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Contrasting effects of nitrogen addition on rhizosphere soil CO_2, N_2O, and CH_4 emissions of fine roots with different diameters from *Pinus tabulaeformis* forest using laboratory incubation

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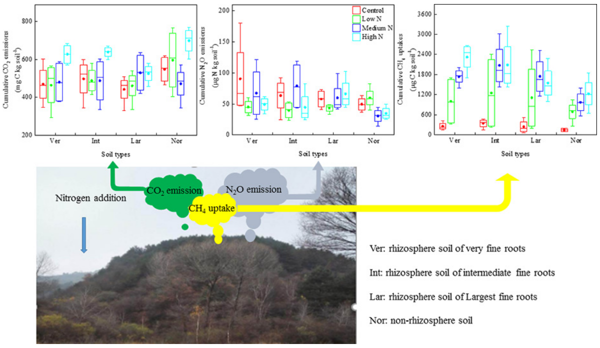
HIGHLIGHTS

- Nitrogen addition has a promoting effect on soil CO_2 emission and CH_4 uptake, and an inhibitory effect on N_2O emission.
- CO_2 and N_2O emissions in rhizosphere soil among different root-size classes had similar responses to N addition.
- CH_4 uptake in rhizosphere soil of very fine roots was more responsive to N addition than that of larger fine roots.
- Soil NH_4^+ and NO_3^- had opposing effects on greenhouse gas emissions.

GRAPHICAL ABSTRACT



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ABSTRACT

Nitrogen (N) addition has variable effects on chemical composition, function, and turnover of roots with different diameters. However, it is unclear whether N addition has variable effects on greenhouse gas (GHG) emission in rhizosphere soil. We performed N addition (0–9 g N m^{-2} y^{-1}) experiment in a *Pinus tabulaeformis* forest and a lab-incubation experiment to determine the effects of N addition on carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4) emissions in rhizosphere soils of roots with different diameters (very fine roots: <0.5 mm, intermediate fine roots: 0.5–1.0 mm, largest fine roots: 1.0–2.0 mm). Nitrogen addition significantly promoted CO_2 emission and CH_4 uptake, with maximum values (CO_2, 623.15 mg C kg $soil^{-1}$; CH_4, 1794.49 μg C kg $soil^{-1}$) in the 6 or 9 g N m^{-2} y^{-1} treatments ($P < 0.05$). Nitrous oxide emissions were inhibited, with the greatest inhibitory effect in the 9 g N m^{-2} y^{-1} treatment (48.63 μg N kg $soil^{-1}$). Total phosphorus (TP) content significantly decreased and increased in rhizosphere soil and non-rhizosphere soil after N addition, respectively, while organic carbon (OC), total N (TN), ammonium (NH_4^+), and nitrate (NO_3^-) contents in rhizosphere soil increased. A greater change in chemical properties occurred in rhizosphere soil of largest fine roots than very fine roots. Carbon dioxide and nitrous oxide emissions in rhizosphere soil among root sizes exhibited similar responses to N addition. While CH_4 uptake was more responsive to N addition in rhizosphere soil with very fine roots than with largest fine roots. Basically, OC, TN, NO_3^-, and NH_4^+ were key soil components driving GHG emissions; NO_3^- promoted CH_4 uptake and N_2O emissions, NH_4^+ inhibited CO_2 emissions. GHG response to N addition varied greatly, particularly in rhizosphere soil with different root sizes mainly related to its chemical properties.

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1. Introduction

Carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4) are the main greenhouse gases (GHGs) that have drastically increased the global temperature in recent years (Oertel et al., 2016). Carbon dioxide emissions produced by soil respiration account for approximately 25% of the annual carbon (C) exchange between atmosphere and land, and are the main flux of the C cycle on a global scale (Post et al., 1988). Although soil produces low concentrations of N_2O and CH_4, the global warming potential (GWP) of these two gases is approximately 298 and 25 times higher than that of CO_2 over a 100-year time frame (IPCC, 2007). In addition, forest soil has a high OC content and exhibits vigorous microbial respiration, which is an important source of CO_2 (Spokas et al., 2005). Therefore, soil plays an important role in controlling global warming. Gas emissions from soil, particularly forest soils, and their driving mechanisms are currently a topic of great interest in ecological research (Volkova et al., 2014; Zhang et al., 2016).

Nitrogen (N) release originating from industries has increased by three to five times over the last century. It is estimated that by 2030, the worldwide N deposition will increase by 50–100% (Reay et al., 2008). Therefore, N deposition has become an important environmental factor affecting the stability of forest ecosystems and the C cycle (Galloway et al., 2004). Simulation studies on the effects of N deposition on soil GHG emissions have revealed varied outcomes regarding GHG emissions such as stimulation, inhibition, and no effect (Brumme and Beese, 1992; Janssens et al., 2010; Micks et al., 2004). For example, increasing N deposition in alpine grassland of the Tianshan Mountains in Central Asia significantly increased soil CO_2 and N_2O emissions (Li et al., 2012). However, 3 years of simulated N deposition in a Korean pine plantation in northeast China had no effect on soil CO_2 emissions (Song et al., 2017). The increased availability of N results in inhibition of CH_4 production in acidic soils but has no effect on the production of CH_4 from Mollisol soil (Chen et al., 2017; Xiao et al., 2018). Nevertheless, these studies have not revealed the mechanisms underlying GHG emission changes in sufficient detail and cannot explain the reasons for these differential results.

We believe that N addition rate and soil type are the main reasons for the different results obtained for GHG emissions in previous studies. First, different rates of N addition exhibit varied effects on soil GHG emissions. For example, N addition of 0.1–0.5 mg N g^{-1} promoted CO_2 emissions by increasing the NH_4^+ content and promoted N_2O emissions by increasing the content of NH_4^+ and NO_3^- in salt-affected soils under different vegetation communities (Zhang et al., 2019). In the forest soil of Changbai Mountain in Northeast China, 5 g N m^{-2} y^{-1} significantly inhibited CO_2 and N_2O emissions, which was predominantly driven by soil OC, TN, and NO_3^- (Chen et al., 2017). Low level N addition facilitates soil biochemical reactions, such as nitrification, denitrification, and C-degradation (Li et al., 2020), which increases soil quality, enzyme activity, and GHG emissions (Zhang et al., 2017; Zhang et al., 2019). In contrast, high level N addition is excessive for soil biochemical reactions and has a negative effect on GHG production. Previous studies have identified a threshold of N addition that distinguishes its promoting and inhibiting effects on many soil functions (Zong et al., 2019; Gu and Wang, 2017; Yao et al., 2017). Soil OC, TN, TP, and pH appear to be the main driving factors for GHG emissions due to their crucial parts during biochemical reactions. Furthermore, experimental treatments can influence GHG emissions by altering these soil chemical properties (Pouyat et al., 2007).

Second, soil type may also have varied effects on soil GHG emissions. On one hand, owing to the different micro-environments, the effects of N addition on the chemical properties of rhizosphere and non-rhizosphere soil are obviously different (Majdi and Bergholm, 1995). For example, N addition significantly increased the content of water-soluble OC and water-soluble organic N in rhizosphere soil of *Bothriochloa ischaemum* but had little effect on non-rhizosphere soil (Xiao et al., 2017). In a pot experiment of apple seedlings, N fertilizers decreased the TP content of rhizosphere soil but did not have a significant effect on the TP content of non-rhizosphere soil (Dong and Shu, 2001). Thus, the chemical properties of rhizosphere soils are more responsive to environmental conditions than those of non-rhizosphere soils. Therefore, GHG emissions in rhizosphere soil may be more susceptible to N addition than those in non-rhizosphere soil.

