

Article

Response of Soil Net Nitrogen Mineralization to a Litter in Three Subalpine Forests

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Abstract: Forest litter accumulation can regulate the soil microclimate and alter nutrient distribution, but the effects of litter quality and seasonal differences on soil nitrogen (N) mineralization are still uncertain. The effects of litter change on the rates of net N mineralization, nitrification, and ammonification were studied through in situ incubation experiments in coniferous, mixed, and broad-leaved forests in the eastern Qinghai–Tibetan Plateau. Two litter treatments were established, one to allow the litter to enter the soil normally (remain litter) and the other to prevent the litter from entering the soil (remove litter). Soil samples were collected at the freezing (FS), thawing (TS), early growing (EGS), late growing (LGS), and early freezing (EFS) seasons during the 1.5-year incubation period. Compared to coniferous forests, the effects of litter removal on the net ammonification, nitrification, and N mineralization rates were more pronounced in broad-leaved forests, mainly during the growing and thawing seasons. Structural equation modeling indicated that microbial biomass N (MBN) was a common factor affecting the net ammonification, nitrification, and N mineralization rates in the three forest soils. The coniferous forest microbial biomass carbon (MBC), mixed forest soil moisture, broad-leaved forest soil N concentration, and C:N ratio were the unique influencing factors of the different forest types. The results showed that the effect of litter distribution on the soil net N mineralization mainly depended on forest type and season, suggesting that the litter composition and productivity in different seasons and forest types may alter the soil N cycling processes in subalpine forest ecosystems.

Keywords: litter; net ammonification; net nitrification; net N mineralization; subalpine forest



Citation: Zhang, L.; Yang, Y.; Jiao, Z.; Chen, Z.; Shen, Y.; Liu, Y.; Zhang, L.; Wang, L.; Liu, S.; Wu, Q.; et al. Response of Soil Net Nitrogen Mineralization to a Litter in Three Subalpine Forests. *Forests* **2022**, *13*, 597. <https://doi.org/10.3390/f13040597>

Academic Editor: George L. Vourlitis

Received: 16 March 2022

Accepted: 7 April 2022

Published: 11 April 2022

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1. Introduction

Soil nitrogen (N) mineralization is a key ecological process in forest ecosystems [1]. The rate of N mineralization determines the availability of N in the soil, which is of great significance for maintaining high forest productivity, stable structure, and function [2–4]. Litter input and decomposition are important for soil N cycling [5], as the returned litter is an important source of nutrient inputs for plant growth [6,7]. Meanwhile, it can also

provide food and energy for soil microorganisms [8,9], which may change the microbial N-use efficiency [10], thereby affecting the soil N cycle. Thus, exploring how litter affects soil N mineralization and its driving factors would help us to better understand the N-cycling mechanism in forest ecosystems.

Litter N returning is mainly controlled by plant litter quality [11] and climatic factors [12,13]. There have been studies showing that forest litter exhibited a specific spatial and temporal distribution pattern, and the litter composition and yield vary considerably among forest ecosystems and seasons [14,15]. It is often dominated by foliar litter in the growing season, and by twigs in the winter [16,17]. The initial quality of litter would affect the soil N mineralization rate [18], and it varies greatly among litter types. Twig litter contains high levels of refractory components, such as lignin and phenols [7]. Previous research found that frequent freeze–thaw cycles and intense leaching in winter would promote the degradation of litter cellulose and lignin [19], change the microbial structure and activity of microorganisms [20], and thus alter the relationship between litter and soil N mineralization. In contrast, high-quality foliar litter (such as broad-leaved litter) that is rich in liable carbohydrates and nutrients may be more likely to stimulate the activities of microbes, especially in the growing season [21]. Therefore, it is necessary to study the influence of litter in different forest types on soil N mineralization during different seasons.

There are various forest types in the subalpine forests of western Sichuan, which play important roles in the regional and national economy, as well as in regulating the climate and conserving water and soil [16,22]. The region has unique microclimatic characteristics, such as seasonal snow cover, frequent freeze–thaw cycles, and long-term freezing cold in the winter [22]. These microclimatic features can directly or indirectly modulate the response of soil N mineralization to litter change. To understand the effects of litter change on soil net N mineralization in different forest types and seasons, we selected coniferous, mixed, and broad-leaved forests in the eastern Tibetan Plateau to study the dynamic changes in the effects of litter removal on the soil net ammonification, nitrification, and N mineralization rates from May 2017 to October 2018. We hypothesized that (1) litter removal would decrease the forest soil N mineralization rates because of reduced carbon (C) and nutrient sources; (2) due to differences in the mass of litter species, the N mineralization rates of broad-leaved forest soils would have a greater response to litter removal; and (3) the effect of litter removal on the soil N mineralization rates varies with seasons due to differences in the soil microclimate and litter yield. Our objective is to assess the properties of soil N mineralization with and without litter input.

