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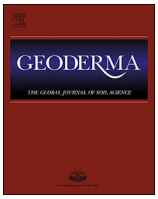

Depth-dependent soil C-N-P stoichiometry in a mature subtropical broadleaf forest

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ABSTRACT

As the balance of multiple chemical substances in ecological interactions, stoichiometry of soil carbon (C), nitrogen (N) and phosphorus (P) is critical for forest sustainability. Soil depth is critical in regulating soil C-N-P stoichiometry; however, the vertical pattern of soil C-N-P stoichiometry remains unclear at the local scale in mature subtropical forests. Here, we sampled 555 soil columns from 185 grids at three soil depths (i.e., 0–20, 20–40, and 40–60 cm) in a mature subtropical evergreen forest of eastern China. We found the C:N, N:P, and C:P ratios decreased with increasing soil depth. However, the strength of bivariate correlations among C, N, and P converged from the top to the deepest soil layer. Such vertical convergence was jointly driven by decreasing C-N (R^2 as 0.84, 0.80, and 0.76) and increasing C-P (R^2 as 0.11, 0.26, and 0.31) correlations from 0–20 to 40–60 cm depths. Further analyses with a structural equation model showed that the spatial variations in C, N, and P were influenced by different environmental factors. For example, the spatial variations in soil organic C and total N in the top soil layer were largely influenced by soil pH, whereas the spatial variation in total P was jointly influenced by topographical, biotic, and soil factors. Our results validate the important impact of soil depth on soil C-N-P stoichiometry at the landscape scale. The converging bivariate correlations between C, N and P along the increasing soil depth indicate the depth-dependent roles of different nutrient elements in soil C cycling.

1. Introduction

Ecological stoichiometry is the balance of energy and multiple chemical elements in ecological processes (Sterner and Elser, 2002), characterizing the coupling of carbon (C), nitrogen (N), and phosphorous (P) cycles in the soil. Thus, understanding soil C-N-P stoichiometry from local to global scales is critical for sustaining soil quality (Walker and Adams, 1956), C sequestration (Alberti et al., 2015), and ecosystem health (Yang et al., 2017). Over the last 10 years, soil C-N-P stoichiometry has been widely studied at regional to global scales (Yu et al., 2018; Yue et al., 2017; Delgado-Baquerizo et al., 2013; Tian et al., 2010; Chen et al., 2016). There is broad consensus among these large-scale studies that soil depth plays an important role in regulating soil C-N-P stoichiometry. For example, a recent study on subtropical soil C-N-P stoichiometry in southern China spanning 60 years showed that the soil C:N:P ratio significantly decreased with soil depth (Yu et al., 2018). Another global synthesis revealed depth-dependent responses of soil C:N ratios to different drivers of global change (e.g., nitrogen deposition, warming, and elevated CO_2) (Yue et al., 2017). However, knowledge remains limited on the depth dependence of soil C-N-P stoichiometry at the local to landscape scale.

The stocks of C, N, and P commonly vary with soil depth, but their driving processes are different. Most of the C substrate to soil organic matter (e.g., above-ground litter) is distributed in topsoil (Jobbágy and Jackson, 2001; Lal, 2009). In wet ecosystems, such as tropical and subtropical forests, fresh dissolved organic C from the upper soil layers leaches to the deep soil layers during rainy events. This leaching process could reinforce the priming effect that promotes the decomposition of soil C in deep soil layers (Kou et al., 2018). Dissolved soil carbon also precipitates in the environment of metal ions (such as Fe^{3+}, Al^{3+}, and Mn^{2+}) (Lützow et al., 2006). N deposition (Goulding et al., 1998), litter degradation (Manzoni et al., 2008), microbial N fixation (Batterman et al., 2013) and rock weathering (Houlton et al., 2018) all provide N to the soil but at different soil depths. In addition to these N input processes varying vertically, the leaching of soil N intensifies the imbalance in N distribution at different soil layers. Rock weathering is the primary source of P in terrestrial ecosystems over the long term (Walker and Syers 1976; Trudgill 1988; Chadwick et al. 1999); however, the

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recycling of P from litter might be important in the short term, especially at fine spatial scales (Xia et al. 2015). The degree of soil weathering in the topsoil is stronger than that in deep soils because it is more exposed to the warm and moist environment (Kelly et al. 1998; Walker and Syers 1976). The accumulation of P in the topsoil is protected by inner-sphere surface complexes, which is formed by phosphate anions with high binding energy and colloids with positive charges (Novais and Smyth, 1999). Besides phosphate anions, bicarbonate and sulphate are also important sources of adsorptive anions in soil solution. The increase in competitive anions and, thus, soil negative charge further facilitates the process of phosphate desorption (Afsar et al., 2012). Certain soil properties, such as soil pH (Barrow, 1984), soil texture (Leclerc et al., 2001) and oxides of iron and aluminum (Toor et al., 1997), also affect the accumulation of soil P. Overall, the vertical variation in soil C-N-P stoichiometry is largely influenced by both biotic and environmental factors.

