agronomy

MDPI

Article

Optimized Nitrogen Fertilization Promoted Soil Organic Carbon Accumulation by Increasing Microbial Necromass Carbon in Potato Continuous Cropping Field

Huidan Lv [1,2,3], Ping He [1,2,3] and Shicheng Zhao [1,2,3,*]

[1] Key Laboratory of Plant Nutrition and Fertilizer, Ministry of Agriculture and Rural Affairs, Beijing 100081, China; lvhuidan1226@163.com (H.L.); heping02@caas.cn (P.H.)
[2] State Key Laboratory of Efficient Utilization of Arid and Semi-Arid Arable Land in Northern China (Institute of Agricultural Resources and Regional Planning), Chinese Academy of Agricultural Sciences, Beijing 100081, China
[3] Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China
* Correspondence: zhaoshicheng@caas.cn; Tel.: +86-10-82105029

Abstract: The form and distribution of organic carbon in soil affect its stability and storage, and nitrogen (N) fertilization can affect the transformation and accumulation of soil organic carbon (SOC), whereas how the N fertilizer rate affects SOC storage by regulating its fractions in a potato continuous cropping system is unknown. A 6-year field experiment was conducted to study the effect of different N fertilizer rates (NE (Nutrient Expert) –N, NE–1/2N, NE, and NE+1/2N) on the changes in SOC and its fractions in a potato continuous cropping system in North China. Soil NO_3^--N gradually increased with increasing N fertilizer rates, whereas the N fertilizer rate had less effect on NH_4^+-N. Compared with the NE−N treatment, the increasing N fertilization increased the SOC and its components, whereas these C fractions did not continue to increase or began to decrease after N fertilization exceeded the rate applied in the NE treatment. While the increase in mineral-associated organic C (MAOC; 16.1–17.2% and 26.1–52.7% in the 0–20 cm and 20–40 cm layers, respectively) was greater than that of particulate organic C (POC; 3.7–7.4% and 11.5–16.4% in the 0–20 cm and 20–40 cm layers, respectively), the increase in bacterial necromass C (BNC; 9.2–21.8% and 28.9–40.4% in the 0–20 cm and 20–40 cm layers, respectively) was greater than that of fungal necromass C (FNC; 6.2–10.1% and 7.1–24.9% in the 0–20 cm and 20–40 cm layers, respectively). Furthermore, the increase in FNC was greater than that of BNC in the 20–40 cm layer of the same treatment. SOC was significantly and positively correlated with MAOC and FNC, and the correlation between SOC and both MNC and FNC was more significant in the 20–40 cm layer than in the 0–20 cm layer. Overall, in the potato continuous cropping system in North China, N fertilization improved SOC storage by increasing MNC to form MAOC, and optimizing N fertilization based on the NE system could better balance the increase and mineralization loss of SOC to achieve high SOC sequestration.

Keywords: nutrient expert; soil organic carbon fraction; microbial residue carbon; soil enzyme activity; optimizing fertilization

Citation: Lv, H.; He, P.; Zhao, S. Optimized Nitrogen Fertilization Promoted Soil Organic Carbon Accumulation by Increasing Microbial Necromass Carbon in Potato Continuous Cropping Field. *Agronomy* **2024**, *14*, 307. https://doi.org/10.3390/agronomy14020307

Academic Editor: José David Flores-Félix

Received: 21 December 2023
Revised: 24 January 2024
Accepted: 25 January 2024
Published: 30 January 2024

1. Introduction

Soil organic matter is a key attribute of soil quality. Increasing soil organic carbon (SOC) sequestration can reduce carbon dioxide emissions and mitigate climate change, while improving soil quality and crop productivity [1]. Crop residues (straw and stubble) are important organic C sources in farmland. After returning crop residues to the field, the labile fractions are rapidly decomposed by soil microorganisms and assimilated into their own organic bodies, while the recalcitrant fractions combine with soil sand particles to form particulate organic carbon (POC) and remain in the soil. The residue after microbial

death is one of the main components of SOC, accounting for more than 50% of total SOC sequestration [2]. Microorganisms selectively utilize plant residues; bacteria preferentially decompose and assimilate labile fractions, such as proteins and cellulose, while fungi primarily assimilate recalcitrant components, such as lignin [3]. These lead to differences in the stability of necromass C from different microbial sources. For example, fungal necromass C (FNC) is generally more stable than bacterial necromass C (BNC) [4]. Microbial necromass can bind to clay minerals or enter micropores, thereby forming mineral-associated organic carbon (MAOC) or stable microaggregates and improving their stability in soil [5]. However, the physically protected necromass with high N and phosphorus contents is an optimal substrate and is easily utilized by microorganisms to meet their C and nutrient demands [6].

Soil physicochemical properties (pH, texture, etc.) and field management measures (fertilization, cultivation, etc.) can significantly affect the transformation and accumulation of SOC [7]. The increase in SOC in clay soil is more significant than that in sandy soil after straw return and the accumulation of organic C in humid areas is greater than that in arid areas [1,8]. The N fertilization can increase soil fertility, crop yield, and plant biomass C, as well as change the soil C/N ratio to affect soil microbial community composition and organic C conversion [1,9–11]. Long-term fertilization can increase SOC by increasing the plant source C input [12], while overapplication of N fertilizers can lead to a large accumulation of soil mineral N, decrease the soil C/N ratio, accelerate SOC decomposition to meet microbial growth needs, and promote mineralization loss of SOC [1]. Therefore, optimizing N fertilization is crucial for maintaining high crop yields and improving SOC sequestration, but the effect of N fertilization on SOC accumulation varies in different crop systems and environmental conditions.

The Nutrient Expert (NE) is a recommended fertilization system built using computer technology based on the relationship between crop yield responses and agronomic efficiencies. It can recommend fertilization based on farmland fertility and yield levels. Fertilization based on the NE system can optimize nutrient usage, improve nutrient utilization efficiency, and maintain or increase crop yields compared with conventional fertilization practices [13,14].

