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Identification of nitrification inhibition in soil by maize to mitigate nitrogen losses

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Abstract: Under aerobic or anaerobic conditions, oxidation and reduction processes of nitrogen dynamics in the soil produce N_2O emissions. To decrease N_2O emissions and conserve nitrogen (N), recent studies have focused on chemicals derived from root exudates that inhibit nitrification. However, selective plant breeding could be used to control nitrification activity in soil instead of fertilisers or synthetic nitrification inhibitors. In this study, we investigated the relationship between nitrification rates (NR) and related N dynamics and plant characteristics for 11 maize cultivars with varying levels of nitrification inhibition (NI) compared to *Brachicaria humidicola* (*Bh*) as the positive control. In a greenhouse experiment, soil concentrations of NI, NR, NH_4^+-N, and NO_3^--N and nitrogen uptake by plants were measured. Six maize cultivars had a 1.1–1.6 times lower NR than *Bh*. Low-NR cultivars had higher NI and lower root-to-shoot ratios. NI was positively correlated with total N and shot N content but not with cumulative N_2O and NH_4^+-NO_3^-. These results show that maize has the capacity to reduce soil nitrification while increasing the total N in the soil and shoot N content in maize.

Keywords: greenhouse gases; root-soil interaction; nitrate production; flux N_2O; climate change

Climate change, a significant global issue, is caused by the rising atmospheric emissions of greenhouse gases (GHGs). Nitrous oxide (N_2O) is a potent greenhouse gas that contributes significantly to the formation of stratospheric ozone holes (Ravishankara et al. 2009). Given the low efficacy of nitrogen fertilisation (< 50%), the soil is the principal source of anthropogenic N_2O emissions, which are primarily caused by nitrogen fertilisation (IPCC 2019).

Oxidation and reduction in the nitrogen cycle in the soil, both under aerobic and anaerobic conditions, produce N_2O. Nitrification is the primary source of N_2O emission under aerobic conditions and the primary source of NO_3^-. In addition, NO_3^- was the principal substrate for denitrification and the dominant N_2O source under limited or no oxygen conditions (anoxic) (Sorai et al. 2007). Therefore, the primary N_2O mitigation strategy should focus on controlling the nitrification process to lower N_2O production.

Soil nitrification is mainly controlled by the activity of nitrifiers, which are microorganisms that release specific enzymes, such as ammonium monooxygenase (AMO), hydroxylamine oxidoreductase (HAO), and nitrite oxidoreductase (NXR), to catalyse nitrification (Sorai et al. 2007). Many studies have proposed the mitigation of N_2O emissions by suppressing nitrification activity in the soil, for example, by ap-

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plying synthetic nitrification inhibitors (SNI) such as nitrapyrin, dicyandiamide (DCD), and 3,4-dimethylpyrazole phosphate (DMPP), which are expensive, difficult to apply, and dangerous to the environment (Subbarao et al. 2006a). However, some plants reduce nitrogen loss and nitrification-denitrification through biological nitrification inhibition (BNI), which is the process of releasing nitrification inhibitors (NI) from the roots (Subbarao et al. 2006a). The maturation zone of a root, which is the main site of allelochemical exudation, and root development both have an impact on the composition of root exudates, including BNI (Badri and Vivanco 2009).

To decrease N_2O emissions, recent studies have focused on the use of chemicals derived from root exudates that inhibit nitrification. Studies on BNI have increased with the development of bioluminescence assays that can identify and quantify BNI. The bioluminescence assay method used by Subbarao et al. (2006b) to discover BNI capability in *Brachicaria humidicola* (*Bh*) used recombinant *Nitrosomonas europaea* for nitrification detection and quantification. Subbarao et al. (2007) confirmed that the high-BNI genotype of *Bh* suppressed nitrification by more than 90% and maintained inorganic N in the NH_4^+ form after 30 days of incubation. BNI was first discovered in rice and wheat by Sun et al. (2016) and O'Sullivan et al. (2016), who demonstrated that BNI could reduce nitrification activity by 0–70% and > 40%, respectively. Additionally, a recent study by Otaka et al. (2021) discovered the BNI capacity of sweet corn for the first time, specifically zeanone from root exudates, and benzoxazinoid 2-hydroxy-4,7-dimethoxy-2H-1,4-benzoxazine-3 (4H)-one (HDMBOA) from the root surface and inside the root. In most terrestrial ecosystems, nitrification is primarily performed by ammonia-oxidising bacteria (AOB) and ammonia-oxidising archaea (AOA). BNI can reduce the abundance and activity of AOA and AOB (Lu et al. 2019). Moreover, plant roots naturally release BNI, making them inexpensive and environmentally friendly (Subbarao et al. 2006a).

The first study that focused on BNI in maize used only the maize cultivar *Zea mays* L. cv. Peter No. 610 (Subbarao et al. 2007) and Otaka et al. (2021) studied BNI capacity only in sweet corn (*Zea may* L. Honey bantam), a maize harvested at the milk stage. The nitrification inhibitor capacity of grain maize, maize harvested at the dent stage, has not been investigated. Grain maize is frequently used as a raw material in the feed industry and food products for human consumption. We hypothesised that grain maize has the capability to mitigate N_2O emission, such as naturally secreting compound(s) to manage soil N known as BNI. Therefore, this study aimed to assess the ability of several maize cultivars to inhibit nitrification. The maize cultivars used in this study were selected from those with different trends in N_2O emissions based on previous research. To assess the nitrification inhibitor ability in grain maize, we used *Bh* as a positive control, which showed the ability to suppress nitrification by up to 90%, with most inorganic soil N remaining in the form of NH_4^+ (substrate for nitrification) (Subbarao et al. 2006b, 2007).

MATERIAL AND METHODS

Experimental design. A greenhouse experiment was established in 2021 with 11 cultivars of maize: (1) Bisi 228, Bisi 79, and Bisi 99, obtained from Bisi International, co, Ltd., Indonesia; (2) NK 007 and NK 7202 were obtained from Syngenta, Ltd., Indonesia; and (3) Pertiwi 3, obtained from Agri Makmur Pertiwi, Ltd., Indonesia (3) Pioneer 35 and 36 were obtained from Dupont Indonesia, Ltd.; and (4) Anoman, Bisma, and Sukmaraga were obtained from the Cereal Plant Research Institution, Indonesia, and the high BNI *Brachicaria humidicola* cv. Tully (*Bh*), obtained from the Faculty of Husbandry, University Gadjah Mada, Indonesia, was used as a positive control. Three replicates were set up using a completely randomised design (CRD). This study used Cambisols soil collected from Sleman Regency, Special Region of Yogyakarta, Indonesia (7°44'47.9"S, 110°25'47.1"E). The soil type was identified with a pH_{H_2O} value of 5.94, total carbon of 3.72% using Walkley and Black method (Walkley and Black 1934), bulk density of 1.11 g/cm^3 (Kurnia et al. 2006), a cation exchange capacity (CEC) of 33.7 mmol$_+$/kg using ammonium acetate extraction method (Eviati 2009), available-P concentration of 9.83 mg/kg using Olsen method (Olsen and Sommers 1982), exchangeable K^+ concentration of 0.25 cmol$_+$/kg using ammonium acetate extraction method (Eviati 2009), NH_4^+-N concentration of 1.97 mg/kg, NO_3^--N concentration of 0.43 mg/kg using colourimetric method (Keeney and Nelson 1983, Kempers and Zweers 1986), and total inorganic-N of 0.16% using N-Kjeldahl method (Kjeldhal 1883). The soil parameters were analysed according to Eviati (2009). As a base fertiliser, potassium in the form of potassium chloride (KCl) and phosphorus in the form of triple superphosphate was treated at rates of 0.023 mg/kg

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(equal to 22.41 kg K^+ per ha) and 0.006 mg/kg (equal to 4.4 kg P per ha) of soil, respectively. At 7 and 35 days after germination (DAG), 0.017 mg N per kg of liquid ammonium sulphate [$(NH_4)_2SO_4$] (equal to 57.5 kg N per ha) was applied as the nitrogen source in two equal split doses.

