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ORIGINAL ARTICLE

High fertilizer nitrogen input increases nitrogen mining in sandy paddy soils

Arjun Pandey · Simon M. Eldridge · Anthony Weatherley · Ian R. Willett · Aung K. Myint · Aung Naing Oo · Kyaw Ngwe · Zoong T. Mang · Deli Chen

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Abstract Excessive nitrogen (N) fertilizer application does not increase rice grain yield and N retention in soils but may lead to higher soil N uptake by plants due to added N interaction (ANI). This study hypothesizes that large doses of fertilizer-N increase native soil N uptake by rice plants and reduce soil N balance. We conducted field experiments in two locations in Myanmar for four consecutive rice cropping seasons to determine grain yield, the source of N in plants, and net soil N balance in sandy loam soils to which 0, 30, 77.6, and 160 kg urea-N ha^{-1} was applied. We used ^{15}N-labeled urea to determine the source of N in plants, ANI and soil N balance. Although rice yield increased with increased N input in the dry seasons, there was minimal yield benefit from N rates above 30 kg ha^{-1} in wet seasons. Fertilizer-N contributed only 30% of the total plant N, seldom exceeding 40%. Nitrogen rates over 30 kg ha^{-1} significantly increased soil N uptake in plants ($p < 0.05$), demonstrating a clear ANI effect of higher N rates. Soil N withdrawal by plants and ANI were the highest in the treatment receiving 160 kg N ha^{-1}, but the fertilizer-N retention in the soil was not enough to compensate for the increased soil N withdrawal, leading to more negative net soil N balance. We demonstrate that excessive N input increases soil N uptake by rice plants, and this combined with low retention of fertilizer-N in sandy paddy soils, leads to more negative soil N balance.

Keywords Soil organic nitrogen · Plant nitrogen uptake · ^{15}N · Rice yield · Added nitrogen interaction

Arjun Pandey and Simon M. Eldridge have contributed equally.

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A. Pandey (✉) · S. M. Eldridge · A. Weatherley · I. R. Willett · D. Chen (✉)
School of Agriculture and Food, The University of Melbourne, Parkville, VIC 3010, Australia
e-mail: arjun.pandey@unimelb.edu.au

D. Chen
e-mail: delichen@unimelb.edu.au

A. K. Myint · A. N. Oo · K. Ngwe · Z. T. Mang
Yezin Agricultural University, Yezin, Nay Pyi Taw, Myanmar

Introduction

Soil nitrogen (N) supply constitutes the majority (60–70%) of rice plant N uptake (Zhang et al. 2012b; Yan et al. 2020). Despite the low use efficiency of N fertilizer, farmers continue to apply large amounts of N fertilizer with the expectation of increased grain production (Ju et al. 2009; Yan et al. 2020). The majority of the applied N fertilizer is lost from the soil system (Ju et al. 2009). Excess and inefficient use of N fertilizer not only creates serious environmental issues, but also has a detrimental effect on natural soil

N retention processes (Pandey et al. 2019), resulting in increased dependency on N fertilizer input (Dobermann et al. 2000).

The N difference method, which is the difference between the amount of plant N uptake in unfertilized plots (also known as control plots) and fertilized plots, is used to determine soil and fertilizer N supply to plants (Wang et al. 2012; Buresh et al. 2019). However, the soil N supply in unfertilized plots may not be the same as in plots receiving N because the application of N increases soil organic nitrogen (SON) mineralization and may lead to more organically bound N being available for plant uptake (Zhang et al. 2012a; Schleuss et al. 2019; Shen et al. 2021). Nitrogen fertilizer input also improves rice root growth (Ge et al. 2015; Kim et al. 2016; Xiao et al. 2019), which enables plants to exploit more soil N. This phenomenon of N fertilizer addition increasing soil N uptake by plants is known as the added N interaction (ANI) or priming effect (Jenkinson et al. 1985). Due to the ANI of N fertilizer, the N difference method overestimates the actual amount of fertilizer-N uptake by rice crops.

An alternative method to estimate the sources of N in plants is to apply ^{15}N labeled fertilizer and determine the uptake of ^{15}N by plants (Rose et al. 2016). However, it is widely debated whether the ^{15}N isotope tracing method underestimates N fertilizer uptake in plants. For example, Schnier (1994) suggested that the higher soil N uptake and lower N fertilizer uptake observed in rice plants when using ^{15}N labeled fertilizer is not due to the priming effect of N addition but is due to 'pool substitution', in which added labeled N displaces native unlabeled soil N which is then taken up by plants, causing a seemingly higher soil N uptake by plants. However, the priming effect would be an artifact only if the added N fertilizer has no influence on SON turnover and plant root biomass (Jenkinson et al. 1985; Schnier 1994). Also, for pool substitution to occur, the applied N fertilizer and soil inorganic N should be in the same pool, which is unlikely under field conditions (Hart et al. 1986).

