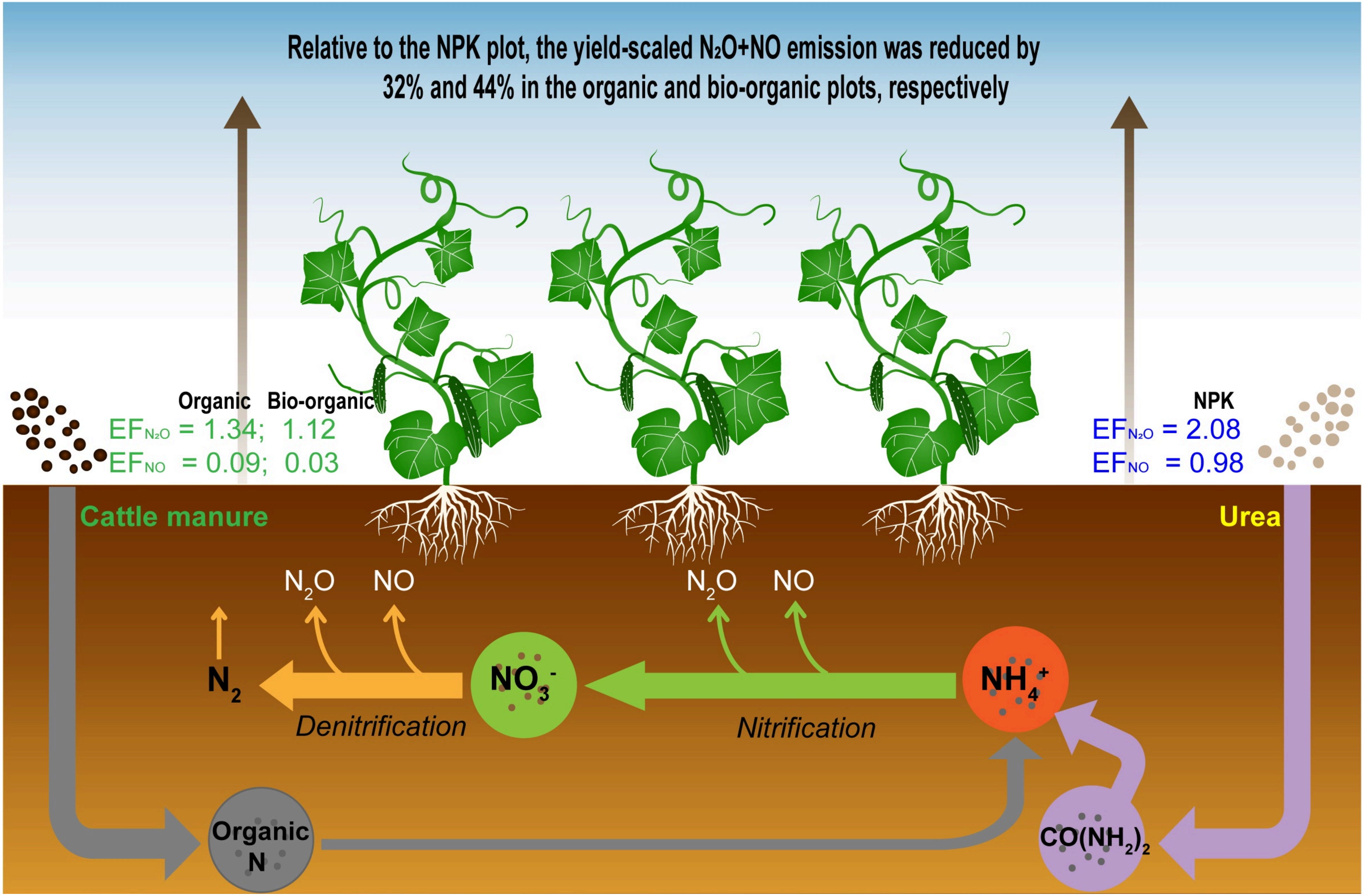
Relative to the NPK plot, the yield-scaled N₂O+NO emission was reduced by 32% and 44% in the organic and bio-organic plots, respectively
Organic Bio-organic
EF$_{N_2O}$ = 1.34; 1.12
EF$_{NO}$ = 0.09; 0.03
NPK
EF$_{N_2O}$ = 2.08
EF$_{NO}$ = 0.98
Cattle manure
Urea
N$_2$O NO
N$_2$O NO
N$_2$
NO$_3^-$
NH$_4^+$
Denitrification
Nitrification
Organic N
CO(NH$_2$)$_2$

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Soil N-oxide emissions decrease from intensive greenhouse vegetable fields by substituting synthetic N fertilizer with organic and bio-organic fertilizers

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Abstract

In order to reduce soil and environmental quality degradation associated with the use of synthetic nitrogen (N), substituting chemical fertilizer with organic or bio-organic fertilizer has become an increasingly popular option. However, components of this fertilizer strategy related to mitigation of soil N-oxide emissions and maintenance of crop yield remain uncertain. Here, we evaluated the effects of three different fertilizer strategies, with equal amounts of N, on nitrous oxide (N_2O) and nitric oxide (NO) emissions, vegetable yield, and yield-scaled N_2O and NO emissions under three consecutive cucumber growing seasons. The three treatments were chemical fertilizer (NPK, urea), organic fertilizer (O, composted cattle manure), and bio-organic fertilizer (O+T, O combined with *Trichoderma*.spp). Results showed that the NPK plot had the highest area-scaled emissions of N_2O (13.1±0.48 kg N ha^{-1} yr^{-1}) and NO (5.01±0.34 kg N ha^{-1} yr^{-1}), which were 1.3–1.4 and 3.1–3.7 times greater than the O and O+T plots, respectively. The annual direct emission factors for N_2O and NO were 2.08% and 0.92% for the NPK plot, which declined to 1.34% and 0.09% in the O plot, and 1.12% and 0.03% in the O+T plot, respectively. The annual vegetable yield was 117±2.9 t ha^{-1} for NPK plot and 122±2.0 t ha^{-1} for O + T plot, which was higher than 111±1.7 t ha^{-1} for O plot. The yield-scaled N_2O+NO emissions differed significantly with fertilization treatment, with the lowest value observed in the O+T plot. We attributed the lower soil N-oxide emissions following organic fertilizer application to the slow release of available N and enhanced denitrification caused by the increase of soil dissolved organic carbon and pH. Compared with the use of organic fertilizer alone, the addition of *Trichoderma*.spp significantly increased the potential denitrification rate but decreased N_2O emissions, which

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may have promoted the reduction of N_2O to N_2. Therefore, our results suggest that adopting composted organic fertilizer mixtures with microbial inoculants could be a win-win practice to mitigate gaseous N losses and simultaneously improve crop yield in intensively managed vegetable cropping systems.

Keywords: Nitrous oxide; Nitric oxide; Static chamber technique; *Trichoderma*; Emission factor; Drip irrigation

1. Introduction

Nitrous oxide (N_2O) and nitric oxide (NO) are two important atmosphere trace gases, directly or indirectly contributing to global climate and environmental changes (IPCC, 2013). N_2O is a potent greenhouse gas and the dominant stratospheric ozone-depleting substance (Ravishankara et al., 2009). NO is a key precursor of tropospheric ozone (O_3) and contributes to the formation of acid rain (Pilegaard, 2013). Agricultural soils are significant sources of N_2O and NO, primarily due to the increased use of chemical or organic nitrogen (N) fertilizers, emitting about 80% and 10% of the total anthropogenic emissions of N_2O and NO, respectively (Davidson, 2009; IPCC, 2013; UNEP, 2019). Emissions of N_2O and NO are predicted to increase in the future, as the global use of N fertilizers is forecast to increase threefold by 2050 to meet the doubling of global food demand (Alexandratos and Bruinsma, 2012; Mueller et al., 2012).

