.78 ± 0.03a	0.17 ± 0.01	10.25 ± 0.38	6.52 ± 0.03b	34.2 ± 0.79b	2.73 ± 1.12	36.9 ± 1.37b
	Compost + Biochar	1.69 ± 0.02ab	0.16 ± 0.01	10.33 ± 0.37	6.64 ± 0.07b	36.2 ± 0.68b	2.05 ± 0.68	38.3 ± 0.01b
	CV (%)	4.12	6.45	7.11	2.16	3.81	76.94	5.23
	Level of significance	*	ns	ns	**	**	ns	**
Aman	Control	1.44 ± 0.03b	0.15 ± 0.02b	9.55 ± 0.34	6.56 ± 0.03c	19.4 ± 0.58e	2.05 ± 0.68b	21.5 ± 0.59d
	Chemical fertilizer	1.51 ± 0.02ab	0.17 ± 0.01ab	8.90 ± 0.24	7.24 ± 0.02b	55.1 ± 1.87a	2.73 ± 1.12b	57.8 ± 2.36ab
	Biochar	1.59 ± 0.05ab	0.18 ± 0.02a	8.82 ± 0.48	7.41 ± 0.04ab	46.4 ± 1.58cd	3.41 ± 0.68b	49.9 ± 2.05bc
	Rice mill ash	1.57 ± 0.03ab	0.18 ± 0.02a	8.96 ± 0.25	7.60 ± 0.07a	53.0 ± 0.94ab	9.56 ± 1.76a	62.6 ± 1.58a
	Compost	1.54 ± 0.08ab	0.18 ± 0.02a	8.78 ± 0.21	7.49 ± 0.13ab	49.2 ± 1.12bc	8.88 ± 0.68a	58.1 ± 0.68a
	Compost + Biochar	1.67 ± 0.03a	0.18 ± 0.01a	9.01 ± 0.15	7.42 ± 0.09ab	42.6 ± 0.68d	6.15 ± 1.31ab	48.7 ± 1.76c
	CV (%)	5.40	5.99	6.28	1.90	5.71	4.17	3.05
	Level of significance	*	**	ns	**	**	**	**

*p < 0.05, **p < 0.01, ***p < 0.001, respectively; ns, not significant. Columns (Mean ± SE) with different letters vary significantly.

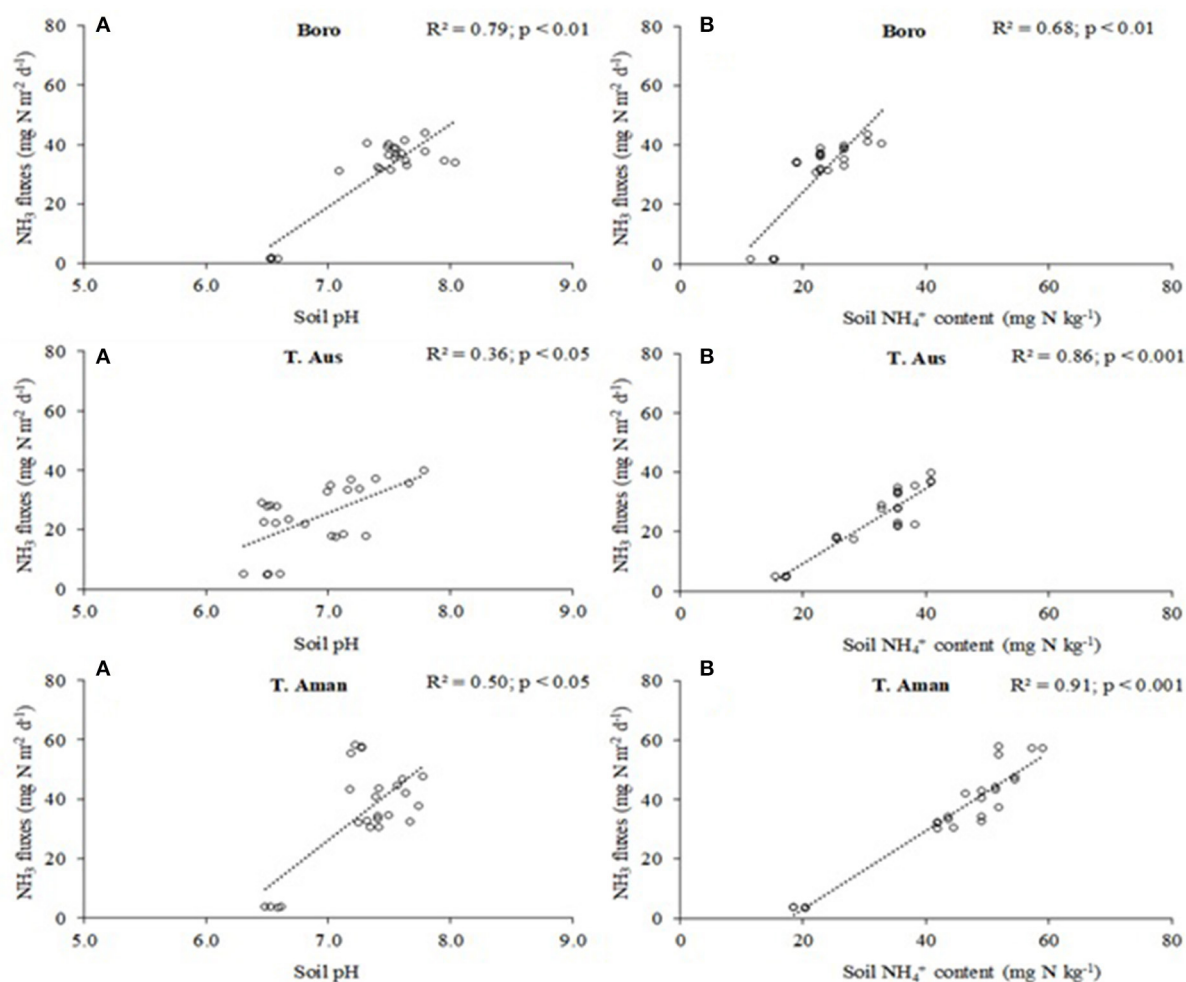


FIGURE 2
Relationship between NH_3 fluxes and soil pH (A) or soil NH_4^+ content (B) in the rice crops; $n = 24$.

be the infiltration of mineral N into the crop rooting zone, and increased nitrification over time (Adviento-Borbe et al., 2010). When the NH_3 fluxes for Boro, Aus and Aman seasons were examined in relation to the soil chemical properties, the closest positive correlation was with soil pH followed by soil NH_4^+ as found in previous studies (Sommer et al., 2004; Rochette et al., 2013).

4.2. Ammonia fluxes, emission factor, and rice yields

While NH_3 volatilization is a major N loss from paddy fields, the rate of N loss is dependent on the fertilization type, time of application, environmental conditions and N application rate (Wang et al., 2016). Pan et al. (2016) stated that about 30% of the applied urea was lost through NH_3 fluxes which

were consistent with our result that the N loss via volatilization ranged from 16% in Boro to 28% in T. Aman rice season. When N supplied in the urea fertilizer was adjusted based on the N content in the organic amendments, NH_3 fluxes were reduced. In this study biochar alone and with compost reduced the NH_3 loss during three rice growing seasons. The NH_3 fluxes of N fertilizer was higher for Aman rice than for Aus and Boro rice which is most likely due to the seasonal variations in temperature being the lowest in Boro season (15–25°C) and the highest in T. Aman season (25–35°C) while in T. Aus the temperature was moderate (20–30°C). High temperature in standing water in rice fields induces rapid urea hydrolysis and higher ammonia volatilization (Sun et al., 2017). While the Aus season in the Indo-Gangetic plain has high rainfall and moderate temperature, the urea application rate in this season was lower than the other two seasons due to lower yield potential, which may lower volatilization.

Biochar was very effective in reducing NH_3 emissions by reducing chemical N input but may also control the N releases. Our results also showed consistency with Sun et al. (2017) and Asada et al. (2002), where their meta-analysis suggested that NH_3 fluxes were reduced with the application of biochar pyrolyzed at $\sim 400^\circ\text{C}$. Ammonia adsorbed onto the biochar surface directly reduces the substrate concentration of NH_3 for the volatilization process (Clough et al., 2013). However, the liming effect of alkaline biochar may increase NH_3 fluxes (Sun et al., 2017; Sha et al., 2019). The pH increase in soil amended with biochar in the present study was not high enough to enhance NH_3 fluxes (Kelly et al., 2015). Among the amended plots biochar required the lowest rate of urea fertilizer to equalize total N input with recommended chemical fertilizer dose, which may explain the lower NH_3 fluxes than in compost amended plots. Co-application of biochar with compost has the potential to reduce NH_3 emissions due to high surface area to adsorb NH_4 , high internal porosity to trap NH_4^+ ions but this will depend on N mineralization rate and their inherent N content which varies among biochar and compost products.

All the treatments, except control without N fertilizer applied, had the same yield in all three rice seasons even though the urea application rates were different. Moreover, the N uptake was also the same in each treatment (data not presented). Therefore, questions arise of how biochar-treated soils provided similar N for plant uptake in comparison to a full dose of urea. A moderate substitution ($<40\%$) of N fertilizer by manure has been reported to significantly increase N use efficiency by 14 and 25% for upland crops and rice, respectively (Xu et al., 2016; Zhang et al., 2020). In the present study, rice plants were initially paler green in biochar-treated plots suggesting that it decreased initial N mineralization rate. In addition, N from urea in biochar-treated plots may have been used more efficiently due to better synchronization of N supply and demand.

Rice husk ash had less efficiency in NH_4^+ retention in all seasons and in controlling NH_3 fluxes than biochar, but still decreased N losses relative to the urea fertilizer alone. While ashes are often alkaline, the present RMA did not alter soil pH and was effective in decreasing NH_4 content in soil except in Aman season and in decreasing NH_3 losses, except in the Boro season. As an abundant biowaste in the Indo-Gangetic Plain, RMA can be used to reduce N fertilizer input and to reduce atmospheric NH_3 emissions. However, as the ashing conditions are likely to vary with farm-produced RMA, more study is needed to determine the consistency of the effects reported here.

4.3. Integrated plant nutrition system effects on soil properties

In addition to their effects on NH_3 losses, organic amendments had significant effects on some soil properties. In the current research, sole biochar application increased soil pH

compared to control treatment, however compost + biochar combination and sole compost application decreased soil pH. Poultry manure biochar may have increased soil pH through its liming effect over the sole compost and chemical fertilizers application but when the mixture of compost and biochar was applied, soil pH decreased relative to the sole biochar application. Herein, soils treated with solitary biochar had the highest pH (7.78) which is not enough to raise NH_3 loss, followed by soils treated with both biochar and compost (7.42). Slight increase in soil pH in biochar treated plots could have increased NH_3 emissions but the lower NH_4^+ contents in soils resulted in lower NH_3 emissions.

Application of biochar solely or in combination with compost at a rate of 3 t ha^{-1} has increased SOC in our study, which is in line with previous research (Liu et al., 2021). Biochar is distinguished from compost by its larger proportion of more stable organic carbon molecules (Mahmoud et al., 2018; Eissa, 2019) making it more efficient in enhancing soil physicochemical parameters (Eissa, 2019). Furthermore, Trupiano et al. (2017) also reported that the application of compost and biochar to soils, either alone or in combination, enhanced soil SOC content compared to un-amended soils, implying that biochar and/or compost is a potential source of soil carbon sequestration.

5. Conclusion

Volatilization loss of N from paddy fields in floodplain soils causes economic losses and is a major concern for air and water quality. Application of biochar alone or in combination with compost on an integrated plant nutrition system basis reduced the rate of N-fertilizer application as well as ammonia volatilization. The NH_3 emission factor ranged from 12% in compost plus biochar to 16% in chemical fertilizer-treated plots in Boro rice, from 21% in biochar to 29% in compost treated plots in Aus rice, and from 22% in biochar to 28% in chemical fertilizer-treated plots in Aman rice. Pooling the three rice growing seasons together, either biochar or biochar plus compost mixture reduced N volatilization by 36–37% while compost alone can reduce it by 23%. All the treatments had same crop yield except the control without N fertilizer. Hence, biochar with or without compost mixture has a great potential for mitigating year-round NH_3 volatilization in the triple rice cropping system along with a decrease in the rate of applied N-fertilizer in floodplain soils without losing crop yield and system productivity.

Data availability statement

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

Author contributions

MJ and MAH worked on research planning and paper editing. CM worked on research planning, calculation, and paper editing. RB worked on planning and paper editing. MZ contributed in research planning and methodological development. MBH worked on research planning. JF conducted field and laboratory work, data processing, analysis, and paper draft preparation. NM worked in draft preparation. MMJ worked on research planning, data interpretation and paper editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2022.1067112/full#supplementary-material>

SUPPLEMENTARY DATA 1

Environmental weather data of the experimental site registered during the experimental period (January 2021 to December 2021).

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Nitrogen balance is a predictor of farm business performance in the English Farm Business Survey

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Global environmental sustainability and food security are fundamental societal issues, and most crop production relies upon inputs from organic or inorganic nitrogen sources. Previous research in the Global North has demonstrated a typical over application of nitrogen across global agriculture with substantial negative impacts on the environment. The objective of this work was to draw on English Farm Business Survey (FBS) data of non-organic General Cropping and Cereal farms to explore the relationship between farm gate nitrogen balance, fertilizer application advice and farm business performance. A mixed effects generalized modeling approach was used to partition the variance into random (such as year, or farm ID) and fixed effects (those of interest). Whilst the financial performance of farm businesses is subject to high variance and multiple drivers, a negative relationship was detected between business performance and farm gate nitrogen balance, we demonstrate that nitrogen lost to the environment of >60 kg per hectare is associated with a significant negative impact on farm performance. Supplier-provided fertilizer advice was also associated with reduced farm performance. These results imply a positive effect on farm performance of enhancing on-farm understanding of crop nutrient requirements through the provision of accredited fertilizer advice. Within the stated bounds our model demonstrates good predictivity on randomly subsetting data, and is presented as a tool for use in scenario modeling of interventions such as agri-environment schemes, Natural Capital and Ecosystems Assessment, and the UN Sustainable Development Goals.

KEYWORDS

nitrogen balance, farm business performance, mixed effects models, nitrogen emissions, fertilizers

1. Introduction

1.1. Broad impacts of nitrogen balance

Global environmental sustainability and food security are fundamental societal issues (Lal, 2006) in the face of climate change and population growth predictions (UNFPA, 2021). Specifically, the UN Sustainable Development Goals 12, 13, and 15 (United Nations Department of Economic and Social Affairs, 2021) highlight the urgent need for action in these areas (Withers et al., 2014). The fundamental basis of crop production to support a growing population relies on crucial crop inputs, arguably one of the most important of which is nitrogen (N) supplied either from organic (crop residues, fixation, manures) or inorganic sources (manufactured fertilizer) (Ball, 2015). Nitrogen is a key ingredient of photosynthesis and the most important yield-limiting factor in agricultural systems (Lin et al., 2016) yet economically optimal crop fertilization (Falk Øgaard, 2014) may result in higher N application than is removed in grain, suggesting an economic distortion underlying N losses. This distortion is exacerbated by the relative inexpensiveness of N, in which externalities are not captured in its market price

(Cherry et al., 2012), and the risk-averse nature of farmers who may over apply N to avoid yield production penalties (Edmeades, 2003).

1.2. Agricultural food production in the UK

Temperate agriculture is a major contributor to global food production (Gornall et al., 2010). Within northern Europe, cereals (wheat, barley) typically dominate arable rotations (Hawkesford, 2014), with high value tuber, root and bulbs forming additional crops of economic importance (Vasco Silva et al., 2021). The drive for enhanced environmental stability to sit alongside food production in UK farming has recently received renewed impetus following the publication of the UK Agriculture Act (2020) and the focus on support for agriculture through the provision of public goods, including climate change adaptation and reducing losses to the environment (Defra, 2018, 2021a).

Comprising 70% of UK land area (Defra, 2021a), the UK agriculture industry is a major determinant of its rural economies and landscapes, is pivotal in a range of ecosystems services (Firbank et al., 2007) and provides about 60% (by economic value) of its domestic food consumption (Defra, 2021c). Using long term research at Rothamsted, MacDonald et al. (2017) demonstrated that the application of fertilizers, herbicides and the use of modern high-yielding varieties can dramatically increase yields, although inputs such as fertilizer N which exceed crop requirements are not economically or environmentally sustainable. This was supported by Dicks et al. (2019)'s poll on farm management practices by agricultural experts, who scored the factors "Use fertilizer more efficiently" and "Benchmark environmental performance" in their top 20 interventions for sustainable intensification.

1.3. Mixed effects modeling

Factorial experimental designs are common in agricultural research, where data are derived from crops blocked under specific growth conditions, each associated with discrete inputs and generating discrete output values (e.g., the long term field experiments at Rothamsted, Johnston, 1994). However, factorial experiments are relatively expensive to undertake and are not suited to the analysis of understanding variation in results derived from more than one source of variation. By contrast, mixed-effect models are suited to such data comprising many sources of variation, some of which may be considered random. Below we establish the methodological approach utilized in this paper that seeks to control for repeated measures over four different years, and repeated farms within these years. This framework enables the incorporation of multiple sources of within-subject variation, which are challenging to represent in a standard regression model.

1.4. Research aims

Here we use a powerful, large and unique data set (the Farm Business Survey) to better understand the relationship between the application of N on Cereal and General Cropping farms and the financial performance of the farm business, as well as the impact

that different sources of fertilizer application advice have on this relationship. Specifically, we answer the following questions:

- Does seeking independent advice about fertilizer application rates impact the amount of N lost to the environment?
- Does seeking independent advice about fertilizer application rates impact farm business performance?
- Is there a relationship between farms which lose more N to the environment and farm business performance?

2. Materials and methods

2.1. Farm business survey variables and metrics

The Farm Business Survey (FBS; Defra, 2021b) is an annual stratified survey of English farms run by the Department for Environment, Food and Rural Affairs. It collects detailed data on the physical, environmental and financial performance of farms, running on a harvest year basis. The Sustainable Intensification Platform (SIP; Defra the Welsh Government, 2018) was a multi-partner research programme comprising farmers, industry experts, academia, environmental organizations, policy-makers and associated stakeholders. As part of this research, metrics were developed to measure aspects of sustainable production from farm business data.

FBS data for the years 2015/16–2018/19 inclusive were analyzed together with the corresponding metrics from the Sustainable Intensification Platform. The final dataset comprises 428 Cereal and 173 General Cropping farms, with most farms replicated in multiple years. As the FBS is a stratified sample of farms in England it is representative of the English Cereal and General Cropping farm population. Detailed methods on how the data are collected can be found at <https://www.gov.uk/government/collections/farm-business-survey>.

The FBS provides detailed financial data on farm businesses annually, with detailed fertilizer usage questions introduced in 2015. The Sustainable Intensification Platform metrics were developed to measure (among other aspects) the volumes of N brought onto or taken off farms, and are available for the years 2015–2018. Additionally the FBS contains data on a range of farm management practices in relation to fertilizer application (e.g., source of fertilizer advice) that we incorporated into our analysis. The analyses herein draws on this range of data alongside the wider set of farm business and production data, in particular the physical crop production of each farm and the N contained within this production.

2.2. Derivation of nitrogen balance and performance ratio

The concept of N balance is well documented (Neuens et al., 2006; Bassanino et al., 2007; Treacy et al., 2008). Here we designate the N balance as the difference between N brought on to the farm (in the form of inorganic fertilizers) and taken off the farm (in the form of agricultural outputs e.g., crops), as a proportion of the total area farmed; our N balance thus represents a farm-gate N balance. The N balance data were subsetting to six discrete bands:

“exports>imports”, “0–20 Kg/Ha”, “20–40 Kg/Ha”, “40–60 Kg/Ha”, “60–80 Kg/Ha”, “>80 Kg/Ha”.

The volume of N brought on to the farm was calculated from FBS input data on quantities of physical nutrient use. It was not possible to quantify N brought on to the farm in the form of organic fertilizers because the sources of organic nutrients were themselves extremely variable in composition (relative to more precise commercially available products). In addition, no robust coefficients on composition could be found. The volume of N taken off the farm was calculated from standard nutrient compositions (RoySocChem, RB209, and AHDB) along with agricultural product production data, to estimate nutrient offtakes in the form of agricultural products. Framed in this context, the difference between N brought onto and taken off a farm can be thought of as the amount of N lost to the environment through, for instance, leeching into watercourses. The N balance was then calculated with the following formula:

$$N \text{ balance} = \frac{(N_{\text{on to farm}} - N_{\text{off farm}})}{A}$$

Where $N_{\text{on to farm}}$ is the volume of nitrogen brought onto the farm in kilograms, $N_{\text{off farm}}$ is the volume of nitrogen taken off the farm in kilograms, and A is the area farmed in hectares.

In addition to understanding N balance we seek to determine the influence of farm management decisions on both N balance and farm business performance. The latter is represented as a ratio, calculated as:

$$\text{performance ratio} = \frac{\text{farm business output}}{(\text{farm business costs} + \text{unpaid labour adjustment})} * 100$$

Where an unpaid labor adjustment is estimated through conversations with the farm manager, and is valued at average local market rates for manual agricultural work. Farm business output is the total value of agricultural produce generated by the farm, for instance as crops such as wheat. Farm business costs are the total costs for the farm, both variable (such as fertilizers or pesticides) and fixed (such as labor or machinery repairs).

2.3. Stratification of farm classes

Within the FBS dataset, farms are classified into farm “types” on the basis of their Standard Outputs (SO). Standard Outputs measure the total value of output of any one agricultural enterprise per hectare. This is the main product (e.g., wheat, barley, peas) plus any by-product that is sold, for example straw. Each farm is assigned a total SO by aggregating the SOs for its agricultural enterprises. The farm is classified into a particular type of farming by evaluating the proportion of its total SO deriving from different enterprises, for instance a farm which generates two thirds or more of its SO from cereal production would be classified as a Cereal farm. Although livestock enterprises were included in the calculation of total SO, only farms which were classified as non-organic, Cereal or General Cropping farm types were used in this analysis (i.e., farms which generated two thirds or more of their SO from Cereal or General Cropping enterprises, and were not organic). This is a standard approach taken to classifying farms with

similar characteristics based on their type of output, and is used by countries across the UK and the EU when calculating national scale farm statistics. For more information see the [technical notes](#) on the [FBS website](#).

Farms were classified into size bands, which are based on the amount of labor used, calculated by applying labor coefficients (known as Standard Labor Requirements, or SLRs) to individual enterprise types. The SLR of a farm represents the normal labor requirement, in Full Time Equivalents, for all the enterprises on a farm under typical conditions. The SLR was then used to classify farms into one of the following size bands; “Spare-time”, “Part-time”, “Small”, “Medium”, “Large”, and “Very large”.

Farms were assigned a tenancy status based on the proportion of their total farm area which is owned or rented: “Owner occupied”, “Mostly owner-occupied”, “Mostly tenanted” and “Tenanted”.

Financial debt was calculated as total net interest payments as a proportion of farm business income (FBI), this measure provides an indication of whether farms can afford to pay the interest on their debts. The following debt bands were used; “No interest”, “FBI negative”, “<5%”, “5–<10%”, “10–<20%”, “20–<50%”, “50%+”.

The Government Office Region of each farm was used to inspect the broad effect of geography in the model; “North East”, “North West”, “Yorkshire & Humber”, “East Midlands”, “West Midlands”, “East of England”, “South East” and “South West”.

The distribution of agricultural area of each farm was significantly skewed so it was transformed to \log_{10} (area) to achieve a normal distribution.

Some farms receive advice about fertilizer application rates, in order to better match their application rates to crop requirements. The Fertilizer Advisers Certification and Training Scheme (FACTS) provides training in an evidence-based approach to fertilizer applications. Farms were classified into one of five bands based on their self-declared main source of advice for fertilizer application; “Own (not FACTS) advice”, “Own (FACTS) advice”, “Independent FACTS advice”, “Supplier” and “none (N/A)”.

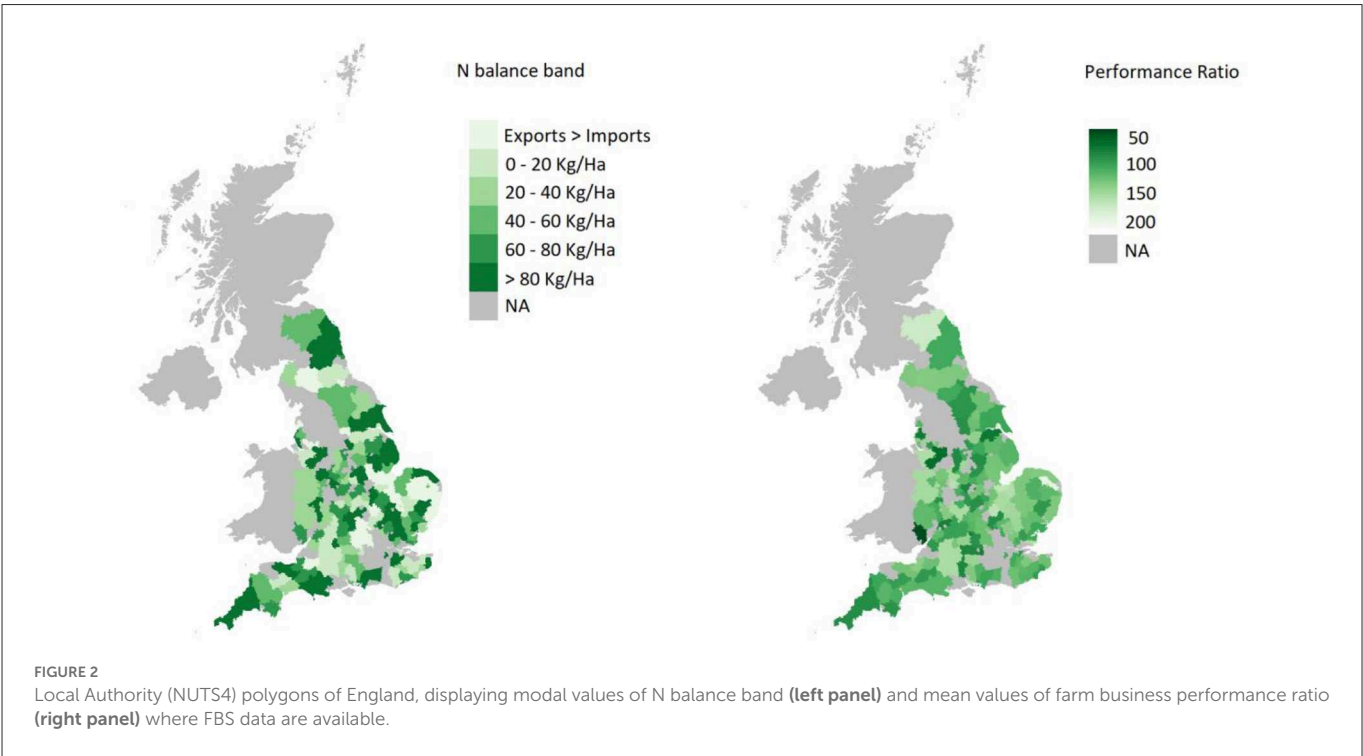
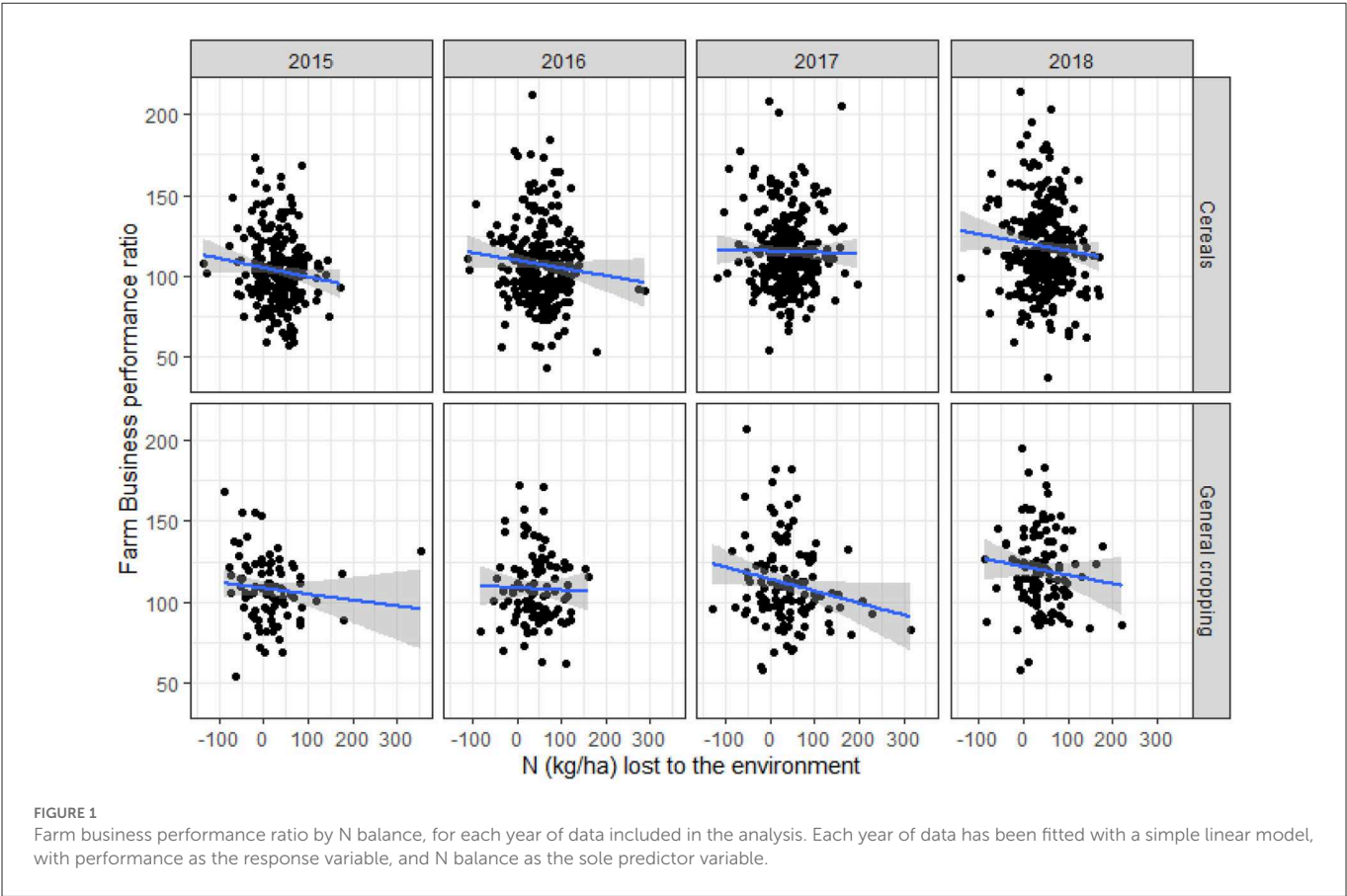
For more details and information about how FBS farms are classified see the [FBS website](#).

2.4. Exploratory data analyses

Preliminary checks for data quality were performed using histograms and QQ plots. Farms with missing data and/or non-respondents were removed, which accounted for only a handful of datapoints. Distributions of all variables were examined to check for any strong deviation from normality and appropriate transformations applied, including log transformation of farm business performance ratio for the final model. A single outlier was detected in the latter and removed to optimize the model’s generality to new datasets. The final working dataset consisted of 1,474 datapoints.

All statistical analyses were undertaken in R (version 4.0.3, [R Core Team, 2020](#)) and the non-spatial figures plotted in ggplot2 ([Wickham, 2016](#)). The final model was built using the lme function in the nlme ([Pinheiro et al., 2021](#)) package to fit linear mixed effects models. Mixed models are an extension of generalized linear models which allow estimation of both fixed and random effects,

being of particular value when there is non-independence (i.e., a hierarchical structure) in the data. Such non-independence could have been tackled simply by aggregating to average values, which yields consistent coefficients and standard errors, but would reduce the sample size as well as not take full advantage of the information value of all the data.



A set of candidate variables were selected for the maximal model, which were informed from previous work investigating the relationship between farm characteristics and business performance (Betts, 2020; Jones C., 2020; Jones N., 2020).

A range of random effects structures were trialed and tested using the *anova* function, including a simple intercept effect and autocorrelation structures to account for temporal pseudo-replication. The final random effects model structure was selected by parsimony to minimize degrees of freedom and Akaike Information Criterion (AIC). Farm ID was fitted to have a random effect on the intercept of the model, and year to have a random effect on the slope.

2.5. Spatial distribution

The spatial distribution of N balance per area and farm business performance ratio was examined in a confidentiality-preserving way by linkage to UK Local Authority polygons (Office for National Statistics ONS, 2021), computing the mean-per-polygon in each case, then plotting in the tmap package (Tennekes, 2018). Hypothesis testing for spatial autocorrelation of these polygon values was performed with a Monte Carlo permutation test for Moran's I statistic, using the spdep package (Bivand and Wong, 2018). This is preferred to an analytical calculation of Moran's I as it makes no assumptions about the dataset, including the shape and layout of each polygon.

