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Short-Term Litter Manipulations have Strong Impact on Soil Nitrogen Dynamics in *Larix gmelinii* Forest of Northeast China

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Abstract: Changes in above-ground litterfall can influence below-ground biogeochemical processes in forests, which substantially impacts soil nitrogen (N) and nutrient cycling. However, how these soil processes respond to the litter manipulation is complex and poorly understood, especially in the N-limiting boreal forest. We aimed to examine how soil N dynamics respond to litter manipulations in a boreal larch forest. A litter manipulation experiment including control, litter exclusion, and litter addition was performed in the *Larix gmelinii* forest on the north of the Daxing'an Mountains in China. Monthly soil inorganic N, microbial biomass and the rate of net N mineralization in both 0–10 cm and 10–20 cm layers, and N_2O flux were analyzed from May 2018 to October 2018. In 0–20 cm soil layer the average soil inorganic N contents, microbial biomass N (MBN) contents, the rate of net N mineralization (Rmin), and the soil N_2O emission in the litter addition plot were approximately 40.58%, 54.16%, 128.57%, and 38.52% greater, respectively than those in the control. While litter exclusion reduced those indexes about 29.04%, 19.84%, 80.98%, and 31.45%, respectively. Compared with the dynamics of the 10–20 cm soil layer, the N dynamics in 0–10 cm soil were more sensitive to litter manipulation. Rmin and N_2O emissions were significantly correlated with MBN in most cases. Our results highlight the short-term effects of litter manipulations on soil N dynamics, which suggests that the influence of litter on soil N process should be considered in the future defoliation management of the boreal larch forest.

Keywords: litter input; soil nitrogen availability; soil nitrogen mineralization; microbial biomass; Soil N_2O flux; boreal forest

1. Introduction

Nitrogen (N) is an important nutrient limiting the productivity of plants in boreal forest ecosystems [1,2]. The dynamics of soil N in forest ecosystem have great effects on N feedback to regional environmental changes as well as on global nutrient cycling [3,4]. Soil N dynamics depended on the situation of N input and output and are controlled by many factors, such as plant litter inputs, soil microbial compositions, N transformations, and stabilization of soil environment [5,6]. Particularly, changes in above-ground litter inputs can influence below-ground biogeochemical processes either directly by modifying organic C and nutrient inputs or indirectly through biotic activities [7]. These below-ground changes have significant effects on soil N availability and N transformation processes (i.e., soil N mineralization, nitrification and denitrification), and ultimately affect plant growth, nutrient cycling, and soil N_2O emission in forest ecosystems. Thus, a better understanding of the influence of plant litter quantity on soil N dynamics is a key to learning carbon (C) and N cycling, plant and soil interactions, and ecosystem structure and functions.

Recently, the effects of litter inputs on soil N cycling have been studied intensively. Numerous studies demonstrated that soil N transformation, availability, and microbial biomass are associated with the amount of litterfall in different ecosystems [8–10]. Generally, litter exclusion leads to reduced surface soil nutrients due to a direct reduction in the input of N to the soil, which may result in lower N concentration [11] and transformation rates [12]. In addition, litter exclusion could also reduce N_2O flux as litter layer is the important contributor to surface emissions of N_2O [13,14]. On the contrary, litter addition could accelerate these processes. However, the neutral and negative effects of litter manipulation were also observed. For instance, some studies showed soil N availability remains unchanged or even decreased with litter addition [15,16]. Deng et al. also reported the descending sequence of the net N mineralization rate: litter exclusion > control > litter addition [17]. Furthermore, some researchers also found litter addition significantly suppressed the emission of N_2O. These observations suggest that amounts of litter inputs may have various influences on soil N dynamics in different ecosystems. Nevertheless, changes in soil N dynamics with different litter manipulations in the boreal forest ecosystem, where N commonly limits plant productivity, were paid less attention. Moreover, the interactive effects of the litter and time on soil N dynamics and the dependence of the process on soil environmental factors have been less addressed in cold temperate zone. These knowledge gaps hinder our ability to predict the biogeochemical cycles of N. Therefore, more information is essential for understanding ecosystem's N dynamics in cold temperature zone.

The north of Daxing'an Mountains is the southern margin of boreal forest in the Eurasian continent and the only boreal forest region of high latitude in China [18]. It is also the most sensitive area to climate change with 3.8×10^5 km^2 of permafrost [19]. As the top community of forest ecosystem in this region, Larch (*Larix gmelinii*) forest plays an important role in the ecological balance of the area [20], covering 80% of the total forest area and accounting for approximately 30% of timber production in China [21]. Due to the slow decomposition rate of litter in cold temperature, the standing stock of litter in natural larch forest is large, and the amount of litter increases significantly over time [22]. Meanwhile, climate change also affects litter production and standing, which will ultimately affect the process of nutrient cycling in the larch forest [23,24]. Despite the importance of this ecosystem in regulating the global climate and its ecological value, the effects of litter manipulation on soil N cycling in the high-latitude boreal larch forests of China have received little attention. Hence, the objective of this study is to reveal the effects of litter manipulations on the soil N dynamics including soil inorganic N (NH_4^+-N + NO_3^--N), net N mineralization rate, and N_2O flux in the permafrost region of the Daxing'an Mountains, Northeast China. Two contrasting manipulations (exclusion vs. addition of harvest residues in the soil surface) were compared to estimate the contribution of the 0–10 cm and 10–20 cm soil layers. We addressed the following specific questions: (1) What are the characteristics of soil N dynamics in the *Larix gmelinii* forest of the Daxing'an Mountains, Northeast China? (2) What are the overall effects of litter manipulation on soil N dynamics in the boreal forest ecosystem?

2. Materials and Methods

2.1. Study Area

Our study field experiment was conducted at the Heilongjiang Mohe Forest Ecosystem Research Station (53°22′ N, 122°07′ E to 53°30′ N, 122°27′ E) in the Daxing'an Mountains, northeast China (Figure 1). The climate at the area is typical cold-temperate continental monsoon, with long and severe winters. The annual average precipitation is 350–500 mm, mainly distributed from June to August. The frost-free period for plant growth lasts approximately 80–90 days of the year, from June to August, and snowpack is more than half a year from October to April The average temperature is −4.9 °C [3]. The zonal soil is Podzol according to FAO soil classification. The dominant tree species are *Larix gmelinii*, *Pinus sylvestris* var. *mongolica*, *Betula platyphylla*, and *Populus davidiana* forest [25]. The understory vegetation is mainly *Rhododendron dauricum* L., *Ledum palustre* L., and *Vaccinium vitis-idaea* L. [3].

