Fertilization and clear-cutting effects on greenhouse gas emissions of pinewood nematode damaged Masson pine plantation

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ABSTRACT

Introduction: Management practices are essential for maintaining forest ecological functions under increasing diseases and pest disasters. The effects of nitrogen fertilization (NF) and clear-cutting (CC) on the soil microbial community structure and greenhouse gas emissions were investigated of pinewood nematodes (Bursaphelenchus xylophilus)-infected Masson pine (Pinus massoniana) plantations.

Outcomes: CC increased the soil microbial biomass carbon (SMBC) and soil microbial biomass nitrogen (SMBN) contents relative to the control (CK). NF increased the SMBN but had no significant effect on the SMBC content. The total fungal and bacterial abundances increased in the CC treatment compared with the CK, but there was no significant difference between the NF and CK. The cumulative soil CO2 emission (2.35 t C·hm-2) was higher than that of CK (1.65 t C·hm-2) in summer, and the cumulative annual N2O emission (16.90 kg N·hm-2·yr-1) of NF was approximately 47 times of CK (0.36 t N·hm-2·yr-1). CC increased the CO2 flux (2.21 t C·hm-2) in summer but have no significantly effects on N2O emission.

Conclusion: These results indicated that NF and CC practices changed forest soil microbial community structure and affected soil greenhouse gas emissions in pinewood nematodes -infected Masson pine plantations. The CO2 emission rates increased in the NF and CC treatments, which reduced the carbon sequestration function of forests and had a negative impact on climate change.

Introduction

Soil respiration in forest ecosystems is an important source of global atmospheric greenhouse gases (Raich and Schlesinger 1992). Soil carbon (C) release through soil respiration is estimated to be 78–98 Gt C yr-1, which exceeds the C released from fossil fuels (Bond-Lamberty and Thomson 2010). Even small changes in soil respiration will have a great impact on atmospheric gas composition and the global climate (Williams et al. 2013). Forest soil respiration is affected by the physical and chemical environment of the soil (Tang et al. 2005), human interferences (López-Díaz, Benítez, and Moreno 2017), nature disasters (Borkhuu et al. 2015), and so on. It is important to study greenhouse gases release and its influential factors in forest soils to scientifically manage forests and maintain and evaluate forest C stocks.

Soil physical and chemical properties are considered the main factors that explain the temporal variations in soil respiration. Among these properties, soil temperature mainly changes the soil respiration rate by stimulating the decomposer community or fine root growth (Ceccon et al. 2016). Some studies have shown that soil temperature is positively correlated with soil microbial activity and root activity within a certain temperature range (Kim 2008). Moreover, a temperature increase can accelerate the decomposition of soil organic matter (Wang et al. 2016), thereby increasing the soil respiration rate. In addition, the effect of soil temperature on the soil respiration rate is limited by soil moisture in a specific environment (Currie et al. 2003).

Different forest management practices may affect the dynamic process of soil respiration by changing the soil micro-environment, aboveground vegetation composition, and soil mineralization rate (Trathan et al. 2015). The effect of human disturbances on the soil respiration rate has also been studied. Fertilization may increase (Gallardo and Schlesinger 1994; Gao et al. 2015), decrease, or even have no effect on soil respiration (Jassal et al. 2011). Clear-cutting is a common forest management practice that has been reported to increase (Pyker and Fredeen 2002), decrease (Zerva and Mencuccini 2005; Wang et al. 2007), or have no effect on soil respiration (Yuichiro et al. 2008). Increasing soil nutrients and clear-cutting have...
different effects on soil respiration, which may be attributed to differences in vegetation and environmental factors.

