XXIX Congreso Argentino de la Ciencia del Suelo
Suelos… Huellas del pasado, desafíos del futuro
San Fernando del Valle de Catamarca, Prov. de Catamarca, Argentina
21 al 24 de mayo de 2024

ARGENTINE RICE AND SORGHUM HAVE PROMISSORY POTENTIAL AS SERVICE CROPS FOR BIOLOGICAL INHIBITION OF NITRIFICATION

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ABSTARCT: Agriculture faces the challenge of regulating soil nitrification which promotes the loss of applied nitrogen (N) as, for example, nitrous oxide (N_2O) emissions. Biological inhibition of nitrification (BIN), a process that suppresses soil nitrifying activity by the release of root exudates from some plants, is a desirable characteristic that can improve N utilization and nitrogen use efficiency (NUE) and mitigate N losses. We aimed to assess the potential BIN of commercial varieties of rice and sorghum to screen the best of them and evaluate their impact on ammonium and nitrite oxidant bacteria. We conducted an experiment to determine the potential nitrification rates in soil with different rice and sorghum varieties. We planted seeds in pots, took soil samples after six weeks, and incubated them for 16 days. We extracted mineral N at different time intervals and calculated potential soil nitrification rates. We also estimated the number of cultivable nitrifiers using the Most Probable Number technique. The nitrification rates varied based on the time period and treatment. Two commercial varieties of rice and sorghum potentially have the ability to inhibit soil nitrification. The MPN method showed that the BIN capacity of rice targeted the nitrite-oxidizing bacteria (NOB) community up to 10 days from N addition. This could probably increase N_2O emissions. Thus, comprehensive field studies are necessary to determine the net nitrogen loss mitigation potential of rice and sorghum varieties.

KEYWORDS: climate smart agriculture, greenhouse gases, nitrogen use efficiency.

INTRODUCTION

One of the major concerns in agriculture is the loss of nitrogen (N) applied to the soil, which occurs due to the conversion of ammonium (NH_4^+) to nitrates (NO_3^-) in a process called nitrification. Nitrates are the most mobile form of N and can be lost through leaching, while also serving as a source for the denitrification process. Both processes are primarily driven by microorganisms in the soil and result in the release of nitrous oxide (N_2O), the most potent greenhouse gas. Therefore, one of the biggest challenges facing agriculture is to regulate soil nitrification.

In this context, the biological inhibition of nitrification (BIN), a natural process of suppression of soil nitrifying activity by the release of root exudates from some plants (Subbarao et al., 2006, 2015), has acquired special importance. Two of the species recently recognized for their BIN capacity were rice (*Oryza sativa* L.) (Sun et al., 2016; Zhang et al., 2019) and sorghum (*Sorghum bicolor* L. Moench) (Tesfamariam et al., 2014). This property of plants is a desired characteristic to improve N utilization and/or nitrogen use efficiency (NUE) (Sadhukhan et al., 2022). However, there is no precedent that characterizes this property in Argentine species. Precisely, this could help to define the potential role of this varieties as a service crop.

We aimed to assess the potential BIN of nine commercial varieties of rice and one of sorghum to screen the best of them and evaluate their impact on ammonium and nitrite oxidant bacteria.

MATERIALS AND METHODS

The potential nitrification rates were determined on soil with different varieties of rice and sorghum (Figure 1) as treatments. The soil used was characterized by having a pH of 6.8 ± 0.1; 2.9 ± 0.1% of organic matter; 42 ± 5.3, 40 ± 3.5 and 18 ± 2% of sand, silt and clay, respectively.

In the case of rice, we assessed nine varieties including seven commercial (C. rice) and two parental (P. rice). The parental varieties were used as positive controls as shown by Sun *et al.* (2016). Whereas, in the case of sorghum, we used a commercial variety able to produce the metabolite ‘sorgoleone’ released from roots that regulate nitrification (Gao et al., 2022). As a negative control, we used soil without plants.

