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Short-Term Effects of N Deposition on Soil Respiration in Pine and Oak Monocultures

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Abstract

Atmospheric nitrogen input has been a severe challenge worldwide. The influences of N deposition on carbon cycling, loss, and storage have been recognized as a critical issue. This study aimed to assess the immediate responses of soil respiration to different N deposition treatments in radiata pine (*Pinus radiata* D. Don) and chestnut-leaved oak (*Quercus castaneifolia* C. A. Mey) plantations within 12 months. N treatments were performed monthly at levels of 0, 50, 100, and 150 kg N ha^{-1} $year^{-1}$ from October 2017 to September 2018. Litterfall was collected and analyzed seasonally for its mass and C content. Within the 0–10 cm depth of mineral soil in both plantations, parameters such as total nitrogen, pH, microbial biomass carbon (MBC), organic carbon (OC), and fine root biomass were measured seasonally. Soil respiration (Rs) was determined through monthly measurements of CO_2 concentration in the field using a portable, closed chamber technique. The control plots exhibited the highest Rs during spring (2.96, 2.85 μmol CO_2 m^{-2} s^{-1}) and summer (2.92, 3.1 μmol CO_2 m^{-2} s^{-1}) seasons in oak and pine plantations, respectively. However, the introduction of nitrogen significantly diminished Rs in both plantations. Moreover, N treatments caused a notable reduction of soil MBC and fine root biomass. Soil microbial entropy and the C/N ratio were also significantly decreased by nitrogen treatments in both plantations, with the most prominent effects observed in summer. The observed decline in Rs in N-treated plots can be attributed to the decrease in MBC and fine root biomass, potentially with distinct contributions of these components in the pine and oak plantations. Our findings suggested that N-induced alteration in soil carbon dynamics was more pronounced in the oak plantation, which resulted in more SOC accumulation with increasing N inputs, while the pine plantation showed no significant changes in SOC.

Keywords: extra nitrogen input; microbial biomass carbon; fine root biomass; soil CO_2 efflux; soil organic carbon; Hyrcanian forests

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1. Introduction

Atmospheric nitrogen (N) inputs have increased due to the widespread use of fuel combustion and the application of fertilizers, along with intensive animal husbandry [1]. Elevated N input has prominent effects on biogeochemical processes in forest soils. To our knowledge, soil respiration [2–4], soil chemistry [5–7], and soil microbial and enzyme activities [8] are important processes in the C cycle, which have been affected by N deposition.

The influences of N deposition on carbon cycling and storage have been recognized as a critical issue, leading to numerous experimental studies in recent decades aimed at evaluating its effects on C cycles in various ecosystems [9–15]. Given that the soil C pool substantially outweighs plant C storage in terrestrial ecosystems [16], its role in the global C cycle is pivotal. Storing soil C comprises the interactions of inputs via litterfall, throughfall, and losses through soil respiration [17], and leaching of dissolved organic C [18], and all these processes might be affected by the availability of nitrogen (for plants and soil microbes).

Since soil respiration (Rs) constitutes a major contributor to atmospheric carbon dioxide levels [19], even slight changes in Rs rates can lead to a significant variation in atmospheric CO_2 concentration [16,20,21]. According to Fang et al. [22], in the past decades, the atmospheric CO_2 concentration has increased by approximately 35% (from 285 to 400 ppm) and is predicted to reach 700–800 ppm at the end of this century [23]. With the rise in anthropogenic N deposition rates, there is an urgent need to understand the relationship between Rs and N inputs [24–26]. Notably, increased N deposition may decrease soil respiration in the naturally N-limited temperate forest ecosystems [3]. Documented studies revealed diverse effects of N additions on soil CO_2 emission, including decline [27,28], rise [29], or no significant change [30–32]. Various factors can affect soil respiration rates in temperate forests, including moisture [33] and temperature [34], both affecting fine root biomass and activity as the source of autotrophic respiration [29,35], and soil microbial activity [36,37] as well. In previous studies, nitrogen addition experiments have demonstrated how variation in N availability affects soil carbon efflux and plant root growth [38]. Furthermore, it is illustrated that elevated N concentration may increase both the quantity and quality of litterfall [39–41]. Additionally, soil acidification resulting from N addition has the potential to alter microbial biomass and diversity [13,42,43] and influence soil enzyme activities [44–46]. As Yuan et al. [14] claimed, the combined effects of biological and microclimatic factors may control Rs under N deposition. Understanding these multifaceted impacts is crucial for unraveling the complexities of nitrogen-induced changes in soil respiration and its broader implications for carbon cycling in temperate forest ecosystems.

Hyrcanian forests are one of the last remnants of natural temperate deciduous forests in the world [47], which were recently inscribed on the UNESCO World Heritage List [48]. Despite this international recognition, the conversion of forest ecosystems to arable land, intensive livestock husbandry, and industrial pollution has led to excessive deposition of reactive N in this unique eco-region [49], potentially disrupting the carbon cycle. The present study was conducted to assess the short-term impact of experimental N additions on the soil CO_2 efflux and its main drivers in pine and oak plantations. Since the provision of surplus available nitrogen via N deposition diminishes soil microbial activity and fine root biomass, we hypothesized that the higher levels of N input would result in lower rates of soil respiration. Specifically, the present work seeks to elucidate the short-term impact of experimental N addition on soil CO_2 emission and to discern the predominant below-ground CO_2 sources in these two distinct plantations.

2. Materials and Methods

This study was carried out in two 34-year-old neighboring monocultures of radiated pine (*Pinus radiata* D. Don) and chestnut-leaved oak (*Quercus castaneifolia* C. A. Mey), situated near Sari city, Mazandaran Province, north of Iran (latitude: 36°30′ N, longitude: 53°2′ E, and elevation 400 m). Both plantations were located in a flat area. The annual average precipitation and temperature are 950 mm and 17 °C, respectively. According to the USDA Soil Taxonomy, the soil type is classified as Alfisols. The average height and diameter of the oak trees were 20 m and 30 cm, and the pine trees were 35 m and 25 cm. The original natural forest was dominated by various native hardwood trees such as ironwood (*Parrotia persica* C. A. M.), chestnut-leaved oak (*Q. castaneifolia* C. A. M.), and hornbeam (*Carpinus betulus* L.), which had been degraded. In 1986, both stands were clear-cut and then reforested with pine and oak trees (plant spacing 3 m × 3 m). Herbaceous species dominated, including perforate woodruff (*Asperula odorata* L.), St. John's wort (*Hypericum perforatum* L.), sweet and ann ala (*Hedera pastuchovii* Woronow), and butcher's broom (*Ruscus hyrcanus* Woronow), which covered less than 10% of both plantations. Chestnut-leaved oak is a high-quality native hardwood tree in the Hyrcanian forests, but pine is an exotic, coniferous species that was introduced to the Hyrcanian region specifically for reforestation purposes [50].

