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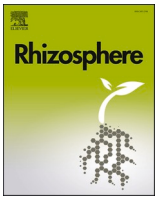

Soil N_2O emissions are more sensitive to phosphorus addition and plant presence than to nitrogen addition and arbuscular mycorrhizal fungal inoculation

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ABSTRACT

Although phosphorus (P) addition and arbuscular mycorrhizal fungal (AMF) colonization have potential for mitigating soil N_2O emission, the effects and mechanisms remain unresolved. We conducted a pot experiment with ryegrass (*Lolium perenne*) growing in a growth chamber for 80 days with three factors: Plant and AMF presence (unplanted, with plants, with plants colonized by AMF), two nitrogen (N) addition levels (0 and 50 mg N kg^{-1} soil) and two P addition levels (0 and 20 mg P kg^{-1} soil). Our results showed that N addition and AMF colonization had insignificant effects on soil N_2O emission. However, the presence of plants decreased soil N_2O emission by 90%–99% (planted vs. unplanted) under P addition. Moreover, P addition increased (+134%) N_2O emission from unplanted soil, but decreased (74%–98%) it from planted soil. Further analysis showed that soil N_2O emission was controlled by soil available P, soil NO_3^--N, plant biomass P and N across all treatments. The lower N_2O emissions from planted soils were mainly due to the lower soil NO_3^--N content which might be immobilized by plant biomass, while the higher N_2O emissions from unplanted soils under P addition were attributed to the increased soil available P content. Furthermore, we found an antagonistic effect between AMF inoculation and P addition on soil N_2O emission because sufficient soil P may inhibit AMF colonization and activity. Taken together, we conclude that plant presence combined with P addition can effectively reduce N_2O emission from P-limited soils.

1. Introduction

Nitrous oxide (N_2O) with a global warming potential 298 times greater than CO_2 is one of the three important greenhouse gases (CO_2, CH_4 and N_2O) contributing to global climate change (Ravishankara et al., 2009). Soil N_2O is mainly produced by nitrification or denitrification processes, which are dependent on a variety of factors, including available nitrogen (N) and carbon (Liang et al., 2015), soil moisture and temperature (Butterbach-Bahl et al., 2013), soil oxygen (Song et al., 2019), soil pH (Wang et al., 2018b), and soil texture (Gaillard et al., 2016). It is well known that fertilizer use and atmospheric N (nitrogen) deposition are important sources for N_2O emissions. The deposited N serves as a critical substrate for nitrifiers and denitrifiers (Liu and Greaver, 2009; Deng et al., 2020), which may increase soil N_2O emission in widely distributed ecosystems (Lund et al., 2009; Wang et al., 2014; Chen et al., 2019; Huddell et al., 2020). In order to mitigate N_2O emission derived from N deposition, a number of practices have been taken, such as reducing the amount of N application (Luo et al., 2010), improving nitrogen use efficiency (Cardenas et al., 2019), and avoiding soil conditions that favor denitrification by improving drainage and reducing compaction (de Klein and Ledgard, 2005).

Plants play vital roles in soil nitrogen cycling as well as N_2O emission. Some studies have revealed that the presence of cover crops can decrease soil N_2O emission compared to bare/fallow soil (Parkin and Kaspar, 2006; Muhammad et al., 2019). This is mainly due to the fact that cover crops often increase N uptake and reduce soil available N. The

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N_2O mitigation effect is also found in the beech and ash saplings planted treatment compared to root-free soil (Fender et al., 2013). On the other hand, plant root exudation or fine root turnover may stimulate soil net N mineralization (Gan et al., 2021). Meanwhile, the root-derived carbon could stimulate denitrification activities and N_2O emissions (Henry et al., 2008). However, a meta-analysis on the effect of cover crops on soil N_2O emission implied that 40% of the observations showed decreased N_2O emission, while 60% of observations showed increased N_2O emission, and this effect was mainly related to the type of crops, period of measurement and soil carbon content (Basche et al., 2014). We assume that the plant effect on soil N_2O emission is dependent on the tradeoff between N uptake-induced substrate depletion and carbon/-nitrogen input-induced denitrification/nitrification stimulation.

Phosphorus (P) addition has been considered to be a promising strategy to alleviate the negative effect of N deposition on N_2O production. Baral et al. (2014) found that P addition led to 50% lower N_2O production in a pot experiment planted with maize. Moreover, this negative effect was reported in other cases (Zhang et al., 2014; Zheng et al., 2016; Chen et al., 2017). Less N_2O emissions with N + P addition compared to N addition was also observed (Baral et al., 2014). This N_2O mitigation with P addition was likely due to decreased soil inorganic N concentration (Chen et al., 2016). Because plant P limitation was alleviated by P addition, most of soil available N was absorbed and utilized by plants (Mori et al., 2014), which together with increased abundance of nitrous oxide reducers (Tang et al., 2019) could result in less N_2O emissions from soil. By contrast, other studies have reported insignificant effect of P addition on N_2O emission from ultrabasic rock soils (Mori et al., 2016), tropical forest soils (Wang et al., 2014; Zheng et al., 2016), and alpine meadow soils (Wang et al., 2016). This might be attributed to the lower water-filled pore space during sampling (Mori et al., 2017), or the short-term P addition (Martinson et al., 2013), which masked the effects of P application on N_2O emission. In contrast, other studies argued that P addition may increase the gaseous loss of N as N_2O in P-limited soils when inorganic N is abundant (Mehnaz and Dijkstra, 2016). Mori et al. (2013) pointed out that P addition stimulated denitrification activity by relieving P shortage for denitrifying bacteria. Tang et al. (2019) showed that the potential nitrification and denitrification activities increased and AOB *amoA* (the genes encoding ammonia monooxygenase in the group of ammonia-oxidizing bacteria) and *nirK* (nitrite reductase genes) genes were more abundant following P addition in a subtropical forest soil. Hence, the P addition effect on soil N_2O emission remains unresolved and requires further research.

