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Brachiaria humidicola Cultivation Enhances Soil Nitrous Oxide Emissions from Tropical Grassland by Promoting the Denitrification Potential: A ^{15}N Tracing Study

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Abstract: Biological nitrification inhibition (BNI) in the tropical grass *Brachiaria humidicola* could reduce net nitrification rates and nitrous oxide (N_2O) emissions in soil. To determine the effect on gross nitrogen (N) transformation processes and N_2O emissions, an incubation experiment was carried out using ^{15}N tracing of soil samples collected following 2 years of cultivation with high-BNI *Brachiaria* and native non-BNI grass *Eremochloa ophiuroide*. *Brachiaria* enhanced the soil ammonium (NH_4^+) supply by increasing gross mineralization of recalcitrant organic N and the net release of soil-adsorbed NH_4^+, while reducing the NH_4^+ immobilization rate. Compared with *Eremochloa*, *Brachiaria* decreased soil gross nitrification by 37.5% and N_2O production via autotrophic nitrification by 14.7%. In contrast, *Brachiaria* cultivation significantly increased soil N_2O emissions from 90.42 μg N_2O-N kg^{-1} under *Eremochloa* cultivation to 144.31 μg N_2O-N kg^{-1} during the 16-day incubation ($p < 0.05$). This was primarily due to a 59.6% increase in N_2O production during denitrification via enhanced soil organic C, notably labile organic C, which exceeded the mitigated N_2O production rate during nitrification. The contribution of denitrification to emitted N_2O also increased from 9.7% under *Eremochloa* cultivation to 47.1% in the *Brachiaria* soil. These findings confirmed that *Brachiaria* reduces soil gross nitrification and N_2O production via autotrophic nitrification while efficiently stimulating denitrification, thereby increasing soil N_2O emissions.

Keywords: biological nitrification inhibition; *Brachiaria humidicola*; N_2O emissions; gross N transformation processes; denitrification

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1. Introduction

Nitrous oxide (N_2O) concentrations in the atmosphere have increased by more than 20% since pre-industrial times and are responsible for 6% of current global warming [1]. N_2O emissions are also an important factor in stratospheric ozone depletion [2], with agricultural soil accounting for approximately 66% of global anthropogenic N_2O emissions, mainly due to the excessive input of synthetic N fertilizers [3,4]. The increasing use of synthetic fertilizers is also causing increased nitrifier activity, transforming modern agricultural systems into high-nitrifying environments [5,6].

Ammonia oxidation is the rate-limiting first step of nitrification, producing N_2O as

a by-product [7]. Biological nitrification inhibition (BNI) is a rhizospheric process whereby specific inhibitors exudated or released from the plant's roots suppress the activity of nitrifying bacteria [8]. This process is widely found in major crops, such as sorghum [9], rice [10], wheat [11,12] and maize [13], as well as in certain forage species [14] and trees [15]. *Brachiaria humidicola*, a tropical grass native to East and Southeast Africa, has a strong BNI capacity due to the release of the specific compound brachialactone in its root exudates [14,16]. Previous studies have shown that soil collected from established *Brachiaria* plots shows a remarkable decrease in the net nitrification rate during incubation compared with soil cultivated with non-BNI plants [16–19]. Meanwhile, Subbarao et al. [14] found that both the soil ammonia oxidation rates and cumulative N_2O emissions were reduced by almost 90% after *Brachiaria* pasture planting compared with soybean or plant-free plots during a three-year field experiment in Colombia. However, in contrast, Vazquez et al. [20] found no apparent differences in the gross nitrification rates in the soil in which different *Brachiaria* genotypes with differing BNI capacities were grown.

N_2O is produced by a number of simultaneous N transformation processes [21]. Denitrification produces N_2O as an intermediate product during the reduction in nitrate (NO_3^-) to N_2 and is considered a much more potent source of N_2O than nitrification in grassland soil [22]. However, it remains unclear whether cultivation of exotic *Brachiaria* in tropical pastures results in a reduction in soil denitrification potential and N_2O emissions due to the decrease in supply of NO_3^- substrates for denitrifiers. In this study, we therefore established an incubation experiment using a ^{15}N tracing technique with soil samples collected from an experimental field cultivated with *Brachiaria* and the native grass *Eremochloa ophiuroide*, which has no BNI capacity. The objectives were to: (1) determine the effect of *Brachiaria* on soil N transformation rates in terms of gross nitrification and denitrification rates; and (2) understand how cultivation of *Brachiaria* affects soil N_2O emissions. We hypothesized that *Brachiaria* cultivation would reduce nitrification by releasing biological nitrification inhibitors, thereby reducing the availability of NO_3^- for denitrification and together with nitrification, decreasing soil N_2O emissions.

2. Materials and Methods

2.1. Field Experiment and Soil Sampling

The field experiment was established in Danzhou, Hainan Province, China (109°29′ E, 19°30′ N), in August 2015. The area has a tropical monsoon climate, with an annual mean air temperature of 23.1 °C and annual precipitation of 1823 mm. The soil was developed from granite and classified as Latosol according to the US soil taxonomy. The field experiment involved eight treatments consisting of two forage grasses and four N application rates, with three replicates each. The two forage species were the introduced exotic grass *Brachiaria humidicola* CIAT679, which has a high-BNI capacity [14], and the native tropical grass *Eremochloa ophiuroide*, which has no BNI capacity. The four N application rates were 0, 150, 300 and 450 kg N ha^{-1} $year^{-1}$. Plot size was 4 × 3 m and all plots were arranged according to a randomized block design. N fertilizer urea was surface applied prior to irrigation. In the first growing season, 60% urea was applied in August 2015 as a basal fertilizer, with the remaining 40% applied in April 2016 as a top-dressing. In the second growing season, 40% urea was applied in August 2016 as a basal fertilizer, while 30% was applied in March and 30% in June 2017 as a top-dressing. The grasses were harvested using a lawnmower one day before each top-dressing. Phosphorus and potassium application rates were 120 kg P_2O_5 ha^{-1} $year^{-1}$ (calcium superphosphate) and 120 kg K_2O ha^{-1} $year^{-1}$ (potassium sulfate), respectively, with both applied annually as a basal fertilizer in August.

