



Full Length Article

Effects of Nitrogen and Nitrification Inhibitors Addition on N₂O Emissions under different Long-Term Fertilization Regimes

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Abstract

Changes in soil systems can occur during the implementation of long-term agronomic practices and consequently result in different N₂O emissions in response to external environment. Therefore, an incubation study was conducted using Fluvisols from a 30-yr fertilization experiment to assess N₂O emissions produced because of the nitrogen (N) and nitrification inhibitor (NI) addition. Different soils were sampled from four fertilization treatments: no fertilizer (NF), chemical NPK fertilizer (NPK), organic manure (M) and chemical NPK fertilizer plus manure (NPKM). The results showed that effects of N and NI additions on N₂O emissions were significantly different among the different soils. The highest stimulation on N₂O emission with N addition was observed in soil with long-term NPK fertilization regime (10.2 times), while the lowest reduction on N₂O emission due to NI addition in soil with long-term M fertilization (27.7%). The regression analysis showed that increase rate of N₂O emission caused by N addition and decrease rate by NI was negatively related to soil organic carbon (SOC) concentration. Our findings indicated that response of N₂O emissions to N and NI additions were different under different long-term fertilization regimes in Fluvisols, mainly resulting from the difference of soil organic matters. © 2022 Friends Science Publishers

Key words: Nitrogen; Nitrification inhibitor; N₂O; Long-term fertilization; Different soil ecosystem

Introduction

Nitrous oxide (N₂O) is the third important greenhouse gas (GHG) which contributes 6~8% to current global warming (Smith *et al.* 2007). Additionally, N₂O concentration can also increase atmospheric PM 2.5 accumulation and aggravate stratospheric O₃ depletion (Ravishankara *et al.* 2009; Huang *et al.* 2014). Agriculture accounting for ~60% of the global anthropogenic N₂O emissions (IPCC 2013), is projected to increase by 60% in 2050 in order to satisfy the food needs of the growing population (FAO 2013). It is necessary to carry out the appropriate agricultural management, which can mitigate GHG emissions and maintain crop production simultaneously.

Soil N₂O is produced mainly by microbial nitrification and denitrification processes (Bouwman 1998; Zhu *et al.*

2013; Zhang *et al.* 2018). The soil physical, chemical and microbial characteristics have been observed to change significantly with different long-term agricultural management practices (García-Orenes *et al.* 2009; Zhang *et al.* 2012), and these changes could affect N₂O emissions in response to the external disturbance, such as temperature (Coudrain *et al.* 2016). Nitrogen (N) and nitrification inhibitor (NI) are both external disturbance that can significantly affect N₂O emissions from agricultural soils. Generally, application of N fertilizer can increase soil N₂O emissions in a nonlinear trend (Hoben *et al.* 2011; Shcherbak *et al.* 2014; Hoa *et al.* 2018). Nitrification inhibitors can inhibit NH₄⁺ oxidation to NO₂⁻ through slowing the genus of nitrifying bacteria and nitrosomonas, reduce NO₃⁻ concentration, and may thus reducing N₂O emissions (Abbasi and Adams 2000; Zhu *et al.* 2019;

Borzouei *et al.* 2021). As reported previously in meta-analysis, response of soil N₂O emissions to the N and NI additions was different along with climate factors, cropping systems or soil conditions (Shcherbak *et al.* 2014; Li *et al.* 2018). To the best of our knowledge, however, information on how the different soil ecosystems affect N₂O emissions in response to N and NI additions in a specific site with the same environment conditions is still limited.

Fertilization is a key agricultural practice that would have a long-term impact and significantly affect the soil ecosystem (Geisseler and Scow 2014; Wen *et al.* 2020). Previously, we found the significant difference in soil microbial biomass, pH, organic carbon and nitrogen under treatments of chemical fertilizer and manure after a 30-yr field experiment (Zhang *et al.* 2017). It was hypothesized that effects of N and NI additions on N₂O emissions would be different in soil after long-term different fertilization. Therefore, a laboratory incubation study was conducted to investigate the differences in N₂O emissions resulting from the additions of N and NI to the soils under different long-term fertilization regimes.