Arbuscular mycorrhizal fungi (AMF) are widely distributed among terrestrial ecosystems (Smith and Read, 2008). Recently, the role of AMF on soil N cycling had drawn increasing attention (Smith and Smith, 2011; Jansa et al., 2019; Thirkell et al., 2019), especially on N_2O emission (Gui et al., 2021; Shen and Zhu, 2021). It is well established that AMF can take up N from the soil and transfer it to their host plants. They can, therefore, reduce soil N concentrations, which is a substrate for N_2O emission (Veresoglou et al., 2012). Bender et al. (2014) showed that AMF reduced N_2O emissions from soil planted with tomato by 33–42% via this mechanism in a greenhouse experiment. Also, AMF can reduce soil total N or NO_3^--N concentration and emissions of N_2O in a meadow grassland (Kang et al., 2020) and a tropical grass mesocosm study (Teutscherova et al., 2019). However, another study showed that the decreased N_2O emissions by AMF were more related to increased soil water utilization than improved N uptake (Lazcano et al., 2014). In addition to enhanced nutrients and water uptake, another possibility is that the presence of AMF tends to alter soil microbial communities. Nitrifiers were found to be suppressed by AMF hyphae as ammonia-oxidizing bacteria are weak competitors for soil NH_4^+-N than AMF (Storer et al., 2018; Veresoglou et al., 2019). AMF abundance has also been shown to correlate negatively with the *nirK* gene abundance, and positively with the *nosZ* gene abundance (Bender et al., 2014). Notably, a few studies found that AMF has little effect on N_2O emissions (Cavagnaro et al., 2012; Okiobe et al., 2020). Moreover, a positive effect of AMF on N_2O emissions was even detected by Okiobe et al. (2019). This might be driven by hyphodeposition and hyphal turnover, although no direct evidence was available. Therefore, the influences of AMF on N_2O emission and the underlying mechanisms remain to be identified.

As mentioned above, P addition, plant and AMF colonization play key roles in mediating soil N_2O emission. Moreover, the role of AMF in P uptake has been extensively studied, and AMF hyphae can absorb available P from the soil and transfer it to their host plant for the exchange of carbon (van der Heijden et al., 2006; Zhang et al., 2018; Wen et al., 2019). Consequently, this may also alleviate plant P limitation, promote N uptake, and have an indirect impact on soil N cycling. Still, limited studies have addressed the potential combined effects of P addition and AMF colonization on soil N_2O emission. Therefore, the objectives of our study are to identify the underlying mechanisms of the effects of P addition and AMF colonization on soil N_2O emission. We hypothesize that: (1) P addition may lead to low N_2O emission from a P-limited soil due to the enhanced N assimilation by plants; (2) Soils under plants with AMF colonization may have a lower N_2O emission compared with soils under plants without AMF colonization, mainly because of the enhanced plant N uptake by AMF presence; and (3) the combined addition of P and AMF may have an additive effect and result in the lowest soil N_2O emission.

2. Materials and methods

2.1. Experiment design

We conducted a three-factorial pot experiment with three levels of plant/AMF presence (presence of None, presence of Plant, and presence of Plant + AMF), two levels of N addition (N0 and N1, with 0 and 50 mg N kg^{-1} soil, equivalent to 0 and 50 kg N ha^{-1} in our experiment, applied in the form of NH_4NO_3) and two levels of P addition (P0 and P1, with 0 and 20 mg P kg^{-1} soil, equivalent to 0 and 20 kg P ha^{-1}, applied in the form of KH_2PO_4) in a growth chamber from December 6th, 2018 to February 24th, 2019. Each treatment had 5 replicates. The soil (Alfisol) was collected from the top 20 cm of the mineral soil layer in an old orchard field located near Beijing, China (40.39° N, 116.67° E), which was not previously fertilized. Other main soil properties are as following: organic C is 8.46 g kg^{-1}, total N is 1.09 g kg^{-1}, available phosphorus is 3.54 mg kg^{-1}, and pH is 7.94. The soil was sieved through 2 mm, and large stones and roots were removed. Then the soil was mixed with quartz sand by a soil:sand ratio of 2:1 (v/v). The mixture was transported to the laboratory and irradiated by gamma ray with a maximum dose of 32 kGy for one week to eliminate indigenous AMF. Then each pot (16 cm diameter, 14 cm height) was filled with the soil: sand mixture (1 kg pot^{-1}) and fertilizer which includes the corresponding rate of N and P mentioned above and other macro-and micronutrients: 120 mg kg^{-1} potassium (K), 10.46 mg kg^{-1} calcium (Ca), 2.76 mg kg^{-1} sodium (Na), 2.92 mg kg^{-1} magnesium (Mg), 3.36 mg kg^{-1} iron (Fe), 2.08 mg kg^{-1} manganese (Mn), 0.15 mg kg^{-1} zinc (Zn), 0.06 mg kg^{-1} cuprum (Cu), 1.50 mg kg^{-1} boron (B) and 0.01 mg kg^{-1} molybdenum (Mo). Also, the AMF inoculum (mixture of seven species: *Rhizophagus intraradices*, *Claroideoglomus etunicatum, Claroideoglomus claroideum, Funneliformis caledonium, Funneliformis geosporum, Rhizophagus manihotis,* and *Funneliformis mosseae,* Huai'an Chaimihe Agriculture Science and Technology Development Co., Ltd., China) was added to corresponding Plant + AMF pots. The fungi were propagated on maize (*Zea mays* L.) marigold (*Tagetes erecta* L.) and white clover (*Trifolium repens* L.) grown in sterilized perlite and vermiculite substrate. The inoculated dosage was 50 mL of inocula per pot containing approximately 3000 spores (Dong et al., 2008). To include native microbes from the soil, 100 mL of a microbial slurry was mixed into the substrate for each pot. The slurry was produced by suspending the fresh field soil in deionized water and subsequent filtering through a filter paper of 2.5 μm pore size to exclude AMF spores.

