Effects of Arbuscular mycorrhizal fungi on leguminous crop growth and soil N_2O emissions

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF), which form symbiotic associations with 80% of land plants, provide host plants with soil phosphorus (P) and nitrogen (N) in exchange for carbon. The AMF often reduce nitrous oxide (N_2O) emissions from soils with non-legume plants, as they acquire N from soils to plants. However, the effect of AMF on soil N_2O emissions is still unknown in the legume system, a major source of this potent greenhouse gas. Therefore, we conducted the field and pot experiments to investigate the effects of AMF on plant growth and soil N_2O emissions in legume systems. The results showed that AMF significantly increased soil N_2O emissions from soils with soybean by 39% in the field experiment and by 63% in the pot experiment. The AMF significantly enhanced the root biomass, aboveground biomass, grain yield, plant N uptake, plant P uptake, and the nodule numbers. The AMF increased soil extractable N concentrations and DOC concentrations, but reduced soil available P concentrations. The AMF stimulated the N_2O-producing microbes (*nirS*- and *nirK*-type) whereas they did not affect the N_2O-consuming (*nosZ*-type) denitrifiers. The AMF reduced the yield-scaled N_2O emissions in the field experiment and increased the net ecosystem economic budget. Our findings indicate that AMF increase soil N_2O emissions from legume system through enhancing P acquisition for biological N_2 fixation and suggest that AMF benefit crop production for higher-yield with less N_2O emissions.

Keywords: N_2O emissions, Greenhouse gas emissions; Arbuscular mycorrhizal fungi; Food security; Phosphorus

1. Introduction

Nitrous oxide (N_2O) is the third most important greenhouse gas with a 265 times greater global warming potential than carbon dioxide (CO_2) (IPCC, 2013). Nitrous oxide is estimated to contribute approximately 6%~8% of global warming (IPCC, 2007) and enhances stratospheric ozone (O_3) depletion (Ravishankara et al., 2009; Millar et al., 2018). The atmospheric N_2O concentration has increased by 20% since industrial times and is predicted to continue to increase in the future (IPCC, 2013). Agricultural soils are responsible for nearly 60% of the anthropogenic N_2O emissions (Syakila and Kroeze, 2011; Jahangir et al., 2021). In agricultural soils, N_2O is often emitted from soil N, N fertilizer, biological N_2-fixation, and crop residue (Rochette and Janzen, 2005; Itakura et al., 2013). Soil N_2O is mainly produced through microbial processes of nitrification (i.e., oxidation of NH_4^+ into NO_3^-) and denitrification (i.e., reduction of NO_3^- to gaseous forms, NO, N_2O, and N_2) (Bouwman, 1998; Barnard et al., 2005; Zhu et al., 2013; Guillermo et al., 2018), which is affected by soil C and N availability, soil moisture, plants species, and soil microbes (Barnard et al., 2005; Abalos et al., 2014; Chen et al., 2016).

Arbuscular mycorrhizal fungi (AMF) are widespread in most terrestrial ecosystems and form associations with roots of ~80% of terrestrial plant species (Kiers et al., 2011; Voriskova et al., 2016; Ghorchiani et al., 2018). AMF often stimulate the uptake of several soil nutrients (i.e. N, P, and Zn) via extensive hyphal networks and transfer of nutrients from the fungus to the plant root in exchange for C (Smith and Read, 2008; Fellbaum et al., 2012; Xu et al., 2018). As AMF often increase plant N uptake (Cheng et al., 2012; Cavagnaro et al., 2015), they are widely expected to reduce soil N_2O emissions (Bender et al., 2014; Storer et al. 2018; Teutscherova et al., 2019; Bender et al., 2019). However, all these studies were conducted using

non-legume species, such as *Lolium multiflorum, Solanum lycopersicum L., Z. mays L., Brachiaria decumbens Stapf., P. australis, and Oryza sativa L.*(Lazcano et al., 2014; Bender et al., 2014; Bender et al., 2015; Zhang et al., 2015; Storer et al. 2018; Liang et al., 2019; Okiobe et al., 2019; Teutscherova et al., 2019). As such, the effects of AMF on N_2O emissions in legume systems are still unknown.