Vegetable cultivation under greenhouse conditions can lead to substantial emissions of soil N-oxides. Nitrogen fertilizer application rates in greenhouse vegetable fields are often several times higher than in other cereal grain cropping systems (Liu et al., 2013; Rashti et al., 2015). Because of frequent irrigation and high temperatures, greenhouse vegetable cropping systems are highly susceptible to N losses, with annual soil N_2O and NO emissions are as high as 60.5 and 10.8 kg N ha^{-1}, respectively (Yao et al., 2019a; Zhang et al., 2016). In agricultural soils, N_2O and NO emissions are mainly produced as by-products through the biotic processes of nitrification, denitrification, and nitrifier denitrification (Firestone and Davidson, 1989; Pilegaard, 2013; Wrage-Mönnig et al., 2018), while their emissions are influenced by the same factors in different ways (Loick et al., 2017). There has been a much stronger focus on N_2O than on NO or combined N_2O+NO emissions from vegetable fields (e.g., De Rosa et al., 2018; Zhang et al., 2018). At the regional or country scale, N_2O or NO estimates generally described using emission factors (EF) to quantify the amount of N_2O-N or NO-N lost as a function of the N inputs, excluding background N_2O or NO emissions (Hergoualc'h et al., 2019). However, studies have found that the N_2O emission factors for upland crops may differ between the crop-growing season (EF_{gs}) and the whole year (EF_{wy}), especially for vegetables (Shang et al., 2020). Different water management practices in agricultural systems can also result in high variability in EFs (Cayuela et al., 2017). Besides, 5.5-20.6% of the annual NO emissions from greenhouse vegetables occurred in the non-growing stage (Yao et al., 2019a), suggesting the importance of annual measurement of NO emissions. Nevertheless, the EF_{gs} has been commonly used to determine national N_2O or NO inventories for vegetable fields (Rashti et al., 2015; Wang et al., 2011). Exploring the EFs of N_2O and NO over an annual period would contribute to a more accurate estimate of N_2O and NO emissions from intensively managed greenhouse vegetable cropping systems.

In the past decade, shifts in fertilization strategies have received increasing attention due to the potential for greenhouse gas mitigation. For example, to address the challenges associated with the use of chemical N fertilizer (e.g., soil quality degradation, soil acidification, and groundwater pollution), substitution of synthetic fertilizer with organic fertilizer is promoted in intensively managed cropping systems (Zhang et al., 2020; Sanz-Cobena et al., 2017). However, the side effects of this fertilization strategy on N_2O and NO emissions are uncertain. Organic fertilizers play multiple roles in microbial-mediated N_2O production, leading to stimulatory or inhibitory effects. For instance, the easily mineralizable carbon (C) supply from poultry manure can stimulate heterotrophic denitrification, resulting in larger N_2O emissions than occur with chemical N fertilization (Hayakawa et al., 2009). In contrast, a higher denitrification rate as a result of organic fertilizer application may promote the production of N_2, with no significant difference in N_2O emissions between organically farmed soils and conventionally farmed soils (Kramer et al., 2006). Several studies have reported that substituting organic for chemical fertilizer can contribute to decreased NO emissions (Akiyama and Tsuruta, 2003a, b; Meijide et al., 2007; Vallejo et al., 2006). Additionally, compared with liquid organic fertilizers, solid and composted organic fertilizers have a low N_2O and NO emission potential due to reduced mineral N concentrations, especially the NH_4^+ content (Aguilera et al., 2013; Bertora et al., 2008; Meijide et al., 2007). The combination of drip irrigation with organic fertilizer reduced N_2O emissions by 28% but had no significant effect on NO emissions when compared with furrow irrigation (Sanchez-Martin et al., 2010). These scenarios could be highly dependent on climate, soil properties, site-specific management practices, as well as the biochemical quality of the organic materials. Nevertheless, emerging evidence suggests that the differences in the rate of N release from synthetic and organic fertilizers can have the potential to significantly influence soil N-oxide emissions (Prosser et al., 2020). This is mainly due to niche preference of ammonia-oxidizing bacteria (AOB) or archaea (AOA) (Hink et al., 2017; 2018; Stein, 2019).

Substituting chemical fertilizer with organic fertilizer may have a neutral or even negative effect on vegetable yield, especially when the replacement rate of synthetic N with organic N exceeds 75% (Xia et al., 2017). The release of available N from composted organic fertilizer is slow and may not be able to meet the high N demand of fast-growing and high-yield vegetables (Berry et al., 2002). Thus, a promising alternative strategy is the use of bio-organic fertilizers (a mixture of organic material with beneficial soil microorganisms). *Trichoderma.* spp, a biological control and plant growth-promoting agent, is commonly used in bio-organic fertilizers (Harman et al., 2004). Colonization of *Trichoderma* on the root surface was shown to promote the development of plant roots and improve N use efficiency (Shoresh et al., 2010). The use of *Trichoderma* enriched bio-organic fertilizer has been shown to enhance plant uptake of soil nutrients and stimulate plant growth when compared with organic or chemical fertilizer (Pang et al., 2017). However, the effect of bio-organic fertilizer on soil N-oxide emissions remains unclear. Meanwhile, the benefits or trade-offs from substituting synthetic fertilizer with organic or bio-organic fertilizers are not well-known in vegetable fields. Therefore, it is necessary to evaluate the yield-scaled N_2O+NO emission (expressed as N_2O+NO produced per unit of crop yield) for balancing soil N losses and food security in agricultural ecosystems (Linquist et al., 2012).

In this study, we conducted an in situ field measurement over a 16-month period to quantify the annual N_2O and NO fluxes under various fertilizer-N regimes and to quantify crop yield benefits on a cucumber monoculture under a drip irrigation system in the greenhouse. We hypothesized that i) the substitution of synthetic N by organic or bio-organic fertilizer would mitigate N-oxide emissions due to the slow N release from added organic fertilizers; and ii) that bio-organic fertilizer would decrease N_2O and NO emissions and increase crop yields, thus resulting in lower yield-scaled N_2O and NO emissions. Specifically, the objectives of our study were to i) quantify the seasonal and annual emission intensity of N_2O and NO and their direct EFs under different fertilizer-N regimes; ii) evaluate the effects of synthetic and organic N management on yield-scaled emissions of N_2O and NO; and iii) clarify the environmental driving factors that regulate N-oxides emissions.

2. Materials and Methods

2.1 *Site description and experimental design*

Field experiments were conducted from August 2015 to December 2016 at a research site (31°95'N, 118°83'E) of Nanjing Agricultural University in suburban Nanjing, Jiangsu province, China. The experimental site has been in continuous vegetable cultivation (e.g., Chinese cabbage, cabbage and cucumber) for >5 years. The climate is characterized by a subtropical monsoon, with hot-rainy summers and mild-less rainy winters. From 2015 to 2016, the annual average temperature is 16.5°C, and summer precipitation accounts for about 52% of the total average annual precipitation of 1786 mm (Fig. S1). From July 1, 2016 to August 12, 2016 (the second fallow stage), the plastic greenhouse was refurbished and open to the air at this time. Rainfall during this period reached 537 mm, accounting for 30% of total annual rainfall. The soil was classified as a Eutric Planosol (FAO, 1981) with 31.1% sand, 17.7% silt, 50.2% clay, and a soil bulk density of 1.33 g cm^{-3}. The surface soil (0-15 cm) contained an organic C content of 13.0 g C kg^{-1}, a total N content of 1.74 g N kg^{-1}, and a pH (1:2.5, soil/water) of 5.63.

Four treatments with different fertilizer-N regimes were arranged in a randomized block design with three replicates (each plot with a size of 2.7 m × 2 m) within a plastic greenhouse (30 m × 6 m). A 0.3 m wide buffer row separated the adjacent plots. The four treatments referred to the unfertilized control (control), urea (NPK), organic fertilizer (O), and bio-organic fertilizer (O+T). The composted organic fertilizer was derived from a mixture of cattle manure and mushroom residue with a mass ratio of 3:1, containing organic matter 401 g kg^{-1}, total N 16.4 g N kg^{-1}, phosphorus 12.3 g P_2O_5 kg^{-1}, and potassium 12.9 g K_2O kg^{-1}. The bio-organic fertilizer was organic fertilizer plus *Trichoderma guizhouense* NJAU 4742 (10^8 CFU g^{-1} dry weight (DW), an antagonist of *Fusarium oxysporum*) containing organic matter 445 g kg^{-1}, total N 14.5 g N kg^{-1}, phosphorus 10.5 g P_2O_5 kg^{-1} and potassium 14.5 g K_2O kg^{-1}. From August 2015 to December 2016, cucumber seedlings were transplanted at the two-leaf stage on August 19, 2015, March 26, and August 12, 2016, respectively. The final harvest dates were on October 17, 2015, June 13 and October 14, 2016, respectively. Each cucumber cropping cycle was divided into the cucumber growing stage and subsequent fallow stage (Table S1). In each cucumber growing stage, each treatment received equal amounts of 180 kg N ha^{-1}, 135 kg P_2O_5 ha^{-1}, and 180 kg K_2O ha^{-1}. The missing P and K in each treatment were supplemented with superphosphate and potassium sulfate, respectively. According to the local practice, basal fertilizers and topdressing were broadcasted on the soil surface, plowed into the soil at a depth of 3-4 cm, and then followed by irrigation. The drip irrigation system was applied in this study. The cucumber planting density is 5 plants m^{-2}. The details of cultivation and fertilization management for each treatment are shown in Table S1.