Two seeds of each cultivar were planted in 7 kg of soil in pots, with a top diameter of 30 cm, a base diameter of 24 cm, and a height of 22 cm, of air-dried soil (ø 2 mm) for each experimental unit, and following germination, the number of seedlings was decreased to one per pot. The plant developed throughout the vegetative stages for approximately 56 days after germination. It was managed at field capacity by irrigation using tap water every two days during the first stage (around 21 DAG) and once a day during the following stage.

Gas sampling. Gas samples were collected 1, 4, and 7 days after fertilisation using a closed polyvinyl chloride (PVC) chamber with a 5 mm thick wall. During the sampling period, three different chambers of varying volumes were used: (1) 0.15 m diameter and 0.5 m height for the first 8 days after plant germination; (2) 0.2 m diameter and 1.5 m height for the following 11 and 14 DAG; and (3) 0.2 m diameter and 1.5 m height for the following 36, 40, and 43 DAG.

A thermometer was installed in the chamber to gauge the temperature, and a portable fan was added to mix the air-three sampling times at 10-min intervals from 7:00 a.m. to 11:00 a.m. comprised of one sample set. A polypropylene syringe (10 mL) was used for the collection and placed inside a vacuum tube (10 mL).

The N_2O concentration was measured using a gas chromatograph (Agilent Technology 7820A, Shanghai, China) equipped with an electron capture detector (ECD). Subsequently, the N_2O flux (F) was calculated using Eq. (1), as reported by Ussiri et al. (2009). Finally, cumulative N_2O emissions were calculated for each pot by linear interpolation of the concentration of N_2O during the sampling period of 7 days following fertilisation (Villegas et al. 2020).

$$\text{Flux } N_2O = \frac{\Delta C}{\Delta t} \times \frac{V}{A} \times \rho \times \frac{273}{273+T} \times \frac{28}{44} \times k \quad (1)$$

where: $\Delta C/\Delta t$ – average of the change in gas concentration within the chamber (mg/m^2/min); ρ – gas density; V – volume of the chamber (m^3); A – surface area around the chamber (m^2); T – temperature in the chamber (°C); 273 – constant to convert Celsius to Kelvin; 28/44 – ratio of molecular weight of N to N_2O; *k* – time of the conversion factor.

Soil sampling. Every week, topsoil (± 5 cm from the soil surface) was collected from each pot to assess the concentration of NH_4^+-N and NO_3^--N using the colourimetric measurement methods described by Keeney and Nelson (1983) and Kempers and Zweers (1986). First, 5 g of fresh soil was extracted with 50 mL of 1 mol/L KCl, shaken for 30 min, and filtered through Whatman filter paper Grade 2. Subsequently, the resulting extract was treated with Keeney and Nelson (1983) and Kempers and Zweers (1986) solutions. The concentration of NH_4^+-N and NO_3^--N was measured using a colourimetric method with a UV-VIS spectrophotometer (Shimadzu A-06-22, Kyoto, Japan) at wavelengths of 655 nm and 540 nm for NH_4^+-N and NO_3^--N, respectively. Following harvest at 56 DAG, the soil was collected to measure NH_4^+-N and NO_3^--N concentrations and total N (TN).

Plant sampling. The maize in each plot was harvested at 56 DAG to measure nitrogen uptake by plant tissues (shoots and roots). The plant was separated into root and shoot, dried for 7 days at 70 °C, and weighed as dry weight (DW). The dried samples were later used to measure the root-to-shoot ratio and nitrogen uptake by the shoots (shoot N content) and roots (root N content).

Nitrification rate and nitrification inhibition in soil. Following the microcosm incubation method described by Karwat et al. (2017), nitrification rates and nitrification inhibition in the soil of the 11 maize cultivars and *Bh* were measured at 56 DAG. A sieve (2 mm) was used to filter the soil, which was then air-dried for 48 h. The incubation period was set for 5 subsamples from each pot experiment (36 pots total); therefore, there were 180 units. A 50 mL amble flask containing 3 g of air-dried soil was treated with 27 mmol $(NH_4)_2SO_4$ and kept moist during incubation in a 60% water-filled pore space (WFPS). The soil was incubated for 8 days at 25 °C, and five different time points were used to extract the mineral N (before incubation and at 1, 2, 4, and 8 days after incubation). NH_4^+-N and NO_3^--N concentrations in the soil were measured following the colourimetric determination method reported by Keeney and Nelson (1983) and Kempers and Zweers (1986).

NR was calculated using the linear regression slope between the NO_3^- concentration and incubation time. Using Eq. (2) published by Bremner and McCarty (1989), nitrification inhibition was calculated based on the NO_3^- concentration in the soil of maize and *Bh*.

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$$\text{Nitrification inhibition} = \frac{(B-A)}{B} \times 100\% \quad (2)$$

where: B – concentration of NO_3^- (mg N per kg soil) produced in the $(NH_4)_2SO_4$ positive control (*Bh*) at days 0 and 1, 2, 4, and 8; A – concentration of NO_3^- (mg N per kg soil) produced in the soil of 11 maize cultivars at days 0 and 1, 2, 4, and 8.

Statistical analysis. The Windows version of the statistical software R (x64.4.1.3. Ink) (R Core Team 2022) was used for statistical analyses. One-way ANOVA and Tukey's test at a 5% confidence level were used to identify statistically significant differences among the maize cultivars. The correlations between nitrification (nitrification rate and nitrification inhibition), N dynamics (NH_4^+, NO_3^-, N_2O), and plant characteristics (shoot N content, root N content, plant N content, and root-to-shoot ratio) at 56 DAG were generated using linear regression analysis of R.

RESULT AND DISCUSSION

Nitrification rate of the soil. In this study, although the cultivar was the only source of variation within the population, we discovered a very high level of variation in the nitrate concentration (mg N per kg of soil) in the soil of 11 maize cultivars at 56 DAG, with a coefficient correlation (*CV*) in the range of 27–60% during the 8 days of incubation (Table 1). During the sampling period, the NO_3^- concentration in the soil increased over time in all soils under the different maize cultivars. Very high variability (*CV* = 63%; Table 1) was found among the maize cultivars for the soil nitrification rate, which varied from 0.44 mg to 2.05 mg NO_3^- per kg of soil per day (Figure 1). Cvs. Bisma, Anoman, Pioneer 35, Pioneer 36, Bisi 228, and Bisi 99 showed a 1.1–1.6 times lower NR than *Bh*, which indicated a higher capacity in reducing nitrification activity compared with that of *Bh*. Cv. Bisi 79 (17.61 mg NO_3^- per kg of soil per day) and cv. Sukmaraga (18.77 mg NO_3^- per kg of soil per day) showed a significantly higher NR than *Bh*, which indicated those cultivars had lower capacity in reducing nitrification in soil.