In March 2017, approximately 2 years after establishment of the field experiment and 6 months after the last fertilization, surface soil (0–20 cm) was collected from 10

different positions in each *Brachiaria* and *Eremochloa* plot treated with 150 kg N ha^{-1} $year^{-1}$. The samples were then pooled to form a composite sample for each treatment. After removal of visible roots and litter, the fresh soil was sieved through a 2 mm mesh then divided into two subsamples, one of which was stored at 4 °C for incubation and the other which was air-dried for further analysis. Soil pH was measured in a 1:2.5 soil:water sample ratio using a DMP-2 mV/pH detector (Quark Ltd., Nanjing, China). Soil organic C (SOC) and total N (TN) were determined by wet-digestion with H_2SO_4-$K_2Cr_2O_7$ and on a CN analyzer (Vario Max CN, Elementar, Hanau, Germany), respectively, while NH_4^+ and NO_3^- were extracted using 2 M potassium chloride (KCl) at a 1:5 soil:solution ratio then analyzed using a continuous-flow autoanalyzer (Skalar, Breda, The Netherlands). Dissolved organic carbon (DOC) was extracted using deionized water at a soil:water ratio of 1:5 with shaking for 0.5 h then analyzed using a TOC analyzer (Vario TOC cube, Elementar, Hanau, Germany). Soil available K^+ was extracted with ammonium acetate and analyzed using a flame photometer (FP640, INASA, China). Soil properties are presented in Table 1.

Table 1. Properties of the test soils after approximately 2 years of cultivation with *Brachiaria* and *Eremochloa*.

	pH	TN ($g\ N\ kg^{-1}$)	SOC ($g\ C\ kg^{-1}$)	NH_4^+-N ($mg\ N\ kg^{-1}$)	NO_3^--N ($mg\ N\ kg^{-1}$)	DOC ($mg\ C\ kg^{-1}$)	Available K^+ ($mg\ K\ kg^{-1}$)
Brachiaria soil	6.40 ± 0.30a	0.55 ± 0.02a	7.32 ± 0.10a	4.24 ± 0.11a	6.57 ± 0.10a	29.79 ± 0.90a	153.13 ± 10.67a
Eremochloa soil	6.10 ± 0.1a	0.54 ± 0.01a	7.01 ± 0.09b	4.38 ± 0.13a	3.70 ± 0.11b	22.64 ± 1.01b	44.28 ± 1.18b

[1.] Values represent means ± standard errors ($n = 3$). Values within the same column with different lowercase letters represent a significant difference at $p < 0.05$. [2.] TN: total N, SOC: soil organic C, DOC: dissolved organic C.

2.2. ^{15}N Tracing Experiment

The soil incubation experiments consisted of two NH_4NO_3 treatments with three repetitions each, with labelling of either ammonium ($^{15}NH_4NO_3$, 10.23 atom % excess) or nitrate ($NH_4{}^{15}NO_3$, 10.28 atom % excess) with ^{15}N. Six sets of 250 mL incubation bottles (six bottles per set) were prepared with 30 g fresh soil (on oven-dried basis). After 24 h pre-incubation, 2 mL of $^{15}NH_4NO_3$ solution or $NH_4{}^{15}NO_3$ solution was then added at a rate of 50 mg NH_4^+-N kg^{-1} soil and 50 mg NO_3^--N kg^{-1} soil, respectively. The bottles were sealed with cling film punctured with seven pin holes to allow gas exchange then incubated for 16 d at a water holding capacity (WHC) of 60% and a temperature of 25 °C in the dark. Water lost during incubation was compensated for by adding deionized water using a mini pipette to maintain a constant weight. Prior to incubation, a pre-experiment was conducted to confirm the optimal incubation time and gas sampling time interval for identifying the N_2O flux peaks and meeting the requirement of data-input for the ^{15}N tracing model.

Gas sampling and destructive soil sampling were carried out 2, 98, 194, 290, and 386 h after NH_4NO_3 application, respectively. At each sampling point, gas samples were collected using a 50 mL syringe from a specific set of bottles at 0 and 6 h after sealing with an air-tight lid. The samples were then immediately injected into two pre-evacuated gas vials with a butyl-rubber stopper for analysis of N_2O concentrations and the isotopic composition of $^{15}N_2O$. In advance of the first gas collection, the bottles were injected with 50 mL of fresh gas to maintain air pressure then after the second collection, the lids were replaced with the punctured cling film. At the same time as gas sampling, NH_4^+ and NO_3^- were extracted from another set of soil samples using 100 mL 2 M KCl. After extraction, the soil was rinsed repeatedly with deionized water to remove any residual inorganic N then oven-dried at 50 °C for soil organic N testing. The soil and solution samples were both stored at −20 °C until use.

N_2O concentrations in the sampled gas samples were measured using a gas chromatograph (Agilent 7890, Agilent Technologies, Santa Clara, CA, USA) equipped with a ^{63}Ni electron capture detector. For isotopic analysis, extracted NH_4^+ was separated by distillation with MgO, thereafter NO_3^- was converted to NH_4^+ with Devarda's alloy in another distillation [23]. Released ammonia was absorbed in boric acid solution, and NH_4^+ concentration was measured using 0.02 M sulfuric acid. After acidification, the solution was dried in an oven at 50 °C and ^{15}N enrichment of NH_4^+ was determined using an isotope ratio mass spectrometry (IRMS 20-22, SerCon, Crewe, UK). While ^{15}N enrichment of N_2O and organic N were measured using a MAT 253 mass spectrometer (Thermo Finnigan, Bremen, Germany).

