forests

MDPI

Article

Enhanced Nitrogen Fertilizer Input Alters Soil Carbon Dynamics in Moso Bamboo Forests, Impacting Particulate Organic and Mineral-Associated Carbon Pools

Haoyu Chu [1], Huijing Ni [2], Wenhui Su [1,*], Shaohui Fan [1], Yongmei Long [1] and Yutong Sun [1]

1 International Center for Bamboo and Rattan, Key Laboratory of National Forestry and Grassland Administration, Beijing 100102, China; chuhaoyu0109@163.com (H.C.); longyongmei1006@163.com (Y.L.); sunyutong1025@163.com (Y.S.)
2 Zhejiang Academy of Forestry, Hangzhou 310023, China; hjni0529@163.com
* Correspondence: swh612@icbr.ac.cn

Abstract: The application of nitrogen fertilizer is crucial in the cultivation of bamboo forests, and comprehending the alterations in soil organic carbon (SOC) due to nitrogen application is essential for monitoring soil quality. Predicting the dynamics of soil carbon stock involves analyzing two components: particulate organic carbon (POC) and mineral-associated organic carbon (MAOC). This study aimed to investigate the impact of high nitrogen inputs on SOC stock in Moso bamboo forests located in southwestern China. The research focused on analyzing changes in soil chemical properties, SOC content, and its components (POC and MAOC), as well as microbial biomass in the surface layer (0–10 cm) under different nitrogen applications (0, 242, 484, and 726 kg N ha^{-1} yr^{-1}). The results indicate that nitrogen application significantly reduced the SOC content, while concurrently causing a significant increase in POC content and a decrease in MAOC content within the Moso bamboo forest ($p < 0.05$). The HM treatment notably increased the NO_3^--N content to 2.15 mg/kg and decreased the NH_4^+-N content to 11.29 mg/kg, although it did not significantly influence the microbial biomass carbon (MBC) and nitrogen (MBN). The LN and MN treatments significantly reduced the MBC and MBN contents (71.6% and 70.8%, 62.5% and 56.8%). Nitrogen application significantly increased the Na^+ concentration, with a peak observed under the LN treatment (135.94 mg/kg, $p < 0.05$). The MN treatment significantly increased the concentrations of Fe^{3+} and Al^{3+} ($p < 0.05$), whereas nitrogen application did not significantly affect Ca^{2+}, Mg^{2+} concentration, and cation exchange capacity ($p > 0.05$). Correlation and redundancy analyses (RDAs) revealed that the increase in annual litterfall did not significantly correlate with the rise in POC, and changes in extractable cations were not significantly correlated with the decrease in MAOC. Soil nitrogen availability, MBC, and MBN were identified as the primary factors affecting POC and MAOC content. In conclusion, the application of nitrogen has a detrimental impact on the soil organic carbon (SOC) of Moso bamboo forests. Consequently, it is imperative to regulate fertilization levels in order to preserve soil quality when managing these forests. Our research offers a theoretical foundation for comprehending and forecasting alterations in soil carbon stocks within bamboo forest ecosystems, thereby bolstering the sustainable management of Moso bamboo forests.

Keywords: nitrogen application; soil organic carbon; soil organic carbon fraction; microbial biomass; soil quality

Citation: Chu, H.; Ni, H.; Su, W.; Fan, S.; Long, Y.; Sun, Y. Enhanced Nitrogen Fertilizer Input Alters Soil Carbon Dynamics in Moso Bamboo Forests, Impacting Particulate Organic and Mineral-Associated Carbon Pools. *Forests* **2023**, *14*, 2460. https://doi.org/10.3390/f14122460

Academic Editor: Frank S. Gilliam

Received: 20 November 2023
Revised: 13 December 2023
Accepted: 14 December 2023
Published: 18 December 2023

1. Introduction

The history of bamboo production and utilization in China is long, and it is one of the countries with the most abundant bamboo resources and the widest grove area in the world [1]. Bamboo plays a pivotal role in mitigating the greenhouse effect and fostering rural development [2]. Moso bamboo (*Phyllostachys edulis* (Carrière) J. Houz) is renowned for its rapid growth, robust regeneration capabilities, and significant potential for carbon sequestration [3,4]. Given its ecological significance and economic value, Moso bamboo has garnered considerable attention from the scientific community [5]. Nitrogen stands out as the most crucial nutrient element required and absorbed during the growth and development phase of Moso bamboo [6]. The judicious application of nitrogen fertilizer can enhance bamboo forest yields while preserving soil organic matter and ensuring soil quality [7]. In actual production processes, operators often increase the yield of bamboo forests by applying substantial amounts of nitrogen fertilizer to maximize profits [8]. However, such extensive nitrogen input not only offers substantial benefits but also impacts soil microbial activity and organic matter content, thereby compromising soil quality [9]. Soil organic carbon (SOC) content serves as a critical indicator of soil quality [10]. While some studies have delved into the effects of fertilization on the SOC pool of Moso bamboo forests, elucidating the impact of nitrogen application on soil quality remains an area of ongoing research [11–13]. Investigating the alterations in the SOC pool due to nitrogen application provides insights into the mechanism through which nitrogen affects soil quality in bamboo forests. It can provide a theoretical basis for the sustainable management of Moso bamboo forests.

The composition of SOC is complex and crucial for preserving soil quality [14]. Its classification, based on formation mechanism (physical, chemical, and biological) stability (easy-to-decompose carbon pool and difficult-to-decompose carbon pool), results in various components [15–18]. However, examining the dynamics of SOC as a unified entity presents significant challenges [19,20]. By classifying soil particle size, SOC can be divided into particulate organic carbon (POC, >0.053 mm) and mineral-associated organic carbon (MAOC, <0.053 mm) [21]. Lavallee et al. assert that POC and MAOC, with their unique formation mechanisms, stabilities, and functions, warrant separate examination to effectively capture changes in the SOC pool [22]. Given the distinct turnover time and stability mechanisms of these two components, their responses to nitrogen application also vary. Chen et al. [23] found in a nitrogen application experiment conducted in Wuyi Mountain, China, that nitrogen application would increase the content of POC in evergreen broad-leaved forests in subtropical areas. Sun et al. [24] also found the same result in a nitrogen application experiment on paddy fields. They proposed that the increase in POC content could be attributed to an increase in plant-derived carbon input due to nitrogen application [25,26]. Beyond augmenting carbon input, reducing carbon loss is another significant factor contributing to the increase in POC content [27]. Increases in nitrogen application frequently result in soil acidification, a process that impedes soil microbial activity (such as microbial biomass and extracellular enzyme activity). This subsequently diminishes the carbon loss caused by microbial decomposition [23,28]. However, this relationship is not universally consistent, with some studies indicating that nitrogen application may reduce the content of POC [29,30]. For instance, Chen et al. [17] observed a reduction in SOC following nitrogen application on the Qinghai–Tibet Plateau alpine meadow. They attributed this reduction to a decline in soil microbial carbon utilization efficiency. Consequently, the impact of nitrogen application on POC depends on the balance between carbon input (derived from plants) and carbon loss (soil microbial decomposition).

In contrast to POC, the response mechanism of MAOC to nitrogen application is greater. Previous research indicates that nitrogen application has both positive [23,31] and negative effects on MAOC [26,32], which is related to the formation mechanism of MAOC [22]. Microbial metabolism, ex situ modification, and the physical fragmentation of plant residue carbon yield low-molecular-weight soluble organic carbon, which serves as the primary source for MAOC formation. This process is significantly influenced by microbial metabolism and extracellular enzyme activities. However, soil acidification induced by nitrogen application can reduce the MAOC source by hindering microbial growth [33–36]. Additionally, adsorption of organic carbon onto soil mineral surfaces is an important mechanism for the stable storage of MAOC. This mechanism encompasses both chemical processes, such as ligand substitution, complexation, hydrogen bonding, and ionic bond bridges combined with organic carbon, as well as physical isolation mechanisms protected by soil aggregates [37–40]. Nitrogen application can influence the stability of MAOC by altering the chemical mechanism of organic carbon and mineral adsorption. Soil acidification causes the leaching of extractable cations (Ca^{2+}, Mg^{2+}) and an increase in the solubility of hydrolyzed cations (Fe^{3+}, Al^{3+}) [18,41,42]. Ye et al. [15] found that nitrogen application reduced the content of MAOC in Mongolian grassland. This was attributed to soil acidification, which reduced MAOC sources and increased Fe-associated C content, collectively contributing to the reduction in MAOC. A study conducted by Sun et al. [24] showed that nitrogen application reduced MAOC content. However, this reduction was primarily due to the weakening of soil minerals' protective effect induced by nitrogen application-induced soil acidification, rather than any significant correlation with microbial activity (microbial biomass and carbon degradation gene abundance). In conclusion, the effects of nitrogen application on both POC and MAOC vary, and the mechanisms affected differ across ecosystems. Current research predominantly focuses on the impact of nitrogen application on POC and MAOC, mainly in alpine meadows, subtropical broad-leaved forests, and agricultural ecosystems. Studies on bamboo forest ecosystems primarily examine the effects of varying nitrogen application amounts and timings of nitrogen application on the characteristics of soil microorganisms related to POC and MAOC [43]. However, there is a limited exploration into the impact mechanism of large-scale nitrogen application on POC and MAOC in bamboo forests.

