RESPONSE OF SOIL MICROBIAL COMMUNITY AND HYDROTHERMAL ENVIRONMENT TO NITROGEN DEPOSITION IN *PINUS MASSONIANA* FOREST IN CENTRAL ASIA

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Abstract*: In order to demonstrate the response of soil microbial community and hydrothermal environment to nitrogen deposition(low nitrogen* N_{20}*:20kg•hm*$^{-2}$*;medium nitrogen* N_{40}*:40kg•hm*$^{-2}$*;high nitrogen*N_{60}*:60kg•hm*$^{-2}$ *and contrast* N_0*:0kg•hm*$^{-2}$*) , a Pinus massoniana forest in Central Asia was chosen to do the nitrogen deposition simulation experiment. This research is aimed to provide a theoretical evidence for the protection of soil ecosystem under different forest types in china. The results showed that: 1) Soil microbes of Pinus massoniana forest were in seasonal changes (spring, autumn, winter, and summer). Differences in different seasons were significant. 2) A very significant quadratic relationship was shown between soil microbes and soil temperature. However, the relationship between soil microbes and the soil water content was not closely related. N deposition reduced the relationship between microbes and temperature but increased the correlation between microbes and water content. 3) Effects of N deposition on soil temperature and soil water content were significant, but the effects were in small scale. 4) The effects of N deposition on soil microbial community structure were significant.*

Key words*: nitrogen deposition; soil microbes; soil temperature; soil water content; Pinus massoniana forest*

1. Introduction

In recent years, human activities such as nitrogen (N) fertilizer application, rapid development of animal husbandry and combustion of fossil fuels have led to be doubled and redoubled of global atmospheric nitrogenous compounds, leading to an increase in atmospheric N deposition [1]. Excessive nitrogen deposition can increase environmental pressure, such as increasing carbon (C) deposition, exacerbating soil acidification, leading to water eutrophication, and reducing soil microbial diversity [2-4]. Soil microbes are a key part of soil ecosystems and their community structure as an indicator of environmental change is gradually drawing people's attention [5-7]. At present, soil environment change caused by nitrogen deposition has gradually become the emphasis of scholars in various fields. Of them, the most commonly used research method is the simulated N deposition test of adding exogenous nitrogen. However, the conclusions of different research areas, research objects and research time are different [8-13]. There is great uncertainty about the effects of nitrogen deposition on soil microbial community structure [14-15]. In addition, and the effects of different seasons and N deposition levels on soil microbial community composition and structural diversity of *Pinus massoniana* forest have not been reported.

Widely distributed *Pinus massoniana* soil in the mid-subtropical regions of China was selected as the research object in this study. In four seasons (spring, summer, autumn and winter), simulated N deposition test of artificially adding exogenous nitrogen was carried out. The effects of different N deposition levels on soil microbial community and related environmental factors in *Pinus massoniana* forest soil were explored and the dynamic characteristics of soil microbial community in *Pinus massoniana* soil were studied to provide a reference for studying the effects of N deposition on the mid-subtropical forest ecosystem.

2. Methodology

2.1. Research area overview

In this study, Jinyun Mountain Nature Reserve, the typical representative area of the mid-subtropical mountainous regions with the wide distribution of *Pinus massoniana*, was selected as the research area. Jinyun Mountain Nature Reserve is located in Beibei District of Chongqing (106°22'E, 29°49'N), with an altitude of 350-951.5 m and a typical mid-subtropical warm and humid monsoon climate. It is mainly acid yellow soil (pH 4.0-4.5) and most are sticky or sandy. The forest coverage rate in the region is 96.6%. There are a wide range of warm coniferous forests and mixed broadleaf-conifer forests. Of them, the coniferous species are mainly *Pinus massoniana* and Chinese fir, especially with the widest distribution of *Pinus massoniana.*

The basic features of three standard plots in *Pinus massoniana* forest soil are shown in table 1。

Table 1: Stand characteritics of *Pinus massoniana* forest

forest type	sample plot	stand age (a)	canopy density (%)	average DBH (cm)	average height (m)	herbage coverage (%)
Pinus massoniana forest	plot 1	45	79	Φ18.6	14.2	53
	plot 2	45	82	Φ18.7	13.8	45
	plot 3	45	79	Φ18.8	14.3	53

Note:D stands for ground diameter(The trunk diameter measureed at a height of 30cm from the ground);Φstands for diameter at breast height(The trunk diameter measureed at a height of 1.3m from the rhizome).