2.6. Model selection

Stepwise reverse model simplification was performed manually and informed by *anova* and AIC. Models were fitted using Maximum Likelihood during model simplification, and Restricted Maximum Likelihood to obtain final coefficient estimates from the final minimum adequate model.

The retained variables in the final model were farm type (factor with two levels), farm size (factor with six levels), tenancy type (factor with four levels), \log_{10} farm area, debt (factor with seven levels), region (factor with eight levels), \log_{10} (agri-environment scheme payments), fertilizer advice (factor with five levels) and N balance (factor with six levels). The fit was inspected using standard model diagnostic plots, and checked for multicollinearity using the car package (Fox and Weisberg, 2019) to ensure that Variance Inflation Factor values were <5 .

A model validation step was performed by fitting the minimum adequate model to a randomly selected (80%) subset of the data, which was then used to predict the performance ratio for the remaining 20%, the predicted values and actual values were regressed against one another and R^2 calculated.

3. Results

3.1. Bivariate analysis

Farm performance ratio is subject to high variance and multiple drivers, yet for every single year studied there is a negative relationship to N balance which is borderline or significant (Figure 1).

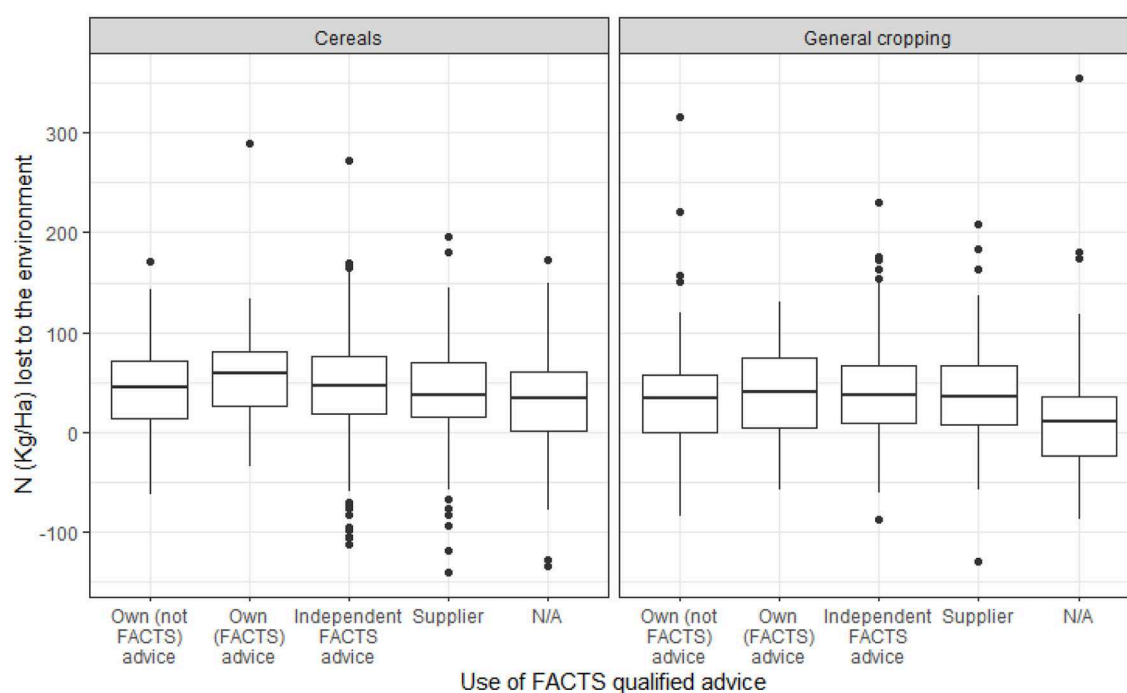


FIGURE 3
Box plots of N balance against the use of FACTS qualified advice, for Cereal and General Cropping farms. Scores of N/A are assigned for pre 2016 when FACTS data were not available.

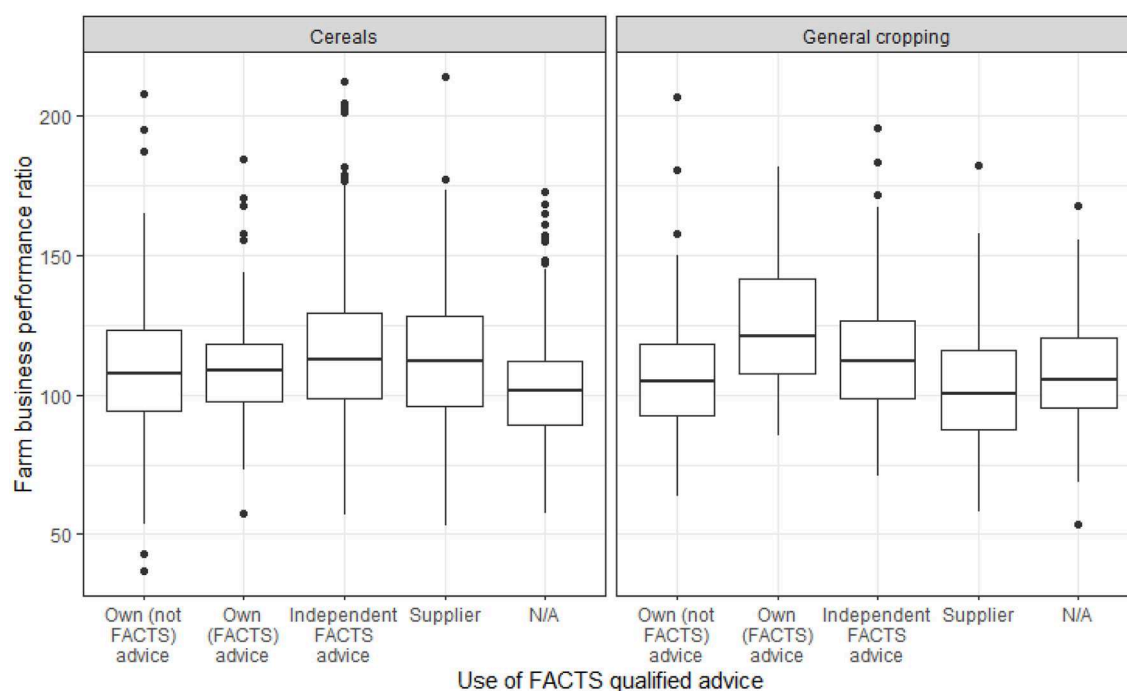


FIGURE 4

Box plots of farm business performance against the use of FACTS qualified advice, for cereals and General Cropping farms. Scores of N/A are assigned for pre 2016 when FACTS data were not available.

The negative slope of every individual model suggests a general pattern that those farms which lose the most N to the environment tend to be poorer performers.

3.2. Spatial distribution

The data were plotted spatially at Local Authority (i.e., 'NUTS4') level in order to explore any regional bias, whilst retaining the confidentiality of farms (Figure 2). The distribution of N balance and farm business performance ratio for Cereal and General Cropping farms shows little spatial clustering across the study area.

Monte Carlo permuted Moran's I tests reported no significant spatial autocorrelation of N balance per area farmed ($I = 0.10$, $p = 0.052$) nor farm business performance ratio ($I = -0.07$, $p = 0.87$). Supplementary Figures 1, 2 show these I scores against the density distribution of Moran I values that we could expect if the variable is randomly distributed across the local authorities.

3.3. Fertilizer use advice

The volume of N lost to the environment was not found to be correlated to the source of fertilizer application advice (Figure 3) for these farm types. However, the relationship between source of fertilizer application advice and farm business performance differed between the two farm types (Figure 4). Whilst no relationship is evident for Cereal farms, General Cropping farms show a pattern, such that farms which used their own (FACTS qualified) advice tended to be better business performers.

3.4. Coefficients of the final model

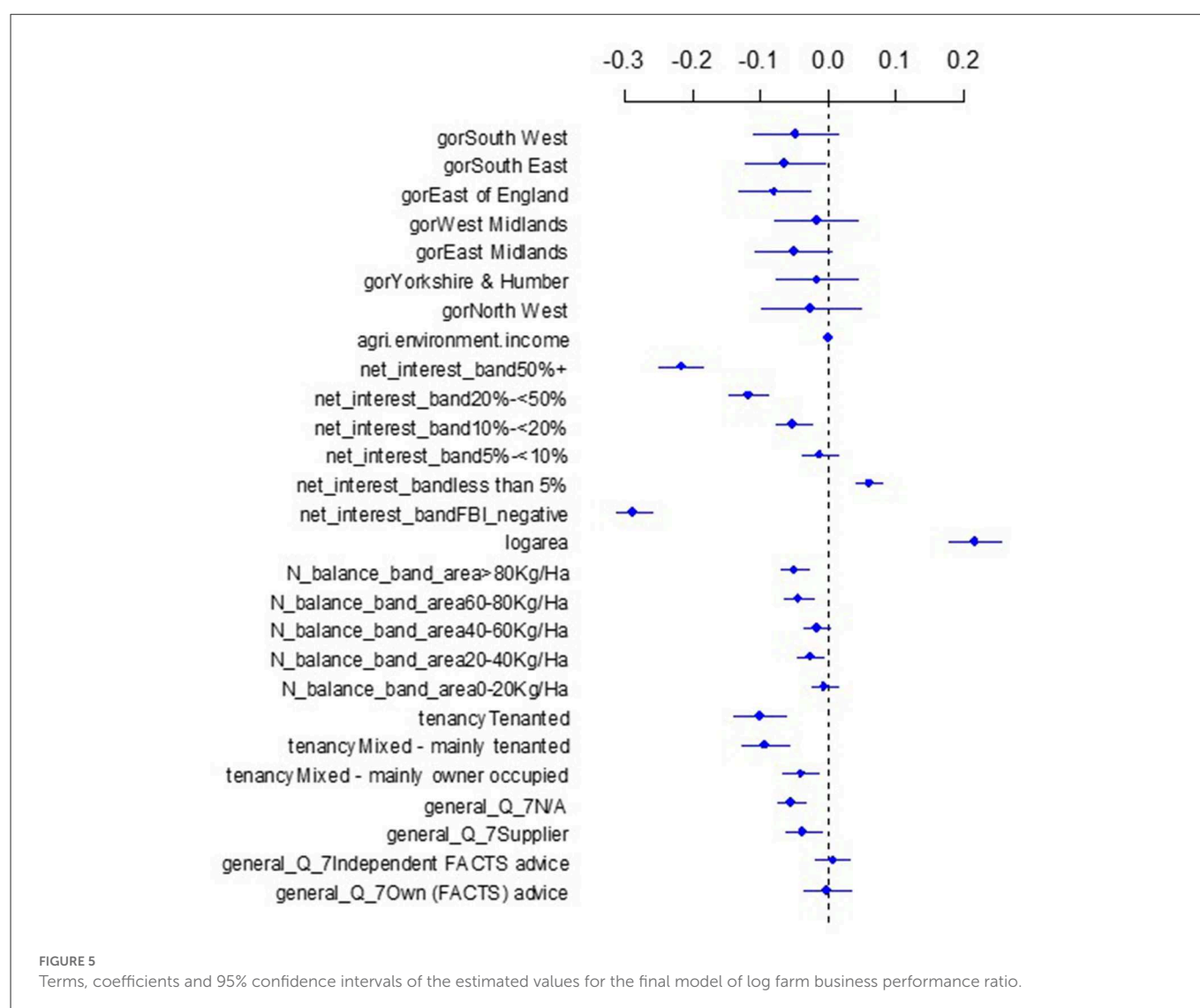
Interestingly the signs and magnitude of the coefficients remained little changed (Supplementary Table 1) through the model simplification process. The variables with the largest coefficient magnitudes (and therefore those that are most influential in the model) are those of area and financial debt.

Examination of the effects structure shows that 60% of variance in the random effects is accounted for by farm ID, reflecting the high degree of variability in farm businesses. Thirty two percent of the variation was explained by year, and the remaining 8% is residual (i.e., the variability that was unexplained by the predictors in the model—the fixed effects).

3.5. Marginal effects

In the final model, N balance was found to be significantly related to log farm business performance (Figures 5, 6, Supplementary Table 1), wherein farms which lost more N to the environment (>60 Kg/Ha) were significantly poorer farm business performers, and those farms having a positive N balance (i.e., exports $>$ imports) were consistently higher business performers than all others.

For model prediction purposes, note that the effects of individual model terms with all others held fixed are interpreted as $e^{(\text{model coefficient})}$. So for example a switch from the N balance class "exports $>$ imports" to "60–80 Kg/Ha" or " >80 Kg/Ha", are significantly associated with a 4% ($e^{-0.04} = 0.96$) and 5% ($e^{-0.05} = 0.95$) reduction in farm business performance ratio.



In addition, fertilizer advice was significantly related to log farm business performance ratio (Figure 7, Supplementary Table 1), wherein farms which took advice from suppliers were significantly poorer business performers than those using independent FACTS, own FACTS, or own non-FACTS advice.

3.6. Validation

This model showed good predictivity on the bulk of randomly subsetting data (Figure 8), suggesting that its wider use in scenario-modeling of farm performance would be justified. Its correlation coefficient of 67% between predictions and actual data is of note given that the performance of the model is constrained by fitting it to only 80% of the available data.

The model underpredicts log performance at the higher end, beyond values of around 5 (i.e., farm business performance of ~150). This suggests that there are a group of high-performing farms, which are insufficiently represented by the drivers which fit well those farms with a performance ratio under ~150. Indeed, if that group

are omitted from Figure 8, the model displays a strong linear fit to the remainder.

4. Discussion

4.1. Nitrogen balance and business performance

In line with previous studies (e.g., Langeveld et al., 2007; Buckley and Carney, 2013) the results presented in Figure 1 demonstrate a negative relationship between over application of N and farm business performance. Specifically, farms which lost more N to the environment (>60 Kg/Ha) were significantly poorer farm business performers, while businesses with a positive N balance (i.e., exports > imports) were consistently higher performers.

As Local Authority means of neither N balance nor farm business performance were significantly auto correlated across England, we have not found evidence for spatial effects (e.g., contagion or repulsion) at work at this spatial level. In other words, the proximity of one Local Authority to another does

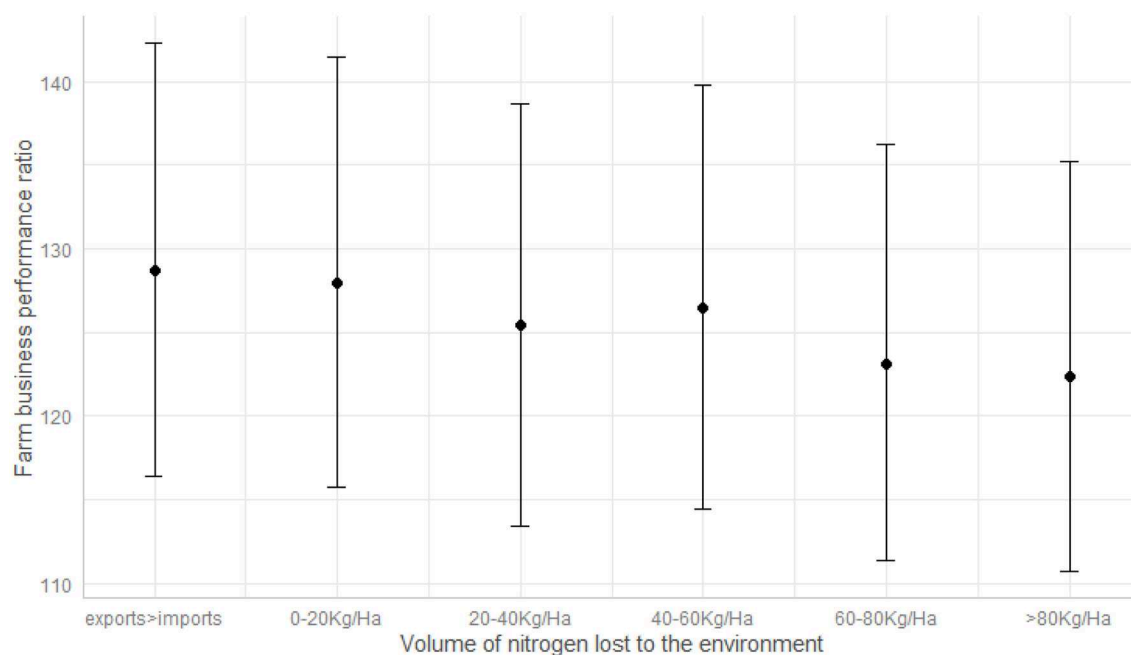


FIGURE 6

Mean and 95% confidence intervals of marginal predicted values of farm business performance ratio (back-transformed from log farm business performance ratio) generated from the final model, by N balance.

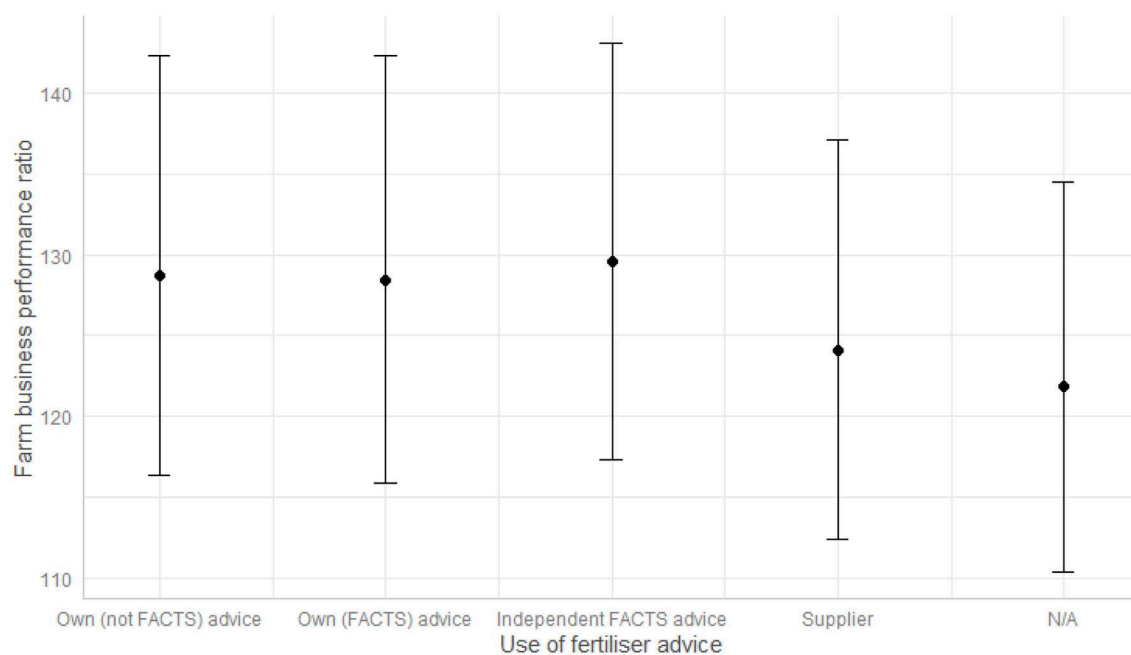


FIGURE 7

Mean and 95% confidence intervals of marginal predicted values of farm business performance ratio (back-transformed from log farm business performance ratio) generated from the final model, by fertilizer advice.

not appear to be explanatory of the N balance nor business performance of its constituent farms. Thus for any policy aiming to target N balance or business performance, farmer take-up may also be anticipated to be dependent on other factors than farm proximities.

4.2. Fertilizer use advice

In contrast to previous studies (e.g., [Williamson, 2011](#)), we have found little relationship between the source of fertilizer advice and N lost to the environment, whereas other research has shown a positive

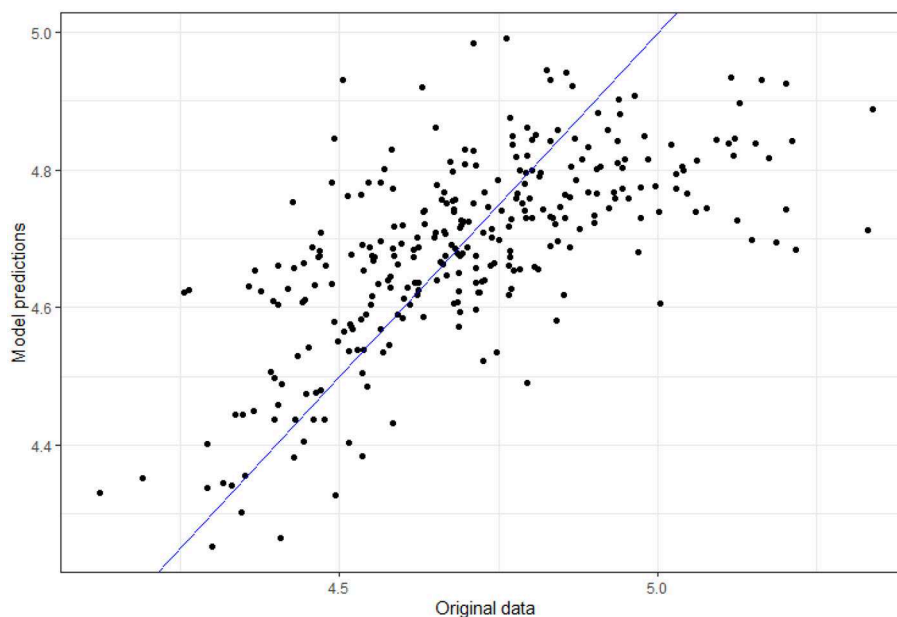


FIGURE 8

Validation of the final model: The same model specification was fit on a random 80% subset of the original data, that model being used to generate predictions for the remaining 20%, plotting those predictions against actual data. $R^2 = 0.67$.

relationship between farm efficiency (Buckley et al., 2016) or farm performance (Lillywhite and Rahn, 2005) and the use of independent advice (e.g., advice not linked to fertilizer sales).

For General Cropping farms however, our results suggest that the direct provision of fertilizer skills to farmers (*via* FACTS) is linked to farm business outcomes, in a similar way to previous studies that have shown the importance of independent advice (Sharpley et al., 2015). Our model results are in line with this previous research (Barnes et al., 2013), suggesting that farms which took advice from suppliers (advice linked to fertilizer sales) were significantly poorer business performers than those using independent FACTS, own FACTS, or own non-FACTS advice. This is the first work of which we are aware explicitly linking the detailed source of fertilizer advice to the financial performance of farm businesses rather than crop enterprise performance.

4.3. Changing policy landscape in the UK

Within the UK, and specifically within England, new agricultural policies will result in lower payment supports to farmers *via* the Basic Payment Scheme, and increased support for the delivery of public goods (UK Agriculture Act, 2020). This represents a fundamental change in the support structures for farming, that will reinforce the need for improved business and agricultural enterprise performance. Efficiency of resource use in food production will thus be of even greater importance to business survival. The current study uses a uniquely powerful dataset to demonstrate a link between environmental and business performance, wherein farms that have lower losses to the environment are associated with improved performance, in agreement with previous work (Wilson et al., 2001).

Whilst it is not possible to attribute causality between on-farm advice and actions with environment and business performance

from our results, these findings are informative for policy recommendations. Specifically, in order to enhance business and environmental (i.e., lower losses to the environment) performance, supporting the delivery of FACTS or similar training to farmers alongside reducing barriers to the uptake of independent fertilizer advice represent clear and actionable policy recommendations.

In contrast to environmental policies that seek to reduce land devoted to food production in order to undertake environmental actions, enhancing farmer control of crop nutrient requirements will arguably aid in the delivery of both food security and environmental sustainability goals. We recognize that this recommendation cannot independently deliver food and environmental sustainability. However, the “win-win” outcome from this represents a tangible deliverable that could be supported with environmental land management schemes. This has the potential to be widely and positively received within the farming sector, provided that the method and means of communicating this outcome are clearly understood by farmers (Wilson et al., 2001).

4.4. Possible data generating mechanisms

The pattern is dominated by the variables of area and financial debt, and the rank order of coefficient values within factors (e.g., net interest band, N balance band, tenancy) is intuitive. The fact that location (at least at the level of Government Office Region) is apparently of significance in explaining farm business performance warrants further investigation and has ramifications for parity in post-Brexit agricultural policy.

That the model under predicts at the higher end ($> \sim 150$) of farm business performance is of interest. Clearly the variables of area, tenancy and debt are critical predisposers of farm business performance, but they do not seem to explain the full story in this

dataset. The implication is of a predictor variable (or variables) that we did not include, and which does not appear to be available even in the vast and comprehensive Farm Business Survey. Assuming that this pattern at the upper end of the scale is not an artifact arising from the calculation of farm business performance, it seems likely that its key predictor(s) may be hard to detect or widely overlooked. This tallies with the fact that farm ID assumes the large majority role in our random effects structure, as well as the narrative of O’Leary (2017) on explanatory factors in the human side of farm management.

Whilst there can be a trade-off between model complexity and interpretability, we suggest that potential alternatives to our approach could include GLMM, Bayesian inference, LASSO regression, mixed-effects Random Forest, or mixed-effects Support Vector Machines.

4.5. Conclusions

Globally, the results presented herein demonstrate the potential for enhancing farm level understanding of matching crop needs to input supply, and the impact of over application of N on farm business performance.

Many countries of the Global South provide farmer extension services, albeit that the market price for N often leads to that advice being directed toward oversupply of nutrients (Ndambi et al., 2019). In the Global North, policy and business structures tend to rely on farm businesses procuring independent advice with an associated businesses cost. Our results imply that through investment in training and/or annual cost of independent fertilizer advice, there is a potential link to both environmental and business performance. Global environmental and food sustainability will not be delivered through a single mechanism or policy, but it is hoped that our findings represent an important aspect of the drive to provide more sustainable outcomes.

Within the stated bounds our model demonstrates good predictivity on randomly subsetting data, and as such is presented as a tool for use in current scenario modeling of interventions such as agri-environment schemes, Natural Capital and Ecosystems Assessment, and the UN Sustainable Development Goals.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: The data used herein is of a sensitive nature and confidential, and as such is not openly available. Anonymous, confidential FBS data are available to bona fide researchers and can be obtained *via* the Economic and Social Data Service, UK Data

Archive, University of Essex. Requests to access these datasets should be directed to <https://www.data-archive.ac.uk>.

Author contributions

PW and MR oversaw data collection. CG and DH conducted the analysis. All authors contributed to formalizing the research questions and contributed to manuscript writing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Multitrait *Pseudomonas* sp. isolated from the rhizosphere of *Bergenia ciliata* acts as a growth-promoting bioinoculant for plants

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Multifunctional plant growth-promoting bioinoculants are used to enhance growth, harvest yields, and add economic value to agricultural crops. In this study, such bioinoculant, BC-II-20 (*Pseudomonas* sp.), was isolated from the rhizospheric soil of a medicinal plant *Bergenia ciliata* from the Garhwal Himalayas, Uttarakhand, India. After characterization, supplementation with *Pseudomonas* sp. was used to study growth stimulation in a commercially important medicinal plant, *Andrographis paniculata* (Kalmegh), and it depicted enhanced physiological growth parameters under controlled conditions. Bacterial seed priming and also supplementation led to early and increased germination and plants displayed better vegetative growth during the entire growth stages. Early initiation of flowers and the appearance of pods occurred in inoculated plants, ultimately leading to the reduction in the life cycle of the plant. At the time of harvesting, there was an increase in the physiological parameters such as shoot length (38%), root length (14%), fresh weight (57%), dry weight (60%), number of panicles, and root branching. Photosynthetic efficiency was also higher, and ultimately, overall plant growth was improved by bacterial inoculation. The eco-friendly and sustainable use of this bioinoculant will provide an alternative to harmful chemical fertilizers and has become increasingly important. In conclusion, we reported a promising bioinoculant having plant growth-promoting traits, which promotes growth and development in *A. paniculata* and may be applied to other plants also.

KEYWORDS

Andrographis paniculata, biofertilizer, plant growth promotion, seed biopriming, PGPR, sustainable development goals (SDGs)

1. Introduction

Medicinal plants have microbial communities with a genetically diverse population having multifunctional growth-promoting properties (Premalatha et al., 2021). Plant roots produce a range of organic substances that serve as food for microorganisms, enhancing beneficial microbial activity in the complex environment termed as rhizosphere (Egamberdieva and Teixeira da Silva, 2015). Plants and soil microbes interact, interrelate, and affect each other in the rhizosphere (Mhlongo et al., 2018). These rhizospheric microbes are plant growth-promoting rhizobacteria (PGPR), which stimulate plant growth and development. There are reports that PGPR supplementation leads to an enhancement in phytochemical content in medicinal plants (Egamberdieva and Teixeira da Silva, 2015) and also acts as potential biocontrol agents in many plant diseases (Sharf et al., 2021). These medicinal plants serve as raw materials for traditional herbal medicine. According to the estimates, traditional medical practices such as herbal remedies, indigenous therapies, and others are used in 88% of all countries (WHO, 2019).

B. ciliata belongs to the family Saxifragaceae and can be found at a height of 800–3,000 m in the temperate Himalayan regions. For centuries, it has been used as medicine for several ailments (Ahmad et al., 2018). Various diseases are treated with *B. ciliata* in the Himalayan region (Kour et al., 2021). Medicinal plants have a distinct microbiome, which produces distinct bioactive secondary metabolites such as flavonoids, terpenoids, glycosides, sterols, and saponins (Ferdosi et al., 2021). Thus, the PGPRs present in the rhizospheric soil associated with *B. ciliata* may prove beneficial for other medicinal plants also.

A. paniculata belongs to the plant family Acanthaceae and is also known as the “King of Bitters.” The leaves and stems of the plant are used as fresh and dried herbal medicine since ancient times and have active phytochemicals. The most medicinally active phytochemical is andrographolide. The plant has been widely used to treat jaundice, digestive disorders, and hepatoprotection as well as liver tonics, antipyretics, antithrombotics, blood purifiers, and febrifuges (Okhuarobo et al., 2014). The plant is revered as a miraculous remedy in tribal societies and ancient Siddha and Ayurvedic medical systems for a number of medicinal uses. It has been extensively cultivated in India and other South Asian countries (Kumar et al., 2011; Khan et al., 2015). As a powerful immune booster, this plant is in high demand (Premalatha et al., 2021). But the herbage yield of *A. paniculata* gets affected by indiscriminate harvesting from natural habitats and suffers the risk of drought (Kalariya et al., 2021). Thus, there is a need to increase the cultivation and growth and development of *A. paniculata* to get more biomass for increased phytometabolite content. Seed dormancy is also a problem during germination in *A. paniculata* and may be resolved by the application of plant growth-promoting rhizobacteria, as is important to promote vegetative growth where leaves are the important plant part for medicinal purposes (Premalatha et al., 2021).

As a result of intensive cultivation practices, chemical fertilizers are typically used extensively to increase medicinal crop yields and quality. Such agricultural practices may also negatively affect the growth of medicinal plants and their secondary metabolites apart from being expensive and environmentally harmful. Nowadays, environment-friendly, sustainable, and organic approaches are becoming increasingly popular for yield enhancement in medicinal plants (Yilmaz and Karik, 2022; Yuan et al., 2022). All of these are finally directed toward the fulfillment of sustainable development goals (SDGs) adopted by the United Nations.

Therefore, this study was primarily designed to isolate and characterize the rhizobacteria associated with *B. ciliata* for PGPR traits and to determine the effects of its supplementation. We investigated the effect of bioinoculant, i.e., *Pseudomonas* sp. on a medicinal plant, *A. paniculata*, for plant growth promotion under controlled conditions. The beneficial effects of this bioinoculant were observed in root development, shoot development, and early flowering, thereby proving its role as a potential biofertilizer.

2. Materials and methods

2.1. Isolation of bacteria from rhizospheric soil

Soil sampling was done from the rhizosphere of *B. ciliata*, a medicinal plant, from district Pauri Garhwal in Uttarakhand, India (30°09′30.5″N 78°51′14.8″E). The intact plant was carefully removed

with a 15-cm slab of soil. For bacterial isolation, the soil clumps that were firmly associated with the roots were carefully preserved in sterile polyethylene bags. Bacterial isolation was done using the serial dilution method. Rhizospheric soil sample (1 g) was mixed with autoclaved distilled water (10 ml) and dilution was prepared to range from 10^{-1} to 10^{-5} . Several bacterial colonies appeared when sterile tryptone soy agar (TSA) plates were subjected to incubation for 24 h (h) at $28 \pm 2^\circ\text{C}$. The distinct colonies were then picked and streaked on nutrient agar plates. The isolates were re-streaked to obtain pure cultures. Later, the selected isolates were characterized for various experiments.