Legumes play a significant role in maintaining global food security and ecosystem resilience, because they can enhance soil N supply and improve crop yields through biological N_2 fixation (Tilman et al., 2002; Siddique et al., 2012; Foyer et al., 2016). The harvested area of grain legumes is around 1/3 of total cereal harvested area (Foyer et al., 2016), and legume systems are estimated to emit around 10% of total agricultural N_2O emissions (Stehfest and Bouwman, 2006). In legume ecosystems, N_2O is also emitted from the degradation of the root nodules. Organic N inside the nodules is mineralized to NH_4^+, followed by nitrification and denitrification that produces N_2O (Rochette and Janzen, 2005; Inaba et al., 2009; Barton et al., 2011; Itakura et al., 2013). AMF often stimulate the biological N_2 fixation through improving P uptake (Wang et al., 2011; Wang et al., 2016; Püschel et al., 2017). Therefore, in legume ecosystems, AMF may improve P restrict and increase root nodules infection, which can indirectly change N substrates and the response of microbial processes related to nitrogen transformation (e.g., N_2O production). Based on these hypotheses, AMF may result in higher N_2O emissions in legume ecosystems.

Here, we conducted pot and field experiments to assess the effects of AMF on N_2O emissions in the legume system. First, soybeans were planted to enable comparison of the soil

N_2O emissions between AMF and non-AMF treatments. Second, we measured the N_2O emissions under field condition that AMF hyphae were either allowed or prevented access to a chamber containing P fertilizer. We tested whether AMF increased N_2O emissions in legume system through improving P uptake. To the best of our knowledge, this is the first study to report on the effects of AMF on N_2O emissions from legume systems.

2. Material and methods

2.1 Experimental design

Pot experiment

We conducted a pot experiment to investigate the effects of AMF on N_2O emissions under open field conditions at the Jiangxi Academy of Agricultural Science (28.6ºN, 115.9ºE), Nanchang, China. This experiment consisted of AMF inoculum and non-AMF inoculum treatments, and three replicates for each treatment.

The test soil was collected from the plow layer of a cropland and was air dried, sieved (6 mm mesh size) and mixed. The main soil properties were as follows: soil organic C 26.2 g kg^{-1}, total N 2.8 g kg^{-1}, and total P 1.0 g kg^{-1}. The soils were sterilized using an autoclave at 120 ºC for 30 min. Plastic pots (diameter, 30 cm; depth, 30 cm) were filled with 9 kg of sterilized soil and 2 kg sterilized sand that were mixed together. To recover the requisite soil microflora, 100 ml of soil microbial solution without AMF was added into each pot. The microbial solutions were produced from the same fresh soil of this experiment. In the AMF treatments, we added 150 g AMF inoculum sand into soil. The sand included six common AMF species (i.e. *Glomus mosseae*, *Gigaspora margarita*, *Acaulospora scrobiculata*, *Corymbiglomus tortuosum*,

109 *Diversispora epigaea* and *Rhizophagus intraradices*). In the non-AMF treatments, 150 g sand
110 without AMF and a solution without AMF prepared from AMF inoculum was added. We also
111 added 5 ml rhizobium solution into each pot and did not apply any fertilizer. The soybean
112 (*Glycine max (Linn.) Merr.*) seeds were surface-sterilized. In a pot, we sowed five soybean
113 germinated seeds. Ten days later, plants were thinned to only three seedlings in each pot. When
114 almost all pods became yellow and the bottom leaves began to fall, the soybean plants were
115 harvested at early full ripening stage (90 days after seeding). We placed an open bottom and top
116 chamber (diameter, 8 cm; height, 10 cm) into the soil (2 cm depth) to measure the emitted N_2O
117 gas.

