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Variation in Soil Denitrification among Fertilization Regimes and Its Microbial Mechanism

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ABSTRACT

A 27-year field experiment with various fertilization regimes was chosen for evaluating how fertilization regimes influence soil denitrification potential (SDP), to determine whether the microbial mechanism of SDP variation varies with fertilization regimes. The results showed that the compost (OM) fertilizer treatment presented the highest SDP followed by half compost plus half chemical fertilizer (NPKOM) treatment. Both SDPs were 3–25 times higher than those of the other treatments, and the SDP was higher in the OM than NPKOM, indicating an increase in SDP with the application of compost. The higher SDPs from OM and NPKOM were closely associated with higher abundances of functional genes, and enrichments of *Rubrivivax gelatinosus* and *Azospirillum* sp. TSO28-1, is mainly determined by the organic carbon (SOC), total nitrogen (TN), and dissolved organic carbon (DOC). For the treatments without compost, SDP mainly followed the order of chemical fertilizers of N and P (NP) > chemical fertilizers of N and K (NK) > unfertilized (Nil) > chemical fertilizers of N, P, and K (NPK) > chemical fertilizers of P and K (PK), in which the variations had a close association with different specific microbial species. That is, relative to Nil, the enhanced SDP in NP and NK was coupled with the enrichment of *Ideonella* sp. NC3L-43b and *R. gelatinosus*, likely due to their higher NO_3^- contents, while the reduced SDP in NPK was linked to the depletions of *R. gelatinosus* and *Azospira* sp. NC3H-14, and that in PK to the depletion of *R. gelatinosus* and *Azospirillum* sp. TSO28-1, probably as a result of their lower N:P ratios. Microbial mechanisms for SDP differences varied with fertilization regimes, and *R. gelatinosus* can be regarded as a universal microbial indicator for SDP differences across the fertilization treatments.

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Composition of *nirS*-type denitrifier community; denitrifying functional genes; long-term fertilization; soil denitrification potential

Introduction

The process of soil denitrification (SD) is a significant way in soil N_2O emission (Bremner 1997). Additionally, the process of denitrification leads to N losses from the soil (Mathieu et al. 2006). Under anaerobic conditions with abundant carbon/nitrate substrates, denitrification rates can be defined as the soil denitrification potential (SDP), which is widely used to examine the SD variation under different environmental conditions (Balasubramanian and Kanehiro 1976). During the process of SD, the reduction of NO_3^- or NO_2^- to nitrogen gas (NO, N_2O, N_2) was mainly driven by denitrifying microorganism (Hallin et al. 2009; Stein 2011). Both bacteria and fungi possess the ability of driving SD (Wu et al. 2021), but the denitrifying bacteria are the dominant microorganism (Hallin et al. 2009). However, denitrifying bacteria is not a specific taxonomic group, but a wide range of different phylogenetic species and genera with the capacity of denitrification (Mounier et al. 2004). 16S rRNA gene-based analysis was not suitable for investigating community and diversity of denitrifying bacteria. Thus, functional genes involved in SD can usually be used as biomarkers (Balows et al. 2013). The reduction of NO_2^- to NO catalyzed by cd1-Nir (encoded by *nirS*) or Cu-Nir (encoded by *nirK*) was not only the most important speed-limiting step but also a marker reaction of soil denitrification that differed from other nitrate metabolism (Cutruzzola et al. 2001; Henry et al. 2004; Sun et al. 2015). Therefore, *nirS* or *nirK* gene was commonly used as a marker molecular to detect denitrifier communities. The *nirS*-type denitrifiers were more widely distributed than the *nirK*-type denitrifiers in the environment (Braker et al. 1998), additionally, the *nirS*-type denitrifiers were more active than the *nirK*-type denitrifiers under anaerobic condition (Yuan et al. 2012). The *nirS*-type denitrifiers have been usually employed to study the abundance, diversity and distribution of soil denitrifiers under different management conditions (Zheng et al. 2015).

Fertilization can increase the quality and quantity of crop production by changing soil properties, which might be connected with denitrifying bacteria (Bassouny and Chen 2016;

