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Biological and synthetic approaches to inhibiting nitrification in non-tilled Mediterranean soils

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Abstract

Background: The increasing demand for food production has led to a tenfold increase in nitrogen (N) fertilizer use since the Green Revolution. Nowadays, agricultural soils have been turned into high-nitrifying environments that increase N pollution. To decrease N losses, synthetic nitrification inhibitors (SNIs) such as 3,4-dimethylpyrazole phosphate (DMPP) have been developed. However, SNIs are not widely adopted by farmers due to their biologically limited stability and soil mobility. On the other hand, allelopathic substances from root exudates from crops such as sorghum are known for their activity as biological nitrification inhibitors (BNIs). These substances are released directly into the rhizosphere. Nevertheless, BNI exudation could be modified or even suppressed if crop development is affected. In this work, we compare the performance of biological (sorghum crop) and synthetic (DMPP) nitrification inhibitors in field conditions.

Results: Sorghum crop BNIs and DMPP prevented an increase in the abundance of ammonia-oxidizing bacteria (AOB) without affecting the total bacterial abundance. Both nitrification inhibitors maintained similar soil NH_4^+ content, but at 30 days post-fertilization (DPF), the sorghum BNIs resulted in higher soil NO_3^- content than DMPP. Even so, these inhibitors managed to reduce 64% and 96%, respectively, of the NO_3^- -N/ NH_4^+ -N ratio compared to the control treatment. Similar to soil mineral N, there were no differences in leaf $\delta^{15}\text{N}$ values between the two nitrification inhibitors, yet at 30 DPF, $\delta^{15}\text{N}$ values from sorghum BNI were more positive than those of DMPP. N_2O emissions from DMPP-treated soil were low throughout the experiment. Nevertheless, while sorghum BNIs also maintained low N_2O emissions, they were associated with a substantial N_2O emission peak at 3 DPF that lasted until 7 DPF.

Conclusions: Our results indicate that while sorghum root exudates can reduce nitrification in field soil, even at the same efficiency as DMPP for a certain amount of time, they are not able to prevent the N pollution derived from N fertilization as DMPP does during the entire experiment.

Keywords: Sorghum, DMPP, *amoA* gene, Soil mineral nitrogen, $\delta^{15}\text{N}$, Nitrous oxide emissions, No-till farming

Background

Sorghum (*Sorghum bicolor* L. Moench) is a widely cultivated cereal, being the fifth most important after wheat, maize, rice and barley [1]. The heat tolerance and drought

resistance that it possesses has made it well adapted to semiarid regions [2, 3]. While Africa and India cultivate sorghum for human food, which accounts for 40% of world sorghum production, countries in North America and Europe use sorghum for biomass production and livestock feed [4, 5]. However, sorghum is becoming relevant for industrialized countries because its grain is safe for celiac and gluten-intolerant people [6]. Therefore, it can meet the growing demand for gluten-free foods and

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beverages from consumers who cannot eat products containing wheat, barley or rye [7]. Besides becoming a safe food product, sorghum is also known for the allelopathy of its root exudates. The molecules that have been characterized in sorghum exudates are sorgoleone [8] and 3,4-hydroxyphenyl propionate (MHPP) [9]. Sorgoleone was initially used for its substantial weed-suppressing capability [10], but later its potential as a biological nitrification inhibitor (BNI) was discovered. More than 80% of the hydrophobic component of sorghum-exuded BNIs is sorgoleone [11], while MHPP comprises the hydrophilic component. Both molecules can reduce nitrification in soil [12, 13]. Subbarao et al. [14] characterize the BNI activity from MHPP and sorgoleone in the soil. Once these molecules are released from sorghum roots, their nitrification inhibitory action seems to be relatively stable over a pH range of 3.0 to 9.0. This is in contrast to BNIs release from other plants, such as *Brachiaria humidicola*, whose molecules are reported to have a total loss of inhibitory function at $\text{pH} \geq 8.0$. Moreover, the inhibitory effect on soil nitrification of MHPP and sorgoleone appears to be stable in the temperature range of 20 to 30 °C.

The use of crops that produce BNIs could be the first step towards a low-nitrifying agronomic environment in agricultural systems [15]. The tenfold increase in the use of nitrogen (N) fertilizers since the Green Revolution [16] has greatly augmented food production, but it has turned agricultural soils into high-nitrifying environments with significant environmental costs [17]. Nitrification is the biological transformation of N in the form of ammonium (NH_4^+) into oxidized N. Chemolithoautotrophic ammonia-oxidizing bacteria (AOB) and archaea (AOA) oxidize NH_4^+ to hydroxylamine (NH_2OH) through the ammonium monooxygenase enzyme (AMO) encoded by the *amoA* gene [18]. NH_2OH is converted to nitrite (NO_2^-) and then, nitrite-oxidizing bacteria (NOB) oxidize it to nitrate (NO_3^-) [19]. MHPP blocks the AMO enzyme, while sorgoleone blocks both the AMO and the HAO enzyme [14]. The chemical structure of sorgoleone, which has a hydroquinone head and a fatty acid tail with a terminal double bond, has the potential to disrupt the electron flow between AMO and HAO enzymes and hence the nitrification activity [20]. Following with the final product of nitrification, as NO_3^- is a negatively charged anion, it is repelled by negatively charged soil colloids and is thus lost through leaching, causing eutrophication and contamination of groundwater supplies [21]. Furthermore, in anoxic conditions, NO_3^- is the substrate for a denitrification process that releases N gases such as nitrous oxide (N_2O) [22]. The global warming potential (GWP) of N_2O is 265 to 298 times higher than CO_2 over a 100-year time horizon [23]. Therefore,

it is necessary to reduce the pollution originating from the application of N fertilizer, particularly as its use is expected to double by 2050 [24].