There is increasing consensus that high anthropogenic N input influences SON turnover and net N mineralization leading to higher soil N uptake in plants, including in rice paddies (Zhang et al. 2012a; Schleuss et al. 2019, 2021; Shen et al. 2021). It has been proven empirically that microbes retain N when they are N limited (high microbial N use efficiency), leading to N immobilization (Mooshammer et al. 2014). However, increased N availability relative to labile carbon as a result of N fertilizer addition, especially in low C soils, results in large amounts of organic N being released as ammonium (low microbial N use efficiency, Mooshammer et al. 2014; Manzoni et al. 2008; Nave et al. 2009; Schleuss et al. 2019, 2021). Therefore, high N input in paddy soils increases SON mineralization and this combined with increased root growth can lead to high soil N uptake by rice plants.

Soil N removal by rice plants is always greater than the N fertilizer retained in soils (Rose et al. 2016; Zhang et al. 2012b), so without accounting for N_2 fixation, N deposition and native soil N loss, the net soil N balance (the difference between fertilizer N retained in soil and soil N taken up by rice plants) is always negative in paddy soils. Retention of applied N fertilizer in paddy soils is significantly lower compared to soils of other cereal production systems (Yan et al. 2020). The low redox potential of continuously flooded paddy soils can increase the release of fixed NH_4^+ from the interlayers of clay minerals due to the reductive dissolution of Fe(III) of Fe oxides coatings on clay surfaces (Akter et al. 2018), resulting in the release and plant uptake of fixed NH_4^+. Moreover, N mineralization is higher than immobilization in continuously flooded paddy soils, leading to low fertilizer N retention (Zhang and Scherer 2000; Said-Pullicino et al. 2014), and the inability of sandy paddy soils to retain a considerable amount of fertilizer-N (Xie et al. 2013) can lead to more negative N balance in sandy paddy soils. We hypothesize that the application of high doses of N fertilizer to sandy paddy soils increases soil N uptake by rice plants and leads to more negative net soil N balance. We conducted field experiments at two locations in Myanmar for four consecutive rice growing seasons to determine how increasing the rate of fertilizer-N application affects the sources of N in rice plants and net soil N balance. We used ^{15}N tracing methods to determine the source of N in plants, ANI and net soil N balance.

Materials and methods

Experimental sites

Field experiments were established at Yezin in Zeyarthiri Township (19°49′55.5″N, 96°15′52.2″E) and at Taungoo Township (18°56′56.8″N, 96°19′51.7″E) in Central Myanmar. The soil was a Gleyic Fluvisol at Yezin and an Orthofluvic Fluvisol at Taungoo (IUSS Working Group 2014) with sandy loam top soils. The sites represent widely distributed soils of the lowlands of Central Myanmar formed from alluvial deposition that are used for rice production. Yezin is north of Taungoo and usually receives less rainfall. Physicochemical properties of the soils are presented in Table 1. The Yezin soil has a higher fraction of coarse sand (0.50–2 mm, 85% of the total sand content) compared to Taungoo soil (62% of the total sand content). The field experiments at both sites ran for the summer (dry, irrigated) seasons and the monsoon (wet) seasons in 2017 and 2018. The Yezin site in Zeyarthiri Township was discontinued after the wet season in 2017 due to difficulties in water management and continued at a location close by (Sein Sar Pin: 19°49′23.4″N, 96°15′45.6″E, Orthofluvic Fluvisol) with loamy sand topsoil (Table 1) and identical treatments to the original trial.

Experimental design

The experiments at Yezin and Taungoo had an identical randomized complete block design with four treatments and three replications. Treatments at Sein Sar Pin, near the Yezin site (referred also as Yezin hereafter) which commenced in the dry season of 2018 only differed by having four replications instead of three. Experimental plots were 5×5 m delineated by double bund walls 40 cm wide and 30 cm high. There was a one meter spacing between plots in each block and three meters spacing between blocks. To avoid any residual effect of fertilizer, the field was left unfertilized in the preceding monsoon season. The four treatments included a control without N fertilizer input (N0), 30 kg N ha^{-1} (N30), 77.6 kg N ha^{-1} (N78) and 160 kg N ha^{-1} (N160). The nitrogen rate in the N78 treatment was set to approximately meet the rice crop N requirement (16 kg N per metric ton of grain yield, IRRI 2017) to achieve the 4.5 t ha^{-1} grain yield target set by Ministry of Agriculture in Myanmar (Thwe et al. 2019), and the N rate in the N160 treatment was set with consideration of 45% N fertilizer recovery in rice plants, and the remaining N to compensate for N loss and N immobilization. Granular urea was applied as a N source by surface broadcasting. Urea was applied as two equal split applications; one at 10 days after transplanting (10 DAT) and the second at the panicle initiation (PI) stage of the crop, which is in accordance with the farmers' practice in the region. A detailed timeline of field activities is presented in Table 2.