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Cui et al. 2016; Motavalli 2012; Yu et al. 2018). The response of SDP to fertilization in black soil was closely related to *nirS*-type denitrifier community structure, influenced by the variation in soil properties, such as pH, total nitrogen, organic matter, and the C:P ratio (Yin et al. 2015). Organic fertilization could provide a sufficient carbon source and electronic donor for denitrifiers (Aljarallah 2002) and change *nirS*-type denitrifiers' community structure (Yin et al. 2014), further affecting the nitrogen transformation process. In addition, Yin et al. (2014) also found that the application of chemical fertilizers decreased SDP, accompanied by a 39.0% reduction in *nirS*-type denitrifier abundance. The 'sustainable fertilization strategy' is usually concurrent fertilization with nitrogen, phosphorus, and potassium, while fertilization without one of the nutrient elements has widely been used (Zhao et al. 2010). Long-term nutrient deficiency results in variations in the microbial biomass, function and composition of the microbial community (Geisseler and Scow 2014; Ma et al. 2019). Ma et al. (2019) demonstrated that the bacterial community structure and composition significantly changed under N deficiency conditions in a lime concreted black soil. The available K content is an important factor influencing the *nirK*-type denitrifying bacterial community in upland soil. Phosphate (P) application was favorable for stimulating denitrifier growth in a P-limited paddy soil by regulating microbial gene responses, resulting in the enhancement of gaseous N loss potential (Wei et al. 2017). Likewise, P addition could stimulate N_2O and NO emissions from *Acacia mangium* plantations under relatively wet conditions (Mori et al. 2010). However, Shi et al. (2012) suggested that the application of P fertilization has no connection with the soil microbial community. To accurately understand the soil N cycle, it is necessary to establish the response of soil denitrification to the fertilization regime.

Fifty-four percent of the Chinese cropland N_2O emissions come from the North China Plain (Gao et al. 2011). Ding et al. (2013) found that the application of compost alone or chemical fertilizers significantly increased background N_2O emissions in a Calcaric Fluvisol, one of the typical soils in the North China Plain. However, systematic comparisons of the involved microbial mechanisms among various fertilization regimes have rarely been reported in the region, which will obstruct us from continuing the stabilization between ecology and crop productivity. Therefore, a long-term fertilization experiment located in the North China Plain was chosen. The SDP, functional genes and composition of the *nirS*-type denitrifier community were measured to (1) study the variations in SDP under different long-term fertilization regimes, (2) determine changes in denitrifying functional genes and the composition of the *nirS*-type denitrifier community as influenced by fertilization regimes, and (3) explore the associated microbial mechanisms.

Materials and methods

Long-term experimental field site and soil sampling

The long-term fertilization experiment started in October 1989; this study was conducted at a site in Fengqiu County, Henan Province (114° 24′ E, 35° 00′ N). The soil was classified as a Calcaric Fluvisol according to the FAO, and derived from the alluvial sediments of the Yellow River with a texture of sandy loam (Li et al. 2018). Before the long-term fertilization experiment, no fertilizer was applied from 1987 to 1989 to obtain a uniform field site. The basic soil properties are shown in Table S1. Winter wheat (*Triticum aestivum* L.) was grown from October to May and summer maize (*Zea mays* L.) from June to September in wheat-corn crop rotation system.

Seven treatments with four replications were included: compost (OM), half compost plus half chemical fertilizers (NPKOM), chemical fertilizers of N and P (NP), chemical fertilizers of N and K (NK), chemical fertilizers of P and K (PK), and unfertilized treatment (Nil). Each replicate plot was 9.5 m × 5 m and separated by cement banks.

The application of chemical fertilizer referred to standard practices of local farmers. The fertilizers for N, P, and K were 150, 32.7, and 124.5 kg ha^{-1} for winter wheat, while 150, 26.2, and 124.5 kg ha^{-1} for summer wheat, respectively. Wheat straw mixed with soybean cake and cotton seed cake was used as the compost (417 g C kg^{-1}, 23.6 g N kg^{-1}, 3.8 g P kg^{-1}, and 5.9 g K kg^{-1}). Half of the compost was applied for the NPKOM. For OM and NPKOM treatments, chemical fertilizers were added to obtain the same quantity of N, P, and K supplied in NPK. Organic compost, P, and K were applied as basal fertilizer. For winter wheat, 60% of N was used as basal fertilizer and 40% of N as topdressing, while for summer maize, 40% of N and 60% of N. Detailed information was shown in Table S2.

Random subsamples were collected at the depth of 0–20 cm. For each plot, a mixture sample was made by mixing the five random samples, then sieved to remove visible plant residues and stones. About 100 g of fresh samples were stored at −80 °C to determine denitrifying functional genes and denitrifying microorganisms, and 50 g of fresh samples were stored at 4 °C for the determination of NO_3^--N, NH_4^+-N, and DOC. The remaining was air-dried and ground to pass a 100-mesh sieve.

