Fungi Contribute More to N2O Emissions than Bacteria in Nitrogen Fertilized Lawn Soil

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Abstract

Lawn is the primary vegetation utilized for urban sporting grounds and garden greenbelts. The N fertilizer addition induced higher N2O emission in various soils including the lawn soil, however, a key gap in knowledge lies in identifying the ecological consequences of the N2O emission and potentially associated microbial mechanisms. N2O is a product of microbial participation in nitrogen transformation processes, which is closely related to the use of nitrogen fertilizer. This study evaluated the effect of N fertilizer on N2O emissions and associated microbial mechanism in lawn soil through incubated experiment under different N application rates [300 kg ha yr-1 (N300), 225 kg ha yr-1 (N225), 150 kg ha yr-1 (N150) and control (N0)]. In addition, the contribution of different microbial communities to N2O emissions was quantified by combining biological inhibitors with high-throughput sequencing. The results indicated that N fertilizer addition induced higher N2O emissions in lawn soil, showed the highest in the N225 treatment. The contribution of fungi to N2O emissions was 45%, significantly higher than that of bacteria (31%). The dominant fungi in the lawn soil included Ascomycota, Basidiomycota, and Mucoromycota. N fertilizer significantly increased the relative abundance of Ascomycota and decreased the relative abundance of Basidiomycota. We found a positive correlation between N2O emission and Ascomycota through RDA analysis. The growth trend of Ascomycota during the four nitrogen fertilizer treatments was consistent with the N2O emission trend in lawn soil. N2O emissions reached their highest levels after the N225 treatment. The relative abundance of Pyrenochaetopsis, Myrothecium, and Humicola was positively correlated with N2O emission. Thus, Pyrenochaetopsis, Myrothecium, and Humicola were found to be the main functional microorganisms leading to N2O production in lawn soil. Our findings can deepen the understanding on N2O emission and associated microbial mechanism in lawn soil with N fertilization.

Abstract

The N fertilizer addition induced higher N_2O emission in various soils including the lawn soil, however, a key gap in knowledge lies in identifying the ecological consequences of the N_2O emission and potentially associated microbial mechanisms. This study evaluated the effect of N fertilizer on N_2O emissions and associated microbial mechanism in lawn soil through incubated experiment under different N application rates. In addition, the contribution of different microbial communities to N_2O emissions was quantified by combining biological inhibitors with high-throughput sequencing. The results indicated that N fertilizer addition induced higher N_2O emissions in lawn soil and the contribution of fungi to N_2O emissions was significantly higher than that of bacteria. We found a positive correlation between N_2O emission and Ascomycota through RDA analysis. The growth trend of Ascomycota during the four nitrogen fertilizer treatments was consistent with the N_2O emission trend in lawn soil. The relative abundance of*Pyrenochaetopsis* , *Myrothecium* , and *Humicola* was positively correlated with N_2O emission. Thus,*Pyrenochaetopsis* , *Myrothecium* , and *Humicola* were found to be the main functional microorganisms leading to N_2O production in lawn soil. Our findings can deepen the understanding on N_2O emission and associated microbial mechanism in lawn soil with N fertilization.

Keywords: lawn soil, N fertilizer, N_2O emission, biological inhibitors, fungi

1. Introduction

Lawn, including urban green space, parks, athletic fields, roadsides and home lawns, is one of the most popular landscapes worldwide and provides numerous ecological, environmental and economic benefits[1]. However, the rapid increase of lawn area has raised concerns about turfgrass breeding, resistance of lawn, and pollution associated with fertilizers and pesticides, which are used for lawn establishment and maintenance. Still, the N_2O emission from urban lawn soil has received less attention. It is unclear how the N_2O emission from lawn soil change along lawn management.

Nitrous oxide (N_2O) is one of the three major greenhouse gases [2]. N_2O can generate 300 times stronger effect on global warming than carbon dioxide in the troposphere and is a stable greenhouse gas. It has the capacity to reacts with ozone and became the dominant substance in ozone depletion[3]. N_2O is also an important part of the nitrogen cycle in terrestrial ecosystems. Not always are high. It depends on the ecosystem and management practice. The impact of those practices (tillage, fertilization, irrigation) is studied aiming to reduce their contribution to N_2O emissions. More attention that the addition of exogenous nitrogen, such as atmospheric nitrogen deposition and fertilization, alters the pathway of N_2O production [4–6]. Meanwhile, previous studies have shown that the addition of a nitrogen fertilizer could increase N_2O emissions[7-10] For instance, compared with unfertilized lawn soil, nitrogen fertilization significantly increased N_2O emission in Baltimore, USA[11]. Further, a fertilization addition experiment in lawn in Phoenix, Arizona, USA also reported urbanization increased N_2O emissions compared to native landscapes, primarily due to large amount of nitrogen fertilizer applied to lawn[12]. However, other studies found that the addition of nitrogen fertilizer did not increase N_2O emission, for instance, studies on sandy loam and grasslands in the UK found that the addition of nitrogen fertilizer did not increase N_2O emissions [13]. Similar results were also found in a farmland soil study in Shandong Province, China [14]. These results indicate that the effect of exogenous nitrogen addition on N_2O emission has not been elucidated yet, and emissions could be either inhibited or promoted, which likely was related to uncertain factors such as nitrogen fertilizer form, vegetation type, soil properties, and microbial action. Thus, it is important to understand the effect of fertilization on soil N_2O emission when formulating the scientific rational for nitrogen regulation.

N_2O is generally produced in soils through microbiological nitrification and denitrification in soils. Therefore, Microorganisms play a key role in soil nitrogen transformation. Previous studies suggested that N_2O emission in soil was caused by bacterial nitrification and denitrification, because the conventional nitrogen transformation is thought to be dominated primarily by bacteria[15]. However, recent studies have shown that fungi are the main players in N_2O emission. In the arid grassland of the southwestern United States, N_2O was mainly produced by the nitrification and denitrification processes of fungi, not bacterial ammonia oxidation [16]. N_2O in the Qinghai-Tibet Plateau within China was also found to be largely produced by fungi [17]. These studies debunked the conventional idea that soil N_2O was produced primarily by bacteria. With the mature application of 15N labelling technology, the contribution of heterotrophic nitrification to N_2O emissions has attracted increasing attention, and fungi are considered the largest functional microorganisms[18,19]. Some studies also suggested that fungi played an even greater role in N_2O emission. In the temperate grassland, fungi contributed to 86-89% of the N_2O emissions [20]. Fungi contributed to 54% of N_2O emissions during the nitrification process and 63% in the denitrification process in an alpine grassland [17]. Moreover, some studies have showed that bacteria are the main contributors to N_2O emissions. For instance, bacterial denitrification was dominant in farmland soils in northern China (winter wheat and summer maize rotation farmland) [21]. Therefore, the microbiological mechanism involved in N_2O emission from different vegetation types and ecological regions varies considerably. Clarifying the microbial mechanism of N_2O emission is a prerequisite o for managing N_2O emissions.

