

Article

Contrasting Effects of Nitrogen Deposition and Phosphorus Addition on Soil Organic Carbon in a Subtropical Forest: Physical Protection versus Chemical Stability

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Abstract: Soil organic carbon (SOC) not only contributes to maintain soil health, but is also important in regulating global climate change. How atmospheric nitrogen (N) deposition and phosphorus (P) addition affects SOC dynamics remains unclear, especially in subtropical forests. The response of SOC in three layers to N deposition and P addition in this study is estimated by analyzing the soil aggregates and C chemical stability composition fertilized with N (100 kg N hm⁻² a⁻¹) and/or P (50 kg P hm⁻² a⁻¹) over 9 years in a Chinese fir (*Cunninghamia lanceolata*) plantation. Treatments involving N deposition increased the SOC concentration, while P addition alone decreased the SOC concentration in soil layers above 10 cm. The addition of N significantly increased the mean diameter of topsoil aggregates, macroaggregates SOC concentration, and the contribution of N to total SOC. P addition decreased the relative abundances of aromatic and aliphatic functional groups while decreasing the chemical stability of SOC in the topsoil. A structural equation model indicated that N deposition promoted SOC concentration by mainly improving the physical protection of soil aggregates, while P addition reduced SOC sequestration by decreasing the chemical stability of SOC. Our research suggested that elevated N deposition might promote the soil C sink, while P fertilization would not be recommended under increased N deposition to protect soil C storage in subtropical forests.

Keywords: chemical stability of organic carbon; nitrogen deposition; phosphorus addition; soil aggregate; soil physical protection



Citation: Wang, X.; Wu, A.; Chen, F.-S.; Fang, X.; Wang, H.; Wang, F. Contrasting Effects of Nitrogen Deposition and Phosphorus Addition on Soil Organic Carbon in a Subtropical Forest: Physical Protection versus Chemical Stability. *Forests* **2024**, *15*, 385. <https://doi.org/10.3390/f15020385>

Academic Editor: Frank S. Gilliam

Received: 19 January 2024

Revised: 12 February 2024

Accepted: 16 February 2024

Published: 19 February 2024



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1. Introduction

Nitrogen (N) deposition has become an important phenomenon in the process of global change due to the influence of anthropogenic N emissions and atmospheric transport processes [1,2]. Due to the high degree of soil weathering, most of the phosphorus (P) is fixed by Fe, Al oxides leading to widespread P limitation in subtropical forests [3]. Meanwhile, excessive N deposition-induced soil acidification leads to reducing of phosphatase activity and cation mobility, which aggravated P limitation in forests [4–6]. The application of exogenous P fertilizer is one of the important measures that is used to alleviate soil P limitation [7]. Meanwhile, P addition was shown to alleviate soil nutrient limitation and change the physical and chemical properties of forest ecosystem soils [8]. Furthermore, N

deposition or P fertilization influenced both nutrient availability and decomposition processes, which determined carbon (C) stocks and global scale C-cycle. However, substantial gaps still exist in our knowledge, particularly about the effects of N and P availability on soil C dynamics in a forest ecosystem [9].

The forest soil C pool is the largest C pool in terrestrial forest systems, dominated by tropical and subtropical forests, which account for 55% of global forest carbon uptake. A small variation of SOC would cause significant impacts on CO₂ concentration in the atmosphere. In contrast, SOC is sensitive to external environmental changes [10]. Continued increase in N deposition would have an effect on forest soil carbon cycling processes [11–13]. Many experiments and meta-analyses documented positive, neutral, and negative effects of nutrient addition on the accumulation of SOC [11,12,14,15]. However, the underlying mechanisms regulating SOC dynamics are still not perfectly understood and integrated. In addition, SOC may generally decrease along with the soil depth [16], but the vertical distribution pattern and mechanisms influencing SOC under N deposition and P addition are still uncertain [17].

Previous research has found that aggregates could isolate microorganisms from decomposing SOC, which were considered to be the physical protection mechanisms for soil C accumulation [18]. Compared with macroaggregates, microaggregates generally have a more long-term protective effect on SOC and are more conducive to facilitating the sequestration of SOC [19]. However, SOC concentration in soil aggregates and their contributions to total SOC are markedly various in different sized particles [20,21]. Generally, larger mean weight diameter (MWD) and geometric mean diameter (GMD) reflect improved stability of soil structure [22]. The addition of N may enhance formation of soil aggregation and MWD by increasing the plant biomass that act as the physical binding agents of aggregates [10,23,24]. In contrast, soil acidification and the leaching of nutrient cations such as Ca²⁺, Mg²⁺ induced by N input [23] which decreased mycelium growth [25], further inhibiting the formation of soil aggregates [10]. However, the stability of soil aggregates and the contribution of different sized aggregates to SOC in light of the additions of both N and P remain unclear.

The chemical composition of various types of SOC (e.g., polysaccharides, aliphatics, aromatics, and phenols) can reflect the stability of organic C [26]. The nature of SOC decomposition is usually related to the properties of different SOC components [12]. In general, polysaccharides are derived from plant residues and root exudates, which can be preferentially decomposed when compared with more decomposition-resistant components [27]. In contrast, lignin-derived aromatic C is generally considered to be a recalcitrant SOC component and thus is more resistant to microbial decomposition [28]. In addition to individual chemical composition of various types of SOC, the ratios of specific chemical components are typically indicative of the chemical stability and degree of decomposition for various SOC [29]. Meanwhile, differences in the functional groups of different SOC have been used to indicate stabilization of organic C under different treatments [29]. The concentrations of labile components of SOC could be increased under N deposition due to an increased C input from plants [30], while previous studies documented the effect of nutrient addition on recalcitrant aromatic C were controversial [12,31]. Nevertheless, further research about how N deposition and P addition affect chemical composition and stability of SOC is required.

The SOC in subtropical forests serve as the world's largest reservoir of forest C sink and may be affected by a changing environment. However, the response pattern of SOC along the soil profile to atmospheric N deposition and P addition need further research. Thus, the present study explored the responses of soil physical structure and the chemical composition of SOC to N deposition and P addition and their roles in the accumulation of SOC in a subtropical Chinese fir (*Cunninghamia lanceolata*) plantation by conducting a 9-year in situ field experiment. The following three hypotheses are proposed: (1) N deposition will result in decreased SOC content due to the changes in soil chemical properties with excess N input; (2) P addition will result in increases in SOC because soil physiochemical

properties are improved with reduced P-limitation especially in the context of N deposition; and (3) N deposition or P fertilization has a stronger effect on SOC and its stability in the 0–5 cm and 5–10 cm than 10–20 cm soil layers. The results will provide data for evaluating potential soil C sink and provide a scientific basis for proposing management strategies for subtropical forests to respond to global change.

