RESEARCH ARTICLE

Differential immediate and long-term effects of nitrogen input on denitrification $N_2O/(N_2O+N_2)$ ratio along a 0–5.2 m soil profile

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Abstract

High nitrogen (N) input to soil can cause higher nitrous oxide (N_2O) emissions, that is, a higher $N_2O/(N_2O+N_2)$ ratio, through an inhibition of N_2O reductase activity and/or a decrease in soil pH. We assumed that there were two mechanisms for the effects of N input on N_2O emissions, immediate and long-term effect. The immediate effect (field applied fertilizer N) can be eliminated by decreasing the N input, but not the long-term effect (soil accumulated N caused by long–term fertilization). Therefore, it is important to separate these effects to mitigate N_2O emissions. To this end, soil samples along a 0–5.2 m profile were collected from a long-term N fertilization experiment field with two N application rates, that is, 600 kg N ha^{-1} $year^{-1}$ (N600) and no fertilizer N input (N0). External N addition was conducted for each subsample in the laboratory incubation study to produce two additional treatments, which were denoted as N600+N and N0+N treatments. The results showed that the combined immediate and long-term effects led to an increase in the $N_2O/(N_2O+N_2)$ ratio by 6.8%. Approximately 32.6% and 67.4% of increase could be explained by the immediate and long-term effects of N input, respectively. Meanwhile, the long-term effects were significantly positively correlated to soil organic carbon (SOC). These results indicate that excessive N fertilizer input to the soil can lead to increased N_2O emissions if the soil has a high SOC content. The long-term effect of N input on the $N_2O/(N_2O+N_2)$ ratio should be considered when predicting soil N_2O emissions under global environmental change scenarios.

Introduction

The $N_2O/(N_2O+N_2)$ ratio reflects the proportion of N_2O accounting for the total main gaseous N emissions by denitrification, and its decrease is beneficial for the mitigation of soil N_2O emissions in a certain extent. High N fertilizer input could cause higher N_2O emissions, that is, a higher $N_2O/(N_2O+N_2)$ ratio. Increase in this ratio could result from the inhibition of soil N_2O reductase activity and/or a decrease in soil pH [1]. We assumed that there should be considered the immediate and long-term effects of N input on N_2O emissions. The immediate

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effect could be eliminated through a decrease in soil nitrate (NO_3^-) content, but it is more difficult to eliminate the long-term effects [2]. Numerous studies have investigated the enhancing effect of N input on soil denitrification and N_2O emission [3–5], and the effect of N input on the end product composition of denitrification [6, 7]. However, these studies did not quantify the relative contributions of the immediate and long-term effects of N input on the $N_2O/(N_2O+N_2)$ ratio.

A separation of the immediate and long-term effects could provide a better understanding of the concurrent effect of continuous N input on soil N_2O emissions and mitigation. In the present study, we collected soil samples along a 0–5.2 m profile from a long-term N fertilization experimental field and conducted an external N addition experiment in a laboratory incubation study. The main aims of this study were to: 1) quantify the immediate and long-term effects of high N addition on the $N_2O/(N_2O+N_2)$ emission ratio and 2) analyze the correlation between soil properties and the $N_2O/(N_2O+N_2)$ ratio.

Materials and methods

Experimental site and soil sampling

The experimental site was located at the Luancheng Agro-Ecosystem Experimental Station (37.90˚N, 114.67˚E; elevation, 50 m) of the Chinese Academy of Sciences, Hebei, China. A long-term N fertilization experiment was established in 1998 with a winter wheat (*Triticum aestivum*) and summer maize (*Zea mays*) double cropping system. The experiment has four N fertilizer treatments each with three replicates: CK (0 kg N ha^{-1} $year^{-1}$), low nitrogen (200 kg N ha^{-1} $year^{-1}$), medium nitrogen (400 kg N ha^{-1} $year^{-1}$) and high nitrogen (600 kg N ha^{-1} $year^{-1}$). Two selected N treatments (0 and 600 kg N ha^{-1} $year^{-1}$) were examined in this study, which are denoted as N0 and N600, respectively. Undisturbed soil columns (43 mm in diameter) of 0–5.2 m depth (13 soil layers of 0.4 m) were collected in triplicates using a Geoprobe drilling rig (Geoprobe® 54DT, USA) from the N0 and N600 treatments in October 2012. The collected composite soils within each layer were mixed and divided into two subsamples: one for soil physicochemical parameter analyses and one for measuring soil denitrification. Fresh soils were stored in poly vinyl chloride bags at 4˚C before analysis. Details of other soil properties and specific field management have been previously described by Yuan *et al.* [8].