Synthetic nitrification inhibitors (SNIs) have been developed to decrease N losses by suppressing soil-nitrifier activity. The dimethylpyrazole-based SNI DMPP (3,4-dimethylpyrazole phosphate) is able to reduce AOB abundance, delaying the oxidation of NH_4^+ while diminishing N_2O emissions [25, 26]. However, the use of SNIs is not widely adopted by farmers [27]. The inhibitory effect does not last more than a few weeks, they have biologically limited stability, and SNI mobility could prevent these molecules from acting on the sites of nitrification [15, 28]. On the other hand, BNIs are exuded directly into the rhizosphere, which is the main site of nitrification due to the great abundance of AOB and AOA [29]. Moreover, sorghum BNIs are known for being stable across a wide range of soil pH and temperature [14]. In addition, BNIs from sorghum can be released until close to physiological maturity of the crop [14], which would ensure the presence of nitrification inhibitors during all the stages of crop development. Nevertheless, BNI exudation is related to the physiological state and development of the plant [30], so biotic or abiotic stresses that affect crop growth might modify the rate of BNI exudation or even prevent its release.

Sorghum allelopathy is highlighted in the framework of sustainable agriculture and its use could drive cultivation systems towards environmentally friendly agronomic practices that allow us to meet global food demand while reducing the loss of reactive N into the environment. For this reason, the aim of this work was to compare under field conditions the performance of biological and synthetic nitrification inhibitors in retaining NH_4^+ in soil for longer periods and reducing AOB growth and its effect on N_2O emissions.

Methods

Experimental design

This work was conducted in Pamplona, northern Spain (42° 47' N, 1° 37' W, 450 m above sea level), from May to October 2019. Table 1 describes the soil characteristics of the upper horizon. Daily precipitation and mean temperatures are shown in Additional file 1: Fig. S1. Sorghum (*Sorghum bicolor* L. var. PR88P68 Pioneer Corteva Agriscience®) was sown under no-tillage at a rate of 15 kg seeds ha^{-1} on 15th May 2019 after a previous hairy vetch (*Vicia villosa* L.) winter cover crop. The vetch was terminated with 1.5 kg ha^{-1} dose of glyphosate on 29th April, rate that is routinely applied in no-till system from this region, and left on the soil surface. This experiment consisted of three randomized N treatments with four replications (5 m × 5 m plots). The N treatments

Table 1 Physical and chemical properties of the soil collected in 0–30 cm depth layer in Pamplona (42° 47'N, 1° 37'W, 450 m above sea level, Navarre, Spain)

Soil texture			Soil chemical properties								
Sand (%)	Silt (%)	Clay (%)	pH ^a	C:N	N ^b (g kg ⁻¹)	Organic matter ^c	Carbonate ^d	Mg ^d (mg kg ⁻¹)	K ^d	Ca ^d	P ^e
38.6	31.8	29.6	8.3	8.9	1.4	21.5	20.3	53.5	2700	2735.7	11.5

^a pH (1:2.5 soil/water)

^b N Kjeldahl digestion, Keeney and Nelson [32]

^c Organic matter, Walkley and Black [33]

^d CaCO₃, Mg, K NH₄ AcO, MAPA [34]

^e P, Watanabe and Olsen [35]

were (1) sorghum without fertilizer (Control); (2) sorghum fertilized with ammonium sulfate (AS) and (3) sorghum fertilized with ammonium sulfate combined with DMPP (AS+D). The fertilizer application rate was 150 kg N ha⁻¹ in the form of ammonium sulfate 21%, with the fertilizer hand broadcast on 7th July 2019 in a single application at the beginning of stem elongation (Z30) according to the Zadoks scale [31]. Fertilizer combined with DMPP inhibitor was provided by EuroChem Agro Iberia S.L. The DMPP rate was 0.8% of the NH₄⁺-N applied with the fertilizer. As the purpose of this experiment was only to measure the effects of the sorghum crop on N losses, sorghum was not harvested and it was terminated on 14th October 2019 and left on the soil surface according to usual management practices.

Plant analysis

The N isotopic composition in sorghum leaves was determined with an elemental analyser (FlashEA1112 ThermoFinnigan) coupled to a mass spectrometer (DELTA^{plus} Finnigan MAT) in the Unidade de Técnicas Instrumentais de Análise, Serviços de Apoio à Investigação (SAI), Universidade da Coruña. To do so, one sorghum plant per plot was taken randomly at 10, 20, 30, and 60 days post-fertilization and dried at 80 °C in a circulation oven for 72 h until a constant dry weight was reached. Later, dry plants were ground with a ball miller (Retsch MM 500) at a frequency of 27 s⁻¹ for 2 min. The values of the isotopic ratio of 100 mg of ground material were expressed as δ¹⁵N, in parts per thousand (‰) relative to atmospheric N₂. The isotope composition values δ (‰) were obtained with the following equation:

$$\delta_{\text{sample}}(\text{‰}) = \left(\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right) \times 1000,$$

where R_{sample} is the ¹⁵N/¹⁴N ratio of the plant sample and R_{standard} is the ¹⁵N/¹⁴N ratio of the atmospheric N₂.

