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ORIGINAL PAPER

Inter-microbial competition for N and plant NO_3^- uptake rather than BNI determines soil net nitrification under intensively managed *Brachiaria humidicola*

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Abstract

Brachiaria humidicola (syn. *Urochloa humidicola*) has been acknowledged to control soil nitrification through release of nitrification inhibitors (NI), a phenomenon conceptualized as biological nitrification inhibition (BNI). Liming and N fertilization as features of agricultural intensification may suppress BNI performance, due to a decrease in NI exudation, increased NH_3 availability and promotion of ammonia oxidizing bacteria (AOB) over archaea (AOA). A 2-year three-factorial pot trial was conducted to investigate the influence of soil pH and soil microbial background (ratio of archaea to bacteria) on BNI performance of *B. humidicola*. The study verified the capacity of *B. humidicola* to reduce net nitrification rates by 50 to 85% compared to the non-planted control, irrespective of soil pH and microbial background. The reduction of net nitrification, however, was largely dependent on microbial N immobilization and efficient plant N uptake. A reduction of gross nitrification could not be confirmed for the AOA dominated soil, but possibly contributed to reduced net nitrification rates in the AOB-dominated soil. However, this putative reduction of gross nitrification was attributed to plant-facilitated inter-microbial competition between bacterial heterotrophs and nitrifiers rather than BNI. It was concluded that BNI may play a dominant role in extensive *B. humidicola* pasture systems, while N immobilization and efficient plant N uptake may display the dominant factors controlling net nitrification rates under intensively managed *B. humidicola*.

Keywords Biological nitrification inhibition · *Urochloa humidicola* · N immobilization · Liming · Ammonia oxidizing archaea (AOA) · Ammonia oxidizing bacteria (AOB)

Introduction

The tropical forage grass *Brachiaria humidicola* (syn. *Urochloa humidicola*) has been acknowledged to control soil nitrification, an attribute contributing to the mitigation of nitrification-related N losses, including NO_3^- leaching and N_2O emissions (Byrnes et al. 2017; Subbarao et al. 2009; Sylvester-Bradley el al. 1988). This functional trait has been proposed to be based on an allelopathic reduction of gross nitrification rates, through release of nitrification inhibitors (NI) into the rhizosphere, a phenomenon termed Biological Nitrification Inhibition (BNI) (Subbarao et al. 2006). For *B. humidicola*, BNI has been attributed to different fusicoccanes described as brachialactones (Egenolf et al. 2020a; Subbarao et al. 2009), as well as phenolic compounds including, but not limited to methyl-coumarate, methyl-ferulate (Gopalakrishnan et al. 2007), and vanillin (Egenolf 2021), as well as different free fatty acids (Subbarao et al. 2008). The first group of NI — brachialactones — are actively exuded from the root under conditions favoring a low rhizosphere pH, i.e. low background soil pH levels and rhizosphere acidification related to plant nutrition (e.g. cation/NH_4^+ uptake) (Egenolf et al. 2020b; Subbarao et al. 2007). Accordingly, it has been argued that BNI of *B.*

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humidicola evolves primarily under acidic soil conditions, reflected in a reduced activity of nitrifiers (Norton 2008) accentuated by increased amounts of nitrification inhibitors released into the rhizosphere.

The majority of *Brachiaria* spp. production areas in tropical Latin America can be characterized as extensively managed grasslands on highly weathered and acidic soils (Miles et al. 2004; Rao et al. 1995). Although viewed critically by parts of the scientific community (Kaimowitz and Angelsen 2008; Kreidenweis et al. 2018), the intensification of these pasture systems is generally considered as alternative to the progressive deforestation of natural habitats and therefore promoted by many governments, e.g. Brazil and Colombia (Cohn et al. 2014; Rudel et al. 2015; Strassburg et al. 2014). Following the introduction of new forage genotypes, lime and fertilizer applications are key strategies to increase productivity and to avoid pasture degradation (Rudel et al. 2015). It remains unclear, however, to what extent such significant alterations in soil chemical properties, as a response of intensifying grassland management, may impact the expression of BNI. Recent field and greenhouse assessments of BNI on a Vertisol with near neutral pH confirmed a plant-mediated and genotype-dependent reduction of net nitrification rates (Byrnes et al. 2017; Nuñez et al. 2018). Yet, additional studies on the same site, emphasizing the underlying soil N dynamics, raised the question whether the observed effects were due to a reduction of gross nitrification (i.e. a real BNI effect) or rather an increase of microbial N (both NH_4^+ and NO_3^-) immobilization rates (Vazquez et al. 2020).

Besides influencing plant NI exudation patterns, soil pH and N availability determine the soil microbial community composition (Zhalnina et al. 2015). With regard to nitrifiers, both factors affect the competitiveness (niche differentiation) of ammonia oxidizing archaea (AOA) and bacteria (AOB). While AOA are able to take up NH_4^+ (Li et al. 2018) and therefore perform better under low pH and NH_3 limited conditions, AOB reveal higher maximum NH_3 turnover rates and, hence, higher competitiveness in neutral to alkaline and N abundant soils (Di et al. 2009; Prosser and Nicol 2012; Thion et al. 2016; Zhang et al. 2012). It could be speculated that conversion of extensively managed tropical grasslands on low pH soils into productive pastures through liming and increased N fertilization leads to a shift from AOA towards AOB populations (Zhang et al. 2017; Zhao et al. 2018). Consequently, an increase of soil pH may not only affect BNI performance of *B. humidicola* in terms of reduced NI exudation rates, but may also induce changes in ammonia oxidizer populations, possibly affecting its sensitivity. This assumption is based on a recently published laboratory study observing that (with the only exception of methyl 3-(4-hydroxyphenyl)propionate) AOA show a higher sensitivity to selected NI than AOB (Kaur-Bhambra et al. 2021). In addition, under field conditions, a plant exerted control of ammonia oxidizer abundance was mainly confirmed for AOA (Byrnes et al. 2017; Nakamura et al. 2020; Nuñez et al. 2018; Srikanthasamy et al. 2018; Subbarao et al. 2009), while only to a lesser extent for AOB (Subbarao et al. 2009).

To verify these assumptions, we hypothesized that (1) *B. humidicola* is capable of reducing soil net nitrification rates under conditions favorable to nitrification facilitated through pH increments and regular N amendments, (2) the reduction of net nitrification rates is the result of a BNI-induced reduction of gross nitrification, and (3) *B. humidicola* controls nitrification under both archaea and bacteria dominated soil environments. Pertaining to the verification of these hypotheses, alternative mechanisms shaping soil N cycling have been conceptualized and a broader concept of plant exerted control of soil nitrification is proposed.

Material and methods

Experimental set-up

A three-factorial, controlled pot experiment was conducted at CIAT headquarters, Cali (Colombia), from March 2016 till March 2018. Pots were fabricated from PVC drainpipes (16.8 cm diameter, 1.20 m length) and the experiment was set up in a randomized complete block design (three replications) in an open greenhouse equipped with a shading net. Experimental factors were (1) Soil: soil microbial background as reflected by the ratio between archaea and bacteria, (2) Liming: soil pH increment, and (3) Genotype: *B. humidicola* accessions.

The first experimental factor (soil) consisted of two soils (A horizon, 0–20 cm) originating from natural savanna sites along a soil texture transect from Villavicencio to Puerto Gaitan (Colombian Llanos region), which were chosen due to their soil texture and organic matter–related contrasting microbial backgrounds (Table S2, Fig. S1). In the following, the soils will be referred to the site of origin, with "Taluma" representing an archaea dominated, sandy soil (4°22′11.03704 N 72°13′31.49378 W), and "Porvenir" a slightly bacteria-dominated, clayey soil (4°13′23.52 N 72°32′40.05 W). Both soils were classified as Ferralsols (IUSS working group WRB, 2006), and physical soil properties are detailed in Table S1. The initial archaeal and bacterial 16S rRNA and *amoA* gene copy numbers of these two soils before experimental start are displayed in Table S2.

The second experimental factor (liming) consisted of a non-limed and limed variant of each soil. Liming rates aimed at reducing aluminum (Al) saturation rates to ~ 5% and increase pH to near neutral range (pH = 6–7). Liming rates were calculated in dependence of Al saturation and cation exchange capacity (CEC), according to Cochrane

et al. (1980): Liming rate (t $CaCO_3$ ha^{-1}) = 1,8 * (Al saturation [cmol kg^{-1}] – (target Al saturation [%] * effective CEC [cmol kg^{-1}]/100)), applying a target Al saturation of 5%. The calculations resulted in liming rates of 1.6 t ha^{-1} (equivalent to 5.4 g kg^{-1} soil) for the Taluma, and 3.8 t ha^{-1} (equivalent to 12.8 g kg^{-1} soil) for the Porvenir soil. Soil pH (H_2O extracts) were verified at the end of the experiment, leveling out in the planted pots at 6.3 versus 6.8 (Taluma soil) and 5.8 versus 6.3 (Porvenir soil) for non-limed and limed treatments, respectively (Fig. S3). Unexpectedly, the pH levels of non-limed soils were above initial soil pH levels (4.6), which was attributed to occasional carbonate input via irrigation water.