2. Materials and Methods

2.1. Study Area and Experimental Treatment

The experimental platform is located at the Qianyanzhou Subtropical Forest Ecosystem Observation and Research Station, Chinese Academy of Sciences (115°13′04″ E, 26°44′52″ N, altitude 102 m), Jiangxi Province, China. The main soil type of this station is red soil, which is a typical strong leaching soil and chalky clay with pH 4.3. The experimental area belongs to the central subtropical monsoon climate, with average annual temperature and precipitation of 18 °C and 1500 mm, respectively. The experimental platform was established in 2011, and the age of the Chinese fir stand was 12 years old, with *Dicranopteris pedata*, *Adinandra millettii*, *Cibotium barometz* as the understory dominant species (Figure S1).

The factorial experiment for simulating N deposition and P addition was established in a Chinese fir plantation. The fertilization experiment was begun from March 2012 to June 2020. Five replicate blocks were randomly conducted to 20 × 20 m plots in Chinese fir plantation, each block including four treatments: control (CK), N deposition (+N, 100 kg N hm^{−2} year^{−1}), P addition (+P, 50 kg P hm^{−2} year^{−1}), N deposition and P addition (N + P, 100 kg N hm^{−2} year^{−1} + 50 kg P hm^{−2} year^{−1}). We mixed with NH₄NO₃ and NaH₂PO₄ in fine sand and applied the fertilizer into each plot every three months, four times for each year (Figure S1).

2.2. Sample Collection

In July 2020, five replicate soil columns were randomly excavated using a steel box (20 × 20 × 20 cm) within each plot. Undisturbed soil samples (0–5, 5–10, and 10–20 cm layers) were collected and stored in sterilized aluminum boxes to prevent breaking aggregates. After soil was sieved through 2 mm screen, soil mixture samples were processed to determine other soil characteristics. Part of each soil sample was used for measuring SOC, total N, total P, available metallic elements, aggregate fractions, and chemical composition of SOC. And fresh soil was used for measuring of soil moisture, pH, mineral N (NH₄⁺-N plus NO₃[−]-N), and available P. Finally, the soils of each of the three soil depths were sampled with a 100 cm³ size cutting ring, which was used to measure bulk density after dried to a constant weight at 105 °C and calculated SOC storage.

2.3. Soil Physicochemical Properties

The SOC were determined using the K₂Cr₂O₇ titration method [32]. Soil pH was measured with a glass electrode (FiveEasy Plus™ FE28, Mettler Toledo, Greisensee, Switzerland). The content of N and P were measured with H₂SO₄-H₂O₂ digestion method [32]. The mineral N were extracted by KCl and available P were extracted by NaHCO₃; the supernatants were measured by automated flow analyzer. Available trace elements were determined by Atomic Absorption Spectrometry (TAS-990, Persee Analytics, Beijing, China) after extracted by DTPA-CaCl₂-TEA.

The SOC storage was calculated using the following equation:

$$SOC\ storage = \sum_{i=1}^n (C_i \times Y_i \times T_i) / 10$$

where SOC storage is the soil organic carbon storage at a specific soil depth, t hm^{−2}; *n* represents the number of soil profile layers; and *C_i*, *Y_i*, and *T_i* are the SOC concentration (g kg^{−1}), soil bulk density (g cm^{−3}), and the depth (cm) of the *i*th layer, respectively [16]. In addition, 10 is the unit conversion coefficient.

2.4. Soil Aggregate Structure

The undisturbed soil was fractionated into two aggregate-size classes through a nest of two sieves (0.25 mm and 0.053 mm), macroaggregates (MA, >0.25 mm) and microaggregates (MI, >0.053 mm, <0.25 mm), using an electric vibrating screen [33]. Briefly, 200 g of air-dried soil samples were placed on the top sieve of 0.25 and 0.053 mm sieve sets, soaked in deionized water for 10 min, and then separated using an oscillating wet sieve apparatus with an upper and lower amplitude of 3 cm, a frequency of 25 times per minute, and vibration for 2 min. Two particle sizes of soil aggregates were separated: macroaggregates (MA, >0.25 mm) and microaggregates (MI, >0.053 mm, <0.25 mm) [34]. The soil mass recovery rate after fractionation averaged 98%. All fractions were determined for SOC.

MWD and GMD were calculated by using Equations (2) and (3), respectively:

$$MWD = \sum_{i=1}^n (D_i M_i)$$

$$GMD = \text{Exp} \left(\sum_{i=1}^n (M_i \ln D_i) / \left(\sum_{i=1}^n M_i \right) \right)$$

where D is the average diameter of the aggregate fraction, and M is the ratio of the each size fraction weight in bulk soil [24].

The contribution of aggregates to SOC was calculated by using Equation (4):

$$P = \frac{C \times W}{Q}$$

where P is the contribution of aggregates to SOC (%); C is the C concentration of aggregates ($\text{g} \cdot \text{kg}^{-1}$); Q is the total SOC ($\text{g} \cdot \text{kg}^{-1}$); and W is the mass proportion of specific aggregates [21].

2.5. Soil Organic Carbon Chemical Composition

The chemical composition of SOC was determined by diffuse reflection Fourier transform mid-infrared spectrometer (Thermo Electron Corporation, Waltham, MA, USA). The air-dried soil sample and dry 97% potassium bromide were mixed and ground with mass ratio of 1:80, and then compacted by the tablet press. Parameter setting: the spectrum range is set to $400 \sim 4000 \text{ cm}^{-1}$ and the scanning frequency is 64 times. Different characteristic relative peak areas (rA, %) were used to assess the relative abundance of each C functional group: alcohol and phenol compounds (3260 cm^{-1}), aliphatic C groups ($2924, 2820, 1460, 1405 \text{ cm}^{-1}$), aromatic C groups ($1650, 920, 840 \text{ cm}^{-1}$), and polysaccharides ($1160, 1076, 1033, 1010 \text{ cm}^{-1}$) [35].