Soil properties determination

Soil moisture was determined gravimetrically by oven drying samples to a constant weight at 105±0.5˚C. Soil particle composition was determined using a laser particle size analyzer (Malvern Mastersizer 3000, UK). Soil pH was measured in a suspension of 1:5 soil to water using a pH meter. Soil NO_3^- was extracted using a 1 M KCl solution in a soil/solution ratio of 1:5 (w/v) and determined using dual-wavelength ultraviolet spectrophotometry (Shimadzu UV2450, Japan) [9]. The total soil organic carbon (SOC) was determined using the dichromate oxidation method [10]. The soil parameters were analyzed in duplicate because of the limited soil volume in each layer.

Laboratory incubation experiment

The laboratory experiment had three triplicate treatments using the composite soils: (I) soil sample N0 without N addition (N0), (II) soil sample N0 with N addition (N0+N) (III) soil sample N600 with N addition (N600+N). Previous studies have shown that dissolved organic carbon (DOC) content differs significantly within different soil depths and N fertilization rates [11]. Therefore, DOC was adjusted to the same level to eliminate the potential effects of DOC on the $N_2O/(N_2+N_2O)$ ratio. The $N_2O/(N_2+N_2O)$ ratio differences between N0+N and N0 were assumed to be the immediate effects, and the N_2O ratio differences between N600+N and N0+N were considered as combined immediate and long-term effects. The procedure of a N0 +N or N600+N was briefly described as follows: an equivalent of 10.0 g field-moist soil was agitated by a magnetic bar after adding 15 ml of 10 mM KNO_3 and glucose mixture in a 120 ml serum flask. The flask was then capped with an air-tight butyl rubber stopper and aluminum crimp seal, and evacuated (0.1 kPa) and filled with high-purity helium gas (99.999%, 120 kPa) five times. The pressure of the headspace was adjusted to 101.3 kPa after the final helium filling. All flasks were anaerobic incubated (with helium filling) in a thermostatic water bath at 25˚C. N_2O and N_2 measurements were started after a 1 hour incubation to establish equilibrium between the soil and headspace. The headspace gas in the flasks was sampled and analyzed for N_2O and N_2 concentrations using a robotized sampling and analysis system according to Molstad et al. [12]. The analysis for N0 sample followed the same procedure as the N0+N and N600+N treatments, with addition of no external N. The N_2O and N_2 emission rates were calculated by linearly regressing the headspace N_2O and N_2 concentrations with incubation time. The data of the initial couple of days were not used to calculate the N_2O emission rate because N addition had obvious priming effects on N_2O emission during this period.

Data processing and statistical analysis

The figures were plotted using OriginPro9.0 (©Origin Lab Corporation) software. Analysis of variance (ANOVA) was performed to determine the difference in the $N_2O/(N_2O+N_2)$ ratio between the different N addition treatments using SPSS for Windows (version 16.0, SPSS Inc., Chicago). Duncan's *post-hoc* test was used to assess the significant effects of the different N treatments on the $N_2O/(N_2O+N_2)$ ratio. Pearson's correlations were analyzed between soil properties and immediate and long-term effects. Statistical significance was set at $p < 0.05$, unless otherwise stated.

Results and discussion

The $N_2O/(N_2O+N_2)$ ratio in the 0–5.2 m soil profile increased after 24 h of incubation with external N addition (N0+N) compared to that under the N0 treatment (Fig 1). Increase in the $N_2O/(N_2O+N_2)$ ratio was more remarkable at 0–2.0 m depth. The ratio increased by 1.9% on average along the entire profile, indicating an immediate effect of N addition on the $N_2O/(N_2O+N_2)$ ratio. Previous studies have observed that N_2O can be completely reduced to N_2 at a low NO_3^- addition (20 mg N kg^{-1}), but is inhibited at a high NO_3^--N addition (> 50 mg N kg^{-1}) [13, 14]. The addition of 210 mg N kg^{-1} as NO_3^--N in the present study, which was much higher than those applied in previous studies, similarly showed an effective immediate inhibition of N_2O reductase activity. On an average, the $N_2O/(N_2O+N_2)$ ratio in the 0–5.2 m soil profile under the N600+N treatment was 4.9% higher than that under the N0+N treatment (Fig 1), which showed the long-term effects of N addition on N_2O reduction. If we regard the combined immediate and long-term effects as unit 1, then the results indicate that, apart from the immediate effect, 67.4% in the $N_2O/(N_2O+N_2)$ ratio resulted from the long-term effect of N addition. This was probably caused by a decrease in pH owing to the large amount of NO_3^--N accumulation in the profile (Fig 2b and 2d).