Potatoes are an important food crop in China and are suitable for growing in sandy or loamy soils with rich organic matter. However, frequent soil disturbance during potato production can easily lead to a rapid loss of SOC [15]. The potato yield in the Inner Mongolia Autonomous Region accounts for about 10% of the total potato yield in China and the northern foothills of Yinshan Mountain are the main potato production areas in Inner Mongolia [16]. In this area, the annual rainfall is relatively low and the soil is sandy chestnut soil with low clay and organic matter content, which is not conducive to SOC accumulation; therefore, enhancing SOC storage and soil quality by fertilizer management is necessary to ensure high potato yields. Yu [17] found that the continuous application of N fertilizers for five years significantly increased SOC accumulation compared with non-N treatment in potato planting. However, the effect and mechanism of different N fertilizer rates after straw return on SOC accumulation are not clear in the sandy soil area of Inner Mongolia. We hypothesized that the optimized N fertilization based on the NE system can improve SOC sequestration, while achieving high potato tuber yields. Therefore, a 6-year field fertilization experiment was conducted in a potato cropping system in North China. Our objectives were to investigate how different N fertilizer rates affect SOC storage by regulating SOC fractions and soil enzyme activities in a potato continuous cropping system, providing a theoretical basis for optimizing N fertilizer management for potato production in sandy soil areas in North China.

2. Materials and Methods

2.1. Experimental Site Description

The field experiment was established in May 2017 at the Field Dry Farming Test Station (41°14′ N, 111°30′ E), Inner Mongolia Autonomous Region, North China. This

area has a typical semi-arid continental monsoon climate in the middle temperate zone with an average annual temperature of 3.0 °C and total rainfall of 280 mm, and the annual evaporation is about 1850 mm. The local soil is classified as Aridisol (FAO classification) and contains 74.2% sand, 17.3% silt, and 8.8% clay. The chemical properties of the initial soil (0–20 cm) in 2017 were as follows: pH, 7.91 (water:soil = 2.5:1); organic matter, 19.0 g kg^{-1}; total N, 1.04 g kg^{-1}; C/N ratio, 10.6; NH_4^+-N, 0.5 mg kg^{-1}; NO_3^--N, 15.1 mg kg^{-1}; available phosphorus (P), 29.8 mg kg^{-1}; and available potassium (K), 150 mg kg^{-1}.

2.2. Experimental Design

The experiment was conducted on a potato continuous cropping system using four treatments with different N fertilizer rates: NE (recommended fertilization based on the NE system of potato farming [18,19]), NE−N (no N fertilization), NE−1/2N, and NE+1/2N with combined P and K fertilization. All treatments were arranged in a randomized block design with three replicates and the plot size was 30 m^2 (7.5 m × 4 m). The annual rates of N, P, and K fertilizers were recommended using the NE system. The annual N fertilizer rates for different treatments are shown in Table 1. The rates of the P fertilizer used were 150, 180, 91, 84, 89, 85, and 86 kg P_2O_5 ha^{-1} and the rates of the K fertilizer used were 234, 270, 238, 134, 135, 140, and 135 kg K_2O ha^{-1} from 2017 to 2023, respectively. The ratio of basal N to topdressing N was 3:7. The base N fertilizer was sprinkled before sowing and after turning over the soil. The topdressing N was applied through drip irrigation and amounts of 20.0%, 25.0%, and 25.0% of N fertilizer were applied with irrigation water at the seeding stage, flowering stage, and starch accumulation stage, respectively. The N, P, and K fertilizers were urea (N, 46%), monoammonium phosphate (P_2O_5, 61%, N12%) (2017) or calcium superphosphate (P_2O_5, 14%) (since 2018), and potassium chloride (K_2O, 60%), respectively.

Table 1. The nitrogen fertilizer rate in different treatments (kg N ha^{-1}).

Treatment	2017	2018	2019	2020	2021	2022	2023
NE−N	0	0	0	0	0	0	0
NE−1/2N	90	120	105	89	89	90	90
NE	180	240	209	178	178	180	180
NE+1/2N	270	360	314	267	267	270	270

NE means N, P, K fertilizers are applied basen on Nutrient Expert (NE) recommendated; NE−N, means the appliation of P and K fertilizers as that in the NE treatment, but no N fertilizer was applied; NE−1/2N, means the application of 1/2 N used in NE, and the rate of P and K fertilizers were same as that in the NE treatment; NE+1/2N, means the application of 3/2 N used in NE, and the rate of P and K fertilizers were same as that in the NE treatment.

The cultivar of potato was Huasong 7, which was planted on high ridges with drip irrigation. Potatoes were generally planted from late Spring to early May, with a space of 100 cm between the rows, in a single ridge and single row, with a plant space of 20 cm, and with 37 plants per row; and was harvested from mid-September to early October. Potatoes were irrigated 5–6 times throughout the whole growth period, with a total irrigation volume of 1500 m^3 ha^{-1}. Diseases and pests were controlled during potato growth. At maturity, potato tubers were harvested and potato residues were left on the surface and incorporated into the soil the following year.

2.3. Soil Sampling

In mid-September 2023, five soil cores were collected with an auger (2 cm in diameter) in each plot at a depth of 40 cm with 20 cm increments. Soil samples of the same soil depth per plot were mixed as composite samples and then sieved to pass through a 2 mm sieve. Fresh soil samples were immediately analyzed for NH_4^+-N, NO_3^--N, and soil enzyme activity; other samples were air-dried for the analysis of SOC fractions and microbial necromass C (MNC).

2.4. Soil Mineral Nitrogen, Organic Carbon, and Enzyme Activity Analysis

Soil NH_4^+-N and NO_3^--N were extracted with 1 M KCl and measured using a flow injection analyzer (SEAL Auto Analyzer 3, SEAL Analytical GbmH, Norderstedt, Germany).

The POC and MAOC were separated according to the method of Sokol et al. [20]. Briefly, 20 g of air-dried soil was shaken with 60 mL of sodium hexametaphosphate (5% mass/volume) for 18 h and then thoroughly rinsed through a 53 μm sieve to separate the POC fraction (>53 μm) and MAOC fraction (<53 μm). The collected components were dried at 65 °C and weighed; the organic C in the air-dried soil, POC, and MAOC was analyzed using an elemental analyzer (Vario MACRO cube, Elementar Analysensystem GmbH, Langenselbold, Germany) after a round in a ball mill.

