Biological nitrification inhibition by rice root exudates in two different soils of Uruguay

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ABSTRACT
Rice root exudates can control nitrification by releasing biological nitrification inhibitors (BNIs), reducing nitrogen losses in agricultural soils. However, the inhibitory effect on nitrification and the abundance of ammonia oxidisers in different soil types remain unclear. Two temperate paddy soils with different organic matter contents were collected to investigate the impact on nitrification rates of two rice cultivar root exudates, El Paso 144 (O. sativa ssp. indica) and Tacuarí (O. sativa ssp. japonica). Root exudates were extracted before the tillering growth stage, and their BNI potential was evaluated in a bioassay with luminescent Nitrosomonas europaea and in a 12-day soil microcosm incubation. While exudates from Tacuarí showed stronger BNI activity in the bioassay, its nitrification inhibition in both soils was similar to that of DCD. El Paso did not show BNI activity in Salto whose organic matter content was higher. The abundance of ammonia oxidisers was not affected by root exudates or DCD, but only ammonia-oxidising bacteria had a significant positive relationship with soil nitrate. Our results demonstrated that although the bioassay showed high BNI activity, its expression in soils varied depending on the rice cultivar and the type of soil, particularly with its organic matter content.

Introduction
Rice (Oryza sativa L.) is a staple food and one of the main products of the Uruguayan agriculturally based economy, achieving average yields of 8 Mg ha⁻¹ using locally developed cultivars (Tseng et al. 2020). Agronomic rice management is associated with environmental concerns, such as increased nitrogen (N) fertiliser application for booting yields. Crop N use is inefficient and particularly low for rice, approximately 44% (Ladha et al. 2005).
The microbial process of nitrification, the aerobic two-step oxidation of ammonia (NH₃) to nitrite (NO₂⁻) and nitrate (NO₃⁻), is responsible for most soil nitrogen losses in agroecosystems (Souri 2010).

In autotrophic nitrification, the most relevant process in soils, the initial oxidation of NH₃ to hydroxylamine is catalysed by the enzyme ammonia monooxygenase (AMO), which is present in ammonia-oxidising bacteria (AOB) and ammonia-oxidising archaea (AOA) (Lan et al. 2018). The amoa gene, encoding a subunit of AMO, is a functional marker used to identify AOB and AOA (McCarty 1999).

Attempts to control nitrification in agricultural soils, blocking NH₃ oxidation by deactivating AMO, have recently become the focus of much research (Souri 2010; Souri 2016; Coskun et al. 2017). Numerous compounds, including nitrapyrin (Parkin and Hatfield 2010), dicyandiamide (DCD) (Di et al. 2009), and 3,4-dimethylpyrazole phosphate (DMPP) (Zerulla et al. 2010), have been identified to slow nitrification in agricultural systems. Despite the high cost of these compounds, their variable effectiveness due to rapid degradation rates in soils or difficulties with application, among others (Fillery 2007), has limited their adoption.

Recent research suggests that some plant roots can release nitrification inhibitory compounds, denominated biological nitrification inhibitors (BNIs) (Subbarao et al. 2006, 2009). These findings offer possibilities for using BNI as a low-cost in situ biological alternative to synthetic nitrification inhibitors. The BNI potential of root exudates was initially estimated by a bioluminescence assay using a recombinant Nitrosomonas europaea strain (harbouring luxAB genes from Vibrio harveyi) (Subbarao et al. 2006). Once checked enough BNI activity on Nitrosomonas sp. in pure cultures, it is necessary to test the effectiveness in the field (Wendeborn 2019). These methodologies were applied to the tropical grass Brachiaria humidicola (Bh), which has the highest reported BNI potential to date (Lu et al. 2019) and decreases AOB and AOA soil population size (Ishikawa et al. 2003; Nuñez et al. 2018).

Field crops have been evaluated for BNI capacity, and cereals showed variable behaviours (Subbarao et al. 2007). Several BNIs exuded from roots have been identified, such as sorgoleone and methyl 3-(4-hydroxyphenyl) propionate (MHPP) in sorghum (Subbarao et al. 2013), but BNI in staple foods such as rice, wheat, and maize has been less investigated (Coskun et al. 2017). Tanaka et al. (2010) showed that only some primitive rice genotype root exudates possessed BNI ability. However, Sun et al. (2016) confirmed BNI potential in both rice subspecies, indica and japonica, and identified 1,9-decanediol as their BNI compound. Lu et al. (2019) found that 1,9-decanediol decreased AOB and AOA abundance in three different soils and changed AOB community structure.

