scientific reports

OPEN

Response of N₂O emission and denitrification genes to different inorganic and organic amendments

Yajun Yang[1,2], Hexiang Liu[1,2] & Jialong Lv[1,2✉]

Denitrification is a key biochemical process in nitrogen cycling and nitrous oxide (N_2O) production. In this study, the impacts of different inorganic and organic amendments (OAs) on the abundance of denitrifying genes (*nirS*, *nirK* and *nosZ*) and the level of N_2O emission were examined with incubation experiments. Six treatments included the indicated applications: (i) no fertilization (CK); (ii) urea application alone (U); (iii) wheat straw plus urea (U + WS); (iv) pig manure plus urea (U + PM); (v) compost product plus urea (U + CP); and (vi) improved compost product plus urea (U + IC). The results indicated that all fertilization treatments increased accumulative N_2O emissions compared with the CK treatment. The U + WS, U + PM and U + CP treatments increased N_2O emissions by 2.12–141.3%, and the U + IC treatment decreased N_2O emissions by 23.24% relative to the U treatment. *nirK* was the dominant denitrification gene rather than *nirS* and *nosZ* found in soil. Additionally, the highest abundance of *nirK* gene was that with the U + PM treatment, and the lowest was that with the U + IC treatment. Additionally, changes in the *nirK* gene were highly correlated with levels of dissolved organic carbon (DOC), dissolved organic nitrogen (DON) and nitrate nitrogen (NO_3^-N). Automatic linear modeling revealed that N_2O emission was closely related to the *nirK* gene, DOC and NO_3^-N. Overall, the use of urea and improved compost as co-amendments retarded N_2O emission to a considerable degree compared with other OA additions.

Nitrous oxide (N_2O) is one of the most prevalent greenhouse gases, and its warming potential is approximately 265 times that of CO_2 on the 100-year scale[1]. In addition, N_2O is an important substance that destroys the ozone layer[2]. Annual average N_2O emissions from global cultivated fields total 6.4×10^{12} g [calculated as nitrogen (N)], which accounts for 25% of total global N_2O emissions[3]. Agricultural fertilization management is the main source of N_2O emissions. Application of nitrogen fertilizer stimulates N_2O emission from cultivated soils[4,5]. Therefore, it is urgent to find an effective strategy for decreasing N_2O emissions.

N_2O is produced mainly through biological and abiotic processes. Denitrification is one of the main ways N_2O is produced under microbe-driven conditions in soil[6]. Denitrification is a process in which nitrate (NO_3^-N) is sequentially reduced to nitrite (NO_2^-N), nitric oxide (NO), nitrous oxide (N_2O), and finally dinitrogen gas (N_2)[7]. It is essentially the inverse process of nitrification. Nitrite-reducing bacteria are encoded by nitrite reductase genes (*nirK* and *nirS*). In addition, reduction of N_2O to N_2 is one of the key steps in the whole process, and is encoded by the *nosZ* gene[8,9]. Nos-catalyzed N_2O reduction plays a significant role in N_2O emissions. Therefore, the abundance of *nirS*, *nirK* and *nosZ* denitrifying genes was used to study soil nitrogen cycling and N_2O emissions[10,11]. At present, nitrogen addition affecting N_2O emissions has attracted wide concern[12,13]. Wang et al.[14] reported that nitrogen addition at 250 kg ha⁻¹ promoted denitrification, thus contributing to comparable stimulation of N_2O emissions. Albanito et al.[15] proposed a linear relationship between N_2O emissions and amounts of nitrogen fertilizer applied in tropical and subtropical regions. To reduce N_2O emissions, combined application of organic materials and inorganic fertilizer has been endorsed by some scholars. The addition of organic material altered the abundance of denitrifiers and thus influenced the denitrification process[16,17]. Wang et al.[18] found that organic materials stimulated the activity of the *nosZ* gene and decreased N_2O emissions. However, some studies have suggested that organic materials (i.e., wastewater sludge, manure and vermicompost) provide carbon sources for denitrifiers and accelerate denitrification, thus increasing N_2O emissions[11,19]. Although many studies have

[1]College of Natural Resources and Environment, Northwest A&F University, Yangling 712100, Shaanxi, People's Republic of China. [2]Key Laboratory of Plant Nutrition and the Agri-Environment in Northwest China, Ministry of Agriculture, Yangling, People's Republic of China. ✉email: ljlll@nwsuaf.edu.cn

been focused on combined application of organic and inorganic fertilizers and its effect on N$_2$O emissions in agricultural soils, the research conclusions are not yet consistent. Therefore, it is necessary to carry out research to determine the drivers of N$_2$O emissions and propose reasonable fertilization strategies.

In addition, the identification of major denitrification-driving genes plays an important role in predicting N$_2$O emissions. Yin et al.[20] indicated that denitrifiers were more sensitive to inorganic fertilization than to organic fertilization. Harter et al.[21] revealed that the *nosZ* gene is the decisive factor for N$_2$O emissions. Hai et al.[22] found that organic fertilizers increased the abundance of *nirS* and *nirK* genes and enhanced N$_2$O emissions. Huang et al.[23] reported that co-addition of urea and cattle manure decreased the abundance of *nirS* and *nirK* but increased N$_2$O emissions. In addition, Xu et al.[24] suggested that *nirS*-type denitrifying genes showed strong correlations with significant increases in N$_2$O emissions from soils undergoing organic fertilization. Henderson et al.[25] questioned whether the relationship between N$_2$O emissions and the abundance of *nirS* and *nosZ* genes was significant. Other studies have focused on the impacts of co-amendment with inorganic and organic fertilizers on the abundance of denitrification bacterial communities and changes in soil properties[21,26–28]. However, there was still a knowledge gap regarding the dominant drivers of N$_2$O emissions, including denitrifiers and environmental factors.