118

119 Field experiment

120 In this experiment, we tested whether AMF increased N_2O emissions in legume systems
121 through improving P uptake under field conditions at Jinxian, Jiangxi province (28.3ºN,
122 116.3ºE). The main soil properties recorded were: soil organic C 13.3 g kg^{-1}, total N 1.2 g kg^{-1},
123 total P 0.5 g kg^{-1} and available P 6.4 mg kg^{-1}.

124 We designed the "phosphorus chamber" (length, 60 cm; width, 20 cm; height, 30 cm) with
125 four mesh windows (Fig. 1). The pore sizes of the meshes were either 20 μm or 0.45 μm. AMF
126 hyphae were allowed access to the chambers with two mesh window pore size of 20 μm
127 (nonprevented) and were prevented access to chambers with two mesh window pore size of
128 0.45 μm (prevented). For each chamber, we put 46 kg air dried soils mixed with 1.44 g P_2O_5.

129 We sowed 12 rows of soybean seeds. For each row, we sowed 20 hills soybean with hill
130 spacing of 0.25 m× 0.15 m. Twenty days after seeding, we selected adjacent two rows with

131 healthy soybean plant and placed a P chamber into the soil close to soybean plant. The distance
132 of two P chamber was more than 1 m. An open bottom and top chamber (diameter, 8 cm; height,
133 10 cm) was placed into the soil (2 cm depth) close to the plant with chamber to collect the N_2O
134 gas. The soybean plants were harvested at early full ripening stage (90 days after seeding) after
135 almost all pods became yellow.
136
137 *2.2 Sampling and measurement methods*
138 In both experiments, the static closed chamber technique was used to measure the N_2O
139 fluxes from 10-20 days after thinning to harvest at 5-10-day intervals. Two gas samples (40 ml)
140 were collected from each static sampling chamber at 0 and 30 min after the chamber was closed
141 (Wu et al., 2017; Qiu et al., 2019). The N_2O concentrations were determined with a gas
142 chromatograph (GC, Agilent 7890A, USA) and the fluxes calculated as follows: $F = \Delta C/\Delta T \times$
143 V/A, where $\Delta C/\Delta T$ is the change rate of N_2O concentration ($\mu g\ L^{-1}\ h^{-1}$) in the chamber. We
144 assumed a linear rate of N_2O accumulation in the chambers over a short period (Wu et al., 2017;
145 Qiu et al., 2019). V is the volume of the chamber (L) and A is surface area of chamber (m^2).
146 Cumulative N_2O emissions were estimated using the trapezoidal method (Jiang et al., 2018).
147 At the harvest, soils were collected to determine dissolved organic carbon (DOC),
148 extractable nitrogen (NH_4^+ and NO_3^-), available P concentrations and the abundances of *nirS*,
149 *nirK* and *nosZ* genes related to N_2O emission. We measured the DOC concentrations by using
150 a TOC analyzer (multi N/C UV, Analytik Jena AG, Germany). The flow autoanalyzer (Auto
151 Analyzer 3, BRAN LUEBBE, Germany) was used to measure the soil NH_4^+ and NO_3^-

concentrations. Soil available P concentrations were measured by the Olsen method for alkaline soils (Olsen et al., 1954). We used a Power Soil DNA Isolation Kit (MoBio, USA) to extract soil DNA from 0.25 g of fresh soil. The quantification of *nirS*, *nirK* and *nosZ* genes was decided by the primer pairs *cd3aF*/*R3cd*, *nirK1F*/*nirK5R* and *nosZF*/*nosZR*, respectively (Throbäck et al., 2004). The *nirS* and *nirK* genes represent the process of N_2O production, and the *nosZ* gene represents the process of N_2O consumption. The quantitative real-time PCR was performed using a QuantStudio 3 (Bio-Rad, USA).