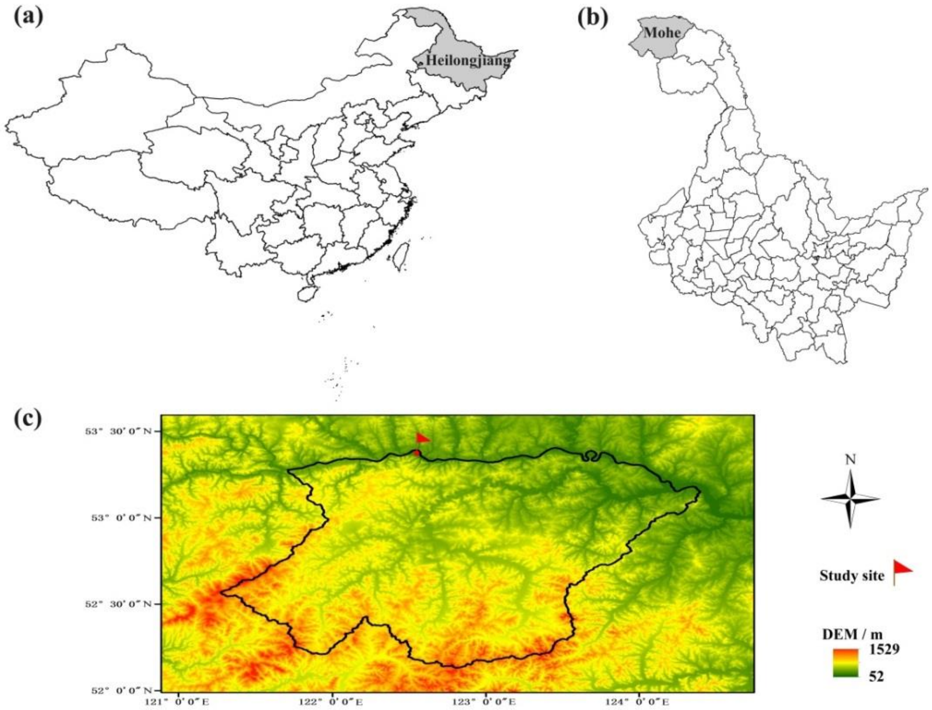

Figure 1. Location of study site in Mohe (**c**), Heilongjiang province (**b**), China (**a**) and the DigitalElevation-Model (DEM) of Mohe (**c**).

We selected *Larix gmelinii* forest to examine continuously the effect of litter on soil N dynamics. The *Larix gmelinii* forest was about 75–90 years old. Its measured tree density, height, and the diameters at breast height were 1266 stems·ha^{-1}, 17.23 m, and 13.78 cm, respectively, of which *Larix gmelinii* shared more than 90% of the total tree density. In May 2018, we established three 20 × 20 m plots as a randomized block design, at intervals of >20 m, which were all located at the same sea level of the same slope. The soil characteristics of the *Larix gmelinii* forest was following as Table 1.

Table 1. Soil characteristics of the *Larix gmelinii* forest.

Layer	Bulk Density (g·cm^{-3})	The Percentage of Stones (%)	Porosity (%)	pH	Carbon Content (g·kg^{-1})	Nitrogen Content (g·kg^{-1})
0–10 cm	0.72 ± 0.03	9.36 ± 2.90	64.46 ± 7.42	5.22 ± 0.21	66.74 ± 2.37	3.86 ± 0.53
10–20 cm	1.58 ± 0.10	22.36 ± 4.56	56.90 ± 5.89	5.46 ± 0.38	14.65 ± 1.54	1.41 ± 0.28

2.2. Litter Production and Quality

In each plot, five 1 m^2 litter traps constructed with nylon netting (1 mm mesh) were randomly laid out and suspended approximately 0.7 m above the ground with four stakes. Litter was collected monthly from May to October. Samples were taken to the laboratory and oven-dried at 70 °C to a constant weight to determine the dry mass. The litter composition of each plot was measured by litter dry weight per hectare (Mg·ha^{-1}). The dried litter samples of each month were ground and screened with a 2.5 mm metal sieve to measure the C and N content. Total C contents in litter samples were measured by the dichromate oxidation method [26]. Total N contents in litter samples were determined on aliquots of 1.0 g of samples using semi-micro-Kjeldahl method [27].

2.3. Litter Layer and Decomposition

In each plot, standing litter crop was sampled from May to October (six times). Five random samples of 50 × 50 cm were collected near the litter traps in each plot. Samples, including the litter and twigs (<2 cm diameter) in each quadrat, were collected and brought to the laboratory. All samples were oven-dried at 70 °C to constant weight per hectare (Mg·ha^{-1}). Litter mesh bags were used to determine the litter breakdown. In late autumn 2017 at each study site, freshly senescent needles that had not touched the ground were collected. These litter substrates were air dried to a constant weight at room temperature and then 20.0 g of substrate was enclosed in 15 × 15 cm nylon bags (1 mm mesh). Three subplots were selected randomly in each sample plot. On 2 May 2018, we placed litterbags in the three subplots under various ground litters to allow them to decompose naturally. A total of 54 litterbags (three plots × three subplots × six sampling times) were prepared and then collected once at the interval of about 30 days. On each sample date, nine bags (three plots × three subplots) were collected, transported to the laboratory, and oven-dried at 70 °C to a constant weight to determine the dry mass of the litterbags and litterbag contents. Litter mass loss rate was calculated as [6]

$$Dt = [\Delta M/M0] \times 100\% \quad (1)$$

where M0 is the quantity of litter before decomposition and ΔM is the quantity of litter mass loss.

2.4. Soil N Mineralization

On 2 May 2018, 15 1 × 1 m subplots were randomly established within each plot for three litter manipulations: litter exclusion (L−, reduced by 100%), litter addition (L+, increased by100%), and intact litter input (L0, no litter alteration). In the L− subplots (1 × 1 m), above-ground litter was collected using a 1 mm nylon mesh, suspended 70 cm above the ground. The collected litter was added to the L+ subplots with gentle raking. Soil N mineralization was then measured using the resin-core technique. Briefly, each resin-core was prepared by hammering into the soil a sharp-edged PVC tube, 5 cm in diameter and 24 cm in length, with the bottom 4 cm soil removed and replaced by a nylon mesh (0.3 mm) bag containing 10 g of an anion-exchange resin (mixture of Dowex 50W-X8 cation and Dowex l-X8 anion exchange resins 1:1). The resin bag was sandwiched between two pieces of filter paper to avoid direct contact with the soil. A gypsum block was placed below the resin bag to prevent the exchange of nitrate from the subsoil with the resin but not disrupt drainage (Figure 2) [28,29]. In each subplot, seven pairs of resin-core tubes were driven 20 cm into the ground. One resin-core tube was immediately removed from the soil for initial inorganic analyses (NH_4^+-N + NO_3^--N) on 2 May 2018, while the rest were left in the field for incubation. One of these tubes was collected at the interval of approximately 30 days from 31 May 2018 to 29 October 2018 (six sampling periods).

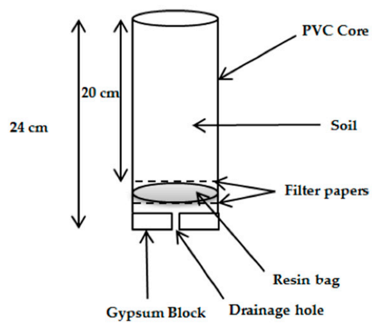

Figure 2. Diagram of the resin core incubation technique [29].