2.1. Experimental Design

An N-addition experiment was set up according to the design explained by Mo et al. [51] and Tian et al. [52]. In October 2017, twelve 200-m^2 (20 m × 10 m) experimental plots were selected as a randomized block design in each plantation. A buffer distance of 10 m between plots was implemented, as suggested by Mo et al. [51] and Ma et al. [53]. Salehi et al. [54] reported that Hyrcanian forests (altitudes < 300 m) receive about 56 kg N ha^{-1} $year^{-1}$ through wet N deposition. Considering these values, four levels of ammonium nitrate (NH_4NO_3) were considered as control treatment (C, zero), low (LN, 50 kg N ha^{-1} $year^{-1}$), medium (MN, 100 kg N ha^{-1} $year^{-1}$), and high (HN, 150 kg N ha^{-1} $year^{-1}$). Each treatment was applied in three replicates. To achieve the desired nitrogen application rates, an aqueous stock solution of NH_4NO_3 was prepared and mixed with 20 L of tap water. This solution was then sprayed monthly under the canopy for 12 months (from October 2017 to September 2018). Control plots received the equivalent of 20 L of tap water without NH_4NO_3 application (see Figure 1 for a visual representation of the experimental setup). Given the lack of reliable dry deposition data for the Hyrcanian region, we based our N application rates on reported wet deposition values, acknowledging that total atmospheric N input may be somewhat higher.

2.2. Soil Sampling

Within each plot, three samples of topsoil (0–10 cm) were collected seasonally using the coring method (10 cm in height; 8 cm inner diameter). The organic layer was removed from the sampling points before the samples were taken. The soil samples were stored at 4 °C for analysis using conventional methods. Soil pH was determined in a 1:2.5 soil-to-water suspension; the Walkley and Black technique was performed to estimate soil organic C (SOC) [55], and the combustion method was applied to measure litter carbon. Total N was determined using the semi-micro Kjeldahl technique by Olsen et al. [56]. The fumigation-extraction technique was employed to estimate soil microbial biomass carbon or MBC [57,58], and microbial entropy or ratio was calculated based on the MBC/SOC [59]. The soil properties prior to the application of nitrogen treatments are presented in Table 1.

Table 1. Physical and chemical properties of the soil before applying N treatments in pine and oak plantations.

Plantation	Soil Properties	
Pinus radiata D. Don	Moisture (%)	18.58
	Bulk density (g cm^{-3})	1.6
	Lime (%)	2.82
	pH (1:2.5 H_2O)	6.6
	EC (dS/m^2)	0.4
	C (%)	5.9

Table 1. *Cont.*

Plantation	Soil Properties	
Quercus castanifolia	Moisture (%)	29.5
	Bulk density (g cm^{-3})	1.35
	Lime (%)	2.85
	pH (1:2.5 H_2O)	6.1
	EC (dS/m^2)	0.49
	C (%)	4.8

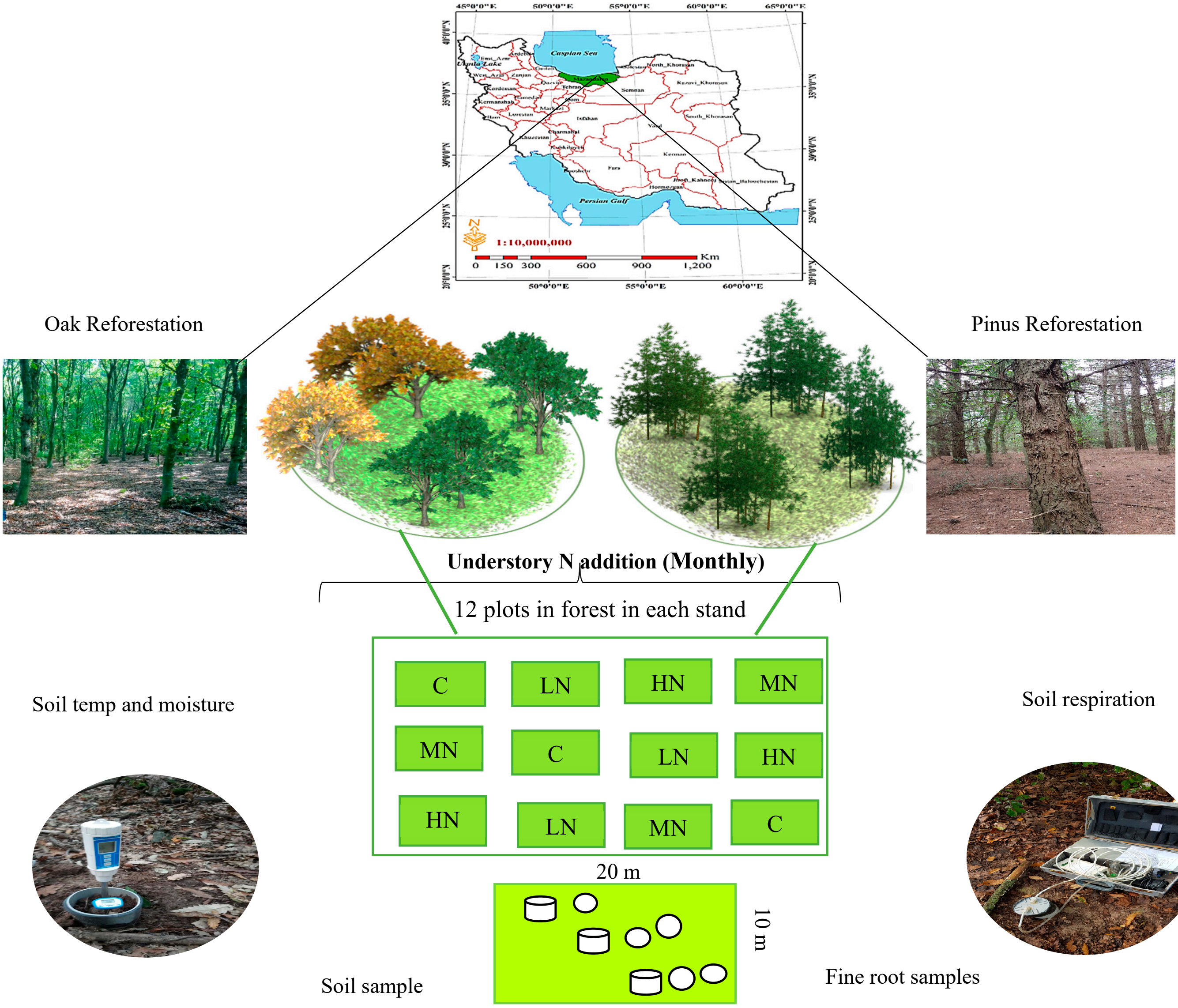

Figure 1. Location of study area in Mazandaran Province, north of Iran, and sampling method in oak and pine plantations in different N treatments.

2.3. Measuring Soil Respiration (Rs)

In September 2017, three PVC cylinders (15 cm inner diameter and 25 cm in height) were installed 10 cm into the soil (far enough from the edge of the plots). By using a portable, closed chamber technique, an infrared absorption sensor with a CO_2PORT device (Messwert Company GmbH, Göttingen, Germany; a developed version of the infrared gas analyzer, Edinburgh Sensors-Gascard II), the concentration of emitted CO_2 was measured as the soil respiration (Rs) monthly from October 2017 to September 2018 [4,60]. All Rs measurements were conducted from 10 am to 3 pm. Simultaneously, soil temperature was recorded at 10 cm depth with a temperature probe in each Rs measurement point.