When At harvest, we counted the nodule numbers and AMF vesicle numbers of plant roots (Phillips and Hayman, 1970) and measured the root biomass, above-ground biomass and grain yield. Plants were oven-dried at 70°C no less than 48 hours to achieve a constant weight. The plant N concentrations were measured using an elementary analyzer (vario PYRO, Elementar, Germany) and plant P concentrations were measured from H_2SO_4-H_2O_2 digestion and molybdenum-antimony colorimetric method (Thomas et al., 1967).

Yield-scaled N_2O emissions and relative net ecosystem economic budget (NEEB) between AMF and non-AMF treatments were calculated as follows:

Yield-scaled N_2O emission = N_2O emission / grain yield

Relative NEEB = (yield income of AMF- agricultural activity of AMF- N_2O cost of AMF)-(yield income of non-AMF- agricultural activity of non-AMF- N_2O cost of non-AMF), where yield incomes were calculated using the gain yield and the price of soybean (0.84 US$ kg^{-1}) and N_2O emission costs were calculated using total N_2O emissions and carbon trade price (17 US$ t^{-1} CO_2-eq). Agricultural activity costs were the same between the AMF and non-AMF treatments in this study.

174
175 *2.5 Statistical analysis*

176 In pot experiment, the data was analyzed by independent sample t-test. In the field
177 experiment, we used the paired sample t-test. All analyses were performed using the SPSS 22.0
178 statistical software package. Differences between treatments were considered significant at $P <$
179 0.05.

180

181 **3. Results**

182 *3.1 Plant traits*

183 In the pot experiment, the vesicle numbers on the roots were two orders of magnitude
184 higher in the AMF treatment than in the non-AMF treatment. The AMF increased root biomass
185 by 38%, and also significantly stimulated above-ground biomass, plant N and P accumulation,
186 and nodule numbers by 37%, 33%, 116% and 192% respectively. The AMF enhanced the grain
187 yield by 41% (Table 1).

188 In the field experiment, the AMF vesicle numbers in the roots were similar between the
189 allowed and prevented AMF hyphal access treatments. The AMF hyphal access significantly
190 stimulated root biomass, above-ground biomass, plant N and P accumulation and nodule
191 numbers by 100%, 146%, 200%, 113% and 213% respectively. The AMF hyphal access
192 increased the grain yield of soybean by 97% (Table 1).

193 *3.2 N_2O emissions and NEEB*

194 In the pot and field experiments, AMF significantly increased the soil N_2O emissions (Fig.
195 2 and 3a). In the pot experiment, AMF increased soil N_2O emissions by 33%. In the field
196 experiment, the AMF hyphae accesses significantly increased soil N_2O emissions by 24%. The

AMF reduced the yield-scaled N_2O emissions by 34% under the field condition, whereas they did not affect the yield-scaled N_2O emissions in the pot experiment (Fig. 3b). The AMF increased the NEEB by 1475 US$ ha^{-1} in the field experiment and by 1005 US$ ha^{-1} in the pot experiment (Fig. 3c).

3.3 Soil properties

In the pot experiment, AMF significantly increased the soil DOC and NH_4^+ concentrations, but did not affect the soil NO_3^- concentrations for soybean (Table 2). AMF reduced the soil available P concentrations by 17%. AMF did not affect the *nirS* and *nosZ* abundances, but increased the *nirK* abundance by 80% and (*nirS*+ *nirK*)/ *nosZ* by 62% (Fig. 4).

In the field experiment, AMF hyphal access increased the soil DOC concentrations and soil NO_3^- concentrations, but did not affect the soil NH_4^+ concentrations. The AMF enhanced the *nirS* abundance by 64% and *nirK* abundance by 34%. The *nosZ* abundance was similar between the allowed and prevented AMF hyphal access treatments. Finally, AMF hyphal access increased the (*nirS*+ *nirK*)/ *nosZ* by 27%.

