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Variability of Aboveground Litter Inputs Alters Soil Carbon and Nitrogen in a Coniferous–Broadleaf Mixed Forest of Central China

Renhui Miao [1], Jun Ma [2], Yinzhan Liu [1], Yanchun Liu [1], Zhongling Yang [1] and Meixia Guo [3,*]

1 Key Laboratory of Plant Stress Biology, State Key Laboratory of Cotton Biology, School of Life Sciences, Henan University, Kaifeng 475004, China; miaorenhui@henu.edu.cn (R.M.); liuyinzhan.1@163.com (Y.L.); yanchunliu@henu.edu.cn (Y.L.); yang_zhl06@126.com (Z.Y.)

2 Ministry of Education Key Laboratory for Biodiversity Science and Ecological Engineering, School of Life Science, Fudan University, Shanghai 200433, China; ma_jun@fudan.edu.cn

3 Henan Joint International Research Laboratory of Environmental Pollution Control Materials, College of Chemistry and Chemical Engineering, Henan University, Kaifeng 475004, China

* Correspondence: guomeixia@vip.henu.edu.cn; Tel.: +86-371-2388-5016

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Abstract: Global changes and human disturbances can strongly affect the quantity of aboveground litter entering soils, which could result in substantial cascading effects on soil biogeochemical processes in forests. Despite extensive reports, it is unclear how the variations in litter depth affect soil carbon and nitrogen cycling. The responses of soil carbon and nitrogen to the variability of litter inputs were examined in a coniferous–broadleaf mixed forest of Central China. The litter input manipulation included five treatments: no litter input, natural litter, double litter, triple litter, and quadruple litter. Multifold litter additions decreased soil temperature but did not affect soil moisture after 2.5 years. Reductions in soil pH under litter additions were larger than increases under no litter input. Litter quantity did not affect soil total organic carbon, whereas litter addition stimulated soil dissolved organic carbon more strongly than no litter input suppressed it. The triggering priming effect of litter manipulation on soil respiration requires a substantial litter quantity, and the impacts of a slight litter change on soil respiration are negligible. Litter quantity did not impact soil total nitrogen, and only strong litter fluctuations changed the content of soil available nitrogen (nitrate nitrogen and ammonium nitrogen). Litter addition enhanced soil microbial biomass carbon and nitrogen more strongly than no litter input. Our results imply that the impacts of multifold litter inputs on soil carbon and nitrogen are different with a single litter treatment. These findings suggest that variability in aboveground litter inputs resulting from environmental change and human disturbances have great potential to change soil carbon and nitrogen in forest ecosystems. The variability of aboveground litter inputs needs to be taken into account to predict the responses of terrestrial soil carbon and nitrogen cycling to environmental changes and forest management.

Keywords: litter inputs manipulation; litter quantity; microbial biomass; priming effects; soil dissolved organic carbon; soil organic carbon

1. Introduction

Aboveground litter plays a critical role in regulating soil carbon and nitrogen cycling between plants and soils in forest ecosystems, and the litter layer mediates the soil microclimate by buffering the soil surface and atmosphere [1]. Terrestrial ecosystems are undergoing changes from human disturbances that coincide with environmental changes [2]. Those alterations may strongly affect forest net primary productivity and consequently change the aboveground litter inputs to soils. Alterations, such as an elevated atmospheric CO_2 concentration [3], climate warming [4], atmospheric nitrogen deposition [5], and increased precipitation [6], were found to increase plant productivity. In contrast, forest thinning [7], elevated O_3 [8], drought [9], and acid deposition [10] can lead to decreased plant productivity. Changes in plant productivity will shift the amounts of aboveground litter moving into soils, which subsequently affects biogeochemical processes by changing soil carbon and nitrogen inputs, soil biotic activity, and the soil microenvironment [1]. In addition, future extreme events (i.e., wildfire, hurricane, sandstorm) can also result in sudden and dramatic changes in litter inputs [11,12].