Many studies have detected decreasing trends of soil C, N, and P stocks with increasing soil depth (Ward et al., 2016; Tashi et al., 2016; Yu et al., 2018). However, inconsistent vertical patterns have been reported for the C:N:P ratio. For example, similar patterns of soil N:P and C:P ratios occur along with the soil profile in the synthesis of the second Chinese soil survey (Tian et al., 2010; Chen et al., 2016). However, larger C:N ratios in the deep soil layer, not the surface soil layer, were detected in a mollisol watershed in northeastern China (Zhang et al., 2016). In comparison, the C:N ratio showed no significant difference across soil depths in a survey of alpine grasslands, including 405 soil profiles from 135 sites on the Tibetan Plateau (Yang et al., 2010). Although the national synthesis of soil survey data has confirmed significant linear relationships for soil C, N and P (Tian et al., 2010), how their stoichiometric relationships change with the soil depth at the local scale remain unclear.

Subtropical forests in China have a high diversity of evergreen broadleaved tree species and provide a large net C sink (Yu et al., 2014). The large net C sink in this ecosystem results from the fast aging of forests and a rapid increase in atmospheric N deposition (Yu et al., 2014; Cui et al., 2019). However, this region has been subject to intense weathering caused by intensive heat and rainfall (Vitousek et al., 2010). These conditions facilitate P loss, making subtropical forests P limited regions (Neufeldt et al., 2000; Huang et al., 2015). Over the last six decades, P limitation has been enhanced in subtropical forests due to elevated N deposition, rising atmospheric CO_2, and regional warming (Yu et al., 2018). Both elevated N deposition and atmospheric CO_2 facilitate plant growth, further stimulating P uptake by plants (Deng et al., 2016; Li et al., 2016). Lower total P content is commonly found in older soils (Walker and Syers, 1976); however, some studies have indicated that the P content of soil increases with forest age (Crews et al., 1995; Frizano et al., 2002). For example, the chronosequence of soils in a subtropical forest of eastern Puerto Rico showed that the available P was larger in late-successional forest soil compared to younger soils due to the combined effects of plants and associated microbes (Frizano et al., 2002). Another study on soil chronosequences, with soil age ranging from 300 years to 4.1×10^6 years, found higher P availability in intermediate-aged compared to younger and older soils (Crews et al., 1995). These contrasting results increase the uncertainty of soil C-N-P stoichiometry in mature and old forests, especially in P limited regions. Thus, it is important to establish whether and how C-N-P correlations change with soil depth in mature evergreen broad-leaved forests.

Here, we studied the distributions of soil C-N-P stoichiometry at three soil depths (i.e., 0–20, 20–40, and 40–60 cm) in a subtropical evergreen broad-leaved forest of eastern China. All of the samples were collected from a long-term 20-ha dynamic forest plot, with a stand age of ~200 years. Due to large variation in biotic and abiotic factors with soil depth, we first hypothesized that the ratios of soil C, N, and P would change with soil depth in this ecosystem. We further hypothesized that the factors controlling spatial variation in soil C, N, and P density are different between soil layers.

2. Materials and methods

2.1. Study area

The study was carried out in the Tiantong National Forest Park (29°48′N, 121°47′E), which is a subtropical evergreen broad-leaved forest in eastern China (Yang et al., 2011) (Fig. S1). A 20-ha (500 × 400 m) dynamic forest plot was selected as our sampling area (hereafter called Tiantong plot). The Tiantong plot is one component of the Forest Global Earth Observatory (ForestGEO) network (https://forestgeo.si.edu/). This subtropical monsoon climate region experiences annual mean precipitation of 1374.7 mm. The mean annual temperature is 16.2 °C, being coldest in January (4.2 °C) and hottest in July (28.1 °C). The soil texture is primarily Ferralosol, with pH ranging from 4.5 to 5.0 (Song and Wang, 1995). The parental material is mainly composed of quartzite and granite (Song and Wang, 1995). The elevation of the Tiantong plot is approximately 300 m on average, ranging from 304.3 to 602.9 m. The dominant tree families are Theaceae, Lauraceae, and Fagaceae (Yang et al., 2011).