2.2 *Measurements of N_2O and NO fluxes*

Flux measurements were taken over the periods of August 17, 2015 to December 19, 2016. Fluxes of N_2O and NO were measured using a static opaque chamber technique comprising PVC chambers (50 cm × 50 cm × 50 cm) (Zhang et al., 2016; Zou et al., 2005). Chamber bases (area 0.25 m^2, height 15 cm, ~10 cm inserted into the soil) were installed on each plot before transplanting. Within each chamber base, one cucumber was grown throughout each growing season. Each cucumber plant in the chamber base was equipped with a rope, which was hung on a bamboo frame. During sampling, the rope was taken off and the cucumber plant was carefully hovered in the chamber. After the gas collection, the cucumber plants were hung on the bamboo frame again. Gas sampling generally occurred once or twice a week except following fertilizer application when it occurred every day or every two days during the cucumber growing stages, and every 7 or 10 days during the fallow periods. Gas sampling events occurred from 08:00 through 10:00 local standard time on each sampling day. On the sampling day, five samples were collected from the headspace with a 1.5-L gas sampling bag at 0, 5, 10, 15, and 20 min after chamber closure.

The N_2O concentrations in the samples were analyzed with a gas chromatograph (Agilent 7890A, Santa Clara, USA) equipped with an electron capture detector (ECD). The carrier gas was a gas mixture of argon-methane (5%:95%) at a flow rate of 40 mL min^{-1}. The temperatures of the ECD detector and column were 300°C and 40°C, respectively. The N_2O calibration standard gas concentration is 0.32×10^{-6} mol mol^{-1}. The gas sample analysis for NO concentrations was performed using a model 42i chemiluminescence NO-NO_2-NOx analyzer (Thermo Environmental Instruments Inc., Franklin, MA, USA), which was calibrated using the TE-146*i* dilution-titration instrument (Zhang et al., 2016). The calibration standard gases for N_2O and NO were provided by the National Center of Standard Matters (Beijing, China). The fluxes (F, μg N m^{-2} h^{-1}) of N_2O and NO were calculated using the following equation:

$$F = \frac{V}{A} \times \frac{M \times P}{R \times (237 + T)} \times \frac{dC}{dt}$$

where, V is the volume of gas sampling chamber (m^3), A is the cover area of gas sample chamber (m^2), M is the molar mass of N_2O (44 g mol^{-1}) or NO (28 g mol^{-1}), P is the air pressure inside the gas sample chamber (1.013 × 105 Pa), R is the universal gas constant (8.314 Pa m^3 mol^{-1} K^{-1}), T is the temperature inside the chamber(°C), dC/dt is calculated by the linear increases of N_2O or NO concentration with time. Note that the volume of the sampling chamber was not corrected for the volume occupied by the cucumber plant.

The average flux of N_2O or NO over each cucumber cropping cycle (growing stage + fallow stage) was the average of all measured fluxes weighted by the intervals between two adjacent measurements. The total seasonal N_2O or NO emissions were approximated by applying the trapezoid rule on time intervals between measured fluxes, assuming constant fluxes per day.

2.3 Measurements of soil physicochemical and biological properties

Parallel to gas sampling, alcohol thermometers were used to record the air temperature and soil temperature (5 cm depth) in the greenhouse. Soil volumetric water content (0-5 cm) was measured using an MP406 Moisture probe with an MPM 160 Moisture Probe Meter (ICT International Pty Ltd, Armidale, Australia). The ratio of measured soil volumetric water content to total porosity was expressed as soil water-filled pore space (WFPS). The total soil porosity was calculated as [1-(soil bulk density (g cm^{-3})/2.65)], assuming a soil particle density of 2.65 (g cm^{-3}). Soil samples were collected from surface layers (0-15 cm) at intervals of 10-15 days during the cucumber growing stages and at intervals of 20-30 days during the fallow stages. Soil samples collected from the three points on the diagonal of each plot were mixed and passed through a 2 mm sieve to remove stones and impurities. The homogenized soil samples were stored at 4°C for analysis of soil physicochemical properties (Lu, 2000). Soil pH and electrical conductivity (EC) were measured using a pH probe (PHS-3C, Shanghai, China) and a conductivity meter (FE-30, Shanghai, China) at the soil to water ratio of 1:2.5 (*w*/*v*), respectively. Soil mineral N (NH_4^+ and NO_3^-) was determined by extracting soil with 2 M KCl (soil: water ratio of 1:10) after shaking for 1 h on a rotary shaker. Soil NH_4^+ and NO_3^- concentrations were measured using the indophenol blue method and two-wavelength approach using a UV-VIS spectrophotometer, respectively (U-2900, Hitachi, Tokyo, Japan). Soil dissolved organic carbon (DOC) was determined using a UV-Persulfate TOC analyzer (Teledyne-Tekmar Phoenix 8000, Mason, Ohio, USA).

The soil potential denitrification rates were performed on soil samples on the final harvest date of each cucumber season (i.e., October 17, 2015, June 13, 2016, and October 14, 2016) using the acetylene inhibition method (Tiedje, 1982). In brief, potassium nitrate (KNO_3 1 mM), chloramphenicol (1 g L^{-1}), and dextrose (1 mM) were added to 5 g of fresh soil in a 50-mL air-tight flask, kept anaerobic by repeated flushing with N_2. Acetylene (10%, *v*/*v*) was injected to inhibit the reduction of N_2O to N_2, and samples were incubated for 3 h at 25°C. A 5 mL headspace sample was collected using a gas-tight syringe for the determination of N_2O concentration.

2.4 Vegetable yield and area/yield-scaled N-oxide emissions

Fresh cucumber yields (g N t^{-1} yield) were weighed in each plot. Due to the staggered maturation of cucumber, the yields of multiple harvests were added to calculate the total yields. The annual cucumber yield across the three cucumber cropping cycles was used to calculate the annual N-oxide emission below.

The annual N-oxide emission (kg N ha^{-1}) was calculated as total emissions of N-oxide over the experimental period (16 months) multiplied by 12/16. The yield-scaled N-oxide emission (g N t^{-1} yield) was calculated as follows (Linquist et al., 2012):

Yield-scaled N_2O or NO emissions=Area-scaled N_2O or NO emissions/Fresh cucumber yield (1)

The N fertilizer-induced direct EF (%) of N_2O and NO were calculated as:

$$EF_{wy} = (ER_{N} - ER_{0})/N \text{ applied} \times 100 \quad (2)$$

$$EF_{gs} = (ER_{N} - ER_{0})/N \text{ applied} \times 100 \quad (3)$$

$$\Delta EF = EF_{wy} - EF_{gs} \quad (4)$$

where, *wy* and *gs* represent the whole year and cucumber growing season periods, respectively. ER_N (kg N ha^{-1}) is the total N_2O or NO emissions from the fertilizer N-applied (NPK, O, or O+T) plots; ER_0 (kg N ha^{-1}) is the total N_2O or NO emissions from the control.

2.5 ***Statistical analyses***

Data were tested for normality (using Shapiro-Wilk's test) and equality of variance (using Levene's test), and parameters with non-normal distributions or unequal variances were either logarithmical or square-root transformed prior to analysis. Differences in seasonal or annual N-oxide emissions, EFs, and vegetable yield between treatments were determined by the LSD test (P<0.05). The effects of fertilization, season, and their interactions on the fluxes of N_2O, NO and N_2O+NO, and soil properties were examined using a repeated-measures analysis of variance (MANOVA) with the R package MANOVA.RM (Friedrich et al., 2019). A linear model was fitted with the logarithmically transformed values of N_2O+NO emission and soil parameters. To assess the relative importance of predictors in the linear model, we used the R package *relaimpo* to calculate the importance of each soil parameter (Gromping, 2006). The Kruskal–Wallis one-way analysis of variance (ANOVA) was used to investigate differences in the potential denitrification rate between treatments of each sampling date and in the N_2O/NO ratio (log-transformed) during the study period. A nonparametric multiple comparison test (Dunn's test) was performed to compare the difference in the sum of ranks between groups with the expected average difference. All statistical analyses were conducted using R 3.6.3 (R Core Team, 2020).

