atmosphere

MDPI

Article

High Level of Ammonium Nitrogen Increases Net Ecosystem Productivity in a *Quercus liaotungensis* Forest in Northern China

Jingcong Qiu [1,2,3], Minghua Song [4], Yun Li [5] and Chunmei Wang [1,2,3,*]

[1] College of Environmental Science and Engineering, Beijing Forestry University, No. 35, Qinghua Dong Road, Haidian District, Beijing 100083, China; qiujingcong0415@163.com
[2] Beijing Key Lab for Source Control Technology of Water Pollution, College of Environmental Science and Engineering, Beijing Forestry University, Beijing 100083, China
[3] Engineering Research Center for Water Pollution Source Control & Eco-Remediation, College of Environmental Science and Engineering, Beijing Forestry University, Beijing 100083, China
[4] Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, A11, Datun Road, Chaoyang District, Beijing 100101, China; songmh@igsnrr.ac.cn
[5] School of Environment, Beijing Normal University, Beijing 100875, China; liyun0825@mail.bnu.edu.cn
* Correspondence: wangcm@bjfu.edu.cn; Tel.: +86-10-6233-6615; Fax: +86-10-6233-8689

Abstract: Forest ecosystems are vital to the terrestrial ecosystem's carbon (C) cycle. The effects of nitrogen (N) addition on C sequestration in forest ecosystems are critical for better understanding C dynamics when facing an increase in N availability. We conducted a six-year field experiment to examine the effects of N addition on C sequestration and net ecosystem productivity (NEP) in a *Quercus liaotungensis* forest in northern China. N addition resulted in a significant increase in biomass C storage (17.54–48.62%) and changed the distribution patterns of above and belowground biomass C storage, resulting in a 9.64 to 23.23% reduction in the proportion of belowground biomass C compared with the control. The annual average heterotrophic respiration was significantly increased by the additional N (by 0.06–0.94 Mg C ha^{-1} yr^{1}). In comparison with the control, the C sequestration efficiency driven by N addition ranged from 7.12 to 33.50 kg C/kg N. High-level N addition exerted stronger effects on ecosystem C sequestration than low-level N addition. NH_4^+-N, rather than NO_3^--N, dominated the increase in ecosystem C sequestration. We found that *Q. liaotungensis* forest acted as a C sink. The increase in NEP in the study forest in northern China was mainly due to an increase in net primary productivity (NPP) caused by N addition. Atmospheric N deposition increased the C sequestration efficiency depending on the rate and form of N deposition.

Keywords: nitrogen addition; nitrogen form; nitrogen level; net ecosystem productivity; north temperate forest; carbon sequestration

check for updates

Citation: Qiu, J.; Song, M.; Li, Y.; Wang, C. High Level of Ammonium Nitrogen Increases Net Ecosystem Productivity in a *Quercus liaotungensis* Forest in Northern China. *Atmosphere* **2022**, *13*, 889. https://doi.org/10.3390/atmos13060889

Academic Editors: Lei Liu, Chao Fang and Zhaozhong Feng

Received: 12 April 2022
Accepted: 23 May 2022
Published: 30 May 2022

1. Introduction

Due to the extensive use of chemical fertilizers and fossil fuels, global inorganic nitrogen deposition increased to 93.6 TG N yr^{-1} by 2016, 3.8 times higher than that in 1960 [1]. Although the ratio of NH_4^+-N to NO_3^--N in bulk deposition has dropped from 5 to 2 since the 1970s, NH_4^+-N is still the dominant form of N in atmospheric N deposition in China [2,3]. The estimated value of the plantation C stock (1.89 ± 0.048 Pg C) was 16.44–18.03% of the total forest biomass C in China (10.48 ± 11.49 Pg C) [4,5]. Thus, it is urgent to elucidate the responses of C dynamics in forest ecosystems to the increased N availability, particularly the response of net ecosystem productivity (NEP) [6,7].

N is considered as a primary limiting factor to net primary productivity (NPP) in most temperate forests, and N addition can usually increase biomass C fixation and ultimately alter C storage in forest ecosystems [8]. A meta-analysis showed that short-term N addition (10–640 kg N ha^{-1} yr^{-1}) led to an increase of 21.9% in the aboveground biomass C storage [9]. The C sequestration efficiency of terrestrial ecosystems driven by atmospheric N deposition is still unknown, but an estimate of 16 to 470 kg C/kg N was made [10,11]. N deposition generally leads to an increase in C sinks in terrestrial ecosystems with N limitation [12]. However, Some researchsynthesized 146 observations and showed that the corresponding increase in NPP to N drastically decreased as the amount of N added became higher than 50–60 kg N ha^{-1} yr^{-1} [13]. Consequently, the effect of N addition on forest C sequestration depends on the total N load [12] and site conditions [14].

Plants have the potential to use all types of N forms, but they often show different preferences for the different chemical forms, such as NH_4^+-N, NO_3^--N, or amino acids. Some plants, such as *Machilus velutina* and *Magnolia officinalis*, are more inclined toward NH_4^+-N when NO_3^--N and NH_4^+-N coexist [15,16]. However, more recent studies indicated that plants may prefer NO_3^- over NH_4^+, especially conifer species in northeast China [17,18]. Evidence of plant preference for NH_4^+ mostly comes from hydroponic studies, which tend to overestimate NH_4^+ uptake due to the lack of microbial competition and soil interception of NH_4^+ [18]. In the field and soil, plants compete with soil microbes to take up available N. The effects of N forms on biomass C storage are mediated by the tree species [19,20], soil properties [21], and N application rate [22], which are complex and the conclusions are inconsistent [15].

Current studies on how the continuous increase in N deposition affects the forest soil C pool remain controversial [22–25]. It has been shown that the addition of N may lead to a decrease in the lignin/N ratio in litter and an increase in labile C input into the soil, which may enhance heterotrophic respiration (R_h) during litter decomposition and release more C from the ecosystem [26,27]. The balance of N-induced inputs (increased above-ground plant biomass) and outputs (soil respiration) may lead to an increase in C storage in the organic soil layer by 17% [28] and short-term stability [29], but a reduction in C storage with long-term exogenous N addition [30,31].

To the best of our knowledge, there is still insufficient discussion of the effects of multiple N forms and the interaction with different N levels on the response of C storage in forest ecosystems [32–34]. We hypothesized that N addition with different chemical forms would (1) increase the NEP of the forest ecosystem; (2) decrease ecosystem C storage under long-term high-level N addition; and (3) show a stronger effect on C storage in the treatment with the application of both NO_3^--N and NH_4^+-N than the treatments with the application of NO_3^--N and NH_4^+-N alone. This study was designed to provide a further understanding of the response of ecosystem C sequestration to N application in northern temperate forests in China.

