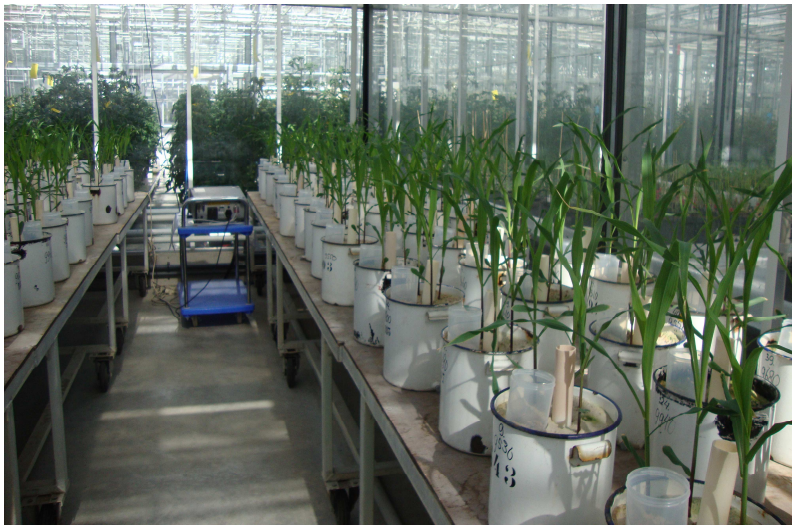
Soil Phosphorus Application Can Reduce N Fertilizer- induced Nitrous oxide Emissions



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Abstract

Soil-applied nitrogen (N) fertilizer is one of main sources of nitrous oxide (N_2O) emissions from agricultural soils. Although it is clear that N application rates are a strong determinant of N_2O emissions, the role which other nutrients can play through stoichiometric relations with N has hardly been studied. Here, we quantify the effects of phosphorus availability and the arbuscular mycorrhizal fungi (AMF) symbiosis on N fertilizer-induced N_2O emissions. We hypothesized that at high N availability but low P availability, reduced plant growth through P limitation would increase N_2O emissions due to higher mineral N concentrations in the soil. Therefore, both P fertilization and AMF symbiosis (which can increase P uptake) could mitigate N_2O emissions in such systems. We tested this in a pot experiment with maize (*Zea mays* L. var LG 11) growing on a P limited mixture of sandy soil and quartz sand. Treatment factors included AMF inoculation (+ or -), P fertilization (0 and 90 kg P ha^{-1}), and N levels (0, 125, 250 and 500 kg N ha^{-1}). N fertilization, P fertilization and their interaction all significantly ($P < 0.01$) affected N_2O emissions. Highest cumulative N_2O fluxes of 2.38 kg N_2O-N ha^{-1} were measured from the treatment without AMF and without P at 500 kg N ha^{-1}. Among the high N levels applied treatments, the lowest cumulative fluxes of 0.71 kg ha^{-1} was measured in the mycorrhizal with P applied treatment. The emission factors, both based on applied N and on yield scaled emissions (N uptake based) reduced with approximately 50% when P fertilization was applied. Although AMF inoculation did not significantly affect N_2O emissions ($P = 0.247$), high N_2O emissions occurred only when colonization levels were below 15%. Our results show that P availability can determine N_2O emissions in cropping systems, and underline the importance of N: P: C stoichiometry in the rhizosphere to understand N_2O emission.

Key words: Nitrous oxide, AMF, cumulative fluxes, emission factor, N uptake

Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas and is the main ozone depleting substance of the 21th century (Ravishankara et al. 2009). The global warming potential of N_2O is 298 times higher than that of CO_2 (Solomon et al. 2007). Atmospheric N_2O concentrations have been rising at a rate of approximately 0.6-0.9 µl m^{-3} yr^{-1} (IPCC 2007) over the last 250 years, with soil being the major N_2O source. It is estimated that anthropogenic activities account ~40% of global N_2O emission of which agricultural soils account ~70% of that anthropogenic N_2O emissions (IPCC 2007; Solomon et al. 2007). Agriculture related N_2O producing major sources are newly fixed synthetic N fertilizer and biologically fixed N (Hall and Matson 1999; Crutzen et al. 2008; Van Groenigen et al. 2010). Although N fertilizer is a source of N_2O, it is essential to global crop production. The worldwide N fertilizer demand is expected to increase from 100 million tons in 2006 to greater than 135 million tons in 2030. Therefore, N_2O emissions are expected to rise even further in the future (FAOSTAT 2008; IFA/FAO 2008).

N_2O is mainly produced by microbial N transformations during the denitrification, nitrification and nitrifier denitrification pathways (Baggs 2008; Kool et al. 2011). The main controlling factors of these microbial pathways are soil available N and C, pH, microbial community composition, soil moisture and its effect on aerobicity (Velthof et al. 1996; Dobbie and Smith 2003; Van Groenigen et al. 2004). The application rate of N fertilizer and manure is probably the main management decision determining N_2O emissions from agricultural soil (Crutzen et al. 2008). Fertilizer application, often leads to situations where soil available N (at least temporarily) exceeds plant demand. The excess soil N can then lead to N_2O production by the activity of nitrifying and denitrifying microorganisms. Therefore, judicious use of N fertilizer doses are the first step towards mitigating N_2O emissions.

Recently, it has been suggested that N_2O emissions should be assessed as a function of crop yield, rather than as a function of N application rate. Moderate N_2O emissions combined with high yields may ultimately be more beneficial than low N_2O emissions with low yields. A meta-analysis study by Van Groenigen et al. (2010), using aboveground N uptake as a proxy for yield, reported that N_2O emissions were smallest at application rate of 180-190 kg N ha^{-1} and increased sharply after that. This indicated that agricultural management practices which influence yield are keys to reduce N_2O emission from agricultural soils.