2. Materials and Methods

2.1. Site Description

The study was conducted in the Wanglang National Nature Reserve, Sichuan Province, which is located at the eastern Qinghai–Tibetan Plateau of China (103°55′–104°10′ E, 32°49′–33°02′ N, 2300–4980 m a.s.l.). The mean annual temperature ranges from 2.5 to 2.9 °C, with maximum and minimum temperatures of 26 °C (July) and −18 °C (January), respectively [17,23]. The annual precipitation ranges from 801 to 825 mm depending on the elevation [24]. The dominant trees are *Picea purpurea*, *Abies faxoniana*, *Sabina saltuaria*, *Betula albosinensis*, and *Betula utilis*, and the dominant shrubs are *Salix cupularis*, *Fargesia denudate*, and *Elaeagnus pungens* [25]. The forest soils are classified as Cambisols [26]. Three forest types with similar elevations and age structures were selected to conduct the in situ experiment, including coniferous, mixed, and broad-leaved forests [17,23].

2.2. Experimental Design

In May 2017, three plots with similar altitudes, slopes, and aspects were established in each forest type (Figure 1). Two in situ incubation boxes (depth 43 cm, length 70 cm, and width 51 cm) were installed at each plot throughout the study period. A perforated plastic sheet matching the incubator size was fixed 3 cm above the bottom of each box. At the same time, small holes were drilled into the bottom of each box to ensure that water

flow could permeate without removing soil. All incubation boxes were left from May 2017 to October 2017 to reach equilibrium. In October 2017, soil samples were collected from the boxes to determine basic indicators. In each plot, three litter-input boxes and three litter-removal boxes (remove litter) were installed. For the litter-retain box, there was no litter interception net above the litter-input boxes to allow continuous litter input. The nylon mesh was secured with a bracket about 50 cm above the litter-removal boxes to capture all fallen litter.

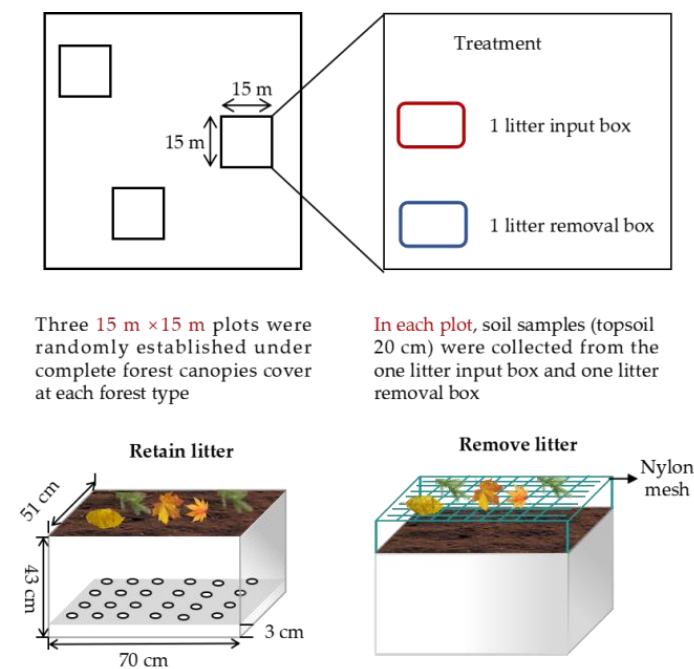


Figure 1. Schematic diagram of sample plot setting and collection.

2.3. Samples Collection

In 2018, based on temperature data and previous studies [27], soil samples were collected from the topsoil in January (freezing season, FS), March (thawing season, TS), May (early growing season, EGS), September (late growing season, LGS), and November (early freezing season, EFS). At each sampling date of each plot, soil samples were collected from both the litter-input and litter-removal boxes. The numbers of in situ incubation days were 80, 67, 66, 96, and 63 days. All soil samples were brought back to the laboratory, passed through a 2 mm sieve after removing the visible roots and then analyzed.

2.4. Microclimate and Soil Biochemical Analyses

The soil temperature (5 cm depth) was measured every 1 h using the Thermometers iButton DS1921G-F5 Recorders (Maxim Dallas Semiconductor Corp, San Jose, CA, USA). The maximum daily mean soil temperature of the coniferous forest, broad-leaved forest, and mixed forest all appeared on 29 July 2018, which were 16.59 °C, 17.06 °C, and 16.88 °C, respectively (Figure S2A). The minimum temperatures appeared on 9 January, 3 February, and 31 January 2018, which were −5.25 °C, −6.24 °C, and −4.83 °C, respectively (Figure S1A). The soil moisture was determined by measuring the moisture loss at 105 °C for 24 h. There was no significant difference in soil moisture between the retaining litter and litter-removal plots for the three forest types (Figure S1B). However, in the litter-removal treatment, the soil moisture of mixed forest and broad-leaved forest was different in the different seasons (Figure S1B).

The soil and litter organic C concentrations were determined using the dichromate oxidation–sulfate–ferrous titration method; the soil and litter total N concentrations were determined by the macro-Kjeldahl method [28,29]. The soil microbial biomass C (MBC) and microbial biomass N (MBN) were extracted with 0.5 mol L^{−1} potassium sulfate (K₂SO₄), and

the concentrations were determined by the chloroform fumigation extraction method [30,31]. Litter removal has little effect on the soil microbial biomass (Table S1). Soil ammonium N ($\text{NH}_4^+\text{-N}$) and nitrate N ($\text{NO}_3^-\text{-N}$) were extracted with 2 M potassium chloride (KCl) solution and then measured using the Indophenol-blue and dual-wavelength colorimetric methods, respectively [32,33]. The total inorganic N is the sum of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$.