Soil analysis

Soil N₂O emissions were measured using the closed chamber method [36]. Gas samples were taken over 60 days post-fertilization at decreasing sampling frequency from three times per week over 2 weeks to twice per week in the subsequent 2 weeks and, finally, once per week until the end of measuring time. N₂O samples were measured as detailed in [37].

Soil mineral N was determined based on the soil NH₄⁺ and NO₃⁻ contents. Three soil subsamples (3 cm diameter × 0.3 m depth) per plot were taken the day before the treatment application, and later at 10, 20, 30 and 60 days post-fertilization. Then, soil subsamples were homogenized with rocks and roots being removed. The

NH₄⁺ and NO₃⁻ contents were determined as described in [37]. Each day of soil and/or gas measurement two additional soil subsamples (3 cm diameter × 0.3 m depth) were taken randomly from the field to determine soil water content. After removing rocks and roots, they were placed into a circulation oven at 80 °C for 72 h until a constant dry weight was reached. Following [38], soil water content was described as the percentage of water-filled pore space (WFPS):

$$\text{WFPS} = (\text{soil gravimetric water content} \times \text{bulk density}) \times (1 - (\text{bulk density}/\text{particle density}))^{-1},$$

where 2.65 Mg m⁻³ was used as particle density. The density of the bulk soil was measured at the beginning of the experiment resulting in 1.0 Mg m⁻³.

Soil samples from mineral N determinations at 20 days post-fertilization were used to quantify the abundance of total bacteria (*16s rRNA*), and nitrifying (bacterial *amoA*) and denitrifying (*nirK*) populations. Quantification was done using quantitative polymerase chain reaction (qPCR) in a StepOne Plus™ Real-Time PCR System. Soil DNA isolation and gene amplification were carried out as explained in [39].

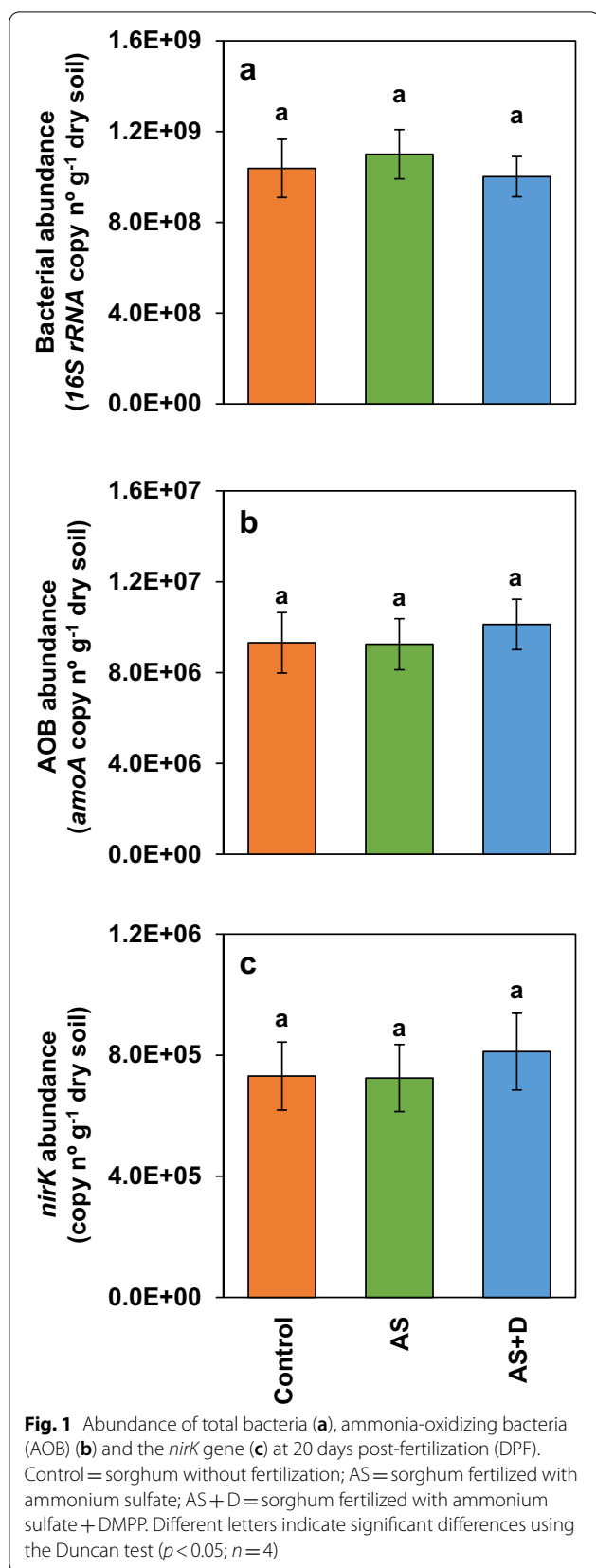
Statistical analysis

Data obtained in this experiment were statistically analysed with one-way ANOVA followed by Duncan's multiple range tests for separation of means between treatments using SPSS statistical software (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp). Significant differences are expressed at $p < 0.05$.