A controlled incubation of soils was performed following Villegas et al. (2020). For this, we first planted four pre-germinated seeds of each variety in 1.9 L pots (Figure 1a). Then, after six weeks of growing (Sun et al., 2016), we took composite soil samples from each pot. Soil samples were air dried by 48 h and sieved with a 2 mm mesh. From each sample, we took 16 subsamples of 10 g of soil and incubated them in 100 ml flasks for 16 days. Just before incubation, we applied an equivalent dose of 100 kg N ha^{-1} as liquid ammonium sulfate ($(NH_4)_2SO_4$). During the incubation, soils were kept with a moisture of 60% of field capacity, at 25 °C and under darkness (Figure 1b).

The extractions of mineral N (NH_4^+ and NO_3^-) were performed at 2, 4, 8 and 16 days after initiating the incubation (DAI). On each of these days, we extracted four flasks of each treatment using 50 ml of potassium chloride (KCl, 1M) per flask and filtered the solution with filter paper after shaking it for 30 minutes. The contents of NH_4^+ and NO_3^- were determined by UV-VIS spectrophotometry (Smartchem 200, Westco). Potential soil nitrification rates were calculated as the slope between NO_3^- concentrations and incubation time.

Because the nitrification rates could vary according to the time considered, we first studied the components of the variance of the nitrification rates by adjusting a general mixed model taking as random effects the consecutive periods (defined by the dates of extractions) and the treatments. Then, we performed a one-way analysis of variance (ANOVA) for the period that contributed more to the total variance. These analyses were performed in InfoStat software (Di Rienzo et al., 2020).

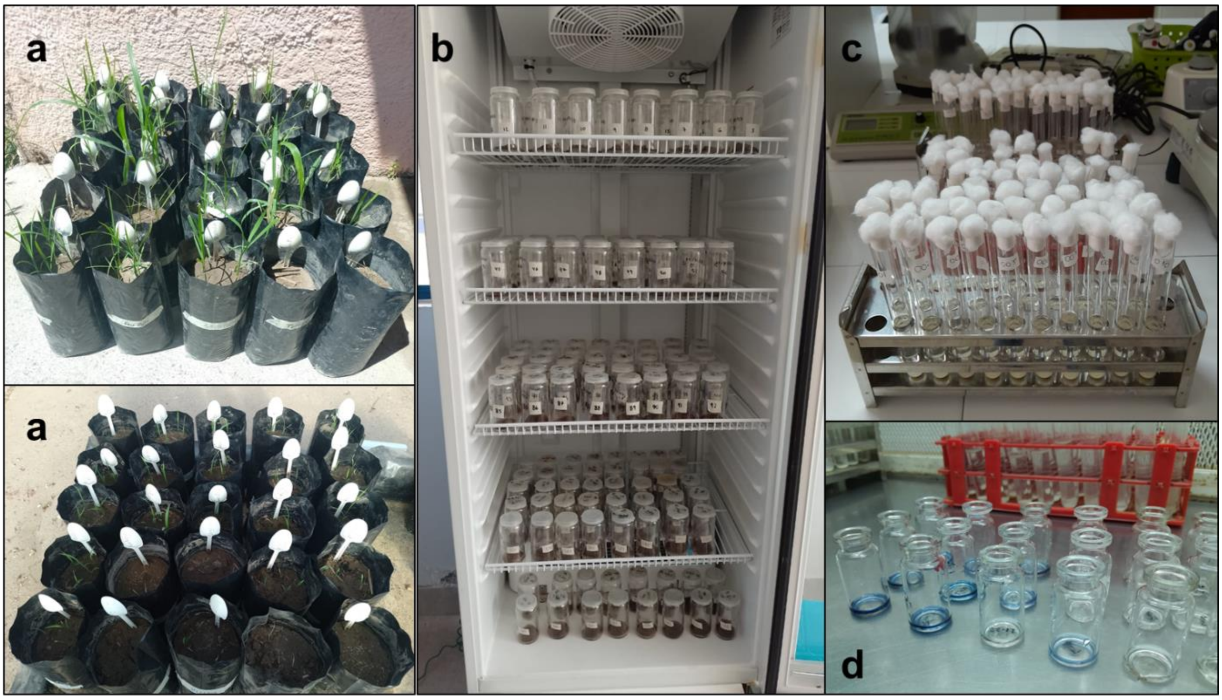

Figure 1. Details of materials and methods applied. Rice and sorghum in pots (a), incubation of soil in 100 ml flasks (b), tubes incubated in specific media for nitrifiers identification (c) and positive tubes in blue containing nitrifiers (d).