Fertilization is an important measure to increase the productivity of plantation forestry. However, fertilization can affect the release of soil greenhouse gases by changing the physical, chemical, and biological characteristics of forest soils. The species composition and the age structure of a plantation are simple, which results in a decline in forest biodiversity, and the frequency of forest fire, diseases, and insect outbreaks is increasing annually. Pinewood nematode (Bursaphelenchus xylophilus) is a pest of pine trees that causes serious damage to forests and endangers ecological security (Borkhuu et al. 2015; Menéndez-Gutiérrez et al. 2018). Research is needed on the effects of the physical and chemical properties of soil and greenhouse gas release under the background of various forest disasters. To maintain the ecological functions of forests, reasonable management measures are essential to improve forest environments or to ensure the establishment of new stands of post damaged plantations.

Masson pine (Pinus massoniana) plantations, with rapid growth and strong adaptability, are one of the most important forests in the southern area of China. Masson pine plantations play important roles in providing wood products and maintaining regional ecological protection functions. However, the decline in biodiversity and the widespread of pinewood nematodes are due to the large area of pure Masson pine forests. Although the factors that affect forest soil respiration have been reported in many studies, it is still unclear whether human management practices affect forest soil respiration and soil C stocks after natural disasters (such as B. xylophilus) (Concilio et al. 2006). It is also unclear whether fertilization affects the biotic and abiotic environment, soil C stocks, and flux, and greenhouse gas release of Masson pine plantations after damage caused by pinewood nematodes. Therefore, post damaged Masson pine forests were selected to research the effects of N application and clear-cutting on the microbial population structure and greenhouse gas emissions, to assess the effect of human activities on the soil C and N cycles of forest ecosystems and to provide a basis for scientifically managing pinewood nematodes-damaged forests.

Materials and methods

Study site description

The study sites are located in Xiangyang City (31°59’S, 112°03’E), Hubei Province, China. This region has a temperate continental and monsoon-influenced climate and acidic yellow-brown soil. The mean annual precipitation is about 820 mm-1100 mm, and the mean annual temperature is 15°C-16 °C. The mean temperature is the highest (28°C) in July and the lowest in January (5.2°C). The highest rainfall was 124.6 mm in April, and the lowest rainfall was 26.2 mm in February. The annual rainfall was 1051.9 mm. The mean tree height in the Masson pine plantation was 13.66 m, and the diameter at breast height was 23.56 cm. The canopy density of the CK and fertilized plots were 0.7, and the coverage of the clear-cutting plots were 0.5 in spring and 0.9 in summer.

Experimental treatment

Twenty-six-year-old Masson pine plantations infected by pinewood nematodes were chosen with similar site conditions under three treatments: control (CK), nitrogen fertilization (NF) and clear-cutting (CC). There were 3 replicate plots (20 m×10 m) of each treatment. Nitrogen was applied from 2014 to 2016 in the NF treatment in April, June and August, i.e., 375 kg·ha⁻¹, 750 kg·ha⁻¹ and 375 kg·ha⁻¹ urea, respectively. Masson pine trees were clear-cutting, and harvest residues of the CC treatment were removed from the sites without destroying the undergrowth vegetation in December 2015.

Soil respiration gases sampling and laboratory analysis

Soil CO₂ flux and soil N₂O flux were sampled and measured twice a month from April 2016 to April 2017 with the closed-chamber method. Three static chambers were installed in each plot. The chambers were made of polyvinyl chloride (PVC) pipes, and the surrounding soil was compacted tightly to prevent air leakage. Sampling was performed from 9:00 am to 11:00 am. Gas samples were collected from the sample port with syringes (100 ml) 2 hours after closing the chamber and were then injected into vacated bottles immediately. The concentrations of CO₂ and N₂O in the gas samples were quantified by gas chromatography (Agilent 7890A, Agilent Technologies, Palo Alto, California, USA) within 24 h.

CO₂ and N₂O fluxes were calculated according to the slope of the linear or nonlinear changes in gas concentrations in the enclosed chamber headspace over time. Annual cumulative CO₂ and N₂O emissions were calculated by linear interpolation between temporally adjacent measurements. Soil temperature and soil moisture at a depth of 5 cm were measured adjacent to each chamber using a temperature and moisture measuring instrument and gas sampling.