Results

Fertilizer treatments did not have any effect on total bacterial abundance (Fig. 1a). Based on the *16S rRNA* gene copy number, bacterial abundance ranged from 1.00·10⁹ to 1.10·10⁹. Nitrifying bacteria were also not affected by N treatments, having an abundance of between 9.31·10⁶ and 1.01·10⁷ *amoA* gene copy numbers g⁻¹ dry soil (Fig. 1b). Alike AOB, denitrifying microorganisms neither were affected by addition of fertilizer. They showed an abundance that varied from 7.31·10⁵ to 8.12·10⁵ *nirK* gene copy numbers g⁻¹ dry soil (Fig. 1c).

After fertilizer application, the soil NH₄⁺ content increased in AS and AS+D treatments maintaining higher values during the first 30 days post-fertilization (DPF) (Fig. 2a). At 30 DPF, the soil NH₄⁺ content of fertilized treatments decreased to levels that were similar to the control treatment. However, it was observed that



the soil NH_4^+ content from the AS and AS + D treatments increased at 60 DPF, which might have been a consequence of mineralization. On the other hand, the AS treatment showed the highest soil NO_3^- content during the 60 days of measurement (Fig. 2b). Although the AS + D treatment showed constant soil NO_3^- content during the first 20 DPF, it was able to diminish its formation to levels of unfertilized treatment until 60 DPF. Control treatment also showed low soil NO_3^- content. Indeed, the highest NO_3^- -N/ NH_4^+ -N ratio throughout the experiment was observed in the control treatment (Fig. 2c). The AS and AS + D treatments had equally low ratios during the first 20 DPF because there were no differences between them in terms of soil NH_4^+ and NO_3^- content. Nevertheless, because the AS treatment did not decrease soil NO_3^- levels at 30 DPF, the NO_3^- -N/ NH_4^+ -N ratio showed a sixfold increase compared to the AS + D treatment. Still, at 30 DPF both treatments were able to reduce the NO_3^- -N/ NH_4^+ -N ratio in AS and AS + D by 64% and 96%, respectively, compared to the control treatment.

Sorghum leaves from the control treatment showed the least negative $\delta^{15}\text{N}$ values (Fig. 3a). The similarity in leaf $\delta^{15}\text{N}$ values between the AS and AS + D treatments until 20 DPF indicated no effect from DMPP up to this point. However, at 30 DPF, both fertilizer treatments showed an increase in $\delta^{15}\text{N}$ values that was greater in the AS treatment than the AS + D treatment, and the same $\delta^{15}\text{N}$ values were maintained until 60 DPF. As expected, the unfertilized treatment possessed the lowest leaf N content (Fig. 3b). Fertilized treatments showed higher N contents that declined throughout the experiment until they reached similar N values to the control treatment at 60 DPF. In this case, there were no differences between the AS and AS + D treatments.

The treatment fertilized with AS showed a substantial N_2O emission peak at 3 DPF with an emission of $38.7 \text{ g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$ (Fig. 4a). Nevertheless, the peak was quickly reduced from 7 DPF, with N_2O emissions in the AS treatment maintained between 3.79 and $0.74 \text{ g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$. In contrast, the N_2O emissions from the control and AS + D treatments were both low throughout the experiment, ranging from 4.23 to $0.67 \text{ g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$ for the control treatment and from 4.01 to $0.31 \text{ g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$ for the AS + D treatment. Although N_2O emissions from AS treatment were reduced to levels similar to the control and AS + D treatments, it had the highest total cumulative N_2O emissions due to the short emission peak (Fig. 4b). There were no differences between control and AS + D treatments in total cumulative N_2O emissions, with reductions of 54% and 59%, respectively, compared to the AS treatment.