Interestingly, the role of nutrients other than N on N_2O emission from cropping systems has so far not been extensively studied. Nevertheless, mineral N concentrations in the soil (a main determinant of N_2O emission) are also determined by stoichiometric relations with other nutrients. For example, it has been shown that N fertilizer rates can be increased without causing an accumulation of soil NO_3^- by applying P fertilizer along with N, thereby avoiding a reduction in N uptake through P limitation (Schlegel et al. 1996). Furthermore, White and Reddy (1999) noticed that P addition influenced the microbial biomass and denitrifying enzyme activity in soil. However, the exact mechanism of P on regulation of N_2O emission is not clear. Hall and Matson (1999) suggested that poor P availability may cause higher emissions of N_2O and NO through decreased N immobilization. In contrast, other studies argued that P enrichment increases N_2O emission due to stimulation of denitrifiers and nitrifiers (Minami and Fukushi 1983; Bauhus et al. 1993; Falkiner et al. 1993). Nevertheless, positive and negative responses of P suggest that soil P availability may play an important role in determining N_2O emissions.

As P is extremely immobile in soil, increasing P availability is a major challenge for agronomists. Plant strategies to improve P uptake include producing P mineralizing enzymes or P-carrier enzymes, as well entering symbiotic association with arbuscular

mycorrhizal fungi (AMF). AMF are known to be effective in increasing nutrient uptake, particularly P, and have been shown to increase plant biomass of many crops in P-limited soils. AMF improve P uptake by increasing the effective 'root' colonization of the plant, thereby increasing the P depletion zone (Bolan 1991; Jakobsen 1995). In addition to the enhanced P acquisition, AMF may contribute to plant N uptake by assimilating inorganic NO_3^- and NH_4^+ into plant roots through glutamine synthetase production (Govindarajulu et al. 2005). This enhanced N uptake in AMF plants has been explained by higher N demand because of enhanced P uptake (Hamel and Smith 1991; Gerorge et al. 1995). Furthermore, some studies suggested that AMF directly alter the number and activity of N transforming microbial community and affect ammonification, nitrification and denitrification (Meyer and Linderman 1986). Amora-Lazcano (1998) found that autotrophic ammonium oxidizers increased with AMF colonization while ammonifying and denitrifying bacterial populations decreased. Considering these facts, we expect that AMF will affect N_2O emissions through a combination of direct and indirect effects, and that these effects vary with P availability.

There is a distinct lack of mitigation practices to minimize N fertilizer-induced N_2O emissions from soil. Therefore, the aim of this study was also to investigate the effects of phosphorus and AMF symbiosis on N fertilizer-induced N_2O emissions, with the ultimate aim of developing future mitigation options that have hitherto not been considered. We hypothesize that (i) at high N availability, plant P-limitation might increase N_2O emissions due to decreased plant N uptake; and (ii) AMF can increase P uptake leading to increased N uptake, thereby reducing N_2O emissions.

Materials and Methods

Experimental set up

A pot experiment was carried out in a greenhouse under controlled conditions. The experiment was laid out as a full factorial randomized block design. The treatment factors were (i) mycorrhizal inoculation (+ or -); (ii) P fertilization (0 or 90 kg P ha^{-1}); and (iii) N fertilization (0, 125, 250 and 500 kg N ha^{-1}). With 5 replicates, this resulted in an experiment of 80 pots.

The soil (*Cambic Podzol*) was collected from Wageningen University experimental farm "Droevendaal" from 0 to 25 cm depth. The collected soil was sieved through 5 mm screen and dried at 70 ºC for a week to sterilize fungal spores present in the soil. The soil and sand mixture was prepared in order to reduce P availability in the soil. To insure P limited soil condition for maize growth, the soil: sand ratio was 5:1 as determined by sample analysis with different combinations of soil and sand. Each mitscherlich pot (0.033 m^2 area and 0.3 m depth) was filled with 7.2 kg of soil-sand mixture. This soil-sand mixture contained 6.3 mg kg^{-1} NO_3^-, 2.2 mg kg^{-1} NH_4^+, 0.8 mg kg^{-1} PO_4^-. The pH of the mixture was 5.9 (all analysis after extraction with 0.01M $CaCl_2$) and the organic matter content was 0.8%.

Sodium biphosphate (NaH_2PO_4) and Ammonium Nitrate (NH_4NO_3) were applied as P and N sources, respectively. For all treatments, 377 kg K ha^{-1} and 73 kg Mg ha^{-1} were applied as KCl and $MgSO_4$. A solution of 10 ml Zn (Zn-EDTA) and other trace nutrients solution were applied for each pot. N fertilizer solution was applied in two splits, one during initial pot filling and the other one month later. All the other nutrient solutions were mixed during pot filling.

Mycorrhiza inoculation and planting

A commercial AMF inoculum mixture of three species *Glomus etunicatum, G. intraradices* and *G. claroideum* (Servaplant BV, 210,000 MPN) was used. An addition of 10 g of inoculum was surface-applied to each pot. For the non-mycorrhiza treatments, sterilized inoculum was applied in order to correct for possible nutrient additions through the inoculum. Inoculum was sterilized by autoclaving for 20 minutes at 121ºC. To correct for possible bacterial inoculation through the inoculum, 10 ml filtrate from the non-sterilized inoculum was applied in all treatments. The filtrate was prepared by adding non-sterilized mycorrhiza inoculum in water (500 g l^{-1}) and that mixture was filtered through a < 5 µm pore size filter, to filter out fungal spores.