Soil amino sugars (e.g., glucosamine, galactosamine, mannosamine, and muramic acid) were determined according to the method of Zhang and Amelung [21]. About 0.5 g of freeze-dried soil was hydrolyzed with 10 mL of 6 M HCl at 105 °C for 8 h. After cooling, 100 μg myoinositol was added as a recovery standard. The hydrolysates were then filtered, evaporated to dryness at 52 °C under reduced pressure, and redissolved in 5 mL of MilliQ water with the pH adjusted to 6.6–6.8 using 0.4 M potassium hydroxide. The supernatant was freeze-dried after centrifugation. Amino sugars were redissolved in methanol and separated from salts by centrifugation. After the addition of a quantitative standard (methyl-glucamine), amino sugars were transformed into aldononitrile derivatives by heating in 0.3 mL of a derivatization reagent containing 32 mg mL^{-1} of hydroxylamine hydrochloride and 40 mg ml^{-1} of 4-(dimethylamino) pyridine in a pyridine and methanol mixture (4:1, *v*/*v*) at 75 °C for 30 min. The derivatives were further acetylated with 1 mL of acetic anhydride at 75–80 °C for 20 min and mixed with 1.5 mL of dichloromethane after cooling. Excessive derivatization reagents were removed by extracting with 1 M HCl and MilliQ water, while the dichloromethane phase containing amino sugar derivatives was dried under N_2 before quantification.

The amino sugar derivatives were separated using a gas chromatograph (GC-2014C, Shimadzu, Kyoto, Japan) equipped with a DB-5 column (60 m × 0.25 mm × 0.25 μm). Fungal and bacterial necromass C were calculated based on Equations (1) and (2), according to Liang et al. [2]

$$\text{Fungal necromass C} = (\text{glucosamine}/179.2 - 2 \times \text{muramic acid}/251.2) \times 179.2 \times 9 \quad (1)$$

$$\text{Bacterial necromass C} = \text{muramic acid} \times 45 \quad (2)$$

where 179.2 and 251.2 in Equation (1) are the molecular weights of glucosamine and muramic acid, respectively; 9 and 45 are the conversion values from fungal glucosamine to FNC and from muramic acid to BNC, respectively [22]. The MNC was estimated as the sum of FNC and BNC.

The activities of β-glucosidase (BG) and leucine-aminopeptidases (LAPs) were measured as described by Saiya-Cork et al. [23]. Briefly, each equivalent of 1.0 g dry mass of fresh soil was homogenized in 100 mL of 50 mM acetate buffer (pH 8.5). The buffer, sample suspension, 10 μM references, and 200 μM substrates (4-methyl-umbelliferone or 7-amino-4-methylocumarin) were dispensed into the wells of a black, 96-well microplate. The microplates were covered and incubated at 25 °C for 4 h in the dark, after which the fluorescence was quantified using a microplate fluorometer (Scientific Fluoroskan Ascent FL, Thermo, MA, USA) with 365 nm excitation and 450 nm emission filters. The enzyme activity was expressed in nmol h^{-1} g^{-1}.

2.5. Statistical Analysis

A one-way analysis of variance (ANOVA) was performed on each variable of soil mineral N, SOC fractions, and MNC fractions using the SPSS software (The SPSS 24.0 for windows (SPSS Inc., Chicago, IL, USA)) to assess their differences among different treatments, followed by Duncan's multiple range test using the least significant difference (LSD) (α = 0.05) to evaluate the significance of their differences. A correlation analysis

was conducted to evaluate the relationship between SOC and its fractions and other soil variables using the "corrplot" package in R.

3. Results

3.1. Soil Mineral N in Different Fertilization Treatments

Compared with the NE−N treatment, soil NO_3^--N in the 0–20 cm and 20–40 cm layers significantly increased with increasing N fertilization rates (Figure 1). Soil NH_4^+-N in the 0–20 cm layer did not significantly change across all treatments, whereas NE and NE+1/2N increased NH_4^+-N of the 20–40 cm soil layer by 21.4 and 25.9%, respectively, compared with NE−N.

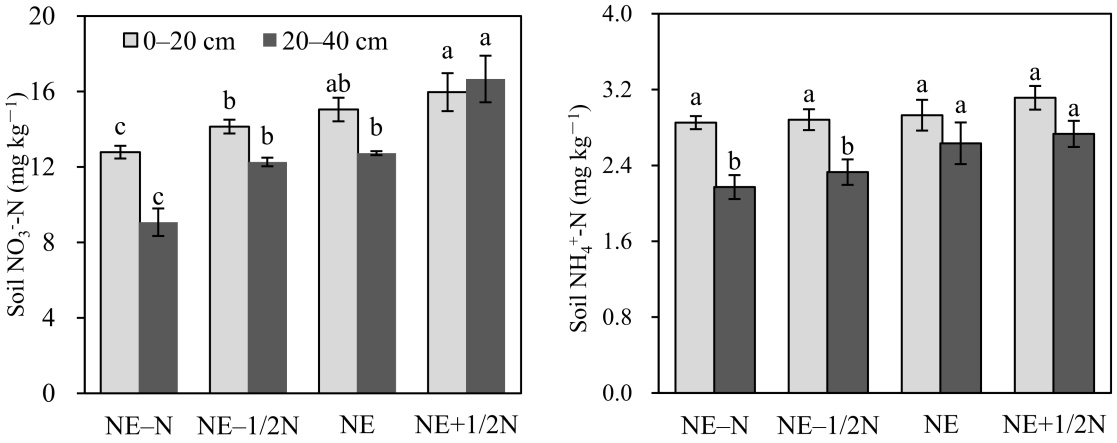

Figure 1. Soil NO_3^--N and NH_4^+-N in different treatments. Different letters at the top of the columns indicate significant differences among fertilization treatments ($p < 0.05$).

3.2. Soil Organic Carbon Fractions in Different Treatments

Compared with the NE–N treatment, the application of the N fertilizer increased SOC, POC, and MAOC in the 0–20 cm soil layer by 10.0–11.0%, 3.7–7.3%, and 16.1–17.2%, respectively, but there were no differences in these SOC fractions among the three N fertilization treatments; in the 20–40 cm layer, N fertilization increased SOC, POC, and MAOC by 3.5–13.9%, 11.5–14.4%, and 26.1–52.7%, respectively, but the SOC and MAOC of NE+1/2N were significantly reduced compared with those of NE (Figure 2a–c). Compared with NE−N, N fertilization decreased the POC/MAOC ratio, with the NE treatment showing the largest decrease (24.3% and 10.2% in the 0–20 and 20–40 cm layers) (Figure 2d).