Therefore, this study was focused on the impacts of co-additions of urea and four organic additions (OAs), including wheat straw, pig manure, compost products and improved compost products, on N$_2$O emissions. Additionally, fluorescence quantitative PCR and 16S rDNA sequencing were used to study changes in the abundance of denitrifying genes in soil caused by OAs and clarify the mechanism for the response of denitrifying gene abundance to added OAs. Particularly, the purposes of this research were (i) to compare the impacts of different OAs with urea on changes in N$_2$O emissions; (ii) to understand the abundance of denitrifiers affected by different OAs; and (iii) to identify the relationships among N$_2$O emissions, soil properties and the abundance of denitrifiers.

Materials and methods

Experimental soil collection.
The tested soil was collected from the experimental station around Northwest A&F University in Shaanxi Province, China. The tested plot lies in a typical semiarid region with average annual precipitation of 533–631 mm. The basic soil properties before the experiment were as follows: pH = 7.53, soil organic carbon (SOC) = 0.79%, total nitrogen (TN) = 0.075%.

Experimental materials collection.
The four OAs included compost product (CP), wheat straw (WS), pig manure (PM) and improved-compost product (IC). PM and WS were collected from the farm and field of the experimental station. CP and IC products were produced in the greenhouse on the campus. CP was manufactured with PM and WS. IC was produced by the addition of bean dregs and biochar to the compost product. Previous studies confirmed that IC products increased SOC and TN contents more than compost products[29,30]. All OA materials were dried at 55 °C and then sieved through a 2 mm mesh. Basic physicochemical properties for all OAs are shown in the Supplementary Material.

Experimental design.
Six incubation experiments, including no fertilization (CK), urea N alone (U), 70% urea N plus 30% N from PM (U + PM), 70% urea N plus 30% N from WS (U + WS), 70% urea N plus 30% N from CP (U + CP) and 70% urea N plus 30% N from IC (U + IC), were conducted with three replicates to understand the responses of N$_2$O emissions and denitrification genes to different fertilization practices. Fresh soil samples were first weighed (700 g for dry weight basis) and placed into plastic bottles. A total of 0.5 mg N g^{-1} of dry soil was added to each treatment. The amounts of urea and OAs were calculated by their total nitrogen content. The content of soil moisture was controlled at 60% of the water-holding capacity. Incubation was performed in a 25 °C environment for 77 days. Each plastic bottle was weighed, and distilled water was added to maintain constant moisture in the soil. All soil samples were divided into three parts after incubation: one was used for determination of soil properties after drying, one was used for measurements of NO$_3^-$-N and NH$_4^+$-N at 4 °C, and the other was used for DNA determination at − 80 °C.

Properties determination.
Soil NO$_3^-$-N and NH$_4^+$-N were determined with an AA3 flow analyzer by extraction with 2 mol L^{-1} KCl. Soil organic carbon (SOC) was measured through K$_2$Cr$_2$O$_7$ digestion. Total nitrogen (TN) was measured by a Kjeldahl nitrogen analyzer[31]. In addition, soil moisture content was determined by weighing after drying. In addition, the soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) contents were determined using the chloroform fumigation-extraction method[32,33]. Briefly, triplicate soil samples were weighed and fumigated with ethanol-free chloroform for 24 h at 25 °C. The three fumigated and unfumigated samples from each treatment were extracted with 100 mL of 0.5 mol L^{-1} K$_2$SO$_4$ on a shaker at 220 rpm for 30 min and then filtered and determined with a TOC/TN analyzer. Dissolved organic carbon (DOC) was determined by a TOC-analyzer after extraction and filtering[34]. Dissolved organic nitrogen (DON) was measured using a flow analyzer after extraction with H$_2$O[35]. N$_2$O samples were collected on days 0, 3, 7, 14, 21, 28, 35, 49, 63 and 77 during incubation. All bottles were sealed for 10 min before sampling to ensure concentration of N$_2$O emissions. N$_2$O samples were determined by gas chromatography. The N$_2$O fluxes and cumulative N$_2$O emissions were calculated as described by Huang et al.[36]. The specific calculation formula is as follows:

$$F = \frac{\Delta C}{\Delta t} \cdot \frac{V}{m} \cdot \frac{M}{22.4} \cdot \frac{273}{273 + T}$$