2.4. Litterfall Collection

Three litter traps (0.5 m × 0.5 m) were installed 0.5 m above the ground in each of the 12 plots for monthly litterfall sampling [61]. The samples were transported to the laboratory and dried at 70 °C for 48 h. Monthly litterfall samples were pooled to create composite seasonal samples (three months), ground with a mill, and passed through a sieve of 0.5 mm. The combustion method was applied to measure the concentration of organic carbon (OC) of each litter sample.

2.5. Fine Root Biomass Measurement

To sample the fine roots, three soil cores (10 cm in length, 8 cm inner diameter) were taken from each plot seasonally (November 2017, February 2018, May 2018, and August 2018). Soil samples were immediately sealed in plastic bags. In the laboratory, fine roots (<2 mm) were removed from soil samples and cleaned with distilled water. The samples were dried to a constant mass and then weighed [62].

2.6. Statistical Analysis

The normality of the data was checked using the Shapiro–Wilk test, and Levene's test was employed for testing the homogeneity of the variances. Repeated measures analysis of variance (ANOVA) was used to estimate the effects of sampling time (seasons) and N treatments on the soil characteristics (Rs, MBC, OC, litterfall mass, and C, fine root biomass). A significance level of $p < 0.01$ was applied throughout. Multiple comparisons were conducted using Bonferroni correction following repeated measures ANOVA. The effect of seasons on the soil characteristics (Rs, MBC, OC, litterfall mass, C, fine root biomass, C/N, and microbial entropy) was analyzed by repeated measurements with Bonferroni correction (pairwise comparisons) in each plantation. A one-way ANOVA followed by an LSD test was performed to compare changes under N treatments on the soil characteristics (Rs, MBC, OC, litterfall mass, and C, fine root biomass). Regression analyses were applied to assess the relationship between Rs and variables potentially influencing soil respiration. All statistical analyses were conducted in the SPSS 19.0 statistical software package.

3. Results

3.1. Microbial Biomass Carbon (MBC)

MBC exhibited a peak during the summer season and reached its minimum levels in both autumn and winter. N treatments significantly decreased the MBC in the soil, also in the order of the applied amounts (C > LN > MN > HN). The most substantial negative effect of the N treatments was observed in summer, which yielded a significant interaction between treatment and season. The pine plantation showed a significantly ($p < 0.01$) higher MBC than the oak plantation and, at the same time, a stronger decrease with the N treatments, making a significant interaction between N treatments and species (Figure 2; Table 2).

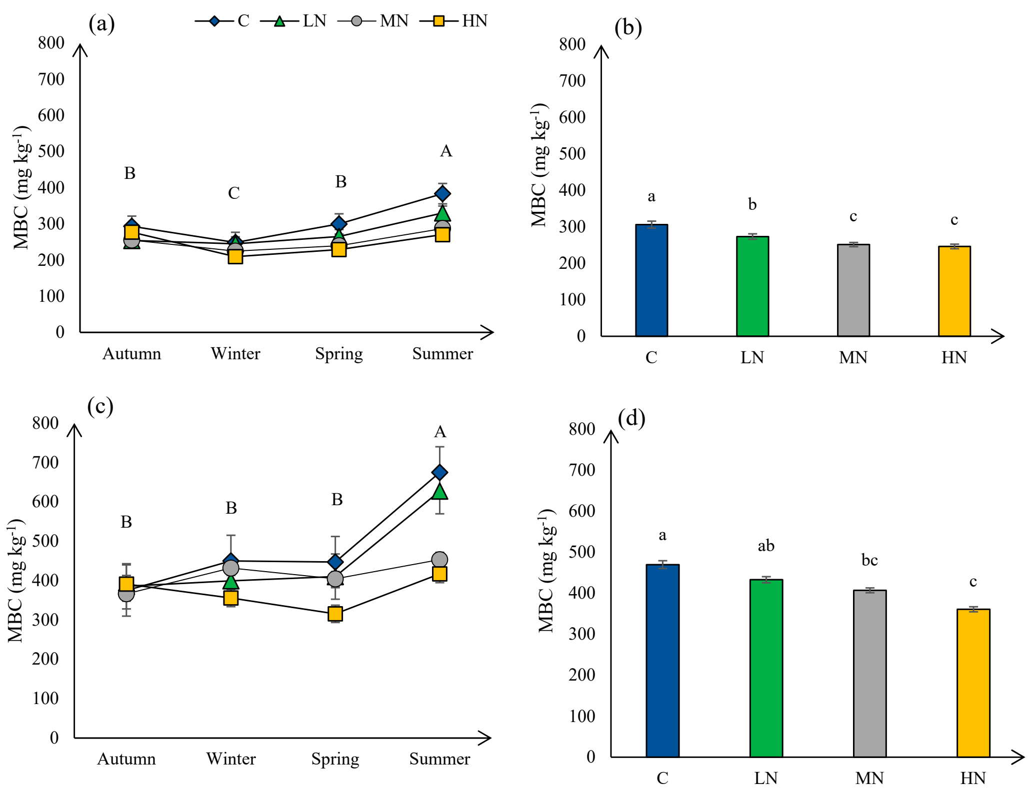

Figure 2. MBC (Mean ± SD) in different seasons and N treatment in oak (**a**,**b**) and pine stands (**c**,**d**); Capital letters represent significant differences between season; Lowercase letters represent significant differences between N treatments (C: control; LN: low nitrogen; MN: medium nitrogen; HN: high nitrogen).

Table 2. Results of repeated measures ANOVAs of the effects of different seasons and N treatments and their interactions in the soil Rs, MBC, soil OC, litter fall OC, litterfall production, and fine root biomass.

Nutrient Concentrations in Soil	Species	Different Seasons		Nitrogen Treatments		Different Seasons × Nitrogen Treatments	
		F	p	F	p	F	p
Rs (mol CO_2 s^{-1} m^2)	oak	95.658	<0.01	50.537	<0.01	5.336	<0.01
	pine	121.007	<0.01	61.115	<0.01	11.359	<0.01
MBC (mg kg^{-1})	oak	64.796	<0.01	23.536	<0.01	5.340	<0.01
	pine	38.509	<0.01	16.586	<0.01	6.092	<0.01
Soil OC (%)	oak	30.039	<0.01	40.529	<0.01	7.754	<0.01
	pine	92.706	<0.01	4.987	<0.01	3.680	<0.01
Litterfall OC (%)	oak	262.287	<0.01	0.485	0.714	0.560	<0.01
	pine	25.048	<0.01	0.436	0.728	0.120	0.999
Litterfall mass (g m^{-2})	oak	2866.858	<0.01	2.208	0.106	2.253	0.025
	pine	2891.092	<0.01	1095	0.365	1.553	0.220
Fine root biomass (g m^{-2})	oak	87.670	<0.01	11.579	<0.01	6.790	<0.01
	pine	39.356	<0.01	17.920	<0.01	6.450	<0.01

3.2. Soil Organic Carbon (SOC)

In the pine plantation, nitrogen additions did not have a statistically significant effect on soil organic carbon (SOC). However, significant differences were observed concerning seasons and the interaction of seasons/treatments. On the other hand, in the oak plantation, N treatments led to higher SOC levels during the summer season, resulting in a significant overall effect of the treatments. The impact of the treatments varied across seasons, with SOC levels being highest in summer, followed by spring, fall, and winter. The interaction between treatments and seasons was also found to be significant (Figure 3; Table 2). Overall, the amount of SOC in the pine plantation was significantly higher compared with the oak one.