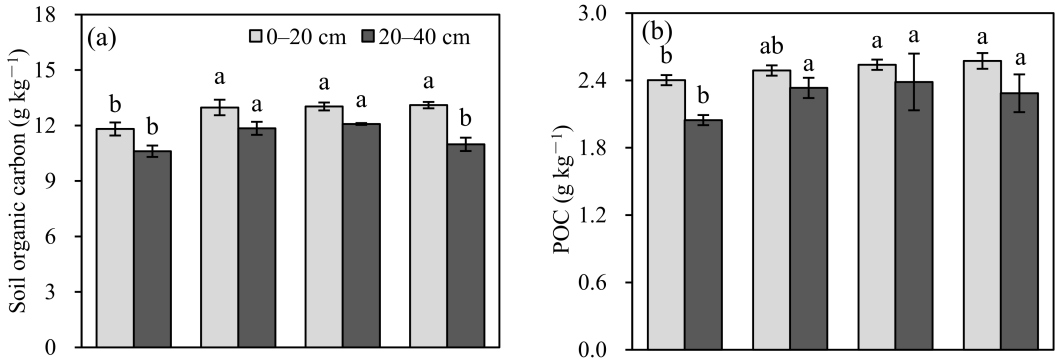

Figure 2. *Cont.*

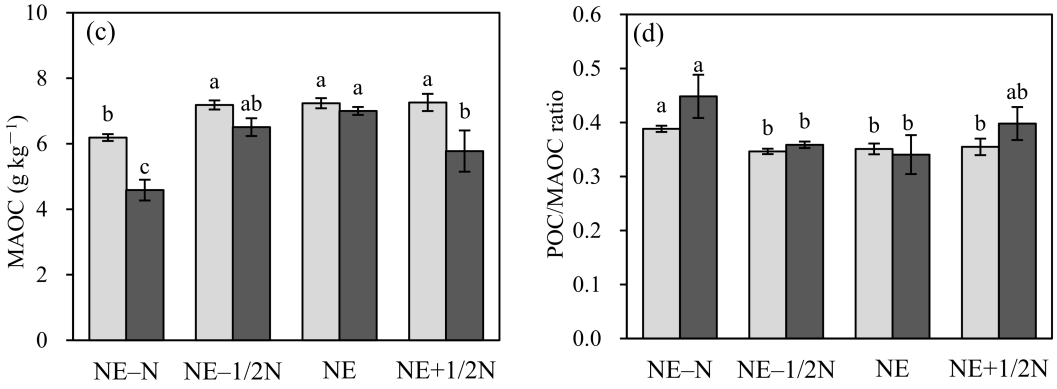

Figure 2. Total soil organic carbon (SOC) (**a**), particulate organic carbon (POC) (**b**), mineral-associated organic carbon (MAOC) (**c**), and the POC/MAOC ratio (**d**) in different treatments. Different letters at the top of the columns indicate significant differences among fertilization treatments ($p < 0.05$).

3.3. Soil Microbial Necromass Carbon in Different Treatments

In the 0–20 cm soil layer, the NE−1/2N, NE, and NE+1/2N treatments increased MNC by 7.0%, 10.2%, and 12.9%; increased BNC by 9.2%, 13.1%, and 21.8%; and increased FNC by 6.2, 9.5%, and 10.1%, respectively, compared with the NE−N treatment. There were no differences in MNC and FNC among the three N fertilization treatments (Figure 3a–c). In the 20–40 cm layer, the NE−1/2N, NE, and NE+1/2N treatments increased MNC by 23.3%, 31.4%, and 13.1%; increased BNC by 28.9%, 40.4%, and 22.1%; and increased FNC by 18.0%, 24.9%, and 7.1%, respectively. However, NE+1/2N decreased MNC, BNC, and FNC by 13.9, 13.0%, and 14.2%, respectively, relative to the NE treatment. The FNC/BNC ratio of the 0–20 cm layer did not change across all treatments but showed a gradually decreasing trend with increasing N fertilization, and the ratio in the 20–40 cm layer was slightly higher than that in the 0–20 cm layer (Figure 3d). There was no difference in the MNC/SOC ratio of topsoil among all treatments; however, NE−1/2N, NE, and NE+1/2N increased the ratio in the 20–40 cm layer by 8.0%, 12.9%, and 8.6% relative to the NE−N treatment, respectively (Figure 3e).

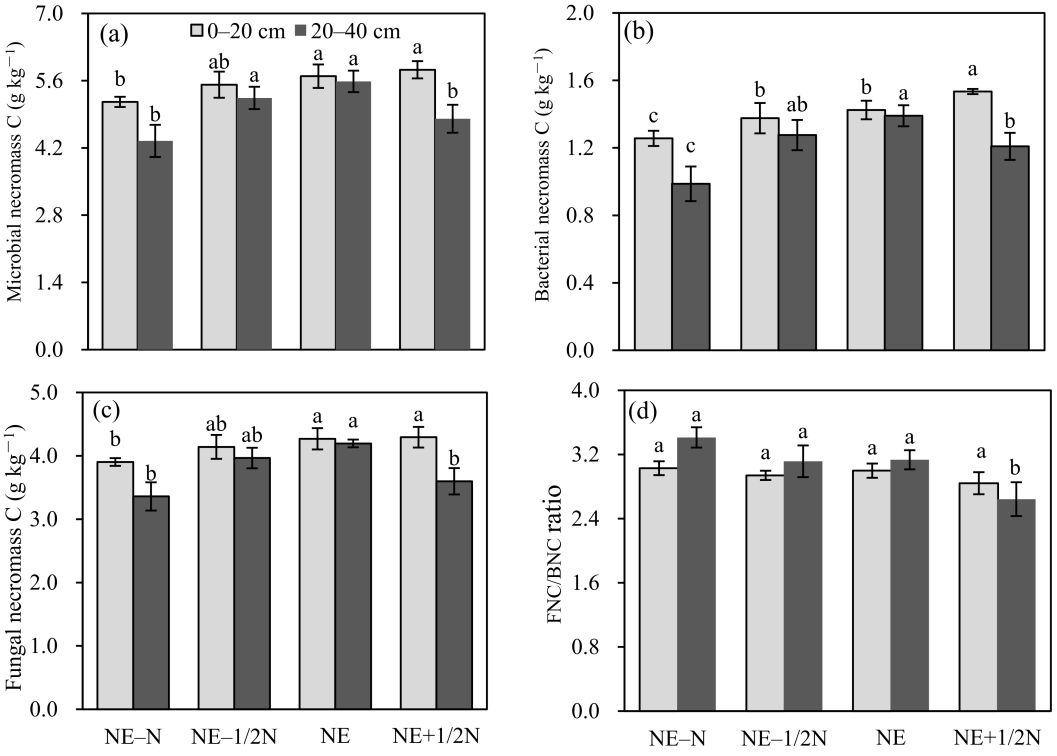

Figure 3. *Cont.*

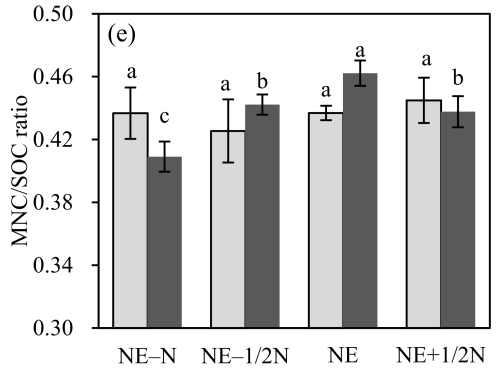

Figure 3. Soil microbial necromass carbon (MNC) (**a**), bacterial necromass carbon (BNC) (**b**), fungal necromass carbon (FNC) (**c**), the FNC/BNC ratio (**d**), and the MNC/SOC ratio (**e**) in different treatments. Different letters at the top of the columns indicate significant differences among fertilization treatments ($p < 0.05$).