2. Materials and Methods

2.1. Site Description

The study location is in a typical temperate forest in the Xi Mountain Forestry Research Station of Beijing Forestry University (39°54′ N, 116°28′ E; 133 m a.s.l.) near Beijing (Figure 1). The study site is in a temperate monsoon humid climate region. The annual mean temperature and annual precipitation at the station are approximately 12.5 °C and 628.9 mm, respectively. The soil type is classified as Chromic Luvisols with a soil thickness of approximately 40 cm. The O horizon is less than 3 cm, and the A horizon is about 3–5 cm. The study was conducted in plantations where *Quercus liaotungensis* is the dominant tree species with a density of 2963 trees ha^{-1}. The age of the plantation is 62 years and it has not experienced any disturbance. Very few shrubs and herbs occurred in the experimental site. Soil samples (0–5 cm deep) at the study site were collected to measure the physicochemical properties at the beginning of the experiment as the background data. The initial tree height and diameter at breast height (DBH) of the sample trees in each plot were recorded. The initial conditions of the plots under different treatments were not statistically significant (Table 1).

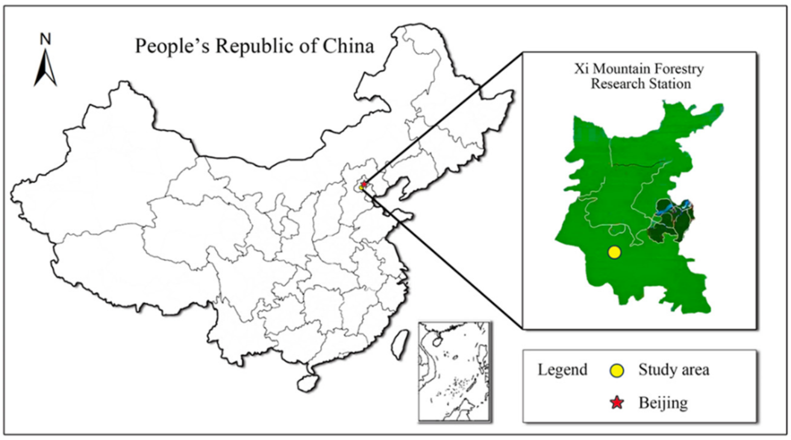

Figure 1. Location of the study area.

Table 1. Initial physicochemical properties of the study site.

Plots	pH	Soil Bulk Density (g cm^3)	Organic C (g kg^{-1})	Total N (g kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Height (m)	DBH (cm)
Control	7.18 ± 0.28 a	1.12 ± 0.062 a	29.97 ± 0.86 a	2.43 ± 0.68 a	2.57 ± 0.46 a	12.93 ± 1.55 a	5.52 ± 0.12 a	11.43 ± 0.38 a
L-$NaNO_3$	7.02 ± 0.39 a	1.19 ± 0.049 a	30.14 ± 0.51 a	2.35 ± 0.84 a	2.51 ± 0.43 a	12.80 ± 1.02 a	5.50 ± 0.19 a	11.78 ± 0.44 a
H-$NaNO_3$	7.16 ± 0.49 a	1.33 ± 0.043 a	29.45 ± 0.72 a	2.36 ± 0.72 a	2.37 ± 0.43 a	12.87 ± 1.18 a	5.53 ± 0.34 a	11.94 ± 0.74 a
L-NH_4NO_3	7.19 ± 0.39 a	1.05 ± 0.048 a	30.33 ± 0.56 a	2.39 ± 0.66 a	2.47 ± 0.44 a	13.06 ± 1.22 a	5.29 ± 0.35 a	11.46 ± 0.47 a
H-NH_4NO_3	7.12 ± 0.21 a	1.20 ± 0.026 a	31.06 ± 0.71 a	2.44 ± 0.48 a	2.55 ± 0.36 a	13.15 ± 1.36 a	5.49 ± 0.11 a	11.46 ± 0.52 a
L-$(NH_4)_2SO_4$	7.01 ± 0.36 a	1.22 ± 0.057 a	28.37 ± 0.82 a	2.40 ± 0.68 a	2.45 ± 0.22 a	13.05 ± 1.06 a	5.38 ± 0.16 a	11.49 ± 0.29 a
H-$(NH_4)_2SO_4$	7.20 ± 0.28 [a]	1.22 ± 0.015 a	29.15 ± 0.52 a	2.54 ± 0.60 a	2.41 ± 0.36 a	12.78 ± 1.05 a	5.32 ± 0.23 a	11.59 ± 0.53 a

Means with different lowercase letters are significantly different at $p < 0.05$ in the column. Capital letters L and H represent nitrogen addition of 50 and 150 kg N ha^{-1} yr^{-1} respectively.

2.2. Experimental Design

The experiment was conducted from March 2011 to March 2017. The total area of the experimental site was about 3500 m^2. Twenty-one 10 m × 10 m plots were set up randomly and divided into three replicate sub-plots. A wide buffer was built among the plots. In each plot, a 1 m × 1 m trench plot was set up before the N treatment in 2010. Each trench plot was surrounded by vertical 10 cm-wide and 100 cm-deep trenches. All of the roots passing through the boundaries were cut off, but not removed, while the aboveground parts of the plants in the trenched plots were cleaned up. Pieces of thin polyethylene sheet were placed in the vertical trenches to prevent living roots from spreading into the trench plots. The N addition experiment was designed using three N chemical forms ($NaNO_3$, $(NH_4)_2SO_4$, and NH_4NO_3) at two levels (low N: 50 kg N ha^{-1} yr^{-1} and high N: 150 kg N ha^{-1} yr^{-1}) to simulate the applications of NO_3^-, NH_4^+, and their combinations. The N levels were about two and five times the amount of N deposition in Beijing [35]. Control plots without N addition were set up to detect the effect of atmospheric N deposition. The N compound was dissolved in deionized water and sprayed with a sprayer during the first few days of each month from March to October in each year. The same dose of deionized water was applied to the control plot.

2.3. Biomass and Net Ecosystem Productivity

The calculations of NPP and NEP were according to previous studies [32,36]. NPP was calculated as the sum of the annual litter input and the increment in plant biomass, while NEP was the difference between NPP and R_h. The height, DBH, and crown width of sample trees were recorded in each plot during March of each year from 2011 to 2017. Before the N addition experiment, the DBH (1.3 m aboveground level) of all the trees in each plot was measured, and then we selected and tagged three sample trees. The biomass calculation was based on the general allometric model of hardwood broad-leaved forest provided by previous studies [37]. The model is as follows:

$$W = a\left(D^2H\right)^b \quad (1)$$

where W is tree biomass (Mg C ha^{-1}), a and b are the parameters (the parameters of different parts of the tree are shown in Figure S1), D is DBH (cm), and H is the tree height (m).