3. Results

3.1 Environmental variables

Soil temperature during the three cropping cycles was comparable, ranging from 10.7 to 33.5°C (mean: 22.4°C), with the highest value occurring in July 2016 (Fig. 1a). Soil WFPS ranged from 20.5 to 80.6% (mean: 40.6%) in the second cropping cycle and was higher than the average of first and third cropping cycles, ranging from 23.6 to 57.3% (mean: 28.4%). The main reason for this difference was that the field was subjected to heavy rainfall during the second fallow stage due to the refurbishment of the greenhouse. Since there was no significant difference in soil temperature and WFPS among all treatments, the average values are shown in Fig. 1a.

Nitrogen fertilization significantly increased soil NH_4^+-N and NO_3^--N contents, with the highest soil NH_4^+-N and NO_3^--N contents in the NPK plot (both $P<0.001$; Fig. 1b and c, Table S2). Urea had a much stronger effect on soil mineral N content than organic or bio-organic fertilizers. During the whole experimental period, soil NH_4^+ contents averaged 10.0±0.1, 51.0±1.3, 15.6±0.7, and 15.5±1.1 mg N kg^{-1} for the control, NPK, O and O+T plots, respectively. Soil NO_3^- contents ranged from 24.3 to 190.5 mg N kg^{-1} over the entire observation period, averaging 52.5±2.1, 131.7±3.5, 87.7±4.4 and 80.8±1.5 mg N kg^{-1} for the control, NPK, O and O+T plots, respectively.

Organic fertilizer application resulted in an increase in soil pH and DOC content ($P<0.001$; Fig. 1d and f, Table S2). The average pH of the control was 5.5±0.0, which increased significantly to 6.0±0.1 and 6.1±0.1 in the O and O+T plots, respectively, but decreased to 5.1±0.0 in the NPK plot. The mean DOC content in the control plot was 122.2±0.3 mg C kg^{-1}, which increased by 19.4% and 16.0% in the O and O+T plots, respectively, but declined by 11.5% in the NPK plot. The EC value in the NPK plot was greater than the other three treatments, where no statistical differences occurred (Fig. 1e, Table S2).

3.2 N_2O fluxes

The seasonal patterns of N_2O fluxes were similar among the fertilization treatments, with frequent N_2O peaks generally driven by fertilizer application and/or irrigation (and/or rainfall) events (Fig. 2a). Throughout the entire experimental period, N_2O fluxes were lower in the control plot than in fertilized treatments, although several relatively small peaks were observed following irrigation or rainfall events. Seasonal N_2O fluxes averaged 61.2±3.5, 173.8±8.1, 120.9±6.9 and 132.8±11.2μg N m^{-2} h^{-1} for the control, NPK, O and O+T plots, respectively (Table S3). Generally, substantial N_2O emissions occurred during the cucumber-growing stages, while N_2O fluxes were relatively low during the fallow stages, except for the second fallow stage. Across all treatments, total N_2O emissions were 0.33–0.56 and 0.23–0.41 kg N ha^{-1} during the first and third fallow stages, accounting for only 15.6–23.0% and 11.5–18.9% of the first and third cucumber cropping cycle, respectively. While for the second fallow stage, total N_2O emissions across all treatments were 2.07–5.91 kg N ha^{-1}, accounting for 55–61% of the second cucumber cropping cycle (Fig. 2a, Table S4). Overall, relative to the NPK plot, seasonal N_2O emissions were significantly reduced by 13.2–34.3% and 17.4–36.6% under O and O+T plots (P<0.01), respectively, but there was no significant difference between the O and O+T plots (Table S5).

The N fertilizer-induced EF of N_2O over the whole year period (EF_{wy}) ranged from 1.12% to 2.08% (Table 1), with an EF_{gs} of 0.61–0.89%, 0.82–1.81% and 0.52–1.19% for the corresponding cropping cycle (Table S4). Both EF_{wy} and EF_{gs} of N_2O for the three fertilizer plots followed the order NPK > O ≈ O+T, such that EF_{wy} under O and O+T plots were significantly reduced by 35.6% and 46.0% relative to the NPK plot, respectively.

*3.3 **NO fluxes***

The seasonal pattern of the soil NO flux was distinct from that of the soil N_2O flux and differed significantly among N fertilized plots (Fig. 1b). During the entire experimental period, NO fluxes under the NPK plot ranged from 11.7 to 216.0 μg N m^{-2} h^{-1}, with pronounced peaks captured following urea application events. In contrast, the NO fluxes in the O and O+T plots remained unaffected. Seasonal mean NO fluxes were 16.0±1.7, 64.2±4.4, 21.3±1.6, and 17.4±1.6 μg N m^{-2} h^{-1} for the control, NPK, O and O+T plots, respectively (Table S3).

Across all treatments, annual NO emissions ranged from 1.28 to 5.01 kg N ha^{-1}, with the corresponding range of 0.51–1.76, 0.68–3.00, and 0.48–1.92 kg N ha^{-1} for the three cucumber cropping cycles (Table1, Table S4). About 56.7% of NO emissions occurred during the fallow periods. Across all treatments, the mean total NO emission during the second fallow period was 0.62±0.03 kg N ha^{-1}, which was slightly higher than that of 0.55±0.04 kg N ha^{-1} (P >0.05) in the first fallow period and significantly higher than that of 0.43±0.03 kg N ha^{-1} (P <0.05) in the third fallow period. Relative to the NPK plot, seasonal NO emissions were significantly reduced by 60.3-70.8% and 69.3-76.2% in the O and O+T plots, respectively, while there was no clear difference between the O and O+T plots. The N fertilizer-induced EF for NO (EF_{gs}) was -0.02–0.28%, -0.01–0.84%, and 0.03–0.50% for the first, second, and third cropping cycle, respectively (Table S4). The EF_{wy}s of NO in the O and O+T plots were significantly decreased by 90.3% and 97.4% relative to the NPK plot, respectively (Table 1).

3.4 ***Combined N_2O+NO emissions and yields***

Annual N_2O+NO emissions totaled 5.98, 18.2, 11.8, and 10.6 kg N ha^{-1} for the control, NPK, O, and O+T plots, respectively (Table 1). The application of N fertilizers enhanced vegetable yields by 6.5–35.6%. Vegetable yields decreased by 2.9–5.8% in the O plot, but increased by 2.8-9.0% in the O+T plot, when compared to the NPK plot. The annual area- and yield-scaled N_2O+NO emissions were significantly higher in the NPK plot than in the O and O+T plots

(Table 1). Compared with the O plot, annual yield-scaled N_2O+NO emissions were significantly reduced by 17.7% in the O+T plot. The N fertilizer-induced emission factor of combined N_2O+NO (EF_{wy}) was 3.00%, 1.45%, and 1.15% for the NPK, O, and O+T plots over the annual scale, respectively (Table 1).

3.5 ***The potential denitrification rate and variable importance analysis***

Soil potential denitrification rates were measured at the end of each cucumber growing season and differed significantly between treatments. Nitrogen fertilization generally increased soil potential denitrification rate relative to the control (Fig. 3). A greater increase in the potential denitrification rate was observed in the O (110.7–222.4%) and O+T plots (219.7–287.5%) compared with the NPK plot (22.5–53.4%), and the O+T plot always had the highest potential denitrification rate.

Results of linear regression analysis showed that the selected variables explained 71.4% to 81.4% of the observed variability in the combined N_2O+NO emissions (Fig. 4). Across all treatments, soil temperature and WFPS were the first two important variables in explaining the variance of N_2O+NO emissions. Soil NH_4^+ content played a greater role in the NPK plot, whereas soil NO_3^- in the O and O+T plots was more important than the remaining variables.