Materials and Methods

Soil sampling and analysis

The long-term fertilization experiment was initiated in 1983 at the Institute of Agricultural Sciences in Xiao County, Anhui Province, China (34°18' N, 116°53' E). The climate and soil characteristics and the experimental design of this site have been described in our previous study (Zhang *et al.* 2017). The fertilization regimes selected in the present study were no fertilizer (NF), chemical NPK fertilizer (NPK), organic manure (M), and chemical NPK fertilizer plus manure (NPKM). The total amount of nitrogen input in each fertilization regime was 240 kg ha⁻¹, while phosphorus and potassium were not unified. Chemical N, P and K fertilizers used in this experiment were urea, superphosphate, and potassium sulphate, respectively, and cattle manure was used for the M and NPKM regimes. The application amounts of different fertilizers in each treatment are shown in Table 1.

We collected fresh soils from 0–20 cm layer in the field after soybean harvest in 2015. In each plot, five randomly sampled soil cores were taken and mixed to one sample. The samples were passed through 2 mm sieve and stored at 4°C for further processing. A portion of the soil samples were air dried for the measurement of basal properties. Part of the air-dried samples was ground for the determination of soil organic carbon (SOC) using the potassium dichromate oxidation-redox titration method (Nelson and Sommers 1982).

Soil incubation and gas sampling

Laboratory incubation experiment was conducted in

Chinese Academy of Agricultural Sciences (40.0°N, 116.3°E), Beijing, China. Six aliquots (100 g) from composite field samples of each plot were drawn and placed in 500-mL glass jars. The incubation treatments were soil only (CK), soil with urea (U), and soil with urea and nitrification inhibitor 3,4-dimethyl pyrazole phosphate (DMPP, UNI). For the U treatment, 0.05 g urea was added into each jar. For the UNI, 0.05 g urea and 0.3 mg DMPP were added in each jar. There were six jars for each treatment, three for gas sampling and other three for soil chemical properties determination. Before the incubation treatment, the soil microcosms were pre-incubated under 25°C in the dark for one week to stabilize the microbial activity (Zhang *et al.* 2015). And then, all of the soil microcosms were kept on incubation in the dark at 25°C after the jars were sealed with air permeable plastic film. During the incubation period, deionized water was added at regular intervals to keep soil moisture at 60% water holding capacity (WHC).

N₂O fluxes in the incubation studies were measured every day for three consecutive days and every 2 or 3 days afterwards, until the fluxes under U and UNI treatments were no different from the CK (12 days totally). On each sampling occasion, three glass jars of each treatment were sealed with airtight rubber plugs and then incubated for 2 h in the dark at 25°C. The rubber plugs were fitted with three-way valves to allow for headspace gas sampling. Before and after the 2-h airtight incubation, a 30-mL gas sample was taken from each jar using an airtight syringe. The sampled headspace N₂O concentrations in the jars were determined with a gas chromatograph (GC, Agilent 7890A, USA). The N₂O fluxes were calculated as the linear increased rate of concentration during the 2 h. Cumulative N₂O emissions over the incubation period were determined by multiplying each gas flux with the interval between sampling dates.

Effects of N and NI additions on N₂O emissions

The effects of N and NI additions on N₂O emissions in the different soils were calculated as the follows:

$$\text{Effect of N addition on N}_2\text{O emissions} = (U - \text{CK})/\text{CK} \times 100\% \quad (1)$$

$$\text{Effect of NI addition on N}_2\text{O emissions} = (U - \text{UNI})/U \times 100\% \quad (2)$$

Soil measurement

On 6th day of the incubation, three soil microcosms for soil properties determination in each treatment was destructively sampled and passed through a 2-mm sieve for the measurement of soil available nitrogen, nitrification and denitrification potential. NH₄-N and NO₃-N concentrations extracted by potassium chloride solution were analyzed with the continuous flow analyzer (TRAACS 2000, Germany). Soil nitrification and denitrification potentials were measured following the techniques described by Šimek and Kalčík (1998) and Chu *et al.* (2007), respectively.