F is the N$_2$O fluxes (µg/kg/day), ΔC/Δt is the variation of concentration per unit time (µg/kg/day), V is the volume of the device (L), m is the dry soil weight (g), M/22.4 is the mass density of standard gases (g/L), and T is the incubation temperature (°C).
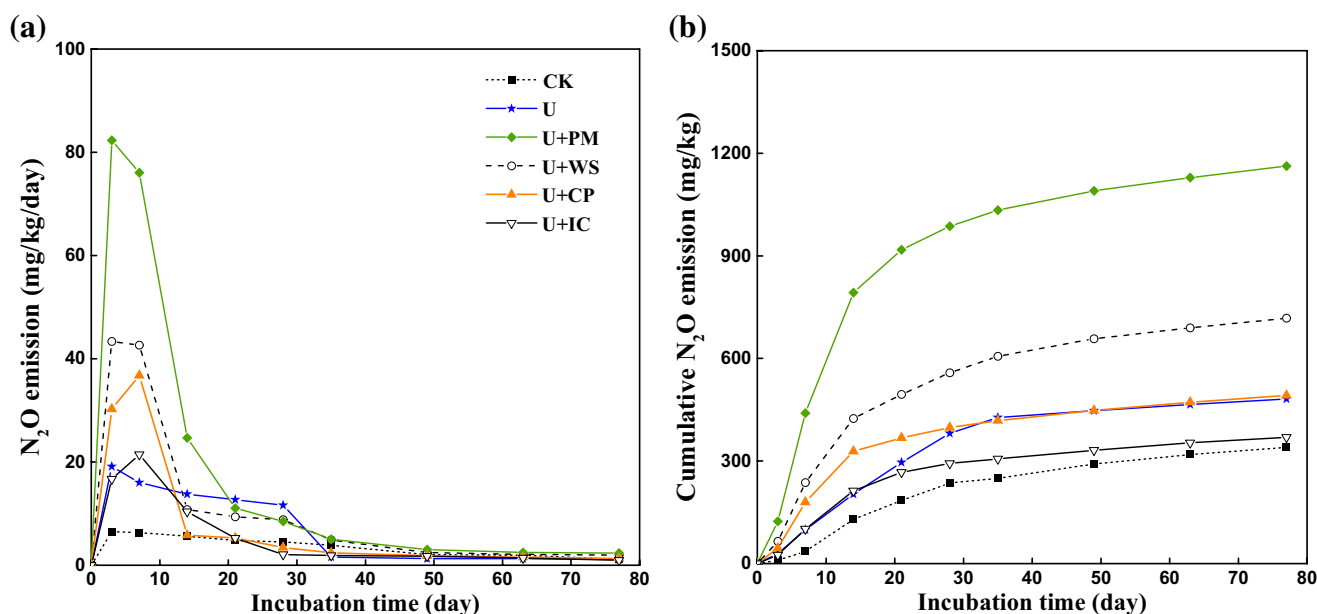
Figure 1. The changes of N₂O emission (**a**) and cumulative N₂O emission (**b**) during the incubation affected by different OAs. *CK* no chemical fertilizer, *U* urea, *U + WS* urea plus wheat straw, *U + PM* urea plus pig manure, *U + CP* urea plus compost, *U + IC* urea plus improved compost.

Cumulative N₂O emission is the accumulation of the N₂O fluxes during the incubation period (μg/kg).

Microbial analyses. Soil DNA was extracted from 0.5 g of fresh soil using a DNA kit (Omega, GA, USA). The DNA solution extracted from each sample was studies with 1% agarose gel electrophoresis. The *nirK*, *nirS* and *nosZ* denitrifying genes were amplified by PCR using primers (Supplementary Materials). The abundances of denitrifiers were quantified by quantitative real-time PCR (qPCR) on an Illumina MiSeq platform in Beijing.

Data analyses. Figures were prepared with Origin 2018. Significant differences among the treatments were analyzed by Duncan's multiple range test using IBM SPSS Statistics (version 20.0). Automatic linear modeling was conducted using IBM SPSS Statistics (version 20.0). Redundancy analyses (RDA) and principal component analyses (PCA) were conducted with Canoco (version 4.5).

Results

Changes in N₂O emission. N₂O emissions influenced significantly by different OAs are shown in Fig. 1a. A similar trend for N₂O emissions among all treatments was observed. N₂O emissions increased within the first 7 days and declined thereafter. N₂O emissions peaked on day 3 for the CK, U, U + WS and U + PM treatments and peaked on day 4 for the U + IC and U + CP treatments. The OAs increased N₂O emissions during the whole incubation process compared with the CK treatment. The maximum peak value for N₂O emissions among all OAs was that seen with the U + PM treatment (76.04 mg/kg/day), and the minimum value of N₂O emissions was that seen with the U + IC treatment (21.44 mg/kg/day). Compared with other OA treatments, compost amendments (U + IC, U + CP) decreased the peak values of N₂O emissions. Furthermore, the highest cumulative N₂O emissions were seen with the U + PM treatment, which were 241.8% higher than the levels seen with the CK sample; this was followed by the U + WS (111.1%), U + CP (44.63%), U (41.61%) and U + IC (8.70%) treatments (Fig. 1b). In addition, cumulative N₂O emissions for the U + WS, U + PM and U + CP treatments were 49.05%, 141.3% and 2.12% higher relative to the U treatment, but the cumulative N₂O emission for the U + IC treatment was 23.24% lower compared with that of the U treatment. These results indicated that relative to application of urea alone, mixing urea with wheat straw, pig manure and compost products promoted N₂O emissions during incubation. In addition, IC retarded N₂O emissions to a greater degree compared with other OA additions.

Changes in soil properties. The basic properties of soils are shown in Table 1. The highest SOC content was observed for the U + WS treatment, followed by the U + IC and U + CP treatments, and these levels were significantly higher than that of the CK treatment (P < 0.05). However, there was no significant difference in SOC contents for the U + PM and CK treatments (P > 0.05). Relative to the CK treatment, OAs significantly increased the levels of DON, NH₄⁺-N and NO₃⁻N. The highest NO₃⁻N, NH₄⁺-N and DON contents were found for the U + PM treatment, and these were higher than those seen for other OA treatments (P < 0.05). However, the DOC contents with all OA treatments except for U + IC differed significantly from that of the CK treatment (P < 0.05). The maximum DOC concentration was observed for the U + WS treatment. In addition, only the U + PM treatment, among all OA treatments, failed to increase the MBN content significantly (P > 0.05). The U treatment did