2.5. Data Calculations and Statistical Analysis

Net N mineralization on a dry mass basis was calculated as the changes in inorganic N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) in the initial and incubated samples [34].

For a time interval $\Delta t = t_{i+1} - t_i$

$$A_{\text{amm}} = c(\text{NH}_4^+\text{-N})_{i+1} - c(\text{NH}_4^+\text{-N})_i$$

$$A_{\text{nit}} = c(\text{NO}_3^-\text{-N})_{i+1} - c(\text{NO}_3^-\text{-N})_i$$

$$A_{\text{inn}} = A_{\text{amm}} + A_{\text{nit}}$$

where t_i and t_{i+1} are the initial and post-incubation times; A_{amm} is the accumulation of $\text{NH}_4^+\text{-N}$; $c(\text{NH}_4^+\text{-N})_i$ and $c(\text{NH}_4^+\text{-N})_{i+1}$ are the mean concentrations of ammonium nitrogen in the initial and incubation samples, respectively; A_{nit} is the accumulation of $\text{NO}_3^-\text{-N}$; $c(\text{NO}_3^-\text{-N})_i$ and $c(\text{NO}_3^-\text{-N})_{i+1}$ are the mean concentrations of $\text{NO}_3^-\text{-N}$ in the initial and incubation samples, respectively; and A_{inn} is the accumulation of total inorganic N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$). Additionally,

$$R_{\text{amm}} = A_{\text{amm}} / \Delta t$$

$$R_{\text{nit}} = A_{\text{nit}} / \Delta t$$

$$R_{\text{min}} = (A_{\text{amm}} + A_{\text{nit}}) / \Delta t$$

where R_{amm} , R_{nit} , and R_{min} are the net ammonification, nitrification, and N mineralization rates, respectively.

Variance inflation factors (VIFs) were calculated to examine the presence of collinearities between environmental variables. Environmental variables were excluded when VIF was >5 . To assess the effects of the different forest types of litter treatment on the soil moisture, N concentration, C:N ratio, MBC, and MBN, and to quantify the relative contributions of the soil moisture, N concentration, C:N ratio, MBC, and MBN on the soil net ammonification, nitrification, and mineralization rates during litter treatment, we created structural equation models (SEMs) to predict the relationship between multiple variables and to determine the direct and indirect effects of variables on the soil net ammonification, nitrification, and mineralization rates in AMOS 23.0 (IBM SPSS, Chicago, IL, USA). According to the explanatory power of the independent variables to the dependent variables, we determined the best model (with a high fit degree) by removing the relationships that were not significant from the conceptual model. We used the Chi-square test, Bentler–Bonett normed fit index (NFI), Bentler’s comparative fit index (CFI), and the root-mean-square error of approximation (RMSEA) to examine the goodness-of-fit of the models [35,36]. The Chi-square test should not be significant, the NFI and CFI should be >0.9 , and the RMSEA should be <0.08 in order for the model to be accepted as a good fit.

Repeated-measures analysis of variance (ANOVA) was used to examine the effects of sampling season, litter treatment, forest type, and their interactions on the soil $\text{NH}_4^+\text{-N}$ concentrations, $\text{NO}_3^-\text{-N}$ concentrations, inorganic N concentrations, ammonification rate, nitrification rate, and mineralization rate. A one-way ANOVA with Tukey’s post hoc test was performed to test the effects of the sampling season or forest type on the soil $\text{NH}_4^+\text{-N}$ concentrations, $\text{NO}_3^-\text{-N}$ concentrations, inorganic N concentrations, net ammonification rate, net nitrification rate, and net N mineralization rate at the same litter treatment and forest type, or the same litter treatment and sampling season, respectively. For individual sampling seasons and forest types, an independent-samples *t*-test was used to compare

the effect of litter treatment on the soil NH_4^+ -N concentrations, NO_3^- -N concentrations, inorganic N concentrations, net ammonification rate, net nitrification rate, and net N mineralization rate. All statistical tests were performed using the Software Statistical Package for the Social Sciences (SPSS) version 27.0 (IBM, Armonk, NY, USA), and the statistically significant differences were determined at $p < 0.05$.

3. Results

3.1. Net Ammonification Rate

The significant effect of litter removal on the soil NH_4^+ -N concentration only appeared in the EGS of the broad-leaved forest (Figure 2a). The soil NH_4^+ -N concentration varied with forest types ($p < 0.001$; Table 1 and Figure 2a) and seasons ($p < 0.001$; Table 1 and Figure 2a). In both litter treatments, the NH_4^+ -N concentration was highest in FS (Figure 2a). The difference in the NH_4^+ -N concentration among different forest types was manifested in the FS, TS, and EGS, in which those in coniferous forests were significantly higher than those in the mixed forest and broad-leaved forest (Figure 2a).