Silage maize (*Zea mays* L. var LG 11) was sown in all pots. Four maize seeds were sown, leaving a small part of the pot open for the flux chamber. One plant was removed one week after germination. A PVC tube was installed 8 cm deep in the middle of the pot to facilitate watering and to prevent possible disturbance in soil during watering. Water content was gravimetrically adjusted when needed (two times a week during the first two weeks; approximately, three times a week for the remainder of the period). To simulate field condition, a rainfall event (22.7 mm) was applied one week after each N application.

N_2O flux measurement

The N_2O flux measurement procedure was followed as described by Van Groenigen et al. (2004). N_2O fluxes were measured daily for the first two weeks, then three times a week for the following six weeks which resulted in 32 measurements during the 53 days that the experiment lasted. The N_2O fluxes were measured using closed flux chambers and a photo-acoustic infra-red gas analyzer. PVC flux chambers (500 ml volume and diameter 6.8 cm) were inserted into the soil to a depth of 5 cm and the remained volume of chamber above the

surface was 300 ml. N_2O concentrations were measured approximately 25 to 30 minutes after closing the chamber. The N_2O concentrations were measured using a photo-acoustic multi-gas monitor (Innova 1312). A soda lime scrubbing filter trap was installed at the inlet of the analyzer to minimize CO_2 interference and concentration of N_2O was corrected for CO_2 and water vapour. The accuracy of analyzer was approximately 5% in the range of 300 to 5000 ppb. The N_2O fluxes were calculated using the difference between ambient N_2O concentration and the N_2O concentration in the closed chamber assuming linear increase over time based on previous study (Velthof and Oenema 1995). The emission factors based on applied N fertilizer and based on N uptake were calculated as described by Van Groenigen et al. (2004).

Plant and soil analysis

The fresh plant stovers were chopped into small pieces (3 to 5 cm), dried at 70^{o}C for 2 days to measure dry matter (DM) and total N and P content analysis. For total N and P content analyses, the dried samples were ground in a Wiley mill. Afterward, a digestion procedure was followed as described by Temminghoff (2000). Total N and P were determined spectrophotometrically using Segment Flow Analyzer (SFA, Skalar, The Netherland).

Soil sampling was done at two occasions, one month after N application and at the end of the experiment. Soil samples were taken with small auger to a depth of 20 cm from two sites of each pot and were mixed to make a composite sample for each pot. Soil moisture content was analysed gravimetrically. Soil samples were extracted with 0.5 M K_2SO_4, after which available NO_3^-, NH_4^+ and total N were analysed spectrophotometrically using a Segment Flow Analyzer (SFA, Skalar, The Netherland). The dissolved organic nitrogen (DON) was calculated by subtracting soluble inorganic N from total soluble N.

Microbial biomass N was assessed by fumigation followed by K_2SO_4 extraction. The fumigation extraction method was followed as described by Brookes et al. (1985). In brief, moist soil samples were fumigated with alcohol free Chloroform ($CHCl_3$) for 24 hours at 25^oC then extracted with 0.5 M K_2SO_4. Simultaneously, subsamples for non-fumigated soil were also extracted with the same methodology as the fumigated soil samples. The filter extracts were analysed for total N in both fumigated and non-fumigated samples. The amount of N released by $CHCl_3$ after 24 hours fumigation (1 day $CHCl_3$-N) was calculated by subtracting total N in K_2SO_4 extracts of non-fumigated from the total N of fumigated samples. Soil biomass was calculated assuming a correction factor of 0.54 (Brookes et al. 1985).

Mycorrhiza staining and colonization counting

Root samples were cleaned with water, cut into small pieces and preserved in 50% ethanol for 3 weeks. The staining procedure was followed as described by (Brundrett et al. 1996). For staining, root samples were cut into 1 to 2 cm pieces and added 10% KOH solution and autoclaved for 20 minutes at 120ºC. Afterward, the samples were acidified by dipping in 2% HCl for 1 hour. The staining was done with 0.01% Trypan Blue dye. To remove extra staining, 50% glycerol was added. Colonization level was assessed using the magnified intersection method (McGonigle et al. 1990) by placing 33 root pieces in microscope slides (3x) and around 3 views per root were performed, leading to around 100 views.

Statistical analyses

Three way ANOVA analysis was performed to test the effects of all three treatment factors (AMF inoculation, P and N fertilization) as well as their interactions. General linear regression was used to relate N_2O emission with different parameters. The ANOVA analysis was carried out using Genstat 13.2. Homogeneity of variance was tested by plotting the

residue vs the predicted values and Levene's test. Normality of the data was checked graphically. For AMF colonization level, the data were analysed after an arcsine transformation. The significant differences among the means were tested using least significance difference test (LSD) at 5% significance level. Graphical figures were presented using Sigma Plot 11.