| Treatments	SOC (g/kg)	NH$_4^+$-N (mg/kg)	NO$_3^-$-N (mg/kg)	MBN (mg/kg)	DON (mg/kg)	DOC (mg/kg)
CK	6.53 ± 0.85a	0.58 ± 0.01a	49.35 ± 10.6a	0.48 ± 0.24a	7.14 ± 3.06a	150 ± 48a
U	6.59 ± 0.80a	0.63 ± 0.01a	80.60 ± 11.2b	0.53 ± 0.18a	7.21 ± 3.01a	155 ± 44a
U + PM	6.91 ± 0.58a	0.37 ± 0.02b	219.4 ± 16.94c	0.85 ± 0.15a	60.53 ± 4.19b	290 ± 22b
U + WS	8.88 ± 0.32a	0.13 ± 0.07c	147.1 ± 10.05d	1.49 ± 0.26b	54.22 ± 3.08c	330 ± 32b
U + CP	8.13 ± 0.65b	0.18 ± 0.03c	182.6 ± 16.25e	0.97 ± 0.15ac	54.79 ± 1.58bc	210 ± 18c
U + IC	8.63 ± 0.55b	0.25 ± 0.02d	178.1 ± 10.45e	0.94 ± 0.25c	48.14 ± 4.22d	180 ± 9.1ac

Table 1. Soil properties in different treatments with OAs. *CK* no urea and OAs, *U* urea, *U + WS* urea plus wheat straw, *U + PM* urea plus pig manure, *U + CP* urea plus compost, *U + IC* urea plus improved compost. Values are mean ± standard deviation (n = 3). The different letters indicate significant differences at the 0.05 probability level.
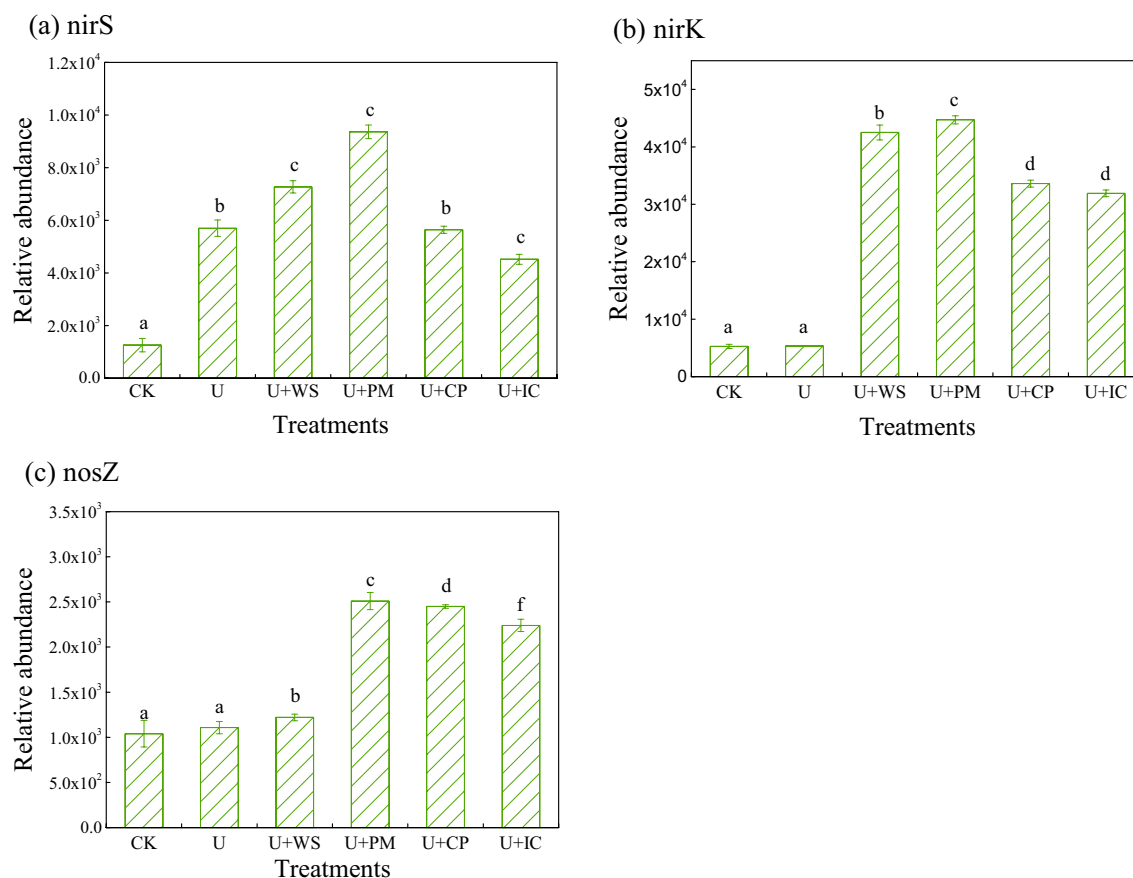
Figure 2. Relative abundance of denitrification genes during the incubation affected by different OAs. Error bars indicate standard deviations (n = 3). The different letters indicate significant differences (P < 0.05) (nirS (**a**); nirK (**b**); nosZ (**c**); *CK* no urea and OAs, *U* urea, *U + WS* urea plus wheat straw, *U + PM* urea plus pig manure, *U + CP* urea plus compost, *U + IC* urea plus improved compost).

not significantly increase any soil properties during incubation relative to the CK treatment except for NO$_3^-$N (P > 0.05).