Lawn, which has the characteristics of high coverage, more roots, and more fertilization, is the main vegetation of sports fields and urban park green spaces and is quite different from natural grassland and farmland soil. In 2001, China's urban lawn area was 0.94 million ha, while in 2010, it grew to 2.13 million ha, an

increase of 1.3 times[22]. With the policy about greenbelt issued, lawn area will continue to increase in China. Therefore, proper management of this ground cover is important for improving urban environmental quality and formulating more accurate emission reduction measures. In a previous study, we found that fertilization promoted N_2O emission of lawn soil, and heterotrophic nitrification contributed to N_2O emission by 63% [23]. Yet, we still know very little on the associated microbial mechanism of N_2O emission from lawn soils. In this study, we investigated N_2O emission from lawn soil and the associated microbial mechanism under three fertilization gradients (N300, N255, and N150). Our study provides insights into the impacts of N fertilizer addition on N_2O emission in lawn soil and the improvement of fertilizer utilize strategy. We hypothesized that N_2O emission increased with the increase of nitrogen application, and fungi contributed more to N_2O emissions than bacteria in lawn soil.

2. Materials and Methods

2.1 Site Description and Experimental Design

The experiment was conducted in Baicao Garden Teaching and Research base of Shenyang Agricultural University, Shenyang City Liaoning Province, northeast China (123°25'E, 41°46'N). The region belonged to a temperate semi-humid continental climate zone, with an annual average temperature of 6.5 , annual precipitation of 700 mm, and brown soil type (Typic Eutrochrepts in the US soil taxonomy). The soil properties were as follows: an organic carbon content of 7.33 g*kg-1, a total nitrogen content of 0.93 g*kg-1, an ammoniacal nitrogen content of 3.21 mg*kg-1, a nitrate nitrogen content of 25.99 mg*kg-1, available P 12.36 mg*kg-1, and available K 98.96 mg*kg-1, pH of 7.0. The lawn was planted in the experimental site on May 7, 2016 and was dominated by Kentucky bluegrass (*Poa pratensis* L.) of the variety Merit.

The experiment was started by applying N fertilizer in 2016. Four gradients were set according to the standard of lawn management and fertilization in north China. The gradients decreased from 300 kg kg*ha*yr-1 to 225 kg*ha*yr-1, 150 kg*ha*yr-1 and control treatments. They were referred to as the N300, N225, N150, and N0 treatments, respectively. In total, 12 plots of 1 x 1 m size were fully randomized throughout the study site, and each treatment was repeated three times. The annual urea equivalent was divided into two parts; fertilization was carried out on May 15 and August 15, continuous fertilization for two years. Sprinkler irrigation was carried out immediately after fertilization.

Five soil cores were randomly taken from each plot from a depth of 0-20 cm and then mixed to form one composite sample on the day after the last fertilization. All soil samples were transported to the laboratory and sieved through a 2 mm mesh within 24 h. The soils were divided into two parts. One part of the soil was used to measure physicochemical properties. The remainder of each sample was stored at -80 for subsequent laboratory tests and total DNA extraction of soil microorganisms.

2.2 N_2O flux measurement

To measure the N_2O flux potential in the soil, 30 g dry weight soil was placed into Erlenmeyer flasks (250 mL) and moistened to 50% of water holding capacity. All flasks were sealed with silicone rubber stoppers with three-way valves. Adjusted soil samples were held for 1 week prior to incubation at 25 and 50% relative air humidity in the dark under aerobic conditions to activate microorganisms. During the incubation period, the samples were stored in an incubator under dark conditions at 25 and 50% relative air humidity and adjusted to 50% of the water-holding capacity (WHC). The soil moisture content was maintained by adding deionized water every 3 days with a micro pipette to compensate for water loss. The samples were aerated by removing the stoppers for 1 h every day. A total of 84 flasks were divided into four treatments (N0, N150, N225, and N300 treatment), with three independent replicates. Gas samples from each treatment was taken in order to determine N_2O flux on 1, 3, 5, 7, 9, 12, and 15 days. Samples were taken using a 50 mL syringe, fitted with a three-way-stopcock and 22G hypodermic needle, and then injected into pre-prepared 50 ml vacuum flasks for determination of the N_2O flux. The remaining soil samples were used for the determination of NH_4+-N and NO_3–N after taking gas samples. The N_2O concentration was determined using a gas chromatograph (GC; Agilent 7890, Agilent technologies, Santa Clara, CA, USA). N_2O gasstandards were supplied by the National Research Center for Certified Reference Materials, Beijing, China. N_2O flux was calculated according to the

following formula (3):

F= {(C - C0) x V x M x [273 / (273 + T)]} / (d x m x 22.4 x 1000) (1)

F represents N_2O flux (μg·kg-1·d-1). C represents N_2O concentration (μL·L-1). C_0represents the gas concentration above the culture bottle at the beginning of the culture (μL·L-1). V represents gas volume (mL). M represents the mass of N_2O per mole (44). T represents incubator temperature (25). The lowercase letter "d" represents incubation time (d/h). The lowercase letter "m" represents dry soil quality (30g).

2.3 Distinguishing between quantifying the contribution of fungi and bacteria to N_2O emission

Biological inhibitors were used to distinguish between the contribution of fungi and bacteria to lawn soil N_2O emission. Four treatments were established according to the results of previous studies[16,24-26]: (i) cycloheximide ($C_{15}H_{23}NO_4$, a fungicide) treatment at 1.5 mg g-1 was used to inhibit the fungal activity, (ii) streptomycin treatment ($C_{42}H_{84}N_{14}O_{36}S_3$, a bactericide) at 3.0 mg g-1 was used to inhibit the bacterial activity, (iii) cycloheximide at 1.5 mg g-1 and streptomycin at 3.0 mg g-1 were used to inhibit both fungal and bacterial activity, (iv) a no-inhibitor control was used to assess the total microbiological activity contribution to N_2O. The contribution of soil fungi to N_2O emission was estimated by the equation (1). The contribution to N_2O emission of soil bacteria was estimated by the equation (2).

Fungal contribution rate = 100 x (A – B) / (A - D) (1)

Bacterial contribution rate = 100 x (A – C) / (A - D) (2)

A represents N_2O fluxes in the no-inhibitor control. B represents N_2O fluxes in the cycloheximide treatment. C represents N_2O fluxes in the streptomycin treatment. D represents N_2O fluxes in the cycloheximide and streptomycin treatment.