The third experimental factor (genotype) consisted of three *B. humidicola* accessions with contrasting BNI ratings (Karwat et al. 2019; Nuñez et al. 2018), which were received from the CIAT germplasm bank: CIAT 16888 (high BNI), CIAT 679 (medium BNI), CIAT 26146 (low BNI). A non-planted bare soil was used as control.

Before filling the pots, soils were homogenized including base fertilization with all plant essential nutrients [mg kg^{-1} soil]: N [11.5], P [6.4], K [25.6], Ca [25.6], Mg [7.2], S [2.6], B [0.1], Zn [0.5], Cu [0.3], Mo [0.03]. To ensure percolation, pots were equipped with a 10-cm drainage layer of sand at the bottom and care was taken to avoid compaction when filling in the soil (100-cm soil horizon). Through the course of the experiment, plants were regularly fertilized with N (80 kg N ha^{-1} as urea at month 6, and 160 kg N ha^{-1} as $(NH_4)_2SO_4$ at month 12) to stimulate soil nitrification potential. Plants were trimmed every 3 months to promote vigorous growth, and irrigation was performed manually according to plant demand. In dependence of the three experimental factors, BNI performance was evaluated at the end of the experiment (2 years after establishment): To simulate grazing, plants were cut back and N was applied at the rate of 150 kg N ha^{-1} in the form of $(NH_4)_2SO_4$ solution (200 ml per pot), allowing topsoil penetration. N cycling was monitored over a period of 14 days after fertilization, before destructively harvesting the experiment to obtain soil and biomass samples.

In situ monitoring of soil solution nitrate concentration

For in situ monitoring of soil NO_3^- concentrations after the application of NH_4^+, soil solution was regularly sampled via micro-suction-tubes (Rhizons Soil Moisture Sampler, Ø 2.3 × 50 mm, hydrophilic polymer, porosity 0.1 µm, Ecotech, Bonn, Germany) installed horizontally at 7.5-cm depth (Karwat et al. 2018). NO_3^- concentration in the soil solution was quantified colorimetrically at 410 nm as yellow nitro-salicylate obtained by salicylic acid nitration in sulfuric acid solution (ISO 7890–3, 1988).

Quantification of mineral N pools

Topsoil NH_4^+ and NO_3^- contents were quantified 7 and 14 days after NH_4^+ fertilization. Seven days after NH_4^+ fertilization, soil samples were taken with a soil auger (Ø = 1.5 cm, 3 punctures per pot, 5-cm depth), whereas 14 days after NH_4^+ fertilization the soil sample consisted of an aliquot of sieved and homogenized topsoil at 0–10 cm (destructive harvesting of the experiment). Extraction was performed immediately with 1 M KCl. NH_4^+ was quantified colorimetrically as green ammonium salicylate complex at 667 nm (Kempers and Zweers 1986) and NO_3^- as yellow nitro-salicylate at 410 nm (ISO 7890–3, 1988).

Quantification of microbial C and N pools

Microbial C and N pools (C_{mic}/N_{mic}) were quantified by chloroform fumigation (ISO 14240–2, 1997), using the sieved and homogenized soil samples taken 14 days after NH_4^+ fertilization. In brief, unfumigated and chloroform fumigated (24 h) soil sample aliquots (10 g) were extracted with 40 ml of a 0.5 M K_2SO_4 solution on a horizontal shaker (30 min, 200 rpm). After centrifugation at 4400 × *g* for 30 min, the supernatant was diluted 1:4 and analyzed for C and N (Multi N/C 2100C, Analytik Jena AG, Jena, Germany). Microbial C pools were obtained by calculating the difference in C content between fumigated and unfumigated aliquots and dividing by $k_{EN} = 0.45$, representing the proportion of extractable microbial C (Jörgensen 1996). Microbial N pools were obtained by calculating the difference in N content between fumigated and unfumigated aliquots and dividing by $k_{EN} = 0.54$, representing the proportion of extractable microbial N (Brookes et al. 1985).

Quantification of soil nitrification and N immobilization potential (*ex situ*)

Soil nitrification and N immobilization potential were estimated based on the soil incubation method described by Karwat et al. (2017). Sieved (2-mm mesh size) and homogenized soil samples taken 14 days after NH_4^+ fertilization were air-dried, and 5-g aliquots were incubated in eightfold replications with 1.5 ml of 27 mM $(NH_4)_2SO_4$ (225 mg N kg^{-1} soil) at 25 °C. To maintain soil moisture but allow aeration, incubation vessels (small glass flasks) were sealed with parafilm, which was punctured three times with a needle. Mineral N was extracted with 50 ml of 1 M KCl solution from 2 replicates each at days 0, 4, 12, and 20 after incubation start. Extracts were filtered immediately and analyzed colorimetrically for NH_4^+ and NO_3^- as described above. Nitrification potentials represent the steepest slope of the NO_3^- curve. In this study, the steepest slope was recorded between Days 4 and 12. The N immobilization

potential was calculated based on mineral N (NH_4^+ and NO_3^-) disappearance from the start till end (Day 20) of incubation. Although it is acknowledged that NH_3 volatilization constitutes a potential N leak during the incubation, volatilization-related losses were judged negligible due to soil pH below seven, incorporation of fertilizer N (application as solution) and poor surface ventilation (parafilm-sealed incubation vessels).

Quantification of gene copy numbers

Archaeal and bacterial 16S rRNA (total archaea and bacteria), as well as archaeal and bacterial *amoA* (archaeal and bacterial ammonia oxidizers) gene copy numbers were quantified for selected treatment combinations, using both soil samples taken prior to fertilization (soil auger, Ø = 1.5 cm, 3 punctures per pot, 5-cm depth), as well as the sieved and homogenized soil samples taken 14 days after NH_4^+ fertilization. Soil samples were frozen and lyophilized prior to transport to University of Hohenheim (Stuttgart, Germany). DNA was extracted with the DNA™ Spin Kit for Soil (MP, Biomedicals Solon, OH, USA), following the manufacturer's instructions along with two additional washings with guanidine thiocyanate solution (5 M) for humic acid removal. Quantification of archaeal and bacterial 16S rRNA and *amoA* genes via quantitative PCR (qPCR) (StepOnePlus™ Real Time PCR System (Thermo Fisher Scientific, Foster City, CA, USA)) was performed according to Rasche et al. (2014) and Nuñez et al. (2018), respectively. For quality check, melting curves of amplicons were generated and reaction efficiency determined (archaeal 16S rRNA gene 80–85%, bacterial 16S rRNA gene 94–107%, archaeal *amoA* gene 79–89%, bacterial *amoA* gene 84–91%) using StepOne™ software version 2.2.2 (Thermo Fisher Scientific).

Quantification of plant N uptake

Plant N uptake was estimated based on N content of aboveground plant biomass re-grown within 14 days after NH_4^+ fertilization. The entire re-grown biomass was harvested, dried, and ball milled. Leaf tissue samples were evaluated for N content by dry combustion (Vario El Cube, Elementar Analysesysteme, Germany).

Statistical analysis

Statistical analysis was performed and plots were created with R version 3.5.3 (R Core Team, 2018), using the packages "lme4", "lsmeans", "multcompView" and "ggplot2". The "lme4" package was used to fit linear models with soil solution NO_3^-, topsoil NH_4^+ and NO_3^-, plant N, N_{mic}, nitrification rates, total archaeal and total bacterial as well as AOA and AOB abundance as response variables and the three factors "soil", "liming" and "genotype" as main effects and "block" as random effect. Studentized residuals were inspected graphically for normality and homogeneity. Package "lsmeans2" was used to perform ANOVA and "multcompView" to perform Tukey HSD tests on the differences of means between treatment levels. Package "ggplot2" was used to create the figures. A correlation analysis (Pearson) between the variables shoot biomass, root biomass, plant N uptake, soil NH_4^+, soil NO_3^- and N_{mic} pools was conducted using the "lm" function.

Results

In situ N dynamics

As both soils revealed similar NO_3^- concentration in the soil solution (no significant soil effect), the displayed values consist of the modeled averages over both soils (Fig. 1). Both liming and genotype influenced NO_3^- levels ($p < 0.001$). Liming boosted NO_3^- evolution within the soil solution, and planted pots revealed lower NO_3^- levels than the non-planted control. Genotypic differences between *B. humidicola* accessions were only observed under limed conditions, with genotype CIAT 26146 revealing lowest NO_3^- evolution ($p < 0.05$).

Topsoil (0–10 cm) N_{min} analysis at 7 and 14 days after NH_4^+ fertilization (Table S3) reflects the patterns observed for soil solution NO_3^- levels, confirming the suitability of the non-destructive monitoring approach via micro suction-cups. Additional N_{mic} analysis and estimates of plant N uptake (based on N in re-grown aboveground biomass) completed the estimation of system N fluxes. Trends were consistent between non-limed and limed treatments but more pronounced for the limed treatment. Figure 2 displays N pool sizes 14 days after NH_4^+ fertilization for the limed treatment. Total fertilizer N recovery (plant and soil) ranged from 33 to 50% in Taluma and 65 to 80% in Porvenir soil. Presence of plants reduced topsoil NO_3^- contents by 65 to 85% compared to the non-planted control. This reduction of soil NO_3^- was reflected by plant N uptake and increased soil N_{mic} pools. Aboveground plant biomass (re-grown) accounted for 12 to 20% of applied N and although not significant genotype CIAT 26146 tended to have highest acquisition rates. N_{mic} pools showed a higher variability, reflecting 10 to 25% of applied N in the planted pots, but lower amounts (0 to 10%) in the non-planted control ($p < 0.05$). N_{mic} pools were largest under genotype CIAT 26146 and always higher than under genotype CIAT 16888 ($p < 0.05$). Soil NH_4^+ levels did not show any difference ($p > 0.05$).