Soil sampling and analysis

Six replicate soil samples at 0–30 cm depth were collected with an 8 cm diameter auger from each plot,
quarterly from April 2016 to April 2017. One part of the soil was air-dried, ground, and then sieved through a 0.15 mm mesh sieve before laboratory analysis. Another part of the fresh soil was used to determine the soil microbial biomass carbon (SMBC), soil microbial biomass nitrogen (SMBN) and microbial community structure. A mixed soil suspension (soil: water = 1:2.5) was used to determine soil pH values with a glass electrode.

Soil organic carbon was determined with the potassium dichromate oxidation method. Soil NH$_4^+$-N and NO$_3^-$ N were analyzed with a flow-injection autoanalyzer (Tecator FIA Star 5000 Analyzer, Foss Tecator, Sweden). SMBC and SMBN were measured with chloroform fumigation extraction method by a TOC analyzer (Wu et al. 2010) (TOC-VWP, Shimadzu Corporation, Japan). The soil microbial community structure was characterized by phospholipid fatty acid (PLFA) analysis as described by the modified Buyer method (Buyer et al. 2010) and was classified by the Microbiological Identification System (Microbial Identification System, MIDI, Newark, DE, USA).

**Statistical analysis**

One-way ANOVA with Tukey’s post hoc test ($p < 0.05$) was used to test for significant differences in environmental factors among different treatments. Correlation analysis was used to investigate the relationships between soil respiration and environmental variables. Univariate regression analysis was used with soil environmental factors in different phases as a response variable and the dynamics of soil CO$_2$ flux or soil N$_2$O flux as predictor variables. SPSS software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

**Results**

**Soil physical and chemical properties**

There were no significant differences in soil temperature among the three treatments in the whole the study period. The soil moisture of the CC treatment was significantly lower than that of the CK from July to September, but there was no significant difference between the NF and the CK treatment. In the CC treatment, the soil mean pH value was higher but the soil NH$_4^+$-N and NO$_3^-$N were lower than those of the CK. The soil organic carbon contents were not significantly different between the CC and CK treatments (Table 1). The soil NH$_4^+$-N and NO$_3^-$N contents in the NF treatment were significantly higher than those of the CK, but there was no significant difference in the soil pH values and soil organic carbon contents (Table 1).

**SMC and SMBN**

The annual mean SMBC and SMBN in the CC treatment were higher than those of CK (Table 1). SMBC in October (Figure 1A) and SMBN in July and October of CC treatment were significantly increased compared with the CK (Figure 1B). The annual mean SMBN in the NF treatment was higher than that in the CK treatment, but there was no significant difference in the SMBC between NF and CK treatments. The SMBC in the NF treatment was significantly lower than that of the CK in July, January and April, while the SMBN increased significantly in the four sampling months compared with CK (Table 1).

**Soil microbial community structure**

The soil total bacterial abundances of the CC treatment increased significantly by 27.7% in January and 26.4% in April compared with CK, including the gram-positive and gram-negative bacteria ($p < 0.05$, Table 2). Actinomycetes and anaerobes abundances of CC treatment were increased significantly in January, about one years after clear-cutting, compared with CK ($p < 0.05$). The soil total fungal abundance of the CC treatment was 134.5% higher in January and 40.2% higher in April compared with that of the CK, in particular, there was a significant increase in AM fungi in July 2016, January 2017 and April 2017 (Table 2). The bacteria/fungi ratios in the CC treatment