4. Discussion

4.1 ***Responses of N-oxide emissions to synthetic and organic N fertilizer applications***

Our results demonstrated that applying organic fertilizers instead of synthetic N fertilizer can significantly decrease soil N_2O and NO emissions in greenhouse cucumber monocultures with a drip irrigation system. For comparison, we compiled data from previous studies as shown in Table 2 and found that the mitigation effects of organic substitution on soil N-oxide emissions also occurred in cereal (Meijide et al., 2007; Yan et al., 2015; Yao et al., 2019b) and other vegetable fields (Akiyama and Tsuruta, 2003a; Vallejo et al., 2006). Several reasons below may contribute to explain lower N-oxide emissions following organic N fertilizer application. First, relative to the organic N fertilization treatments, significantly higher mineral N concentrations in the NPK plot may have contributed to greater N-oxide emissions, which is the major factor driving microbial formation of N-oxide (Davidson et al., 2000). This is consistent with the expectations of the conceptual hole-in-the-pipe model, which hypothesizes that the total N-oxide gas flux is proportional to the rates of microbial N transformation processes (Firestone and Davidson, 1989). The lower WFPS in this study (usually <60%) suggested that the nitrifying communities may play an important role in soil N_2O and NO production (Bateman and Baggs, 2005; Russow et al., 2008). Hence, the second possible reason is that niche specialization of AOB and AOA associated with NH_4^+ supply may result in differences in N_2O emission between synthetic and organic fertilization treatments (Hink et al., 2017; 2018). As suggested by Hink et al. (2018), low NH_4^+ supply from the organic fertilization treatments may favor AOA growth with low N_2O yield (N_2O-N produced per NO_2^--N generated from ammonia oxidation), while high NH_4^+ supply after urea application can lead to growth of AOA and AOB, thereby resulting in greater N_2O production because of high N_2O yield by AOB. Furthermore, we probably attributed the reduced emission of N-oxide following organic fertilizers application to the enhanced soil denitrification and the promoted reduction of N_2O to N_2. Support for this assumption comes from recent evidence suggesting that organic fertilizer application can promote the consumption of N_2O to N_2 in global agricultural soils, despite increased soil denitrification following synthetic or organic fertilizer application (Wang et al., 2018). The rationale for this is that organic C addition can stimulate heterotrophic microbial activity and enhance soil O_2 consumption, which thereby favors the development of anaerobic conditions for denitrification (Dambreville et al., 2006; Kramer et al., 2006).

Nevertheless, organic fertilizer application has divergent impacts on soil N_2O emissions (Table 2). For example, some studies have shown that organic fertilizer increased N_2O emissions 6-fold compared to synthetic N fertilizer (Akiyama and Tsuruta, 2003b; Hayakawa et al., 2009). Differential responses of N_2O emissions to organic fertilizer application are associated with their decomposability, that is, organic matter with a relatively low C/N ratio can be important source, but organic matter with a relatively high C/N ratio is less likely to emit N_2O (Akiyama and Tsuruta 2003a). A recent meta-analysis indicated that the threshold of C/N ratios for organic fertilizers was 8.6 (95% confidence interval: 4.5–22.3) in terms of the positive or negative responses of N_2O emissions from acid soils (He et al., 2019). In our study, organic fertilizers with a C/N ratio of ~15 contributed to lower N_2O emissions relative to the chemical N application.

Regarding NO, it is an essential metabolite in AOA that is produced and immediately consumed with tight control, whereas AOB tend to produce and release it (Kozlowski et al., 2016; Stein, 2019). Support for this explanation comes from a global meta-analysis where it was found that organic or mixed N fertilizer application is less effective at stimulating soil NO emissions when compared to synthetic N fertilizer application (Liu et al., 2017). Unlike

N_2O, the consistent and negative response of soil NO emission to organic fertilizer applications as shown in Table 2 is likely due to i) as discussed above, the dominant AOA emit less NO when organic fertilizer is applied (Kozlowski et al., 2016; Stein, 2019); and ii) the addition of organic matter can promote some heterotrophic bacteria to oxidize NO through aerobic co-oxidation, rather than reducing NO to N_2O by denitrifying bacteria (Dunfield and Knowles, 1998).

4.2 Responses of yield-scaled N-oxide emissions to synthetic and organic N fertilizer applications

When linking N-oxide emissions to vegetable yields, our results show that organic and bio-organic fertilizer applications significantly decreased yield-scaled N_2O+NO emissions when compared to the synthetic N fertilizer. However, there was a yield penalty when organic fertilizer was used, but the use of bio-organic fertilizer slightly increased vegetable yields. In agreement with our findings, plant growth and crop yield were better in the *Trichoderma* enriched bio-organic treatment than in the organic fertilizer treatment (Pang et al., 2017). *Trichoderma*.spp were reported to be able to produce auxin-like phytohormones and several sparingly soluble nutrients, thereby promoting the development of the plant root system and uptake of soil nutrients (Cai et al., 2013). Enriched bio-organic fertilizer can also maintain a more diverse and stable soil microbiome for plant growth (Pang et al., 2017). Additionally, a meta-analysis of 143 studies showed that the full substitution of chemical N by manure in short-or medium-term (<10 years) will decrease crop yields, but the negative impact on yields will disappear over time (≥10 years), probably due to the various positive effects of manure in soil remediation (Zhang et al., 2020). Thus, our results demonstrate that the combination of organic fertilizers with microbial inoculants present a potentially pragmatic option for maintaining crop yield and decreasing yield-scaled N-oxide emissions.

4.3 ***Emission factors of N_2O and NO***

The N fertilizer-induced EF of N_2O and NO may be underestimated without considering their emissions during the fallow period. Our results showed that EF_{gs} of N_2O and NO were, on average, 41% and 58% lower, respectively than those calculated for the whole cycle. The underestimation of NO by more than half indicates that the fallow period is an important period for NO emissions in this vegetable system. It has been well acknowledged that N-oxide emissions from agricultural land are spatially and temporally variable (Smith, 2017). In this study, the EFs of N_2O and NO varied seasonally because of changes in environmental conditions (Table S4). The greater N-oxide emissions were captured during the second cucumber cropping cycle when soil moisture ranged from 40 to 80% WFPS and soil temperature became gradually elevated. Thus, there is a need to measure soil N-oxide emissions over the whole year if we are to obtain the reliable EFs for their emission estimates using the Tier 2 method (Shang et al., 2020; Hergoualc'h et al., 2019).

Across all fertilized treatments, the mean value of EF_{wy} of N_2O is 1.51%, which is very close to the mean EF of N_2O (1.41%, 95% confidence interval: 1.19–1.64%) for vegetable cropping systems (Yang et al., 2019). The EF_{wy} of N_2O also falls within the IPCC reported default range of 0.1–1.8% (Hergoualc'h et al., 2019). However, our results are notably higher than the earlier estimates of 0.49–0.55% for Chinese vegetable fields (Wang et al., 2011), 0.51% for drip irrgation systems in Spain (Cayuela et al., 2017), and 0.94% for global vegetable fields (Rashti et al., 2015). For NO, the EF_{wy} of 0.92% in the NPK plot was largely higher than 0.03% and 0.09% for O and O+T plots. These values were markedly lower than the reported mean value of 1.71% for global vegetable fields (Liu et al., 2017). Similarly, the EF_{wy} of N_2O+NO was estimated to be 1.15–3.00%, which was lower than the global average of 4.13% for vegetable fields (Liu et al., 2017). Collectively, these results suggest that there is great uncertainty in estimating N-oxide emissions from specific cropping system using default EFs.