The precise impact of substantial nitrogen input on soil quality alterations in Moso bamboo forests remains unclear. This study focused on Moso bamboo forests in subtropical regions of China, conducting nitrogen application experiments at four different levels (0, 242, 484, and 726 kg ha^{-1}). Following a two-year nitrogen application experiment, we analyzed soil physicochemical properties, POC and MAOC content, microbial biomass, cation exchange capacity, and basic cation concentration. We aimed to investigate the mechanisms underlying the impact of nitrogen application on POC and MAOC, as well as the dynamic changes in organic carbon in Moso bamboo forest soils. To this end, we proposed the following hypotheses:

Hypotheses 1 (H1). *Nitrogen application has no significant effect on SOC but increases POC and reduces MAOC.*

Hypotheses 2 (H2). *The reason for the increase in POC content is that the reduction in microbial biomass caused by nitrogen application inhibits the decomposition of POC by microorganisms.*

Hypotheses 3 (H3). *The reason for the decrease in MAOC content is that the reduction in microbial biomass and extractable cations caused by nitrogen application leads to a decrease in microbial carbon input and a decrease in mineral adsorption.*

2. Materials and Methods

2.1. Study Sites

The nitrogen application experiment in this study was conducted in Hushi Town, Chishui City, northern Guizhou Province, China (28°23′, 105°54′). The region has a subtropical humid monsoon climate, with an average annual temperature of 18.1 °C and an annual precipitation of approximately 1195.7 mm. The soil in this area has a total carbon content of 19.8 g/kg, a bulk density of 1.25 g/kg, and a pH of 4.75. The bamboo forests exhibit an average diameter at breast height of 9.86 cm, an average height of 12.07 m, and a density of 3866 plants/ha, with noticeable on–off year variations.

Pure stands of Moso bamboo were selected based on identical growing conditions and freedom from pests or diseases. The experiment consisted of four fertilization gradients (CK, LN, MN, and HN) with rates of 0, 242, 484, and 728 kg/ha, respectively. Each plot measured 15 × 15 m, and the experiment was conducted with 12 plots, with each treatment being repeated three times. The experiment followed a randomized block design, with a 5 m isolation zone established between each plot. Previous studies by the research team have proposed an appropriate amount of nitrogen fertilizer for bamboo forests in southern China (242 kg/ha, LN treatment). The nitrogen fertilizer amounts for the MN and HN treatments were chosen to simulate the overuse of nitrogen fertilizer in production practices.

The nitrogen application experiment was conducted in October 2020 and 2021, using urea (contains 46% N) as the nitrogen fertilizer. Along with the nitrogen application, 178 kg/ha of phosphate fertilizer and 147 kg/ha of potassium fertilizer were applied to each plot, with calcium superphosphate (contains 12% P_2O_5) as the phosphate fertilizer and potash chloride (contains 60% K_2O) as the potassium fertilizer. To ensure uniform fertilization, all the fertilizers were dissolved in 50 L of deionized water and added by spraying the solution. The amount of nitrogen, phosphorus, and potassium fertilizer applied can be determined based on Su's research results [6].

After applying nitrogen in October 2020, six square frames measuring 1 m $\times$ 1 m were uniformly established within each plot to gather litterfall. The litterfall within these frames was then collected on a monthly basis to determine the annual litterfall within the bamboo forest after nitrogen application. The data collection for the litterfall was conducted until October 2022.

2.2. Collection and Determination of Soil Samples

2.2.1. Soil Sample Collection

Soil samples were collected in October 2022, with 10 sampling points set up for each plot using the "S"-shaped sampling method. Ten sampling points were set up for each plot, and the surface litter was cleaned before drilling a soil core from the 0–10 cm soil layer using a soil auger with an inner diameter of 38 mm. A composite soil sample was formed by mixing 10 soil cores collected from each plot. A total of 12 soil samples were collected. The freshly collected soil was immediately stored in a portable insulated box and refrigerated for preservation. A portion of the fresh soil was then sieved through a 2 mm screen to remove stones, plant debris, roots, and other impurities. This was carried out to determine the soil enzyme activity, microbial biomass carbon and nitrogen, nitrate nitrogen, and ammonium nitrogen. Some soil was air-dried, ground, and sieved to determine the soil pH, organic carbon, total nitrogen, and extractable cations in the soil. Additionally, a portion of the fresh soil was carefully broken into 1 cm pieces along the cracks of the soil block, air-dried, and used to determine the POC and MAOC.

2.2.2. Soil Basic Chemical Properties

The SOC was determined using the potassium dichromate heating method. Total nitrogen (TN) was measured using an automatic Kjeldahl nitrogen analyzer (K986, Hai Neng, China). Ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) were first extracted from the soil with a potassium chloride solution, and then the contents of NH_4^+-N and NO_3^--N were determined via indophenol blue colorimetry and ultraviolet spectrophotometry, respectively. Prior to determining the pH, the soil sample is mixed with distilled water. This process was executed using the glass electrode method (ST2100, Ohaus, Parsippany, NJ, USA).

2.2.3. Soil Cation Exchange Capacity and Extractable Cations

The soil cation exchange capacity (CEC) was determined by extracting a cobalt hexaammonium trichloride solution and comparing the difference in absorbance of the extract before and after leaching. Extractable cations (Fe^{3+}, Al^{3+}, Ca^{2+}, Mg^{2+}, Na^+) were determined using the method outlined by Bowman et al. [44]. The air-dried soil was extracted with a 0.1 M $BaCl_2$ solution, and after centrifugation, the supernatant was determined via inductively coupled plasma mass spectrometry (ICP-MS; Thermo Fisher, Waltham, MA, USA).

2.2.4. Soil Microbial Biomass Carbon and Nitrogen

The chloroform fumigation extraction method was employed to determine the soil microbial biomass [45]. Initially, 30 g of fresh soil was taken and fumigated with ethanol-free $CHCl_3$ for 24 h at 25 °C in dark conditions. Another 30 g of fresh soil was taken without fumigation. The fumigated and unfumigated soils were extracted with 40 mL of 0.5 M K_2SO_4 in a shaker for 30 min and then filtered, respectively. The filtered extract is directly used to determine its total carbon and nitrogen content with a carbon and nitrogen analyzer (TOC-LCSH/CPH, Shimadzu, Shanghai, China). The difference between the TC and TN content of the fumigated and unfumigated soil extracts is multiplied by 2.2 and 1.85, respectively, which are the contents of microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN).

2.2.5. Soil Organic Carbon Fractions

The soil particulate organic matter (POM) and mineral-associated organic matter (MAOM) were obtained using the soil wet-sieving method, as improved by Marriott and Wander [46]. To execute this method, 20 g of air-dried soil was placed in a 50 mL centrifuge tube, mixed with 10 mL of a 5% sodium hexametaphosphate solution, and shaken at 25 °C for 18 h (90 rpm). The soil suspension was sieved through a 53 μL sieve and repeatedly rinsed with distilled water. The fraction retained on the sieve is particulate organic matter (POM), while the fraction that passed through the sieve is mineral-associated organic matter (MAOM). The organic carbon content, which includes both POC and MAOC, was measured after drying the samples at 60 °C.