The dominant plants in subtropical forests are shown in table 2.

Table 2: Dominant plant species of *Pinus massoniana* forest in the study area

Plant Type andcommon names	Plant Names
superiority magaphanerophytes	*Cunninghamia lanceolata (Lamb.) Hook.*
	Rhus chinensis Mill.
	Symplocos setchuensis Brand
	Ficus heteromorpha Hemsl.
	Cyclobalanopsis glauca (Thunb) Oersted
	Mallotus japonicus (Thunb.) Muell. Arg.
	Mallotus barbatus (Wall.) Muell. Arg.
	Celtis sinensis Pers.
	Symplocos laurina (Retz.) Wall.
	Eurya obtusifolia H. T. Chang
	Quercus fabri Hance
	Neosinocalamus affinis (Rendle) Keng f.
herbaceous species	*Microlepia marginata (Houtt.) C. Chr.*
	Hicriopteris chinensis (Ros.)Ching
	Setaria palmifolia (Koen.) Stapf
	Smilax china Linn.
	Rubus corchorifolius Linn. f.
	Sabia swinhonei Hemsl.
	Millettia dielsiana Harms

In May 2014,We adopted the "five point method" in each fixed plots,and taked soil sample using a 10cm diameter earth drill.the sampling depth is 20cm.and removed plant and animal residues in *Pinus massoniana* forest soil.After bringing back to the laboratory for sift,We mixed the soil on plastic film and taked about 1kg soil by quartering for analysis of the physiochemical properties of soil .the average physical chemical properties of original soil in three plots of *Pinus massoniana* forest are shown in the following table 3.

Table 3: Soil physical chemical properties of *Pinus massoniana* forest

Forest Type	pH	Available Kalium/g•kg^{-1}	SOC /g•kg^{-1}	Sand grains 1~0.05/%	Silt 0.05~0.005/%	Clay <0.005/%
Pinus massoniana forest	4.34	0.32	23.64	88.17	4.81	7.02

2.2. Test designs

Three standard and fixed plots with an area of 20 m × 20 m were selected in the natural forest of *Pinus massoniana* in Jinyun Mountain. The interval between the two standard plots was greater than 10 m. Determining three points along the diagonal line of each plot, four small plots of 1 m × 1 m were selected at each point, shown in Figure 1. Three N deposition levels with low N (N_{20}: 20 kg·hm^{-2}), medium N (N_{40}: 40 kg·hm^{-2}) and high N (N_{60}: 60 kg·hm^{-2}) were set for N deposition treatment on plots. Each level of N treatment in *Pinus massoniana* forest was repeated 9 times and each treatment amount was divided into 4 times (including control: N_0: 0 kg·hm^{-2}).

One week after the N deposition treatment, soil samples (spring, summer, autumn, and winter) at the depth of 0-20 cm in PVC pipes were collected and mixed three soil samples with the same N deposition treatment on the same standard plot, respectively. Soil samples were brought back to the laboratory on the same day. Animal and plant residues were removed and then the samples were screened with a 2 mm sieve. The samples were stored in a preservation tank at 4°C for the determination of phospholipid fatty acid (PLFA). The modified Bligh-Dyer method was used in PLFA [16]. Two soil temperature resistance thermometers and two soil moisture SM300 sensors equipped with the ACE Automated Soil CO_2 Exchange System were used to measure the temperature and water content of the soil at a depth of 10 cm.