2.2. Soil physicochemical properties

Soil physicochemical properties such as pH, electrical conductivity, organic carbon, and elemental analysis were tested. pH was measured using a glass electrode on a digital pH meter by preparing soil water suspension of ratio (1:2) following the method of Estefan et al. (2017). Electrical conductivity (EC) was measured following the protocol of Bower and Wilcox (1965), in which soil water suspension of ratio (1:2) was measured using a conductivity meter. Organic carbon (OC) was determined by the method of Walkley (1947). Furthermore, the concentrations of macro elements such as phosphorus, potassium, and sulfur and microelements such as zinc, iron, copper, and boron present in soil samples were analyzed after digestion, using atomic absorption spectroscopy (AAS) and standard calibration curves of the above elements.

2.3. Morphological and biochemical characterization of bacterial isolate

Pure cultures of rhizobacterial isolates were incubated for 24 h on TSA plates to study their morphological features. As the colonies appeared, the colony morphology, size, shape, and coloration were observed using Bergey's Manual of Determinative Bacteriology (Holt, 1994). Gram staining was done to determine whether the isolate is gram-positive or gram-negative. Biochemical characterization of the bacterial isolate was conducted, which included a catalase test which was performed according to the method of Reiner (2010), an oxidase test by the method of Tarrand and Gröschel (1982), a citrate utilization test by the method of MacWilliams (2009), and Methyl Red (MR), Voges Proskauer (VP) test according to the method of McDevitt (2009). Different carbon sources including sucrose, dextrose, and lactose were used to determine carbohydrate utilization by the bacterial isolate (Reiner, 2010).

2.4. Characterization of bacteria for plant growth-promoting traits

2.4.1. Indole acetic acid production

Bacterial isolate was tested for the production of IAA by the method described by Ehmann (1977). IAA produced by the selected isolate was detected and quantified using tryptone soy broth (TSB) supplemented with 1 g L^{-1} tryptophan. The culture was then incubated at $28 \pm 2^\circ\text{C}$ for 2–3 days, IAA production was determined

by mixing Salkowski reagent in the bacterial culture supernatant, and absorbance was taken at 530 nm using a spectrophotometer.

2.4.2. Screening for nitrogen fixing ability

Screening the bacterial isolate for its ability to fix nitrogen was carried out using Burk's Nitrogen-free medium (HiMedia) with the protocol of [Park et al. \(2005\)](#). Before autoclaving at 121°C for 15 min (min), the medium's pH was adjusted to 7 ± 0.1 . Indicator dye bromothymol blue (BTB) was used for the detection of nitrogen-fixing bacteria. After inoculation, the plates were incubated overnight at $28 \pm 2^\circ\text{C}$. The blue-colored zone production around the colony served as a marker for isolates having the nitrogen-fixing ability.

2.4.3. Production of hydrogen cyanide

Production of HCN by the bacterial isolate was determined using the method of [Bakker and Schippers \(1987\)](#). Nutrient agar supplemented with glycine was used for the bacterial culture. Whatman filter paper soaked in a solution of 2% (w/v) Na_2CO_3 and 0.5% (w/v) picric acid was placed on the lid of Petri plates. Plates were then sealed with parafilm and incubated for 48 h at $28 \pm 2^\circ\text{C}$. The presence of volatile HCN was confirmed by a change in color (from yellow to reddish brown) of the soaked Whatman filter paper.

2.4.4. Phosphate solubilization assay

To determine the phosphate-solubilizing ability of the bacterial isolate, bacterial culture was inoculated in Pikovskaya's agar plates containing insoluble tricalcium phosphate, which were then incubated at $28 \pm 2^\circ\text{C}$ for 7 days ([Pikovskaya, 1948](#)). Potential phosphate solubilizers were the bacterial colonies that produced distinct transparent halos. The phosphate solubilization index (PSI) was calculated using the following formula:

$$\text{PSI} = [\text{colony diameter (cm)} + \text{halo zone diameter (cm)}] / \text{colony diameter (cm)}.$$

2.4.5. Calcite solubilization assay

The calcite solubilization was done using DB agar medium in the following composition/L: glucose 5 g, yeast extract 1 g, peptone 1 g, K_2HPO_4 0.4 g, MgSO_4 0.01 g, NaCl 5 g, $(\text{NH}_4)_2\text{SO}_4$ 0.05 g, CaCO_3 5 g, and agar 15 g ([Cacchio et al., 2004](#)). The pinpoint inoculation was done on the agar plates and the plates were placed in an incubator at $28 \pm 2^\circ\text{C}$ for 5–7 days. Clear zone formation around the colony confirmed calcite solubilization by bacteria ([Tamilselvi et al., 2016](#)). By calculating the ratio of the halo zone to colony size, the solubilization index (SI) of the isolate was determined:

$$\text{SI} = [\text{colony diameter (cm)} + \text{halo zone diameter (cm)}] / \text{colony diameter (cm)}$$

2.4.6. Ammonia production test

Ammonia production by the bacterial isolate was done in peptone water as described by [Cappuccino and Sherman \(1996\)](#). Overnight

grown fresh bacterial culture was inoculated in 10 ml of peptone water and incubated for 48 h at $28 \pm 2^\circ\text{C}$. Nessler's reagent (0.5 ml) was added to each tube after incubation and the color change from yellow to brown marked ammonia production in the culture medium.

2.4.7. Protease production test

On skim milk agar or SMA medium (HiMedia), the bacterial isolate was tested for its proteolytic enzyme production ([Masi et al., 2021](#)). The appearance of transparent zones around the colonies after 48 h of incubation at $28 \pm 2^\circ\text{C}$ indicates protease production.

2.5. Drought stress tolerance

The selected bacterial isolate was tested for tolerance against drought stress. Drought tolerance was tested on TSB having different concentrations [0, 10, 15, 20, and 25% (w/v)] of polyethylene glycol (PEG) 6000 ([Michel and Kaufmann, 1973](#)). A total of 100 μl of overnight grown bacterial culture [10^7 colony-forming units (CFUs) mL^{-1}] was inoculated in TSB and incubated at $28 \pm 2^\circ\text{C}$ followed by visual examination for the growth for 2–3 days. Tolerance to varying concentrations of PEG was examined by streaking the isolate on TSA plates and incubated at $28 \pm 2^\circ\text{C}$. Viable cells in the medium that grew on the TSA plates showed tolerance against drought stress.

2.6. Antimicrobial susceptibility tests

A diffusion agar assay was used to assess the antimicrobial resistance of the bacterial isolate ([Armalyte et al., 2019](#)). TSB was used to test for resistance to selected antibiotics. In total, six antimicrobial discs (HiMedia, India) were used, including streptomycin, penicillin, chloramphenicol, kanamycin, tetracycline, and erythromycin. After overnight incubation at 30°C , antibiotic susceptibility was determined by the presence of clear zones around the antibiotic discs, which indicated antibiotic susceptibility.

2.7. Molecular identification of the bacterial isolate

Genomic DNA was isolated from the bacterial cells according to the method of [Wright et al. \(2017\)](#). Bacterial isolate was identified based on the 16S rRNA gene sequence. Briefly, the 16S rRNA gene was amplified using universal bacterial primers 16SF-5' AGAGTTTGATCCTGGCTCAG 3' and 16SR-5' AAGGAGGTGATCCAGCCGCA 3'. The polymerase chain reaction (PCR) was performed with a 50 μl reaction mixture comprising 5 μl of Taq buffer (10 X), 1 μl of dNTPs (12.5 mM), 1 μl of template genomic DNA, 200–250 $\mu\text{mol L}^{-1}$ of forward and reverse primers (1 μL each), 0.1 μl of Taq DNA polymerase, 1 μl of MgSO_4 (50 mM), and the remaining volume was made up with water. DNA amplification was performed in a thermocycler programmed as initial denaturation for 2 min at 98°C , primer annealing for 1 min at 64°C , primer extension for 1 min at 72°C , and a final extension of 10 min at 72°C up to 35 cycles. The 16S rRNA gene amplicons were visualized in 0.8% agarose gel under UV light using a UV

transilluminator. The PCR product was purified and subjected to sequencing.

2.7.1. Bioinformatic analysis

The sequence of the isolate was subjected to bioinformatic analysis for the identification and phylogenetic relationships. Using a standard nucleotide basic local alignment search tool (BLAST) search, the isolate was identified by the comparison of its 16S rRNA gene sequences with nucleotide sequences present in the National Center for Biotechnology Information (NCBI) database. The 16S rDNA sequence of the isolate was aligned with similar sequences retrieved from the NCBI database using the MUSCLE (MEGA 11), and a phylogenetic tree was constructed to determine the evolutionary relationship of the bacterial isolate using the neighbor-joining method of MEGA 11 based on the 500 bootstraps (Tamura et al., 2021).

2.8. Plant growth promotion studies on *A. paniculata*

2.8.1. Seed germination assay

First, surface sterilization of the seeds of *A. paniculata* was done with 0.1% HgCl₂ solution for 3 min, washed with sterilized distilled for 4–5 times, again sterilized with 70% ethanol for 3 min, and thereafter washed with sterilized distilled water for 2–3 times. Furthermore, the seeds were primed with a bacterial culture that was pelleted down after centrifugation at 1,000 rpm and suspended in sterile water. After bacterization, the seeds were placed on the Petri plates containing layers of moist filter paper. The seeds were then allowed to germinate at 28 ± 2°C. The appearance of a 2–5 mm radicle was considered an initiation of seed germination (Iida and Takemoto, 2018).

2.8.2. Pot experiments

A pot experiment was conducted to study the effect of bioinoculant on plant growth parameters of *A. paniculata*. Bacterized seeds were sown and allowed to germinate in plastic pots containing sterilized artificial soil having no nutrients (cocopeat 60% + vermiculite 20% + perlite 20%). The uninoculated condition was marked as control has only sterilized artificial soil without any addition. Plants were grown in the controlled conditions of light (14-/10-h light/dark cycle), temperature (25 ± 2°C), and relative humidity (~70%). The seedlings supplemented with bioinoculant served as experimental, while the control seedlings had only sterile water. Bioinoculant supplementation was done every 15-day interval till the flowers appeared. The pots were arranged in random order and the experiments were replicated as 10 pots per treatment: one plant per pot and replicated three times. At various growth stages, observations were recorded related to the plant's growth metrics such as vegetative, flowering, and maturation. Then, 70 days after germination, the plants were carefully removed with intact roots from the pots and then washed with distilled water to remove the soil contents. Parameters such as shoot length, root length, leaf number, node number, whole plant fresh, and dry weight were measured, which are treated as standard for plant growth and development.

During the above course, only the bacterial inoculum was used as natural fertilizer instead of chemical fertilizers, and pesticides were also avoided.

2.8.3. Leaf chlorophyll and carotenoid content

Chlorophyll and carotenoid contents from the leaves were calculated using acetone extract (Arnon, 1949). A total of 10 ml of 80% acetone was used to grind the fresh leaves (0.1 g). It was then centrifuged for 5 min at 5,000 rpm (Biehler et al., 2010). It was repeated until the residue was colorless after removing the supernatant. The absorbance of the supernatant was measured at 470, 645, and 663 nm against a blank solvent (acetone). The following equations were used to calculate the chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content in the plant as per the method of Arnon (1949):

$$\text{Chlorophyll a (mg/g)} : [(12.7 \times A_{663} - 2.69 \times A_{645})v/w]$$

$$\text{Chlorophyll b (mg/g)} : [(22.9 \times A_{645} - 4.68 \times A_{663})v/w]$$

$$\text{Total Chlorophyll (mg/g)} : [20.2(A_{645}) + 8.02(A_{663})v/w]$$

$$\text{Carotenoid (mg/g)} : \{[(1,000 \times A_{470}) - 3.27$$

$$\times \text{Chlorophyll a} + 1.04 \times \text{Chlorophyll b}]/227v/w\}$$

2.8.4. Evaluation of morphophysiological characteristics

Morphophysiological comparison between control and inoculated plants was done carefully after 7, 21, 28, 35, 42, 48, 56, and 60 days after germination. Evaluation of shoot and root morphology, number of tillers (shoots), root length, shoot length, number of branches, and average number of compound leaflets were analyzed and counted in each plant after ~70 days in control and inoculated plants.

2.8.5. Growth and biomass yield

The plants were harvested and washed with sterile water after ~70 days of germination. The length of the shoots and roots was measured and recorded. To obtain the total biomass of roots and shoots, the plants were weighed for the fresh weight (FW) and plants were oven-dried for 12 h at 80°C to obtain dry weight (DW).

2.9. Statistical analysis

One-way analysis of variance (ANOVA) was done to confirm the variability of data and the validity of the results. Student's *t*-test was performed to measure the significance of data at a 95% confidence level.

3. Results

3.1. Isolation and screening of rhizospheric bacteria

The soil from which the sampling was done showed a pH of 6.4, optimum organic carbon content, and average distribution of

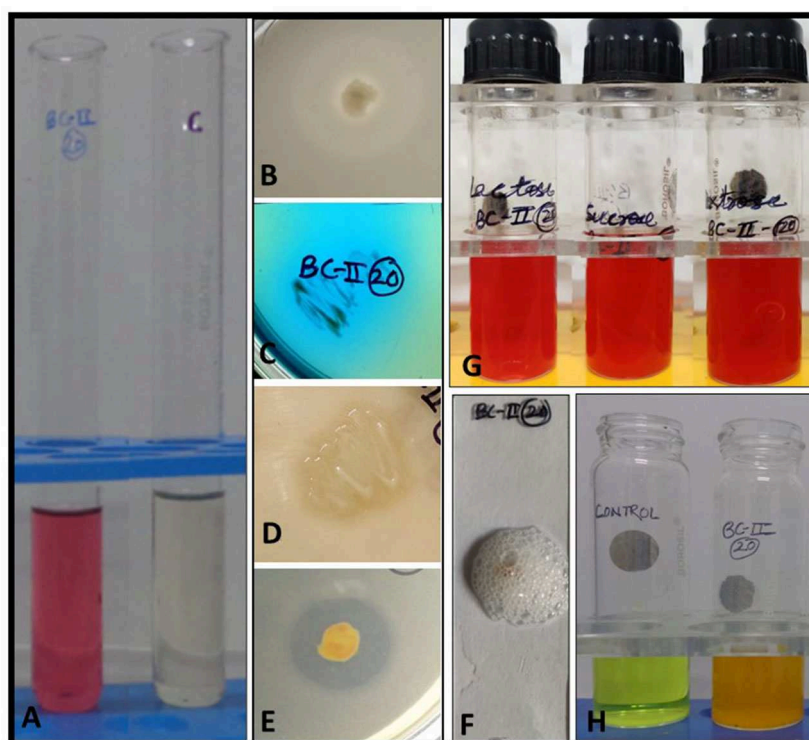


FIGURE 1

In vitro biochemical and plant growth-promoting traits of bacterial isolate BC-II-20. (A) IAA production, (B) phosphate solubilization, (C) citrate utilization, (D) protease production, (E) calcite solubilization, (F) catalase test, (G) carbohydrate fermentation tests, and (H) ammonia production.

macro- and micro-elements. The rhizobacteria from the rhizospheric soil of the medicinal plant *B. ciliata* have been isolated. The isolate was rod-shaped and motile when examined under a microscope and was negative for gram reaction. Based on the morphological and biochemical characteristics, the isolate was found to be positive for oxidase, catalase, citrate utilization, and protease production. Isolate does not show any acid production when tested for the utilization of different carbohydrates (sucrose dextrose and lactose) (Figure 1G). It showed tolerance growth on PEG up to 25%. Moreover, the antibiogram profile of the isolate showed its resistance to penicillin, erythromycin, and chloramphenicol whereas its sensitivity to kanamycin, streptomycin, and tetracycline (Figure 2). The morphological and biochemical characteristics of the isolate are summarized in Table 1.

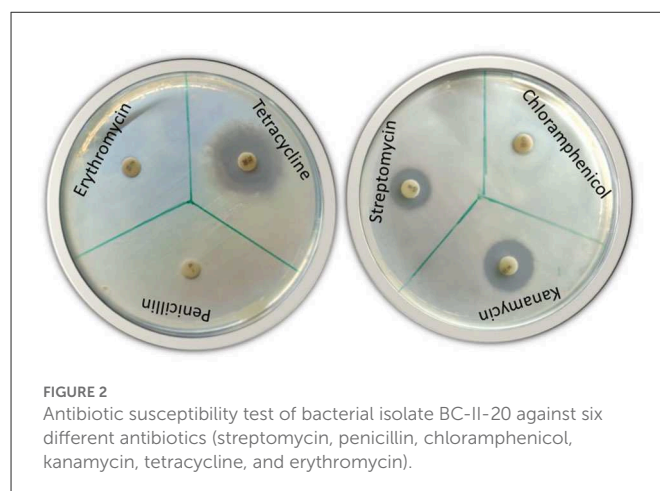


FIGURE 2

Antibiotic susceptibility test of bacterial isolate BC-II-20 against six different antibiotics (streptomycin, penicillin, chloramphenicol, kanamycin, tetracycline, and erythromycin).

3.2. *In vitro* plant growth-promoting attributes of the bacterial isolate

The isolate was initially screened for its *in vitro* plant growth-promoting (PGP) activities such as indole acetic acid (IAA) production, nitrogen fixation, HCN production, ammonia production, phosphate and calcite solubilization, and siderophore production. The isolate exhibited multiple PGP traits and was positive for IAA production ($120 \mu\text{g ml}^{-1}$ of culture filtrate), solubilized tricalcium phosphate and calcite, produced ammonia and protease, and was negative for chitinase and siderophore production (Figure 1; Table 2).

3.3. Molecular identification of the isolate and phylogenetic tree

Molecular identification of the bacterial isolate was done and sequence analysis was performed. After nucleotide BLAST against 16S ribosomal DNA sequence database, a high similarity was observed with the *Pseudomonas* sp. A phylogenetic tree was generated using the 16S rDNA sequence with 10 different representative sequences from the NCBI database (Figure 3). Then, there were three broad groups: BC-II-20 showed maximum similarity with *P. fluorescens*, *P. proteolytica*, and *P. brenneri*.

TABLE 1 Biochemical characteristics, antibiogram profile and drought stress tolerance of the isolate BC-II-20.

BC-II-20		
Biochemical characteristics		
Gram's test		Gram –ve
Shape		Bacillus
Motility	Swimming	+
	Swarming	+
Catalase test		+
Oxidase test		+
Carbohydrate fermentation	Sucrose	–
	Dextrose	–
	Lactose	–
Citrate utilization		+
Protease production		+
Amylase production		–
Chitinase production		–
MR		–
VP		–
Antibiotic resistance		
Kanamycin		–
Tetracycline		–
Chloramphenicol		+
Streptomycin		–
Penicillin		+
Erythromycin		+
Drought tolerance		25% w/v PEG

+, activity present; –, activity absent.

MR, methyl red; VP, Voges-Proskauer.

TABLE 2 Qualitative analysis of plant growth-promoting traits of bacterial isolate BC-II-20.

BC-II-20	
Plant growth promoting traits	
IAA production	+++ (120 µg/ml)
Nitrogen fixation	++
Phosphate solubilization	+++
	PSI-2.3
Ammonia production	++
Hydrogen cyanide production	–
Calcium solubilization	+++
	CSI-1.4
Chitinase activity	–
Siderophore production	–

Activity = +, slight; ++, medium; + + +, good.

and were grouped together, *P. fidesensis*, *P. meridiana*, and *P. antarctica* were placed in the second group, and the third group comprised *P. extremaustralis*, *P. petroselini*, *P. gremontii*, and *P. rodesiae*.

3.4. Effects of bioinoculant on seed germination

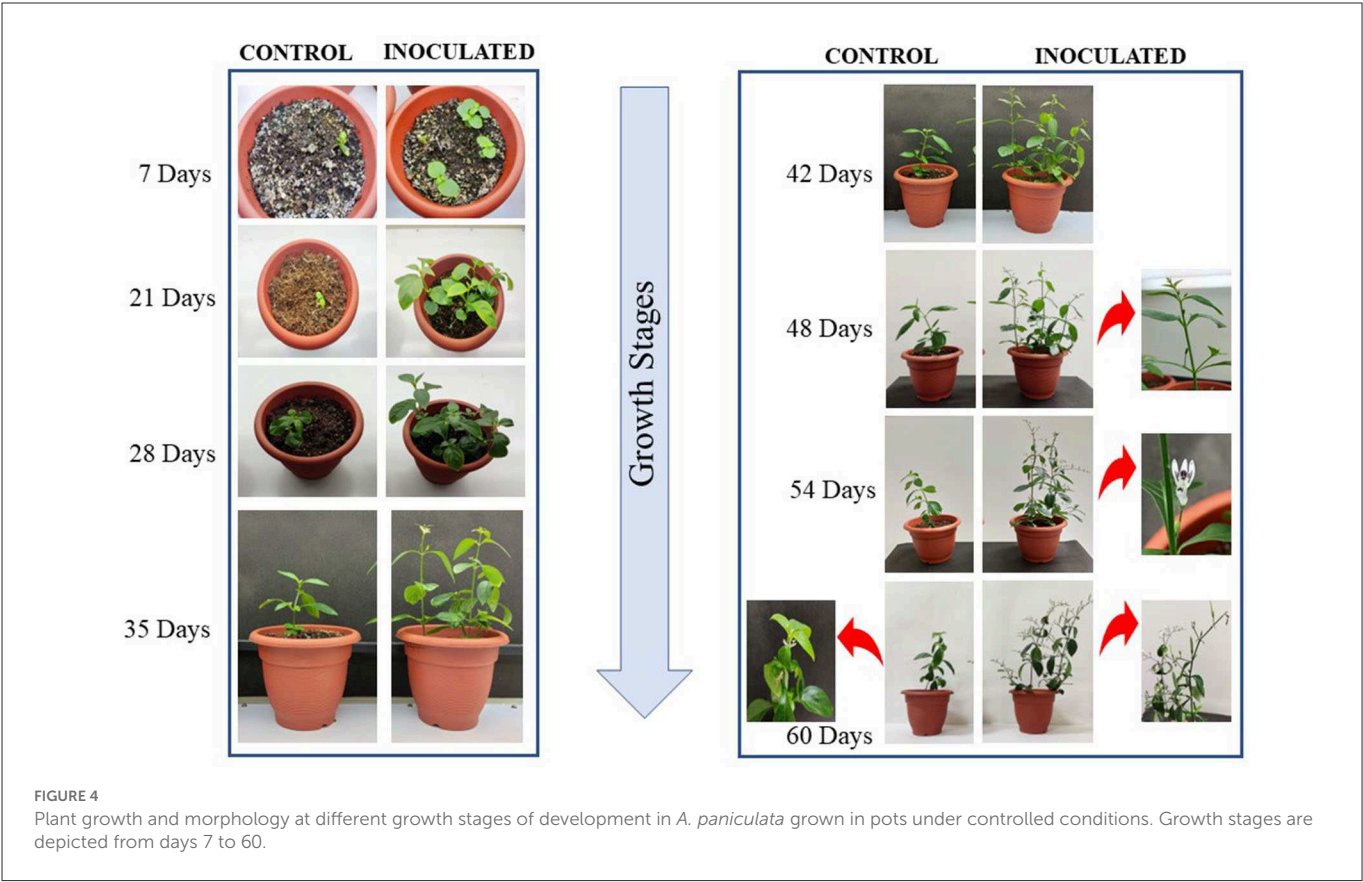
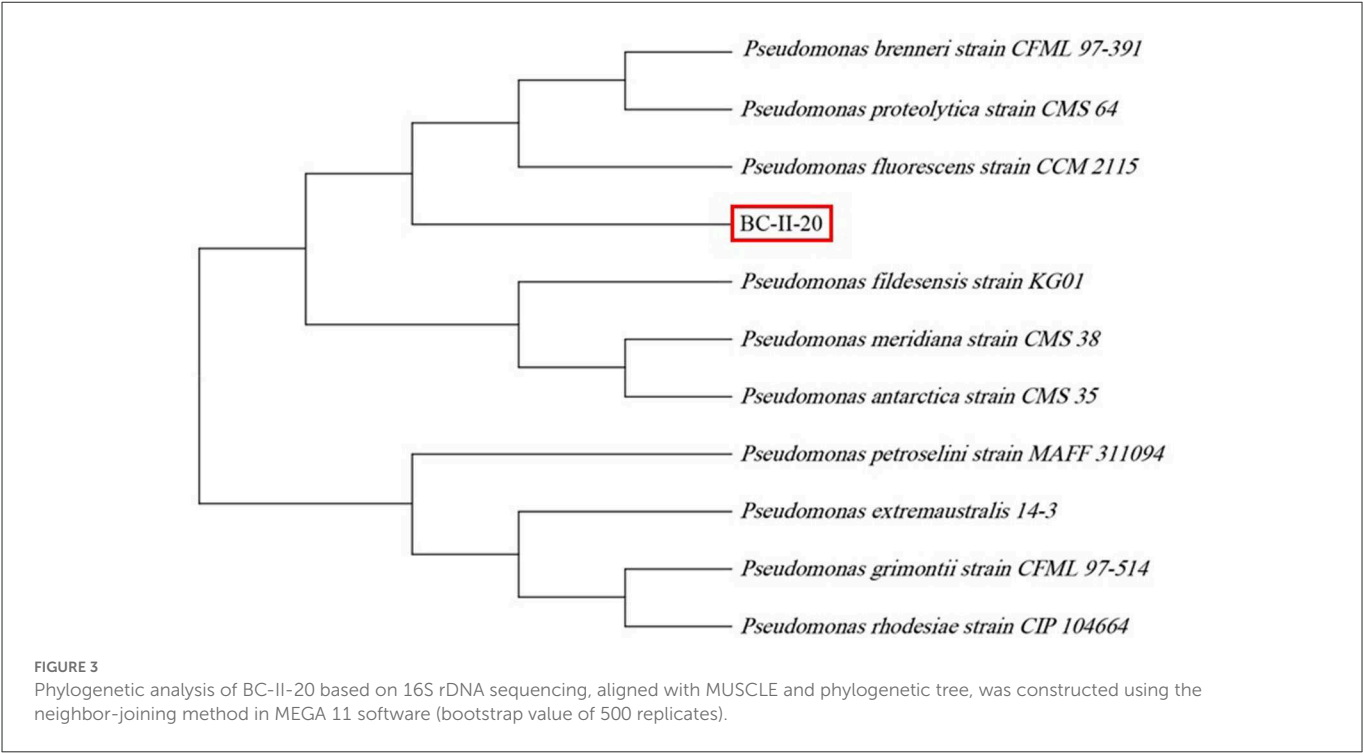
The *A. paniculata* bioprimered seeds with the bioinoculant (BC-II-20) showed good germination efficiency, and 100% germination of treated seeds was observed, but less germination rate was seen in the control experiment, which was about 90%. The equivalent concentration of bacterial suspension was further chosen for the pot experiments based on the results of the seed germination experiment. In summary, the isolate displayed a stimulating effect on *A. paniculata* seed germination.

3.5. Evaluation of bioinoculant for plant growth potential and root morphology of *A. paniculata*

When the isolate was evaluated for its potential for plant growth in pot experiments on *A. paniculata*, it significantly improved its agronomic performance. The growth of inoculated plants was much better as observed on days 7, 21, and 28 as compared to the control plants, which showed poor growth. The control plant growth was improved from day 35 onward (Figure 4), but inoculated plants displayed a much better response with increasing growth stages. The growth performance of inoculated plants can be summarized as follows: plant height (~34%), the total number of panicles, panicle length, number of pods and flowers and fresh weight (~57%), and total dry matter (~60%) as compared to the un-inoculated control plants (Figure 5; Table 3). As compared to the control plants, the treated plants also significantly enhanced root development, including root length, root surface area, lateral roots, and root volume (Figure 6). Bioinoculant also significantly enhanced leaf chlorophyll a content by ~22%, chlorophyll b content by ~4%, carotenoid content by ~12%, and total chlorophyll content by ~12% (Figure 7; Table 3). For comparative growth analysis, the leaves from control and inoculated plants (60 days) were arranged in acropetal order (older at the bottom and younger at the top). Visibly, the inoculated plants were more green in color and leaf size was also better than the control plants. A very interesting difference was seen in the panicle as the panicle length was more and well-differentiated in the inoculated plants with the presence of flowers and pods (Figure 6B). Figure 6F represents the arrangement of growth stages from immature bud to mature flower and finally the appearance of the pod.

3.6. Effect of bioinoculant on the onset of early flowering

The inoculation of the bacteria also positively influenced the onset of early flowering and the overall growth performance of the plants. Flower bud initiation was observed on the 48th day after germination, after a week (54th day—Figure 4 inset), bud initiation fully bloomed flowers of white color were seen, and this observation was absent in uninoculated/control plants. Also, in the inoculated plants, fully mature panicles with a clear distinction from flower bud initiation to pod formation were seen as compared to the control ones, which showed delayed flowering, and overall growth was slow in control plants (Figure 4). The flower appeared after ~60 days in a less prominent panicle in the uninoculated plants.



4. Discussion

This study deals with bioinoculant supplementation on *A. paniculata* plants and its growth-promoting effects. Bioinoculants

are naturally occurring soil microbes that reside around the roots to encourage plant growth and development (Paré et al., 2011). Developing healthy plants resilient to abiotic stresses requires an enhancement of soil functions to promote and ensure sustainable

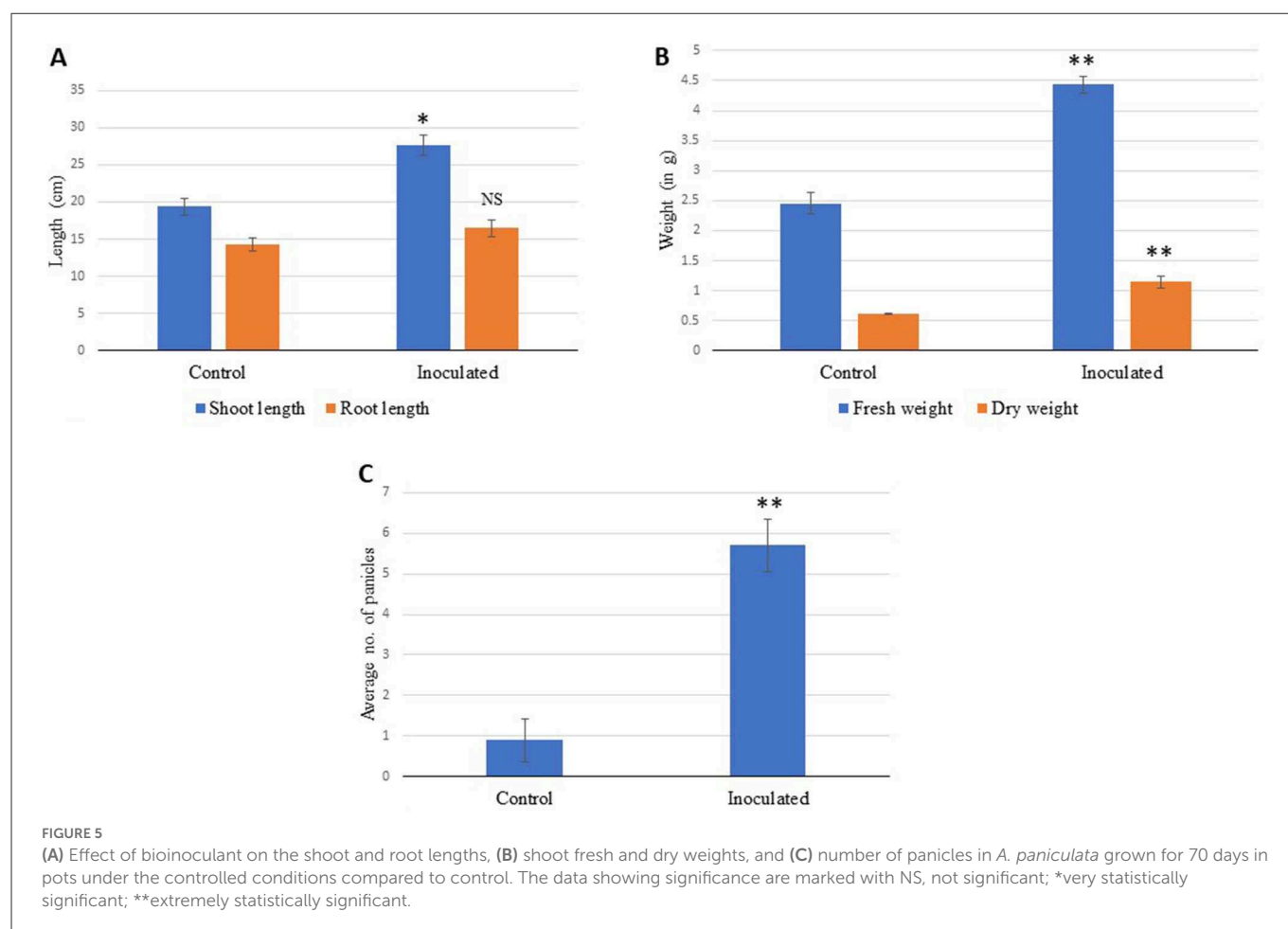


TABLE 3 Effect on different growth parameters of *A. paniculata* plants treated with bacterial isolate BC-II-20 at the time of harvesting (after ~70 days post germination).

