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Inorganic Nitrogen Addition Affects Soil Respiration and Belowground Organic Carbon Fraction for a *Pinus tabuliformis* Forest

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Abstract: The capability of forest ecosystems to sequester carbon from the atmosphere largely depends on the interaction of soil organic matter and nitrogen, and thus, this process will be greatly influenced by nitrogen deposition under the future scenario of global change. To clarify this interaction, the current study explored the variations in soil carbon fraction and soil respiration with different levels of nitrogen deposition. NH_4NO_3 was added at concentrations of 0, 50, 100, 200, and 400 kg N ha^{-1} year^{-1} separately on twenty 100 m^2 plots in a *Pinus tabuliformis* Carr forest in northern China. Soil samples were analyzed for their nutrient content and biophysical properties two years after nitrogen application, and the soil respiration rate was measured every month during the study period. Seasonal variation and nitrogen addition significantly affected soil respiration rate. On average, nitrogen addition significantly reduced the annual soil respiration rate by 23.74%. Fine root biomass significantly decreased by an average of 43.55% in nitrogen treatment plots compared to the control plot. However, the average proportions of autumn and winter soil respiration rates out of the annual cumulative soil respiration rate greatly increased from 23.57% and 11.04% to 25.90% and 12.18%, respectively. The soil microbial biomass carbon content in the control plot was 342.39 mg kg^{-1}, 23.50% higher than the average value in nitrogen treatment plots. The soil dissolved organic carbon was reduced by 22.60%, on average, following nitrogen addition. Significant correlations were detected between fine root biomass and the annual cumulative soil respiration rate, soil microbial biomass carbon content, and soil dissolved organic carbon content. This demonstrates that nitrogen addition affects soil organic carbon transformation and carbon emission, mainly by depressing fine root production.

Keywords: nitrogen addition; soil respiration; organic carbon fraction; fine root biomass

1. Introduction

The intensified nitrogen deposition associated with dramatically increased fossil fuel combustion and the overuse of nitrogen fertilizer is an important component of global change [1,2]. Atmospheric nitrogen deposition has increased by three to five times over the past century [3], and the annual rate of nitrogen deposition in China increased from 9.43 kg ha^{-1} year^{-1} in 1980 to 21.76 kg ha^{-1} year^{-1} in 2010 [4], a rate that is much higher than the natural biological nitrogen fixation rate of 7.91 kg ha^{-1} year^{-1} [1].The negative effect of surplus nitrogen input into terrestrial ecosystems has received much research attention in recent decades [2,4–6]. However, the future effects of nitrogen input on belowground carbon transformation in temperate forests in northern China are still unknown.

Soil respiration results from a complex process of soil organic carbon mineralization, soil microbial activity, root exudation, and root autotrophic metabolism [6,7]. It represents the primary pathway for regulating carbon emissions from the soil carbon pool to the atmosphere, with a global flux rate of 50–98 Pg C year^{-1} [7–9]. In temperate forests, about 50% of carbon emissions result from the decomposition of different kinds of soil organic matter by the soil microbial community [10,11]. The easy accessibility of soil organic carbon by soil microorganisms has been proposed to trigger the fast carbon cycling process [12,13]. Normally, the soil organic carbon pool is partitioned into recalcitrant organic carbon and labile organic carbon [14]. Labile organic carbon mainly comprises soil microbial biomass carbon (MBC), dissolved organic carbon (SDOC), and readily oxidative organic carbon (ROC) [15,16]. The transformation dynamics of these carbon fractions not only determine the magnitude of soil carbon storage but also influence the role of the forest ecosystem in coping with future climate change. The root system is the interactive bridge connecting the aboveground part of a plant with the soil [6]. The production and exudate of forest root systems are the major sources of support for soil microbial metabolism, and they regulate soil organic carbon mineralization [17–19]. So far, anthropogenic nitrogen input has changed much of the belowground process, including the availability of labile organic carbon [20,21], soil microbial activity [22,23], and the allocation of photosynthetic production to root systems [17,24]. The annual cumulative soil respiration rate (R_A) has been reduced by 17% from nitrogen input in temperate forests [6], but the explicit contributor for this large reduction is still a controversial issue. Thus, researchers should pay much more attention to answering the following question: How will fine root production and the soil carbon fraction respond to future increased anthropogenic nitrogen input in temperate forests?

It has been proposed that temperate forest ecosystems in the median-to-high latitudinal areas of the northern hemisphere will be threatened by higher nitrogen input from the atmosphere under the scenario of future global change [4,6], but related studies on the precise biogeochemical cycle by which this will occur are constrained by the uncertainties in the responses of soil respiration and the soil carbon fraction to nitrogen deposition. *Pinus tabuliformis* Carr is an endemic Chinese temperate coniferous forest tree that is distributed in 16 provinces with a total forest cover area of 2.28×10^6 ha [25]. A simulated atmospheric nitrogen deposition experiment was conducted by adding NH_4NO_3 in a *Pinus tabuliformis* forest in northern China. One of the objectives of the current study was to quantify variation in the magnitude of soil respiration and the belowground organic carbon fraction in response to nitrogen input in *Pinus tabuliformis* forests. The second objective was to clarify which belowground processes drive the abovementioned effects of the deposited nitrogen. Five different rates of inorganic nitrogen were applied to twenty plots in a *Pinus tabuliformis* forest in northern China, and the responses of soil respiration rate, soil carbon fraction, and fine root biomass were monitored. We hypothesized that soil respiration would be affected by inorganic nitrogen addition, mainly through changes in fine root production and labile organic carbon.