2.3. ^{15}N Tracing Model

A full process-based ^{15}N tracing model (Figure 1) was used to simultaneously quantify the gross N transformation rates in each soil sample [24]. Average NH_4^+ and NO_3^- concentrations and ^{15}N excess values (average ± standard deviations) from the two ^{15}N-labeled treatments were included in the model. The model calculated the gross N transformation rates by simultaneously optimizing the kinetic parameters for the various N transformation processes to minimize misfit between the modeled and observed NH_4^+ and NO_3^- concentrations and respective ^{15}N enrichments. A Markov chain Monte Carlo metropolis algorithm (MCMC-MA) was used for parameter optimization, since it is known to be efficient to simultaneously estimate a large number of parameters [25,26]. This algorithm performed a random walk in model parameter space in order to find the global minimum and was shown to be robust against local minima [24]. The optimization procedure produced a probability density function for each model parameter, from which the mean and standard deviation of three parallel sequences were then calculated [25]. To obtain the best parameter set for ^{15}N tracing analysis that was able to simulate the observed data, various combinations of kinetic settings of individual processes were evaluated (Table 2 shows the final version of the parameter set). The most appropriate model to describe the measured N dynamics was then selected according to the Akaike information criterion for each model version [25]. The ^{15}N tracing model was performed using MatLab (Version 7.2, The MathWorks Inc., Natick, MA, USA), which used models individually constructed in Simulink (Version 6.4, The MathWorks Inc., Natick, MA, USA).

Table 2. Descriptions and average gross N transformation rates (mean ± standard deviation, μg N g^{-1} soil d^{-1}) in the *Brachiaria* and *Eremochloa* soils.

Parameter	Description	Kinetics	Gross N Transformation Rates	
			Brachiaria Soil	*Eremochloa* Soil
M_{Nrec}	Mineralization of N_{rec} to NH_4^+	0	2.02 ± 0.05 a	1.57 ± 0.03 b
$I_{NH4\text{-}Nrec}$	Immobilization of NH_4^+ to N_{rec}	1	2.94 ± 0.06 b	3.52 ± 0.07 a
M_{Nlab}	Mineralization of N_{lab} to NH_4^+	1	0 ± 0	0 ± 0
$I_{NH4\text{-}Nlab}$	Immobilization of NH_4^+ to N_{lab}	1	0 ± 0	0 ± 0
O_{Nrec}	Oxidation of N_{rec} to NO_3^-	0	0.002 ± 0.001 b	0.006 ± 0.007 a
I_{NO3}	Immobilization of NO_3^- to N_{rec}	1	0.20 ± 0.03 b	0.88 ± 0.01 a
O_{NH4}	Oxidation of NH_4^+ to NO_3^-	1	1.44 ± 0.02 b	1.98 ± 0.04 a
D_{NO3}	Dissimilatory NO_3^- reduction to NH_4^+	1	0.0005 ± 0.0002 b	0.0011 ± 0.0007 a
A_{NH4}	Adsorption of NH_4^+	1	0.07 ± 0.06 b	35.43 ± 4.65 a
R_{NH4}	Release of adsorbed NH_4^+	1	0.71 ± 0.10 b	35.76 ± 3.36 a
A_{NO3}	Adsorption of NO_3^-	1	0 ± 0	0 ± 0
R_{NO3}	Release of adsorbed NO_3^-	1	0 ± 0	0 ± 0

[1.] Values followed by different lowercase letters within the same row indicate a significant difference between treatments (no overlap of 85% confidence intervals). [2.] N_{lab}: soil labile organic N, N_{rec}: soil recalcitrant organic N. [3.] Kinetic types: 0 = zero order, 1 = first order.

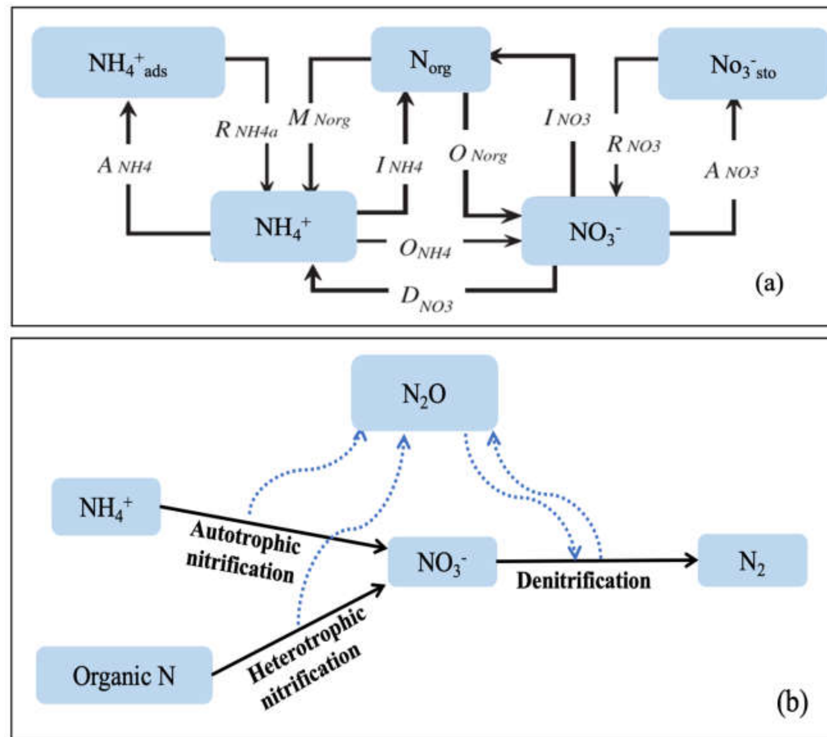


Figure 1. The ^{15}N tracing model used to determine gross N transformation rates (**a**) [24] and N_2O production pathways from specific N pools (**b**). N_{org}: soil organic N (including soil labile organic N and recalcitrant organic N), NH_4^+: ammonium, NO_3^-: nitrate, $NH_4^+{}_{ads}$: ammonium adsorbed to soil, $NO_3^-{}_{sto}$: stored nitrate, SOM: soil organic matter. Abbreviations for the transformations are as in Table 2.