Bowatte et al. (2016) investigated the potential NR in the soil associated with 126 cultivars from 26 species, representing three functional groups used in temperate grassland management. They found variation in the NR among plant species and between cultivars related to the different root-zone soils, which provided breeding opportunities to accentuate differences in this trait that had not previously been selected. Moreover, Mwafuliwa et al. (2021) stated that various interactions between the soil and root system, including the effects of root diameter, biomass, length, and density on the com-

Table 1. Nitrate concentration in the soil of 11 maize cultivars 56 days after germination at 1, 2, 4, and 8 days of incubation using a microcosm incubation system

Cultivar	NO_3^- production in soil (mg N per kg of soil)				
	0 day	1 day	2 days	4 days	8 days
NK 7202	5.10 ± 0.08^{a}	6.78 ± 0.80^{a}	8.45 ± 0.65^{ab}	8.38 ± 0.27^{bcde}	14.08 ± 0.27^{b}
Bisi 228	2.46 ± 0.01^{bcde}	0.71 ± 0.15^{d}	6.23 ± 0.35^{bc}	6.39 ± 0.69^{def}	13.83 ± 0.52^{b}
Bisi 99	1.21 ± 0.01^{e}	6.05 ± 0.09^{ab}	5.97 ± 2.15^{bc}	5.56 ± 0.14^{ef}	14.66 ± 0.85^{b}
Anoman	3.64 ± 0.00^{ab}	0.88 ± 0.28^{d}	8.56 ± 0.62^{ab}	9.23 ± 0.13^{abcd}	12.19 ± 2.71^{bc}
Pertiwi 3	1.39 ± 0.01^{de}	5.23 ± 0.05^{ab}	10.22 ± 0.09^{a}	11.92 ± 0.31^{a}	14.23 ± 0.53^{b}
Pioneer 35	2.89 ± 0.03^{bcd}	1.04 ± 0.27^{d}	2.12 ± 0.47^{d}	1.70 ± 0.09^{g}	14.64 ± 0.91^{b}
Bisma	3.24 ± 0.06^{bc}	4.48 ± 0.68^{b}	8.61 ± 2.90^{ab}	10.27 ± 2.44^{ab}	20.51 ± 2.89^{a}
NK 007	2.31 ± 0.03^{bcde}	4.86 ± 1.26^{ab}	10.16 ± 0.07^{a}	11.22 ± 0.06^{ab}	13.43 ± 0.07^{b}
Sukmaraga	4.77 ± 0.12^{a}	4.16 ± 0.30^{bc}	5.40 ± 0.57^{bcd}	5.26 ± 1.02^{f}	7.47 ± 0.10^{d}
Pioneer 36	2.82 ± 0.07^{bcd}	1.99 ± 0.58^{cd}	3.11 ± 0.33^{cd}	9.60 ± 1.19^{abc}	15.17 ± 0.76^{b}
Bisi 79	2.11 ± 0.03^{cde}	5.42 ± 1.76^{ab}	7.33 ± 0.75^{ab}	6.82 ± 1.05^{cdef}	9.26 ± 1.34^{cd}
Control-Brachicaria	1.64 ± 0.02^{de}	1.85 ± 0.60^{d}	6.34 ± 0.00^{bc}	8.71 ± 1.50^{bcd}	8.21 ± 1.52^{cd}
CV (%)	44	60	37	37	27

Different letters indicate significant differences among the maize cultivars treated at $P < 0.05$, as determined by Tukey's test. *CV* is the coefficient of variability among maize cultivars for each parameter

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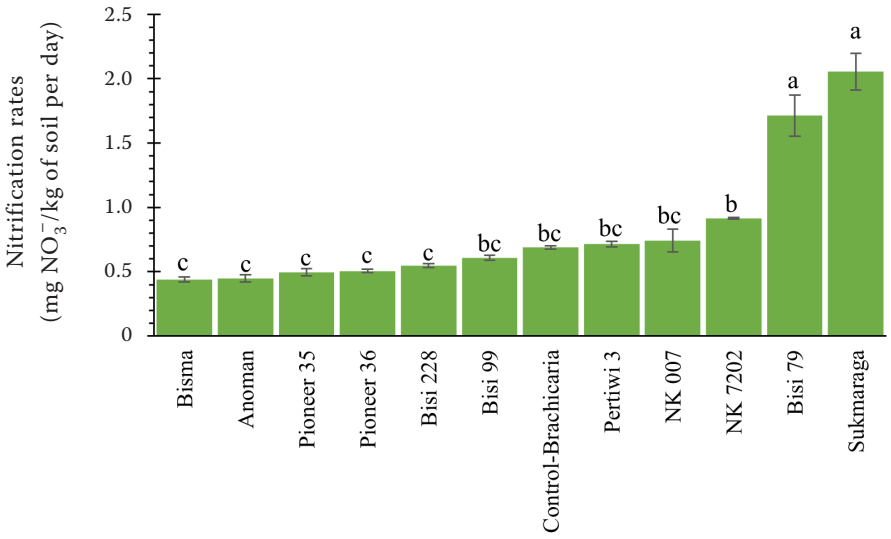

Figure 1. Nitrification rates in the soil of 11 maize cultivars 56 days after germination and planting in the greenhouse. Nitrification rates are represented as the slope of a linear regression between concentrations of NO_3^- overtime after eight days of incubation (n = 3). Different letters indicate significant differences among the maize cultivars treated at $P < 0.05$, as determined by Tukey's test

position and abundance of microbial communities, affect the amount and composition of rhizodeposits, which affect the soil rhizosphere zone (Mwafuliwa et al. 2021). Our result, in which we only measured root dry weight and calculated the root-to-shoot ratio, revealed that NR was positively correlated with the root-to-shoot ratio (r^2 = 0.2252, $P < 0.01$) (Figure 2A). According to Snyder et al. (2009), the growth of maize was the primary factor influencing the nitrification process in soil because soil N was one of the major nutrients absorbed by maize for growth. Plants naturally maintain nitrogen in the soil rhizosphere by producing substances through the root system to suppress nitrification, thereby increasing the availability of nitrogen and reducing nitrogen loss. This is referred to as BNI (Subbarao et al. 2006a). Our findings showed that NR and NI had a negative relationship (r^2 = 0.18, $P < 0.05$) (Figure 2A). According to Subbarao et al. (2007), BNI was a genetically controlled characteristic, and highly adapted accessions to low-N production environments demonstrated the highest BNI capacity, while those that were adapted to high-N input and intensive production systems demonstrated the lowest BNI capacity. Furthermore, *Bh* genotypes with high BNI produced three to five times as much BNI as those with low BNI, and high BNI genotypes had low nitrification and NO_3^- formation, with the majority of the mineralised N being in the form of NH_4^+.