We harvested twelve sample trees and then calculated the dry weights of the trunks, branches, leaves, and roots to fit the equation to obtain specific parameters. We calculated the biomass C storage based on the C concentration of 49% of the tree's biomass.

In each sample plot, the collection of litter was completed by using 25 litter traps, which were 1 m high and 0.5 m in diameter. The litter traps were randomly placed under the forest canopy from May to December. During the study period, the traps were emptied monthly. Litter decomposition was determined by a decomposition experiment using the burry-bag method. At the beginning of the test, 25 × 25 cm nylon mesh bags with a pore size of 0.5 mm at bottom and 2.0 mm at top were prepared, and each bag was filled with about 10 g of air-dried leaves. Then, the mesh bags with litter were evenly placed under the litter layer in the forest and above the soil layer. The residue was measured every three months. Since the decomposition of roots or the secretions of roots was not included in the NPP calculation, the NPP in this study might have been underestimated.

2.4. Soil Sampling

Before soil sampling, litter and vegetation were first removed from the surface soil. At each site, three soil profiles were dug to a depth of about 40 cm. In the soil profile, the undisturbed soil in each layer was collected with a 100 cm^3 metal core to test the soil bulk density. The soil samples were collected from 0–10, 10–20, and 20–40 cm, with three replicate samples per layer. The soil samples were air-dried, sieved with a 2 mm sieve, and then sealed in a glass bottle for the subsequent measurements. Soil C storage (Mg C ha^{-1}) was calculated as:

$$S = \frac{\sum_{i=1}^{n}(1-\delta_i\%)C_i d_i D_i}{10} \quad (2)$$

where I is the soil layer, n is the number of layers in the soil profile, S (Mg C ha^{-1}) is the SOC storage in the 40 cm soil layer, δ_i (%) is the volume fraction of gravel in layer i, d_i (g cm^{-3}) is the soil bulk density in layer i, C_i (g kg^{-1}) is the soil organic C (SOC) content in layer i, and D_i (cm) is the thickness of each soil layer (Table 2).

Table 2. Soil inorganic nitrogen and organic carbon (SOC) in the study site after the experiment.

Plots	pH	NH_4^+	NO_3^-	SOC (g kg^{-1})		
Soil Depth (cm)	0–10	0–10	0–10	0~10	10~20	20~40
Control	7.11 ± 0.05 a	3.06 ± 0.26 d	12.29 ± 1.03 d	17.92 ± 0.92 b	11.50 ± 0.61 d	7.59 ± 0.40 de
L-$NaNO_3$	6.94 ± 0.05 b	4.11 ± 0.57 c	28.75 ± 3.61 c	17.92 ± 0.61 b	11.99 ± 0.41 cd	7.81 ± 0.27 ce
H-$NaNO_3$	6.94 ± 0.05 b	5.13 ± 0.69 a	48.52 ± 2.52 a	18.53 ± 0.39 b	11.29 ± 0.23 d	9.27 ± 0.19 b
L-NH_4NO_3	6.86 ± 0.05 b	4.67 ± 0.39 b	26.97 ± 3.56 c	18.35 ± 0.97 b	11.36 ± 0.60 d	7.99 ± 0.42 cd
H-NH_4NO_3	6.48 ± 0.05 d	5.20 ± 0.37 a	42.03 ± 3.96 a	19.18 ± 0.42 a	13.19 ± 0.29 a	9.52 ± 0.19 ab
L-$(NH_4)_2SO_4$	6.83 ± 0.05 b	5.14 ± 0.42 a	24.16 ± 2.74 c	18.71 ± 0.37 b	12.51 ± 0.25 c	8.15 ± 0.16 c
H-$(NH_4)_2SO_4$	6.66 ± 0.09 c	5.80 ± 0.43 a	35.48 ± 1.58 b	19.91 ± 0.32 a	13.40 ± 0.19 b	10.00 ± 0.14 a

Means with different lowercase letters are significantly different at $p < 0.05$. Capital letters L and H represent nitrogen addition of 50 and 150 kg N ha^{-1} yr^{-1}, respectively.

2.5. Measurement of Soil Respiration

Gas samples were collected three times monthly inside and outside of the trenched subplots using the static opaque chamber method from March 2011 to February 2017. The chamber consisted of a fixed stainless steel base (50 × 50 × 20 cm) and a detachable top

(50 × 50 × 50 cm). Four gas samples were collected with a plastic syringe at intervals of 0, 5, 10, and 20 min for each plot. A digital thermometer was used to record the air temperature and soil temperatures at a depth of 5 cm near the chamber when collecting the gas samples. The gas samples were measured within 12 h after gas collection using a gas chromatograph (PE Clarus 500, PerkinElmer, Inc., Waltham, MA, USA). Detailed gas collection methods and soil respiration flux calculations can be found inour previous work [38,39], respectively. The total amount of CO_2 emitted from the plots without trenching was treated as the total soil respiration, as published in the article of previous studies [39], and the CO_2 emitted from the trenched subplot was considered to be R_h.

2.6. Statistical Analyses

The differences in the initial soil properties and the C storage in different pools of the ecosystem (biomass, litter, soil, etc.) under the N addition treatments were analyzed using one-way analysis of variance (ANOVA) and least significant difference (LSD) (IBM Corp, Armonk, NY, USA). One-way ANOVA was used to show the overall estimates of the N addition effects on the increase in ecosystem C storage. Univariate analysis and LSD testing were used to explore the effects of N forms, N levels, and their interactions on the biomass, litter, and NEP of the forest ecosystem. All results were considered significant at the $p < 0.05$ level.

3. Results

3.1. Plant Response

In the control plot, biomass C storage increased at an average rate of 2.50 Mg C ha^{-1} yr^{-1} from 2011 (19.12 ± 0.43 Mg C ha^{-1}) to 2017 (34.10 ± 2.99 Mg C ha^{-1}), while the average rate of increase in biomass in the plots treated by N addition was 4.01 Mg C ha^{-1} yr^{-1} (Figure 2). The highest biomass C storage increment (5.11 Mg C ha^{-1} yr^{-1}) occurred in the H-$(NH_4)_2SO_4$ treatment after 6 years of N addition, and the annual increase rate of biomass in the N addition plots gradually decreased from the beginning to the end of the experiment (Figure 2). Low and high levels of N addition increased biomass C storage by 24.01% and 34.66%, respectively. The biomass C storage in the $NaNO_3$, NH_4NO_3, and $(NH_4)_2SO_4$ treatments were increased by 18.17, 30.80, and 39.05%, respectively. Both the N level and N form had a significant influence on biomass C storage ($p < 0.01$, Table 3), while the interactive effect between them was insignificant (Table 3).