Results

Soil and plant analysis

Table 1 shows the soil N analyses at the two harvesting dates. Fertilization with N and P, as well as their interaction, significantly affected most N levels on both days. The available NO_3^- remained at the harvest was < 0.2 mg kg^{-1} in most of the treatments except at 500 kg N ha^{-1} applied without P (Table 1). The highest available NO_3^- of 28.23 mg kg^{-1} was observed in 500 kg N ha^{-1} applied without P in non AMF. The amount of soil available NH_4^+ remaining at harvest showed similar patterns as NO_3^- (Table 1). AMF inoculation did not significantly affect soil available NO_3^-. AMF did not affect the NO_3^-: NH_4^+ ratio at harvest, however, an effect was observed one month after N application (Table 1). The NO_3^-: NH_4^+ ratio significantly ($P < 0.01$) reduced with P application. After one month of N application, the DON content was significantly varied with AMF inoculation ($P < 0.05$), P ($P < 0.05$) and N ($P < 0.01$) while at harvest was only differed with N application. The maximum microbial biomass N of 36 kg N ha^{-1} was observed at 250 kg N ha^{-1} applied with P. Only a small proportion of the applied N was recovered in the microbial biomass N.

Highest plant N uptake of 474 kg h^{-1} was found at 500 kg N ha^{-1} applied with P (Table 2). There was significant ($P < 0.001$) interaction between N and P level for total N uptake. Total N uptake was increased with P application only at higher N level. AMF plant had slightly ($P = 0.06$) higher N uptake especially when P was not applied. Total P uptake was increased with increasing N levels. Plant uptake N: P ratio varied from 1.4 to 20.1 (Table 2). The uptake N: P ratio was highly affected by P application rather than N application. The highest uptake N: P ratio of 20.1 was observed at 500 kg N ha^{-1} without P fertilization.

There was significant interaction ($P < 0.001$) between N and P for DM yield (Table 2). AMF inoculation marginally ($P = 0.07$) increased DM in comparison to un-inoculated. The

highest DM of 31.8 ton ha^{-1} was produced in 500 kg N ha^{-1} level with P and inoculated treatment. P application significantly ($P < 0.01$) increased DM in all N levels except for 0 kg N. N applied up to 250 kg N ha^{-1} significantly increased DM, after that not anymore. The mass fraction N in DM was always higher in P not applied condition except for 0 Kg N (Table 2). Highest mass fraction N of 2.6% was observed with high N level in P not applied treatment. P applied with N up to 250 kg N ha^{-1} resulted low (< 1%) mass fraction N in DM.

Arbuscular mycorrhiza colonization

Root length colonized by AMF varied from 2.8 to 21.0% (Table 2). All the non-inoculated treatments were free from mycorrhiza colonization. P application significantly increased AMF colonization. The highest colonization of 21% was found at 125 kg N ha^{-1} with P applied. The lowest colonization level of 2.8% was found at 500 kg N ha^{-1} with P not applied. In general, AMF colonization decreased with increased N levels, however these values did not differ significantly (Table 1).

Nitrous oxide emissions

N and P fertilization, as well as their interaction, significantly affected cumulative N_2O emissions ($P < 0.001$; Table 2). The cumulative N_2O fluxes over the period of 53 days varied from -0.03 to 2.38 kg N_2O-N ha^{-1}. Highest N_2O fluxes occurred in the treatment without P applied and without AMF at 500 kg N ha^{-1} level (Fig. 1). Emission peaks did not exclusively occur directly after N application. The first N_2O peak fluxes were observed two weeks after the first N application, in combination with the simulated rain event. The second peak occurred one week after the second split N fertilization. During the first week after both events, no differences in N_2O fluxes were observed, and the fluxes were not different from the background fluxes (Fig. 1). Highest daily peak fluxes of 325 g N_2O-N ha^{-1} day^{-1}

were measured in the treatment without P and an un-inoculated treatment at 500 kg N ha^{-1} level.

Emission factors

Emission factor based on percentage applied N varied from 0.01% to 0.47% (Table 2). The highest emission factor of 0.47% was observed at 500 kg N ha^{-1} without P and AMF. Within 500 kg N ha^{-1} applied treatments, the lowest emission factor of 0.14% was found with P and AMF. Emission related to N uptake (Fig. 2b) followed roughly the same trend as factors based on applied N (Fig. 2a). In general, the emission factors were < 0.2% in P applied all treatments (Table 2). The higher emission factors were observed at 500 kg N ha^{-1}as P not applied. The emissions were correlated ($r^2 = 0.68$) with surplus N in soil (Fig. 3a). At a surplus of more than 90 kg N ha^{-1}, the N_2O emissions increased exponentially.

N_2O emissions measured based on DM production ("yield-scaled") were significantly ($P < 0.01$) affected by N and P fertilization and their interaction (Table 2). Application of P significantly decreased N_2O emission for DM production only at 500 kg N ha^{-1}. All the treatments with P applied had < 20 g N_2O emission for Mg^{-1} DM production (Table 2). The highest emission of 162 g N_2O-N for Mg^{-1}DM was observed at 500 kg N ha^{-1} applied without P and AMF. Our result showed the NUE varied from 70.4 to 88.7% based on applied N fertilizer recovery (Table 2). The lowest NUE of 70.4% was recorded on high N level treatment without P applied and AMF. Application of P increased NUE especially at 500 kg N level.

Discussion

Effects of P availability on N_2O emission

Our first hypothesis stated that at high N availability plant performance would be P-limited, and that P application would increase uptake of N, thereby reducing N_2O emissions. Our results conform this: we found significantly lower cumulative N_2O emission after 53 days of N application in the treatments with P fertilizer application (Fig. 1; Table 2). These results indicate that P availability can play an important role in determining soil N_2O emission. Our results are in line with Hall and Matson (1999), who suggested that poor P availability can cause higher N_2O emission at higher N fertilizer inputs. The mechanism behind this role can be either plant mediated or microbial-mediated. These two possibilities are discussed below.