Nitrification inhibition capacity. Biological nitrification inhibition can decrease nitrification by up to 70%, lowering nitrate loss through runoff and leaching, as well as nitrous oxide loss through the gas. However, BNI has not been demonstrated in all plants. The first BNI study by Subbarao et al. (2006) used a bioassay method. In contrast to the major crops grown worldwide – maize, wheat, and rice, sorghum could produce BNI. The discovery of BNI in rice, specifically 1,9-decane diol, by Sun et al. (2016) after exploring 19 cultivars, and the discovery of BNI in wheat by O'Sullivan et al. (2016) by exploring 96 landraces contributed to the advancement of research on BNI as a natural N_2O emission mitigation strategy, which is sustainable and environmentally friendly. In this study, the NO_3^- concentration in the soil of 11 maize cultivars and *Bh* (at 56 DAG) gradually increased over an 8-day incubation period. When NO_3^- concentrations in soil were compared between maize and *Bh*, we discovered that eight maize cultivars, including NK 7202, Bisi 228, Bisi 99, Anoman, Pioneer 35, Sukmaraga, Pioneer 36, and Bisi 79, had lower NO_3^- concentrations, ranging from 2% to 80% (Figure 3), indicating that they had a nitrification inhibitor ability that was approximately 2–80% higher than that of *Bh*. This finding was consistent with that of Otaka et al. (2021). The presence of N, especially NH_4^+, and the physiological effects associated with its uptake in the root environment appeared to play an important role in the synthesis and release of BNI-compounds from the root, according to Subbarao et al. (2007) who reported on the regulatory role of plants in the expression of BNI attribute in *Bh*. Our results demonstrated a positive correlation between NI and soil TN, indicating that higher soil TN was associated with higher NI in soil. Additionally, Subbarao et al. (2007) stated that the regulatory role of NH_4^+ in releasing BNI compounds seems to protect NH_4^+ from nitrifiers. These were in

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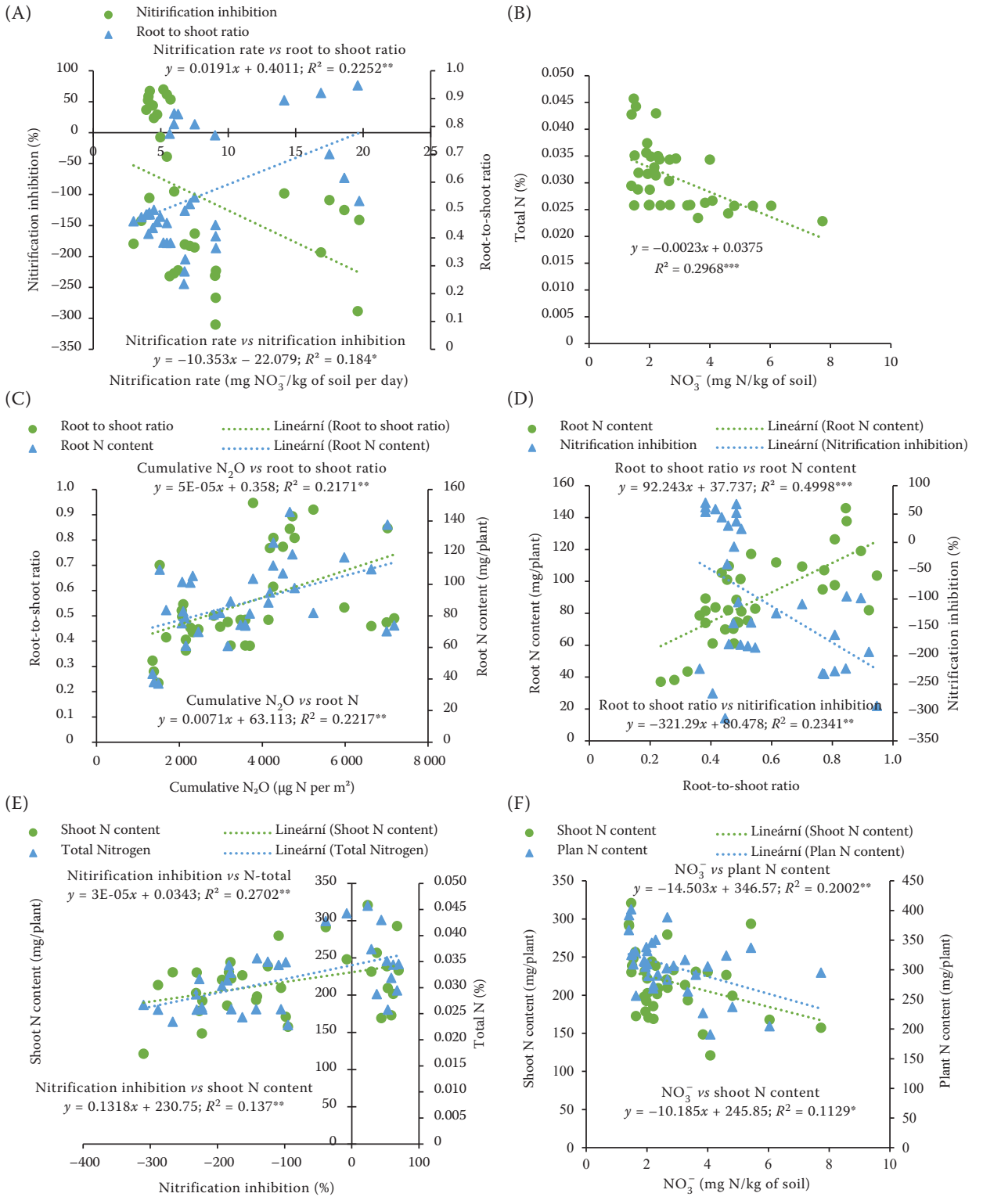

Figure 2. Significant ($P < 0.05$) relationship (A) between root-to-shoot ratio and root nitrogen (N) content and nitrification inhibition; (B) between nitrification rate and nitrification inhibition and root-to-shoot ratio between N-total and NO_3^-; (C) between cumulative N_2O emission and root N content and root-to-shoot ratio; (D) between root-to-shoot ratio and nitrification inhibition and root N content; (E) between nitrification inhibition and shoot N content and N-total, and (F) between NO_3^- and shoot N content and plant N content. Symbols represent different maize cultivars