4. Discussion

The results showed that AMF significantly increased N_2O emissions from soils with soybean. However, several previous studies indicated that AMF significantly reduced soil N_2O emissions from non-legume system (e.g. Bender et al., 2014; Storer et al. 2018; Teutscherova et al., 2019). AMF played an important role in plant N uptake (Smith and Read, 2008; Cavagnaro et al., 2015). The extraradical mycelium of AMF takes up NH_4^+ and NO_3^- from the soils and transfers them to the host plants (Hodge and Fitter, 2010; Cheng et al., 2012; Fellbaum

et al., 2012). The AMF hyphae can extend more than 10 cm beyond the root surface, which allows AMF to transfer N extensively and quickly (Li et al., 1991; Cavagnaro et al., 2015). Thus, the presence of AMF increased plant N accumulation and reduced soil N availability in non-legume systems (Bender et al., 2014; Liang et al., 2019). The denitrification is primarily limited by the soil N availability (Zhu et al., 2013), resulting in lower N_2O emissions of AMF treatments. Besides reducing soil N availability, AMF can decrease N_2O emissions from nonlegume systems through other underlying mechanisms. For instance, Bender et al. (2014) indicated that increased N immobilization by the soil microbial biomass can contribute to lower N_2O emissions because of AMF. Lazcano et al. (2014) found that control over N_2O emissions by AMF seemed to be driven by a higher use of soil water. Storer et al. (2018) found that AMF hyphae directly reduced N_2O production because it may be outcompeting slower-growing nitrifiers for ammonium.

In the legume system, N_2O is mainly emitted as a consequence of biological N_2 fixation (Itakura et al., 2013). The rhizobia symbiosis imposes great P demand on the host plants, probably due to nitrogenase demand for ATP and high P concentration in microbial tissue (Jakobsen, 1985; Divito and Sadras et al., 2014; Kleinert et al., 2014). AMF can stimulate P uptake via their extensive hyphal networks (Smith and Read, 2008, Püschel et al., 2017), as indicated by lower availability of P concentrations from soil and higher plant P accumulation in the AMF treatments. Furthermore, Zhang et al. (2016) showed that AMF increased soil P mobilization. Thus, AMF increased the weight of nodules in both pot and field experiments, indicating that AMF stimulated biological N_2-fixation (Püschel et al., 2017). Higher biological N_2-fixation can release more N into soils through exudates and decomposition of root nodules (Itakura et al., 2013), as indicated by higher NH_4^+ or NO_3^- concentrations in AMF treatments.

242 Moreover, AMF increased root growth and soil DOC concentrations, which may provide more
243 C for denitrification. Higher soil C and N availability can stimulate the microbial role in
244 nitrification and denitrification (Barnard et al., 2005; Zhu et al., 2013). The results also showed
245 that AMF stimulated the *nirS* and/or *nirK* abundances in the soils for soybean. In other words,
246 AMF stimulated soil C and N availability in the legume systems, through improving biological
247 N_2-fixation and plant growth, and resulted in higher N_2O emissions.

248 Because of symbiotic atmospheric nitrogen fixation, grain legumes benefit the agricultural
249 sustainability in developed and developing countries. In this study, AMF stimulated the grain
250 yields of soybean, in accordance with several previous studies (e.g. Lazcano et al., 2014; Liu et
251 al., 2018; Liang et al., 2019). The global meta-analysis showed that AMF inoculation increased
252 crop grain yield by about 20% (Lekberg and Koide, 2005; Pellegrino et al., 2015). These results
253 suggest that AMF may benefit to break the yield ceiling for ensuring food security. Furthermore,
254 the results showed that AMF reduced the yield-scaled N_2O emissions under field condition and
255 increased the NEEB, indicating that benefits of crop yield improvement of AMF can cover the
256 enhancement of N_2O emissions.