On the other hand, the chemical composition and physiological characteristics of roots with different diameters in a root system respond differently to N addition. For example, increasing N availability significantly enhanced the production and turnover of very fine and intermediate fine roots of *B. ischaemum* but had no effect on the largest fine and thicker roots (Wang et al., 2017a). The OC and TN contents of very fine roots of *P. tabulaeformis* seedlings increased upon N addition, while those of thicker roots showed little change (Jing et al., 2017). Furthermore, secretion of organic acids, carbohydrates, and other macromolecular substances by very fine roots is more responsive to N addition than that of thicker roots (Li et al., 2018). The chemical composition and physiological functions of the root system are closely related to the chemical properties of the rhizosphere soil. Therefore, the chemical properties of the rhizosphere soil of very fine roots are more responsive to N addition than those of the rhizosphere soil of thicker roots. Accordingly, GHG emissions in rhizosphere soil with different root diameters show variable responses to N addition. Thus, N addition has different effects on GHG emissions depending on the soil type in an ecosystem, including non-rhizosphere soil and rhizosphere soil with different root diameters. Understanding the effects of N addition on GHG emissions from different types of soils and evaluation of the driving forces of soil chemical properties not only explain the reasons for the discrepant results in previous studies but may also help to elucidate the mechanisms by which N deposition alters the emission of GHG from soils.

The aim of this study was to investigate the influence of multilevel N addition on the emission/uptake of CO_2, N_2O, and CH_4 in different types of soils and to identify the main driving factors of soil chemical properties during this process. The soil chemical properties were selected because of their unique role in biochemical reactions and decisive functions in gas emissions. We proposed the following hypotheses: (1) Low N addition has a promoting effect on soil GHG emissions, whereas high N addition has an inhibitory effect, and there is a threshold value of N addition between promotion and inhibition. (2) GHG emissions in rhizosphere soil are more responsive to N addition than those in non-rhizosphere soils, and the variation of GHG emissions in the rhizosphere soil of very fine roots is more apparent than that of the largest fine roots. (3) Soil chemical properties of OC, NH_4^+, and NO_3^- are the main driving factors that determine the effect of N addition on GHG emissions. We used a field sampling method combined with laboratory incubation experiments to eliminate the interference of other environmental factors and revealed the corresponding changes in GWP.

2. Materials and methods

2.1. Study area description

Soil samples were collected from a *P. tabulaeformis* forestland on the Loess Plateau, Shaanxi Province, China (35°5834′N, 110°0538.1′E). The elevation of the region is 1000–1200 m, the slope is 20–25°, and the slope faces eastward. The forestland has a classic monsoon-type climate. The average annual precipitation in this region is 574.4 mm, the annual average temperature is 9.8 °C, and the frost-free period is 180 days. The soil is gray forest soil (Gray Luvisols, FAO soil classification), and the nutrient status is poor. The *P. tabulaeformis* forestland was planted in 1966. In March 2014, we investigated the vegetation characteristics before the N addition experiment and determined a current forest density of 1400–1800 plants ha^{-1}. The average canopy density is 0.7, the average diameter at breast height is 10.0 cm, the average tree height is 11.2 m, the forest volume is 75.5 m^3 hm^{-2}, and the leaf area index is 6.34. The understory vegetation of the forestland mainly includes *Elaeagnus pungens* Thunb, *Rosa xanthina* Lindl, *Spiraea salicifolia* L, *Lonicera japonica* Thunb, *Viburnum dilatatum* Thunb, and *Carex lanceolata* Boott. Accordingly, the plant diversity index of the community in the experiment area was 0.51 (calculated using the Simpson method).

2.2. N addition experiment

In many studies, 0–12 g N m^{-2} y^{-1} have been added to alter the soil N content (from limitation to saturation for forest ecosystems) on the Loess Plateau (Jing et al., 2017; Wang and Zheng, 2018). Meanwhile, the average ambient N deposition rate in the region is approximately 2.2 g N m^{-2} y^{-1} (Yang et al., 2010). Based on the ambient N deposition rate and N additions in previous studies, we designed four levels of N addition treatments, namely, 0, 3, 6, and 9 g N m^{-2} y^{-1} (control, low, medium, and high N addition, respectively). Each N addition treatment had four replicates (each plot 10 × 10 m), with a total of 16 plots. The plots were arranged according to the N addition levels, and the distance between any two adjacent plots was 5 m. The N addition experiment was initiated in March 2014, following the protocol described by Jing et al. (2019). We dissolved ammonium nitrate (NH_4NO_3) in distilled water (10 l for each plot) and sprayed the solution evenly on each plot annually before rainfall in early April, June, August, and October. Therefore, the concentrations of ammonium nitrate solution each time are 0.0, 21.0, 41.1, 60.4 g NH_4NO_3 kg $water^{-1}$, and the corresponding total amount of N added are 0, 75, 150, 225 g N respectively. Only distilled water was sprayed in the control treatment without the addition of NH_4NO_3.

2.3. Soil sampling

After 6 years of N addition, rhizosphere and non-rhizosphere soil of *P. tabulaeformis* were collected from the 0–20 cm soil layer in October 2019. Non-rhizosphere soil was collected from the locations without trees or any vegetation (canopy gap) in each plot. Root-free soils (i.e., non-rhizosphere soils) were sampled from the topsoil (0–20 cm) using an auger (5 cm in diameter and 20 cm long). Rhizosphere soil around three classes of roots, which were classified according to root diameter as follows, were collected: very fine roots, < 0.5 mm; intermediate fine roots, 0.5–1.0 mm; and largest fine roots, 1.0–2.0 mm. Thus, there were four types of soil in each plot. First, a 30 cm-deep soil profile was excavated with many cut surfaces of different diameter roots: the upper 0–20 cm soil profile was used for soil sample collection, and the 20–30 cm soil profile was used for standing and avoiding operational disturbances. Second, after measuring the root diameter with a Vernier caliper, rhizosphere soil was collected within 5 mm distance from a root with a specific diameter by using a small spoon. The fresh weight of soil in one point is very small, and only mixed masses in multiple points meet the requirement of laboratory experiment. Four soil subsamples were randomly collected from each soil type in each plot and mixed to form a composite soil sample. In total, 64 soil samples (4 soil types × 4 N addition treatments × 4 replicates) were included in our experiment, and the fresh weight of each sample was 200 g. The harvested soil samples were transported to the laboratory, where stones and litter were removed using tweezers. The cleaned samples were sieved through a 2.0-mm mesh and separated into two groups. One group was air-dried to a constant weight for chemical analysis, and the other was stored at 4 °C for incubation experiments and gas measurements.

2.4. Incubation experiment and gas measurements

The incubation experiment included pre-incubation in the dark at 25 °C for 10 days to reduce the interference from sieving and packing (Li et al., 2019). We then conducted a formal incubation in the dark at 25 °C for 30 days to determine the temporal variations in GHG fluxes (Duval and Radu, 2017). Soil nutrient contents were consumed and microbial activities decresed duirng incubation period, resulting in a gradual slowdown of gas emissions (Lang et al., 2011). Therefore, dynamic measurements were conducted to evaluate the effect of treatment on soil gas emissions (Zhang et al., 2016). Gas samples were measured after 10, 20, and 30 days of formal incubation according to the methods followed in previous studies (Liu et al., 2017; Zhu et al., 2018). First, soil samples (equivalent of 20 g dry weight with moisture content of 60% of the water-holding capacity) were transferred to 500 ml Mason jars (8 cm in diameter and 10 cm in height). Second, all Mason jars were covered with a sterile and breathable sealing film that prevented moisture loss but allowed gaseous exchange. Third, the CO_2 emission rate (mg C kg $soil^{-1}$ d^{-1}), N_2O emission rate (μg N kg $soil^{-1}$ d^{-1}), and CH_4 emission rate (μg C kg $soil^{-1}$ d^{-1}) were measured by calculating the change in gas concentration after the jar was sealed for 2 h, following the procedure by Li et al. (2020). During each measurement, the Mason jar was closed with a cap and then connected to the analyzer via inlet and outlet valves, forming a closed system. The measurements were conducted for 60 s per sample. Each Mason jar was sealed and returned to the original incubator immediately after the starting gas concentrations were measured. Another measurement was then recorded after 2 h. We assumed a linear emission of gas during the sealing process. Gas concentrations were measured using a Picarro G2508 N_2O/CO_2/NH_3/CH_4/H_2O gas concentration analyzer (Picarro G2508 Environmental, Picarro Inc., CA, USA). The operating range of the Picarro analyzer were 0–400 ppm (N_2O), 0.5–15 ppm (CH_4), and 0.02–2% (CO_2). The precisions of the Picarro analyzer were <10 ppb + 0.05% (N_2O), <7 ppb + 0.05% (CH_4), and < 300 ppb + 0.05% (CO_2). The gas emission rate was calculated as the change in gas concentration during the 2-h period after the jars were sealed.