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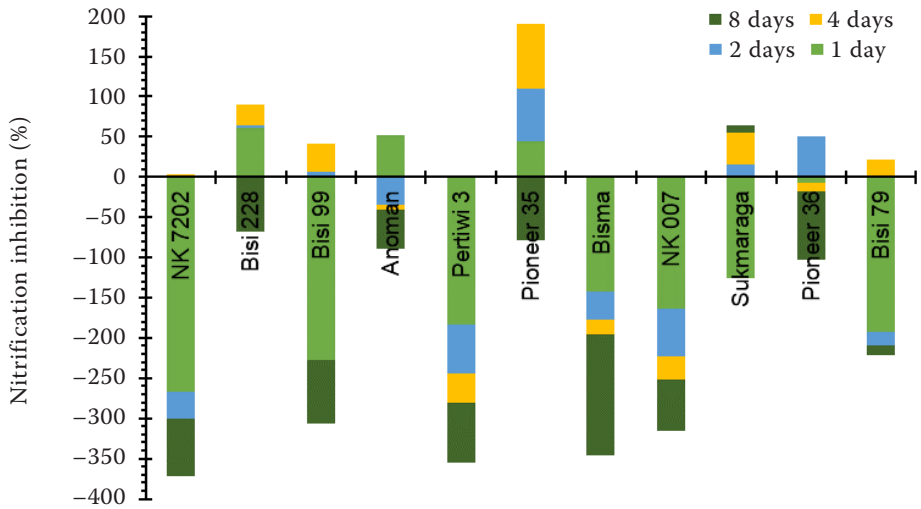

Figure 3. Inhibition of nitrification in the soil of 11 maize cultivars after 56 days of germination in a greenhouse. Nitrification inhibition based on the NO_3^- in soils during 8 days of incubation (n = 3)

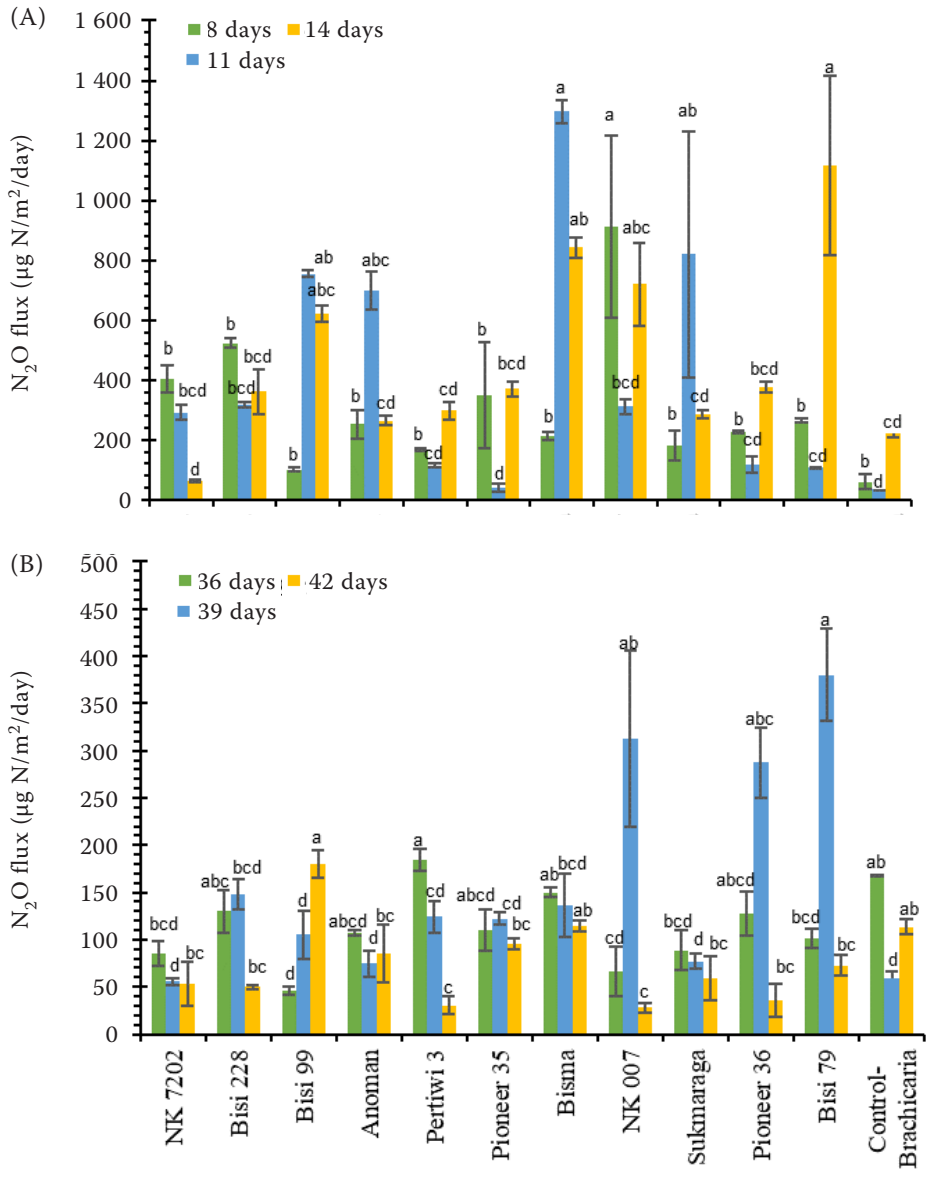

Figure 4. Nitrous oxide (N_2O) emissions from the soil of 11 maize cultivars at (A) 1, 4, and 7 days after the first fertilisation (days 8, 11, and 14) and (B) after the second fertilisation (days 36, 39, and 42). Different letters indicate significant differences among the maize cultivars treated at $P < 0.05$, as determined by Tukey's test

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line with our finding that TN negatively correlated with NO_3^--N (r^2 = 0.30, P < 0.001) (Figure 2B).

However, the inhibitory effect was only observed during the first four days of incubation, and only cvs. Pioneer 35 and Bisi 228 consistently demonstrated an inhibitory effect during this period. Cv. Pioneer 35 showed an increasing trend in NI with 8 days of incubation, and cv. Bisi 228 inversely showed a decreasing trend in NI with 8 days of incubation. Compared to *Bh*, cvs. Pertiwi 3, Bisma, and NK 007 did not perform NI over 8 days. According to Subbarao et al. (2007), a threshold level of BNI of approximately 5 Allylthiourea (AT) unit/g soil was required before the inhibitory effect took effect. Genotypes with high BNI could maintain inorganic N in the form of NH_4^+ longer than genotypes with low BNI, which contributed to reaching the threshold level much earlier than low BNI and influenced nitrification rates longer than low-BNI genotypes. Additionally, Lu et al. (2019) reported that the decreased inhibition rate and NH_4^+ immobilisation in the BNI 1,9-decanediol treatment in rice between days 7 and 14 suggested microbial degradation and uptake after 7 days of incubation. This loss of activity in the rice field might be recovered by releasing BNI 1,9-decanediol continuously or intermittently throughout the growing season. A better match for the dynamics of ammonia oxidisers and the NH_4^+ substrate itself might also be made by the spatiotemporal properties of BNIs due to their continuous release. Moreover, earlier research has shown that allelochemicals could release unexpectedly large amounts of soil-bound chemicals in the field (Lu et al. 2019).

Based on our results, the inhibition of maize nitrification was negatively associated with the nitrification rate (r^2 = 0.18, P < 0.05) (Figure 2A). Subbarao et al. (2007) reported that nitrification inhibitors by plants could slow down nitrification by up to 90%, as shown by the total nitrogen in the soil remaining as NH_4^+ during an incubation period of 30 days, whereas a higher nitrification rate accompanied lower nitrification inhibition. Villegas et al. (2020) also discovered that different NR accessions of *Megathyrsus maximus* statistically performed similar total BNI potentials with low variability for specific BNI. They assumed that the variability in root biomass was the reason for the differences in the total nitrification inhibition potential. Our findings also show that nitrification inhibition increased as the root-to-shoot ratio increased (r^2 = 0.2341, P < 0.01) (Figure 2D). Similarly, Bowatte et al. (2016) demonstrated the release of nitrification-inhibiting compounds to increase with root mass, particularly in the presence of ammonium.