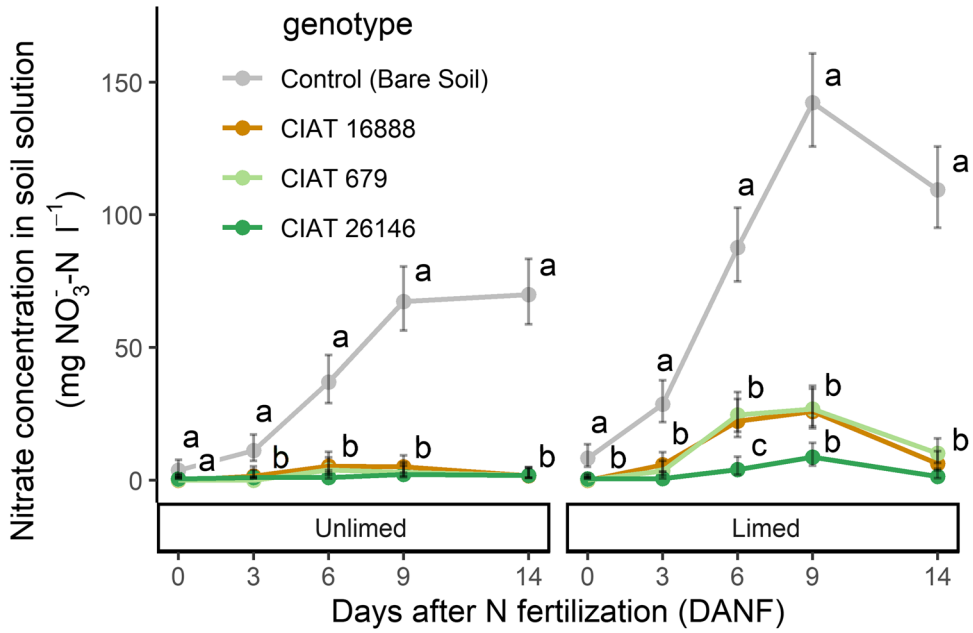

Fig. 1 Nitrate concentration in the soil solution of non-limed (left) and limed (right) soils in dependence of *B. humidicola* genotype planted over 14 days after N fertilization. Depicted values are model-based means over both soils (genotype: $p < 0.0001$). Points that share a common letter do not differ significantly at $\alpha = 0.05$ level. (Error bars = S.D.)

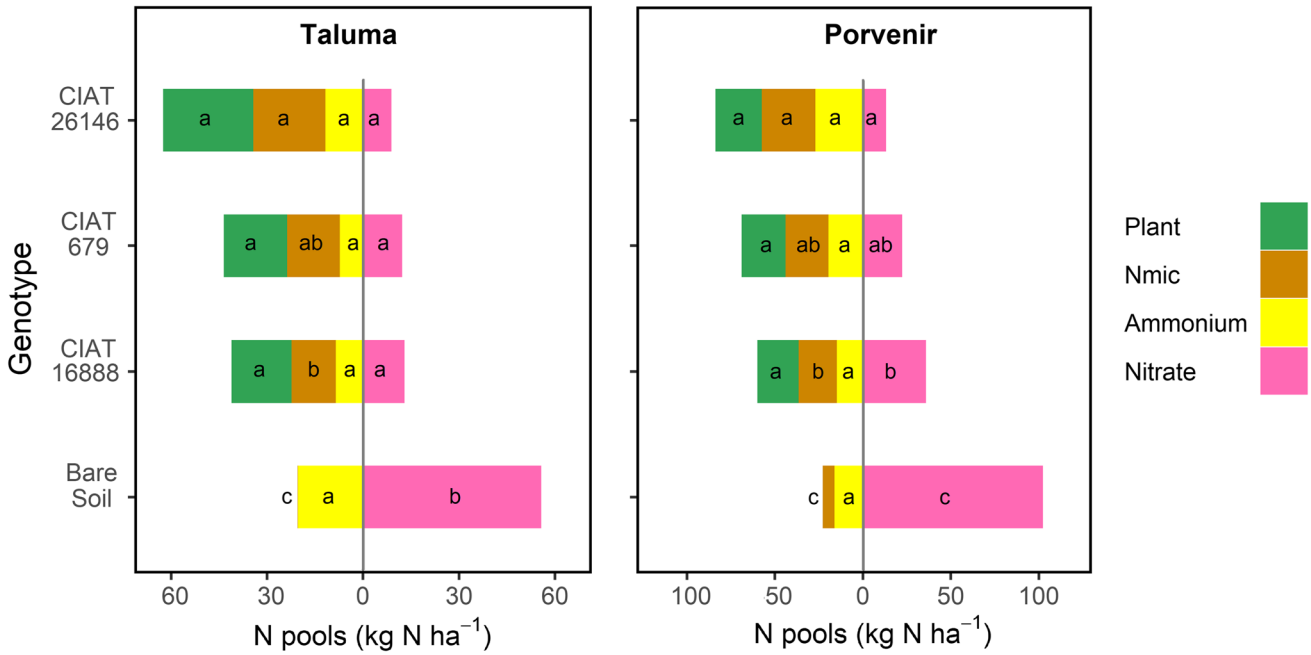

Fig. 2 Nitrogen pools (kg N ha^{-1}) 14 days after NH_4^+ fertilization at the rate of 150 kg NH_4^+-N ha^{-1} (limed soils). Pools illustrated to the left (plant N, N_{mic} and NH_4^+) represent stabile N, pools illustrated to the right (NO_3^-) represent nitrogen prone to leaching and denitrification. Depicted values are model-based means. Genotypes that share a common letter do not differ significantly in respective N pool size at $\alpha = 0.05$ level

Ex situ vs. in situ soil nitrification and N immobilization potential

To segregate direct plant competition effects (plant uptake) from plant-mediated alterations of the microbial N cycle (nitrification, immobilization), both soil nitrification and N immobilization potentials were assessed without the influence of plant roots (ex situ). This was achieved through a soil incubation approach with simultaneous monitoring of NH_4^+ and NO_3^- levels (Table 1). Soil incubation (ex situ) confirmed the results obtained from the in situ evaluation. While soils did not differ in nitrification potential, both

Table 1 In situ (pot trial) and ex situ (incubation study) estimation of nitrification and N immobilization potential. Depicted values are model-based means. Genotypes that share a common letter do not differ significantly at α = 0.05 level. Percentage values in parenthesis refer to the share of applied fertilizer N encountered in the respective pool. DANF — Days after NH_4^+ fertilization. n.d. — not detected

		Nitrification potential				Immobilization potential			
		In situ net nitrifications rates (Pot trial, 0–7 DANF) [mg N kg^{-1} soil d^{-1}]		Ex situ net nitrification rates (Incubation study) [mg N kg^{-1} soil d^{-1}]		In situ N_{mic} pools (Pot trial, 14 DANF) [kg N ha^{-1}]		Ex situ N immobilization (Incubation study) [mg N kg^{-1} soil]	
		Taluma	Porvenir	Taluma	Porvenir	Taluma	Porvenir	Taluma	Porvenir
Non-limed treatments	Bare soil	3.2 a	7.3 a	1.6 a	3.3 a	−1.7 (~0%) a	14.2 (9%) a	n.d	0.0 (0%) a
	CIAT 16888	1.4 a	1.0 b	1.5 a	1.2 b	13.2 (9%) b	29.1 (19%) b	n.d	5.3 (2%) b
	CIAT679	1.4 a	0.9 b	1.3 a	0.9 b	15.7 (10%) bc	31.6 (21%) bc	n.d	6.7 (3%) b
	CIAT 26146	1.0 a	0.8 b	0.8 a	0.2 b	21.8 (15%) c	37.7 (25%) c	n.d	3.0 (1%) b
Limed treatments	Bare soil	9.5 a	8.6 a	6.0 a	8.1 a	−1.1 (~0%) a	6.8 (5%) a	n.d	3.3 (~1%) a
	CIAT 16888	2.6 c	4.3 b	5.8 a	5.4 b	13.8 (9%) b	21.7 (14%) b	n.d	27.0 (12%) b
	CIAT 679	4.9 b	3.6 bc	6.1 a	6.2 ab	16.4 (11%) bc	24.3 (16%) bc	n.d	46.4 (20%) b
	CIAT 26146	2.0 c	1.3 c	5.0 a	3.0 c	22.5 (15%) c	30.4 (20%) c	n.d	37.0 (16%) b

liming ($p < 0.001$) and genotype ($p = 0.02$) had an influence upon nitrification. Nitrification potentials were increased by factor 2 to 10 (depending on soil and genotype) in the limed compared to the non-limed treatments. Genotype-dependent differences in nitrification potential (ex situ) were only detectable for the limed treatment of the clayey, bacteria dominated Porvenir soil, but not for the sandy, archaea dominated Taluma soil, indicating that in the limed Taluma soil differences in net nitrification observed in situ were probably related to differences in plant N uptake (Fig. 2). In the Porvenir soil, genotype CIAT 26146 showed lowest net nitrification rates ($p < 0.05$).