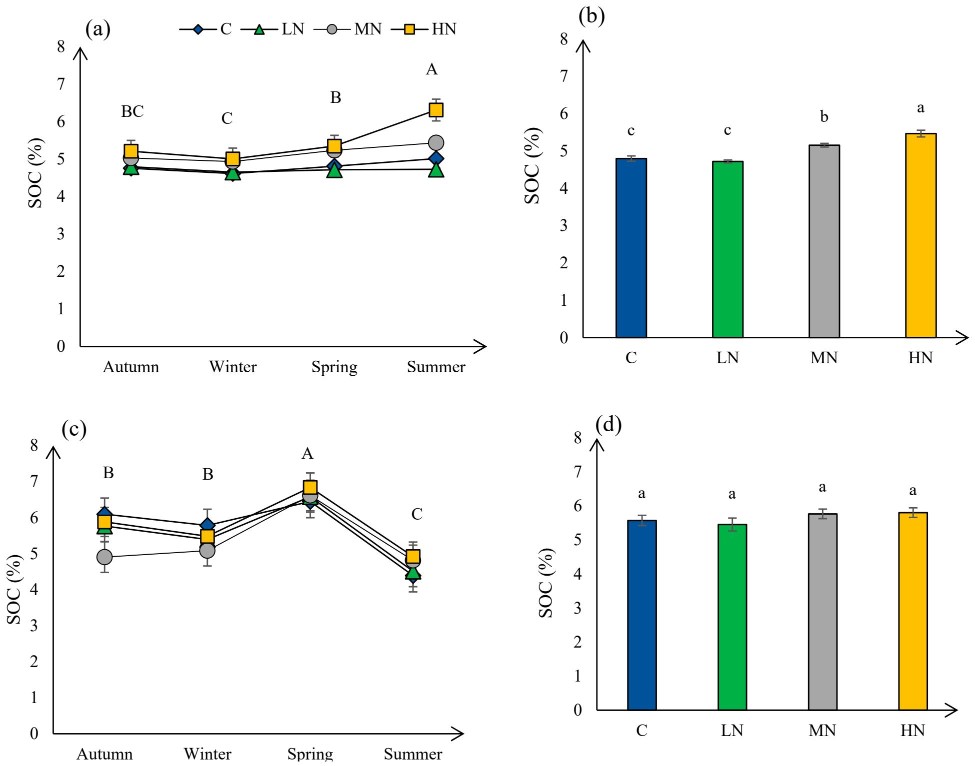

Figure 3. SOC (Mean ±SD) in different seasons and N treatments (mean value of four seasons) in oak (**a**,**b**) and pine plantations (**c**,**d**); capital letters illustrate significant differences between seasons; lowercase letters illustrate significant differences between N treatments.

3.3. Soil Microbial Entropy and C/N Ratio

Both soil microbial entropy and soil C/N ratio were significantly higher ($p < 0.01$) in the pine plantation compared to the oak plantation (Figure A1). After one year of N addition, soil microbial entropy decreased by 24% and 33% in MN and HN treatments in the pine plantation, respectively. Concurrently, medium and high N additions resulted in a decrease in soil C/N ratio by 20% and 26%, respectively, compared to the control. In the oak plantation, soil microbial entropy showed reductions of 23% and 29% in the MN and HN treatments, while the soil C/N ratio decreased by 14% in the HN treatment compared to the other treatments.

3.4. Soil Respiration (Rs)

The lowest Rs was observed in winter, while the highest one was recorded in summer. Both seasons and nitrogen (N) treatments, along with their interaction, exerted significant effects on soil CO_2 emission. Nitrogen addition led to a decrease in soil respiration, particularly in the spring and summer, towards the end of the experiment. The Rs values in plots with different N levels followed the order of the amount of added nitrogen: C > LN > MN > HN, resulting in a reduction of up to 27% at high and medium N levels (Figure 4). There was also a significant difference ($p < 0.01$) in Rs between the two plantations and an interaction between treatment and species, as the pine plantation showed slightly higher respiration rates and a more pronounced decrease in Rs with increasing N inputs (Table 2).

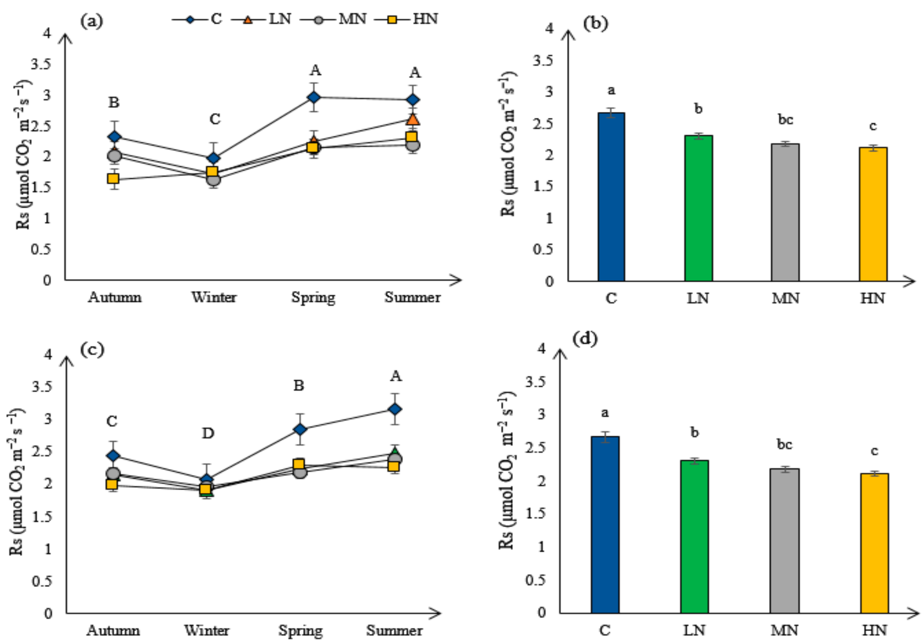

Figure 4. Rs (Mean ± SD) in different seasons and N treatment (mean value of four seasons) in oak (**a**,**b**) and pine plantations (**c**,**d**); capital letters illustrate significant differences between seasons; lowercase letters illustrate significant differences between N treatments.