N dynamics: ammonium, nitrate, and nitrous oxide. There was significant variation (P < 0.0001) between maize cultivars in N_2O emissions, with a *CV* of approximately 36–97% (Figure 4). During the sampling period, different trends were observed for the 11 maize cultivars, and emissions peaked at different sampling times. N_2O emissions varied more after the first fertilisation than after the second fertilisation, and the degree of variation varied with the growth stages of maize and *Bh*. Wang et al. (2019) demonstrated that soil N_2O emissions from maize fields with various nitrogen treatments peaked after fertilisation and demonstrated varying degrees of change as plants became older and began to decline, as well as varying degrees of impact of maize growth on N_2O emissions with plant growth. In this study, the soil of 11 maize cultivars emitted 39.42–1 296.23 μg N per m^2 per day, which was higher N_2O relative to *Bh* (37.15–220.26 μg N per m^2 per day) (Figure 4). Four days after first fertilisation (49 DAG), cv. Bisma peaked at 1 296.23 μg N per m^2 per day, which was 35 times higher than *Bh*, representing the highest N_2O, and *Bh* (37.15 μg N per m^2 per day) had the lowest N_2O emission during sampling time (Figure 4). *In situ* nitrification rates and emissions of soil-derived N_2O during the early growing season were likely to have been stimulated briefly by N fertiliser application. This was demonstrated by a rise in soil NO_3^- concentrations and an increase in nitrification potential, which was probably caused by a rise in the production of the enzyme ammonia monooxygenase (which we did not measure) in response to the high soil NH_4^+ concentrations after fertilisation (Lam et al. 2017). Considering the whole sampling period, the cumulative N_2O emission of each maize cultivar was 1.5–5 times higher than that of *Bh* (2 070–6 939 μg N per m^2 *vs.* 1 409 μg N per m^2) (Figure 5). Bisma (6 939.27 μg N per m^2) presented the highest cumulative N_2O emission, and cv. Pertiwi 3 (2 070.93 μg N per m^2) presented the lowest. Although some maize cultivars had lower nitrification rates than *Bh*, all maize cultivars significantly outperformed *Bh* in terms of cumulative N_2O, indicating that *Bh* is the strongest suppressor of N_2O. These findings agreed with earlier research. Subbarao et al. (2007) reported that *Bh* had the capacity to suppress NO_2^- production by up to 90%, and Otaka et al. (2021) demonstrated that maize could suppress only approximately 45%.

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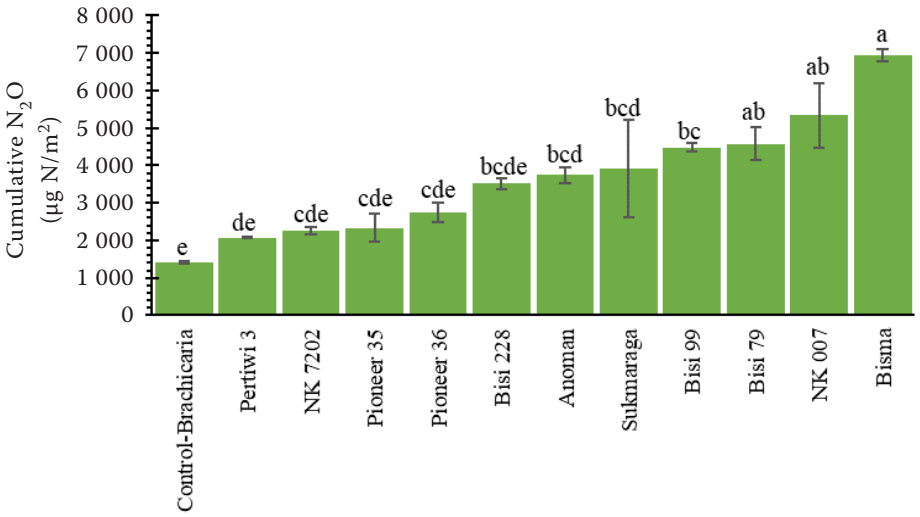

Figure 5. Cumulative nitrous oxide (N_2O) from the soil of 11 maize cultivars during a sampling period of seven days following fertilisation. Different letters indicate significant differences among the maize cultivars treated at $P < 0.05$, as determined by Tukey's test

This might be attributed to the different soil-root interactions that arise from the different strategies those plants use to absorb, recover, and use their nitrogen. It was in line with a previous investigation (Subbarao et al. 2006a), which discovered that the potential for soil nitrification varied significantly between ecological systems and that some plants could influence soil nitrification by releasing BNI activity from their roots.

Maize cultivars significantly influenced the availability of NH_4^+ and NO_3^- in the soil during the vegetative stages (56 DAG), with high variation during the sampling period ($P < 0.001$) (Figure 6). NH_4^+ strongly increased 7 days after the first fertilisation by 15.91–104.99 mg N per kg of soil and further sharply decreased by 20.29–99.32 mg N per kg of soil (56–95% decrease) in the following week, then continuously decreased until second fertilisation (35 DAG) (Figure 6A). This was accompanied by the decreasing trend of NO_3^- in the soil of maize and *Bh* at 7 days after fertilisation by 1.05–8.45 mg N per kg of soil (6.6–55%), except cvs. Bisi 228, Pioneer 35, and NK 007 which showed a significant increase of NO_3^- by 28.08, 7.90, and 13.34 mg N per kg of soil, respectively (Figure 6B). NO_3^- in soil sharply increased in the range of 1.09–41.97 mg N per kg of soil during the following week (21 DAG) and further strongly decreased by 13.23–58.88 mg N per kg of soil (40–99% decrease) at 28 DAG, whereas *Bh* showed the lowest decrease rate of NO_3^- (40%). Marsden et al. (2016) reported that the ammonium concentration in the soil significantly decreased under favourable conditions, such as 60% WFPS, where nitrification peaked. This encouraged nitrate production to rise quickly. Nitrification generated N_2O emissions as a by-product, in addition to NO_3^- as a substrate for denitrification.

Seven days after the second fertilisation, NH_4^+ in the soil slightly increased by 1.91–18.31 mg N per kg of soil, then continuously decreased in the following time, and it was slightly accompanied by an increasing trend of NO_3^- by 0.28–6.87 mg N per kg of soil. Wang et al. (2019) demonstrated that differences in NH_4^+ and NO_3^- revealed the degree of distinction between growth stages and among maize cultivars. The amount of nitrogen required by maise cultivars at different developmental stages may affect nitrogen absorption efficiency. This has an indirect impact on NH_4^+ and NO_3^- as well as on the release of N_2O from the soil. In addition, maize and *Bh* had different NO_3^- at 28 DAG, with maize exhibiting a rapid reduction in NO_3^- loss in soil (71–99%) after fertilisation and *Bh* exhibiting a considerably lower decrease in soil NO_3^- loss (40%). Crop variability has a varied impact on crop residue production, particularly in the rhizosphere, which has different effects on the interaction among soil, plant roots, and microbes, such as nitrifiers, thereby influencing nitrification (Snyder et al. 2009). This has an indirect impact on the form of N in the environment as well as the absorption, utilisation, and dispersion of N for plant productivity and environmental quality (Subbarao et al. 2006a). Furthermore, plant nutrient absorption capacity varies across growth stages, caused by differences in root morphology and mechanical properties, resulting in differences in root lodging, which directly affects soil NH_4^+ and NO_3^- concentrations (Wang et al. 2019).