To establish the relationship between functional group composition and SOC quality, we calculated the two ratios of the areas of specific wavelength peaks of each functional group as shown in Equations (5) and (6). These two indices were used to indicate indicators of relative SOC decomposition and resistance [29,36]:

$$\text{SOC decomposition degree} = \frac{rA_{1650+920+840}}{rA_{2429+2850+1470+1405}}$$

$$\text{SOC chemical stability} = \frac{rA_{2419+2850+1470+1405+1650+920+840}}{rA_{1160+1076+1033+1010}}$$

where rA represents the peak area, and each number represents the characteristic peak wavelength (cm^{-1}) corresponding to the functional group.

2.6. Data Analyses

Normality of distributions of all data were tested with Levene's test prior to statistical analysis. Two-way ANOVA was used to examine the effects of N deposition, P addition, and their interactions on all soil properties. Meanwhile, one-way ANOVA was used to analyze the variability of soil physicochemical properties, soil carbon chemical composition, and soil aggregate structure among four treatments. We used Pearson's correlation analysis to

assess the relationships among SOC, chemical composition, aggregate protection properties in different soil layers. Finally, structural equation modeling (SEM) was used layer by layer to test how influencing factors (aggregate protection and chemical composition) mediated the response of SOC storage to N and P addition. SPSS 18.0 and Amos 21.0 (SPSS Inc., Chicago, IL, USA) were used to perform all statistical analyses.

3. Results

3.1. SOC Concentration and Other Physicochemical Properties

Nitrogen and P addition treatments generally altered SOC concentration and storage at the 0–5 cm and 5–10 cm soil layers but not at the 10–20 cm soil layer (Figures 1 and S2). In the 0–10 cm soil layer, N deposition treatments (+N, N + P) significantly increased SOC, but the treatment using P addition alone (+P) significantly decreased SOC storage and concentration. Nutrient addition treatments had no effect on SOC storage and concentration in the 10–20 cm soil layer (Figures 1 and S2).

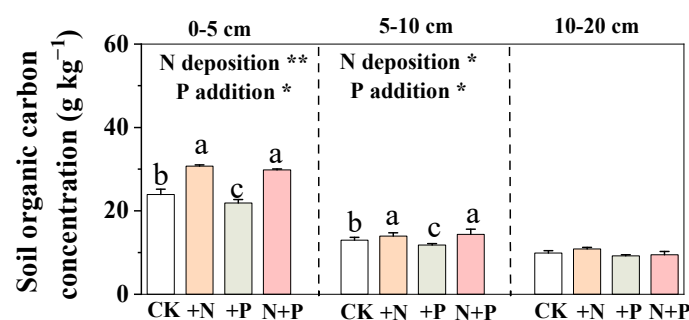


Figure 1. Soil organic carbon concentration (mean $\pm$ standard error) in the 0–5, 5–10, and 10–20 cm layers in response to N and P addition. The significant among four treatments differences were characterized by lower case letters on the error bars ($p < 0.05$). The nitrogen deposition, +N, orange bars; phosphorus addition, +P, gray bars; both nitrogen and phosphorus addition, N + P, pink bars; control, CK, white bars. The two-way analysis of variance results are presented as an inset, * $p < 0.05$ and ** $p < 0.01$.

Nitrogen and/or P addition treatments almost affected all of the physicochemical properties of soil, except for the bulk density, in the three soil layers analyzed here (Table 1). In three soil layers, soil moisture, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and TN significantly increased in N deposition treatments (+N and N + P) ($p < 0.05$; Table 1) but did not change under the P addition treatment (+P). In contrast, soil pH and available Ca and Mg generally decreased under N deposition treatments (+N and N + P; Table 1 and Figure S3). The soil available P and TP in all soil layers were increased by P addition (+P and N + P; Table 1).

Table 1. Results of analysis of variance (ANOVA) comparing the effects of both N and P addition on soil properties in the three soil layers of a subtropical Chinese fir (*Cunninghamia lanceolata*) plantation.

Soil Depths	Variables	Control	+N	+P	N + P	F Value of ANOVA		
						N Deposition	P Addition	Interaction
0–5 cm	Soil moisture (%)	15.57 $\pm$ 0.65 b	20.19 $\pm$ 0.13 a	14.83 $\pm$ 0.73 b	18.25 $\pm$ 0.91 a	20.25 ***	2.24	0.45
	Bulk density (g cm^{-3})	1.48 $\pm$ 0.33 a	1.47 $\pm$ 0.27 a	1.56 $\pm$ 0.25 a	1.49 $\pm$ 0.22 a	0.45	1.21	0.12
	Soil pH	4.62 $\pm$ 0.06 a	4.21 $\pm$ 0.02 b	4.52 $\pm$ 0.02 a	4.25 $\pm$ 0.03 b	64.67 ***	0.76	2.80
	$\text{NH}_4^+\text{-N}$ (mg kg^{-1})	10.20 $\pm$ 0.22 b	15.26 $\pm$ 0.30 a	9.81 $\pm$ 0.28 b	16.27 $\pm$ 0.66 a	350.26 ***	3.13	2.01
	$\text{NO}_3^-\text{-N}$ (mg kg^{-1})	2.66 $\pm$ 0.28 b	4.73 $\pm$ 0.31 a	2.81 $\pm$ 0.27 b	4.36 $\pm$ 0.30 a	261.57 ***	6.62 **	1.77
	Available P (mg kg^{-1})	4.33 $\pm$ 0.4 c	4.69 $\pm$ 0.8 c	94.92 $\pm$ 1.2 a	60.90 $\pm$ 1.01 b	198.71 ***	3552 ***	193.38 ***
	Total N (g kg^{-1})	0.84 $\pm$ 0.02 b	1.43 $\pm$ 0.09 a	0.84 $\pm$ 0.02 b	1.44 $\pm$ 0.09 a	1557 ***	2.81	0.81
	Total P (g kg^{-1})	0.29 $\pm$ 0.02 c	0.31 $\pm$ 0.02 c	0.44 $\pm$ 0.03 a	0.39 $\pm$ 0.03 b	1.46	57.45 ***	0.10

Table 1. Cont.