Soils were amended with glucose (2 mg C g-1 soil) and KNO_3 (100 μg N g-1 soil). All chemicals were dissolved in 2 mL distilled water and added drop to soil to ensure sample homogenization. Deionized water was added to 50% of the soil water holding capacity. The flasks were pre-incubated in a dark climate chamber (25 , 50% relative air humidity, 50%WHC) for 24 h to ensure that biocides had taken effect, and flasks were capped to prevent water evaporation. The flasks were then uncapped for 1 h and resealed for the start of the 48h incubation period in a dark climate chamber (25 , 50% relative air humidity, 50%WHC). During the incubation period, gas samples were collected at 1, 3, 7, 12 and 24 h.

2.4 Soil property analysis

Soil NH_4+-N and NO_3–N were extracted with a 2 M KCl solution at a soil/water ratio of 1:5 at 25 and measured using a SmartChem140 Automatic Chemistry Analyzer. Soil organic carbon (SOC) was determined by the potassium dichromate digest method. Total soil nitrogen (TN) was measured by the micro-Kjeldahl method. Soil pH was determined by pHS-3C acidity meter.

2.5 Microbial community structure analysis

Total soil microbial DNA was extracted from 0.5 g fresh soil. DNA SPIN extraction kit (Felix Bio-Tech, USA). The fungal ITS1 region was amplified using the following primers: ITS1F: (5'-TCCGTAGTGAACCTG-3'), ITS2-Rev: (5'-GCTGCGTTCTTCATCGATGC-3'). In the present study, soil samples were sent to Shanghai Personal Biotechnology Co., Ltd. for high-throughput sequencing.

2.6 Statistical analysis

Statistical analyses were performed using the SPSS 22.0 software package for Windows (SPSS Inc., Chicago, IL, USA). All statistical tests performed in this study were considered significant at $P< 0.05$. The effects of different treatments on soil properties and microbial richness indices were calculated using one-way analysis of variance (ANOVA) with Tukey's multiple comparison test. Normal distribution and homogeneity of variance were verified using the Bartlett's and Dunnett' tests. We used Pearson's correlation coefficient analyses to

determine whether there was significant correlation between soil properties with fungal relative abundance and diversity indices.

A principal component analysis (PCA) was performed on the first 20 dominant fungal genera (the relative abundance of the major 20 dominant fungal genera reached 98%) and used to visualize in composition and structure of the fungal communities among the four treatments. The correlation of multiple variations between soil properties and community composition was shown by a redundancy analysis (RDA) using CANOCO 5.0 [27]. The manual forward-selection procedure was used in the RDA to determine significance of environmental variables ($P < 0.05$) using a Monte Carlo test with 499 permutations. The relationship between N_2O emission and microbial abundance was further validated by regression analysis.

3. Results

3.1 Effects of N fertilization on inorganic nitrogen content

The NH_4+-N concentration was very low, at approximately 3 mg N kg-1, in the four treatments (Table S1). There were no significant differences in soil NH_4+-N concentration between N fertilization treatments and N non-fertilization treatment in the first seven days (Fig. 1a). The soil NH_4+-N concentration in the N225 treatment was significantly lower ($P< 0.05$) than that in the control treatment on the 9_{th} and 12_{th} day.

The NO_3–N concentration in all treatments had a significantly upward trend with N addition ($P < 0.05$). The NO_3–N concentration in the N150, N225, and N300 treatment treatments was 36.46, 38.22, and 41.65 mg N kg-1, respectively, approximately 1.4 to 1.6 times higher than that in the N0 control (Table S1). Soil NO_3–N concentration showed significant increase in all treatments by 15 days of incubation (Fig. 1b). The NO_3–N concentration in the nitrogen treatments was significantly ($P < 0.05$) higher than that in the control treatment, and the accumulation of NO_3–N in the N225 treatment was higher than that in the N300 treatment.

3.2 Effects of fertilization on N_2O emission

N_2O flux in the N300 treatment was significantly higher than that in other treatment treatments ($P < 0.05$) on Day 1 ($P < 0.05$), however, it did not reach a peak value. The daily N_2O flux reached the highest value in the N225 and N300 treatment treatments on the 5th day, at 10.29 μg N g-1 d-1 and 9.78 μg N g-1 d-1, respectively, which was 1.6 and 1.5 times higher, respectively, than in the N0 treatment; however, there was no significant difference between the two treatments (Fig. 2a). There was no significant difference between the N150 treatment and control treatment. After 7 days of incubation, the daily N_2O flux remained stable, and there was no significant difference among the treatments.

During the 15-day incubation, there were significant differences in cumulative N_2O emissions between different treatments. Cumulative N_2O emission was 49.65 mg N kg-1 in the N225 treatment, which was significantly ($P < 0.05$) higher than that in the other treatments and was up to 1.09 times higher than that in the control treatment (Fig. 2b). The N300 treatment also significantly ($P < 0.05$) increased N_2O emissions, relative to the control treatments. In the initial 7 days during the 15 days of incubation, cumulative N_2O emission in the N225 and N300 treatments was 30.46 and 29.48 mg N kg-1 soil, which accounted for 61.3% and 60.8%, respectively, of the total amount during the incubation period. However, there was no significant difference between the N150 and control treatments. This result indicated that the high application rate of N fertilizer efficiently stimulated N_2O emission within a short time.

3.3 Contribution of microorganisms to N_2O emission

Compared with the control without biological inhibitors (control treatment), the treatment with bacterial inhibitors (streptomycin treatment) and fungal inhibitors (cycloheximide treatment) significantly reduced soil N_2O emission ($P < 0.05$) (Fig. 3a). The N_2O emission after bacterial inhibitor treatment was significantly higher than that after fungal inhibitor treatment ($P < 0.05$), and significantly lower after fungal and bacterial inhibitor treatment ($P < 0.05$). The N_2O production was reduced to 30.9% by fungal inhibitor treatment, 45.4% by bacterial inhibitor treatment, and 23.7% by fungal and bacterial inhibitor treatment as compared

to that in the control treatment. These results suggeste d that fungi may play a larger role in N_2O production in lawn soil.

Moreover, the contribution rate of fungi to N_2O emission was significantly higher than that of bacteria and other microorganisms ($P < 0.05$) (Fig. 3b). Fungi contributed to 45% of total N_2O emission, while bacterial contribution only reached 68% of that of the fungi. This also indicated that the contribution of fungi to N_2O emission was significantly higher than that of bacteria in lawn soil.

3.4 Analysis of fungal community structure

A total of 491602 high-quality fungal ITS gene sequences generated from the lawn soil samples were clustered into 2698 OTUs at 97% sequence similarity. The dominant fungal phyla across lawn soil samples were Ascomycota (21.1–36.6%), Basidiomycota (6.7–10.1%), and Mucoromycota (0.7–5.4%). The highest relative abundance of *Ascomycota* was found in the N225 treatment, while the lowest was found in the N150 treatment. In contrast, the relative abundance of Basidiomycota was the highest in the N150 treatment, and the lowest in the N300 treatment. The highest relative abundance of Mucoromycota was found in the N300 treatment and the lowest was found in the N225 treatment (Fig. 4a). N fertilizer significantly increased the relative abundance of*Ascomycota* , yet significantly decreased the relative abundance of Basidiomycota.