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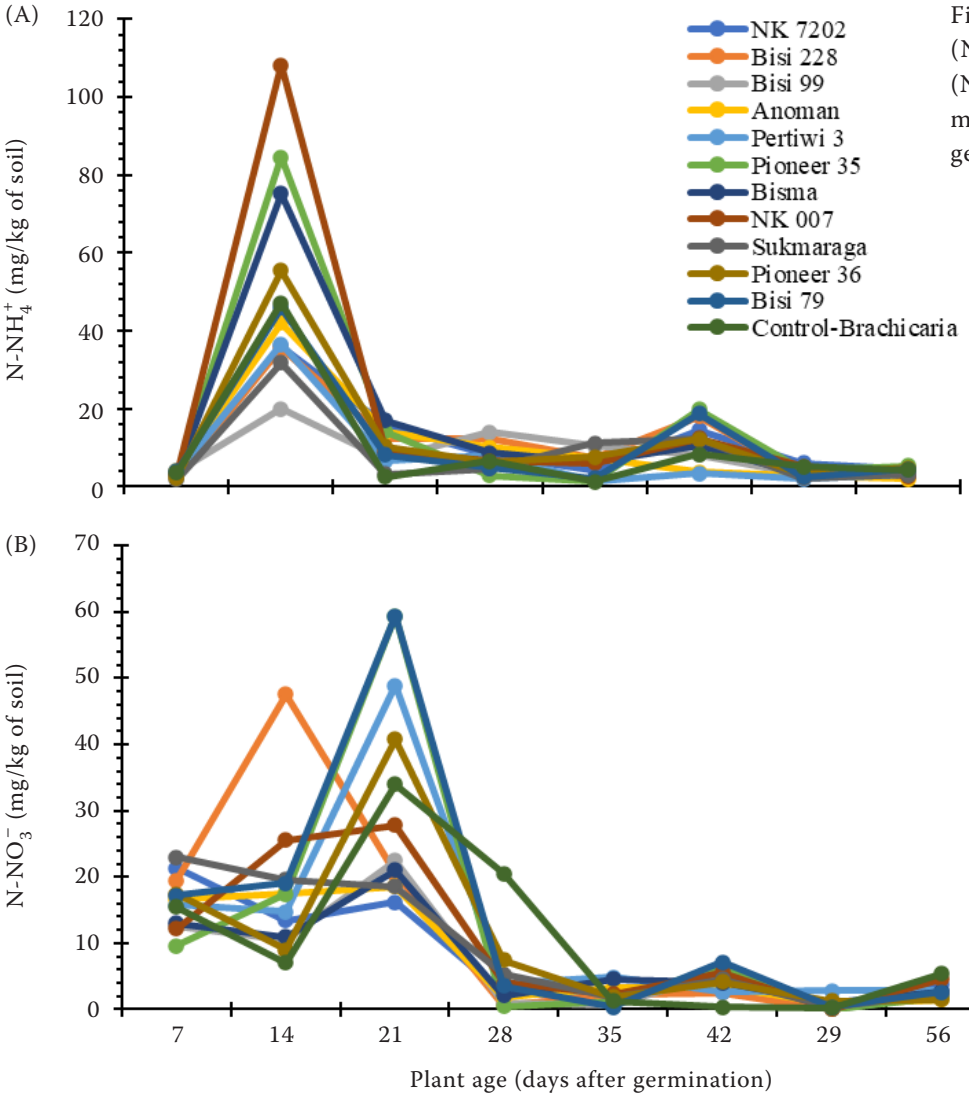

Figure 6. (A) Ammonium (NH_4^+-N) and (B) nitrate (NO_3^--N) in the soil of 11 maize cultivars 56 days after germination

In this study, NO_3^- negatively affected total nitrogen in the soil at 56 DAG ($r^2 = 0.2002$, $P < 0.01$) (Figure 2B), whereas cvs. Pioneer 35 and Pioneer 36 had the highest NH_4^+ and lowest NO_3^- in the soil, respectively, and both displayed the highest levels of total nitrogen in the soil at approximately 0.04% N and 0.05% N at 56 DAG (Figure 7). This indicates that cvs. Pioneers 35 and 36 were better able to manage nitrogen in the soil. Additionally, total nitrogen was positively correlated with nitrification inhibition at 56 DAG ($r^2 = 0.2702$, $P < 0.01$) (Figure 2E), which is in line with a study by Nan et al. (2016) in which the primary factors affecting soil nitrification, which in turn were the primary factors affecting soil N_2O emissions, were determined by the nitrogen content in the soil.

Relationship between nitrification and nitrogen dynamic and nitrogen uptake by the plant. In the present study, there was significant variation ($P < 0.05$) in plant N content and root-to-shoot ratio among the maize cultivars measured at 56 DAG (Table 2). Shoot N content and plant N content tissue showed moderate variability among the maize cultivars with a variation coefficient of 15% and 12%, respectively, whereas shoot N content ranged from 166.82 to 286.66 mg per plant and plant N content ranged from 236.64 to 367.68 mg per plant for plant N content. Root N content performed high variability (*CV* = 26%) varying from 69.82 to 126.37 mg per plant, and very high *CV* was performed by root-to-shoot ratio (36%) ranging from 0.38 to 0.92 mg per plant. This indicates that the maize cultivars had a greater impact on the roots than on the shoots. *Bh* performed 1.4–3.3 times worse in root-to-shoot ratio than maize, which could be attributed to a significant difference in the proportion of fine roots. Based on the study by Redin et al. (2018), the proportion of

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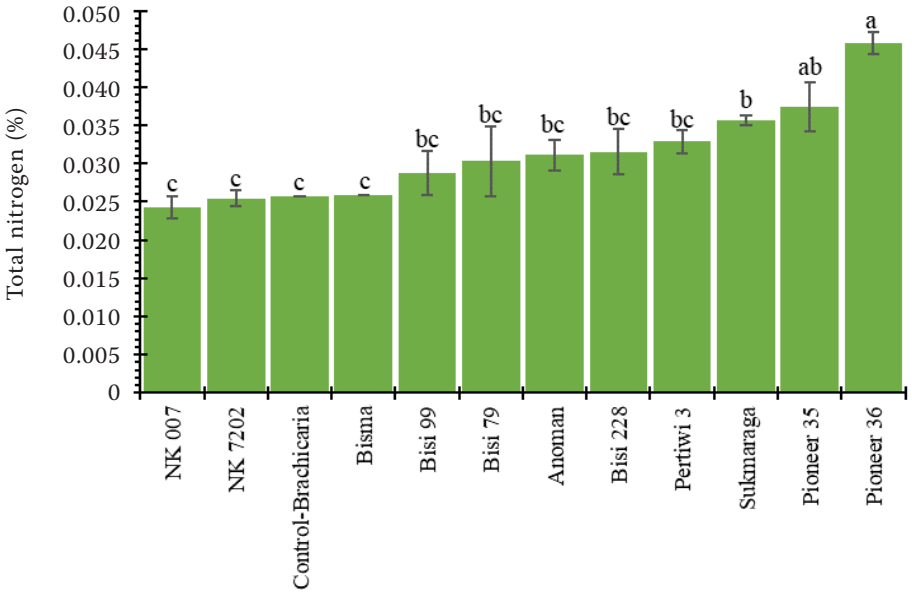