The litter layer was removed before sampling, and samples were transported to the laboratory in coolers inside polyethylene bags. We classified 0–10 cm and 10–20 cm soil depths into the upper layer and the lower layer, respectively. Soil samples from the five tubes obtained from the same manipulation subplots were mixed to form a composite sample that was then sieved through a 2 mm mesh. Then, samples were transported in an insulated box (Esky) and stored at 4 °C for subsequent chemical analysis. Inorganic N content, soil water content, soil microbial biomass nitrogen (MBN), and microbial biomass carbon (MBC) were then measured for the composite samples.

For soil inorganic N (NH_4^+-N and NO_3^--N), 10 g of fresh soil was extracted with 100 mL 1 mol·L^{-1} KCl by shaking 1 h and filtering through a 0.45 pore-diameter syringe filter. The extracts of soil inorganic N were analyzed using a Lachat flow-injection auto-analyzer (Seal Analytical AA3, Norderstedt, Germany). Soil water content was determined by drying the composite samples at 105 °C for 24 h. The microbial biomass was determined by the chloroform fumigation-extraction method for soil MBC [30] soil MBN [31]. The resin bags were washed with distilled water and dried at room temperature (28–32 °C). The content determination method used for resin NH_4^+-N and NO_3^--N was the same as for the soil inorganic N. Soil net N mineralization (including ammonification and nitrification) rates during the incubation period were calculated based on the difference between soil and resin NH_4^+-N, NO_3^--N, and inorganic N contents at the pre- and post-incubation. The rates of soil rates of ammonification (Ramm), nitrification (Rnit), and net N mineralization (Rmin) calculation method were described by Bhogal et al. [29].

2.5. N_2O Gas Sampling and Analysis

In May 2018, nine 1 × 1 m subplots were randomly established in each plot and manipulations were divided into L$^-$, L+, and L0. N_2O flux was measured using the static opaque chamber technique. The static opaque chamber was made of a polypropylene square-framed box and consisted of two parts, the open-bottom removable box (50 × 50 × 50 cm) and an open base collar (50 × 50 × 20 cm high, with 10 cm of bottom permanently inserted into the soil). The removable box was inserted directly into the base collar during sample collection. From May 2018 to October 2018, one gas sampling for measuring N_2O flux was conducted monthly between 9:00 a.m. and 11:00 a.m. A 50 mL plastic syringe was used to collect gas samples at time intervals of 0, 15, 30, and 45 min after chamber closure. Air temperature and soil temperature at 10 cm below the soil surface were measured while gas samples were being retrieved. The gas samples were taken to the laboratory and analyzed by gas chromatograph (Shimadzu GC2010, Shimadzu Analytical and Measuring Instruments Division, Kyoto, Japan). The N_2O flux was calculated using the method of Gao et al. [32].

2.6. Statistical Analysis

A two-way ANOVA was performed with incubation sampling time and litter manipulation as the main factors for the following variables: soil properties, soil net N mineralization rates, and N_2O emissions. One-way ANOVA was used to compare the differences between variables among litter manipulations, sampling time, and soil layers. Significant differences between litter manipulations were tested using post hoc Duncan tests. Relationships among soil net N mineralization rates, N_2O emissions, and soil properties were tested using Pearson's test. Statistical significance was set at $p < 0.05$ or 0.01. All data analyses were performed using SPSS 19.0 for Windows (SPSS Institute, Inc., Chicago, IL, USA).

3. Results

3.1. Basic Litter Characteristics

Litter production in this forest displayed significant variations throughout the research period (Table 2). The proportion of litter in these months ranged from 0.06 to 1.28 Mg·ha^{-1} and September was the litter production peak. Litter quality also varied significantly ($p < 0.05$). September and October had slightly higher C and N contents than the other months. The C/N ratio was greater in July than that in other months (Table 2).

Table 2. Monthly dynamics of litter production and quality of the *Larix gmelinii* forest.

Month	Litter Production (Mg·ha^{-1})	Litter Quality		
		C Content (g·kg^{-1})	N Content (g·kg^{-1})	C/N Ratio
May	0.14 ± 0.02c	475.19 ± 4.23c	7.23 ± 0.09c	65.75 ± 0.23b
June	0.08 ± 0.04d	473.00 ± 4.82c	7.39 ± 0.11c	63.99 ± 1.24c
July	0.06 ± 0.02e	510.63 ± 1.12b	6.54 ± 0.14d	78.10 ± 1.55a
August	0.10 ± 0.02d	430.27 ± 8.13d	9.41 ± 0.11b	45.71 ± 1.09f
September	1.28 ± 0.04a	576.74 ± 5.36a	10.51 ± 0.20a	54.86 ± 1.13e
October	0.38 ± 0.06b	580.81 ± 7.60a	9.99 ± 0.21a	58.12 ± 1.04d

Mean ± Standard deviation, n = 9. Different lowercase letters in the same column indicate significant differences across months (p < 0.05).

A significant difference (p < 0.05) of litter standing crop was found across different months (Figure 3). The average litter standing crop was 5.65 Mg·ha^{-1}, with the highest value (7.64 Mg·ha^{-1}) in October and the lowest value (4.45 Mg·ha^{-1}) in May. Figure 3 shows a higher rate of mass loss in August than in other months.

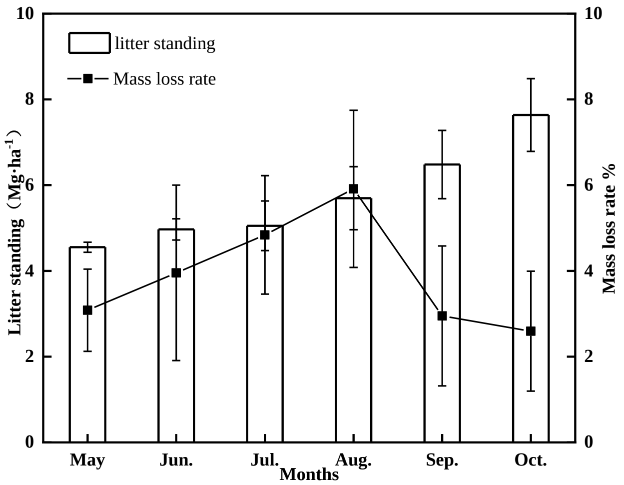

Figure 3. Monthly variations of litter standing crop and litter decomposition during the study period (n = 9).

3.2. Soil Inorganic N and Microbial Biomass

Our study showed that both litter manipulation and sampling time had significant effects on the NH_4^+-N, NO_3^--N and inorganic N contents (p < 0.001, Figure 4). In both soil layers, soil NH_4^+-N, NO_3^--N, and inorganic N contents increased significantly in L+ but decreased significantly in L$^-$ in relation to L0. However, these effects varied across soil layers. In the upper layer, the average contents of soil NH_4^+-N, NO_3^--N, and inorganic N increased by 48.65%, 27.36%, and 47.10% in L+, respectively, while those in L$^-$ decreased by 32.62%, 14.38%, and 31.29%, respectively (Table 3). However, those in the lower layer only increased by 37.02%, 8.13%, and 34.06% in L+ but decreased by 28.37%, 12.76%, and 26.77% in L$^-$. Soil NH_4^+-N, NO_3^--N, and inorganic N contents also showed significant monthly variations during sampling time in both soil layers, increasing first and then decreasing, with the peak on 29 July and 26 August (p < 0.05, Figure 4). Higher contents of them were found in the upper layer rather than the lower layer (p < 0.05, Figure 4). Meanwhile, significant interaction effects between sampling time and litter manipulations on soil NH_4^+-N, NO_3^--N, and inorganic N contents were also detected in our study (Table A1).