2.5. Soil chemical analysis

The OC, TN, and TP contents (mg g^{-1}) were determined through traditional methods, namely, potassium dichromate and sulfuric acid heating (Mebius, 1960), Kjeldahl method (Page et al., 1982), and molybdenum antimony colorimetry (Lu, 2000), respectively. The content of NH_4^+ and NO_3^- was determined using a continuous flow analyzer (TRAACS 2000, Bran and Luebbe, Norderstedt, Germany). The soil samples (5 g) were extracted with 2 M KCl (25 ml) for 2 h, and then filtered with quantitative filter paper. Finally, we determined the soil pH of the filtrate using an automatic titrator (Metrohm 702, Switzerland) with a soil/water ratio of 1:2.5.

2.6. Statistical analysis

Cumulative gas emissions after 30 days of incubation (CO_2, mg C kg $soil^{-1}$, N_2O, μg N kg $soil^{-1}$, CH_4, μg C kg $soil^{-1}$) were calculated using an integrating method by multiplying the instantaneous gas emission rate with the duration of the incubation (Wetterstedt et al., 2010). We assumed that the flux rate is constant from the date of each gas sampling to the next gas sampling. The GWP after 30 days of incubation was calculated as follows (Xiao et al., 2018):

$$\mathrm{GWP}\left(\mathrm{mg\ CO_2\ eq\ kg\ soil^{-1}}\right) = CO_2 \times 1 + CH_4 \times 25 + N_2O \times 298 \quad (1)$$

where CO_2, N_2O, and CH_4 represent the individual total cumulative emissions after 30 days of incubation.

Two-way analyses of variance were used to verify the effects of N addition and root diameter on soil chemical properties, gas emissions, and GWP values. Least Significance Difference (LSD) was used for the post-hoc test. The data were presented as means ± standard error (SE) for four replications. Normality and homogeneity were determined using

Table 1
Rhizosphere and non-rhizosphere soil properties of Chinese pine before the incubation. (mean ± SE).

	Root diameters	Treatments	OC (mg g^{-1})	TN (mg g^{-1})	TP (mg g^{-1})	NH4$^+$ (mg g^{-1})	NO3$^-$ (mg g^{-1})	pH
Rhizosphere soil	Very fine roots	Control	26.979 ± 1.656aA	1.260 ± 0.055aA	0.553 ± 0.013aA	2.137 ± 0.113aA	0.697 ± 0.132bAB	8.298 ± 0.017aA
		Low N	26.980 ± 4.148aA	1.253 ± 0.175aA	0.566 ± 0.026aA	2.619 ± 0.318aA	1.594 ± 0.770abA	8.264 ± 0.020abA
		Medium N	28.456 ± 0.794aAB	1.338 ± 0.026aAB	0.491 ± 0.006bB	2.735 ± 0.101aAB	3.267 ± 0.452aA	8.205 ± 0.006bB
		High N	28.113 ± 2.093aA	1.290 ± 0.083aA	0.488 ± 0.008bB	2.225 ± 0.250aA	3.238 ± 0.663aAB	8.241 ± 0.042abA
	Intermediate fine roots	Control	19.844 ± 1.643cB	1.000 ± 0.087bB	0.553 ± 0.014aA	2.061 ± 0.112bA	0.424 ± 0.033bB	8.346 ± 0.031aA
		Low N	25.927 ± 0.816bA	1.174 ± 0.050bA	0.565 ± 0.018aA	2.115 ± 0.028bB	1.071 ± 0.303bA	8.255 ± 0.026aA
		Medium N	31.481 ± 2.109aA	1.424 ± 0.082aA	0.485 ± 0.016bB	2.922 ± 0.243aA	2.168 ± 0.438abA	8.230 ± 0.022aAB
		High N	19.243 ± 1.515cB	0.996 ± 0.084bB	0.497 ± 0.010bB	2.122 ± 0.081bA	3.418 ± 1.343aA	8.291 ± 0.057aA
	Largest fine roots	Control	21.327 ± 1.714aAB	1.039 ± 0.078aAB	0.586 ± 0.015aA	2.339 ± 0.294aA	0.434 ± 0.049bB	8.303 ± 0.061aA
		Low N	20.091 ± 2.228aA	0.971 ± 0.085aA	0.563 ± 0.016aA	1.893 ± 0.067aB	1.139 ± 0.339bA	8.275 ± 0.023aA
		Medium N	24.257 ± 1.166aB	1.170 ± 0.068aB	0.500 ± 0.017bB	2.368 ± 0.096aB	2.697 ± 0.448aA	8.283 ± 0.019aA
		High N	19.577 ± 2.718aB	1.010 ± 0.109aB	0.488 ± 0.011bB	2.297 ± 0.247aA	4.027 ± 0.699aA	8.265 ± 0.032aA
Non-rhizosphere soil		Control	20.643 ± 2.774aAB	0.873 ± 0.088abB	0.590 ± 0.017bA	0.781 ± 0.051aB	1.083 ± 0.275aA	8.225 ± 0.054aA
		Low N	22.553 ± 1.089aA	1.033 ± 0.031aA	0.601 ± 0.006abA	0.667 ± 0.018aC	0.770 ± 0.076aA	8.230 ± 0.016aA
		Medium N	15.570 ± 2.728aC	0.740 ± 0.091bC	0.586 ± 0.005bA	0.621 ± 0.047aC	0.626 ± 0.138aB	8.250 ± 0.036aAB
		High N	21.168 ± 1.312aB	1.018 ± 0.057aB	0.623 ± 0.007aA	0.770 ± 0.115aB	0.708 ± 0.080aB	8.229 ± 0.027aA

Note: Values followed by different lowercase letters indicate significant difference among the N addition treatments, values followed by different uppercase letters indicate significant difference among the root diameter classes and non-rhizosphere soil. ($n = 4$ $P < 0.05$). OC, organic carbon; TN, total nitrogen; TP, total phosphorus. Control, low, medium, high N are 0, 3, 6, and 9 g N m^{-2} y^{-1} respectively. Very fine, intermediate fine, largest fine roots are <0.5 mm, 0.5–1.0 mm and 1.0–2.0 mm respectively.

Table 2
Two-way ANOVA F values for the effects of nitrogen addition, root diameter and their interaction on soil properties before the incubation.

Factors (df)	OC	TN	TP	NH4$^+$	NO3$^-$	pH
Nitrogen addition (3)	1.796	1.571	19.816**	3.856*	14.795**	1.641
Root diameter (3)	10.520**	13.702**	27.839**	94.457**	6.065**	1.890
Interaction (9)	3.030**	2.670*	3.656**	2.453*	2.553*	0.734

Note: **($P < 0.01$) and * ($P < 0.05$) indicate significant differences among the treatments based on a two-way ANOVA test. OC, soil organic carbon; TN, total nitrogen; TP, total phosphorus.

the Shapiro-Wilk's and Levene's tests, respectively. $P < 0.05$ was considered the statistical level of significance. Principal coordinate analysis (PCA) on the Bray–Curtis distance matrices was performed to test the effects of N addition and root diameter on GHG fluxes using the "vegan" package of the R software, version 3.2.1. Multiple stepwise regression analyses were used to test the relationships between gas cumulative emissions and soil chemical properties. All analyses not specifically mentioned were performed using the integral functions in R software and Origin Pro 7.5 (Origin Lab Corp., USA).