Regarding N immobilization, the incubation study reflected only partially the soil N_{mic} pools quantified in situ. N immobilization was not verifiable for the sandy Taluma soil. For the Porvenir soil, however, N immobilization was detectable, with a considerable magnitude in the limed variant (up to 20% of applied N), although similar in situ N_{mic} pools were determined for both non-limed and limed treatments. Generally, soils from planted pots had a higher N immobilization rate than the non-planted control ($p < 0.05$). A summary of in situ estimates of net nitrification rates and N_{mic} pools, as well as ex situ estimates of net nitrification rates and N immobilization potential is provided in Table 1.

AOA and AOB abundance

Soils were chosen to compare the influence of contrasting microbial backgrounds on nitrification dynamics. To capture functional niche occupation of both soils, abundance of archaeal (AOA) and bacterial (AOB) nitrifier groups were quantified 14 days after NH_4^+ fertilization and related to the soil N_{min} pools 7 days after NH_4^+ fertilization. In the case of Taluma, soil NH_4^+ levels were negatively correlated with AOA ($R^2 = -0.74$, $p < 0.001$, Fig. 3), whereas soil NO_3^- levels showed a strong positive correlation with AOA ($R^2 = 0.91$, $p < 0.001$, Fig. 3). In the Porvenir soil, however, NO_3^- correlated only slightly with AOA ($R^2 = 0.44$, $p = 0.051$), but strongly with AOB ($R^2 = 0.81$, $p < 0.001$). These tendencies were confirmed with AOA to AOB ratios of 5:1 versus 1:5 for the Taluma and Porvenir soil, respectively.

Correlation between plant biomass, soil microbial and mineral N pools

Root biomass showed a strong positive correlation with N_{mic}, especially in the Porvenir soil ($R^2 = 0.84$, $p < 0.01$) (Fig. 4a), and a strong negative correlation ($R^2 = -0.68$, $p < 0.001$) with soil NO_3^- (Fig. 4b). Root biomass did not correlate with soil NH_4^+ and shoot biomass and plant N uptake did not correlate with any factor ($p > 0.05$, data not shown).

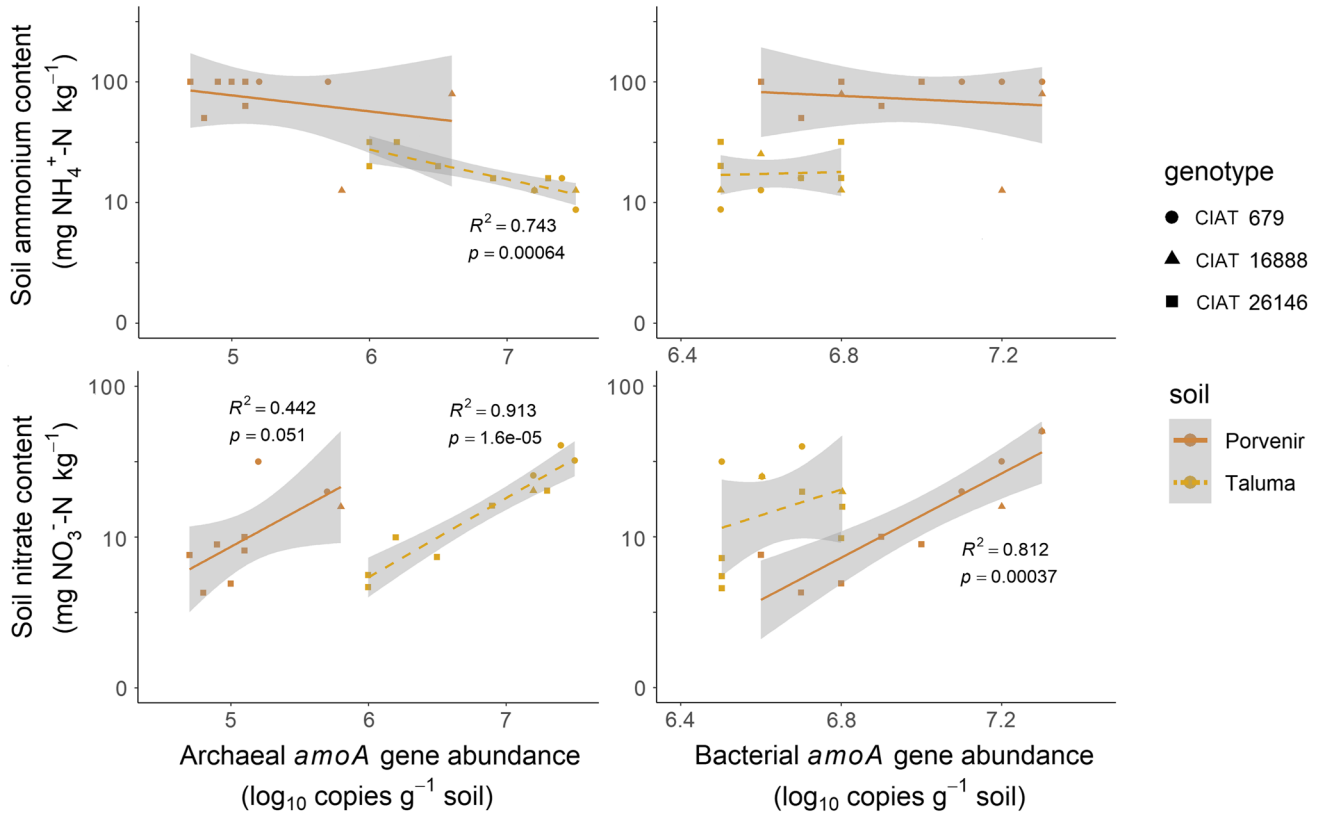

Fig. 3 Logarithmic presentation of regressions between AOA (left) or AOB (right) abundances 14 days after NH_4^+ fertilization and soil ammonium (top) and nitrate (bottom) content 7 days after NH_4^+ fertilization for Taluma (dashed line) and Porvenir (continuous line) soil. R^2 and p-values are only displayed for correlations that were statistically significant at $\alpha = 0.05$. Gray area represents 95% confidence interval

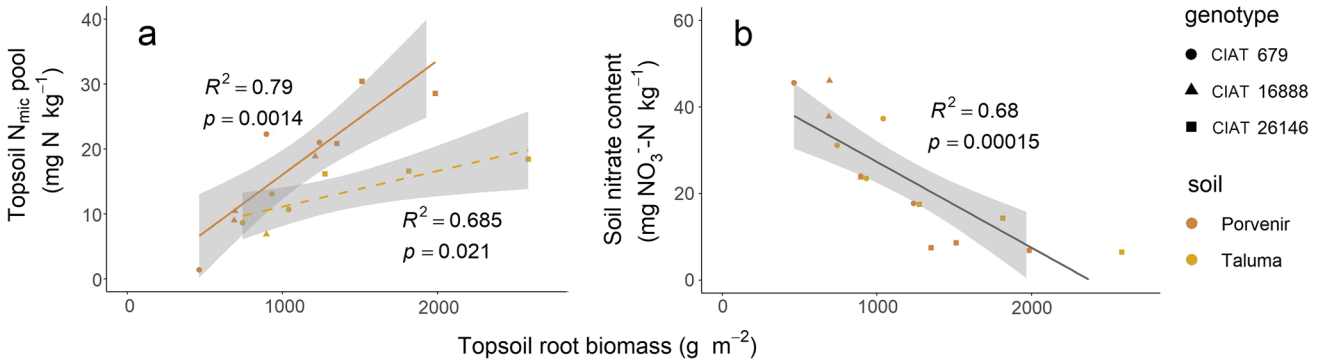

Fig. 4 Regression between topsoil (0–10 cm) root biomass (g m^{-2} DM), and (**a**) topsoil microbial N pool 14 days after NH_4^+ fertilization, and (**b**) topsoil NO_3^- content 7 days after NH_4^+ fertilization for planted pots under lime treatment. Gray shading represents 95% confidence interval

Discussion

Microbial N immobilization and plant NO_3^-uptake control net nitrification rates

The evaluation of soil nitrification dynamics confirmed the capacity of *B. humidicola* to reduce net nitrification rates in both soils. Although liming boosted overall nitrification dynamics by a factor of 3 to 4, the genotype dependent reduction of net nitrification by 50 to 85% compared to the non-planted control (in situ evaluation) remained relatively constant, irrespective of liming. These findings confirm our first hypothesis that *B. humidicola* is capable

of reducing net nitrification rates even under conditions favorable to nitrification.

