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Decreased nitrous oxide emissions associated with functional microbial genes under bio-organic fertilizer application in vegetable fields

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ABSTRACT

Bio-organic fertilizers enriched with plant growth-promoting microbes (PGPMs) have been widely used in crop fields to promote plant growth and maintain soil microbiome functions. However, their potential effects on N_2O emissions are of increasing concern. In this study, an *in situ* measurement experiment was conducted to investigate the effect of organic fertilizer containing *Trichoderma guizhouense* (a plant growth-promoting fungus) on soil N_2O emissions from a greenhouse vegetable field. The following four treatments were used: no fertilizer (control), chemical fertilizer (NPK), organic fertilizer derived from cattle manure (O), and organic fertilizer containing *T. guizhouense* (O+T, referring to bio-organic fertilizer). The abundances of soil N cycling-related functional genes (*amoA*) from ammonium-oxidizing bacteria (AOB) and archaea (AOA), as well as *nirS*, *nirK,* and *nosZ*, were simultaneously determined using quantitative PCR (qPCR). Compared to the NPK plot, seasonal total N_2O emissions decreased by 11.7% and 18.7% in the O and O+T plots, respectively, which was attributed to lower NH_4^+-N content and AOB *amoA* abundance in the O and O+T plots. The *nosZ* abundance was significantly greater in the O+T plot, whilst the AOB *amoA* abundance was significantly lower in the O+T plot than in the O plot. Relative to the organic fertilizer, bio-organic fertilizer application tended to decrease N_2O emissions by 7.9% and enhanced vegetable yield, resulting in a significant decrease in yield-scaled N_2O emissions. Overall, the results of this study suggested that, compared to organic and chemical fertilizers, bio-organic fertilizers containing PGPMs could benefit crop yield and mitigate N_2O emissions in vegetable fields.

Key Words: chemical fertilizer, gene abundance, greenhouse vegetable, N cycle-related genes, plant growth-promoting microbe, *Trichoderma guizhouense*, yield-scaled N_2O emission

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INTRODUCTION

Nitrous oxide (N_2O) is a potent greenhouse gas that has a 298-fold greater warming potential than carbon dioxide (CO_2), and is involved in the depletion of stratospheric ozone (Ravishankara *et al*., 2009). The concentration of N_2O in the atmosphere reached 330 μg L^{-1} in 2017 and increased at a rate of 0.80 μg L^{-1} $year^{-1}$ (NOAA, 2018). Agriculture contributes to nearly 80% of the global anthropogenic N_2O emissions, making agriculture the largest anthropogenic source of N_2O (UNEP, 2019). The N_2O emissions from vegetable fields account for 9% of the total anthropogenic emissions (Rezaei *et al*., 2015).

China is one of the largest vegetable producers in the world. The vegetable harvest area in China reached 23.9 million hectares in 2017, accounting for 41% of the world's total vegetable harvest area (FAO, 2018). In China, vegetable cultivation under simple plastic solar greenhouse conditions totaled 4.7 million hectares (Fei *et al*., 2018). The greenhouse vegetable cropping systems in China are typically characterized by intensive cropping rotation and frequent irrigation. In particular, fertilizers are applied at a rate of 1 000–2 000 kg N ha^{-1} $year^{-1}$ for greenhouse vegetables, which is much more than for non-vegetable crops (Xiong *et al*., 2006; Yao *et al*., 2019). As a result, greenhouse vegetable cropping systems are highly vulnerable to N loss, with N_2O emission as high as 31.1–60.5 kg N ha^{-1} $year^{-1}$ (Yao *et al*., 2019). Therefore, optimizing fertilizer management, such as by replacing chemical fertilizers with organic fertilizers, has been suggested as a method to reduce fertilizer-induced N_2O emissions from greenhouse vegetable fields in China.

Fertilizer application can affect soil physicochemical properties and functional microbial genes that are involved in N_2O production through nitrification and denitrification (Sun *et al*., 2015). Nitrification is the process of oxidizing

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ammonia to nitrite, with N_2O being the byproduct of the first step in the cycle, which is performed by ammonium oxidizing bacteria (AOB) and archaea (AOA) (Taylor *et al*., 2012). Denitrification is the stepwise process of sequentially reducing NO_3^-/NO_2^- to N_2. The four steps involved are sequentially catalyzed by nitrate reductase (encoded by *narG*), nitrite reductase (encoded by *nirK*/*nirS*), nitric oxide reductase (encoded by *norB*), and nitrous oxide reductase (encoded by *nosZ*). N_2O is the final product or is an intermediate of the denitrification cascade (Canfield *et al*., 2010). It is well-documented that these functional genes respond differently to chemical and organic fertilizers (Sun *et al*., 2015). For instance, meta-analysis has revealed that AOB populations have stronger responses to chemical N fertilizers relative to AOA populations (Carey *et al*., 2016), while organic fertilizers have positive impacts on soil functional microbial growth (Pereg *et al*., 2018).

Recently, several mitigation strategies have been proven effective at mitigating N_2O emissions from vegetable fields through regulating microbial-related N_2O production or reduction pathways. For example, biochar application can facilitate N_2O consumption by increasing the population and transcription of the *nosZ* gene (Xu *et al*., 2014); while nitrification inhibitors are able to constrain N_2O production by inhibiting autotrophic ammonia-oxidizing bacteria (Chen *et al*., 2015). Although these synthetic methods could effectively mitigate N_2O emissions, high costs and the potential for environmental contamination still limit their widespread application and development (Lam *et al*., 2017). Therefore, the development of ecofriendly approaches is anticipated to mitigate N_2O emissions from vegetable fields.

Plant growth-promoting microbes (PGPMs) are a type of free-living microorganism in soils (Vacheron *et al*., 2013), which have been increasingly used to improve plant growth and N use efficiency in agriculture (Pang *et al*., 2017; Fiorentino *et al*., 2018). Furthermore, recent experiments have found that inoculation with PGPMs such as *Bacillus amyloliquefaciens* or *Trichoderma viride* significantly reduced fertilizer N-induced N_2O emissions from acid tea fields (Wu *et al*., 2018; Xu *et al*., 2018). Unfortunately, these selected plant-beneficial strains often suffer from low survival rates in soils (Saravanan *et al*., 2003). Instead, commercial bio-organic fertilizers, which combine the advantages of beneficial microbial inoculants and organic substrate, were found to be more beneficial to plant growth (Pang *et al*., 2017; Rodrigues *et al*., 2018). Although the widespread use of bio-organic fertilizers has been approved to promote vegetable growth and maintain diverse soil microbiome functions (Zhang *et al*., 2018), their potential roles in mitigating N_2O emissions from greenhouse vegetable fields have yet to be addressed. Here, we conducted a field experiment to measure N_2O fluxes from greenhouse vegetable cropping systems in Southeast China. First, we predicted that seasonal N_2O emissions were lower in organic and bio-organic fertilizer plots than in chemical fertilizer plot. Second, we also revealed that, relative to chemical and organic fertilizers, bio-organic fertilizers containing *T. guizhouense* would produce higher vegetable yields and lower yield-scaled N_2O emissions. Finally, we proposed that the addition of *T. guizhouense* would decrease AOB *amoA* gene abundance but increase *nosZ* gene abundance. The objective of this study was to gain an insight into the potential role of bio-organic fertilizers containing *T. guizhouense* in mitigating N_2O emissions from vegetable cropping systems. We also attempted to explore the associated mechanisms by linking N_2O emissions to key soil biogeochemical factors and functional microbial genes.