3.5. Litterfall

The highest litterfall production and the associated C input to the soil were recorded during autumn and winter. The oak plantation exhibited a significantly higher mass and C flux via litterfall. N additions in both plantations showed no significant effect on litterfall production and the corresponding C input to the soil (Figures 5 and 6; Table 2). These findings indicate that the litterfall dynamics and C input were primarily influenced by seasonal variations rather than short-term N treatments in the studied plantations.

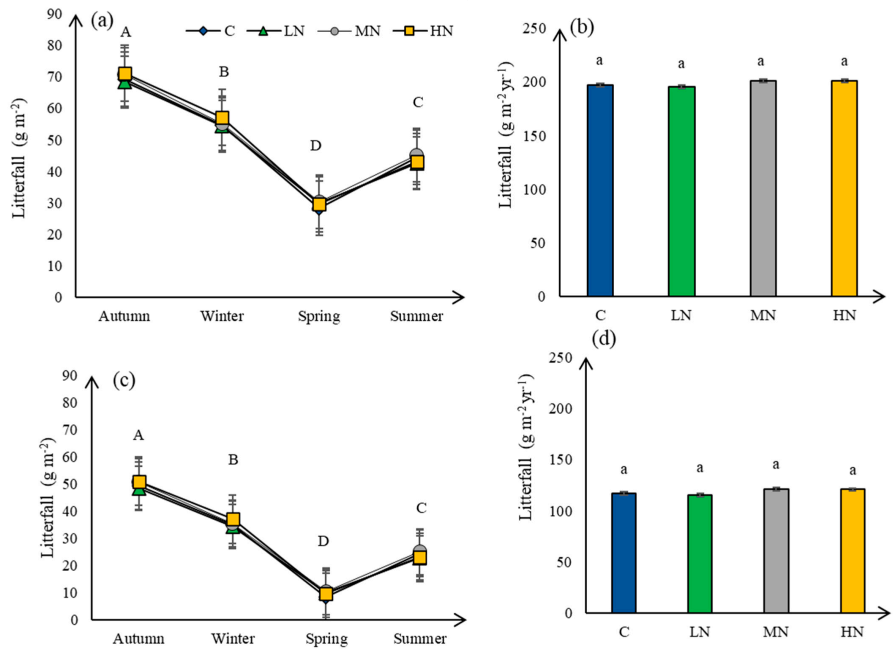

Figure 5. Litterfall production (Mean ± SD) seasonally and annually in different N treatments in oak (**a**,**b**) and pine stands (**c**,**d**); capital letters illustrate significant differences between seasons; lowercase letters illustrate significant differences between N treatments.

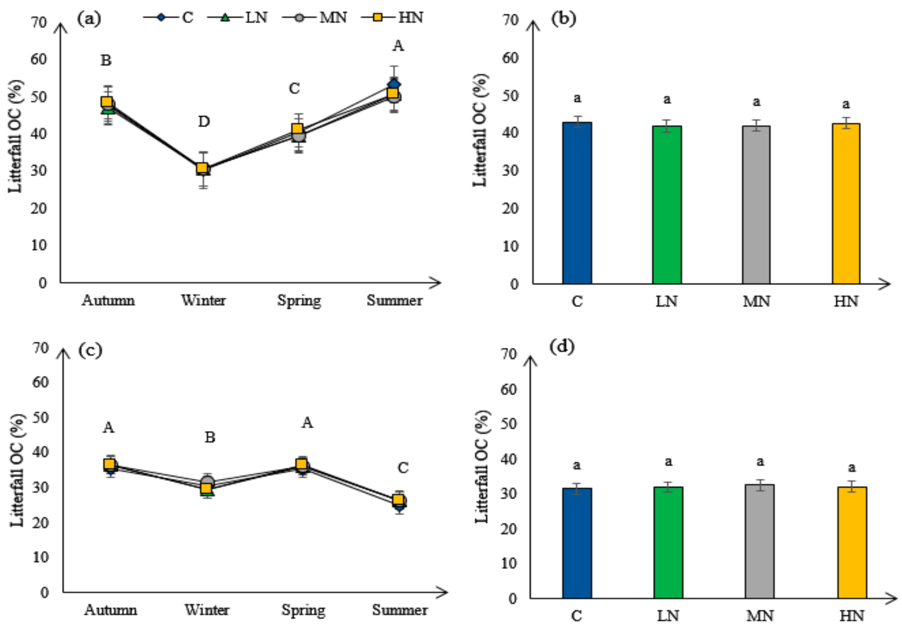

Figure 6. Litterfall OC (Mean ± SD) in different seasons and N treatment in oak (**a**,**b**) and pine stands (**c**,**d**); capital letters illustrate significant differences between seasons; lowercase letters illustrate significant differences between N treatments.

3.6. Fine Root Biomass

In both plantations, higher fine root biomass was recorded during autumn (Figure 7; Table 2). Across all seasons and N treatments, the fine root biomass was significantly ($p < 0.01$) greater in the pine plantation compared to the oak one. N treatments had a significant ($p < 0.01$) effect on fine root biomass in both populations. The N addition reduced the fine root biomass, which was more pronounced in the summer (toward the end of the experiment). The impact of different N levels on the fine root biomass was in the order of C > LN > MN > HN (Figure 7).

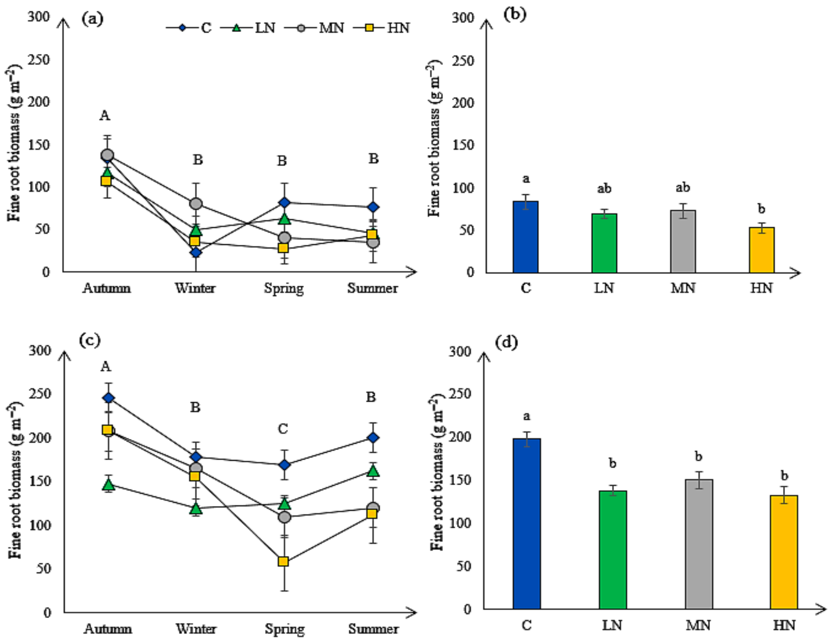

Figure 7. Fine root biomass (Mean ± SD) in different seasons and N treatment in oak (**a**,**b**) and pine stands (**c**,**d**); capital letters illustrate significant differences between seasons; lowercase letters illustrate significant differences between N treatments.