Table 3. Effects of N chemical form and N level and their interaction on the carbon storage and NEP.

		Biomass		Soil		Ecosystem		NEP	
	d.f.	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
N Form	2	11.523	0.001	12.638	0.001	21.859	0.001	3.989	0.043
N Level	1	8.855	0.010	78.796	<0.001	61.363	0.001	4.499	0.049
N Form × N Level	2	2.100	0.159	8.640	0.004	7.796	0.005	0.068	0.935

NEP = Net ecosystem productivity.

N addition significantly promoted the above- and belowground biomass C storage by 29.00 and 16.11%, respectively, and resulted in a 9.64–23.23% reduction in the proportion of belowground biomass relative to the control. Compared with the control, low and high levels of N addition caused decreases in the proportion of belowground biomass C of 13.04 and 17.91%, respectively (Table 4). N forms significantly altered the proportion of belowground biomass C storage. $(NH_4)_2SO_4$ had the strongest effect on the proportional decrease among the N forms (Table 4).

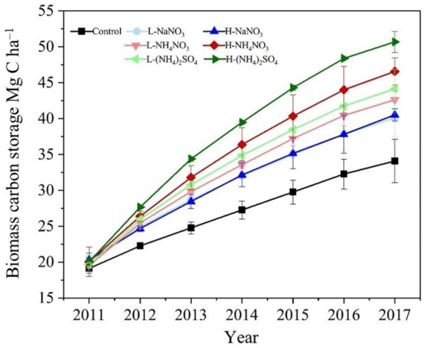

Figure 2. Biomass carbon storage in the N addition plots from 2011 to 2017. Capital letters L and H represent nitrogen addition of 50 and 150 kg N ha^{-1} yr^{-1}, respectively. Vertical bars represent ± SE (n = 3).

Table 4. Proportion of belowground biomass carbon storage under different N forms and N levels.

	Proportion of Belowground Biomass C (%)	**Decrease From Control**		**Proportion of Belowground Biomass C (%)**	**Decrease From Control**
Control	18.47 ± 0.93 a	-	Control	18.47 ± 0.93 a	-
Low	16.06 ± 0.49 b	13.04%	$NaNO_3$	15.93 ± 0.06 bc	13.75%
High	15.16 ± 0.72 c	17.91%	NH_4NO_3	16.07 ± 0.41 b	13.03%
			$(NH_4)_2SO_4$	14.84 ± 0.62 c	19.65%

$NaNO_3$ = average proportion of belowground biomass C storage in both low and high-$NaNO_3$-addition plots ($(NH_4)_2SO_4$ and NH_4NO_3 were calculated in the same way). Low = average proportion of belowground biomass C storage in L-$NaNO_3$, L-$(NH_4)_2SO_4$, and L-NH_4NO_3 plots (high was calculated in the same way). Means with different lowercase letters in the same column are significantly different at $p < 0.05$.

The litter C storage after six years of N addition showed an increase of 2.56 Mg C ha^{-1} and 3.36 Mg C ha^{-1} in the low- and high-N-addition plots, respectively, as compared with the control (7.15 ± 1.60 Mg C ha^{-1}, Figure 3a). The litter C storage in the $(NH_4)_2SO_4$ treatment had a higher increase, with an increasing rate of 48.30%, than that of 36.96% in NH_4NO_3 and of 38.92% in $NaNO_3$ treatment (Figure 3a).

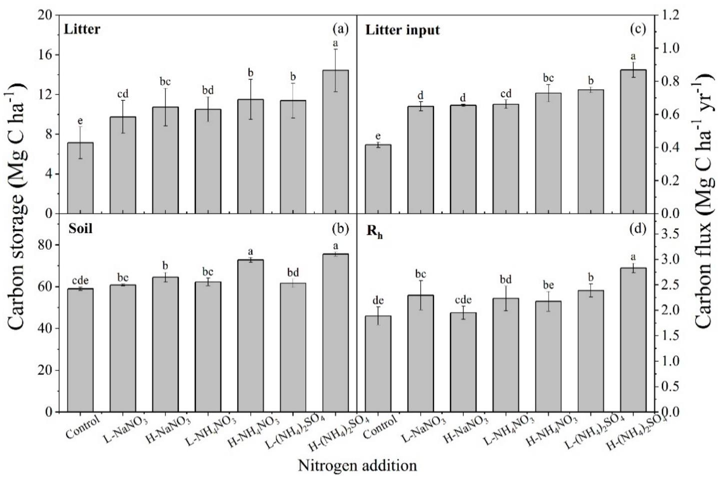

Figure 3. Carbon storage of litter (**a**) and soil (**b**) in 2017, the annual average carbon input from litter decomposition (**c**), and the annual average soil heterotrophic respiration (R_h), (**d**). Capital letters L and H represent nitrogen addition of 50 and 150 kg N ha^{-1} yr^{-1}, respectively. Means with different lowercase letters in the same column are significantly different at $p < 0.05$. Vertical bars represent ± SE (n = 3 in (**a**,**b**); n = 6 in (**c**,**d**)).

3.2. Soil Response

The annual average litter decomposition rate ranged from 29.15 to 36.14%, and the effect of the addition of N on the decomposition rate was not significant. However, N addition with different forms significantly increased litter C storage—the litter C input was enhanced in the N-addition treatments (Figure 3c). Litter C input was increased by 29.60, 40.37, and 64.80% in $NaNO_3$, NH_4NO_3, and $(NH_4)_2SO_4$, respectively, as compared with that of 0.42 ± 0.096 Mg C ha^{-1} yr^{-1} in the control. Meanwhile, litter C input was increased by 37.91 and 51.94% in the low- and high-N-addition plots, respectively (Figure 3c). N addition promoted the annual average heterotrophic respiration by 0.06–0.94 Mg C ha^{-1} yr^{-1} as compared with the control (1.89 ± 0.17 Mg C ha^{-1} yr^{-1}, Figure 3d). The N form, not N level, significantly affected the annual average heterotrophic respiration. The annual average heterotrophic respiration in the $NaNO_3$, NH_4NO_3, and $(NH_4)_2SO_4$ addition plots significantly increased by 12.37, 16.63, and 38.11%, respectively. Moreover, the N form, N level, and their interaction significantly affected soil C storage ($p < 0.01$, Table 3). The high-N treatments consistently significantly promoted soil C storage, while low N addition had an insignificant effect. Compared with the control (57.92 ± 0.78 Mg C ha^{-1}), the soil C storage in the H-$NaNO_3$, H-NH_4NO_3, and H-$(NH_4)_2SO_4$ treatments was significantly increased by 9.29, 23.35, and 28.02%, respectively (Figure 3b). The effects of $(NH_4)_2SO_4$ and NH_4NO_3 on soil C storage were stronger than those of $NaNO_3$.