Physio-morphological parameters		Control	BC-II-20 inoculated	% increase
Shoot length (cm)		19.4 ± 1.11	27.6 ± 1.35*	34.89
Root length (cm)		14.30 ± 0.9	16.50 ± 1.11 ^{NS}	14.28
Fresh weight (g)		2.45 ± 0.18	4.43 ± 0.13**	57.55
Dry weight (g)		0.61 ± 0.01	1.14 ± 0.10**	60.57
Average number of panicles		0.9 ± 0.53	5.7 ± 0.64**	57.14
Chlorophyll content (mg/g fresh wt.)	Chlorophyll a	15.17 ± 0.14	18.97 ± 0.32**	22.26
	Chlorophyll b	20.22 ± 0.97	21.05 ± 0.15 ^{NS}	4.02
	Total chlorophyll	35.38 ± 0.83	40.01 ± 0.83**	12.28
	Carotenoids	4.79 ± 0.01	5.41 ± 0.01**	12.15

Data presented are mean ± SD from three replicates: Each replicate consisted of ten plants. One-way ANOVA significant at $p \leq 0.01$. Student's *t*-test was performed to test the significance at 95% confidence level. The data showing significance is marked with NS, not significant; *very statistically significant; **extremely statistically significant.

crop production. This may be achieved *via* complex, labor, and cost-intensive genetic engineering approaches, but the use of greener tools such as bioinoculants offers economical and eco-friendly better alternatives. This involves the application of plant growth-promoting bacteria as a sustainable agronomic practice, thereby enhancing plant growth development and yield.

We have isolated and characterized multitrait *Pseudomonas* sp., which displayed plant growth-promoting attributes such as

IAA production, nitrogen-fixing ability, triphosphate solubilization, calcite solubilization, and ammonia production. IAA production is correlated with better root growth, and nitrogen-fixing ability, phosphate and calcite solubilization to provide nitrogen, mineral phosphates, and a source of calcium available to plants. Many microbes have the innate ability to release organic acids, resulting in their phosphate-solubilizing activity. *Pseudomonas* is one of the most powerful genera having the capability to solubilize

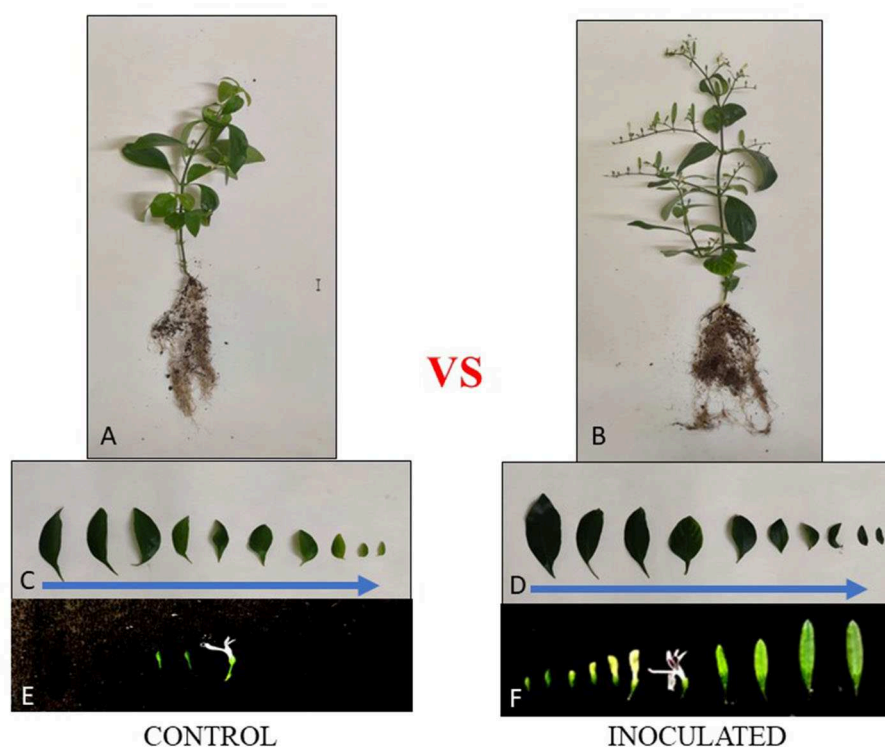


FIGURE 6

Effect of bacterial isolate BC-II-20 inoculation in the morphology of root, shoot, and leaves in *A. paniculata* plants (A) control plants, (B) plants inoculated with bioinoculant (BC-II-20), and (C, D) comparison of leaf size in control (A) and inoculated (B) plants in the same plane in acropetal order. The representative construct/representation of a panicle in the inoculated plant (F) compared to the control plant (E), the growth stages of flowers were arranged from top to bottom in an acropetal manner (older flowers at the bottom and younger at the top) in 60 days old plants.

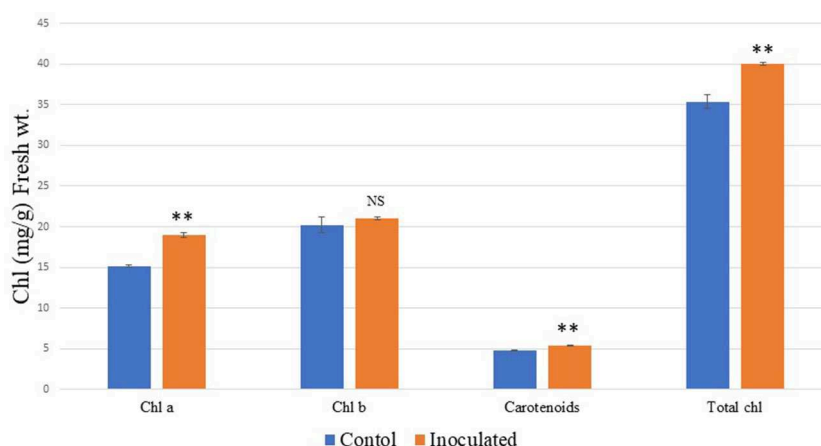


FIGURE 7

Effects of bioinoculant BC-II-20 on *A. paniculata* plant chlorophyll content. The data showing significance are marked with NS, not significant; **extremely statistically significant.

phosphate (Kalayu, 2019). As a result, phosphate-solubilizing microbes are widely used in plant growth promotion and maintain soil fertility while reducing the increasing costs of hazardous phosphate fertilizers. Ahmed et al. (2014) also isolated bacteria from the rhizosphere of some medicinal plants and reported their plant growth-promoting attributes. *P. brenneri* (Hayat et al., 2013) and *P. fluorescens* (Lally et al., 2017)

also displayed tricalcium phosphate solubilization and nitrogen-fixing ability.

Out of six antibiotics, the bacterial isolate seemed to be resistant to only three antibiotics such as penicillin, erythromycin, and chloramphenicol. According to Ferjani et al. (2019), *Pseudomonas* rhizobacteria expressed a low rate of antibiotic resistance as a result of various pathogens and human activities. Because of low antibiotic

resistance, the noxious effects and the evolution of resistance in the environment are limited. Bioinoculant *Pseudomonas* sp. under study can grow under drought conditions as it showed tolerance at 25% PEG. PGPR application enhanced drought tolerance in potato plants as reported by Batool et al. (2020), thereby proving the role of such beneficial bacteria in abiotic stress management.

Multitrait bacterial isolate BC-II-20 was identified as *Pseudomonas* sp. based on the sequence of 16S rRNA gene amplification. BC-II-20 *Pseudomonas* sp. showed a close phylogenetic relationship with *P. brenneri* and *P. fluorescens*, which are reported PGPRs. Inoculation of wheat plants with *P. brenneri* enhanced its shoot, root and overall growth (Hayat et al., 2013) and *P. fluorescens* acts as efficient PGPRs in many crops (Sah et al., 2021). *Pseudomonas* is diverse and has been isolated from the rhizosphere of several plants and reported to have plant growth-promoting traits (Qessaoui et al., 2019).

Plants undergo diverse changes that are induced by PGPR, and growth is the result of complex and interrelated pathways (Bharti et al., 2016). Inoculation of *A. paniculata* with our bacterial isolate significantly increased germination and root branching as compared to the control. The greater root surface that arises from root development can consequently have a favorable impact on the absorption of nutrients and water. According to Premalatha et al. (2021), the availability of nutrients has a major impact on plant productivity and quality. IAA is a phytohormone associated with root development (Ortiz-Castro et al., 2014). The bacterial isolate used in this study is a good IAA producer, thereby promoting root growth of primary roots and subsequent development of secondary roots, thus helping plants to absorb nutrients more effectively as also reported by Patten and Glick (2002). There is some evidence that direct interactions between plants and IAA-producing bacteria can have various outcomes, including phytostimulation, based on the biochemical pathways of bacterial IAA synthesis and regulation (Spaepen et al., 2007).

Photosynthetic ability is increased when growth and nutrition are proper, and this was observed in our study as the inoculated plants had better root development, so more nutrient acquisition and water absorption promoted plant growth and increased plant biomass. Bioinoculant BC-II-20 induced photosynthetic pigments viz. 22% more chlorophyll a content, 12% more carotenoid content, and a 12% increase in total chlorophyll content. Better growth performance after germination was observed in inoculated plants on 7, 21, 28, and 35 days after germination, thereby proving the role of PGPR-mediated growth enhancement. When fully mature plants were compared, we can figure out very prominent panicles in inoculated *A. paniculata* plants. The presence of more photosynthetic pigments imparted a more greenish color in leaves as observed visually, and the size of leaves was also bigger as compared to the control. Growth and development of many plant species were increased after inoculation with PGPRs, and Cappellari et al. (2013) also reported an increase in plant growth parameters in marigold (*Tagetes minuta*) after inoculation with *P. fluorescens*.

In our study, the inoculated plant had early initiation of flowering, and the number of flowers increased, as compared to the uninoculated plant. Early flowering might be due to the indirect effect of PGPR, which can increase the availability of nutrients in the soil and enhance different physiological processes.

Redondo-Gómez et al. (2022) reported flower bud induction in strawberry plants after inoculation with PGPRs and colonization by *Paenibacillus lentimorbus* also resulted in more flowers and seeds in tobacco, as reported by Kumar et al. (2016). It may be hypothesized that early flowering in healthy plants of *A. paniculata* may reduce its life cycle. These observations were also supported by the results of Poupin et al. (2013), which show that the whole life cycle of the plant may be impacted by *Burkholderia phytofirmans*, a PGPR which showed accelerated growth and reduced vegetative stages.

5. Conclusion

Overall enhancement in the growth and physiological parameters of *A. paniculata* was observed by the application of bioinoculant, BC-II-20 (*Pseudomonas* sp.) plant growth-promoting traits were displayed by the beneficial microbe in this study, and its direct and indirect effects positively correlated with the increased biomass and better photosynthetic efficiency. Bioinoculant assisted and promoted the growth of the plant in a sustainable manner, and ultimately, its early flowering and maturation led to a reduction in its life cycle, thereby increasing commercial applications. Further experiments are needed to ensure and achieve maximum positive effects using this PGPR, also in other crops. The underlying molecular basis of the observed phenomenon also needs to be undertaken.

Data availability statement

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

Author contributions

SY conceived and designed the research study. RT conducted the experiments. RT and SY analyzed the results and wrote the article. SS performed the statistical analyses and editing. All authors reviewed the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Regional differences in nitrogen balance and nitrogen use efficiency in the rice–livestock system of Uruguay

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The reintegration of crops with livestock systems is proposed as a way of improving the environmental impacts of food production globally, particularly the impact involving nitrogen (N). A detailed understanding of processes governing N fluxes and budgets is needed to design productive and efficient crop–livestock systems. This study aimed to investigate regional differences in N balance (NBAL, defined as all N inputs minus outputs), N use efficiency (NUE, defined as N outputs/inputs × 100), and N surplus (NSURP, defined as all N inputs minus only outputs in food products) in the rice–livestock system of Uruguay. Three regions across Uruguay are distinguished based on soil fertility and length of pasture rotation. The northern region has high soil fertility and short length of rotation (HFSR); the central region has medium soil fertility and medium length of rotation (MFMR); the eastern region has low fertility and long pasture rotation (LFLR). Results for the last 18 years show a very high NUE (90%) for the rice component in all rotations, associated with negative NBALs ranging from $-35 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in HFSR to $-3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in LFLR. However, the livestock component, which overall had low animal productivity ($<2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), had low NUE ($<10\%$) but positive NBALs in all the rotations, sustaining N supply in the rice component. At the system level, NUE was high (60%) and NBAL was slightly positive in all rotations (from $+2.8 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in HFSR to $+8.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in LFLR). Because of a recent increase in the N fertilizer dose in rice, NSURP for the overall system was intermediate ($40 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) and should be monitored in the future. Efforts to improve the system's efficiency should focus on the livestock component.

KEYWORDS

rice–pasture rotations length, nitrogen budgets, nutrient balance, full-chain NUE, NUE development pathway

Introduction

Over the past many decades, production systems in most parts of the world have adapted to the growing global food demand and changes in diets by specialization (Russelle et al., 2007; Lassaletta et al., 2014). Specialized systems frequently rely on large amounts of external inputs of which fertilizers, particularly N, play a key role. This has caused environmental damage including a major contribution to global greenhouse gas (GHG) emissions (Galloway et al., 2008; Hilimire, 2011). Nitrogen use efficiency (NUE) in global food production is low with an average of $<20\text{--}25\%$ of N inputs reaching the final consumable product (Sutton et al., 2013; Zhang, 2020). In general, crop systems have higher NUE than livestock systems, which are associated with high animal waste and GHG emissions (Uwizeye et al., 2020). Specialization has broken

a virtuous circle between livestock and crops, whereby the forage, fiber, and grains for animal feed were provided by cropping while nutrients and organic matter were returned from animals to crops (Thorne, 2007; Wolfe, 2011). A return to integrated crop–livestock production systems are increasingly discussed as a way of achieving high production while avoiding the negative externalities of specialized systems (Baiyeri et al., 2019; Peterson et al., 2020; Vogel et al., 2021).

There are many variants of the integrated crop–livestock systems, from those managed in separate farms but sharing by-products and residues to those in which crops and animals are on the same farm, sometimes in rotation on the same land, but this scenario is currently quite rare, representing in the best case <50% of the total system agricultural area (Wolfe, 2011; Garrett et al., 2017; Brewer and Gaudin, 2020). In all cases, regardless of the degree of integration, the common denominator is the use of animals for what they are good at converting fibrous feeds (e.g., forage) and by-products from the food system into high-value products and manure (Van Zanten et al., 2019). Recoupling crops and livestock at least through the inclusion of annual forages for direct animal grazing between cash crops are being considered in the Rio de la Plata region of South America. Despite remaining incipient, regarding the total region area, diverse ecosystem services have been observed (i.e., soil restoration, nutrient cycling, better adaptation to climate variation) near after starting that management practice (De Faccio Carvalho et al., 2021). In contrast, the particular case of the Uruguayan rice–livestock system could be seen as an example of such a circular farming system, with the whole country's rice area integrated into a systematic pasture–livestock rotational scheme (García et al., 2009; Lanfranco et al., 2018). The system has been operated for four to six decades depending on the region, with a constant yield increase over time of 90 kg ha⁻¹ yr⁻¹ (Blanco et al., 2010) and with relatively low use of N fertilizers (Tseng et al., 2021). In an earlier study (Castillo et al., 2021), we analyzed the system at a national level and found complementarity through N transfer from animal deposition to rice, biological N fixation during the pasture phase, and N recycling in rice bran to livestock. We found the N balances are tight (< 3.5 kg N ha yr⁻¹ in both the components and the system), and N surpluses are low but increasing. Nitrogen use efficiency is high in rice (65%) but much lower in livestock (13%) and the system (23%). National rice yields of 10 Mg ha⁻¹ are now targeted by farmers, potentially requiring more N fertilizer. Over time, this could lead to a decline in NUE and potentially increased N surplus up to undesirable values (Dobermann et al., 2022). At that point, adjustments in fertilizer technology and regulations would be needed. There are regional differences in management across the rice–livestock system, mainly in terms of the length of pasture rotations related to the level of natural soil fertility. These are likely to be linked to differences in NUE and N surplus and their progression over time, which need to be understood to improve the overall system.

Our objectives were to assess N balance, NUE, and their components in rice–livestock rotations across Uruguay and follow their changes from 2004 to 2022. Based on our national scale assessment (Castillo et al., 2021), we hypothesize that even with relatively small N fertilizer additions to rice, the NBAL has been around neutrality while NUE has reached high values in all the rotations over the period investigated. However, we expect differences among rotations due to different pasture lengths and management practices. Because of small N outputs in animal products, we hypothesize that the livestock component reached positive and stable

NBALs and medium to low NUE across the period, resulting in positive NBALs and medium NUE in the whole system. We also explore different production scenarios to identify the more sensitive aspects of NBAL and NUE for improving management practices.

Materials and methods

Cropping system characteristics and data sources

The rice–livestock system of Uruguay consists of ~163,000 ha of rice and 570,000 ha of pastures integrated into a stable rotation divided into three regions (Table 1). The main region is in the east (LFLR in Table 1), accounting for 70% of the national rice area. The northern area (HFSR) accounts for 20% and the central area (MFSR) accounts for 10%. The eastern region is characterized by a flat landscape with slopes of ~0.1%, medium to low soil fertility, and river water sources for flood irrigation. In the northern and central regions, rice is grown on more fertile soils, which includes sloped areas of <5% (nearly 60% in the north and 25% in the central region), and irrigation water is sourced from artificial dams. Despite those particularities, the main differences among the regions are soil fertility and pasture phase length after rice. On average, after two or three consecutive rice crops (the latter mainly associated with the northern region), 4, 3, or 2 years of perennial pastures grazed by livestock complete the rotation in the eastern, central, and northern regions, respectively (García et al., 2009; Giménez et al., 2011; Lanfranco et al., 2018).

Following rice crops, ~31% of HFSR, 33% of MFSR, and 38% of LFLR are mixed pastures, including legume species, seeded into the rice stubble. The combination of these factors means that the ratios of rice seeded into (a) rice stubble, (b) improved pastures including legumes or (c) native grassland are 60–17–23 for HFSR, 50–18–32 for MFSR, and 35–21–44 for LFLR. However, there are a few differences in crop management and the amount of fertilizer and agrochemical products added. The system as a whole is stable and based on land agreements in which the rice farmers rent land for long periods or on an annual basis.

We analyzed data from the Agricultural and Livestock Ministry (MGAP), the Agricultural Statistics Department (DIEA), the National Institute of Meat (INAC), the National Institute for Agricultural Research (INIA), and the rice milling industry (Supplementary Table 1). The original data are available at different scales. For example, while rice data are available from the farm to the county level, livestock and pasture information are only available at the county level. However, calculated cattle stocking rates for each region (0.79, 0.75, and 0.81 livestock units ha⁻¹) were similar to the 0.76 livestock units ha⁻¹ reported in previous studies of a typical rice–livestock rotation district (Simeone et al., 2008).

Rice data

Annual information on rice yield and seeded area were collected from governmental agencies (DIEA Estadísticas Agropecuarias, 2005, 2022). Crop management data are presented annually by the rice milling companies and summarized by INIA, covering ~85–90% of the total rice area. Crop parameters and characteristics associated with each variety were taken from internal records of INIA.

TABLE 1 Components of the rice–livestock system of Uruguay at a regional level.

Sites Rice livestock system parameter	HFSR		MFMR		LFLR		Units
	Mean	Range	Mean	Range	Mean	Range	
Area of rice harvested annually	0.33	0.25–0.40	0.15	0.10–0.23	1.15	1.0–1.38	ha × 10 ⁵
Area of natural pasture	0.44	0.41–0.53	0.30	0.27–0.40	2.90	2.78–3.22	ha × 10 ⁵
Area of improved pasture	0.22	0.12–0.31	0.15	0.12–0.29	1.7	1.12–2.21	ha × 10 ⁵
Rice: rotation ratio	1:2	–	1:3	–	1:4	–	–
Stock density (bovine + ovine)	0.75	0.61–0.80	0.79	0.73–0.9	0.81	0.77–0.84	LU ha ⁻¹ *
Main soil properties (0–20 cm)**							
pH	6.3	5.8–7.6	5.7	5.1–6.0	5.8	5.1–6.0	1:1 H ₂ O
Cation exchange capacity	33.3	13.1–43.9	21.5	8.3–43.7	11.8	8.1–30.7	cmol _c kg ⁻¹
Organic carbon	28.1	15.5–43.3	21.1	15.3–34.4	18.3	9.1–39.9	g kg ⁻¹
Total soil N	2.5	1.6–4.3	2.3	1.0–3.7	1.8	0.7–4.1	g kg ⁻¹
Sand	200	130–270	260	110–560	290	150–460	g kg ⁻¹
Silt	340	250–460	440	200–530	390	300–480	g kg ⁻¹
Clay	460	220–610	300	180–450	320	150–560	g kg ⁻¹
Bulk density***	1.25	–	1.31	–	1.35	–	g cm ⁻³

HFSR represents the high fertility and short rotation of the northern region, MFMR represents the medium fertility and medium rotation of the central region, and LFLR represents the low to medium fertility and low rotation of the eastern region. Values are averages and ranges for the 2004–2005 to 2021–2022 growing seasons.

*Livestock unit. 1 LU = 380 kg animal live weight ha⁻¹. **Average data obtained from soil samplings of 52 experiments conducted over 3 years in the main rice production locations at each region.

***Estimated values using the SPAW software (Saxton and Willey, 2006), based on soil type, percentage of sand, clay, and organic carbon.

Approximately 75% of the exported or internally consumed rice is white rice (Observatory of Economic Complexity, 2020), so we assumed that all the bran after milling was returned to the rice–livestock system as animal feed. In addition, soil information for the dominant rice systems in each region was collected from a multi-year-location field trial network of N response conducted by INIA (Table 1).

Pasture data

The country forage base is composed of native grassland, semi-natural pastureland, and temporary pastureland, averaging 90, 4, and 6%, respectively, following Allen et al. (2011) classification. Native grassland comprises native grass species, and the other two pasture categories include legumes (*Trifolium spp.* and/or *Lotus spp.*) and grasses (*Lolium spp.* or *Festuca spp.*). No N fertilizer is applied. We refer to the semi-natural pastureland and temporary pastureland as improved pastures. Natural grassland forage productivity was estimated based on 16 years of remote sensing data for the main ecological regions of the country (Asuaga et al., 2019) and 10 years of remote sensing data for improved pastures (Martínez, 2011). Additional information on dry matter production and botanical pasture composition at different pasture stages and years was taken from a long-term experiment on rice-improved pasture rotations at INIA facilities.

Livestock data

We estimated animal meat production (beef and sheep) and the N accumulated in the animal body as follows. We used long-term data of county annual livestock stock (Dirección Nacional de

Contralor de Semovientes, 2004; Sistema Nacional de Información Ganadera, 2022), and monthly reports of the livestock category and live weight of animals received at the abattoir from each county (Instituto Nacional de Carnes, 2020). The latter also includes records of on-farm self-consumption on an annual and county basis. These records were used for animal meat production and N retention calculations. In addition, wool production was included in the meat production calculations under the equivalent meat concept (FAO, 2018). Wool was also included in the N retention calculations considering the country's average wool production of 4 kg animal⁻¹ yr⁻¹ (DIEA Oficina de Estadísticas Agropecuarias, 2020), adjusted to a dry and clean basis and a literature N concentration value of 16% (ARC, 1980). We calculated the animal N recycling as a function of the animal species, the botanical pasture composition, and production, as well as the forage utilization efficiency (including rice straw) and animal internal N use efficiency.

Modeling of missing N data

Despite having good long-term records for calculating the main N pool fluxes, data on soil N losses are scarce and partial in the country. We have recently parameterized and tested the DeNitrification–DeComposition (DNDC) model for different rice rotations (including the rice–pasture–livestock rotation) on a typical rice soil of Uruguay (Castillo et al., 2022). Results showed good agreement between simulated and observed crops and pasture yields, cumulative N rice uptake, and soil NH₄-N during flooded conditions, as well as acceptable estimates of N₂O emissions during aerobic and anaerobic soil conditions. For this study, we used DNDC to simulate N losses (gaseous NH₃ and N₂O, and NO₃⁻ in leaching and runoff) in rice–pasture + livestock

rotations in each region over the study period. Considering all the rotation phases present in 1 year, we started the modeling for 2004–2005 with first-year rice and second-year rice or pasture, varying the pasture duration as appropriate for each region. Both natural grassland and improved pasture were simulated. The crop parameters set in the DNDC model were as in our previous study (Godinot, Leterme, Vertés, Favardin, and Carof, Godinot et al.), and the soil data according to the region as in Table 1. Climatic data were obtained from INIA's weather stations in each region.

Data analysis

We conducted simple NBAL analyses following a mass conservation approach, and a full chain NUE analysis for both the component and the system level, as well as for each rotation. For rice, inputs were N in fertilizers, atmospheric N deposition, biological N fixation (BNF), and animal N deposition (AND) occurring during the 6 months before the crop, and outputs of N in grain, gaseous NH_3 and N_2O , and leached NO_3^- . Nitrogen inputs for the livestock component included N from pasture BNF, atmospheric deposition, and rice bran, while outputs were N in animal tissue, gaseous NH_3 and N_2O , and leached NO_3^- . The N output from AND corresponded to feces and urine from the livestock-pasture component of the 6 months before land preparation or chemical fallow. Rice bran is the main feed input used in commercial farms of the rice–livestock system area, so we assumed all the annual production was returned to the livestock component in the same proportion as regionally produced. These N inputs were not considered when analyzing the entire system because they act as an intermediate product between components. Mineralization of soil N and N in the forage was considered in constant recycling and not included in the calculations.

We assessed the trajectories of NUE over the study period using a graphical approach (EU Nitrogen Expert Panel, 2015). The resulting values of N outputs in edible food products in relation to inputs were plotted against defined low and high NUE thresholds and a desirable N target in food products. For rice, defined NUE thresholds were <90 and >50%, and the crop N target was 80 kg N in grain $\text{ha}^{-1} \text{yr}^{-1}$ (EU Nitrogen Expert Panel, 2015). This crop N target value is in accordance with the average rice yield of the period (8.1 Mg ha^{-1} , 130 g kg^{-1} humidity) and with the high-yielding rice pasture systems of South America in general (Singh et al., 2017). For the livestock component, NUE thresholds were <25 and >10, as stated by Gerber et al. (2014), and the defined target N in food products was 3 kg N $\text{ha}^{-1} \text{yr}^{-1}$. This targeted N value is reasonable for extensive grazing systems (Oenema et al., 2016) and similar to the values stated by Kanter et al. (2016) as attainable values for Uruguayan extensive conditions. We set the system boundary as the farm gate given the negligible food import and low product industrialization that typify Uruguay as a net commodity exporter.

For all the assessed parameters, rotations were compared using multiple *t*-tests with a significance level of 5%. The Satterthwaite procedure was used if variances were not homogenous. Adjusted regressions were analyzed using auxiliary variables to test the equality mean effect of the different groups and the homogeneity regression slope. Analyses were conducted using InfoStat (Di Renzo et al., 2017).

Uncertainties and scenarios analysis

We analyzed data for the average situation of the rice and livestock components and the whole system. The pasture component has the greatest variability, which in turn influences livestock production (forage offer) and the rice component (N recycling), giving uncertainty to our estimations. For example, a survey of different rice–livestock systems in Uruguay (Simeone et al., 2008) has shown that the percentage of improved pastures considered in those systems ranged from 8 to 84% of the total pasture grazing area. The animal productivity of those scenarios ranged from 54 to 355 kg live weight $\text{ha}^{-1} \text{yr}^{-1}$ (148 kg $\text{ha}^{-1} \text{yr}^{-1}$ on average). This indicates that calculations for this study with 34% of improved pastures (Table 1) could be under or overestimated when different percentages of improved pastures are considered. Another source of uncertainty is the amount of N applied to rice.

To assess the effects of these uncertainties, we analyzed three scenarios. First, rice–livestock production rotation on regenerated natural grasslands after rice crop (SGR). Second, the same scenario but with 40 kg N ha^{-1} fertilizer to rice (SGRN). Third, with 80% improved pastures (SIP). For SGR, we considered a decrease of N output in animal products by 25% based on the stocking rate of extensive livestock systems (Soares de Lima, 2009), which also decreases the N transferred from the livestock component to the rice. For the SIP scenario, we assumed a high meat production of 355 kg live weight $\text{ha}^{-1} \text{yr}^{-1}$ (Simeone et al., 2008) and an extraction rate (ratio of sold animal weight to total animal weight in stock) of 40% (Soares de Lima, 2009). Increased N in rice bran fed to livestock after higher rice yields were allowed.

Results

Rice yield and nitrogen balance

Rice yield reached $8,100 \pm 727 \text{ kg ha}^{-1} \text{yr}^{-1}$ with yield gain rates from 66 kg $\text{ha}^{-1} \text{yr}^{-1}$ (HFSR) to 110 kg $\text{ha}^{-1} \text{yr}^{-1}$ (MFMR and LFLR) over the period (Figure 1A). The N fertilization rate also showed increasing trends of 3.6, 2.7, and 2.3 kg N $\text{ha}^{-1} \text{yr}^{-1}$ in HFSR, MFMR, and LFLR, respectively (Figure 1B). The annual increases in N rate were 4.7 (HFSR), 1.2 (MFMR), and 0.9 (LFLR) times the annual increase of N removed in grain yield.

Total N input to rice was greater in LFLR than in HFSR and MFMR (Table 2). The main N input to rice was in fertilizers (73, 70, and 68% of the total N inputs for HFSR, MFMR, and LFLR, respectively), with smaller contributions from BNF and atmospheric deposition. Differences in total N inputs among rotations were due to AND transferred to rice. Total N inputs to pastures were the greatest in HFSR followed by MFMR and LFLR due to the BNF from pastures. This BNF value is linked to the entire pasture area of each region (native grasslands + improved pastures); on average 46 kg $\text{ha}^{-1} \text{yr}^{-1}$ of N was fixed in improved pastures. Similarly, differences in N input from bran are mainly explained by the total area of rice in each region. On average, atmospheric N deposition was very similar among rotations averaging 6 kg N $\text{ha}^{-1} \text{yr}^{-1}$. At the rice–livestock system level, total N inputs for each rotation differed in the order HFSR > MFMR > LFLR.

Total N outputs for the rice component were greater in HFSR than in MFMR and LFLR. Nitrogen in grain was the main output

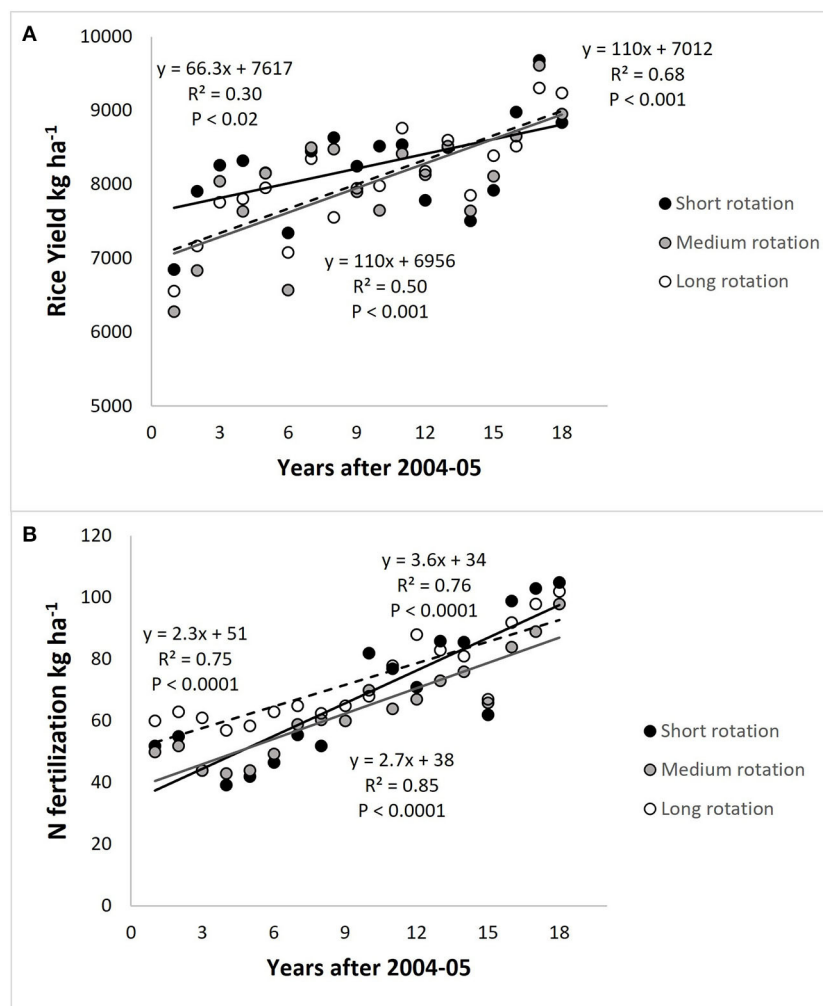


FIGURE 1

(A) Rice yield and (B) nitrogen fertilization trajectories from 2004–2005 to 2021–2022 growing seasons. HFSR, high fertility and short rotation; MFMR, medium fertility and medium length rotation; LFLR, low to medium fertility and long rotation.

and was similar among rotations, averaging $84.6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, i.e., $\sim 72\%$ of the total N output. Differences in total N output were associated with N losses, which represented 31, 28, and 24% of total N output in HFSR, MFMR, and LFLR, respectively. Volatilization was the main N loss process (97, 91, and 87% in HFSR, MFMR, and LFLR, respectively), followed by denitrification (2, 7, and 8%) and leaching plus runoff (1, 2, and 5%).