2. Materials and Methods

2.1. Site Description and Experimental Design

This study was carried out at the Taiyueshan Long-Term Forest Ecosystem Research Station (36°04′ N, 112°06′ E; 1450 m a.s.l.), which is located 190 km southwest of Taiyuan in the Shanxi Province of China. The study area is located in the continental monsoon climate zone and has a mean annual precipitation of about 600 mm and a mean annual air temperature of about 11 °C. The soil type is categorized as eutric cambisols (FAO classification) or cinnamon soil (Chinese classification), with a mean depth between 30 and 50 cm. The dominant tree species in the forest are *Pinus tabuliformis* Carr, *Quercus wutaishanica* Mayr, and *Larix gmelinii* var. *principis-rupprechtii* Mayr. The understory layer mainly consists of *Rosa xanthine* Lindl, *Corylus mandshurica* Maxim, *Corylus heterophylla* Fisch, *Lespedeza bicolor* Turcz, *Carex lanceolata* Boott, *Spodiopogon sibiricus* Trin, and *Thalictrum petaloideum* Linn.

An 80-year-old *Pinus tabuliformis* Carr forest on the hillside of a north-facing slope 5 km northwest of the research station was selected for the inorganic nitrogen addition experiment. Nitrogen in the form of NH_4NO_3 was mixed with 10 kg of local surface soil particles and evenly scattered over the forest floor of 100 m^2 plots in early spring 2015 and 2016. Taking the background atmospheric nitrogen deposition in northern China and the large amount of fossil fuel production in Shanxi province into consideration [4,26–28], inorganic nitrogen was added at five levels, i.e., 0 (N0), 50 (N5), 100 (N10), 200 (N20), and 400 (N40) kg N ha^{-1} $year^{-1}$. Each addition level had four replicates and their spatial distribution is shown in Figure 1. This nitrogen gradient was based on the annual increase rate (0.42 kg N ha^{-1} $year^{-1}$) [4] to represent future levels of anthropogenic atmospheric nitrogen deposition over the next 300–500 years. Adjacent plots were separated by a buffer strip of 10 m in width to avoid cross-contamination of neighboring plots. Base characteristics of the soil and plants are shown in Table 1. The forest community had an overstory height of 17.50 m, and the maximum diameter at breast height (DBH) of the trees within the 20 forest plots was 36.2 cm.

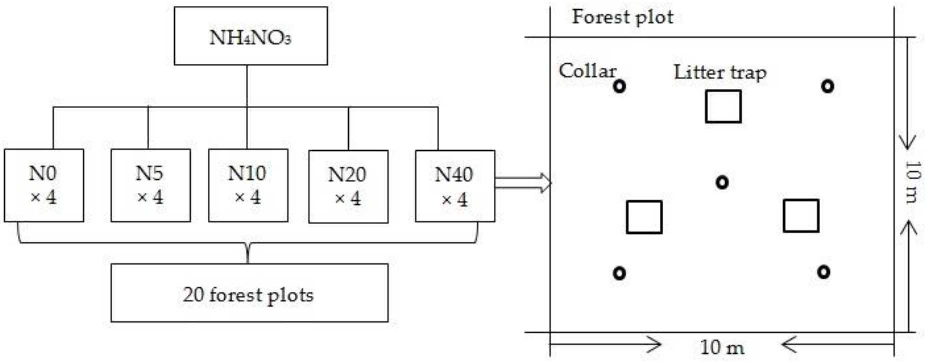

Figure 1. The experimental design at the study site.

Table 1. Basic characteristics of forest soil and plants in different treatment plots before nitrogen addition.

Treatment	SOC (g kg^{-1})	TN (g kg^{-1})	SBD (g cm^{-3})	pH	PD (stem ha^{-1})	DBH (cm)
N0	22.53 ± 2.30 a	1.47 ± 0.20 a	1.23 ± 0.03 a	6.01 ± 0.23 a	1650 ± 119 a	19.39 ± 0.52 a
N5	21.19 ± 3.51 a	1.47 ± 0.16 a	1.32 ± 0.03 a	6.39 ± 0.25 a	2250 ± 348 a	17.41 ± 0.75 a
N10	20.10 ± 2.87 a	1.38 ± 0.12 a	1.22 ± 0.03 a	6.14 ± 0.05 a	1950 ± 284 a	18.22 ± 1.48 a
N20	19.68 ± 1.19 a	1.40 ± 0.10 a	1.23 ± 0.02 a	6.38 ± 0.30 a	2000 ± 245 a	18.96 ± 1.41 a
N40	20.75 ± 1.17 a	1.45 ± 0.24 a	1.32 ± 0.02 a	6.93 ± 0.42 a	2000 ± 108 a	18.10 ± 0.92 a

SOC, TN, SBD, PD, and DBH denote soil organic carbon, soil total nitrogen, soil bulk density, plant density, and plant diameter at breast height, respectively. Different lowercase letters in the same row represent a significant difference ($p < 0.05$, $n = 4$) among the five nitrogen addition rates.

2.2. Soil Respiration Measurements

The soil instantaneous respiration rate (*Ri*) was measured monthly using a LI-8100 Automated Soil CO_2 Flux System (LI-COR Inc., Lincoln, NE, USA) from April 2016 to May 2017. Five polyvinyl chloride (PVC) collars were systematically installed on each plot, with one collar at the middle point and the other four in the direction of the four corners (Figure 1). Living plants inside the PVC collars were clipped at the ground surface one week before each measurement. Concurrently, the soil temperature at a depth of 10 cm close to each soil collar was monitored using a thermocouple probe attached to the LI-8100 system. Soil temperature was measured hourly and automatically logged during the period from 1 June 2016 to 31 May 2017 by a temperature monitor (HOBO U22-001, Onset, MA, USA) at a depth of 10 cm in each forest plot. The average soil respiration rate and soil temperature were calculated from the five collars in each plot.