Soil Depths	Variables	Control	+N	+P	N + P	F Value of ANOVA		
						N Deposition	P Addition	Interaction
5–10 cm	Soil moisture (%)	14.94 ± 0.37 bc	18.86 ± 0.55 a	16.13 ± 0.68 b	19.07 ± 0.55 a	99.90 ***	4.10	2.07
	Bulk density (g cm ^{−3})	1.58 ± 0.04 a	1.39 ± 0.09 a	1.56 ± 0.08 a	1.52 ± 0.09 a	0.33	1.01	0.25
	Soil pH	4.51 ± 0.07 a	4.26 ± 0.06 b	4.51 ± 0.10 a	4.33 ± 0.06 b	32.14 ***	0.89	1.10
	NH ₄ ⁺ -N (mg kg ^{−1})	8.75 ± 0.90 b	12.70 ± 0.82 a	8.30 ± 0.59 b	14.02 ± 0.61 a	271.12 ***	3.31	4.15
	NO ₃ [−] -N (mg kg ^{−1})	1.89 ± 0.20 bc	4.53 ± 0.11 a	2.43 ± 0.09 b	4.05 ± 0.12 a	1969 ***	0.43	86.11 ***
	Available P (mg kg ^{−1})	3.34 ± 0.25 c	3.26 ± 0.53 c	52.71 ± 4.12 a	36.45 ± 2.98 b	41.26 ***	1084.2 ***	41.07 ***
	Total N (g kg ^{−1})	0.78 ± 0.04 b	1.06 ± 0.09 a	0.69 ± 0.04 c	1.03 ± 0.07 a	179.49 ***	6.44 *	0.00
	Total P (g kg ^{−1})	0.20 ± 0.02 c	0.22 ± 0.03 c	0.31 ± 0.04 a	0.25 ± 0.02 b	5.27 *	69.50 ***	22.20 ***
10–20 cm	Soil moisture (%)	14.81 ± 0.79 c	16.60 ± 0.40 b	14.96 ± 0.80 c	17.06 ± 0.55 a	77.90 ***	3.00	1.07
	Bulk density (g cm ^{−3})	1.50 ± 0.07 a	1.61 ± 0.01 a	1.59 ± 0.01 a	1.53 ± 0.04 a	0.35	1.21	0.01
	Soil pH	4.46 ± 0.03 a	4.28 ± 0.02 b	4.56 ± 0.02 a	4.37 ± 0.03 b	32.14 ***	0.33	0.31
	NH ₄ ⁺ -N (mg kg ^{−1})	7.13 ± 0.39 b	11.34 ± 0.28 a	7.17 ± 0.20 b	12.16 ± 0.42 a	100.12 ***	3.11	2.79
	NO ₃ [−] -N (mg kg ^{−1})	1.48 ± 0.06 c	4.06 ± 0.08 a	2.03 ± 0.12 c	3.86 ± 0.08 b	324.40 ***	0.23	10.12 **
	Available P (mg kg ^{−1})	1.63 ± 0.21 c	1.78 ± 0.18 c	15.19 ± 0.15 a	10.54 ± 0.31 b	41.26 ***	356.25 ***	22.56 ***
	Total N (g kg ^{−1})	0.59 ± 0.03 b	0.81 ± 0.03 a	0.59 ± 0.03 b	0.88 ± 0.03 a	39.49 ***	1.44	0.00
	Total P (g kg ^{−1})	0.14 ± 0.01 b	0.18 ± 0.01 b	0.21 ± 0.02 a	0.17 ± 0.01 b	3.27 *	31.79 ***	1.10 **

The data shown in the left columns indicate mean ± one standard error. The data shown in the right columns indicate *F* and *p* values. Significance is indicated by an asterisk: * *p* < 0.05, ** *p* < 0.01, and *** *p* < 0.01. The significant among four treatments' differences were characterized by lower case letters on the error bars (*p* < 0.05). N deposition (+N), P addition (+P), and N plus P addition (N + P).

3.2. Soil Aggregates and Organic Carbon in Different Aggregates

Macroaggregates dominated the soil aggregates, accounting for more than 90% of the total aggregates, and soil aggregate structure was mainly affected by N deposition, especially at the top 10 cm depths (Figure 2). N deposition (+N and N + P) treatments notably increased the proportion of macroaggregate mass, MWD, and GMD in the 0–5 and 5–10 cm soil layers (Figure 2a,c,d). In addition, +N treatment and +P treatment decreased the mass of microaggregates in the 5–10 cm soil layer, respectively (Figure 2b). +N + P treatment increased the proportion of microaggregate mass in the 10–20 cm soil layer, while no significant differences were detected for aggregate MWD and GMD among the four treatments (Figure 2a–d).

Nitrogen deposition significantly altered the macroaggregate OC concentration and its contribution to total SOC. These values were higher when the soil was treated with +N and N + P than with +P and the control in the three soil layers, except for similar contributions of macroaggregate OC to total SOC among four treatments in the 10–20 cm soil layer (Figure 2e,g). In contrast, the microaggregate OC concentration has no significant response to N deposition and P addition in all soil layers. However, the contribution of microaggregates C to total SOC was influenced by +N treatment (+N and N + P) in the 0–5 cm and 5–10 cm soil layer, and +P treatment in the 10–20 cm soil layer (Figure 2 h).