The average total N output of the livestock–pasture component was 12% of that of the rice component. Nitrogen losses were the main output averaging $7.2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, followed by the N transferred from the livestock to rice ($5.1 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), and both outputs varied a little among rotations. Output in animal tissue was only $1.8 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. At the system level, total N input and output values were close to each other, resulting in a slightly positive balance. However, the system N balance differed among rotations over the study period. The system NBAL for HFSR increased from -8.5 in 2004/2005–2009/2010 to $+2.8$ in 2010/2011–2015/2016 and $+14.1 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in 2016/2017–2021/2022. By contrast, system N balance for MFMR and LFLR was always positive but decreased over time from 5.7 to 3.8 to $4.2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in MFMR and from 9.7 to 8.7 to $7.1 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in LFLR over the same periods. For all rotations,

the system NBAL was highly correlated with total N inputs of the livestock–pasture component ($r = 0.87, 0.82$, and 0.75 , $p < 0.001$ for HFSR, MFMR, and LFLR, respectively), mainly due to the amount of N fixed during the pasture phase ($r = 0.73, 0.70$, and 0.66 , $p < 0.01$ for the same rotations). In addition, the system NBAL was strongly associated with N fertilizer inputs in HFSR ($r = 0.86$, $p < 0.0001$).

Full chain-NUE and N surplus analyses

Rice component

The NUE of the rice component was higher in HFSR and MFMR (98 and 94%, respectively), than in LFLR (79%) averaged over the 18 years. The NUE trajectory had two stages in HFSR: first where NUE values exceeded the upper threshold (average 115%), and then when NUE was in the target zone (Figure 2A). This shift happened because of an increase in the N fertilization rate (50 vs. $86 \text{ kg N ha}^{-1} \text{ yr}^{-1}$; Figure 1). In the second phase, during 2018 only, the NUE exceeded the threshold due to less N fertilizer application. On average, the total N removed in grain was higher than the desirable N target ($80 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). For MFMR, 50% of the records were above or around

TABLE 2 Nitrogen balance of each system component and the entire rice–pasture–livestock system.

Component level	Balance factor	Rice–pasture rotation (Rice: rotation ratio)		
		HFSR (1:2)	MFMR (1:3)	LFLR (1:4)
	Inputs (kg ha ⁻¹)			
	Fertilizers	67.6 ^a	63.8 ^a	72.9 ^a
	Animal direct deposition	16.6 ^b	18.7 ^b	26.1 ^a
	Atmospheric deposition	6 ^a	6.4 ^a	5.8 ^a
	BNF free living + symbiotics	2.5 ^a	2.5 ^a	2.5 ^a
Rice	Total N inputs	92.8 ^b	91.5 ^b	107.2 ^a
	Outputs (kg ha ⁻¹)			
	Grain	86.1 ^a	83.6 ^a	84.1 ^a
	Total N losses*	39.4 ^a	32.6 ^{ab}	26.3 ^b
	Total N outputs	125.5 ^a	116.2 ^b	110.4 ^b
	N balance	−32.7 ^b	−24.7 ^b	−3.2 ^a
	Inputs (kg ha ⁻¹)			
	Pasture BNF	20.2 ^a	17.7 ^{ab}	16.0 ^b
	Rice bran	10.2 ^a	6.5 ^b	4.9 ^c
	Atmospheric deposition	6 ^a	6.4 ^a	5.8 ^a
Livestock	Total N inputs	36.3 ^a	30.7 ^b	26.7 ^c
	Outputs (kg ha ⁻¹)			
	N in animal tissue	1.7 ^a	1.8 ^a	1.8 ^a
	Total N losses*	6.2 ^b	8.6 ^a	6.9 ^b
	Animal direct deposition	5.5 ^a	4.7 ^a	5.2 ^a
	Total N outputs	13.3 ^a	15.1 ^a	14.0 ^a
	N balance	23.1 ^a	15.6 ^b	12.7 ^c
	Total N inputs (kg ha ⁻¹)**	49.5 ^a	41.2 ^b	37.6 ^c
System	Total N outputs (kg ha ⁻¹)**	46.7 ^a	36.8 ^b	29.1 ^c
	N Balance (kg ha ⁻¹)	2.8 ^c	4.4 ^b	8.5 ^a

Values are averaged over the 2004/2005–2021/2022 growing seasons. HFSR, high fertility and short rotation (northern region); MFMR, medium fertility and medium length rotation (central region); LFLR, low to middle fertility and long rotation (eastern region).

Means followed by the same letter within rows are not statistically different ($p = 0.05$).

*Total N losses considered NH₃, N₂O, N leached and runoff.

**Total N inputs and outputs at a system level did not include the animal direct deposition factor. Presented N inputs and outputs values were adjusted by the proportion of each component (rice and livestock) on an annual base.

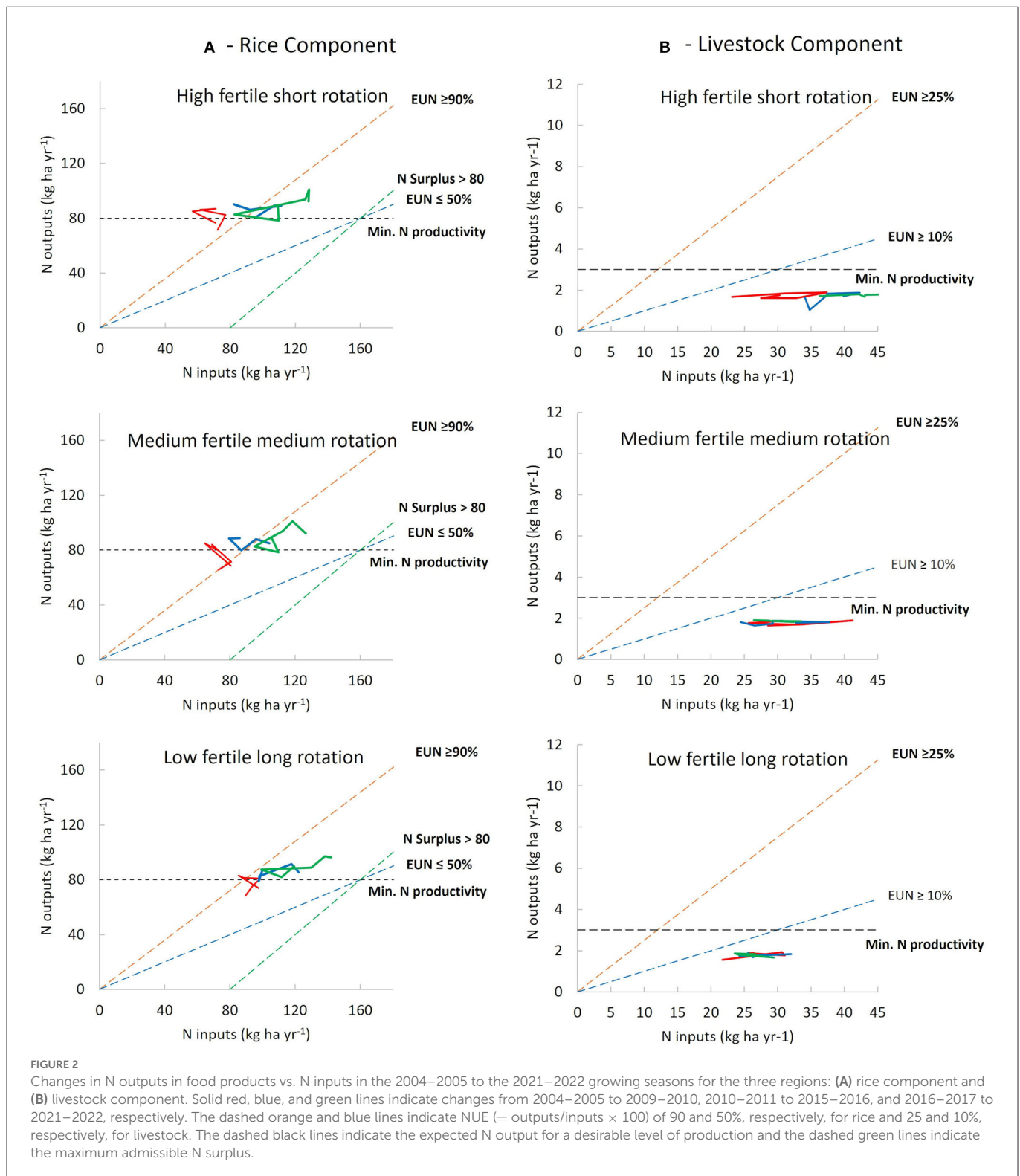
the upper threshold, and the remaining data were in the target zone. Again, the increase in N fertilizer dose explained a constant offset of NUE into the desirable zone ($r = -0.73$, $p < 0.001$, Figure 2A).

Unlike the other rotations, 90% of NUE values for LFLR were in the target zone, with an average of 84 kg N ha⁻¹ yr⁻¹ in grain. Here again, the increase in the N fertilizer dose strongly influenced NUE each year ($r = -0.80$, $p < 0.0001$), shifting values toward the lower NUE threshold (50%) in the last few years of the study. On average, NSURP in LFLR was higher (23 kg N ha⁻¹ yr⁻¹) than in MFMR and HFSR (8 kg N ha⁻¹ yr⁻¹ and 7 kg N ha⁻¹ yr⁻¹, respectively). However, positive values for NSURP in MFMR and HFSR were observed around the middle of the study period when NUE fell below 100% (Figure 3A). At the end of the study period, NSURP reached 36, 33, and 46 kg N ha⁻¹ for HFSR, MFMR, and LFLR, respectively. As expected, NSURP in rice was positively correlated with N fertilizer in addition to all regions ($r > 0.90$, $p < 0.0001$). A negative correlation

between NUE and NSURP was found for all rotations ($r = -0.97$, $p < 0.0001$). The decline in NUE across the period differed ($p = 0.005$) between HFSR and LFLR, while MFMR was intermediate. Similarly, the rate of increase in NSURP differed between HFSR and LFLR, with MFMR intermediate (Figure 3A). The different downward trends of NUE and associated upward trends of NSURP matched the different stages of the generalized pathways, as shown in Figure 3C.

Livestock component

Livestock NUE values were much lower than those in rice. For the 18-year period, NUE values were 6.8, 6.0, and 4.8% for LFLR, MFMR, and HFSR, respectively. Nitrogen output in animal tissue was almost the same for the different rotations, so differences in NUE were associated inversely with the total N inputs, mainly by pasture BNF ($r = -0.80$, $p < 0.0001$), followed by rice N bran ($r = -0.72$,

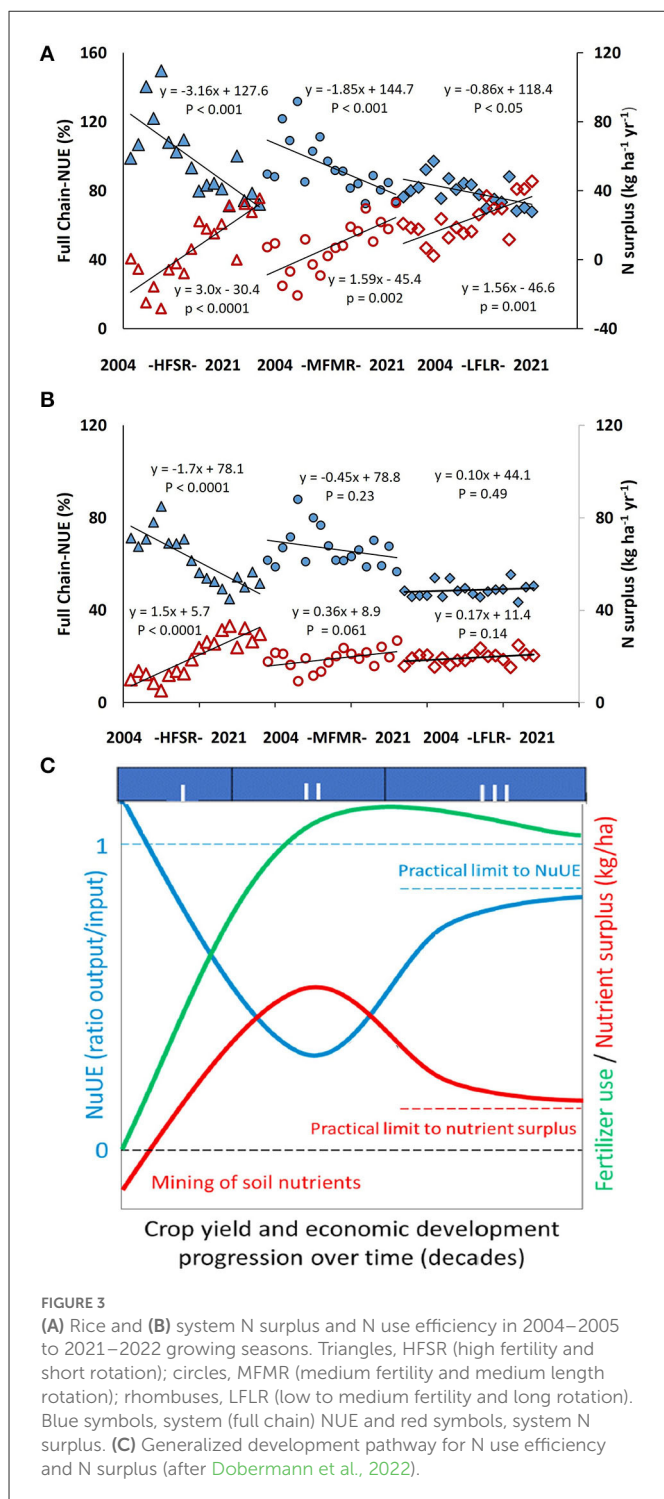


$p < 0.0001$). While NUE values for LFLR and MFMR remained flat over time, values for HFSR decreased by $\sim 20\%$ (Figure 2B), explained by an increase in pasture BNF linked to a greater area of improved pastures over time. Both NUE and N in animal products were below the targets (10% NUE and 3 kg N ha⁻¹ animal products, respectively), in all rotations. Records were closer to the lower NUE threshold during the first years in LFLR but more distant in the last few years of HFSR. Unlike rice, the NUE of the livestock component was not

associated with NSURP, and both variables remained steady over the study period.

Rice–livestock system

The average annual NUE at the system level was higher in HFSR and MFMR (62 and 67%, respectively), than in LFLR (49%), following the same trend as for the rice component (Figures 3A, B). The annual



rate of decrease was higher in HFSR (-1.7% , $p < 0.0001$) than in MFMR (-0.45%) and LFLR (0.1%), the latter being basically flat during the study period. The NUE was positively correlated with rice NUE in HFSR ($r = 0.91$, $p < 0.0001$) and MFMR ($r = 0.80$, $p < 0.0001$), and with livestock NUE in LFLR ($r = 0.70$, $p < 0.001$). For HFSR, there was also a negative correlation with the addition of N fertilizer ($r = -0.84$, $p < 0.0001$). For NSURP, the annual increase was higher in HFSR ($+1.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) than in MFMR ($+0.36 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) and LFLR ($+0.17 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). For all rotations, NSURP was positively correlated with rice N fertilizer addition ($r = 0.55$, $p = 0.018$; $r = 0.57$, $p = 0.013$, and $r = 0.91$, $p < 0.0001$ for

LFLR, MFMR, and HFSR, respectively). As for the rice component, the NUE was negatively correlated with NSURP ($r = -0.78$, -0.92 , and -0.98 , $p < 0.0001$ for LFLR, MFMR, and HFSR, respectively).

Scenario analysis

For all rotations, the SGR scenario generated the most negative NBAL and a higher NUE than the original situation in the rice component (Figure 4). That was due to a greater reduction in N inputs (less AND in the absence of improved pastures) than the decrease in N outputs (mainly N in grain and N losses). In the HFSR and MFMR, the NUE was shifted beyond the upper NUE threshold but not in the LFLR rotation. Adding more N fertilizer in the SGRN scenario not only increased N inputs but also increased N outputs, mainly due to greater N losses which increased by 52, 60, and 80% over the original values for HFSR, MFMR, and LFLR, respectively. This resulted in an even more negative NBAL, and all three rotations reached NUE values between 80 and 87%. Total N inputs of the SIP scenario were almost the same as for SGRN but with more N from pasture BNF. However, N losses were lower than in SGRN because less N was added as fertilizer. The NBAL for the SIP scenario was the least negative among the three scenarios, increasing on average by $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for HFSR and LFLR and not changing in MFMR. The resulting NUE was 84, 79, and 70% for HFSR, MFMR, and LFLR, respectively. The NSURP was higher in SIP (23 , 27 , and $43 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for HFSR, MFMR, and LFLR, respectively) than in the other scenarios and rotations (all $< 26 \text{ kg N ha}^{-1} \text{ yr}^{-1}$).

For the livestock component, the fall in N inputs of the SGR scenario (-40% on average) was explained mostly by the absence of N inputs from pasture BNF. Nitrogen outputs also decreased mainly because of the reduced AND transference to rice (-50% on average), followed by a fall in animal N products (-25%). Because the decrease in N inputs was greater than the decrease in N outputs, NBAL and NSURP decreased in all rotations and NUE increased, reaching values above the lower NUE livestock threshold for MFMR and LFLR, and close to it for HFSR. Increased N fertilization in the SGRN scenario only affected the input from rice bran, which increased the NBAL compared with SGR but was still smaller than in the original situation. As expected, there were greater changes in the SIP scenario due to a substantial increase of N inputs from pasture BNF. However, N outputs from animal N products and N losses also increased, resulting in increases in NBAL and NUE (41 , 38 , and 43% for NBAL and 14 , 11 , and 15% for NUE in HFSR, MFMR, and LFLR, respectively).

At a system level and for all rotations, NBAL was negative in SGR and SGRN and positive in SIP. By contrast, NUE was higher in SGR and SGRN and lower in SIP, in the latter case being even below the original situation. The NUE values in the SGR scenario were 32 , 30 , and 44% higher than in the original situation, while they were decreased by 15 , 10 , and 14% at SIP for HFSR, MFMR, and LFLR, respectively.

Discussion

Nitrogen balance

We have found regional differences in the N balance of the rice and livestock components as well as of the whole system. The negative N balance in the rice in the more fertile HFSR and MFMR regions

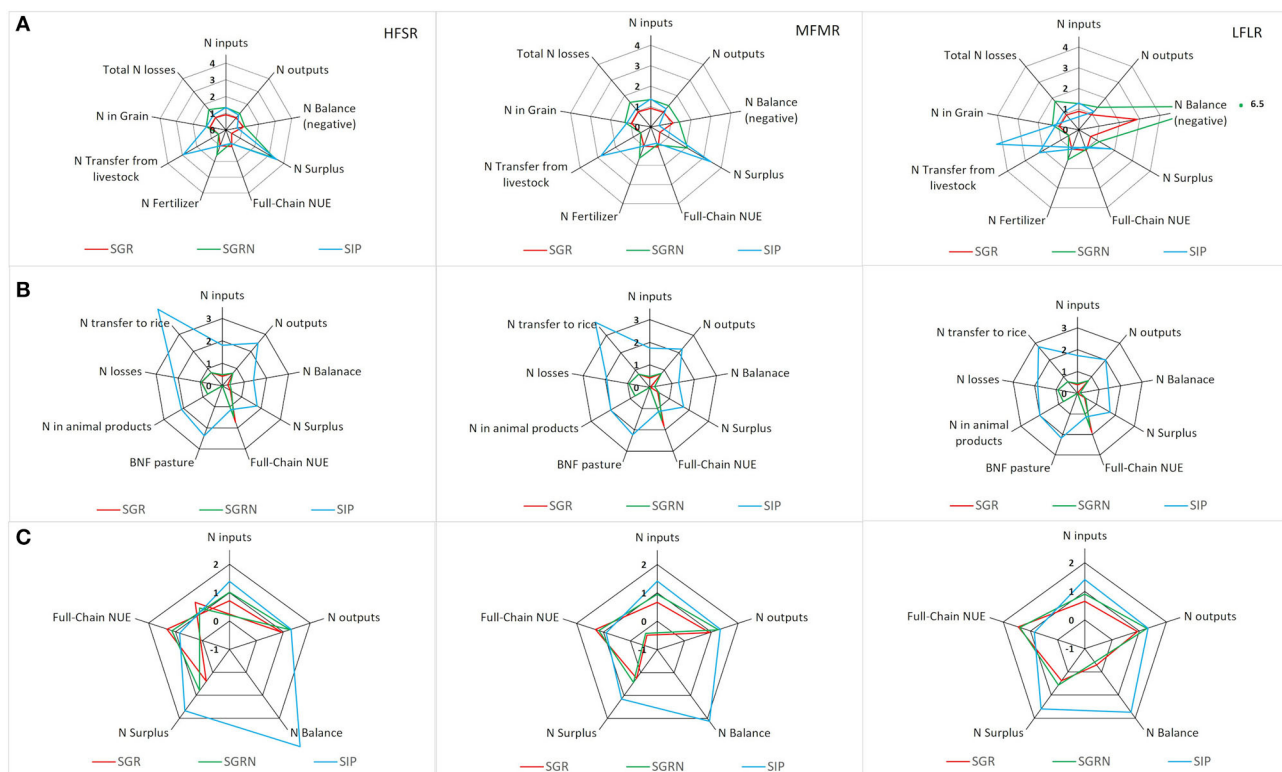


FIGURE 4 Relative changes of N balance, N inputs, N outputs, N surplus, and N use efficiency for three simulated scenarios. The red line represents a rice rotation with grassland (SGR), the green line represents a rice rotation with grassland but adding extra N to the rice crop (SGRN), and the blue line represents a rice rotation with improved pasture on 80% of the total forage area (SIP). (A) Rice component, (B) livestock component, and (C) the entire system. HFSR, high fertility and short rotation; MFMR, medium fertility and medium length rotation; LFLR, low to middle fertility and long rotation.

differed from the slightly positive balance at the country level in our earlier study (Castillo et al., 2021). But the country-level estimates relied on the literature data and some of these, particularly N volatilization losses, might have been underestimated. The HFSR and MFMR regions should have greater N volatilization losses because of the greater amounts of N cycling from the higher natural soil fertility and proportionally greater N transfer from the livestock component to rice. The N balance was far more negative in HFSR and MFMR rotations when N fertilizer use was lower during the first 5 years (-57 and -30 kg N ha $^{-1}$ yr $^{-1}$, respectively). Therefore, the greater precision of this regional analysis is important for correctly understanding the system.

It is likely that the accumulated NBAL before our study period was highly negative because of much lower or no N fertilizer use after the introduction of the rice component. Linking the negative NBAL with the inferred initial N stock based on the soil data, we estimate an average depletion of 10, 8, and 1% of the total N (0.20 m depth) for HFLR, MFMR, and LFLR, respectively. Such mining of soil N is typical of the agriculture of developing countries at the early stages of intensification, but this can be partially reversed with increased N fertilizer doses over time, in turn leading to increased losses and environmental hazards in the long term (Quemada et al., 2020).

How has the Uruguayan rice system been in operation for more than 50 years with consistently high yield levels but only a relatively small addition of N fertilizer? The answer is linked to efficient N cycling from the livestock component. The contributions of N fixed by pastures and N returned in rice bran exceed the relatively low

N outputs from the system. The main output was the N lost by volatilization, which was at similar rates to previous reports for Uruguay (Perdomo et al., 2009; FAO, 2018). All rotations reached positive NBALs for the livestock component, which resulted also in positive NBALs for the whole system. Therefore, the livestock component plays a key role in supporting the rice component by offsetting its negative NBAL. Such complementarity between components has been reported in other systems. For example, García-Préchac et al. (2004) showed that during 46 years of the upland crop–pasture rotation in a long-term experiment, soil organic C was depleted during the upland crop phase but recovered in the pasture phase. In each crop–pasture cycle, soil C rose to near the initial C level. Similar results were reported by Macedo et al. (2021), and Carlos et al. (2020) also found the presence of animal pastures in rice rotations was the key to maintaining soil organic C and total N levels.

In the following sections, we discuss how different N balances in each component and the entire system is related to their N use efficiencies and N surpluses, and how the simulated scenarios can inform future improvements of the system.

The whole system N use efficiency and N surplus

In general, the less positive the NBAL, the higher the NUE, reaching values greater than the upper threshold (90%), indicating soil N mining. For HFSR (98%) and MFMR (94%), this is mainly

explained by low N fertilizer use during the early years. Some studies in European countries (EU Nitrogen Expert Panel, 2015; Erisman et al., 2018) have shown a trajectory opposite to this, with the NUE moving from very low values toward the desirable target after reducing N inputs and improving N recovery by the crops. In our study, the shift to the target NUE zone in HFSR and MFMR regions was associated with higher N fertilizer rates. In the LFLR region, which had a slightly negative average NBAL, NUE was in the desirable zone for the whole period. The high yield reached by rice in all years and rotations meant that the minimum N target in grain ($80 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) was achieved, indicating a high contribution of indigenous soil N. However, the trend of increasing N fertilizer rates across the three regions resulted in NUE in rice of 75, 80, and 70 for HFSR, MFMR, and LFLR, respectively, over the last 3 years of the series, which is very close to the desirable 70% NUE value for crop systems (Scientific Panel on Responsible Plant Nutrition, 2020).

By contrast to the rice, the positive NBAL in the livestock component corresponded to a very low NUE (6% on average), much below the defined thresholds ($25\% > \text{NUE} > 10\%$) but similar to reports from extensive livestock systems, which ranged from 4 to 7% (Gameiro et al., 2019). In addition, the amount of N captured in animal food products ($1.8 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) was low compared with the target ($3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). In our previous study (Castillo et al., 2021), the livestock NUE was within the thresholds because the pasture area data exceeded the typical rice-to-pasture ratio. First, the percentage of the improved pasture area was lower than in this study, and with it, the amount of N fixed by improved legume pastures; and second, the amount of rice bran per hectare was lower because of the greater total pasture area considered. Therefore, lower amounts of both N inputs explained the higher livestock NUE of the previous study. But even though a “too low” NUE is associated with inefficient resource use and could be linked to high N losses to the environment, our study shows how a low-efficiency component (livestock) helps the other system component (rice) reach a very high NUE record. When combined, the entire system reached a high average NUE (62, 67, and 49% for HFSR, MFMR, and LFLR, respectively, for the entire period). These values are higher than reported for other mixed systems, which were $\sim 35\text{--}45\%$ (Godinot, Leterme, Vertés, Favardin, and Carof, Godinot et al.; Westhoek et al., 2014). However, in the last third of the time span analyzed here, system NUE values decreased considerably (51, 63, and 48%) due to greater N fertilizer use.

Increased N fertilizer applications to the rice increase NSURP and decrease NUE in the rice and the complete system in all the rotations. Given that N applications are still increasing, it is possible that NSURP will continue to increase and NUE will decrease. This matches the theoretical trajectory of NUE shown in the scheme in Figure 3C (after Dobermann et al., 2022). It seems that the three rotations are at different parts of Stages I and II in Figure 3C based on the slope of the adjusted regression for NUE and NSURP. While HFLR seems to be in the left upper zone of Stage I for NUE and the bottom zone for NSURP, MFMR, and LFLR are likely to be in the first and approaching middle zone of Stage II. The rate of increase in N fertilizer use over time was $\text{HFLR} > \text{MFMR} > \text{LFLR}$, while NSURP in the last few years of the series was in the order of $\text{LFLR} > \text{MFMR} = \text{HFLR}$. The system-level analysis followed the same trends for the rice component. Because the three rotations were apparently in different stages within the NUE development scheme, management changes should consider the initial situation to shift the current scenario to Stage III, trying to avoid Stage II as much as possible. Such an analysis

could help to identify the best management practices to be adopted in each region and also be applied to other regions or systems if data of N inputs and outputs be available, as mentioned by Dobermann et al. (2022) when comparing different countries.

Scenario analysis

In some areas, improved pastures provide biologically fixed N to the system, compensating for N exported in grains (Pittelkow et al., 2016; Tseng et al., 2021). However, much of the area has no or very low inclusion of improved pasture species. This increases the importance of N contributed to the rice crop from livestock depositions. Removal of the improved pasture in the SGR scenario caused a greater decrease of N inputs (-9%) than N outputs (-5%) in the rice component, mainly due to less N transferred to rice as animal direct deposition, especially in HFLR (-50%) because of a shorter pasture phase. As a result, NBAL was even more negative and NUE more positive, especially in LFLR. However, the results of this simulation are incomplete to the extent that a continuing negative NBAL would reduce crop yields in the future. In that case, less N removed in grain will decrease NUE and the NBAL will be less negative.

When the NUE indicates N mining, a strategy of N replenishment is recommended (Quemada et al., 2020). Our simulations with increased N application rates (SGRN scenario) showed that after the N fertilizer was increased by 60%, the NUE was improved, shifting the efficiency values from mining into the desirable zone, which also increased the rice yield. However, NBAL and NSURP reached the minimum and maximum values, respectively, associated with a significant increase of 63% in N losses, indicating that a strategy of N replenishment through N fertilizer addition is not a good alternative. Finally, the SIP scenario maintained a similar amount of N input to SGRN but with N fertilizer replaced by BNF. This allowed a higher rice yield than in the original situation (17% on average), lower N losses (15% less on average), and less negative NBALs for all rotations. The only negative trend was the increase of NSURP, as for SGRN but without increased N losses.

The scenario analysis for the livestock component showed similar trends to the rice but differences for SGR and SIP. For SGR, the removal of improved pastures decreased N inputs (40% on average) resulting in reductions in all other parameters related to the NBAL. However, the greatest change was increased NUE ($+72$, $+82$, and $+91\%$ for HFLR, MFMR, and LFLR, respectively), into or around the targeted efficiency zone ($25\% < \text{NUE animal systems} > 10\%$). This indicates that if pastures are improved through the inclusion of legumes, an increase in animal productivity brings the NUE within the desirable zone. That was what happened in the SIP scenario where a greater percentage of improved pastures (80%) increased meat productivity by 100%. But because of the higher N inputs from biological fixation, NUE values were just 14% higher on average, reaching values below the lower threshold (10%). In those cases, alternative management toward increasing animal productivity must be applied while avoiding risks associated with very high stocking rates (Lezama and Paruelo, 2022). However, there is still an opportunity to improve animal productivity because the stocking rate of the SIP scenario (1.4 livestock units of $380 \text{ kg live weight ha}^{-1}$) is still far below the standard of improved pastures (Rovira et al., 2020).

In summary, the scenario exploring a stable and greater use of improved legume pastures seems to improve the productivity and N budget of each component and the system. This is close to the proposal of Kanter et al. (2016) and Soares de Lima (2009) who identified improved practices to increase livestock productivity and indirectly the crop component. But we also believe that there is room for improved integrated management of the rice–livestock system to lead the system into an intensified and sustainable future.

Conclusion

The Uruguayan rice–livestock system is highly efficient and productive, with relatively low N fertilizer inputs and low N surpluses across the regions and rotations. In all the regions, this system is sustainable in terms of N balance because of the complementarity of the livestock and rice components. This could be challenged if either or both components were to intensify without considering the whole system. For this, a good quantification of all the components of the N balance combined with modeling tools can help to design future strategies. Improvements in livestock productivity and efficiency could be achieved by adjusting pasture lengths in regions with shorter pasture rotations and increasing the proportion of improved legume pastures. This could also contribute to greater rice yields without more N fertilizer use. Fine-tuning the system could also help to reduce greenhouse gas emissions and other costs associated with fertilizers use.

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

JC: conceptualization, visualization, data acquisition, curation, interpretation, analysis, modeling, and drafting of the manuscript. GK, SH, MR, and JC: critical thinking and discussion, and writing-review and editing. All authors listed contributed directly to the manuscript and approve this work for publication.