MATERIALS AND METHODS

Study site description

The field experiment was carried out at an experimental site of Nanjing Agricultural University, Nanjing, Jiangsu Province, China (31°95′ N, 118°83′ E). The region possesses a subtropical monsoon climate, with cool winters and warm summers. The study was conducted from August to October 2015, during which total precipitation was 292.0 mm and an average air temperature was 22.9 °C. The field site was overwhelmingly dominated by polyethylene plastic cropping fields, in which there was no extra heating or lighting. The soil at the experimental site was classified as a yellow brown soil, consisting of 50.17% clay, 31.15% sand, and 17.68% silt. The initial properties of the surface soil (0–15 cm depth) were as follows: initial organic C, 13.02 g kg^{-1}; total N (TN), 1.74 g kg^{-1}; available P, 10.09 mg kg^{-1}; available K, 227.5 mg kg^{-1}; soil pH, 5.63 (1:2.5 soil to water ratio); soil bulk density, 1.33 g cm^{-3}.

Experimental design

In 2015, the field experiment was conducted in a polyethylene plastic film-covering greenhouse vegetable cropping system with a more than 5-year history of continuous vegetable cultivation. In a 180-m^2 grid of the greenhouse vegetable cropping system, four fertilizer treatments with three replicates were established using a randomized block design, with each plot covering 5.4 m^2. The four fertilizer treatments were: i) control, unfertilized; ii) chemical fertilizer (NPK, 180 kg N ha^{-1}, 135 kg P_2O_5 ha^{-1}, and 180 kg K_2O ha^{-1}; iii) organic fertilizer (O, 180 kg N ha^{-1}, 135 kg P_2O_5 ha^{-1}, and 180 kg K_2O ha^{-1}); iv) organic fertilizer containing *T. guizhouense* (O+T), referring to bio-organic fertilizer (180 kg N ha^{-1}, 135 kg P_2O_5 ha^{-1}, and 180 kg K_2O ha^{-1}). The organic fertilizer was made up of cattle manure and mushroom residue in a mass ratio of 3:1, which contained 40.10%, 1.64%, 1.23%, and 1.29% of organic matter, TN, phosphorus (P_2O_5), and potassium (K_2O), respectively. The bio-organic fertilizer was made by direct inoculation with *Trichoderma guizhouense* NJAU 4742 (*Harzianum* clade,

10^8 colony-forming units (CFU) g^{-1} dry weight), which is an effective bio-antagonist of *Fusarium oxysporum* f.sp. *cubense* 4 *in situ* in the vegetable crop rhizosphere (Zhang J *et al*., 2016). The bio-organic fertilizer contained 44.49%, 1.45%, 1.05%, and 1.45% of organic matter, TN, P_2O_5, and K_2O, respectively. Greenhouse-grown seedlings of the "Jinyou 1" cucumber cultivar were transplanted into the field. All fertilizers were divided into two parts, with one half used as a basal fertilizer before seedlings were transplanted and the other half used as topdressing on the 35th day after transplanting. Following the local practices, basal fertilizers and topdressings were spread on the soil surface and then plowed into the soil, followed by irrigation. There were four irrigation events to meet crop growth demand over the entire growing season (Fig. 1).

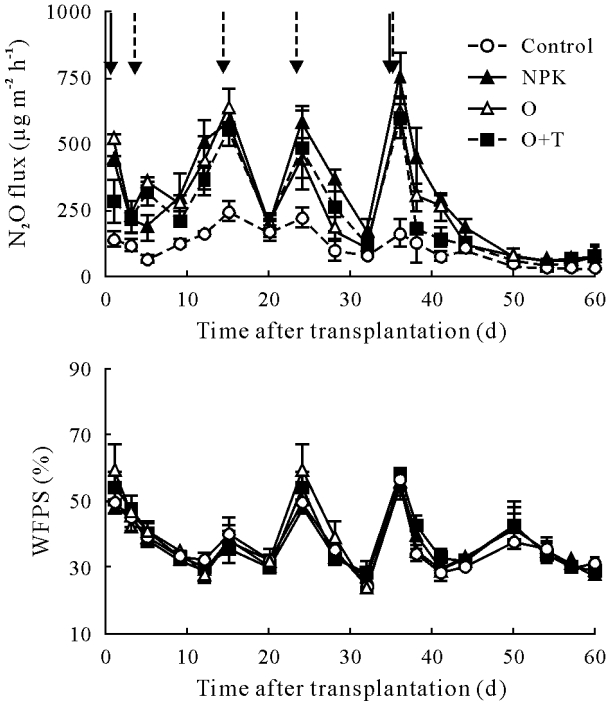

Fig. 1 Seasonal dynamics of N_2O fluxes and soil water-filled pore space (WFPS) under different fertilizer treatments over the cucumber growing season. Solid and dotted arrows represent fertilization and irrigation events, respectively. Vertical bars represent standard errors of means ($n = 3$). Control = unfertilized; NPK= chemical fertilizer; O = organic fertilizer; O+T = organic fertilizer containing *Trichoderma guizhouense*, referring to bio-organic fertilizer.

Measurement of N_2O emissions

Soil N_2O fluxes were measured using the static opaque chamber method, and the protocol details were described in our previous studies (Zou *et al*., 2005; Zhang Y J *et al*., 2016). During the vegetable growing season, square PVC collars were installed (50 cm long × 50 cm wide × 15 cm high) in the center of each plot before the cucumber seedlings were transplanted. The upper sampling chamber (50 cm long × 50 cm wide × 50 cm high) was wrapped with a layer of sponge and aluminum foil to ensure dark conditions and to minimize air temperature changes inside the chamber. During gas collection, the top groove of the collar was filled with water to prevent air exchange, then the chamber was securely inserted into the collar. The air inside the chamber was homogenized with the help of a small fan fixed inside the chamber. Soil temperature and air temperature inside the chamber were simultaneously recorded during the time of sampling. Gas measurements were collected twice a week, but this was increased to every other day or every two days following fertilizer application. Gas samples were collected with an air pump every morning from 8:00 to 10:00, and samples were taken from the headspace chamber 0, 5, 10, 15, and 20 min after the chambers were closed.

The N_2O concentrations were analyzed by a gas chromatograph fitted with an electron capture detector (ECD; Agilent 7890A, USA) (Zou *et al*., 2005). The carrier gas was an argon-methane (1:19, volume:volume) mixture at a flow rate of 40 mL min^{-1}. The temperatures of the column and ECD detector were 40 and 300 °C, respectively.

The N_2O flux was calculated using the following equation:

$$F = H \times (M \times P)/[R \times (273 + T)] \times (\mathrm{d}C/\mathrm{d}t) \qquad (1)$$

where F is the N_2O flux (μg m^{-2} h^{-1}), H is the height of the static opaque chamber (m), M is the molar mass of N_2O (44 g mol^{-1}), P is the atmospheric pressure at the sampling site, which is regarded as the standard atmospheric pressure here (1.013×10^5 Pa), R is the universal gas constant (8.314 Pa m^3 mol^{-1} K^{-1}), T is the temperature inside the static opaque chamber (°C), and $\mathrm{d}C/\mathrm{d}t$ is the rate at which the N_2O concentration changes with time (μL L^{-1} min^{-1}). Total N_2O emissions were sequentially accumulated from every two consecutive measurements. The N_2O emission factors (EFs) of the fertilizers were calculated as the difference in total N_2O emissions between the fertilized and unfertilized treatments divided by the amount of N applied (Zou *et al*., 2005).

Soil sampling

The soil samples (0–15 cm) were collected at 3, 13, 36, 41, and 57 d after cucumber seedlings were transplanted. Each sample was passed through a 2-mm sieve and was then divided into three parts. One part was air dried and stored at room temperature for later analyses of chemical properties. The second part was stored at 4 °C to determine soil mineral N and dissolved organic carbon (DOC). The remaining part was stored at −80 °C for molecular analysis.