3.4. The Activity of Soil Enzymes in Different Treatments

Compared with NE−N, the BG activity in the 0–20 cm soil gradually increased with increasing N fertilization rates, while a decrease occurred in the 20–40 cm layer after peaking in the NE treatment (Figure 4a). Compared with NE−N, the LAP activity in the 0–20 cm and 20–40 cm soil layers gradually decreased with increasing N application (Figure 4b). The change in the ratio of BG/LAP activity was similar to that observed for the BG activity across all treatments (Figure 4c).

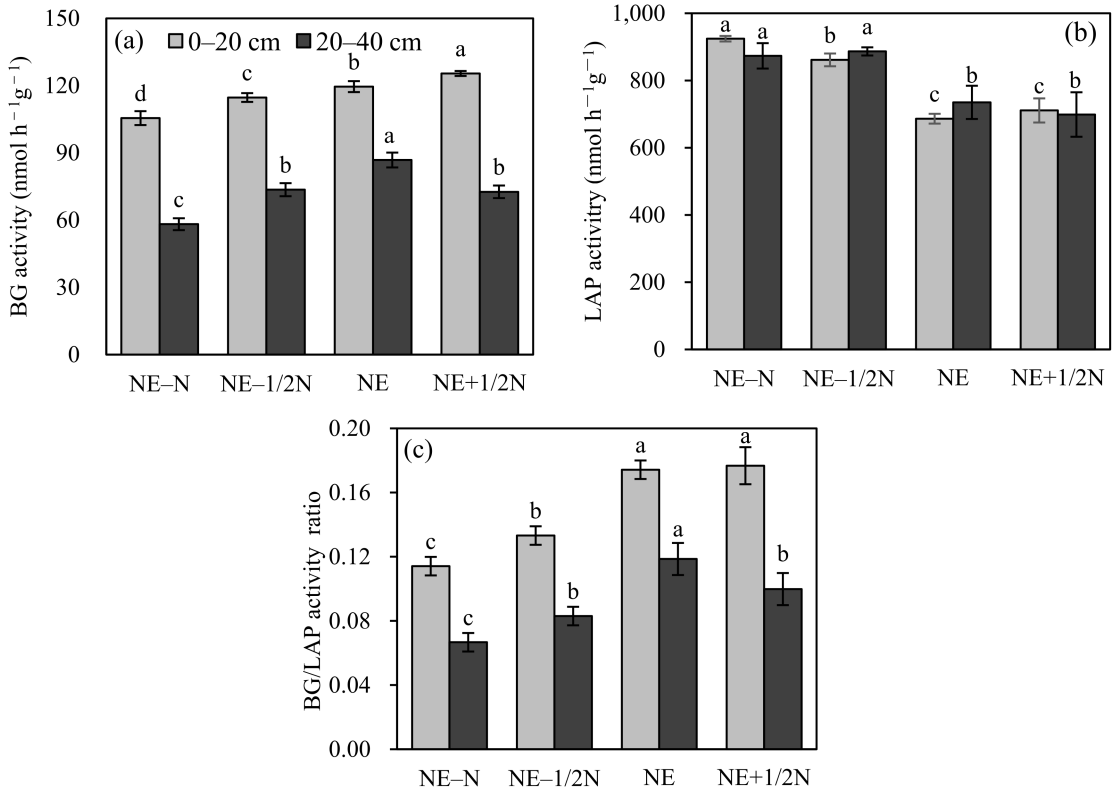

Figure 4. The activity of β-glucosidase (BG) (**a**) and Leucine-aminopeptidases (LAP) (**b**) and the ratio of BG/LAP activity (**c**) in different treatments. Different letters at the top of the columns indicate significant differences among fertilization treatments ($p < 0.05$).

3.5. Correlations between Total Soil Organic Carbon and Other Variables across Different Treatments

The correlation analysis showed that in the 0–20 cm layer, SOC was significantly and positively correlated with MAOC and the BG activity ($p < 0.001$) and MAOC was significantly and positively correlated with the BG activity, NBC, MNC, TN, and NO_3^--N ($p < 0.001$) (Figure 5a). In the 20–40 cm layer, SOC was positively correlated with MAOC, BNC, FNC, MNC, and the activity of BG ($p < 0.001$) and MAOC was significantly and positively correlated with the BG activity and MNC ($p < 0.001$).

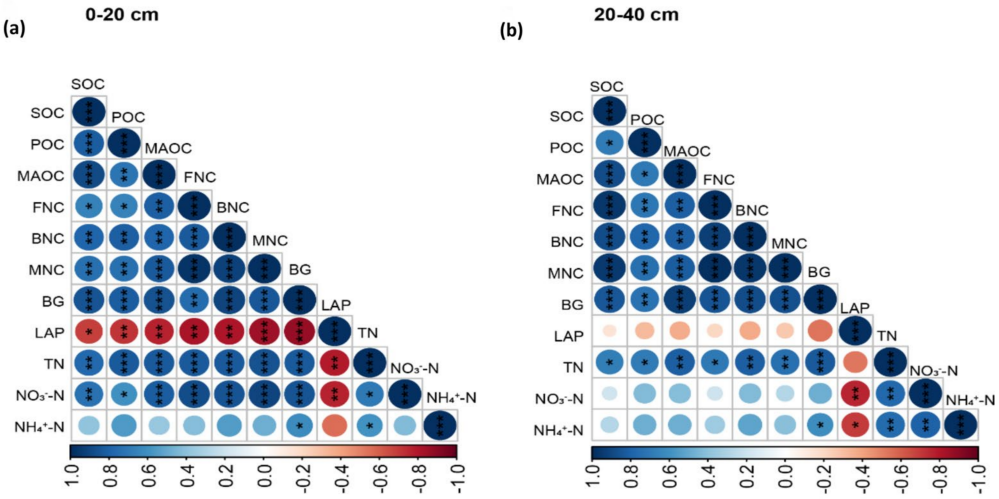

Figure 5. Correlation between soil organic carbon (SOC) and other variables in the 0–20 cm (**a**) and 20–40 cm layers (**b**) across all treatments. POC, particulate organic carbon; MAOC, mineral-associated organic carbon; BNC, bacterial necromass carbon; FNC, fungal necromass carbon; MNC, microbial necromass carbon; TN, total nitrogen; BG, β-glucosidase; LAPs, leucine-aminopeptidases. Significant differences were evaluated at *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$.