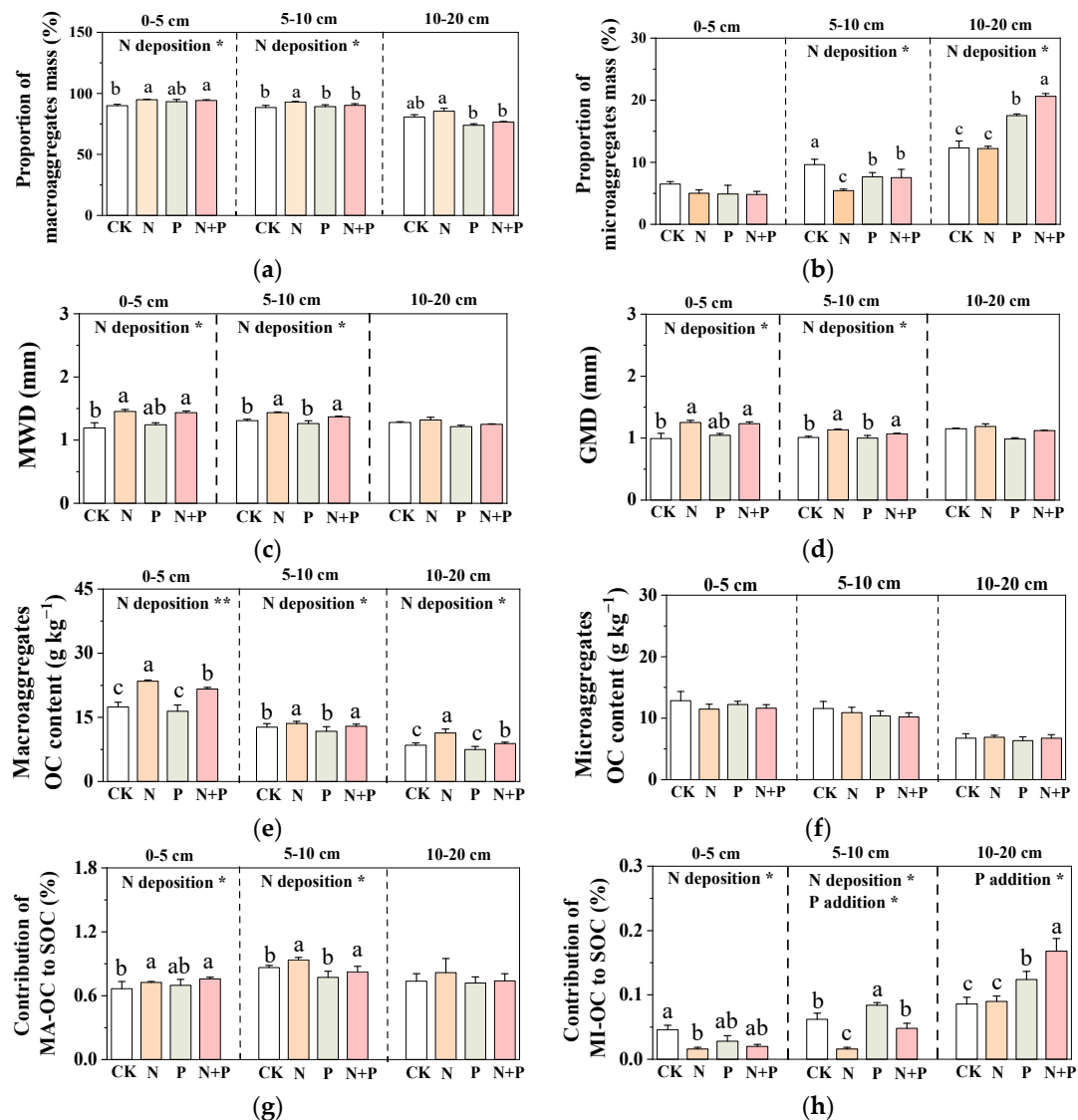


Figure 2. Indices (mean $\pm$ standard error) related to soil aggregate structure in different soil layers in response to N deposition and P addition. Proportions of (a) macroaggregate and (b) microaggregate mass; (c) MWD; (d) GMD; (e) macroaggregate and (f) microaggregate organic carbon (OC) content; and (g) contributions of macroaggregate OC and (h) microaggregate OC to SOC. The significant among four treatments' differences were characterized by lower case letters on the error bars ($p < 0.05$). The nitrogen deposition, +N, orange bars; phosphorus addition, +P, gray bars; both nitrogen deposition and phosphorus addition, N + P, pink bars; control, CK, white bars. The two-way analysis of variance results are presented as an inset, * $p < 0.05$ and ** $p < 0.01$.

3.3. Chemical Composition of OC and Their Stability and Decomposability

The chemical composition and stability of SOC were mainly affected by P addition but not by N deposition (Figure 3). Functional SOC groups were dominated by polysaccharides (41–56%), followed by alcohol and phenols, and with low levels of aromatic and aliphatic compounds (Figure 3a–d). Compared with the control, the proportions of aromatic and aliphatic compounds to SOC decreased when treated with +P in the soil layer above 10 cm, while the proportion of alcohols, phenols, and polysaccharides to SOC did not change under N and P addition in all soil layers (Figure 3a–d) and was low in the +P treatment but had no significant difference among treatments. Both the degree of decomposition of SOC and chemical stability of SOC were generally higher when soils were treated with +N than control treatments in the soil layer above 10 cm (Figure 3e,f).