3. Results

3.1. Soil properties

Nitrogen addition, root diameter, and their interaction had a significant effect on soil chemical properties (Tables 1, 2; $P < 0.05$). In non-rhizosphere soil, N addition significantly increased the TP content by 5.59%. In rhizosphere soils, N addition increased the NO_3^- content by 19.03% and decreased the TP content by 12.88%. The content of OC, TN, and NH_4^+ in rhizosphere soil of intermediate fine roots initially increased and then decreased with increasing N addition treatments, with the maximum value being observed at the medium N treatment (31.48 mg g^{-1}, 1.42 mg g^{-1}, and 2.92 mg g^{-1}, respectively). An interaction between N addition and root diameter was observed; this shows that the OC, TN, and NO_3^- contents in rhizosphere soil of the largest fine roots were more responsive to N addition than that in the rhizosphere soil of very fine roots. In contrast, the NH_4^+ content in the rhizosphere soil of very fine roots was more responsive.

3.2. Temporal variations in GHG fluxes

The average CO_2 emission rate was 17.565 mg C kg $soil^{-1}$ d^{-1}. During the entire incubation period, CO_2 emission was rapid at 0–20 days and decreased at 20–30 days (Fig. 1). Nitrogen addition significantly increased the CO_2 emission rate with the maximum values observed in the medium N or high N treatments at day 10 and 30 (Fig. 1, $P < 0.01$). Root diameter and its interaction with N addition did not have a significant effect on soil CO_2 emission rates.

The average N_2O emission rate was 1.833 μg N kg $soil^{-1}$ d^{-1}. The N_2O emission rate remained stable during the entire incubation period (Fig. 2). Nitrogen addition significantly affected the N_2O emission rate at day 30 (Fig. 2, $P < 0.01$). In particular, the N_2O emission rate decreased with N addition with the maximum value at control or low N treatment during most incubations. Root diameter and its interaction with N addition did not have a significant effect on soil N_2O emission rates.

The average CH_4 uptake rate was 38.98 μg C kg $soil^{-1}$ d^{-1}. The CH_4 uptake rate decreased with incubation time (Fig. 3). Nitrogen addition significantly increased the uptake rate of CH_4, with the maximum value at medium N or high N treatments (Fig. 3, $P < 0.01$). A significant difference in CH_4 uptake rate was observed among the root diameters at 10 days of incubation. The CH_4 uptake rate in rhizosphere soil was faster than that in non-rhizosphere soil, and the CH_4 uptake rate in the

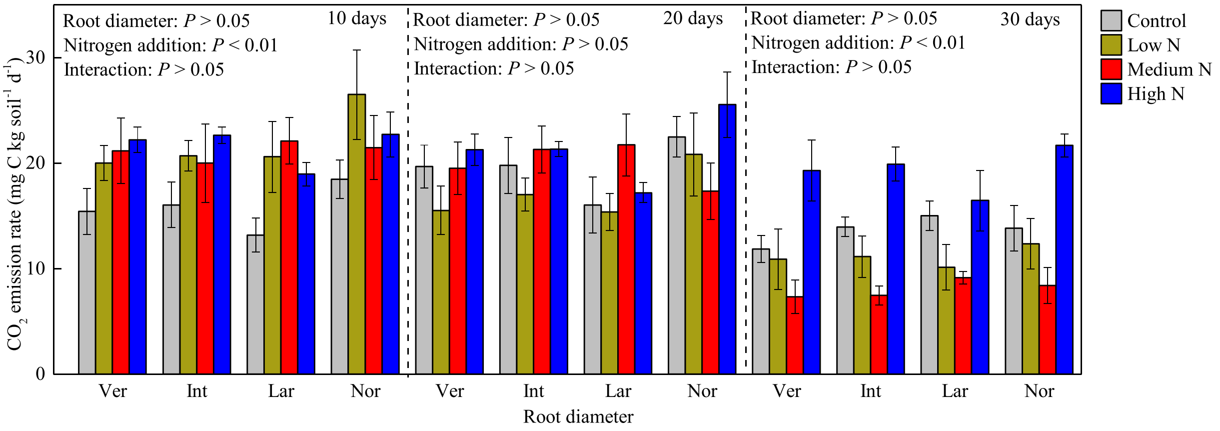

Fig. 1. Temporal variations of CO_2 emission rates among nitrogen addition levels and root diameters. Note: The bars represent the means, and the error bars indicate the standard errors of means (n = 4). Ver, rhizosphere soil of very fine roots (diameter < 0.5 mm); Int, rhizosphere soil of intermediate fine roots (0.5 mm < diameter < 1 mm); Lar, rhizosphere soil of largest fine roots (1 mm < diameter < 2 mm); Nor, non-rhizosphere soil. Control, low, medium, high N are 0, 3, 6, and 9 g N m^{-2} y^{-1} respectively.

rhizosphere soil of very fine roots was faster than that in the rhizosphere soil of the largest fine roots.

3.3. Cumulative GHG emissions

After 30 days of incubation, the cumulative emission/uptake of CO_2, N_2O, and CH_4 was 526.939 mg C kg $soil^{-1}$, 54.990 μg N kg $soil^{-1}$, and 1169.44 μg C kg $soil^{-1}$, respectively. Nitrogen addition significantly increased the cumulative emission of CO_2, with the maximum value in the high N treatment (average of 623.15 mg C kg $soil^{-1}$, Fig. 4, $P < 0.01$). Nitrogen addition inhibited the cumulative N_2O emissions by 37.8%. The cumulative emissions of CO_2 and N_2O were not significantly different among the root diameters, and no interaction was identified between N addition and root diameter. Nitrogen addition significantly increased the CH_4 cumulative uptake with the maximum values observed in the medium N or high N treatment (average of 1794.49 μg C kg $soil^{-1}$). The cumulative uptake of CH_4 in rhizosphere soil was higher than that in non-rhizosphere soil by 73.1%, and cumulative uptake of CH_4 in the rhizosphere soil of very fine roots was higher than that in the rhizosphere soil of the largest fine roots by 14.6%. An interaction was identified between N addition and root diameter, indicating that the cumulative uptake of CH_4 in the rhizosphere soil of very fine roots was more responsive to N addition than that in the rhizosphere soil of the largest fine roots.

3.4. Discrepancies in GWP and total GHG emissions among treatments

The average GWP was 508.77 mg CO_2 eq kg $soil^{-1}$. Nitrogen addition significantly increased the GWP, which showed a maximum value with high N treatment (average of 585.79 mg CO_2 eq kg $soil^{-1}$, Fig. 5, $P < 0.05$). A significant difference in GWP was observed among root diameters. The GWP in rhizosphere soil was less than that in non-rhizosphere soil, and the GWP of rhizosphere soil of the largest fine roots was less than that of the rhizosphere soil of very fine roots. No interaction was identified between N addition and root diameter on GWP. CO_2 emissions mainly contributed to the GWP (Fig. 6). The samples of the different root diameters were scattered, and GHG emission/uptake revealed few differences among root diameters. In the N addition treatments, the samples of the control and low N treatments were combined on the left of the vertical axis (Fig. 6), and the samples of the medium and high N treatment were combined on the right of the vertical axis. Large differences among N treatments were observed with respect to GHG emission/uptake.