Previous studies have shown that long-term N fertilization results in a decrease in soil pH and a consequent increase in the $N_2O/(N_2O+N_2)$ ratio [15, 16]. The pH across the profiles was about 0.5 units lower under the N600 treatment than that in the N0 treatment (Fig 2b), that is, the soil pH indeed decreased under the 15-year fertilizer N input. Pearson correlation analysis was performed to further test the correlation between soil properties and N_2O ratio increase.

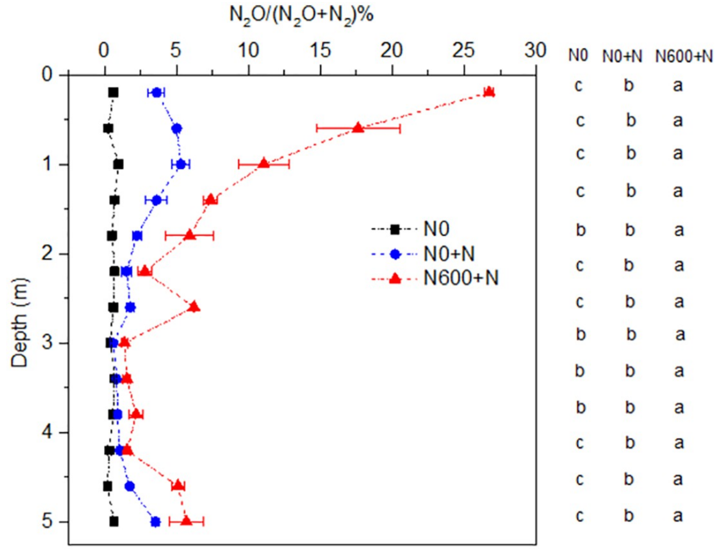

Fig 1. Soil $N_2O/(N_2O+N_2)$ emission ratio in different depths across the 0–5.2 m soil profile in the N0, N0+N and N600+N treatments. N0 and N0+N represent laboratory anaerobic incubation using the soil N0 and N0+N, respectively. N600+N is the same as the N0+N treatment except for using the soil N600. N0 and N600 represent fertilizer N input rates of 0 and 600 kg N ha^{-1} $year^{-1}$, respectively. Bars represent standard deviations of the means (n = 3). Different letters indicate significant difference at $p < 0.05$ between different treatments.

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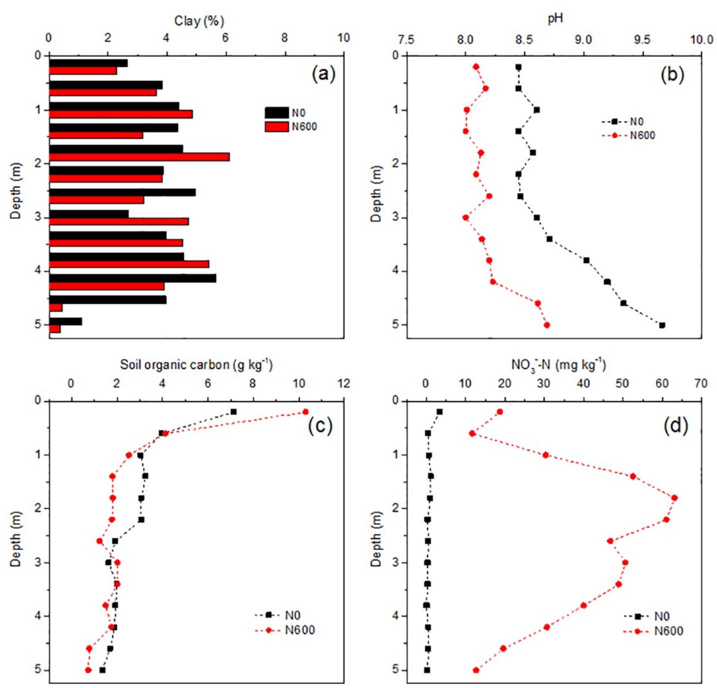

Fig 2. Soil chemical properties in different soil depths across the 0–5.2 m soil profiles in the N0 and N600 treatments. Soil clay content (a), pH (b), soil organic carbon (c) and nitrate content (d) in different soil depths across the 0–5.2 m soil profiles in the N0 and N600 treatments. N0 and N600 represent fertilizer N input rates of 0 and 600 kg N ha^{-1} $year^{-1}$, respectively. Relative errors were less than 0.05 for all the measured parameters (n = 2).