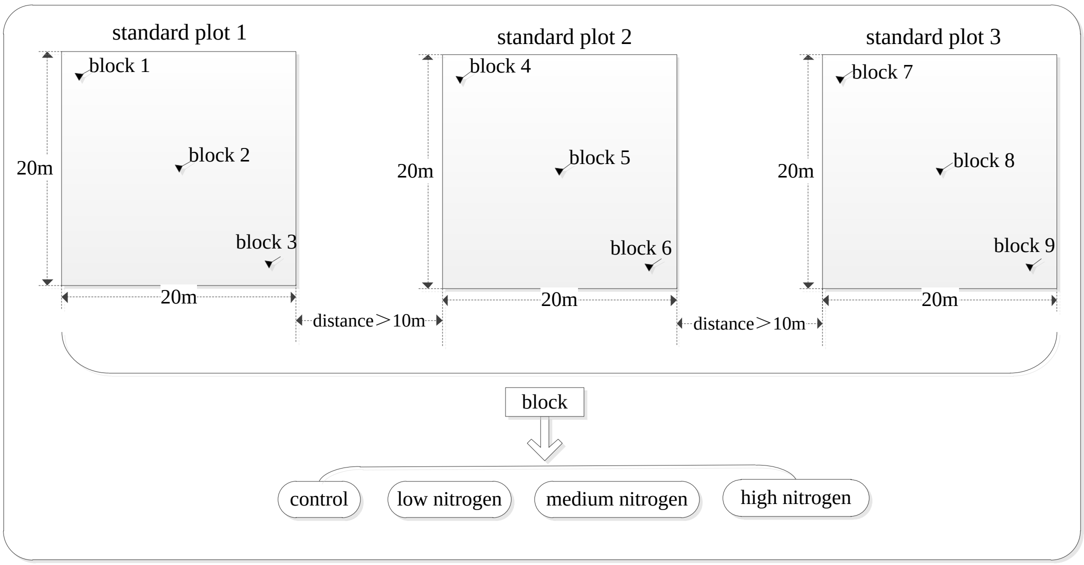

Figure 1: Sketch map of sample plot setting.

2.3. Data processing and statistical analyses

SPSS18.0, Origin 9.2, Microsoft Excel 2007, Microsoft Visio 2010 and other software were used for statistical analyses. The least significant difference (LSD) method was used to compare the differences between the data sets under different N deposition treatment. A linear model was used to describe compound relation between the total PLFA and soil water content, and between total PLFA and soil temperature and water content.

3. Results and analyses

3.1. Effects of N deposition treatment in different seasons on PLFA of soil microbes

The effects of N deposition on soil microbes in *Pinus massoniana* forest were significant ($P<0.05$), and the effects of different seasons were different (Table 4). The results of simulated nitrogen deposition in spring showed that bacteria, fungi and total PLFA presented a linear decline trend while actinomycetes presented a trend of increasing first and then decreasing with the increase of N concentration. It indicated that the content of soil bacteria, fungi, actinomycetes and total PLFA was decreased with the N deposition treatment in spring. After the N treatment in summer, the content of bacteria, fungi, actinomycetes and total PLFA was firstly increased and then decreased. It showed that the content of soil bacteria, fungi and total PLFA was increased with the N treatment. The content of actinomycetes was increased with the low N treatment but it was decreased with the medium and high N treatment. N deposition in summer increased the content of soil microbes on overall. In autumn, the content of fungi and actinomycetes was increased first and then decreased after N deposition. The content of soil fungi and bacteria was increased but the content of bacteria and total PLFA was decreased with low N treatment. The content of actinomycetes was increased but the content of fungi, bacteria and total PLFA was decreased. The content of fungi, bacteria, actinomycetes and total PLFA was all decreased with high N treatment. In winter, the content of bacteria, fungi actinomycetes, and total PLFA was all increased firstly and then decreased. In addition, the maximum value was in the medium N group. The minimum value of bacteria, actinomycetes and total PLFA was in the high N group. However, the group with the minimum value of fungi was the control group. It showed that the content of soil microbes was increased with the low and medium N treatment in winter, while it was decreased with the high N treatment.