The N-addition treatments had positive effects on ecosystem C storage, and the average rate of increase was 7.76 Mg C ha^{-1} yr^{-1} compared with the control (136.045 ± 5.67 Mg C ha^{-1}). The increase in ecosystem C storage under high-level N addition (39.87%) was significantly higher than that of the low level (22.91%). A significant difference in ecosystem C storage among the N forms was observed, and the increases in ecosystem C storage in $NaNO_3$, NH_4NO_3, and $(NH_4)_2SO_4$ were 20.87%, 31.05%, and 42.25%, respectively.

3.3. Response of NEP

Both N form and N level significantly affected NPP and NEP compared with the control (Figures 4 and 5). The NEPs under $NaNO_3$, NH_4NO_3, and $(NH_4)_2SO_4$ were 1.74 ± 0.23, 2.52 ± 0.34, and 2.68 ± 0.35 Mg C ha^{-1} yr^{-1}, respectively. $NH_4{}^+$-N, instead of $NO_3{}^-$-N or their combination, showed stronger effects on the NEP increments. The response of forest ecosystem C sequestration to N addition was 18.55 kg C/kg N in $(NH_4)_2SO_4$, 15.41 kg C/kg N in NH_4NO_3, and 6.68 kg C/kg N in $NaNO_3$ after 6 years of N addition. Compared with the control, low- and high-level N addition increased the NEP by 0.98 and 1.59 Mg C ha^{-1} yr^{-1}, respectively. The response of ecosystem C sequestration under low-level N addition was 27.30 kg C/kg N, while it was 13.13 kg C/kg N under high-level N addition. However, it is necessary here to mention that the C–N response was lower in the high-N treatment, implying lower N use efficiency in the high-N treatment.

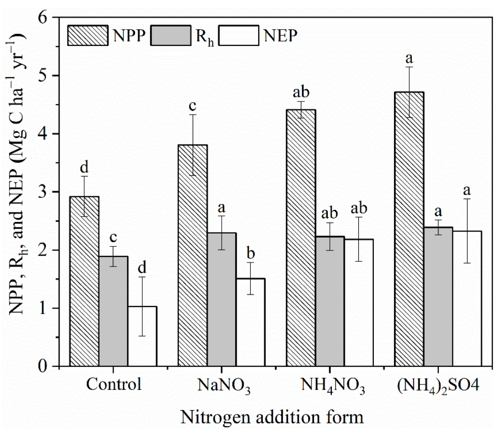

Figure 4. Comparison of net ecosystem productivity (NEP), soil heterotrophic respiration (R_h), and net primary productivity (NPP) under different nitrogen forms. Values labeled with the same lowercase letter do not differ significantly ($p < 0.05$). Vertical bars represent ± SE (n = 3).

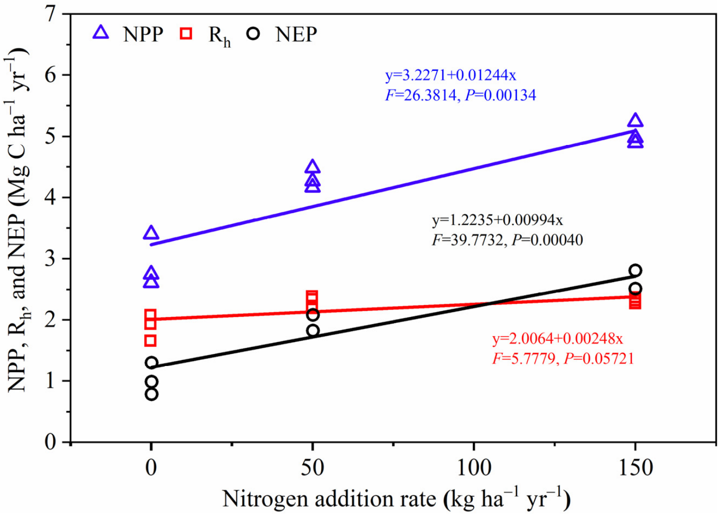

Figure 5. Relationships between the changes in net primary productivity (NPP, triangle), heterotrophic respiration (R_h, square), and net ecosystem productivity (NEP, circle) and nitrogen addition level.

4. Discussion

In this study, we quantified the effects of multiple N chemical forms and N levels on forest C storage, which included the C allocation to the above- and belowground biomass, NEP, and heterotrophic respiration. Our findings showed that N addition in different chemical forms led to an increase in NEP, which supported our first hypothesis. The N-induced increase in NPP exceeded the increase in R_h, and thus led to the increase in NEP (Figure 5). The ecosystem C sequestration was increased by 1.73–4.08 Mg C ha^{-1} yr^{-1} after N addition, which corresponded to an increase of 7.12–33.50 kg C/kg N. A comparable range, that is, 20–30 kg C/kg N, was found in an evaluation of a global data set of 80 forest sites [40]. The other meta-analysis concluded that temperate forests responded strongly to N addition and sequestered on average an additional 10–20 kg C/kg N in woody biomass, with approximately 60% retained in tree wood and 40% in the soil [41]. Furthermore, the C–N response (defined as the additional mass unit of C sequestered per additional mass unit of N addition) was affected by forest productivity, experimental N addition rate, and rate of ambient N deposition [42]. Model predictions indicated that the N deposition in northern and tropical forests will offset 3 billion tons of additional CO_2 emissions each year in the next few decades [43]. With the increase in N addition, the response of C sequestration dropped from 27.30 kg C/kg N (low-level N addition) to 13.13 kg C/kg N (high-level N addition). High-level N addition could lead to higher N losses via volatilization and leaching, and result in relatively low N use efficiency [44]. The response of forest ecosystem C sequestration was more susceptible to multiple nutrient restrictions with the increase in the N addition level [45]. Further detailed experiments are needed to determine the threshold of the N application rate that maximizes the efficiency of C sequestration in the boreal temperate forest ecosystem.

We also found that N addition altered the C allocation pattern of above- and belowground biomass. The increase in above- and belowground biomass C storage caused by N addition was 29.00% and 16.11%, respectively, which was comparable to the result of previous work [29], who conducted a meta-analysis that indicated that N addition significantly increased above- and belowground biomass C storage by 35.7% and 23.0%, respectively. Differences in the extent of the responses between above- and belowground biomass to N addition resulted in a decrease in the proportion of belowground biomass C (Table 3). These findings could be explained by the functional equilibrium hypothesis [46,47], which indicated that root growth is beneficial when local resources are limited. N treatment may cause an increase in the available N in the soil so that plants do not have to allocate too much C to the belowground parts to obtain more N sources [48,49].