Prior to transplanting the rice seedlings, a basal dose of phosphorus (40 kg P ha^{-1} as triple superphosphate), potassium (25 kg K ha^{-1} as muriate of potash) and sulfur (25 kg S ha^{-1} as gypsum) was applied to all the treatments, including the control (N0). Two additional split doses of K (25 kg K ha^{-1} each) were applied during the later rice growth stages (i.e., 75 kg K ha^{-1} in total). Before transplanting, the roots of the rice seedlings were dipped in a 2% Zn solution (as zinc sulfate) to prevent any possibility of Zn deficiency. The experimental plots were puddled to incorporate the basal fertilizers prior to manually transplanting 20 day old rice seedlings at a 20 cm hill-to-hill spacing with four seedlings planted on each hill. A common dry season variety grown by the local farmers, '*Yadanar Toe*', was transplanted in the 2017 dry season. The rice variety '*Sin Thukha*', popular with farmers, was grown during all other seasons. The plots were irrigated when required to keep the water depth above 5 cm.

Table 1 Physicochemical properties of soils (0–10 cm) at the experiment sites

Site	pH_w	EC	TC	TN	Olsen P	Particle size analysis		
		dS m^{-1}	%	%	mg kg^{-1}	Clay (%)	Silt (%)	Sand (%)
Taungoo	5.8	0.04	0.6	0.06	4	9	16	75
Yezin	5.5	0.19	1.1	0.09	7	9	16	75
Sein Sar Pin	6.5	0.08	1.0	0.18	10	5	12	83

EC, TC and TN represent electrical conductivity, total carbon and total nitrogen, respectively

Table 2 Timeline of activities for the field experiment in the dry and wet seasons at both locations in 2017 and 2018

Sites	Activities	Dates			
		Dry 2017	Wet 2017	Dry 2018	Wet 2018
Taungoo	Seedling transplant	26 Feb	15 Jul	2 Feb	16 Jul
	First split fertiliser application (10DAT)	8 Mar	4 Aug	12 Feb	26 Jul
	Second split fertiliser (PI stage)	27 Apr	28 Sep	3 Apr	19 Sep
	Harvest	13 Jun	23 Nov	28 May	7 Nov
Yezin*	Seedling transplant	5 Mar	1 Aug	9 Feb	23 Jul
	First split fertiliser application (10DAT)	15 Mar	11 Aug	19 Feb	02 Aug
	Second split fertiliser (PI stage)	4 May	5 Oct	10 Apr	26 Sep
	Harvest	26 Jun	26 Nov	17 Jun	14 Nov

*A separate site, Sein Sar Pin, was used for the experiment in 2018 dry and wet seasons in Yezin

^{15}N microplot setup

We used the ^{15}N tracing method to determine the source of N in the plants and the fate of N fertilizer. Stainless steel microplots (80 cm L × 40 cm W × 40 cm H) were installed within the main plots 10 days after seedling transplanting. The microplots were driven to 20 cm depth and each contained 8 plant hills (i.e., 2 rows of 4 plant hills). The microplots received ^{15}N labeled granular urea (10.1 atom% ^{15}N) on the same day and same rate as urea-N application to the main plots (10 DAT and PI stage). The water within all the microplots was maintained at the same level as the main plots by topping the microplots with irrigation water when required. The microplot within each plot was moved to a new location following sampling at harvest to eliminate carry over of residual soil ^{15}N into the following crop. The ^{15}N microplots were discontinued in the wet season of 2018 due to technical difficulties.

Crop and soil sample management

The central 1.8 × 1.8 m of each main plot comprising of 81 plant hills was harvested manually by cutting the plants at ground level. Grains were separated from the straw, and these samples were weighed and a subsample of both grain and straw was air dried to constant mass at 65 °C. Subsamples of the dried rice straw and grain were finely ground (< 50 µm) for later analysis. Following the main plot harvest, all the rice plants in microplots were sampled carefully by cutting at ground level. Rice straw and grain samples from the microplots were initially milled down to 2 mm, avoiding cross contamination, and subsampled for fine grinding (< 50 µm) for later analysis. The soil within a microplot was sampled at 5 random points using a 5 cm diameter sampling core after crop harvest. From each point, a 20 cm long core was taken and cut into 0–10 cm and 10–20 cm depths. The 5 cores for each depth within a microplot were then composited and homogenized to make one representative sample. These soil samples were air-dried to constant mass and sieved to < 2 mm and finely ground (< 50 µm) with a tissuelyser for analysis. Bulk density cores were collected from four randomly selected locations within each plot at 2–7 cm and 13–18 cm depths at harvest, when paddy surface water was drained, to represent the 0–10 cm and 10–20 cm soil depths for later soil nutrient conversion to per hectare basis. Crop residue (straw) was not returned to plots after harvest to align with farmers' practice; farmers practice stubble burning.

Laboratory analysis

The ^{15}N atom% was quantified on weighed subsamples of dried ground soil (~40 mg), rice straw (~4 mg) and grain materials (~4 mg) from the ^{15}N microplots using an isotope ratio mass spectrometer (Sercon Hydra 20–20). The corresponding plant and soil samples from the control plots were used as the natural abundance control samples for the analyses and subsequent calculations. Total carbon in soil was determined by combustion. Nitrogen concentrations in plant tissue (straw and grain) and soil samples taken from the harvested areas were determined using the Kjeldahl digestion method (Horneck and Miller 1998). Soil pH (water), soil electrical conductivity and Olsen-P was analyzed using the methods of Rayment and Lyons (2011). Soil particle size analysis (PSA) was determined using the 'pipette method' (Bowman and Hutka 2002).