Table 4: Seasonal effects of different nitrogen deposition treatments on the PLFA content of different microbial groups (mean±SD) /nmol·g^{-1}

PLFA	nitrogen deposition treatments	Spring	Summer	Summer	Winter
fungal PLFA	N_0	1.017±0.011 Ab	0.402±0.015 Dd	0.805±0.034 Bb	0.549±0.010 Cd
	N_{20}	1.000±0.012 Ac	0.595±0.027 Cb	0.825±0.039 Ba	0.573±0.021 Dc
	N_{40}	0.901±0.026 Ad	0.613±0.089 Da	0.783±0.031 Bc	0.681±0.025 Ca
	N_{60}	1.082±0.021 Aa	0.527±0.022 Dc	0.642±0.018 Cd	0.666±0.028 Bb
bacterial PLFA	N_0	10.732±0.370 Aa	7.395±0.265 Cd	8.373±0.476 Ba	7.133±0.252 Dc
	N_{20}	10.475±0.383 Ab	8.667±0.377 Bb	8.333±0.471 Cb	7.491±0.279 Db
	N_{40}	9.555±0.492 Ac	8.702±0.390 Ba	7.525±0.415 Dc	7.756±0.410 Ca
	N_{60}	8.522±0.375 Ad	7.546±0.303 Bc	5.881±0.356 Dd	6.506±0.375 Cd
actinomycetic PLFA	N_0	0.527±0.013 Ab	0.139±0.047 Db	0.147±0.015 Cc	0.222±0.019 Bc
	N_{20}	0.557±0.066 Aa	0.247±0.064 Da	0.333±0.024 Ca	0.356±0.004 Bb
	N_{40}	0.393±0.074 Bc	0.077±0.002 Dc	0.275±0.009 Cb	0.513±0.028 Aa
	N_{60}	0.382±0.038 Ad	0.052±0.006 Dd	0.142±0.003 Cd	0.192±0.006 Bd
total PLFA	N_0	13.283±0.514 Aa	8.462±0.501 Cd	10.551±0.419 Ba	8.356±0.512 Dc
	N_{20}	12.523±0.598 Ab	10.071±0.385 Ca	10.283±0.401 Bb	9.055±0.374 Db
	N_{40}	11.454±0.554 Ac	9.921±0.482 Bb	9.329±0.384 Dc	9.618±0.337 Ca
	N_{60}	10.432±0.557 Ad	8.657±0.499 Bc	7.192±0.340 Dd	8.247±0.344 Cd

Note: Lowercase letters indicate significant differences between treatments (P＜0.05);Capital letters indicate significant difference between different seasons (P＜0.05).

3.2. Effects of N deposition treatment in different seasons on soil microbial community

The effects of seasonal changes on microbial richness, diversity and evenness were different (Table 5).The richness index (S) was changed with the seasonal changes of spring>summer>autumn>winter. The Shannon-Wiener diversity index (H') was changed with the seasonal changes of spring>autumn>summer>winter, Pielou evenness index (J) showed the seasonal change trend of autumn>spring>winter>summer. It indicated that soil microbial community diversity was significantly changed with seasonal changes. The richness index and diversity index were the highest in spring. The evenness index was the highest in autumn. In addition, the effects of the N deposition level on soil microbial community structure were significant (P<0.05). In spring, soil microbial richness index and diversity index were increased but the evenness index of microbial distribution was decreased with the low and medium N deposition, while the richness index, diversity index and evenness index were all decreased with the high N deposition treatment. In summer, the soil microbial diversity index and richness index were increased but the richness index was decreased with the low and medium N deposition treatment. Microbial evenness index was increased but the richness index and diversity index were decreased with the high N deposition treatment. In autumn, microbial richness index and diversity index were increased with the low and medium treatment. Evenness index was increased with the medium N deposition treatment but was decreased with the low N deposition treatment. Microbial richness index, diversity index and evenness index were all decreased with the high N deposition treatment. In winter, the soil microbial richness index, diversity index and evenness index were all increased with high, medium and high N deposition treatment.