4.4 Implications

This study reveals several key implications for soil N-oxide emissions within intensively managed greenhouse vegetable cropping systems. First, substantial increases in the magnitude of soil N-oxide emissions can be expected following N application from this cropping system under drip irrigation, independent of the forms of N fertilizer. In order to avoid significant N-oxide emissions, it is necessary to avoid maintaining plastic greenhouses in the hot-rainy summers in the subtropical regions. Second, the shift from synthetic N fertilizers to organic fertilizers can mitigate soil N-oxide emissions under greenhouse vegetable cultivation conditions. Our results demonstrate that organic fertilizers not only contribute greatly to reduced N-oxide emissions but also result in a very low emission intensity for NO. Third, from an agronomic perspective, bio-organic fertilizer is a promising option for fertilizer management in the current cropping system because of the significantly lower yield-scaled N-oxide emissions. We found that the fresh vegetable yield in bio-organic fertilizer plot was comparable to and slightly higher than that of the synthetic fertilization plot. Finally, our findings highlight the importance of the year-round measurement of soil N-oxide emissions toward a reliable estimate of their emissions. Taken together, combining soil beneficial microbial inoculants with organic fertilizers is a promising strategy to reduce N losses and ensure yield in intensive greenhouse vegetable cropping systems. Further research is required to explore the effectiveness of microbial strains inoculate with organic fertilizer under a wider range of soil and environmental conditions.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1 Total annual N_2O, NO, N_2O+NO emissions (kg N ha^{-1}) and their direct emission factors (EF, %), cucumber yield (t ha^{-1}), and yield-scaled N_2O, NO and N_2O+NO emissions (g N t^{-1} yield).

	N_2O				NO				N_2O+NO			
	Control	NPK	O	O+T	Control	NPK	O	O+T	Control	NPK	O	O+T
Area-scaled	4.71±0.28c	13.1±0.48a	10.1±0.78b	9.26±0.59b	1.28±0.13b	5.01±0.34a	1.64±0.12b	1.37±0.12b	5.98±0.36 c	18.2±0.13a	11.8±0.79b	10.6±0.68b
Yield	100±1.33c	117±2.85ab	111±1.71b	122±1.98a								
Yield-scaled	47.0±2.30c	113±6.99a	91.0±5.68b	75.9±3.62b	12.8±1.35b	42.9±1.93a	14.8±1.18b	11.3±0.81b	59.8±3.14d	156±5.07a	106±5.61b	87.1±4.13c
EF_{wy}		2.08±0.12a	1.34±0.19b	1.12±0.12b		0.92±0.08 a	0.09±0.03b	0.03±0.03b		3.00±0.03 a	1.43±0.19b	1.15±0.17b
EF_{gs}		1.30±0.05a	0.76±0.08b	0.65±0.08b		0.54±0.06 a	0.03±0.01b	0.01±0.01b		1.84±0.02 a	0.79±0.07b	0.66±0.09b
ΔEF		0.79	0.58	0.47		0.38	0.06	0.02		1.17	0.64	0.49

Values are means ± SEM (*n*=3). Different letters within the row for each variable indicate significant differences between treatments at $P<0.05$. Control, without fertilization; NPK, chemical fertilizer-urea; O, organic fertilizer derived from composted cattle manure with mushroom residue; O+T, bio-organic fertilizer consisting of organic fertilizer plus *Trichoderma guizhouense* NJAU 4742.

Table 2 An overview of studies simultaneously investigating the impacts of synthetic and organic fertilizer application on soil N_2O and NO emissions

Site/ climate	Crop/ soil	Day (d)	N fertilizer		N emissions (kg N ha^{-1})				% of change relative to synthetic N					
			Type	N input (kg N ha^{-1})	N_2O	NO	N_2O+NO	EF_c	N_2O	NO	N_2O+NO	Yield-scaled	EF_c	
Jiangsu, China/	Vegetable/	130	CMT	180	4.12	0.61	4.73	1.15	-30	-73	-41	-44	-62	This study
Subtropical-	Clay		CM	180	4.5	0.73	5.23	1.43	-23	-67	-35	-32	-52	
monsoon			U	180	5.84	2.23	8.07	3.00						
Hubei, China/	Rice	135	50%U+50%PM	150	0.87	0.07	0.94	0.48	-77	-42	-76	-76	-80	Yao et al.,
Subtropical-	Silt loam		U	150	3.74	0.12	3.86	2.42						2019 b
monsoon			50%U+50%PM	150	1.05	0.07	1.12	0.52	-77	-59	-76	-76	-81	
			U	150	4.47	0.17	4.64	2.87						
Madrid, Spain	Maize	365	67%U+33%CPS	180	0.66	3.5	4.16	0.90	-15	13	7	61	21	Guardia et
Temperate	Sandy-		67%U+33%LFPS	180	0.53	3.9	4.43	1.05	-32	26	14	55	41	al., 2017
	clay loam		U	180	0.78	3.1	3.88	0.75						
Shandong, China	Wheat	240	70%(U+DP)+30%M	180	0.74	0.3	1.04	0.38	-16	-54	-32	-10	-42	Yan et al.,
Subtropical	Silt loam		70%CRU+30%M	180	0.89	0.35	1.24	0.49	1	-46	-19	-3	-25	2015
monsoon			U+DP	180	0.88	0.65	1.53	0.65						
Madrid, Spain	Maize	142	UPS	175	8.27	0.13	8.4	1.38	-4	-43	-5	-	-14	Meijide et
Subtropical	Sandy-		DPS	175	7.7	0.13	7.83	1.05	-10	-43	-11	-	-34	al., 2007
	loam		71% PS+29%U	175	9.28	0.1	9.38	1.94	8	-57	7	-	20	
			71% SW+29%U	175	7.13	0.03	7.16	0.67	17	-87	-19	-	-58	
			U	175	8.57	0.23	8.8	1.61						

Madrid, Spain	Vegetable	150	UPS	175	5.62	0.1	5.72	1.15	-23	-58	-24	-	-48	Vallejo et
Temperate	Clay loam		DPS	175	4.69	0.1	4.79	0.63	-36	-58	-37	-	-71	al., 2006
			CPS	175	6.41	0.17	6.58	1.65	-12	-29	-13	-	-25	
			71%SW+ 29%U	175	5.65	0.07	5.72	1.16	-23	-71	-24	-	-48	
			U	175	7.31	0.24	7.55	2.21						
Tsukuba, Japan	Vegetable	42	PM	150	1.71	0.12	1.83	-	494	-92	3	-	-	Akiyama
Temperate	Sandy-		SM	150	0.47	0.16	0.63	-	63	-89	-64	-	-	and Tsuruta,
marine	loam		U	150	0.29	1.49	1.78	-						2003 b
Tsukuba, Japan	Vegetable	48	OC	150	0.35	0.23	0.58	-	223	-57	-8	-	-	Akiyama
Temperate	Sandy-		CM	150	0.03	0.02	0.05	-	-77	-95	-92	-	-	and Tsuruta,
marine	loam		U	150	0.11	0.52	0.63	-						2003 a
		47	FM	150	0.35	0.21	0.55	-	64	-80	-56	-	-	
			DC	150	0.06	0.08	0.14	-	-70	-93	-89	-	-	
			U	150	0.21	1.05	1.26	-						
Japan	Vegetable	365	PM	240	0.70	0.79	1.49	-	84	-50	-23	-	-	Hayakawa et
Temperate	Sandy-		PPM	240	2.72	0.69	3.41	-	616	-56	76	-	-	al., 2009
marine	loam		AS	240	0.38	1.56	1.94	-						

EF_c, emission factor of N_2O+NO; CMT, Cattle manure+*Trichoderma. spp*; CM, Cattle manure; U, Urea; PM, Poultry manure; CPS, Composting solid fraction of pig slurry; LFPS, Liquid fraction of pig slurry; DP, Diammonium phosphate; M, Manure; CRU, Controlled-release urea; UPS, Untreated pig slurry; DPS, Digested pig slurry; PS, Pig slurry; SW, Solid waste; SM, Swine manure; OC, Oilcake; FM, Fishmeal; DC, Dried cattle excreta; PPM, Pelleted poultry manure; AS, Ammonium sulfate.