Basal parameters of soil respiration were estimated using the empirical correlation of the instantaneous rate of soil respiration with soil temperature. The measured soil respiration rate and soil temperature data were fitted to Equation (1) [29]. The annual and seasonal cumulative soil respiration rates were calculated using the basal parameters and the hourly measured soil temperature according to Equation (2) [30]:

$$R_i = R_{10} \times Q_{10}(T_i - 10)/10, \quad (1)$$

$$R_A = \sum R_i \times 3600 \times 12 \times 10^{-6} = \sum R_{10} \times Q_{10}(T_i - 10)/10 \times 3600 \times 12 \times 10^{-6}, \quad (2)$$

where R_i is the instantaneous rate of soil respiration (μmol CO_2 m^{-2} s^{-1}), R_{10} is the basal rate of soil respiration at 10 °C, Q_{10} is the temperature sensitivity of soil respiration, which represents the change in soil respiration rate with every 10 °C increase in temperature, T_i is the hourly measured soil temperature (°C) at a depth of 10 cm, and R_A refers to the annual cumulative soil respiration rate (g C m^{-2} $year^{-1}$). Relevant abbreviations of soil respiration variables were shown in Table 2.

Table 2. List of abbreviations.

R_A	Annual cumulative soil respiration rate	ROC	Soil readily oxidizable organic carbon
R_S	Seasonal cumulative soil respiration rate	HEOC	Hot-water-extractable organic carbon
R_i	The instantaneous soil respiration rate	MBC	Soil microbial biomass carbon
R_{ai}	Annual average instantaneous soil respiration rate	SDOC	Soil dissolved organic carbon
R_0	Soil respiration rate at 0 °C	TN	Soil total nitrogen
R_{10}	Basal soil respiration rate at 10 °C	MBN	Soil microbial biomass nitrogen
Q_{10}	Temperature sensitivity of soil respiration	SDON	Soil dissolved nitrogen
SOC	Soil organic carbon	HEON	Hot-water-extractable nitrogen

2.3. Fine Root, Litter, and Soil Sampling

Fine roots (diameter < 2 mm) were sampled in August 2016 using an auger of 10 cm in diameter and 10 cm in height (each area being 78.54 cm^2), with four replicates randomly taken from each plot [31]. The sampled roots were washed with a 0.5 mm sieve, manually picked up, and then oven-dried at 60 °C to a constant mass and weighed. The fine root biomass average was calculated from four soil cores for each plot. The foliage was regularly collected every three months by litter traps of 1 m^2, with three traps set in each forest plot. The average annual leaf litter input was calculated from the annual cumulative leaf litter input of three samples. The root and fresh leaf litter samples were separately and mechanically ground by a ball milling machine before chemical analysis. Five soil samples were taken using a cylinder soil auger of 4.5 cm in diameter and 10 cm in height from each plot, and each sample was separated into two portions. One subsample was air-dried after the manual removal of any visible litter and debris and then ground to pass through a 0.149 mm sieve screen for chemical analysis. Another subsample was transported in an ice box to the laboratory of Beijing Forestry University to analyze the soil microbial biomass.

2.4. Chemical Analysis

Soil organic carbon content was measured using the wet combustion method via dichromate oxidation and the titration of ferrous ammonium sulfate [32]. Soil, fine root and leaf litter nitrogen contents were analyzed using the Kjeldahl digestion procedure [33]. Readily oxidizable organic carbon (ROC) was determined by oxidation of the soil sample with $KMnO_4$ [34,35]. Soil microbial biomass C (MBC) was measured using the chloroform fumigation extraction technique, where the MBC equals the DOC of fumigated soil minus that of the non-fumigated soil with a conversion factor of 0.38 [36]. Hot-water-extractable organic carbon (HEOC) and dissolved soil organic carbon (SDOC) were separately extracted using boiling water [37,38] and a solution of 0.5 M K_2SO_4 [31], and after filtering through a 0.45 μm cellulose nitrate membrane, the supernatants were tested by a Multi 3100 (TOC/TN) analyzer (Analytik Jena, Germany) to determine the organic carbon and nitrogen concentrations. Fine root nitrogen content, leaf litter nitrogen content, and soil parameters were averaged from the sample replicates of each plot. Relevant abbreviations of soil variables were shown in Table 2.

2.5. Statistical Analysis

The data presented in the figures and tables for each parameter were averaged across four treatment plot replicates for each nitrogen addition rate. Differences in the measured properties were tested at the level of $\alpha = 0.05$ across different treatments using the one-way analysis of variance (ANOVA) with tests for normal distribution and homogeneity of variance. A two-way analysis of variance was implemented to examine the effects of nitrogen addition and seasonal variation on the seasonal cumulative soil respiration rate (R_S). An exponential and linear regression analysis was conducted to measure the relationships between parameters in our study. The statistical analysis was conducted using R version 3.50 (R Core Team, 2018 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria) [39]. The figures were made using SigmaPlot 12.0 (https://sigmaplot.en.softonic.com).

3. Results

3.1. Soil Respiration Rate over Time

Following the addition of inorganic nitrogen, the annual average instantaneous soil respiration rate (R_{ai}) measured in field plots decreased by an average of 16.71%, varying from 2.19 μmol CO_2 m^{-2} s^{-1} in the control (N0) plot to 1.74 μmol CO_2 m^{-2} s^{-1} in the N40 plot (Table 3). The estimated R_A averaged 640.17 g C m^{-2} $year^{-1}$ across all forest plots. The average R_S values across all five treatment levels were 74.04, 369.21, 160.73, and 36.20 g C m^{-2} in spring, summer, autumn, and winter, respectively (Figure 2a). Nitrogen addition significantly reduced the R_A by 23.74 %, on average (Table 3). The N40 plot had the lowest R_A at 542.78 g C m^{-2} $year^{-1}$, 31.32% lower than that of the N0 plot. The Rs in summer accounted for almost 57.41% of the R_A across all plots, on average, ranging from 301.67 g C m^{-2} in the N40 plot to 481.24 g C m^{-2} in the N0 plot (Figure 2a,b). The second-largest R_S, about 25.22% of the R_A, on average, occurred in autumn (Figure 2a,b). Although the magnitude of autumn soil respiration decreased, its average contribution to R_A significantly increased following nitrogen addition, from a minimum value of 23.57% in the N0 plot to a maximum value of 25.90% in the N20 plot (Figure 2b). In addition, nitrogen addition significantly increased the proportion of winter soil respiration from 11.04% in the N0 plot to 12.18% in the N40 plot (Figure 2b). The two-way analysis of variance suggested that seasonal variation and nitrogen addition significantly affected R_S (Figure 2, Table 4). R_{10} was also significantly reduced by 23.95% in the N40 plot (Table 3). The Q_{10} and soil respiration rate at 0 °C (R_0) were not affected by nitrogen addition and were maintained at around 5.18 and 0.38 μmol CO_2 m^{-2} s^{-1}, respectively, across different nitrogen addition rates (Table 3).