2.2. Field sampling and laboratory analysis

Soil sampling was conducted based on 185 grids in the Tiantong plot (Fig. 1a) during May to June 2016. The area of each grid was 20 × 20 m. In each grid, three soil cores (5 cm diameter) were sampled and pooled at each soil depth (i.e., 0–20 cm, 20–40 cm, 40–60 cm). Before sampling the soil, the litter layer was removed. Soil temperature was monitored every 30 min at 0–10 cm depth from March 2016 to March 2017 using temperature data loggers (iButton, DS1922, Wdsen Electronic Technology C_o., Shanghai, China). Soil moisture at 0–10 cm depth was monitored three times a week from March 2016 to March 2017 using a portable soil moisture detector (TZS, Zhejiang, China). The mean values of soil temperature and soil moisture were calculated based on these data. Litter quantity, litter C:N:P ratios, convexity, and topographic slope were measured in every grid. In each grid, the aboveground litter was collected with three replications in May 2016. Litter was sampled in an area of 50 cm × 50 cm. The litter samples were dried at 65 °C for 48 h using an oven and then weighed and ground to a fine powder (< 100 mm). Litter C concentration was measured using the potassium dichromate volumetric method (Bao, 2010). Litter N concentration was determined using the Kjeldahl method (Miller and Keeney, 1982). Litter P concentration was measured by the molybdenum blue colorimetric method (Murphy and Riley, 1962).

Roots and stones were removed from all collected soil samples, which were further homogenized and air-dried. Subsequently, some of the soil samples were sieved at 0.25 mm for the soil organic carbon (SOC), total nitrogen (TN) and total phosphorus (TP) analyses. The other soil samples were sieved at 2 mm for pH analyses. SOC was measured by the dichromate heating-oxidation (Liu, 1996). TN concentration was determined by using the modified Kjeldahl method (Miller and Keeney, 1982). TP concentration was determined by the molybdenum blue colorimetric method, as described by Murphy and Riley (1962). Soil pH was measured with a pH meter. The soil subsamples were dried at 105 °C for 24 h in an oven to acquire dry soil results.

2.3. Statistical analyses

The non-parametric tests of multiple paired samples (i.e. *Friedman* test) were used to analyze the vertical distribution of SOC, TN, and TP concentration, as well as their ratios, at different soil depths using SPSS 21.0. The reduced major axis regression was used to analyze the relationship of SOC with TN and TP at each soil depth (i.e., 0–20 cm, 20–40 cm, and 40–60 cm) by R (version 3.3.2, R Development Core Team, 2017) based on the log-transferred data. Reduced major axis