Table 1: The application amounts of fertilizers and their total pure nutrient contents of N, P, and K under different long-term fertilization regimes

Treatments	Application amounts of fertilizers in kind (kg ha ⁻¹)				Pure nutrient contents in total (kg ha ⁻¹)		
	Manure	Urea	Superphosphate	Potassium sulphate	N	P	K
NF	0	0	0	0	0	0	0
NPK	0	522	1338	267	240	335	120
M	75000	0	0	0	240	188	113
NPKM	37500	261	669	134	240	261	116

Treatments of NF, NPK, M and NPKM represent no fertilizer application, sole organic manure, balanced chemical fertilizer and chemical NPK plus manure, respectively

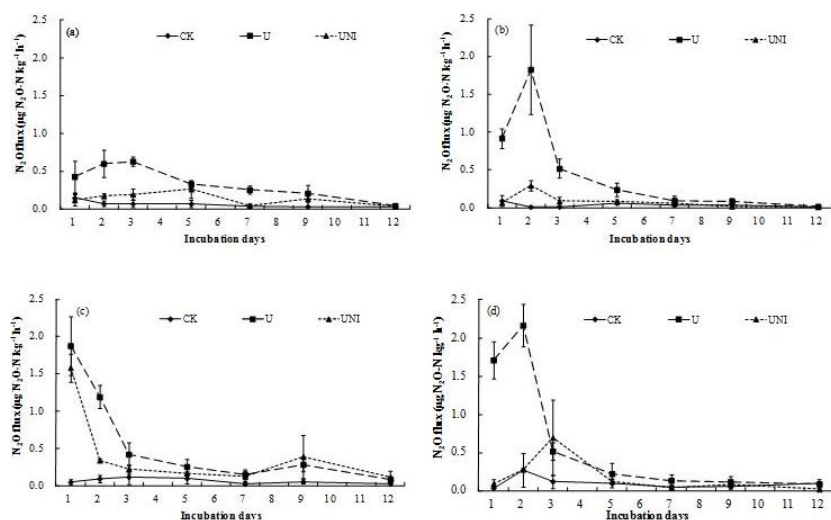


Fig. 1: Response of N₂O fluxes to N and nitrification inhibitor (NI) additions in soils under different long-term fertilization: (a) NF; (b) NPK; (c) M; (d) NPKM. Vertical bars indicate the standard error (n = 3)

Statistical analysis

The means and standard error for each data set were calculated from triplicate plots while Microsoft Excel 2003 was used for basic data calculation and drawing of graphs. All statistical analyses were carried out using SAS system (SAS 9.2, USA). Differences in treatments were evaluated using analysis of variance (Proc Anova) and significance among treatment means using the least significant difference (LSD). Proc Reg was used to do the linear regression analysis of N₂O emissions in response to N and NI additions upon soil organic carbon.

Results

Dynamics of N₂O fluxes during the incubation period

The application of urea increased N₂O fluxes from the fertilizer treatments as compared to CK (Fig. 1). Urea application increased N₂O fluxes significantly in the 1st and 2nd day, and subsequently decreased continuously till no difference were observed in comparison with the CK. The application of urea with nitrification inhibitor (*i.e.*, UNI treatment) decreased N₂O fluxes compared to urea (U) treatment under all the fertilization regimes, with almost similar time trends to N₂O fluxes in urea (U) treatment.