Table 3. Soil respiration variables among five nitrogen addition rates.

Treatment	R_A (g C m^{-2} $year^{-1}$)	R_0 (μmol CO_2 m^{-2} s^{-1})	R_{10} (μmol CO_2 m^{-2} s^{-1})	R_{ai} (μmol CO_2 m^{-2} s^{-1})	Q_{10}	T °C
CK	790.25 ± 30.85 a	0.41 ± 0.02 a	2.24 ± 0.08 a	2.19 ± 0.09 a	5.41 ± 0.12 a	7.41 ± 1.59a
N5	644.64 ± 28.29 ab	0.39 ± 0.01 a	2.03 ± 0.09 ab	1.99 ± 0.07 ab	5.26 ± 0.12 a	7.41 ± 1.63a
N10	628.45 ± 44.48 b	0.34 ± 0.03 a	1.86 ± 0.14 ab	1.78 ± 0.07 ab	5.55 ± 0.14 a	7.56 ± 1.66a
N20	594.73 ± 39.45 b	0.39 ± 0.02 a	1.89 ± 0.11 ab	1.80 ± 0.11 ab	4.84 ± 0.33 a	7.49 ± 1.63a
N40	542.78 ± 38.80 b	0.36 ± 0.03 a	1.71 ± 0.07 b	1.74 ± 0.13 b	4.81 ± 0.29 a	7.48 ± 1.66a

R_A, R_0, R_{10}, R_{ai}, Q_{10}, and T denote the annual cumulative soil respiration rate, the soil respiration rate at 0 °C, the basal soil respiration rate at 10 °C, the annual average instantaneous soil respiration rate, the temperature sensitivity of soil respiration, and the annual average soil temperature, respectively. Different lowercase letters in the same column represent significant differences ($p < 0.05$, $n = 4$) among the five nitrogen addition rates. Data are mean ± SE.

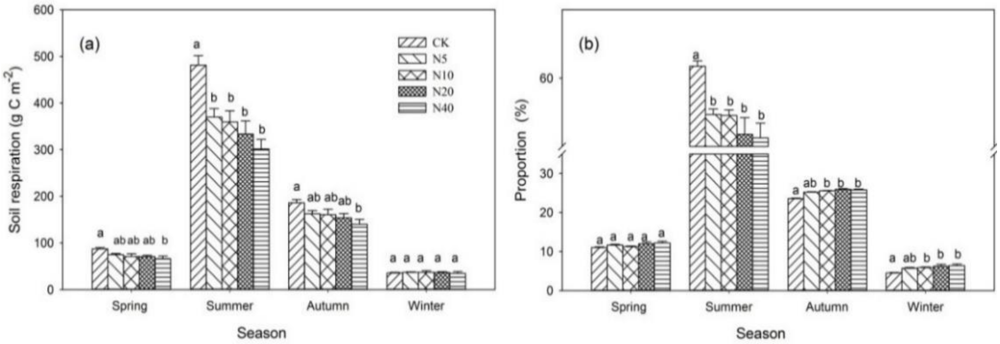

Figure 2. Seasonal cumulative soil respiration (R_S) (**a**) and variation in the seasonal proportion of R_S (**b**) under different nitrogen rates. Different lowercase letters in the same column indicate a significant difference ($p < 0.05$, $n = 4$) among the five nitrogen addition rates.

Table 4. Significance of the effects of nitrogen addition and seasonal variation on seasonal cumulative soil respiration (R_S) as assessed by two-way ANOVA.

Variables	Df	Sum Sq	Mean Sq	F	P
T	4	32743	8186	11.596	4.86×10^{-7}
S	3	1342413	447471	633.862	$<2 \times 10^{-16}$
T × S	12	44167	3681	5.214	6.53×10^{-6}

T and S denote the nitrogen addition rates and seasonal variation, respectively.

3.2. *The Effect of Inorganic Nitrogen Addition on Soil Physicochemical Properties*

Following nitrogen addition, the MBC was reduced by an average of 23.50%, with the N40 plot having the lowest value at 247.90 mg kg^{-1} (Table 5). Similarly, the SDOC was reduced by an average of 22.60%, with the N20 plot having the lowest value at 120.43 mg kg^{-1} (Table 5). Nitrogen addition significantly reduced the values of SOC/TN and SDOC/SDON by 8.80 % and 29.34%, on average, respectively (Table 5). Although no significant differences were found at the statistical level of 0.05, SOC and MBN showed slight decreasing trends with increased nitrogen addition rates (Table 5). The ROC, HEOC, TN, hot-water-extractable nitrogen (HEON), soil dissolved nitrogen (SDON), and HEOC/HEON were not influenced by inorganic nitrogen addition (Table 5).

Table 5. Variations in soil variables at different nitrogen additions rates.