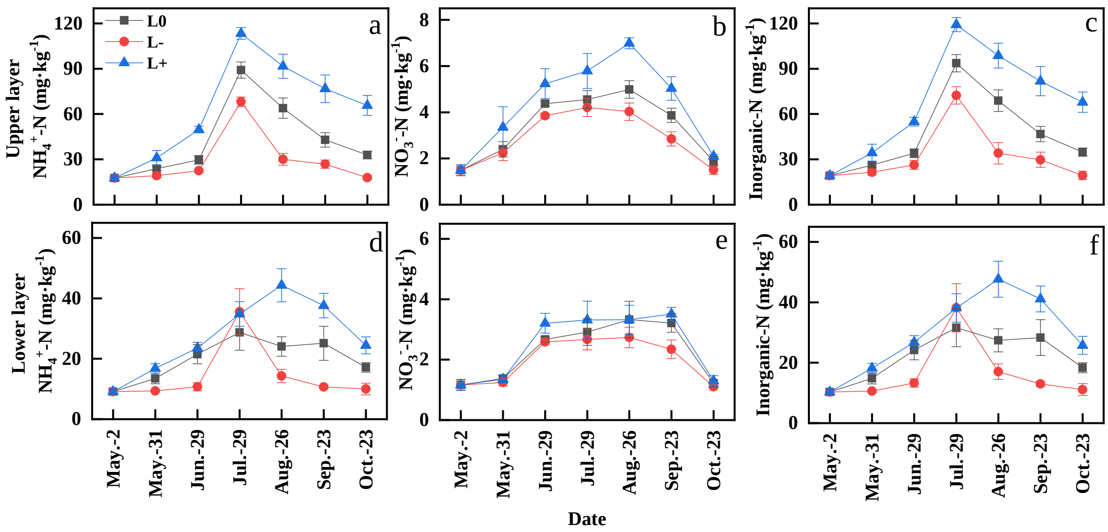

Figure 4. Monthly dynamics of the contents of soil NH_4^+-N, NO_3^--N, and inorganic N of different litter manipulation in varied soil layers of the *Larix gmelinii* forest (n = 9).

Table 3. Average soil NH_4^+-N, NO_3^--N, inorganic N, MBC, MBN contents, and MBC/MBN ratio over the sampling time under different litter manipulations in varied soil layers of the *Larix gmelinii* forest.

Litter Manipulation		NH_4^+-N Content (mg·kg^{-1})	NO_3^--N Content (mg·kg^{-1})	Inorganic N Content (mg·kg^{-1})	MBC Content (mg·kg^{-1})	MBN Content (mg·kg^{-1})	MBC/MBN Ratio
L0	Upper layer	42.84 ± 0.61Ba	3.36 ± 0.11Ba	46.21 ± 0.71Ba	338.38 ± 10.25Ba	135.35 ± 12.48Ba	2.71 ± 0.30Ab
	Lower layer	19.88 ± 0.62Bb	2.27 ± 0.13Bb	22.15 ± 0.49Bb	161.15 ± 3.56Bb	54.81 ± 1.56Bb	3.96 ± 0.41ABa
L$^-$	Upper layer	28.87 ± 0.50Ca	2.88 ± 0.15Ca	31.75 ± 0.52Ca	285.70 ± 13.01Ca	112.83 ± 2.27Ca	2.72 ± 0.32Aa
	Lower layer	14.24 ± 0.77Cb	1.98 ± 0.02Cb	16.22 ± 0.79Cb	150.44 ± 9.27Bb	42.18 ± 1.90Cb	4.48 ± 0.66Ab
L+	Upper layer	63.69 ± 2.76Aa	4.28 ± 0.19Aa	67.97 ± 2.92Aa	470.31 ± 40.78Aa	221.09 ± 23.21Aa	2.28 ± 0.21Aa
	Lower layer	27.24 ± 0.42Ab	2.45 ± 0.03Ab	29.69 ± 0.45Ab	192.13 ± 5.06Ab	68.06 ± 2.62Ab	3.14 ± 0.27Bb

Mean ± Standard deviation, n = 9. Different capital letters indicate significant differences between manipulations in each soil layer (p < 0.05). Different lowercase letters indicate significant differences between soil layers.

Similarity, litter manipulation, sampling time, and the interaction between sampling time and litter manipulation had significant effects on soil MBC, MBN contents, and MBC/MBN ratio (p < 0.01 Figure 5 and Table A1) in our study. The soil MBC and MBN contents in both soil layers significantly increased in L+ while significantly decreased in L$^-$ (p < 0.01). Soil MBC, MBN contents, and MBC/MBN ratio all showed significant monthly variations during sampling time in both soil layers, but these trends showed significant differences. The soil MBC content and MBC/MBN ratio were significantly higher on 29 June and 23 September than other sampling times (p < 0.05, Figure 5). The soil MBN content was significantly higher on 26 August than other sampling times (p < 0.05, Figure 5). Meanwhile, the soil MBC and MBN contents in the upper layer were greater than those in the lower layer (p < 0.01, Table 3 and Figure 5), but the MBC/MBN ratio in the lower layer was greater than that in the upper layer (p < 0.01, Table 3 and Figure 5).

3.3. Soil Net N Mineralization

The monthly dynamics of Ramm, Rnit, and Rmin were similar, showing a trend of positive first and then negative; the maximum values of Ramm, Rnit, and Rmin for both soil layers in three litter manipulations occurred mainly in June and July (Figure 6). Results of two-way ANOVA indicates that the sampling time, litter manipulation, and the interaction between sampling time and litter manipulation have significant effects on Ramm, Rnit, and Rmin in the upper layer soils, but only the sampling time and the interaction between sampling time and litter manipulation have significant effects on Ramm, Rnit, and Rmin in the lower layer soils (p < 0.001, Tables 4 and A2).

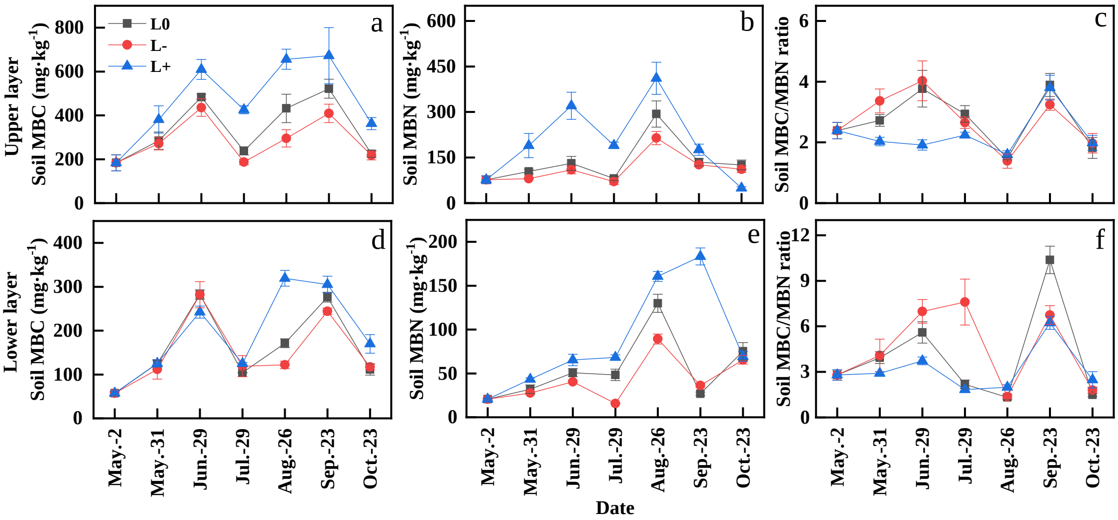

Figure 5. Monthly dynamics of the contents of soil MBC, MBN, and MBC/MBN ratio of different litter manipulation in varied soil layers of the *Larix gmelinii* forest (n = 9).