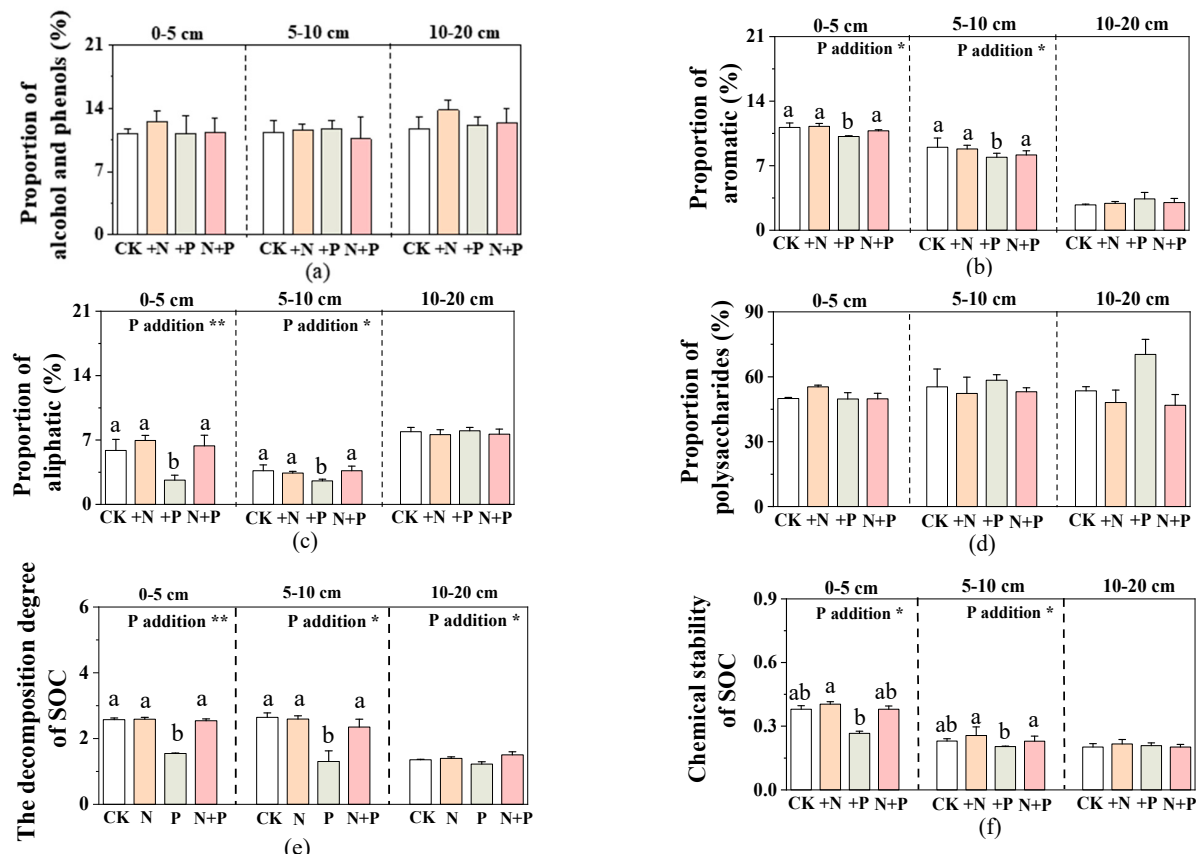


Figure 3. Chemical composition and stability of SOC (mean $\pm$ standard error) in different soil layers in response to N deposition and P addition. Proportions of (a) alcohol and phenols, (b) aromatic, (c) aliphatic, (d) polysaccharides; (e) the decomposition degree of SOC; (f) chemical stability of SOC. The significant among four treatments' differences were characterized by lower case letters on the error bars ($p < 0.05$). The nitrogen deposition, +N, orange bars; phosphorus addition, +P, gray bars; both nitrogen deposition and phosphorus addition, N + P, pink bars; control, CK, white bars. The two-way analysis of variance results are presented as an inset. * $p < 0.05$ and ** $p < 0.01$.

3.4. Influencing Factors on SOC Storage Driven by N and P Addition

In the soil layer above 10 cm, the SOC concentration was positively correlated with $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$, TN, microaggregate OC, the relative abundance of aromatic compounds, aggregate MWD or GMD, and the chemical stability of SOC but negatively correlated with available P and the degree of decomposition of SOC (Figure 4). Meanwhile, the macroaggregate OC concentration was positively correlated with $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and TN. The degree of decomposition of SOC was positively correlated with available P, but the chemical stability of SOC was negatively correlated with available P (Figure 4). In contrast, SOC was not significantly correlated with any physical and chemical properties in the 10–20 cm soil layer (Figure S4).

According to the structural equation model, N deposition played a positive direct role in aggregate MWD and GMD as well as on macroaggregate OC, all of which significantly and positively altered the SOC storage. However, P addition showed a neutral effect on soil aggregate composition, structure, and OC storage (Figure 5a). In contrast, N deposition and P addition had positive and negative effects, respectively, on the aromatic proportion of SOC. Meanwhile, P addition inhibited the degree of decomposition and chemical stability of SOC. Next, among the aromatic proportion to SOC, the degree of SOC decomposition and chemical stability of SOC were positive, while the degree of SOC decomposition was negatively correlated with SOC storage. Thus, all these three chemical properties together led to a decrease in SOC in top soils (Figure 5b). The result in the 10–20 cm layer is not shown due to the absence of the expected pathway.

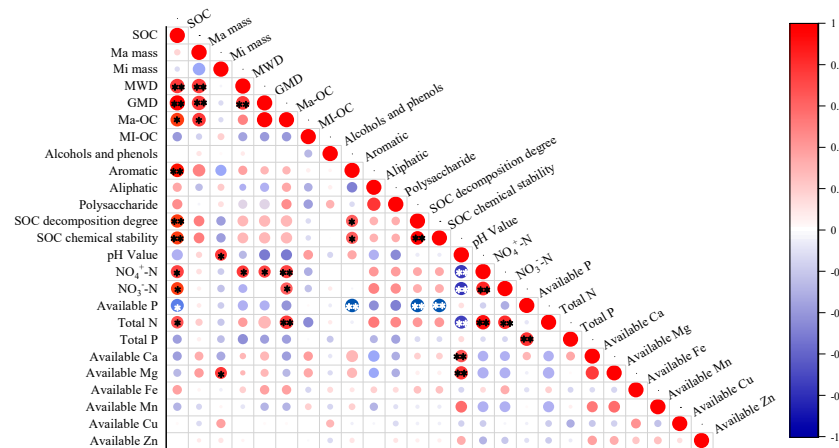


Figure 4. A matrix heatmap of Pearson's correlation among the organic carbon concentration, chemical composition, stability of soil organic carbon, as well as soil physical and chemical properties of soil aggregates in 0–10 cm soil layer. Note: MA mass = the proportion of macroaggregate mass; MI mass = the proportion of microaggregate mass; Ma-OC = macroaggregate organic carbon content; MI-OC = microaggregate organic carbon content; MWD = aggregate mean weight diameter; GMD = geometric mean diameter; AP = available soil phosphorus. The red circle represents a positive correlation, the blue circle represents a negative correlation, the darker the color, the stronger the correlation. An asterisk indicates a significant correlation. * $p < 0.05$ and ** $p < 0.01$.