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Conflict of interest

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Supplementary material

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Measured and modeled nitrogen balances in lowland rice-pasture rotations in temperate South America

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Rotational rice systems, involving pastures, other crops and/or livestock, are common in temperate South America, exemplified by the rice-pasture-livestock system of Uruguay which combines very high rice yields with tight nitrogen (N) balances. The generally good nutrient use efficiency in these systems provides a template for nutrient management in other mixed farming systems, if the underlying processes can be sufficiently well quantified and understood. Here, we studied N balances in rice–non-rice rotations in a long-term experiment in Uruguay, with the aim of parameterizing and testing the DNDC model of N dynamics for such systems for use in future work. The experiment includes three rotations: continuous rice (RI-CONT), rice-soybean (RI-SOY) and rice-pasture (RI-PAST). We considered 9 years of data on N balances (*NBAL*), defined as all N inputs minus all N outputs; N surplus (*NSURP*), defined as all N inputs minus only N outputs in food products; and N use efficiency (*NUE*), defined as the fraction of N inputs removed in food products. We parameterized DNDC against measured yield and input and output data, with missing data on N losses inferred from the N balance and compared with literature values. The model performance was assessed using standard indices of mean error, agreement and efficiency. The model simulated crop yields and rice cumulative N uptake very well, and soil N reasonably well. The values of *NBAL* were +45 and –20 kg N ha^{–1} yr^{–1} in RI-CONT and RI-SOY, respectively, and close to zero in RI-PAST (–6 kg N ha^{–1} yr^{–1}). Values of *NSURP* decreased in the order RI-CONT >> RI-SOY > RI-PAST (+115, +25 and +13 kg N ha^{–1} yr^{–1}, respectively). Values of *NUE* (84, 54, and 48% for RI-SOY, RI-PAST, and RI-CONT, respectively) decreased as *NBAL* increased. The sensitivity of DNDC's predictions to the agronomic characteristics of the different crops, rotations and water regimes agreed with expectations. We conclude that the DNDC model as parameterized here is suitable for exploring how to optimize N management in these systems.

KEYWORDS

nitrogen use efficiency, DNDC model, nitrogen budgets, nutrient balances, long-term experiment

Introduction

Increased global trade of crops and livestock as well as the separation of crop and livestock production has led to large imbalances in nutrient budgets across the globe (Grote et al., 2008; Uwizeye et al., 2020). To improve local and global nutrient balances, future food systems should include a return to mixed farming systems with integrated crop and livestock production (Asai et al., 2018; Garrett et al., 2020; Peterson et al., 2020). Such systems have a greater potential for efficient nutrient use and cycling than the intensive single commodity systems that have become dominant globally over the last decades (Martin et al., 2016; Ghimire et al., 2021). For example, until recently rice production in Argentina, Paraguay and southern Brazil was predominantly a monoculture, shifting in the last decade to more complex systems with the inclusion of soybeans or short pastures with livestock (Denardin et al., 2020; Ribas et al., 2021; Macedo et al., 2022). This diversification has helped improve soil conditions, weed control, and farm income, though nutrient management still needs to be improved (De Faccio Carvalho et al., 2021).

An example of a well-integrated system is the national rice-livestock system in Uruguay. This has been practiced for over 50 years, attaining a high level of production for rice (mean grain yields $> 8 \text{ Mg ha}^{-1} \text{ y}^{-1}$) and an average level for livestock ($120 \text{ kg liveweight gain ha}^{-1} \text{ y}^{-1}$) (Castillo et al., 2021) for the prevailing production conditions (e.g. climate, pasture management). Analysis of country-level statistics over the last 16 years showed tight positive nitrogen (N) balances of $+2\text{--}3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for both the rice and livestock components as well as the whole system (Castillo et al., 2021). This is remarkable given that N inputs to agricultural systems globally range from greatly excessive to inadequate, generating imbalances from environmental pollution to soil N mining (Ladha et al., 2020).

To understand how to optimize nutrient management in such systems, a detailed understanding of local and regional variation in system properties and processes is needed. A good assessment requires detailed data on nutrient inputs and outputs, as well as on loss processes. Regional and national datasets on nutrient balances are inevitably incomplete, especially for gaseous emissions, which are expensive and hard to quantify in detail (Katayanagi et al., 2012). Despite having good data on rice, pasture and livestock production and N budgets (Kanter et al., 2016; Pittelkow et al., 2016), data on gaseous N losses and process modeling of such losses are scarce for the Uruguayan rice-pasture system. Irisarri et al. (2012) and Tarlera et al. (2016) reported very low nitrous oxide (N_2O) emissions ($< 2 \text{ kg N-N}_2\text{O ha}^{-1} \text{ season}$) from rice across the country. However, the main gaseous N loss process in rice systems is generally volatilization of ammonia (NH_3), which can reach up to 50–60% of the applied N (Chowdary et al., 2004; Xu et al., 2013; Chen et al., 2015; Wang et al., 2018). Reliable modeling of NH_3 volatilization is therefore needed to fill-in missing data, and to interpret and extrapolate results. Models for this purpose need to capture all the relevant processes equally well.

There are particular challenges in modeling N dynamics in rice-pasture systems, because very different processes operate in the flooded rice phase compared with the non-flooded pasture phase. Under flooded conditions, high rates of loss can occur by (1) NH_3 volatilization from the floodwater layer due to daytime

increases in floodwater pH (by up to 2 units) as dissolved CO_2 is removed in photosynthesis of floodwater algae; and (2) nitrification-denitrification processes in adjacent oxic and anoxic regions in the soil-floodwater system (Kirk, 2004; Buresh et al., 2008). In the alternating pasture phase, N dynamics depend particularly on (1) biological N fixation (BNF) associated with legume species; (2) the effects of grazing animals; and (3) the retention of fixed N in crop residues and soil organic matter for following crops (Peoples and Craswell, 1992; Ledgard, 2001).

Potential models of rice field N dynamics include DayCent, but it does not calculate NH_3 volatilization (Del Grosso et al., 2015; Necpálová et al., 2015; Gurung et al., 2021), and CERES-Rice, which does calculate NH_3 volatilization but its application to N balances in Uruguayan rice-pasture systems was not promising (Pravia, 2009). The DeNitrification-DeComposition (DNDC) model (Li, 2000) is a widely used process-oriented simulation model of soil C and N biogeochemistry with a focus on agro-ecosystems. Originally developed for simulating GHG emissions from agricultural systems in the USA, the DNDC model has been calibrated and used worldwide (Kesik et al., 2005; Abdalla et al., 2022). The model is dynamic and can capture complex agro-ecosystem interactions for simulating GHG emissions from croplands and other ecosystems. The DNDC model has also been used to simulate crop grain yield and N uptake in lowland rice systems (Babu et al., 2006; Katayanagi et al., 2013; Zhao et al., 2020) as well as in aerobic “upland” crops (Zhang et al., 2018; Jiang et al., 2021; Abdalla et al., 2022).

Our objectives were to parametrize and test the DNDC model for characterizing N dynamics in rice-pasture rotations in Uruguay, and to use the model to examine the components of the N balance in these rotations. We parameterized and tested the model against data from a no-till long-term experiment on direct seeded rice-pasture rotations in Uruguay, with nine years of measurements of yields and the components of the N balance. To the best of our knowledge, this is the first time the DNDC model has been used in multi-cropping systems including perennial pastures and livestock, alternating between dry and flooded soil conditions. If a good fit between predicted and observed data can be achieved, the model will allow us to predict the trajectory of the existing systems and the effects of altering the current management, such as by the intensification of rice cultivation and introduction of non-traditional crops such as soybeans. The results should apply to rice production across temperate South America (Argentina, Brazil, Paraguay, Uruguay) where 1.5 M ha of land is currently used for rice and potentially could shift to more complex systems. The results could help define optimal N fertilization management strategies for such systems.

Materials and methods

The long-term experiment

The experiment is located at the Instituto Nacional de Investigación Agropecuaria (INIA) Treinta y Tres, Paso de la Laguna Experimental Station in the East of Uruguay ($33^\circ 16' 22.2''\text{S}$; $54^\circ 10' 23.1''\text{W}$). The climate is mesothermic and humid. Daily mean temperatures is $22.6 \pm 0.54^\circ\text{C}$ in summer and $12.0 \pm 0.82^\circ\text{C}$ in winter. Annual mean rainfall is $1,354 \pm 283 \text{ mm}$ and total potential

evapotranspiration $1,048 \pm 196$ mm. The dominant soil type is an Argialboll (main properties in [Supplementary Table S1](#)). In the 30 years before the experiment, the area was under a rice-pasture rotation with 2 years of rice followed by 3 years of improved legume pasture. The experiment occupies an area of 7.2 ha, comprising 60 plots, each 60 m long and 20 m wide. There are six rotations in a randomized complete block design with three replications (full details in [Supplementary Text S1](#), [Supplementary Table S2](#)). For this study, we selected the three most contrasting rotations in terms of the frequency of rice cultivation and N additions: (a) continuous rice-legume pasture cover crop each year (RI-CONT), (b) rice-pasture cover crop-soybean-legume pasture cover crop every two years (RI-SOY) and (c) rice-pasture cover crop-rice-grazed pasture for 3.5 years every 5 years (RI-PAST) ([Table 1](#)).

Field measurements

Data on all management variables for crops and pastures were recorded annually. Rice and soybean yields, nutrient removals with the grain and seasonal pasture production and botanical composition were measured each season. Biological N fixation by the pasture and soybean was estimated based on crop and pasture measurements and literature data. Similarly, N removal in animal tissue were estimated from animal production and literature data ([Supplementary Table S3](#)). Climate data (daily maximum and minimum air temperature, precipitation, solar radiation, wind speed and air humidity) were obtained from a weather station at the site.

Aboveground rice N uptake and KCl-extractable $\text{NH}_4\text{-N}$ in the soil during flooding were measured in sub-plots in 2019–2020 and 2020–2021. For rice N uptake, above-ground plant tissue was sampled seven times during the growing season each year, from 15 d after flooding about every 15 d up to harvest. The plant samples were dried at 60°C for 48 h and N concentrations measured by the Dumas method. The soil was sampled seven times from immediately after flooding to 15 d before harvest by inserting a 30-mm diameter scaled tube to 15 cm depth. Six soil samples were taken per plot and bulked. Extractable $\text{NH}_4\text{-N}$ was measured by shaking the wet soil in 2 M KCl for 2 h and analyzing colorimetrically ([Nelson, 1983](#)), and allowing for the soil water content. Emissions of N_2O were measured in the main plots by the closed chamber technique ([Rochette and Eriksen-Hamel, 2008](#); [Minamikawa et al., 2012](#)) every 15 d on average, starting after flooding in the rice crop and 1 week after establishment in pasture and soybean crops and up to the day of the rice drainage, in both crops.

Parameterizing DNDC

DNDC is a process-based model representing C and N biogeochemical cycles in agricultural systems on daily time steps, with four ecological drivers: climate, soil, vegetation and cultural practices ([Li, 2000](#); [Simmonds et al., 2015](#)). One component calculates crop growth and soil temperature, moisture, pH, redox potential and substrate (dissolved organic C, hereinafter expressed

TABLE 1 Components of the three selected rotations in the long-term experiment in spring-summer (SS) and autumn-winter (AW) seasons from 2012 to 2021.

	2012		2013		2014		2015		2016		2017		2018		2019		2020		2021	
	SS	AW	SS	AW	SS	AW	SS	AW	SS	AW	SS	AW	SS	AW	SS	AW	SS	AW	SS	AW
RI-PAST ^a	Rice1	RG	Rice2								Rice1	RG	Rice2							
RI-SOY ^b	Rice	RG	Soy	EC	Rice	RG	Soy	EC	Rice	RG	Soy	EC	Rice	RG	Soy	EC	Rice	RG	Soy	EC
RI-CONT	Rice	EC	Rice	EC	Rice	EC	Rice	EC	Rice	EC	Rice	EC	Rice	EC	Rice	EC	Rice	EC	Rice	EC

RG, Ryegrass (*Lolium multiflorum* L.); TF, Tall fescue (*Festuca arundinacea* L.); WC, White clover (*Trifolium repens* L.); BT, Birdfoot trefoil (*Lotus corniculatus* L.); EC, Egyptian clover (*Trifolium alexandrinum* L.).
^aRice1, Rice 2 and the first year of a TF + WC + BT pasture were grown in SS from 2012 to 2021.
^bRice and soya crops were grown in the Rice-Soybean and Soybean-Rice sequences in SS from 2012 to 2021.

TABLE 2 DNDC cropping parameter settings used for calibration.

	Rice	Soybean	Egyptian clover	Ryegrass	Mixed perennial pasture
Range of maximum grain production (kg ha ⁻¹ yr ⁻¹)	7,790–13,908	1210–3,170	23–46	17–43	63–155 ^a
					80–224 ^b
					69–92 ^c
					14–46 ^d
Biomass fraction (grain/leaf/stem/root)	0.45/0.21/0.22/0.12	0.31/0.25/0.24/0.2	0.01/0.4/0.4/0.19	0.01/0.4/0.4/0.19	0.02/0.35/0.35/0.28
C to N ratio (grain/leaf/stem/root)	35/60/80/85	10/45/45/24	15/10/21/30	15/25/25/30	19/19/19/19
Thermal degree days (TDD)	2,800–3,140 ^e	2,700	1,700	1,700	4,000
N fixation index (N plant/N from soil)	1.05	4	10	1	1.34

^{a,b,c,d}Based on measured aboveground biomass fraction for pastures in its 1st, 2nd, 3rd and 3.5th year.

^eRange of thermal degree days for rice for the different used rice varieties across the years.

as DOC, NH₄⁺, NO₃⁻, CO₂, and H₂) concentration profiles. A second component calculates nitrification, denitrification and fermentation, simulating CO₂, CH₄, NH₃, NO, N₂O, and N₂ emissions from the plant-soil system.

For the climate driver, we used on-site data of temperature (maximum and minimum), solar radiation, wind speed, air humidity and precipitation over the study period. The default atmospheric CO₂ value was adjusted to 390 ppm for 2012, increasing by 2.5 ppm each year thereafter (NOAA, 2022). Nitrogen concentration in rainfall was set at 0.35 mg l⁻¹ (Zunckel et al., 2003). Soil clay content, pH and organic carbon content were as measured at the start of the experiment (Supplementary Table S1). The depth of the water retention layer (0.6 m) and the drainage efficiency (50%) were estimated based on the site conditions. Bulk density, porosity, soil hydraulic conductivity and available water potential values were calculated by the model from the Input soil property variables.

Annual crop and cultural practices were included in the cropping management sub-model. Grazing by sheep was allowed for in the RI-PAST rotation with grazing frequency (rotational grazing averaging 8 d occupancy and 20 d of regrowth) and stocking rate (28 animals ha⁻¹ on average) as managed in the experiment. To simulate the start of a chemical fallow period in pasture cover crops and pastures in the final productive year, we selected the “crop termination tillage” option, which does not alter the soil surface.

The model was calibrated by adjusting the crop parameters listed in Table 2 to obtain best fits between measured and simulated crop and pasture yields and N balances (total N uptake, grain and straw N partitioning, and soil NH₄-N concentrations) in the different rotations over the nine growing seasons. All other parameter values were either as determined above or the DNDC default values.

We lacked measurements of NH₃ volatilization, but it is an important part of the N budget (Results). We therefore assessed the sensitivity of simulated NH₃ volatilization and grain yields to the main soil parameters affecting volatilization (carbon and clay contents and pH) and N fertilization rate. We varied each parameter by –30 to +30 % of the standard value with an

increment of 10%, consistent with variability across the main rice regions in Uruguay. We also checked the sensitivity of NH₃ volatilization and grain yield to the main crop development parameters (Table 2) by varying values by –15 and +15%. That range covers the expected variability in biomass fraction and C to N ratio in all rice plant components, and thermal degree days accumulation.

Model performance

Model accuracy during calibration and validation stages was tested through three indices following (Yang et al., 2014): mean error (ME), index of agreement (IA) and modeling efficiency (MEF):

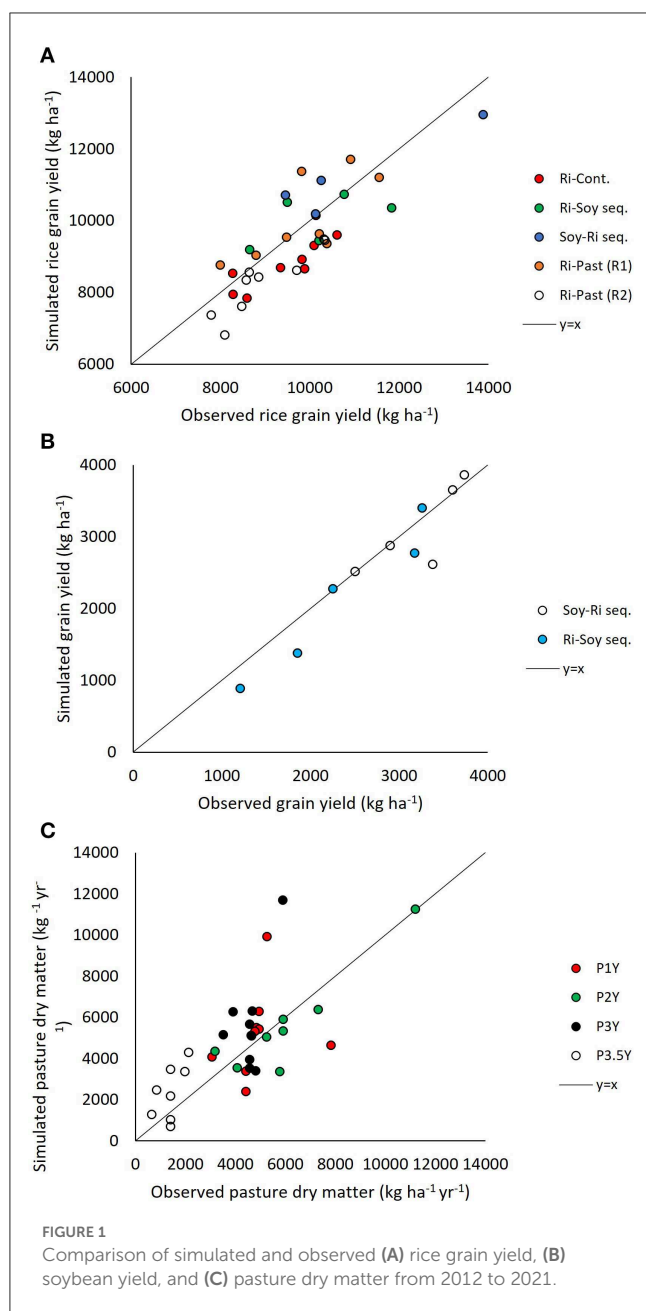
$$ME = \frac{\sum_{i=1}^n (P_i - O_i)}{n} \quad (1)$$

$$IA = 1 - \frac{\sum_{i=1}^n (P_i - O_i)^2}{\sum_{i=1}^n (|P_i - \bar{O}| + |O_i - \bar{P}|)^2} \quad (2)$$

$$MEF = 1 - \frac{\sum_{i=1}^n (P_i - O_i)^2}{\sum_{i=1}^n (O_i - \bar{O})^2} \quad (3)$$

where P_i and O_i are the predicted and observed values respectively in season i , \bar{P} and \bar{O} are the respective mean values, and n is the number of seasons. If ME is above or below 0, the model underestimates or overestimates the observed data, respectively. The dimensionless IA index ($0 \leq IA \leq 1$) is used to represent the degree of deviation from zero. The MEF values ($-\infty$ to 1), also dimensionless, assess the goodness-of-fit of the model, with $MEF = 1$ indicating a perfect fit and 0–1 denoting acceptable fit. For $MEF < 0$, goodness of fit must be assessed with a t -test.

The good agreement (see results) when comparing simulated and measured field data during the two parametrization stages gave us confidence to use other N parameters simulated by the DNDC model but not measured, to conduct a more complete N budget analysis in the different crop rotation systems.



Nitrogen balance, surplus and use-efficiency

The N balance (*NBAL*) was calculated as:

$$NBAL = \sum N_{inputs} - \sum N_{outputs} \quad (4)$$

where N_{inputs} = N in fertilizers, BNF, atmospheric N deposition, and $N_{outputs}$ = N in food products, N gas losses (NH_3 , N_2O , NO_2 , and NO), and N leached.

All N inputs not retained in food products were considered as potential N loss to the environment and was defined as

surplus (*NSURP*):

$$NSURP = \sum N_{inputs} - N_{food\ products} \quad (5)$$

Components of N_{inputs} = as in Equation 1 and $N_{food\ products}$ = amount of N in grain or meat or both.

The N use efficiency was calculated as the fraction of N retained in food products considering all inputs:

$$NUE\% = \frac{N_{food\ products}}{\sum N_{inputs}} \times 100 \quad (6)$$

where *NUE%* = N use efficiency expressed in percentage while N in food products and N inputs are the same items as in Equation 5.

Analyses of variance for *NBAL*, *NSURP* and *NUE%* were conducted with a general linear model. In the model, rotation was considered a fixed effect while the block effect nested in year was considered random. A significance level of $P \leq 0.05$ was defined and all data was tested for normality and variance homogeneity. Comparisons of the assessed soil parameters during the sensitivity analysis were conducted using multiple t-tests ($P \leq 0.05$). All the statistical analyses were performed in the Infostat software (Di Rienzo et al., 2008).

Results

Rice yield

Yields were highest in the RI-SOY and in the first rice of the RI-PAST rotations and lowest in the second rice of the RI-PAST and RI-CONT rotations (Figure 1A). Supplementary Table S4 gives the results of the model assessment using Equations 4–6. The model simulated rice yields well for all rotations over the entire period, with a small average underestimation of $-409 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (Figure 1A). Analysis within rotations also showed a high association between simulated and observed yields for the first rice of the RI-PAST rotation, the RI-CONT rotation, and the RI-SOY Rotation depending on the RI-SOY rotation sequence (rice-soybean or soybean-rice, respectively). In both cases, the model under or overestimated rice yield on average by -397 and $558 \text{ kg ha}^{-1} \text{ yr}^{-1}$, respectively. In contrast, the simulation for the second rice of the RI-PAST rotation was poor with an average yield underestimation of $-2,937 \text{ kg ha}^{-1} \text{ yr}^{-1}$. This was associated with an average difference in total N uptake of around 25 kg N ha^{-1} compared with the average N uptake of the first rice in the RI-PAST rotation. Based on that N uptake difference we added the mentioned amount as fertilizer and assuming 50% of fertilizer recovery and repeated the model run.

Soybean yield

Yields tended to be lower and more variable during the first years of the experiment because of dry summers and lack of supplementary irrigation, which started in 2015. As with the rice, soybean yield was predicted well by the DNDC model (Figure 1B). On average, predicted values slightly underestimated

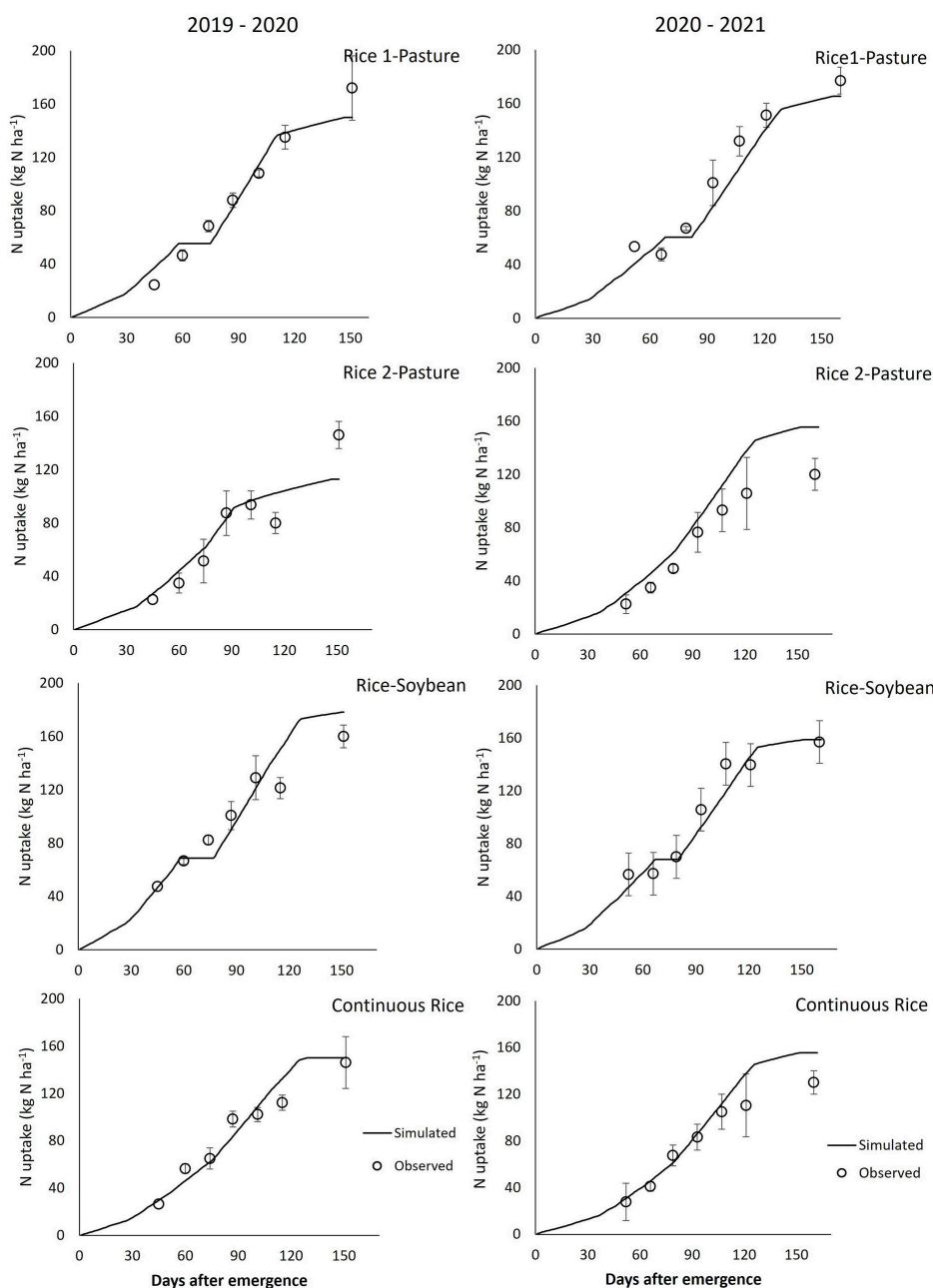


FIGURE 2

Comparison of simulated and observed rice N uptake (kg N ha^{-1}) in rice-pasture (first and second rice), rice-soybean and continuous rice in the 2019–2020 and 2020–2021 growing seasons. Observed data are means \pm standard errors ($n = 3$).

yield ($-195 \text{ kg ha}^{-1} \text{ yr}^{-1}$) but efficiency indices values indicated good agreement. Within the RI-SOY rotation, soybean yield predictions were off by -290 and $118 \text{ kg ha}^{-1} \text{ yr}^{-1}$ for the rice-soybean and soybean-rice sequences, respectively, with indices values confirming a good simulation (Supplementary Table S4).

Pasture yield

The greatest pasture production was during the second year and the least during the last cycle which considered

only half a year. Dry matter production during the first and third year were similar. Pasture dry matter production in the RI-PAST rotation was well predicted by the model for the yearly average and the pasture growing season. However, this changed when analyzing each growing season separately (Figure 1C). Except for model prediction of second-year pastures, the simulations showed only intermediate values for the goodness of fit index values (Supplementary Table S4). t -tests did not detect differences between predicted vs. observed values except for the third-year pastures ($P = 0.04$).

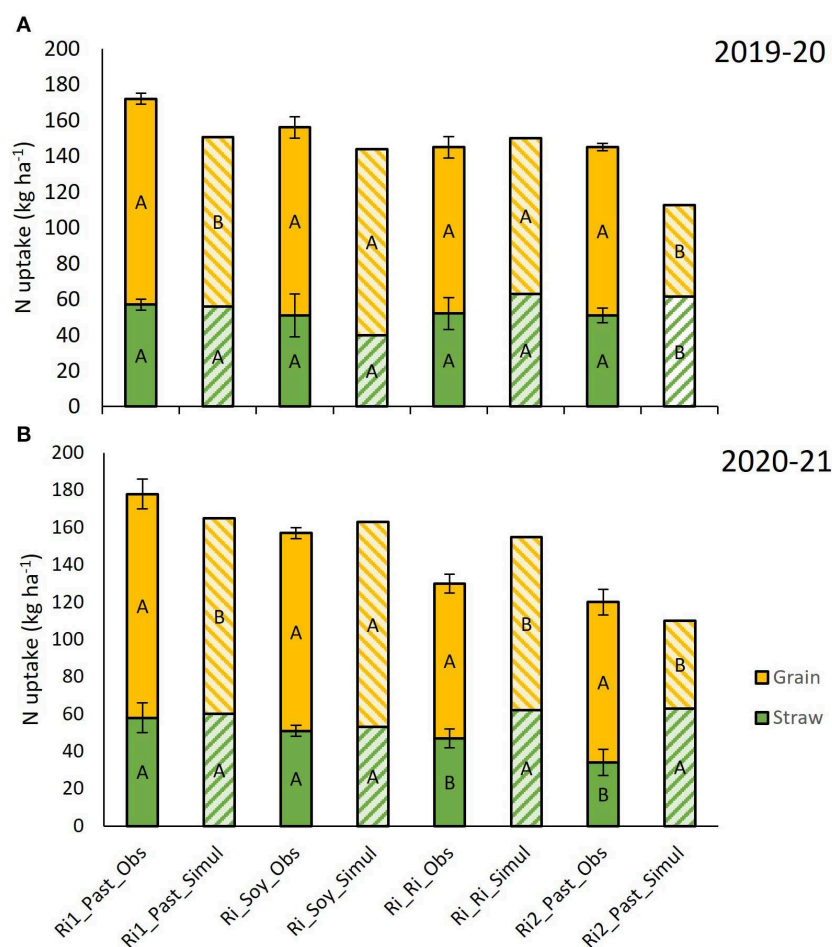


FIGURE 3

Comparison of simulated and observed rice grain and straw N uptake (kg N ha^{-1}) at physiological maturity in the rice-pasture (first and second rice), rice-soybean and continuous rice in (A) 2019–2020 and (B) 2020–2021 growing seasons. Observed data are means \pm standard errors ($n = 3$). Letters next to bars indicate differences (Fisher 5%) between simulated and observed grain and straw N values for each rotation.

Nitrogen uptake and soil N concentration

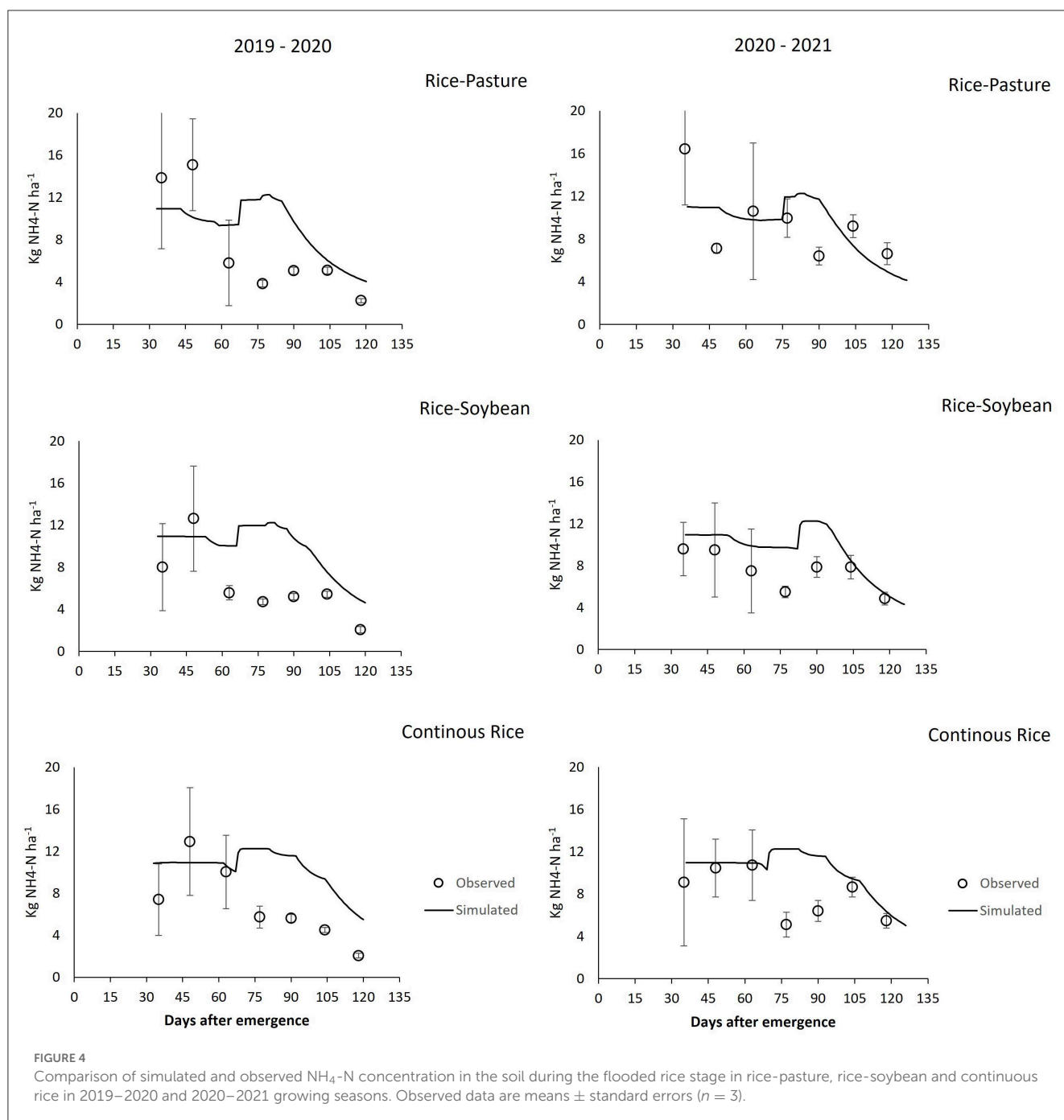
In both 2019–20 and 2020–21 growing seasons, total rice N uptake was greater in the first rice of the RI-PAST (172 and 177 kg N ha^{-1}) and the RI-SOY (160 and 157 kg N ha^{-1}) systems compared with the second rice of RI-PAST (146 and 120 kg N ha^{-1}) and RI-CONT rotations (146 and 130 kg N ha^{-1}), following the same trends as observed for rice yield. The results of the evaluation index values for cumulative N uptake are shown in [Supplementary Table S4](#). These values indicate that the DNDC model simulated the cumulative rice N uptake in the different rotation systems very well (Figure 2), with a low deviation between predicted and simulated N uptake values. The model could also satisfactorily simulate the amount of N kept in straw and rice grain in the different rotations and years (Figure 3).