The ability of BNI depends not only on the plant variety but also on the growth stage of the plant, which determines root exudation, and on the soil type (Gopalakrishnan et al. 2009). However, the effects of soil type and its organic matter (OM) content have been scarcely investigated (Ipinmoroti et al. 2008; Subbarao et al. 2012).

The aim of this study was to determine whether the root exudates of the main rice cultivars sown in Uruguay, one indica and one tropical japonica, have BNI abilities in different rice paddy soils with different OM contents and decrease the abundance of AOB or AOA. We used the bioluminescence assay and a soil microcosm experiment to evaluate the BNI of root exudates of different rice growth stages over the potential soil nitrification activity and abundance of AOB and AOA.

We, therefore, hypothesised that both high-yielding rice cultivars possess different BNI activities, and that this activity differs with the soil type and is linked to the AOB population.

Materials and methods

Soil samples

The soils used in this study were collected from the top 15 cm of two temperate lowland paddy fields two months before rice sowing. The two sites represent the two main geographical zones where rice is cultivated in Uruguay. One soil was collected at Paso de la Laguna Experimental Station of the Instituto Nacional de Investigación Agropecuaria (INIA) (33°16′10″ S; 54°10′04″ W), and the other soil was collected from a commercial paddy field (31°22′10″ S; 57°27′45″ W). In this study, they are referred to as Treinta y Tres and Salto, respectively. Treinta y Tres was a Typic Argiudoll, with pH 5.7, 3.4% OM, 13 mg/g P Bray I, and 10 mg/g NO₃-N. Salto soil was a Typic Hapludert, with pH 5.7, 5.8% OM, 12 mg/g P Bray I, and 10 mg/g NO₃-N. The stones, roots, and debris were removed from the soils, partially dried for a day before being passed through a 2 mm sieve. A subsample of the sieved soils was used to determine the moisture content by drying at 105°C for 24 h. Other soil subsamples were used to determine the soil water holding capacity.

Cultivation of rice plants and collection of root exudates

Seeds of the two rice cultivars, El Paso 144 (O. sativa ssp. indica) and Tacuarí (O. sativa ssp. japonica), hereafter El
Paso and Tacuarí, were surface sterilised and pregerminated in water agar. Three germinated seeds were sown in each plastic bag (n = 4), corresponding to a cultivar and a soil type. The plastic bag (4 kg capacity) contained a mixture of each soil and washed and sterilised sand (1:1 ratio) and was fertilised with (NH₄)₃PO₄ (0.49 g per plastic bag). The plants were grown in a greenhouse for 56 days, and 60% water holding capacity was maintained with distilled water. The plants of both cultivars were harvested at 32 and 56 days after sowing, which corresponds to seedling (4 leaves) and tillering rice growth stages. According to other studies that obtained root exudates from plants growing on soil (de Vries et al. 2019), root manipulation was carefully controlled to remove the soil without injuring the roots. Following the procedure of Aulakh et al. (2001), the plastic bags were cut open, the soil around the roots was washed off with gentle water spray, and the last washing was given distilled water. The root system of each replicate was rinsed consecutively with distilled water and trap solution (1 mM NH₄Cl and 200 µM CaCl₂) and transferred to jars, where their roots were incubated in a 500 ml dark bottle containing aerated trap solution for 24 h to collect root exudates (Tanaka et al. 2010). After that, the roots were saved for dry matter determination. The trap solution was evaporated to dryness using a rotary evaporator (Buchi, R-114, Switzerland) at 40°C, resuspended in 5 ml 100% methanol, passed through a syringe-driven 0.22 µm membrane filter (Ministat, Millipore, USA) and re-evaporated twice. Finally, the concentrated sample was suspended in 25 µl of dimethyl sulfoxide (DMSO). These rice root exudates were used for the bioluminescence assay.

