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Effect of nitrogen and phosphorus addition on soil aggregation and its associated organic carbon

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ABSTRACT

Although the response of plant productivity and soil biogeochemical processes to nutrient addition has been widely investigated, little is known about the effects of N and P addition on soil aggregate fractions, an important indicator of soil organic C stability. We investigated the effects of exogenous N and P inputs on soil aggregates and their associated organic C in a 6-year fertilisation experiment in a subtropical Chinese fir (*Cunninghamia lanceolata*) plantation. A wet sieving method was used to determine the aggregate size distribution. Our results showed that the most abundant aggregate was the macroaggregate, ranging between 59% and 74%. The least abundant fraction was the smallest (silt and clay), ranging between 10% and 15%. Neither the soil aggregate fractions nor their associated organic C differed significantly after 6 years of N and P addition at the 0.05 level. In contrast, the oxalate-extractable iron Fe and pyrophosphate-extractable Fe of 0–10 cm soil were significantly decreased by P addition; this may alter aggregate formation over longer periods. Thus, future studies should examine the effects of nutrient addition on aggregates over a longer period. In particular, more attention should be paid to the effects of P addition in subtropical forests.

Introduction

Both N and P are key nutrients for plant growth [1,2]. Primary production may be limited by N, P, or both in most ecosystems worldwide [3]. The main biological sources of N are legume nitrogen fixation and mineralisation of organic matter, while P is naturally provided via mineral weathering [4,5]. In recent decades, anthropogenic sources of N and P, including artificial addition and deposition from the atmosphere, have become increasingly significant and even dominant in some ecosystems [4,5]. Human activities, such as agriculture and fossil fuel combustion, convert large amounts of gaseous N into reactive N

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that can be used by vegetation and microorganisms [6]. For example, the average annual bulk deposition of N in China increased from 13.2 kg ha^{-1} in the 1980s to 21.1 kg ha^{-1} in the 2000s [7]. The main sources of atmospheric P include combustion and primary biogenic and biomass-burning aerosols [8]. The total annual transfer of P to and from the atmosphere is estimated to be 3.7 Tg [5]. However, P deposition is less easily quantified than N. Large P-carrying particles are most likely redeposited near their origin, potentially causing significant local redistribution from fertilised farmland to P-poor ecosystems, such as forests. Additionally, long-distance transportation activities usually lead to the deposition of fine dust that contains high levels of P [5]. One of the most limiting nutrients in subtropical forests is usually P [9]; it becomes depleted in subtropical regions owing to biological uptake and other losses. In addition, N deposition can sometimes reduce the availability of soil P and lead to an imbalance in the N:P ratio [10,11]. The interactions between N and P, and the effects of their addition require further research.

Soil is the largest source of terrestrial organic C, storing more C than plants and the atmosphere [12]. Small changes in organic C stored in the soil cause large fluctuations in atmospheric carbon dioxide concentrations and affect the rate of climate change [13]. Therefore, understanding the factors controlling soil C turnover processes is essential for comprehending the changing global C cycle. Anthropogenic changes to the cycling of N and P may directly affect plant productivity and soil properties such as pH, nitrate and ammonium nitrogen, and microbial growth, thus, affecting the soil C cycle [14,15]. The increase in N and P availability in forests may increase C sequestration [16] by promoting plant growth and increasing the accumulation of soil organic matter (SOM) through increased litter production. Meanwhile, as the composition of soil is very complex and contains a variety of substrates, N addition has been found to have positive [17], negative [18], or neutral [19,20] effects on soil organic carbon (SOC) decomposition rates. Compared to N addition, research on P addition is still scarce. Given that P is often limited within subtropical systems, the effects of increased N availability on SOC storage are likely to be dependent on the availability of P [21], interactions between these two elements should therefore be identified.