On the other hand, it was deduced that the observed effects reflect dynamics expected for ammonia oxidizer control through effective resource competition rather than allelopathic inhibition (BNI). In this sense, especially the large differences in net nitrification between planted and non-planted soils on the one hand, and low inter-genotypic variation inverting commonly suggested BNI ratings (Karwat et al. 2019; Moreta et al. 2014; Nuñez et al. 2018) on the other, suggested that reduced soil NO_3^- levels could predominantly be attributed to a net increase of heterotrophic N immobilization (confirmed both in situ and ex situ) and plant competition for N (confirmed by in situ data). This assumption aligns with the ongoing controversy on the ecological reasons for reduced net nitrification in certain climax ecosystems, i.e. grasslands and coniferous forests (Davidson et al. 1992; Nardi et al. 2020; Vazquez et al. 2020). Precisely, it reflects our second hypothesis whether reduced net nitrification rates can be attributed to a BNI-induced reduction of gross nitrification rates. Several researchers favor a plant exerted control of gross nitrification through allelopathic inhibition of ammonia oxidizers, i.e. BNI, as explanation for low soil NO_3^- levels (Lodhi and Killingbeck 1980; Subbarao et al. 2009; Ward et al. 1997). Others emphasize heterotrophic competition for N and efficient plant NO_3^- uptake as the dominant factors (Davidson et al. 1992; Stark and Hart 1997; Vazquez et al. 2020). The latter position is corroborated by the higher competitiveness of microbial heterotrophs for mineralized or deposited NH_4^+-N (Hodge et al. 2000; Kuzyakov and Xu 2013; Verhagen et al. 1992) and dominant (or even preferential) NO_3^- feeding as described for many plants adapted to nitrifying ecosystems, i.e. dryland and calcareous soils (Li et al. 2013; Marschner 1995; Zhang et al. 2018) and certain grasslands (Jackson et al. 1989; Schimel et al. 1989).

How effectively soil heterotrophs immobilize and hence prevent mineralized or applied NH_4^+ from nitrification, depends on the size and activity of the heterotrophic community, which in turn is controlled by plant derived C inputs, e.g. root exudation and turnover (Karwat et al. 2017; Leptin et al. 2021; Vazquez et al. 2020). This fundamental relationship was confirmed by the strongly positive correlations between root system size (approximated through topsoil root biomass) and soil microbial N pools, highlighting the importance of an active soil microbial community in preventing NH_4^+-N from being nitrified (Fig. 5 — mechanism 1 "*Plant facilitated heterotrophic N immobilization*"). Interestingly, the incubation study proved high net immobilization, despite overall low soil microbial biomass (C_{mic} quantified in situ, data not shown). This observation was corroborated by Kaye

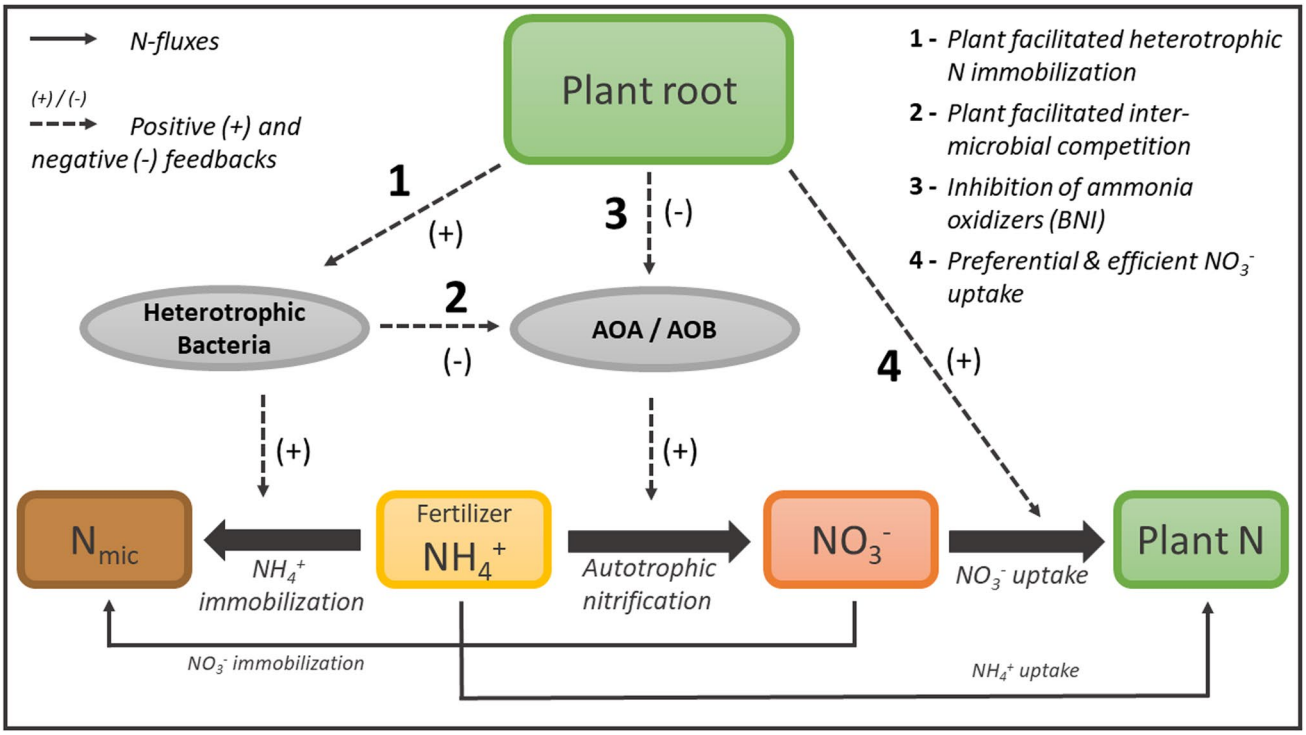

Fig. 5 Conceptualized mechanisms leading to reduced soil NO_3^- levels (net nitrification rates): (1) plant facilitated heterotrophic N immobilization, (2) plant facilitated inter-microbial competition, (3) plant exerted inhibition of ammonia oxidizers (BNI), and (4) preferential and efficient NO_3^- uptake by plants

and Hart (1997) reporting long-term net N immobilization ranging between 6 and 15% of applied N in a temperate grassland soil. In our experiment, net immobilization under *B. humidicola* amounted up to 20% of applied N (both in situ and ex situ), despite high N amendments (150 kg ha^{-1} in situ, 225 mg kg^{-1} soil in the incubation study). Notably, immobilization (ex situ) increased with liming (Porvenir soil), a result in line with Garbuio et al. (2011) reporting increased activity and growth of heterotrophs after liming of Ferralsols.

B. humidicola fulfills all characteristics of a calcifuge plant adapted to low-nitrifying environments (Li et al. 2013). It is adapted to humid and low pH soil conditions with low redox potential (Miles et al. 2004), and feeds on NH_4^+ as sole N source (Castilla and Jackson 1991). The concluded NH_4^+ preference has, however, been recently questioned. Using hydroponic studies, Egenolf et al. (2020b) verified that under mixed N nutrition *B. humidicola* captured 90% of its N uptake from the NO_3^- pool. This obvious preference of NO_3^- over NH_4^+ finds support in the high affinity of the NO_3^- transporter systems described for *Brachiaria* spp. (Nakamura et al. 2005; Silveira et al. 2014). In the case of *B. humidicola* for instance, the extraordinarily low K_m value (Michaelis constant) for NO_3^- uptake (4.4 µmol l^{-1}) has been reported to lie far below the K_m value for NH_4^+ uptake (36.7 µmol l^{-1}), indicating efficient depletion of the soil NO_3^- pool and — at least under N limited conditions — preference for NO_3^- (Nakamura et al. 2005). It could be further hypothesized that this physiological affinity for NO_3^- is accentuated in a soil-based system, in which higher mobility of NO_3^- compared to NH_4^+ and mass flow movement towards the root discriminates against NH_4^+. This assumption finds support by Karwat et al. (2019) verifying that under field conditions *B. humidicola* readily feeds on nitrification derived NO_3^- with uptake rates responding linearly to soil NO_3^- concentrations. Consequently — for the special case of this study simulating an intensified grassland system — it was assumed that *B. humidicola* was almost exclusively feeding on NO_3^-, due to the following reasons: (1) the above-described physiological affinity towards NO_3^-, (2) a high NO_3^- availability in the soil solution within days after fertilization, and (3) disabled NH_4^+ uptake due to energy shortage in the root as a result of plant pruning (Castilla and Jackson, 1991). Accordingly, it was concluded that beyond altering soil microbial N fluxes, *B. humidicola* directly affected net nitrification rates by strongly depleting the soil NO_3^- pool. Preferential and efficient NO_3^- feeding is thus proposed as additional factor pivotal to low net nitrification (Fig. 5 — mechanism 4 "*Preferential & efficient NO_3^- uptake*"). As for heterotrophic immobilization, root system size is fundamental for an effective reduction of soil NO_3^- levels and hence net nitrification rates. This was confirmed by the negative correlation between root biomass and topsoil NO_3^- levels, a finding in line with Nuñez et al. (2018).