The number of ammonium- and nitrite-oxidizing bacteria (AOB and NOB, respectively) was estimated for the most contrasting (in nitrification rates) commercial varieties of rice and the control without N fertilizer by the Most Probable Number (MPN) technique. For this, 10 g of soil (from each treatment) were suspended in 90 ml of sterile distilled water (SDW) from which serial dilutions of 1 in 10 were made in tubes containing 9 ml of SDW (Figure 1c). Then, 1 ml of homogenized sample of each dilution was seeded, in triplicate, in tubes containing 10 ml of Ammonium Broth mineral medium for nitrifying bacteria (Schmidt & Belser, 1982). The tubes were incubated in an oven at 30 ± 2 °C for 21 days. On days 10 and 21, aliquots were taken from each tube to determine the presence of NO_2^- formed from the oxidation of NH_4^+, adding sulfuric acid and diphenylamine in sulfuric acid (DAS), and to determine the presence of NO_3^- formed from the oxidation of NO_2^-, adding sulfuric acid, urea and DAS. In both cases, the positive tubes showed an intense blue coloration (Figure 1d). To calculate the MPN, the positive tubes of each dilution were counted, and the Mac Crady table was used for 3 replications. The number of cultivable AOB and NOB was expressed as colony forming units per gram of soil (CFU.gr soil^{-1}).

RESULTS AND DISCUSSION

The highest variation of nitrification rates (93.4 %) was attributed to the period of time from fertilization considered (green portion of Figure 2 a). The variability of treatments within periods was higher than residual (Figure 2 a); thus, there was a differentiation of nitrification rates according to treatments. Within the period factor, most variability was found for the 2-4 DAI period. This was consistent with changes in NO_3^--N concentrations that were more notable until the 4th DAI (Figure 2b).

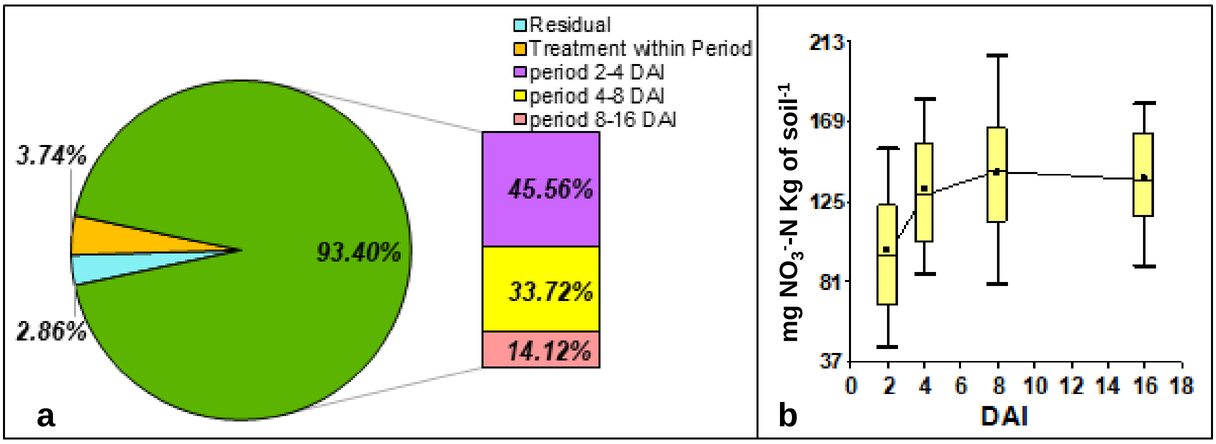

Figure 2. Components of variance for soil nitrification rates (*a*) and summary box plot showing NO_3^--N concentration through day after incubation initiation (DAI) (*b*). The green portion of *a* represents the period of time considered to calculate nitrification rates.