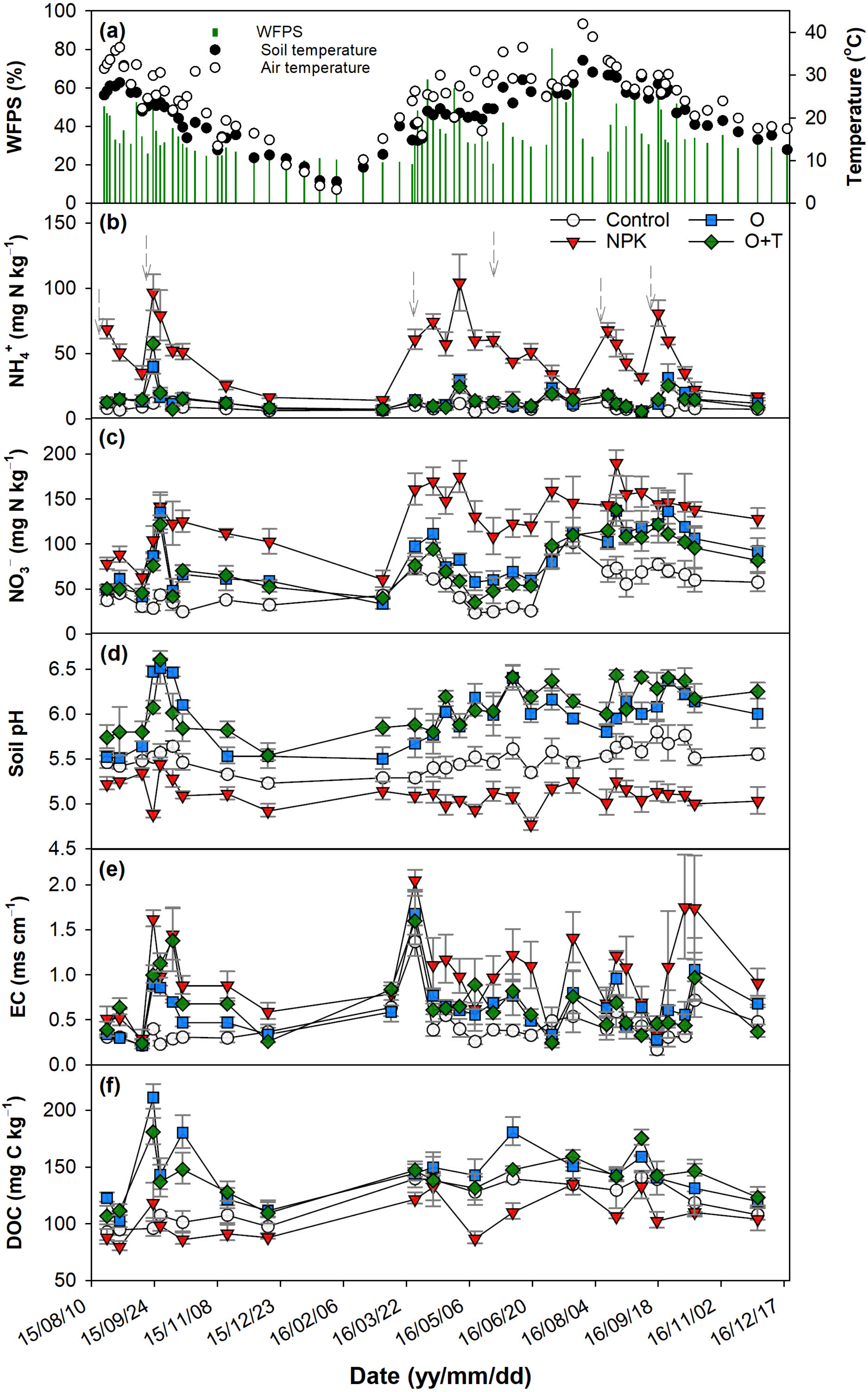
Figure captions

Fig. 1 Soil water-filled pore space (WFPS), soil and air temperature (**a**), NH_4^+ (**b**), NO_3^- (**c**), pH (**d**), electrical conductivity (EC; **e**), dissolved organic carbon (DOC; **f**) from a greenhouse mono-cucumber field under different fertilization treatments. The gray dashed arrows indicate fertilization. Values are means ± SEM (*n*=3). Control, without fertilization; NPK, chemical fertilizer-urea; O, organic fertilizer derived from composted cattle manure with mushroom residue; O+T, bio-organic fertilizer consisting of organic fertilizer plus *Trichoderma guizhouense* NJAU 4742.

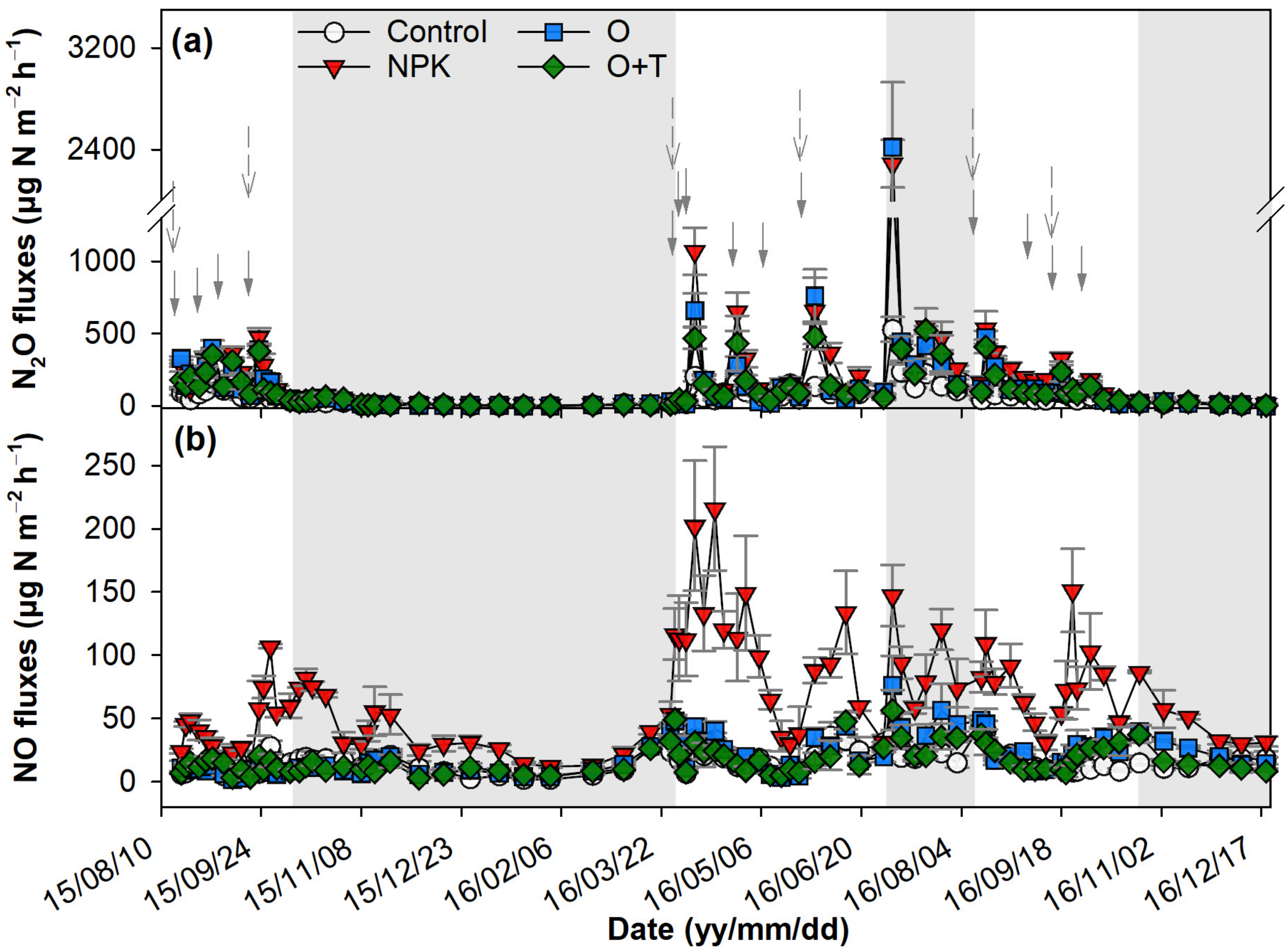
Fig. 2 Year-round fluxes of soil N_2O (**a**) and NO (**b**) from a greenhouse mono-cucumber field under different fertilization treatments. The gray dashed and solid line arrows indicate fertilization and irrigation, respectively. Grey shading marks the fallow period between the cucumber growing seasons. The field during the second fallow period was open-air because of the refurbishment of the greenhouse. Values are means ± SEM (*n*=3). Control, without fertilization; NPK, chemical fertilizer-urea; O, organic fertilizer derived from composted cattle manure with mushroom residue; O+T, bio-organic fertilizer consisting of organic fertilizer plus *Trichoderma guizhouense* NJAU 4742.