| Table 1. Soil physical and chemical properties (Mean±SE). Different letters in the same row indicate significant differences at $P < 0.05$ level. |
|---------------------------------------------|
| **Soil properties** | **Treatment** | **n** |
|---------------------------------------------|--------------|-----|
| Soil temperature (°C) | 15.60 ± 1.03 a | 16.64 ± 1.06 a | 15.74 ± 1.01 a | 39 |
| Soil moisture (%) | 19.58 ± 0.56 a | 19.06 ± 1.07 b | 19.85 ± 0.75 a | 39 |
| pH | 4.57 ± 0.06 b | 4.95 ± 0.08a | 4.56 ± 0.07 b | 12 |
| NO$_3^-$ N (mg·kg$^{-1}$) | 28.23 ± 1.23 b | 21.72 ± 1.65 b | 91.94 ± 7.69 a | 27 |
| NH$_4^+$-N (mg·kg$^{-1}$) | 7.43 ± 0.58 b | 7.07 ± 0.84 b | 115.89 ± 26.88a | 8 |
| Organic C (g·kg$^{-1}$) | 17.18 ± 0.99 a | 16.74 ± 1.26 a | 15.34 ± 1.79 a | 12 |
| MBC (mg·kg$^{-1}$) | 91.83 ± 14.61 a | 106.24 ± 12.55 a | 64.98 ± 10.64 a | 12 |
| MBN (mg·kg$^{-1}$) | 23.09 ± 1.32 b | 30.27 ± 2.30 b | 62.22 ± 5.05 a | 12 |

Note: MBC: Microbial biomass carbon; MBN: Microbial biomass nitrogen. CK: Control, CC: Clear-cutting, NF: Nitrogen fertilization; the same in the tables below.
ranged from 11.32 to 24.29 and decreased significantly in January and April compared with CK (Table 2).

There was no significant difference between NF and CK treatments in the gram-positive, gram-negative, anaerobes, actinomycetes, and the total bacterial abundances. All kinds of bacteria abundances of NF treatment almost lower than that of CC treatment ($p > 0.05$, Table 2). The total fungi abundances of the NF treatment were lower than, but no significantly difference when compared with CK (Table 2). The fungal/bacterial ratios in the NF treatment ranged from 10.98 to 37.26 and increased in July, but decreased in January significantly compared with CK ($p < 0.05$, Table 2).

**Soil CO$_2$ flux and soil N$_2$O flux**

The soil N$_2$O flux ranged from 2.38 to 9.96 $\mu$g N m$^{-2}$ h$^{-1}$ in the CK treatment, from 0.74 to
6.64 µg N·m\(^{-2}\)·h\(^{-1}\) in the CC treatment, and from 54.89 to 631.35 µg N·m\(^{-2}\)·h\(^{-1}\) in the NF treatment. Clear-cutting had no significant effect on the soil N\(_2\)O flux and cumulative emissions, but N application significantly increased the soil N\(_2\)O flux and cumulative emissions (Figure 2, Figure 3). Soil N\(_2\)O emissions exhibited an increasing trend from May to September. There was no significant difference between the CK and CC treatments, but the soil N\(_2\)O emission of the NF treatment was significantly higher than that of the CK treatment, and the peak emissions occurred in July (Figure 2(a)). The cumulative annual N\(_2\)O emissions (16.90 kg N·hm\(^{-2}\)·yr\(^{-1}\)) in the NF treatment were approximately 47 times those of the CK (0.36 kg N·hm\(^{-2}\)·yr\(^{-1}\)). The cumulative soil N\(_2\)O emissions showed no significant difference between the CC (0.19 kg N·hm\(^{-2}\)·yr\(^{-1}\)) and the CK treatments (Figure 3(a)).

The soil CO\(_2\) flux ranged from 15.83 to 54.27 mg C·m\(^{-2}\)·h\(^{-1}\) in the CK treatment, from 16.95 to 84.23 mg C·m\(^{-2}\)·h\(^{-1}\) in the CC treatment, and from 13.21 to 85.33 mg C·m\(^{-2}\)·h\(^{-1}\) in the NF treatment. Both CC and NF treatments increased soil CO\(_2\) emissions (Figure 2, Figure 3). The maximum CO\(_2\) emission fluxes occurred from June to August in all treatments, and significant dynamic changes occurred from May to September. The soil CO\(_2\) emission rates of the NF and CC treatments were

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Monthly variations in soil CO\(_2\) (A) and N\(_2\)O (B) fluxes (n = 8).
soil communities affected by plantations, a different microbial community was observed. In the shrub type, the annual cumulative soil CO₂ emissions were approximately 1.4 times those of the CK (1.65 t C·hm⁻²) in summer (Figure 3(b)).