Aboveground litter manipulation experiments, including litter addition and litter removal, have been conducted to examine the impacts of changing litter on soil carbon, nitrogen, and the microenvironment in various terrestrial ecosystems [13–16]. Aboveground litter and its decomposition represent the main pathway of carbon and nitrogen from plants to soils [17]. The amount of accumulated litter can influence the soil organic carbon content and underground processes. In a meta-analysis of 70 manipulative experiments, Xu et al. (2013) found that litter addition increased soil total carbon, soil respiration, and soil microbial biomass carbon, whereas contrary results were found under litter exclusion [18]. However, the effects of litter addition and removal on soil carbon cycling vary with the type of ecosystem. For example, neutral effects of litter addition and removal on soil organic carbon are detected in some temperate forests [13,19], but positive or negative effects of litter treatments on soil organic carbon are also found in other forest ecosystems [15,20]. No impact of no litter input conditions on soil microbial biomass carbon in a pine plantation and secondary forest has been found [21]; however, litter addition can increase, and no litter input decrease, soil microbial biomass in a subtropical forest of China [22] and a temperate deciduous forest of North America [16]. Litter addition and no litter input can respectively enhance and decrease soil respiration in most forest ecosystems [13,15,21,22], but both litter addition and no litter input can increase soil respiration in a temperate forest in Germany [19].

Coupled with soil carbon, soil nitrogen may also be changed under litter addition and removal because litter treatment can change the amount of N gained, lost, or retained in soils. The synthesis by Xu et al. (2013) also found that soil total nitrogen decreased under no litter input conditions but was not influenced by litter addition [18]. In addition, litter could help maintain a favorable microenvironment for the decomposition process in forests [1]. However, previous reports neglected the effect of variability in litter depths on regulating soil carbon and nitrogen in natural conditions. The dynamics of soil carbon and nitrogen may increase exponentially with litter depth due to the "priming effect". The priming effect is a complex and poorly understood plant–soil relationship and could play an important role in soil carbon and nitrogen dynamics under global change. Priming effects occur when there is an increase in the litter input of fresh organic matter into soil, which can stimulate litter decomposition of older stored soil carbon [23]. As the main pathway of soil carbon, the CO_2 fluxes often increase disproportionately with litter depth due to priming effects [24]. Therefore, experiments with varying litter depths in nature are urgently needed to determine the response of soil carbon and nitrogen cycling to changing litter depths.

No studies have reported how the variability of litter inputs affect soil carbon and nitrogen cycling in the subtropical–warm temperate climate transition zone in Central China. This region is sensitive to environmental changes [25]. Here, the overall objective was to assess the responses of soil carbon and nitrogen to variations in litter depth in Central China. To achieve this goal, a manipulative field experiment with a variability in litter depth was conducted for 2.5 years in a coniferous–broadleaf mixed forest in Central China. The specific objectives were to examine whether: (1) soil carbon and nitrogen concentrations resulting from the priming effect vary with litter depth, and (2) the effects of litter depth on soil temperature and moisture—the dominant abiotic factors regulating soil carbon and nitrogen cycling—vary with litter quantity.

2. Materials and Methods

2.1. Study Site

The litter manipulation experiment was conducted in a coniferous–broadleaf mixed forest (32°6′53″ N, 114°1′52″ E, 215 m a.s.l.), from of the Xinyang Forest Ecosystem Research Station, Xinyang, Henan, China. This region belongs to a subtropical–warm temperate monsoon climate. The long-term (1951-2014) mean annual temperature is 15.2 °C, ranging from 1.9 °C in January to 33.6 °C in July. Mean annual precipitation is 1063 mm, of which approximately 70% occurs in the growing season (from May to October). The soil is classified as a Haplic Luvisol [26]. The forest is a natural secondary forest because the original forest was clear-cut in 1987. The dominant trees are German oak (*Quercus acutissima*) and Masson pine (*Pinus massoniana*), with densities of 0.053 and 0.073 individuals m^{-2}, respectively. Shrubs are dominated by *Vitex negundo*, *Lindera glauca*, *Rubus corchorifolius*, and *Symplocos chinensis*, with a total density of 3.24 individuals m^{-2}. Mean soil bulk density, pH, litter mass, and litter moisture are 0.99 g cm^{-3}, 4.5, 788 g m^{-2} dry mass, and 7.8%, respectively. Mean annual dry aboveground litter production was 703.91 ± 59.29 g m^{-2} $year^{-1}$ from 2014 to 2017. The litter contains 119.92 ± 30.09 g m^{-2} $year^{-1}$ of needle leaves, 350.54 ± 50.55 g m^{-2} $year^{-1}$ of broad leaves, 20.31 ± 5.21 g m^{-2} $year^{-1}$ of shrub leaves, 130.23 ± 29.88 g m^{-2} $year^{-1}$ of branches, 22.06 ± 1.73 g m^{-2} $year^{-1}$ of reproductive organs, and 60.84 ± 5.95 g m^{-2} $year^{-1}$ of other debris. The C:N ratios of the litter are 32.77, 33.53, and 29.26 in the leaves of *Q. acutissima*, *P. massoniana*, and *L. glauca*, respectively. In this forest ecosystem, the litter depth is 1.2 cm and varies from 0 cm to 3 cm.