Table 5 Seasonal effects of different nitrogen deposition treatments on soil microbial PLFA community's diversity

time	nitrogen deposition treatments	The richness index (S)	Shannon-Wiener (H')	Pielou (J)
Spring	N_0	31 Aa	2.925 Aa	0.852 Bb
	N_{20}	23 Cd	2.717 Dc	0.867 Ca
	N_{40}	28 Bb	2.755 Cb	0.827 Dc
	N_{60}	25 Ac	2.653 Bd	0.824 Dd
Summer	N_0	27 Ba	2.675 Cc	0.812 Dd
	N_{20}	26 Bab	2.902 Ba	0.891 Aa
	N_{40}	25 Cb	2.725 Db	0.847 Cb
	N_{60}	23 Bc	2.638 Cd	0.841 Cc
Summer	N_0	22 Cb	2.747 Bc	0.889 Ab
	N_{20}	24 Ca	2.809 Ca	0.884 Bc
	N_{40}	22 Db	2.762 Bb	0.894 Aa
	N_{60}	20 Cc	2.609 Dd	0.871 Ad
Winter	N_0	22 Cd	2.586 Dd	0.837 Cc
	N_{20}	30 Ab	2.915 Ab	0.857 Db
	N_{40}	32 Aa	2.990 Aa	0.863 Ba
	N_{60}	25 Ac	2.782 Ac	0.864 Ba

Note:Lowercase letters indicate significant differences between treatments (P＜0.05)；Capital letters indicate significant difference between different seasons (P＜0.05)

3.3. Characteristics of soil temperature and water content in different treatments with seasonal changes

The soil temperature and water content under different treatments showed significant seasonal variation rules (Table 6). The temperature in summer was the highest, followed by autumn and spring, and the lowest in winter. The soil water content presented the change trend of spring>summer>winter>autumn. The effects of fertilization treatment on soil temperature and soil water content were significant. However, the effects were in small scale.

Table 6: Seasonal dynamics of soil temperature and soil water content at 0~10cm depth under different nitrogen deposition treatments (mean±SD)

environmental factors	nitrogen deposition treatments	Spring	Summer	Autumn	Winter
Soil temperature at 0~10cm depth	N_0	20.194±0.939 Cd	27.773±1.575 Ab	21.375±0.159 Bd	13.765±0.429 Dd
	N_{20}	20.488±1.009 Cb	27.589±1.091 Ad	21.482±0.928 Bb	14.131±0.239 Db
	N_{40}	20.250±0.786 Cc	27.668±1.250 Ac	21.472±0.214 Bc	14.142±0.103 Da
	N_{60}	20.954±0.710 Ca	27.820±1.562 Aa	21.578±0.308 Ba	13.981±0.333 Dc
Soil Water content at 0~10cm depth	N_0	0.464±0.027 Ab	0.354±0.097 Bb	0.131±0.039 Dc	0.185±0.038 Cc
	N_{20}	0.443±0.077 Ad	0.340±0.101 Bc	0.136±0.044 Db	0.199±0.239 Ca
	N_{40}	0.500±0.039 Aa	0.354±0.044 Bb	0.140±0.037 Da	0.188±0.010 Cb
	N_{60}	0.458±0.007 Ac	0.365±0.083 Ba	0.136±0.032 Db	0.171±0.027 Cd

Note:Lowercase letters indicate significant differences between treatments(P＜0.05)； Capital letters indicate significant difference between different seasons(P＜0.05).

3.4. Relationship between the PLFA of soil microbes and soil temperature and water content

It showed that the total PLFA of soil microbes and soil temperature with various treatments presented extremely significant axial symmetry relationship ($P<0.05$), and the fitting relationship between soil temperature and soil microbes was decreased with N deposition(Table 7).With the increase of soil temperature, the PLFA of soil microbes was increased firstly and then decreased after reaching the maximum value. From the curve trend, it showed that the optimal microbial growth temperatures of N_0, N_{20}, N_{40} and N_{60} were 20.72 °C, 21.55 °C, 20.68 °C and 21.38 °C, respectively.