257 The results indicated that AMF increased N_2O emissions for legume systems due to higher
258 biological N_2-fixation. Yet, higher biological N_2-fixation may reduce N fertilizer input of the
259 next season, which can directly and indirectly reduce N_2O emissions (Shcherbak et al., 2014).
260 AMF also affect the soil organic matter decomposition (Cheng et al., 2012), which may affect
261 soil organic C stock. Thus, we suggest that the effect of AMF on net greenhouse gas emissions
262 should be investigated in future research.

263 5. Conclusion

In conclusion, we found that AMF increased the soil N_2O emissions from soybean. The AMF increased plant biomass, grain yield, plant N and P uptake, and biological N_2 fixation. AMF stimulated soil NH_4^+ or NO_3^- concentrations, DOC concentrations, and the abundances of *nirS* and/or *nirK* involved in N_2O production. AMF reduced the yield-scaled N_2O emissions under field condition and increased the NEEB. Our findings indicate AMF stimulate N_2O emissions from legume plants mostly through improving P acquisition for biological N_2 fixation, and suggest AMF benefit soybean production for higher-yield with less N_2O emissions.

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452
453 **Tables and figures:**

454 **Table 1** Root biomass, above-ground biomass, yield, plant N and P accumulation, nodule
455 numbers and AMF vesicle numbers.

Table 2 Soil dissolved organic C (DOC), NH_4^+, NO_3^- and available P concentrations. **Fig. 1** "Phosphorus chamber" - a device for controlling the absorption of phosphorus by arbuscular mycorrhizal fungi (AMF) in the field experiment.

Fig. 2 N_2O emissions flux as affected by AMF in the pot (a) and field (b) experiments. AMF, with AMF inoculum; non-AMF, without AMF inoculum; non-prevented, allowed AMF hyphal access; prevented, prevented AMF hyphal access. Error bars indicate standard errors (n=3).

Fig. 3 Cumulative N_2O emissions (a), yield-scaled N_2O emissions (b) and relative net ecosystem economic budget (c) as affected by AMF. AMF, with AMF inoculum; non-AMF, without AMF inoculum; non-prevented, allowed AMF hyphal access; prevented, prevented AMF hyphal access. * indicates significant differences at $P < 0.05$. Error bars indicate standard errors (n=3).

Fig. 4 The abundance of *nirS* (a), *nirK* (b), *nosZ* (c) and (*nirS* + *nirK*)/*nosZ* (d) as affected by AMF. AMF, with AMF inoculum; non-AMF, without AMF inoculum; non-prevented, allowed AMF hyphal access; prevented, prevented AMF hyphal access. * indicates significant differences at $P < 0.05$. Error bars indicate standard errors (n=3).

Table 1 Root biomass, above-ground biomass, grain yield, plant N and P accumulation, nodule numbers and AMF vesicle numbers.

	Pot		Field	
	non-AMF	AMF	Prevented	non-prevented
Root biomass (g plant^{-1})	1.3±0.1	1.8±0.3	0.6±0.0*	1.2±0.1
Aboveground biomass (g plant^{-1})	18.0±0.2*	24.6±0.2	3.9±0.2*	9.6±0.9
Yield (g plant^{-1})	7.8±0.4*	11.0±0.1	1.7±0.1*	3.3±0.4
N accumulation (g plant^{-1})	0.6±0.0*	0.8±0.1	0.1±0.0*	0.3±0.1
P accumulation (mg plant^{-1})	30.6±2.9*	66.1±3.9	10.0±0.8*	21.3±1.4
Nodule number (No. plant^{-1})	6.4±2.9*	18.7±2.0	8.0±1.0*	25.0±5.1
AMF vesicle (No. cm^{-1})	0.3±0.1*	31.2±4.7	18.1±0.8	20.7±6.7