2.2. Experimental Design

Four 20 m × 20 m plots were established in a *P. massoniana*–*Q. acutissima* mixed forest in October 2015 and have been maintained ever since. Five manipulative litter treatments were randomly and evenly designed in each plot, including no litter input (NL), natural litter (L), double litter (DL), triple litter (TL), and quadruple litter (QL). In each plot, each treatment had three 2 m × 2 m quadrats, and the distance between any two adjacent quadrats was at least 3 m, resulting in 60 quadrats (five treatments × four replicates × three parallels) in this experiment. Aboveground litter was cleared in the no litter input quadrat at the beginning of the experiment. Aboveground litter was collected in the no litter input quadrat and non-manipulative plots using a 1 mm nylon mesh (1 m × 1 m) suspended 70 cm above the ground. The collected litter was added to the litter addition quadrats and distributed with gentle raking, to avoid disturbing the quadrat and to maintain litter depth monthly. from October 2015 to May 2018.

2.3. Measurements

2.3.1. Soil Respiration, Temperature, and Moisture

The locations were observed to study the responses of soil respiration, temperature, and moisture to litter quantity [27]. Soil respiration was measured three times a month using a Li-8100 portable soil CO_2 flux system (Li-Cor, Inc., Lincoln, NE, USA) between June 2017 and May 2018. Two permanent polyvinyl chloride (PVC) collars with an 11 cm inside diameter and 8 cm height were inserted 6 cm into the soil at two opposite corners of each quadrat in April 2017. Living plants inside the collars were removed by hand at least 24 h before measurement to eliminate the respiration of the aboveground plants. The Li-8100 soil chamber was put on the collar to detect the change in CO_2 concentration for 2 min to calculate the soil respiration of each collar. Each measurement was conducted between 8:30 a.m. and 12:00 p.m. local time on sunny days.

Soil temperature at the 10 cm depth was measured using a thermocouple probe (Li-8100-201, Li-Cor, Inc., Lincoln, NE, USA) connected to the Li-8100 to simultaneously measure soil respiration. Soil volumetric moisture (0–5 cm) was measured with portable Time Domain Reflectometer (TDR) equipment (Soil Moisture Equipment Corp. Santa Barbara, CA, USA) at three points close to each collar.

2.3.2. Soil Chemical Properties

Two soil cores (7 cm diameter, 0–10 cm deep) were randomly collected from each quadrat, and six collected soil cores for each litter treatment in each plot were combined into one composite soil sample in August 2018. After homogeneous mixing and removal of roots, the soil was passed through a 2 mm mesh sieve and divided into two parts. One part was maintained fresh at 4 °C for the determination of soil microbial biomass carbon (MBC), nitrogen (MBN), and dissolved organic carbon. The other part was air-dried for the determination of soil pH, total organic carbon, total nitrogen, nitrate nitrogen, and ammonium nitrogen. Soil pH values were determined by a glass electrode using a soil–water ratio of 1:2.5. Soil total organic carbon was measured with the $K_2Cr_2O_7$ volumetric dilution heating method [28]. Total nitrogen was determined by elemental analysis. Soil dissolved organic carbon (DOC) was extracted from 10 g of fresh soil in 40 ml deionized water at 20 °C and shaken for 1 h using an end-to-end shaker [29]. The mixture was centrifuged at 3500 rpm for 20 min and filtered through a 0.45 µmol filter membrane. The organic carbon concentrations in the water extracts were determined by a total organic carbon (TOC) 5000 analyzer (Shimadzu Ltd., Kyoto, Japan). Soil samples were shaken three times with 20 mL solutions of 2 mol L^{-1} KCl for 60 min each time. The three extracts were combined and measured with a flow-injection Lachat automated colorimetry system (FIAstar 5000 Analyzer; Foss Tecator, Foss Ltd., Hillerød, Denmark) to determine the concentration of nitrate nitrogen and ammonium nitrogen.

2.3.3. Soil Microbial Biomass Carbon and Nitrogen

Soil microbial biomass C and N were measured using the chloroform fumigation extraction method [30]. The microbial biomass C (MBC) and soil microbial biomass N (MBN) were calculated as the difference in extractable C and N contents between the fumigated and unfumigated samples using the conversion factors of 0.45 and 0.54 [31,32], respectively.