3.5. Regression analysis between gas emissions and soil chemistry

Soil cumulative CO_2 emissions were significantly determined by NH_4^+ and NO_3^- contents, with coefficients of determination of −50.8 and 20.9, respectively (Table 3, $P < 0.05$). In particular, NH_4^+ had a

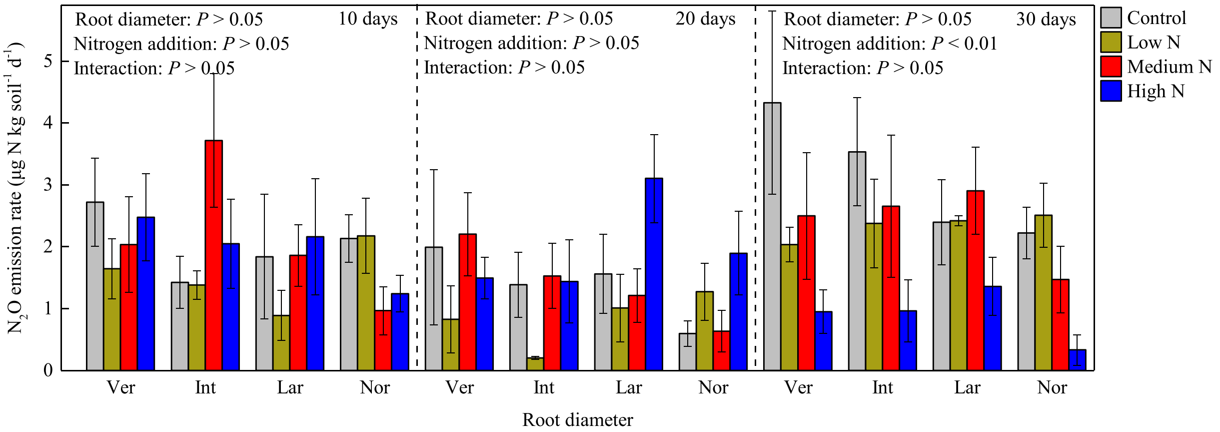

Fig. 2. Temporal variations of N_2O emission rates among nitrogen addition levels and root diameters. Note: The bars represent the means, and the error bars indicate the standard errors of means ($n = 4$). Ver, rhizosphere soil of very fine roots (diameter < 0.5 mm); Int, rhizosphere soil of intermediate fine roots (0.5 mm < diameter < 1 mm); Lar, rhizosphere soil of largest fine roots (1 mm < diameter < 2 mm); Nor, non-rhizosphere soil. Control, low, medium, high N are 0, 3, 6, and 9 g N m^{-2} y^{-1} respectively.

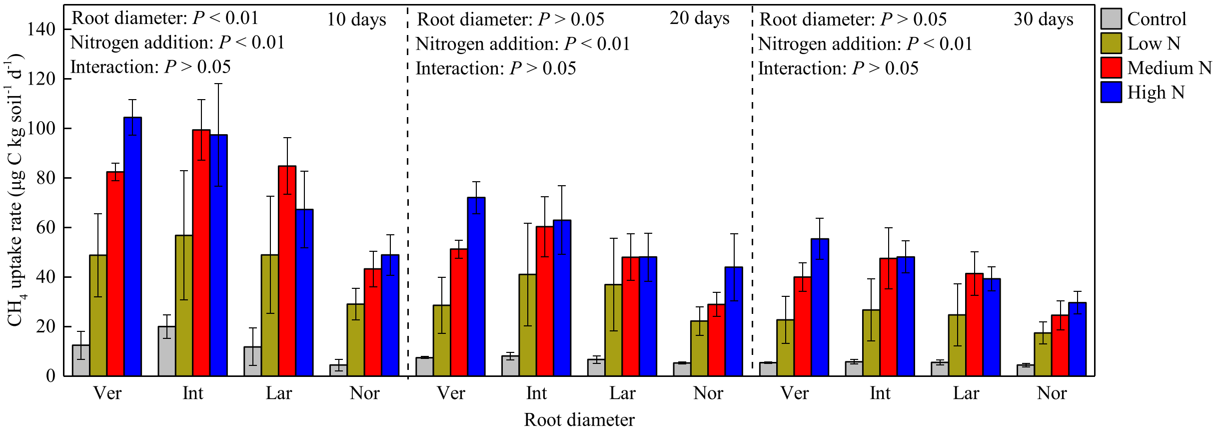

Fig. 3. Temporal variations of CH_4 uptake rates among nitrogen addition levels and root diameters. Note: The bars represent the means, and the error bars indicate the standard errors of means (n = 4). Ver, rhizosphere soil of very fine roots (diameter < 0.5 mm); Int, rhizosphere soil of intermediate fine roots (0.5 mm < diameter < 1 mm); Lar, rhizosphere soil of largest fine roots (1 mm < diameter < 2 mm); Nor, non-rhizosphere soil. Control, low, medium, high N are 0, 3, 6, and 9 g N m^{-2} y^{-1} respectively.

significant inhibitory effect, while NO_3^- had a significant stimulatory effect. Soil OC and NO_3^- had significant positive effects on cumulative N_2O emissions, with coefficients of 1.6 and 7.6, respectively. The cumulative CH_4 uptake was determined based on OC content, NO_3^- content, and pH, with coefficients of 70.0, 501.2, and 4160.6, respectively, and they all exhibited a significant stimulatory effect.

4. Discussion

4.1. Chemical properties of different rhizosphere soils exhibited varied responses to N addition

The effect of N addition on the chemical properties of rhizosphere soil and non-rhizosphere soil was different, which is in line with our hypothesis. In previous studies, the nutrient content of OC, TN, and TP in the roots of *P. tabulaeformis* increased first and then decreased with N addition and had a maximum value at 3 g N m^{-2} y^{-1}, while soil microbial biomass had a maximum value at 6 g N m^{-2} y^{-1} (Jing et al., 2017; Li et al., 2020). Soil quality was affected by the root chemical composition and soil microorganisms. The mean content of OC, TN, and NH_4^+ in the rhizosphere soil of *P. tabulaeformis* had a maximum value at 6 g N m^{-2} y^{-1}. N addition has the same promotion effect on soil microbial biomass, TN, ammonium cycle genes and nitrification genes, with the maximum values observed at 6 g N m^{-2} y^{-1} treatment. Soil organic C, NO_3^- were the main factors driving these positive effects. Li et al. (2020)'s study was carried out in 2018, which has the same N addition treatments and sample plots with us. His soil microbial results fully support our study. Based on these studies in the *P. tabulaeformis* forest, we concluded that soil chemistry is a direct driving factor that affects microorganisms and gas emissions. Exeperiment treatments can change soil biochemical reactions by affecting chemical properties. Non-rhizosphere soil had no direct substance input from the roots. Rhizosphere and non-rhizosphere soils had different responses to N addition due to different mechanisms. Previous studies have focused on rhizosphere soils, and only few studies have been conducted on non-rhizosphere soils. Organic carbon in non-rhizosphere soil is an important part of the soil C pool, which is stable and difficult to decompose (Feudis et al., 2019). However, after activation by non-biological processes (such as N addition), it will be utilized by microorganisms to generate GHGs, which in turn exacerbate climate change (Kemmitt et al., 2008).

Nitrogen addition had variable effects on the chemical properties of rhizosphere soil with different root diameters. Very fine roots have high physiological activity and rapid turnover rates, resulting in an apparent change in the OC content of rhizosphere soil (Wang et al., 2017b). *P. tabulaeformis* forests on the Loess Plateau of China were limited by soil available N (Wang and Zheng, 2018). Nitrogen addition can accelerate the absorption and secretion of N and NH_4^+ by very fine roots and improve the consumption and utilization of N by microorganisms. The largest fine roots have a slow turnover rate and limited function of absorption, resulting in the accumulation of specific soil nutrients under N addition. NO_3^- content increased by 368.7% and 827.9% in the rhizosphere soil of very fine roots and the largest fine roots after N addition,