Calculations and statistical analysis

Total N and ^{15}N uptake in straw and grain were calculated separately and combined to obtain values for total aboveground plant biomass. Likewise, total N content in soil samples from the 0–10 cm to 10–20 cm depths were calculated separately and combined to obtain final values. Total N uptake in dry plant tissue (TNuptake) was calculated by adding the N uptake in grains and straw as follows:

$$\text{N uptake in grains}\left(kg\ \ N\ \ ha^{-1}\right) = \text{N content in grains}\left(kg\ \ kg^{-1}\right) \times \text{total grain yield}\left(kg\ \ ha^{-1}\right) \quad (1)$$

$$\text{N uptake in straw}\left(kg\ \ N\ \ ha^{-1}\right) = \text{N content in straw}\left(kg\ \ kg^{-1}\right) \times \text{total straw yield}\left(kg\ \ ha^{-1}\right) \quad (2)$$

$$\text{TN uptake}\left(kg\ \ N\ \ ha^{-1}\right) = \text{N uptake in grain}\left(kg\ \ N\ \ ha^{-1}\right) + \text{N uptake in straw}\left(kg\ \ N\ \ ha^{-1}\right) \quad (3)$$

The calculations for ^{15}N labeled fertilizer recovery in plant and soil systems were based on Wang et al. (2018). The percent N in the plant tissue or soil derived from the ^{15}N-labeled urea (%Ndff) was calculated using the following equation:

$$\%Ndff = \frac{(a-b)}{(c-b)} \times 100 \quad (4)$$

where a is the atom% ^{15}N in plant or soil sample receiving ^{15}N fertilizer, b is the atom% ^{15}N in plant or soil sample from the control plot, c is the atom% ^{15}N in labeled urea-N (10.1%). The amount of N derived from fertilizer in plants (Plant Ndff, kg N ha^{-1}) was then calculated as follows:

$$\text{Plant Ndff}\left(kg\ \ N\ \ ha^{-1}\right) = \frac{\text{TNuptake}\left(kg\ \ N\ \ ha^{-1}\right) \times \%\ \text{Ndff in plant}}{100} \quad (5)$$

The amount of N derived from native soil N in plants in zero N plots (Plant $Ndfs_0$) and N fertilized plots (Plant Ndfs, kg N ha^{-1}) were calculated as follows:

$$\text{Plant } Ndfs_0\left(\text{kg N ha}^{-1}\right) = \text{TN uptake in zero N plots}\left(\text{kg N ha}^{-1}\right) \quad (6)$$

$$\text{Plant Ndfs}\left(\text{kg N ha}^{-1}\right) = \text{TN uptake}\left(\text{kg N ha}^{-1}\right) - \text{Plant Ndff}\left(\text{kg N ha}^{-1}\right) \quad (7)$$

The amount of N fertilizer recovered in soil (Soil Ndff, kg N ha^{-1}) was calculated as follows:

$$Soil\ Ndff\left(kg\ \ N\ \ ha^{-1}\right) = \frac{Total\ soil\ N\left(kg\ \ N\ \ ha^{-1}\right) \times \%Ndff\ in\ soil}{100} \quad (8)$$

The amount of unaccounted N fertilizer in plant and soil was considered as N lost from the system. Net soil N balance was calculated as follows:

$$Net\ soil\ N\ balance\left(kg\ \ N\ \ ha^{-1}\right) = Soil\ Ndff\left(kg\ \ N\ \ ha^{-1}\right) - Plant\ Ndfs\left(kg\ \ N\ \ ha^{-1}\right) \quad (9)$$

The net soil N balance in this study does not account for N fertilizer retained in root residue, the input of N through biological N_2 fixation, and loss of native soil N. We assumed the input of N through deposition to be very small and our analysis found irrigation water N concentrations to be negligible at all sites. The increase in soil N uptake in plants due to added N interaction (ANI) was calculated as the difference between soil N uptake in fertilized plots (Plant Ndfs) and soil N uptake in zero N plots (Plant $Ndfs_0$).

Two-way ANOVA was used to test the effect of treatments and sites on grain yield and crop N uptake, N loss and N balance using a generalized linear model with block as a random effect in Minitab 18. There was no significant interaction effect between treatments and sites on grain yield and N parameters so the results are presented separately for each site. Fisher's LSD at 95% confidence level was used to compare differences between the treatment means.

