Effect of Nitrogen Deposition on Soil CO_2 Emission During Freezing-Thawing Incubation Period

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ABSTRACT: *Due to the traditional analysis method on the influence of soil nitrogen deposition on soil CO_2 emissions during the freezing and thawing period, the initial impact of nitrogen deposition on soil CO_2 emissions during the freezing and thawing incubation period was not analyzed, resulting in insufficient accuracy of the later analysis results. A new method was proposed to analyze the effect of nitrogen deposition on Soil CO_2 emission during freeze-thaw cultivation. On this basis, the contents of soil temperature, moisture, inorganic nitrogen, and soluble carbon were determined. Three freeze-thaw models of nitrogen deposition levels were established. The influence of nitrogen deposition on Soil CO_2 emission and the effect of nitrogen deposition on CO_2 emission in alpine wetlands were studied by multivariate variance analysis. The effect of nitrogen deposition on CO_2 emission of alpine wetlands was studied. The results showed that different soil temperatures and moisture content had a great influence on the seasonal variation of soil flux, which was generally consistent with the single peak of soil temperature, but highly consistent with the variation of soil moisture content in different growth periods. Nitrogen treatment changed the DOC content of soil organic matter. DOC content in the mineral layer and organic layer increased significantly in low and medium nitrogen treatments. The CO_2 emission of soil in the freezing period is lower than that in a normal temperature period, and that in multiple freezing periods is less than that in one freezing period. The CO_2 emission rate of soil under freeze-thaw conditions is the smallest, and the CO_2 emission rate of soil after thaw is the largest. Appropriate nitrogen deposition can promote soil CO_2 emission, while high nitrogen deposition can inhibit CO_2 emission.*

KEYWORDS: *Nitrogen deposition; Freezing-thawing; Incubation period; Soil; CO_2; Emission.*

INTRODUCTION

Nitrogen is one of the basic elements of biological composition, and the nitrogen cycle is also an important part of the biogeochemical cycle [1]. Nowadays, with the increasing impact of human activities on nature, nitrogen deposition has become one of the main driving forces of man-made natural changes in the production of forests,

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wetlands, farmland, cities, and other systems. The results show that a certain concentration of nitrogen deposition can improve ecosystem productivity and promote soil microbial activity, but a high concentration of nitrogen deposition can produce microbial activity on ecosystem productivity[2], and a high concentration of nitrogen deposition can produce a negative feedback on ecosystem productivity and inhibit soil microbial growth. Some studies have shown that nitrogen deposition has no significant effect on soil microbial activity [3]. Soil microbial and enzyme activities can directly affect soil respiration, and nitrogen deposition can affect soil respiration. The present research shows that nitrogen deposition can promote or inhibit soil respiration, but it has little effect on soil respiration [4].

Reference [6] proposed the impact of farming and nitrogen sources on soil N2O emissions. In the experiment, two farming systems and five fertilization treatments were used as sub-blocks, of which 140 kgnha 1 was used as urea (UR), pig manure (RS), anaerobically digested pig manure (ads), or compost cement slurry (CS). No nitrogen was applied as a control (CTR). N_2O emissions are measured using a static indoor method and are compared with soil temperature (0 ~ 0.1M), Water-Filled Pore Space (WFPS), Dissolved Organic Carbon (DOC), ammonium (NH_4 +-n), nitrate (NO_3-N)) and specific nitrification and denitrification biomarker genes (amoA, nag, NIRS, qnorb, and nitrite). However, the above two traditional methods will affect the physical and chemical properties of soil and microbial activity during the freezing and thawing process, thereby affecting the decomposition of soil organic matter and soil respiration. Literature [7] proposes a countermeasure to control carbon dioxide emissions in the near future. Including prioritizing the development of clean energy, adjusting the energy supply structure to promote economic structural upgrading, developing a low-carbon economy, implementing energy saving and consumption reduction, promoting the construction of a low-carbon society, relying on technological progress, and promoting the reduction of carbon dioxide in accordance with the laws of the social market, strengthening international exchanges, and actively developing low-carbon Technical cooperation. An improved Divisia decomposition method with logarithmic average weight is proposed. Its core idea is to decompose the change of a dependent variable in the system into the sum of various changes of related independent variables to measure the contribution of each variable to the change of the dependent variable. In his paper, using the data of Singapore, China, and South Korea, respectively, and comparing with three other existing decomposition methods, it is concluded that the log-average weight Divisia decomposition method is superior to the other three methods, and contributes to energy and the environment. The conclusion of the factor decomposition study. Studies have shown that compared with the ablation stage, the freezing and thawing process can reduce soil emissions. The main reason is that the soil microorganisms and enzyme activities are low during the freezing period, the soil organic matter is decomposed slowly, the soil particles are covered by ice film, and the soil permeability is reduced. It is unclear whether nitrogen deposition during freezing and thawing will promote soil emissions.

In response to the above problems, this paper proposes a new method to analyze the effects of nitrogen deposition during the freezing incubation period on soil CO_2 emissions. Its innovation lies in the study of the effect of nitrogen deposition on soil CO_2 emissions through freeze-thaw and freeze-thaw simulation experiments. The carbon source used gradually decreases and the emission rate of microorganisms gradually decreases. It provides a basis for further understanding of soil carbon emission dynamics and carbon cycle models under climate change and also provides a basis for wetland protection and management in climate change negotiations [9].