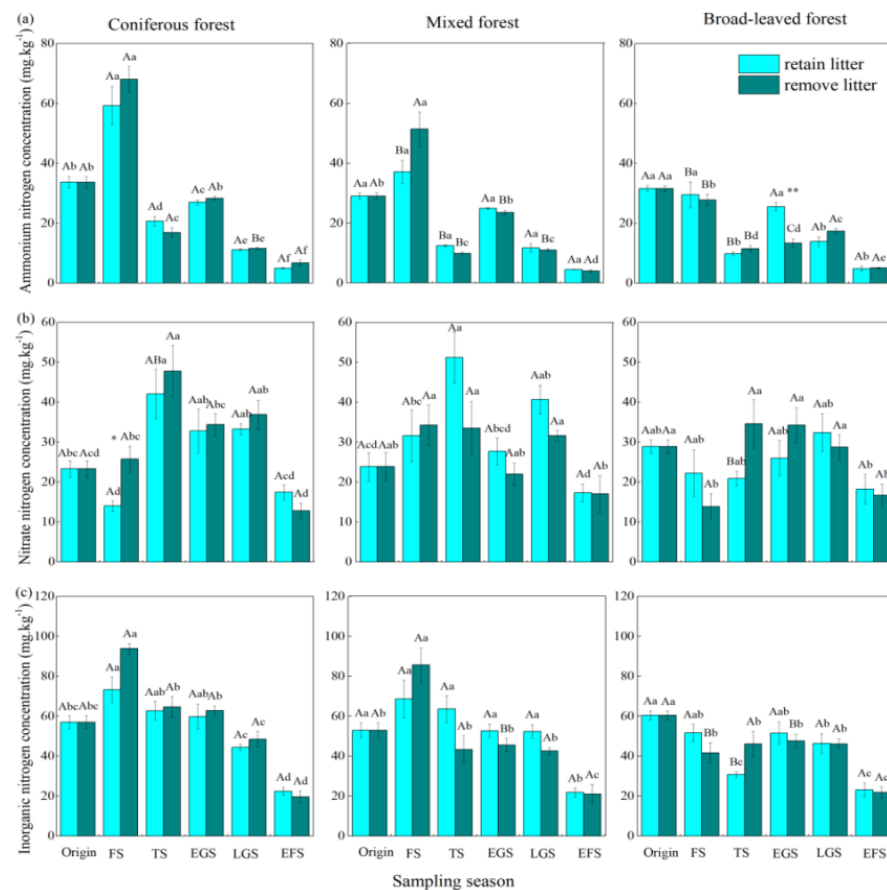


Figure 2. Concentrations of soil ammonium nitrogen, nitrate-nitrogen, and inorganic nitrogen as affected by litter treatment at different sampling seasons in coniferous (a), mixed (b), and broad-leaved (c) forests. Values indicate the means $\pm$ standard error, $n = 3$. FS: freezing season, TS: thawing season, EGS: early growing season, LGS: late growing season, EFS: early freezing season. Asterisks represent the significant differences between different litter treatments in the same forest type and sampling season. Lowercase letters represent significant differences among different sampling seasons in the same litter treatment and forest type. Uppercase letters represent significant differences among different forest types in the same litter treatment and sampling season.

Table 1. Repeated-measures ANOVA results for the effects of sampling season (SS), litter treatment (LT), forest type (FT), and their interactions on the soil ammonium ($\text{NH}_4^+ - \text{N}$), nitrate ($\text{NO}_3^- - \text{N}$) and inorganic N concentration, and ammonification (R_{amm}), nitrification (R_{nit}) and N mineralization (R_{min}) rates.

Factors	$\text{NH}_4^+ - \text{N}$		$\text{NO}_3^- - \text{N}$		Inorganic N		R_{amm}		R_{nit}		R_{min}	
	F	P	F	P	F	P	F	P	F	P	F	P
LT	0.44	0.52	0.01	0.93	0.02	0.90	3.05	0.10	0.23	0.64	1.34	0.26
FT	42.68	0.00 ***	0.99	0.39	6.99	0.01 *	3.95	0.04 *	0.89	0.43	0.09	0.91
SS	304.55	0.00 ***	43.86	0.00 ***	115.49	0.00 ***	193.82	0.00 ***	46.83	0.00 ***	42.22	0.00 ***
LT × FT	1.587	0.24	0.98	0.40	0.83	0.46	1.49	0.265	2.36	0.137	0.43	0.66
LT × SS	4.845	0.03 *	0.72	0.55	2.26	0.06	4.94	0.03 *	0.30	0.804	1.96	0.15
FT × SS	22.88	0.00 ***	7.94	0.00 ***	12.79	0.00 ***	20.70	0.00 ***	7.25	0.00 ***	8.48	0.00 ***
LT × FT × SS	2.673	0.08	4.29	0.00 ***	4.55	0.00 ***	3.95	0.03 *	4.58	0.00 **	6.41	0.00 ***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Compared to coniferous and mixed forests, the effect of litter removal on the net soil ammonification rate was more significant in the broad-leaved forest (Figure 3a). Litter removal significantly reduced and increased the net soil ammonification rate of the broad-leaved forest in the EGS, EFS, and LGS, respectively (Figure 3a). The net soil ammonification rate varied with seasons ($p < 0.001$; Table 1 and Figure 3a) and forest types ($p < 0.05$; Table 1 and Figure 3a), with an interaction between the two factors ($p < 0.001$; Table 1). In both litter treatments, the net ammonification rate of coniferous forest and mixed forest was highest in FS and lowest in TS, while broad-leaved forest was highest in EGS and lowest in TS (Figure 3a). The difference in the net soil ammonification rate between forest types was mainly observed in the coniferous forest and broad-leaved forest (Figure 3a).