Results

Crop yield

There was no significant difference in rice grain yield between the N0 (control) and N30 treatments in any of the seasons and locations (Fig. 1a and b). There

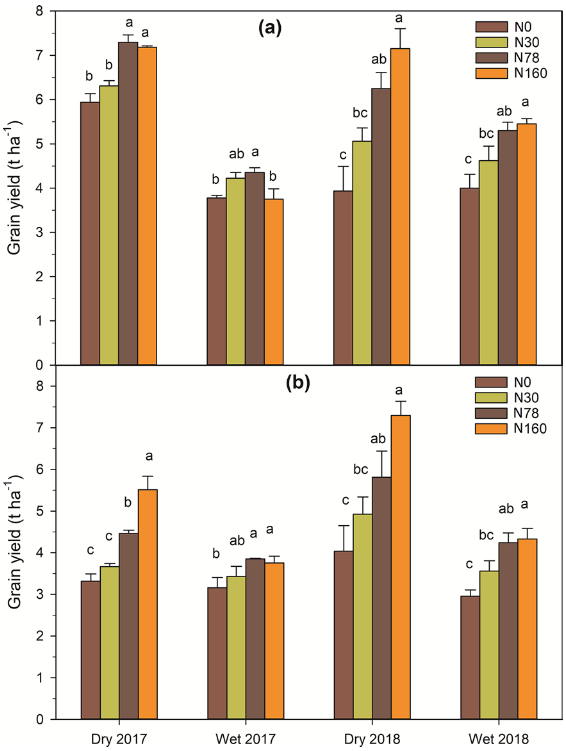

Fig. 1 Rice grain yield (tonnes ha^{-1}) at Yezin (a) and Taungoo (b) in Dry and Wet seasons of 2017 and 2018. N0, N30, N78 and N160 represent N addition of 0, 30, 77.6 and 160 kg ha^{-1}, respectively. Column bars with different letters within a season are significantly different ($p < 0.05$). Error bars represent ± 1 standard error of the mean

was an increasing trend in grain yield with increasing levels of N applied, although the differences were not always statistically significant ($p > 0.05$). The N78 treatment showed higher yield ($p < 0.05$) compared to the N30 treatment only in the dry season of 2017 at both sites. The N160 treatment achieved a higher yield ($p < 0.05$) than the N30 treatment except in the wet season of 2017 and higher yield than the N78 only in the dry season of 2017 at Taungoo. Grain yields in the wet seasons were considerably lower than in the dry seasons. The plant yield response to N application over 30 kg N ha^{-1} was very low in wet seasons compared to dry seasons. Although there was some yield response to N application over 30 kg N ha^{-1} in the 2018 wet season, the yield increase was less than 0.8 t ha^{-1} with 130 kg ha^{-1} additional N input.

Source of nitrogen in plants

The total N uptake in rice plants (TNuptake = Ndff + Ndfs$_0$ + ANI) increased with increasing rates of N fertilizer inputs at both locations (Fig. 2). Also, the amount of N derived from N fertilizer in plants (Plant Ndff) significantly increased ($p < 0.05$) with increasing rates of N fertilizer input. Application of 30 kg N ha^{-1} generally had minimal effects on ANI, but further increases in N fertilizer inputs significantly increased ANI ($p < 0.05$) at both locations. Application of 160 kg N ha^{-1} always led to significantly higher ANI ($p < 0.05$) in plants than in the control and N30 treatments. Recoveries of N fertilizer in plants mostly remained around 30% and seldom exceeded 40%.

Recovery of N fertilizer in soil

There was no consistent pattern in the recovery of N fertilizer in soil (Soil Ndff) between the treatments and seasons at any of the locations (Fig. 3). Generally, the N30 treatment had lower N fertilizer recovery in soils compared to the N78 and N160 treatments. The highest amount of N fertilizer recovered in soil was in the N160 treatment; it was 25 kg N ha^{-1} in the dry season of 2018 at Yezin and 37 kg N ha^{-1} in the wet season of 2017 at Taungoo.

Nitrogen fertilizer loss and net soil N balance

The amount of N fertilizer loss (N fertilizer unaccounted for in plant and soil) from the N160 treatment (58–95 kg N ha^{-1}) was always the highest ($p < 0.05$) compared to other treatments (Fig. 4a and b). The proportion of N fertilizer loss was between 34 and 45%, 35% and 48%, and 36% and 59% in the N30, N78 and N160 treatments, respectively. Net soil N balance was negative in all the treatments at both locations (Fig. 4c and d). The N160 treatment always resulted in significantly more negative net soil N balance ($p < 0.05$) than the N0 and N30 treatments, regardless of the season and location.