Abundances of *nirK*, *nirS* and *nosZ* denitrifying genes. The relative abundances of denitrification genes (*nirK*, *nirS* and *nosZ*) were determined during incubation for all treatments (Fig. 2). The copy numbers for *nirK*, *nirS* and *nosZ* varied within the ranges 5.26×10^3–4.47×10^4, 1.26×10^3–9.36×10^3 and 1.04×10^3–2.51×10^3, respectively. The *nirK*-type denitrification genes exhibited the highest abundance after incubation. Compared with the CK treatment, all OA treatments (U + CP, U + IC, U + PM and U + WS) significantly increased the abundance of the *nirK* gene. The highest copy number of the *nirK* gene was seen for the U + PM treatment, followed by the U + WS, U + CP and U + IC treatments. In addition, there were no significant differences between the U + IC and U + CP treatments. However, the *nosZ* gene had the lowest abundance after incubation. Compared with the CK treatment, all OA treatments except the U + WS treatment significantly increased the copy number

(a)

(b)
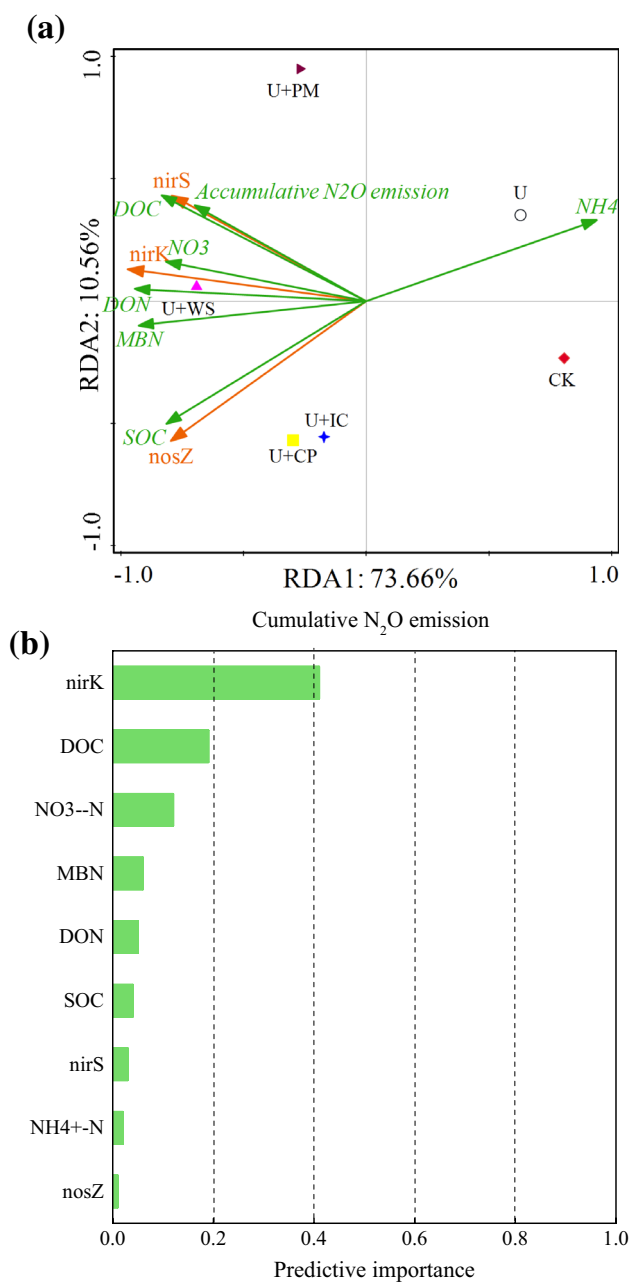
Figure 3. Principal component analysis (PCA) of soil properties, cumulative N$_2$O emission and abundance of denitrification genes (**a**); predictive importance of denitrifier gens abundance, and soil properties on cumulative N$_2$O emission as determined by automatic linear modeling (**b**) (*DOC* dissolved organic carbon, *MBN* microbial biomass carbon, *DON* dissolved organic nitrogen, *SOC* soil organic carbon, *CK* no urea and OAs, *U* urea, *U + WS* urea plus wheat straw, *U + PM* urea plus pig manure, *U + CP* urea plus compost, *U + IC* urea plus improved compost).

of the *nosZ* gene. The maximum abundance of the *nosZ* gene was observed for the U + PM treatment. There was no significant difference between the CK and U treatments. For abundance of the *nirS* gene, the U and OA treatments all significantly increased the abundance relative to the CK treatment. The highest *nirS* gene abundance was seen for the U + PM treatment, followed by the U + WS, U + CP and U + IC treatments. However, there was no significant difference between the U and U + CP treatments. Among all denitrification genes, the U treatment significantly increased only the abundance of *nirS* genes. These results indicated that co-additions of urea and OAs increased the abundance of *nirK* genes more, while urea alone increased the abundance of *nirS* genes more.

Correlations of denitrifying gene abundance and soil properties. PCA was used to analyze the abundances of denitrifiers among all treatments (Fig. 3a). The results showed that the explanations of Axis 1 and Axis 2 contributed 73.66% and 10.56%, respectively, to changes in denitrifying genes (*nirS, nirK* and *nosZ*).

The OA treatments were largely separated from the CK and U treatments along PCA1. Additionally, the U + PM and U + WS treatments were segregated from the U + CP and U + IC treatments along PCA2. The results suggested that OA application significantly influenced the abundance of the three types of denitrifiers, and OAs had stronger effects on the abundance of denitrifiers than urea application alone. Correlations among soil properties, accumulative N_2O emissions and denitrifying gene abundance showed that the abundance of the *nirS* gene was significantly associated with accumulative N_2O emissions (r = 0.876, P < 0.05) and DOC (r = 0.859, P < 0.05). The abundance of the *nirK* gene was correlated with DON (r = 0.977, P < 0.1), NO_3^--N (r = 0.880, P < 0.05), DOC (r = 0.865, P < 0.05), MBN (r = 0.852, P < 0.05) and NH_4^+-N (r = −0.880, P < 0.05). In addition, the abundance of the *nosZ* gene was mainly related to SOC (r = 0.965, P < 0.1), MBN (r = 0.836, P < 0.05) and NH_4^+-N (r = −0.931, P < 0.1). In addition, the results of automatic linear modeling showed that *nirK* abundance, DOC, NO_3^--N, MBN and DON were the main factors influencing N_2O emissions (Fig. 3b).