EXPERIMENTAL SECTION

Experimental setup

The soil samples were collected from a typical alpine wetland soil wetland in the M National Nature Reserve in September 2020. The size of the sample plot is 40 m × 40 m. A small plot of 0.6 m × 0.6 m is set every 6 m, and the sampling depth is The soil of 0-20 cm is collected, frozen, and sealed, placed in an insulation bag, and taken back to the laboratory.

The small stones and roots in the soil were screened out and then mixed evenly with the quartering method. A total of 40 parts of 80 g wetland soil (adjusted to 80% of the field capacity, then cultivated in 600 mL plastic flasks, supplemented with distilled water regularly to ensure the same water content) were pre-cultured at 16 ℃ for 2 days. Among these samples, 4 samples were used to determine soil pH, organic carbon, soluble organic carbon, nitrate nitrogen, ammonium nitrogen, and another basic physical and chemical indicators. The remaining 36 samples were used for pumping experiments. The samples were divided into four groups: the first group was frozen at - 1 °C for 7 days, and then frozen at 15 °C for 23 days; the second group was frozen at 16 °C for 7 days, and then frozen at - 18 °C for 7 days in the freezer, and finally thawed once for 2 times; The fourth group was incubated at - 15 °C for 30 days and at 16 °C for 7 days. Each treatment was repeated four times.

Formation of soil freezing and thawing

The frozen soil includes permafrost and seasonally frozen soil. Seasonal freezing-thawing soil refers to warm soil freezing whose annual average temperature is higher than 0 °C. In the process of winter season change, it has a frequent influence on the soil. The periodic variation of meteorological factors results in the difference in thermal properties between seasonal freezing and thawing layers, which leads to seasonal freezing and thawing of the soil-water system [10]. In the north of China, the frozen soil layer gradually melts with the increase in temperature and snow melting; In November, with the decrease in temperature, the seasonal thawing layer freezes, and the frozen layer deepens. The soil freezing period begins, and there is shallow freezing at night, but it is thawing in the daytime. With a further decrease in temperature, the frozen layer deepens. Even in the daytime, the layer does not melt, and the seasonally frozen layer begins to appear and thickens [11]. During the initial melting period, the soil thaws during the day and frozen at night. With the further increase in temperature, the soil thaws during the day would not freeze even at night. At this time, the soil begins to appear in the melting layer and the melting layer increased continuously [12]. Therefore, in the process of forming a seasonally frozen soil layer and seasonally thawed layer, freezing and thawing is not instantaneous, but experiences repeated freezing and thawing processes due to the changes in solar radiation and surrounding environmental conditions [13]. The time gradient of shallow soil temperature varies greatly during the freezing and thawing periods when the average daily temperature approaches 0 °C. The diurnal variation of soil temperature has a significant impact on the freezing and thawing process [14].

Setting of experiment

During the freezing-thawing incubation period, the size of the soil sample plot was 20m × 20m. A small sample plot of 0.5m × 0.5m was set every 5 m, and the sampled depth of soil was 0-15 cm. After collection, the soil was frozen and sealed in a heat preservation bag and brought back to the laboratory.

After screening the small stones and roots in the soil, four samples were used to mix the soil sample [15]. The soil was weighed at 60 g (70% of field water holding capacity allocated, cultured in 500 mL plastic flask, supplemented with distilled water regularly to ensure constant water content), and 40 copies were pre-cultured at 15 °C for two days. Four samples were used to determine the basic physical and chemical properties of soil, such as pH, organic carbon, soluble organic carbon, nitrate nitrogen, and ammonium nitrogen[16]. The other 36 samples were used for the culture experiment. There are three levels of nitrogen deposition: high nitrogen sedimentation (1.286 mg/g), low nitrogen sedimentation (0.429 mg/g), control (without nitrogen sedimentation), corresponding to the local nitrogen deposition of 30 $kghm^{-2}a^{-1}$, 10 $kghm^{-2}a^{-1}$ and 0 $kghm^{-2}a^{-1}$ [17]. The samples were divided into three groups: the first group was frozen and thawed for 7 days at - 18 °C, then thawed for 23 days at 15 °C the second group was thawed for 7 days at 15 °C then frozen for 7 days at – 18 °C, and then repeatedly thawed for 1 time, total 2 times; the third group was isothermal culture, that is, sample placement in incubate at 15 °C constant temperature for 30 days. Each treatment was processed with 4 replicates [18].

Determination of CO_2 emission velocity

The concentration of CO_2 was determined by static chamber-gas chromatography on the 1st, 2nd, 7th, 9th, 10th, 16th, 23rd and 30th day of culture respectively[19].

The emission amount of CO_2 is calculated as follows:

$$F = M / m - V / V_0 \cdot T_0 / T - P / P_0 \times dC_t / dt \qquad (1)$$

Where, F is the emission rate of the measured gas, M is the molar mass of the measured gas, m is the soil mass in the bottle, V is the volume of the extracted gas, V_0, T_0, P_0 are the gas mole in the standard state, air thermodynamic temperature (273.15K) and air pressure (1.013×10^5 Pa), T and P are the thermodynamic temperature at sampling time and the pressure of sampling point respectively, C_t is the gas concentration (PPm), dC_t/dt is the linear slope of gas concentration varying with time during sampling[20].

Table 1: Results of two-factor variance analysis for measuring the effects of time, nitrogen application levels and their interactions on soil variables and CO_2 flux.