The ryegrass (*Lolium perenne* L.), a common grass species in

agricultural and natural grasslands was seeded on December 6th, 2018 and then thinned to keep 30 individuals for each pot. All the 60 pots were randomly placed in a growth chamber (16 h, 25 °C during daytime, and 8 h, 19 °C during nighttime, relative humidity was 75%) and relocated every ten days to minimize the effect of spatial heterogeneity inside the growth chamber. During the experiment, each pot was weighed daily and added with deionized water to maintain soil moisture at 55% water-filled pore space (WFPS). During the experiment period we noticed nitrogen deficiency in plant leaves, so we applied additional 50 mg N kg^{-1} soil on the 37th day and 70 mg N kg^{-1} soil on the 64th day after ryegrass sowing. Also, the pots without plants received an equivalent level of nitrogen.

2.2. Soil and plant sampling and chemical analysis

Plants and soils were destructively sampled at the end of the experiment (February 24th, 2019, 80 days after sowing, when ryegrass was at its late jointing stage). Mycorrhizal colonization rate was determined from root samples after staining with trypan blue (Koske and Gemma, 1989) and using a colonization grade intensity method under a microscope (Sraj-Krzic et al., 2006). Root segments were ranked into six classes of mycorrhizal colonization: 0%, <1%, 1–10%, 11–50%, 51–90%, and >90%. AMF colonization was calculated as:

$$\text{AMF colonization} = \frac{95 \times n_{>90\%} + 70 \times n_{51-90\%} + 30 \times n_{11-50\%} + 5 \times n_{1-11\%} + n_{<1\%}}{n} \times 100\%$$

where n is the number of root segments.

Plant biomass was determined by drying at 65 °C to a constant mass. Both soil and plant samples were grinded to pass through a 0.15 mm mesh for total carbon (TC), total nitrogen (TN), and total phosphorus (TP) analysis. TC and TN were determined using a CHNOS elemental analyzer (Vario El III, Elementar Analysensysteme GmbH, Germany). TP was analyzed by X-Ray Fluorescence (XRF) spectrometer (AxiosmAX, Malvern Panalytical, Netherlands).

Soil moisture content was determined by drying at 105 °C for 48 h. Fresh soils were sieved through 2 mm and extracted with 2 mol L^{-1} KCl solution, then the concentrations of NO_3^--N and NH_4^+-N were determined using a flow injection analyzer (AutoAnalyzer 3, SEAL Analytical GmbH, Germany). Soil available P (AP) was measured by the Olsen method. Soil pH was determined with a glass electrode in a 1:2.5 soil:water solution (w/v). Soil microbial biomass C and N (MBC and MBN) were measured by fumigation-extraction (Vance et al., 1987) and extractable organic carbon were extracted by 0.5 mol L^{-1} K_2SO_4 solution then analyzed using a carbon and nitrogen analyzer (TOC/TNb analyzer, Elementar Analysensysteme GmbH, Germany).

2.3. N_2O emission

Fluxes of N_2O from soil surface were measured manually near the end of the experiment (on February 22nd, 2019, 78 days after sowing) using static chambers-gas chromatography method. The pot was put into a plastic chamber (29.2 cm in diameter and 32.5 cm in height) with a lid sealed with the chamber during flux measurements. A three-way stopcock attached to the lid was used for gas sampling. Samples were collected at 0, 20, 40, and 60 min after the lids were attached by a plastic syringe, and 30 mL gases were transferred immediately to pre-evacuated 12 mL exetainer vials with gas-tight rubber stoppers. All gas samples were analyzed by a gas chromatograph (Model 7890 B, Agilent, USA) equipped with an electron capture detector. The hourly fluxes (F, μg m^{-2} h^{-1}) for N_2O, were calculated by Shen et al. (2018):

$$F = \frac{M}{V_0} \frac{V}{A} \frac{dc}{dt} \frac{T_0}{T} \times 10^3$$

where M is the relative molecular mass of N_2O (44 g mol^{-1}), V_o is the volume of an ideal gas, V (m^3) and A (m^2) are the volume and bottom area of the chamber, respectively, dc/dt (ppm h^{-1}) is the slope change of gas concentration in the chamber, T is the temperature (K) in the chamber, T_o is the temperature of an ideal gas.

2.4. Statistical analysis

The effects of Presence, N fertilization, P fertilization, and their interactions on the different parameters were analyzed using a three-way analysis of variance (ANOVA) to test for significant differences. In order to determine the effect of AMF inoculation, we made an additional three-way ANOVA analysis with the data excluding None treatment. When F values indicated significant differences, a Student's t-test or post-hoc test (LSD) was conducted to compare the difference among treatments. We used Random Forest Package (Liaw and Wiener, 2002) in R 4.03 to make a variable importance rank among 13 variables to predict N_2O emission rate, as this method has the advantage over maximum likelihood model-selection method that can handle many potential predictors and their interactions, and considers nonlinear relationships. The predictor variables included 10 soil and plant parameters: soil ammonium (NH_4^+-N), soil nitrate (NO_3^--N), soil available P (AP), soil pH, soil water content (SWC), microbial biomass nitrogen (MBN), microbial biomass carbon (MBC), extractable organic carbon (EOC), plant biomass phosphorous (PBP), plant biomass nitrogen (PBN), and 3 treatment factors: Presence, N addition, and P addition. The values of plant parameters in None treatment (i.e. PBN and PBP) were assigned to "zero" because of no plant existence. The random forest algorithm estimated the importance of a variable by looking at how much prediction error increased when (out-of-bag, OOB) data for that variable was permuted while all others were left unchanged. The higher the increased mean square error was, the more important the variable. Then 6 selected predictive variables (PBP, NO_3^--N, AP, PBN, P addition and Presence) were identified using 10-fold cross-validation, implemented using the '*rfcv*' function in Random Forest Package (Liu et al., 2020). Regression analysis was used to explore the relationships between the N_2O emission rate and selected variables.

3. Results

3.1. AMF colonization

We found that AMF colonization rate (indicated by the rate of mycorrhizal colonization in the root system) varied from 19.46% to 35.32% in AMF + Plant treatment, significantly higher than the non-AMF inoculated treatment (ranged from 0.23% to 0.33%). This result indicated that the inoculation of AMF achieved our expectations. We also detected that fertilization had no significant effect on AMF colonization rate, though the treatments with P addition (P and NP) showed a lower AMF colonization than those without P addition (CK and N, Fig. S1).