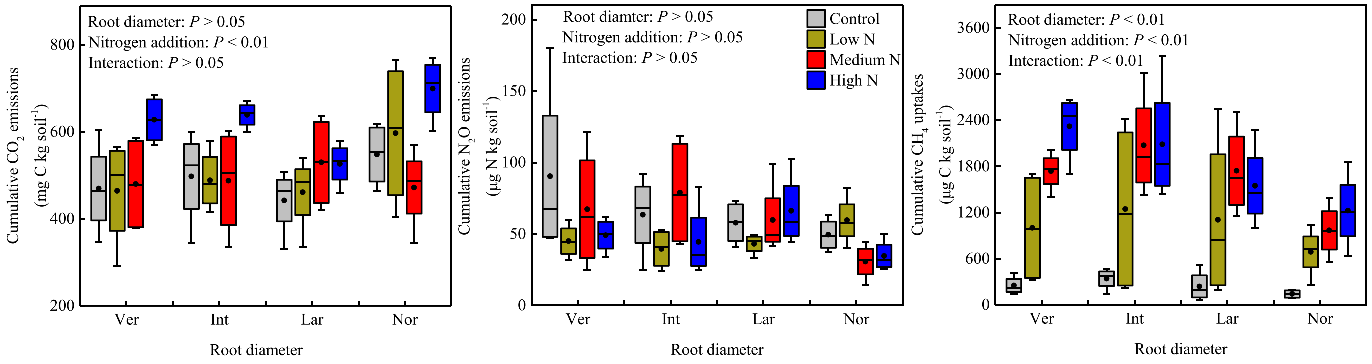

Fig. 4. Cumulative emissions of greenhouse gas among nitrogen addition levels and root diameters (n = 4). Note: Lines inside the boxes represent medians, dots represent means, and the upper and lower edges of the boxes represent the 25th percentile and 75th percentile, respectively. The top bars show the maximum values and the bottom bars show the minimum values. Ver, rhizosphere soil of very fine roots (diameter < 0.5 mm); Int, rhizosphere soil of intermediate fine roots (0.5 mm < diameter < 1 mm); Lar, rhizosphere soil of largest fine roots (1 mm < diameter < 2 mm); Nor, non-rhizosphere soil. Control, low, medium, high N are 0, 3, 6, and 9 g N m^{-2} y^{-1} respectively.

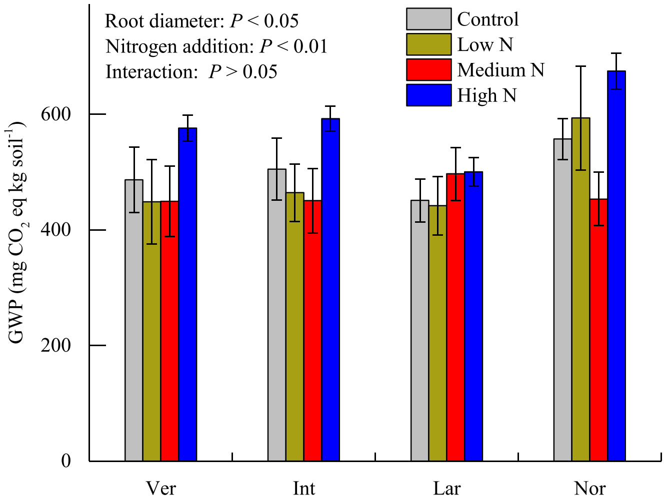

Fig. 5. Global warming potentials (GWP) among nitrogen additions and root diameters. Note: The bars represent the means, and the error bars indicate the standard errors of means (n = 4). Ver, rhizosphere soil of very fine roots (diameter < 0.5 mm); Int, rhizosphere soil of intermediate fine roots (0.5 mm < diameter < 1 mm); Lar, rhizosphere soil of largest fine roots (1 mm < diameter < 2 mm); Nor, non-rhizosphere soil. Control, low, medium, high N are 0, 3, 6, and 9 g N m^{-2} y^{-1} respectively.

respectively. The effect of N addition on NO_3^- content was greater in the rhizosphere soil of the largest fine roots than of very fine roots. Plants exhibit different utilization efficiencies for different N forms (Wang et al., 2015). Bailey (1999) found that *Agrostis stolonifera* easily utilize

Table 3
Multiple stepwise regression analysis of gas cumulative emissions and soil chemistries.

Gases	Selected independent variables	Coefficients	Standard error	t value	P	R^2 (P)
CO_2	Intercept	588.678	35.063	16.789	<0.001	0.13
	NH_4^+	−50.815	17.994	−2.824	<0.05	(<0.05)
	NO_3^-	20.864	9.607	2.172	<0.05	
N_2O	Intercept	−956.755	566.968	−1.687	>0.05	0.12
	OC	1.624	0.730	2.225	<0.05	(<0.05)
	NO_3^-	7.616	3.322	2.293	<0.05	
CH_4	Intercept	−35,874.74	12,275.00	−2.923	<0.05	0.57
	OC	69.99	17.02	4.113	<0.001	(<0.01)
	NO_3^-	501.22	62.69	7.995	<0.001	
	pH	4160.58	1462.91	2.844	<0.05	

Note: OC, organic carbon content; NH_4^+, ammonium content; NO_3^-, nitrate content.

NH_4^+-N, but retain NO_3^--N in the rhizosphere soil. Thus, the utilization efficiency of NH_4^+-N was higher than that of NO_3^--N. In this study, N was added in the form of NH_4NO_3. We speculate that very fine roots absorbed low level of NO_3^--N, while NO_3^--N accumulated only in the rhizosphere soil of the largest fine roots. Therefore, NO_3^- content in the rhizosphere soil of the largest fine roots was more responsive to N addition than that in the rhizosphere soil of very fine roots. Studies have shown the variable effects of N addition on the chemical composition and physiological function of root systems with different diameters (Wang et al., 2017a; Jing et al., 2017). However, the effect of N addition on the chemical properties of rhizosphere soil from different root diameters remains unclear, and this is not conducive to the determination of specific root–soil relationships. Our results confirmed that N addition

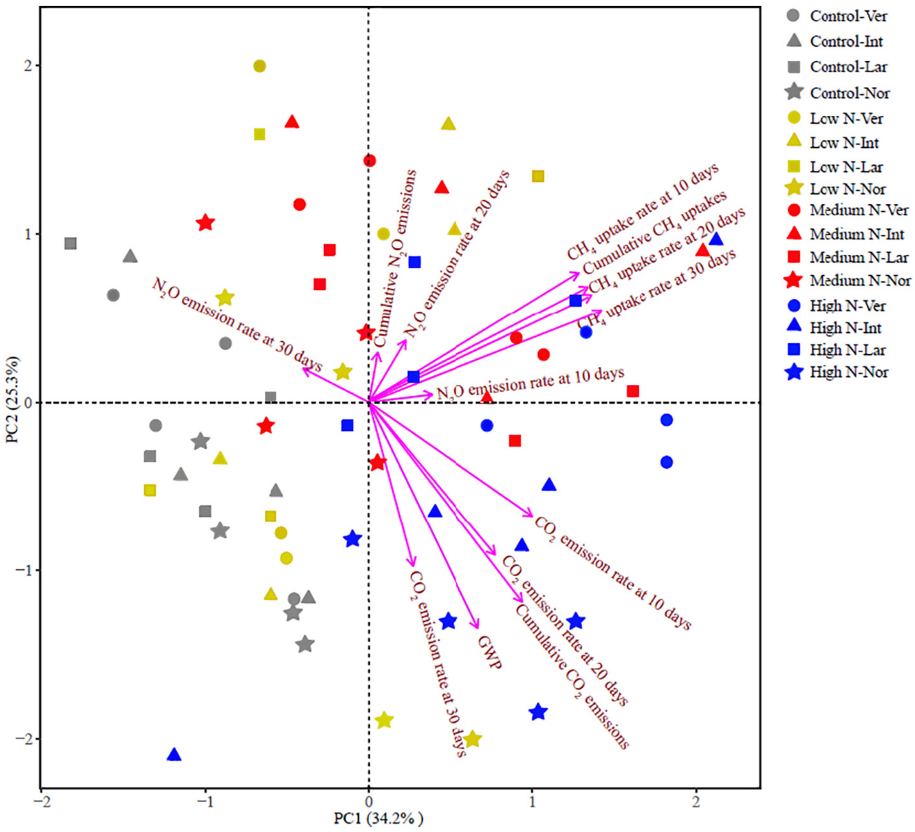

Fig. 6. Principal component analysis (PCA) plot based on Bray–Curtis Distances depicting greenhouse gas fluxes (CO_2 and N_2O emissions and CH4 uptake) and global warming potential (GWP) across the different nitrogen additions and root diameters. Note: The variance explained by each PC axis is given next to the axes. Note: Ver, rhizosphere soil of very fine roots (diameter < 0.5 mm); Int, rhizosphere soil of intermediate fine roots (0.5 mm < diameter < 1 mm); Lar, rhizosphere soil of largest fine roots (1 mm < diameter < 2 mm); Nor, non-rhizosphere soil. Control, low, medium, high N are 0, 3, 6, and 9 g N m^{-2} y^{-1} respectively.

had variable effects on the chemical properties of rhizosphere soil as well.