Source of variation	Temperature (°C)	Soil moisture content (m^3/m^3)	Soil CO2 flux ($m^{-1}h^{-1}$)	Soil DIC content		Soil DOC content		Soil NO content		Soil NH content	
	P	P	P	Organic layer	Mineral layer	Organic layer	Mineral layer	Organic layer	Mineral layer	Organic layer	Mineral layer
Month	<0.001	<0.001	<0.001	<0.001	<0.001	<0.009	0.72	0.17	0.001	0.001	<0.01
Nitrogen application levels	0.05	0.46	0.05	0.01	0.15	0.001	0.02	0.31	0.005	0.86	0.14
Month * nitrogen application level	0.99	0.94	0.06	0.26	0.97	0.37	0.98	0.67	0.99	0.43	0.73

Sample analysis

The pH value of the soil was measured by pH meter (Shanghai LeiCiCompany), and its value was 7.07±0.17. Soil Organic Carbon (SOC) and Soil Soluble Organic Carbon (DOC) were detected by MultiN/C3100 TOC analyzer (Analytik Jena AG, Germany), with values of (450.96±15.95) g/kg and (9.06±3.88) g/kg, respectively. Soil ammonium nitrogen and nitrate nitrogen contents were measured by AA3 continuous flow analyzer (SFAL Company, Germany), with values of (0.51±0.09) mg/kg and (28.79±1.13) mg/kg, respectively.

Data analysis

The effects of nitrogen deposition, freezing-thawing, and measurement time on soil CO_2 emission rate and cumulative emissions were analyzed by multivariate variance analysis (SPSS17.0) [21]. The differences in soil temperature, soil water content, inorganic nitrogen [22], soluble carbon content, and soil carbon flux under different nitrogen application levels and months were compared by two-factor variance analysis [23].Sigmaplot12.0 software was used to map [24].

RESULTS AND DISCUSSION

The initial effect of nitrogen deposition on soil CO_2 emission during the freezing-thawing incubation period

(1) Changes in soil temperature, moisture, and CO_2 flux

Table 1 shows the results of a two-way ANOVA of the effects of time, nitrogen level, and their interaction on soil variables and CO_2 flux. The soil temperature of the soil above 10cm of different treatments changed in a single peak season during the whole growing season, the highest and the lowest appeared at the end of July and the end of September respectively[25]. Soil volumetric water content in 0-10 cm layer fluctuates. During the freezing-thawing period in early June and the precipitation concentration period in July and mid-August, there are three peaks of soil water content. In a dry period with relatively less precipitation in late June and early September, soil water content is lower. During the whole growing season, the measured values of the soil temperature at 10cm under different treatments are significantly different (P=0.05,), but the average change range of soil temperature in each month is 8.81-9.30℃, with no significant difference (Table 2). The average soil water content of 10cm in the surface layer of different treatments ranges, 11.07-13.62 m^3/m^3, and there is no significant difference among different treatments (P=0.46).

Analysis of Table 1 shows that there are significant differences in soil temperature of 10 cm and CO_2 in different treatments during the whole growing season (P=0.05), and the results of variance showed that there are significant differences between different nitrogen treatments and the control (P=0.05).

Table 2 shows the average and standard error results of major soil environmental variables and soil CO_2 flux under different treatments.

Table 2 shows that the average change range of soil temperature in each month is 8.81-9.30 °C, and the difference is not obvious. The average change range of soil CO_2 flux in different treatments is 357.33-422.53 mgCO_2 m^{-2}/h, which shows an increasing trend with the increase of nitrogen application dose.

Table 2: Average and standard error of major soil environmental variables and soil CO_2 fluxes under different treatment.

Nitrogen application levels	Soil temperature/℃	Soil moisture content (m^3/m^3)	Soil CO_2 flux($m^{-1}h^{-1}$)	Soil DIC content/mg/kg		Soil DOC content/mg/kg		Soil NO content/mg/kg		Soil NH content/mg/kg	
				Organic layer	Mineral layer	Organic layer	Mineral layer	Organi c layer	Minera l layer	Organic layer	Mineral layer
Contrast	8.88a	12.09a	357.33a	384.22ab	113.73a	859.18a	218.03a	2.05a	0.27a	100.37a	17.23a
	-0.35	-1.15	-18.72	-26.97	-2.58	-57.34	-6.52	-0.29	-0.03	-12.59	-1.49
Low nitrogen	8.81a	13.62a	387.08a	420.85b	119.70a	1212.34b	263.60b	3.05a	0.48b	100.23a	19.15a
	-0.59	-2.66	-40.37	-38.88	-6.74	-148.63	-24.72	-1.79	-0.07	-26.31	-2.21
Medium Nitroge n	8.82a	11.07a	383.80a	414.52b	116.81a	1208.69b	260.23b	1.85a	0.31a	95.63a	18.54a
	-0.64	-1.51	-35.38	-64.52	-5.35	-168.45	-13.67	-0.46	-0.05	-22.44	-3.04
High nitrogen	9.30a	12.55a	422.53a	347.56a	11.42a	961.51ab	277.05ab	2.88a	0.24a	121.40a	11.92a
	-0.6	-1.53	-33.45	-46.02	-4.28	-91.47	-9.51	-1.25	-0.06	-38.75	-1.78
Average	8.93	12.23	378.11	391.42	114.94	995.22	234.58	2.44	0.31	101.98	17.18
	-0.25	-0.78	-14	0.26	0.97	0.37	0.98	0.67	0.99	0.43	0.73