Determination of physical and chemical analysis

Soil pH was determined at soil/deionized water ratio of 1:5 (w/v) by pH meter (FE20, Mettler Toledo, Germany). Total nitrogen (TN) was measured by Kjeldahl method and soil organic carbon (SOC) by dichromate oxidation (Page et al. 1982). DOC was extracted at soil/deionized water ratio of 1:5 (w/v) and measured by TOC analyzer (Multi N/C 3100 TOC/TN, Jena, Germany). Total phosphorus (TP) and total potassium (TK)was extracted by HF-$HClO_4$ and measured by molybdenum-blue method and flame photometer (FP640, Huayan, Shanghai, China), respectively (Olsen 1954). Available phosphorus (AP) was extracted with 0.5 mol/l $NaHCO_3$ and measured with the molybdenum blue method (Olsen 1954). Available potassium (AK) was extracted with CH_3COONH_4 and measured by flame photometer (Page et al. 1982). NO_3^--N and NH_4^+-N were extracted by 2 mol/l

KCl and determined with a flow injection autoanalyzer (Page et al. 1982).

Anaerobic incubation and measurement of SDP

The acetylene (C_2H_2) inhibition technique is commonly used to measure the SDP (Li et al. 2020; Song et al. 2017). During the whole incubation period, the N_2O emission rate per hour was used to express the SDP (Zhang et al. 2009). Soil samples were maintained in 40% content of water-filled pore space (WFPS) and pre-incubated for 7 days under 25 °C and dark conditions. A detailed incubation experiment was carried out according to Wang et al. (2020). Briefly, pre-incubated soil (equivalent to 6 g dry weight), 6 mg C (CH_3COONa) and 1.2 mg N (KNO_3) were added into a 120-ml of serum culture flask and the sample was adjusted to 70% WFPS. The flasks were vacuumed and flashed with pure argon three times. Then the flasks were vacuumed and filled with 108 ml pure argon and 12 ml (10% v/v) acetylene. The flasks were shaken at 180 rpm and 25 °C for 15 minutes. Under 25 °C and dark conditions, soil incubation was carried out for 24 h. Gas samples were collected at 6, 12, 18, and 24 h, respectively. After collecting gas samples, the flasks were vacuumed and flashed three times again. Gas samples were determined with gas chromatograph (Shimadzu GC-14B, Kyoto, Japan).

DNA extraction and quantitative real-time PCR (qPCR)

DNA was extracted using the Fast®DNA spin kit for soil (MP Biomedicals, Santa Ana, CA). Three successive extractions were mixed in order to reduce the bias in DNA extraction (Feinstein et al. 2009). Quantity and quality of the extracted DNA were checked with NanoDrop spectrophotometer (Nanodrop Technologies, Wilmington, DE). Before further assays, extracted DNA was stored at −80 °C.

Functional genes (*narG*, *nirS*, *nirK*, and *nosZ*) were measured by the qPCR (Bio-Rad Laboratories, Hercules, CA). To enable the comparison of functional genes, the amplification efficiencies were 90.4–93.1%, 86.0–87.8%, 84.1–92.8%, and 89.8–89.0% for *narG*, *nirK*, *nirS*, and *nosZ* genes, respectively. The primers were 1960 m2f/2050 m2r, nirK1F/nirK5R, cd3aF/R3cd, and nosLb/nosRb for *narG*, *nirK*, *nirS*, and *nosZ* genes, respectively. Standard curves were made with serial dilutions of plasmid. Reaction mixtures and amplification conditions of each genes are consistent with Wang et al. (2020).

Illumina MiSeq sequencing and analyses

The composition of the *nirS*-type denitrifier was evaluated by the Illumina MiSeq platform (Illumina, San Diego, CA, USA). PCR reactions were carried out in a 25 µl reaction solution consisting of 5 µl of 5 × reaction buffer, 1 µl of forward/reverse primer (10 mmol/l), 5 µl of 5 × GC buffer, 2 µl of DNA (20 ng/µl) template, 0.25 µl of Q5 DNA Polymerase (M0491L, NEB), 2 µl of dNTP (2.5 mmol/l), and 8.75 µl of ddH_2O. The PCR amplifications were performed according to Wang et al. (2020). Products of PCR were purified using Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN) and quantified by PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After the individual quantification step, the amplicons were pooled in equal amounts, and paired-end 2 × 300 bp sequencing was carried out with MiSeq Reagent Kit v3 (Shanghai Personal Biotechnology Co., Ltd).

Barcodes and low quality sequences were eliminated from the raw pyrosequencing by QIIME (Caporaso et al. 2010). Paired-end reads were established with FLASH (Magoc and Salzberg 2011). Operational taxonomic units (OTUs) at 97% identity were gathered based on UCLUST (Edgar 2010). In order to get the similar published sequences, OTUs were analyzed using BLAST with NCBI database (Gu et al. 2019). Raw sequence data have been submitted to NCBI database with the BioProject accession number PRJNA647978.