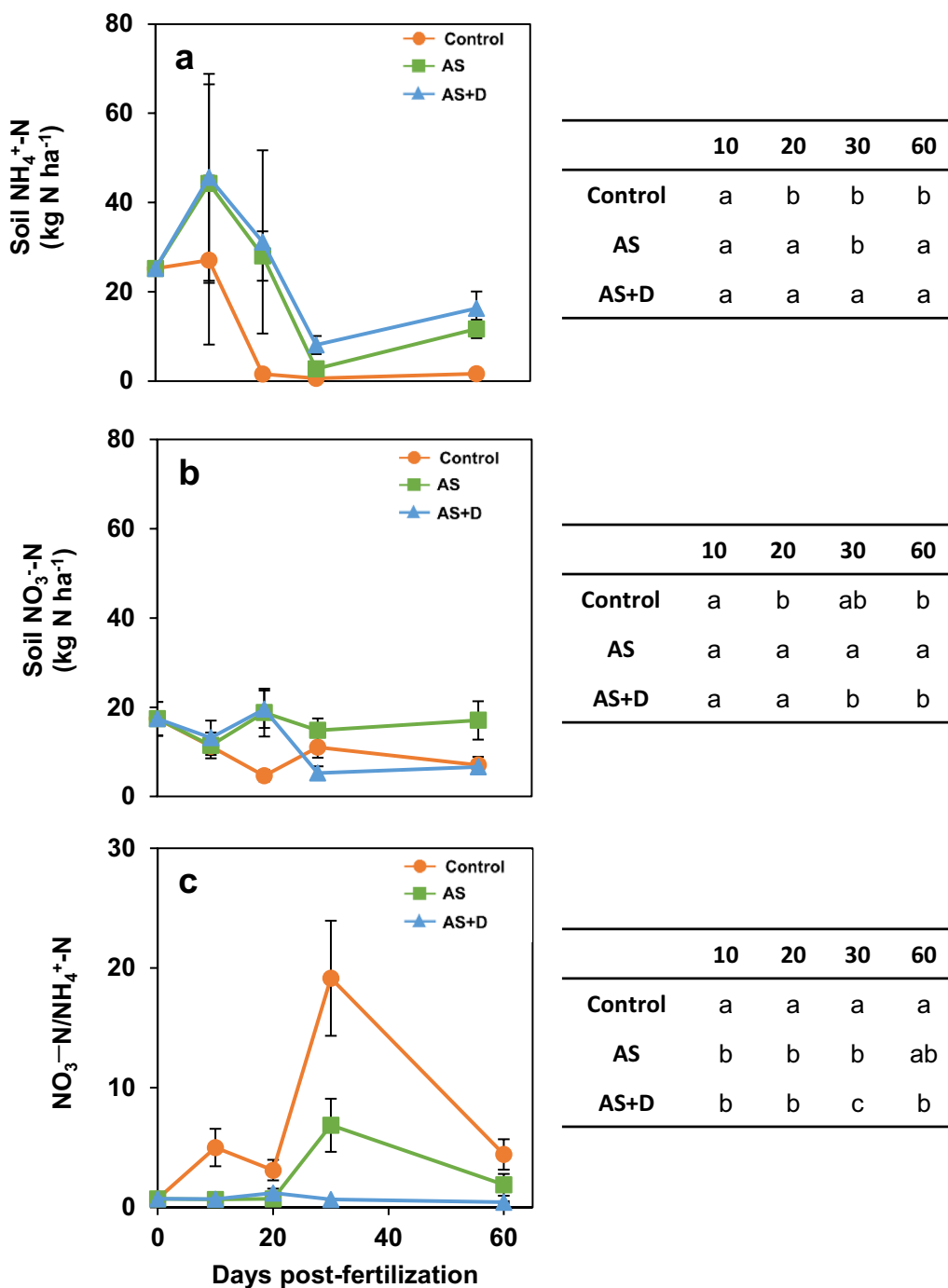
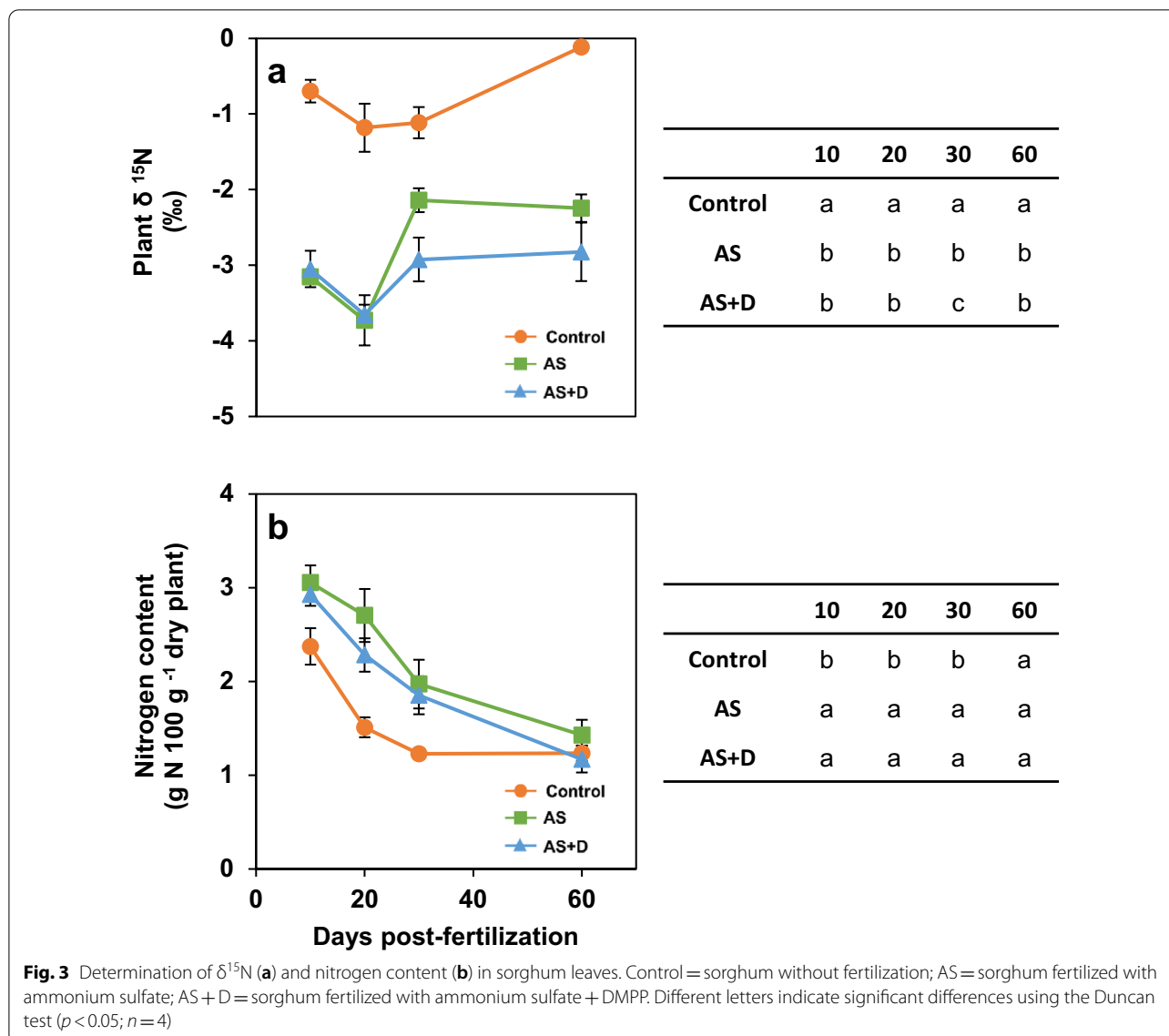