Soil Variable	CK	N5	N10	N20	N40
SOC (g kg^{-1})	19.18 ± 3.11 a	18.20 ± 2.10 a	18.76 ± 2.27 a	16.70 ± 1.04 a	16.65 ± 0.58 a
ROC(g kg^{-1})	4.94 ± 0.94 a	4.19 ± 0.50 a	5.10 ± 0.42 a	4.29 ± 0.30 a	4.20 ± 0.35 a
MBC(g kg^{-1}) *	342.39 ± 31.16 a	251.58 ± 10.64 b	275.23 ± 12.46 ab	273.07 ± 16.98 ab	247.90 ± 19.07 b
SDOC(mg kg^{-1}) *	159.43 ± 6.45 a	126.43 ± 8.57 ab	122.54 ± 9.49 b	120.43 ± 8.70 b	124.19 ± 6.26 b
HEOC(mg kg^{-1})	741.95 ± 64.17 a	765.79 ± 25.86 a	816.39 ± 95.62 a	761.86 ± 49.36 a	693.87 ± 46.89 a
TN (g kg^{-1})	1.35 ± 0.20 a	1.36 ± 0.13 a	1.44 ± 0.14 a	1.27 ± 0.08 a	1.37 ± 0.03 a
MBN(mg kg^{-1})	57.88 ± 8.50 a	45.52 ± 4.82 a	45.48 ± 1.76 a	50.03 ± 4.16 a	45.02 ± 2.05 a
SDON(mg kg^{-1})	26.44 ± 0.64 a	29.94 ± 0.78 a	29.97 ± 1.77 a	28.51 ± 1.41 a	27.95 ± 0.60 a
HEON (mg kg^{-1})	67.46 ± 6.20 a	65.59 ± 2.56 a	69.35 ± 3.37 a	66.32 ± 2.98 a	66.59 ± 2.45 a
pH	6.55 ± 0.23 a	6.60 ± 0.21 a	6.51 ± 0.18 a	6.48 ± 0.28 a	6.53 ± 0.25 a
SOC/TN *	14.11 ± 0.32 a	13.30 ± 0.41 ab	12.90 ± 0.30 ab	13.13 ± 0.27 ab	12.16 ± 0.30 b
SDOC/SDON **	6.03 ± 0.18 a	4.23 ± 0.31 b	4.12 ± 0.35 b	4.23 ± 0.29 b	4.46 ± 0.31 b
HEOC/HEON	11.02 ± 0.08 a	11.68 ± 0.13 a	11.65 ± 0.91 a	11.46 ± 0.33 a	10.39 ± 0.33 a

SOC, ROC, MBC, SDOC, HEOC, TN, MBN, SDON, and HEON denote soil organic carbon, soil readily oxidizable organic carbon, soil microbial biomass carbon, soil dissolved organic carbon, soil hot-water-extractable organic carbon, soil total nitrogen content, soil microbial biomass nitrogen, soil dissolved nitrogen, and soil hot-water-extractable nitrogen, respectively. Different lowercase letters in the same row represent a significant difference ($p < 0.05$, $n = 4$) among the five nitrogen addition rates. * represents $p < 0.05$ and ** represents $p < 0.01$. Data are mean ± SE.

3.3. The Effect of Inorganic Nitrogen Addition on Litter Production and Its Nitrogen Content

Litter production and nitrogen content were affected differently by inorganic nitrogen addition (Figure 3). The amount of fine root biomass was significantly reduced by almost half (Figure 3a), but its nitrogen content was not significantly affected (Figure 3b). The nitrogen content of the fine root averaged 8.08 g kg^{-1} for nitrogen-addition plots, compared with 6.65 g kg^{-1} in the N0 plot. Nitrogen addition also had no effect on foliage production (Figure 3c), but it significantly increased the nitrogen content of fresh leaf litter (Figure 3d) by an average of 15.73%, with the largest concentration being 15.71 g kg^{-1} in the N20 plot (Figure 3d). Foliage production slightly declined (by 8.22%) from the N0 value of 634.44 g m^{-2} in the plots with nitrogen added (Figure 3c).

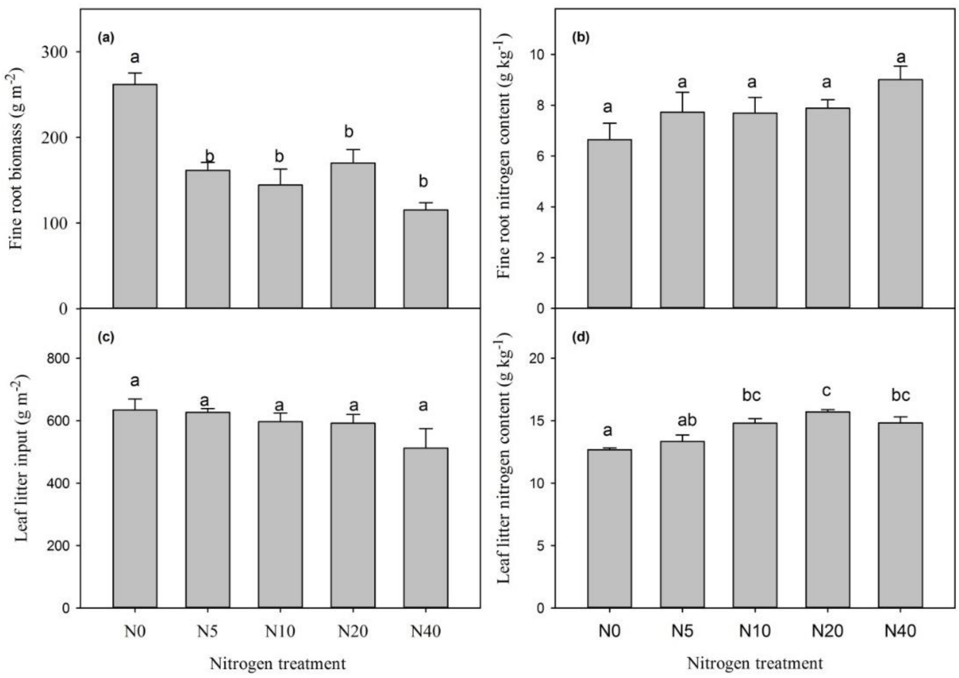

Figure 3. Variations in fine root biomass (**a**), fine root nitrogen content (**b**), leaf litter input (**c**), and leaf litter nitrogen content (**d**). Different lowercase letters above bars represent a significant difference ($p < 0.05$, $n = 4$) among the five nitrogen addition rates.