4. Discussion

4.1. Effects of Different Rates of Nitrogen Fertilization on Crop Carbon Input

The application of the N fertilizer increased soil N supply and promoted aboveground potato plant biomass accumulation and tuber yield, which increased with increasing N rates (Figure 6). High plant biomass also improved the production of root exudates and returning these straw residues to the field and stubble retention increased organic C input [24].

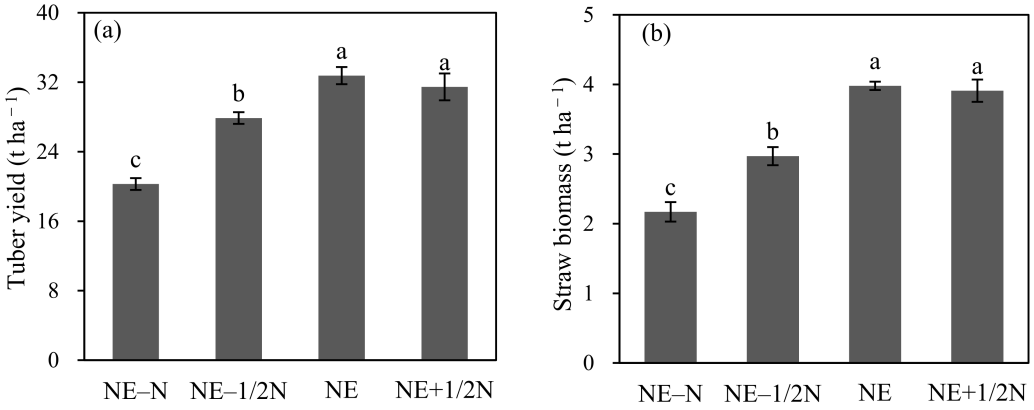

Figure 6. The mean tuber yield (**a**) and aboveground dry straw biomass (**b**) of potato in different treatments (2017–2022). The mean annual content of N, P, K, and organic C and the C/N ratio were 18.8 (14.8–22.5), 1.7 (1.2–2.4), 28.4 (17.1–40.3), 434.5 (398.3–461.4) g kg^{-1}, and 23.1 (17.8–31.2) in potato straw (2017–2022). Different letters at the top of the columns indicate significant differences among fertilization treatments ($p < 0.05$).

4.2. Effect of Different Rates of Nitrogen Fertilization on Microbial Residue Carbon Components

The MNC, FNC, and BNC all increased with increasing N application rates (except for in the 20–40 cm layer of NE+1/2N). The increased input of mineral N and organic C promoted microbial growth and enhanced the retention of microbial residue C. Compared with the no N treatment, the increase in BNC was greater than that in FNC with the higher rate of N fertilization, indicating that bacteria were more sensitive to N fertilization than fungi. Breulmann et al. [9] pointed out that soil microbial communities evolved from fungal dominance to bacterial dominance when soil fertility increased. Soil FNC presented higher content than BNC in the same treatment. The FNC/BNC ratio showed a decreasing trend with increasing N application rates and the decrease was more significant in the 20–40 cm soil layer. These results indicated that fungi contributed more to the MNC accumulation than bacteria, especially in low N soils and subsoils, and the increase in the contribution of BNC to MNC was greater than that of FNC with increasing N fertilization. The correlation analysis also showed that the correlation between FNC and MNC was greater than that between FNC and BNC. Yang et al. and Buckeridge [5,25] also pointed out that FNC was more stable and contributed more to total SOC sequestration than BNC. Xu et al. [26] reported that straw incorporation contributed to FNC accumulation in low fertility soils, while it contributed to increased BNC in high fertility soils.

4.3. Effect of Different Rates of Nitrogen Fertilization on Soil Particle Carbon Components

The application of the N fertilizer increased MAOC, POC, and total SOC compared with the no N treatment (except for that in the 20–40 cm layer in the NE+1/2N treatment). The increase in POC was smaller than that of MAOC with N fertilization and the POC/MAOC ratio showed a decreasing trend with increasing N application rates. In farmland soil, increased organic C and N supply can promote microbial growth. However, soil is generally C-limited for microbial growth and the high N input further increases the microbial C limitation. Therefore, soil microorganisms need to decompose SOC to meet their growth demands for C [27]. POC is formed by the combination of semi-decomposed straw residues and sand particles; it lacks the physical protection of clay and aggregates, is easily decomposed by external microorganisms, and has a fast turnover rate [28]. Therefore, it is preferentially decomposed and utilized by microorganisms under conditions of C limitation. On the other hand, MAOC is formed by combining microbial residues with clay particles or entering soil particle micropores and is more stable than POC and not easily decomposed [29]. Thus, MAOC constitutes the main body of SOC, which was evidenced by the high correlation between MAOC and SOC in this study. Soil hydrolases are pioneers during the microbial decomposition of organic residues. Under conditions of C limitation, microorganisms may increase the secretion of C-cycling enzymes to improve their activities and decompose SOC, such as BG, while reducing the secretion of N-cycling enzymes [30]. This was consistent with our findings in this study; the activity of BG, which dominates the decomposition of cellulose, gradually increased with increasing N application rates, while the activity of LAPs, which dominate the decomposition of N fractions, such as proteins and chitin, gradually decreased with the increasing N rates. The increase in the ratio of BG/LAP activity proved that the higher N fertilization rates promoted soil C limitation because this ratio can reflect the resource (C) limitation of the soil for microbial growth [30]. These results demonstrated that the high N inputs accelerated soil C limitation for microbes, which, in turn, enhanced the activity of C-cycling enzymes to decompose SOC, especially for the labile POC fraction. The changes in soil MAOC and MNC/SOC ratio were similar among different treatments because N fertilization increased MNC, which facilitated MAOC formation by combining with soil clay particles, and further enhanced SOC storage.