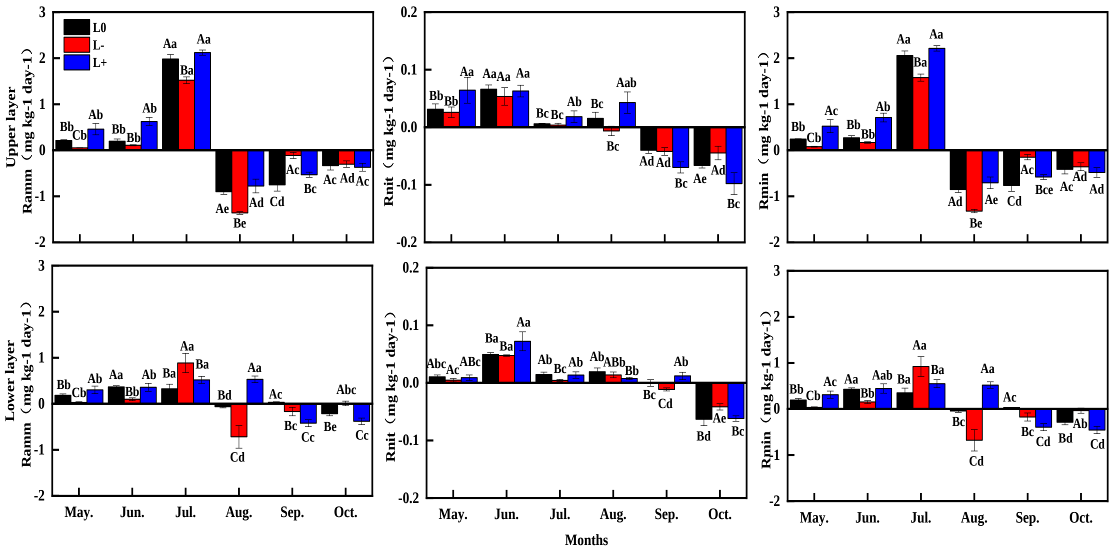

Figure 6. Monthly dynamics of the contents of soil net N mineralization of different litter manipulation in varied soil layers of the *Larix gmelinii* forest. Different capital letters indicate significant differences between manipulations in each soil layer, and different lowercase letters indicate significant differences among different months ($p < 0.05$) (n = 9).

Table 4. Pearson correlation coefficients (r) for soil characteristics and Rmin (rate of net mineralization) for L0, L^{-}, and L+.

Soil Characteristics	L0		L^{-}		L+	
	Upper Layer Rmin	Lower Layer Rmin	Upper Layer Rmin	Lower Layer Rmin	Upper Layer Rmin	Lower Layer Rmin
Soil water content	0.247	−0.187	−0.141	0.329	0.350	−0.290
Soil temperature	0.200	−0.136	0.252	−0.178	0.020	−0.133
MBC	−0.504 *	0.272	−0.279	−0.049	−0.362	−0.131
MBN	−0.648 **	−0.421	−0.828 **	−0.791 **	−0.242	0.380
MBC/MBN ratio	0.218	0.202	0.436	0.644 **	−0.157	−540 *
Ramm	0.999 **	0.995 **	0.999 **	0.998 **	0.998 **	0.995 **
Rnit	0.310	0.836 **	0.263	0.051	0.429	0.621 **

* Indicates significance at the p = 0.05 level. ** Indicates significance at the p = 0.01 level. n = 9.

The effect of litter manipulation on soil net nitrification, ammonification and mineralization accumulation and rates were significant in the 0–20 cm soil layer ($p < 0.05$, Figure 7). The cumulative net nitrification, ammonification, and mineralization were the highest in the L+, and then in the L0 followed by the L^{-} ($p < 0.05$, Figure 7). The cumulative Ramm, Rnit, and Rmin values for the 0–20 cm in the L+ were significantly higher than those in the L0 and L^{-} ($p < 0.05$, Figure 7).

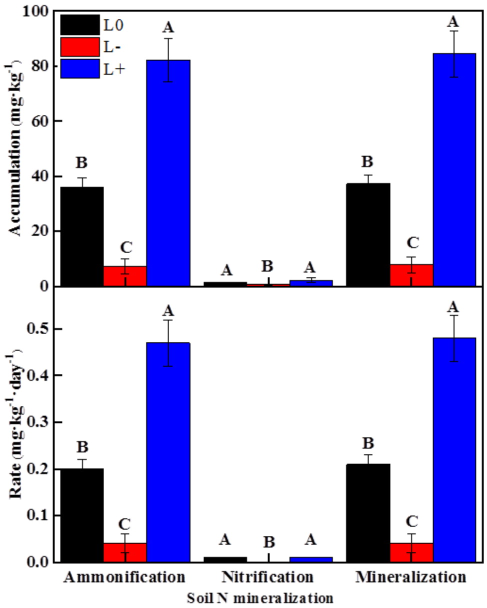

Figure 7. Soil net N mineralization accumulation and rate of different litter manipulations in 0–20 cm soil layers of the *Larix gmelinii* forest. Different capital letters indicate significant differences between manipulations in each index ($p < 0.05$) ($n = 9$).

Table 4 showed that in the upper layer soils of the three plot types, Rmin was negatively correlated with soil MBC and MBN, but was not significantly correlated with soil water content and soil temperature. Rmin was significantly correlated with Ramm in the upper and lower layer soils of the three plot types ($p < 0.01$), while Rmin values did not exhibit a close relationship with Rnit (Table 4).