Soil $\text{NH}_4\text{-N}$ concentrations were small (6.3 ± 2.6 and $9.2 \pm 5.4 \text{ kg N ha}^{-1}$ during the rice flooded stage in 2019–20 and 2020–21, respectively) and decreased over time. There were no differences between rotations within a year. Index values for soil $\text{NH}_4\text{-N}$ concentration were $ME = 1.38$ and 0.68 ; $IA = 0.48$ and 0.54 ; $MEF = -0.27$ and 0.05 , for the first and second rice

growing season, respectively. Although observed and predicted $\text{NH}_4\text{-N}$ values were very close (10 vs. 6.8 kg ha^{-1} and 9.8 vs. 8 kg ha^{-1} in the predicted vs. observed for 2019–2020 and 2020–2021, respectively), index values of model accuracy showed intermediate results ([Supplementary Table S4](#)). A paired t -test did not show differences between the predicted and observed values for this variable in the RI-CONT and the first rice of the RI-PAST rotation in both years, but differences were detected for the RI-SOY rotation in 2019–2020 ($P = 0.02$) and 2020–2021 ($P = 0.04$). But the trend in simulated and observed soil $\text{NH}_4\text{-N}$ values was close, corresponding to the small range of observed values for this variable (Figure 4).

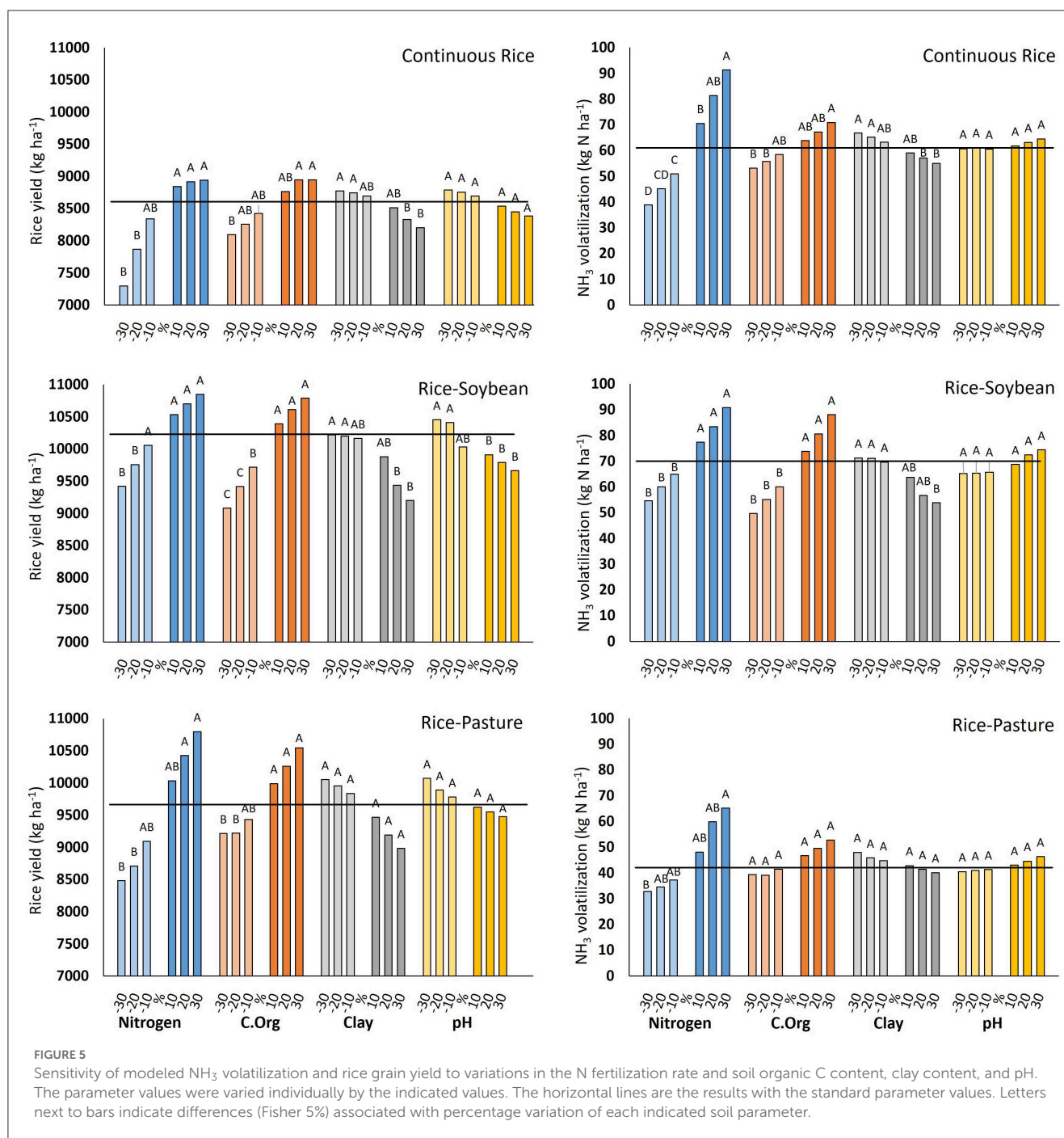
Ammonia emission

Although we do not have measurements of NH_3 volatilization against which to test the model predictions directly, the good agreement between measured and modeled N uptake and soil N concentrations suggests that NH_3 volatilization is modeled satisfactorily, given that it is the main source of N losses from



the soil. We assessed the sensitivity of simulated volatilization to the main model parameters affecting it varied by $\pm 30\%$ of the measured field values (Figure 5). Simulated volatilization and rice yield were moderately sensitive to all the soil parameters tested over this range, and the effects were consistent with expectations. Volatilization increased by 25% with N addition on average across the rotations, by 13% with soil organic C content and by 7% with soil pH. The effect of pH was greater during periods when the soil was drained (around 35 days from rice seeding to the soil being flooded), increasing by 29% with higher soil pH values. Conversely, volatilization decreased by 10% with a 30% increase

in soil clay content. The effects of crop parameters on NH_3 volatilization ranged from -6 to $+8\%$. In all rotations, rice yields increased slightly with N addition and soil organic C content and decreased with soil clay content and pH. Crop parameters also had small effects on rice yield. In summary, the sensitivity of NH_3 volatilization to variations in soil parameters was greater than for crop parameters, and rice yield was little affected by any of the tested parameters. We conclude that the model is well corroborated by the observed crop and pasture growth and N dynamics, and therefore suitable for predicting unmeasured N loss components.



Nitrous oxide emission

Measured N₂O-N emissions were small in the flooded and dry soil stages (0.54 ± 2.6 and 0.37 ± 2.8 g N₂O-N ha⁻¹ day⁻¹, respectively). During the pasture phase of the RI-PAST and for rice in RI-CONT rotation, measured and simulated N₂O-N values were zero in both growing seasons. Simulated N₂O emissions underestimated measured records during the first rice of the RI-PAST rotation (0.54 and 0.13 g N₂O-N ha⁻¹ day⁻¹ for 2019–2020

and 2020–2021 respectively) and overestimated for soybean in the RI-SOY rotation (0.5 g N₂O-N ha⁻¹ day⁻¹). According to the calculated model accuracy indices values, predicted N₂O emissions showed low to intermediate similarity to the observed values (Supplementary Table S4). On average, the range of predicted and measured N₂O-N emission values was very narrow, and the values were low (0.45 and 0.48 g N₂O-N ha⁻¹ day⁻¹, respectively). There were no significant differences between predicted and observed values assessed by t-tests in all rotations.

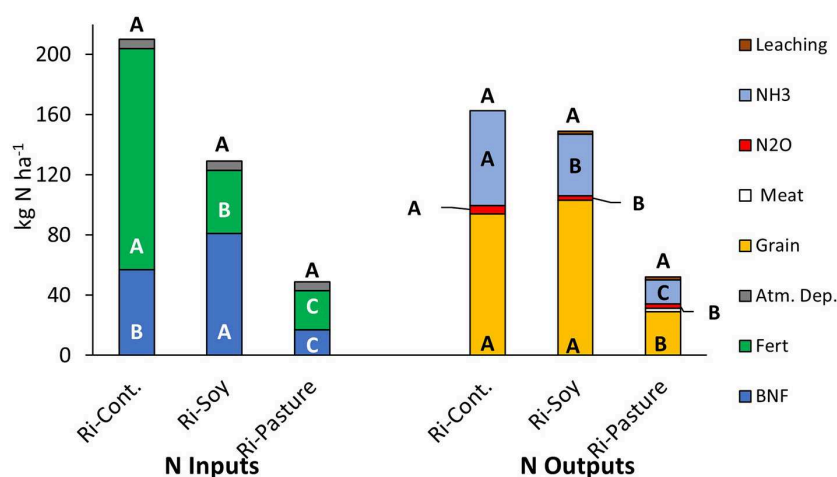


FIGURE 6

Composition of N inputs and outputs and average N amounts for rice-pasture, rice-soybean and continuous rice in 2019–2020 and 2020–2021 growing seasons. Letters next to bars indicate differences (Fisher 5%) between the rotations for each indicated component.

TABLE 3 Calculated N balance, N surplus and % N use efficiency (defined in Equations 4–6) for the three rotations from 2012 to 2020.

Rotation	N balance	N surplus	NUE
	kg N ha ⁻¹ yr ⁻¹	kg N ha ⁻¹ yr ⁻¹	%
RI-PAST	−6	13	53
RI-SOY	−23	25	84
RI-CONT	45	115	48
LSD ^a	22	16	18

^aLeast significant difference.

Nitrogen balance, surplus and use-efficiency

Total N inputs accounted for 209, 139 and 51 kg N ha⁻¹ yr⁻¹ in RI-CONT, RI-SOY and RI-PAST respectively. The main N source was fertilizer for RI-CONT (70%) but biological N fixation (BNF) for RI-SOY (66%). In RI-CONT BNF (57 kg N ha⁻¹ yr⁻¹) was due to the Egyptian clover used as cover pasture crop, and in RI-SOY it was due to soybean (136 kg N ha⁻¹ yr⁻¹) and Egyptian clover (47 kg N ha⁻¹ yr⁻¹). The RI-PAST rotation had both the smallest inputs of N fertilizer (26 kg N ha⁻¹ yr⁻¹) and the least BNF (17 kg N ha⁻¹ yr⁻¹). Although N fertilizer rates for RI-SOY (42 kg N ha⁻¹ yr⁻¹) and RI-PAST were small, the average amounts applied to the rice per season over the study period were 84 kg N ha⁻¹ in RI-SOY (4.5 rice crops) and 71 kg N ha⁻¹ in RI-PAST (3.3 rice crops).

The RI-PAST rotation had the smallest N outputs (Figure 6). Nitrogen retained in grain crops accounted for 72, 58 and 51% of total N outputs for RI-SOY, RI-CONT and RI-PAST, respectively, while gaseous N losses represented 30, 42 and 34% of N outputs, respectively. For the three rotations, NH₃ volatilization was the main N loss process (39 kg NH₃-N ha⁻¹ yr⁻¹). Both N₂O emission (4 kg N ha⁻¹ yr⁻¹) and N leaching (2 kg N ha⁻¹ yr⁻¹) were negligible by comparison. Volatilization of NH₃ was mainly associated with the rice phase of each rotation (93% on average). Cumulative NH₃ volatilization losses were different

among rotations being 62, 41 and 17 kg NH₃-N ha⁻¹ yr⁻¹ for RI-CONT, RI-SOY and RI-PAST, respectively. The greatest amount of N volatilized was found during the rice phase in the RI-SOY rotation (74 kg NH₃-N ha⁻¹) followed by RI-CONT and RI-PAST (61 and 44 kg NH₃-N ha⁻¹, respectively), all statistically different. In the absence of N fertilizer addition, simulated volatilization was on average 18, 9 and 15 kg N-NH₃ ha⁻¹ for the same rotations.

Differences between N inputs and outputs generated differences in N balances, N surpluses and NUE (Table 3). NBAL ranged from +45 kg N ha⁻¹ yr⁻¹ in RI-CONT to −23 kg N ha⁻¹ yr⁻¹ in RI-SOY, with RI-PAST having an intermediate value (−6 kg N ha⁻¹ yr⁻¹). Because of the high fertilizer N input in RI-CONT, which is similar to the total output of this rotation, the positive NBAL values are close to the BNF and atmospheric deposition inputs (Table 3). In contrast, NBAL was negative in RI-SOY even though one of the rotation components fixed N. The amount of N derived from the atmosphere to the soybean was defined in 75% of the total N uptake, explaining partly that negative N balance. The RI-PAST rotation reached a very tight N balance of −6 kg N ha⁻¹ yr⁻¹. In the RI-CONT rotation, the amount of N not retained in grain from all the N inputs (NSURP) was 115 kg N ha⁻¹ yr⁻¹. This value represented around 79% of the N added as fertilizer. Both, RI-SOY and RI-PAST showed low NSURP values (Table 3). The NUE % was higher in RI-SOY (84%) compared with RI-CONT (48%). The NUE % in RI-PAST was 53%, similar to RI-SOY. Higher NUE% values were associated with less positive NBAL.

Discussion

Simulation of crop yields and N dynamics

In general, the agreement between observed and simulated rice yield, N uptake and soil N concentration in the different rotations was very good. Given the complexity of the model and the diverse processes simulated, this is good evidence that the important processes are satisfactorily simulated and that the model gives a reliable description of the system.

Yields were somewhat under-predicted (by 9%) in the second rice of the RI-PAST rotation. This was evidently linked to over-prediction of N immobilization in the decomposition of crop residues at the start of the rice season, leading to under-prediction of N uptake. This was reflected in the simulated dissolved organic carbon (DOC) production, heterotrophic respiration and N assimilation by microbes (data not presented). The use of herbicides in the “chemical” fallow of the RI-PAST rotation improves N availability for the subsequent crop through mineralization of residues. Our simulation of this was evidently effective for a legume pasture cover crop or mixed pastures (legume + graminea), but not for Ryegrass, especially when the window between the cover crop and the rice crop was narrow.

Yield of the non-rice crops were slightly underestimated, and pasture production overestimated. This could be explained by the selective grazing of more palatable forage by lambs, tending to degrade the pasture, which is not accounted for in the model (Rutter, 2006; Cuchillo-Hilarario et al., 2017).

Observed and predicted N uptake data followed the same trend as the yields. Thus, when observed rice yields showed some degree of deviation from the predicted values, the same occurred for N uptake. However, only the N uptake in the RI-SOY rotation showed a slight underestimation in the predicted N uptake values, and in all cases predicted values followed the observed trend.

Gaseous N losses

The main gaseous N loss was *via* NH_3 volatilization. The DNDC model was mainly developed to estimate GHG emissions, and has been widely used to simulated N_2O and CH_4 emissions from rice systems but less so for NH_3 (Li, 2000; Zhao et al., 2020). In our study, the average rate of volatilization simulated during the rice phase was comparable to measured rates in well-fertilized high-yielding rice systems (Liu et al., 2015). Simulated volatilization in the RI-PAST rotation was comparable to that measured by Shang et al. (2014) in a rice-legume pasture system with a similar N fertilizer dose. On average, the simulated volatilization rate for all the rotations in the absence of N fertilizer addition was 15 kg N ha^{-1} , which is in the range of several studies (Shang et al., 2014; Liu et al., 2015, 2018). The good match between our predicted and published gaseous N losses, and the good agreement between observed and simulated N uptake and soil $\text{NH}_4\text{-N}$ concentrations, gives us confidence that the model satisfactorily describes volatilization.

We found that NH_3 volatilization was sensitive to N fertilizer additions and the soil organic C content and, to lesser extents, to soil clay content and pH. Volatilization losses increased in the order RI-SOY > RI-CONT > RI-PAST, matching the increase in soil $\text{NH}_4\text{-N}$ concentration at the beginning of each rice crop with N fertilizer additions and legume BNF. Likewise simulated losses increased with soil organic C content and associated organic N mineralization.

The observed decrease in volatilization with soil clay content is explained by greater NH_4^+ retention on soil surfaces, lowering the concentrations in the soil solution and gaseous NH_3 in equilibrium with it (Sommer et al., 2001).

The relatively modest effect of the initial soil pH on NH_3 volatilization is explained as follows. The main NH_3 losses occur when the soil is flooded. The effects of the initial soil pH are small then because of (a) the moderating effect of the biogeochemical changes following soil flooding, which cause the pH of acid and alkali soils to converge on near neutral, and (b) the dominant effect of the alkalinity released in urea hydrolysis in the floodwater [$\text{CO}(\text{NH}_2)_2 + 3\text{H}_2\text{O} = 2\text{NH}_4^+ + \text{HCO}_3^- + \text{OH}^-$], the floodwater pH being only weakly buffered, independent of the soil pH. Hence the floodwater pH increased from near neutral to pH 9.5 immediately after the third N fertilization.

We found no or only small N_2O emissions. There was no significant emission during the pasture phases, but there were small emissions in the first rice of the RI-PAST rotation and in the soybean crop of the RI-SOY rotation with a reasonable match between observed and simulated values given the low rates. Other studies in rice in Uruguay have found similarly low N_2O emissions (Tarlera et al., 2006; Irisarri et al., 2012; Illarze et al., 2018). Greater N_2O losses are expected in dry-seeded rice at the beginning of the flooding period, at field drainage (around harvest), and after heavy rains during the dry crop phase. Recent report of very high N_2O emissions from flooded rice (Kritee et al., 2018) cannot be generalized for all forms of water management (Wassmann et al., 2019).

Nitrogen balance, surplus and use-efficiency

The RI-PAST system had near neutral N balance ($-6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), as it did in our earlier evaluation of the rice-livestock system in Uruguay at the national level ($+2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$; Castillo et al., 2021). The slightly negative balance was explained by greater N removal in animal tissues with higher stocking rates than at the national scale. The forage base of the experiment was sown pastures whereas at the national scale it is almost 100% natural pastures. Although there were differences in N inputs and outputs between the studies, the relations between the main inputs and outputs were similar. Comparing the two studies, ratios of rice grain N/fertilizer N were 1.19 vs. 1.23 and gaseous N losses/total N inputs were 0.30 vs. 0.21.

Nitrogen balance in the RI-CONT system was strongly positive ($+45 \text{ kg N ha yr}^{-1}$), despite the high rice yield and high cropping frequency, and was associated with large N surpluses. This was due both to more N fertilizer use and more BNF in the legume cover crop. More positive N balances with smaller N surpluses could be achieved by using improved legume species in the cover crop to increase BNF and reduce N fertilizer use. The model sensitivity analysis showed that less fertilizer N use would reduce NH_3 volatilization losses without reducing rice yield. In the last two years of the RI-CONT rotation in the experiment, blooms of the floating macrophyte *Lemna minor* L. have been observed during rice phase at high N inputs (Supplementary Image S1). Although this phenomenon is not common in the Uruguayan rice system, it is in aquatic environments worldwide with high nutrient load (Goopy and Murray, 2003; Kiage and Walker, 2009). By sequestering N in rice field floodwater, it may reduce NH_3 volatilization losses (Li et al., 2009; Sun et al., 2019).

By contrast, N balance in the RI-SOY rotation was negative. This was despite inputs from BNF in both the pasture cover crop and soybean. The N balance of the soybean itself was slightly positive, so the negative balance of the whole rotation was due to soil N mining during the rice phase. This is important because soybean is increasingly important in rice-pasture rotation in temperate South America (Oficina de Estadísticas Agropecuarias, 2018; Ribas et al., 2021) due to higher economic margins and rice yields. The latter is associated by farmers with a contribution of soybean N to the rice. But, as in maize-soybean rotations in the US, the positive effect on rice yield seems to be more related to less N immobilization when rice is grown after soybean than after rice or another crop with high C to N ratio, such as maize (Green and Blackmer, 1995). Therefore, the negative N balance in our results is concerning for this rotation type in the long term.

These results agree with previous results obtained from this experimental platform (Macedo et al., 2021), where 19 % less N was found in the particulate soil organic matter in RI-SOY compared with RI-PAST, even at early stages of the long-term experiment. Soil N depletion due to negative balances has been reported in pure rice-soybean rotations (Benintende et al., 2008; Nishida et al., 2013; Hall et al., 2019). In our study, NBAL in RI-SOY was negative even when the rotation included Egyptian clover as a cover-crop. If that cover crop was not considered in the rotation, the NBAL would be even more negative. To avoid this, a better N nutrition from BNF has to be ensured, particularly by maintaining N fixing pasture species in the rotation. Results from a 6 year study (Landriscini et al., 2019) showed that positive NBAL was achieved in almost all the years and soybean-cover crops treatments. The explanation for the difference compared with our results was the N yields of the used cover crops (118 kg N ha^{-1}), which at least doubled our results. If increasing the rice yields rely only in the addition of more N fertilizer, our model sensitivity analysis suggests there would be no improvement in the negative NBAL. An increase of around $30 \text{ kg N fertilizer ha}^{-1}$ which apparently turns the NBAL of this rotation slightly positive (from -23 to $+7 \text{ kg ha}^{-1} \text{ yr}^{-1}$), will generate an extra N output of around 7 and 19 kg ha^{-1} in grain yield and N volatilization, maintaining the negative NBAL.

A clear trend in our results was that, in all three rotations, the higher the NBAL and NSURP values were, the lower was the resulting NUE. A well-managed system should have NUE between 50 and 90% (Oenema et al., 2014). As in Castillo et al. (2021), NUE of the RI-PAST rotation was in this range. However, the higher N inputs in RI-CONT caused a lower NUE of 48%. The opposite was observed in the RI-SOY which reached the highest NUE value, close to the upper threshold. Efficiency values around or above this threshold potentially indicate soil N mining and the negative NBAL values observed for this rotation.

Conclusions

1. The DNDC model successfully simulated crop responses and system N dynamics over the nine years of the rice rotational systems studied. Given the complexity of the model, this is good evidence that the important processes are satisfactorily simulated and that the model gives a reliable description of the system.

2. Though we lacked measurements of NH_3 volatilization, which was the main N loss process, our modeled values agreed with literature values for equivalent systems and were sensitive to relevant variables in expected ways, giving us confidence that the modeled volatilization was realistic.
3. Nitrogen management must be carefully optimized if rice rotations are intensified, as exemplified by the RI-CONT rotation where there were large N surpluses and RI-SOY where there were negative N balances, compared with the RI-PAST rotation which had only small N surpluses, neutral N balances and good N use efficiencies.
4. The DNDC model as parameterized here is suitable for exploring how to optimize N management in rice-pasture-livestock systems at regional scales, which is the subject of the companion paper: Castillo et al. (2023).

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

JC, GF, AR, PI, WA, and JT: data acquisition and curation. JT and AR: LTE project managers and funding acquisition. JC: conceptualization, visualization, interpretation, analysis, modeling, and drafting of the manuscript. GK, SH, MR, and JC: critical thinking and discussion, writing—review, and editing. GF, AR, PI, WA, and JT: manuscript review. All authors listed contributed directly to the manuscript. All authors approved this work for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2023.1103118/full#supplementary-material>

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Integrated nutrient management for improving crop yields, soil properties, and reducing greenhouse gas emissions

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Recently, most agrarian countries have witnessed either declining or stagnant crop yields. Inadequate soil organic matter (SOM) due to the poor physical, chemical, and biological properties of the soil leads to an overall decline in the productivity of farmlands. Therefore, the adoption of integrated nutrient management (INM) practices is vital to revive sustainable soil health without compromising yield potential. Integrated nutrient management is a modified nutrient management technique with multifarious benefits, wherein a combination of all possible sources of plant nutrients is used in a crop nutrition package. Several studies conducted in various parts of the world have demonstrated the benefits of INM in terms of steep gain in soil health and crop yields and at the same time, reducing greenhouse gas emissions and other related problems. The INM practice in the cropped fields showed a 1,355% reduction in methane over conventional nutrient management. The increase in crop yields due to the adoption of INM over conventional nutrient management was as high as 1.3% to 66.5% across the major cropping systems. Owing to the integration of organic manure and residue retention in INM, there is a possibility of significant improvement in soil aggregates and microbiota. Furthermore, most studies conducted to determine the impact of INM on soil health indicated a significant increase in overall soil health, with lower bulk density, higher porosity, and water-holding capacity. Overall, practicing INM would enhance soil health and crop productivity, in addition to decreasing environmental pollution, greenhouse gas emissions, and production costs.

KEYWORDS

agroecosystem, crop nutrition, greenhouse gas, nutrient management, soil health

1. Introduction

The increasing human population and their consequent need for food, combined with the depletion of healthy soil, have led to unprecedented damage to natural resources, making it difficult to meet the global demand for food. In the developing world, achieving food security through sustainable systems is a big task, yet it is vital for poverty alleviation. To get around this problem, farmers have resorted to overusing specific inputs like chemical fertilizers and

pesticides, which have already begun to harm the ecosystem. During the initial days of fertilizer usage, the impact of crop fertilization with inorganic fertilizers has been prominent in world agriculture (Hossain and Singh, 2000). In most countries of agrarian background, manufacturing and service of chemical fertilizers have been practiced as prime agenda in securing nations' food and nutritional security. India, a populous agrarian country, is the world's third-largest producer and consumer of chemical fertilizers (Tandon and Tiwari, 2007). As per recent reports, the Indian fertilizer market reached a value of Rs. 887 billion and is expected to grow at a compound annual growth rate of 5.5% by 2026 [IFA (International Fertilizer Association), 2020]. Food production must increase significantly while agriculture's environmental impact must decrease greatly to fulfill the world's future food security and sustainability needs (Foley et al., 2011).

India is a country with diverse cropping practices. The nutrient mining by various crops and cropping systems is far higher than the nutrient additions annually through fertilizers (Kumar et al., 2015). For the past 40 years, a nutrient gap of 8 to 10 million tons of nitrogen (N), phosphorus (P), and potash (K) per year have been documented (Tandon, 2004). This condition is analogous to depleting the soil's nutritional reserve. Long-term negative impacts of imbalance and indiscriminate use of inorganic fertilizers, particularly NPK-based formulations, have irreversibly damaged the soil resource base of many agroecosystems (Prasad et al., 2002; Singh et al., 2014). Despite the tremendous improvement in crop productivity in numerous crops due to improved varieties and increased use of agrochemicals, the goal of ensuring food and nutritional security remains challenging (Nath et al., 2018). The ever-increasing food demands of the burgeoning population have continuously exerted pressure on the agroecosystems. In the process of increasing agricultural production, the agriculture production units significantly contribute to environmental pollution (Wheeler and von Braun, 2013; Wu and Ma, 2015). For future generations, the goal is no longer to enhance agricultural production but also to optimize nutrients, energy, and water usage while minimizing environmental impact. Over-exploitation of nutrients from the soil and poor nutrient loss replenishment, depleted nutrients from the soil are often unable to be replenished by artificial crop fertilization, resulting in an imbalance in the soil nutrients pool (Paramesh et al., 2013a,b, 2014, 2020). Hence, the huge increase in global greenhouse gas (GHG) emissions by the agriculture sector, primarily due to the use of synthetic fertilizers and pesticides in recent years, both of which are rapidly expanding. One of the primary causes of environmental pollution, such as eutrophication and GHG emissions is the injudicious use of fertilizer, particularly nitrogen (Davidson et al., 2014). Thus, it is high time to search for innovative practices that guarantee higher yields with the least amount of additional environmental damage possible, especially for developing countries.

No single source of plant nutrients, such as chemical fertilizers, organic manures, crop residues, and bio-fertilizers, can meet the entire nutrient need of a crop in today's intensive agriculture systems (Mahajan and Gupta, 2009). In this context, the integration of all possible sources of nutrients is ideal for enhancing the soil resource base besides contributing to crop productivity (Nath et al., 2018). Findings of various studies (Selim and Al-Owied, 2017; Selim, 2018; Wang et al., 2019; Song et al., 2020) suggest integrated nutrient management (INM) is a tool that can offer good options and economic choices to supply macro and micronutrients of plants and also

contribute to reducing the dependence on externally purchased chemical fertilizers besides protecting soil health. Physical properties related to soil structure, are greatly influenced by adding organic manures (Das et al., 2014). An increasing number of research have suggested that INM has an impact on crop production, soil quality, and the environment while balancing food security and GHG mitigation. Numerous earlier studies largely examined how INM affected crop productivity, soil quality, or environmental performance. It is currently unknown how INM will affect crop productivity, soil bulk density, microbial biomass carbon, and the environment as a whole. This review addresses the synergistic effects of different nutrient sources and their combinations. This includes exploring the interactions between organic and inorganic fertilizers, biofertilizers, and crop residues on crop productivity and soil quality. By understanding how different nutrient sources interact, the researcher can develop more effective nutrient management strategies that optimize nutrient use efficiency and minimize environmental impacts. Further, this review highlights the long-term impacts of INM on soil quality parameters such as SMBC, bulk density, and dehydrogenase activity. This review article helps in understanding how INM affects soil quality, productivity, and environment. The researchers can develop more sustainable soil management practices that protect and improve soil health, and can develop more effective fertilizer recommendations and minimize nutrient losses. Finally, we can help farmers and policymakers make informed decisions about nutrient management.

2. Fertilizer consumption in India and the need for INM

Global use of inorganic fertilizers have increased almost fivefold since 1960 and have significantly supported population growth (FAO, 2017). The present world's demography will change in many folds in the near future and will require the production of 70% extra food, fodder and fuel to meet the demands of the ever-growing population (FAO, 2017). On the contrary, the natural base resource required for additional food production is shrinking. A study conducted by FAO (2017) indicated that the growth of agriculture between 1960 and 2015 exhibited about a 28% increase in the production of 174 crops cultivated all over the world. This phenomenal gain in production over the past few decades has not only tripled production but also paved the way for land degradation by hampering the soil's physical, chemical, and biological resource base (Power, 2013). High-input-driven and resource-intensive farming practices have caused massive and irreparable damage to soil and water resource in the agro-ecosystem and deteriorations in soil health besides contributing enormously to GHG emissions. Among agricultural practices, nutrient management is one such practice that plays a critical role in crop productivity and soil health. Additionally, after the green revolution, an over-reliance on fertilizers led to a decline in the efficiency of nutrient use and an increase in GHG emissions. The use of fertilizers can have implications for the accumulation of heavy metals in the soil and plants, which can subsequently enter the food chain, leading to potential pollution of water, soil, and air. Some mineral/chemical fertilizers contain low quantities of heavy metals and radionuclides, and their excessive application in agriculture can pose environmental problems. For example, the excessive use of urea has been identified

as a major contributor to increased nitrate (NO_3^-) levels in drinking water and river systems. Studies in agricultural districts of Srikakulam in Andhra Pradesh, located in the Vamsadhara river basin, revealed elevated nitrate concentrations ranging from traces to $450 \text{ mg NO}_3^-/\text{L}$ of water, particularly following fertilizer applications (Rao, 2006).

The current consequences of nitrate pollution in freshwater bodies in India reflect the detrimental effects of historical and ongoing excessive use of fertilizers and manures. The transport of phosphatic fertilizers through surface water flow can also contribute to increased phosphate content in drinking water and rivers. The rising levels of dissolved N and P loads are primarily associated with increased losses from agricultural and sewage systems. Moreover, the excessive application of nitrogen fertilizers has implications for GHG emissions. The relative contribution of fertilizer nitrogen application to total GHG emissions has increased from 8% in 1970 to 23% in 2010. NH_3 volatilization from rice fields is higher when N fertilizers are surface-broadcasted, leading to reduced nitrogen use efficiency (Ladha et al., 2005). Additionally, excessive N fertilizer application can negatively affect soil health by degrading soil carbon and affecting the structure and function of soil biological communities. Long-term application of N fertilizer alone has been shown to significantly reduce soil pH at various experimental sites in India.