Compared with the NE treatment, soil NO_3^--N increased, whereas the SOC and MNC components did not change in the 0–20 cm layer and significantly decreased in the 20–40 cm soil layer of the NE+1/2N treatment. The potato straw biomass did not significantly increase after the N fertilization exceeded the rate of the NE treatment; however, the high

soil N supply resulted in a more severe microbial C limitation, thereby promoting microbial mineralization and the reuse of labile C fractions, such as POC, which could be proved by the increased BG activity observed with high N fertilization. These results suggested that the recommended N fertilization based on the NE system could promote crop growth and yield by optimizing the N fertilizer supply, while promoting the sequestration of organic C from both crops and microbial sources, and higher N fertilizer inputs also promoted microbial decomposition of SOC under conditions of C limitation. However, SOC and its fractions presented an increased change, although BG activity gradually increased with increasing N fertilization rates, indicating that the accumulation of SOC from crop C and microbial C far exceeded SOC losses by microbial decomposition. SOC and its fractions were generally lower in the 0–20 cm layer compared with the 20–40 cm layer because crop residues and fertilizer nutrients were generally incorporated into the 0–20 cm layer, the potato roots were also mainly distributed in the 0–20 cm layer, and the distribution of organic C and fertilizer N were less in the 20–40 cm layer, which led to lower microbial abundance and residue accumulation; similar results were reported by Chen. [31]. In contrast, the contribution of FNC to MNC and SOC was greater in the 20–40 cm layer than in the 0–20 cm layer, according to the correlation analysis.

Compared with the NE treatment, soil mineral N in the 20–40 cm layer was higher in NE+1/2N, and the amount of crop straw return was similar in these two treatments; however, BG activity and SOC and its fractions in NE+1/2N were significantly lower compared with those in the NE treatment. The reason for this needs to be investigated further. Soil microorganisms are crucial decomposers of soil organic matter and their abundance and community compositions are affected by changes in soil physicochemical properties resulting from fertilization and straw incorporation, thereby affecting the transformation and storage of SOC [3,32]. Therefore, the relationship between soil microbial community compositions, their functions, and changes in organic C components needs to be further studied to reveal the mechanism of N fertilization regulating SOC sequestration.

5. Conclusions

In continuous potato cropping systems in North China, the application of N fertilizer combined with straw return increased SOC and soil fertility, but the high rate of N inputs also accelerated soil microbial C limitation and increased the activity of C-degrading enzymes. For microbial necromass C, BNC increased at a higher rate than FNC with increasing N application, but FNC contributed more to MNC accumulation than BNC, especially in the deeper soil layers. The MNC combined with soil clay to form MAOC, which increased its C stability and then improved the total SOC accumulation. Overall, this study demonstrated that optimized N fertilization based on the NE system could better balance the increase and mineralization loss of SOC and achieve high SOC sequestration while attaining high tuber yield, whereas overapplication of the N fertilizer did not consistently increase SOC storage.

Author Contributions: Conceptualization, P.H. and S.Z.; methodology, H.L.; formal analysis, H.L.; investigation, H.L.; writing—original draft preparation, H.L.; visualization, H.L.; supervision, P.H. and S.Z.; project administration, P.H. and S.Z. All authors have read and agreed to the published version of the manuscript.

Funding: The research was funded by the National Key Research & Development Program of China (2022YFD1700605) and the Science and Technique Plan of Inner Mongolia Autonomous region of China (2021GG0010).

Data Availability Statement: Data can be made available from the corresponding author upon reasonable request.

Acknowledgments: We are grateful to Jun Zhang and Xiya Wang for their great help during field experiment, and we are grateful to three anonymous reviewers for their constructive comments and suggestions on this manuscript.