2.4. Statistical Analysis

Data were tested for normality and homogeneity of variance prior to the statistical analysis. Two-way ANOVAs were performed to test the main and interactive effects of month and litter treatment on soil respiration, soil temperature, and soil moisture. Duncan's test at $p < 0.05$ was used to examine the significant differences among mean values of soil respiration, soil temperature, and soil moisture in each month and the soil pH, total organic carbon, dissolved organic carbon, total nitrogen, C:N ratio, nitrate nitrogen, ammonium nitrogen, MBC, and MBN of different treatments. All analyses were conducted with the SPSS 19.0 software package (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Soil Microenvironment

Soil temperature changed significantly across months ($p < 0.001$, Figure 1A). Litter variability marginally affected soil temperature ($p < 0.1$), and soil temperature in TL and QL plots was 0.17 °C and 0.18 °C lower than in the L plot, respectively. The effects of litter quantity on soil temperature varied with month ($p < 0.001$). In addition, soil temperatures in DL, TL, and QL were significantly ($p < 0.05$) lower than in NL, and soil temperature in QL was lower than in L in spring. Soil temperatures in DL, TL, and QL were significantly lower than in NL and L in summer. Soil temperatures in DL, TL, and QL were significantly higher than in NL in autumn. Soil temperature in QL was significantly higher than in NL in winter. Soil moisture fluctuated greatly between months ($p < 0.001$, Figure 1B). No main effect or interactions of litter treatment and month on soil moisture were detected. Soil pH in DL, TL, and QL was 0.65, 0.65, and 0.73 lower than in NL, and 0.64, 0.64, and 0.72 lower than in L, respectively ($p < 0.05$, Figure 1C).