Discussion

Different fertilization practices influenced N_2O emissions from soil. Soil N_2O emissions peaked on day 3 for the CK, U, U + WS and U + PM treatments, while those for the U + CP and U + IC treatments peaked on day 7. This may be attributed to the fact that WS and PM provided more rapidly available N than CP and IC[37]. In addition, compared with urea application alone, the U + CP, U + WS and U + PM treatments increased cumulative N_2O emissions by 2.13–49.06%, which was within the increase range of 27–74% seen with pig slurry and compost amendments[38]. However, the U + IC treatment decreased cumulative N_2O emissions by 23.24% relative to urea alone. Therefore, the combined application of IC and urea reduced N_2O emissions in soil because IC was more stable than CP, PM and WS. Additionally, OA treatment increased cumulative N_2O emissions during incubation relative to the CK treatment. This might be because OAs provided more substrates for denitrification through mineralization. Denitrification could result in a rapid reduction in NO_3^--N content and promote the emission of N_2O. Previous studies showed that the NO_3^--N concentration was an important factor affecting the denitrification rate and N_2O release[39–43]. Increasing the concentration of NO_3^--N could significantly increase N_2O release. Compared with urea application alone, the OA additions increased the content of NO_3^--N in soil; furthermore, PM application provided more NO_3^--N than WS, CP and IC. Therefore, PM application promoted changes in N_2O emissions, which was consistent with the variations in NO_3^--N. In addition, SOC increased by addition of OAs to the soil. The importance of SOC as a factor affecting denitrification and N_2O emissions has been reported by Chen et al.[44]. However, in this study, the effect of SOC on denitrifiers and N_2O emissions was lower than that of DOC due to the narrow range of SOC changes occurring in a short-term incubation experiment. Carbon availability was the key controlling factor for denitrification in soil[45]. Compared with the U + PM treatment, the U + WS treatment produced a higher DOC content but lower N_2O emissions, which may be due to the higher C/N ratio in WS. Microbial fixation of carbon in the straw treatment limited carbon effectiveness. In addition, of all treatments involving addition of organic material, the modified compost addition treatment produced the lowest DOC content due to its higher degree of humification, which reduced the abundance of *nirK* genes and therefore reduced N_2O emissions from the soil[37].

N_2O emissions were highly related to soil physiochemical properties and the abundance of denitrifiers, which was consistent with the findings of Sun et al.[46]. Denitrifying bacteria are active in soil biological denitrification[11,47]. Many studies showed that fertilization increased the number of denitrifying microorganisms in the soil by providing substrates and energy for denitrifying bacteria and promoting their growth and reproduction[37,46,48]. In this study, the OA addition increased the abundance of denitrifying genes in soil. The contributions of *nirK* to N_2O emissions were higher than those of *nirS* and *nosZ*. In addition, application of pig manure significantly increased *nirK* gene abundance and thus promoted N_2O emissions more than other OAs. Yoshida et al.[49] also found that application of organic manure increased the abundance of *nirK* genes in rice paddy soil more than that of *nirS* genes. However, there are some studies showing the opposite results. For example, Yin et al.[50] found that organic manure changed the abundance of the *nirS* gene community in black soil during long-term treatment but not that of *nirK*. Barrett et al.[51] confirmed that a higher abundance of *nirS*-type genes was observed in carbon-amended soil relative to other genes. This may be related to the different sources and nature of available carbon in amended soil. Environmental factors significantly influenced the abundance of denitrifiers in this study[52,53]. Positive correlations were observed between NO_3^--N, DON, DOC and the abundance of the *nirK* gene. These findings suggested that NO_3^--N, DON and DOC levels were important factors affecting the abundance of the soil denitrifying bacterial community, thus affecting denitrification with different OA applications. This may be due to differences in the availability of C and N in OA-amended soil.

Conclusion

Fertilization treatments increase denitrification and N_2O emissions relative to the CK treatment. In addition, compared with urea alone, combined application of pig manure and urea provided more available N (DON and NO_3^--N) and increased the abundance of *nirK* genes, thus increasing cumulative N_2O emissions from the soil. However, combined applications of improved compost products and urea reduced accumulated N_2O emissions by decreasing the abundance of *nirK* genes. Overall, the different fertilization practices affected the abundance of denitrifiers, denitrification and soil properties. From the perspective of soil N_2O emission reduction, we recommend the application of improved compost products and urea to the soil.