Date analysis

Statistical analysis was carried out using SPSS 20 and R 3.6.1. Differences in soil properties, SDP and functional genes between different treatments were assessed using ANOVA analysis. The relationships between SDP and soil properties and functional genes were tested by spearman correlation. While the relationships between *nirS*-type denitrifier bacterial community and soil properties and SDP were identified by redundancy analysis (RDA). Monte Carlo permutations with 1000 times were used to assess the correlation between *nirS*-type denitrifier communities and soil properties. The significant changed OTUs between Nil and NPKOM, OM, NPK, NP, NK and PK were identified using Volcano plot (R package 'DESeq2') (Edwards et al. 2015). The importance of soil properties was conducted by Stepwise multiple regression (Tang et al. 2019).

Results

Soil physicochemical properties

The influences of fertilization regimes on soil properties are shown in Table 1. Compared with the other treatments, the Nil, NK, and PK treatments had higher pH values ($p < 0.05$, Pearson correlation test). The SOC, TN, and DOC ranged from 4.14 to 11.61 g kg^{-1}, 0.46 to 1.37 g kg^{-1}, and 19.21 to 60.05 mg kg^{-1}, respectively, and they usually followed a similar order of OM > NPKOM > NPK, NP > PK > NK, Nil. The highest NO_3^- content was in the NK and NP, while the lowest NO_3^- content was in the PK and unfertilized Nil. For the TP and AP contents, the highest values in the PK were observed, while the highest AK content value was in the NK. The lowest N:P ratio in the PK was observed, followed by NPKOM, OM, NPK, NP, Nil and NK. In contrast to the N:P ratio, the NK treatment had the lowest C:N ratio.

Table 1. Effect of 27-year fertilizer treatment on soil physicochemical properties.

	Nil	NK	PK	NP	NPK	NPKOM	OM
pH	8.58 ± 0.11a	8.20 ± 0.07bc	8.29 ± 0.09b	8.03 ± 0.07d	8.07 ± 0.04 cd	8.04 ± 0.05d	8.03 ± 0.10d
SOC (g kg^{-1})	4.17 ± 0.14f	4.14 ± 0.24f	5.26 ± 0.03e	6.11 ± 0.11d	6.44 ± 0.26c	9.36 ± 0.32b	11.61 ± 0.39a
TN (g kg^{-1})	0.46 ± 0.01e	0.52 ± 0.01de	0.59 ± 0.01d	0.77 ± 0.08c	0.74 ± 0.04c	1.05 ± 0.07b	1.37 ± 0.08a
TP (g kg^{-1})	0.52 ± 0.04e	0.50 ± 0.02e	1.11 ± 0.02a	0.90 ± 0.03b	0.87 ± 0.01bc	0.82 ± 0.03 cd	0.78 ± 0.01d
TK (g kg-1)	19.28 ± 0.42b	19.95 ± 0.60ab	20.48 ± 0.15a	19.52 ± 0.29b	19.58 ± 0.77b	19.86 ± 0.23ab	19.5 ± 0.13b
NO_3^-(mg kg^{-1})	8.61 ± 1.10d	70.93 ± 5.07a	7.29 ± 1.50d	62.46 ± 6.32a	50.25 ± 5.08b	34.19 ± 1.65c	42.36 ± 4.65b
NH_4^+ (mg kg^{-1})	1.67 ± 0.04a	0.33 ± 0.02d	1.50 ± 0.13b	0.65 ± 0.04c	0.33 ± 0.03d	0.43 ± 0.08d	0.46 ± 0.10d
AP (mg kg^{-1})	1.57 ± 0.22d	2.79 ± 0.19d	26.61 ± 4.57a	15.51 ± 3.44c	15.59 ± 0.79c	18.64 ± 0.32bc	20.27 ± 0.08b
AK (mg kg^{-1})	65.76 ± 5.24d	448.01 ± 35.45a	378.83 ± 12.25b	54.64 ± 0.02d	218.35 ± 6.73c	224.35 ± 17.24c	248.05 ± 11.16c
DOC (mg kg^{-1})	19.62 ± 0.11 cd	19.21 ± 1.18d	20.66 ± 1.37 cd	22.14 ± 0.60 cd	24.01 ± 3.07c	42.08 ± 1.45b	60.05 ± 4.76a
C:N	8.94 ± 0.33a	7.84 ± 0.19c	8.81 ± 0.26a	8.06 ± 0.62bc	8.56 ± 0.19a	8.94 ± 0.21a	8.50 ± 0.17ab
N:P	5.84 ± 0.98b	24.95 ± 3.24a	0.28 ± 0.07e	4.64 ± 1.33bc	3.31 ± 0.47 cd	1.72 ± 0.31de	2.07 ± 0.20de

Values are mean ± standard deviations ($n = 4$). Different letters in a same row indicate significant difference between fertilizer treatments ($p < .05$, Duncan's multiple range test). SOC: Soil organic carbon; TN: total N; TP: total P; TK: total P; AP: available P; AK: available K; DOC: dissolved organic C; C:N: SOC:TN ratio; N:P: NO_3^-:AP ratio.