2.2.6. Soil Extracellular Enzyme Activities

The activities of soil catalase (CAT), peroxidase (POD), urease (urease), and acid phosphatase (AP) were determined using a double-antibody one-step sandwich enzyme-linked immunosorbent assay (ELISA). Taking POD as an example, 1 g of fresh soil was first extracted with 9 mL of phosphate buffer, thoroughly mixed, and then centrifuged at 2500 rpm for 25 min at a constant temperature of 4 °C. The supernatant was collected for the determination of extracellular enzymes in the soil. After incubation and washing, the OD value of each sample was measured using an enzyme marker. By plotting the concentration of standard substances against the OD value, the concentration of the sample was calculated. The methods for determining the remaining extracellular enzymes in the soil were the same. The specific operation steps can be found in the study by Xia et al. [47].

2.3. Data Analysis

This study utilized a one-way ANOVA to assess differences in soil chemical properties, microbial biomass carbon and nitrogen, extracellular enzyme activity, and organic carbon components ($p < 0.05$), followed by Duncan's post hoc test. A Pearson correlation analysis was used to assess the relationship between the soil chemical properties, microbial biomass carbon and nitrogen, extracellular enzyme activity, POC, and MAOC. The soil nitrogen availability was expressed as the sum of NO_3^--N and NH_4^+-N. A redundancy analysis (RDA) was used to investigate the most significant environmental factors that affect SOC, POC, and MAOC. Prior to conducting the RDA, significant environmental factors related to SOC, POC, and MAOC were selected using the Pearson correlation analysis. A principal component analysis (PCA) was performed on extractable cations, microbial biomass carbon and nitrogen, and the soil extracellular enzyme activity to create new variables for each (extractable cations, microbial biomass, and soil extracellular enzyme activity), and all data from the environmental factors were standardized before the RDA. All data organization in this study was completed using Excel 2021 (Microsoft Corp., Redmond, WA, USA); the one-way ANOVA and Pearson correlation analysis were performed using SPSS 21.0 (SPSS, Chicago, IL, USA); and the RDA was performed using Canoco 5 (Canoco, Ithaca, NY, USA) (Monte Carlo test, 499 times). All figures were created using the "ggplot2" package in R Studio (version 3.4.2; R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Effect of Nitrogen Application on Soil Organic Carbon Fractions (POC and MAOC)

It can be seen from Figure 1 that nitrogen application decreased the SOC content significantly ($p < 0.05$) under the LN and MN treatments, which were 29.1% and 24.7% lower than that under CK, respectively. However, the reduction under the HN treatment was not significant ($p > 0.05$). In addition, the SOC content increased with the increase in the nitrogen application gradient (12.62–17.68 g/kg). The SOC content was significantly higher under the HN treatment than under the LN treatment ($p < 0.05$). Although nitrogen application reduced the SOC content, it resulted in a significant increase in the POC content and a significant decrease in the MAOC content. It can be seen from Figure 1B that the POC content under the LN and MN treatments significantly increased ($p < 0.05$) from 1.07 g/kg under CK to 1.79 and 1.61 g/kg, respectively, while the increase under the HN treatment was not significant (1.42 g/kg, $p > 0.05$), and the POC content decreased with the increase in the nitrogen application gradient. The MAOC content was significantly reduced ($p < 0.05$) by nitrogen application, approximately by 24.7%–35.1%, and it increased with the increase in the nitrogen application gradient.

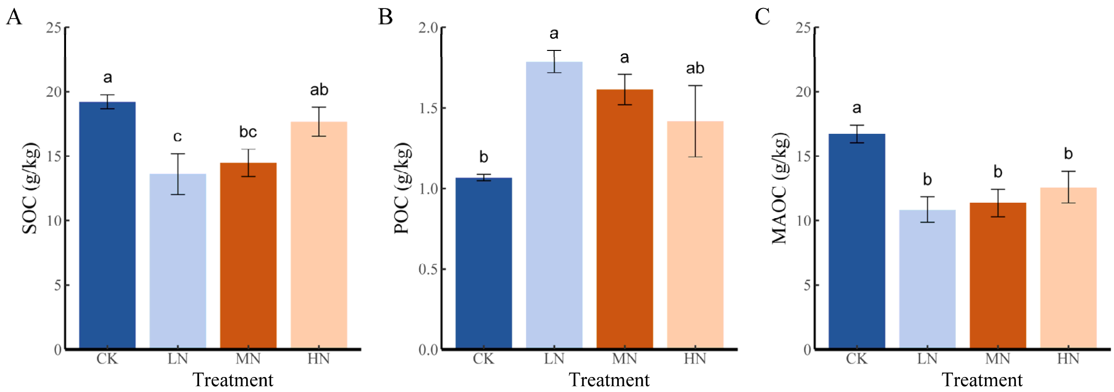

Figure 1. Effects of nitrogen application on soil organic carbon fractions in Moso bamboo forests. Lowercase letters in each column indicate significant differences ($p < 0.05$) between the different nitrogen application treatments (CK, LN, MN, and HN). (**A**–**C**) indicate the response of SOC, POC and MAOC to nitrogen application, respectively. POC, soil particulate organic carbon; MAOC, soil mineral-associated organic carbon.

3.2. Effects of Nitrogen Application on Soil Basic Chemical Properties

Nitrogen application increased the NO_3^--N content in the soil. The MN and HN treatments showed significant increases of 39.5% and 88.6%, respectively, compared to CK ($p < 0.05$, Table 1). Conversely, nitrogen application decreased the NH_4^+-N content in the soil, with a significant decrease in the HN treatment, which was 11.29 mg/kg, and a 29.2% reduction compared to CK ($p < 0.05$, Table 1). The soil TN, pH, and soil C/N were not significantly affected by nitrogen application ($p > 0.05$, Table 1). Nitrogen application had no significant effect on the CEC and Ca^{2+} concentration ($p > 0.05$). However, as the nitrogen gradient increased, the Fe^{3+} concentration was significantly increased in the MN and HN treatments ($p < 0.05$), and the Al^{3+} concentration was significantly increased in the HN treatment ($p < 0.05$). Nitrogen application did not affect the difference between the Mg^{2+} concentration and the control ($p > 0.05$), but the Mg^{2+} concentration increased with the increasing nitrogen gradient, and the difference between the LN and HN treatments was significant (0.59 and 1.05 mg/kg, $p < 0.05$). Nitrogen application significantly elevated the concentration of Na^+ ($p < 0.05$), with the highest concentration observed in the LN treatment (135.94 mg/kg). Additionally, a positive correlation was noted between the amount of nitrogen applied and a decrease in the Na^+ concentration (Figure 2).

Table 1. Effects of nitrogen application on soil chemical properties of Moso bamboo forests. Lowercase letters in each column indicate significant differences ($p < 0.05$) between different nitrogen application treatments (CK, LN, MN, and HN). TN denotes total soil nitrogen.

	TN (g/kg)	NO_3^--N (mg/kg)	NH_4^+-N (mg/kg)	pH	Soil C/N
CK	1.41 ± 0.09 a	1.14 ± 0.11 c	15.96 ± 1.39 a	4.57 ± 0.03 a	13.73 ± 0.86 a
LN	1.17 ± 0.04 a	1.53 ± 0.08 cb	13.93 ± 0.85 ab	4.58 ± 0.03 a	12.1 ± 1.23 a
MN	1.25 ± 0.11 a	1.59 ± 0.66 b	14.44 ± 0.93 ab	4.68 ± 0.07 a	12.04 ± 0.47 a
HN	1.45 ± 0.12 a	2.15 ± 0.1 a	11.29 ± 1.05 b	4.61 ± 0.09 a	13.08 ± 0.78 a

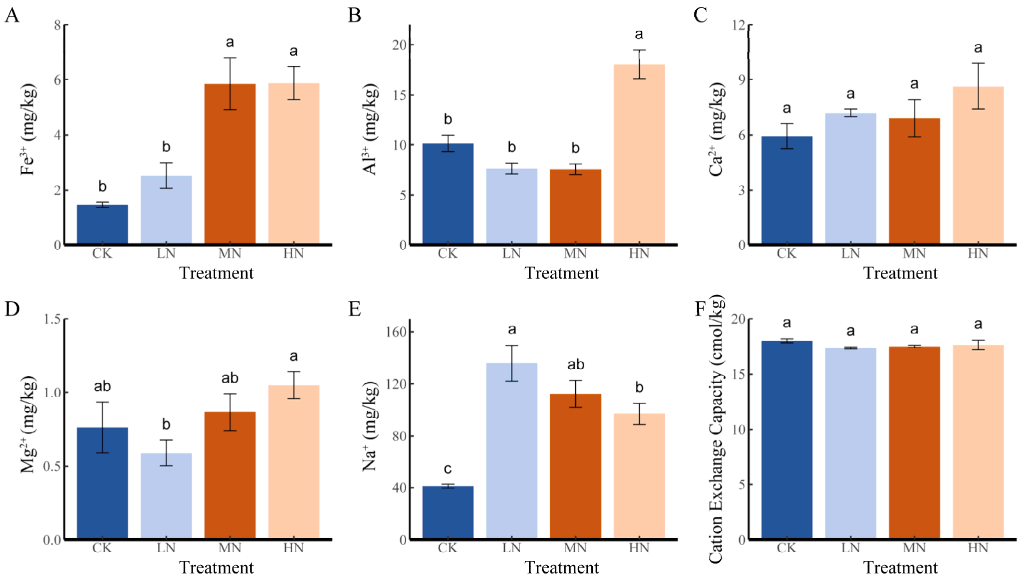

Figure 2. Effect of nitrogen application on extractable cations of Moso bamboo forests. Lowercase letters in each column indicate significant differences between different nitrogen application treatments (CK, LN, MN, and HN) ($p < 0.05$). (**A**–**F**) indicate the response of Fe^{3+}, Al3$^+$, Ca^{2+}, Mg^{2+}, Na^+, CEC to nitrogen application, respectively. CEC, cation exchange capacity.