Fig. 3 Potential denitrification rates in different sampling periods under different fertilization treatments. Different letters refer to significant differences between treatments at the P<0.05. Control, without fertilization; NPK, chemical fertilizer-urea; O, organic fertilizer derived from composted cattle manure with mushroom residue; O+T, bio-organic fertilizer consisting of organic fertilizer plus *Trichoderma guizhouense* NJAU 4742.

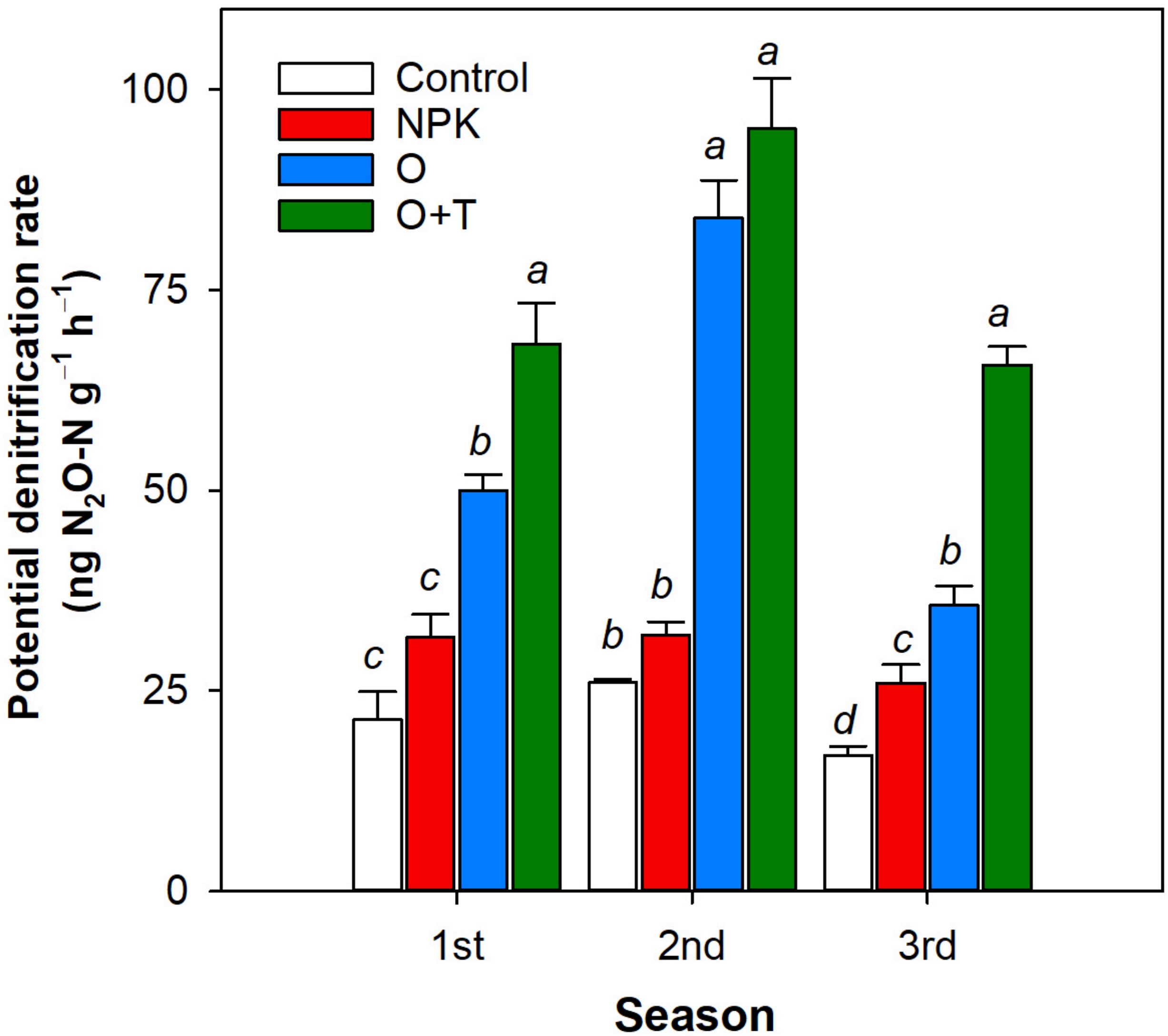
Fig. 4 The relative importance of environmental factors in predicting N_2O+NO emissions with a linear regression model under different fertilization treatments. Control, without fertilization; NPK, chemical fertilizer-urea; O, organic fertilizer derived from composted cattle manure with mushroom residue; O+T, bio-organic fertilizer consisting of organic fertilizer plus *Trichoderma guizhouense* NJAU 4742.



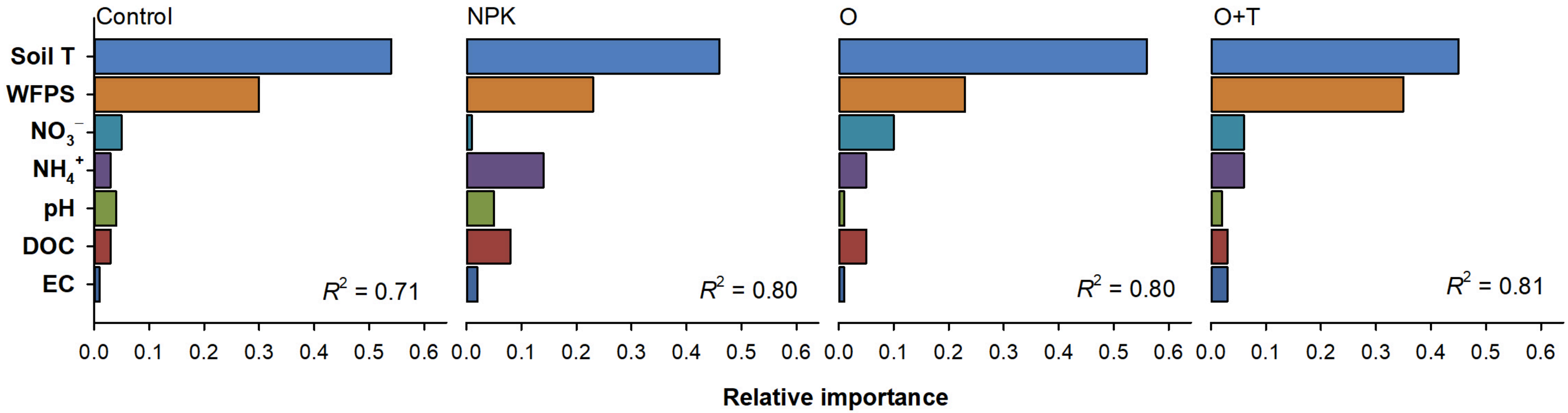
(a)
WFPS
Soil temperature
Air temperature
WFPS (%)
Temperature (°C)
(b)
Control
O
NPK
O+T
NH4+ (mg N kg-1)
(c)
NO3- (mg N kg-1)
(d)
Soil pH
(e)
EC (ms cm-1)
(f)
DOC (mg C kg-1)
15/08/10
15/09/24
15/11/08
15/12/23
16/02/06
16/03/22
16/05/06
16/06/20
16/08/04
16/09/18
16/11/02
16/12/17
Date (yy/mm/dd)



(a)
Control
NPK
O
O+T
3200
2400
1000
500
0
N_2O fluxes (μg N m^{-2} h^{-1})
(b)
250
200
150
100
50
0
NO fluxes (μg N m^{-2} h^{-1})
15/08/10
15/09/24
15/11/08
15/12/23
16/02/06
16/03/22
16/05/06
16/06/20
16/08/04
16/09/18
16/11/02
16/12/17
Date (yy/mm/dd)



Control
NPK
O
O+T
Potential denitrification rate
(ng N_2O-N g^{-1} h^{-1})
100
75
50
25
0
a
a
a
a
b
b
b
b
b
c
c
c
c
d
1st
2nd
3rd
Season



Control
NPK
O
O+T
Soil T
WFPS
NO3−
NH4+
pH
DOC
EC
R2 = 0.71
R2 = 0.80
R2 = 0.80
R2 = 0.81
0.0 0.1 0.2 0.3 0.4 0.5 0.6
Relative importance

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Soil N-oxide emissions decrease from intensive greenhouse vegetable fields by substituting synthetic N fertilizer with organic and bio-organic fertilizers

Geng, Yajun

2020-09-24

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