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Table 1. Pearson correlation coefficients (r) between soil clay content, pH, and soil organic carbon (SOC) and the immediate and long-term effects of N addition on N_2O emission ratio.

ΔN_2O ratios†	Soil parameters		
	Clay	pH	SOC
Immediate effects	-0.113	-0.278	0.519
Long-term effects	-0.273	-0.350	0.911**

** Significant at $p < 0.01$ levels.

† Immediate effects = $R_{[N0+N]} - R_{N0}$; long-term effects = $R_{[N600+N]} - R_{[N0+N]}$. N0+N and N600+N represent anaerobic incubation using the soil from zero and 600 kg N ha^{-1} year^{-1} fertilizer N treatment with external N addition, respectively. N0 was the same treatment, except for using the soil from the no-fertilizer N treatment.

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The results showed that only the long-term effect of N addition on the $N_2O/(N_2O+N_2)$ ratio was significantly positively correlated with SOC content rather than clay and pH (Table 1). These results imply that more N_2O is likely to be emitted in soils with excessive NO_3^- and concurrent organic C enrichment. On the contrary, pH showed a negative correlation with the immediate or long-term effects of N input on N_2O ratio increase (r = –0.28 and –0.35, respectively), but this was not statistically significant. This may be because even though the pH under N600 tended to decrease, the pH value was still in the alkaline range in this study due to the considerable calcium ion buffering capacity [17]. Nevertheless, N addition has significantly reduced pH by 0.26 pH-units on average globally (Tian and Niu, 2015). Under the trend of increasing atmospheric N deposition and continuous large N input for higher crop yields, soil acidification becomes more serious [18], which would thus have a greater impact on soil N_2O emissions.

Conclusions

Using soil samples along a 0–5.2 m soil profile from N0 and N600 fertilizer plots, we investigated the immediate and long-term effects of N addition on the $N_2O/(N_2O+N_2)$ ratio. High N fertilizer input (600 kg N ha^{-1} year^{-1}) increased the $N_2O/(N_2O+N_2)$ emission ratio by 6.8%. Approximately 32.6% of the increase was due to the N_2O reductase activity inhibition, and 67.4% of the increase was caused by comprehensive long-term effects, such as pH decrease or soil microbial community shift. There was a significant positive correlation between the long-term effects and SOC content. Future studies are needed to investigate soil acidification caused by N addition on the denitrifying community and end-product partition, especially in N-saturated and low-pH fields. Our results suggest that N addition-induced soil pH decrease should be included in models that predict biota communities and linkages to carbon and nitrogen cycling in terrestrial ecosystems under global environmental change scenarios, such as N deposition and soil acidification.

Supporting information

S1 Data.
(XLSX)

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Author Contributions

Data curation: Haijing Yuan.

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Writing – original draft: Haijing Yuan.

Writing – review & editing: Xinhua He, Stuart Lindsey.