DNA extraction and quantitative PCR (qPCR) gene analysis

DNA samples were extracted from 0.25 g of soil using PowerSoil™ DNA isolation kits (Mo Bio, USA). The concentration and quantity of the DNA samples were determined

using a Nanodrop spectrophotometer (Thermo Scientific, USA). The quantification of *amoA* from AOA and AOB, and *nirK*, *nirS*, and *nosZ* was carried out in the StepOne™ real-time PCR system (Applied Biosystems, Germany). Amplifications were performed in a reaction mixture with a total volume of 20 μL, consisting of 10 μL SYBR® Premix Ex Taq (Takara, China), 0.4 μL each primer™ (10 μmol L^{-1}), 0.4 μL ROX reference dye II (50×), 6.8 μL sterile water, and 2 μL template DNA. The DNA samples were diluted to 10 ng of DNA μL^{-1} with sterile water prior to assays. The standard curves of all genes were created using triplicate 10-fold dilutions (10^{-1}–10^{-7}) of linear plasmid DNA. The amplification efficiencies for all genes ranged from 94.6% to 100.5%, with R^2 values ranging from 0.997 to 0.999. Each qPCR run also included corresponding standards and duplicate no-template controls. Melting curve analyses were used at the end of runs to confirm amplicon specificity. Details of the primer sets and qPCR cycling conditions for each primer set are summarized in Table I.

Auxiliary measurements

Soil volumetric water content was monitored with a portable rod probe (MPM-160, ICT International Pty Ltd., Armidale, Australia) and was converted into water-filled pore space (WFPS) according to the following equation: WFPS = soil volumetric water content/(1 – soil bulk density/2.65) × 100%, assuming 2.65 Mg m^{-3} as the soil particle density (Liu *et al*., 2013). Cucumber yields under different treatments were measured, and the yield-scaled N_2O emissions were calculated as seasonal total N_2O emissions divided by vegetable fresh yield (kg N_2O-N t^{-1} yield).

The soil physicochemical properties were determined according to the guidelines of the Chinese Soil Society (Lu, 2000). Soil NH_4^+ and NO_3^- were extracted from 2 mol L^{-1} KCl solution (soil:water ratio of 1:10) after shaking for 1 h on a rotary shaker. Soil NH_4^+ contents were determined by the indophenol blue method, and NO_3^- contents were measured by dual-wavelength (220 and 275 nm) ultraviolet spectroscopy (HITACHI, U-2900, Japan).

Total nitrogen was determined with an auto elemental analyzer (Vario EL III, Elementar, Germany). Soil pH and electrical conductivity (EC) were determined with a soil to water volume ratio of 1:2.5 (Lu, 2000). Soil dissolved organic carbon (DOC) was determined using a total organic C analyzer (Elementar, Laurel, Germany) after 5 g soil was extracted using 25 mL deionized water.

Statistical analysis

One-way analysis of variance (ANOVA) was used to test for significant differences (significance level $P = 0.05$) in seasonal total N_2O emissions, vegetable yield, and yield-scaled N_2O emissions. Pearson correlation analysis was conducted to examine the correlations between N_2O fluxes and soil physicochemical parameters and between N_2O fluxes and functional gene abundances. All functional gene copy numbers were log-transformed. A linear model with the personality of ordinary least squares (OLS) was used to fit the relationship of N_2O fluxes with the copy number ratio of *nirS* plus *nirK* to *nosZ* ((*nirK* + *nirS*)/*nosZ* ratio). Statistical analyses were performed in SPSS Statistics 21 (IBM Software, Chicago, USA).

RESULTS

N_2O and yield-scaled N_2O emissions

The seasonal dynamics of the N_2O fluxes under the different fertilizer treatments showed similar patterns, except that the control remained at a relatively low level over the entire experimental period (Fig. 1). Four N_2O flux peak events were detected at 1, 15, 24, and 36 d after seedlings were transplanted, generally following fertilizer application and/or water irrigation practices (Fig. 1). Thereafter, N_2O fluxes decreased at 41 d after transplanting and then stabilized

TABLE I

Primers used for quantitative PCR in this study

Target gene[a)]	Prime name	Sequence[b)]	Product size bp	Reference
AOA *amoA*	CrenamoA23fd	ATGGTCTGGCTWAGACG	593	Tourna *et al*., 2008
	CrenamoA616r	GCCATCCATCTGTATGTCCA		
AOB *amoA*	amoA1F	GGGGHTTYTACTGGTGGT	491	Stephen *et al*., 1999
	amoA2R	CCCCTCKGSAAAGCCTTCTTC		
nirK	nirKF1acu	ATCATGGTSCTGCCGCG	473	Henry *et al*., 2004
	nirKR3cu	GCCTCGATCAGRTTGTGGTT		
nirS	nirSCd3aF	GTSAACGTSAAGGARACSGG	425	Braker *et al*., 1998
	nirSR3cd	GASTTCGGRTGSGTCTTGA		
nosZ	nosZ-F	AGAACGACCAGCTGATCGACA	380	Scala and Kerkhof, 1998
	nosZ-R	TCCATGGTGACGCCGTGGTTG		

[a)] Amplifications of these genes were performed in triplicates under the following cycling conditions: 95 °C for 30 s, sequenced by 40 cycles for 5, 30, and 60 s at 95, 55, and 72 °C, respectively, and eventually a dissociation stage (15 s at 95 °C, 60 s at 60 °C, and 15 s at 95 °C). AOB = ammonium-oxidizing bacteria; AOA = ammonium-oxidizing archaea.

[b)] W = A/T; H = A/C/T; Y = C/T; K = G/T; S = C/G; R = A/G.

at lower release rates. The highest peak of N_2O flux was found under the NPK treatment, with an average of 749.1 ± 95.6 μg m^{-2} h^{-1}.

Over the experimental period, seasonal N_2O fluxes averaged 121.1 ± 14.6, 296.7 ± 7.7, 261.9 ± 7.6, and 241.3 ± 6.2 μg m^{-2} h^{-1} for the control, NPK, O, and O+T treatments, respectively. Compared to the NPK treatment, seasonal total N_2O emissions were significantly decreased by 11.7% and 18.7% under the O and O+T treatments, respectively (Table II, $P < 0.01$). Relative to the organic fertilizer treatment, the NPK and O+T treatments enhanced vegetable yield by 4.0% and 8.4%, respectively (Table II). By relating N_2O emissions to vegetable yields, the yield-scaled N_2O emissions were the lowest under the O+T treatment across all treatments. The direct emission factors of fertilizer N for N_2O were 0.89%, 0.72%, and 0.61% under the NPK, O, and O+T treatments, respectively.

Soil physicochemical properties

Over the experimental period, soil moisture (WFPS) was primarily regulated by irrigation events, ranging from 25.9% to 56.1% (mean: 37.6%), with no significant differences among treatments (Fig. 1). Soil NH_4^+-N content in the NPK treatment increased following the application of basal and topdressing fertilizers, and varied from 51.1 to 97.0 mg N kg^{-1} (Fig. 2). Compared to the NPK treatment, on average, soil NH_4^+-N content was 70.4% lower in the O treatment and was 64.7% lower in the O+T treatment over the experimental period ($P < 0.01$) (Table SI, see Supplementary Material for Table SI). The soil NO_3^--N content showed similar seasonal variation among the N-fertilized treatments, ranging from 50.5 to 142 mg N kg^{-1} (Fig. 2). Relative to the NPK treatment, mean soil NO_3^--N content decreased by 25% in the O plot and by 31% in the O+T plot over the experimental period ($P < 0.01$). The NH_4^+ and NO_3^- contents were relatively stable in the control and remained at low levels. The N fertilizer practices significantly increased TN content, but TN did not significantly differ among the NPK, O, and O+T treatments (Fig. 2). Compared to the control, mean TN contents were 8.0%, 6.3%, and 6.5% greater in the NPK, O, and O+T plots over the vegetable growing season, respectively ($P < 0.01$) (Table SI, see Supplementary Material for Table SI).