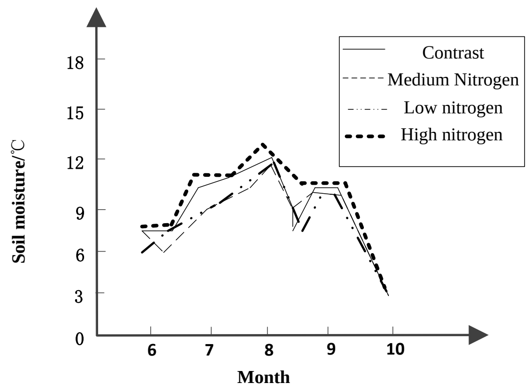
Fig. 1 is the seasonal variation of soil temperature, water content, and CO_2 content at 10 cm and its response to nitrogen increase.

From the analysis of Fig. 1, it can be seen that the seasonal variation of soil temperature is unimodal in general. The highest and lowest values appear at the end of July and September, respectively, and the lowest values of soil temperature can reach 3℃ at the end of September. From the point of view of soil moisture content, there are three peaks, namely, early June, mid-July, and mid-August. And soil moisture content is relatively low in late June and early September when the dry season is relatively low. From the point of view of the same amount of CO_2 in soil, the changing pattern is consistent with the soil temperature as a whole, showing a single peak seasonal change. The changing trend of soil CO_2 emission fluxes is highly similar to that of soil water content in mid-July and mid-August. In the two observation dates in July and mid-August, the double-peak characteristics also appear.

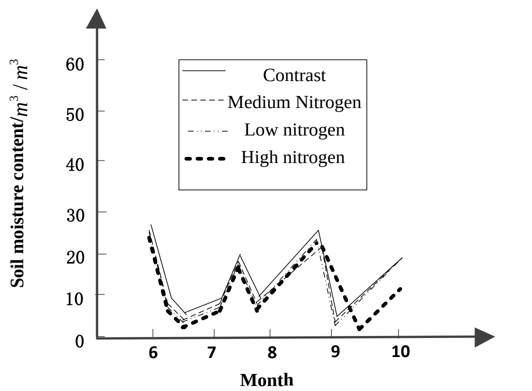
The changing pattern of soil CO_2 emission flux is consistent with soil temperature as a whole, showing a single peak seasonal variation. At the same time, in the growing season (July-August), the changing trend of soil CO_2 emission flux is highly similar to soil water content and also shows a double peak in July and mid-August. The average change range of soil CO_2 flux in different treatments is 357.33-422.83 $mgCO_2/m^2.h$, showing an increasing trend with the increase of nitrogen application dose; the results of variance show that different nitrogen treatments and control are significant (P=0.05).

(2) Changes in soil soluble carbon content

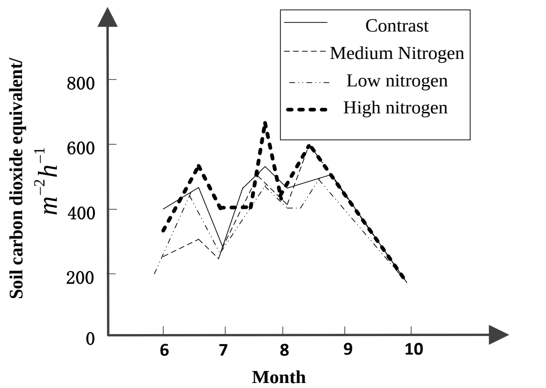
The seasonal variation of DIC (inorganic carbon) content in organic layer soil is significant, the lowest in June and the highest in August. The seasonal variation of DIC content in the mineral layer is similar to that in the organic layer, but the fluctuation range is relatively small ($P < 0.001$). In general, the DIC content of the organic layer varies from 347.56 mg/kg to 420.85 mg/kg under different treatments. In low nitrogen treatment, it is tended to increase, while in medium nitrogen and high nitrogen treatment, it is tended to decrease the DIC content of the organic layer(P=0.01). However, the DIC content in different treatments ranges from 110.42 mg/kg to 119.70 mg/kg,



(a) Changes in soil moisture



(b) Changes in soil moisture content



(c) Soil CO_2 equivalence

Fig. 1 Seasonal change of soil temperature, water content, and CO_2 equivalent in 10cm and its response to nitrogen increase10cm.

with few changes, and nitrogen application does not significantly change the DIC content in mineral soils (P=0.15).

The seasonal variation of DOC (organic carbon) content in the organic layer soil of the control is not consistent with that of DIC, which is the lowest in June and the highest in July. There is a significant difference in DIC content in different months ($P < 0.001$,); the variation of DOC in the mineral layer soil is not significant (P=0.72,). The ranges of DOC content in organic and mineral layers are 859.18-1212.334 mg/kg and 218.03-263.60 mh/kg, respectively. The former is 4.2 times that of the latter. Nitrogen application changes the DOC content of mineral soils in organic layers. Low and medium nitrogen treatment significantly increases the DOC content of mineral and organic layers (P=0.02 and 0.001, Fig. 2).

Fig. 2 is the monthly variation of soil inorganic carbon and soluble carbon contents in organic and mineral layers and their response to nitrogen increase.