Inter-microbial competition rather than BNI determine AOA and AOB abundances

Unlimed soils revealed constantly low soil NO_3^- levels both 7 and 14 days after NH_4^+ fertilization, indicating uniformly low net nitrification rates within these soils. The inter-genotypic differences between soil NH_4^+ levels, especially 7 days after NH_4^+ fertilization were therefore probably not related to differences in net nitrification, but to differences in heterotrophic NH_4^+ immobilization. In contrast, in the limed variants, decreasing soil NH_4^+ levels were mirrored by increasing soil NO_3^- levels, indicating inter-genotypic differences in net nitrification. Based on the presented dataset, it could not be concluded with confidence, whether these differences in net nitrification were based on differences in gross nitrification. However, the tendency for higher topsoil NH_4^+ pools — despite high N immobilization (N_{mic} pools, in situ) and higher (Taluma) or constant (Porvenir) plant N uptake rates — were taken as indication for reduced gross nitrification rates under genotype CIAT 26146, as compared to genotypes CIAT 16888 and CIAT 679. This reasoning is based on the argument that alternative explanations, e.g. increased gross mineralization or DNRA rates, seemed improbable under the given circumstances. Interestingly, these differences in nitrification were probably not related to BNI, as the observed genotype performance disagreed with previous BNI ratings (Karwat et al. 2019; Moreta et al. 2014; Nuñez et al. 2018). In this sense, the poor BNI performance of genotypes CIAT 679 and CIAT 16888 (in situ), both generally considered as high BNI cultivars, suggested that the BNI effect was impaired under regular N fertilization. Accordingly, our second hypothesis could not be confirmed. This finding is in line with Castoldi et al. (2013) and was presumably attributed to (1) low NI exudation as a result of reduced plant terpene and especially phenolics synthesis due to constant rejuvenation through pruning (Burney and Jacobs 2012) and N inputs (Bryant et al. 1987; Hussain 2016; Lavola and Julkunen-Tiitto 1994) and (2) a reduced effectiveness of plant allelopathics and especially nitrification inhibitors in N abundant (high nitrifying) soil environments (Blum et al. 1993; White 1991).

Interestingly, our data indicated inverse genotypic effects on the abundance of bacterial heterotrophs on the one hand, and abundances of both archaeal heterotrophs and ammonia oxidizers (AOA, AOB) on the other. In both soils, the abundance of heterotrophic bacteria increased with roots system size (CIAT 16888 < CIAT 679 < CIAT 26146), whereas those of archaea, AOA and AOB decreased (Fig. S1 and S2). Hence, it could be speculated that the plant-dependent reduction of nitrifier populations

was due to increased inter-microbial competition between bacterial heterotrophs (stimulated by the plant) and nitrifiers. A possible explanation for the latter theory would be long-term competition for substrate N, proposed to be the predominant control of ammonia oxidizer abundance (Xiao et al. 2020). In our experiment, especially the concomitant reduction of *amoA* and archaeal 16S rRNA gene abundances favors the conceptualized theory of inter-microbial competition (Fig. 5 — mechanism 2 "*Plant facilitated inter-microbial competition*") rather than BNI (Fig. 5 — mechanism 3 "*Inhibition of nitrifiers (BNI)*") as determinant of nitrifier abundance. These findings are in line with Subbarao et al. (2009), confirming a reduction of both *amoA* and archaeal 16S rRNA gene abundances under *Brachiaria* spp.

B. humidicola suppresses both AOA and AOB abundance

The third hypothesis in this study addressed the question whether *B. humidicola* is able to control nitrification under both archaea and bacteria dominated soil microbial backgrounds. The contrasting soil microbial community compositions (Fig. S1, Table S2) between both soils were mainly attributed to differences in soil texture and soil organic matter content (Girvan et al. 2003; Rasche et al. 2006; Sessitsch et al. 2001). Although both factors have been acknowledged to influence soil N dynamics (NH_4^+ sorption to clay minerals, N mineralization dynamics), the soil microbial community composition was considered as the primary determinant of N immobilization and nitrification. The presented correlations between the abundance of nitrifier groups (AOA, AOB) and soil N pools as well as the changes of nitrifier community size within 14 days after NH_4^+ fertilization (Fig. S1) suggested that nitrification was predominantly controlled by AOA in the archaea dominated Taluma soil, whereas by AOB in the bacteria dominated Porvenir soil. This was unexpected, as pH correction by liming and regular N inputs generally favor AOB over AOA (Di et al. 2009; Prosser and Nicol 2012; Thion et al. 2016) and have been reported to provoke community shifts towards AOB (Zhao et al. 2018). Furthermore, genotypic effects on soil microbial populations were consistent, irrespective of the soil microbial environment. Genotype CIAT 26146 always favored the proliferation of bacteria, along with a suppressive effect on archaea and both nitrifying groups. Hence, under the described conditions, hypothesis 3 could be accepted. Due to the ambiguity regarding the underlying mechanism of ammonia oxidizer control (BNI versus plant facilitated inter-microbial competition, Fig. 5), we remain cautious to infer any conclusion towards BNI in general.

Conclusions

This study confirmed the capacity of *B. humidicola* to reduce soil net nitrification rates, irrespective of soil pH, microbial background and genotype. The reduction of net nitrification, however, was largely dependent on microbial N immobilization and efficient plant N (probably NO_3^-) uptake, reflected by an equal N partitioning of NH_4^+-N towards the microbial N pool, the plant N pool and the soil NO_3^- pool. If the reduction of net nitrification was at least partially attributable to a reduction of gross nitrification cannot be answered with certainty. Furthermore, it remains unclear, whether a putative reduction of gross nitrification under genotype 26146 can be attributed to a plant exerted control of nitrifiers through BNI or rather to the facilitation of bacterial heterotrophs and subsequent suppression of ammonia oxidizers via competition.

Therefore, we generally conclude, that reduced net nitrification rates under *B. humidicola* were the result of several, interacting mechanisms: (1) heterotrophic N immobilization, (2) plant facilitated inter-microbial competition, (3) BNI, and (4) preferential and efficient NO_3^- uptake by plants. Consequently, we proposed a broader concept on the plant exerted control of soil nitrification (Fig. 5). Which of the proposed mechanisms predominates depends strongly on the management intensity. Accordingly, under intensification of *B. humidicola* pasture systems (soil liming, N fertilization), heterotrophic N immobilization and efficient plant N uptake rather than BNI represent the dominant factors controlling net nitrification rates, irrespective of the soil microbial background. This hypothesis should be considered upon future studies, advising a thorough investigation and dissection of the different N pathways affecting soil net nitrification rates via ^{15}N stable isotope approaches.

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Data availability Not applicable.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

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References

Blum U, Gerig TM, Worsham AD, King LD (1993) Modification of allelopathic effects of p-coumaric acid on morning-glory seedling biomass by glucose, methionine, and nitrate. J Chem Ecol 19:2791–2811. https://doi.org/10.1007/BF00980584

Brookes PC, Kragt JF, Powlson DS, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: the effects of fumigation time and temperature. Soil Biol Biochem 17:831–835. https://doi.org/10.1016/0038-0717(85)90143-9

Bryant JP, Clausen TP, Reichardt PB, McCarthy MC, Werner RA (1987) Effect of nitrogen fertilization upon the secondary chemistry and nutritional value of quaking aspen (Populus tremuloides Michx.) leaves for the large aspen tortrix (Choristoneura conflictana (Walker)). Oecologia 73:513–517. https://doi.org/10.1007/BF00379408

Burney OT, Jacobs DF (2012) Terpene production and growth of three Pacific Northwest conifers in response to simulated browse and nutrient availability. Trees 26:1331–1342. https://doi.org/10.1007/s00468-012-0709-4

Byrnes RC, Nùñez J, Arenas L, Rao I, Trujillo C, Alvarez C, Arango J, Rasche F, Chirinda N (2017) Biological nitrification inhibition by Brachiaria grasses mitigates soil nitrous oxide emissions from bovine urine patches. Soil Biol Biochem 107:156–163. https://doi.org/10.1016/j.soilbio.2016.12.029

Castilla CE, Jackson WA (1991). Nitrogen uptake mechanism in two Brachiaria species: Y-315. In: McBride, T.P. (Ed.), Tropsoils Technical Report, 1988–1989. Raleigh NC, pp. 164–167.