Relative to the NPK plots, organic N application consistently increased soil dissolved organic carbon (DOC) and pH values in the O and O+T plots (Fig. 2). Over the vegetable growing season, DOC content increased from 87.3–119.5 mg kg^{-1} in the NPK plots to 103.8–212.3 mg kg^{-1} in the O and O+T plots. Similarly, pH values increased from 4.89–5.45 in the NPK plot to 5.52–6.61 in the O and O+T plots. Compared to the NPK treatment, DOC content and pH were 70.4% and 16.9% greater in the O plot, respectively, and were 64.7% and 15.9% greater in the O+T plots, respectively, over the experimental period ($P < 0.01$) (Table SI, see Supplementary Material for Table SI). Electrical conductivity (EC) ranged from 0.35 to 1.63 mS cm^{-1} in the N-fertilized treatments and was highest in the NPK treatment (Fig. 2).

Abundances of AOA amoA, AOB amoA, nirK, nirS, and nosZ genes

The abundances of nitrification and denitrification functional genes were determined by qPCR (Fig. 3). The log-transformed copy numbers of AOA and AOB *amoA* genes ranged from 6.12 to 6.48 and from 7.23 to 7.97 copies g^{-1} dry matter (DM), respectively, across all treatments (Fig. 3). The dynamic patterns of AOA *amoA* gene abundance were similar across all treatments in the cucumber growing season, and there were no significant differences in the AOA *amoA* gene abundance among all treatments (Table SII, see Supplementary Material for Table SII). In contrast, the AOB *amoA* gene abundance was more sensitive to chemical fertilizer application. Compared to the O and O+T treatments, chemical fertilizer application significantly increased AOB *amoA* gene abundance (Fig. 3, Table SII, see Supplementary Material for Table SII).

The log-transformed *nirK*, *nirS*, and *nosZ* gene copy numbers ranged from 5.94 to 6.27, from 6.10 to 6.75, and from 6.70 to 7.21 copies g^{-1} DM, respectively (Fig. 3). The *nirK* and *nosZ* abundances in the O and O+T treatments were significantly higher than in the control and NPK treatments (Table SII, see Supplementary Material for Table SII). Compared to the O treatment, in addition, the inoculation of

TABLE II

Seasonal total N_2O emissions, vegetable yield, yield-scaled N_2O emissions, and the direct emission factors (EF) under various fertilizer treatments

Treatment[a)]	N_2O emission	Vegetable yield	Yield-scaled N_2O emission	EF
	kg N ha^{-1}	t ha^{-1}	kg N t^{-1} yield	%
Control	1.10 ± 0.14[b)]c[c)]	56.05 ± 1.32b	0.020 ± 0.002c	
NPK	2.72 ± 0.07a	62.08 ± 3.91ab	0.044 ± 0.002a	0.89
O	2.40 ± 0.07b	59.00 ± 1.98ab	0.040 ± 0.001a	0.72
O+T	2.21 ± 0.06b	64.68 ± 2.12a	0.033 ± 0.001b	0.61

[a)] Control = unfertilized; NPK= chemical fertilizer; O = organic fertilizer; O+T = organic fertilizer containing *Trichoderma guizhouense*, referring to bio-organic fertilizer.

[b)] Means ± standard errors ($n = 3$).

[c)] Means followed by different letters in a column are significantly different at $P < 0.05$.

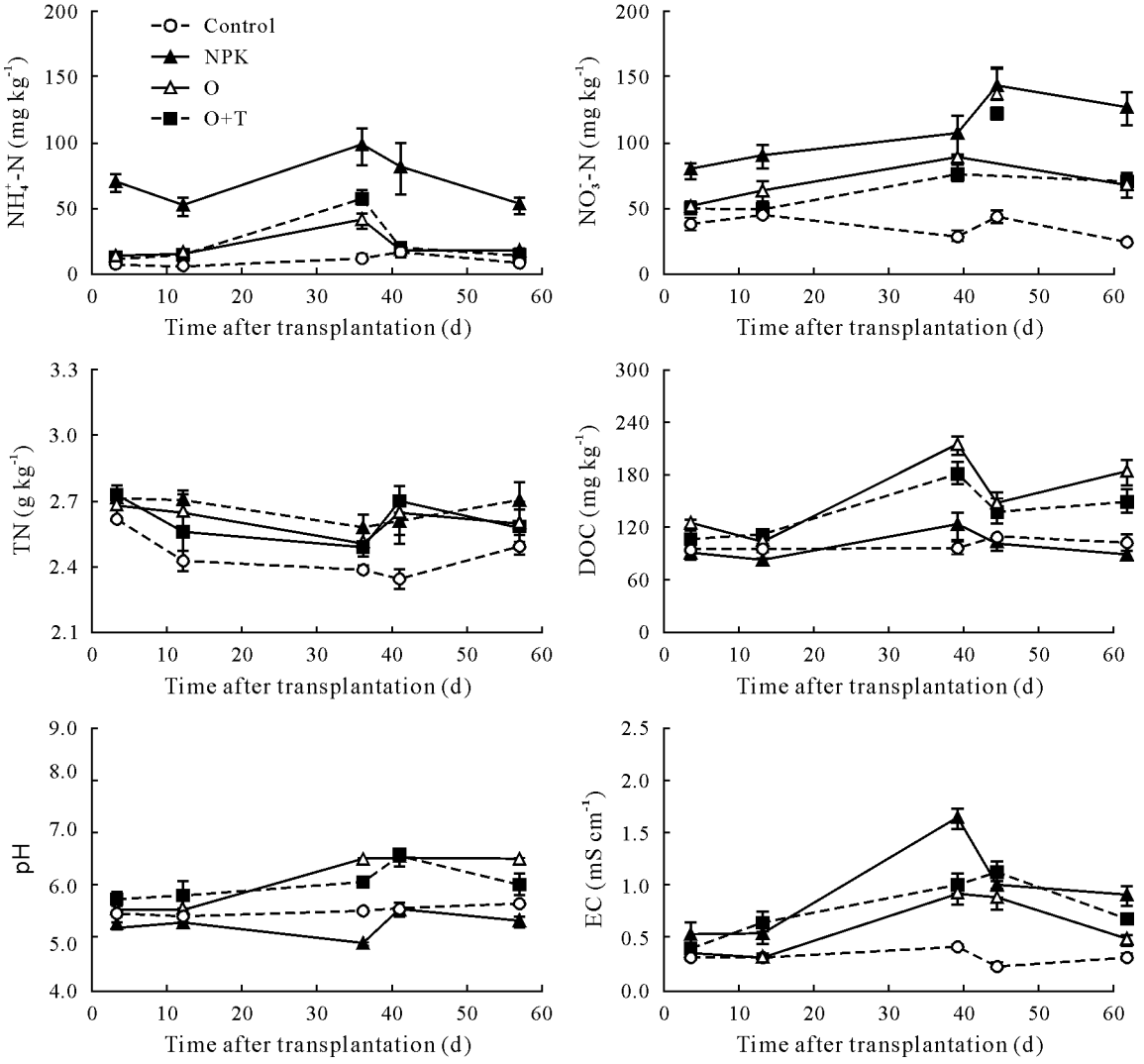

Fig. 2 Dynamics of soil NH_4^+-N, NO_3^--N, total N (TN), dissolved organic C (DOC), pH, and electrical conductivity (EC) under different fertilizer treatments over the cucumber growing season. Vertical bars represent standard errors of means ($n = 3$). Control = unfertilized; NPK= chemical fertilizer; O = organic fertilizer; O+T = organic fertilizer containing *Trichoderma guizhouense*, referring to bio-organic fertilizer.