Soil denitrification potential

As shown in Figure 1, the SDPs in the treatments with compost application (NPKOM and OM) were 3.28–24.64 times higher than those in the treatments without compost application (Nil, NK, PK, NP, and NPK). In addition, the SDP in OM was significantly higher than that in NPKOM (959 *vs.* 734 μg N_2O kg^{-1} dry soil h^{-1}) ($p < 0.05$, Pearson correlation test).

The SDPs in those treatments without considering OM and NPKOM were significantly different ($p < 0.05$, Pearson correlation test), which followed the order of NP > NK > Nil > NPK > PK. Compared with the Nil treatment, significantly higher SDPs were detected in the NP and NK treatments ($p < 0.05$, Pearson correlation test), while the SDP in the NPK and PK treatments significantly decreased by 28% and 55%, respectively.

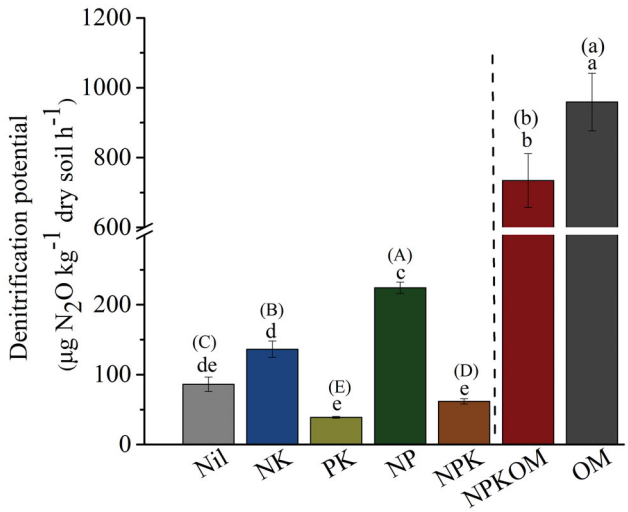

Figure 1. Effect of 27-year fertilizer treatment on soil denitrification potential. Different lowercase letters indicate significant differences among the seven fertilization treatments, and different uppercase letters in brackets indicate significant differences among the five fertilization regimes that contained no compost (i.e., Nil, NK, PK, NP, and NPK treatments) ($p < .05$, Duncan's multiple range test) or difference between the OM and NPKOM treatment (Independent sample T test).

Functional genes involved in SDP

The highest functional gene abundances of *narG*, *nirS*, *nirK* and *nosZ* were in the OM, and the lowest functional gene abundances were in the Nil (Figure 2). The abundances from the treatments with compost application (i.e., OM and NPKOM) were approximately 1.25–6.25 times higher than those without compost application (i.e., Nil, NK, PK, NP, and NPK).

For those treatments without compost, the functional gene abundances in the NPK were significantly higher than those in the NP, PK, NK, and Nil ($p < 0.05$, Pearson correlation test). Abundances of *narG*, *nirS* and *nirK* followed the order of NPK > NP > PK > NK > Nil, while the *nosZ* gene followed the order of NPK > NP > PK > Nil > NK.

Composition of the nirS–type denitrifier community and its determinants

A total of 326,116 high-quality sequences obtained were clustered into 1598 OTUs at 97% identity. The *nirS*-type denitrifier was mainly from the phylum *Proteobacteria* (93.94%). The major *nirS*-type denitrifier genera across the seven fertilizer treatments were *Rubrivivax* (15.8–41.5%), *Azospirillum* (15.8–19.3%), *Cupriavidus* (5.4–21.1%), *Ideonella* (2.5–17.9%), *Azoarcus* (2.8–9.5%), *Azospira* (2.1–4.1%), *Sulfuritalea* (2.2–3.6%), *Dinoroseobacter* (0.7–5.4%), *Bradyrhizobium* (0.9–2.3%), and *Aromatoleum* (0.4–2.1%) (Figure S1 and Table S3).

PERMANOVA indicated that the composition of the *nirS*-type denitrifier community under the various fertilization regimes substantially differed from that under the Nil treatment (Table 2). Moreover, there was a significant difference in the compost treatments (NPKOM and OM) and those without compost application (Nil, NK, PK, NP, and NPK).