The increment in ecosystem C storage under the high-level N addition was significantly higher than that under the low-level N addition, which did not support our second hypothesis. In a shorter period, high-level N addition had a stronger effect on the improvement of ecosystem C storage compared with low-level N addition [50,51]. However, over time, such an increase might be insignificant under high-level N addition or even inhibit C storage [12,52]. This indicated that the total amount of N loading was an important factor in determining the effect of different levels of N addition on ecosystem C storage. Long-term N input could induce a decrease in soil pH, and lead to soil acidification, which would further influence plant growth and soil microbe composition and activity [52]. Meanwhile, continuous N addition can lead to the loss of soil exchangeable base cations, such as Ca^{2+}, Mg^{2+}, K^+, and Na^+, which also influence plant growth [53]. In our study, the soil pH in the temperate forest was around 7.2 in the plots without N addition (control). We indeed observed a consistent decrease in soil pH in the N-addition treatment as compared with the control in 2017 (Table 2), indicating the occurrence of N-induced soil acidification. However, we did not find a significant difference in soil base cations (Ca^{2+}, Mg^{2+}, and Na^+) between the N-addition treatment and the control. Although $SO_4{}^{2-}$ and Na^+ were introduced with N addition, such cation inputs did not cause an alteration of soil base cations. This finding suggests that N-induced soil acidification can still be buffered by the soil base cations for now. Despite the continuous increase in the tree biomass with N addition, we found a decrease in the increasing rate of biomass with time. The results indicate that N-induced soil acidification could have been influencing tree growth. Therefore, the effect of N-induced alteration in the soil properties on plant growth needs to be tested in the future.

In our study, ecosystem C storage under different N forms showed significant differences. $NH_4{}^+$-N elicited a higher increase in ecosystem C storage than the other two forms, which did not support our third hypothesis. Our findings are consistent with the results of the isotope experiments conducted by previous studies [19], whose results showed that, when glycine, $NH_4{}^+$-N, and $NO_3{}^-$-N coexisted in the soil, $NH_4{}^+$-N was preferentially absorbed by the trees, which accounted for more than 80% of the total N absorbed. The possible reason was that $NH_4{}^+$-N can directly be used to synthesize amino acids, which can be used by plants [19]. The absorption and utilization of $NH_4{}^+$-N require less energy and are relatively more economical compared with those of $NO_3{}^-$-N. It is worth mentioning that Li et al., 2016, also used hydroponic experimental settings, which tend to overestimate $NH_4{}^+$ uptake due to a lack of microbial competition and soil interception of $NH_4{}^+$ [18]. The soil N form and availability appear to be important factors for the plant N uptake form. A large amount of available $NH_4{}^+$-N was absorbed, promoting plant biomass, litter, and soil carbon storage, and then improved the carbon sequestration efficiency. In our site, $NO_3{}^-$ was much higher than $NH_4{}^+$, which could have been the result of microbial and plant $NH_4{}^+$ uptake. On the other hand, high $NO_3{}^-$ implied high nitrification potential in this site, and also may have induced more plant uptake of $NO_3{}^-$.

5. Conclusions

Our study explored the effects of 6 years of N addition on the ecosystem C sequestration in a temperate plantation forest dominated by *Q. liaotungensis*. We found that the *Q. liaotungensis* forest in northern China acted as a C sink. A high level of ammonium nitrogen had a significant positive impact on the increase in net ecosystem productivity in the *Q. liaotungensis* forest. The increase in ecosystem C storage under high-level N addition was higher than that under low-level N addition. $NH_4{}^+$-N contributed more to the increase in ecosystem C storage than $NO_3{}^-$-N. NEP was increased by N addition mainly due to the higher increase in NPP than the increase in R_h. Finally, the mechanism underlying the N-induced alteration of the ecosystem C storage and threshold of the N efficiency in promoting C sinks need to be further investigated.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/atmos13060889/s1, Figure S1. the parameters of different parts of the tree.

Author Contributions: Conceptualization, J.Q. and Y.L.; methodology, M.S., Y.L. and C.W.; software, J.Q.; validation, M.S., Y.L. and C.W.; formal analysis, Y.L.; investigation, J.Q. and C.W.; resources, M.S.; data curation, Y.L.; writing—original draft preparation, J.Q. and Y.L.; writing—review and editing, M.S., Y.L. and C.W.; visualization, J.Q.; supervision, C.W.; project administration, M.S.; funding acquisition, C.W. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Natural Science Foundation of China (Nos. 41971024 and 41373069) and Beijing Municipal Education Commission through the Innovative Transdisciplinary Program "Ecological Restoration Engineering (Nos. GJJXK210102).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to extend their gratefulness to all individuals who contributed to the successful accomplishment of this study.

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

Abbreviations

Nitrogen: N; Carbon: C; Net primary productivity: NPP; Net ecosystem productivity: NEP; Diameter at breast height: DBH; Soil organic carbon: SOC.