The initial pool sizes for soil NH_4^+-N and NO_3^--N were estimated by extrapolating the first two sets of data back to the time point zero [27]. Based on the kinetic settings and the final parameters set, average gross transformation rates were then calculated over the whole incubation period and presented in units of μg N g^{-1} soil d^{-1} (Table 2).

2.4. Calculations

The N_2O flux (F, μg N_2O-N kg^{-1} h^{-1}) was calculated as follows:

$$F = \frac{\rho \times \Delta C \times V \times 273}{W \times \Delta t \times T} \quad (1)$$

where ρ is the density of gas under standard conditions (1.25 kg N_2O-N m^{-3}); ΔC is the variation in gas concentrations during the 6 h gas sampling period (ppbv); V is the volume of the flask (m^{-3}); T is the incubation temperature; Δt is the incubation time (h); and W is the dry weight of the soil (kg).

Cumulative N_2O emissions (E, μg N_2O-N kg^{-1}) were calculated as follows:

$$E = \sum \frac{(F_i + F_{i+1})}{2} \times (t_{i+1} - t_i) \times 24 \quad (2)$$

where F is the N_2O flux (μg N_2O-N kg^{-1} h^{-1}); i is the ith measurement; and t_{i+1}–t_i represents the time interval between the two adjacent measurements.

N_2O is thought to be derived from three N transformation process: autotrophic nitrification, heterotrophic nitrification, and denitrification. The relative contributions of each process to the N_2O emissions were therefore calculated as follows [28]:

$$a_{N_2O} = f_{AN} \times a_a + f_{HN} \times a_h + f_{DN} \times a_d \quad (3)$$

$$\boldsymbol{f_{AN} + f_{HN} + f_{DN} = 1} \quad (4)$$

where ***AN***, ***HN*** and ***DN*** represent autotrophic nitrification, heterotrophic nitrification and denitrification, respectively; $\boldsymbol{a_{N2O}}$, $\boldsymbol{a_a}$, $\boldsymbol{a_h}$ and $\boldsymbol{a_d}$ represent the ^{15}N atom % excess of N_2O-N, NH_4^+-N, organic N and NO_3^--N from the paired $^{15}NH_4NO_3$ and $NH_4{}^{15}NO_3$ treatments, respectively; and $\boldsymbol{f_{AN}}$, $\boldsymbol{f_{HN}}$ and $\boldsymbol{f_{DN}}$ represent the respective fractions of N_2O derived from ***AN***, ***HN***, and ***DN***.

The average rate of N_2O production from heterotrophic nitrification (N_2O_h), autotrophic nitrification (N_2O_a), and denitrification (N_2O_d) were then calculated as follows:

$$\boldsymbol{N_2O_h = f_{HN} \times N_2O_T} \quad (5)$$

$$\boldsymbol{N_2O_a = f_{AN} \times N_2O_T} \quad (6)$$

$$\boldsymbol{N_2O_d = f_{DN} \times N_2O_T} \quad (7)$$

where N_2O_T is the total N_2O production rate during the entire incubation time.

2.5. Statistical Analyses

Statistical analysis was not applied to the parameter results since the ^{15}N tracing model contained plenty of iterations [24]. Accordingly, differences between treatments were considered significant at an alpha level of 0.05 if the 85% confidence intervals did not overlap. Differences in soil properties and N_2O emissions between treatments were determined using an independent *t*-test. All statistical analyses were carried out using SPSS Statistics (version 26.0, IBM corp., Armonk, NY, USA) for Windows.

3. Results

3.1. Soil N Pool Sizes and ^{15}N Enrichment

NH_4^+ concentrations decreased while NO_3^- concentrations increased during incubation of both the *Brachiaria* and *Eremochloa* soils (Figure 2). NH_4^+ concentrations decreased more rapidly in the *Eremochloa* soil, with a decrease of 95.0% during the first 8 d of incubation, with a reduction of only 49.8% in the *Brachiaria* soil at the end of the 16-day incubation period (Figure 2a). NO_3^- concentrations in the *Eremochloa* soil reached a maximum on day 8 after the application of NH_4NO_3, while a continuous increase was observed up until the end of the incubation in the *Brachiaria* soil (Figure 2b).

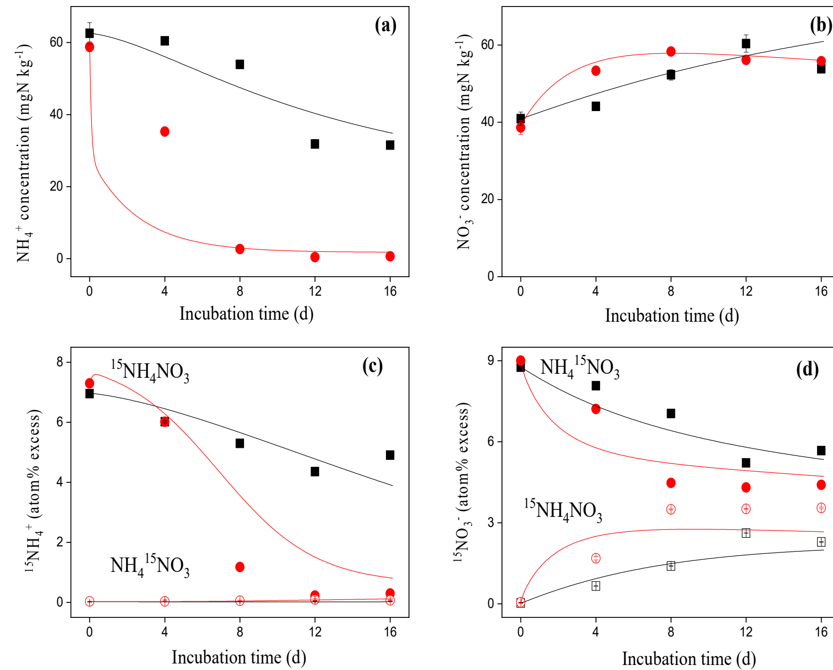


Figure 2. Measured (points) and modeled (lines) concentrations (a, b) and ^{15}N enrichment of NH_4^+ and NO_3^- (c, d) in the *Brachiaria* (squares) and *Eremochloa* soil (circles) treated with either $^{15}NH_4NO_3$ or $NH_4{}^{15}NO_3$. Vertical bars denote the standard deviation of the mean (n = 3).