3.3. Effects of Nitrogen Application on Soil Microbial Biomass and Extracellular Enzyme Activities

It can be seen from Figure 3 that the application of nitrogen significantly affected the MBC and MBN contents. The LN and MN treatments significantly reduced the MBC content by 71.6% and 70.8%, respectively ($p < 0.05$), while the HN treatment had no significant effect on the MBC content ($p > 0.05$). The MBN content responded similarly to nitrogen application as MBC, with the LN and MN treatments causing significant reductions of 62.5% and 56.8% ($p < 0.05$), respectively. The HN treatment had no significant effect on the MBN content ($p > 0.05$, Figure 3A). In addition, the MBC and MBN contents increased with the increase in the nitrogen application gradient (117.24–224.98 mg/kg, 20.67–58.09 mg/kg) (Figure 3B). Nitrogen application significantly reduced MBC/MBN ($p < 0.05$), and there was no significant difference among various nitrogen application treatments ($p > 0.05$, Figure 3C). Nitrogen application decreased the activity of CAT, and the reduction in CAT activity from the LN and MN treatments was significant ($p < 0.05$). The urease activity was significantly affected by nitrogen application ($p < 0.05$), which decreased by approximately 5.3%–12.5%. There was no significant effect of nitrogen application on the POD and AP activities ($p > 0.05$). The effects of nitrogen application on soil extracellular enzymes are shown in Figure 4.

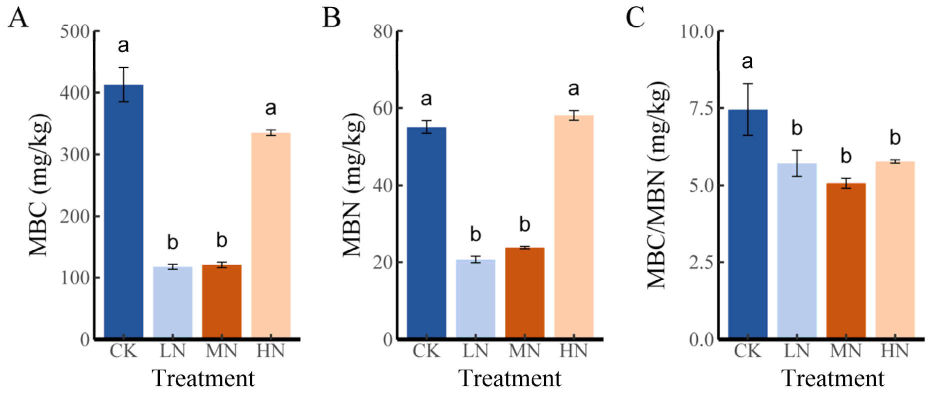

Figure 3. Effects of nitrogen application on soil microbial biomass in Moso bamboo forests. Lowercase letters in each column indicate significant differences ($p < 0.05$) between different nitrogen application treatments (CK, LN, MN, and HN). (**A**–**C**) indicate the response of MBC, MBN and MBC/MBN to nitrogen application, respectively. MBC, microbial carbon; MBN, microbial nitrogen; MBC/MBN, the ratio of microbial carbon to microbial nitrogen.

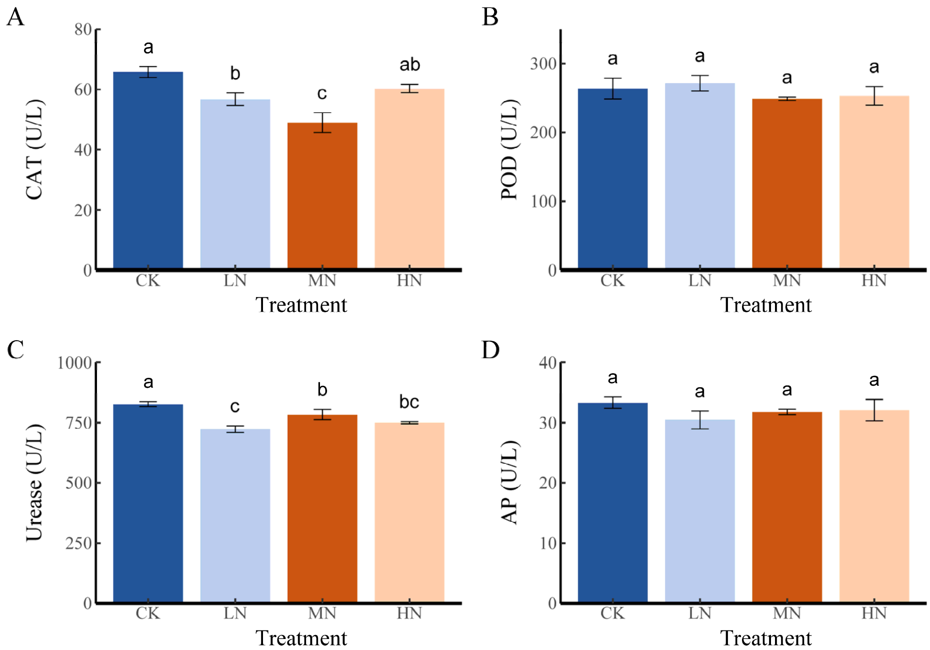

Figure 4. Effect of nitrogen application on enzyme activities in Moso bamboo forests. Lowercase letters in each column indicate significant differences ($p < 0.05$) between different nitrogen application treatments (CK, LN, MN, and HN). (**A**–**D**) indicate the response of CAT, POD, Urease and AP to nitrogen application, respectively. CAT, catalase; POD, peroxidase; AP, acid phosphatase.

3.4. Main Factors Affecting Soil Organic Carbon Fractions in Moso Bamboo Forests

From the correlation analysis, it can be seen that POC had a negative correlation with CEC, MBC, and MBN ($p < 0.05$) and a positive correlation with Na^+ ($p < 0.05$). Meanwhile, MAOC had a significant correlation with MBC, MBN, and CAT activity ($p < 0.05$) and a negative correlation with Na^+ ($p < 0.05$). The annual litterfall of Moso bamboo forests increased with nitrogen application (Figure S2). The increase was positively correlated with POC and negatively correlated with MAOC, but the relationship was not statistically significant ($p > 0.05$, Figure S1). The RDA used the soil nitrogen availability, extractable cations, microbial biomass, soil enzyme activity, and annual litterfall as explanatory variables and POC and MAOC as response variables. The results showed that the explanation rates of changes in POC and MAOC under nitrogen application conditions of the first and second axes were 77.62% and 0.56%, respectively (Figure 5A). Through the ranking of explanatory variables, it was found that the explanation rate of microbial biomass and nitrogen availability reached a significant level ($p < 0.05$) (Figure 5B).