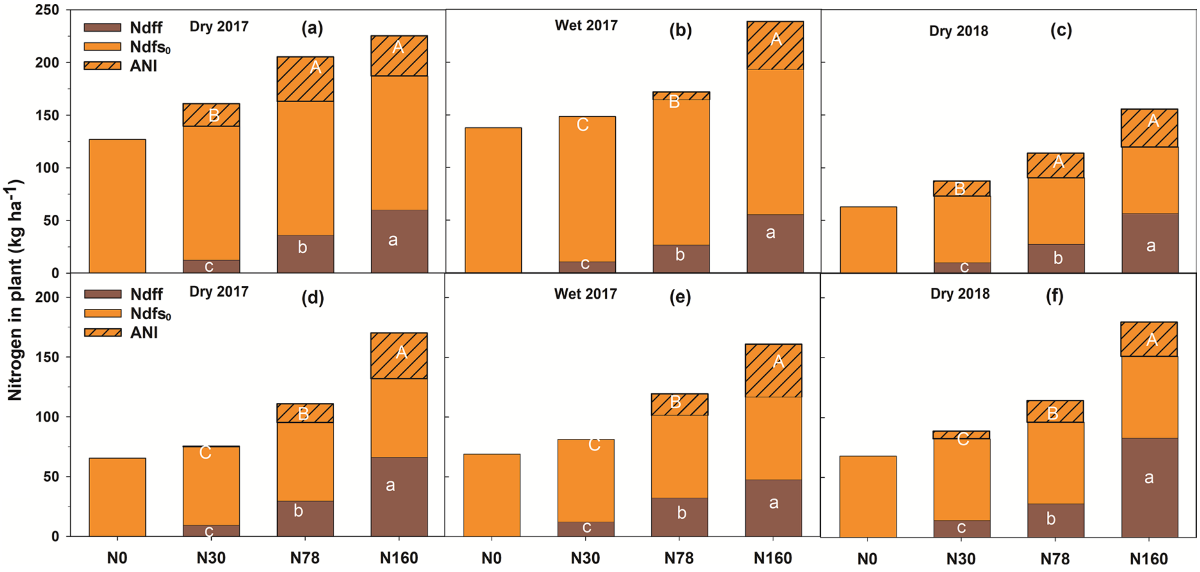

Fig. 2 Nitrogen content in plants (kg N ha^{-1}) at Yezin (a, b and c) and Taungoo (d, e, and f) in the Dry and Wet season of 2017 (Dry 2017 and Wet 2017) and Dry season of 2018 (Dry 2018). Each column bar for a treatment represents total N uptake (TNuptake) in plants. Ndff, N derived from fertilizer (kg N ha^{-1}) in plant; Ndfs$_0$, N derived from native soil N from zero N plots (kg N ha^{-1}) in plants; ANI, increase in native soil N uptake due to added N interaction (kg N ha^{-1}). Ndfs$_0$ plus ANI in each column bar represents total native soil N uptake (Ndfs) in plants. Different uppercase letters within a graph represent significantly different ($p<0.05$) ANI and different lowercase letters within a graph represent significantly different ($p<0.05$) Plant Ndff

Discussion

Excessive N input leads to high soil N uptake by rice with limited yield benefits

Rice grain yields and soil N uptake in plants at the Yezin site were higher than that at the Taungoo site, which is due to the higher soil fertility levels at Yezin compared to Taungoo (Table 1). We observed similar trends of higher soil N uptake in plants in the treatments receiving high N fertilizer input with limited yield benefit at both sites, which confirmed the occurrence of ANI. High N uptake by rice plants does not necessarily translate into increased grain yield because of the rice plant's physiological response leading to greater vegetative growth and smaller grain filling and grain yield (Ohnishi et al. 1999; Fageria and Baligar 2001; Zhong et al. 2017). This was apparent during the wet seasons, in particular, in this study where N uptake by rice was at similar levels in both seasons (Fig. 2), but the harvest index was always lower (<0.40) for the wet season compared to the dry season (>0.40) crops (Table S1). This point is further reinforced by the declining agronomic N use efficiency (kg grain yield increase per kg N applied) with N input above 30 kg ha^{-1} of the wet season rice at both sites (Table S2). In southeast Asia, there is lower direct beam solar radiation (solar radiation that reaches the Earth's surface without being diffused by clouds and other atmospheric components) during wet seasons due to more cloudy days compared to dry seasons, which reduces the grain to biomass ratio in rice, leading to lower grain yield in wet seasons than in dry seasons (van Der Gon et al. 2002; Dobermann et al. 2003). It should be noted that the rice cultivars used in this study are grown by local farmers due to marketability, so they may not be as responsive to N input as some high yielding varieties grown in other parts of the world.

Significantly higher ANI with increasing N fertilizer application rates observed in this study may be due to a combination of increased root growth enabling more exploration and uptake of N from soil (Ge et al. 2015; Xiao et al. 2019), and enhanced soil organic N mineralization, making more soil N available for plant uptake (Zhang et al. 2012a; Schleuss