Soil aggregates affect soil fertility and C stability by rearranging the distribution and localisation of SOC and nutrients [22]. Some studies have shown no significant change to bulk soil C; however, different soil C fractions with distinct physical and biochemical properties respond variously to N addition [19], highlighting a limitation inherent to the single-pool approach to investigating soil C responses to disturbances. Therefore, it is necessary to separate bulk soil into different aggregate fractions and to investigate their response to nutrient addition separately [23].

Changes to bulk soil C are slow and sometimes unobserved in the short term, while organic C stability responds faster to interference [19]. Moreover, SOC attached to iron oxides or minerals is usually more stable than that protected by aggregates. For example, in acidic soils, attachment to iron oxides is the most effective protection mechanism [24]. Attachment to minerals plays the most important role in SOC protection in some ecosystems [25]. Stabilisation mechanisms for SOC are very complicated, and there remains considerable uncertainty regarding the response of SOC to nutrient addition.

Chinese fir (*Cunninghamia lanceolata*) is one of the most important tree species in plantations in southern China. We selected a Chinese fir plantation that had been fertilised for

6 years to examine the effects of N and P addition on soil aggregate fractions and their associated organic C, iron oxides, and clay content. To the best of our knowledge, this study is among the first to examine the effects of nutrient addition on each aggregate fraction and relative soil properties. We hypothesised that (1) N and P addition would change aggregate fractions and their associated organic C levels, and (2) P addition may have a stronger effect on SOC and soil aggregates than N because subtropical plantations are generally limited by P. We hope our results will provide new insights into the mechanisms of SOC stability under global change and provide a scientific basis for forest management.

Materials and methods

Site description

The experimental fields were located at the Qianyanzhou Ecological Research Station (QYZ, 26°4446″ N, 115°0405″ E) in Jiangxi Province, southern China. The study site was maintained by the Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences. The field study did not involve endangered or protected species and all necessary permits were obtained. The site is a typical red soil hilly region with a subtropical monsoon climate. The mean air temperature near the site was 18 °C. The coldest and warmest months were January and July, respectively. The region receives an average annual precipitation of 1509 mm. The clear-sky duration per year was 1306 h, and the solar radiation was 4349 MJ m^{-2}. The elevation ranges from 30 to 200 m above sea level. The soil is weathered from red sandstone and mudstone and is classified as dystrudepts by the USDA system.

Experimental design

In 2011, a 12-year-old Chinese fir plantation was selected to simulate N deposition and exogenous P addition. There were six treatment groups: control (0P+0N), low N addition (0P + LN: 50 kg N ha^{-1} yr^{-1}), high N treatment (0P + HN: 100 kg N ha^{-1} yr^{-1}), P addition (P+0N: 50 kg P ha^{-1} yr^{-1}), low N with P addition (P + LN: 50 kg P ha^{-1} yr^{-1} + 50 kg N ha^{-1} yr^{-1}), and high N with P addition (P + HN: 50 kg P ha^{-1} yr^{-1} + 100 kg N ha^{-1} yr^{-1}). Each treatment was replicated four times. Each plot comprised an area of 400 m^2 (20 m × 20 m), and the buffer zones between the plots were >10 m wide. N was added in the form of NH$_4$NO$_3$, and P was added in the form of NaH$_2$PO$_4$. N and P were applied four times each year: March (30%), June (30%), September (20%), and December (20%). The fertiliser was weighed, mixed with 8 kg of clean and dry fine sand, and applied to each plot surface. The control plots received fine sand without N and P addition.

Soil sampling

In February 2018, soil samples were collected from each of the 24 plots. Five soil cores (four corners and the centre) were taken from the plots at depths of 0–10 cm and 10–20 cm from the vertical profiles. The five soil cores were combined to form one soil sample and brought to the laboratory. Obvious roots, stones, and residues were manually

removed from soil samples. Then, part of the soil was gently broken to pass through an 8-mm sieve and air-dried for the further analysis of aggregates, iron oxides, proportions of sand, silt and clay, SOC, total N (TN) content, and pH. The rest of the soil samples were stored at 4 °C to determine ammonium nitrogen (NH_4^+-N), nitrate-nitrogen (NO_3^--N), and dissolved organic carbon (DOC). A separate sample was taken in each plot with cylinders of 100 cm^3 volume and 5 cm length to determine the bulk density and soil water content.