3.4. The Correlation of R_A with the SOC Fraction and Litter Input

Statistical results showed that R_A was positively correlated with the SOC fraction and litter input (Figure 4), and 62%, 41%, 26% and 32% of its variation was explained by the fine root biomass (Figure 4a), leaf litter input (Figure 4b), MBC content (Figure 4c), and SDOC content (Figure 4d), respectively, across all five inorganic nitrogen addition rates. There was a positive and exponential correlation between foliage input and R_A (Figure 4b).

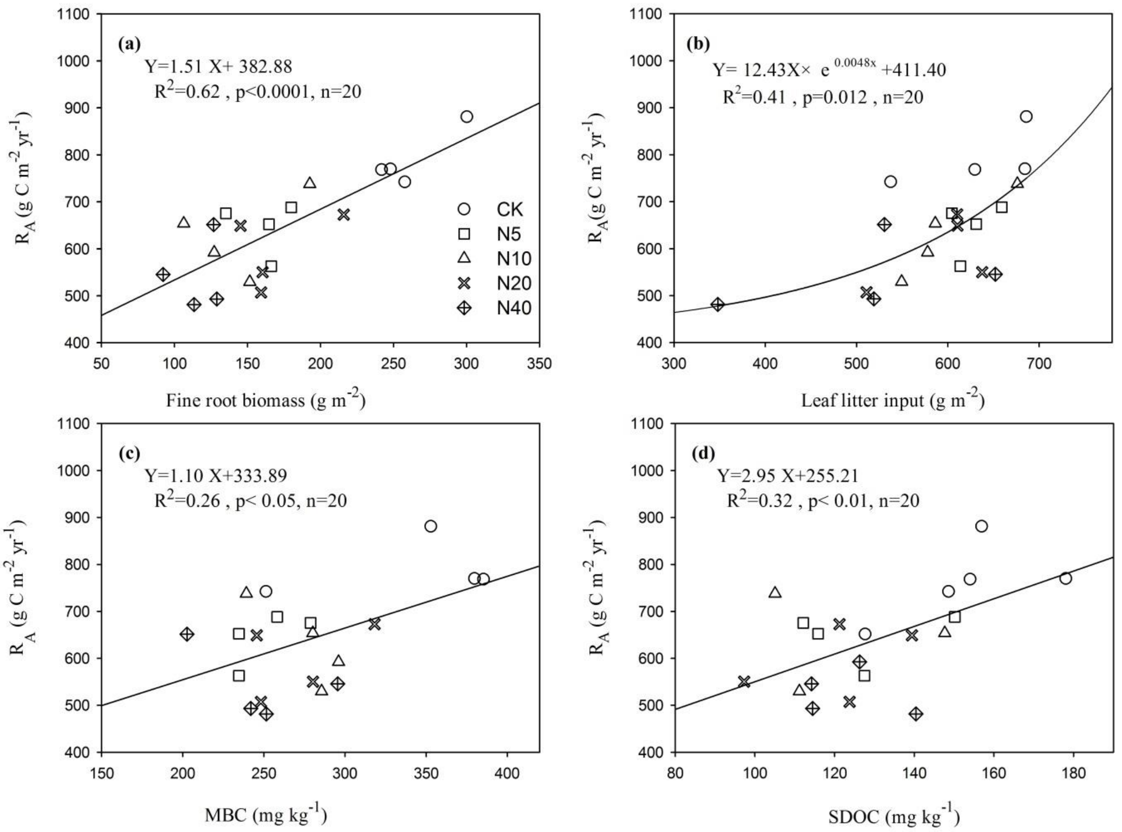

Figure 4. Relationship between annual cumulative soil respiration rate (R_A) and fine root biomass (**a**), leaf litter input (**b**), MBC content (**c**), and SDOC content (**d**).

3.5. Variation of Soil Organic Carbon by Litter Input

The belowground litter input played an important role in controlling the SOC fraction. Correlation analysis showed that fine root biomass was exponentially and positively correlated with MBC and SDOC contents (Figure 5a,b), respectively. Fine root biomass explained 36% and 33% of the variation in MBC and SDOC contents, respectively.

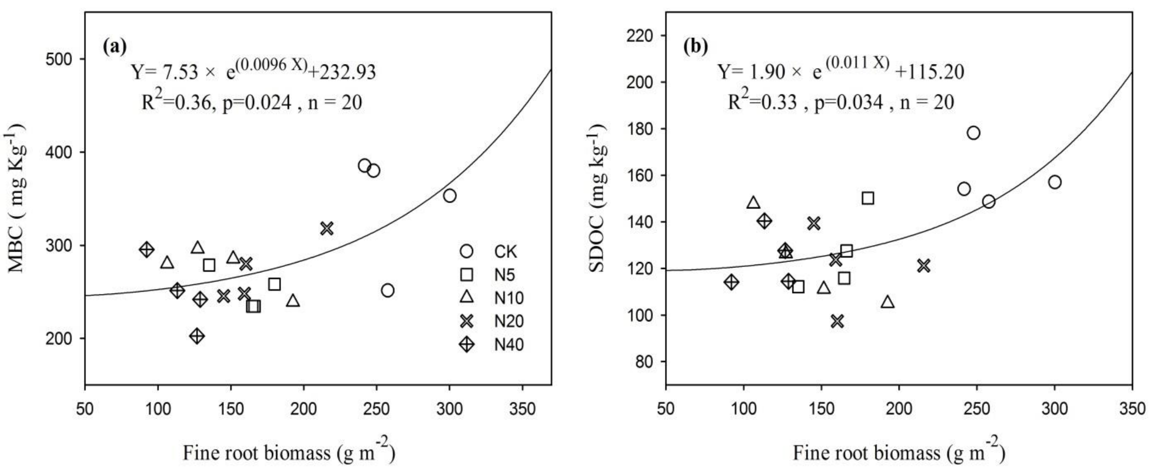

Figure 5. Relationship between fine root biomass and MBC (**a**) and SDOC (**b**) content.