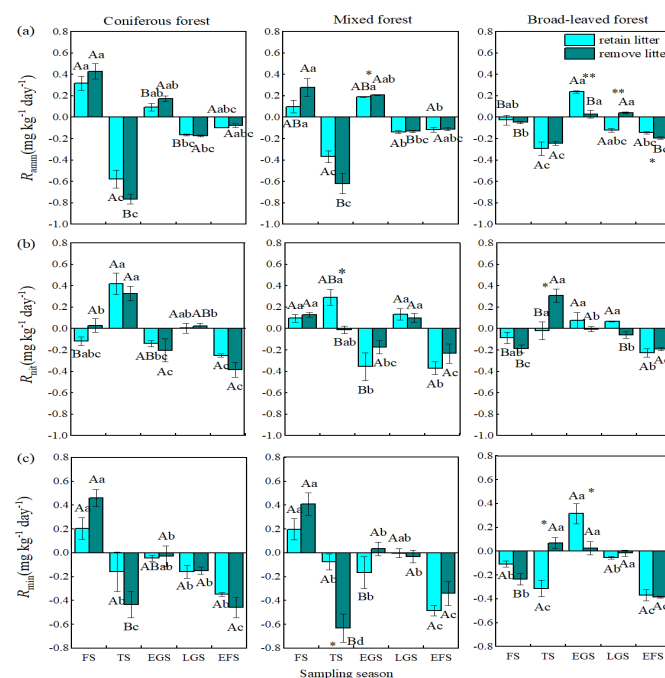


Figure 3. Effects of litter treatment on the net ammonification (a), nitrification (b) and mineralization (c) rates at different incubation stages in coniferous, mixed, and broad-leaved forests. Values indicate the means $\pm$ standard error, $n = 3$. FS: freezing season, TS: thawing season, EGS: early growing season, LGS: late growing season, EFS: early freezing season. Asterisks represent the significant differences between different litter treatments in the same forest type and sampling season. Lowercase letters represent the significant differences among different sampling seasons in the same litter treatment and forest type. Uppercase letters represent significant differences among the different forest types in the same litter treatment and sampling season.

3.2. Net Nitrification Rate

Litter removal significantly increased the soil NO_3^- -N concentration of the coniferous forest during the FS (Figure 2b). Seasons significantly affected the NO_3^- -N concentration changes ($p < 0.001$; Table 1 and Figure 2b), which increased in the TS and then decreased with the sampling season (Figure 2b). Forest types had no significant effect on the NO_3^- -N concentration (Table 1 and Figure 2b).

Litter removal significantly affected the net soil nitrification during the TS of the mixed forest and broad-leaved forest (Figure 3b). The net soil nitrification rate of the three forest types all varied with seasons, showing a trend of increasing first in the TS and then decreasing in the EFS ($p < 0.001$; Table 1 and Figure 3b). Differences in the net soil nitrification rate between forest types were mainly observed between the broad-leaved forests and the other two forests (Figure 3b).

3.3. Net Nitrogen Mineralization Rate

Litter removal had no significant effect on the soil inorganic N concentration of the three forest types (Table 1 and Figure 2c). Forest types ($p < 0.05$; Table 1 and Figure 2c) and seasons ($p < 0.001$; Table 1 and Figure 2c) significantly affected the soil inorganic N concentration. The inorganic N concentration was highest in the FS (Figure 2c). Compared with coniferous forests and mixed forests, broad-leaved forests had relatively lower inorganic N concentrations in the FS and TS (Figure 2c).

Litter removal had no significant effect on the net soil N mineralization rate in the coniferous forest (Table 1 and Figure 3c). In the mixed forest, litter removal significantly reduced the rate of net soil N mineralization during the TS (Figure 3c). In the broad-leaved forest, litter removal significantly increased and decreased the rate of net soil N mineralization in the TS and EGS, respectively (Figure 3c). The net soil N mineralization rate varied with seasons ($p < 0.001$; Table 1 and Figure 3c). In both litter treatments, the net N mineralization rate of the coniferous and mixed forest was the highest in the FS and the lowest in the TS and EFS, respectively (Figure 3c). However, in the broad-leaved forest, the highest net N mineralization rate was found in the EGS (Figure 3c). Consistent with the net ammonification rate, a difference in the net N mineralization rate was found between the coniferous and broad-leaved forests (Figure 3c).