Aggregate separation

Soil samples were physically fractionated into different aggregate sizes (>0.25 mm, 0.053–0.25 mm, and <0.053 mm) according to the procedure described by Elliott (1986) [26]. A subsample of 100 g air-dried soil was immersed in deionised water on top of a 0.25 mm sieve for 5 min. Then, sieving was performed manually by moving the sieve up and down 50 times over 3 cm in 2 min. The fraction remaining on the 0.25 mm sieve (macroaggregate) was collected. Water plus soil (<0.25 mm) was poured through a 0.053 mm sieve, and the sieving procedure was repeated. Then, the fraction remaining on the 0.053 mm sieve (microaggregate) was collected. The fraction that passed through the 0.053 mm sieve (the silt and clay fraction) was collected by centrifugation at 5000 rpm for 30 min at 48 °C. All aggregate fractions were gently back-washed into an aluminium pan, dried at 50 °C for 12 h, and then weighed.

Iron oxide analysis

Dithionite-citrate-bicarbonate-extractable Fe (Fe_d) was measured as described by Mehra and Jackson (1960) [27], oxalate-extractable Fe (Fe_{ox}) by Schwertmann's (1964) [28] method, and pyrophosphate-extractable Fe (Fe_p) using the methods of Sheldrick (1984) [29].

Measurement of soil physical and chemical properties

Soil samples collected with cylinders were oven-dried to calculate the bulk density and soil water content. Ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N) were extracted from 20 g of fresh soil with 1 mol L^{-1} KCl (soil:extract, 1:5) and analysed using a flow-injection autoanalyzer ((CFA)-AA3, SEAL, Germany). Soil DOC was extracted using deionised water (soil:water = 1:5) and analysed using a TOC analyser (Elementar, Vario TOC cube, Hanau, Germany). Soil pH was measured at a soil:water ratio of 1:2.5 using a pH meter. Soil TN was measured using a C/N analyser (Elementar, Vario Max CN, Germany) with a combustion temperature of 900 °C. The SOC of the original soil and each aggregate fraction was measured using the sulfuric acid potassium dichromate oxidation method. Sand, silt, and clay were separated by mild dispersion in water, followed by centrifugation [30].

Statistical analyses

We used a randomised complete block design analysis of variance to test the effects of N addition, P addition, soil depth, and their possible interactions on soil physicochemical properties, aggregate fractions, iron oxides, and clay contents. The least significant difference test was used to compare the differences among the three N addition levels.

Statistical significance was set at $P < 0.05$. All statistical analyses were performed using SPSS (version 19.0; SPSS Inc., Chicago, USA) and Origin 7.0 (Origin Lab Corporation, USA).

Results

Soil physical and chemical properties

SOC was not significantly ($P > 0.05$) affected by 6 years of nutrient addition. Other soil physical and chemical properties were not significantly altered by P addition. Only NH_4^+-N and NO_3^--N were significantly affected by N addition (Table 1). N addition significantly increased soil NH_4^+-N at 0–10 cm and NO_3^--N in both soil layers (Table 2). Moreover, the effects varied with the amount of N added. HN increased NH_4^+-N and NO_3^--N levels more than LN. In plots without P addition, LN and HN increased NH_4^+-N by 50% and 103.4%, respectively. In plots with P addition, LN and HN addition increased NH_4^+-N by 24.2% and 48.5%, respectively. The effect of N addition on NO_3^--N increased with P addition (Table 2), although the interaction effect of N and P was not significant (Table 1).