3.4. N_2O Emissions

During the study period, the N_2O flux ranged from 3.68 to 23.79 $\mu g \cdot m^{-2} \cdot h^{-1}$ in L0, from 2.51 to 17.80 $\mu g \cdot m^{-2} \cdot h^{-1}$ in L^{-}, and from 7.38 to 27.81 $\mu g \cdot m^{-2} \cdot h^{-1}$ in L+ (Figure 8). Results of two-way ANOVA indicated that the sampling time, litter manipulation and the interaction between sampling time and litter manipulation had significant effects on the N_2O fluxes ($p < 0.001$, Table A3). The N_2O fluxes were significantly higher in June and August than other sampling times in the three litter manipulations ($p < 0.05$, Figure 8). The mean value of N_2O flux in the L+ (17.58 $\mu g \cdot m^{-2} \cdot h^{-1}$) was significantly higher than that in L0 (12.69 $\mu g \cdot m^{-2} \cdot h^{-1}$) and L^{-} (8.70 $\mu g \cdot m^{-2} \cdot h^{-1}$) ($p < 0.05$). The observed N_2O fluxes were positive so that the cumulative N_2O emissions continued to increase over time. The cumulative N_2O emissions were 0.56, 0.38, and 0.77 $kg \cdot ha^{-1}$ from L0, L^{-}, and L+, respectively (Figure 8).

Examination of the correlations between the surface N_2O flux and upper layer soil characteristics (Table 5) showed that all the three plot types were significantly and positively correlated with NO_3^{-}-N and MBN, and only L0 and L+ had a significantly positive correlation with soil temperature and soil MBC ($p < 0.01$). N_2O flux exhibited a positive correlation with Rnit in the L0, and while N_2O flux exhibited a negative correlation with Ramm and Rmin in the L^{-} ($p < 0.05$, Table 5).

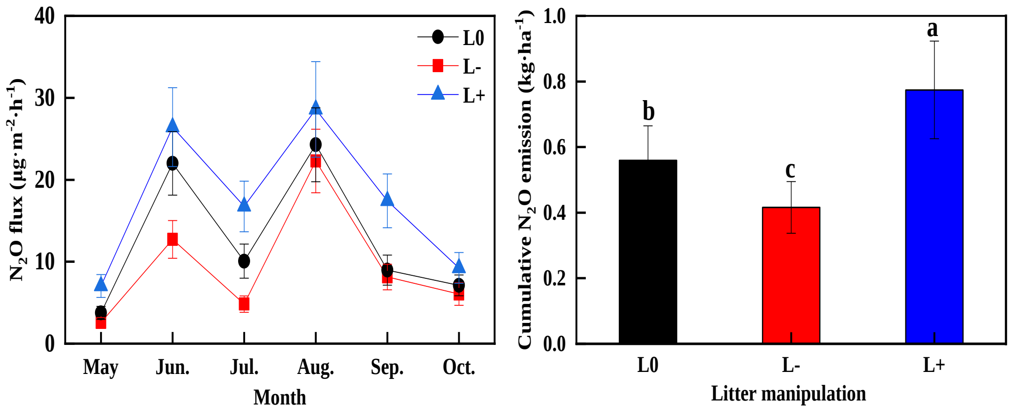

Figure 8. N_2O fluxes and cumulative N_2O emissions of different litter manipulation in the *Larix gmelinii* forest. Mean ± standard deviation. Different lowercase letters indicate significant differences between manipulations in each soil layer ($p < 0.05$) ($n = 9$).

Table 5. Pearson correlation coefficients between upper layer soil characteristics and the monthly flux of N_2O for L0, L$^-$, and L+ in the *Larix gmelinii* forest.

Upper Layer Soil Characteristics	L0	L$^-$	L+
Soil water content	0.334	0.185	0.270
Soil temperature	0.603 **	0.382	0.637 **
$NH_4{}^+$-N	0.211	−0.148	0.371
$NO_3{}^-$-N	0.725 **	0.480 *	0.766 **
Inorganic N	0.240	−0.116	0.403
MBC	0.563 **	0.406	0.781 **
MBN	0.710 **	0.801 **	0.806 **
MBC/MBN ratio	−0.127	−0.316	−0.135
Ramm	−0.244	−0.631 **	−0.078
Rnit	0.462 *	0.140	0.334
Rmin	−0.211	−0.605 **	−0.043

* Indicates significance at the $p = 0.05$ level. ** Indicates significance at the $p = 0.01$ level. $n = 9$.

4. Discussion

To determine whether litter manipulations alter soil N dynamics in the boreal larch forest, we analyzed the effects of different litter manipulations on soil N dynamics by field observation and laboratory experiments. We found that short-term alterations in the amount of surface litter of the larch forest significantly affected soil N dynamics through the complex biotic and abiotic processes along with the variations of soil N availability and N transformation (Figure 9). Besides, the effects varied at different soil depths. This section discusses these results in more detail.

4.1. Impact of Litter Manipulations on Soil N Availability

Litter plays a vital role in regulating nutrient retention in forest ecosystem, and litter decomposition is an important pathway to transform nutrient from vegetation to surface soil [33]. Thus changes in litter input could influence surface soil substrate and nutrient availability [34]. Our results showed that litter manipulations had strong effects on soil N availability. The soil inorganic N contents showed strong responses to short-term litter manipulations, increasing by 46% in L+ but decreasing by 29% in L$^-$ (Figure 9). This result is different with other studies which showed litter manipulations had strong effects on soil inorganic N in a long-term [35] or no significant effects [36]. A reasonable explanation for these different results may be from the difference of litter decomposition. Inconsistent with other study area, the litter decomposition mainly concentrates on May to September in our study area when the litter decomposition rate is higher, while it is stagnant in winter. The litter decomposed at the study period, losing more than 23% of the initial mass (Figure 3), which is close to previous study in temperate region [37,38]. The rapid mass loss and leaching at this study period may have significant effects on soil N dynamics. Therefore, in this period, litter addition may rapidly increase soil substrate and nutrient availability, and ultimately affect soil inorganic N [39]. Likewise, litter removal reduces the inorganic N content in the soil by slowing down the decomposition of N in the soil and inhibiting the activity of soil enzymes [40]. Meanwhile, the rapid litter decomposition in our study period also can significantly influence soil microbial biomass by supplying growth substrate directly for microbial [41,42]. Similar with previous studies, soil microbial biomass showed very vulnerable to litter manipulations [15,43]. Soil MBN contents increased significantly in L+ but significantly decreased in L−. Except the higher litter decomposition, litter manipulations also can change the soil microbial biomass production and activities by changing the microclimate conduction of soil [20,44]. Especially for the cold-temperate forest ecosystem in our study, its higher sensitivity to environmental changes may make it easier to have strong responses of soil microbial biomass to litter manipulation.

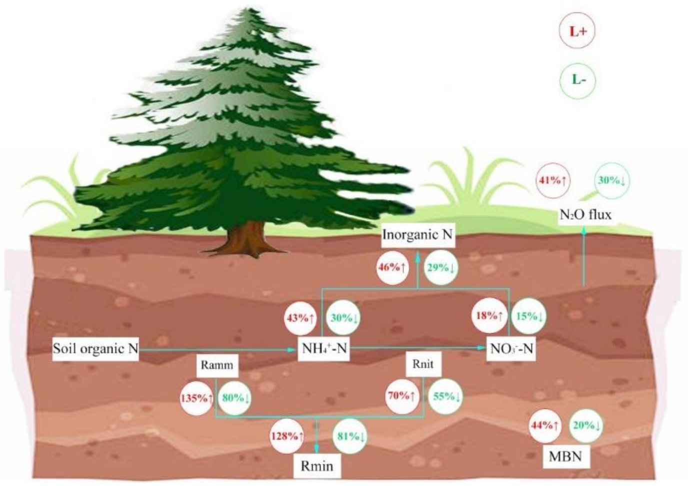

Figure 9. Potential mechanisms of the effects of litter manipulations on 0–20 cm layer soil N dynamics in the *Larix gmelinii* forest in the Daxing'an Mountains, Northeast China.