3.7. Relationship Between Rs and Soil Properties Under N Treatments

Rs exhibited a positive correlation with soil temperature, elucidating 70% of the variations (R^2) of soil respiration in control plots within the pine plantation. Corresponding R^2 were 65%, 39%, and 29% in low, medium, and high N treatments in the pine plantation, respectively (Figure A2a). In the oak plantation, soil temperature explained 66%, 65%, 34%, and 25% of the variations (R^2) in soil respiration in control, low, medium, and high N treatments, respectively (Figure A2b).

At the end of the N addition experiment, Rs was positively correlated with soil pH, microbial entropy, MBC, and fine root biomass under different N treatments in both plantations. Soil pH explained 47% and 26% of the variations in Rs under oak and pine plantations, respectively. The relationship between Rs and MBC was stronger (R^2 = 44% and 58%) in both stands. Rs was positively correlated with fine root biomass in two plantations; fine root biomass explained 50% and 48% of the variations in Rs under pine and oak canopies, respectively. Rs was positively correlated with microbial entropy, but the correlation between Rs and microbial entropy was much weaker in the pine plantation compared with the oak one (Figures A3 and A4). There was a significant linear relationship between fine root biomass and MBC in both plantations. Fine root biomass explained 46% and 40% of the variation in MBC in pine and oak plantations, respectively (Figure A5).

4. Discussion

Litterfall serves as a crucial pathway for the input of organic matter into the forest soils as the primary source of nutrients and energy [61]. The simulated short-term N depositions had no significant effect on litterfall production and its organic carbon content in our experimental plots. In line with Zhang et al. [28] and Peng et al. [3], short-term N additions did not affect litterfall amount and organic carbon input. Previous long-term studies showed higher N concentrations in above- and below-ground biomass in response to nitrogen addition [63,64]. However, litterfall decreased in response to the chronic or long-term extra nitrogen inputs in a temperate forest when the soil became N-saturated [65,66]. Thus, in different forest ecosystems, the quantity and quality of litterfall response following N input may vary according to the intensity and duration of N addition experiments [3]. Consistent with previous studies, an increase in forest soil OC content can occur owing to the increase in biomass production and litterfall by alleviating the N limitation [67] and/or decelerating litter decomposition [68]. However, in this study, litterfall was not affected by N deposition; thus, an increase in soil carbon content in the oak plantation might have been due to the reduction of the litter decomposition rate [3]. Although the pine plantation had a higher initial SOC content, the oak plantation demonstrated a significant increase in SOC under high N treatment, while the pine stand showed no significant change. This finding is explained by the N-induced suppression of microbial activity [37,69]. The added N led to soil acidification, which significantly reduced microbial biomass carbon (MBC) and microbial entropy via inhibiting organic matter decomposition rather than by increasing litter input, which was more pronounced in the oak plantation.

According to our findings, in both plantations, Rs showed a clear seasonal pattern in all treatments, with a peak in the summer (warm season) and the lowest rates recorded in winter. Similar results were obtained in many previous studies in coniferous [70,71] and temperate deciduous forests [30,72,73]. Our results imply that N treatments significantly reduced soil respiration. Similar findings (Rs reduction) were reported in a *Larix mastersiana* forest during the first year of the N additions experiment [74]. These results are similar to those found in several temperate forests [28,37,74,75]. Soil respiration originates from different processes (root respiration, faunal respiration, and microbial respiration) and is regulated by temperature, soil moisture, pH, and SOM amount and quality, the latter being influenced by vegetation type (land use) and disturbances [76,77]. In the present investigation, soil respiration was positively correlated with soil pH, MBC, microbial entropy, and fine roots in both reforested areas. These results are in accordance with those of other studies, such as Lee and Jose [78], Zhou et al. [75], and Zhang et al. [28].

The observed reduction in soil respiration after N additions may be attributed to changes in both autotrophic and heterotrophic components, constituting approximately 50% of the total CO_2 emission (e.g., [29]). Firstly, autotrophic respiration (Ra) originating from roots and rhizosphere microorganisms may decrease after N additions [52]. In our study, fine root biomass declined with increasing N application in both plantations. Bowden et al. [30], Wang et al. [79], and Zhang et al. [28] also demonstrated that fine root biomass was significantly reduced in response to N treatments. In temperate forests, when N limitation is alleviated by N addition, trees tend to allocate less photosynthetic product into root systems [52,80]. Secondly, heterotrophic respiration arising from microbial communities may be decreased in N-treated plots. Our findings revealed that MBC significantly decreased in the N-treated plots, which was also claimed by Liu et al. [81] and Zhang et al. [28]. According to Janssens et al. [37], the reduction in MBC may be directly linked to the formation of stable compounds via the rapid reaction of $NH_4.NO_3$ with the low-molecular-weight organic matter, which led to a lower decomposition rate. In addition, Baath et al. [82] implied that there is a positive relationship between soil pH and microbial biomass production. Therefore, in line with the findings of Smolander et al. [83], the activity and abundance of the soil microbial community change due to variations in soil organic matter and soil pH. In our investigation, the reduced MBC is largely explained by the observed reduction in soil pH. Finally, N deposition can also lead to a decrease in the C/N ratio and the soil organic matter, making it less decomposable for microbes [84,85]. In our N treatment plots, soil N increased much more than C, indicating that the litter decomposition rate could be reduced due to the decrease in the C/N ratio of the substrate [7]. Nitrogen addition significantly lowered the C/N ratio compared with the control plots. The decline in the C/N ratio is attributed to the increase in nitrogen input during the nitrogen addition experiment. However, in the pine plantation, no significant effect of deposited N on the SOC was found, possibly because short-term N treatment was not sufficient to lead to a considerable increase in the SOC content [28] because needle leaves may be less immediately affected by N-induced acidification [83]. It's worth noting that the SOC content was more variable in the pine plantation, making changes more challenging to detect.

In both plantations, soil respiration had high correlations with fine root biomass and MBC, which is consistent with the results of Zhou et al. [86] and Lee and Jose [78]. The MBC, SOC, and litter mass flux have been related to heterotrophic respiration [87,88]. Since N treatments clearly led to reductions in both fine root biomass and MBC, it can be inferred that these are the two dominating mechanisms explaining how N additions negatively impact soil respiration.

The weaker correlation between soil respiration (Rs) and microbial entropy in the pine plantation compared with the oak one (R^2 = 0.199 vs. R^2 = 0.566) likely reflects differences in belowground substrate quality and microbial community. Pine soils receive lignin-rich and recalcitrant litter, which reduces labile substrate availability, while oak soils receive more nutrient-rich, easily decomposable litter, fueling microbial activity and linking more strongly to Rs [89–91].