T. guizhouense in the O+T treatment resulted in 38% and 22% increases in *nirS* and *nosZ* gene abundances, respectively (Table SII, see Supplementary Material for Table SII). The (*nirS* + *nirK*)/*nosZ* ratio was slightly higher in the NPK treatment than in the O and O+T treatments during the N_2O peak flux periods (Fig. 3).

N_2O emissions in relation to soil properties and functional gene abundances

Seasonal N_2O fluxes were significantly positively correlated with soil NH_4^+-N content, WFPS, and EC (Table III). In addition, N_2O fluxes were also positively correlated with AOB *amoA* gene abundance (Table III). The gene abundance of AOB *amoA* was positively associated with NH_4^+-N and NO_3^--N contents and with EC (Table III). The copy numbers of the denitrifying functional genes (*nirK*, *nirS*, and *nosZ*) were all positively associated with soil pH and DOC. Although no significant correlations were found between N_2O flux and the abundances of the denitrifying genes, the (*nirK* + *nirS*)/*nosZ* ratio was positively correlated with N_2O flux (Fig. 4, $P < 0.01$).

DISCUSSION

Greenhouse vegetable cropping systems have been recognized as an important source of agricultural N_2O emissions in China, mainly due to the considerable N fertilizer input (Riya *et al.*, 2012; Yao *et al.*, 2015). In the present study, four N_2O flux peaks were observed, generally following fertilizer N application and/or water addition events (Fig. 1). Indeed, numerous studies have revealed that N fertilizer application and water addition events induce significant N_2O emissions (Yao *et al.*, 2015; ZhangY J *et al.*, 2016). This is probably because rewetting dry soil stimulates soil microbial activity and thereby increases N_2O emissions (Williams and Xia, 2009).

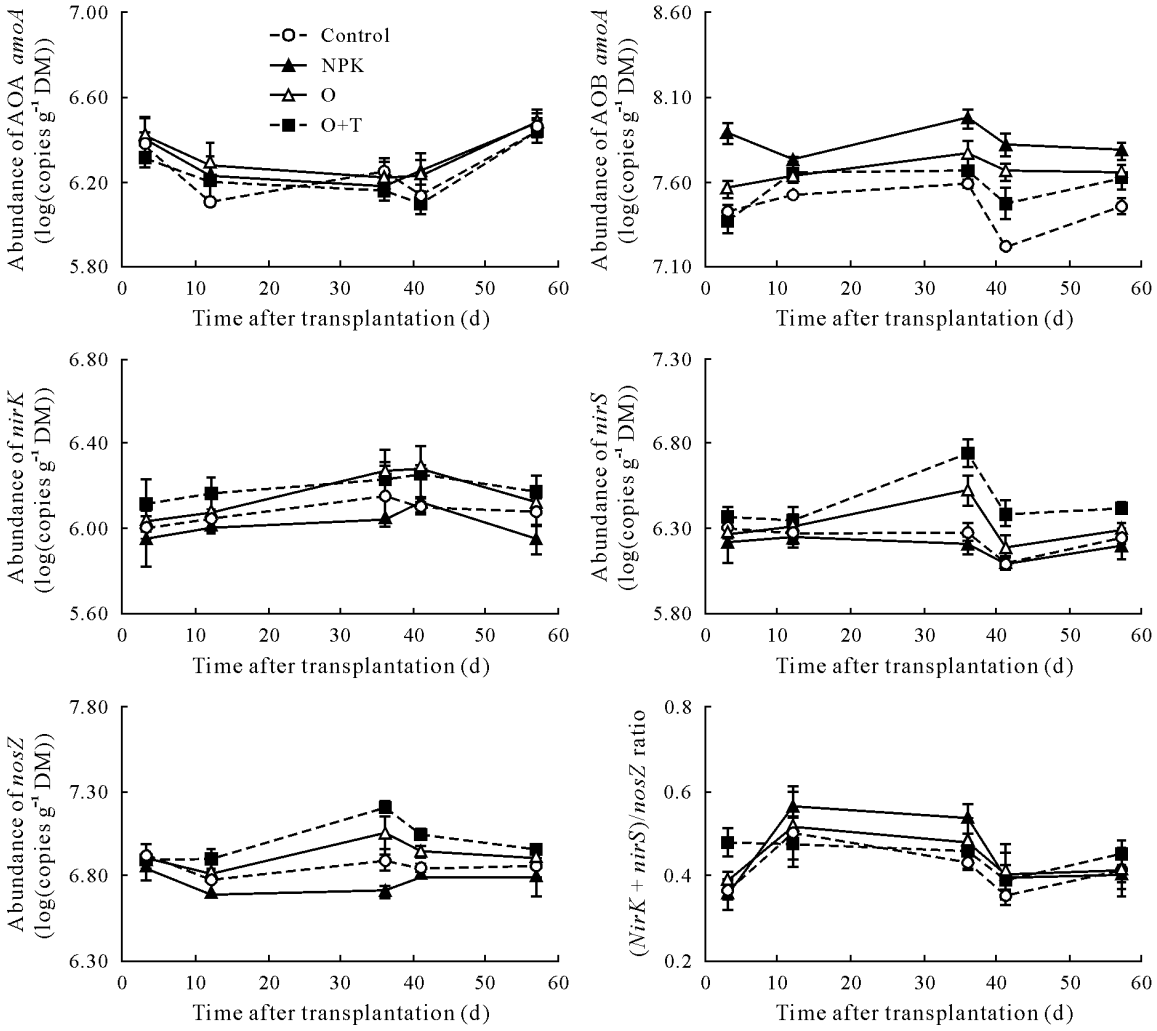

Fig. 3 Dynamics of abundances of *amoA* from ammonium-oxidizing bacteria (AOA) and archaea (AOB) and *nirK*, *nirS*, and *nosZ*, and the copy number ratio of *nirS* plus *nirK* to *nosZ* ((*nirS* + *nirK*)/*nosZ* ratio) under different treatments over the cucumber growing season. Vertical bars represent standard errors of means ($n = 3$). Control = unfertilized; NPK= chemical fertilizer; O = organic fertilizer; O+T = organic fertilizer containing *Trichoderma guizhouense*, referring to bio-organic fertilizer; DM = dry matter.

TABLE III

Correlation coefficients (r) for relationships between N_2O fluxes, functional gene abundances, and soil properties[a)]

Parameter	N_2O flux	WFPS	NH_4^+-N	NO_3^--N	TN	pH	EC	DOC
N_2O	1[b)]	0.469*	0.595**	0.27	0.01	−0.12	0.522*	0.29
AOA *amoA*	−0.48	−0.11	−0.12	−0.17	0.38	−0.12	−0.04	−0.10
AOB *amoA*	0.559*	0.21	0.787**	0.569**	0.28	−0.23	0.717**	0.10
nirK	0.19	0.08	−0.23	0.19	−0.26	0.841**	−0.26	0.723**
nirS	0.352	0.486*	−0.11	−0.17	−0.08	0.471*	−0.26	0.639**
nosZ	0.04	0.36	−0.22	−0.01	−0.13	0.746**	−0.43	0.722**

*,** Significant at $P < 0.05$ and $P < 0.01$, respectively.
[a)] WFPS = water-filled pore space; TN = total N; EC = electrical conductivity; DOC = dissolved organic C.