3.4. Driving Factors Difference among Different Forest Types

The structural equation models (SEMs) showed that the effect of litter treatment in different forest types on the rates of net soil ammonification, nitrification, and net N mineralization rates was different (Figure 4). In the coniferous forest, the increase in soil MBC and MBN after litter removal had a directly positive effect on the net soil ammonification rate (Table 2 and Figure 4a). The increase in soil MBC and MBN after litter removal had directly negative and positive effects on the net soil nitrification rate, respectively (Table 2 and Figure 4a). The increases in soil MBC and MBN after litter removal had an indirectly positive effect on the net soil mineralization rate by changing net ammonification or nitrification rates (Table 2 and Figure 4a). In the mixed forest, the increase in soil MBN after litter removal had a direct positive effect on the net soil nitrification rate (Table 2 and Figure 4b). The increase in soil moisture after litter removal had an indirectly positive effect on the net soil mineralization rate by changing the MBN and net nitrification rate (Table 2 and Figure 4b). In the broad-leaved forest, the increase in the soil N concentration and MBN after litter removal had directly positive and negative effects on the net soil ammonification rate, respectively (Table 2 and Figure 4c). The increase in the soil MBN after litter removal had a directly positive effect on the net soil nitrification rate (Table 2 and Figure 4c). The increase in the soil N concentration, C:N ratio, and MBN after litter removal had a directly positive effect on the net soil mineralization rate (Table 2 and Figure 4c).

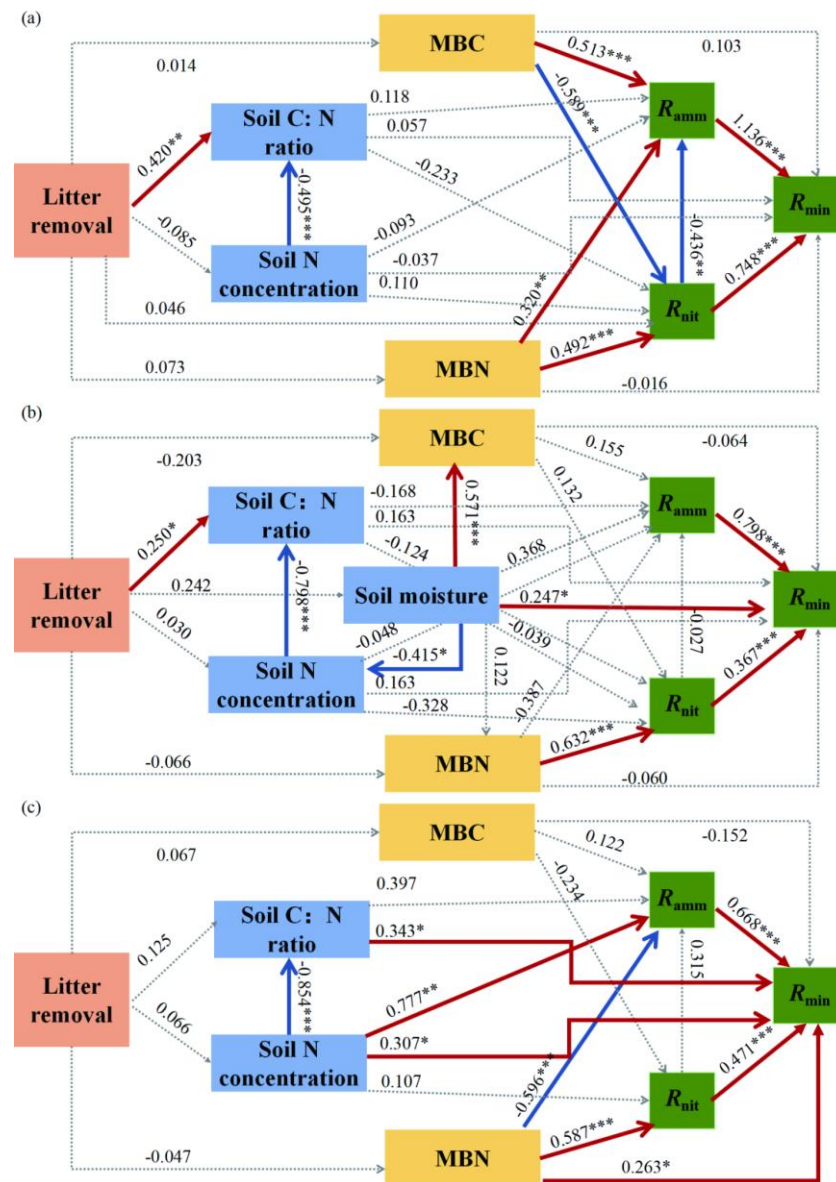


Figure 4. Structural equation models (SEMs) showing the effects of the litter treatment, soil moisture, MBC, MBN, N concentration, and C:N ratio on the net soil ammonification (R_{amm}), nitrification (R_{nit}), and N mineralization (R_{min}) rates in coniferous (a), mixed (b), and broad-leaved (c) forests. Red (positive) and blue (negative) indicate significant and gray indicate insignificant ($p > 0.05$) effects, respectively. (a) Coniferous forest model satisfactorily fit the data based on the $\chi^2 = 6.911$, $df = 7$, $\chi^2/df = 0.987$, NFI = 0.955, CFI = 1.000, and RMSEA = 0.000. (b) Mixed forest model satisfactorily fit the data based on the $\chi^2 = 10.767$, $df = 9$, $\chi^2/df = 1.196$, NFI = 0.928, CFI = 0.983, and RMSEA = 0.078. (c) Broad-leaved forest model satisfactorily fit the data based on the $\chi^2 = 10.118$, $df = 9$, $\chi^2/df = 1.124$, NFI = 0.931, CFI = 0.990, and RMSEA = 0.062. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 2. Standardized total, direct, and indirect effects were estimated by the structural equation models (SEMs) to assess the effects of litter treatment, soil moisture, MBC, MBN, N concentration, and C:N ratio on the net soil ammonification (R_{amm}), nitrification (R_{nit}), and N mineralization (R_{min}) rates in subalpine forests.