To test the effect of rice root exudates in soils, larger quantities of exudates were necessary. To obtain the exudates for the microcosm assay, 20 rice seedlings were hydroponically grown in each plastic box (40 × 60 × 32 cm) for a month at 25°C and a photoperiod of 16:8 h, light:dark with aerated Yoshida mineral solution (Subbarao et al. 2006). There were three replicates (boxes) for each rice cultivar whose roots were submerged in the trap solution for 24 h. The solution was filtered (0.22 µm pore) and lyophilised. The samples were resuspended in 10 ml methanol, rotoevaporated twice, and finally resuspended in 1.5 ml of distilled water (Tanaka et al. 2010). The same procedure was employed with the trap solution as a control.

**Nitrification inhibition potential bioassay**

The bioassay was performed with a recombinant luminescent *N. europaea* strain (Iizumi et al. 1998) standarised to estimate BNI potential by Subbarao et al. (2006). The strain produces bioluminescence due to the expression of the luxAB gene, which is reduced when exposed to a nitrification inhibitor. The *Nitrosomonas* strain was grown in P-medium (KH₂PO₄ 5.14 mM, Na₂HPO₄ 95.1 mM, (NH₄)₂SO₄ 18.91 mM, NaHCO₃ 5.95 mM, CaCl₂-2H₂O 0.034 mM, MgSO₄-7H₂O 0.041 mM, Fe(III) EDTA 0.0027 mM, pH 7.8) for 7 days at 150 RPM and 28°C in darkness, supplemented with kanamycin 50 mg ml⁻¹. After that, the culture was centrifuged, and the pellet obtained was resuspended in 50 ml P-medium. A mix of 2 µl of root exudate (suspending in DMSO) with 198 µl of distilled water and 250 µl of bacteria (DO 0.6) was incubated for 15 min at 15°C with continuous shaking at 700 RPM (Thermomixer Eppendorf 3420). Luminescence was measured with a Glomax 20/20 luminometer (Promega) with the injection of 25 µl of 1% n-decil-aldehyde (luciferase substrate). The luminescence was registered as the integration time between 2 and 10 s. Each sample had 4 independent replications. Root exudates from *Bh CIAT16888* with recognised nitrification inhibition activity (Arango et al. 2014) were used as a positive control (Nuñez et al. 2018).

The allylthiourea (ATU) units were calculated considering an inhibition of 80% of luminescence of 0.22 mM of ATU, according to Subbarao et al. (2006).

**Microcosm setup and measurement of potential nitrification inhibition in soils**

Soil microcosms were established as described by Nuñez et al. (2018) with some modifications. They consisted of 25 ml vials containing the equivalent to 5 g of dry homogenised soil whose 60% water holding capacity, considered optimum for nitrification (Mosier 1998), was maintained with the addition of the different treatments.

The treatments were as follows: (1) nitrogen, 0.2 g N kg⁻¹ soil provided as an (NH₄)₂SO₄ solution, (2) (NH₄)₂SO₄ plus 0.5 ml root exudate of Tacuarí, (3) (NH₄)₂SO₄ plus 0.5 ml root exudate of El Paso, (4) (NH₄)₂SO₄ plus 20% DCD, a synthetic nitrification inhibitor and (5) (NH₄)₂SO₄ plus trap solution. All treatments were prepared to contain the same concentration of N, and (NH₄)₂SO₄ solution was adjusted according to exudates and DCD N content to create nonlimiting conditions to estimate the nitrification potential. To monitor NH₄⁺ and NO₃⁻ dynamics without added N, vials with only soil and distilled water were prepared.

The microcosms were covered with Parafilm™ to allow gaseous exchange and kept at 25°C in the dark for 12 days. The NO₃⁻ content of three technical replicates was analysed at 0, 6, and 12 days to determine the nitrification dynamics, taking destructive samples for all the treatments.
Soil suspensions, 4 g of soil with 40 ml of 2 M KCl shaken for 30 min at 200 RPM, were filtered through a Whatman filter N°42. The N-NO₃⁻ content was determined colorimetrically after reduction through a Cd column (Griess-Ilosvay reaction, Mulvaney (1996)). N-NO₃⁺ was determined colorimetrically (660 nm) according to the Berthelot method (Rhine et al. 1998).

The nitrification rate in each replicate was calculated as the slope of the increase in N-NO₃⁻ concentration during the 12 days of incubation. The relative nitrification rate to the nitrogen treatment was calculated as the nitrification inhibition (%) for DCD and root exudates.