Fig. 2 Soil mineral nitrogen evolution over 60 days post-fertilization in the form of NH₄⁺ (a), NO₃⁻ (b) and the NO₃⁻-N/NH₄⁺-N ratio (c). Control = sorghum without fertilization; AS = sorghum fertilized with ammonium sulfate; AS + D = sorghum fertilized with ammonium sulfate + DMPP. Different letters indicate significant differences using the Duncan test (*p* < 0.05; *n* = 4)

Discussion

During the last few decades, root exudates from sorghum have been well studied due to the presence of allelopathic substances [40]. Lately, investigations have focused on

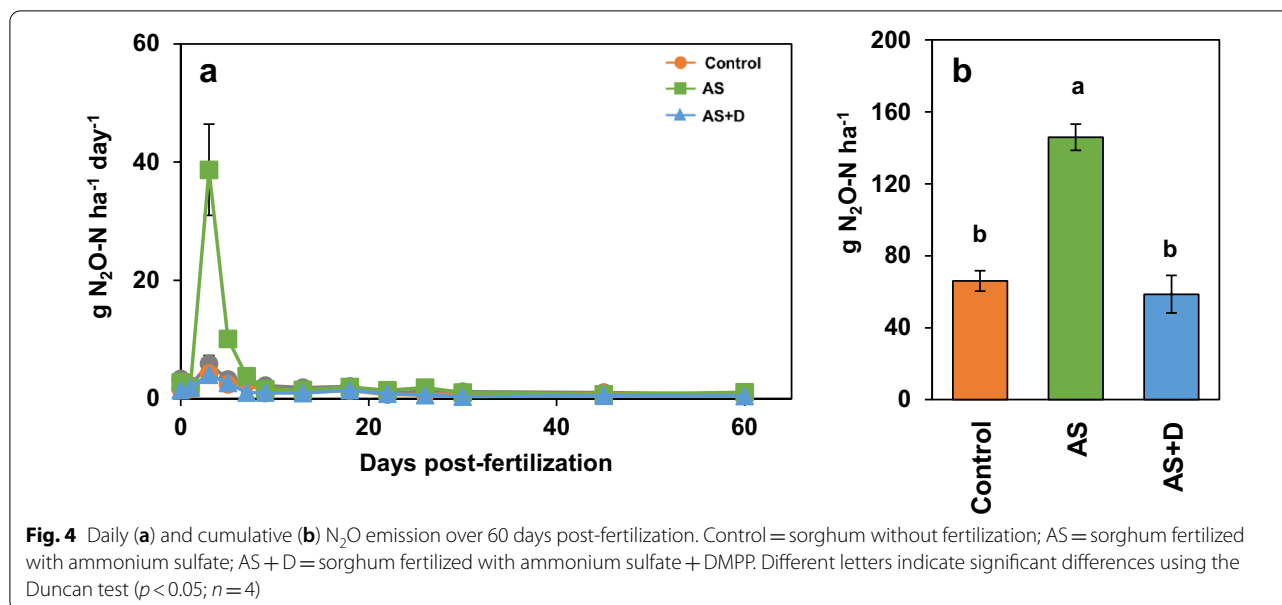
their ability to inhibit the nitrification pathway. There are several greenhouse and microcosm studies where molecules such as sorgoleone and MHPP have been characterized and their potential as BNIs investigated [8, 9, 12,



14]. One of the main aspects to consider in the attempt to improve agricultural sustainability is whether these substances could have a negative effect on soil health. Encouragingly, while MHPP molecules reduce AOB abundance, they do not exert a general negative impact on the soil bacterial community, as indicated by maintenance of *16S rRNA* gene abundance in microcosm experiments [12]. In the same manner, no effects on total bacterial abundance have been observed in soil from pot-grown sorghum that release different quantities of sorgoleone [13]. Here, we corroborate that sorghum plants do not alter total bacterial abundance under field conditions (Fig. 1a). This is a confirmation that both sorghum root exudates and the synthetic nitrification inhibitor (SNI) DMPP do not produce general deleterious effects because

DMPP also had no effect on *16S rRNA* abundance, as reported here (Fig. 1a) and in several other studies [41, 42]. Nevertheless, previous work with DMP-based nitrification inhibitors and with sorgoleone has shown that there are some shifts in non-target bacterial abundance, even when the total bacterial abundance is not altered, with SNIs associated with decreases in bacterial diversity [43] and BNIs associated with changes in bacterial networks (BNIs) [44]. These studies are still preliminary, so further work should expand these analyses to determine exactly what effects are exerted by these compounds on the soil microbiota.