In plots without P addition, N addition significantly decreased bulk density and significantly increased DOC in the 0–10 cm soil layer, while the effects were not significant in plots with P addition (Table 2). Soil physical and chemical properties were significantly different between the two soil layers (Table 1). The soil at 0–10 cm had lower bulk density and higher water, NH_4^+-N, TN, and SOC content than the soil at 10–20 cm (Table 2). In addition, soil depth had a significant interaction effect with N and P addition (Table 1). For example, soil NH_4^+-N at 0–10 cm was significantly increased, but at 10–20 cm, it was not significantly affected by N addition (Table 2).

Soil aggregates and their associated organic C content

The proportions of the aggregate fractions were in the order of (>0.25 mm) > (0.053–0.25 mm) > (<0.053 mm) in all treatments (Figure 1). Macroaggregates were the most abundant aggregate, comprising between 59% and 74%. The microaggregates comprised between 16% and 26%, whereas the smallest fraction (<0.053 mm, the silt and clay fraction) ranged between 10% and 15%. Nutrient addition did not alter the proportion of soil aggregate fractions (Table 3). Soil depth had a significant effect on the proportion of the

Table 1. Three factor randomised complete block design ANOVA of N addition, P addition, soil depth effects on soil physical and chemical properties in a Chinese fir plantation.

| Source of variation	Bulk density g cm^{-3} F	Soil water content % F	NH_4^+-N mg kg^{-1} F	NO_3^--N mg kg^{-1} F	DOC mg kg^{-1} F	pH F	TN g kg^{-1} F	SOC g kg^{-1} F
N addition (N)	0.91	1.21	35.62***	16.34**	3.61	3.55	0.31	1.83
P addition (P)	0.14	2.18	4.33	0.80	3.55	5.28	3.20	5.46
N × P	0.47	2.42	2.37	0.31	0.01	0.26	0.05	0.12
Soil depth (D)	32.86*	300.65***	104.82**	7.92	8.78	6.97	19.14*	24.38*
D×N	0.39	0.56	8.43*	4.32	1.83	0.09	12.42**	8.20*
D×P	0.33	2.16	2.11	0.01	0.06	0.01	29.40*	52.58**
D×N×P	3.15	0.90	0.53	0.28	0.46	0.77	0.19	0.19

Note: ns, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.
Variables: NH_4^+-N: ammonium nitrogen; NO_3^--N: nitrate nitrogen; DOC: dissolved organic carbon; TN: total nitrogen; SOC: soil organic carbon.

Table 2. Soil properties after nutrient addition in a Chinese fir plantation.

Soil layer	Treatment		Bulk density g cm^{-3}	Soil water content %	NH$_4^+$-N mg kg^{-1}	NO$_3^-$N mg kg^{-1}	DOC mg kg^{-1}	pH	TN g kg^{-1}	SOC g kg^{-1}
0–10 cm	0P	+0N	1.27±0.03a	22.75±0.89a	16.57±0.84b	3.34±0.41b	43.97±5.62b	4.32±0.06a	1.02±0.09a	15.42±1.85a
		+LN	1.11±0.03b	22.57±0.53a	24.85±2.71ab	6.78±1.00a	65.25±6.84a	4.17±0.06a	1.19±0.12a	19.42±2.28a
		+HN	1.13±0.04b	23.36±0.64a	33.70±5.50a	7.57±0.68a	62.19±5.77ab	4.16±0.07a	1.03±0.10a	17.54±2.24a
	P	+0N	1.11±0.11a	21.93±0.22a	16.25±0.79c	3.47±1.0a	43.34±3.14a	4.42±0.08a	0.92±0.09a	12.01±1.82a
		+LN	1.19±0.11a	21.07±0.72a	20.18±0.50b	5.86±1.83a	61.37±3.33a	4.35±0.05a	1.00±0.15a	15.73±3.07a
		+HN	1.21±0.05a	21.29±0.77a	24.13±1.39a	6.38±0.79a	53.41±9.60a	4.28±0.06a	0.95±0.06a	14.40±1.20a
10–20 cm	0P	+0N	1.35±0.01a	18.70±0.36a	16.33±0.66a	2.70±0.40b	51.93±6.68a	4.29±0.11a	0.71±0.06a	8.72±0.65a
		+LN	1.37±0.02a	18.34±0.17a	19.76±2.45a	7.53±1.17a	56.49±7.37a	4.28±0.08a	0.64±0.06a	6.89±1.23a
		+HN	1.31±0.08a	20.46±1.10a	20.97±1.72a	9.05±1.87a	49.35±1.59a	4.25±0.07a	0.73±0.12a	11.64±3.41a
	P	+0N	1.35±0.02a	19.73±0.87a	16.53±0.77a	2.94±1.04b	42.89±6.66a	4.57±0.15a	0.67±0.07a	8.59±0.94a
		+LN	1.28±0.09a	17.87±0.82a	15.74±0.51a	5.57±1.27ab	49.08±4.13a	4.36±0.06a	0.66±0.05a	7.24±0.55a
		+HN	1.32±0.04a	18.55±0.74a	16.24±0.88a	8.66±1.28a	47.36±4.47a	4.33±0.03a	0.73±0.06a	8.82±1.05a