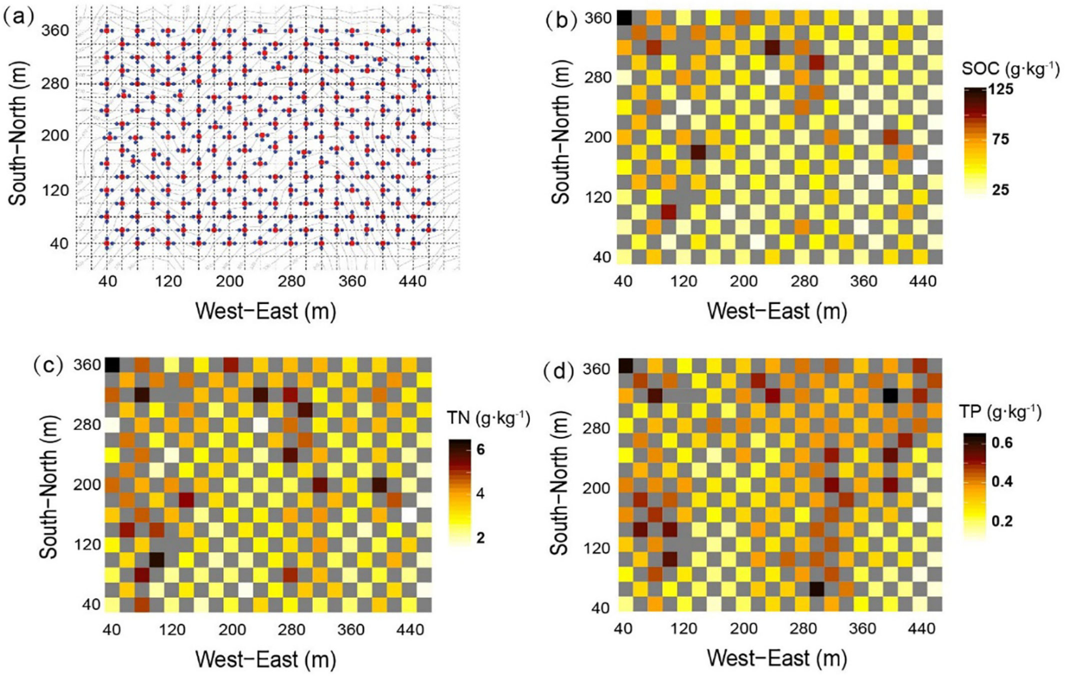

Fig. 1. Locations of the sampling grids (a) and spatial distribution of SOC (b), TN (c), and TP (d). Data shown in panels (b), (c), and (d) are the mean values of three soil layers (0–20 cm, 20–40 cm, and 40–60 cm). The red dots in panel (a) show the center of each grid. while the blue dots surrounding them show the sampling locations. The gray grid cells in panels b–d indicate no data. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

regression analysis was applied using the 'lmodel2′ R package. We used the coefficient of determination (i.e., R^2) of the reduced major axis regression to quantify the bivariate correlation strength of between C, N, and P at different soil depths. Structural equation modeling (SEM) was used to determine the factors regulating SOC, TN, TP concentration, and C-N-P ratios along the soil depth gradients. The biotic factors used in the SEM included litter quantity and litter C:N:P ratios. We also linked topographical factors (convexity and slope) and soil factors (pH, soil temperature, and soil moisture) to variation in soil C-N-P stoichiometry in the SEM analyses. The difference in average elevation between a given grid and the average elevation of its adjacent eight grids is defined as convexity (Valencia et al., 2004). The standardized path coefficients were used to indicate correlations between factors in SEM. The model χ^2 test and root mean square error of approximation (RMSEA) were used to evaluate the fitness of the model. The model fit was considered to be good when the χ^2 test was not significant ($P > 0.05$) and the RMSEA was near 0 (Schermelleh-Engel et al., 2003). SEM analyses were performed using AMOS 21.0 (Amos Development Co., Greene, ME, USA).

3. Results

3.1. Spatial distributions of C, N, and P concentrations

All of SOC, TN, and TP concentration showed large spatial variability in the 20-ha forest plot (Fig. 1). SOC, TN, and TP concentration ranged from 17.50 ± 10.51 (mean ± SD) to 129.30 ± 51.66 g kg^{-1} (Fig. 1b), 1.58 ± 0.75 to 6.50 ± 1.74 g kg^{-1} (Fig. 1c), and 0.10 ± 0.04 to 0.64 ± 0.09 g kg^{-1} (Fig. 1d), respectively. SOC concentration significantly decreased with soil depth (Fig. 2a, Table 1); the means at 0–20 cm, 20–40 cm, and 40–60 cm depth were 68.39 g kg^{-1}, 42.29 g kg^{-1}, and 31.72 g kg^{-1}, respectively. TN and TP concentration showed similar decreasing trends from the surface to deep soil layers (Fig. 2b, c). All of the C:N, N:P, and C:P ratios decreased with soil depth (Fig. 2d-f). Specifically, the mean ratios of C:N, N:P, and C:P decreased from 14.88 (0–20 cm) to 12.76 (40–60 cm) (Fig. 2d), 12.43 to 9.25 (Fig. 2e), and 189.93 to 120.00 (Fig. 2f), respectively.

Significant linear relationships for SOC with TN, SOC, and TP, TN, and TP concentrations were detected at the three soil depths (Fig. 3). R^2 between SOC and TN in the three soil layers slightly changed from 0.84 (0–20 cm) and 0.80 (20–40 cm) to 0.76 (40–60 cm) (Fig. 3d). R^2 between TN and TP concentrations increased from 0.27 (0–20 cm) to 0.52 (40–60 cm) (Fig. 3d). Similarly, the R^2 between SOC and TP concentrations increased from 0.11 (0–20 cm) to 0.31 (40–60 cm) (Fig. 3d).