The top three dominant fungi belonged to the genera*Pyrenochaetopsis* (2.1–10.8%), *Chaetomium* (1.0%–7.0%), and *Mortierella* (0.7–4.3%). Specifically, the relative abundance of *Pyrenochaetopsis* in the N225 treatment (10.81%) was significantly higher than that in the other three treatments ($P < 0.05$) (Fig. 4b). N fertilizer significantly increased the relative abundance of *Pyrenochaetopsis* , *Penicillium* ,*Talaromyces* , *Humicola* , *Guehomyces* , and*Thermomyces* , while significantly decreased the relative abundance of *Chaetomium, Mortierella* , *Tuber* , and *Simplium* .

The fungal community structure was used as a variable, and PCA was performed in 12 treatment plots. The axis PC1 and PC2 explained 85.44% of the variation information of the fungal community structure. According to the analysis, except for the control plot, the 9 plots treated with N150, N225, and N300 had obvious distribution areas on PC1 and PC2 and were distributed centrally for the same treatment (Fig. 5). This indicated that the fungal community structure was altered by N fertilizer.

3.5 Correlation between N_2O emission and the fungal community and soil properties

Associations between N_2O emission, soil properties and community structures of the fungal phyla and genera were analyzed by RDA. For the fungal phyla, axis1 and axis2 explained 60.7% and 7.5% of the total variation, respectively, in the community structure (Fig. 6a). N_2O emission was positively correlated with fungal*Ascomycota* , with the highest correlation ($R = 0.726$, $P = 0.008$). The dominant phylum of fungi in the tested soil was Ascomycota, which accounted for 21.1-36.6% of the total fungal microbial abundance. The trend of change in the abundance of Ascomycota in the four treatments was consistent with that of N_2O emission, which meant that Ascomycota was an important contributor to N_2O emission.

For the fungal genus community structure, RDA analysis explained 73.2% of the total variation, and axis1 and axis2, respectively, explained 46.6% and 26.6% of the total variation (Fig. 6b). N_2O emission showed a positive correlation with the abundance of*Pyrenochaetopsis* , *Myrothecium* , *Zopfiella* ,*Humicola* , *Bullera* , and *Conocybe* . The genera*Pyrenochaetopsis* , *Myrothecium* , *Zopfiella* , and*Humicola* belong to the phylum Ascomycota, and *Bullera* and*Conocybe* belong to the phylum Basidiomycota. Further quantitative analysis of the correlation between N_2O emission and the relative abundance of the fungal genera revealed that *Myrothecium*($R2 = 0.556$, $P = 0.005$), *Pyrenochaetopsis* ($R2 = 0.478$, $P = 0.013$), and *Humicola* ($R2 = 0.372$, $P = 0.035$) were positively correlated with N_2O emission, while *Cryptococcus*($R2 = 0.551$, $P = 0.006$) was negatively correlated with N_2O emission (Fig. 7). *Pyrenochaetopsis* ,*Myrothecium* , and *Humicola* were also found to be the main functional fungi in N_2O emission. However,*Zopfiella* , *Bullera* , and *Conocybe* were not significantly correlated with N_2O emission.

In addition, N_2O emission was positively correlated with soil NO_3–N and negatively correlated with soil pH and NH_3+-N (Fig. 6). The influence of soil properties on fungal community structure decreased in the order

of pH > NO_3–N > NH_3+-N > SOC > TN. N fertilizer use led to changes in soil properties and in the fungal community structure, and ultimately affected N_2O emission.

4. Discussion

In this study, we specifically studied the effects of different fertilization amounts on N_2O emission from lawn soil and microbes, and further analyzed the community changes within the major functional microorganisms and their correlation with N_2O emissions. The results showed that N fertilizer significantly increased the N_2O emission from lawn soil (Fig. 2b). This was consistent with most studies reporting that exogenous nitrogen addition significantly enhanced soil N_2O emission[28-31]. The contribution of fungi to the N_2O emissions in lawn soil was the highest of the three evaluated microbial communities, accounting for 45%, which was significantly higher than that of bacteria (31%) and other microorganisms (24%) (Fig. 3). Therefore, our results showed that fungi played a larger role than bacteria in N_2O emission in lawn soil. This result supported that soil N_2O was produced primarily by fungi and emphasized the importance of fungi on N_2O emissions in the lawn soil. This result was also similar to results found by other studies. For instance, it has been found that fungi are the major contributors to N_2O emission of soil in grazing grasslands in Tibet[17,41], grazing grasslands in New Zealand[35], tea in southern China[36], and croplands in China[42]. Meanwhile, our result was consistent with results from the traditional agricultural ecosystem, crop-livestock integrated ecosystem, organic agricultural ecosystem, and the artificial forest ecosystem in which fungi contributed 40-51% to N_2O emission [17,42]. Therefore, it is of great significance to explore the microbial mechanism of N_2O production by fungi for reducing N_2O emission from lawn soil.

In addition, fungi are better adapted to the environment than bacteria. N fertilizer significantly increased the NO_3–N concentration and decreased pH in lawn soil (Table. S1). Bacteria preferred ammonia oxidation at high NH_3+-N concentrations [21]. This also proved that fungi contributed more to the N_2O emissions in the lawn soil. In our lawn soil, the NH_4+- N was low, while the NO_3–N concentration was high, indicating that the lawn soil environment was more suitable for the growth of fungal communities.

Our results showed that the N_2O emissions of the N300 treatment which represented the highest level of N fertilizer addition, did not produce the highest emission levels, and they were instead significantly lower than that of the N225 treatment (Fig. 2b). The accumulation of N_2O began to decline after the highest N225 treatment, indicating that high N fertilizer application could effectively promote N_2O emission, but it was not the case that the higher the nitrogen application, the higher the N_2O emission. We speculated that it might be related to soil microorganisms with N transformation function. Thus, increasing the number of microorganisms involved should result in increased production of N_2O emissions. Further analysis revealed that the dominant fungi in the lawn soil accounted for the top three fungal communities, namely Ascomycota, Basidiomycota, and Mucoromycota. Nitrogen fertilizer significantly increased the relative abundance of Ascomycota, while it significantly decreased the relative abundance of Basidiomycota (Fig. 4a). We found a positive correlation between N_2O emission and Ascomycota through RDA analysis (Fig. 6a). Moreover, the growing trend of Ascomycota during the four nitrogen fertilizer treatments was consistent with the N_2O emission trend in lawn soil. N_2O emissions reached their highest levels in the N225 treatment, rather than the N300 treatment. We hypothesized that this might be related to the microbial biomass and nitrogen transformation in lawn soil. We found that the relative abundance of Ascomycota in the N225 treatment (36.6%) was higher than that of the N300 treatment (35.1%). This result not only explained why N_2O emission in the N225 treatment was higher than that in the N300 treatment, but also indicated that Ascomycota played an important role in the N_2O emissions in lawn soil. It has been reported that among fungi, Ascomycota and Basidiomycota preferred to use soil nitrate for denitrification and released N_2O[32]. 90% percent of the fungi reported to produce N_2O belong to the phylum Ascomycota, followed by fungi in the Basidiomycota and Mucoromycota which account for 7% and 3%, respectively. Representative N_2O-producing Ascomycota [33,34]. Ascomycota preferred to grow with nitrogen than Basidiomycota. Meanwhile, high nitrogen could inhibit N_2O reductase activity [35]. These results supported our conjecture.