A plant-mediated mechanism behind the P effect is plausible, as both plant growth and N uptake were significantly increased by P fertilization (Table 1). The nearly doubled DM yield at high N levels with P fertilization probably led to faster N uptake during the experiment (Table 2). The increased mass fraction N in DM with high N level when P was not applied also supports this. The plants had luxury N uptake when P was limited, but P fertilization lead to N limitation in plant to reach the maximum DM respond limit. This higher plant N uptake (probably earlier during the experiment) could lead to decrease N availability in soil for microbial nitrification and denitrification that would be lost from the soil as N_2O. The second, microbial-mediated mechanism to explain the P effect on N_2O emissions could be associated with increased microbial immobilization of N after P fertilization, decreasing the N substrate for nitrification and denitrification. Our analysis of soil NH_4^+ and NO_3^- dynamics provides some indications for such a mechanism: the increased NO_3^-: NH_4^+ ratio after P fertilization (Table 1) may indicate a decrease in

nitrification rates (Gerards et al. 1998). Similar findings were reported that P addition to P-limited soil reduces N_2O emission by removing P limitation on microbial N immobilization and decreasing nitrification and denitrification (Sundareshwar et al. 2003; Mori et al. 2010). Conversely, some studies argue that P addition increases N_2O emission due to stimulation of denitrifiers and nitrifiers (Minami and Fukushi 1983; Bauhus et al. 1993; Falkiner et al. 1993). However, we did not directly measure nitrification and denitrification in this study. The microbial biomass N was low (< 36 kg N ha^{-1}; data not shown), that was not indicating factor for the dynamics of N_2O emissions. Therefore, it is unlikely that an immobilization effect would explain the general trend in our results.

Dynamics of N_2O emissions for different N levels

Our results confirmed that increased N_2O emission related to N application (Fig. 1). In our study both NO_3^- and NH_4^+ analyses show that N_2O emissions increased with high soil availability of NO_3^- and NH_4^+ (Table 1). Our results indicate that soil available NH_4^+ and NO_3^- were the main driving factors for nitrification and denitrification and consequently directly influenced N_2O emission in line with previous findings (Hou et al. 2000; Merbach et al. 2001; Trujillo-Tapia et al. 2008). We found that more than 250 kg N ha^{-1} application significantly increased N_2O emissions. Our results on emissions of N_2O were in lined with others results in relation to N application (Velthof et al. 1996; Kim et al. 2010). The negative fluxes often observed at the control (without N) were likely due to the soils acting as an N_2O sink particularly when soil moisture is high (Flechard et al. 2005). One of the properties of N_2O is that it is easily dissolved in water. Therefore, when soil is wet it may be denitrified by microbes to N_2 or dissolved in moist surface soil.

In our study, the peak fluxes persisted for only two weeks after each N fertilizer application. Those peaks were measured one week after N application, directly following a

rain shower event. This is a strong indication that denitrification may have been the dominant N_2O production process. Furthermore, denitrification follows a competitive Michaelis-Menten type kinetics so larger concentrations of NO_3^- limit reduction of N_2O to N_2 (Betlach and Tiedje 1981). Our study underlines the previous findings (Mosier 1994; Bouwman 1996; Dobbie and Smith 2003; Ambus 2005; Jones et al. 2007) that increasing N_2O emissions following N fertilization are short term responses only and decline to background level thereafter because surplus N no longer provide for microbial substrate.

Effect of AMF on N_2O emissions

Our second hypothesis stated that the effect of AMF inoculation would be largely identical in nature to P fertilization: increased P uptake and thereby indirectly increased N uptake, resulting in reduced surplus soil N and N_2O emissions. We did not find such a significant effect of AMF on N_2O emissions. This might partly be due to low AMF colonization percentages in the inoculated treatments, e.g. in comparison with a previous study on AMF in maize (Azcon et al. 1982). P application significantly increased AMF colonization percentages (Table 2), which runs contrary to conventional arguments. The positive effect of P in colonization percentage may be due to unbalance of N and P in plant. However, there are some indications for an AMF effect on N_2O emissions when emissions are plotted against actual colonization levels (Fig. 3b). No significant N_2O emissions occurred above colonization levels of 15% (Fig. 3b). These results indicate that an indirect relation may be between AMF and N_2O emissions. However, these relations are more indirect than hypothesized, and deserve further study.

Emission factors

The emission factors based on applied N and above ground N uptake were more than two times higher in the absence of P fertilizer at higher N application rates. Our emission factor

based on percentage of applied N ranged between 0.01 to 0.47%, which is lower than the tier 1 IPCC default emission factor of 1.25% (IPCC 2007). This might be due to the short duration of the experiment (53 days) and the high plant density compared to field practices (~9 plant m^{-2}).

We found no significant difference in DM yield between 500 kg N ha^{-1} and 250 kg N ha^{-1} with P application; however, the yield-scaled emission (N uptake based) factor was significantly lower at 250 kg N ha^{-1} (Table 2). Therefore, our findings suggest that plant growth respond to applied N increases when applied with P. Our results were in line with Van Groenigen et al. (2010) and Rafique et al. (2011), as they suggested that within the range of N deficit (< 300 kg N ha^{-1}) no significant difference shown in N_2O emissions. Similarly, McSwinery and Robertson (2005) suggested that an emission factor is more appropriate only when crops are fertilized at N rates less than or equal to N required for maximum yields, because the percentage of fertilizer N that is emitted as N_2O become more variable at higher N rates. In addition, higher N level without P application resulted in higher N_2O emissions (2.38 kg N_2O-N ha^{-1}) led to low NUE (70%). This suggests that P application increases NUE mainly at higher N level. This is in line with Van Groenigen et al. (2010) as they observed that yield scale emission factor reduced by 0.05% when NUE increased from 19 to 75%. However, our NUE values were higher (> 70%) which indicate that most of the applied N taken up by the crop, less remained in the soil and emitted as N_2O. Therefore, we conclude that when N input exceeds an optimum crop requirement consequently decreases NUE and increasing surplus N produces additional N_2O.