Figure 7. Total nitrogen (TN) in the soil of 11 maize cultivars during the vegetative stage (56 days after germination). Different letters indicate significant differences among the maize cultivars treated at $P < 0.05$, as determined by Tukey's test

fine roots varied greatly among plants, with grasses having primarily fine roots and non-grasses having a root system composed of approximately 60% of root dry matter coarse root (ø ≥ 2 mm). Exceptions among the grass species studied were maize, sorghum, and millet, which have root systems composed of approximately 45% coarse roots. In addition, they noted that the ratio of roots to shoots varies across families, species, and phenological stages. In contrast, the different distribution of biomass from the roots to the shoots reveals the resource-acquisition strategy of plants. The wide range in the C:N ratio of the roots is explained by differences in the N content of the root tissue. A very different plant strategy regarding plant N allocation and its ultimate fate was implied by variation in shoot N among families and species.

In this study, nitrification showed a relationship between soil N dynamic and plant N content. There was a significant correlation ($r^2 = 0.18$, $P < 0.05$) between the decline of NI and an increase of NR (Figure 2A),

Table 2. Root-to-shoot ratio, shoot nitrogen (N) content, root N content, and plant N content of 11 maize cultivars 56 days after germination

Cultivar	Root-to-shoot ratio	Root N content	Shoot N content	Plant N content
		(mg/plant)		
NK 7202	0.41 ± 0.03^{e}	69.82 ± 5.04^{bc}	166.82 ± 32.89^{b}	236.64 ± 29.47^{b}
Bisi 228	0.38 ± 0.00^{ef}	81.52 ± 4.40^{bc}	214.62 ± 9.49^{ab}	296.14 ± 5.41^{ab}
Bisi 99	0.81 ± 0.02^{b}	126.37 ± 11.22^{a}	191.23 ± 6.97^{ab}	317.60 ± 10.38^{ab}
Anoman	0.48 ± 0.00^{de}	79.09 ± 4.67^{bc}	262.66 ± 15.94^{ab}	341.74 ± 12.78^{ab}
Pertiwi 3	0.52 ± 0.01^{bc}	86.53 ± 7.75^{ab}	220.16 ± 17.65^{ab}	306.68 ± 22.26^{ab}
Pioneer 35	0.44 ± 0.01^{de}	90.29 ± 7.52^{ab}	191.09 ± 20.24^{ab}	281.38 ± 16.83^{ab}
Bisma	0.47 ± 0.01^{de}	84.59 ± 12.53^{ab}	208.35 ± 8.34^{ab}	292.94 ± 20.20^{ab}
NK 007	0.81 ± 0.02^{b}	110.06 ± 13.83^{ab}	204.74 ± 23.70^{ab}	314.80 ± 9.87^{ab}
Sukmaraga	0.62 ± 0.05^{c}	112.72 ± 2.32^{ab}	238.74 ± 23.53^{ab}	351.46 ± 21.26^{a}
Pioneer 36	0.48 ± 0.01^{de}	81.01 ± 11.56^{bc}	286.66 ± 21.12^{a}	367.68 ± 29.44^{a}
Bisi 79	0.92 ± 0.02^{a}	101.47 ± 10.75^{ab}	201.53 ± 15.55^{ab}	303.00 ± 7.87^{ab}
Control-Brachicaria	0.28 ± 0.02^{f}	39.54 ± 1.94^{c}	220.21 ± 37.81^{ab}	259.75 ± 39.74^{ab}
CV (%)	36	26	15	12

Different letters indicate significant differences among the maize cultivars treated at $P < 0.05$, as determined by Tukey's test. *CV* is the coefficient of variability among maize cultivars for each parameter

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which was attributed to the high NO_3^- formation. The relatively immobile NH_4^+ was converted to highly mobile NO_3^- during nitrification with N_2O as a by-product, which had a great influence on plant N utilisation (Subbarao et al. 2006a). Our result showed that the shoot N had a positive correlation with nitrification inhibition (r^2 = 0.137, P < 0.01) (Figure 2E) and a negative correlation with NO_3^- (r^2 = 0.113, P < 0.01) (Figure 2F). The primary form of N uptake for many plants is the NO_3^- form, despite its high susceptibility to loss from the root zone due to leaching and/or denitrification (Subbarao et al. 2006a). However, it is also highly available to plants. This result was consistent with our observation that lower NI in soil was accompanied by low TN. Additionally, NI had a bad correlation with root-to-shoot (r^2 = 0.2341, P < 0.01), whereas the root-to-shoot ratio had a good correlation with the root N content (r^2 = 0.4998, P < 0.0001) (Figure 2D) and cumulative N_2O (r^2 = 0.2171, P < 0.001) (Figure 2C). The growth of maize is an essential factor for the nitrification process in soil, considering the large amount of N in soil absorbed by plants during the planting season; therefore, maize potentially controls soil N_2O emission levels (Snyder et al. 2009, Wang et al. 2019).

The relationship between resource supply and demand change might affect shoot and root growth. Mašková and Herben (2018) demonstrated that plants encourage tissue growth in areas where energy costs yield the greatest functional benefits. Under unfavourable conditions, plants are predicted to shift resource allocation toward root growth and nutrient capture rather than carbon fixation. These processes affect the root-to-shoot ratios, which could potentially explain some of the variations in the root-to-shoot ratios.

In conclusion, maize cultivars had diverse effects on nitrification, N dynamics, and plant characteristics (i.e., root-to-shoot ratio, shoot N, root N, and plant N content) during the vegetative stages (56 DAG). This study is the first to show that grain maize can inhibit nitrification, and it found that some maize cultivars (i.e., Bisi 228, Bisi 99, Pioneer 35, Pioneer 36, Anoman, and Bisma) had 1.1–1.6 times lower NR compared to that of *Bh*, and had lower NO_3^- formation ranging from 2% to 80%. The inhibitory effect varied depending on the maize cultivars and incubation times. This might demonstrate that there was a threshold BNI amount for grain maize to inhibit nitrification of grain maize relative to *Bh*. Root-to-shoot ratio and root N accumulation decreased with the decrease in NR and increased in NI, in contrast to soil TN and shoot N accumulation, which tended to increase. These comparisons revealed that maize plants preferred greater growing and N accumulation in above-ground biomass under high TN, which is attributed to high NI and low NR.

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