^{15}N enrichment of the NH_4^+ pool decreased, while that of the NO_3^- pool increased following the addition of $^{15}NH_4^+$, suggesting that mineralization of soil organic N and NH_4^+ oxidation occurred simultaneously (Figure 2c,d). Meanwhile, ^{15}N enrichment of NO_3^- decreased after the application of $NH_4{}^{15}NO_3$, suggesting that natural or a low abundance of NO_3^- entered this pool. In contrast, ^{15}N enrichment of NH_4^+ increased slightly after the application of $NH_4{}^{15}NO_3$, suggesting that the direct conversion from $^{15}NO_3^-$ to $^{15}NH_4^+$ was negligible.

3.2. Gross N Transformation Rates

The ^{15}N tracing model described the measured data in the test soil with a correlation coefficient (R^2) of 0.99. The estimated gross rates of the 12 N transformation processes are shown in Table 2. The dynamic rates of labile organic N (labile organic N mineralization into NH_4^+ and immobilization of NH_4^+ into labile organic N) and adsorbed NO_3^- (adsorption of NO_3^- and release of adsorbed NO_3^-) were negligible in both test soils. Meanwhile, the gross mineralization rate of recalcitrant organic N in the *Brachiaria* soil was 2.02 μg N g^{-1} soil d^{-1}, which was significantly higher than that in the *Eremochloa* soil. In contrast, the gross rate of mineral NH_4^+ immobilization in the *Brachiaria* soil decreased to 2.94 μg N g^{-1} soil d^{-1} from 3.57 μg N g^{-1} soil d^{-1} in the *Eremochloa* soil (Figure 3), and as a result, *Brachiaria* planting increased the mineralization–immobilization turnover of NH_4^+ (M/INH_4^+) from 44.6% in the *Eremochloa* soil to 68.7%. Both the adsorption rate of NH_4^+ and the release rate of adsorbed NH_4^+ were significantly lower in the *Brachiaria* compared to *Eremochloa* soil. Meanwhile, the net exchange (release minus adsorption) of mineral NH_4^+ between these two pools was 0.64 μg N g^{-1} soil d^{-1} in the *Brachiaria* soil, almost double that in the *Eremochloa* soil (0.33 μg N g^{-1} soil d^{-1}), suggesting an increase in NH_4^+ supply.