Data availability

All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

References

1. IPCC. *Climate change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (Cambridge University Press, 2013).
2. Pachauri, R. K. *et al.* Climate change 2014: Synthesis report. Contribution of Working Groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change (IPCC, 2014).
3. Reay, D. S. *et al.* Global agriculture and nitrous oxide emissions. *Nat. Clim. Change* **2**, 410–416 (2012).
4. Jassal, R. S., Black, T. A., Roy, R. & Ethier, G. Effect of nitrogen fertilization on soil CH_4 and N_2O fluxes, and soil and bole respiration. *Geoderma* **162**, 182–186 (2011).
5. Hu, H. W., Chen, D. & He, J. Z. Microbial regulation of terrestrial nitrous oxide formation: Understanding the biological pathways for prediction of emission rates. *FEMS Microbiol. Rev.* **39**, 729–749 (2015).
6. Bateman, E. J. & Baggs, E. M. Contributions of nitrification and denitrification to N_2O emissions from soils at different water-filled pore space. *Biol. Fertil. Soils* **41**, 379–388 (2005).
7. Yang, Y. D., Hu, Y. G., Wang, Z. M. & Zeng, Z. H. Variations of the nirS-, nirK-, and nosZ-denitrifying bacterial communities in a northern Chinese soil as affected by different long-term irrigation regimes. *Environ. Sci. Pollut. Res.* **25**, 14057–14067 (2018).
8. Pan, Y., Ye, L., Ni, B. & Yuan, Z. Effect of pH on N_2O reduction and accumulation during denitrification by methanol utilizing denitrifiers. *Water Res.* **46**, 4832–4840 (2012).
9. Hallin, S., Philippot, L., Loffler, F. E., Sanford, R. A. & Jones, C. M. Genomics and ecology of novel N_2O-reducing microorganisms. *Trends Microbiol.* **1485**, 43–55 (2017).
10. Yang, L., Zhang, X. & Ju, X. Linkage between N_2O emission and functional gene abundance in an intensively managed calcareous fluvo-aquic soil. *Sci. Rep.* **7**, 43283 (2017).
11. Cui, P. Y. *et al.* Long-term organic and inorganic fertilization alters temperature sensitivity of potential N_2O emissions and associated microbes. *Soil Biol. Biochem.* **93**, 131–141 (2016).
12. Gerber, J. S. *et al.* Spatially explicit estimates of N_2O emissions from croplands suggest climate mitigation opportunities from improved fertilizer management. *Glob. Change Biol.* **22**, 3383–3394 (2016).
13. Shcherbak, I., Millar, N. & Robertson, G. P. Global metaanalysis of the nonlinear response of soil nitrous oxide (N_2O) emissions to fertilizer nitrogen. *Proc. Natl. Acad. Sci.* **111**, 9199–9204 (2014).
14. Wang, J., Chadwick, D. R., Cheng, Y. & Yan, X. Global analysis of agricultural soil denitrification in response to fertilizer nitrogen. *Sci. Total Environ.* **616**, 908–917 (2018).
15. Albanito, F. *et al.* Direct nitrous oxide emissions from tropical and sub-tropical agricultural systems—A review and modelling of emission factors. *Sci. Rep.* **7**, 44235 (2017).
16. Wolsing, M. & Priemé, A. Observation of high seasonal variation in community structure of denitrifying bacteria in arable soil receiving artificial fertilizer and cattle manure by determining T-RFLP of nir gene fragments. *FEMS Microbiol. Ecol.* **48**, 261–271 (2004).
17. Akiyama, H., McTaggart, I. P., Ball, B. C. & Scott, A. N_2O, NO, and NH_3 emissions from soil after the application of organic fertilizers, urea and water. *Water Air Soil Pollut.* **156**, 113–129 (2004).
18. Wang, Y. Y. *et al.* Responses of N_2O reductase gene (*nosZ*)-denitrifer communities to long-term fertilization follow a depth pattern in calcareous purplish paddy soil. *J. Integr. Agric.* **16**, 2597–2611 (2017).
19. Fernandez-Luqueno, F. *et al.* Emission of CO_2 and N_2O from soil cultivated with common bean (*Phaseolus vulgaris* L.) fertilized with different N sources. *Sci. Total Environ.* **407**, 4289–4296 (2009).
20. Yin, C. *et al.* Different denitrification potential of aquic brown soil in Northeast China under inorganic and organic fertilization accompanied by distinct changes of nirS-and nirK-denitrifying bacterial community. *Eur. J. Soil Biol.* **65**, 47–56 (2014).
21. Harter, J. *et al.* Linking N_2O emissions from biochar-amended soil to the structure and function of the N-cycling microbial community. *ISME J.* **8**, 660–674 (2013).
22. Hai, B. *et al.* Quantification of key genes steering the microbial nitrogen cycle in the rhizosphere of sorghum cultivars in tropical agroecosystems. *Appl. Environ. Microbiol.* **75**, 4993–5000 (2009).
23. Huang, R. *et al.* Nitrous oxide emission and the related denitrifier community: A short-term response to organic manure substituting chemical fertilizer. *Ecotoxicol. Environ. Saf.* **192**, 110291 (2020).
24. Xu, X. *et al.* NosZ clade I rather than clade II determine in situ N_2O emissions with different fertilizer types under simulated climate change and its legacy. *Soil Biol. Biochem.* **150**, 107974 (2020).
25. Henderson, S. L. *et al.* Changes in denitrifier abundance, denitrification gene mRNA levels, nitrous oxide emissions, and denitrification in anoxic soil microcosms amended with glucose and plant residues. *Appl. Environ. Microbiol.* **76**, 2155–2164 (2010).
26. Palmer, K., Biasi, C. & Horn, M. A. Contrasting denitrifier communities relate to contrasting N_2O emission patterns from acidic peat soils in arctic tundra. *ISME J.* **6**, 1058–1077 (2012).
27. Dandie, C. E. *et al.* Abundance, diversity and functional gene expression of denitrifier communities in adjacent riparian and agricultural zones. *FEMS Microbiol. Ecol.* **77**, 69–82 (2011).
28. Avrahami, S., Conrad, R. & Braker, G. Effect of soil ammonium concentration on N_2O release and on the community structure of ammonia oxidizers and denitrifiers. *Appl. Environ. Microbiol.* **68**, 5685–5692 (2002).
29. Yang, Y. J. *et al.* Compost supplementation with nitrogen loss and greenhouse gas emissions during pig manure composting. *Bioresour. Technol.* **297**, 122435 (2019).
30. Yang, Y. J. *et al.* Exploring the microbial mechanisms of organic matter transformation during pig manure composting amended with bean dregs and biochar. *Bioresour. Technol.* **313**, 123647 (2020).
31. Yang, J. H., Wang, C. L. & Dai, H. L. *Agricultural Soil Analysis and Environmental Monitoring* (China Land Press, 2008) **(in Chinese)**.
32. Wang, Q. R., Li, Y. C. & Klassen, W. Changes of soil microbial biomass carbon and nitrogen with cover crops and irrigation in a tomato field. *J. Plant Nutr.* **30**, 623–639 (2007).
33. Moore, J. M., Klose, S. & Tabatabai, M. A. Soil microbial biomass carbon and nitrogen as affected by cropping systems. *Biol. Fertil. Soils* **31**, 200–210 (2000).
34. Jones, D. L. & Willett, V. B. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. *Soil Biol. Biochem.* **38**, 991–999 (2006).
35. Ghani, A., Dexter, M. & Perrott, K. W. Hot-water extractable carbon in soils: A sensitive measurement for determining impacts of fertilization, grazing and cultivation. *Soil Biol. Biochem.* **35**, 1231–1243 (2003).
36. Huang, R. *et al.* Variation in N_2O emission and N_2O related microbial functional genes in straw- and biochar-amended and non-amended soils. *Appl. Soil Ecol.* **137**, 57–68 (2019).
37. Yang, Y. J. *et al.* Soil organic carbon transformation and dynamics of microorganisms under different organic amendments. *Sci. Total Environ.* **750**, 141719 (2021).
38. López-Fernández, S. *et al.* Effects of fertiliser type and the presence or absence of plants on nitrous oxide emissions from irrigated soils. *Nutr. Cycl. Agroecosyst.* **78**, 279–289 (2007).