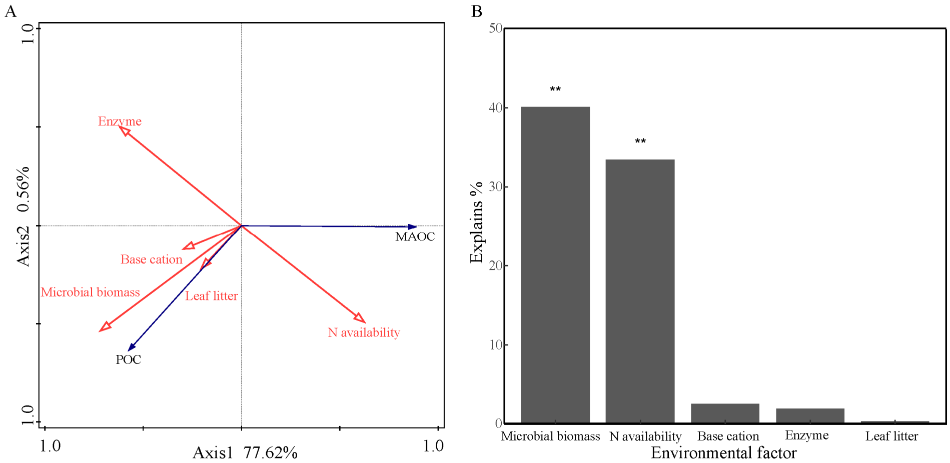

Figure 5. Redundancy analysis of soil environmental factors on POC and MAOC in Moso bamboo forests under nitrogen application. (**A**) shows the redundancy analysis, and (**B**) shows the degree of explanation of environmental factors. ** $p < 0.01$.

4. Discussion

4.1. The Effect of Nitrogen Application on the Basic Chemical Properties of Soil

After applying nitrogen to Moso bamboo forests, we discovered that the soil NO_3^--N significantly increased while the NH_4^+-N decreased. This phenomenon may be attributed to several factors. First, it could be linked to the rise in nitrification (Table 1), which promotes substrate consumption and product generation [48,49]. Furthermore, this phenomenon may be attributed to nitrogen runoff. Zhao et al. discovered that the application of nitrogen amplified soil nitrogen loss in a Moso bamboo forest after establishing a runoff field. They observed that NH_4^+-N loss exceeded that of NO_3^--N [50]. The slope of the study area was approximately 25°, and the nitrogen was applied through water spraying. These factors can increase the loss of soil nitrogen through runoff, resulting in a decrease in NH_4^+-N. The application of nitrogen did not significantly affect soil acidity in this study. According to the "charge balance theory", soil with a high initial pH is more sensitive to acidification than soil with a low initial pH [51]. The initial acidity of the soil in the study area was 4.57 (close to four), which may not be sensitive to soil acidification caused by nitrogen application (Table 1). In addition, Moso bamboo has a large demand for N elements and a strong ability of the ecosystem to absorb nitrogen, which can, to some extent, reduce the aggravation of soil acidification caused by nitrogen application. The soil C/N ratio was minimally affected by nitrogen application, similar to the results of nitrogen application experiments in other subtropical regions [23,52]. (Table 1).

The application of nitrogen significantly increased the extractable Fe^{3+} and Al^{3+} but had little effect on Ca^{2+} and Mg^{2+} (Figure 2). Soils with different initial pH levels have different buffering capacities for soil acidification. Generally, when soil acidification occurs, low-valence cations in soil with a high initial pH are consumed first. As the soil pH decreases and low-valence cations are depleted, high-valence cations are used to alleviate soil acidification [53]. The study area's soil entered the Al^{3+} buffer stage due to a high concentration of applied nitrogen, which could potentially cause the toxicity of Al^{3+} to the soil Al^{3+}. Unlike most studies where there was no significant effect of applied nitrogen on Na^+ [54], applied nitrogen significantly increased the extractable Na^+ in this study (Figure 2). This increase may be related to the decomposition of MAOC, which releases the Na^+ bound to it. The application of nitrogen had a slight effect on the CEC of the soil in this region. The small changes in the soil pH suggest a high CEC, which is associated with higher levels of carbon in the soil [55].

4.2. Effect of Nitrogen Application on Soil Microbiota and Soil Extracellular Enzyme Activities

The application of nitrogen reduced the activity of MBC, MBN, MBC/MBN, CAT, and urease, but had no significant effect on the activity of POD and AP (Figure 3). The mechanism behind the nitrogen-induced reduction in MBC and MBN is complex and can be attributed to nitrogen toxicity itself, soil acidification caused by nitrogen application, and a decrease in belowground carbon input. Furthermore, carbon limitation caused by increased nitrogen availability also contributes to limiting the growth of soil microorganisms [15,56,57]. The decrease in microbial stoichiometry MBC/MBN also suggests that nitrogen application may exacerbate microbial carbon limitation and inhibit microbial biomass [58]. The MN treatment had no significant effect on MBC and MBN, which may be related to the loss of nitrogen. Studies have shown that there is a univariate quadratic regression relationship between the amount of nitrogen applied and microbial biomass carbon and nitrogen [59]. Due to runoff losses, the actual amount of nitrogen retained in the soil is lower than the amount of nitrogen applied, which may cause the amount of nitrogen retained in the soil not to reach its peak, affecting microbial biomass carbon and nitrogen, resulting in the phenomenon observed in the experiment. The response of CAT to nitrogen application is the same as the results of Tu exploring the effects of nitrogen application on the extracellular enzyme activity in bamboo forest soil [60]. According to “resource allocation theory” and the “optimal allocation principle”, nitrogen application will change soil extracellular enzyme activity, and soil microorganisms alleviate nutrient limitation by increasing the production of resource-deficient enzymes. Conversely, when resources are enriched, it will reduce the production of related enzymes to supply its own growth [61–63]. The application of urea in this study led to an increase in the substrate for nitrification, leading to substrate enrichment that caused microorganisms to reduce the allocation of urease production, thereby lowering urease activity. Nitrogen application had little effect on the POD and AP activities, which is similar to the study of Jing et al. [64].

4.3. Effect of Nitrogen Application on Soil Organic Carbon and Its Components

Contrary to Hypothesis 1, nitrogen application reduced SOC, in agreement with some previous research conclusions [65,66] (Figure 1A). The chemical components of POC are mostly complex compounds with a high activation energy and cannot be directly absorbed and utilized by microorganisms [22]. In this study, POC was increased by nitrogen application (Figure 1B), which is consistent with the research on POC in some nitrogen application experiments [17,23,24]. Most studies attributed the increase in POC by nitrogen application to carbon input from the above- and belowground parts of plants and/or the inhibition of POC decomposition by soil microorganisms [67,68]. Through redundancy and correlation analyses, we can find that (Figures 5 and S1), although nitrogen application increased the annual litterfall in bamboo forests, it had no significant correlation with changes in POC and had a low explanation rate for changes in POC. In addition, we also found that POC was negatively correlated with MBC and MBN, the explanation rate of microbial biomass for POC was high, and the decrease in microbial biomass may lead to its reduction in the decomposition of POC. This indicates that the main reason for the increase in POC in this study may be that nitrogen application inhibited the decomposition of POC by microorganisms. This finding is consistent with Hypothesis 2.

Some previous studies have shown that nitrogen application increased MAOC; in contrast, our study found that nitrogen application significantly reduced MAOC (Figure 1C). The formation and stabilization mechanism of MAOC is more complex than that of POC, and soil microorganisms play a vital role in the turnover process of MAOC [36,43]. Microbial residues are considered to be the main carbon source for MAOC [69], and the stoichiometric imbalance caused by nitrogen application in this study may lead to nutrient limitation for soil microorganisms. This limitation could inhibit microbial growth and reduce the process of microbial residues binding with minerals to form MAOC, and the decrease in carbon input may contribute to the reduction in MAOC. Zeng et al. found that nitrogen application exacerbated microbial carbon and phosphorus limitations when exploring nitrogen application on soil microbial nutrient limitation in bamboo forests, and this result verified our reasoning well [70]. In addition, MAOC is difficult for microorganisms to decompose due to its chemical and physical protection mechanisms, but most of its chemical components are simple compounds with a low activation energy and are easier for microorganisms to absorb [22]. Studies have shown that when there is a stoichiometric imbalance in soil nutrient resources, microorganisms will decompose desorbed MAOC relatively easily (MAOC formed by cation binding or van der Waals adsorption) for their own growth under nutrient limitation conditions, and the increase in microbial decomposition may be a potential reason for the reduction in MAOC [71–73]. Furthermore, the mechanisms of soil mineral protection play a crucial role in maintaining the stability of MAOC. Some existing research results show that the loss of Ca^{2+} caused by soil acidification is an important mechanism for reducing MAOC [23,24], but our study did not find that Ca^{2+} and soil pH were significantly affected by nitrogen application. According to the results of the correlation and redundancy analyses (Figure S1 and Figure 5), except for Na^{+}, the other cations did not have a significant correlation with MAOC. The explanation rate of basic cations for changes in MAOC was low, and CEC was little affected by nitrogen application in this study. The changes in base soil cations were relatively stable, indicating that nitrogen application had little effect on the adsorption between SOC and soil minerals. In conclusion, the reduction in MAOC in this study may be attributed to the soil stoichiometric imbalance caused by nitrogen application, leading to nutrient limitation for microorganisms, resulting in decreased microbial carbon input and increased microbial decomposition. This finding differs from Hypothesis 3.