At the fungal genus level, the relative abundance of*Pyrenochaetopsis* , *Myrothecium* , *Zopfiella* , and*Humicola* increased significantly after nitrogen fertilizer treatment, while the relative abundance of *Chaetomium* ,*Simplicillium* , *Cryptococcus* , *Mortierella* and*Phoma* significantly decreased (Fig. 4b). *Myrothecium* ,*Zopfiella* , *Pyrenochaetopsis* , *Humicola* of Ascomycota and *Bullera* and *Conocybe* of Basidiomycota were positively correlated with N_2O emission (Fig. 6b). Regression analysis showed *Pyrenochaetopsis* , *Myrothecium* , and*Humicola* of Ascomycota were positively correlated with N_2O emission, while *Bullera* and *Conocybe*of *Basidiomycota* were not significantly correlated with N_2O emission. These results also indicated that Ascomycota might be the key microbial population driving nitrogen transformation in lawn soil. Moreover, we found that *Myrothecium*was the fungal genus with the highest correlation coefficient for N_2O emission in lawn soil through correlation analysis (Fig. 7). According to the results of previous studies,*Myrothecium* had a strong ability to produce N_2O. The N_2O production capacity of *Myrothecium* was 21.4 nmol N_2O $mL^{-1}d^{-1}$, and the efficiency was far higher than that of*Pyrenochaetopsis* and *Humicola* , respectively 3.5 and 5.1 nmol N_2O $mL^{-1}d^{-1}$[34]. Meanwhile, the relative abundance of*Myrothecium* in Ascomycota was the highest in the N225 treatment (1.88%) and significantly higher than that in the other three treatments (0.37%–0.82%). We found that the adaptability of*Myrothecium* was stronger under the condition of nitrogen addition, but the condition of high nitrogen decreased, which was consistent with the previous research results[32,34]. We concluded that *Myrothecium*played an important role in the increased N_2O emission in lawn soil. Therefore, it is of strategic significance to study the mechanisms related to *Myrothecium* and resultant N_2O emissions in order to reduce increases in N_2O emissions from the urban lawn soil in future.

During the incubation period, we found that N fertilizer increased N_2O flux in the lawn soil (Fig. 2a). Further, the N_2O flux of the N225 and N300 treatment showed the highest emission peaks on the 5th day, while N0 and N150 treatments did not show emission peaks, indicating that high nitrogen fertilizer input could significantly stimulate the N_2O emission of lawn soil, which was consistent with the results of previous studies[36-37]. This indicated that N fertilizer increased the N_2O emissions in lawn soil, and also strengthened the soil nitrogen stimulation effect. Meanwhile, some laboratory experiments showed that N_2O could rapidly reach the emission peak within a short period after N fertilizer addition, and the cumulative emissions accounted for more than half of the total emissions and then rapidly declined[8,38]. Mate analysis showed that the proportion of nitrogen addition was linearly correlated to N_2O emissions [39]. However, the low N fertilizer application (Urea) did not significantly enhance N_2O emission, which was because the fact that the lawn was irrigated after low urea application, leading to urea hydrolysis into inorganic nitrogen which was directly used by plants [7,40].

5. Conclusions

Nitrogen fertilizer significantly promoted N_2O emissions in lawn soil, although this result was not linearly related to the amount of fertilizer applied. When the amount of fertilizer applied was 225 kg·ha·yr-1, N_2O emission was the highest, but it decreased when the amount of fertilizer applied increased to 300 kg·ha·yr-1. N fertilizer significantly altered the soil microbial community structure. Through biological inhibitor treatment, we found that fungi were the main contributors to N_2O emission in lawn soil, accounting for 45% of the total N_2O emissions. *Pyrenochaetopsis* , *Myrothecium* , and*Humicola* of Ascomycota were significantly positively correlated with N_2O emission and were the predominant contributors to N_2O emissions within lawn soil. These findings will help to draw up appropriate measures for mitigation of N_2O emissions in lawn soil.

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Conflicts of interest: The authors declare no conflict of interest.

Data Availability Statement :

- Relative abundances of the main fungal phyla and genus in the lawn soil of all treatments, and soil properties at 0 - 20 cm soil depth sampling: Dryad Doi https://doi.org/10.5061/dryad.nvx0k6dnv.

-All data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Table S1. Soil properties at 0 - 20 cm soil depth sampling (mean +- standard error, n = 3)

Table and figure caption

Fig. 1 Dynamic variation of NH_3+-N (a) and NO_3–N (b) contents in lawn soils with or without urea in a 15-day incubation. Different letters denote significant differences between treatments ($P < 0.05$). Vertical bars denote standard errors of the mean (n = 3)

Fig. 2 Temporal variation of N_2O flux (a) and cumulative N_2O emissions (b) from lawn soils with or without urea over a 15-day incubation. Different letters denote significant differences between treatments ($P < 0.05$). Vertical bars denote the standard error of the mean (n = 3)

Fig. 3. Contributions of fungi and bacteria on N_2O emissions in the lawn soil. Different letters denote significant differences between treatments ($P < 0.05$). Vertical bars denote the standard error of the mean (n = 3)

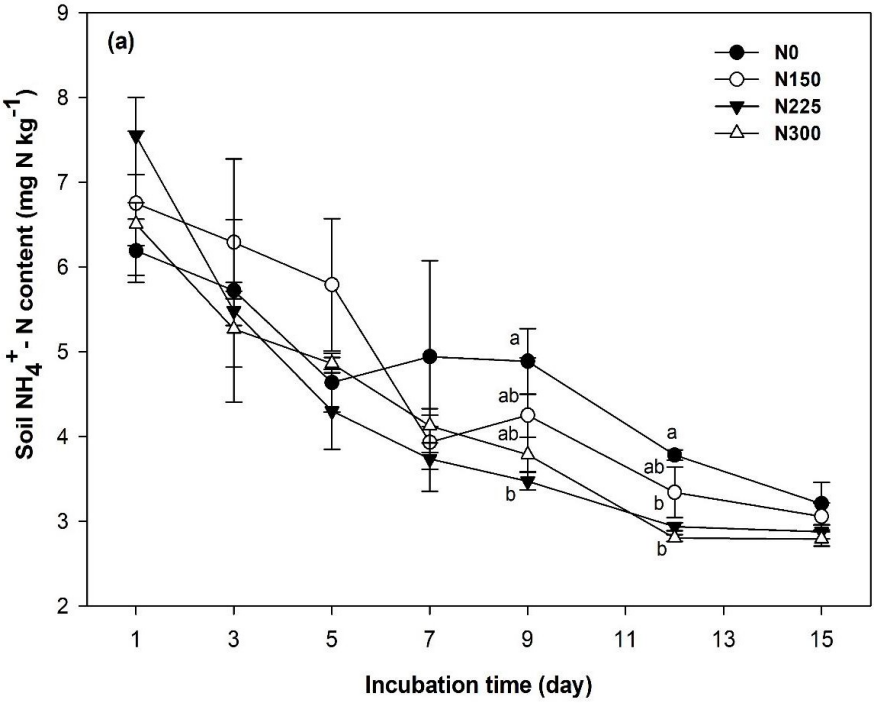
Fig. 4 Relative abundances of the main fungal phyla (a) and genus (b) in the lawn soil of all treatments. Vertical bars denote the standard error of the mean (n = 3)