3.2. N_2O emission

The mean N_2O emission rate was 11.04 μg m^{-2} h^{-1} among all

Table 1
The effects of Presence (None, Plant and Plant + AMF), N (N0 and N1) and P (P0 and P1) fertilization on soil N_2O emission, soil, and plant variables. *P* values of three-way ANOVA results were shown. N_2O: N_2O emission rate, AP: soil available phosphorous content, NO_3^--N: soil nitrate content, PBP: plant biomass phosphorous, PBN: plant biomass nitrogen, EOC: soil extractable organic carbon, NH_4^+-N: soil ammonium content, SWC: soil water content, pH: soil pH, TC: soil total carbon content, TN: soil total nitrogen content, TP: soil total phosphorous content, MBC: microbial biomass carbon, MBN: microbial biomass nitrogen, AMFC: arbuscular mycorrhizal fungi colonization, AGB: plant aboveground biomass, BGB: plant belowground biomass, PB: plant biomass, Root:Shoot, root and shoot ratio.

Variables	Presence	P	N	Presence × P	Presence × N	P × N	Presence × P × N
N_2O	**<0.001**	0.123	0.212	**<0.001**	0.257	0.804	0.171
AP	**<0.001**	**<0.001**	0.101	**<0.001**	0.281	0.550	0.153
NO_3^--N	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.845	**<0.01**	**<0.001**
PBP	**<0.001**	**<0.001**	0.109	0.454	**<0.001**	0.204	**<0.001**
PBN	**<0.001**	**<0.001**	**<0.001**	0.533	0.424	**<0.01**	0.109
EOC	**<0.01**	0.088	0.179	0.394	**<0.001**	0.771	0.221
NH_4^+-N	**<0.01**	0.065	**<0.05**	**<0.05**	**<0.05**	0.177	0.090
SWC	0.332	**<0.01**	0.303	0.369	0.547	0.995	0.773
pH	**<0.05**	**<0.05**	0.104	0.116	0.094	0.215	0.058
TC	0.087	**<0.01**	0.163	0.577	0.352	0.387	0.443
TN	**<0.001**	**<0.01**	**<0.01**	0.934	0.212	**<0.05**	0.156
TP	**<0.01**	0.688	0.763	**<0.05**	0.988	0.701	0.842
MBC	**<0.05**	0.397	0.091	0.736	0.329	0.225	0.318
MBN	**<0.001**	0.177	0.704	0.442	0.446	0.816	0.947
AMFC	**<0.001**	**<0.001**	0.686	**<0.01**	0.696	0.740	0.805
AGB	**<0.05**	**<0.001**	**<0.001**	0.910	0.202	**<0.05**	0.369
BGB	0.464	**<0.05**	**<0.001**	**<0.001**	0.101	**<0.05**	**<0.001**
PB	0.115	**<0.001**	0.342	**<0.05**	**<0.05**	0.714	**<0.001**
Root:Shoot	**<0.01**	**<0.01**	**<0.001**	**<0.001**	0.274	**<0.001**	**<0.001**

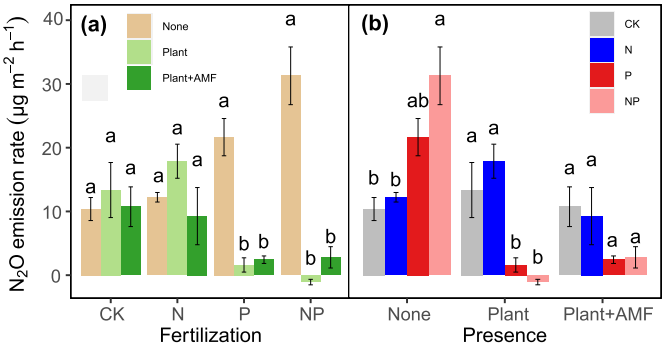

Fig. 1. N_2O emission rates among different fertilization and presence treatments. CK: no N/P addition; N: only 50 mg N kg^{-1} soil was added without P; P: only 20 mg P kg^{-1} soil was added without N; NP: 50 mg N kg^{-1} soil and 20 mg P kg^{-1} soil were added. None: no plant and AMF; Plant: only plant; Plant + AMF: plant with AMF inoculated. Data were analyzed by one-way ANOVA and followed by least square difference (LSD) post hoc test. Values are Mean ± SE (n = 5). Different letters within each group (e.g. NP or None) depict statistical difference, $P < 0.05$. The three-way ANOVA results are shown in Table 1 and Table S1.

treatments with the lowest emission (−1.08 μg m^{-2} h^{-1}) from Plant × NP treatment and the highest (31.27 μg m^{-2} h^{-1}) from None × NP treatment. An interactive effect of Presence × P addition ($P < 0.001$, Table 1) on N_2O emission was observed. The presence of None, Plant, and Plant + AMF showed no differences in N_2O emission ($P > 0.05$, Fig. 1) among each other in the treatments without P addition (CK and N). However, N_2O emission in None treatment was much higher ($P < 0.05$, Fig. 1) than those in Plant and Plant + AMF treatments under P addition (P and NP). Compared with non-P-addition (averaged by CK and N) treatment, P addition (averaged by P and NP) significantly increased N_2O emission (+134%) from unplanted soil (None), but decreased it from planted soils (−98% for Plant and −74% for Plant + AMF, Fig. 1).

Though P addition led to lower N_2O emission rate than non-P addition among planted treatments (Plant and Plant + AMF), this effect was much more pronounced for Plant treatment (Fig. 1). In addition, we found an interactive effect of AMF × P addition when excluding the None treatment ($P < 0.05$, Table S1).

3.3. Factors affecting N_2O emission

The N_2O emission rate was positively related to most soil parameters but negatively related to plant parameters (Fig. S2; Fig. S3). Furthermore, we selected soil NO_3^--N, AP, PBP, and PBN as the most important variables to predict N_2O emission in our experiment based on the random forest model (Fig. 2). We also found that P addition and Presence were the additional significant factors for N_2O emission (Fig. 2). This was consistent with the three-way ANOVA results (Table 1).