		Coniferous Forest			Mixed Forest			Broad-Leaved Forest		
		R_{amm}	R_{nit}	R_{min}	R_{amm}	R_{nit}	R_{min}	R_{amm}	R_{nit}	R_{min}
Litter treatment	Total	0.112	−0.043	0.125	0.047	−0.056	0.122	0.104	−0.036	0.074
	Direct	0.000	0.046	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Indirect	0.112	−0.089	0.125	0.047	−0.056	0.122	0.104	−0.036	0.074
Soil moisture	Total	-	-	-	0.368	0.208	0.559	-	-	-
	Direct	-	-	-	0.368	−0.039	0.247	-	-	-
	Indirect	-	-	-	0.000	0.247	0.313	-	-	-
Soil N concentration	Total	−0.250	0.225	−0.181	0.092	−0.228	0.022	0.471	0.107	0.380
	Direct	−0.093	0.110	−0.037	−0.048	−0.328	0.163	0.777	0.107	0.307
	Indirect	−0.157	0.115	−0.144	0.140	0.099	−0.141	−0.306	0.000	0.073
Soil C:N ratio	Total	0.220	−0.233	0.133	−0.164	−0.124	−0.013	0.397	0.000	0.609
	Direct	0.118	−0.233	0.057	−0.168	−0.124	0.163	0.397	0.000	0.343
	Indirect	0.102	0.000	0.076	0.004	0.000	−0.176	0.000	0.000	0.266
MBC	Total	0.770	−0.589	0.538	0.151	0.132	0.105	0.049	−0.234	−0.229
	Direct	0.513	−0.589	0.103	0.155	0.132	−0.064	0.122	−0.234	−0.152
	Indirect	0.257	0.000	0.435	−0.004	0.000	0.169	−0.073	0.000	−0.077
MBN	Total	0.105	0.492	0.471	−0.404	0.632	−0.150	−0.411	0.587	0.265
	Direct	0.320	0.492	−0.016	−0.387	0.632	−0.060	−0.596	0.587	0.263
	Indirect	−0.215	0.000	0.487	−0.017	0.000	−0.090	0.185	0.000	0.002
R_{amm}	Total	-	-	1.136	-	-	0.798	-	-	0.668
	Direct	-	-	1.136	-	-	0.798	-	-	0.668
	Indirect	-	-	0.000	-	-	0.000	-	-	0.000
R_{nit}	Total	−0.436	-	0.252	−0.027	-	0.345	0.315	-	0.682
	Direct	−0.436	-	0.748	−0.027	-	0.367	0.315	-	0.471
	Indirect	0.000	-	−0.496	0.000	-	−0.021	0.000	-	0.210

4. Discussion

Litter is a key source for terrestrial N cycles [6]. Studies have shown that, once the litter returns to the ground, the lignin will undergo a condensation reaction with N, and approximately 26–38% of the N will be fixed in the lignin to form humus components [7]. These N components will return to the soil through mineralization, thereby regulating the supply and release of plant nutrients [37]. As active N can be directly absorbed and utilized by plants, inorganic N is the important component during soil N transformation, and its supply rate is often controlled by the soil N mineralization processes [3,38]. We utilized a litter treatment experiment to test the effects of litter on the net soil N mineralization in three typical subalpine forests. The results partially support the hypothesis that the effects of litter changes on soil net N mineralization, nitrification, and ammonification rates are inconsistent across forest types and seasons. After 1.5 years of continuous litter removal, we found that the response of soil net N mineralization to litter removal was greater in the broad-leaved forest than that in the coniferous forest, which may be caused by differences in the vegetation types and microorganisms in different forest ecosystems [39]. In this study, the litter type of coniferous forest is dominated by needles, and the broad-leaved forest is dominated by broad leaves [17], which may lead to different litter C:N ratios. The litter C:N ratio is a good inverse predictor of decomposition rates. Studies have shown that a lower litter C:N ratio will enhance the soil N cycle [5]. Compared with the broad-leaved forest, the higher litter C:N ratios in coniferous forests may lead to lower soil N mineralization and availability [4,17]. At the same time, the litter composition and yield are different among different forest types, resulting in different microbial activities and community structures [40,41]. In forest ecosystems, fungi and bacteria play different roles in N mineralization [42]. Compared with bacteria, fungi may be more competitive in utilizing NO_3^- -N [43], which would inevitably lead to different soil N mineralization characteristics.

The subalpine forests in western Sichuan are characterized by obvious seasonal variations [16], and the types of forest litter also change dynamically with the seasons [14]. They are often dominated by foliar litter in the growing season, and by twigs in winter [16,17].