3.2. Factors controlling spatial variation in C, N, and P concentrations

Structural equation models (SEM) were applied to explore the factors controlling spatial variation in SOC, TN concentration, and TP concentration (Fig. 4). The goodness-of-fit test for SEM indicated that our data fitted the priori models well, as both the P-value and root mean square error of approximation (RMSEA) met the good-fitting criterion ($P > 0.05$, RMSEA = 0.00). SEM analyses showed that the model explained 49% of the variance in SOC (Fig. 4a). At 0–20 cm soil depth, soil pH showed significant direct-negative effects on SOC, with a standardized path coefficient of −0.70 ($P < 0.001$). Convexity enhanced SOC by reducing soil pH. The model explained 49% of the variance in TN (Fig. 4b). Similar to SOC, TN was significantly affected by pH (standardized path coefficient of −0.69, $P < 0.001$). Convexity also indirectly affected TN by directly affecting soil pH, with a standardized path coefficient of −0.30. In addition, litter N and litter N:P had significant direct effects on TN with a standardized path coefficient of 0.13 and −0.20, respectively. The explained variance for TP was 45% based on our model (Fig. 4c). Different to SOC and TN, which were mostly affected by soil factors (i.e., pH), TP was negatively affected by biotic factors (i.e., litter N:P), with a standardized path coefficient of −0.37 ($P < 0.001$). Topographical factors (i.e., convexity and slope) negatively affected TP, with a standardized path coefficient of −0.34 and −0.22 ($P < 0.001$). Soil factors (e.g., pH) also negatively affected TP, with a standardized path coefficient of −0.30.

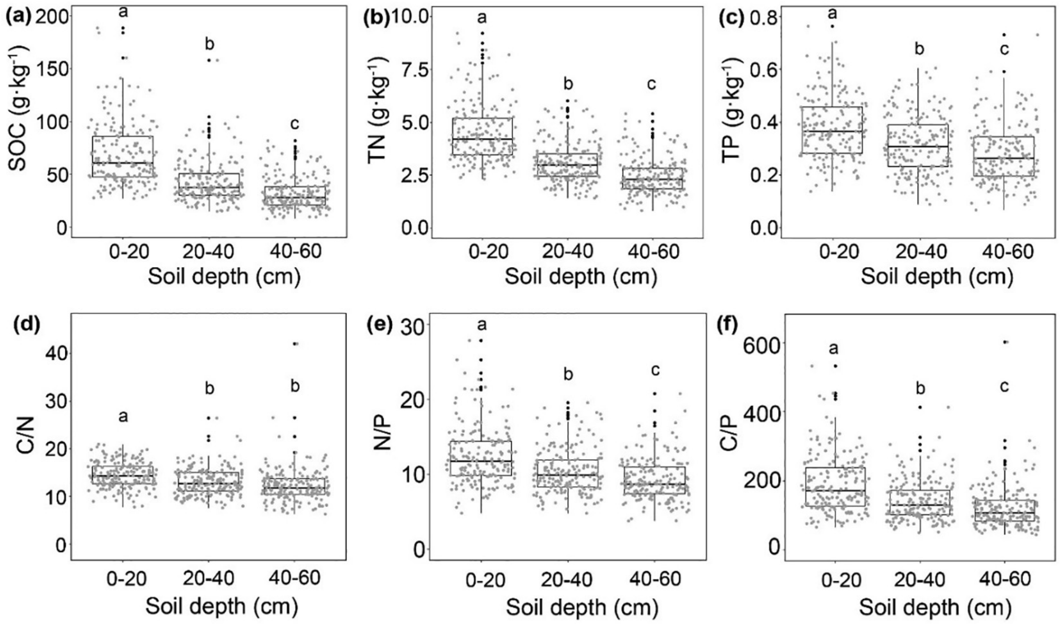

Fig. 2. Vertical distributions of SOC (a), TN (b), and TP (c), as well as the ratios of C:N (d), N:P (e), and C:P (f) at different soil depths (0–20 cm, 20–40 cm, and 40–60 cm). Different lowercase letters above the boxes show the significant differences among different soil depths ($P < 0.01$).

Table 1

Soil C, N, and P storage and their ratios at different depths. Significant differences among different soil depths are represented by different lowercase letters (LSD, $P < 0.01$) after the mean value ± standard deviation (SD).