Consistent with our first prediction, organic or bio-organic fertilizer significantly decreased seasonal N_2O emissions by 11.7%–18.7%, relative to chemical fertilizer (Table II, $P < 0.01$). Presumably, slow mineral N release from organic fertilizer limits the N available for denitrification and nitrification (Gutser *et al.*, 2005). This is supported by the evidence that NH_4^+-N content was lower in the O and O+T treatments than in the NPK treatment (Fig. 2). In this study, N_2O flux was positively correlated with NH_4^+-N content, which is in agreement with previous study (Cui *et al.*, 2016). In addition, organic fertilizer application significantly increased DOC content (Fig. 2), which generally enhanced soil respiration and consumed oxygen, and thereby formed anaerobic conditions favorable for denitrification (Kramer *et al.*, 2006). Similarly to our results, previous studies have shown that organic fertilizer application decreases N_2O emissions,

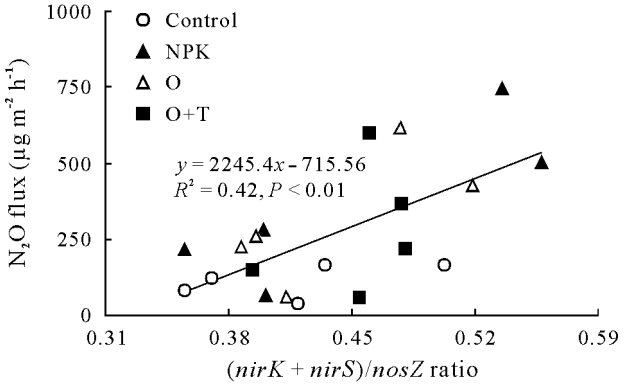

Fig. 4 Dependence of N_2O fluxes on the copy number ratio of *nirS* plus *nirK* to *nosZ* ((*nirK* + *nirS*)/*nosZ* ratio) under different fertilizer treatments over the cucumber growing season. Control = unfertilized; NPK= chemical fertilizer; O = organic fertilizer; O+T = organic fertilizer containing *Trichoderma guizhouense*, referring to bio-organic fertilizer.

as the final product of denitrification is N_2 rather than N_2O (Kramer *et al*., 2006; Tao *et al*., 2018), although the opposite finding has also been reported (Hayakawa *et al*., 2009; Wei *et al*., 2014). Nevertheless, the effect of fertilizers on N_2O emissions depends on the soil properties and fertilizer characteristics (Huang *et al*., 2004; Meijide *et al*., 2007).

In support of our second prediction, vegetable yield-scaled N_2O emissions were significantly lower in the O+T treatment than in the O and NPK treatments, while there was no significant difference between the O and NPK treatments (Table II). The plant growth benefits of bio-organic fertilizer containing *Trichoderma* spp. have been well documented in previous studies (Pang *et al*., 2017; Zhang *et al*., 2018), and are due to modifications to soil nutrient availability, soil microbial diversity, and root growth (Harman *et al*., 2004; Fiorentino *et al*., 2018). Therefore, bio-organic fertilizers may have win-win effects on vegetable yield enhancement and N_2O mitigation.

In this study, the abundances of the functional microbial AOA and AOB *amoA* genes differed in response to fertilizer application (Fig. 3). The application of both chemical and two organic fertilizers increased AOB *amoA* abundance, while these fertilizers had no significant effect on AOA *amoA* abundance in soils (Fig. 3). The abundance of AOB *amoA* was consistently higher than that of AOA *amoA* over the experimental season. The dynamic patterns of the abundances of the AOB and AOA *amoA* genes in response to nutrient input were consistent with recent findings that AOB dominates in high nutrient soils while AOA is well-adapted to low nutrient environments (Di *et al*., 2010; Taylor *et al*., 2012). Similarly, Ouyang *et al*. (2016) also observed that AOB population was significantly modified by ammonium fertilizers, but AOA population was unaffected by ammonium or organic fertilizers in an agricultural soil. Our study indicated that AOB responded more strongly to the chemical fertilizer than to organic or bio-organic fertilizers. Previous studies have also confirmed that AOB abundance is significantly increased under high ammonium levels (Taylor *et al*., 2012; Hink *et al*., 2018). Therefore, the microbial AOB *amoA* gene could play an important role in soil N_2O production through nitrification when a chemical fertilizer is applied (Hayakawa *et al*., 2009).

The abundances of the denitrifying genes (*nirK*, *nirS*, and *nosZ*) were consistently lower in the NPK treatment than in the O and O+T treatments in this study (Fig. 3). As an important indicator for predicating N_2O emissions, the (*nirS* + *nirK*)/*nosZ* ratio was greater in the NPK treatment than in the organic fertilizer treatments during the N_2O peak flux period (Fig. 3). Organic fertilizers have been shown to stimulate heterotroph growth in the short term due to higher C availability (Tatti *et al*., 2013). Similarly, Hallin *et al*. (2009) and Pereg *et al*. (2018) reported that long-term application of organic amendments significantly increased *nirK*, *nirS*, and *nosZ* gene abundances compared to chemical fertilizers, supporting the hypothesis that organic carbon is one of the most important factors in terms of explaining the abundances of denitrifier genes (Cui *et al*., 2016; Tao *et al*., 2018). Soil pH is considered to be another important factor affecting denitrifier gene abundance (Saleh-Lakha *et al*., 2009; Čuhel *et al*., 2010). Pearson correlation analysis showed that the abundances of denitrifier genes were significantly correlated with soil DOC and pH (Table III, $P < 0.01$). Field experiments have also indicated that higher copy numbers of all denitrifier genes appeared in neutral pH soil (Čuhel *et al*., 2010). In the present study, no significant correlations were found between N_2O flux and the abundance of any single denitrified gene, while the ratio of (*nirK* + *nirS*)/*nosZ* was positively correlated with N_2O flux (Fig. 4, $P < 0.01$). Therefore, the net production of N_2O was simultaneously catalyzed by N_2O-derived nitrite reductase and N_2O-consumed nitrous oxide reductase, the denitrifiers functional as a group could be a good proxy for N_2O emission (Morales *et al*., 2010).

A slight decrease in N_2O emissions in the O+T plot relative to O plot may be associated with lower AOB *amoA* abundance due to nitrification and/or higher *nosZ* gene abundance through denitrification. Consistent with our prediction, the bio-organic fertilizer containing *T. guizhouense* significantly decreased AOB *amoA* abundance relative to organic fertilizer, but increased *nosZ* gene abundance (Table SII, see Supplementary Material for Table SII). Similarly, Wu *et al*. (2018) found that inoculation with the PGPM *Bacillus amyloliquefaciens* decreased AOB abundance, thereby inhibiting nitrification and decreasing N_2O emissions. On the other hand, in this study, the addition of *T. guizhouense* increased *nosZ* gene abundance compared to the application of organic fertilizer alone, which may also have contributed to the decline in N_2O emissions. Recently, beneficial microorganisms have been increasingly used as microbial inoculants to promote plant growth and even to reduce environmental risks (Rodrigues *et al*., 2018; Liu *et al*., 2019). Calvo *et*

al. (2016) found that inoculating soils planted with corn with plant growth-promoting *Bacillus* strains decreased N_2O emissions in soils when some kinds of mineral nitrogen fertilizers were applied. Xu *et al*. (2018) reported that the use of urea amended with *Trichoderma viride* reduced N_2O emissions from tea plantation soil. These studies on PGPMs have confirmed their potential roles in reducing N_2O emissions, but the extent of this reduction depends on the type of N fertilizer and on the species or strain of PGPM. Therefore, additional research should focus on the effects of combinations of PGPMs and different N substrates on soil N_2O emissions. Moreover, isotope tracer technology should be used to explore the interplay between PGPM strains and the N cycle-related microbial community.