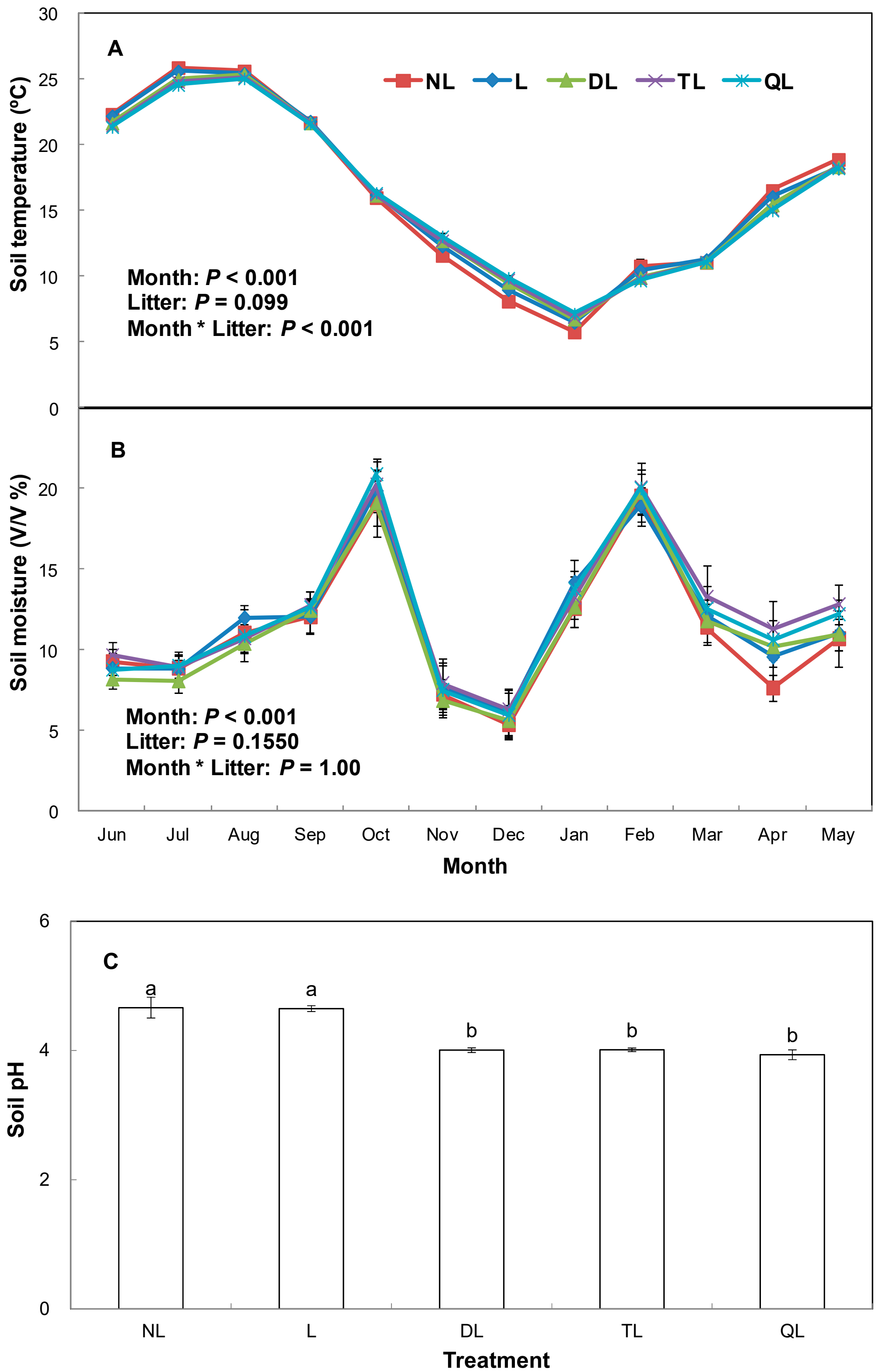

Figure 1. Effects of litter quantity on soil temperature (**A**), moisture (**B**), and pH (**C**). Different letters indicate significant differences between treatments at $p < 0.05$. Values are presented as the means ± standard error. NL: no litter input; L: natural litter; DL: double litter; TL: triple litter; QL: quadruple litter.

3.2. Soil Carbon and Nitrogen Content

Soil dissolved organic carbon in DL, TL, and QL was 269.7, 260.0, and 295.0 mg kg^{-1} higher than in NL, and 239.3, 229.6, and 264.7 mg kg^{-1} higher than in L, respectively (all $p < 0.05$, Figure 2B). Nitrate nitrogen and ammonium nitrogen in QL were 6.34 mg kg^{-1} and 4.0 mg kg^{-1} higher than in NL, respectively (both $p < 0.05$, Figure 2E,F). No differences in soil total organic carbon, total nitrogen, and C:N ratios were found between litter treatments.