In the case where the trap solution had a nitrification inhibition effect per se, nitrification inhibition was compared to that of the trap treatment. This BNI effect of the exudate trap solution has been reported previously (Sun et al. 2016).

**Soil DNA extraction and ammonia-oxidising abundance**

The AOA and AOB abundances were estimated through real-time quantitative PCR (qPCR) using the amoA gene marker on samples of days 0 and 12 of the microcosm assay. Total genomic DNA was isolated from 0.25 g of soil using PowerSoil DNA isolation kits (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions. The extracted DNA was checked on a 0.8% agarose gel. The DNA concentration and purity were determined with NanoDrop® 2000c UV–vis spectrophotometry (USA). qPCR was performed on a Rotor-Gene 6000 thermocycler (Corbett Research Ltd., UK) using the fluorescent dye SYBR-Green I. All samples and standards were quantified in triplicate. The reaction mixture for AOA (12.5 µl) contained 6.25 µl of 2 Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific Inc.), 10 µg of bovine serum albumin, 2.5% (v/v) DMSO and 0.5 µM each primer: Arch-amoAF (5’-STA ATG GTC TGG CTT AGA CG-3’) and Arch-amoAR (5’-GCC GCC ATC CAT CTG TAT GT-3’) (Francis et al. 2005). Thermal cycling was as follows: 95°C for 10 min and 40× (95°C, 45 s; 53°C, 45 s; 72°C, 45 s; and 79°C, 15 s for data collection), and the program ended with a melt curve from 72°C to 90°C. Standard curves were generated by amplifying 10-fold dilutions of linearised pJET1.2/blunt plasmid containing the amoA gene from N. europaea. The PCR efficiency ranged from 85% to 104%, averaging 98%, and the correlation coefficient ranged from 0.91–0.99, averaging 0.96.

Gene abundances were standardised by the mass of DNA that was extracted per gram of dry soil and log10 transformed before analysis.

**Statistical analyses**

The differences between treatments were assessed by analysis of variance (ANOVA) after analysing the homogeneity of variance and the residuals by Shapiro–Wilks and Levene’s tests and verifying interactions among factors. Tukey’s HSD post hoc analysis was used to determine the significance of soil type and treatment effect on nitrification inhibition. InfoStat software was used for statistical analysis (Di Renzo et al. 2018). The significance level was α = 0.05.

Regressions were performed to understand the relationships between soil NO₃⁻ content and AOB and AOA populations.

**Results**

**BNI potential of rice root exudates**

The root exudates of the two rice cultivars grown in the two types of soil presented potential BNI activity (Figure 1) when using a bioluminescence assay with a recombinant N. europaea strain. There were no differences in the BNI activities of root exudates obtained from 36- or 56-day plants for either cultivar or type of soil. The type of soil (Salto and Treinta y Tres) did not significantly affect BNI activity. When considering all the results for the two dates and the two soil types, the rice cultivar Tacuarí presented higher BNI activity than the cultivar El Paso, with averages of 354 and 209 ATU g⁻¹ dry weight of root, respectively (Figure 1). There was no interaction between cultivar and soil type. The BNI activity expressed as inhibition percentage was 45 ± 9% for Tacuarí and 38 ± 12% for El Paso. Both rice root exudates resulted in less BNI activity than the positive
control Bh CIAT 16888, 491 ATU g⁻¹ root exudates (Figure 1).

**Nitrification inhibition in two different soils**

The nitrification rates of the two soils for the different treatments plus (NH₄)₂SO₄ amendment are shown in Figure 2. The rate of increase in N-NO₃⁻ concentration varied within the 12 days of soil incubation between Salto (higher OM) and Treinta y Tres (lower OM) soils. The mean nitrification rate with only (NH₄)₂SO₄ incubation was 0.709 and 0.623 mg N-NO₃⁻ kg⁻¹ soil dry weight day⁻¹ for Treinta y Tres and Salto soils, respectively. The addition of root exudates from both rice cultivars, El Paso and Tacuarí, and DCD significantly decreased the nitrification rate for Salto- and Treinta y Tres soils. Although not significant, the mean nitrification rates for Tacuarí were lower than those for El Paso in both soils (Figure 2). The nitrification rate of N plus the trap solution used to collect the exudates was lower than that of the N treatment only in the Salto soil (0.119 mg N-NO₃⁻ kg⁻¹ soil dry weight day⁻¹), meaning an unspecific nitrification inhibition of the trap solution in this soil. In all the treatments, the N-NH₄⁺ content remained constant over the 12 days of the experiment (133 ± 9 mg g⁻¹ soil) for Treinta y Tres soil. For Salto soil, the N-NH₄⁺ content remained constant in the N treatment (112 mg g⁻¹ soil) but increased by approximately 20% for the treatments with root exudates and DCD.