CONCLUSIONS

Organic and bio-organic (containing *T. guizhouense*) fertilizers significantly decreased N_2O emissions compared to a conventional chemical fertilizer in greenhouse vegetable cucumber cropping fields. The decrease in N_2O emissions was strongly associated with lower soil NH_4^+-N contents and higher soil pH and DOC contents in organic or bio-organic fertilizer treatments. Moreover, the bio-organic fertilizer containing *T. guizhouense* tended to decrease N_2O emissions relative to the organic fertilizer applied alone, which could be due to lower AOB *amoA* abundances inhibiting N_2O production through nitrification and/or higher *nosZ* abundances facilitating the reduction of N_2O to N_2 through denitrification. The significant linear positive correlation between the (*nirK* + *nirS*)/*nosZ* ratio and N_2O flux over the cucumber growing season highlighted that the (*nirK* + *nirS*)/*nosZ* ratio could be a good proxy for N_2O emissions. In addition, the bio-organic fertilizer enhanced vegetable yields and reduced yield-scaled N_2O emissions, suggesting that PGPM inoculation in organic fertilizers could be used as a win-win strategy to enhance vegetable yields and reduce N_2O emissions in vegetable cropping systems.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article can be found in the online version.

REFERENCES

Braker G, Fesefeldt A, Witzel K P. 1998. Development of PCR primer systems for amplification of nitrite reductase genes (*nirK* and *nirS*) to detect denitrifying bacteria in environmental samples. *Appl Environ Microbol.* **64**: 3769–3775.

Calvo P, Watts D B, Kloepper J W, Torbert H A. 2016. The influence of microbial-based inoculants on N_2O emissions from soil planted with corn (*Zea mays* L.) under greenhouse conditions with different nitrogen fertilizer regimens. *Can J Microbiol.* **62**: 1041–1056.

Canfield D E, Glazer A N, Falkowski P G. 2010. The evolution and future of earth's nitrogen cycle. *Science.* **330**: 192–196.

Carey C J, Dove N C, Beman J M, Hart S C, Aronson E L. 2016. Meta-analysis reveals ammonia-oxidizing bacteria respond more strongly to nitrogen addition than ammonia-oxidizing archaea. *Soil Biol Biochem.* **99**: 158–166.

Chen Q H, Qi L Y, Bi Q F, Dai P B, Sun D S, Sun C L, Liu W J, Lu L L, Ni W Z, Lin X Y. 2015. Comparative effects of 3,4-dimethylpyrazole phosphate (DMPP) and dicyandiamide (DCD) on ammonia-oxidizing bacteria and archaea in a vegetable soil. *Appl Microbiol Biotechnol.* **99**: 477–487.

Čuhel J, Šimek M, Laughlin R J, Bru D, Cheneby D, Watson C J, Philippot L. 2010. Insights into the effect of soil pH on N_2O and N_2 emissions and denitrifier community size and activity. *Appl Environ Microb.* **76**: 1870–1878.

Cui P Y, Fan F L, Yin C, Song A L, Huang P R, Tang Y J, Zhu P, Peng C, Li T Q, Wakelin S A, Liang Y C. 2016. Long-term organic and inorganic fertilization alters temperature sensitivity of potential N_2O emissions and associated microbes. *Soil Biol Biochem.* **93**: 131–141.

Di H J, Cameron K C, Shen J P, Winefield C S, O'Callaghan M, Bowatte S, He J Z. 2010. Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. *FEMS Microbiol Ecol.* **72**: 386–394.

Fei C, Zhang S R, Liang B, Li J L, Jiang L H, Xu Y, Ding X D. 2018. Characteristics and correlation analysis of soil microbial biomass phosphorus in greenhouse vegetable soil with different planting years. *Acta Agric Boreali-Sin* (in Chinese). **33**: 195–202.

Fiorentino N, Ventorino V, Woo S L, Pepe O, De Rosa A, Gioia L, Romano I, Lombardi N, Napolitano M, Colla G, Rouphael Y. 2018. *Trichoderma*-based biostimulants modulate rhizosphere microbial populations and improve N uptake efficiency, yield, and nutritional quality of leafy vegetables. *Front Plant Sci.* **9**: 743.

Food and Agriculture Organization of the United Nations (FAO). 2018. FAOSTAT Online Database. Available online at http://www.fao.org/faostat/en/#data (verified on Dec. 17, 2018).

Gutser R, Ebertseder T, Weber A, Schraml M, Schmidhalter U. 2005. Short-term and residual availability of nitrogen after long-term application of organic fertilizers on arable land. *J Plant Nutr Soil Sci.* **168**: 439–446.

Hallin S, Jones C M, Schloter M, Philippot L. 2009. Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. *ISME J.* **3**: 597–605.

Harman G E, Howell C R, Viterbo A, Chet I, Lorito M. 2004. *Trichoderma* species-opportunistic, avirulent plant symbionts. *Nat Rev Microbiol.* **2**: 43–56.

Hayakawa A, Akiyama H, Sudo S, Yagi K. 2009. N_2O and NO emissions from an Andisol field as influenced by pelleted poultry manure. *Soil Biol Biochem.* **41**: 521–529.

Henry S, Baudoin E, López-Gutiérrez J C, Martin-Laurent F, Brauman A, Philippot L. 2004. Quantification of denitrifying bacteria in soils by *nirK* gene targeted real-time PCR. *J Microbiol Methods*. **59**: 327–335.

Hink L, Gubry-Rangin C, Nicol G W, Prosser J I. 2018. The consequences of niche and physiological differentiation of archaeal and bacterial ammonia oxidisers for nitrous oxide emissions. *ISME J.* **12**: 1084–1093.

Huang Y, Zou J W, Zheng X H, Wang Y S, Xu X K. 2004. Nitrous oxide emissions as influenced by amendment of plant residues with different C: N ratios. *Soil Biol Biochem*. **36**: 973–981.

Kramer S B, Reganold J P, Glover J D, Bohannan B J M, Mooney H A. 2006. Reduced nitrate leaching and enhanced denitrifier activity and efficiency in organically fertilized soils. *Proc Natl Acad Sci USA*. **103**: 4522–4527.

Lam S K, Suter H, Mosier A R, Chen D L. 2017. Using nitrification inhibitors to mitigate agricultural N_2O emission: A double-edged sword? *Glob Change Biol.* **23**: 485–489.

Liu G, Ma J, Yang Y T, Yu H Y, Zhang G B, Xu H. 2019. Effects of straw incorporation methods on nitrous oxide and methane emissions from a wheat-rice rotation system. *Pedosphere.* **29**: 204–215.

Liu Q H, Qin Y M, Zou J W, Guo Y Q, Gao Z L. 2013. Annual nitrous oxide emissions from open-air and greenhouse vegetable cropping systems in China. *Plant Soil.* **370**: 223–233.

Lu R K. 2000. Methods of Soil and Agro-Chemical Analysis (in Chinese). China Agricultural Science and Technology Press, Beijing.

Meijide A, Díez J A, Sánchez-Martín L, López-Fernández S, Vallejo A. 2007. Nitrogen oxide emissions from an irrigated maize crop amended with treated pig slurries and composts in a Mediterranean climate. *Agric Ecosyst Environ*. **121**: 383–394.

Morales S E, Cosart T, Holben W E. 2010. Bacterial gene abundances as indicators of greenhouse gas emission in soils. *ISME J*. **4**: 799–808.

National Oceanic and Atmospheric Administration (NOAA). 2018. Nitrous oxide (N_2O)—combined data set. Available online at https://www.esrl.noaa.gov/gmd/hats/combined/N2O.html (verified on May10, 2018).