4. Discussion

4.1. The Effect of Nitrogen Addition on Soil Respiration Rate at Temporal Scales

The soil temperature and instantaneous rate of soil respiration were used as the two fundamental parameters for estimating cumulative soil respiration. Because all 20 experimental plots were distributed in the same forest stand, soil temperature did not display a marked difference between different treatment plots, regardless of the amount of inorganic nitrogen received. In contrast, R_{ai} showed an obvious decreasing trend with increased nitrogen addition (Table 3). Inorganic nitrogen addition significantly changed R_A (Table 3); summer R_S was reduced by 29.10%, whereas spring and autumn R_S only declined by 18.89% and 17.11%, on average, across all four nitrogen-addition plots (Figure 2a). The marked decrease in soil respiration in the main growing season is consistent with many previous studies [6,40,41] but differs from a previous experiment showing that soil respiration was affected by organic nitrogen (urea) in the same region [42]. The different results may derive from the contrasting effects of inorganic and organic nitrogen; organic nitrogen addition could promote the transformation of recalcitrant organic compounds into easily digestible forms, improve the microbial community metabolism, and strengthen the activity of relevant extracellular enzymes [43]. In contrast to the effect during the growing season, the four nitrogen levels had little effect in winter, as previously reported by Fang et al. (2017) [44]. In contrast to the negative effect of nitrogen on the magnitude of R_S, nitrogen addition significantly increased the proportions of autumn and winter R_S in R_A (Figure 2b). This is because soil respiration is inhibited by nitrogen addition to a greater extent in the warm season than in the cold season [45,46]. The independent and interactive effects of seasonal variation and nitrogen addition on R_S (Figure 2a, Table 4) and the variable inhibition magnitudes of different seasons (Figure 2a,b) illustrate that a warming climate could enhance the inhibition of inorganic nitrogen. This result is consistent with studies by Liu et al. (2017) and Tao et al. (2013), who proposed that nitrogen deposition could mitigate increased soil respiration due to warming temperatures [45,47].

In order to avoid uncertainty in the comparability of the field-measured rate of soil respiration, R_{10} was also calculated [48,49], and a similar changing trend to R_{ai} was shown (Table 3) across all the different treatments. R_{10} varied from 2.24 μmol CO_2 m^{-2} s^{-1} in the N0 plot to 1.71μmol CO_2 m^{-2} s^{-1} in the N40 plot. This significant decrease in R_{10} at the same ambient temperature is a better indication that inorganic nitrogen addition inhibited soil respiration. No statistical difference in the Q_{10} values of different treatments were discovered (Table 3). This result is consistent with some short-term studies in a *Pleioblastus amarus* Keng plantation [50], a semi-arid alfalfa-pasture of the Loess Plateau [44], and a young subtropical plantation [45]. The Q_{10} values in our study are similar to those reported by a previous study, which calculated an average value of 5.2 ± 2.4 in boreal and tundra forests at high latitudes [51]. The variation in soil respiration and seasonal respiration proportions supports the hypothesis that soil respiration would be affected by inorganic nitrogen addition.

4.2. The Effect of Nitrogen Addition on the Soil Carbon Fraction and Plant Litter Input

Soil organic carbon, which was measured via the wet combustion method, represents the total content of all organic carbon forms contained in mineral soil particulates, and it is resistant to slight disturbance. Consistent with Chen et al. (2012) [35] and Liu et al. (2017) [45], SOC and TN were not influenced by inorganic nitrogen addition in the current study (Table 5), possibly because short-term nitrogen treatment was not sufficient to induce a significant variation in SOC and TN content [50]. However, a compartment carbon, soil labile organic carbon, was predicted to be sensitive to changing environmental variables and was applied to interpret the inherent transformation dynamics of SOC [34,35,52]. Soil labile organic carbon refers to organic matter that is easily available to the soil microbial communities, including litter detritus and root exudates, SDOC, ROC, HEOC, and soil microbial lysates [15,38,53]. Soil MBC and SDOC content both decreased following the inorganic nitrogen input (Table 5). Soil microbial communities, as a part of labile organic carbon, play a vital role in SOC decomposition processes. In this study, on average, inorganic nitrogen addition decreased MBC content by 23.50%, which is higher than the average N-induced suppression of MBC content of 20.00% [5] and 15.00% [22] found by related meta-analyses. The average SDOC content reduction of 22.60% in the present study is similar to that found by Du et al. (2014) [43] and Frey et al. (2014) [20]. In addition, the rate of organic carbon with nitrogen in total soil organic matter (SOC/TN) and soil dissolved organic matter (SDOC/SDON) were significantly decreased (Table 5) in inorganic nitrogen addition plots. This may be ascribed to the nitrogen form (NH_4NO_3) added to forest plots. Ammonium nitrate is easily dissolvable, is absorbed quickly by organic matter with a low molecular weight, and stimulates the formation of recalcitrant compounds [6]. Meanwhile, inorganic nitrogen is chemically immobilized by mineral soil particles [54]. Previous studies indicate that the interaction of deposited nitrogen with soil particles will produce toxic aluminum and will limit magnesium and calcium availability, thereby depressing soil microbial community activity [22,55,56]. The reduction of labile organic carbon bioavailability and environmental stress by inorganic nitrogen addition are two important factors that limit soil decomposers' activities [57].