The nitrification inhibition percentage of root exudates and DCD for the two soils after subtracting the nonspecific inhibition of the trap solution is presented in Figure 3. There was an interaction between soils and cultivars, so all results were analysed together. The percentage decrease in the nitrification rate within 12 days of incubation was similar for the two rice cultivars and the synthetic nitrification inhibitor DCD (ranging from 50 to 96%) for Treinta y Tres soil. For Salto, the soil with higher OM content, the range of inhibition of exudates from Tacuarí and of DCD was similar to those in Treinta y Tres (ranging from 63 to 82%). However, considering the nonspecific nitrification inhibition in trap solution for Salto soil, root exudates from cultivar El Paso did not inhibit nitrification (Figure 3).

**Effect of root exudates on AOA and AOB abundance**

The total bacterial amoA gene copy number in Salto at the beginning ranged from 1.2 (± 0.1) to 2.1 (± 1.1) × 10⁶, whereas in Treinta, y Tres ranged from 3.7 (± 0.9) to 6.5 (± 2.3) × 10⁵ g⁻¹ dry soil weight (Figure 4). Therefore, Salto soil, with a higher OM content, had a larger AOB population size than Treinta y Tres. However, considering the nonspecific nitrification inhibition in trap solution for Salto soil, root exudates from cultivar El Paso did not inhibit nitrification (Figure 3).

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AOA gene abundance was an order of magnitude higher compared to bacterial amoA, with a mean of 1.5 × 10⁷ for Salto and 1.1 × 10⁵ g⁻¹ dry soil weight for Treinta y Tres (Figure 4). The AOA gene copy number fluctuated less than that of their bacterial counterparts in this experiment. For bacteria, the AOA population was higher in Salto soil than in Treinta y Tres (p <
The copy number of archaeal amoA increased twice after 12 days of incubation. There was no significant effect on AOA population abundance among treatments in any of the soils (Figure 4).

A significant linear correlation was observed between the soil N-N\textsubscript{3}\textsuperscript{-} concentration and AOB abundance but not with AOA (Figure 5).

**Discussion**

**Root exudates of Tacuari and El Paso rice cultivars inhibit nitrification depending on soil type**

In this work, two temperate lowland rice cultivars planted in Uruguay were evaluated for their BNI potential.

Using the luminescent *N. europaea* bioassay, the two rice cultivars presented specific BNI activity, although lower than that of the control pasture grass *Bh CIAT1688* (Figure 1). Their registered BNI activity was in the range of the highest BNI observed by Tanaka et al. (2010) for other rice varieties. Tacuari ssp. *japonica* had more BNI potential than El Paso ssp. *indica* and did not vary with the plant stages of growth tested or seedling and tillering initiation (Figure 1). However, other authors found that the inhibition of nitrification increased (Zakir et al. 2008; Sun et al. 2016) or decreased with plant maturity (Tanaka et al. 2010; Subbarao et al. 2013). These different results might be caused by the different varieties used and different growth and exudate collection conditions (Sun et al. 2016). A soil-hydroponic-hybrid approach was used for this bioassay to more realistically estimate root exudation under natural soil growth conditions (Nardi et al. 2020). The two soils compared in this work, with contrasting OM contents, did not influence rice root exudate BNI ability under the conditions of this assay (Figure 1).