5. Conclusions

Our findings highlight the significant role of microorganisms in the dynamic changes in soil carbon pools in Moso bamboo forests. The regulation of POC (increase) and MAOC (decrease) responses to nitrogen application via microbial nutrient limitation underscores the influence of microbial activity on these soil carbon components. The decrease in SOC was a comprehensive reflection of the dynamic changes in POC and MAOC. The subdivision of SOC into POC and MAOC components provides valuable insights into understanding the nuanced response of soil carbon pools to nitrogen application. In conclusion, the decline in stable MAOC stocks due to nitrogen application underscores that excessive nitrogen application is detrimental to the long-term stability of soil carbon stocks in Moso bamboo forests. This not only compromises soil quality but also poses challenges for the sustainable management of these ecosystems. Our research can provide a theoretical basis for understanding and predicting changes in soil carbon pools in bamboo forest ecosystems and support the sustainable management of Moso bamboo forests.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14122460/s1, Figure S1: Correlation analysis among basic chemical properties, soil microbial biomass and extracellular enzyme activities, and soil organic carbon fractions in Moso bamboo forests under nitrogen application conditions. Figure S2: Effect of nitrogen application on annual litterfall in Moso bamboo forests.

Author Contributions: Conceptualization, S.F. and W.S.; formal analysis H.N.; methodology, H.N. and S.F.; data curation, H.C., Y.L. and Y.S.; writing—original draft, H.C.; writing—review and editing, H.C. and W.S.; visualization, H.C., Y.L. and Y.S.; funding acquisition, W.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by Basic Scientific Research Funding of the International Center for Bamboo and Rattan (1632023008) and Research Projects on Special Forestry Industries in Guizhou Province (2020-01).

Data Availability Statement: The original contributions presented in the study are included in the article and Supplementary Materials. Further inquiries can be directed to the corresponding author.

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

References

1. Jiang, Z. *Bamboo and Rattan in the World*; China Forest Publishing House: Beijing, China, 2002.
2. Zhao, J.; Cai, C. Physiological Integration Improves Nitrogen Use Efficiency of Moso Bamboo: An Isotopic (^{15}N) Assessment. *For. Ecol. Manag.* **2023**, *542*, 121073. [CrossRef]
3. Vogtlander, J.G.; van der Lugt, P. *The Environmental Impact of Industrial Bamboo Products: Life-Cycle Assessment and Carbon Sequestration*, 2nd ed.; INBAR Technical Report; The International Network for Bamboo and Rattan: Beijing, China, 2015; ISBN 978-92-95098-89-3.
4. Song, X.; Peng, C.; Ciais, P.; Li, Q.; Xiang, W.; Xiao, W.; Zhou, G.; Deng, L. Nitrogen Addition Increased CO_2 Uptake More than Non-CO_2 Greenhouse Gases Emissions in a Moso Bamboo Forest. *Sci. Adv.* **2020**, *6*, eaaw5790. [CrossRef]
5. Song, X.; Zhou, G.; Jiang, H.; Yu, S.; Fu, J.; Li, W.; Wang, W.; Ma, Z.; Peng, C. Carbon Sequestration by Chinese Bamboo Forests and Their Ecological Benefits: Assessment of Potential, Problems, and Future Challenges. *Environ. Rev.* **2011**, *19*, 418–428. [CrossRef]
6. Su, W. *Fertilization Theory and Practice for Phyllostachys Edulis Stand Based on Growth and Nutrient Accumulation Rules*; Chinese Academy of Forestry: Beijing, China, 2012.
7. Zhao, J. *Study on the Rule of Nitrogen Utiliztion and Its Influence Factors in Phyllostachys edilis Forests*; Chinese Academy of Forestry: Beijing, China, 2016.
8. Su, W.; Fan, S.; Zhao, J.; Cai, C. Effects of Various Fertilization Placements on the Fate of Urea-15N in Moso Bamboo Forests. *For. Ecol. Manag.* **2019**, *453*, 117632. [CrossRef]
9. Singh, B. Are Nitrogen Fertilizers Deleterious to Soil Health? *Agronomy* **2018**, *8*, 48. [CrossRef]
10. Babur, E.; Dindaroğlu, T.; Roy, R.; Seleiman, M.F.; Ozlu, E.; Battaglia, M.L.; Uslu, Ö.S. Chapter 9—Relationship between Organic Matter and Microbial Biomass in Different Vegetation Types. In *Microbial Syntrophy-Mediated Eco-Enterprising*; Singh, R.P., Manchanda, G., Bhattacharjee, K., Panosyan, H., Eds.; Developments in Applied Microbiology and Biotechnology; Academic Press: Cambridge, MA, USA, 2022; pp. 225–245. ISBN 978-0-323-99900-7.
11. Davidson, E.A.; Janssens, I.A. Temperature Sensitivity of Soil Carbon Decomposition and Feedbacks to Climate Change. *Nature* **2006**, *440*, 165–173. [CrossRef]
12. Zhou, G.; Xu, J.; Wu, J.; Jiang, P. Changes in soil active organic with history of intensive management of *Phyllostachy pubescens* forest. *Sci. Silvae Sin.* **2006**, *42*, 124–128.
13. Ma, S.; Li, Z.; Wang, G.; Liu, R.; Fu, M. Effects of intensive and extensive management on soil active organic carbon in bamboo forests in China. *Chin. J. Plant Ecol.* **2011**, *35*, 551–557. [CrossRef]
14. Mao, X.; Zheng, J.; Yu, W.; Guo, X.; Xu, K.; Zhao, R.; Xiao, L.; Wang, M.; Jiang, Y.; Zhang, S.; et al. Climate-Induced Shifts in Composition and Protection Regulate Temperature Sensitivity of Carbon Decomposition through Soil Profile. *Soil Biol. Biochem.* **2022**, *172*, 108743. [CrossRef]
15. Ye, C.; Chen, D.; Hall, S.J.; Pan, S.; Yan, X.; Bai, T.; Guo, H.; Zhang, Y.; Bai, Y.; Hu, S. Reconciling Multiple Impacts of Nitrogen Enrichment on Soil Carbon: Plant, Microbial and Geochemical Controls. *Ecol. Lett.* **2018**, *21*, 1162–1173. [CrossRef] [PubMed]
16. Rowley, M.C.; Grand, S.; Verrecchia, É.P. Calcium-Mediated Stabilisation of Soil Organic Carbon. *Biogeochemistry* **2018**, *137*, 27–49. [CrossRef]
17. Chen, Y.; Liu, X.; Hou, Y.; Zhou, S.; Zhu, B. Particulate Organic Carbon Is More Vulnerable to Nitrogen Addition than Mineral-Associated Organic Carbon in Soil of an Alpine Meadow. *Plant Soil* **2021**, *458*, 93–103. [CrossRef]
18. Wang, S.; Jia, Y.; Liu, T.; Wang, Y.; Liu, Z.; Feng, X. Delineating the Role of Calcium in the Large-Scale Distribution of Metal-Bound Organic Carbon in Soils. *Geophys. Res. Lett.* **2021**, *48*, e2021GL092391. [CrossRef]
19. Lu, X.; Hou, E.; Guo, J.; Gilliam, F.S.; Li, J.; Tang, S.; Kuang, Y. Nitrogen Addition Stimulates Soil Aggregation and Enhances Carbon Storage in Terrestrial Ecosystems of China: A Meta-analysis. *Glob. Chang. Biol.* **2021**, *27*, 2780–2792. [CrossRef] [PubMed]
20. Lugato, E.; Lavallee, J.M.; Haddix, M.L.; Panagos, P.; Cotrufo, M.F. Different Climate Sensitivity of Particulate and Mineral-Associated Soil Organic Matter. *Nat. Geosci.* **2021**, *14*, 295–300. [CrossRef]
21. Gosling, P.; Parsons, N.; Bending, G.D. What Are the Primary Factors Controlling the Light Fraction and Particulate Soil Organic Matter Content of Agricultural Soils? *Biol. Fertil. Soils* **2013**, *49*, 1001–1014. [CrossRef]
22. Lavallee, J.M.; Soong, J.L.; Cotrufo, M.F. Conceptualizing Soil Organic Matter into Particulate and Mineral-Associated Forms to Address Global Change in the 21st Century. *Glob. Chang. Biol.* **2020**, *26*, 261–273. [CrossRef] [PubMed]
23. Chen, J.; Xiao, W.; Zheng, C.; Zhu, B. Nitrogen Addition Has Contrasting Effects on Particulate and Mineral-Associated Soil Organic Carbon in a Subtropical Forest. *Soil Biol. Biochem.* **2020**, *142*, 107708. [CrossRef]
24. Sun, T.; Mao, X.; Han, K.; Wang, X.; Cheng, Q.; Liu, X.; Zhou, J.; Ma, Q.; Ni, Z.; Wu, L. Nitrogen Addition Increased Soil Particulate Organic Carbon via Plant Carbon Input Whereas Reduced Mineral−associated Organic Carbon through Attenuating Mineral Protection in Agroecosystem. *Sci. Total Environ.* **2023**, *899*, 165705. [CrossRef]
25. Schulte-Uebbing, L.; De Vries, W. Global-Scale Impacts of Nitrogen Deposition on Tree Carbon Sequestration in Tropical, Temperate, and Boreal Forests: A Meta-analysis. *Glob. Chang. Biol.* **2018**, *24*, e416–e431. [CrossRef]