Effects of N and NI additions on N₂O emissions and their relationship with SOC under different fertilization regimes

Application of urea (U) significantly increased N₂O emissions in comparison with CK, while nitrification inhibitor application (UNI) significantly decreased N₂O emissions compared to urea (U) treatment (Fig. 2a, $P < 0.05$). Under different fertilization regimes, various effects of N and NI additions on N₂O emissions were observed. Compared to CK, N₂O emissions were increased by 543, 1023, 537 and 365%, under fertilization treatments of NF, NPK, M and NPKM (Fig. 2b), respectively. Moreover, the increase rate of N₂O emission under NPK treatment was significantly higher than other fertilization treatments ($P < 0.05$). Compared to urea (U) treatment, decrease rate of N₂O emissions in UNI were 56.9, 79.9, 27.7 and 60.3%, respectively, under fertilization treatments of NF, NPK, M and NPKM (Fig. 2c). In addition, the decrease rate of N₂O emission under M treatment was significantly lower than those under other fertilization treatments ($P < 0.05$).

Increase rate of N₂O emissions in U compared to CK was negatively related to SOC (Fig. 3a), though the relationship was not significant. Decrease rate of N₂O emissions in UNI compared to U treatment was negatively related to SOC (Fig. 3b, $P < 0.01$).

Effects of N and NI additions on soil NO₃-N content, nitrification and denitrification potential under different fertilization regimes

Urea (U) treatment significantly increased NO₃-N content in comparison with CK, and urea + nitrification inhibitor (UNI) treatment significantly decreased NO₃-N content in comparison with urea (U) (Fig. 4b, $P < 0.05$), with various impacting amplitudes under different fertilization regimes. Increase of NO₃-N content under NPK treatment was higher than both M and NPKM treatments when urea was added.

Urea (U) treatment significantly increased nitrification potential in comparison with CK, while nitrification inhibitor (UNI) treatment significantly decreased nitrification potential in comparison with urea (U), with various impacting amplitudes under different fertilization regimes (Fig. 4c, $P < 0.05$). Increase of nitrification potential under M treatment was lower compared to other fertilization treatments when urea was added. Although fertilization treatments of M and NPKM significantly increased soil denitrification potential, N and NI additions had no effect on soil denitrification (Fig. 4d).

Discussion

The results showed that application of urea significantly increased N₂O emissions in comparison with CK, with the various effects under different fertilization regimes. According to Geisseler and Scow (2014) and Zhang *et al.* (2017) and Yang *et al.* (2019), long-term different fertilization would change the physical, chemical and microbial characters of soil, and may affect the response of N₂O emissions to N addition. In the present study, significant differences in soil organic carbon, nitrogen and pH were observed among the fertilization regimes (data not shown), indicative of variation in the soil ecosystems after long-term fertilizer application. Moreover, regression analysis showed the increase rate was negatively related to SOC. The observed increase in N₂O emission under NPK was higher than under M and NPKM fertilization regimes. It can be attributed to the sorption of NH₄⁺ onto soil organic matters (Fernando *et al.* 2005). Soil organic matter content was significantly higher in fertilization regimes of organic amendment (*i.e.*, M and NPKM regimes) compared to NPK regime. When urea added to soil, NH₄⁺ hydrolyzed from urea might be absorbed by soil organic matters, then the NO₃⁻ would thereupon decrease, which is confirmed by the lower increase rate of NO₃-N content in urea (U) treatment compared to CK under fertilization regimes of M and NPKM (Fig. 4b).

It was also found that application of nitrification inhibitor significantly reduced soil N₂O emissions, as previously reported in earlier studies of upland field (Tian *et al.* 2015; Guardia *et al.* 2017; Recio *et al.* 2019), with different reduction rate under various fertilization regimes.