Data labeled with different letters indicated soil properties were significantly different at $P < 0.05$ by Duncan test.
Treatments: 0P: treatments without P addition; P: treatments with P addition (50 kg P ha^{-1} yr^{-1}); +0N: treatments without N addition; +LN: treatments with low N addition (50 kg N ha^{-1} yr^{-1}); +HN: treatments with high N addition (100 kg N ha^{-1} yr^{-1}).
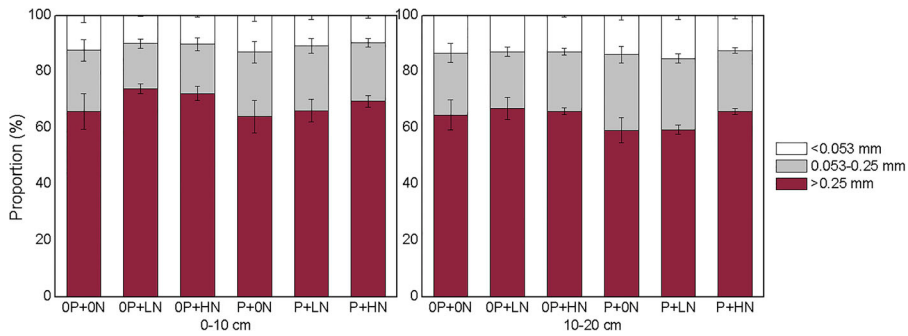
Figure 1 Proportions of the > 0.25, 0.053–0.25, and < 0.053 mm aggregate fractions under nutrient addition treatments, including control (0P+0N), low N addition (0P+LN: 50 kg N ha-1 yr-1), high N treatment (0P+HN: 100 kg N ha-1 yr-1), P addition (P+0N: 50 kg P ha-1 yr-1), low N and P addition (P+LN: 50 kg P ha-1 yr-1+50 kg N ha-1 yr-1) and high N with P addition (P+HN: 50 kg P ha-1 yr-1 +100 kg N ha-1 yr-1).

<0.053 mm class (Table 3), the average proportions of which were 11% for the 0–10 cm soil layer and 13% for the 10–20 cm soil layer.

The organic C content of each aggregate fraction was not altered by N and P addition but significantly changed with soil depth (Table 3). Soil aggregates from 0–10 cm had higher organic C content than those from 10–20 cm (Table 4). Organic C contents of the 0–10 cm soil aggregate fractions were 14.73, 12.39, and 14.76 g kg^{-1} for >0.25 mm, 0.053–0.25 mm, and <0.053 mm classes, respectively. The organic carbon contents of the same soil aggregate fractions from 10–20 cm were 8.85, 7.19, and 8.11 g kg^{-1}, respectively.