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

References

1. Wang, Y.L.; Wu, P.N.; Mei, F.J.; Ling, Y.; Qiao, Y.B.; Liu, C.S.; Leghari, S.J.; Guan, X.K.; Wang, T.C. Does continuous straw returning keep China farmland soil organic C continued increase? A meta-analysis. *J. Environ. Manag.* **2021**, *288*, 9. [CrossRef]
2. Liang, C.; Amelung, W.; Lehmann, J.; Kastner, M. Quantitative assessment of microbial necromass contribution to soil organic matter. *Glob. Change Biol.* **2019**, *25*, 3578–3590. [CrossRef] [PubMed]
3. Paterson, E.; Sim, A.; Osborne, S.M.; Murray, P.J. Long-term exclusion of plant inputs to soil reduces the functional capacity of microbial communities to mineralize recalcitrant root- derived C sources. *Soil Biol. Biochem.* **2011**, *43*, 1873–1880. [CrossRef]
4. Deng, F.B.; Liang, C. Revisiting the quantitative contribution of microbial necromass to soil C pool: Stoichiometric control by microbes and soil. *Soil Biol. Biochem.* **2022**, *165*, 108486. [CrossRef]
5. Yang, Y.L.; Xie, H.T. Fungi determine increased SOC more than bacteria through their necromass inputs in conservation tillage croplands. *Soil Biol. Biochem.* **2022**, *167*, 108587. [CrossRef]
6. Cui, Y.X.; Wang, X.; Zhang, X.C.; Ju, W.L.; Duan, C.J.; Guo, X.B.; Wang, Y.Q.; Fang, L.C. Soil moisture mediates microbial carbon and phosphorus metabolism during vegetation succession in a semiarid region. *Soil Biol. Biochem.* **2020**, *147*, 107814. [CrossRef]
7. Feng, Q.; An, C.J.; Chen, Z.; Wang, Z. Can deep tillage enhance carbon sequestration in soils? A meta-analysis towards GHG mitigation and sustainable agricultural management. *Renew. Sustain. Energy Rev.* **2020**, *133*, 110293. [CrossRef]
8. Gentile, R.; Vanlauwe, B.; Kavoo, A.; Chivenge, P.; Six, J. Residue quality and N fertilizer do not influence aggregate stabilization of C and N in two tropical soils with contrasting texture. *Nutr. Cycl. Agroecosyst.* **2010**, *88*, 121–131. [CrossRef]
9. Breulmann, M.; Schulz, E.; Weißhuhn, K.; Buscot, F. Impact of the plant community composition on labile soil organic C, soil microbial activity and community structure in semi-natural grassland ecosystems of different productivity. *Plant Soil* **2012**, *352*, 253–265. [CrossRef]
10. Frey, S.D.; Ollinger, S.; Nadelhoffer, K.; Bowden, R.; Brzostek, E.; Burton, A.; Caldwell, B.A.; Crow, S.; Goodale, C.L.; Grandy, A.S.; et al. Chronic nitrogen additions suppress decomposition and sequester soil carbon in temperate forests. *Biogeochemistry* **2014**, *121*, 305–316. [CrossRef]
11. Luo, M.; Moorhead, D.L.; Ochoa-Hueso, R.; Mueller, C.M.; Ying, S.C.; Chen, J. Nitrogen loading enhances phosphorus limitation in terrestrial ecosystems with implications for soil carbon cycling. *Funct. Ecol.* **2022**, *36*, 2845–2858. [CrossRef]
12. Li, J.; Wen, Y.C.; Li, X.H.; Li, Y.T.; Yang, X.D.; Lin, Z.A.; Song, Z.Z.; Cooper, J.M.; Zhao, B.Q. Soil labile organic C fractions and soil organic C stocks as affected by long-term organic and mineral fertilization regimes in the North China Plain. *Soil Till. Res.* **2018**, *175*, 281–290. [CrossRef]
13. Huang, X.M.; Xu, X.P.; Wang, X.B.; Yang, L.F.; He, P.; Qiu, S.J.; Zhao, S.C.; Zhou, W. Availability of fertilizer recommendation for winter wheat based on Nutrient Expert System in Yangtze River Valley. *J. Plant Nutrie. Fertil.* **2020**, *26*, 1430–1439.
14. Pampolino, M.F.; Witt, C.; Pasuquin, J.M.; Johnston, A.; Fisher, M.J. Development approach and evaluation of the nutrient expert software for nutrient management in cereal crops. *Comput. Electron. Agric.* **2012**, *88*, 103–110. [CrossRef]
15. Liu, X.; Qiu, H.Z.; Zhang, W.M.; Zhang, C.H.; Zhu, J.; Ma, X.; Cheng, W.L. The effects of potato continuous cropping on soil chemical and biological properties in the Yellow River irrigation area of central Gansu. *Chin. J. Eco Agric.* **2017**, *25*, 581–593.
16. Ministry of Agriculture, PRC. *China Agric Statis. Report*; China Agriculture Press: Beijing, China, 2016.
17. Yu, X.B. The Effect of Fertilization on Soil Characteristics and Yield of Potato Farmland. Master's Dissertation, Inner Mongolia Agricultural University, Hohhot, Mongolia, 2016.
18. He, P.; Xu, X.P.; Zhou, W.; Smith, W.; He, W.T.; Grant, B.; Ding, W.C.; Qiu, S.J.; Zhao, S.C. Ensuring future agricultural sustainability in China utilizing an observationally validated nutrient recommendation approach. *Europ. J. Agron.* **2022**, *132*, 126409. [CrossRef]
19. Jiang, L.L.; Qiu, S.J.; Ding, W.C.; Xu, X.P.; He, P. Synergizing potato productivity and environmental performance with Nutrient Expert recommendation approach in northern China. *J. Clean. Product.* **2023**, *382*, 135258. [CrossRef]
20. Sokol, N.W.; Kuebbing, S.E.; Karlsen-Ayala, E.; Bradford, M.A. Evidence for the primacy of living root inputs, not root or shoot litter, in forming soil organic carbon. *New Phytol.* **2019**, *221*, 233–246. [CrossRef]
21. Zhang, X.D.; Amelung, W. Gas chromatographic determination of muramic acid, glucosamine, mannosamine, and galactosamine in soils. *Soil Biol. Biochem.* **1996**, *28*, 1201–1206. [CrossRef]
22. Joergensen, R.G. Amino sugars as specific indices for fungal and bacterial residues in soil. *Biol. Fertil. Soils* **2018**, *54*, 559–568. [CrossRef]
23. Saiya-Cork, K.R.; Sinsabaugh, R.L.; Zak, D.R. The effects of long-term N deposition on extracellular enzyme activity in an Acer saccharum forest soil. *Soil Biol. Biochem.* **2002**, *34*, 1309–1315. [CrossRef]
24. Bertin, C.; Yang, X.; Weston, L.A. The role of root exudates and allelochemicals in the rhizosphere. *Plant Soil* **2003**, *256*, 67–83. [CrossRef]
25. Buckeridge, K.M.; Mason, K.E.; Mcnamara, N.P.; Ostle, N.; Puissant, J.; Goodall, T.; Grif, R.I.; Stott, A.W.; Whitaker, J. Environmental and microbial controls on microbial necromass recycling, an important precursor for soil carbon stabilization. *Commun. Earth Environ.* **2020**, *1*, 1–9. [CrossRef]
26. Xu, Y.D.; Gao, X.D.; Liu, Y.L.; Li, S.G.; Liang, C.; Lal, R.; Wang, J.K. Differential accumulation patterns of microbial necromass induced by maize root vs. shoot residue addition in agricultural Alfisols. *Soil Biol. Biochem.* **2022**, *164*, 108474. [CrossRef]

27. Rousk, J.; Bååth, E. Fungal and bacterial growth in soil with plant materials of different C/N ratios. *FEMS Micro Ecol.* **2007**, *62*, 258–267. [CrossRef]
28. Wang, B.R.; Huang, Y.M. Initial soil formation by biocrusts: N demand and clay protection control microbial necromass accrual and recycling. *Soil Biol. Biochem.* **2022**, *167*, 108607. [CrossRef]
29. Yang, J.Q.; Zhang, X.; Bourg, I.C.; Stone, H.A. 4D imaging reveals mechanisms of clay- carbon protection and release. *Nat. Commun.* **2021**, *12*, 622. [CrossRef]
30. Zheng, H.F.; Vesterdal, L. Ecoenzymatic stoichiometry can reflect microbial resource limitation, substrate quality, or both in forest soils. *Soil Biol. Biochem.* **2022**, *167*, 108613. [CrossRef]
31. Chen, J.; Luo, Y.Q.; Katterer, T.; Olesen, J.E. Depth-dependent responses of soil organic carbon stock under annual and perennial cropping systems. *Proc. Natl. Acad. Sci. USA* **2022**, *119*, 28–29. [CrossRef] [PubMed]
32. Ridgeway, J.R.; Morrissey, E.M.; Brzostek, E.R. Plant litter traits control microbial decomposition and drive soil carbon stabilization. *Soil Biol. Biochem.* **2022**, *175*, 108857. [CrossRef]