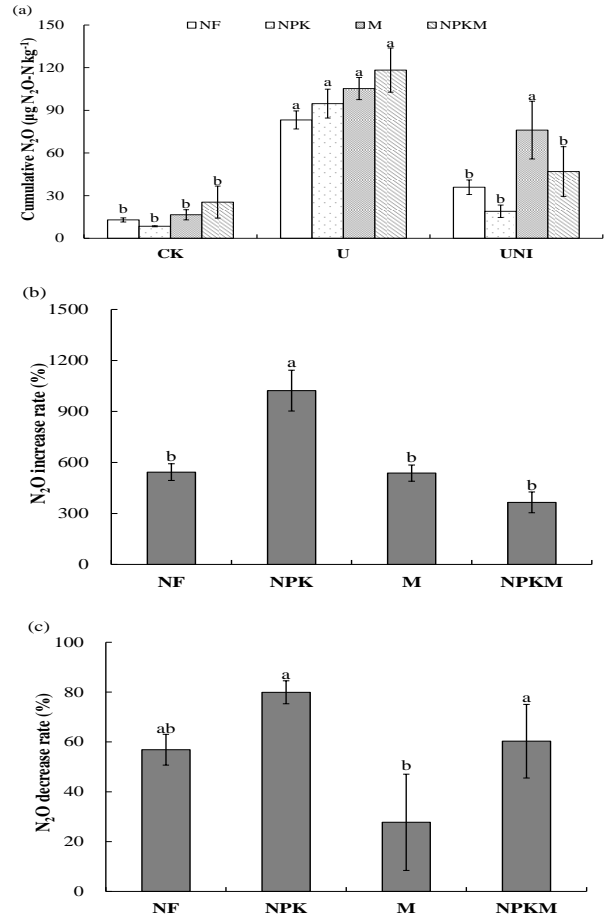


Fig. 2: Effects of N and NI additions on N₂O emissions in soils under different long-term fertilization regimes. (a) Cumulative N₂O emissions; (b) Increase rate of N₂O emissions in response to N addition; (c) Decrease rate of N₂O emissions in response to NI addition. Vertical bars indicate the standard error (n = 3). Different lowercase letters indicate significant difference between incubation treatments at $P < 0.05$

Regression analysis showed the decrease rate was negatively related to SOC content. The decrease rate of N₂O emission under regime with manure was significantly lower than under other fertilization regimes ($P < 0.05$). One of the mechanisms can be that high organic matter could null the nitrification inhibitor through adsorption (Jacinte and Pichtel 1992; Asgedom *et al.* 2014). Fertilization regime of manure has the highest organic matter, which can greatly hinder the nitrification inhibitor, and thus got lower reduction rate of N₂O emission. Another reason might be the difference of soil microbes among different fertilization treatments. The nitrification and denitrification potential under fertilization regime of manure was higher than NPK, indicating greater microbial activities related to N₂O emissions. After application of nitrification inhibitor, decrease rate of these microbial properties was lower in manure regime, which in turn caused lower reduction of N₂O emissions.

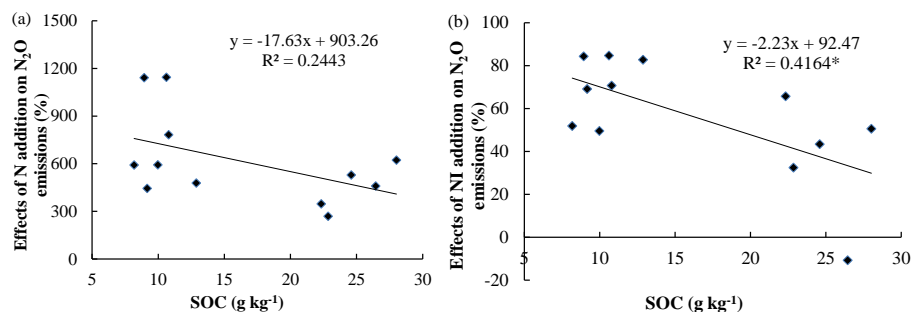


Fig. 3: Linear regressions of N₂O emissions in response to N (a) and NI (b) additions upon soil organic carbon (SOC) content. * represents the significant regression at $P < 0.05$