Conclusion and recommendation

This study shows that P dynamics can reduce N_2O emissions from P limited soils. The N_2O emission in this study was mainly affected by the N, P and their interaction, plant N, P uptake and N surplus in soil. The peak N_2O emission flux occurred for short period of two week only after N application and rain shower event. AMF inoculation marginally affect on DM, N and P uptake. AMF did not significantly affect on N_2O emission, possibly due to low colonization levels. The results of this study suggest that the N_2O emission can be reduced with the best agronomic management practices with balanced application of N and P to increase N uptake and minimize surplus N in soil. Our findings suggest further study on N: P: C stoichiometry in the rhizosphere and AMF effect on N_2O.

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Table 1. Soil N status during and at the end of the experiment. Codes refers to mention in figure 1 . [a]Significance level: *****P* **< 0.01, ****P*** **< 0.05, NS = not significant, LSD = Least significant difference at *P* < 0.05 value are in between brackets. AMF colonization (%) was analyzed for AMF (+M) with two way ANOVA.**

Treatment			One month after soil N				Final soil N				AMF Colonization
AMF	P	N (kg ha^{-1})	NO_3^- (mg kg^{-1})	NH_4^+ (mg kg^{-1})	DON (mg kg^{-1})	NO_3^-/ NH_4^+	NO_3^- (mg kg^{-1})	NH_4^+ (mg kg^{-1})	DON (mg kg^{-1})	NO_3^-/ NH_4^+	(%)
-M	-P	0	0.14	0.27	5.61	0.52	0.20	0.28	4.51	0.66	0
		125	0.65	0.56	6.57	1.13	0.09	0.19	4.15	0.37	0
		250	7.95	2.09	7.53	4.04	0.14	0.25	4.91	0.64	0
		500	38.07	18.86	9.83	2.12	28.23	12.16	5.13	3.34	0
	+P	0	0.19	0.74	7.23	0.49	0.16	0.22	5.21	1.06	0
		125	0.22	0.43	6.71	0.55	0.18	0.33	5.26	0.48	0
		250	0.26	0.75	6.87	0.37	0.19	0.38	5.17	0.86	0
		500	10.87	10.23	9.37	0.97	0.16	1.11	4.55	0.14	0
+M	-P	0	1.04	0.83	6.32	2.44	0.09	0.19	2.91	0.52	14.2
		125	1.09	0.48	6.92	2.52	0.11	0.22	4.86	0.51	11.8
		250	9.38	2.25	8.16	4.79	0.14	0.41	4.59	0.27	8.2
		500	43.98	22.12	8.15	2.16	21.02	4.33	6.54	5.01	2.8
	+P	0	0.22	0.31	5.38	0.76	0.07	0.18	3.98	0.36	16.6
		125	0.21	0.33	6.03	0.63	0.09	0.22	4.64	0.37	21.0
		250	0.37	0.54	6.26	0.78	0.12	0.39	4.73	0.29	14.8
		500	6.21	6.89	7.62	0.88	0.02	1.08	5.60	0.02	9.0
Sign./LSD ($p < 0.05$)[a]											
M			NS	NS	*(0.45)	*(0.56)	NS	NS	NS	NS	-
P			**(2.19)	**(1.54)	*(0.45)	**(0.56)	**(1.88)	**(1.25)	NS	**(0.41)	*(1.9)
N			**(3.10)	**(2.18)	**(0.64)	*(0.80)	**(2.66)	**(1.77)	*(0.75)	**(0.58)	NS
M*P			NS	NS	*(0.64)	NS	NS	NS	NS	NS	-
M*N			NS	NS	*(0.91)	NS	NS	NS	**(1.06)	NS	-
P*N			**(4.39)	**(3.08)	NS	**(1.13)	**(3.76)	**(2.50)	NS	*(0.82)	NS
M*P*N			NS	NS	NS	NS	NS	NS	NS	NS	-

Table 2. Effects of AMF, P and N on above-ground DM, N and P uptake, N_2O emission and emission factors. Codes [a]Emission factor as percentage of applied fertilizer N, [b]Emission factor as percentage based on above ground N uptake, [c] Nitrogen use efficiency based on applied fertilizer N, [d]Significance level: **$p < 0.01$, *$p < 0.05$, NS = not significant, LSD = Least significant difference at $p < 0.05$ value are in between brackets