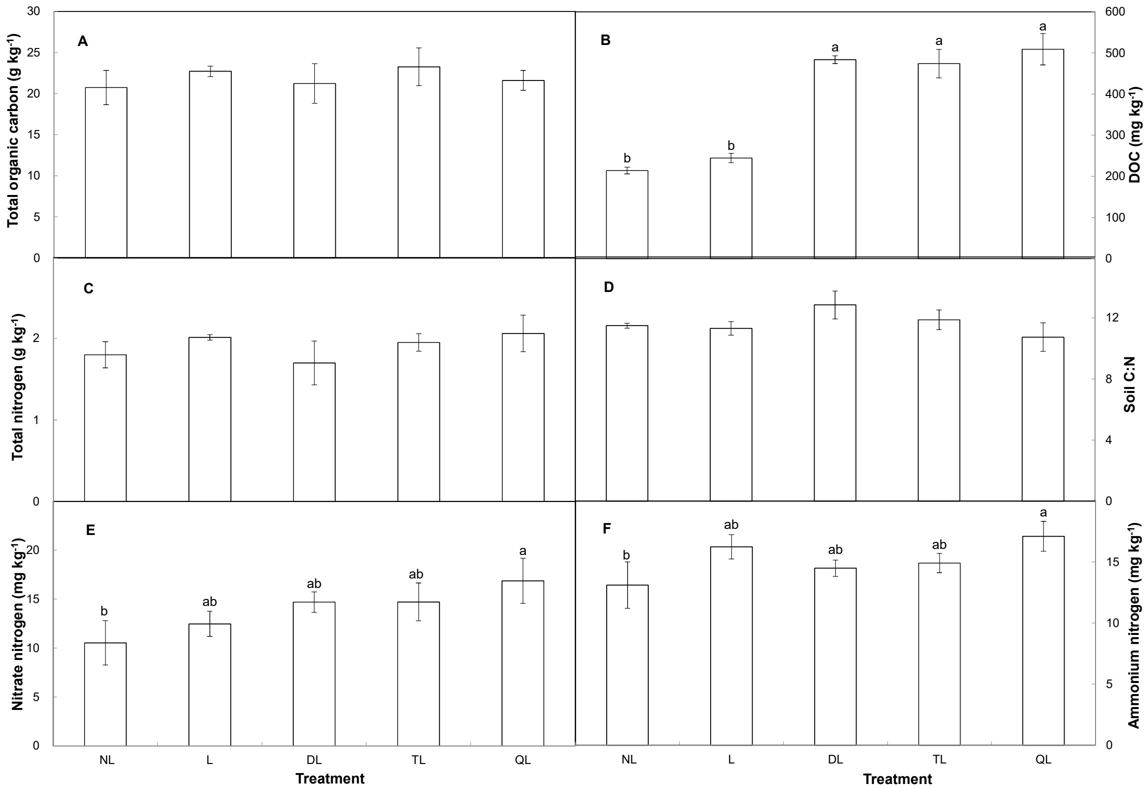

Figure 2. Effects of litter quantity on soil total organic carbon (**A**), dissolved organic carbon (DOC) (**B**), total nitrogen (**C**), the C:N ratio (**D**), nitrate nitrogen (**E**), and ammonium nitrogen (**F**). Different letters indicate significant differences between treatments at $p < 0.05$. Values are presented as the means ± standard error. See Figure 1 for abbreviations.

3.3. Soil Microbial Biomass Carbon and Nitrogen

Soil microbial biomass carbon in DL, TL, and QL was 542.2, 521.8, and 626.4 mg kg^{-1} higher than in NL, and 458.3, 437.8, and 542.4 mg kg^{-1} higher than in L, respectively (all $p < 0.05$, Figure 3A). Soil microbial biomass nitrogen in DL and QL was 28.8 and 42.3 mg kg^{-1} higher than in NL, respectively, and that in DL, TL, and QL was 37.3, 32.8, and 50.7 mg kg^{-1} higher than in L, respectively (all $p < 0.05$, Figure 3B). No differences in soil microbial biomass carbon or nitrogen were detected between other treatments.

3.4. Soil Respiration

Soil respiration varied greatly with month ($p < 0.001$, Figure 4). Litter depth significantly ($p < 0.001$) affected soil respiration. Soil respiration in the QL plot was 1.14, 1.25, 1.22, and 1.11 times higher than in the NL, L, DL, and TL plots, respectively (all $p < 0.05$). Soil respiration in the QL plot was 1.38, 1.52, 1.52, and 1.18 times higher, respectively, than in the NL, L, DL, and TL plots in August (all $p < 0.05$). No differences in soil respiration between litter treatments were detected in other months.

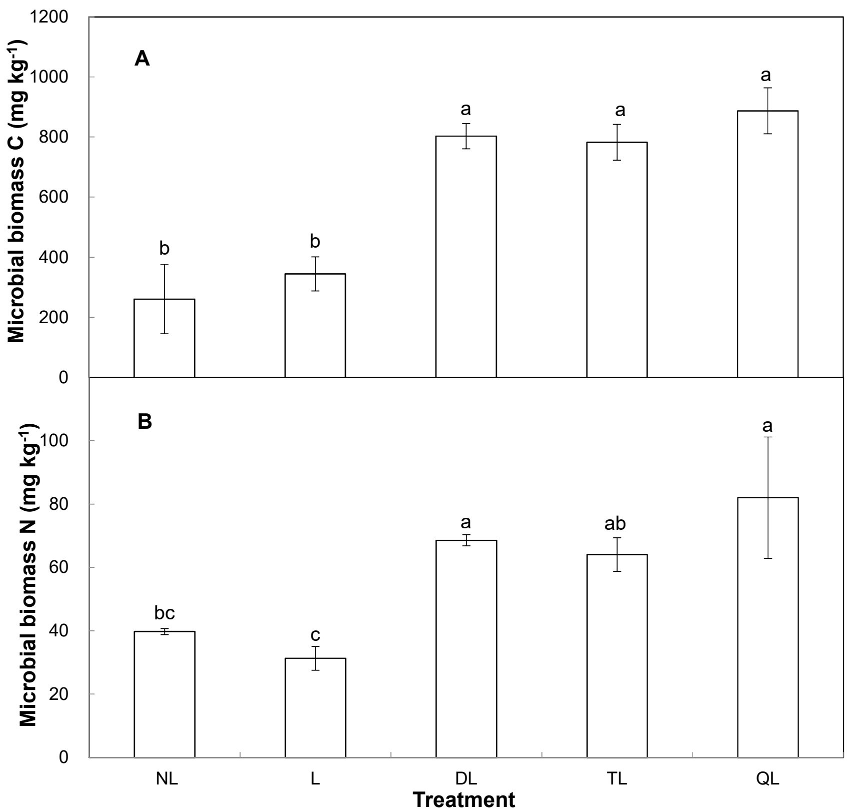

Figure 3. Effects of litter quantity on soil microbial biomass carbon (**A**) and nitrogen (**B**). Different letters indicate significant differences between treatments at $p < 0.05$. Values are presented as the means ± standard error. See Figure 1 for abbreviations.