References

1. Ackerman, D.; Millet, D.B.; Chen, X. Global estimates of inorganic nitrogen deposition across four decades. *Glob. Biogeochem. Cycles* **2019**, *33*, 100–107. [CrossRef]
2. Gu, F.; Zhang, Y.; Huang, M.; Tao, B.; Yan, H.; Guo, R.; Li, J. Nitrogen deposition and its effect on carbon storage in Chinese forests during 1981–2010. *Atmos. Environ.* **2015**, *123*, 171–179. [CrossRef]
3. Sheng, W.; Yu, G.; Jiang, C.; Yan, J.; Liu, Y.; Wang, S.; Wang, B.; Zhang, J.; Wang, C.; Zhou, M.; et al. Monitoring nitrogen deposition in typical forest ecosystems along a large transect in China. *Environ. Monit. Assess.* **2013**, *185*, 833–844. [CrossRef] [PubMed]
4. Tang, X.; Zhao, X.; Bai, Y.; Tang, Z.; Wang, W.; Zhao, Y.; Wan, H.; Xie, Z.; Shi, X.; Wu, B.; et al. Carbon pools in China's terrestrial ecosystems: New estimates based on an intensive field survey. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 4021–4026. [CrossRef] [PubMed]
5. Xu, L.; Yu, G.; He, N.; Wang, Q.; Gao, Y.; Wen, D.; Li, S.; Niu, S.; Ge, J. Carbon storage in China's terrestrial ecosystems: A synthesis. *Sci. Rep.* **2018**, *8*, 2806. [CrossRef]
6. Fang, J.; Chen, A.; Peng, C.; Zhao, S.; Ci, L. Changes in Forest Biomass Carbon Storage in China Between 1949 and 1998. *Science* **2001**, *292*, 2320. [CrossRef]
7. Winjum, J.; Schroeder, P. Forest plantations of the world: Their extent, ecological attributes, and carbon storage. *Agric. For. Meteorol.* **1997**, *84*, 153–167. [CrossRef]
8. Du, Z.; Wang, W.; Zeng, W.; Zeng, H. Nitrogen deposition enhances carbon sequestration by plantations in northern China. *PLoS ONE* **2014**, *9*, e87975. [CrossRef]
9. Chen, H.; Li, D.; Gurmesa, G.A.; Yu, G.; Li, L.; Zhang, W.; Fang, H.; Mo, J. Effects of nitrogen deposition on carbon cycle in terrestrial ecosystems of China: A meta-analysis. *Environ. Pollut.* **2015**, *206*, 352–360. [CrossRef]
10. Galloway, J.N.; Townsend, A.R.; Erisman, J.W.; Bekunda, M.; Cai, Z.; Freney, J.R.; Martinelli, L.A.; Seitzinger, S.P.; Sutton, M.A. Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science* **2008**, *320*, 889–892. [CrossRef]
11. Magnani, F.; Mencuccini, M.; Borghetti, M.; Berbigier, P.; Berninger, F.; Delzon, S.; Grelle, A.; Hari, P.; Jarvis, P.G.; Kolari, P.; et al. The human footprint in the carbon cycle of temperate and boreal forests. *Nature* **2007**, *447*, 848–850. [CrossRef] [PubMed]
12. Hyvönen, R.; Persson, T.; Andersson, S.; Olsson, B.; Ågren, G.; Linder, S. Impact of long-term nitrogen addition on carbon stocks in trees and soils in northern Europe. *Biogeochemistry* **2007**, *89*, 121–137. [CrossRef]
13. Tian, D.; Wang, H.; Sun, J.; Niu, S. Global evidence on nitrogen saturation of terrestrial ecosystem net primary productivity. *Environ. Res. Lett.* **2016**, *11*, 024012. [CrossRef]
14. Feng, Z.; Rutting, T.; Pleijel, H.; Wallin, G.; Reich, P.B.; Kammann, C.I.; Newton, P.C.; Kobayashi, K.; Luo, Y.; Uddling, J. Constraints to nitrogen acquisition of terrestrial plants under elevated CO_2. *Glob. Chang. Biol.* **2015**, *21*, 3152–3168. [CrossRef]

15. Cao, C.; Li, S. Effect of N Form on Crop Physiological Characteristics and Growth. *J. Huazhong Agric. Univ.* **2004**, *23*, 000581–000586. [CrossRef]
16. Li, B.; Li, G.; Kronzucker, H.J.; Baluška, F.; Shi, W. Ammonium stress in Arabidopsis: Signaling, genetic loci, and physiological targets. *Trends Plant Sci.* **2014**, *19*, 107–114. [CrossRef]
17. Zhou, X.; Wang, A.; Hobbie, E.A.; Zhu, F.; Qu, Y.; Dai, L.; Li, D.; Liu, X.; Zhu, W.; Koba, K.; et al. Mature conifers assimilate nitrate as efficiently as ammonium from soils in four forest plantations. *New Phytol.* **2021**, *229*, 3184–3194. [CrossRef]
18. Zhu, X.; Liu, D.; Yin, H. Roots regulate microbial N processes to achieve an efficient NH4+ supply in the rhizosphere of alpine coniferous forests. *Biogeochemistry* **2021**, *155*, 39–57. [CrossRef]
19. Li, C.; Li, Q.; Qiao, N.; Xu, X.; Li, Q.; Wang, H. Inorganic and organic nitrogen uptake by nine dominant subtropical tree species. *iForest Biogeosci. For.* **2016**, *9*, 253–258. [CrossRef]
20. Zhang, Y.; Bai, S. Effects of nitrogen forms on nutrient uptake and growth of trees. *Chin. J. Appl. Ecol.* **2003**, *14*, 2044–2048. [CrossRef]
21. Stadler, J.; Gebauer, G. Nitrate reduction and nitrate content in ash trees (*Fraxinus excelsior* L.): Distribution between compartments, site comparison and seasonal variation. *Trees* **1992**, *6*, 236–240. [CrossRef]
22. Shelden, M.C.; Dong, B.; de Bruxelles, G.L.; Trevaskis, B.; Whelan, J.; Ryan, P.R.; Howitt, S.M.; Udvardi, M.K. Arabidopsis ammonium transporters, AtAMT1;1 and AtAMT1;2, have different biochemical properties and functional roles. *Plant Soil* **2001**, *231*, 151–160. [CrossRef]
23. Li, Y.; Zhang, J.; Chang, S.; Jiang, P.; Zhou, G.; Fu, S.; Yan, E.; Wu, J.; Lin, L. Long-term intensive management effects on soil organic carbon pools and chemical composition in Moso bamboo (*Phyllostachys pubescens*) forests in subtropical China. *For. Ecol. Manag.* **2013**, *303*, 121–130. [CrossRef]
24. Thomas, R.Q.; Canham, C.D.; Weathers, K.C.; Goodale, C.L. Increased tree carbon storage in response to nitrogen deposition in the US. *Nat. Geosci.* **2010**, *3*, 13–17. [CrossRef]
25. Tu, L.; Hu, T.; Zhang, J.; Li, X.; Hu, H.; Liu, L.; Xiao, Y. Nitrogen addition stimulates different components of soil respiration in a subtropical bamboo ecosystem. *Soil Biol. Biochem.* **2013**, *58*, 255–264. [CrossRef]
26. Berg, B.; Laskowski, R. *Litter Decomposition: A Guide to Carbon and Nutrient Turnover*; Academic Press: Cambridge, MA, USA, 2006; Volume 38, pp. 1–421. [CrossRef]
27. Liu, J.; Wu, N.; Wang, H.; Sun, J.; Peng, B.; Jiang, P.; Bai, E. Nitrogen addition affects chemical compositions of plant tissues, litter and soil organic matter. *Ecology* **2016**, *97*, 1796–1806. [CrossRef] [PubMed]
28. Liu, L.; Greaver, T.L. A global perspective on belowground carbon dynamics under nitrogen enrichment. *Ecol. Lett.* **2010**, *13*, 819–828. [CrossRef]
29. Lu, M.; Zhou, X.; Luo, Y.; Yang, Y.; Fang, C.; Chen, J.; Li, B. Minor stimulation of soil carbon storage by nitrogen addition: A meta-analysis. *Agric. Ecosyst. Environ.* **2011**, *140*, 234–244. [CrossRef]
30. Kaštovská, E.; Šantrůčková, H. Comparison of uptake of different N forms by soil microorganisms and two wet-grassland plants: A pot study. *Soil Biol. Biochem.* **2011**, *43*, 1285–1291. [CrossRef]
31. Yue, K.; Peng, Y.; Peng, C.; Yang, W.; Peng, X.; Wu, F. Stimulation of terrestrial ecosystem carbon storage by nitrogen addition: A meta-analysis. *Sci. Rep.* **2016**, *6*, 19895. [CrossRef]
32. Fan, H.; Wu, J.; Liu, W.; Yuan, Y.; Huang, R.; Liao, Y.; Li, Y. Nitrogen deposition promotes ecosystem carbon accumulation by reducing soil carbon emission in a subtropical forest. *Plant Soil* **2014**, *379*, 361–371. [CrossRef]
33. Gao, J.; Mo, Y.; Xu, X.; Zhang, X.; Yu, F. Spatiotemporal variations affect uptake of inorganic and organic nitrogen by dominant plant species in an alpine wetland. *Plant Soil* **2014**, *381*, 271–278. [CrossRef]
34. Jampeetong, A.; Konnerup, D.; Piwpuan, N.; Brix, H. Interactive effects of nitrogen form and pH on growth, morphology, N uptake and mineral contents of *Coix lacryma-jobi* L. *Aquat. Bot.* **2013**, *111*, 144–149. [CrossRef]
35. Liu, X.; Ju, X.; Zhang, Y.; He, C.; Kopsch, J.; Fusuo, Z. Nitrogen deposition in agroecosystems in the Beijing area. *Agric. Ecosyst. Environ.* **2006**, *113*, 370–377. [CrossRef]
36. Gower, S.T.; Krankina, O.; Olson, R.J.; Apps, M.; Linder, S.; Wang, C. Net Primary Production and Carbon Allocation Patterns of Boreal Forest Ecosystems. *Ecol. Appl.* **2001**, *11*, 1395–1411. [CrossRef]
37. Li, Z.; Kurz, W.A.; Apps, M.J.; Beukema, S.J. Belowground biomass dynamics in the Carbon Budget Model of the Canadian Forest Sector: Recent improvements and implications for the estimation of NPP and NEP. *Can. J. For. Res.* **2003**, *33*, 126–136. [CrossRef]
38. Yang, X.; Wang, C.; Xu, K. Response of soil CH_4 fluxes to stimulated nitrogen deposition in a temperate deciduous forest in northern China: A 5-year nitrogen addition experiment. *Eur. J. Soil Biol.* **2017**, *82*, 43–49. [CrossRef]
39. Wang, C.; Yang, X.; Xu, K. Effect of chronic nitrogen fertilization on soil CO_2 flux in a temperate forest in North China: A 5-year nitrogen addition experiment. *J. Soils Sediments* **2018**, *18*, 506–516. [CrossRef]
40. Liu, L.; Greaver, T.L. A review of nitrogen enrichment effects on three biogenic GHGs:the CO_2 sink may be largely offset by stimulated N_2O and CH_4 emission. *Ecol. Lett.* **2009**, *12*, 1103–1110. [CrossRef]
41. Vries, W.; Du, E.; Butterbach-Bahl, K. Short and long-term impacts of nitrogen deposition on carbon sequestration by forest ecosystems. *Curr. Opin. Environ. Sustain.* **2014**, *9*, 90–104. [CrossRef]
42. Schulte, U.L.; Vries, W. Global-scale impacts of nitrogen deposition on tree carbon sequestration in tropical, temperate, and boreal forests: A meta-analysis. *Glob. Chang. Biol.* **2017**, *24*, e416–e431. [CrossRef] [PubMed]