Variable	n	0–20 cm	20–40 cm	40–60 cm
SOC	185	68.39 ± 29.45a	42.29 ± 19.05b	31.72 ± 15.07c
TN	185	4.49 ± 1.37a	3.07 ± 0.93b	2.44 ± 0.86c
TP	185	0.38 ± 0.12a	0.32 ± 0.11b	0.28 ± 0.11c
SOC:TN	185	14.88 ± 2.58a	13.48 ± 2.69b	12.76 ± 3.52b
TN:TP	185	12.43 ± 3.89a	10.35 ± 2.94b	9.25 ± 2.74c
SOC:TP	185	189.93 ± 81.83a	142.06 ± 57.47b	120.59 ± 60.96c

The influence of the main environmental factor (i.e., pH) on SOC decreased from the top (0–20 cm) to the deep (40–60 cm) soil layer, with standardized path coefficients ranging from −0.70 ($P < 0.001$) to −0.38 ($P < 0.001$) (Fig. 4 a, d). The quantity of litter had a significant direct-negative effect on SOC, with a standardized path coefficient of −0.19 ($P < 0.01$) at 40–60 cm soil depth. The influence of the main environmental factor (i.e., pH) on total N decreased from the top soil layer (0–20 cm) to the deep soil layer (40–60 cm), with standardized path coefficients ranging from −0.69 to −0.38 (Fig. 4 b, e). The negative effect of litter N:P on total N increased along the soil depth gradient, with standardized path coefficients ranging from −0.20 ($P < 0.01$) in the top soil layer (0–20 cm) to −0.31 ($P < 0.001$) in the deep soil layer (40–60 cm; Fig. 4b, e). Litter N did not significantly influence total N in the deeper soil layer. Similar to 0–20 cm soil depth, TP was mainly influenced by litter N:P and soil pH at 40–60 cm soil depth, with a standardized path coefficient of −0.53 and −0.22, respectively (Fig. 4f). The influence of topographical factors (i.e., convexity and slope) on TP decreased in the deep soil layer. The influence of convexity on TP decreased from the top to the deep soil layer, with standardized path coefficients ranging from −0.34 ($P < 0.001$) to −0.18 ($P < 0.05$) (Fig. 4 c, f). Slope did not significantly influence TP in the deeper soil layer.

3.3. Factors controlling spatial variation in C:N, N:P, and C:P ratios

Structural equation models (SEM) were applied to explore the factors controlling spatial variation in C:N, N:P, and C:P ratios (Fig. 5). SEM analyses showed that the model explained 40% (0–20 cm soil depth) and 23% (40–60 cm soil depth) of the variance in C:N (Fig. 5a, d). At both 0–20 cm and 40–60 cm soil depths, convexity and pH significantly affected C:N (Fig. 5a, d). The quantity of litter had a significant negative effect on C:N, with standardized path coefficients of −0.15 ($P < 0.05$) at 40–60 cm soil depth (Fig. 5d), which was consistent with the SEM analysis of SOC (Fig. 4d). SEM analyses showed that the model explained 42% (0–20 cm soil depth) and 22% (40–60 cm soil depth) of variance in N:P (Fig. 5b, e). Litter N:P and convexity were the main factors influencing N:P at 0–20 cm (standardized path coefficients of 0.18, 0.30) and 40–60 cm (standardized path coefficients of 0.29, 0.20) soil depth (Fig. 5b, e). SEM analyses showed that the model explained 50% (0–20 cm soil depth) and 24% (40–60 cm soil depth) of the variance in C:P (Fig. 5c, f). Similar to the results of the SEM on SOC and TP, convexity, slope, and pH were the main factors influencing C:P in the soil profile (Fig. 5c, f).

4. Discussion

4.1. Different factors control soil C, N, and P concentrations across soil depths

The decreasing C, N, and P concentrations with increasing soil depth in the subtropical forest in our study site (Fig. 2a, b, c) are consistent with some previous findings in other ecosystems, including tropical forests (Stone and Plante, 2014), croplands (Panda et al., 1988), shrublands (Yu et al., 2018) and grasslands (Yu et al., 2018). However, to the best of our knowledge, this study firstly uses multiple factors (soil, biology and topography) to explore the different driving factors of soil C, N, and P concentrations at the landscape scale of a subtropical forest. The spatial distribution of soil C concentration was negatively influenced by soil pH in both of surface and deep soils. Unexpectedly, litter quantity negatively influenced SOC concentration at 40–60 cm soil depth (Fig. 4d). One possible reason is that the higher litter quantity provides more dissolved organic C from the topsoil layers to stimulate microbial activity (Fontaine et al., 2007) and promotes the decomposition of soil C in deep soils (Kou et al., 2018). However, further experimental studies are still needed to validate this hypothesized mechanism in this region.