A volcano plot was employed to visualize the variation in *nirS*-type denitrifiers that significantly differed between treatments (Figure S2). Compared with the Nil, the variation in the composition of the *nirS*-type denitrifier community in the NP and NK treatments was mainly reflected by the enrichment of *Rubrivivax gelatinosus* and *Ideonella* sp. NC3L-43b; in the PK treatment was ascribed to the depletion of *R. gelatinosus* and *Azospirillum* sp. TSO28-1; and in the NPK was attributed to the depletion of *R. gelatinosus* and *Azospira* sp. NC3H-14 (Figure S2a–d and Table S4). Meanwhile, the differences between the treatments with

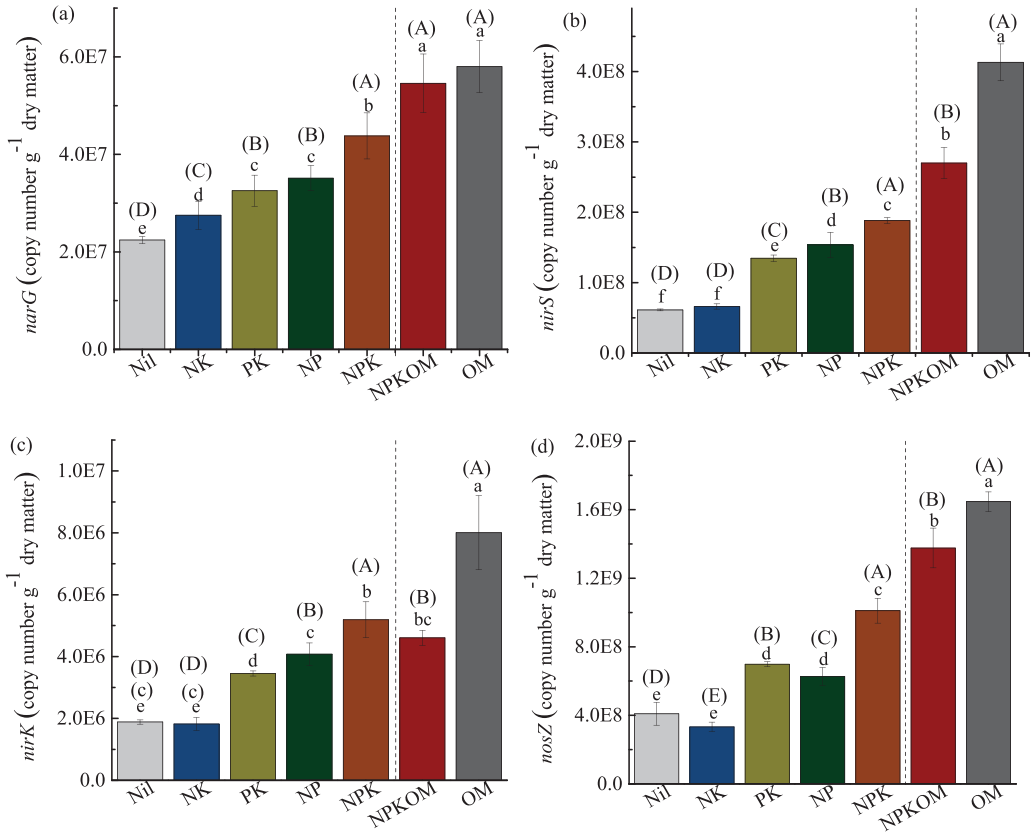

Figure 2. Abundances of *narG* (a), *nirS* (b), *nirK* (c), and *nosZ* (d) genes as influenced by fertilizer treatments. Different lowercase letters indicate significant differences among the seven fertilization treatments ($p < .05$, Duncan's multiple range test), and different uppercase letters in brackets indicate significant differences among the five fertilization regimes that contained no compost (i.e., Nil, NK, PK, NP, and NPK treatments) ($p < .05$, Duncan's multiple range test) or difference between the OM and NPKOM treatment (Independent sample T test).

Table 2. PERMANOVA analysis revealing difference in the *nirS*-type denitrifier community between treatments.

Treatments			
Group A	Group B	*F*	*p*
NK	Nil	13.49	0.029
PK	Nil	2.68	0.032
NP	Nil	5.56	0.040
NPK	Nil	5.28	0.024
NPKOM	Nil	12.70	0.027
OM	Nil	21.49	0.023
OM + NPKOM	Nil + NK + PK + NP + NPK	16.81	0.032

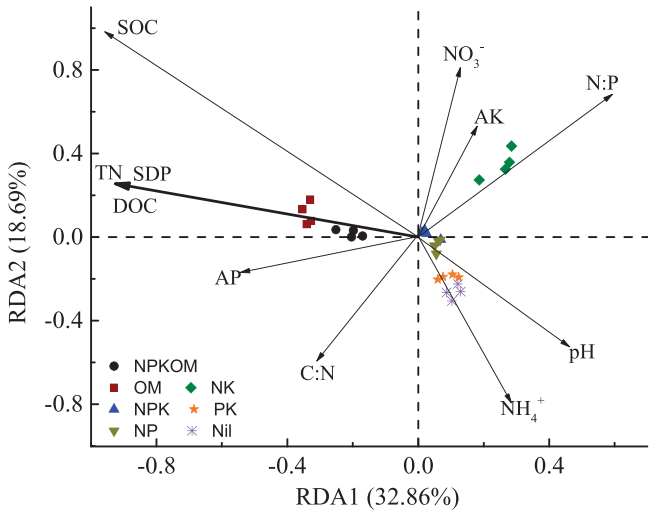

Figure 3. Redundancy analysis (RDA) correlation plot showing variance in *nirS*-type denitrifier community composition explained by SDP and soil physicochemical properties.