Fig. 5 Principal coordinate analysis (PCA) of fungal community structure in all treatments. The first two principal coordinate axes together explained 85.44% of the fungal variation

Fig. 6 Correlations between N_2O emission, soil properties and the community structure of fungal phyla (a) and genus (b) as determined by redundancy analysis (RDA)

Fig. 7 Regression analysis of N_2O emissions with fungal genus in different nitrogen treatments (n=12)

Fig. 1



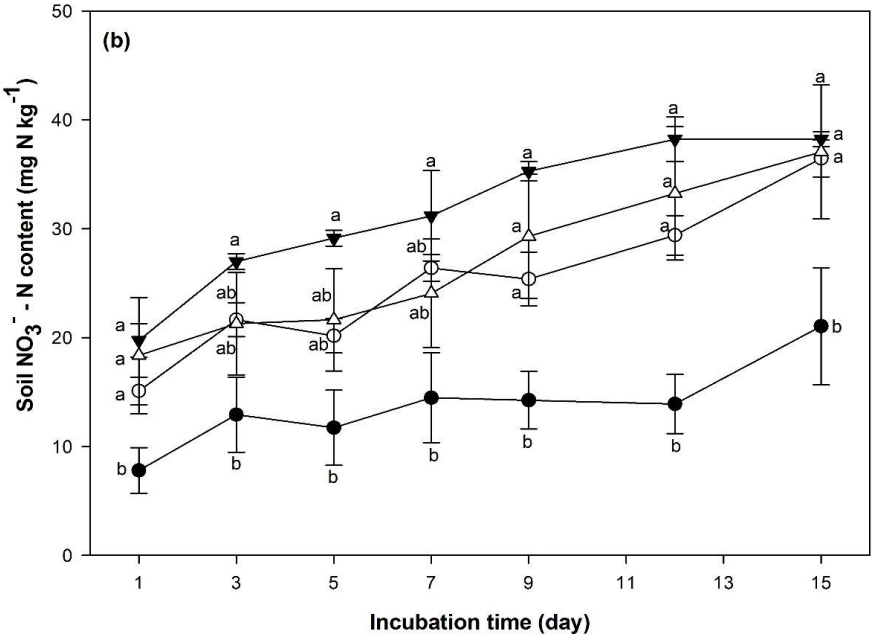
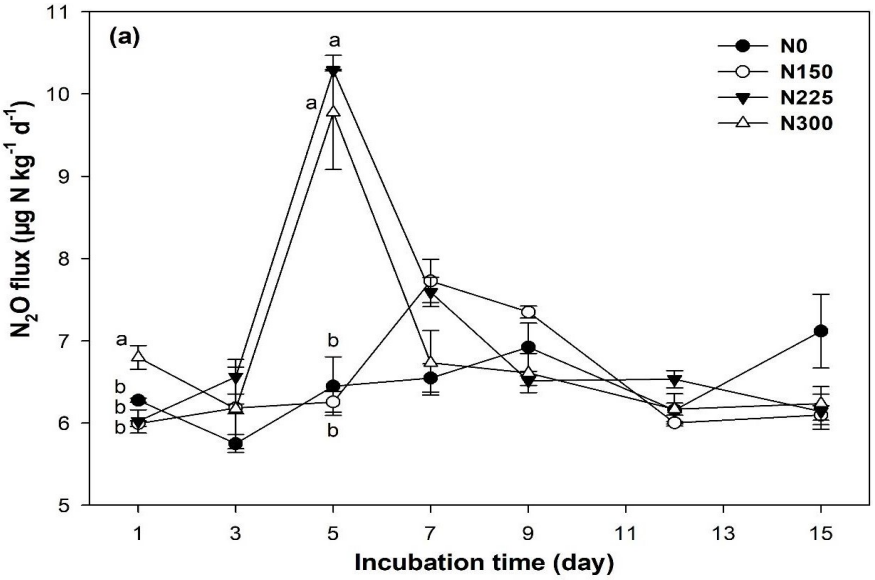
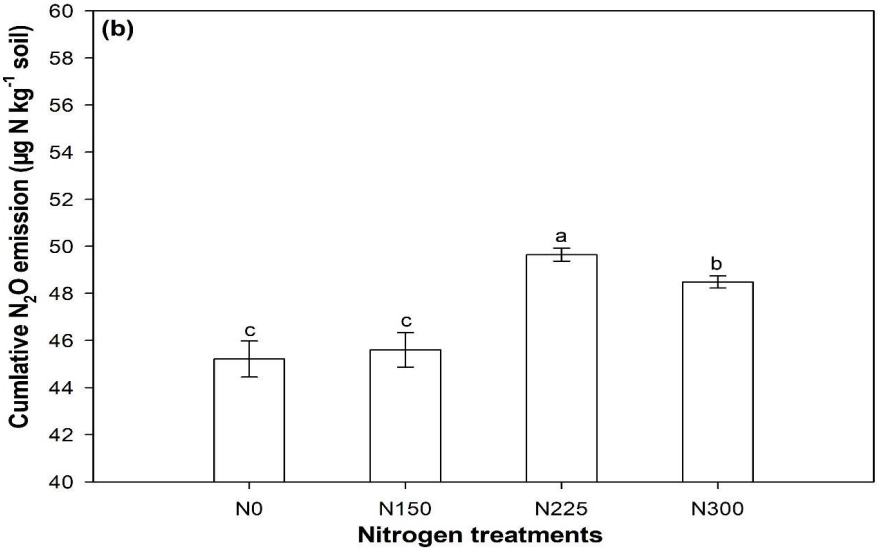