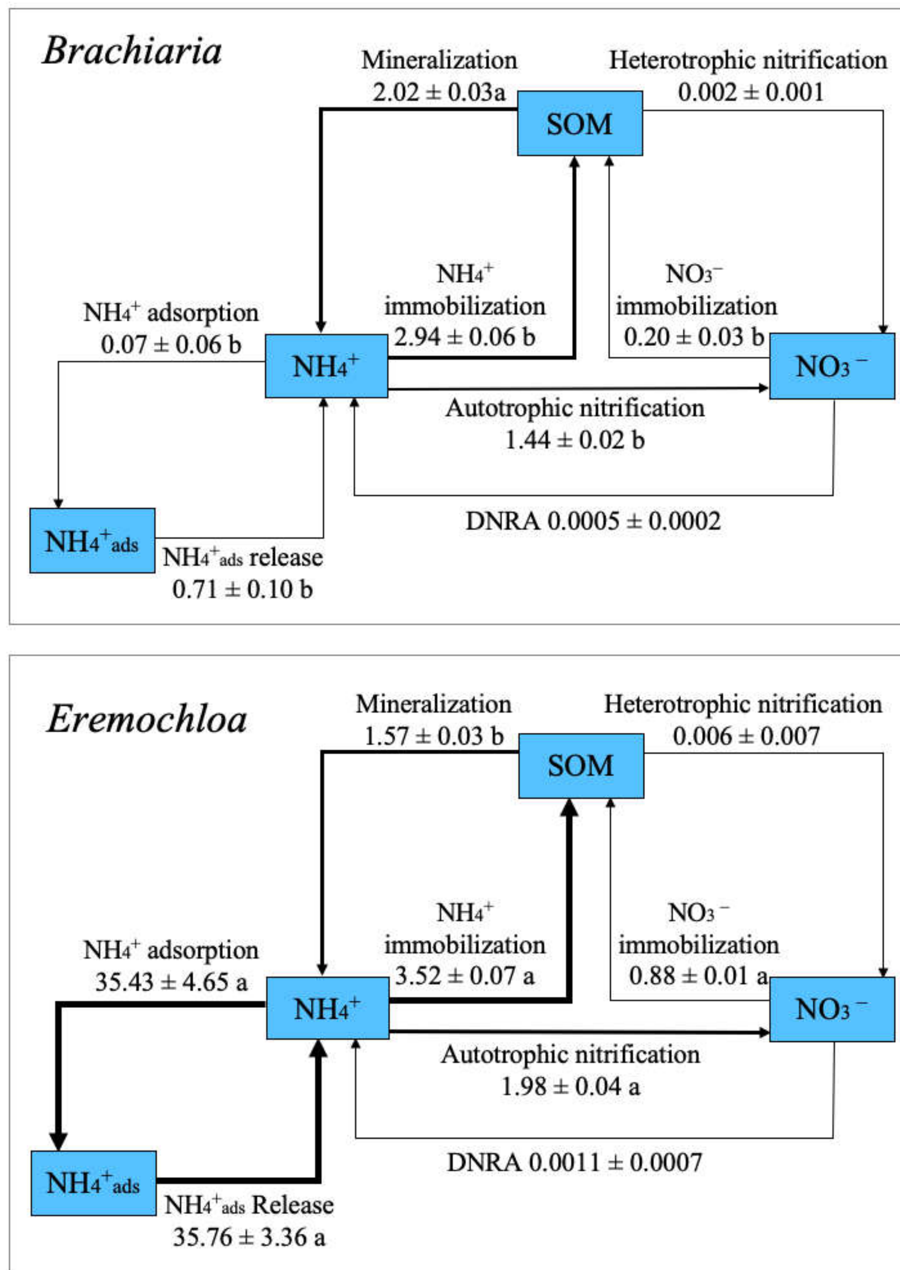


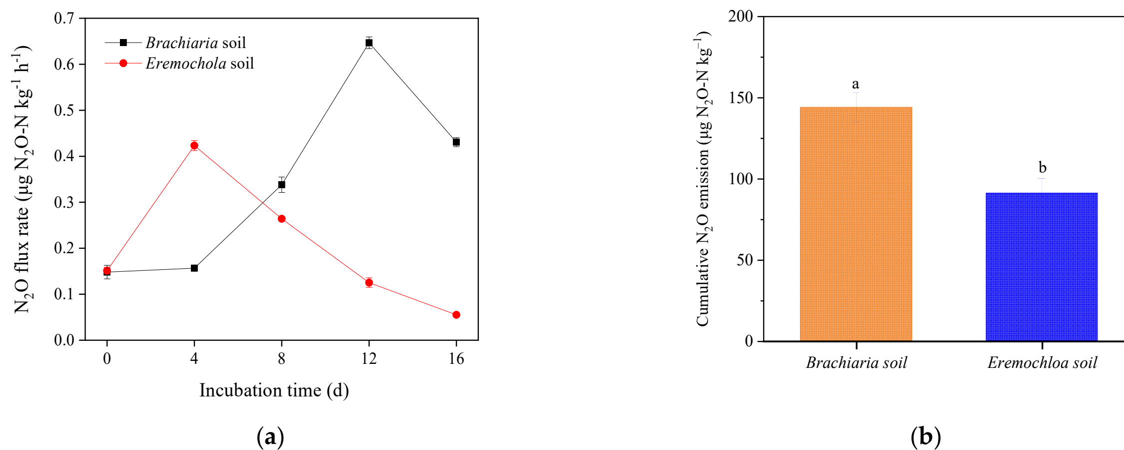
Figure 3. Nitrogen cycles in the *Brachiaria* and *Eremochloa* soil. Values shown represent gross N transformation rates (mean ± SD, μg N g^{-1} d^{-1}). The thickness of the arrows indicates the strength of each process. Different lowercase indicates denote a significant difference between treatments at $p < 0.05$. $NH_4^+{}_{ads}$: ammonium adsorbed to soil.

Autotrophic nitrification was a dominant NO_3^- production process in both sets of soils, while heterotrophic nitrification was negligible. *Brachiaria* planting reduced the autotrophic nitrification rate to 1.44 μg N g^{-1} soil d^{-1} from 1.98 μg N g^{-1} soil d^{-1} in the *Eremochloa* soil. The *Brachiaria* soil also showed a lower nitrification capacity (ONH_4^+/M) than the *Eremochloa* soil (71.3 vs. 126.0%, respectively). Immobilization of NO_3^- overwhelmingly surpassed the rate of dissimilatory nitrate reduction to ammonium

(DNRA) in both sets of soil, representing the primary NO_3^- consumption process under our experimental conditions. The NO_3^- immobilization rate in the *Eremochloa* soil was 0.88 μg N g^{-1} soil d^{-1}, nearly four times greater than that in the *Brachiaria* soil. Meanwhile, the NO_3^- retention capacity and availability in the *Eremochloa* soil, expressed as the ratio of NO_3^- consumption to production, was significantly higher than in the *Brachiaria* soil (44.4 vs. 13.9%, respectively).

3.3. N_2O Production Pathways and Emissions

The N_2O flux peak occurred on day 4 of the incubation in the *Eremochloa* soil and on day 12 in the *Brachiaria* soil (Figure 4), although there was no apparent difference in the N_2O production rates from heterotrophic nitrification between the *Eremochloa* and *Brachiaria* soil (Table 3). The average N_2O production rate of autotrophic nitrification was 3.19 μg N_2O-N kg^{-1} d^{-1} in the *Eremochloa* soil, while it was significantly lower at 2.78 μg N_2O-N kg^{-1} d^{-1} in the *Brachiaria* soil. In contrast, the average N_2O production rate during denitrification increased sharply from 0.55 μg N_2O-N kg^{-1} d^{-1} in the *Eremochloa* soil to 4.25 μg N_2O-N kg^{-1} d^{-1} in the *Brachiaria* soil, representing a 7.7-fold increase.



(**a**) (**b**)

Figure 4. Fluxes (**a**) and cumulative emissions (**b**) of N_2O in the *Brachiaria* and *Eremochloa* soil during the 16-day incubation. Different lowercase indicates denote a significant difference between treatments at $p < 0.05$. Vertical bars denote the standard deviation of the mean ($n = 3$).

Table 3. Average N_2O production rates, the relative contribution of each nitrogen transformation process, and the ratio of N_2O emissions from heterotrophic and autotrophic nitrification and denitrification in the *Brachiaria* and *Eremochloa* soils.