In Fig. 2, in May and June, DIC content in the organic layer, DIC content in the mineral layer, DOC content in the organic layer, DOC content in the mineral layer, NO_3^--N content in the organic layer, NO_3^--N content in the mineral layer, NO_4^--N content in the organic layer, and NO_4^--N content in the mineral layer all reached the highest level. In July, the contents of DIC in soil, DIC in mineral soil, DOC in organic soil, NO_3^--N in mineral soil, NO_4^--N in organic soil, and NO_4^--N in mineral soil are the highest at low nitrogen deposition, and DOC in mineral soil and NO_3^--N in organic soil are the highest at medium nitrogen and high nitrogen deposition, respectively. In August, DIC content in organic layer soil, DOC content in organic layer soil, DOC content in mineral layer soil, NO_4^--N content in organic layer soil, and NO_4^--N content in mineral layer soil are the highest in medium nitrogen deposition, DIC content in mineral layer soil is the highest in low nitrogen and medium nitrogen deposition, and the content of NO_3^--N in organic soil and the content of NO_3^--N in the mineral layer is the highest in low nitrogen deposition.

In September, the DIC content in the organic layer reaches the highest at low nitrogen and high nitrogen deposition. The DIC content in the mineral layer of soil, NO_3^--N content in the mineral layer, NO_4^--N content in the mineral layer, and DOC content in the mineral layer reach the highest at low nitrogen deposition. The DOC content in the organic layer reaches the highest at medium and high nitrogen deposition, and the DOC content of the organic layer reaches

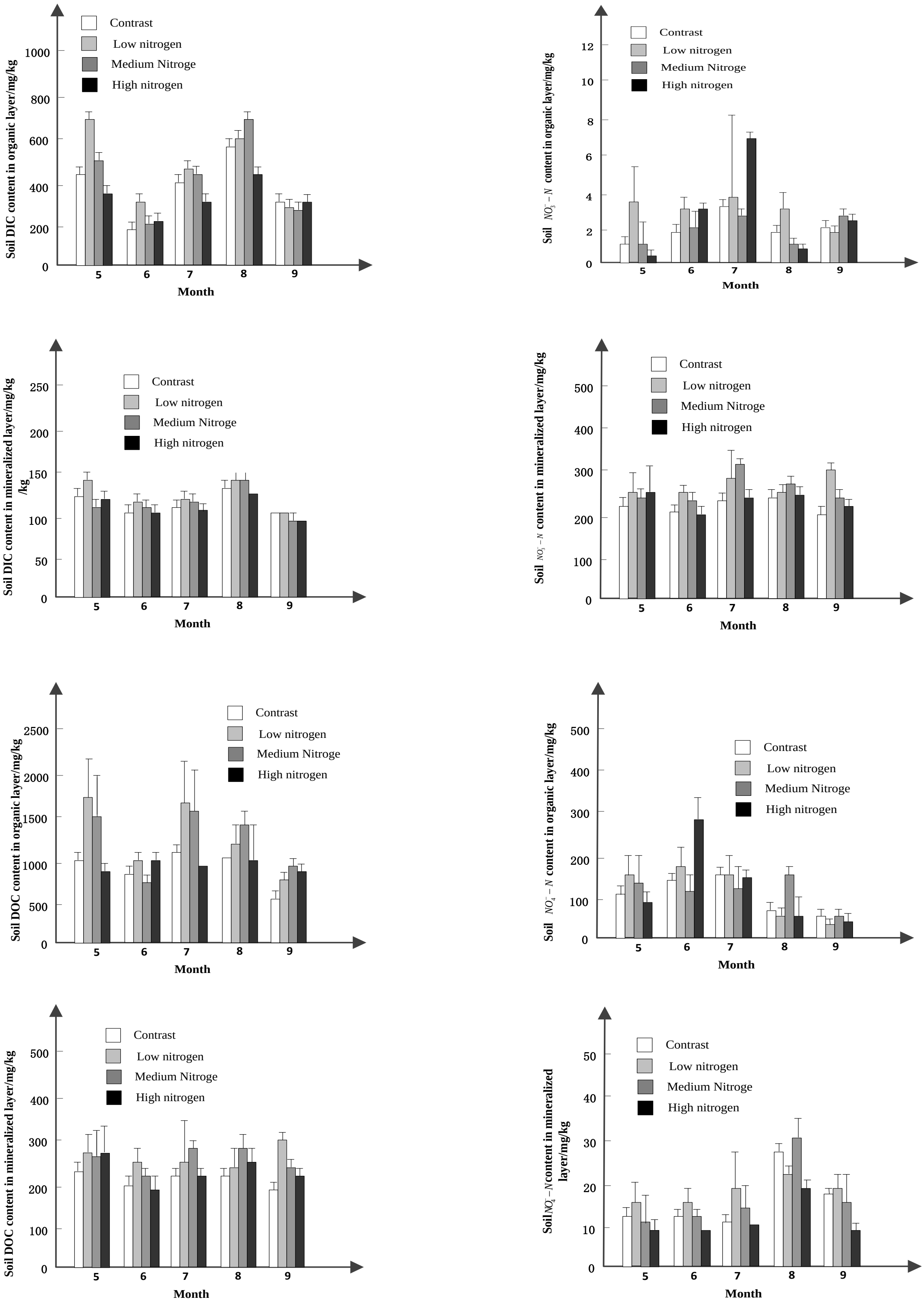


Fig. 2: Monthly change of inorganic carbon and soluble carbon content in soil of organic layer and mineral layer and its response to nitrogen increase.