Our study found that the effect of L+ and L− on the soil N availability was asymmetric. Compared with litter exclusion, both the soil inorganic N and soil microbial biomass are more sensitive to litter addition in present study. This result suggests that adding litter may cause a priming effect—increased litter input induces the accelerated native soil organic matter degradation [45]. The priming effect in L+ could release the N rapidly stored in soil organic matter, which induces higher N availability in L+. Besides, the larch forest in our study is an N-limited forest ecosystem. Litter addition provides abundant substrates and nutrients to soils, which would promote microbial growth. However, the litter removal may stimulate the N-conserving mechanism and ultimately induce a lower variation of soil inorganic N and soil MBN in L−. This result provides evidence for a key role of litter in regulating nutrients cycling in the boreal forest as well.

In our study, the result show that there are significant differences across different forms of soil N in their responses to litter manipulations, the responses of soil NH_4^+-N to litter manipulations in our study (increased by 43% in L+, and decreased by 30%in L−) is more sensitive than soil NO_3^--N (increased by 18% in L+ and decreased by 15% in L−) (Figure 9), which is opposite to previous studies findings the responses of soil NH_4^+-N to litter manipulations in our study (increased by 43% in L+ and decreased by 30% in L−) is more sensitive than soil NO_3^--N (increased by 18% in L+ and decreased by 15% in L−) (Figure 9), which is opposite to previous studies findings [35,36,46]. The differences in findings may result from the differences in litter decomposition rate, plant net N uptake, and leaching in different ecosystems. There might be two causes for the significant difference of NH_4^+-N and NO_3^--N in response to litter manipulations. First, the increase of litter reduces the air circulation to a certain extent, resulting in an oxygen-deficient environment, which is not conducive to the progress of nitrification [47], hence the accumulation effect of NH_4^+-N as a substrate of nitrification is higher than that of NO_3^--N. Second, plants preferentially absorbed NO_3^--N from the soil in our study area [48,49], and NO_3^--N as an anion is easily lost in the soil through soil eluviation and denitrification [50], leading to less impact of litter manipulations on NO_3^--N. Meanwhile, we also found that litter manipulations had a significantly higher effect on surface soil inorganic N and MBN than on deep soil for a given litter manipulation (Table 3), possibly because abundant plant roots and litters and lower soil bulk density of surface soil could enhance soil microbial activities so that more soil inorganic N and MBN accumulates in the surface soil [51].

4.2. Impact of Litter Manipulations on Soil N Transformation

Litter has been proved as an important source of soil organic matter accumulation to sustain soil N transformations through litter decomposition, mineralization, and assimilation [6]. Our study also showed short-term litter manipulation had strong effect on soil N transformation, including soil net N mineralization and soil N_2O emission. Litter addition significantly increased soil net N mineralization and soil N_2O emission by 128% and 41%, respectively, while litter exclusion decreased them by 81% and 30%, respectively (Figure 9). The significant effect can be attributed to the following reasons. Firstly, the N mineralized and N_2O emission from litter are usually assumed to be a part of soil N transformation [52–54], and hence the soil net N mineralization rate and soil N_2O emission increased in L+ are higher than in L0. Secondly, litter manipulation resulting in the variation of soil microbial biomass is another important reason. Although cold-temperature forest is recognized as the lower nutrients turnover and microbial activity forest because of its lower temperature, our study found the variations of soil N transformation was mainly due to the alteration of soil microbial biomass resulting from litter manipulation in May to October (Tables 4 and 5). The higher soil microbial biomass in L+ induced higher N transformation. Thirdly, the variation of litter input will affect the soil environment, and then lead to the change of soil N transformation. Previous studies also confirmed that the increase of litter will form anaerobic environment of soil, promote denitrification, and increase N_2O emission [55,56]. Moreover, our study also found the asymmetric effects of litter addition and removal on soil N transform. Both soil net N mineralization and soil N_2O emission were more sensitive to litter addition. This is probably because although litter exclusion could decrease the organic matters and nutrients input to soil, root turnover, and exudates could also support the microbial growth and N transforms [57]. Therefore, soil net N mineralization and N_2O emission were only weakly affected by litter removal. Although litter layer as buffer also can consume N_2O [29,53], our results showed that surface litter and its enhanced anaerobic environment could promote N_2O production rather than consumption in larch forest. These results further deepen understanding of the response of soil N transform in N-limited forest to changes in external litter input.

Although litter manipulation had significant effects on soil N transform, the rates of soil net N mineralization and N_2O emission all showed similar monthly variation among three litter manipulation treatments during our study period. In present study, the soil net N mineralization showed significant monthly variations in both soil layers, showing positive from May to July but negative from August to October These results correspond to our previous study [25]. One possible explanation for the positive soil net N mineralization from May to July is that, with the rapid growth of vegetation, more organic N in the soil is transformed into inorganic N for vegetation to absorb. Another reason is that the increase in soil temperature may lead to an increase in soil net N mineralization. On the contrary, from August to October, with the end of the growing season, the uptake of N by vegetation decreases and soil temperature drops, causing negative soil net N mineralization. Moreover, our study shows that soil ammoniation largely determined soil net N mineralization in three litter manipulations (Table 4). Thus, the variations of soil NH_4^+-N can also explain the monthly variations of soil net N mineralization rate. Soil NH_4^+-N showed a single peak variation during the study period, increasing from May to August and decreasing from August to October (Figure 4), leading to the positive ammoniation rate from May to July but negative ammoniation rate from August to October, which results in the similar monthly variations of soil net N mineralization. The variations of soil net N mineralization between two soil layers also showed similar trends across three litter manipulations, with the net N mineralization in 0–10 cm soil depth being higher than that in 10–20 cm. On the one hand, more organic matter and nutrients in surface can provide more abundant energy and substrate to be mineralized [58], but the energy and substrate deficiency in subsoil may limit the net N mineralization [59]. On the other hand, higher soil microbial biomass was found in the upper layer in our study, which is favor to N transform [60], and thus inducing higher net N mineralization in 0–10 cm soil depth.