26. Feng, X.; Qin, S.; Zhang, D.; Chen, P.; Hu, J.; Wang, G.; Liu, Y.; Wei, B.; Li, Q.; Yang, Y.; et al. Nitrogen Input Enhances Microbial Carbon Use Efficiency by Altering Plant–Microbe–Mineral Interactions. *Glob. Chang. Biol.* **2022**, *28*, 4845–4860. [CrossRef]
27. Janssens, I.A.; Dieleman, W.; Luyssaert, S.; Subke, J.-A.; Reichstein, M.; Ceulemans, R.; Ciais, P.; Dolman, A.J.; Grace, J.; Matteucci, G.; et al. Reduction of Forest Soil Respiration in Response to Nitrogen Deposition. *Nat. Geosci.* **2010**, *3*, 315–322. [CrossRef]
28. Zhang, T.; Chen, H.Y.H.; Ruan, H. Global Negative Effects of Nitrogen Deposition on Soil Microbes. *ISME J.* **2018**, *12*, 1817–1825. [CrossRef]
29. Cusack, D.F.; Silver, W.L.; Torn, M.S.; McDowell, W.H. Effects of Nitrogen Additions on Above- and Belowground Carbon Dynamics in Two Tropical Forests. *Biogeochemistry* **2011**, *104*, 203–225. [CrossRef]
30. Wang, X.; Cammeraat, E.L.H.; Cerli, C.; Kalbitz, K. Soil Aggregation and the Stabilization of Organic Carbon as Affected by Erosion and Deposition. *Soil Biol. Biochem.* **2014**, *72*, 55–65. [CrossRef]
31. Shen, D.; Ye, C.; Hu, Z.; Chen, X.; Guo, H.; Li, J.; Du, G.; Adl, S.; Liu, M. Increased Chemical Stability but Decreased Physical Protection of Soil Organic Carbon in Response to Nutrient Amendment in a Tibetan Alpine Meadow. *Soil Biol. Biochem.* **2018**, *126*, 11–21. [CrossRef]
32. Wang, X.; Feng, J.; Ao, G.; Qin, W.; Han, M.; Shen, Y.; Liu, M.; Chen, Y.; Zhu, B. Globally Nitrogen Addition Alters Soil Microbial Community Structure, but Has Minor Effects on Soil Microbial Diversity and Richness. *Soil Biol. Biochem.* **2023**, *179*, 108982. [CrossRef]
33. Bradford, M.A.; Keiser, A.D.; Davies, C.A.; Mersmann, C.A.; Strickland, M.S. Empirical Evidence That Soil Carbon Formation from Plant Inputs Is Positively Related to Microbial Growth. *Biogeochemistry* **2013**, *113*, 271–281. [CrossRef]
34. Liang, C.; Schimel, J.P.; Jastrow, J.D. The Importance of Anabolism in Microbial Control over Soil Carbon Storage. *Nat. Microbiol.* **2017**, *2*, 17105. [CrossRef]
35. Samson, M.-E.; Chantigny, M.H.; Vanasse, A.; Menasseri-Aubry, S.; Royer, I.; Angers, D.A. Management Practices Differently Affect Particulate and Mineral-Associated Organic Matter and Their Precursors in Arable Soils. *Soil Biol. Biochem.* **2020**, *148*, 107867. [CrossRef]
36. Wu, J.; Zhang, H.; Pan, Y.; Cheng, X.; Zhang, K.; Liu, G. Particulate Organic Carbon Is More Sensitive to Nitrogen Addition than Mineral-Associated Organic Carbon: A Meta-Analysis. *Soil Tillage Res.* **2023**, *232*, 105770. [CrossRef]
37. Schneider, M.P.W.; Scheel, T.; Mikutta, R.; Van Hees, P.; Kaiser, K.; Kalbitz, K. Sorptive Stabilization of Organic Matter by Amorphous Al Hydroxide. *Geochim. Cosmochim. Acta* **2010**, *74*, 1606–1619. [CrossRef]
38. Jilling, A.; Keiluweit, M.; Contosta, A.R.; Frey, S.; Schimel, J.; Schnecker, J.; Smith, R.G.; Tiemann, L.; Grandy, A.S. Minerals in the Rhizosphere: Overlooked Mediators of Soil Nitrogen Availability to Plants and Microbes. *Biogeochemistry* **2018**, *139*, 103–122. [CrossRef]
39. Cotrufo, M.F.; Ranalli, M.G.; Haddix, M.L.; Six, J.; Lugato, E. Soil Carbon Storage Informed by Particulate and Mineral-Associated Organic Matter. *Nat. Geosci.* **2019**, *12*, 989–994. [CrossRef]
40. Keeler, B.L.; Hobbie, S.E.; Kellogg, L.E. Effects of Long-Term Nitrogen Addition on Microbial Enzyme Activity in Eight Forested and Grassland Sites: Implications for Litter and Soil Organic Matter Decomposition. *Ecosystems* **2009**, *12*, 1–15. [CrossRef]
41. Yu, G.; Xiao, J.; Hu, S.; Polizzotto, M.L.; Zhao, F.; McGrath, S.P.; Li, H.; Ran, W.; Shen, Q. Mineral Availability as a Key Regulator of Soil Carbon Storage. *Environ. Sci. Technol.* **2017**, *51*, 4960–4969. [CrossRef] [PubMed]
42. Qin, S.; Kou, D.; Mao, C.; Chen, Y.; Chen, L.; Yang, Y. Temperature Sensitivity of Permafrost Carbon Release Mediated by Mineral and Microbial Properties. *Sci. Adv.* **2021**, *7*, eabe3596. [CrossRef]
43. Wu, Y.; Zeng, Q.; Su, X.; Zheng, W.; Zhang, Q.; Chen, Y. The Different Factors Driving SOC Stability under Different N Addition Durations in a Phyllostachys Edulis Forest. *Forests* **2023**, *14*, 1890. [CrossRef]
44. Bowman, W.D.; Cleveland, C.C.; Halada, Ĺ.; Hreško, J.; Baron, J.S. Negative Impact of Nitrogen Deposition on Soil Buffering Capacity. *Nature Geosci.* **2008**, *1*, 767–770. [CrossRef]
45. Babur, E.; Dindaroğlu, T.; Solaiman, Z.M.; Battaglia, M.L. Microbial Respiration, Microbial Biomass and Activity Are Highly Sensitive to Forest Tree Species and Seasonal Patterns in the Eastern Mediterranean Karst Ecosystems. *Sci. Total Environ.* **2021**, *775*, 145868. [CrossRef]
46. Marriott, E.E.; Wander, M.M. Total and Labile Soil Organic Matter in Organic and Conventional Farming Systems. *Soil Sci. Soc. Am. J.* **2006**, *70*, 950–959. [CrossRef]
47. Xia, H.; Riaz, M.; Ming, C.; Li, Y.; Wang, X.; Jiang, C. Assessing the Difference of Biochar and Aged Biochar to Improve Soil Fertility and Cabbage (*Brassica oleracea* var. *capitata*) Productivity. *J. Soils Sediments* **2023**, *23*, 606–618. [CrossRef]
48. Lu, M.; Yang, Y.; Luo, Y.; Fang, C.; Zhou, X.; Chen, J.; Yang, X.; Li, B. Responses of Ecosystem Nitrogen Cycle to Nitrogen Addition: A Metaanalysis. *New Phytol.* **2011**, *189*, 1040–1050. [CrossRef] [PubMed]
49. Zhang, Y.; Cheng, X.; Van Groenigen, K.J.; García-Palacios, P.; Cao, J.; Zheng, X.; Luo, Y.; Hungate, B.A.; Terrer, C.; Butterbach-Bahl, K.; et al. Shifts in Soil Ammonia-Oxidizing Community Maintain the Nitrogen Stimulation of Nitrification across Climatic Conditions. *Glob. Chang. Biol.* **2023**, *30*, e16989. [CrossRef] [PubMed]
50. Zhao, J.; Su, W.; Fan, S.; Cai, C.; Su, H.; Zeng, X. Ammonia Volatilization and Nitrogen Runoff Losses from Moso Bamboo Forests after Different Fertilization Practices. *Can. J. For. Res.* **2019**, *49*, 213–220. [CrossRef]
51. Tian, D.; Jiang, L.; Ma, S.; Fang, W.; Schmid, B.; Xu, L.; Zhu, J.; Li, P.; Losapio, G.; Jing, X.; et al. Effects of Nitrogen Deposition on Soil Microbial Communities in Temperate and Subtropical Forests in China. *Sci. Total Environ.* **2017**, *607–608*, 1367–1375. [CrossRef]