Fig. 2





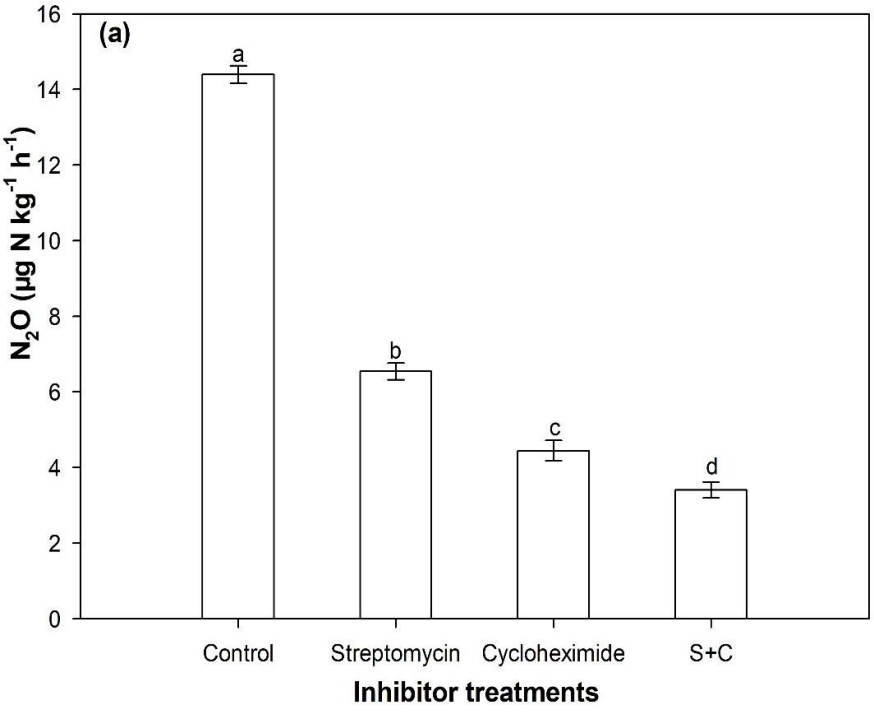


Fig. 3

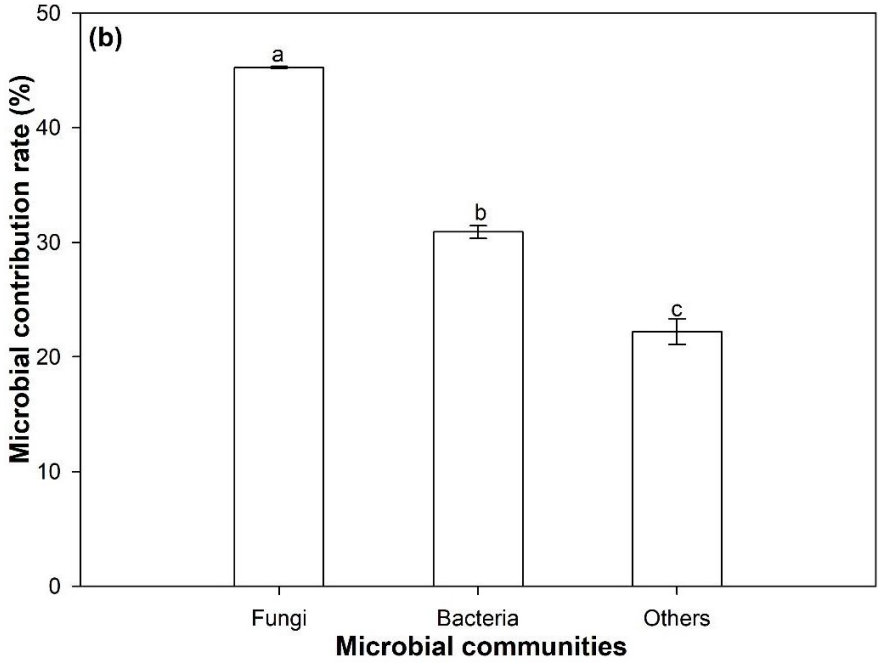
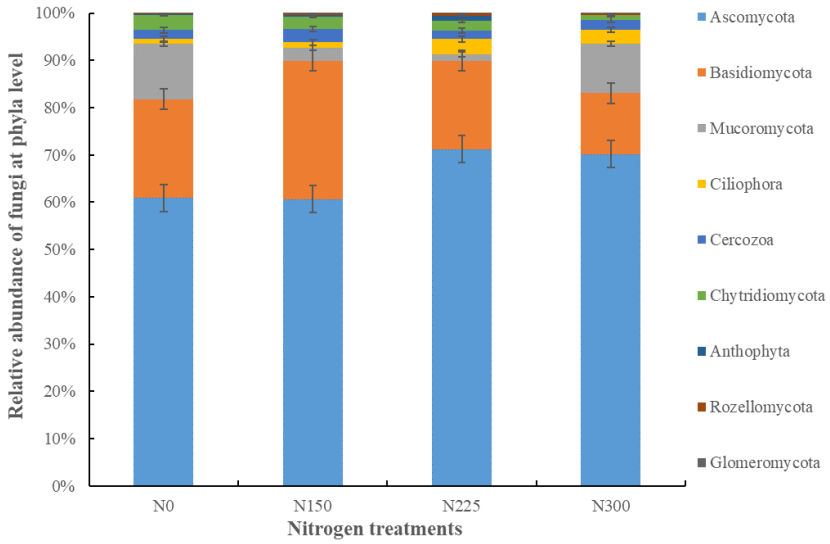