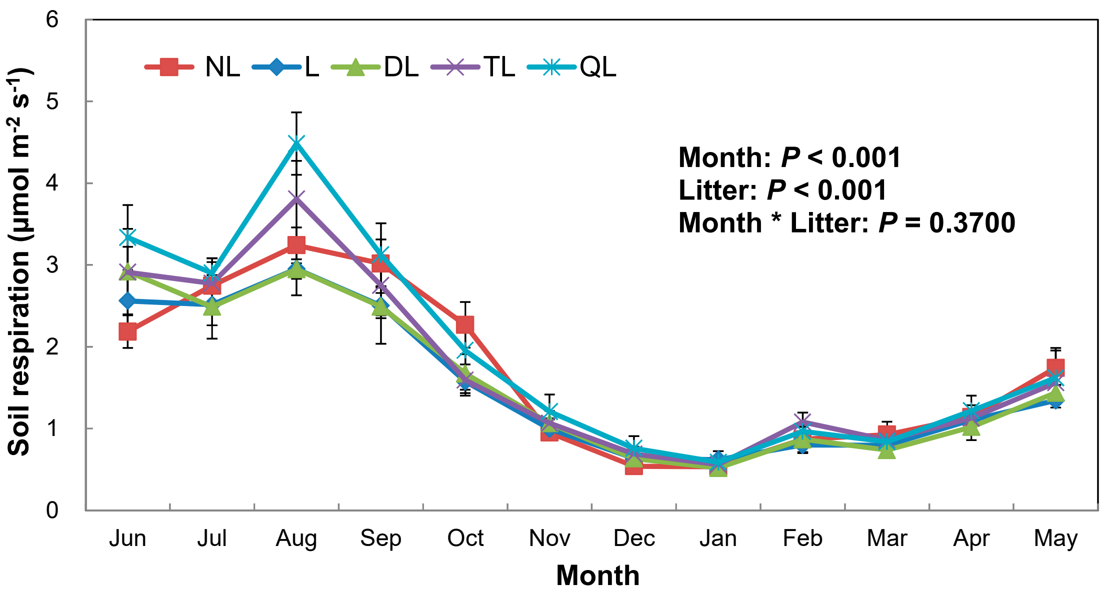

Figure 4. Effects of litter quantity on soil respiration. Values are presented as the means ± standard error. See Figure 1 for abbreviations.

4. Discussion

4.1. Effects of Variability of Litter Inputs on Soil Microenvironment

The litter layer forms a protective layer on the soil surface that also mediates soil microenvironments, such as soil temperature, moisture, and pH [1]. There was a greater decrease in soil temperature under TL and QL compared to L, but no differences in soil temperature between other litter treatments were detected. These findings are consistent with reports of a low-strength litter manipulation experiment in central South China [33], but a meta-analysis detected that litter addition decreased soil temperature, whereas litter removal increased it [18]. The lack of difference in soil moisture with litter variability is also in line with studies conducted in the Costa Rican rainforest [34] and in central South China [33]. The discrepancies between our findings and other reports of soil microclimate may be dependent on the degree of solar heat and precipitation intercepted by the branches and foliage in different forests [24,35,36]. These results indicate that single litter addition has minor effects, whereas multifold litter disturbance has a significant influence on soil temperature. Soil moisture was not a critical factor for the soil biogeochemistry cycle in this forest. Soil pH was lower in DL, TL, and QL than in NL and L, which can be explained by the changing process of the release of organic acids and the litter decomposition [37].

4.2. Effects of Variability of Litter Inputs on Soil Carbon

The different litter treatments had no effects on the concentration of soil total organic carbon, indicating that soil total organic carbon in the topsoil is not very sensitive to changes in litter inputs. The findings are in line with studies conducted in some temperate forests [1,19,38] and tropical rainforests [34,39,40]. In contrast, increases or decreases in soil total organic carbon have been reported in many temperate and tropical forests [15,41–44]. Slight changes in total organic carbon under litter variability can be explained by the alteration of soil dissolved organic carbon and respiration that offset the enhanced soil carbon entering into soil from the organic layer [43,45,46].