During the experimental period, there was no significant linear relationship between the soil microbial biomass and the corresponding soil water content in the control group. However, with the increase of N deposition, the soil microbial biomass and soil water content reached extremely significant levels and the fitting degrees of N_{20}, N_{40} and N_{60} were 0.555, 0.881 and 0.837, respectively. The compound relation between soil microbes and soil temperature and water content in the control group was not in a significant level. With the increase of N deposition, fitting degrees between the total PLFA of soil microbes and soil temperature and water content in N_{20}, N_{40} and N_{60} were 0.457, 0.950, and 0.997, respectively. Fitting degrees between the equation of total PLFA of soil microbes and soil water content and the equation of total PLFA, temperature and water content compound equation were increased with N deposition. It showed that soil microbes were affected by soil temperature and soil water content under the certain N concentration. In addition, with the increase of N concentration, the effects of temperature and water content would be more significant.

Table 7: Regression function among soil microbial PLFA, soil temperature and soil water content under different nitrogen deposition treatments

Indicators	nitrogen deposition treatments	Regression models	Determination coefficient	significance
Temperature (T)	N_0	$y=-0.072T^2+2.984T-18.952$	0.768	0.001
	N_{20}	$y=-0.041T^2+1.767T-7.674$	0.588	0.019
	N_{40}	$y=-0.014T^2+0.579T-4.254$	0.145	0.494
	N_{60}	$y=-0.008T^2+0.342T-5.039$	0.032	0.863
Water content (W)	N_0	$y=7.971W+7.903$	0.279	0.077
	N_{20}	$y=7.870W+8.283$	0.555	0.005
	N_{40}	$y=5.410W+8.482$	0.881	0.000
	N_{60}	$y=7.998W+6.373$	0.837	0.000
relationship among PLFA, T and W	N_0	$y=-0.102T+9.403W+9.621$	0.187	0.160
	N_{20}	$y=-0.004T+7.928W+8.354$	0.457	0.026
	N_{40}	$y=-0.050T+5.971W+9.370$	0.950	0.000
	N_{60}	$y=-0.109T+9.902W+8.126$	0.997	0.000

4. CONCLUSION

In this test, soil microbial biomass in *Pinus massoniana* forest was slightly increased with the low, medium and high N in winter and low and medium N deposition in winter. Soil microbial biomass was slightly decreased with the low, medium and high N deposition in spring, low, medium and high N deposition in autumn, and high N deposition in winter. The results of this study are consistent with the results of some studies in China. However, some of the results are inconsistent [17-23]. It fully shows that the effects of nitrogen deposition are related to soil texture, vegetation type, temperature, precipitation and topography.

There is an obvious seasonal variation in soil microbes. The secondary relationship between soil microbes with soil temperature is extremely significant ($P<0.05$) but the relationship between soil microbes with soil water content is not significant. However, the correlation between soil microbes and soil temperature were decreased with N deposition and the correlation between soil microbes and soil water content were increased. It suggests that the effects of soil water content on soil microbes are more significant in areas with severe N deposition.

The evenness index, richness index and diversity index of *Pinus massoniana* forest were decreased with high N treatment in four seasons (spring, summer, autumn, winter), while the evenness index increased only during high N treatment in summer. The effects of low and medium N deposition treatment on soil microbial diversity index varied in different seasons. In general, soil microbial diversity was decreased with low and medium N deposition in spring and winter, and increased in summer and autumn. However, soil microbial diversity was decreased with the high N treatment in four seasons. It indicates that high N inhibits the growth of most microbial populations.

Acknowledgments

The authors acknowledge the Chongqing Social Science Planning Project (Grant: 2017QNGL56), the Science and Technology Research Project of Chongqing Municipal Education Committee (Grant: KJ1706178), the Scientific Research Olatform Open Projects of Chongqing Technology and Business University (Grant: KFJJ2018081) .

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