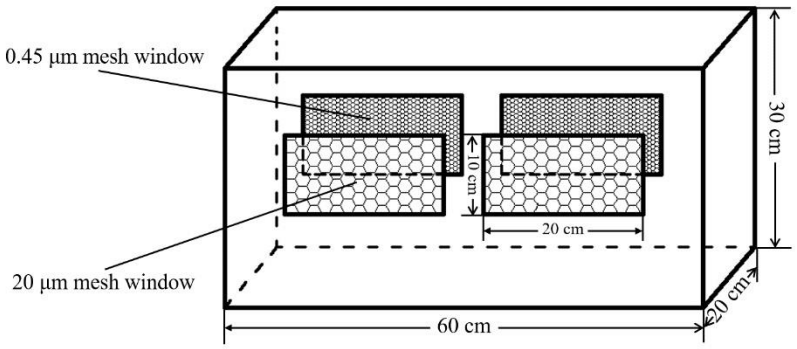
473 Notes. Mean ± standard error (n=3). AMF, soil with AMF inoculum; non-AMF, soil without AMF
474 inoculum; non-prevented, allowed AMF hyphal access; prevented, prevented AMF hyphal access. *
475 indicates significant differences within AMF at $P < 0.05$.

476

477 **Table 2** Soil dissolved organic C (DOC), NH_4^+, NO_3^- and available P concentrations.

	Pot		Field	
	non-AMF	AMF	Prevented	non-prevented
DOC (mg kg^{-1})	53.4±1.8*	66.8±2.2	28.7±1.7*	35.8±3.3
Soil NH_4^+ (mg kg^{-1})	7.1±0.4*	10.4±0.9	4.3±0.1	4.5±0.1
Soil NO_3^- (mg kg^{-1})	7.0±0.4	7.3±1.4	16.5±0.7*	19.5±0.4
Available P (mg kg^{-1})	31.9±1.2	26.5±0.4*	NA	NA

478 Notes. Mean ± standard error (n=3). AMF, soil with AMF inoculum; non-AMF, soil without AMF
479 inoculum; non-prevented, allowed AMF hyphal access; prevented, prevented AMF hyphal access. NA,
480 not acquirement. * indicates significant differences within AMF at $P < 0.05$.



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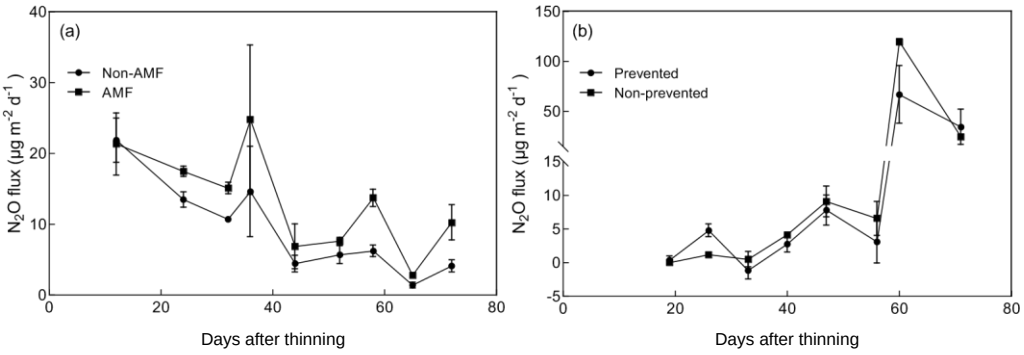
484 **Fig. 1** "Phosphorus chamber" - a device for controlling the absorption of phosphorus by 485 arbuscular mycorrhizal fungi (AMF) in the field experiment.