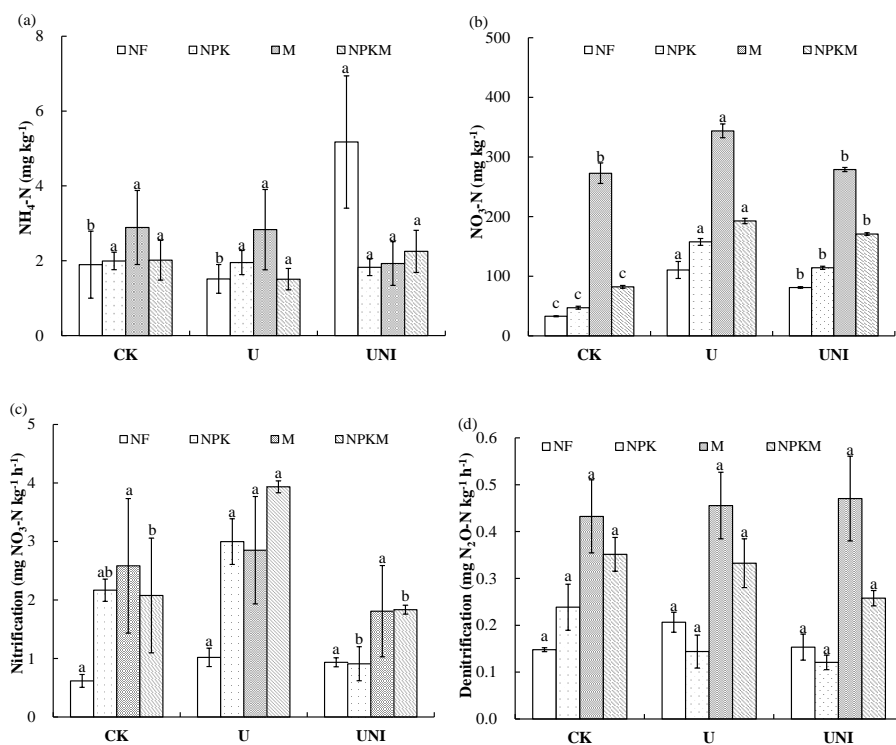


Fig. 4: Impacts of N and NI additions on NH₄-N concentration (a), NO₃-N concentration (b), nitrification potential (c) and denitrification potential (d) in soils under different long-term fertilization. Vertical bars indicate the standard error (n = 3). Different lowercase letters indicate significant difference between incubation treatments at $P < 0.05$

In the present study, N and NI additions had significant effects on N₂O emissions. However, the response of related nitrifiers and denitrifiers in soils from different long-term fertilization was not clear, which needs further investigation. Besides, it is considered that gaseous N is closely related to global warming, *i.e.*, N₂O in this study. However, there is other important gaseous N such as N₂, also being product of denitrification process (Poeth 1986), which need to be considered. Moreover, the leaching of NO₃-N during N conversion should not be neglected. After addition of N or inhibitor, the turnover of external and endogenous N can be further investigated by ¹⁵N isotope labeling.

Conclusion

A significantly different response of N₂O emissions to N and NI additions from Fluvisols under different long-term fertilization regimes. N addition significantly increased N₂O emissions, with the highest increase rate in the soil of long-term NPK fertilization and the lowest increase rate in the soil of long-term NPKM fertilization. NI addition significantly decreased N₂O emissions, with the lowest decrease rate in the soil of long-term M fertilization. Those differences of N₂O emissions in response to N and NI additions were mainly resulted from the difference of soil organic matters. It can be concluded that for soils with lower

organic matter content, chemical N fertilizer addition would cause more N₂O emissions; nevertheless, addition of NI had higher effects on N₂O emissions from these soils. Therefore, the application of nitrification inhibitor in field with lower soil organic matter (e.g., soils after long-term chemical NPK fertilization) is recommended, to better mitigate the global warming potential.

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Author Contributions

XZ: methodology, data curation and writing-original draft preparation, HQ: methodology and validation, SL: resources, FX: resources, YJ: writing-review and editing, FD: writing-review and editing, AD: writing-review and editing, ZS: writing-review and editing, HC: resources, writing-review and editing, WZ: supervision, CZ: Conceptualization.

Conflicts of Interest

The author declares no conflict of interest

Data Availability

All new research results were presented in this article

Ethics Approval

Not applicable.

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