compost application (i.e., NPKOM and OM) and those without compost application (i.e., Nil, NK, PK, NP, and NPK) could be assigned to the enriched *R. gelatinosus* and *Azospirillum* sp. TSO28-1 species in the NPKOM and OM treatments (Figure S2 and Table S4), and the abundances of these enriched species were usually higher in the OM treatment than in the NPKOM treatment (Figure S2e–f). Spearman rank correlation analyses further showed positive associations between these significantly changed *nirS*-type denitrifier species and SDP ($p < 0.05$) (Table S5).

The Monte Carlo permutation test suggested that the composition of the *nirS*-type denitrifier community was closely related to the SDP and soil properties (pH, SOC, TN, NO_3^-, NH_4^+, AP, AK, DOC, C:N ratio, and N:P ratio) (Table S6). Over 76.5% of the variation in the composition of *nirS*-type denitrifier community can be explained by the selected parameters, 51.6% of which is explained by RDA1and RDA2. Figure 3 further demonstrates that the SOC, TN, DOC, the N:P ratio and NO_3^- might have been mainly associated with the variance in the composition of the *nirS*-type denitrifier community, as important variables

are represented by longer arrows, agreeing with the higher correlation coefficients shown in Table S6.

Discussion

Variations in the SDP between the treatments with and without compost application

Organic compost increased SDP, which increased with the application rate of organic compost (Figure 1), which is in agreement with Yin et al. (2014) and Cui et al. (2016). N_2O emissions can be influenced by the *narG*, *nirS*, *nirK* and *nosZ* genes (Chen et al. 2012; Liu et al. 2012). The OM and NPKOM treatments had higher abundances of *narG*, *nirS*, *nirK*, and *nosZ* genes than those treatments receiving no compost, and the gene abundances were higher in the OM treatment than in the NPKOM treatment (Figure 2), indicating that functional genes involved in denitrification can be stimulated by compost application, and the stimulation increased with compost application rate. It thus can be inferred that compost-induced functional genes likely contributed to the enhanced SDP observed in the treatments with compost application (Figures 1 and 2). The application of organic manure alters soil properties, thus affecting functional genes (Cui et al. 2016; Kandeler et al. 2006; Tatti et al. 2013). For example, the soil TN significantly correlated with *nirS* and *nirK* abundances (Ellen et al. 2009; Levy-Booth and Winder 2010). SOM is considered as an important factor influencing *nirK*, *nirS*, and *nosZ* abundance (Petersen et al. 2012), and DOC shows a positive correlation with the *nirK*/*nirS* ratio (Bárta et al. 2010). The ratio of *nirS* + *nirK* to *nosZ* (0.23) in treatments with compost was higher than those without (0.20). These results suggested that compost could enhance the SDP in the OM and NPKOM by increasing the ratio of (*nirS* + *nirK*) to *nosZ*. The contents of SOC, DOC, and TN in the OM and NPKOM were greater than those without compost application (Table 1), suggesting that these changed soil properties may attributed to the enhanced functional gene abundance observed in the OM and NPKOM treatments.

The variation in SDP showed a significant association with the composition of the *nirS*-type denitrifier community (Figure 3), which agreed with previous studies (Cuhel et al. 2010; Yin et al. 2014). Cui et al. (2016) reported that an increase in N_2O emissions induced by organic manure was mainly attributed to the structure of *nirS*-denitrifying bacteria in black soil. The application of organic compost increased denitrifier abundance (Duan et al. 2018) and shifted the composition of the denitrifier community by increasing C availability (Hou et al. 2018). The variation in the composition of the *nirS*-type denitrifier community between the treatments with compost application (i.e., OM and NPKOM) and those without (i.e., Nil, NK, PK, NP, and NPK) was primarily reflected by the enrichment of *R. gelatinosus* and *Azospirillum* sp. TSO28-1 species in the OM and NPKOM treatments (Figure S2g, 4 and Table S4), suggesting that the enhanced SDP from the OM and NPKOM treatments may mainly be associated with the enrichment of *R. gelatinosus* and *Azospirillum* sp. TSO28-1. *Rubrivivax gelatinosus* can use various organic substrates as sources of carbon and energy (Kantachote et al. 2005), reduce nitrite to N_2, and enhance denitrification under anaerobic conditions (Nagashima et al. 2011; Zhang et al. 2016). *Azospirillum* is able to reduce nitrate as a denitrifier in addition to being a nitrogen fixer (Danneberg et al. 1986). Previously, Abdullahi et al. (2013) reported that poultry manure could be a source of organic matter for *Azospirillum* growth; therefore, SOC, TN, and DOC were important variables for regulating the composition of *nirS*-type denitrifier community (Table A6 and Figure 4), which were higher in OM and NPKOM than in those without compost application (Table 1), suggesting that soil properties would be helpful to improve abundance of *R. gelatinosus*.