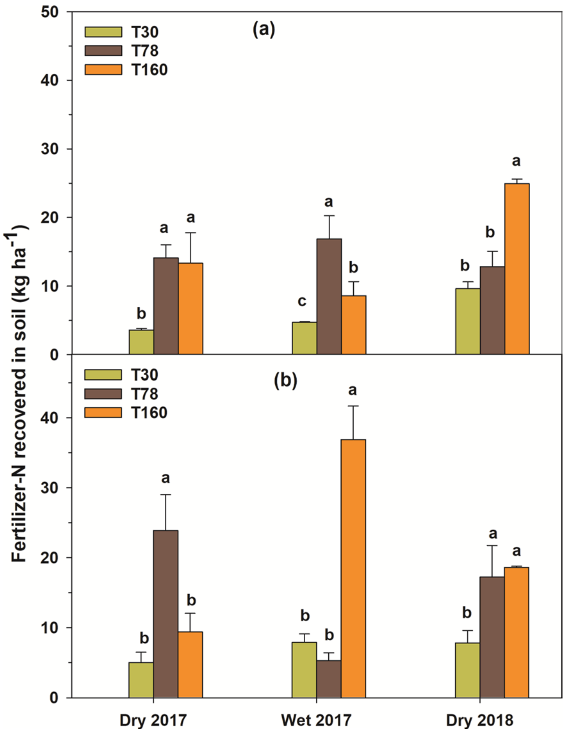

Fig. 3 Nitrogen fertilizer recovered in soil (kg N ha^{-1}) at Yezin (a) and Taungoo (b). N30, N78 and N160 represent an N addition of 30, 77.6 and 160 kg ha^{-1}, respectively. Column bars with different letters within a season are significantly different ($p<0.05$). Error bars represent ± 1 standard error of the mean

et al. 2019). Schleuss et al. (2021) observed 75% to 134% higher net N mineralization when N was added to a diverse range of soils. Although it has been demonstrated that organic N is chemically stabilized in phenolic lignin residue, which helps in maintaining soil N status in anaerobic paddy soils (Olk et al. 2006), microbial biomass N is released as NH_4^+ by soil microbes following fertilizer N addition to achieve stoichiometric C:N balance, thereby reducing microbial N use efficiency (Mooshammer et al. 2014) and potentially increasing soil N supply to crops. Also, due to continuous flooding and reducing conditions of paddy soils, the fixed N in clay minerals is released during the reduction of Fe(III) of Fe oxides on clay surfaces (Akter et al. 2018). Increased ANI with increasing rates of N input observed in our study shows that the N difference method, used by many studies to recommend site specific N management (Buresh et al. 2019; Wang et al. 2012), overestimates fertilizer-N recovery in plants and does not account for ANI.

High N fertilizer input results in large N loss and negative net soil N balance in sandy soils

Increasing N fertilizer inputs do not increase N retention in paddy soils (Wang et al. 2011; Zhong et al. 2017; Chen et al. 2021). Studies conducted in clay soils have shown greater amounts of fertilizer N recovery in soils with increasing amounts of N input (Zhao et al. 2009; Zhang et al. 2012b; Rose et al. 2016). However, such recovery is limited in sandy soils which are known to retain smaller amounts of N fertilizer compared to clay soils (Xia et al. 2018). This is due to the ability of clay minerals to fix NH_4^+ in clay-interlayers, which plays an important role in adsorption and release of N from fertilizer (Said-Pullicino et al. 2014). Nonetheless, N fertilizer retained in soil is generally not enough to compensate for native soil N uptake by rice plants. In addition, the N retained in soil from N fertilizer at harvest is subject to loss during the fallow season, and barely contributes N (<3%) to the following season crop (Phongpan and Mosier 2003).

The very high negative net soil N balances observed in this study, however, could be minimized by returning straw to the soil. We did not quantify N fertilizer recovered in rice roots but previous studies have demonstrated it is less than 3% of the applied N (Zhang et al. 2012b; Yao et al. 2018). We expect that the exclusion of N fertilizer recovered in roots will not change the N balance observed in our study significantly. Returning straw in combination with the fertilizer-N recovered in soil could balance the N removed from soil (Table S3). Farmers practice stubble burning to quickly prepare their fields for planting the next crop (Abdurrahman et al. 2020), even though recent studies have suggested that returning stubble in these low organic carbon soils could prove beneficial for soil fertility improvement and maintaining grain yields in the long-term (Xia et al. 2014; Zhang et al. 2021). Stubble retention can also improve N fertilizer conversion into soil organic N (Said-Pullicino et al. 2014), leading to improvement in soil N supply to following crops. Straw incorporation may lead to limited bioavailable N in the short-term because of the immobilization of N by microbes to achieve stoichiometric balance of C and N when there is excess C relative to