References

1. Qu Z, Wang JG, Almoy T, Bakken LR. Excessive use of nitrogen in Chinese agriculture results in high $N_2O/(N_2O+N_2)$ product ratio of denitrification, primarily due to acidification of the soils. Global Change Biology. 2014; 20(5):1685–98. https://doi.org/10.1111/gcb.12461 PMID: 24249526
2. Qin SP, Ding KR, Clough TJ, Hu CS, Luo JF. Temporal in situ dynamics of N_2O reductase activity as affected by nitrogen fertilization and implications for the $N_2O/(N_2O + N_2)$ product ratio and N_2O mitigation. Biology and Fertility of Soils. 2017; 53(7):723–7. https://doi.org/10.1007/s00374-017-1232-y
3. Abbasi MK, Adams WA. Gaseous N emission during simultaneous nitrification-denitrification associated with mineral N fertilization to a grassland soil under field conditions. Soil Biol Biochem. 2000; 32(8–9):1251–9. https://doi.org/10.1016/S0038-0717(00)00042-0
4. Ciarlo E, Conti M, Bartoloni N, Rubio G. Soil N_2O emissions and $N_2O/(N_2O+N_{(2)})$ ratio as affected by different fertilization practices and soil moisture. Biology and Fertility of Soils. 2008; 44(7):991–5. https://doi.org/10.1007/s00374-008-0302-6
5. Xu HJ, Yang XR, Li S, Xue XM, Chang S, Li H, et al. Nitrogen inputs are more important than denitrifier abundances in controlling denitrification-derived N_2O emission from both urban and agricultural soils. Science of the Total Environment. 2019; 650:2807–17. https://doi.org/10.1016/j.scitotenv.2018.10.001 PMID: 30373058
6. Weier KL, Doran JW, Power JF, Walters DT. Denitrification and the Dinitrogen/ NitrousOxide Ratio as Affected by SoilWater, Available Carbon, and Nitrate. Soil Sci Soc Am J. 1993; 57(1):66–72. https://doi.org/10.2136/sssaj1993.03615995005700010013x
7. Bizimana F, Timilsina A, Dong WX, Uwamungu JY, Li XX, Wang YY, et al. Effects of long-term nitrogen fertilization on N_2O, N_2 and their yield-scaled emissions in a temperate semi-arid agro-ecosystem. Journal of Soils and Sediments. 2021; 21(4):1659–71. https://doi.org/10.1007/s11368-021-02903-4
8. Yuan HJ, Qin SP, Dong WX, Hu CS, Manevski R, Li XX. Denitrification Rate and Controlling Factors for Accumulated Nitrate in the Deep Subsoil of Intensive Farmlands: A Case Study in the North China Plain. Pedosphere. 2019; 29(4):516–26. https://doi.org/10.1016/S1002-0160(17)60472-7
9. Norman RJ, Edberg JC, Stucki JW. Determination of Nitrate in Soil Extracts by Dual-Wavelength Ultraviolet Spectrophotometry. Soil Sci Soc Am J. 1985; 49(5):1182–5. https://doi.org/10.2136/sssaj1985.03615995004900050022x
10. Ciavatta C, Antisari LV, Sequi P. Determination of OrganicCarbon in Soils and Fertilizers. Communications in Soil Science and Plant Analysis. 1989; 20(7–8):759–73. https://doi.org/10.1080/00103628909368115
11. Chen SM, Wang FH, Zhang YM, Qin SP, Wei SC, Wang SQ, et al. Organic carbon availability limiting microbial denitrification in the deep vadose zone. Environmental Microbiology. 2018; 20(3):980–92. https://doi.org/10.1111/1462-2920.14027 PMID: 29266729
12. Molstad L, Dorsch P, Bakken LR. Robotized incubation system for monitoring gases (O_2, NO, N_2O N_2) in denitrifying cultures. J Microbiol Meth. 2007; 71(3):202–11. https://doi.org/10.1016/j.mimet.2007.08.011 PMID: 17904668
13. Blackmer AM, Bremner JM. Inhibitory Effect of Nitrate on Reduction of N_2O to N_2 by SoilMicroorganisms. Soil Biol Biochem. 1978; 10(3):187–91. https://doi.org/10.1016/0038-0717(78)90095-0
14. Senbayram M, Chen R, Budai A, Bakken L, Dittert K. N_2O emission and the $N_2O/(N_2O + N_2)$ product ratio of denitrification as controlled by available carbon substrates and nitrate concentrations. Agr Ecosyst Environ. 2012; 147:4–12. https://doi.org/10.1016/j.agee.2011.06.022
15. Raut N, Dorsch P, Sitaula BK, Bakken LR. Soil acidification by intensified crop production in South Asia results in higher $N_2O/(N_2+N_2O)$ product ratios of denitrification. Soil Biol Biochem. 2012; 55:104–12. https://doi.org/10.1016/j.soilbio.2012.06.011
16. Tian DS, Niu SL. A global analysis of soil acidification caused by nitrogen addition. Environmental Research Letters. 2015; 10(2). Artn 024019 https://doi.org/10.1088/1748-9326/10/2/024019

17. Dong WX, Duan YM, Wang YY, Hu CS. Reassessing carbon sequestration in the North China Plain via addition of nitrogen. Science of the Total Environment. 2016; 563:138–44. https://doi.org/10.1016/j.scitotenv.2016.04.115 PMID: 27135576

18. Zhu QC, de Vries W, Liu XJ, Hao TX, Zeng MF, Shen JB, et al. Enhanced acidification in Chinese croplands as derived from element budgets in the period 1980–2010. Science of the Total Environment. 2018; 618:1497–505. https://doi.org/10.1016/j.scitotenv.2017.09.289 PMID: 29089131