Ouyang Y, Norton J M, Stark J M, Reeve J R, Habteselassie M Y. 2016. Ammonia-oxidizing bacteria are more responsive than archaea to nitrogen source in an agricultural soil. *Soil Biol Biochem*. **96**: 4–15.

Pang G, Cai F, Li R X, Zhao Z, Li R, Gu X L, Shen Q R, Chen W. 2017. *Trichoderma*-enriched organic fertilizer can mitigate microbiome degeneration of monocropped soil to maintain better plant growth. *Plant Soil.* **416**: 181–192.

Pereg L, Morugán-Coronado A, McMillan M, García-Orenes F. 2018. Restoration of nitrogen cycling community in grapevine soil by a decade of organic fertilization. *Soil Till Res.* **179**: 11–19.

Ravishankara A R, Daniel J S, Portmann R W. 2009. Nitrous oxide (N_2O): The dominant ozone-depleting substance emitted in the 21st century. *Science.* **326**: 123–125.

Rezaei R M, Wang W J, Moody P, Chen C R, Ghadiri H. 2015. Fertiliser-induced nitrous oxide emissions from vegetable production in the world and the regulating factors: A review. *Atmos Enviorn*. **112**: 225–233.

Riya S, Min J, Zhou S, Shi W M, Hosomi M. 2012. Short-term responses of nitrous oxide emissions and concentration profiles to fertilization and irrigation in greenhouse vegetable cultivation. *Pedosphere.* **22**: 764–775.

Rodrigues M Â, Ladeira L C, Arrobas M. 2018. *Azotobacter*-enriched organic manures to increase nitrogen fixation and crop productivity. *Eur J Agron.* **93**: 88–94.

Saleh-Lakha S, Shannon K E, Henderson S L, Goyer C, Trevors J T, Zebarth B J, Burton D L. 2009. Effect of pH and temperature on denitrification gene expression and activity in *Pseudomonas mandelii*. *Appl Environ Microbiol.* **75**: 3903–3911.

Saravanan T, Muthusamy M, Marimuthu T. 2003. Development of integrated approach to manage the fusarial wilt of banana. *Crop Prot.* **22**: 1117–1123.

Scala D J, Kerkhof L J. 1998. Nitrous oxide reductase (*nosZ*) gene-specific PCR primers for detection of denitrifiers and three *nosZ* genes from marine sediments. *FEMS Microbiol Lett.* **162**: 61–68.

Stephen J R, Chang Y J, Macnaughton S J, Kowalchuk G A, Leung K T, Flemming C A, White D C. 1999. Effect of toxic metals on indigenous soil β-subgroup proteobacterium ammonia oxidizer community structure and protection against toxicity by inoculated metal-resistant bacteria. *Appl Environ Microbiol.* **65**: 95–101.

Sun R B, Guo X S, Wang D Z, Chu H Y. 2015. 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.

Tao R, Wakelin S A, Liang Y C, Hu B W, Chu G X. 2018. Nitrous oxide emission and denitrifier communities in drip-irrigated calcareous soil as affected by chemical and organic fertilizers. *Sci Total Environ.* **612**: 739–749.

Tatti E, Goyer C, Zebarth B J, Burton D L, Giovannetti L, Viti C. 2013. Short-term effects of mineral and organic fertilizer on denitrifiers, nitrous oxide emissions and denitrification in long-term amended vineyard soils. *Soil Sci Soc Am J.* **77**: 113–122.

Taylor A E, Zeglin L H, Wanzek T A, Myrold D D, Bottomley P J. 2012. Dynamics of ammonia-oxidizing archaea and bacteria populations and contributions to soil nitrification potentials. *ISME J.* **6**: 2024–2032.

Tourna M, Freitag T E, Nicol G W, Prosser J I. 2008. Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms. *Environ Microbiol.* **10**: 1357–1364.

United Nations Environment Programme (UNEP). 2019. UN Environment Releases Frontiers 2018/19: Emerging Issues of Environmental Concern. UNEP, Nairobi.

Vacheron J, Desbrosses G, Bouffaud M L, Touraine B, Moënne-Loccoz Y, Muller D, Legendre L, Wisniewski-Dyé F, Prigent-Combaret C. 2013. Plant growth-promoting rhizobacteria and root system functioning. *Front Plant Sci.* **4**: 356.

Wei W, Isobe K, Shiratori Y, Nishizawa T, Ohte N, Otsuka S, Senoo K. 2014. N_2O emission from cropland field soil through fungal denitrification after surface applications of organic fertilizer. *Soil Biol Biochem.* **69**: 157–167.

Williams M A, Xia K. 2009. Characterization of the water soluble soil organic pool following the rewetting of dry soil in a drought-prone tallgrass prairie. *Soil Biol Biochem.* **41**: 21–28.

Wu S H, Zhuang G Q, Bai Z H, Cen Y, Xu S J, Sun H S, Han X G, Zhuang X L. 2018. Mitigation of nitrous oxide emissions from acidic soils by *Bacillus amyloliquefaciens*, a plant growth-promoting bacterium. *Glob Change Biol.* **24**: 2352–2365.

Xiong Z Q, Xie Y X, Xing G X, Zhu Z L, Butenhoff C. 2006. Measurements of nitrous oxide emissions from vegetable production in China. *Atmos Environ.* **40**: 2225–2234.

Xu H J, Wang X H, Li H, Yao H Y, Su J Q, Zhu Y G. 2014. Biochar impacts soil microbial community composition and nitrogen cycling in an acidic soil planted with rape. *Environ Sci Technol.* **48**: 9391–9399.

Xu S G, Feng S G, Sun H S, Wu S H, Zhuang G Q, Deng Y, Bai Z H, Jing C Y, Zhuang X L. 2018. Linking N_2O emissions from biofertilizer-amended soil of tea plantations to the abundance and structure of N_2O-reducing microbial communities. *Environ Sci Technol.* **52**: 11338–11345.

Yao Z S, Liu C Y, Dong H B, Wang R, Zheng X H. 2015. Annual nitric and nitrous oxide fluxes from Chinese subtropical plastic greenhouse and conventional vegetable cultivations. *Environ Pollut.* **196**: 89–97.

Yao Z S, Yan G X, Wang R, Zheng X H, Liu C Y, Butterbach-Bahl K. 2019. Drip irrigation or reduced N-fertilizer rate can mitigate the high annual N_2O+NO fluxes from Chinese intensive greenhouse vegetable systems. *Atmos Enviorn.* **212**: 183–193.

Zhang F G, Huo Y Q, Xu X X, Hu J, Sun X, Xiao Y, Zhang Y J. 2018. *Trichoderma* improves the growth of *Leymus chinensis*. *Biol Fert Soils.* **54**: 685–696.

Zhang J, Bayram Akcapinar G, Atanasova L, Rahimi M J, Przylucka A, Yang D Q, Kubicek C P, Zhang R F, Shen Q R, Druzhinina I S. 2016. The neutral metallopeptidase NMP1 of *Trichoderma guizhouense* is required for mycotrophy and self-defence. *Environ Microbiol.* **18**: 580–597.

Zhang Y J, Lin F, Jin Y G, Wang X F, Liu S W, Zou J W. 2016. Response of nitric and nitrous oxide fluxes to N fertilizer application in greenhouse vegetable cropping systems in southeast China. *Sci Rep.* **6**: 20700.

Zou J W, Huang Y, Jiang J Y, Zheng X H, Sass R L. 2005. A 3-year field measurement of methane and nitrous oxide emissions from rice paddies in China: Effects of water regime, crop residue, and fertilizer application. *Glob Biogeochem Cycles.* **19**: GB2021.