Soil iron oxides and clay content

Three forms of iron oxide were identified; our results showed that Fe_d in soil was the most abundant form and Fe_p was the lowest (Figure 2(a, b, and c)). Fe_d did not change significantly with nutrient addition in any soil layer (Figure 2(a)), while Fe_{ox} and Fe_p of 0–10 cm soil were significantly lower in the P addition treatments (Figure 2(b and c)). The average values of Fe_{ox} of 0–10 cm soil with and without P addition were 0.68 and 0.84 g kg^{-1}, respectively, while the

Table 3. Three factor randomised compete block design ANOVA of N addition, P addition, soil depth effects on the proportions of soil aggregate fractions and their organic carbon contents (SOC) in a Chinese fir plantation.

	Aggregate fractions (%)			SOC (g kg^{-1})		
	>0.25 mm	0.053-0.25 mm	<0.053 mm	>0.25 mm	0.053-0.25 mm	<0.053 mm
Source of variation	F	F	F	F	F	F
Block	1.89	1.70	3.28	0.60	0.64	0.67
P addition (P)	3.93	9.25	0.66	0.26	0.20	0.05
N addition (N)	0.98	0.95	1.54	0.08	0.67	0.06
N × P	1.28	1.65	1.05	0.66	0.01	0.42
Soil depth (D)	6.0	3.12	13.57*	152.69**	49.80**	20.50*
D× P	0.003	0.02	0.08	0.23	0.02	0.00
D×N	4.40	3.66	2.64	4.33	1.75	0.18
D×N×P	0.71	1.09	0.47	2.15	0.17	0.69

Note: *, $P<0.05$; **, $P<0.01$.

Table 4. Soil organic carbon contents (SOC) of different aggregate fractions after nutrient addition in a Chinese fir plantation.

Soil layer	Treatment		SOC (g kg^{-1})		
			>0.25 mm	0.053-0.25 mm	<0.053 mm
0–10 cm	0P	+0N	13.27±0.76a	12.63±1.12a	14.09±1.32a
		+LN	17.66±2.29a	12.60±1.80a	16.18±2.19a
		+HN	11.14±3.97a	11.35±1.36a	13.69±1.91a
	P	+0N	15.17±1.72a	12.99±0.97a	15.16±1.81a
		+LN	14.95±3.54a	12.45±2.34a	13.50±3.59a
		+HN	16.23±0.93a	12.29±0.46a	15.96±2.26a
10–20 cm	0P	+0N	9.90±0.70a	7.68±0.62a	8.14±0.24a
		+LN	7.18±0.55a	5.99±0.46a	7.44±0.48a
		+HN	9.21±1.73a	7.54±1.64a	8.36±1.41a
	P	+0N	8.70±0.54a	7.67±0.61a	8.67±0.85a
		+LN	8.78±0.52a	6.99±0.36a	7.92±0.33a
		+HN	9.31±1.05a	7.27±1.00a	8.15±1.28a

Data labeled with the same letter indicated soil properties were not significantly different at $P < 0.05$ by Duncan test. Treatments: 0P: treatments without P addition; P: treatments with P addition (50 kg P ha^{-1} yr^{-1}); +0N: treatments without N addition; +LN: treatments with low N addition (50 kg N ha^{-1} yr^{-1}); +HN: treatments with high N addition (100 kg N ha^{-1} yr^{-1}).

values of Fe$_p$ were 0.43 and 0.58 g kg^{-1}, respectively. Moreover, when there was no P addition, LN addition increased Fe$_p$ in the 0–10 cm soil layer (Figure 2(c)).

The proportions of soil sand, silt, and clay were not significantly different among the different treatments or between the two soil layers (Figure 2). Among the three particles, silt was the most abundant (67% on average), and the proportion of clay was the least abundant (12% on average).