Treatment												
AMF	P	N	DM yield	DM fraction Plant N	N uptake	P uptake	N:P uptake	N_2O-N	N_2O-N g	EF-A[a]	EF-B[b]	NUE[c]
		(kg ha^{-1})	(Mg ha^{-1})	(%)	(kg ha^{-1})	(kg ha^{-1})		(kg ha^{-1})	Mg^{-1} DM	(%)	(%)	(%)
-M	-P	0	7.98	0.58	47	15.7	3.2	-0.03	-3.9	-	-0.07	-
		125	16.23	0.90	147	20.5	8.3	0.10	6.8	0.10	0.09	80.4
		250	19.14	1.31	248	22.9	12.6	0.39	20.5	0.17	0.16	80.4
		500	15.87	2.60	400	22.8	20.1	2.38	162.1	0.47	0.61	70.7
	+P	0	8.20	0.55	46	34.1	1.4	-0.03	-3.8	-	-0.07	-
		125	21.83	0.67	146	56.6	2.8	0.11	5.5	0.11	0.09	79.2
		250	29.73	0.81	239	63.0	4.1	-0.01	-0.2	0.01	-0.01	76.9
		500	30.17	1.58	474	71.4	7.1	0.87	29.4	0.18	0.18	85.4
+M	-P	0	8.03	0.61	49	16.6	3.2	0.02	3.3	-	0.05	-
		125	17.42	0.92	158	19.8	9.3	0.11	6.0	0.06	0.07	88.7
		250	18.83	1.38	255	25.8	11.6	0.31	17.5	0.12	0.12	83.2
		500	17.52	2.43	424	24.8	19.3	1.46	82.0	0.29	0.35	75.3
	+P	0	7.92	0.55	43	32.8	1.7	0.00	0.1	-	0.00	
		125	21.95	0.67	148	57.5	2.7	0.05	2.7	0.05	0.04	80.7
		250	31.23	0.75	234	67.7	3.7	0.04	1.5	0.02	0.02	74.9
		500	31.83	1.43	454	73.9	6.8	0.71	21.9	0.14	0.16	81.4
Sign./LSD ($p < 0.05$)[d]												
M			NS	NS	NS	NS	NS	NS	NS		NS	
P			**(0.76)	**(0.08)	NS	**(1.8)	**(0.51)	**(0.21)	**(14.9)		**(0.07)	
N			**(1.07)	**(0.12)	**(12.7)	**(2.6)	**(0.72)	**(0.31)	**(21.1)		**(0.10)	
M*P			NS	NS	NS	NS	NS	NS	NS		NS	
M*N			NS	NS	NS	NS	NS	NS	NS		NS	
P*N			**(1.52)	**(0.17)	**(18.0)	**(3.8)	**(1.02)	**(0.43)	**(29.8)		*(0.14)	
M*P*N			NS	NS	NS	NS	NS	NS	NS		NS	

Figure legends

Figure 1: Cumulative nitrous oxide emission measured during 53 days after N fertilizer applications. (a) –M –P, treatment code refers to without arbuscular mycorrhizal fungi inoculation and without P addition (b) -M +P, code refers to the treatment without AMF inoculation and with P (c) +M –P, code refers to the treatments with AMF inoculation and without P (d) +M +P, code refers to treatment with AMF inoculation and with P. Error bar indicates standard errors (n=5).

Figure 2: Nitrous oxide emission from soil in relation to (a) N fertilizer application and (b) N uptake. Code –P refers to the treatment without Phosphorus applied and code +P refers to the treatment with Phosphorus applied. The data points represent each treatment mean (n=10). Error bars denote standard error (n=10).

Figure 3: Relation of N_2O emission with (a) surplus N in soil and (b) AMF colonization percentage. The data points in (a) represents individual measurement (n=80) and in (b) represents treatment mean only AMF inoculated (n=5).

Figure 1: Cumulative nitrous oxide emissions measured during 53 days after N fertilizer applications