Factors related to soil N₂O and CO₂ emissions

Soil N₂O flux was positively correlated with the SMBN (P < 0.01) and NO₃⁻ N (P < 0.05) contents, but there were no significant correlations with soil temperature and soil moisture (Table 3). Soil CO₂ flux was positively correlated with SMBC, soil temperature, and soil organic C (P < 0.01) but negatively correlated with soil moisture (P < 0.05) (Table 3).

Discussion

Effect of forest management practices on soil microbial communities

Studies have shown that multiple factors, including forest type, climate, soil condition and management practices, affect the soil microbial communities of plantations (Burton et al. 2010; Lladó, López-Mondéjar, and Baldrian 2018). The conversion of native Masson pine plantations to Eucalyptus plantations influences the structure and metabolic activity of soil microbial communities because shrub and herb coverage decrease significantly (Chen et al. 2013).

Our results showed that the clear-cutting treatment improved the soil microbial community abundances, including gram-negative bacteria, gram-positive bacteria, soil anaerobic bacteria, eukaryotic organisms, actinomycetes, arbuscular mycorrhizal fungi, and total fungi. Fertilization may alter soil microbial communities directly or indirectly (Compton et al. 2004; Frey et al. 2004) and may limit soil microbial biomass and activity (Allen and Schlesinger 2004). N fertilizer had an effect on the total fungal population but no significant effect on bacterial communities in this study. Research has shown that understory vegetation plays an important role in maintaining the soil microbial community and in driving litter decomposition processes. The coverage of shrubs and herbs increased after clear-cutting treatment of pine-wood nematodes-damaged Masson pine plantations, which improved the soil microclimate to a certain extent and changed the plant communities and their effects on soil nutrients and habitats, it will further change the composition and function of soil microbes. In this study, the understory vegetation coverage increased significantly after clear-cutting (0.9) compared with CK (0.4), but fertilization had no significant impact on understory vegetation coverage, which may be the reason why clear-cutting had more impact on microbial community abundances than that of fertilization treatment.

Soil bacteria and fungi can decompose organic matter, minerals and nitrogen fixation. The plant symbiosis microbial can provide nitrogen, phosphorus and other mineral elements directly to plants. In this study, clear-cutting improved microbial abundances, which was conducive to the decomposition of organic matter. In addition, the improved microbial abundances, such as AM fungi and fungi etc., providing more nutrients for plant growth. Therefore, we predicted that clear-cutting treatment was conducive to the forests' rapid regeneration.

Effect of N fertilization on soil N₂O and CO₂ fluxes

Soil NH₄⁺-N and NO₃⁻ N were significantly higher in the NF treatment than in the CK, by approximately 3 and 16 times, respectively. The cumulative annual N₂O emissions (16.90 kg N-hm⁻²·yr⁻¹) in the NF treatment
Table 3. Correlation analysis between soil CO₂ fluxes, soil N₂O fluxes and soil environmental factors.