Castoldi G, Reis JGd, Pivetta LA, Rosolem CA (2013) Soil nitrogen dynamics after Brachiaria desiccation. Rev Bras Ciênc Solo 37:1620–1627. https://doi.org/10.1590/S0100-06832013000600018

Cochrane T, Salinas J, Sánchez P (1980) An equation for liming acid mineral soils to compensate crop aluminium tolerance. Trop Agric 57(2):133–140

Cohn AS, Mosnier A, Havlík P, Valin H, Herrero M, Schmid E, O'Hare M, Obersteiner M (2014) Cattle ranching intensification in Brazil can reduce global greenhouse gas emissions by sparing land from deforestation. Proc Natl Acad Sci USA 111:7236–7241. https://doi.org/10.1073/pnas.1307163111

Davidson EA, Hart SC, Firestone MK (1992) Internal cycling of nitrate in soils of a mature coniferous forest. Ecology 73:1148–1156. https://doi.org/10.2307/1940665

Di HJ, Cameron KC, Shen JP, Winefield CS, O'Callaghan M, Bowatte S, He JZ (2009) Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. Nature Geosci 2:621–624. https://doi.org/10.1038/ngeo613

Egenolf K, Conrad J, Schöne J, Braunberger C, Beifuß U, Walker F, Nuñez J, Arango J, Karwat H, Cadisch G, Neumann G, Rasche F (2020) Brachialactone isomers and derivatives of Brachiaria humidicola reveal contrasting nitrification inhibiting activity. Plant Physiol Bioch 154:491–497. https://doi.org/10.1016/j.plaphy.2020.06.004

Egenolf K, Verma S, Schöne J, Klaiber I, Arango J, Cadisch G, Neumann G, Rasche F (2020) Rhizosphere pH and cation-anion balance determine exudation of nitrification inhibitor 3-epi-brachialactone suggesting release via secondary transport. Physiol Plant 172:116–123. https://doi.org/10.1111/ppl.13300

Egenolf K (2021) Biochemical and ecophysiological characterization of BNI (Biological Nitrification Inhibition) by Brachiaria humidicola, PhD Dissertation submitted to the Faculty of Agricultural Sciences, University of Hohenheim

Garbuio FJ, Jones DL, Alleoni LRF, Murphy DV, Caires EF (2011) Carbon and nitrogen dynamics in an oxisol as affected by liming and crop residues under No-Till. Soil Sci Soc Am J 75:1723–1730. https://doi.org/10.2136/sssaj2010.0291

Girvan MS, Bullimore J, Pretty JN, Osborn AM, Ball AS (2003) Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. Appl Environ Microbiol 69:1800–1809. https://doi.org/10.1128/AEM.69.3.1800-1809.2003

Gopalakrishnan S, Subbarao GV, Nakahara K, Yoshihashi T, Ito O, Maeda I, Ono H, Yoshida M (2007) Nitrification Inhibitors from the root tissues of Brachiaria humidicola, a tropical grass. J Agric Food Chem 55:1385–1388. https://doi.org/10.1021/jf062593o

Hodge A, Robinson D, Fitter A (2000) Are microorganisms more effective than plants at competing for nitrogen? Trends Plant Sci 5:304–308. https://doi.org/10.1016/S1360-1385(00)01656-3

Hussain SM (2016) Primary and secondary metabolites production in signal grass around the year under nitrogen fertilizer. https://www.teses.usp.br/teses/disponiveis/74/74131/tde-14072016-094209/en.php

ISO 7890–3 (1988) Water quality — determination of nitrate — part 3: spectrometric method using sulfosalicylic acid. https://www.iso.org/standard/14842.html

ISO 14240–2 (1997) Determination of soil microbial biomass. Part 2: fumigation-extraction method. https://www.iso.org/standard/23951.html

IUSS working group WRB (2006) World reference base for soil resources: a framework for international classification, correlation and communication. World soil resources reports, vol 103. Food and Agriculture Organization of the United Nations FAO, Rome

Jackson LE, Schimel JP, Firestone MK (1989) Short-term partitioning of ammonium and nitrate between plants and microbes in an annual grassland. Soil Biol Biochem 21:409–415. https://doi.org/10.1016/0038-0717(89)90152-1

Jörgensen RG (1996) The fumigation-extraction method to estimate soil microbial biomass: calibration of the K_{EC} value. Soil Biol Biochem 28:25–31. https://doi.org/10.1016/0038-0717(95)00102-6

Kaimowitz D, Angelsen A (2008) Will livestock intensification help save Latin America's tropical forests? J Sustain Forestry 27:6–24. https://doi.org/10.1080/10549810802225168

Karwat H, Moreta D, Arango J, Núñez J, Rao I, Rincón Á, Rasche F, Cadisch G (2017) Residual effect of BNI by Brachiaria humidicola pasture on nitrogen recovery and grain yield of subsequent maize. Plant Soil 420:389–406. https://doi.org/10.1007/s11104-017-3381-z

Karwat H, Egenolf K, Nuñez J, Rao I, Rasche F, Arango J, Moreta D, Arevalo A, Cadisch G (2018) Low 15N natural abundance in shoot tissue of Brachiaria humidicola is an indicator of reduced N losses due to biological nitrification inhibition (BNI). Front Microbiol 9:2383. https://doi.org/10.3389/fmicb.2018.02383

Karwat H, Sparke M-A, Rasche F, Arango J, Nuñez J, Rao I, Moreta D, Cadisch G (2019) Nitrate reductase activity in leaves as a plant physiological indicator of in vivo biological nitrification inhibition by Brachiaria humidicola. Plant Physiol Biochem 137:113–120. https://doi.org/10.1016/j.plaphy.2019.02.002

Kaur-Bhambra J, Wardak DLR, Prosser JI, Gubry-Rangin C (2021) Revisiting plant biological nitrification inhibition efficiency using multiple archaeal and bacterial ammonia-oxidising cultures. Biol Fert Soils. https://doi.org/10.1007/s00374-020-01533-1

Kaye JP, Hart SC (1997) Competition for nitrogen between plants and soil microorganisms. Trends Ecol Evol 12:139–143. https://doi.org/10.1016/S0169-5347(97)01001-X

Kempers AJ, Zweers A (1986) Ammonium determination in soil extracts by the salicylate method. Commun Soil Sci Plant 17(7):715–723. https://doi.org/10.1080/00103628609367745

Kreidenweis U, Humpenöder F, Kehoe L, Kuemmerle T, Bodirsky BL, Lotze-Campen H, Popp A (2018) Pasture intensification is insufficient to relieve pressure on conservation priority areas in open agricultural markets. Glob Chang Biol 24:3199–3213. https://doi.org/10.1111/gcb.14272

Kuzyakov Y, Xu X (2013) Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. New Phytol 198:656–669. https://doi.org/10.1111/nph.12235

Lavola A, Julkunen-Tiitto R (1994) The effect of elevated carbon dioxide and fertilization on primary and secondary metabolites in Birch, Betula pendula (Roth). Oecologia 99:315–321

Leptin A, Whitehead D, Anderson CR, Cameron KC, Lehto NJ (2021) Increased soil nitrogen supply enhances root-derived available soil carbon leading to reduced potential nitrification activity. Appl Soil Ecol 159:103842. https://doi.org/10.1016/j.apsoil.2020.103842

Li S-X, Wang Z-H, Stewart BA (2013) Responses of crop plants to ammonium and nitrate N. Adv Agron 118:205–397. https://doi.org/10.1016/B978-0-12-405942-9.00005-0

Li P-N, Herrmann J, Tolar BB, Poitevin F, Ramdasi R, Bargar JR, Stahl DA, Jensen GJ, Francis CA, Wakatsuki S, van den Bedem H (2018) Nutrient transport suggests an evolutionary basis for charged archaeal surface layer proteins. ISME J 12:2389–2402. https://doi.org/10.1038/s41396-018-0191-0

Lodhi MAK, Killingbeck KT (1980) Allelopathic inhibition of nitrification and nitrifying bacteria in a ponderosa pine (Pinus ponderosa Dougl) Community. Am J Bot 67:1423. https://doi.org/10.2307/2442870

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic Press, London

Miles JW, Do Valle CB, Rao IM, Euclides VPB (2004) Brachiaria-grasses. In: Moser LE, Burson BL, Sollenberger LE (Eds) Warm-season (C4) grasses. American Society of Agronomy; Crop Science Society of America; Soil Science Society of America, Madison, WI, pp 745–783. https://doi.org/10.2134/agronmonogr45.c1

Moreta DE, Arango J, Sotelo M, Vergara D, Rincón A, Ishitani M, Castro A, Miles J, Peters M, Tohme J, Subbarao GV, Rao IM (2014) Biological nitrification inhibition (BNI) in Brachiaria pastures: a novel strategy to improve eco-efficiency of crop-livestock systems and to mitigate climate change. Trop Grass - Forr Trop 2:88. https://doi.org/10.17138/TGFT(2)88-91

Nakamura T, Miranda CHB, Ohwaki Y, Valéio JR, Kim Y, Macedo MCM (2005) Characterization of nitrogen utilization by Brachiaria grasses in Brazilian savannas (Cerrados). Soil Sci Plant Nutr 51:973–979. https://doi.org/10.1111/j.1747-0765.2005.tb00136.x

Nakamura S, Saliou PS, Takahashi M, Ando Y, Subbarao GV (2020) The contribution of root turnover on biological nitrification inhibition and its impact on the ammonia-oxidizing archaea under Brachiaria cultivations. Agronomy 10:1003. https://doi.org/10.3390/agronomy10071003

Nardi P, Laanbroek HJ, Nicol GW, Renella G, Cardinale M, Pietramellara G, Weckwerth W, Trinchera A, Ghatak A, Nannipieri P (2020) Biological nitrification inhibition in the rhizosphere: determining interactions and impact on microbially mediated processes and potential applications. FEMS Microbiol Rev 44:874–908. https://doi.org/10.1093/femsre/fuaa037

Norton JM (2008) Nitrification in agricultural soils. In: Schepers JS, Raun W (eds) Nitrogen in agricultural systems. American Society of Agronomy, Madison, WI, pp 173–199