The increase in dissolved organic carbon under litter addition and lack of change in dissolved organic carbon under no litter input are in agreement with some previous reports [15,43,47]. However, dissolved organic carbon is unaffected by litter inputs in a subtropical forest of China [48] and decreased by litter addition in an old-growth forest of America [46]. Dissolved organic carbon was mineralized by soil microbial biomass or adsorbed by the soil mineral matrix connected with soil organic matter [49]. Increased fresh litter inputs can result in priming effects and an increase in dissolved organic carbon flux through the decomposition of old soil carbon [45]. An increase in soil dissolved organic carbon under litter addition may, at least in part, reflect rapid leaching and movement from litter into soils [50]. For instance, the heavy soluble fraction in litter could move into soils rapidly and stay in mineral soils [50,51], which may enhance soil microbial biomass or activity [15,18,51], thus resulting in a subsequent increase in soil respiration.

Soil respiration was significantly higher in QL plots than in other litter treatments, whereas no differences were detected between other treatments, which is contrary to previous reports showing increased soil respiration under double litter [15,24,43,46]. An increase in soil respiration with increased litter inputs may be partially ascribed to the corresponding increases in soil microbial biomass carbon and dissolved organic carbon [15,18,48]. Priming effects may have occurred in our study, which could accelerate the decomposition of old soil organic carbon and cause the increase in soil respiration (cf. [24,41]). In addition, the priming effect induced by limited litter addition was negligible, and the trigger for the priming effect needs a substantial quantity of litter. A possible reason for the negligible priming effect under the single litter treatment was the low humidity at our study site, which can constrain decomposition and leaching [52]. Crow et al. (2009) reported that the rates of priming effects were not directly induced by litter addition but were attributed to the cumulative added litter over a long time, higher soil temperature and enzyme activity, and greater root activity [46]. Soil respiration was significantly exponentially correlated with soil temperature across all litter depths (cf. [53]) (Figure S1). These findings suggest that the seasonality of soil respiration was possibly induced by the thermal regulation of soil microbial activity [22,54]. Soil respiration is increased in moderate soil moisture; it is limited in soil moisture that is too high or too low. Soil microbial biomass carbon was higher in DL, TL, and QL than in NL and L, which is in line with previous reports that litter addition increases soil microbial biomass carbon [39,43,55]. In contrast, litter removal had little impact on soil microbial biomass carbon (cf. [56]). It is generally accepted that microbes are C-limited and that fresh litter can increase soil microbial biomass and activity [56]. Thus, microbes may rely on fresh litter supply in this area.

4.3. Effects of the Variability of Litter Inputs on Soil Nitrogen

Litter, an important pathway for nutrient transfer from plants to soil, is critical for soil nutrient retention in some systems. No change in soil total nitrogen and C:N ratios under litter variability was observed, but there was higher soil available nitrogen (nitrate nitrogen and ammonium nitrogen) under QL than under NL. These findings are inconsistent with studies conducted in a temperate deciduous forest in Germany [19], a subarctic heath in Sweden [57], and a meta-analysis [18]. Changes in these parameters under litter variability in this study may be ascribed to low total nitrogen concentrations following litter addition, induced by slower litter decomposition and greater nitrogen immobilization in the soils at our study site (cf. [58]). Our findings indicate that soil total nitrogen is insensitive to the variability of litter inputs, and changes in soil available nitrogen need strong litter fluctuations. There was higher soil microbial biomass nitrogen in three litter addition treatments than in L, which is in accordance with the changes in soil microbial biomass carbon under litter variability. These parameters may be used to explain the alteration of soil available nitrogen.

5. Conclusions

In this study, we highlighted the short-term effects of litter manipulation on the fractions of soil total organic carbon and total nitrogen in a natural secondary forest. Litter addition enhanced soil dissolved organic carbon and microbial biomass carbon and nitrogen most likely due to the increased decomposition of old soil carbon, although litter addition had no effects on soil total organic carbon and total nitrogen. Soil nitrate and ammonium nitrogen content were merely higher under quadruple litter treatment than those under litter removal in this experiment. This may be mainly caused by great nitrogen immobilization and the increase of soil microbial biomass after litter additions in the soils in our study site. Our findings suggest that changes in aboveground litter quantity induced by global environmental change and human disturbances have great potential to affect the turnovers of soil organic carbon and total nitrogen by altering labile fractions of soil carbon and nitrogen in forest ecosystems.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/10/2/188/s1, Figure S1: Relationship between soil respiration and soil temperature among different litter quantities.

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