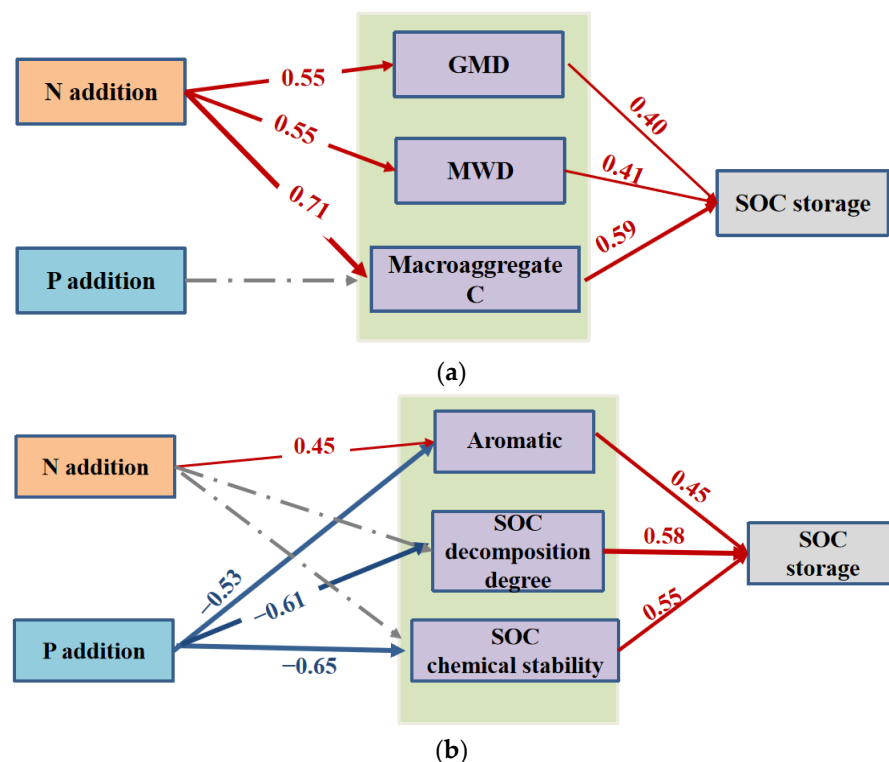


Figure 5. Structural equation model showing the effects of N deposition and P addition on C storage via aggregate structure and chemical composition as well as the stability of organic carbon in the (a) 0–5 cm and (b) 5–10 cm layers. (a) Note: results of model fitting, $\chi^2 = 22.327$, $p < 0.05$, $df = 15$, CFI = 0.969, RMSEA = 0.035. (b) Note: results of model fitting, $\chi^2 = 22.514$, $p < 0.05$, $df = 15$, CFI = 0.980, RMSEA = 0.034. The numbers are standardized path coefficients which are next to the arrows. Deep red and light blue arrows indicate positive and negative relationships, respectively ($p < 0.05$). The grey dashed arrows represent non-significant ($p > 0.05$) relationships; these definitions also apply to supplementary table and figures.

4. Discussion

In total disagreement with our hypotheses, N deposition promoted the accumulation of SOC and increased the SOC concentration due to the increasing of the aggregate diameter so that the aggregate exerted physical protection to soil nutrients, while P addition inhibited SOC storage by decreasing the chemical stability decomposability of organic C. Clearly, the effect of N and P addition on SOC varies under different pathways, which requires further discussion.

4.1. Nitrogen Deposition Increases SOC Content via Increasing Physical Protection

Contrary to our expectation, we found N deposition increased SOC (SOC concentration, storage, or both) in the 0–10 cm soil depth. The increase in SOC was primarily caused by the physical protection of SOC by soil aggregates. A previous study showed a positive correlation between the stability of soil aggregates and SOC concentration [37]. Strong correlations among macroaggregate C, MWD, GMD, and SOC were observed in our study (Figures 4 and 5), which support the idea that soil aggregates can improve the stability and sequestration of SOC in our study.

The soil aggregates are often related to binding agents, which include temporary binding agents such as roots, litter, mycelium that stabilize macroaggregates, and persistent agents including metal oxides and hydroxides that are associated with microaggregates [24,38,39]. Our result was consistent with a recent study [24], which reported that the proportion of macroaggregate mass increased with N deposition in all soil layers. N deposition increased the mass of macroaggregates probably due to the fact that N deposition promoted the production of temporary binding agents. N enrichment resulted in increased aboveground and belowground plant biomass, which facilitated aggregate stability [33]. However, the proportion of microaggregates were decreased by N deposition (Figure 2), which may be related to the reduction of metal cations by a lower pH value under N deposition [11]. Our study also found a strong positive correlation among soil pH, Ca, and Mg (Figure 4). N depositions exacerbated soil acidification which induced the leaching of metal cations and nitrates, thus preventing the formation of microaggregate fractions by persistent agents [24,40]. Moreover, we observed that macroaggregate material, which comprised more than 90% of total soil aggregates, was the dominant soil aggregate in a subtropical forest. In addition, N input improved the MWD and GMD (Figure 2) in this study, indicating that N deposition favors the formation of stable structures in soil aggregates [22].

N deposition enhanced the accumulation of macroaggregate SOC in the soil layer above 10 cm (Figure 2). However, a combination of N and P addition did not change the concentration of OC in the microaggregate (Figure 2). Surprisingly, it was found that a combination of +N and +P treatments had similar effects on OC in aggregates and aggregate mass in all soil layers. The preferential storage of SOC in the macroaggregate under N deposition was mainly caused by a change in microbial diversity and hydrolase activity. N deposition attenuated C cycling in the macroaggregate by inhibiting the activity of macroaggregate-related enzymes [24] and changing the microbial community composition, especially the relative abundance of *Acidobacteria*, leading to C accumulation in the macroaggregate [41,42]. Meanwhile, the contribution of OC in the macroaggregate to SOC was increased by N deposition (Figure 2). The concentration of SOC is always considered the important factor mediating the formation of macroaggregate and, in turn, is affected by the stability of the soil macroaggregate [43]. Although N deposition reduced the contribution of OC in the microaggregate to SOC (Figure 2), the contribution of OC in the microaggregate to SOC was negligible because the proportion of microaggregate mass was much lower than that of the macroaggregate (Figure 2).

4.2. Phosphorus Addition Decreases SOC via Decreasing Chemical Stability

Luo et al. (2022) recently suggested that the increase in SOC driven by P fertilization might alleviate P limitation on soil soluble OC and the contribution of microbial necromass

to SOC, resulting in SOC accumulating in a subalpine forest [15]. However, the present study found that decreasing of SOC in the soil layer above 10 cm after P addition, a strong positive correlation between SOC (SOC concentration, storage, or both) and chemical stability, indicating that the decreasing of SOC under N deposition and P enrichment was primarily caused by a decrease in chemical stability in subtropical forests (Figures 4 and 5). The chemical composition of SOC affects the formation and stability of SOC by providing chemical protection from the breakdown of SOC and is one of the most important indicators that can be used to determine the stability of SOC [44].

The FT-IR analysis revealed how the chemical structure of SOC changes under the addition of N, P, or combined N and P addition. The polysaccharide compounds were the dominant functional groups of SOC among all treatments (Figure 3). The addition of P reduced the relative abundance of aromatic and aliphatic groups in the 0–10 cm soil layer. These results were related to the increased microbial diversity resulting from P addition. Additionally, P addition may decrease the chemical composition of SOC, which is difficult to decompose, by promoting microbial decomposition. It is not difficult to find that a certain correlation exists between the chemical composition of SOC and microorganisms in terms of the mechanism of N and P addition regulating changes in the chemical composition of SOC in soil [30]. Together, previous studies as well as the present study suggested that the addition of N, P, or combined N and P addition could alter the chemical composition of SOC by altering microbial properties and their utilization preferences for the molecular composition of SOC [12,45].