	N_2O Production Rate (μg N_2O-N kg^{-1} d^{-1})				Relative Contribution to N_2O (%)		
	Total	**Autotrophic Nitrification**	**Heterotrophic Nitrification**	**Denitrification**	f_{AN}	f_{HN}	f_{DN}
Brachiaria soil	9.02 ± 0.05a	2.78 ± 0.1b	1.99 ± 0.10a	4.25 ± 0.14a	30.90 ± 0.60b	22.00 ± 1.40b	47.10 ± 1.20a
Eremochloa soil	5.65 ± 0.22b	3.19 ± 0.28a	1.91 ± 0.11a	0.55 ± 0.06b	56.30 ± 2.80a	33.90 ± 3.20a	9.70 ± 0.80b

[1.] Values represent means ± standard deviation ($n = 3$). Different lowercase letters within the same column denote a significant difference between treatments at $p < 0.05$. [2.] f_{AN}, f_{HN} and f_{DN} denote the contribution of autotrophic nitrification, heterotrophic nitrification and denitrification to N_2O production, respectively.

Cumulative N_2O emissions during incubation were significantly higher in the *Brachiaria* soil (144.31 μg N_2O-N kg^{-1}) than that estimated as 90.42 μg N_2O-N kg^{-1} in the *Eremochloa* soil. Meanwhile, denitrification contributed to 47.1% of the emitted N_2O, exceeding the contributions of autotrophic and heterotrophic nitrification in the

Brachiaria soil (Table 3). In contrast, only 9.7% of the produced N_2O was derived from denitrification in the *Eremochloa* soil.

4. Discussion

4.1. Brachiaria Humidicola Cultivation Enhanced the Soil NH_4^+ Supply

This study revealed that the gross mineralization rate of soil recalcitrant organic N was significantly enhanced by 28.7% under cultivation of *Brachiaria* compared with *Eremochloa*, accelerating the renewal of soil organic N due to its slight increase. This is consistent with the findings of Teutscherová et al. [29,30] who revealed a positive priming effect of high-BNI *Brachiaria* on native soil organic N decomposition in Colombian pastures compared with a low-BNI genotype. It is suggested that grasses with a dense root system stimulate organic N mineralization by enhancing microbial biomass and activity through the release of large amounts of dead roots and exudates into the soil [31,32]. It is thought that the increase in organic C accelerates the formation of aggregates and reduces the effective diffusion coefficient of oxygen in the soil, in turn inducing a shift in dominant microbes from aerobes (Gram-negative bacteria) to facultative and/or anaerobic microbes (Gram-positive bacteria and fungi) [33–36]. In general, Gram-positive bacteria and fungi preferentially utilize soil recalcitrant organic matter [37,38]. It is therefore likely that the increase in soil organic C observed here was mainly due to the increase in organic C input from the high biomass of dead roots and exudates under *Brachiaria* cultivation, resulting in more efficient growth of Gram-positive bacteria and fungi and an increase in the turnover of recalcitrant organic C compared with *Eremochloa* cultivation.

In contrast, the immobilization rate of NH_4^+ in the *Brachiaria* soil decreased compared with the *Eremochloa* soil. This differs from the results of Vazquez et al. [20] who showed that gross NH_4^+ immobilization was enhanced in the soil cultivated with high-BNI *Brachiaria* genotypes, while the NO_3^- concentration and N losses remained low. It has been reported that a high C/N ratio in high-BNI plant soil reduces N availability for microbial N immobilization when no N fertilizers are added or when only limited (animal) urine deposition occurs [14,29,39,40]. In contrast, cultivation of high-BNI *Brachiaria* genotypes in the same field experiment results in a significant reduction in microbial NH_4^+ immobilization rates at 7 and 21 days after application of N fertilizers at a rate 50 kg N ha^{-1} [41]. These results suggest that microbial NH_4^+ immobilization is dependent on soil NH_4^+ availability.

Compared with *Eremochloa*, *Brachiaria* more efficiently reduced the gross rate of soil NH_4^+ adsorption than the release rate of adsorbed NH_4^+, causing an increase in the net release rate of adsorbed NH_4^+. This may have been due to two possible reasons. Firstly, roots of *Brachiaria* can distribute within the 20–40 cm soil layer, allowing effective absorption of non-exchangeable K^+ from deeper soil layers [42], dramatically increasing available K^+ in the surface soil. The higher availability of K^+ also outcompetes NH_4^+ for soil adsorption sites, thereby reducing soil adsorption of NH_4^+ since both have a similar ionic radius and physical properties [43,44]. To date, however, the influence of *Brachiaria* planting on the soil available K^+ is less studied. Further study is required to evaluate how *Brachiaria* cultivation affects the soil available K at the different K application rates. Secondly, the adsorption capacity of NH_4^+ in soil is also affected by the content of clay and organic matter [45,46]. Organic matter with plenty of polar atom groups, such as carboxyl and phenolic hydroxyl, contribute to the negative charge and is the main source of variable soil charge. It is therefore likely that NH_4^+ as a cation is not as efficiently adsorbed, while NH_4^+ from decomposed organic N is released during the mineralization of native recalcitrant organic C under cultivation of *Brachiaria*.

Overall, cultivation of *Brachiaria* therefore reduced the rates of NH_4^+ immobilization and adsorption and enhanced the rates of organic N mineralization and adsorbed NH_4^+ release, in turn increasing soil NH_4^+ availability and supply compared with *Eremochloa*

cultivation. These results suggest that in the *Brachiaria* soil, reduced application rate of K fertilizer may increase the adsorption of NH_4^+ from the test soil.