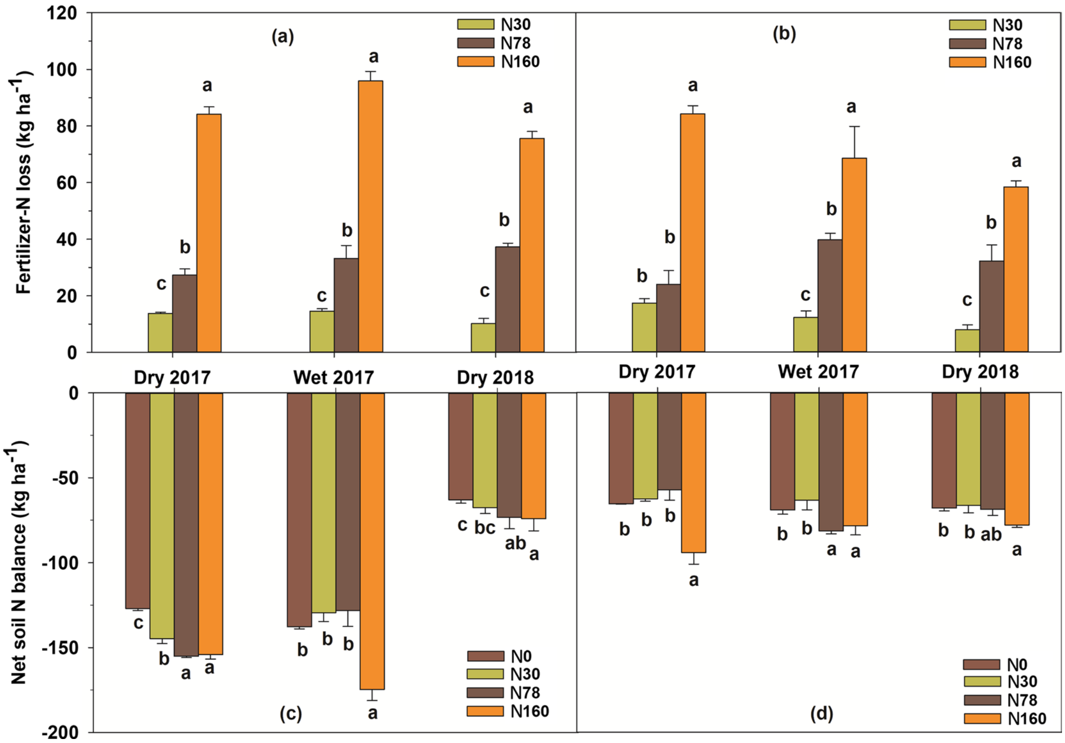

Fig. 4 Nitrogen fertilizer loss (kg N ha^{-1}) and net soil N balance (kg N ha^{-1}) at Yezin (a and c) and Taungoo (b and d). N30, N78 and N160 represent N addition of 30, 77.6 and 160 kg ha^{-1}, respectively. Column bars with different letters within a season are significantly different ($p < 0.05$). Error bars represent ± 1 standard error of the mean

N supply from straw (Shibahara et al. 1998). Unlike in upland soils where most of the microbially immobilized N after crop residue incorporation is released during the cropping season, most of the immobilized N is stabilized in soil organic N under the reducing soil conditions in paddy soils (Said-Pullicino et al. 2014). However, straw incorporation will benefit crops in the longer term as the microbially immobilized N undergoes mineralization, improving rice N uptake (Shibahara et al. 1998; Takahashi et al. 2003). It is clear from this study that N fertilizer input decisions should be made on the basis of the effects of N input on the native soil N reserve and the seasonal effect on grain yield to avoid high native soil N mining.

Perspectives

This study highlights the need for a paradigm shift from an emphasis on N fertilizer use efficiency to soil organic N enhancement-oriented research to minimize environmental risks of excessive use of N fertilizer. Higher soil N uptake by plants in high N input systems has consequences for soil fertility and grain yield over time. Cassman et al. (1995) reported a decline in rice grain yield over time in long-term field trials at the International Rice Research Institute, even though there was no apparent decline in total soil N content. This was suggested to be due to the declining soil N supply over time as a result of the reduced bioavailability of soil organic N in continuously flooded systems (Olk et al. 2006; Kögel-Knabner et al. 2010). The reduced soil N supply over time may also be due to the priming effect of N fertilizer on mineralizable soil organic N. In our study, the amount of N recovered in the plant from fertilizer (%Ndff in plant) rarely exceeded 40% and mostly remained below 30%. This is in agreement with the N uptake patterns of cereal crops, including rice, where almost 2/3 of the total plant N uptake is derived from native soil N regardless of the measurement techniques used, i.e. N difference method or ^{15}N tracing (Yan et al. 2020). It is clear from this and other studies

(Yan et al. 2020; Zhang et al. 2012b) that the majority of the N uptake in rice plants comes from sources other than fertilizer, so it is important to focus on replenishing soil organic N in intensive rice cropping systems to help sustained N supply to rice crops over time.

Conclusions

Our study shows that excessive N input to sandy paddy soils does not increase rice grain yield but it does increase native soil N uptake by plants, which confirms the occurrence of ANI. It is recommended to apply not more than 30 kg N ha^{-1} to wet season rice crops, and 78 kg N ha^{-1} in dry seasons, to rice paddies of central Myanmar with existing rice cultivars and management practices. A combination of increasing ANI with increasing N input rates, N export with straw removal and relatively low N fertilizer recovery in these sandy soils led to more negative net soil N balance across all the seasons and at both locations. Our findings show that the native soil N contributes to the majority of the rice plant N uptake, regardless of the rate of N fertilizer application, and therefore, plays an important role in the sustainability of rice crop production.

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Data Availability Not applicable.

Declarations

Conflict of interest The authors declare no conflict of interest.

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