3.4. Soil and plant properties

As shown in Fig. 3a, P addition (10.74 mg P kg^{-1}, averaged by P and NP) significantly increased ($P < 0.05$) soil AP compared with non-P addition (6.22 mg P kg^{-1}, averaged by CK and N) across all three Presence groups. No significant difference was detected between P and NP, as well as between CK and N. Unplanted treatments (11.62 mg P kg^{-1}) had a significantly higher ($P < 0.05$) soil AP than the other two treatments (Plant and Plant + AMF, with the mean value 7.06 and 6.77 mg P kg^{-1}, respectively) across all fertilization groups. Moreover, there was no remarkable difference in soil AP between Plant and Plant + AMF (Fig. 3a). Notably, the N_2O emission rate was positively related to soil AP ($P < 0.001$, R = 0.81) from None treatment; however, a negative relationship ($P < 0.001$, R = −0.61) between them was found from Plant and Plant + AMF treatments (Fig. 3b).

The fertilizer application showed no effect on soil NO_3^--N from None treatment with an average value of 114.75 mg N kg^{-1}, which was remarkably higher compared to those from Plant and Plant + AMF treatments (averaged 23.66 and 24.01 mg N kg^{-1}, respectively). Unlike None treatment, soil NO_3^--N was significantly lower from P-added treatments (14.05 mg kg^{-1}, averaged across P and NP) than that of non-P-added treatments (33.62 mg kg^{-1}, averaged across CK and N) at the presence of Plant and Plant + AMF (Fig. 3a). Moreover, NO_3^--N was not a good predictor of N_2O emission for None treatment, but positively correlated ($P < 0.001$, R = 0.64) with N_2O emission from Plant and Plant + AMF treatments (Fig. 3b).

We found that AMF inoculation tended to increase PBP and PBN in most cases, except in the NP treatment (Fig. 3a). Phosphorus addition (P and NP) significantly increased ($P < 0.05$) PBP compared to CK and N. There was a significant difference in PBP between P and NP, but the trend was reversed between Plant and Plant + AMF groups (Fig. 3a).

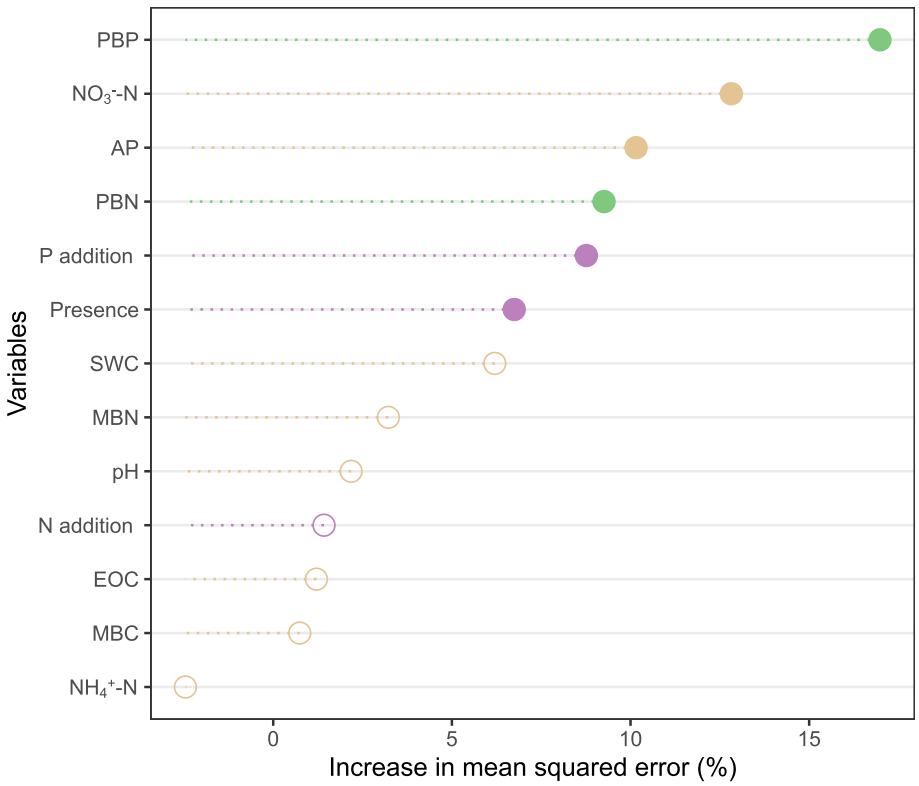

Fig. 2. Cleveland dot plot of the soil variables, plant variables and treatment factor importance as measured by a Random Forest model. The variables were ranked in descending order of the importance to the accuracy of the models, and the solid circles indicated the selected predictive variables for N_2O emission rate by 10 fold cross-validation variable selection strategy, and the blank circles indicated no selected variables. The different color indicated soil (brown), plant variables (green), and treatment factors (Purple). PBP: plant biomass phosphorous, NO_3^--N: soil nitrate content, AP: soil available phosphorus content, PBN: plant biomass nitrogen, SWC: soil water content, MBN: microbial biomass nitrogen, pH: soil pH, EOC: soil extractable organic carbon, MBC: microbial biomass carbon, NH_4^+-N: soil ammonium content.

Moreover, N, P, and NP treatments all showed a significant increase in PBN compared to CK. Both N addition (with 121.17 g N pot^{-1} averaged by N and NP) and P addition (with 123.87 g N pot^{-1} averaged by P and NP) had a higher PBN compared to non-N (with 103.12 g N pot^{-1} averaged by CK and P) and non-P addition (with 100.54 g N pot^{-1} averaged by CK and N) treatments, respectively. Notably, both PBP and PBN were negatively correlated with N_2O emission across all treatments (Fig. 3b).