43. Reay, D.S.; Dentener, F.; Smith, P.; Grace, J.; Feely, R.A. Global nitrogen deposition and carbon sinks. *Nat. Geosci.* **2008**, *1*, 430–437. [CrossRef]
44. Xu, K.; Wang, C.; Yang, X. Five-year study of the effects of simulated nitrogen deposition levels and forms on soil nitrous oxide emissions from a temperate forest in northern China. *PLoS ONE* **2017**, *12*, e0189831. [CrossRef] [PubMed]
45. Fisk, M.; Ratliff, T.; Goswami, S.; Yanai, R. Synergistic soil response to nitrogen plus phosphorus fertilization in hardwood forests. *Biogeochemistry* **2014**, *118*, 195–204. [CrossRef]
46. Brouwer, R. Functional equilibrium: Sense or nonsense? *Neth. J. Agric. Sci.* **1983**, *31*, 335–348. [CrossRef]
47. Poorte, H.; Nagel, O. The role of biomass allocation in the growth response of plants to different levels of light, CO_2, nutrients and water: A quantitative review. *Funct. Plant Biol.* **2000**, *27*, 595–607. [CrossRef]
48. Darby, F.A.; Turner, R.E. Below- and Aboveground Biomass of *Spartina alterniflora*: Response to Nutrient Addition in a Louisiana Salt Marsh. *Estuaries Coasts* **2008**, *31*, 326–334. [CrossRef]
49. Johnson, I.; Thornley, J. A Model of Shoot: Root Partitioning with Optimal Growth. *Ann. Bot.* **1987**, *60*, 133–142. [CrossRef]
50. Zhang, W.; Zhu, X.; Liu, L.; Fu, S.; Chen, H.; Huang, J.; Lu, X.; Liu, Z.; Mo, J. Large difference of inhibitive effect of nitrogen deposition on soil methane oxidation between plantations with N-fixing tree species and non-N-fixing tree species. *J. Geophys. Res. Biogeosci.* **2012**, *117*, G00N16. [CrossRef]
51. Zhao, Y.; Zhang, C.; Zhao, H.; Xu, X. Effects of N and P addition on soil nitrogen mineralization in a subtropical evergreen broadleaved forest. *Chin. J. Ecol.* **2013**, *32*, 1690–1697. [CrossRef]
52. Chen, D.; Lan, Z.; Hu, S.; Bai, Y. Effects of nitrogen enrichment on belowground communities in grassland: Relative role of soil nitrogen availability vs. soil acidification. *Soil Biol. Biochem.* **2015**, *89*, 99–108. [CrossRef]
53. Bowman, W.D.; Cleveland, C.C.; Halada, Ĺ.; Hreško, J.; Baron, J.S. Negative impact of nitrogen deposition on soil buffering capacity. *Nat. Geosci.* **2008**, *1*, 767–770. [CrossRef]