Fig. 4a



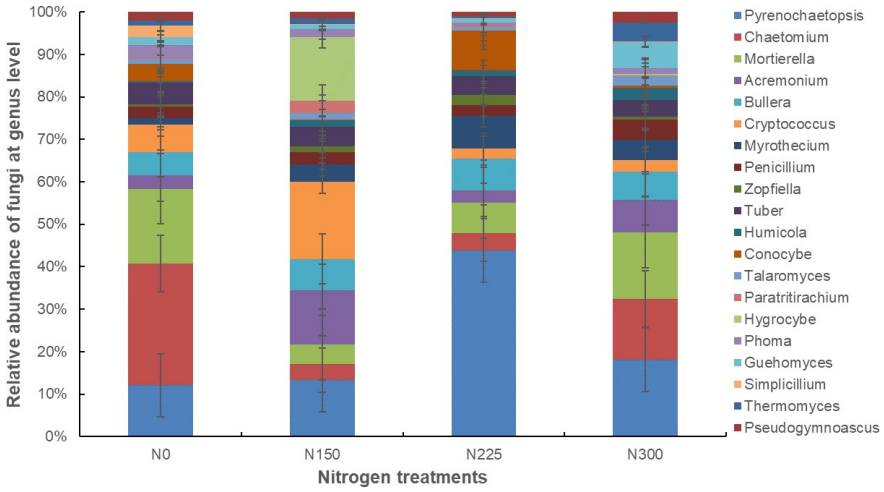


Fig. 4b

Fig. 5

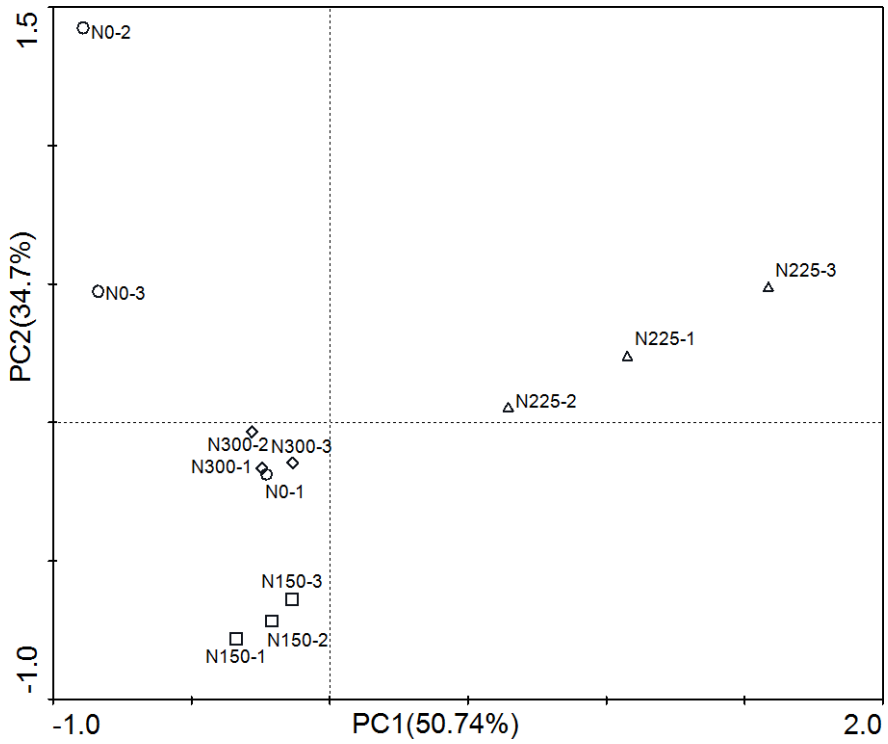


Fig. 6a

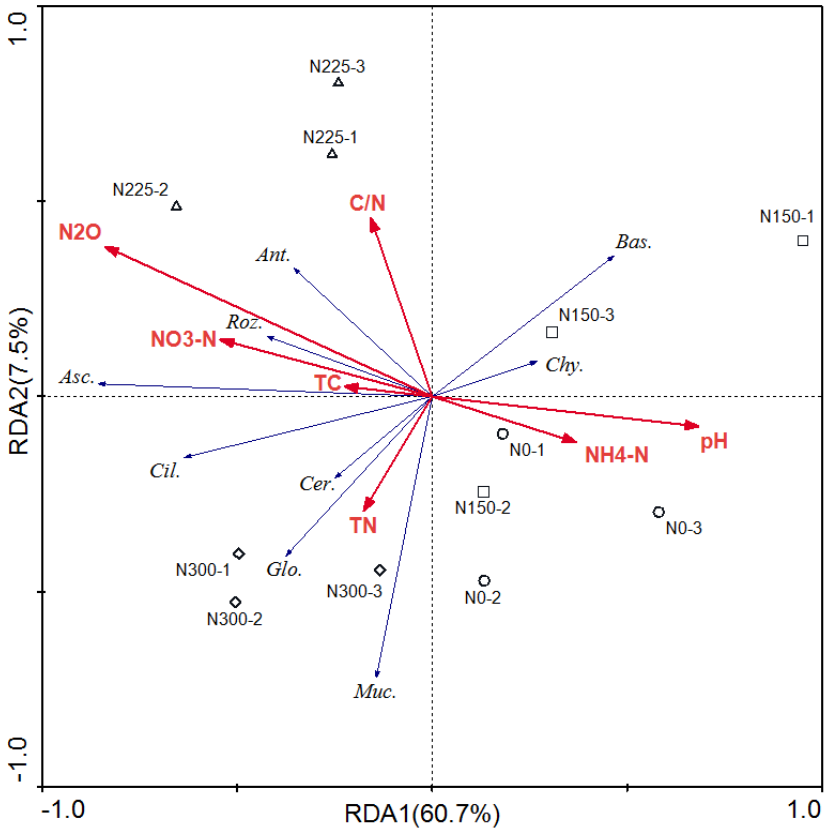


Fig. 6b

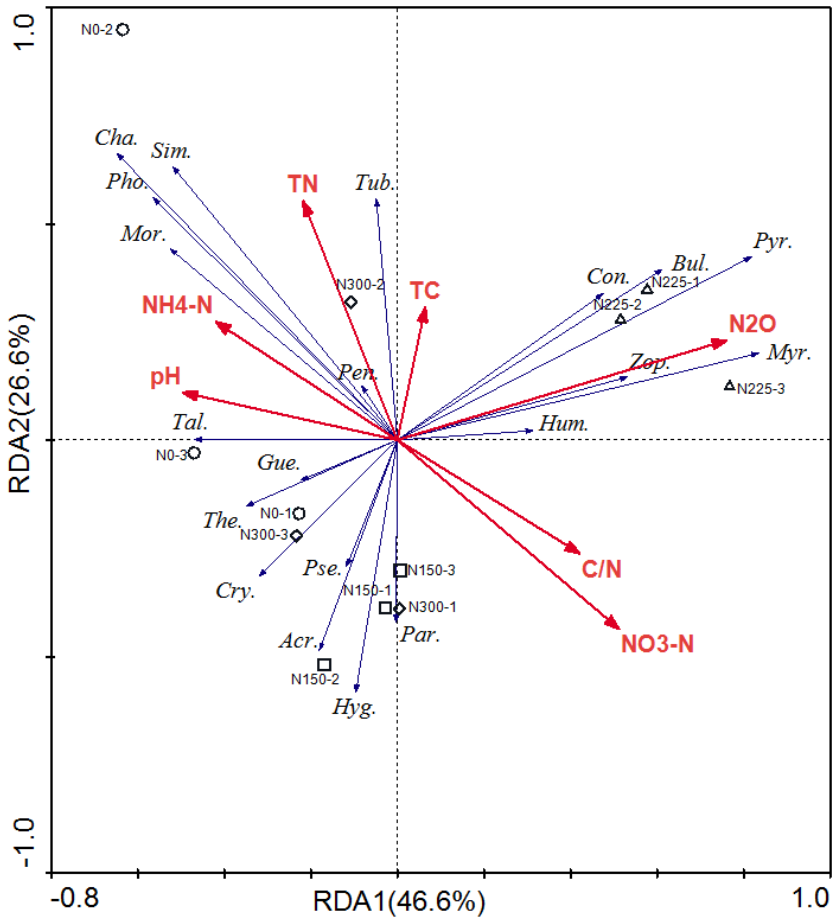


Fig. 7