Norton JM, Firestone MK (1996) N dynamics in the rhizosphere of Pinus ponderosa seedlings. Soil Biol Biochem 28:351–362. https://doi.org/10.1016/0038-0717(95)00155-7

Nuñez J, Arevalo A, Karwat H, Egenolf K, Miles J, Chirinda N, Cadisch G, Rasche F, Rao I, Subbarao G, Arango J (2018) Biological nitrification inhibition activity in a soil-grown biparental population of the forage grass, Brachiaria humidicola. Plant Soil 426:401–411. https://doi.org/10.1007/s11104-018-3626-5

Prosser JI, Nicol GW (2012) Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche specialisation and differentiation. Trends Microbiol 20:523–531. https://doi.org/10.1016/j.tim.2012.08.001

R Core Team (2018) R: a language and environment for statistical computing. https://www.R-project.org

Rao IM, Ayarza MA, Garcia R (1995) Adaptive attributes of tropical forage species to acid soils I. Differences in plant growth, nutrient acquisition and nutrient utilization among C4 grasses and C3 legumes. J Plant Nutr 18:2135–2155. https://doi.org/10.1080/01904169509365052

Rasche F, Hödl V, Poll C, Kandeler E, Gerzabek MH, van Elsas JD, Sessitsch A (2006) Rhizosphere bacteria affected by transgenic potatoes with antibacterial activities compared with the effects of soil, wild-type potatoes, vegetation stage and pathogen exposure. FEMS Microbiol Ecol 56:219–235. https://doi.org/10.1111/j.1574-6941.2005.00027.x

Rasche F, Musyoki MK, Rohl C, Muema EK, Vanlauwe B, Cadisch G (2014) Lasting influence of biochemically contrasting organic inputs on abundance and community structure of total and proteolytic bacteria in tropical soils. Soil Biol Biochem 74:204–213. https://doi.org/10.1016/j.soilbio.2014.03.017

Rudel TK, Paul B, White D, Rao IM, van der Hoek R, Castro A, Boval M, Lerner A, Schneider L, Peters M (2015) LivestockPlus: forages, sustainable intensification, and food security in the tropics. Ambio 44:685–693. https://doi.org/10.1007/s13280-015-0676-2

Schimel JP, Jackson LE, Firestone MK (1989) Spatial and temporal effects on plant-microbial competition for inorganic nitrogen in a California annual grassland. Soil Biol Biochem 21:1059–1066. https://doi.org/10.1016/0038-0717(89)90044-8

Sessitsch A, Weilharter A, Gerzabek MH, Kirchmann H, Kandeler E (2001) Microbial population structures in soil particle size

fractions of a long-term fertilizer field experiment. Appl Environ Microbiol 67:4215–4224. https://doi.org/10.1128/aem.67.9.4215-4224.2001

Silveira CP, Lima JE, Lavres Jr. J, Bendassoli JA, Figueira AVO, Abdalla AL (2014) Nitrogen uptake and expressions of nitrate transporter genes in two tropical forage grasses. 51st Reunião Annual da Sociedade Brasileira de Zootecnia, Barra dos Coqueiros, Sergipe, Brasil

Stark JM, Hart SC (1997) High rates of nitrification and nitrate turnover in undisturbed coniferous forests. Nature 385:61–64. https://doi.org/10.1038/385061a0

Strassburg BBN, Latawiec AE, Barioni LG, Nobre CA, da Silva VP, Valentim JF, Vianna M, Assad ED (2014) When enough should be enough: Improving the use of current agricultural lands could meet production demands and spare natural habitats in Brazil. Global Environ Chang 28:84–97. https://doi.org/10.1016/j.gloenvcha.2014.06.001

Srikanthasamy T, Leloup J, N'Dri AB, Barot S, Gervaix J, Koné AW, Koffi KF, Le Roux X, Raynaud X, Lata J-C (2018) Contrasting effects of grasses and trees on microbial N-cycling in an African humid savanna. Soil Biol Biochem 117:153–163. https://doi.org/10.1016/j.soilbio.2017.11.016

Subbarao GV, Ishikawa T, Ito O, Nakahara K, Wang HY, Berry WL (2006) A bioluminescence assay to detect nitrification inhibitors released from plant roots: a case study with Brachiaria humidicola. Plant Soil 288:101–112. https://doi.org/10.1007/s11104-006-9094-3

Subbarao GV, Wang HY, Ito O, Nakahara K, Berry WL (2007) NH4+ triggers the synthesis and release of biological nitrification inhibition compounds in Brachiaria humidicola roots. Plant Soil 290:245–257. https://doi.org/10.1007/s11104-006-9156-6

Subbarao GV, Nakahara K, Ishikawa T, Yoshihashi T, Ito O, Ono H, Ohnishi-Kameyama M, Yoshida M, Kawano N, Berry WL (2008) Free fatty acids from the pasture grass Brachiaria humidicola and one of their methyl esters as inhibitors of nitrification. Plant Soil 313:89–99. https://doi.org/10.1007/s11104-008-9682-5

Subbarao GV, Nakahara K, Hurtado MP, Ono H, Moreta DE, Salcedo AF, Yoshihashi AT, Ishikawa T, Ishitani M, Ohnishi-Kameyama M, Yoshida M, Rondon M, Rao IM, Lascano CE, Berry WL, Ito O (2009) Evidence for biological nitrification inhibition in Brachiaria pastures. Proc Natl Acad Sci USA 106:17302–17307. https://doi.org/10.1073/pnas.0903694106

Sylvester Bradley R, Mosquera D, Méndez JE (1988) Inhibition of nitrate accumulation in tropical grassland soils: effect of nitrogen fertilization and soil disturbance. J Soil Sci 39:407–416. https://doi.org/10.1111/j.1365-2389.1988.tb01226.x

Thion CE, Poirel JD, Cornulier T, de Vries FT, Bardgett RD, Prosser JI (2016) Plant nitrogen-use strategy as a driver of rhizosphere archaeal and bacterial ammonia oxidiser abundance. FEMS Microbiol Ecol 92:1–11. https://doi.org/10.1093/femsec/fiw091

Vázquez E, Teutscherova N, Dannenmann M, Töchterle P, Butterbach-Bahl K, Pulleman M, Arango J (2020) Gross nitrogen transformations in tropical pasture soils as affected by Urochloa genotypes differing in biological nitrification inhibition (BNI) capacity. Soil Biol Biochem 151:108058. https://doi.org/10.1016/j.soilbio.2020.108058

Verhagen FJM, Duyts H, Laanbroek HJ (1992) Competition for ammonium between nitrifying and heterotrophic bacteria in continuously percolated soil columns. Appl Environ Microb 58:3303–3311. https://aem.asm.org/content/58/10/3303.short

Ward BB, Courtney KJ, Langenheim JH (1997) Inhibition of Nitrosomonas europaea by monoterpenes from Coastal Redwood (Sequoia sempervirens) in whole-cell studies. J Chem Ecol 23:2583–2598. https://doi.org/10.1023/B:JOEC.0000006668.48855.b7

Wessén E, Söderström M, Stenberg M, Bru D, Hellman M, Welsh A, Thomsen F, Klemedtson L, Philippot L, Hallin S (2011) Spatial distribution of ammonia-oxidizing bacteria and archaea across a 44-hectare farm related to ecosystem functioning. ISME J 5:1213–1225. https://doi.org/10.1038/ismej.2010.206

White C (1991) The role of monoterpenes in soil nitrogen cycling processes in ponderosa pine. Biogeochemistry 12. https://doi.org/10.1007/BF00002625

Xiao R, Qiu Y, Tao J, Zhang X, Chen H, Reberg-Horton SC, Shi W, Shew HD, Zhang Y, Hu S (2020) Biological controls over the abundances of terrestrial ammonia oxidizers. Global Ecol Biogeogr 29:384–399. https://doi.org/10.1111/geb.13030

Zhalnina K, Dias R, de Quadros PD, Davis-Richardson A, Camargo FAO, Clark IM, McGrath SP, Hirsch PR, Triplett EW (2015) Soil pH determines microbial diversity and composition in the park grass experiment. Microb Ecol 69:395–406. https://doi.org/10.1007/s00248-014-0530-2

Zhang L-M, Hu H-W, Shen J-P, He J-Z (2012) Ammonia-oxidizing archaea have more important role than ammonia-oxidizing bacteria in ammonia oxidation of strongly acidic soils. ISME J 6:1032–1045. https://doi.org/10.1038/ismej.2011.168

Zhang K, Chen L, Li Y, Brookes PC, Xu J, Luo Y (2017) The effects of combinations of biochar, lime, and organic fertilizer on nitrification and nitrifiers. Biol Fert Soils 53:77–87. https://doi.org/10.1007/s00374-016-1154-0

Zhang J, Cai Z, Müller C (2018) Terrestrial N cycling associated with climate and plant-specific N preferences: a review. Eur J Soil Sci 69:488–501. https://doi.org/10.1111/ejss.12533

Zhao W, Zhang J-b, Müller C, Cai Z-c (2018) Effects of pH and mineralisation on nitrification in a subtropical acid forest soil. Soil Res 56:275. https://doi.org/10.1071/SR17087

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