| Factor       | CO₂ flux | N₂O flux | MBC   | MBN   | Temperature | Moisture | Total N | Organic C | NO₃⁻N | NH₄⁺-N | Actinomycetes | Fungi  | Bacteria |
|--------------|----------|----------|-------|-------|-------------|----------|---------|-----------|--------|--------|--------------|--------|----------|
| CO₂ flux     | 1        |          |       |       |             |          |         |           |        |        |              |        |          |
| N₂O flux     | 0.362*   |          |       |       |             |          |         |           |        |        |              |        |          |
| MBC          | 0.719**  | 0.039    | 1     |       |             |          |         |           |        |        |              |        |          |
| MBN          | 0.36     | 0.855**  | 0.089 | 1     |             |          |         |           |        |        |              |        |          |
| Temperature  | 0.855**  | 0.267    | 0.754** | 0.454 | 1           |          |         |           |        |        |              |        |          |
| Moisture     | −0.355*  | 0.191    | −0.054 | 0.094 | −0.358*     | 1        |         |           |        |        |              |        |          |
| Nitrogen     | −0.347   | −0.028   | −0.139 | −0.419 | −0.429     | −0.037   | 1       |           |        |        |              |        |          |
| Organic C    | 0.727**  | 0.284    | 0.756** | 0.087 | −0.602*    | −0.309   | 0.618*  | 1         |        |        |              |        |          |
| NO₃⁻N        | −0.204   | 0.943*   | 0.127 | 0.189 | 0.968**    | −0.661   | 0.402   | −0.131    | 1    |        |              |        |          |
| NH₄⁺-N       | 0.068    | 0.592    | 0.436 | 0.012 | 0.992**    | −0.732*  | 0.454   | 0.14      | 0.915** | 1      |              |        |          |
| Actinomycetes| 0.343    | −0.229   | 0.614* | −0.257 | 0.264     | −0.269   | 0.415   | 0.588*    | −0.121 | 0.108  | 1            |        |          |
| Fungi        | −0.160   | −0.474   | 0.132 | −0.521 | −0.371    | −0.289   | 0.430   | 0.111     | −0.462 | −0.287 | 0.702*       | 1      |          |
| Bacteria     | 0.227    | −0.223   | 0.472 | −0.267 | 0.105     | −0.314   | 0.541   | 0.447     | −0.096 | 0.126  | 0.978**      | 0.769** | 1        |

*: significant correlation at the 0.05 level based on a two-sided test, **: significant correlation at the 0.01 level based on a two-sided test.
were approximately 47 times those of the CK (0.36 t N·hm$^{-2}$·yr$^{-1}$). The NF treatment caused a significant increase in the N$_2$O flux, which is consistent with previous studies (Gallardo and Schlesinger, 1994; Jassal et al. 2011). Soil nitrification and denitrification are the main pathways for N$_2$O release. High N leads to the enhancement of soil nitrification and denitrification and is the key factor that increases soil N$_2$O flux. The SMBN in the NF treatment was higher than that of the CK, which accelerated the decomposition rate of organic N, leading to an increase in the soil N$_2$O flux. Studies have also shown that rhizosphere respiration increases because of fertilization, which increases soil respiration (Truong and Marschner 2018). For example, soil respiration increased through an increase in the autotrophic respiration rate in a subtropical evergreen forest to which N was applied (Gao et al. 2014).

Greenhouse gas emissions (CO$_2$, N$_2$O) are affected by various factors. The N content is particularly important for soil respiration (Chen et al. 2017; Truong and Marschner 2018). In our study, N application increased not only the soil N$_2$O flux but also the soil CO$_2$ flux, which is an important driver of global warming. Research also showed that increased nutrient inputs have the potential to alter the carbon storage function of these ecosystems through enhanced microbial respiration of previously sequestered carbon (Geoghegan et al. 2018). Therefore, it is essential to CK the soil N levels in forest management practices to reduce greenhouse gas emissions, maintain forest C stocks, and achieve sustainable forest ecosystems.

**Effect of clear-cutting on soil N$_2$O and CO$_2$ fluxes**

Although Masson pine trees will wither and die quickly after attack by pine wood nematodes, clear-cutting post disaster changes the microclimatic characteristics of the forest, accelerates the renewal of the undergrowth layer, and thus affects the microbial activity of the soil and the release of greenhouse gases. In this study, however, there was no significant difference between clear-cutting treatments and the CK treatments in terms of the N$_2$O flux. The correlation analysis showed that the soil N$_2$O flux was positively related to the NO$_3^-$N and MBN contents in the CC treatment. In addition, the soil microbial populations increased in the CC treatment, and these microbes may compete with plants for soil N. At the same time, soil microbial activity, nitrification, and denitrification may be affected by increased soil pH, but the mechanism deserves to be studied further.