4. Discussion

In this study, we found that soil N_2O emissions were more sensitive to phosphorus addition and root presence than to nitrogen addition and arbuscular mycorrhizal fungal inoculation. Moreover, we demonstrated contrasting effects of P addition on N_2O emission from unplanted and planted soils. Specifically, N_2O emission rate was promoted by P addition in unplanted P-poor soils. However, P addition increased soil AP and alleviated plant P limitation in the planted P-poor soil, and thus enhanced plant N uptake and resulted in lower NO_3^--N concentration and N_2O emission. In addition, AMF inoculation and N addition showed insignificant effect on N_2O emission because of the minor changes of soil physico-chemical properties. Moreover, we found an antagonistic effect between AMF inoculation and P addition on soil N_2O emission because sufficient soil P may inhibit AMF colonization and activity.

4.1. The interaction effect of P and plant on N_2O emission

We hypothesized that P addition may result in lower N_2O emissions. This was only confirmed in the treatments with plants (Plant and Plant + AMF), but not in the unplanted soil (None) (Fig. 1). On the other hand, the hypothesis that plant plays a vital role in reducing N_2O emission was only verified under P added soils (Fig. 1), which demonstrated an interaction effect between P addition and plant presence (Table 1). The unplanted (None) treatment had a relatively high soil NO_3^--N content (114.75 mg N kg^{-1}), indicating that soil N was in excess. Also, a high soil N:P ratio of 12.6 ± 6.5 (data not shown) in this unplanted soil was considered to be microbe P-limited because soil N:P ratio was more than 6.3 ± 1.0 (Capek et al., 2018). We suppose that the microbes related to N cycling are limited by P rather than N in this unplanted treatment. This was also supported by the positive relationship between N_2O emission and soil AP content, but not between N_2O emission and soil NO_3^--N content (Fig. 3b). Therefore, an enhanced N_2O emission occurred when P was added (Fig. 1). Mehnaz and Dijkstra (2016) also showed that P addition increased N_2O emission from P-poor soils through microbial denitrification when inorganic N is abundant. Furthermore, we noticed that MBN in P-added treatments was higher (averaged by 36%, though not significant) than that in treatments without P addition (Table S2, Table 1). This suggested that increased soil AP content might enhance microbial biomass in the unplanted soil. It was also possible that P-enrichment may stimulate the activity of denitrifiers and nitrifiers (Wang et al., 2018a) or alter microbial community composition (Liu et al., 2012) for nitrous oxide production. However, we do not have microbial community composition data in our experiment, which should be investigated in future work.

As for the two planted treatments (Plant and Plant + AMF), we found a significantly positive relationship between N_2O emission and soil NO_3^--N content rather than NH_4^+-N content, indicating denitrification might be the main process to produce N_2O (Smith, 2017). Soil available NO_3^--N from planted treatments was averaged at 23.84 mg kg^{-1} at the end of the experiment, which might cause substrate limitation for N_2O emission (Senbayram et al., 2012). Meanwhile, soil AP was averaged at 6.91 mg kg^{-1} across planted treatments, which is much lower than the optimal level for crop growth (10.9 mg kg^{-1} to 21.4 mg kg^{-1}) (Bai et al., 2013). Therefore, the plant was probably P-limited in our experimental conditions. As shown in Fig. 3, N_2O emission was negatively correlated to soil AP content, while positively related to soil NO_3^--N content. It is possible that P addition alleviated plant P-limitation, promoted plant N uptake, and decreased soil NO_3^--N content which was a decisive substrate for N_2O production in our experimental conditions. Thus a significant decline in N_2O emission following P addition was observed.