4.2. Promoting effect of N addition on CO_2 emissions

Multi-level N addition significantly altered the emission rate and accumulation of CO_2, which had maximum values at medium N or high N treatments. These results confirmed our hypothesis and indicated that 6–9 g N m^{-2} y^{-1} have the best promotion effect on soil CO_2 emissions in this study. Nitrogen deposition had a threshold effect on stimulation and inhibition when it affected the composition and function of ecosystems (Zong et al., 2019; Yao et al., 2017). However, the threshold values vary for different ecosystems (Tang et al., 2017). For example, in the northern Qinghai–Tibet Plateau, the threshold values of N addition in four alpine grasslands along a precipitation transect were as follows: 10.3, 11.5, 13.6, and 15.6 g N m^{-2} y^{-1} (Zong et al., 2019). In other studies at this field, N addition significantly affected plant growth, soil microbial community, and soil quality, and 6 g N m^{-2} y^{-1} was the threshold value (Jing et al., 2019; Li et al., 2020). Our results were consistent with previous studies, and the threshold effects of 6–9 g N m^{-2} y^{-1} on soil CO_2 emissions in *Pinus tabulaeformis* forest on the Loess Plateau of China need more verification.

The CO_2 emissions in different rhizosphere soils had the same response to N addition, which was inconsistent with our hypothesis. Deng et al. (2016) reported that the CO_2 emissions in three types of vegetation soil had the same response to experimental treatments, including the acceleration response to vegetation restoration and the reduction response to N deposition. Similarly, the CO_2 emission rate from the rhizosphere soil of different understory vegetation had the same response to environmental changes in an intensively managed Chinese chestnut plantation (Zhang et al., 2014). These results are related to small variations in chemical properties among rhizosphere soils and similar responses to environmental conditions. Experimental treatments have a significantly varied effect on CO_2 emissions in different rhizosphere soils only when the initial chemical properties are sufficiently different. For example, soil chemical properties and crop yields vary greatly in different tillage management regimes. Nitrogen addition (20 g N m^{-2}) significantly increased CO_2 emissions in no-tillage and low-tillage soils but not in traditional tillage soils (Pareja et al., 2019). Likewise, cover cropping promoted soil CO_2 emissions from subsurface drip irrigation treatments but had no effect on soil CO_2 emissions during furrow irrigation treatments (Kallenbach et al., 2010). In the present study, the variations in the chemical properties of rhizosphere soil among root-size classes were 106.6% (OC), 80.9% (TN), 18.0% (TP), 73.7% (NH_4^+), 66.7% (NO_3^-), and 2.3% (pH) less than the variations among N addition treatments. This may be the reason CO_2 emissions in different rhizosphere soils exhibited the same response to N addition. These results indicate that the variation in soil chemical properties among ecosystems is an important basis for evaluating the effect of N deposition on soil CO_2 emissions.

4.3. CH_4 uptake in the rhizosphere soil of very fine roots was more responsive to N addition

Nitrogen addition simultaneously promoted CO_2 emission and CH_4 uptake, which is consistent with the results of previous studies (Stiles et al., 2018; Wang et al., 2018). For example, N addition had a positive effect on soil CO_2 emissions and CH_4 uptake during the growing season in a temperate forest (Yan et al., 2019). Meanwhile, N addition had an inhibitory effect on soil CO_2 emission and CH_4 uptake in an alpine meadow in the Qinghai–Tibet Plateau, China (Jiang et al., 2010). Methane oxidation and respiration of the microbial community exhibited the same response to soil nutrient changes. On one hand, increased soil N availability promoted nutrient accumulation and increased the activity of microorganisms (including methanotrophs), thereby accelerating CO_2 emissions and CH_4 uptake (Yan et al., 2019). On the other hand, N addition induced NO_3^- accumulation indirectly affected the synthesis of enzymes involved in CH_4 oxidation in N starved cells, thereby affecting the CH_4 flux in the soil (Geng et al., 2017).

The CH_4 uptake in rhizosphere soils of different root-size classes exhibited varied responses to N addition, which is inconsistent with the response of CO_2 emissions. Zeng and Gao (2016) found that drying and rewetting did not have a significant effect on soil CO_2 emissions but reduced CH_4 emissions in high-altitude peatlands in the Tibetan Plateau. Biochar addition increased soil CH_4 emissions by 37%, but it had no effect on soil respiration during the rice planting season (Wang et al., 2012). These results are related to the poor effect of short-term experimental treatments on soil properties. Many soil microorganisms respire, and the effect of soil nutrient changes on these microorganisms is relatively limited. However, only a specific group of microorganisms can perform CH_4 oxidation, and the effect of soil nutrient changes on these specific microorganisms is relatively large. Therefore, microorganisms that oxidize CH_4 are more sensitive to soil nutrient changes than other microorganisms. In the present study, variations in the chemical properties of rhizosphere soil among roots of different diameters were not sufficient to cause variable CO_2 emission responses to N addition, but they caused different changes in CH_4 uptake. This indicates that attention should be paid to the changes in soil CH_4 flux when studying the effects of small-scale or short-term experimental treatments on GHG emissions. In addition, CH_4 uptake in the rhizosphere soil of very fine roots was more sensitive to N addition than that in the rhizosphere soil of the largest fine roots. This is consistent with the results of previous studies on the chemical composition, turnover, and exudates of fine roots with different diameters. Hierarchical changes in GHG emissions have been confirmed in rhizosphere soils.

4.4. N_2O emissions showed no N addition threshold

Nitrogen addition inhibited soil N_2O emission, which contradicts our hypothesis. The effect of N addition on N_2O emissions depends on the soil type. For example, N addition significantly promoted N_2O emissions from the surface soils of an apple orchard but did not affect N_2O emissions from the surface soils of grasslands and forestlands (Kong et al., 2013). Moreover, NO_3^--N addition decreased the emission rate of N_2O in an experimental grassland (Bender et al., 2015). The inhibitory effect of N addition may be related to mycorrhizal hyphae (Barrett et al., 2011; Herman et al., 2012). Mycorrhizal fungi form symbiotic relationships with most land plants (Smith and Read, 2008). They provide N to the host plant and themselves by absorbing a large amount of N from the soil (Hodge and Fitter, 2010). These fungi compete effectively with other N_2O producing bacteria for these inorganic N forms, resulting in a reduction in N_2O emissions. Storer et al. (2018) found that N addition reduced soil N_2O emissions when mycorrhizal hyphae were used in microcosm experiments. In contrast, when mycorrhizal hyphae were absent, N addition accelerated the soil N_2O emission rate. Therefore, N addition enhanced the consumption and utilization of soil inorganic N by improving the activity of mycorrhizal fungi, which also reduced N_2O emissions. The soil of *P. tabulaeformis* forests contains numerous mycorrhizal hyphae, leading to an inhibitory effect of N addition on N_2O emissions. The high N treatment had the greatest inhibitory effect, which is in line with the hypothesis that arbuscular mycorrhizae exhibit a high demand for inorganic N. Nitrogen addition for 6 years significantly inhibited N_2O emissions from the surface soil in a mixed Korean pine forest (Chen et al., 2017). In our study, N addition was conducted for 6 years on the surface soil in a Chinese pine forest, and the experimental conditions were similar to those of Chen et al. (2017). These results indicate that N deposition may play a negative role in soil N mineralization in the forests of northern China and reduce the contribution of N_2O emissions to global warming. There is no threshold value for N addition affecting N_2O emissions, which is different from the effect of N addition on CO_2 emissions. Generally, environmental conditions have the same effect on soil microbial respiration and N_2O production. Therefore, many studies have shown a strong positive correlation between

N_2O and CO_2 emissions (Liang et al., 2015; Zhang et al., 2019). However, the number of microorganisms involved in respiration is far greater than that of nitrifying and denitrifying bacteria. Had the response of N_2O production to environmental changes been different from that of respiration, there would be no correlation between N_2O and CO_2 emissions, and environmental conditions would have inconsistent effects (Kong et al., 2013). Nitrogen addition significantly accelerated CO_2 emissions but inhibited N_2O production in our study because the different mechanisms of N addition affected GHG emissions.