The positive correlation between Rs and soil temperature in our control and LN plots is in accord with the temperature sensitivity of soil respiration as a well-established principle of soil biogeochemistry [20,92]. However, the weakening of this relationship under medium and high N treatments suggests that extra N availability shifts microbial compositions and functions towards a slower decomposition process and consequently dampens the response of respiration to temperature [29,93]. This finding aligns with other studies [32,37,94] where N addition reduced the Q_{10} of soil respiration, likely by limiting labile carbon availability for microbes and shifting microbial community function.

In the HN plots of the oak plantation, soil organic C significantly increased, while N treatments did not impact soil carbon content in the pine plantation. The observed decline in soil MBC and the concomitant reduction in soil respiration are certainly the primary cause of SOC accumulation, as also claimed by Bowden et al. [30], Gundersen et al. [95], and He et al. [96]. Similar to our findings in the oak plantation, Nava et al. [97], Wei et al. [98], Ma et al. [53], and Chen et al. [99] also reported that increasing the N input led to an elevation in SOC.

An important contribution of our study is the contrasting responses observed between oak and pine plantations. Oak soils, receiving nutrient-rich and labile litter, accumulated more SOC under N enrichment due to reduced microbial decomposition, consistent with patterns reported in broadleaf forests [28,30,69]. In contrast, pine soils with recalcitrant litter showed no short-term SOC increase, reflecting slower decomposition and stronger long-term stabilization potential [3,70]. Microbial biomass declined more sharply in pine, suggesting higher sensitivity to N-induced acidification, while oak soils exhibited greater resilience [13,42,83]. Fine root biomass was higher in pine but decreased more strongly with N, indicating vulnerability in belowground carbon allocation [30,79]. Oak appears to favor short-term carbon sequestration, while pine supports longer-term stability, reinforcing the importance of species identity in managing C dynamics under elevated N deposition [10,86].

These contrasting mechanisms between a native broadleaf species (oak) and an exotic conifer (pine) emphasize the importance of species selection in forest management under the condition of elevated nitrogen deposition. In the Hyrcanian forests, where N inputs are projected to rise further due to both wet and dry deposition, understanding these species-specific responses is critical.

5. Conclusions

In conclusion, our study demonstrates that even short-term N additions can significantly affect soil pH, microbial biomass carbon, microbial entropy, fine root biomass, and soil respiration in pine and oak plantations. The reduction of soil respiration was driven by declines in both fine root biomass and microbial abundance, highlighting the role of belowground processes in mediating ecosystem C dynamics. We also found species-specific differences, with oak exhibiting higher SOC accumulation under N additions compared to pine, likely due to differences in litter quality and decomposition dynamics. These results have important implications for forest carbon cycling under continued N deposition, suggesting that native broadleaf species may contribute more labile carbon inputs while exotic conifers may promote long-term stabilization. Future long-term experiments are needed to verify these short-term responses and to better understand the cumulative effects of N deposition on forest soil C storage and ecosystem functioning.

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Conflicts of Interest: The authors declare that there are no conflicts of interest.

Appendix A

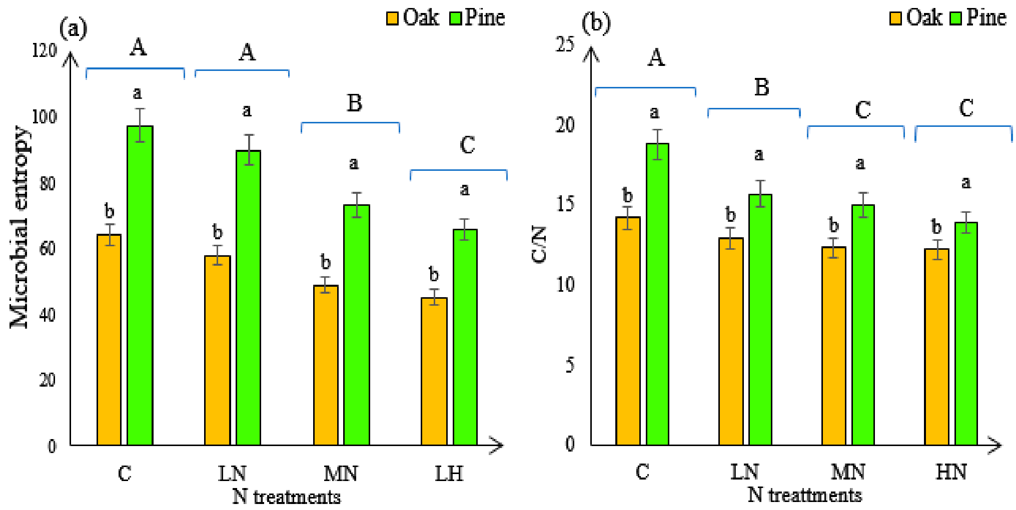

Figure A1. Mean (±ED) of microbial entropy (**a**) and C/N ratio (**b**) in different stands (lowercase letters) and N treatments (capital letters).

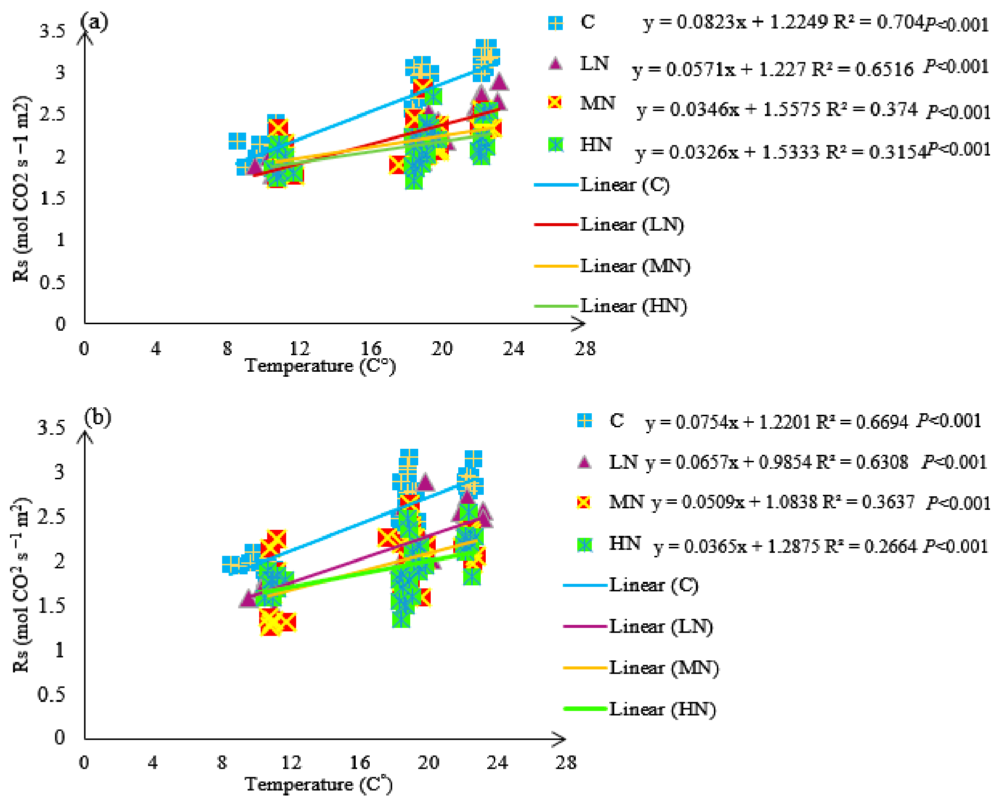

Figure A2. Relationship between soil respiration rate (Rs) and soil temperature in N treatments in oak (**a**) and pine (**b**) plantations.