Fertilizer stimulates root development, changes the soil pH and increases the availability of nutrients for microorganisms and, consequently, the soil bacterial consortia



are greatly influenced [45–47]. Specifically, the AOB population exhibits a strong response to N fertilizers [48]. Shortly after the fertilizer application, soils tend to show a large increase in *amoA* abundance [39, 49]. This growth can be avoided by applying SNIs such as DMPP, and the AOB population size is maintained at the level of unfertilized soils (Fig. 1b) [50, 51]. Furthermore, the objective of BNIs is to suppress soil nitrification by decreasing the ammonia-oxidizing microorganisms' populations [52]. Interestingly, in the absence of DMPP (AS treatment) we also observed no increases in *amoA* abundance in soils. The lack of AOB growth may be associated with the use of glyphosate as an herbicide to terminate winter vetch crop. Several studies have examined the effect of glyphosate on soil microbiology, but the results are highly variable. While some authors described that the use of glyphosate cause negative impacts on microbial community structure [53, 54], others affirmed that glyphosate is able to increase soil microbial biomass and respiration [55, 56] or, at least, have no significant impact at all [57, 58]. Nevertheless, it has been reported that the application of glyphosate at higher rates than in this experiment (1.5 kg ha^{-1}) had no effect on AOB and AOA abundances [59, 60]. Moreover, glyphosate is routinely applied in no-till systems from this region. Therefore, it is reasonable that we could conclude that the inhibition of AOB growth was due to the action of BNI molecules present in sorghum root exudates. This is the first field demonstration that sorghum can avert AOB growth with the same efficiency as SNIs.

Soil mineral N is a useful tool to monitor the activity of AOB based on the oxidation of NH_4^+ to NO_3^- .

The use of SNIs such as DMPP maintains soil NH_4^+ for longer periods due to a delay in NH_4^+ oxidation as a consequence of AOB inhibition [61]. When ammonium-based fertilizers are applied without nitrification inhibitors, soil NH_4^+ increases substantially followed by a rapid decrease and the appearance of NO_3^- [62, 63]. In our work, AS treatment kept soil NH_4^+ content in parallel with the AS + D treatment (Fig. 2a). This could be a consequence of BNIs released by sorghum, demonstrating the ability to maintain NH_4^+ content at the same level as DMPP, which aligns with the equal AOB populations in both soils (Fig. 1b). Although 20% of total N losses from field-applied N occur through volatilization of ammonia, the great majority of N losses occur after microbial reactions transform NH_4^+ in soils into NO_3^- [64]. Therefore, it seems that the use of BNI could be a good option to reduce soil N losses due to its ability to withhold NH_4^+ oxidation derived from the inhibition of AOB growth. Nonetheless, the capacity of BNIs to diminish nitrification seemed to decline over time, as suggested by the daily evolution of soil mineral N (Fig. 2a, b). Although soil NH_4^+ and NO_3^- contents were equivalent between AS- and AS + D-treated soils, differences arose after 20 DPF. The NO_3^- -N/ NH_4^+ -N ratio under AS treatment increased relative to the AS + D treatment (Fig. 2c), which may indicate that the efficiency of BNIs in reducing NH_4^+ oxidation only lasted until 20 DPF. It would be interesting to track AOB abundance over time for longer in further studies to examine the effect of this possible decline in BNI activity on AOB growth. These differences in the NO_3^- -N/ NH_4^+ -N ratio were also associated with an effect on sorghum leaf $\delta^{15}\text{N}$ values. The natural

variation in the heavy N isotope (^{15}N) has now being used with increasing frequency in physiological studies related to N metabolism [65–67]. For example, N fixation processes, both free and biological, tend to impoverish $\delta^{15}\text{N}$ because the value of the atmospheric N_2 is zero. Thus, because the sorghum crop was sown after winter vetch, which is an N-fixing crop, the leaf $\delta^{15}\text{N}$ values of the sorghum control treatment were below zero (Fig. 3). In addition, [66] noted that plants grown with NH_4^+ as the sole source of N demonstrated a decrease in $\delta^{15}\text{N}$. Therefore, as suggested by [68], $\delta^{15}\text{N}$ can be used as an indicator of the origin of the main N source available to the plant during its development. The lower $\delta^{15}\text{N}$ values of the AS and AS+D treatments indicated that the sorghum crops were exposed to a dominating ammonium nutrition for a longer period than the control treatment. Nevertheless, at 30 DPF, the NO_3^- -N/ NH_4^+ -N ratio in soils from the AS treatment showed an increase compared to the AS+D treatment (Fig. 2c), probably due to the aforementioned decline in BNI effectivity. This means that sorghum plants under AS had greater access to NO_3^- than sorghum under the AS+D treatment. As a consequence, the $\delta^{15}\text{N}$ values of the AS treatment were less negative than those of AS+D at 30 DPF (Fig. 3a). In addition, $\delta^{15}\text{N}$ values of the fertilized treatments did not attain the values of the control without fertilization. This indicates that while BNIs are less efficient than the synthetic inhibitor DMPP, they still promote a certain amount of plant NH_4^+ nutrition.