The N_2O flux also showed significant monthly variations among three litter manipulations during study period in our study, with higher emission of N_2O measured in June and August. The correlation analysis conducted in our study showed that soil N_2O emission was significantly affected by soil temperature, soil NO_3^--N content, and soil microbial biomass (Table 5). Our study showed significant positive effects of soil temperature on soil N_2O emission, especially in the L0 and L+, which is in agreement with previous studies [32,53]. Soil temperature may affect soil N_2O emission by affecting soil microbial biomass and activities and litter decomposition rates. The soil temperature showed a single peak trend in our study period, with the highest measured in August. Thus, higher soil temperature in August could induce higher N_2O emission. Meanwhile, the frequent precipitation and higher litter decomposition may result in higher soil N_2O emission in August [53]. However, we found lower N_2O emissions in July than that in June. The higher emissions of N_2O in June may have contributed to the higher soil water contents after soil thawing, which can form a better anaerobic environment for denitrification and ultimately promote N_2O emission [20]. Another reason is that N_2O enclosed in the soil in winter burst out into the atmosphere after thawing [61], which may result in higher N_2O emission in this period. Thus, the burst out of N_2O in June from winter obscures the effects of temperature on N_2O emission. Furthermore, soil NO_3^--N content also had significantly positive effect on N_2O emission in our study (Table 5), suggesting that soil NO_3^--N availability exerts dominant control over N_2O production [62,63]. This result further confirmed that soil NO_3^--N content plays an important role in regulating soil N_2O emission in our study area, where the N_2O from denitrification is recognized as an important source of soil N_2O production [64]. Additionally, similar to previous studies [65,66], we found positive correlations between soil microbial biomass and soil N_2O flux in our study, showing that the variations of N_2O emissions followed the soil MBC and MBN contents during the entire period. It has been suggested that patterns of N cycling and loss appear to be dominated by the microflora [67]. Soil microbial biomass can reflect the soil microbial production and activity [44], and the consumption of soil MBN fosters the flow of NH_4^+-N to nitrifiers and NO_3^--N to denitrifies, facilitating N_2O production by nitrification and denitrification [65]. Thus, higher soil microbial biomass could induce higher soil N_2O emission.

4.3. Relevance for Climate Change

Study on soil N dynimics, including the variations of soil inorganic N pools and associated processes, is a key for better understntding climate changes and managing future climate [68]. In our study, we confirmed that the variation of plant litter input have significant effect on soil N dynimics in larch forest. Previous studies have shown that global changes largely alter the plant litter input into soil [69]. Meanwhile, the quantity of plant litter in larch forests has increased over the years because its lower decomposition rate in the cold temperature zone [22]. Thus, the larch forest has been experiecing a natural plant litter addition process in our study area. Our study showed that the increasing of litter input can increase soil N availability and soil transform rates in a short term. Nevertheless, unlike other nutrients, N almost never accumulates in soils in inorganic form for any length of time, and especially in N-limited systems [70]. Therefore, the increasing N availability in litter addition can be consumed rapidly by plant and soil microbe [71], which will induce the higher N_2O emission and soil respiration [69]. Moreover, as the N-limited forest ecosystem, the increasing soil N availability could provide abandant nutrients for plant growth, which could increase the plant productivity.

However, in boreal region ecosystem, researchers found higher productivity can reduce the soil C stock, ultimately resulting in the net loss of C from ecosystem [72]. Hence, the increasing of plant litter input has positive effect on climate warming in a shor term in larch forest ecosystem. However, this does not mean that litter exclusion will have a negative impact on climate warming because of its the relatively lower effect on soil N dynamics in our study. Although we did more meticulous research about the effect of litter layer on soil N dynamics, the underlying mechanisms controlling the N cycle responses to aboveground litter manipulation treatments are not fully understood. Meanwhile, consideration of that greatly alters the plant litter input to soil affected by global warming is a long-term processes, thus more long-term work is needed to better reveal the responses of soil N cycle on litter input alterations under global climate change.

5. Conclusions

Our study found that the short-term above-ground litter manipulation had significant effects on soil N dynamics in the cold-temperate larch forest. Litter addition significantly increased the contents of soil inorganic N and microbial biomass, net N mineralization rate, and N_2O flux, whereas litter exclusion significantly decreased these indices. However, these effects of litter manipulation on soil N were asymmetric compared with control. Litter addition had stronger effects on soil N dynamics than litter exclusion. The soil N dynamics are primarily induced by the variations of soil microbial biomass affected by litter manipulations. Our study proved that litter manipulation has a substantial impact on soil N dynamics in N-limited boreal forests, but the potential mechanism still needs further exploration. Meanwhile, considering the effects of global warming on plant litter input in the future climate change, a long-term study is necessary for a better understanding of the response of soil N to litter input.

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

Appendix A

Table A1. Results of two-way repeated measures ANOVA concerning the effects of sampling time, litter manipulation, and the interaction between sampling time and litter manipulation on the contents of soil NH_4^+-N, NO_3^--N, inorganic N, MBC, MBN, and MBC/MBN ratio in the upper layer and the lower layer of the *Larix gmelinii* forest.

Factors		Litter			Time			Time × Litter		
		df	*F*-Value	*p*-Value	df	*F*-Value	*p*-Value	df	*F*-Value	*p*-Value
Upper layer	NH_4^+-N	2	345.960	<0.001	6	294.808	<0.001	12	21.453	<0.001
	NO_3^--N	2	66.449	<0.001	6	129.520	<0.001	12	4.848	<0.001
	Inorganic N	2	363.026	<0.001	6	312.492	<0.001	12	22.056	<0.001
	MBC	2	90.168	<0.001	6	78.601	<0.001	12	5.120	<0.001
	MBN	2	128.605	<0.001	6	92.632	<0.001	12	9.646	<0.001
	MBC/MBN ratio	2	13.349	<0.001	6	49.175	<0.001	12	8.087	<0.001
Lower layer	NH_4^+-N	2	78.283	<0.001	6	55.165	<0.001	12	10.847	<0.001
	NO_3^--N	2	13.210	<0.001	6	88.312	<0.001	12	1.752	0.090
	Inorganic N	2	80.434	<0.001	6	63.492	<0.001	12	10.719	<0.001
	MBC	2	49.516	<0.001	6	312.051	<0.001	12	25.022	<0.001
	MBN	2	146.294	<0.001	6	461.537	<0.001	12	24.668	<0.001
	MBC/MBN ratio	2	29.070	<0.001	6	129.607	<0.001	12	23.819	<0.001

Table A2. Results of two-way repeated measures ANOVA concerning the effects of sampling time, litter manipulation and the interaction between sampling time and litter manipulation on the rates of soil net N mineralization in the upper layer and lower layer of the *Larix gmelinii* forest.

Factors		Litter			Time			Time × Litter		
		df	*F*-Value	*p*-Value	df	*F*-Value	*p*-Value	df	*F*-Value	*p*-Value
Upper layer	Ramm	2	52.200	<0.001	5	1349.333	<0.001	10	27.404	<0.001
	Rnit	2	0.941	0.400	5	177.078	<0.001	10	9.409	<0.001
	Rmin	2	53.030	<0.001	5	1369.620	<0.001	10	28.205	<0.001
Lower layer	Ramm	2	8.222	<0.001	5	90.156	<0.001	10	37.278	<0.001
	Rnit	2	3.976	0.028	5	293.194	<0.001	10	7.502	<0.001
	Rmin	2	7.646	0.002	5	106.204	<0.001	10	35.317	<0.001

Table A3. Results of two-way repeated-measures ANOVA concerning the effects of sampling time, litter manipulation, and the interaction between sampling time and litter manipulation on the N_2O fluxes of the *Larix gmelinii* forest.

Factors	Litter			Time			Time × Litter		
	df	*F*-Value	*p*-Value	df	*F*-Value	*p*-Value	df	*F*-Value	*p*-Value
N_2O fluxes	2	126.671	<0.001	5	170.113	<0.001	10	6.087	<0.001

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