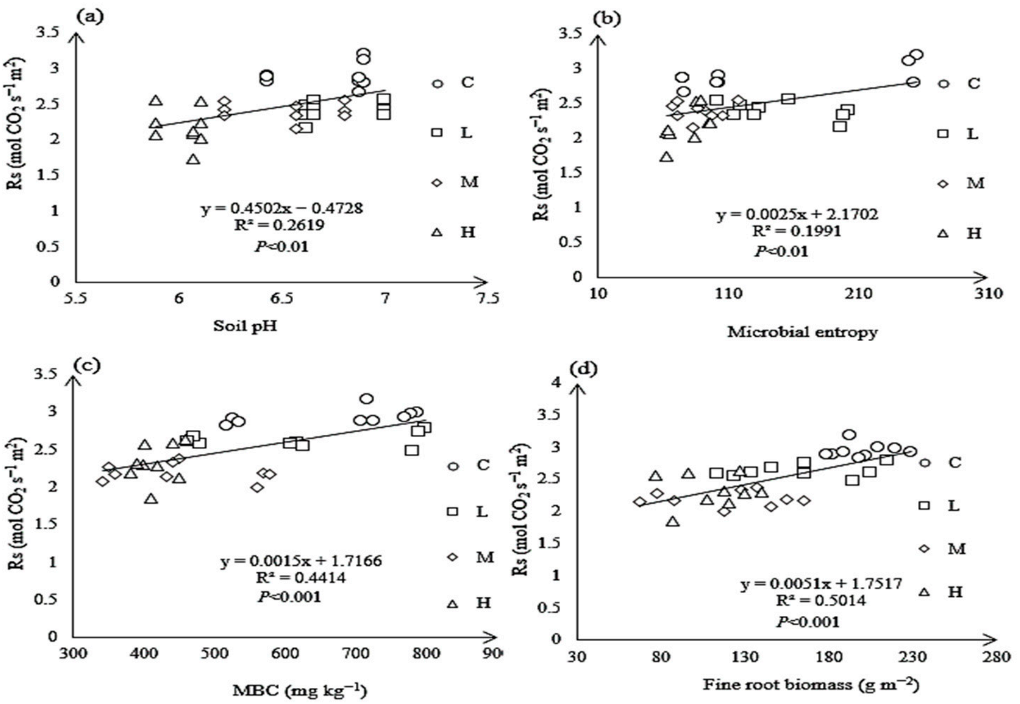

Figure A3. Relationship between respiration rate (Rs), soil pH (**a**), microbial entropy (**b**), MBC (**c**), and fine root biomass (**d**) in a pine plantation.

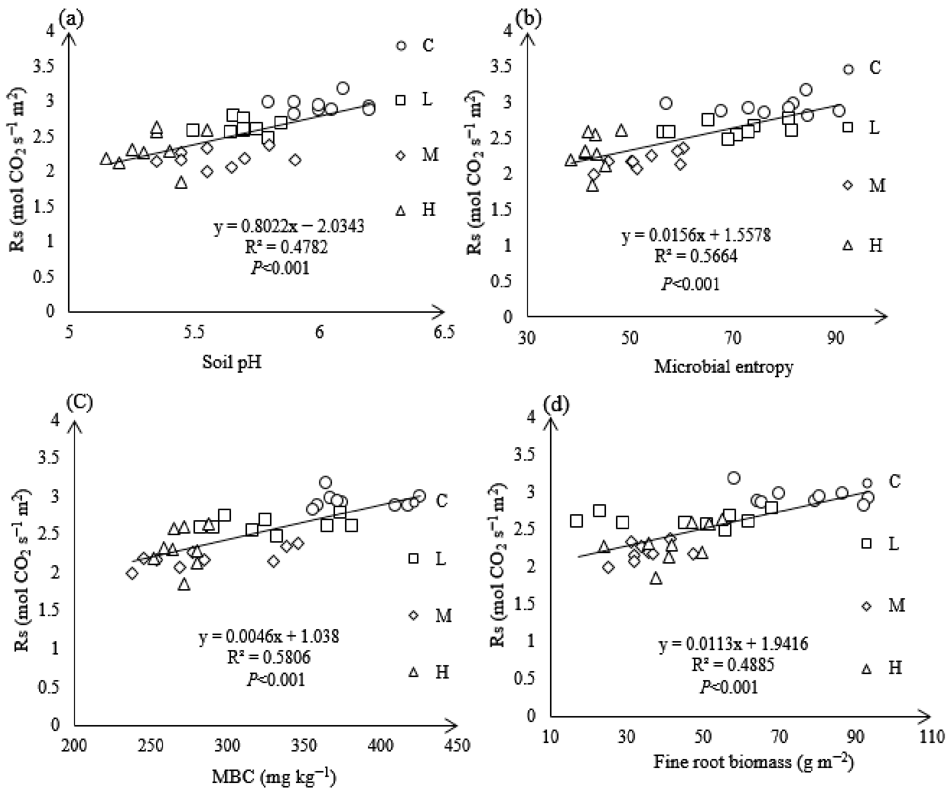

Figure A4. Relationship between soil respiration rate (Rs), soil pH (**a**), microbial entropy (**b**), MBC (**c**), and fine root biomass (**d**) in an oak plantation.

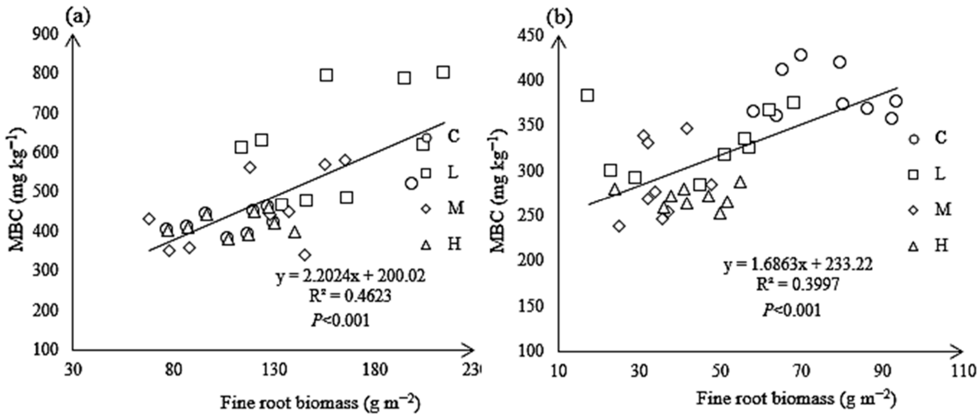

Figure A5. Relationship between fine root biomass with MBC in pine (**a**) and oak (**b**) plantations.

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