Soil nitrification rates determined through microcosm incubation represent a complementary methodology to the in vitro bioassay for assessing the expression of BNI potential in soil and evaluating the behaviour of rice exudates in front of other ammonia-oxidisers apart from a sole *N. europaea* strain. The net nitrification rate was estimated from the dynamics of the N-N\textsubscript{3}\textsuperscript{-} concentration during soil incubation. The relative gross nitrification rate would have been better estimated using the \textsuperscript{15}N dilution technique (Nardi et al. 2020; Vázquez et al. 2020), as nitrate and ammonium are produced and consumed by several processes. For the soil microcosm incubation, an increase in N-NH\textsubscript{4}\textsuperscript{+} content was observed in Salto soil in the root exudate and DCD treatments, suggesting that microbial mineralisation was greater than immobilisation. On the other hand, the high inhibition of nitrification registered for root exudates of both cultivars in Treinta y Tres soil (Figure 3) might not be an indirect effect of net N immobilisation due to...
the high NH$_4^+$ concentration applied to the microcosms. In contrast, Vázquez et al. (2020) reported under a low N environment that the BNI effect was not only the result of gross nitrification suppression but also mainly the product of higher inorganic N immobilisation by microbes.

The two soil types evaluated presented similar textures, although Salto had almost twice the OM content. Soil OM might reduce the effectiveness of nitrification inhibitors either by stimulating microbial degradation activity or by reducing the bioactivity of inhibitors through absorption on the soil colloids (Rajendran 2011). However, in this case, for both soil types, the addition of rice exudates from both cultivars significantly slowed nitrification (Figure 2). However, in the Salto soil, the trap solution used for collecting rice root exudates had a nitrification rate similar to that of the exudates of El Paso (Figure 3). This nonspecific inhibition effect has been previously reported by Tanaka et al. (2010). These authors attributed the effect to the high electric conductivity of the trap solution due to the remnant NH$_4$Cl. Sun et al. (2016) proposed using only water to collect exudates to avoid this effect, and other authors used a column to remove inorganic salts (Li et al. 2021). The soil physico-chemical and biological properties of the Salto soil could have interfered with the trap solution and with El Paso root exudates. Ipinmoroti et al. (2008) suggested that in soils with high OM content, such as Salto soils, the organic compound soil matrix could interfere with BNI. However, in this work, the cultivar Tacuari had a high BNI activity in the soil with a higher OM content. The interaction between soil and cultivar for Salto soil, points out the need to characterise the inhibitory compounds exuded by these high-yielding rice cultivars and their mechanism of action.

To consider BNI as a valid alternative to regulate nitrification in rice paddy soil, the high concentration of exudates and N used in the microcosm assay must be taken into account. Souri and Neumann (2017) claimed that in the case of Bh, the release of BNI substances from root exudates may not be an active process, but a passive phenomenon induced by NH$_4^+$ used to collect them during a long period (24 h) that may have produced membrane damage. Furthermore, Sullivan et al. (2016) demonstrated that under hydroponic conditions, not all the BNI potential observed in the root tissue is exuded. However, the BNI brachilactone was identified in root exudates from Bh collected either with NH$_4$Cl or with water either by chromatography or mass spectrometry (Arango et al. 2014). In contrast, the results in Salto soil with the trap solution underline that care may be taken with exudate extraction conditions.

The nitrification inhibitor DCD was used as a positive control for nitrification inhibition. DCD is an inhibitor of...
the AMO enzyme widely used to reduce nitrification rates in soils, decrease N losses and improve efficient N use by crops (Meng et al. 2020). Inhibition of nitrification rates of approximately 99% has been reported in soil incubation experiments with DCD (Gopalakrishnan et al. 2009). Nitrification inhibition by DCD was approximately 50–60% for the two soils, similar to the inhibition produced by root exudates, except for Salto soil and the El Paso cultivar (Figure 3).

The high-yielding cultivars evaluated in this work are planted in dry soil but flooded from tillering to harvest. Root exudates were collected before the rice tillering stage (approximately 30 days after emergence) because during the first period after basal fertiliser application, BNI activity is desirable to prevent N\(\text{2O}\) emissions, a side product of nitrification. The mitigation of N\(\text{2O}\) emissions by Brachiaria grasses with high BNI potential has already been reported (Byrnes et al. 2017).