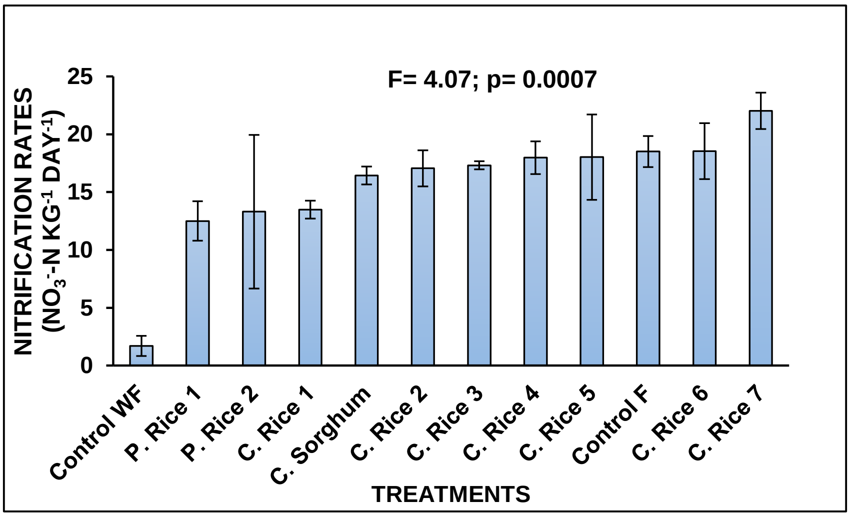

Figure 3. Mean nitrification rates (mg NO_3^--N kg of soil^{-1}.day^{-1}) for the period between days 2 and 4 after the beginning of incubation. n= 4; bars indicate standard errors. WF, F, P and C stand for without fertilizer, fertilized, parental and commercial, respectively.

According to ANOVA, treatments showed different nitrification rates for the period between days 2 and 4 after the beginning of incubation (Figure 3). Indeed, two commercial varieties, one of rice and other of sorghum, presented similar nitrification rates than the positive controls (parental varieties of rice) studied by Sun et al. (2016). Accordingly, these varieties had the ability to inhibit soil nitrification.

Overall, the MPN method showed that cultivable bacteria that oxidized NH_4^+ to NO_2^- and NO_2^- to NO_3^- were lower at day 10 than at day 21. At day 10, cultivable bacteria that oxidized NH_4^+ to NO_2^- was the highest for the sample with low nitrification rates (C. Rice 1). However, at this moment, cultivable bacteria that transformed NO_2^- to NO_3^- in C. Rice 1 was 10-fold lower than C. Rice 7. This could explain why the observed nitrification rates in Figure 3 could be associated with a last-step inhibition of the nitrification process. It is important to note that at day 21 bacteria that transformed NO_2^- to NO_3^- in C. Rice 1 was 20-fold higher than C. Rice 7. This was likely a response of high NO_2^- concentration accumulated. Therefore, the BIN capacity of rice was temporally detected until the day 10. Although this could partially delay the formation of NO_3^--N and prevent N losses, high NO_2^- concentration could increase substantially N_2O emissions (Maharjan & Venterea, 2013; Venterea et al., 2015). For this, to determine the net nitrogen (N) loss mitigation potential of rice and sorghum varieties, it is important to conduct field studies that comprehensively evaluate N uptake, NUE, and N_2O emissions (e.g. Chalco Vera et al., 2022). This information could be valuable as these crops may be utilized as service crops.

Table 1. Most probable number of nitrifiers (CFU gr of soil^{-1}) for the most contrasting in treatments and the control. Values obtained from a composite soil sample.

Day	Nitrifiers	Control WF	C. Rice 1	C. Rice 7
10	$NH_4^+ \rightarrow NO_2^-$	85500	136300	40900
	$NO_2^- \rightarrow NO_3^-$	0	818	8636
21	$NH_4^+ \rightarrow NO_2^-$	2272000	2272000	855000
	$NO_2^- \rightarrow NO_3^-$	136300	409000	18180

CONCLUSIONS

Two commercial varieties (one of rice and the other of sorghum) showed potential to regulate soil nitrification. The main differences in nitrification rates between varieties were observed up to 4 days after N addition during incubation. The BIN capacity of rice targeted the NOB community up to 10 days from N addition.

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