Table 3: Variance Analysis of Effects of Nitrogen Settlement and Freezing on CO_2 Emission Rate and Accumulation.

Effect	DF	CO2 emission rate		Cumulative CO2 emissions	
		F	P	F	P
Nitrogen settlement treatment (N)	2.33	2.2	0.025	2.0	0.038
Freezing-thawing treatment (F)	2.33	42.1	<0.001	50.5	<0.001
Time (T)	7.280	52.9	<0.001	254.5	<0.001
N×F	4.33	1.2	0.294	1.0	0.527
N×T	14.280	2.0	0.045	2.4	0.015
F×T	14.280	20.7	<0.001	27.1	<0.001
N×T×F	28.280	1.3	0.216	0.8	0.797

the highest at low nitrogen deposition. NO_3^--N content in the organic layer is the highest at the medium nitrogen deposition, while NO_4^--N content in the organic layer is the highest at the low and medium nitrogen deposition.

Effect of freezing-thawing on soil CO_2 emission from alpine wetlands

Freezing and thawing had a significant effect on soil CO_2 emission rate and CO_2 cumulative emissions ($P>0.05$, Table 3). Under freezing-thawing and thawing-freezing treatments, the emission rate of CO_2 is significantly lower than that during the thawing period and the emission rate of CO_2 decreases with the increase of time during the thawing period, that is, the peak value of CO_2 emission appears after the thawing period. At room temperature, the emission rate of CO_2 decreases slightly with time (Fig. 3). The length of the freezing period also affects the cumulative emission of CO_2. The longer the freezing period is, the smaller the cumulative emission of CO_2 from the soil is.

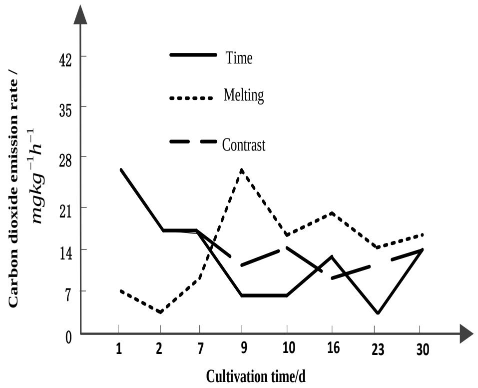
Table 3 is the variance analysis on the effects of nitrogen deposition and freezing-thawing on CO_2 emission rate and its accumulation.

Table 3 shows that freeze-thaw treatment has a significant effect on soil emission rate and cumulative CO_2 emission ($P > 0.05$). When DF of both treatments was 2.33, CO_2 emission of nitrogen deposition treatment was 2.2, and CO_2 emission of freeze-thaw treatment was 42.1. Although there was no interaction between freeze-thaw and nitrogen deposition on Soil CO_2 emission, the effect of freeze-thaw treatment on CO_2 emission was more significant than that of nitrogen deposition treatment on CO_2 emission.

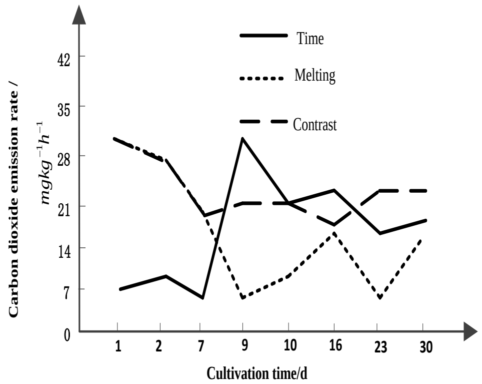
Fig. 3 is the effect of freezing-thawing treatment on CO_2 emission rate under different nitrogen deposition levels.

Fig. 3 shows that the incubation time of the maximum CO_2 emission rate in the freezing-thawing process is 9 h under high nitrogen deposition, low nitrogen deposition, and no nitrogen deposition; the incubation time of the maximum CO_2 emission rate in the freezing-thawing process is 1 h; and the incubation time of maximum CO_2 emission rate appeared in control is also 1 h.

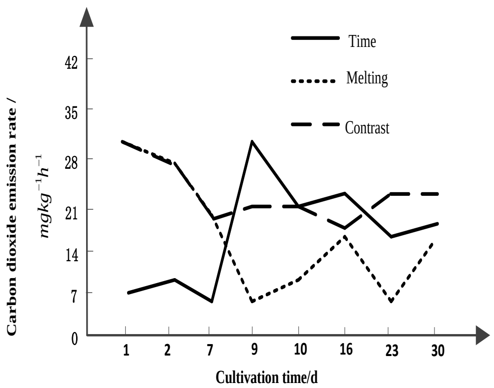
Soil CO_2 emission during the freezing-thawing period is lower than that during the normal temperature period, and multiple freezing-thawing processes lead to lower CO_2 emission, which is similar to previous studies on soil CO_2 emission caused by freezing-thawing. This may be due to the influence of the freezing-thawing process on soil microorganisms and soil enzyme activities, thus affecting soil CO_2 emission. When the temperature is below freezing point, the activity of soil microorganisms decreases greatly and the mineralization of soil weakens. At the same time, soil enzymes are also in non-optimal reaction conditions, so the decomposition rate of soil organic matter is greatly reduced. Although the microbial community in the alpine wetlands is mainly psychrophilic bacteria, the physical effect of freezing and thawing will destroy the cell structure and release internal organic matter. The instantaneous freezing and thawing will lead to the death of some microorganisms, thus increasing the content of organic carbon in the soil. Activated carbon, which is easy to be recovered at room temperature and survives by microorganisms. Changes in soil physical and chemical properties may also have an impact on soil CO_2 emissions. During the freezing period, the physical properties of the soil are mainly reflected in the increase of soil porosity, but because the "small particles" encapsulate a layer of ice film, the permeability of the soil is reduced,