Discussion

N and P addition did not alter SOC or soil aggregate-associated SOC

We found that 6 years of nutrient addition did not significantly increase or decrease SOC (Table 2). Although nutrient addition may promote plant growth and leaf litter, the decomposition of SOC can also be accelerated by the supply of fresh C [31]. Moreover,
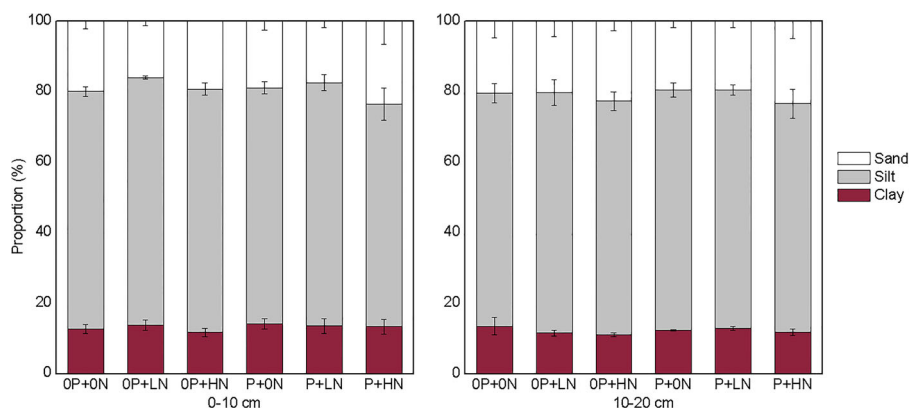
Figure 2. Proportions of sand, silt and clay under nutrient addition treatments, including control (0P +0N), low N addition (0P+LN: 50 kg N ha-1 yr-1), high N treatment (0P+HN: 100 kg N ha-1 yr-1), P addition (P+0N: 50 kg P ha-1 yr-1), low N and P addition (P+LN: 50 kg P ha-1 yr-1+50 kg N ha-1 yr-1) and high N with P addition (P+HN: 50 kg P ha-1 yr-1+100 kg N ha-1 yr-1).
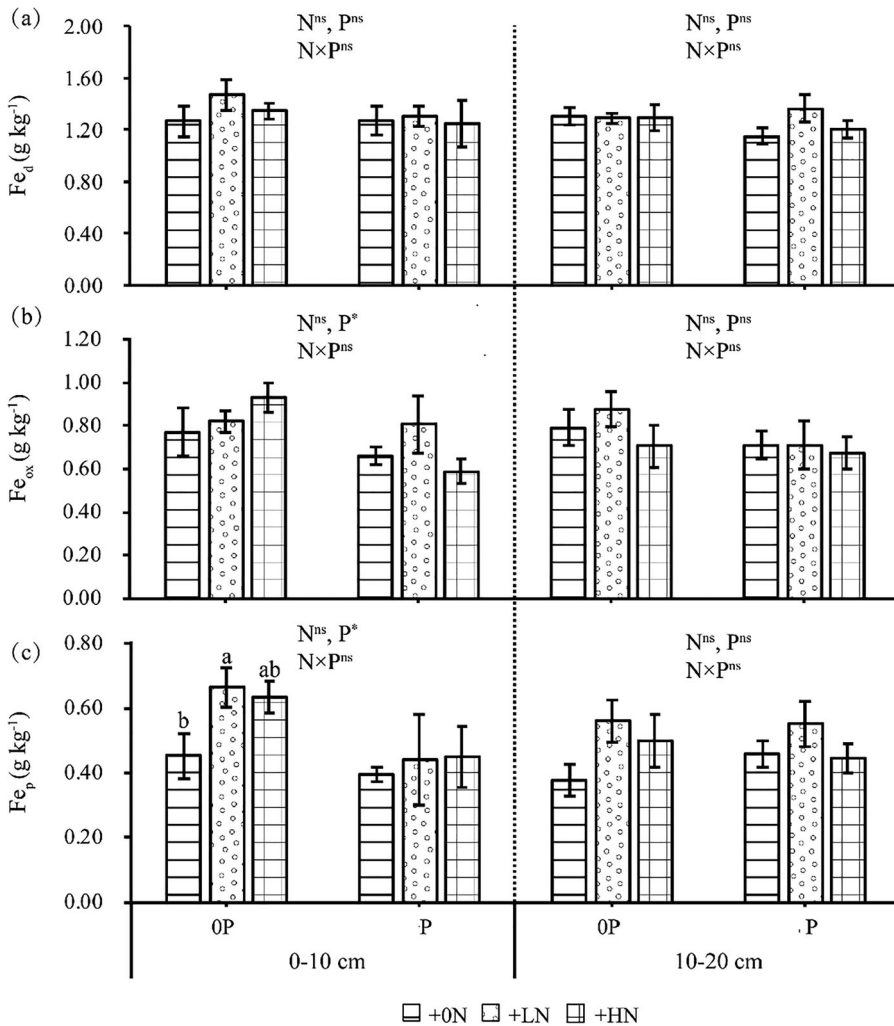
Figure 3. Different forms of iron (Fe) oxides (Fed is the dithionite-citrate-bicarbonate-extractable Fe; Feox is the oxalate-extractable Fe; Fep is the pyrophosphate-extractable Fe.) under nutrient addition treatments, including control (0P+0N), low N addition (0P+LN: 50 kg N ha-1 yr-1), high N treatment (0P+HN: 100 kg N ha-1 yr-1), P addition (P+0N: 50 kg P ha-1 yr-1), low N and P addition (P+LN: 50 kg P ha-1 yr-1+50 kg N ha-1 yr-1) and high N with P addition (P+HN: 50 kg P ha-1 yr-1+100 kg N ha-1 yr-1). Bars with different letter at the top are significantly different at P < 0.05 by LSD test.