39. Wallenstein, M. D., Myrold, D. D., Firestone, M. & Voytek, M. Environmental controls on denitrifying communities and denitrification rates: insights from molecular methods. *Ecol. Appl.* **16**, 2143–2152 (2006).
40. Ciarlo, E., Conti, M., Bartoloni, N. & Rubio, G. Soil N₂O emissions and N₂O/(N₂O+N₂) ratio as affected by different fertilization practices and soil moisture. *Biol. Fertil. Soils* **44**, 991–995 (2008).
41. Dandie, C. E. *et al.* Changes in bacterial denitrifier community abundance over time in an agricultural field and their relationship with denitrification activity. *Appl. Environ. Microbiol.* **74**, 5997–6005 (2008).
42. Francis, C. A., O'Mullan, G. D., Cornwell, J. C. & Ward, B. B. Transitions in *nirS*-type denitrifier diversity, community composition, and biogeochemical activity along the Chesapeake Bay estuary. *Front. Microbiol.* **4**, 237 (2013).
43. Lin, W. *et al.* Evaluation of N₂O sources after fertilizers application in vegetable soil by dual isotopocule plots approach. *Environ. Res.* **188**, 109818 (2020).
44. Chen, M. M. *et al. Nitrosospira* cluster 3 lineage of *AOB* and *nir*K of Rhizobiales respectively dominated N₂O emissions from nitrification and denitrification in organic and chemical N fertilizer treated soils. *Ecol. Indic.* **127**, 107722 (2021).
45. Malghani, S., Kim, J., Lee, S. H., Yoo, G. Y. & Kang, H. Application of two contrasting rice-residue-based biochars triggered gaseous loss of nitrogen under denitrification-favoring conditions: a short-term study based on acetylene inhibition technique. *Appl. Soil Ecol.* **127**, 112–119 (2018).
46. Sun, R., Guo, X., Wang, D. & Chu, H. Effects of long-term application of chemical and organic fertilizers on the abundance of microbial communities involved in the nitrogen cycle. *Appl. Soil. Ecol.* **95**, 171–178 (2015).
47. Philippot, L., Andert, J., Jones, C. M., Bru, D. & Hallin, S. Importance of denitrifiers lacking the genes encoding the nitrous oxide reductase for N₂O emissions from soil. *Glob. Change Biol.* **17**, 1497–1504 (2011).
48. Chen, Z. *et al.* Differentiated response of denitrifying communities to fertilization regime in paddy soil. *Microb. Ecol.* **63**, 446–459 (2012).
49. Yoshida, M., Ishii, S., Otsuka, S. & Senoo, K. *nirK*-harboring denitrifiers are more responsive to denitrification-inducing conditions in rice paddy soil than *nirS*-harboring bacteria. *Microbes Environ.* **25**, 45–48 (2010).
50. Yin, C. *et al.* Denitrification potential under different fertilization regimes is closely coupled with changes in the denitrifying community in a black soil. *Appl. Microbiol. Biotechnol.* **99**, 5719–5729 (2015).
51. Barrett, M. *et al.* Carbon amendment and soil depth affect the distribution and abundance of denitrifiers in agricultural soils. *Environ. Sci. Pollut. Res.* **23**, 7899–7910 (2016).
52. Yoshida, M., Ishii, S., Otsuka, S. & Senoo, K. Temporal shifts in diversity and quantity of *nirS* and *nirK* in a rice paddy field soil. *Soil Biol. Biochem.* **41**, 2044–2051 (2009).
53. Kandeler, E., Deiglmayr, K., Tscherko, D., Bru, D. & Philippot, L. Abundance of narG, *nirS*, *nirK*, and *nosZ* genes of denitrifying bacteria during primary successions of a glacier foreland. *Appl. Environ. Microbiol.* **72**, 5957–5962 (2006).

Acknowledgements

Author contributions

Y.Y. wrote the main manuscript text and H.L. prepared Figs. 1, 2 and 3. All authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-022-07753-9.

Correspondence and requests for materials should be addressed to J.L.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.