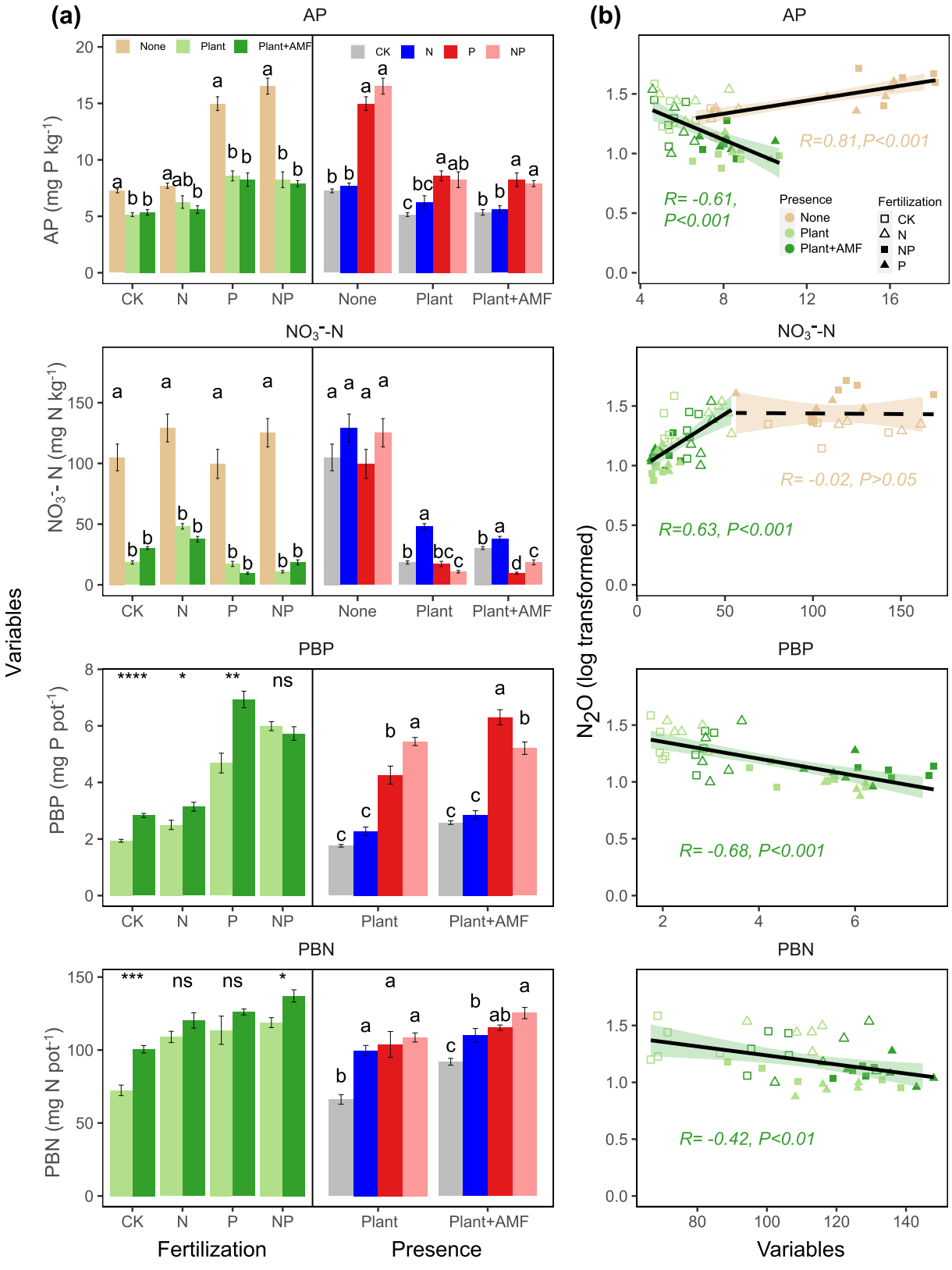

Fig. 3. Selected variables (A) and the regression plots of N_2O emission with the predictors (B). AP, Soil available phosphorus content (mg P kg^{-1}); NO_3^--N, Soil nitrate content (mg N kg^{-1}); PBP, Plant biomass phosphorous (mg P pot^{-1}); PBN, Plant biomass nitrogen (mg N pot^{-1}). Data were analyzed by one-way ANOVA and followed by LSD post hoc test or Student's t-test. Values are Mean ± SE (n = 5). Different letters within each group (e.g. NP or None) depict statistical difference, $P < 0.05$. The three-way ANOVA results are shown in Table 1 and Table S1.

Other studies also found that higher root-uptake of N in P-fertilized plots may result in lower N_2O emission (Nielsen et al., 2009; Blanes et al., 2012; Yu et al., 2017). This P-induced N deficiency may be more detectable at the end of the experiment, thus we measured N_2O emissions at this time. Soil N_2O emissions were likely larger in the early period before the gas flux measurement especially after N and water was added, and more frequent N_2O measurements were needed to get an overall picture of N_2O emission during the whole experimental period in our future work.

It seems that P addition not only promoted plant parameters in planted treatments (e.g. biomass, PBP and PBN), but also enhanced soil microbial activities. However, no significant effect on microbial biomass was detected in the planted soils, which was partly in contrast with the results of unplanted soil (Table S2). We supposed that plants and microbes may compete for soil available P and N following P addition. A number of studies have demonstrated that soil microorganisms are particularly strong competitors for N and P in the short term (i.e. hours to days) due to their high surface area, rapid growth rates and distribution throughout the soil (Clemmensen et al., 2008; Xu et al., 2011; Wang et al., 2020). However, data from longer-term studies imply that plants eventually capture most N because of the longer lifespan of root systems compared with most soil microorganisms (Hodge et al., 2000; Barnard et al., 2006; Kuzyakov and Xu, 2013).

4.2. AMF effect on N_2O emission

There was no significant difference in the fluxes of N_2O between Plant and Plant + AMF treatments. This is in disagreement with our hypothesis that AMF would reduce N_2O emissions, but supports the studies of Cavagnaro et al. (2012) and Okiobe et al. (2020). Likely, this is because we didn't detect a remarkable difference in soil AP and NO_3^--N between the two treatments (Fig. 3), which are two important factors affecting N_2O emission under our experimental conditions (Fig. 2). Although PBP and PBN were both higher in AMF-inoculated plants (Plant + AMF) than in non-inoculated plants (Plant), this did not result in changed soil available N and P pools. One potential explanation might be that the increased N or P assimilated from the soil by AMF accounted for a small proportion of soil available N or P pool. The reduced leaching of inorganic N by AMF (Bender et al., 2015) should not matter in our experiment, because our pots were daily watered to 55% WFPS and leaching was not observed during our experiment.

4.3. Combined P and AMF effect on N_2O emission

The third hypothesis that the combination of P addition and AMF inoculation has an additive effect and results in the lowest soil N_2O emission was not supported in our experiment. First, we found a significant interaction of P addition and AMF inoculation on soil N_2O emission (Table S1), indicating that the effect of P addition depended on the presence of AMF. Second, AMF partly offset the mitigation effect of P addition on soil N_2O emission, because P addition significantly reduced N_2O emission under Root treatment (without AMF), but had a minor effect under Root + AMF treatment (with AMF) (Fig. 1). Consequently, the lowest N_2O emission rate did not occur in the Plant + AMF × P treatment as originally hypothesized. Additionally, no significant interaction effects of P and AMF were detected in other N_2O related parameters (i.e. AP, NO_3^--N, PBP and PBN) (Table S1). We supposed that the AMF may show their N_2O mitigation effect under a P-poor soil. This is partly supported by our result that AMF colonization was higher under P-limited conditions (Fig. S1; Table S1). Other studies also revealed that the AMF colonization rate could be suppressed by P fertilization (Liu et al., 2000; Treseder, 2004; Delavaux et al., 2017), because sufficient available P for host plant would decrease its dependence on AMF (Grman and Robinson, 2013). However, more research is needed to determine N_2O emission under the combined effect of AMF inoculation and P addition.

4.4. N effect on N_2O emission

Soil NO_3^--N content was much higher than NH_4^+-N in our experiment (Table S2), despite equal parts having been added in the form of NH_4NO_3. This was mainly because soil NH_4^+-N was more likely to volatilize through NH_3 as the pH in our soils was above 7 (Table S2) (Rochette et al., 2013).