Nitrous oxide emissions from rhizosphere soils with different root diameters exhibited the same response to N addition, which was also the same as the result of CO_2 emissions. However, the reasons for these two results may be different. The first reason may be the limited variations in the chemical properties of rhizosphere soil among different root diameters. Soil chemistry affects both CO_2 and N_2O emissions, but CO_2 emissions are more sensitive to chemical changes than N_2O emissions. For example, soil nutrient content decreased with incubation time, which markedly slowed down the emission rate of CO_2, but the change in the emission rate of N_2O was small. Soil CO_2 emissions are more dependent on soil quality than N_2O emissions. If the variations in the chemical properties of rhizosphere soil among different root diameters cannot affect the response of CO_2 emissions to N addition, these variations also cannot affect the response of N_2O emissions. The second reason could be that arbuscular mycorrhizae mainly form symbiotic associations with fine roots, which will affect the soil N_2O emission around the fine roots (Smith and Read, 2008). The root diameter in this study was less than 2 mm, which falls within the definition of fine roots (King et al., 2005). The third reason for the lack of a variable response of N_2O emissions to N addition could be the similar traits of arbuscular mycorrhizae among different root-size classes.

4.5. GWP and total GHG emissions were the largest in medium N and high N treatments

Nitrogen addition promoted soil CO_2 emissions and helped intensify the warming effect. Meanwhile, N addition accelerated the soil CH_4 uptake and decreased N_2O emissions, which has the potential to reduce the warming effect. The GWP combines the changes in CO_2, N_2O, and CH_4 at the same time and comprehensively reflects the effect of soil GHG emissions on global warming. Our results showed that N addition increased the GWP, which is consistent with the results of previous studies (Chen et al., 2017; Li et al., 2012). Soil CO_2 emissions mainly contributed to the GWP, and the negative effects of N_2O and CH_4 emission/uptake were relatively small. The PCA demonstrated that the control and low N treatments exhibited similar soil GHG emissions, and medium N and high N treatments exhibited similar soil GHG emissions. It has been found that 6–9 g N m^{-2} y^{-1} have threshold effects on plant nutrients, litter decomposition, and soil physical characteristics in *P. tabulaeformis* forests (Jing et al., 2019; Gu and Wang, 2017; Yao et al., 2017). The present study confirmed the corresponding changes of CO_2 and CH_4 emission/uptake with plant and soil traits, but the threshold effect of N addition on GHG emissions in forest ecosystem need further researches.

4.6. Soil NH_4^+ and NO_3^- exhibited opposing effects with respect to GHG emissions

Soil OC, NO_3^-, NH_4^+, and pH are the main driving factors that affect GHG emissions from the soil of *P. tabulaeformis* forests. This is consistent with the results of previous studies (Kong et al., 2013; Li et al., 2019; Wang et al., 2012). Soil OC and TN provide reaction substrates and essential nutrients that enable the microorganisms to perform respiration, nitrification, and CH_4 oxidation (Lang et al., 2011; Zheng et al., 2012). Li et al. (2020)'s study proved that N addition has a same effect on soil microbial characteristics with our study, Soil OC and NO_3^- play important role during these processes. Other studies in this area have also confirmed the fundamental driving effect of soil chemical properties on biochemical reactions (Yao et al., 2017; Sun et al., 2018). Therefore, it is possible to explore the change mechanism of gas emission by evaluating the driving effect of soil chemical properties. However, different forms of N may have different effects on soil ecological processes. For example, the soil C cycle was inhibited upon the addition of inorganic N, but increased upon the addition of organic N (Wei et al., 2017). Soil NH_4^+ promotes root secretion of H^+ and reduces rhizosphere pH, while NO_3^- promotes OH^- and/or HCO_3^- secretion and increases rhizosphere pH (Dong and Shu, 2001). Therefore, NH_4^+ and NO_3^- have opposing effects on soil microbial communities and biochemical reactions (Wei et al., 2017). This may be why NH_4^+ and NO_3^- had differential effects on GHG emissions in this study. Soil microorganisms have different preferences for the substrate. For example, NH_4^+ reduced the abundance of fungi, while bacterial growth rate was determined by NO_3^- (Birgander et al., 2014; Naoise et al., 2006). $NaNO_3$ addition significantly increased the soil CO_2 emissions, while NH_4Cl addition had no significant effect on soil CO_2 emissions and reduced the fungal biomass in a subtropical coniferous plantation (Wang et al., 2015). Regression analysis in our study confirmed the positive effect of NO_3^- and the negative effect of NH_4^+ on GHG emissions. The form and flux of atmospheric N deposition varies greatly in different regions, which may have different effects on soil GHG emissions. The deposition of NO_3^--N mainly occurs through the combustion of petroleum, living organisms, and lightning; the migration distance can reach more than several thousand kilometers (Aneja et al., 2001). In comparison, NH_4^+-N deposition occurs through volatilization of soil NH_3, fertilizer and livestock manure, and combustion of biomass and fossil fuels; the migration distance is generally within 100 km (Asman and Vanjaarsveld, 1992). Therefore, natural ecosystems are easily affected by the deposition of N in the form of NO_3^--N, which could be increased by soil CO_2 emissions. Artificial ecosystems are affected by the deposition of N in the form of NH_4^+ and NO_3^-, and the actual GHG emission rates rapidly change owing to the large deposition flux. In general, N deposition promoted soil GHG emissions, but the influence of the form of N deposition and the threshold effect need to be considered for the development of a prediction model and exploration of changing mechanisms in the future.

5. Conclusion

Forests are one of the ecosystems severely affected by N deposition. Nitrogen deposition affects not only the phytochemical composition, growth, litter decomposition, and soil microbial characteristics but also the soil GHG emissions and GWP. The CO_2 emissions and CH_4 uptake were highly dependent on soil nutrients. Nitrogen addition accelerated their flow, and they had the same responses. Owing to the different ecological processes of rhizosphere soil among roots with different diameters, the nutrient contents of different rhizosphere soils had varied responses to N addition. This phenomenon was verified in results associated with CH_4 uptake as well, but not in results associated with CO_2 emission and N_2O emission. Variations in the chemical properties of rhizosphere soil among root-size classes were not enough to cause the variable responses of CO_2 and N_2O emissions to N addition. Further studies are necessary to determine the range of chemical properties among different rhizosphere soils that can cause variable responses in GHG emissions. The N_2O emissions were less dependent on soil nutrients, and N addition had an inhibitory effect on it. Moreover, NH_4^+ and NO_3^- were the main forms of N deposition, and they showed opposite effects on soil GHG emissions. This may be one of the reasons why previous studies on N deposition affecting soil GHG emissions showed varied results. The present study evaluated the effects of N addition on rhizosphere soil gas emissions of fine roots with different diameters, and the changes of other biochemical reactions need to be tested in future studies.

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Author statement

GW conceived and designed research; HJ collected data and conducted research; HJ wrote the initial paper; YL and GL revised the paper. All authors read and approved the final manuscript.

Declaration of competing interest

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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