trees decrease C allocation to roots when soil nutrient availability is high [32], leading to reduced root exudates to the soil. Some studies have shown that N and P addition reduces SOC in topsoil [33], while many studies have shown that manure application, instead of inorganic fertilisation, increases SOC storage [34]. Another possible reason for our findings is that the duration of nutrient addition was not long enough for SOC storage to be affected. A previous 26-year study found that inorganic fertilisation with or without manure improved soil SOC storage, soil aggregation, and associated organic C [35].

Under the control and nutrient addition treatments, the proportions of macroaggregates (>0.25 mm) were all higher than those of the other two aggregate sizes (Figure 1); this is consistent with the results of previous studies on red soil using the wet sieving procedure [34,35]. Our results showed that the proportions of soil aggregate fractions and their associated organic C contents were not altered by N or P addition (Table 3), contrasting with our hypothesis. This may be because we used inorganic fertiliser instead of manure. Manure application has a significant effect on soil aggregate fractions as organic matter is an important binding agent for aggregation: Huang et al. (2010) showed that manure application increased the proportion of large macroaggregates (>2 mm) in treated soil [36]. Previous research conducted at the same site showed that tree physiology [37] and soil microorganisms [38] were significantly altered by nutrient addition. However, we found no significant changes in SOC and soil aggregate fractions.

P addition had a significant effect on soil iron oxides

As Fe is closely linked to the biogeochemical cycle of SOC, it is considered a key factor in the long-term storage of organic C [39]. Soil iron oxides have been observed to improve SOC stability by sorption, entrapment, or complexation [40]. We found that Fe_{ox} and Fe_p were significantly decreased by P addition in the 0–10 cm soil layer (Figure 3). Our results partially verify the second hypothesis that P addition may have a stronger effect on soil aggregates than N in subtropical forests that are limited by P. Iron oxides have been found to stimulate the formation of aggregates [41]. Although no significant change in aggregates was observed in the present study, the change in Fe_{ox} and Fe_p by P addition may affect the formation of aggregates in the future, thereby changing the stability of SOC. We did not measure the contents of aluminium and manganese, which are also important for the formation of aggregates [42]. Future research should consider these factors.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Declaration of competing interest

Authors declare that there is no conflict of interest.

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