Variations in SDP among the treatments without compost

The order of SDP in those treatments without compost application were NP > NK > Nil > NPK > PK (Figure 1), with the highest abundances of functional genes in the NPK, and the lowest in the NK and Nil (Figure 2), indicating that there was no association between the SDP and the abundances of the four measured functional genes.

The *nirS*-type denitrifier community composition of each fertilizer treatment significantly differed from the Nil (Table 2), consisting with some previous reports showing that mineral fertilizers considerably affect the composition of denitrifier community, as denitrifier community was sensitive to environmental disturbances (Azziz et al. 2017; Dang et al. 2009; Hu et al. 2020). However, the shift direction of the composition varied with specific fertilizer treatment (Figure S2; Table S4). NO_3^- content was regarded as an important factor regulating denitrifying bacterial communities (Liang and MacKenzie 1997; Xu and Cai 2007) since NO_3^- was the reaction substrate for denitrification (Francis et al. 2013). This suggested that higher NO_3^- content observed in the NP and NK treatments largely contributed to the shift in the *nirS*-type denitrifier community composition (Tables 1, 2, and S4). In addition, the highest AK content in NK may further regulate the composition of *nirS*-type denitrifier community. Xue et al. (2013) also demonstrated that there was a significant correlation between denitrifier community composition and AK content. As for the PK and NPK treatments, the shifts in the *nirS*-type denitrifier community might associate with their lower N:P ratio (Table 1). The variation in N:P ratio was significantly associated with denitrifier community composition (Wei et al. 2017; Zhang et al. 2018), altering the gaseous N loss potential (Wei et al. 2017), as P additions can lead to the change in N cycling (Tang et al. 2016), via largely altering the abundances and community structures of denitrifiers (He and Dijkstra 2015; Mori et al. 2010).

In conclusion, the treatments with long-term compost application (OM and NPKOM) had higher SDP than those without, which is regulated by the enhanced abundance of functional genes and the enrichments of *R. gelatinosus* and *Azospirillum* sp. TSO28-1. For those treatments without

compost application, the variations in SDP showed no correlation with functional genes, while had close association with the *nirS*-type denitrifier community composition to different directions. That is, relative to the Nil, the induced SDP from the NP and NK treatments that was largely ascribed to the enrichments of *R. gelatinosus* and *Ideonella* sp. NC3L-43b, and the reduced SDP from the NPK treatment was attributed to the depletions of *R. gelatinosus* and *Azospira* sp. NC3H-14 and that from the PK treatment was attributed to the depletions of *R. gelatinosus* and *Azospirillum* sp. TSO28-1. Combined with the important contribution of *R. gelatinosus* to the *nirS*-type denitrifier community composition, *R. gelatinosus* can be used as an indicator microbial for the SDP variation across the fertilization regimes in a Calcaric Fluvisol.

Conclusions

Compared with the fertilization treatments without organic compost, the higher SDPs in the OM and NPKOM treatments were associated with functional genes and the composition of the *nirS*-type denitrifier community, adjusted by the SOC, TN, and DOC contents. In addition, enhanced abundances of *R. gelatinosus* and *Azospirillum* sp. TSO28-1 in the OM and NPKOM were the main determinants of the composition of the *nirS*-type denitrifier community.

Related to the Nil, the composition of *nirS*-type denitrifier contributed to the enhanced SDPs in the NP and NK treatments, which is mainly controlled by NO_3^- content. The variation in the composition of the *nirS*-type denitrifier community in the NP and NK treatments could be mainly ascribed to *R. gelatinosus* and *Ideonella* sp. NC3L-43b. The lower SDPs in the NPK and PK treatments were induced by the variation in the composition of *nirS*-type denitrifiers by the depletion of *nirS*-type denitrifiers (*R. gelatinosus* and *Azospira* sp. NC3H-14 for NPK treatment; *R. gelatinosus* and *Azospirillum* sp. TSO28-1 for PK treatment), primarily regulated by the N:P ratio.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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