Further, phenomenal loss in nutrient use efficiency of various crops and cropping systems has gradually encouraged farmers to apply higher doses of nutrients (Figure 1). As a result, this injudicious practice of nutrient management has paved the way to impair soil health due to less or no use of organic manure, residue retention, and bioinoculant-mediated fertilizer management. This has strongly distracted the complementarity between bio-geo cycles of the agroecosystem and is the main reason for multiple nutrient deficiencies, declining fertilizer response, and crop productivity. To address the current challenges associated with excessive fertilizer use, including the potential accumulation of heavy metals, pollution of water sources, GHG emissions, and adverse effects on soil health. Implementing sustainable nutrient management practices that optimize fertilizer use, minimize

losses, and protect both the environment and human health is imperative. Hence, INM is necessary to bring back harmony in the agroecosystem besides sustaining the productivity of crop and soil health.

The INM primarily refers to the judicious, efficient, and integrated use of all available sources of organic, inorganic, and biological components to combine traditional and modern techniques of nutrient management into an environmentally sound and economically optimal agricultural system (Janssen, 1993). To synchronize nutrient demand by the crop and its release in the environment, it optimizes all elements of the nutrient cycle, including N, P, K, and other macro- and micronutrient inputs and outputs. The INM techniques reduce losses due to leaching, runoff, volatilization, emissions, and immobilization while maximizing nutrient use efficiency (Zhang et al., 2012). Additionally, INM aims to improve the physical, chemical, biological, and hydrological aspects of the soil to increase agricultural production and reduce land degradation (Janssen, 1993; Esilaba et al., 2005). There is now a deeper understanding that INM may concurrently and very invisibly protect soil resources while also increasing crop yield. Farmyard manures, farm wastes, soil amendments, crop residues, chemical fertilizers, green manures, cover crops, intercropping, crop rotations, fallows, conservation tillage, irrigation, and drainage are all used in its methods to increase plant nutrition and preserve water (Janssen, 1993). The INM practice also promotes methods designed to reduce nutrient losses and enhance plant uptakes, such as the deep placement of fertilizers and the use of inhibitors or urea coatings (Zhang et al., 2012). Instead of merely concentrating on yield-scaled profit, these techniques urge farmers to focus on long-term planning and offer scope for the reduction of environmental impact.

2.1. Nutrient mobilization/mineralization in soil under INM practice

Farmers generally apply significant amounts of N fertilizer at the time of sowing or planting under conventional practice. Typically, 80% of the total N fertilizer is applied as a basal dressing, and the remaining

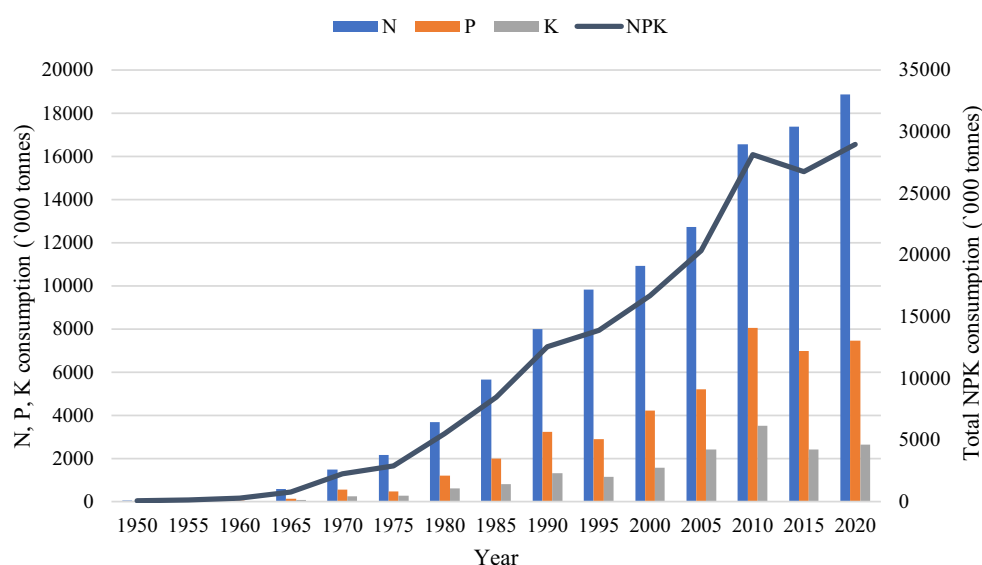


FIGURE 1
Annual fertilizer consumption in India (Fertilizer Association of India, 2021).

N is applied within the first 10 days after transplanting for rice or within the first 30-days after the date of seeding for wheat, maize, and sorghum (Peng et al., 2002; Chen et al., 2011). Due to the inefficient synchronization between the soil's supply and crop demand caused by this N application pattern, a significant amount of inorganic N is available in the soil before it is needed by rapidly growing crops (Chen et al., 2006). However, a material with a high N content (fertilizers) favors net mineralization, whereas one with a low N concentration (manures) results in net immobilization. Interestingly, Reddy et al. (2008) stated that organic manures (farmyard manure-FYM) with a C:N ratio of 29% to 33% immobilized N during the initial 40 days of incubation. This caused net N mineralization to begin during the first week of incubation, and then urea fertilizer has been added to lower the C:N ratio to 18%–22%. Therefore, a rapid increase in the mineral N near the end of incubation was the result of the combined application of fertilizer with FYM. The combined usage of organic manures with that of chemical fertilizer assures the continuous supply of nutrients by acting as a multi-nutrient pool, further during organic matter mineralization nutrients release slowly and gradually so that the crop enjoys the soil nutrient pool throughout its requirement. Consequently, the continual application of inorganic fertilizers in combination with FYM or lime, significantly altered soil microbial biomass carbon, soil N and P, fulvic acid (FA), and humic acid (HA; Srinivasarao et al., 2020). In addition, integrated use of fertilizer, manure, and lime application in soybean-wheat rotation led to improvements in soil water retention, soil aggregates, microporosity, and water holding capacity as well as a decrease in the soil's bulk density (BD) in the top 30 cm of the soil when compared to fertilizer application alone (Hati et al., 2008). Thus, the use of organic manures enhances fertilizer use efficiency and serves as an alternative source of nutrients (Dwivedi et al., 2016). The INM system synchronizes the nutrient demand set by plants, both in time and space, with the supply of nutrients from the labile soil pool and applied nutrient sources (Cassman et al., 2002). As INM encourages the split application of N fertilizers during critical stages of crop growth in small quantities it has the potential to increase crop productivity and quality and also reduce nutrient losses (Tilman et al., 2002; Witt and Dobermann, 2004).

2.2. Data collection and rice equivalent yield

The key data collected from the publications that qualified for review included grain yield, soil bulk density (BD), SOC, soil microbial biomass carbon (SMBC), dehydrogenase (DHA), GHG emission. In this analysis, change (δ) in measured variables between the conventional and INM practice was expressed as a percentage, i.e., $\delta = 100 \times (\text{INM} - \text{Conventional}) / \text{Conventional}$ because of its ease of interpretation. To compare different monocrops and cropping systems, rice equivalent yield (REY; equation 1) was determined by converting the economic yield of different crops on the basis of their marketable price prevailing during the period for each crop, including rice, and expressed in ton per unit area.

$$\text{REY (kg)} = \frac{\text{Yield of component crop (kg)} \times \text{Price of component crop (Rs kg}^{-1}\text{)}}{\text{Price of rice (Rs kg}^{-1}\text{)}} \quad (1)$$

3. Effect of INM on crop productivity, soil bulk density, microbial biomass carbon, and reducing environmental impact

3.1. Crop yield

Yield is the utmost concern in agriculture production systems. To achieve a desirable yield, a plant nutrient management system is one of the prime agro-practices. The impact of integrated nutrient management on rice equivalent yield was synthesized by taking into consideration of yield obtained under INM-treated plots against the conventional nutrient management of various crops and cropping systems and presented in Table 1. The results of rice equivalent yield indicated the gain in productivity of most of the cropping systems. The increment in crop yields under INM over conventional nutrient management across the cropping system studied was 1.3% to 66.5%. Furthermore, significant improvement in crop yields was more noticeable in field crops, especially rice, wheat, and soybean. However, vegetable crops like okra, tomato, and onion crops also demonstrated increased yields under INM. A long-term field experiment in India from 1973 to 2004, observed a substantial decrease trend in soybean yield for both the control and NPK treatments whereas the NPK + FYM treatment had a statistically significant ($p < 0.05$) increase in yield over time (Bhattacharyya et al., 2008). Increased microbial activity, better supply of macro- and micronutrients like S, Zn, C, and B, which are not supplied by NPK (straight) fertilizers, and lower nutrient losses from the soil are some of the additional benefits of organic matter over N, P, and K supply that may have contributed to the higher yields of soybean and wheat obtained with the FYM + NPK treatment. Therefore, INM system enhances the yield potential of crops over and above achievable with recommended fertilizers. In the future, there are several promising areas of work that can be explored in INM to further enhance crop yield. One avenue is the development and utilization of precision nutrient management techniques. As precision nutrient management uses advanced technologies such as remote sensing, geospatial analysis, and machine learning algorithms to assess crop nutrient requirements at a fine-scale level and deliver precise and site-specific nutrient applications. This approach minimizes nutrient loss and ensures that crops receive nutrients when and where they are most needed. Another important issue to be addressed is the exploration of microbial interventions in nutrient management. Microbial interventions in nutrient management use beneficial microorganisms like mycorrhizal fungi, rhizobacteria, and other plant growth-promoting microbes to improve nutrient availability and uptake efficiency, and enhance crop productivity. Scope of INM should be extended to the use cover crops and crop rotations to build soil fertility, enhance nutrient cycling, and crop yield.

3.2. Greenhouse gas emission

Globally, agriculture and its associated systems are viewed as a massive contributor of GHG emissions (Ravikumar et al., 2021). To reduce its contribution, reduction in excess nutrient application and balanced application are the key mitigation strategies (Sapkota et al.,

TABLE 1 Comparison of grain yield in conventional and integrated nutrient management system.

S no.	Country	Crop/cropping system	Nutrient management system	Rice equivalent yield	% change from conventional system	References
1	India	Maize	Conventional	8.2	39.9	Damse et al. (2014)
			INM	13.7		
2	India	Maize	Conventional	7.7	1.2	Verma et al. (2018)
			INM	7.7		
3	India	Maize	Conventional	5.1	29.7	Kalhapure et al. (2013)
			INM	7.3		
4	Zimbabwe	Maize	Conventional	1.5	1.3	Nyamadzawo et al. (2017)
			INM	1.5		
5	China	Maize	Conventional	9.2	−4.4	Nyamadzawo et al. (2017)
			INM	8.8		
6	India	Wheat	Conventional	3.4	32.9	Singh et al. (2019)
			INM	5.1		
7	India	Wheat	Conventional	3.4	32.9	Singh et al. (2018)
			INM	5.1		
8	India	Wheat	Conventional	5.3	14.5	Sharma et al. (2013)
			INM	6.2		
9	India	Wheat	Conventional	2.1	54.1	Argal (2017)
			INM	4.5		
10	India	Wheat	Conventional	1.3	66.5	Sharma U. et al. (2016)
			INM	3.8		
11	China	Wheat	Conventional	6	7.7	Zhang et al. (2012)
			INM	6.5		
12	India	Wheat	Conventional	4.3	8.4	Majumdar et al. (2002)
			INM	4.7		
13	China	Wheat	Conventional	7.2	8.1	Nyamadzawo et al. (2017)
			INM	7.8		
14	India	Rice	Conventional	5.9	16.2	Swarup and Yaduvanshi (2000)
			INM	7		
15	India	Rice	Conventional	5.4	8.0	Garai et al. (2014)
			INM	5.9		
16	China	Rice	Conventional	8.6	21.6	Zhang et al. (2012)
			INM	11		
17	West Bengal	Rice	Conventional	5.4	8.0	Garai et al. (2014)
			INM	5.9		
18	India	Rice	Conventional	3.9	23.1	Das and Adhya (2014)
			INM	5		
19	India	Rice	Conventional	4.5	38.6	Sharma U. et al. (2016)
			INM	7.3		
20	China	Rice-wheat	Conventional	7.7	54.6	Ma et al. (2010)
			INM	16.9		
21	India	Soybean	Conventional	3	3.4	Verma et al. (2017)
			INM	3.1		
22	India	Soybean	Conventional	2.1	44.9	Chaudhari et al. (2019)
			INM	3.8		
23	India	Soybean	Conventional	4.5	26.6	Farhad et al. (2017)
			INM	6.1		
24	India	Soybean	Conventional	6.5	20.2	Chaturvedi et al. (2012)
			INM	8.1		
25	India	Lime tree	Conventional	8.9	26.9	Lal and Dayal (2014)
			INM	12.2		
26	India	Cauliflower	Conventional	31.5	4.25	Sangeeta et al. (2014)
			INM	32.9		

(Continued)

TABLE 1 (Continued)

S no.	Country	Crop/cropping system	Nutrient management system	Rice equivalent yield	% change from conventional system	References
27	India	Sapota	Conventional	11.9	62.40	Baviskar et al. (2011)
			INM	31.7		
28	India	Okra	Conventional	3.5	12.0	Jat et al. (2017)
			INM	4		
29	India	Onion	Conventional	30.3	6.8	Jat et al. (2017)
			INM	32.5		
30	India	Guava	Conventional	193.1	10.9	Dwivedi (2013)
			INM	216.8		
31	India	Tomato	Conventional	34.9	15.7	Prativa and Bhattarai (2011)
			INM	41.3		
32	India	Mustard	Conventional	2.6	25.9	Pati and Mahapatra (2015)
			INM	3.5		
33	India	<i>Brassica napus</i>	Conventional	2	25.9	Nyamadzawo et al. (2014)
			INM	2.7		
34	India	Cotton	Conventional	61.3	11.8	Marimuthu et al. (2014)
			INM	69.4		
35	China	Vegetable species	Conventional	69.5	19.0	Zhang et al. (2012)
			INM	85.9		

TABLE 2 Comparison of GHG emissions from the conventional and integrated nutrient management system.

S no.	Country	Crop/cropping system	Nutrient management system	GHG emission (kg CO ₂ eq./ha)	% change from the conventional system	References
1	Zimbabwe	Maize	Conventional	110	−17.1	Nyamadzawo et al. (2017)
			INM	94		
2	China	Maize	Conventional	338	−75.0	Nyamadzawo et al. (2017)
			INM	193		
3	India	Rice	Conventional	90	−20.2	Sharma S. K. et al. (2016)
			INM	75		
4	China	Wheat	Conventional	252	−10.6	Nyamadzawo et al. (2017)
			INM	228		
5	India	Wheat	Conventional	383	−43.2	Majumdar et al. (2002)
			INM	268		
6	China	Rice-wheat	Conventional	9,300	−1267.6	Ma et al. (2010)
			INM	680		
7	India	Mustard	Conventional	30,016	−1354.5	Nyamadzawo et al. (2014)
			INM	2,064		

2021). Enhancing crop yields by application of plant nutrients become an ambiguous practice in most farmlands (Garnett et al., 2013). On the contrary, the imbalanced application of fertilizer in croplands is a major source of anthropogenic greenhouse gas emissions (Sutton et al., 2013). Therefore, to keep these anthropogenic GHG emissions under control, devising, and practicing proper fertilizer management is essential (Carlson et al., 2017). Of the several means of nutrient management, INM is proven better at minimizing GHG emissions. The work conducted by Nyamadzawo et al. (2017) in Zimbabwe in maize indicated practicing INM reduced about 17.1% GHGs over conventional nutrient management (Table 2). Another study in China demonstrated a significant ($p < 0.05$) reduction (1,268%) in GHG emissions against conventional nutrient management in the rice-wheat cropping system (Ma et al., 2010). Similarly, several studies

conducted in India indicated a considerable reduction in GHG emissions (20%–1,355%) in rice, wheat, and mustard crops (Majumdar et al., 2002; Nyamadzawo et al., 2014; Sharma et al., 2019).

Uncontrolled use of fertilizers increases emissions into the atmosphere and groundwater leaching of nutrients. The INM promotes high agricultural yields while reducing N losses and associated detrimental consequences on the environment (Gruhn et al., 2000). The ultimate fate of applied fertilizers is a combined effect of crop nutrient intake, immobilization, and soil residues, as well as nitrogen losses to the environment as ammonia volatilization, NO_x emissions, denitrification, N leaching, and runoff (Wu and Ma, 2015). Additionally, the pattern of N application, crop features, soil characteristics, climate, and management approaches affect the efficacy of applied fertilizers. INM thus recommends deep urea

placement, which can greatly boost N-use efficiency with low NH_3 volatilization and reduces nitrate-leaching (Jambert et al., 1997). Because nitrification occurs mostly following fertilizer N application (Ma et al., 2010) and irrigation, the use of nitrification inhibitors can also minimize N_2O emissions (Ju et al., 2011). Additionally, INM supports the use of organic nutrient sources since they provide both greater potential for agriculture's sustainability and more immediate environmental advantages. Combining organic manure with other management techniques, such as incorporating agricultural residues and creating conservation tillage (such as no-till or reduced-tillage practices), can also help to lower GHG emissions, enhance soil quality, and boost carbon sequestration (Huang and Sun, 2006).

Reducing environmental pollution can be achieved by developing precision nutrient management techniques, promoting nutrient recycling and reuse strategies, exploring the use of cover crops and diversified cropping systems. Precision nutrient management techniques minimize nutrient losses to the environment by refining nutrient application methods, such as incorporating controlled-release fertilizers or using site-specific technologies to target nutrient placement. In INM, nutrient recycling and reuse strategies efficiently capture and recycle nutrients from various sources, such as agricultural residues, livestock manure, and wastewater. Practices like anaerobic digestion, composting, and biochar production can be used to convert these nutrient-rich materials into valuable organic amendments. Intercropping and crop rotation can also be made an integral part of INM systems that incorporate nitrogen-fixing crops can also reduce the need for synthetic nitrogen fertilizers and decrease nitrogen runoff.

3.3. Carbon sequestration

Soil OM is the most important indicator of soil fertility, quality, and productivity, and is usually estimated by determining SOC (Rasmussen et al., 1998). It is generally believed that fertilizer application increases residues, including roots, returned to the soil, and as a result, can increase SOM content and C sequestration (Lu et al., 2009; Paustian et al., 2019). The INM through green manuring, crop residue incorporation and other animal-based manures has a profound influence on soil carbon stock. From their study, Sujata et al. (2007) revealed application of inorganic fertilizers in combination with organic manure has a positive influence on all soil properties, especially soil organic carbon. Similar observations were also made by Singh et al. (2009) where the addition of inorganic fertilizers with various organic manures in rice-wheat system enhanced the soil particle aggregation which in turn resulted in higher storage of soil organic carbon content. For instance, several studies conducted in India to know the effect of integrated nutrient supply on soil organic carbon status indicated significant gain in soil organic carbon, especially under intensive cropping systems like rice-wheat (Singh et al., 2000; Nayak et al., 2012; Ramteke et al., 2017), maize-wheat (Paramesh et al., 2014, 2020), maize-mustard (Saha et al., 2010; Moharana et al., 2012), and rice-groundnut (Prasad et al., 2002; Table 3).

The direct application of organic manure to the soil, which encouraged the development and activity of microorganisms, as well as improved root growth, which led to increased biomass output, crop stubbles, and residues, are both associated with increasing the amount of organic carbon in the INM (Yilmaz and Alagöz, 2010; Singh et al.,

TABLE 3 Variation in soil organic carbon due to conventional and integrated nutrient management system in India.

S no.	Crop/cropping system	Nutrient management system	SOC (%)	References
1	Rice	Conventional	3.02	Yaduvanshi (2017)
		INM	3.70	
2	Rice	Conventional	11.1	Bharali et al. (2017)
		INM	17.8	
3	Rice-wheat	Conventional	1.25	Kumar et al. (2015)
		INM	1.33	
4	Rice-wheat	Conventional	5.10	Yaduvanshi (2017)
		INM	6.80	
5	Rice-wheat	Conventional	5.50	Nayak et al. (2012)
		INM	6.30	
6	Rice-wheat	Conventional	5.60	Nayak et al. (2012)
		INM	7.70	
7	Rice-wheat	Conventional	8.40	Nayak et al. (2012)
		INM	9.90	
8	Rice-wheat	Conventional	0.37	Walia et al. (2010)
		INM	0.54	
9	Maize-wheat	Conventional	3.67	Hazra et al. (2019)
		INM	4.50	
10	Pearl millet-wheat	Conventional	8.50	Moharana et al. (2012)
		INM	11.08	
11	Maize-mustard	Conventional	2.07	Saha et al. (2010)
		INM	2.41	
12	Field pea	Conventional	0.45	Kumari et al. (2012)
		INM	0.49	
13	Guava	Conventional	0.67	Sharma et al. (2013)
		INM	0.71	

2011; Moharana et al., 2012). The increased carbon content of the soil have been caused by the eventual decomposition of these components. Additionally, the inclusion of FYM facilitated the synthesis of humic acid, which subsequently raised the soil's organic carbon content (Bajpai et al., 2006). Further, they opined that increased residue return, and minimum or zero tillage practices as the contributing factors to increasing SOC under INM practice.

A review of the long-term fertilizer experiment in China by Lu et al. (2009) observed linear relationships between the amount of N application and straw incorporation to soil C sequestration. Contrary to this belief, Khan et al. (2007) observed a decline in SOC after 50 years of chemical fertilizer application despite crop residue incorporation due to excessive N removal by crops to the tune of 60%–190%. The possible reasons for the decrease in SOC under continuous chemical fertilizer application were (i) acid forming ammonium fertilizers application delay soil C decomposition, (ii) enhancement in the activities of heterotrophic soil microorganisms that use C derived from crop residues (Mack et al., 2004; Khan et al., 2007), and (iii) decrease in C:N ratio lead to bacterial-dominated microbial communities, this further results in faster decomposition of SOC (Moore et al., 2003). Increased decomposition of SOC will result in more dissolved organic C, which can be lost through leaching (Mack et al., 2004; Van Kessel et al., 2009).

Enhancing carbon sequestration through integrated nutrient management can be achieved by exploring soil management practices, investigating the role of plant–soil–microbial interactions, exploring the integration of agroforestry practices, developing precision nutrient management techniques, and using modeling tools and decision support systems. Soil management practices that promote carbon sequestration include the use of organic amendments, cover crops, and crop rotations. These practices can help to increase soil organic matter, which is a major sink for atmospheric carbon. Beneficial soil microorganisms, such as mycorrhizal fungi, can help to improve nutrient uptake, promote root growth, and enhance soil aggregation. These activities can create conditions that are favorable for carbon sequestration. Agroforestry systems can provide multiple benefits, including increased biomass production and carbon storage. Precision nutrient management techniques can indirectly contribute to carbon sequestration by improving nutrient use efficiency and minimizing nutrient losses. Adaption of modeling tools can consider factors such as soil type, climate conditions, and crop management practices to estimate carbon sequestration rates and provide recommendations for optimizing nutrient management strategies that enhance carbon sequestration.

3.4. Soil properties

Crops grow better under friable and well-aggregated soils with optimum soil bulk density as it greatly influencing crop root growth and nutrient uptake. Practicing long-term INM brings a favorable difference in soil bulk density (Saha et al., 2010). The main reason for decreasing bulk density under INM was aggregation of soil particles due to increasing organic matter as well as stability of aggregates which leads to an increase in the total pore space in the soil. Islam et al. (2012) also concluded that the addition of organic matter through organic manure decreases the bulk density of soil. Integration of organic sources of nutrients such as crop residue and organic manures has a significant effect on soil bulk density (Celik et al., 2004). Nevertheless, studies undertaken to know the influence of integrated nutrient management on soil bulk density indicated favorable and convincing results. A study conducted by Nayak et al. (2012) in rice-wheat cropping systems at various places indicated significantly lower values of soil bulk density in the plots treated with integrated nutrient management systems over conventional chemical fertilizers. Similarly, Saha et al. (2010) and Salahin et al. (2011) also revealed the positive benefits of integrated nutrient management on soil bulk density in maize-mustard and tomato-mung-toria cropping sequence (Table 4). A long-term fertilizer experiment conducted in a rice-wheat cropping system at Chattisgarh, India ascertained that the incorporation of organic sources with chemical fertilizer application decreased bulk density, increased infiltration rate, and available NPK status of the soil (Bajpai et al., 2006). Significant reduction of bulk density in INM may be due to better soil aggregation (Singh et al., 2000), higher organic carbon, and more pore space (Selvi et al., 2005). A similar reduction in bulk density of soil due to the application of FYM with 100% NPK was also observed by Bellakki et al. (1998) and Bhattacharyya et al. (2010).

Table 5 shows an increase in SMBC and DHA under INM over conventional chemical fertilization. The increase in microbial biomass is mostly driven by the microbial biomass found in the organic byproducts and the addition of carbon from the substrate, both of which encourage the naturally occurring soil microbiota. The

TABLE 4 Variation in soil bulk density due to conventional and integrated nutrient management system of India.

S no.	Crop/cropping system	Nutrient management system	B.D (Mg/m ³)	References
1	Maize	Conventional	1.54	Kannan et al. (2013)
		INM	1.44	
2	Maize	Conventional	1.33	Kalhapure et al. (2013)
		INM	1.31	
3	Rice-wheat	Conventional	1.41	Kannan et al. (2013)
		INM	1.35	
4	Rice-wheat	Conventional	1.42	Nayak et al. (2012)
		INM	1.41	
5	Rice-wheat	Conventional	1.46	Nayak et al. (2012)
		INM	1.38	
6	Rice-wheat	Conventional	1.37	Nayak et al. (2012)
		INM	1.35	
7	Rice-wheat	Conventional	1.46	Bharali et al. (2017)
		INM	1.38	
8	Rice-wheat	Conventional	1.48	Sandhu et al. (2020)
		INM	1.31	
9	Rice-wheat	Conventional	1.34	Sandhu et al. (2020)
		INM	1.25	
10	Maize-mustard	Conventional	1.45	Saha et al. (2010)
		INM	1.38	
11	Sorghum-wheat	Conventional	1.30	Kharche et al. (2013)
		INM	1.21	
12	Potato	Conventional	1.55	Nath et al. (2012)
		INM	1.40	
13	Tomato-moong-toria	Conventional	1.35	Salahin et al. (2011)
		INM	1.40	

combined effect of FYM and chemical fertilizers in raising the SMBC under the maize-wheat system was also highlighted by Verma and Mathur (2009). The application of biofertilizers is known to create a variety of growth-promoting compounds in addition to their basic effects, which help explain the rapid expansion of microbial growth. Additionally, the crops' rhizodeposition (Jones et al., 2009) contributed to the elevated SMBC under INM practice. Nayak et al. (2007) similarly demonstrated enhanced dehydrogenase activity with both compost and inorganic fertilizer application under continuous rice-growing situations and explained a significant relationship between SMBC. The addition of fly ash and FYM to a rice-wheat cropping system in Alfisol and Vertisol significantly enhanced the microbial biomass carbon and dehydrogenase activity in soil (Ramteke et al., 2017). Kanchikerimath and Singh (2001) found that the inorganic fertilizer (NPK) increased crop yield, SOC, total N, mineralizable C and N, microbial biomass C and N, and dehydrogenase, urease, and alkaline phosphatase activities, while manure applied together with inorganic fertilizer increased these parameters more strongly. However, when more organic residues are added to the soil, they undergo microbial decomposition, which releases organic compounds like polysaccharides, which act as a strong binding agent in the formation of large and stable aggregates that help to improve the physical properties of soil (Manickam, 1993). Accordingly, Yuhui et al. (2004)

TABLE 5 Variation in SMBC and dehydrogenase activity due to conventional and integrated nutrient management system.

S no.	Crop/cropping system	Nutrient management system	SMBC (kg/m ³)	Dehydrogenase (μg TPF g ⁻¹ h ⁻¹)	References
1	Wheat	Conventional	51.7	12.43	Argal (2017)
		INM	62.9	45.49	
2	Cabbage	Conventional	318.5	-	Swami et al. (2020)
		INM	414.8		
3	Onion	Conventional	80	6.42	Gupta et al. (2019)
		INM	143	11.42	
4	Sorghum	Conventional	266.9	0.31	Sharma U. et al. (2016)
		INM	303.9	0.33	
5	Rice	Conventional	80	-	Bharali et al. (2017)
		INM	170		
6	Rice-niger	Conventional	75.8	-	Gogoi et al. (2010)
		INM	136.2		
7	Rice-toria	Conventional	124	198.0	Nath et al. (2012)
		INM	222.8	257.30	
8	Rice-wheat	Conventional	49.6	64.63	Nath et al. (2011)
		INM	167.7	152.94	
9	Rice-wheat	Conventional	147.9	-	Borase et al. (2021)
		INM	250		
10	Rice-chickpea	Conventional	161.5	-	Borase et al. (2021)
		INM	258		
11	Rice-wheat-moong	Conventional	203.1	-	Borase et al. (2021)
		INM	292.3		
12	Rice-wheat-rice-chickpea	Conventional	167.3	-	Borase et al. (2021)
		INM	241.2		
13	Maize-mustard	Conventional	266.8	-	Saha et al. (2010)
		INM	317		

SMBC, soil microbial biomass carbon; INM, integrated nutrient management.

highlighted the significance of organic manure addition with chemical fertilizers to improve yield, and soil health, and enhance the earthworm population in the soil. In another long-term experiment in Germany, Marhan and Scheu (2005) observed an increase in earthworm biomass by 42.8% in NPK + FYM treatment, with a decrease of 9.4% in NPK treatment. They ascertained an increase in earthworm biomass in NPK + FYM could be due increased utilizable SOM pool.

Future research in integrated nutrient management can prioritize enhancing soil quality, a critical aspect of sustainable agriculture. One promising area is the exploration of practices that enhance soil organic matter content and composition. Efforts can focus on identifying effective organic amendments, cover crops, and crop rotations that facilitate the accumulation of stable organic matter in soils. Understanding the interactions among nutrient management practices, organic matter dynamics, and soil microbial communities will provide valuable insights for optimizing soil quality enhancement. Additionally, research should emphasize nutrient management strategies that promote soil nutrient cycling and enhance nutrient use efficiency. This involves investigating the synergistic relationships between organic and inorganic fertilizers, biofertilizers, and soil microorganisms in nutrient

cycling processes. Developing nutrient management approaches that minimize nutrient losses through leaching, volatilization, and runoff, while simultaneously ensuring sufficient nutrient availability for crops, will improve nutrient use efficiency and contribute to soil quality enhancement. Moreover, such practices will help mitigate environmental pollution associated with nutrient runoff.

3.5. Integrated nutrient management on soil health and crop quality

The current review has shown a positive effect of Integrated nutrient management (INM) on yield, soil microbial activity, SOC, and BD over conventional chemical fertilizer application. These summarized results further highlighted reduction in use of chemical fertilizer under INM practices, this in turn resulting in reduction of environmental burden associated with fertilizer production, transport, and its use. The main objective of INM is to wisely utilize its three primary components. These include harnessing the existing synergy between dual-purpose microbes (which promote growth and control

soil-borne pathogens), limiting the use of chemical fertilizers, promoting the multiplication of native soil microbial diversity through organic substrates, and maintaining a nutrient inflow that exceeds outflow. Additionally, the aim is to ensure that production economics are favorable in the market. Nevertheless, there are still several crucial areas that require urgent attention to make INM a globally dynamic nutrient management approach. Through enhancement of SOC and soil microbial activity INM increases soil nutrient availability and it is having positive impact on crop quality.

4. Conclusion

Farmers have rich experiences in integrated and efficient utilization of different sources of organic materials to produce modest crop yields and maintain soil fertility using traditional farming practices. However, during the last two decades, to achieve food security with declining land and other resources, this practice is gradually being abandoned, and nutrient management is being shifted to over-reliance on chemical fertilizers. Over-application of N has become more common in intensive agricultural regions, leading to low nutrient-use efficiency and environmental pollution, which threaten the long-term sustainability of the agricultural system. Many factors might have contributed to the over-application problems including obtaining higher yields as a top priority, small land holding, lack of nitrogen management, and lack of effective extension systems. A review of experiments conducted globally indicates that chemical fertilizers alone are not enough to improve yield and soil quality at high levels. Further, the review also highlighted the negative effects of the continuous use of synthetic N fertilizers on soil organic carbon, bulk density, soil enzymatic activity, SMBC, and the environment. Crop yield responses to nutrient management may vary significantly from year to year due to variations in weather conditions and indigenous N supply, and thus the commonly adopted prescriptive approach to N management needs to be replaced by a responsive

in-season management approach based on the diagnosis of crop growth and N status and demand.

Author contributions

VP, RM, SG, GR, AN, ST, and YM designed the research, data collection, and analysis. VP, RM, SB, AN, DJ, and SG were involved in the interpretation of results, data analysis, draft manuscript preparation, and final manuscript preparation and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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