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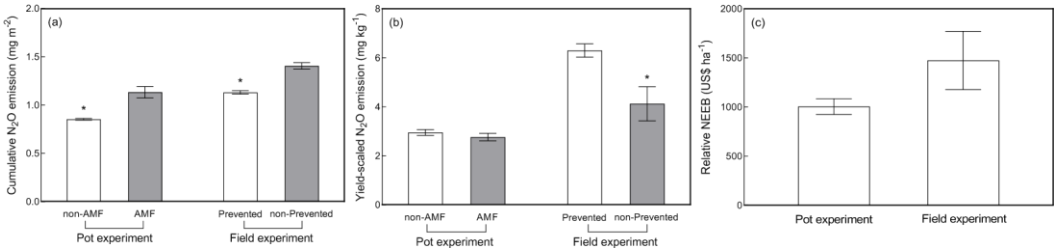
487 **Fig. 2** N_2O emissions flux as affected by AMF in the pot (a) and field (b) experiments. AMF, with AMF inoculum; non-AMF, without AMF inoculum; non-prevented, allowed AMF hyphal 489 access; prevented, prevented AMF hyphal access. Error bars indicate standard errors (n=3).



490

491 **Fig. 3** Cumulative N_2O emissions (a), yield-scaled N_2O emissions (b) and relative net ecosystem economic budget (NEEB, c) as affected by AMF. AMF, with AMF inoculum; non493 AMF, without AMF inoculum; non-prevented, allowed AMF hyphal access; prevented,

prevented AMF hyphal access. * indicates significant differences at $P < 0.05$. Error bars indicate standard errors (n=3).

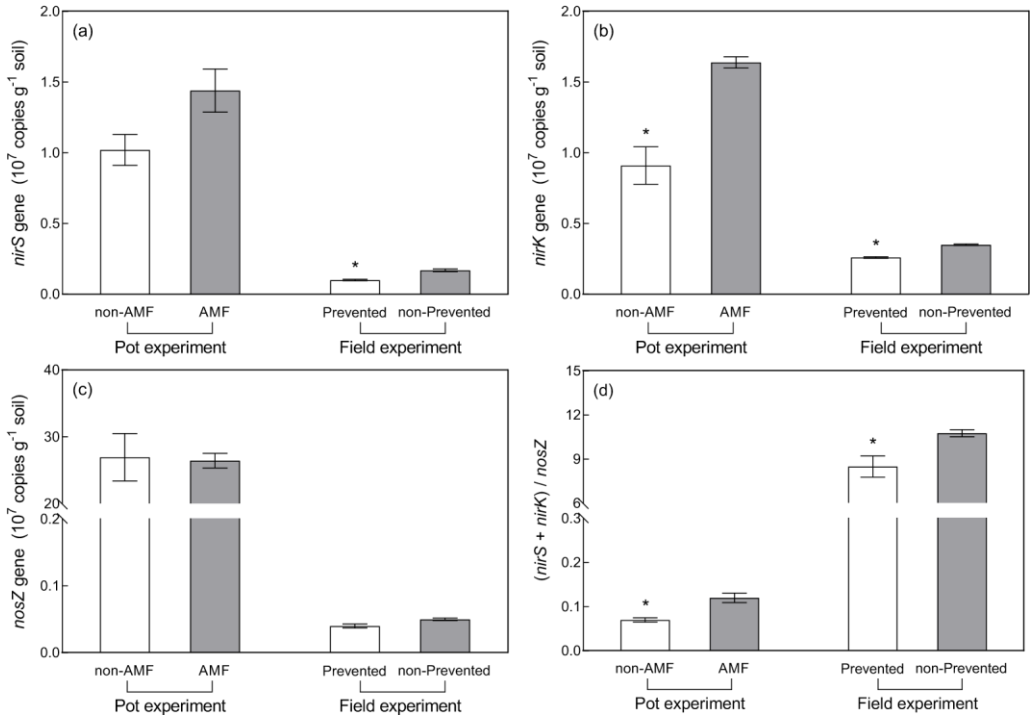


Fig. 4 The abundance of *nirS* (a), *nirK* (b), *nosZ* (c) and (*nirS* + *nirK*)/*nosZ* (d) as affected by AMF. AMF, with AMF inoculum; non-AMF, without AMF inoculum; non-prevented, allowed AMF hyphal access; prevented, prevented AMF hyphal access. *indicates significant differences at $P < 0.05$. Error bars indicate standard errors (n=3).