(a) High nitrogen deposition



(b) Low carbon deposition



(c) Nitrogen-free deposition

Fig. 3: Effects of freezing-thawing treatment on CO_2 emission rates at different nitrogen settlement levels.

until the ice film melts, CO_2 can be released in large quantities. The freezing destroys the aggregate structure of the soil. The larger aggregates "fragmented" into smaller particles, increasing the contact area between microorganisms and soil, making more carbon sources utilized by microorganisms, and increasing the emission of CO_2. Therefore, the CO_2 emission peak will occur during the initial stage of soil thawing. However, with time, the carbon sources that are easy to be used by microorganisms gradually decrease, and the CO_2 emission rate of microorganisms gradually decreases.

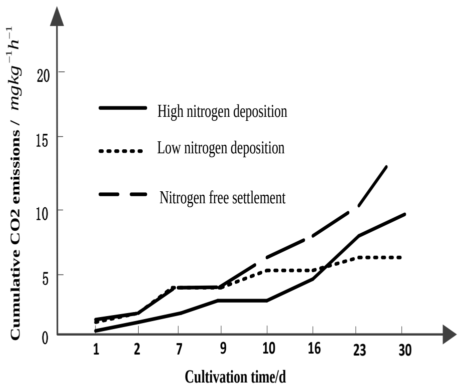
Effect of nitrogen deposition on soil CO_2 emission from alpine wetlands

Nitrogen deposition has a significant effect on soil CO_2 emission rate and CO_2 cumulative emissions ($P<0.05$). Under the same freezing-thawing condition, the cumulative emission of CO_2 under low nitrogen deposition is higher than that of the control group, while the cumulative emission of CO_2 under high nitrogen deposition is higher than that of the control group, and the cumulative emission of CO_2 under high nitrogen deposition (except freezing-thawing treatment) is the lowest (Fig. 4).

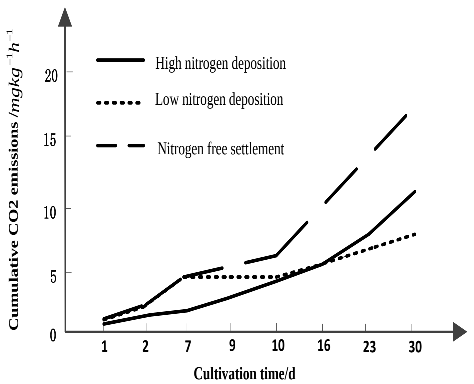
Fig. 4 is the effect of freezing-thawing treatment on CO_2 emissions under different nitrogen deposition levels.

Analysis of Fig. 4 shows that the incubation time of maximum CO_2 emission accumulation in freezing-thawing, thawing-freezing, and control is 30 hours under high nitrogen deposition, low nitrogen deposition, and no nitrogen deposition.

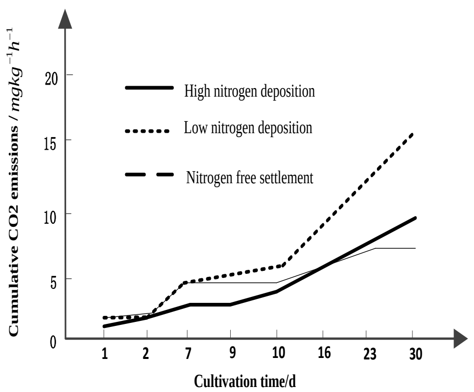
Nitrogen deposition can affect soil microbial growth and enzyme activity and also change soil's physical and chemical properties. Many studies have shown that CO_2 responds differently to nitrogen deposition, with increasing, decreasing, and ineffective results. The results show that proper nitrogen deposition can slightly promote soil CO_2 emissions, while high nitrogen deposition can inhibit soil CO_2 emissions to a certain extent. Appropriate nitrogen application can increase the nitrogen source in soil, especially the microbial activity in alpine wetland soil. The intrinsic changes include the acceleration of physiological processes such as microbial metabolism and reproduction, thus increasing the soil respiration rate. Excessive nitrogen deposition will change the physical and chemical properties of the soil, such as a decrease in soil pH value, but also



(a) Freezing-thawing



(b) Melting



(c) Comparison

Fig. 4 Effects of freezing-thawing treatment on CO_2 emissions accumulation at different nitrogen deposition levels.

changes the soil biological community, and reduce the activity of enzymes in the soil, thus reducing soil respiration and inhibiting the emission of CO_2. The results confirmed the hypothesis that nitrogen deposition had an effect on Soil CO_2 emission.