Unlike polysaccharides, lignin and tannin represent the main source of aromatic compounds in soil; hence, aromatic compounds are relatively resistant to microbial decomposition [12,46]. Therefore, an increase in the volume of aromatic groups in the soil indicates that SOC becomes more stable and less likely to be decomposed by microorganisms. According to previous studies, a higher SOC decomposition degree index (the ratio of aromatic to aliphatic compounds) indicates that SOC in those conditions has a stronger resistance to microbial decomposition and is more stable [47]. Furthermore, a higher degree of structure stability of SOC (measured as the ratio of C- to O-functional groups) was associated with more recalcitrant SOC and increased SOC stability [29]. The degree of SOC decomposition was significantly decreased by P addition, which demonstrated that P addition caused the decomposition of SOC in the 0–20 cm soil layer (Figure 3). Meanwhile, P addition decreased the stability of the chemical structure of SOC, leading to a decrease in SOC stability and consequently SOC concentration in the 0–10 cm soil layer (Figures 3 and 5). In other words, the degree of decomposition in SOC and the chemical stability of SOC appeared to be more appropriate indices of SOC stability of the surface soil in this subtropical forest.

4.3. Responses of SOC and Its Stability to N and P Addition Varied with Soil Depth

SOC and its physical and chemical stability depended on soil depth. Soil nutrients, aggregates, and the chemical composition of SOC decreased along with the soil profile depth [48]. Compared with previous studies [17], we found that the largest decrease in SOC storage occurred in the 10–20 cm soil layer (Figure 1). In addition, SOC, aggregate, protection, and the chemical stability of SOC exhibited asynchronous responses to N and P inputs with increasing soil profile depth. The strong sensitivity of SOC and its physical and chemical stability to N and P addition only appeared in the 0–10 cm soil layer, suggesting that SOC and its stability mechanism were depth-dependent under N deposition and P addition, which supported our hypothesis. The results may be explained in several ways. First, compared with the 0–10 cm soil, the deeper soil had a smaller amount of fresh C input through dead roots and root sediments [16]. Second, deep soil has limited C input from aboveground plant residues [49]. Third, OC decomposes more slowly in deep soil because the oxygen concentration is lower at depth than that in topsoil, which inhibits microbial activity. Finally, since N and P addition is mainly performed in the surface soil, the degree of the response in deep soil to the fertilization process is much smaller than that of the surface soil. Our findings highlight the mechanistic interactions among SOC, soil

aggregates, and SOC chemical composition that vary with soil depth. Increasing soil depth will weaken the response of SOC and its stability to N and P addition.

5. Conclusions

Our research revealed that soil aggregate protection and SOC chemical composition were important for the preservation of SOC under N deposition and P addition in a subtropical Chinese fir plantation. The results revealed that (1) N deposition is conducive to an increase in SOC storage and concentration, while P addition has the opposite effect on SOC; (2) N deposition promoted the stabilization and sequestration of SOC by increasing the physical protection of soil aggregates; (3) P addition inhibited the accumulation of SOC by reducing the chemical stability of SOC; and (4) SOC and its stability mechanism are depth-dependent under N deposition and P addition. The present study demonstrated that the change of SOC under N deposition and P addition treatment were driven by a combination of physical and chemical stability mechanisms in a subtropical Chinese fir plantation. The physical protection of macroaggregates and resistance of chemical C groups may be the main driving mechanisms allowing for the sequestration of SOC in a subtropical forest. Considering the sensitive response of SOC in the plantation to environmental change, the protection of aggregates and the chemical structure stability of SOC should be considered to strengthen the soil C sink in a subtropical forest in the context of the “double carbon” goal of China defined as the twin goals of reaching a “carbon peak” and becoming “carbon neutral” in China.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f15020385/s1>, Figure S1. Illustrations of the studied subtropical Chinese fir (*Cunninghamia lanceolata*) plantation: (a) location of the study areas within China and (b) Jiangxi Province; (c) hemispherical photo of the canopy within the study area; and (d) layout of the study site on an aerial photo and a photo of the study site; (e) graphic illustration of the four treatments. Figure S2. Soil organic carbon storage (mean $\pm$ standard error) in response to N deposition and P addition. Different lowercase letters on the error bars indicate significant differences ($p < 0.05$) among four treatments: nitrogen deposition, +N, orange bars; phosphorus addition, +P, green bars; both N deposition and P addition, N+P, pink bars; and control, CK, white bars) within each soil layer. The two-way analysis of variance results are presented as an inset. * $p < 0.05$. Figure S3. Soil available trace elements concentration in response to N deposition and P addition. Different lowercase letters on the error bars indicate significant differences ($p < 0.05$) among four treatments: nitrogen deposition, N, pink bars; phosphorus addition, P, green bars; both N deposition and P addition, N+P, blue bars; and control, CK, white bars within each soil layer. The two-way analysis of variance results are presented as an inset. * $p < 0.05$. Figure S4. A matrix heatmap of Pearson’s correlation among organic carbon content, chemical composition, stability of soil organic carbon, as well as soil physical and chemical properties of soil aggregates in the 10–20 cm layers. Note: MA mass = the proportion of macroaggregate mass; MI mass = the proportion of microaggregate mass; Ma-OC = macroaggregate organic carbon content; MI-OC = microaggregate organic carbon content; MWD = aggregate mean weight diameter; GMD = geometric mean diameter; AP = available soil phosphorus. The red block represents a positive correlation, the blue block represents a negative correlation, the darker the color, the stronger the correlation. The number at the bottom left represents the correlation coefficient. * $p < 0.05$ and ** $p < 0.01$.

Author Contributions: Conceptualization, F.-S.C.; Investigation, X.F.; Resources, H.W.; Writing—original draft, X.W. and A.W.; Writing—review & editing, F.W.; Funding acquisition, F.W. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Natural Science Foundation of China (grant numbers: 31930070, 32171759, 32201533) and the Double Thousand Plan of Jiangxi Province (grant number jxsq2023201058).

Data Availability Statement: Data will be made available on request.

Acknowledgments: We thank Chunyu Chen, Ren Liu, and Qiao Liu for the help with field sampling and laboratory analysis.

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

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