Nitrogen addition showed no significant effect on N_2O emission, which was beyond our expectation but similar to previous findings (Zhang et al., 2008). This unexpected result might be explained as follows. First, the level of N addition was 50 mg N kg^{-1} (equivalent to 50 kg N ha^{-1}), which might make the N effect insignificant with this amount. Peng et al. (2018) reported that no significant effect on N_2O emission was detected until the rate of 40–80 kg N ha^{-1} yr^{-1} in an alpine grassland. Second, we found N deficiency symptoms in plant leaf during the experiment period, so an extra 120 mg N kg^{-1} was added (to all pots) to meet plant demand: 50 mg N kg^{-1} on 37th day and 70 mg N kg^{-1} on 64th day after sowing. This amount was more than 2 fold of the 50 mg N kg^{-1} (the level of N addition in our experiment), making soil N an excessive nutrient in unplanted treatment. Then soil AP (rather than AN) becomes a limiting factor for microbes to produce N_2O (Fig. 3b). This was supported by other studies that P addition only significantly increased N_2O emission in the NO_3^--N saturated but AP limited soil (Mehnaz and Dijkstra, 2016). In contrast, soil NO_3^--N in both Plant and Plant + AMF treatments decreased below 20 mg N kg^{-1} due to plant uptake, which effectively makes soil NO_3^--N a limited substrate for nitrifiers and denitrifiers (Abalos et al., 2018; Bowatte et al., 2018). Thus, practices aimed to change soil available N content (e.g. P addition) could also influence N_2O emission. In this study, the rate of N addition (50 mg N kg^{-1} soil) showed an insignificant effect on soil N_2O emission, but P addition (at a rate of 20 mg P kg^{-1} soil) which promoted plant N uptake in this P-poor soil was a promising strategy to mitigate soil N_2O emission.

4.5. Limitation

This study has two major limitations. First, we measured soil N_2O flux only once at the end of the experiment. Although the relevant soil and plant samples were collected right after N_2O sampling which aided the link between N_2O flux and soil/plant variables, more frequent N_2O samplings and plant/soil measurements during the plant growth period should be considered in further research. Second, we irradiated the soil by gamma-ray before the experiment to eliminate indigenous AMF, which may have killed the whole microbial community simultaneously, especially nitrifiers and denitrifiers. Although we added fresh soil filtrate (without AMF) to reestablish these native microbes, they may not fully recover to the original level at the time of measurement. As all treatments had the same background microbial community at the beginning of the experiment, we are confident that the likely incomplete recovery of microbial community may not strongly affect our conclusions. However, future work can consider an alternative approach by using an AMF-defective mutant plant together with its wild type (Cavagnaro et al., 2012), which has minimal impact on soil microbial community.

5. Conclusion

This study investigated the implications of AMF inoculation and P addition on the mitigation of N_2O emission under the context of N fertilization. We found no evidence to support the hypothesis that AMF can reduce the risk of N loss as N_2O. Meanwhile, we found an interaction effect of P addition and the presence of plants on soil N_2O emission. The presence of plants (with or without AMF inoculation) showed no effect on soil N_2O emission when no P was added, but significantly decreased soil N_2O emission with P addition (Fig. 4). We also demonstrated that the effect of P addition on soil N_2O emission depends on the soil N status.

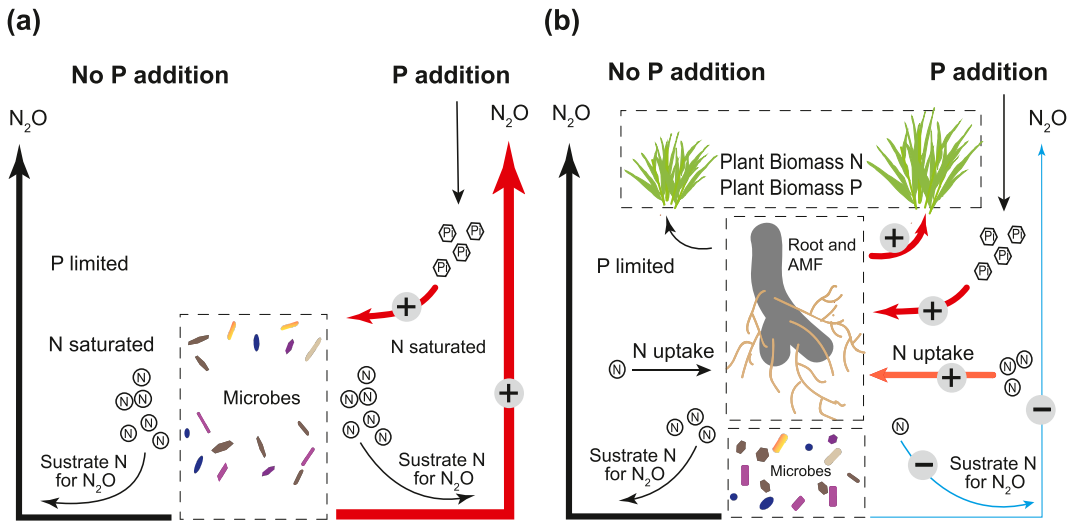

Fig. 4. Conceptual diagram of contrasting effects of P addition on N_2O emission from unplanted treatment and planted treatment. Red arrows or "+" refer to positive effects, blue arrows or "-" refer to negative effects. (a) P addition alleviated microbial P limitation and then affected N_2O emission in unplanted soil. (b) P addition increased soil P availability for plant and then increased plant biomass phosphorus (PBP) and plant biomass nitrogen (PBN), thus leading to decreased soil nitrogen for N_2O production.

Specifically, P addition promoted microbial biomass in the N-saturated unplanted soil (None treatment), but alleviated plant P limitation, enhanced plant N uptake, and then caused lower soil NO_3^--N content and N_2O emission in planted soils (Fig. 4). In addition, we found an antagonistic effect between AMF inoculation and P addition on soil N_2O emission because sufficient soil P may inhibit AMF colonization and activity. Overall, our work provides a mechanistic understanding of soil N_2O emission under the addition of P and N, the presence of AMF and plant, and their interactions. We also provide evidence that the combination of plant presence and P addition might be a promising practice to mitigate N_2O emission from P-poor soils.

Authors' contributions

Yawen Shen: obtained funding, designed the experiment, performed the laboratory work, analyzed the data, wrote the manuscript Tianle Xu: obtained funding, designed the experiment, performed the laboratory work, analyzed the data, wrote the manuscript Baodong Chen: designed the experiment Biao Zhu: supervised the project, obtained funding, designed the experiment, wrote the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rhisph.2021.100414.

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