Leaf and root detritus and exudates are major sources of soil organic carbon, and their production and nutrient content could be influenced by nitrogen input [19,24,58,59]. The fine root biomass was on average reduced by 114.07 g m^{-2} by nitrogen addition and as 43.55 % lower in all nitrogen addition plots compared to the N0 plot. (Figure 3a). This is because the fine root system is mainly responsible for the absorption of nutrients from the rhizospheric environment [24,60], and nitrogen is the limiting factor for forest growth in northern China [54,61,62]. The input of inorganic nitrogen could alleviate the inhibition of plant growth due to soil nitrogen scarcity [6,24]. The ammonium cation in soil solution could be quickly transported by the root system to meet the requirements of the aboveground plant metabolism [63–66]. Although no obvious difference was measured in the aboveground litter input (Figure 3c), the nitrogen content of fresh leaf litter was found to increase with enhanced nitrogen addition rates, and the increase was 23.90% greater in the N20 plot than in the N0 plot (Figure 3d). The stable nitrogen concentration in soil total organic matter and soil labile organic matter (Table 5) and the significant increase in leaf litter nitrogen content suggest that a plant assimilates the deposited nitrogen as an important mechanism to maintain a stable forest soil nitrogen content under intensified atmospheric nitrogen deposition [63,66,67]. Increased nitrogen availability alleviates plants' carbon allocation to their root system and reduces root biomass, root litter, and root exudate production [60,68]. Additionally, nitrogen enrichment could inhibit lignin-rich leaf litter decomposition [69]. These factors will reduce the carbon input from aboveground organic matter transformation and belowground plant allocation.

4.3. The Correlation of R_A with SOC Fraction and Plant Litter Input

Soil respiration is derived from a complicated process of above- and belowground organic matter turnover [10,49]. Soil organic matter decomposition and carbon released from plant litter input accounted for almost equal proportions of soil respiration [11,58,70]. The correlation analysis indicated that R_A was positively correlated with fine root biomass, leaf litter input, MBC content, and SDOC content (Figure 4). These relationships suggest that inorganic nitrogen addition may influence soil heterotrophic respiration by reducing MBC and soil labile organic carbon fractions [20,21,71] and affect soil autotrophic respiration by suppressing soil fine root production, mycorrhizae, and other types of microorganisms metabolism associated with root systems and belowground plant carbon allocation [60,72]. This result is consistent with the hypothesis that inorganic nitrogen addition affects soil respiration by influencing fine root production and the labile organic carbon fraction.

SOC storage and soil nutrient content play important roles in affecting the magnitude of soil respiration [71,73]. However, the transient change in soil respiration is most strongly invoked by the soil organic carbon fraction, which is easily affected by biophysical variables [21,53,74]. In the present research, R_A was significantly correlated with MBC and SDOC content (Figure 4c,d). These two organic carbon fractions consisted of low molecular weight organic matter, including microbial lysates, cellulose, glucose, and amino acids [13,75], and the added inorganic nitrogen proved to be quickly assimilated by these biochemical products, enhancing the ability to resist biological decomposition [6,22]. In addition, the MBC content represents the decomposition intensity of organic carbon by soil decomposers. Organic matter with a low molecular weight can undergo complete turnover during a period of only several hours [21,76]. Thus, we predicted that the labile organic carbon fractions would account for the effect of inorganic nitrogen addition on soil respiration.

Above- and belowground litter input not only influenced SOC content but also controlled soil respiration [10,17,58]. The role of the fine root system in absorbing nutrients could be weakened in a soil entity with sample nutrient provision, which subsequently induces less fine root production [24,60,68]. Because autotrophic respiration comprised almost 50% of total soil CO_2 emissions, the reduction in fine root biomass was another contributor to R_A (Figure 4a). Although differences in foliage input did not reach a significant level ($p > 0.05$), 41% of the variation in R_A was still explained by leaf litter input (Figure 4b). Generally, it was the above- and belowground litter input that jointly affected R_A after inorganic nitrogen addition.

Fine root production indirectly affected soil respiration by influencing the transformation of the soil organic carbon fraction [19,60] and priming soil organic mineralization [18]. The decomposition of root detritus by exocellular enzymes and root exudates produces a large amount of low molecular weight organic matter [13,77,78]. Complex organic compounds are degraded into simple ones, which are the components of the labile organic matter. Meanwhile, the root-derived soil labile organic fraction is the major food resource driving soil microorganisms catabolism [18,78]. Both SDOC and MBC content displayed significant correlation with fine root biomass (Figure 5a,b). The inhibition of fine root biomass production by inorganic nitrogen addition perhaps lowered the labile organic carbon content and soil microbial biomass metabolism concurrently [79].

5. Conclusions

The addition of nitrogen in the form of NH_4NO_3 affected the turnover of soil organic matter, decreasing both the R_A and the fine root biomass. A similar effect of nitrogen addition was found for soil MBC and SDOC content. However, nitrogen addition markedly enhanced the proportion of cold season soil respiration in R_A. The fine root biomass had a significant correlation with R_A, MBC content, and SDOC content, and is presumed to play a core role in untangling the interaction of nitrogen deposition and soil organic matter turnover. Therefore, the effect of inorganic nitrogen addition on the forest plant root system and the biogeochemical interaction of the root system with the soil organic carbon fraction and soil decomposers should be investigated further to determine the variation in forest soil organic carbon following intensified anthropogenic nitrogen deposition.

Author Contributions: H.Z. performed the field and laboratory experiments and analyzed the data; Y.L. and Z.Z. designed the study; Z.Z. and H.Z. wrote the manuscript; Y.Z. took part in the field experiment and provided the relevant material.

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