The main process responsible for N_2O production in soils is denitrification [69]. N_2O emissions are related to soil water content [70], but the humid Mediterranean climate is characterized by a hot and dry summer. The threshold between water-limited and aeration-limited microbial processes is supposed to occur at soil moisture levels of 60% WFPS [70]. Therefore, since the soil WFPS of the present study did not exceed 25% most of the time (Additional file 2: Fig. S2), it is possible that denitrifiers were not responsible for N_2O emissions. This may have been due to the lack of variation between treatments in the abundance of the *nirK* gene at 20 DPF (Fig. 1c). On the other hand, nitrifying microorganisms can also produce N_2O via nitrifiers' denitrification processes [71]. Nevertheless, although nitrifying populations might have been responsible for the emission peak in the AS treatment at 3 DPF (Fig. 4a), at the same time there were no differences in AOB abundance at 20 DPF between AS and AS+D (Fig. 1b). We hypothesized that the presence of the N_2O emission peak at 3 DPF and the inhibition of AOB growth at 20 DPF is related to the amount of BNIs released by the sorghum roots. The release of BNIs is influenced by soil NH_4^+ content, which at higher concentrations has been shown to stimulate greater BNI release

in sorghum roots [72]. Therefore, the limited soil NH_4^+ content before the N_2O measurements did not promote the release of enough BNIs to reduce nitrification in the first few days after fertilizer application. Nonetheless, BNI release increased in sorghum after the addition of ammonium-based fertilizer, inhibiting AOB growth at 20 DPF, and this occurred despite a lack of complete inhibition of nitrification during the first 7 DPF. The fact that the N_2O emission peak in the AS treatment was reduced before 7 DPF, while the N_2O emission peaks of fertilized treatments without nitrification inhibitors lasted more than 15 days [51, 73] is in line with this hypothesis. During the rest of the experiment, sorghum exudates were able to maintain low N_2O emissions similar to the AS+D treatment, which showed great efficiency in reducing them, as described in other studies [74–76]. Nonetheless, this indicates that even though BNIs have a similar efficiency to SNIs in reducing N_2O emissions, the delay in BNI release due to the absence of high soil NH_4^+ content does not prevent N_2O emissions in the short-term.

Conclusions

The use of allelopathic substances from plants to reduce nitrification in the soil is a topic of increasing interest. BNI inhibition could be a nature-based solution to diminish N losses, avoiding reliance on new technologies that are not widely adopted. BNIs from sorghum were able to prevent an increase in *amoA* after N fertilization with the same efficiency as DMPP. Moreover, total bacterial abundance was not affected by either the presence of sorghum roots exudates or by DMPP. In addition, both BNIs and SNIs maintained similar soil NH_4^+ contents throughout the experiment. However, sorghum root exudates could not prevent the appearance of soil NO_3^- after 20 DPF, which might indicate that the BNI effect decreases in efficiency after a certain amount of time. While DMPP maintained low N_2O emissions throughout the experiment, the AS treatment presented one peak at 3 DPF that lasted until 7 DPF. Since the release of BNIs is related to the soil NH_4^+ concentration, we hypothesize that the limited soil NH_4^+ concentration before the N_2O measurements did not allow release of enough BNIs to avoid this emission peak. Therefore, although sorghum root exudates can reduce nitrification in field soil, even with the same efficiency as DMPP for a certain amount of time, they are not able to prevent the N pollution derived from N fertilization as DMPP does during the entire experiment.

Abbreviations

MHPP: 3,4-Hydroxyphenyl propionate; BNI: Biological nitrification inhibitor; N: Nitrogen; AOB: Ammonia-oxidizing bacteria; AOA: Ammonia-oxidizing archaea; NOB: Nitrite-oxidizing bacteria; N_2O : Nitrous oxide; SNI: Synthetic

nitrification inhibitors; DMPP: 3,4-Dimethylpyrazole phosphate; WFPS: Water-filled pore space.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40538-021-00250-7>.

Additional file 1: Fig S1. Precipitation (blue bars) and mean air temperature (red line) of summer growing season. Discontinue bar indicates the application of the fertilizer treatments.

Additional file 2: Fig S2. Evolution of soil temperature (0–10 cm) (red line) and soil WFPS (0–30 cm) (blue line) during GHG emissions measurements in sorghum crop.

Acknowledgements

Not applicable.

Authors' contributions

ABL conducted the experiment on the field and was the main contributor in the data processing and interpretation, also writing the manuscript. MCM participated in formal analysis, writing, reviewing and editing. LMA prepared the experimental field site, sowed the sorghum crop and participated in formal analysis, writing, reviewing and editing. PAT and CGM supervised all phases of data analysis and interpretation and reviewed the entire manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Raw data used to generate the presented results are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

MCM is an associate editor of Chemical and Biological Technologies in Agriculture. The rest of the authors have no conflicts of interest to declare.

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