**Rice root exudates did not impair AOB and AOA abundance after 12 days of soil incubation**

Previous studies showed that root exudate BNI activity decreased nitrification rates and the abundance of ammonia oxidisers without affecting other soil microorganisms (Gopalakrishnan et al. 2009; Subbarao et al. 2009). In this work, no change in AOB abundance could be detected after 12 days of soil incubation (Figure 4). Salto and Treinta y Tres soils presented similar patterns independent of their different copy numbers of bacterial amoA genes (Figure 4). Glaser et al. (2010) found that amoA copy number did not change until 21 days after NH\(\text{4}^+\) increase, so 12 days of incubation would have been enough to show nitrification inhibition effects but not to detect changes in this low growth rate bacteria abundance. In contrast, the rapid growth of AOB in response to the addition of inorganic N has been observed in agricultural soils (Lan et al. 2018).

AOA abundance was higher than AOB abundance for all treatments and types of soil (Figure 4). Similar results were reported for other paddy soils (Jiang et al. 2015), while other authors found opposite trends (Wu et al. 2011). Previous findings suggested that soil pH, acidic in this case, is a key factor driving the niche partitioning of AOA and AOB (Hu et al. 2014), and in general, acidic soils nitrification is dominated by nitrifying archaea (He et al. 2012). The copy number of bacterial amoA genes was in the order of that reported by Azziz et al. (2016) for nearby soils. Salto soil, with a higher OM content, had approximately ten times more AOB abundance (Figure 4). Similar results were registered elsewhere (Gong et al. 2013). AOA abundance did not correlate with NO\(\text{3}^-\) content (Figure 5). Their abundance increased after 12 days of incubation in all treatments (Figure 4). Bello et al. (2019) found that AOA were very sensitive to water stress (matric or osmotic potential), which would explain its growth after water addition with the different treatments.

The abundance of both ammonia oxidisers did not decrease after 12 days of addition of DCD, as reported in other works, although with different lapses of exposure to N fertilisers (Gong et al. 2013). O’Callaghan et al. (2010) and Li et al. (2021) did not observe a metabolic response of AOA to DCD addition. Although with these results we cannot discard AOA increase in response to NH\(\text{4}^+\) addition, this fact was not correlated with nitrification activity (Figure 5), and AOA were not inhibited by DCD or root exudates (Figure 4).

In this study, AOB, but not AOA, were significantly correlated with the NO\(\text{3}^-\) soil content (Figure 5), agreeing with other results (Di et al. 2009). Recently, Li et al. (2021) reported that sorghum root exudates significantly reduce AOB but not AOA, and Lan et al. (2018) suggested that the different compositions of the cell membranes of AOB and AOA may be responsible for their permeability to nitrification inhibitors.

Some of our results need further discussion, as the BNI effect measured for 12 days may be transitory, as has been reported by different authors (Subbarao et al. 2006; Tanaka et al. 2010). On the other hand, Moreta et al. (2014) and Karwat et al. (2017) found that the BNI effect of \(Bh\) on soil nitrifier activity could persist for at least one year after replacement of the pasture with maize.

In conclusion, these rice high-yielding cultivars showed high BNI activity for \(N\) europeae, but their potential nitrification inhibition in soil varied with the soil type and the rice cultivar. Rice cv Tacuari showed high BNI activity under the experimental conditions, while El Paso only showed BNI activity in the soil with lower OM content and lower abundance of ammonia oxidisers. This work suggests a differential genotype capacity of BNI for the rice cultivars evaluated and that BNI activity differs with soils mainly different in their OM content.

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Jacobo Arango is an Environmental Biologist at the Tropical Forages Program at CIAT in Cali, Colombia. Jacobo’s research focuses on how forages and efficient land use in the livestock sector can reduce nitrogen and carbon emissions. He leads the LivestockPlus project, supported by the CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS). The project facilitates and implements a National Mitigation Action Plan to reduce nitrogen and carbon emissions. He has a Ph.D. in plant science from Freiburg University in Germany and did postdoctoral research at Michigan State University and CIAT. He has worked with the IPCC since 2019 and is a co-author of the IPCC working group for long-term mitigation goals and climate scenarios.

Jonathan Núñez was a researcher at the Tropical Forages Program at CIAT under J. Arango supervision when the bioluminescence assay for BNI was performed. He is now at Manaaki whenua-Landcare research in New Zealand during his Ph.D. studies.

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**Disclosure statement**

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**Data availability statement**

The datasets generated during the current study are available on reasonable request.

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