4.2. Effect of Brachiaria humidicola Cultivation on Soil N_2O Emissions

As expected, cultivation of *Brachiaria* significantly reduced autotrophic nitrification and related N_2O production, compared with *Eremochloa*. This is consistent with the findings of Subbarao et al. [14] who reported that *Brachiaria* soil reduced the ammonia oxidation rate by 90% during a 3-year field experiment compared with soybean and plant-free soil. Subbarao et al. [47] revealed that the release of brachialactone exudate by *Brachiaria* blocked the activities of both ammonia monooxygenase and hydroxylamino oxidoreductase, thereby reducing ammonia oxidation in pure culture with the ammonia oxidizer *Nitrosomonas europaea*. In line with this, a reduction in the abundance of ammonia oxidizers was observed in a previous field study of *Brachiaria* cultivation [18,40]. It was also revealed that BNI compounds were able to persist for a long time and tended to accumulate over time, remaining effective even after *Brachiaria* pasture was subsequently replaced with maize [48–50]. However, Vazquez et al. [20] and Teutscherová et al. [41] found no comparable differences in gross nitrification rates between *Brachiaria* genotypes with differing BNI capacities in soil with a high organic C content in the tropical savanna in Colombia. Moreover, they attributed the reduced inhibition of potential net nitrification rates to strong microbial immobilization of NH_4^+, and a subsequent reduction in soil NH_4^+ availability for ammonia oxidizers.

In the present study, the reduction in N_2O production in the *Brachiaria* soil via autotrophic nitrification was at the lower end of the range of 16.8–90.0% reported by previous studies [51]. This suggests that less NH_4^+ was converted into N_2O during autotrophic nitrification in test acidic soil. In general, competition for available NH_4^+ exists between autotrophic nitrification and microbial immobilization or adsorption of NH_4^+ [25,52]. As discussed above, reduced rates of NH_4^+ immobilization and NH_4^+ adsorption together with higher mineralization rates of organic N provided more NH_4^+ substrates for ammonia oxidizers in the *Brachiaria* compared to *Eremochloa* soil. This suggests that the suppression effect of high-BNI *Brachiaria* on N_2O emissions may also depend on soil NH_4^+ availability, with greater suppression in soil with relatively low available NH_4^+ [20,29,53,54].

In contrast to autotrophic nitrification, *Brachiaria* did not alter the N_2O production rate via heterotrophic nitrification. In general, heterotrophic nitrification is carried out by a large variety of bacteria and fungi [55], with heterotrophic nitrifiers using both organic and inorganic N as a substrate, and possibly producing more N_2O than autotrophic nitrifiers [56,57]. In the present study, we supposed that heterotrophic nitrifiers would use organic N as a unique substrate since the model only can select one substrate pool for running. Thus, it is very likely that the gross rate of heterotrophic nitrification in the *Brachiaria* and *Eremochloa* soil was underestimated. In general, fungal nitrification in acidic soil is not inhibited by popular synthetic nitrification inhibitors such as acetylene (C_2H_2), dicyandiamide (DCD) and nitrapyrin [58]. Therefore, our results suggest that the 2-year cultivation with *Brachiaria* had no effect on N_2O production via heterotrophic nitrification.

As unexpected, cumulative N_2O emissions were significantly higher in the *Brachiaria* soil compared to the *Eremochloa* soil as measured in the field (4.30 and 1.54 kg N_2O-N ha^{-1} under *Brachiaria* and *Eremochloa* cultivation, respectively), although N_2O emissions via autotrophic nitrification decreased. This is in contrast with previous results in which *Brachiaria* establishment was found to reduce soil N_2O emissions by 20–90% compared with plants without BNI capacity [14,47]. In the present study, the N_2O production rate in the *Brachiaria* soil during denitrification increased 6.7-fold, while the contribution ratio of denitrification to emitted N_2O dramatically increased compared with *Eremochloa*. These results clearly suggest that the enhanced N_2O emissions in the *Brachiaria* soil were primarily due to an increased denitrification potential. There were

two suggested possibilities. Firstly, compared with *Eremochloa*, *Brachiaria* cultivation sharply reduced the NO_3^- immobilization rate, which outnumbered the rate of DNRA as the primary consumption process of NO_3^-. This suggests that cultivation of *Brachiaria* increased soil NO_3^- availability by reducing the ratio of total NO_3^- consumption through microbial immobilization of NO_3^- and dissimilatory NO_3^- reduction to NH_4^+ (I_{NO3}+D_{NO3}) to total NO_3^- production (O_{NH4} + O_{Nrec}), thereby providing more NO_3^- for denitrification. It was previously suggested that higher NO_3^- concentrations in the soil tend to result in a higher N_2O/N_2 ratio during denitrification, thereby favoring N_2O emissions [59]. Secondly, in this study, *Brachiaria* cultivation significantly enhanced soil organic C, notably dissolved organic C, due to the increase in plant biomass and especially biomass of roots and exudates. Increases in soil organic C were also found to promote the formation of anaerobic microsites for denitrification by stimulating aggregation and soil respiration [60,61]. Meanwhile, an increase in organic C was also found to reduce the minimum soil moisture threshold for the occurrence of denitrification [62,63]. For example, Wan et al. [64] found that the addition of starch to sandy loam soil treated with nitrate-based fertilizers stimulated N_2O production through denitrification. The results of this study therefore suggest that although cultivation of *Brachiaria* suppressed autotrophic nitrification, it significantly increased the soil denitrification potential and subsequent N_2O production by increasing soil organic C, notably labile organic C, through an increase in plant biomass, thereby stimulating soil N_2O emissions.

5. Conclusions

This study examined the effect of the exotic tropical grass *Brachiaria*, which has a high-BNI capacity, on soil N transformation processes and N_2O emissions. Cultivation of *Brachiaria* significantly decreased the gross rate of autotrophic nitrification and N_2O production during nitrification. In contrast, *Brachiaria* also increased the gross mineralization rate of soil recalcitrant organic N and reduced microbial NH_4^+ immobilization and NH_4^+ adsorption, thereby increasing the NH_4^+ supply for nitrification compared with native *Eremochloa*. *Brachiaria* planting caused a significant increase in soil N_2O emissions, primarily due to an increased denitrification potential as a result of reductions in NO_3^- immobilization and an increase in soil labile organic C. Further studies are now required to determine the effects of K fertilizers on the adsorption and availability of NH_4^+. The effect of synthetic nitrification inhibitors together with the biological nitrification inhibitors released from *Brachiaria* on the mitigation of N_2O emissions in tropical pastures also requires further clarification.

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