Different soil micro-environments and litter types may lead to differences in the soil microbial structure and activity [20,21], resulting in different mineralization characteristics. We found that, in the broad-leaved forest, the influence of litter removal on the soil ammonification rate was more significant in the growing season (Figure 3a). During the growing season, the sprouting and utilization of spring plants and the rapid degradation of dead biological residues result in a decrease in the concentration of effective nutrients in the soil [44]. Studies have shown that, compared with nitrate N, ammonium N is more easily absorbed and utilized by organisms [45], which may promote the rate of soil N ammonification. The effect of litter removal in the broad-leaved forest on the rate of nitrification and mineralization is more significant in the thawing season (Figure 3b,c). This is consistent with previous results that the rates of net nitrification and N mineralization were insensitive to low temperatures [34]. The constant low temperature in winter may directly or indirectly kill microorganisms through the limitation of soil water and nutrient availability [46,47] and inhibit the activity of soil microbes [13], thus restricting N mineralization. However, when it comes to the thawing season, the frequent freeze–thaw cycles can increase the soil water content and enhance microbial activity, promote litter decomposition, and improve effective resources [44].

The study found that the soil moisture, microbial biomass properties, and soil C:N ratio are the main influencing factors in the three forest types (Figure 4). Among them, MBN is the common influencing factor (Figure 4). Although the MBN is a small N pool compared to the N contained in plants and other organic forms of N in the soil, the turnover of the MBN pool is very rapid [48], which can participate well in the process of soil N mineralization and provide available N for the demand of plants [49]. The unique impact factors of coniferous, mixed, and broad-leaved forests are the MBC, soil moisture, soil N concentration, and C:N ratio, respectively (Figure 4). Previous studies indicated that MBC positively affected soil N mineralization [50]. In coniferous forests, changes in litter removal may change the ratio of labile fractions and recalcitrant fractions, resulting in higher MBC in the labile fractions than the recalcitrant fractions [50]. In addition, soil moisture variation exerts strong effects on soil N mineralization in forests [51]. It can affect N mineralization via increasing soil substrates or cause a shift in the plant community composition and associated input of litterfall [51]. Consistent with previous studies [52], within a certain humidity range, there is a significant positive correlation between the soil moisture content and mineralization in the mixed forest. This may be related to the most suitable soil moisture content for soil microbial activities [53]. Soil N is a major determinant of microorganism growth, which will affect soil N mineralization [4]. The increasing soil N concentration will increase the N mineralization rate in the broad-leaved forest (Figure 4c), which is consistent with previous studies [48,54]. The soil C:N ratio has also been used as a predictor of the N mineralization rate; a higher soil C:N ratio is generally thought to be responsible for a slower rate of soil mineralization [55]. However, inconsistently, we found that an increase in the soil C:N ratio led to an increase in the N mineralization rate of broad-leaved forests (Figure 4c). When microbes decompose organic matter with a high C:N ratio, they will immobilize inorganic N to meet their N requirement [56]. The critical value of the substrate C:N ratio used to switch between N mineralization and immobilization processes is about 25 [48]. In this study, the C:N ratio was less than 25 (Table S2), which may have been the cause of its positive effect on soil N mineralization. At the same time, microbial, temperature, moisture, and vegetation properties may also be influencing factors [57–60].

5. Conclusions

This study examined the influence of litter on the soil ammonification, nitrification, and net N mineralization rates in three subalpine forests on the Qinghai–Tibetan Plateau. The results showed that the impacts of litter removal on broad-leaved forests were more obvious and mainly focused on the growing and the thawing seasons. The variations in soil moisture, MBC, MBN, N concentration, and soil C:N ratio may partially explain

the differences in N mineralization between forest types and seasons. These findings suggest that the effects of short-term litter changes on soil N mineralization are related to the litter types and soil microclimate, while the effects of long-term litter changes on soil N mineralization require further study. This study provides some basic evidence for understanding plant–soil interactions in subalpine forests and helps to clarify the potential influence of plant litter accumulation on soil N cycling.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f13040597/s1>, Figure S1: Soil temperature from Oct 2017 to Dec 2018 (A) and soil moisture affected by litter treatment at different times in three forests (B); Table S1: Soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) in different litter treatment and sampling season of three forest types; Table S2: Soil C:N, C:P, and N:P ratios in different sampling season of the three forest types.

Author Contributions: Conceptualization, L.Z. (Li Zhang), Y.Y. and H.L.; software, Y.Y. and Y.S.; validation, Z.J., Z.C., Y.L. and L.Z. (Linhui Zhang); data curation, L.Z. (Li Zhang); writing—original draft preparation, L.Z. (Li Zhang), Y.Y. and Z.J.; writing—review and editing, L.W., S.L. and H.L.; visualization, L.Z. (Li Zhang), Y.Y. and Z.J.; funding acquisition, L.Z. (Li Zhang), S.L., Q.W. and H.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Open Fund of Ecological Security and Protection Key Laboratory of Sichuan Province, Mianyang Normal University (ESP1704), and the National Natural Science Foundation of China (31901295, 32001165, 32071747, and 31700542).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We are grateful to the Long-term Research Station of Alpine Forest Ecosystems and the Collaborative Innovation Center of Ecological Security in the Upper Reaches of the Yangtze River.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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