52. Liu, L.; Greaver, T.L. A Global Perspective on Belowground Carbon Dynamics under Nitrogen Enrichment. *Ecol. Lett.* **2010**, *13*, 819–828. [CrossRef]
53. Tian, D.; Niu, S. A Global Analysis of Soil Acidification Caused by Nitrogen Addition. *Environ. Res. Lett.* **2015**, *10*, 024019. [CrossRef]
54. Lu, X.; Mao, Q.; Mo, J.; Gilliam, F.S.; Zhou, G.; Luo, Y.; Zhang, W.; Huang, J. Divergent Responses of Soil Buffering Capacity to Long-Term N Deposition in Three Typical Tropical Forests with Different Land-Use History. *Environ. Sci. Technol.* **2015**, *49*, 4072–4080. [CrossRef]
55. Parfitt, R.L.; Giltrap, D.J.; Whitton, J.S. Contribution of Organic Matter and Clay Minerals to the Cation Exchange Capacity of Soils. *Commun. Soil Sci. Plant Anal.* **1995**, *26*, 1343–1355. [CrossRef]
56. Rasmussen, C.; Heckman, K.; Wieder, W.R.; Keiluweit, M.; Lawrence, C.R.; Berhe, A.A.; Blankinship, J.C.; Crow, S.E.; Druhan, J.L.; Hicks Pries, C.E.; et al. Beyond Clay: Towards an Improved Set of Variables for Predicting Soil Organic Matter Content. *Biogeochemistry* **2018**, *137*, 297–306. [CrossRef]
57. Riggs, C.E.; Hobbie, S.E. Mechanisms Driving the Soil Organic Matter Decomposition Response to Nitrogen Enrichment in Grassland Soils. *Soil Biol. Biochem.* **2016**, *99*, 54–65. [CrossRef]
58. Rosinger, C.; Rousk, J.; Sandén, H. Can Enzymatic Stoichiometry Be Used to Determine Growth-Limiting Nutrients for Microorganisms?—A Critical Assessment in Two Subtropical Soils. *Soil Biol. Biochem.* **2019**, *128*, 115–126. [CrossRef]
59. Fu, X.; Yang, S.; Liu, D.; Liu, Y. Effects of nitrogen application on soil microbial biomass carbon and nitrogen of intercropping wheat-corn in Hetao irrigation area. *Ecol. Environ. Sci.* **2018**, *27*, 1652–1657. [CrossRef]
60. Turner, R.E.; Bodker, J.E. The Effects of N, P and Crude Oil on the Decomposition of *Spartina alterniflora* Belowground Biomass. *Wetl. Ecol. Manag.* **2016**, *24*, 373–380. [CrossRef]
61. Olander, L.P.; Vitousek, P.M. Regulation of Soil Phosphatase and Chitinase Activityby N and P Availability. *Biogeochemistry* **2000**, *49*, 175–191. [CrossRef]
62. Sinsabaugh, R.L.; Hill, B.H.; Follstad Shah, J.J. Ecoenzymatic Stoichiometry of Microbial Organic Nutrient Acquisition in Soil and Sediment. *Nature* **2009**, *462*, 795–798. [CrossRef]
63. Moorhead, D.L.; Sinsabaugh, R.L.; Hill, B.H.; Weintraub, M.N. Vector Analysis of Ecoenzyme Activities Reveal Constraints on Coupled C, N and P Dynamics. *Soil Biol. Biochem.* **2016**, *93*, 1–7. [CrossRef]
64. Jing, X.; Chen, X.; Tang, M.; Ding, Z.; Jiang, L.; Li, P.; Ma, S.; Tian, D.; Xu, L.; Zhu, J.; et al. Nitrogen Deposition Has Minor Effect on Soil Extracellular Enzyme Activities in Six Chinese Forests. *Sci. Total Environ.* **2017**, *607–608*, 806–815. [CrossRef]
65. Mack, M.C.; Schuur, E.A.G.; Bret-Harte, M.S.; Shaver, G.R.; Chapin, F.S. Ecosystem Carbon Storage in Arctic Tundra Reduced by Long-Term Nutrient Fertilization. *Nature* **2004**, *431*, 440–443. [CrossRef]
66. Neff, J.C.; Townsend, A.R.; Gleixner, G.; Lehman, S.J.; Turnbull, J.; Bowman, W.D. Variable Effects of Nitrogen Additions on the Stability and Turnover of Soil Carbon. *Nature* **2002**, *419*, 915–917. [CrossRef]
67. Qi, P.; Chen, J.; Wang, X.; Zhang, R.; Cai, L.; Jiao, Y.; Li, Z.; Han, G. Changes in Soil Particulate and Mineral-Associated Organic Carbon Concentrations under Nitrogen Addition in China—A Meta-Analysis. *Plant Soil* **2023**, *489*, 439–452. [CrossRef]
68. Chen, H.; Li, D.; Feng, W.; Niu, S.; Plante, A.; Luo, Y.; Wang, K. Different Responses of Soil Organic Carbon Fractions to Additions of Nitrogen. *Eur. J. Soil Sci.* **2018**, *69*, 1098–1104. [CrossRef]
69. Liang, C.; Amelung, W.; Lehmann, J.; Kästner, M. Quantitative Assessment of Microbial Necromass Contribution to Soil Organic Matter. *Glob. Chang. Biol.* **2019**, *25*, 3578–3590. [CrossRef] [PubMed]
70. Zeng, X.; Zhang, Q.; Lin, K.; Zhou, J.; Yuan, X.; Mei, K.; Wu, Y.; Cui, J.; Xu, J.; Chen, Y. Enzyme Stoichiometry Evidence Revealed That Five Years Nitrogen Addition Exacerbated the Carbon and Phosphorus Limitation of Soil Microorganisms in a *Phyllostachys pubescens* Forest. *Chin. J. Appl. Ecol.* **2021**, *32*, 521–528. [CrossRef]
71. Ekschmitt, K.; Liu, M.; Vetter, S.; Fox, O.; Wolters, V. Strategies Used by Soil Biota to Overcome Soil Organic Matter Stability—Why Is Dead Organic Matter Left over in the Soil? *Geoderma* **2005**, *128*, 167–176. [CrossRef]
72. Fernandez, C.W.; Langley, J.A.; Chapman, S.; McCormack, M.L.; Koide, R.T. The Decomposition of Ectomycorrhizal Fungal Necromass. *Soil Biol. Biochem.* **2016**, *93*, 38–49. [CrossRef]
73. Possinger, A.R.; Zachman, M.J.; Enders, A.; Levin, B.D.A.; Muller, D.A.; Kourkoutis, L.F.; Lehmann, J. Organo–Organic and Organo–Mineral Interfaces in Soil at the Nanometer Scale. *Nat. Commun.* **2020**, *11*, 6103. [CrossRef]