Effects of freezing-thawing and nitrogen deposition on soil CO_2 emissions from alpine wetlands

Multivariate statistical analysis shows that although freezing-thawing and nitrogen deposition have no interaction effect on soil CO_2 emissions, the effect of freezing-thawing treatment on CO_2 emissions is more significant than that of nitrogen deposition treatment. This may be due to the low-temperature limitation of microorganisms during the freezing-thawing process and the activity of microorganisms is low, resulting in less demand and consumption of nitrogen, so nitrogen deposition has less impact on microorganisms' decomposition of organic matter and release of CO_2. A freezing environment usually reduces the number of soil bacteria and actinomycetes, resulting in the reduction of nitrogen demand. Higher nitrogen has a strong inhibitory effect on soil fungi, which decreases the number of soil fungi and decreases their decomposition ability. During the thawing period, bacteria are suitable to grow and reproduce in soils with high nitrogen content, and the growth rate may compensate for the decreased rate of fungi. Therefore, under freeze-thaw conditions, although nitrogen application can change the proportion of soil microbial community composition, the community has the characteristics of redundancy and complementarity, so the impact on Soil CO_2 emission is relatively limited. In conclusion, freezing-thawing is the dominant factor of nitrogen deposition on CO_2 emissions in the alpine wetlands, and nitrogen deposition has a certain impact on this process.

Comparison results of different methods

On this basis, the three-year simulation of nitrogen deposition on the soil nitrogen dynamics and greenhouse gas emissions of the Northeast Korean pine plantation and the effect of farming and nitrogen sources on soil N_2O emissions are two traditional methods as experimental comparison methods. Comparing the test, the final accuracy results of the three methods are shown in Fig. 5.

From the stability test results of the different methods shown in Fig. 5, it can be seen that after the number of experiments continues to increase, the accuracy of

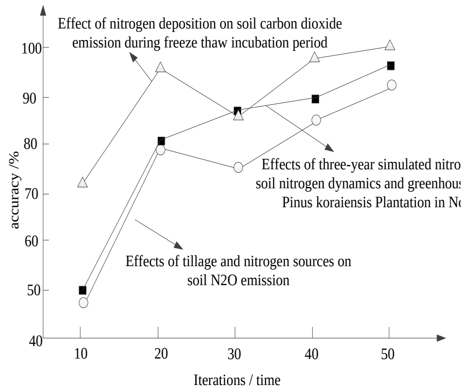


Fig. 5: Accuracy test results of three different methods.

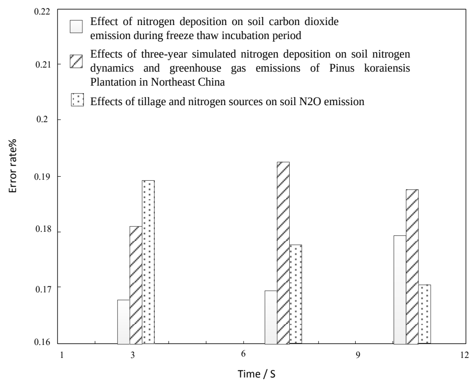


Fig. 6: Error rate test results for three different methods.

the three-year simulation of nitrogen deposition on the soil nitrogen dynamics and greenhouse gas emissions of the Northeast Korean pine plantation is about 78%. The accuracy of the method for the influence of farming and nitrogen sources on soil n2o emissions is about 82%, while the accuracy of this method is about 93%, which proves that the accuracy of this method is higher than that of the two traditional methods. The final error results for the three methods are shown in Fig. 6.

The error rate test results of the different methods shown in Fig. 6 show that the accuracy of three years of simulated nitrogen deposition soil nitrogen dynamics and greenhouse gas emissions is about 0.182%.The method affects agricultural and nitrogen sources on soil nitrogen emissions and about 0.167%, demonstrating that the error rate is lower than the two conventional methods.

CONCLUSIONS

In this paper, the effects of nitrogen deposition on soil CO_2 emission during freezing-thawing incubation are analyzed. The main conclusions are as follows:

(1) The seasonal variation pattern of soil CO_2 flux is controlled by soil temperature and soil water content, which is the same as the single peak pattern of soil temperature on the whole, but highly consistent with the change of soil water content in the growing season.

(2) Nitrogen application changes the DOC content of mineral soils in organic layers. Low and medium nitrogen treatments significantly increase the DOC content in mineral and organic layers.

(3) During the freezing-thawing period, soil CO_2 emission is lower than that during the normal temperature period, and the multiple freezing-thawing periods is smaller than that of the single freezing-thawing period. Freeze-thaw and the freeze-thaw process had no significant effect on cumulative CO_2 emission, indicating that the freeze-thaw sequence had no effect on Soil CO_2 emission.

(4) Under freezing-thawing simulation, the rate of soil CO_2 emission is the lowest in the freezing period, and there is a peak value after thawing in the freezing period.

(5) Nitrogen deposition affects soil CO_2 emission during the freezing-thawing period. Appropriate nitrogen deposition promotes soil CO_2 emissions, while high nitrogen deposition inhibits CO_2 emissions to some extent.

This study revealed the effect of nitrogen deposition on Soil CO_2 emission in the alpine wetlands during freezing and thawing, but the effect of soil freezing and thawing and nitrogen deposition on greenhouse gas emission has not been simulated under actual conditions such as snow cover. Therefore, long-term field in situ experiments and soil microorganisms during the freezing-thawing period under nitrogen deposition need to be strengthened in the future. The study of soil dynamics provides a basis for further understanding the impact of nitrogen deposition in soil on greenhouse gas emissions.

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