The soil CO$_2$ flux in the CC treatment was significantly higher than that of the CK, and the SMBC also increased, similar to the results of Shabaga et al. (2015). The solar radiation intensity reaching the ground increased in the absence of aboveground vegetation because the canopy density decreased after clear-cutting, which potentially increased the soil temperature and accelerated the mineralization rate of soil organic matter (Wang et al. 2018). The soil CO$_2$ flux increased through an increase in the residual organic matter after clear-cutting, and soil microbial biomass and activity increased, which accelerated the decomposition rate of the forest residues after clear-cutting (Guo et al. 2010; Lavoie, Kellman, and Risk 2013; Kulmala et al. 2014). Although the clear-cutting treatment reduced the supply of C sources from above- to belowground and autotrophic respiration decreased due to root death, the increase in soil microbial activity and improved soil C stocks accelerated the CO$_2$ flux. Therefore, clear-cutting post damaged forests will also accelerate the release of greenhouse gases and reduce the C stocks in forest soils.

**Main factors affecting soil N$_2$O and CO$_2$ fluxes**

The correlation analysis showed that SMBN and the soil nitrate-nitrogen content were related factors that affected the N$_2$O emission rate of Masson pine plantations. According to the stepwise regression analysis, SMBN was the main controlling factor of soil N$_2$O flux. First, SMBN is a key factor that affects the soil N mineralization rate. Nitrification and denitrification performed by soil microorganisms are important factors that affect soil N$_2$O emissions. Second, there was a significant positive correlation between soil N$_2$O flux and soil NO$_3^-$N. NO$_3^-$N was the respiratory substrate for N$_2$O emissions, similar to the results of Smith et al. (2003). However, there was no significant correlation between the N$_2$O flux and NH$_4^+$-N probably because NH$_4^+$-N was absorbed by plant roots (Yang et al. 2013).

SMBC, soil organic C, soil temperature, and soil moisture are important factors that affect soil CO$_2$ flux. According to the stepwise regression analysis, soil temperature was the main factor that controlled the soil CO$_2$ flux, followed by the fungal abundance. It was further confirmed that the influence of soil temperature on the soil CO$_2$ emission rate was the most critical among all impacting factors. In addition, CC and NF practices increased soil temperature sensitivity in the order of NF>CC>CK (Table 5), indicating that increased soil nutrients and clear-cutting treatments improved the response of soil CO$_2$ flux to soil temperature. Soil microbial biomass and microbial activity inhibition may decrease due to soil nutrient changes (increasing N applied) (Treseder 2008), which reduce soil C mineralization rates and enhance the response of soil respiration to soil temperature changes. However, clear-cutting will potentially increase soil temperature, decrease soil moisture, and accelerate the mineralization rate of soil.
organic matter, thus enhancing the sensitivity of soil CO$_2$ flux to soil temperature.

**Conclusion**

This study showed that clear-cutting treatment increased the SMBC and SMBN contents, increased the microbial abundances and the CO$_2$ emission of pinewood nematodes damaged Masson pine plantation. Nitrogen fertilization increased the SMBN content, had no significant effect on the SMBC content and microbial abundances. Nitrogen fertilization increased the cumulative annual N$_2$O emission and the cumulative CO$_2$ emission in summer. These results indicated that not only clear-cutting but also nitrogen fertilization management measures interfered with forest soil physicochemical and microbial environmental, resulted in the increasing of soil greenhouse gas emissions in pinewood nematodes-infected Masson pine plantations.

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**Availability of data and materials**

The data sets supporting the results of this article are included within the article and its additional files.

**Authors’ contributions**

Li Mei conceived of and designed the study, Xue Zhang and Lihao Song and Tianyu Zhao collected data for the study. Li Mei, Xue Zhang, Zeyao Zhao and Tong Chen analyzed the results and wrote the manuscript. All authors have read and approved the final version of this manuscript.

**Disclosure statement**

The authors declare that they have no competing interests.

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