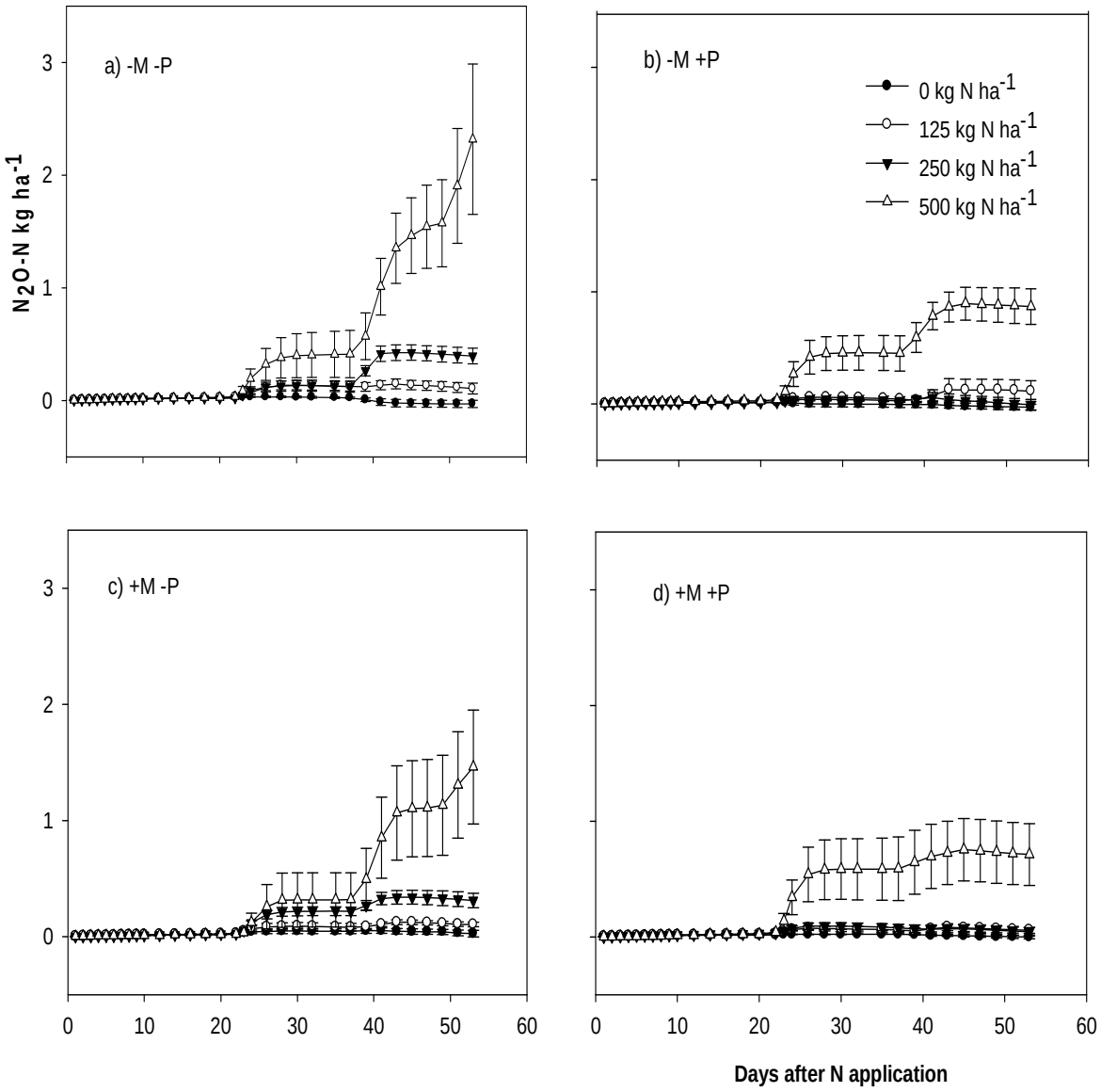

Figure 2: Nitrous oxide emission from soil in relation to (a) N fertilizer application and (b) N uptake.

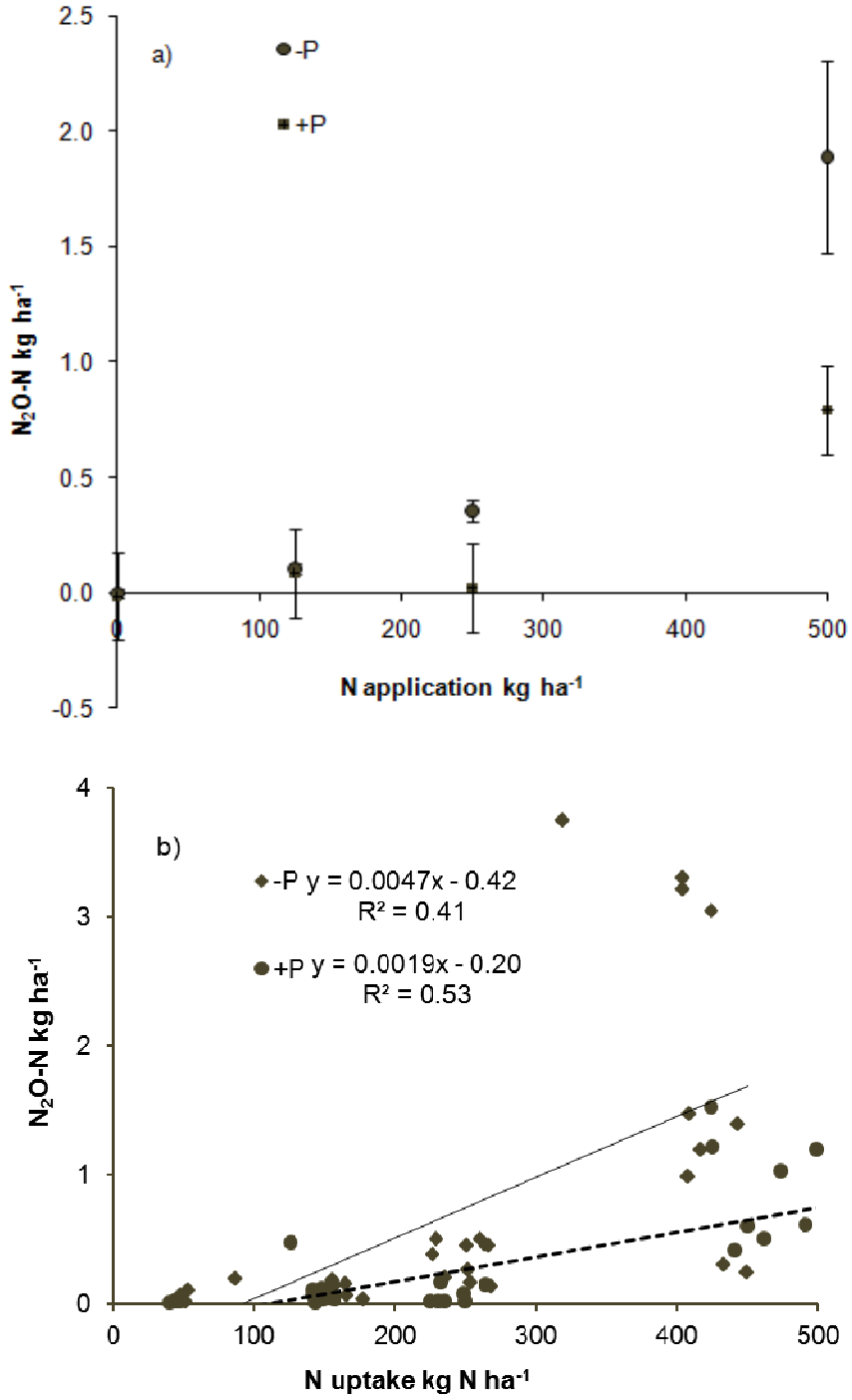

Figure 3. Relation of N_2O emissions with (a) surplus N in soil and (b) AMF colonization percentage.