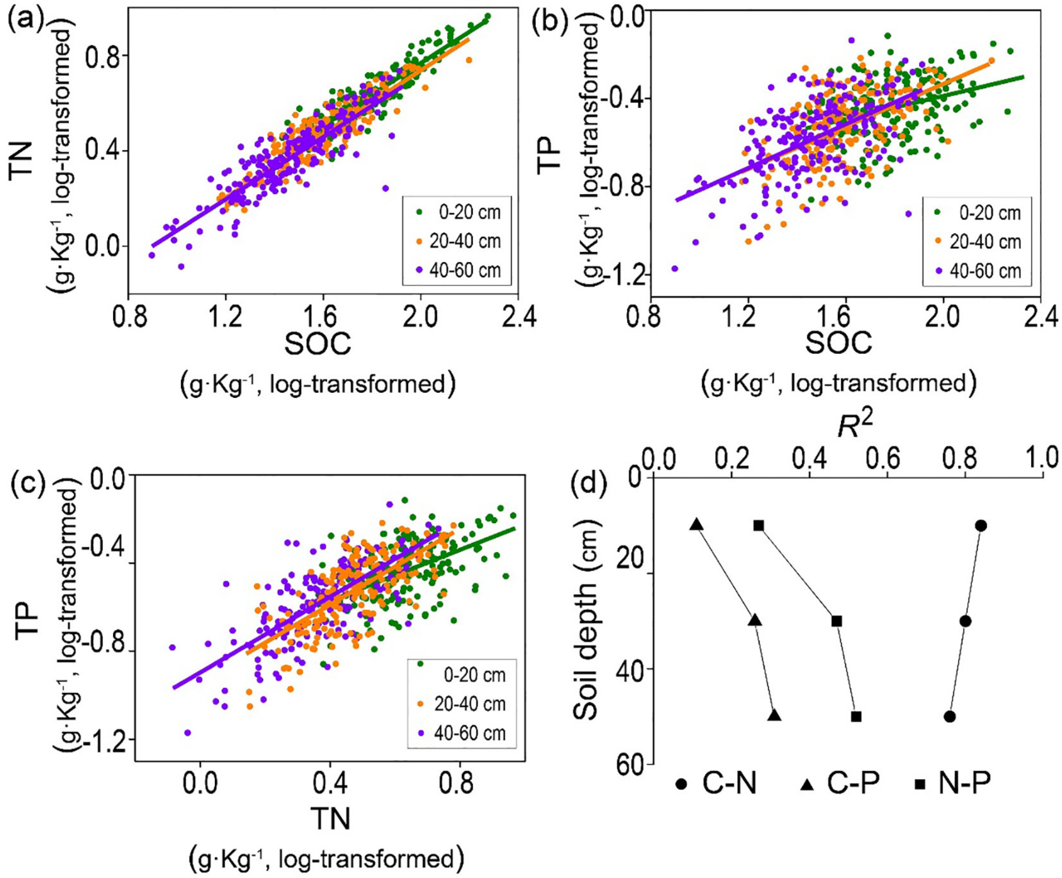

Fig. 3. Relationship of SOC with TN and TP at depths of 0–20 cm, 20–40 cm, and 40–60 cm (a-c). The linear regressions of SOC with TN and TP are significant, at $P < 0.01$. The coefficient of determination (R^2) of the regressions at 0–20 cm, 20–40 cm, and 40–60 cm soil depths (d).

Interestingly, the factors driving SOC variation (i.e., pH and litter quantity) at the landscape scale in this study differed to those at the regional scale. For example, a recent analysis across the forest ecosystems of China showed that the C:N ratio of litter and climate wetness primarily drive spatial variation in SOC accumulation (Zhou et al., 2019). The weak influence of the C:N ratio of litter and climate wetness in this study could be ascribed to their lower spatial variation at local scales compared to regional scales. For example, the C:N ratio of litter ranges from 10 to 100 across the forests of China (Fig. S2 in Zhou et al., 2019), but varies by 15–35 and 17–52 for dead leaves and stems, respectively, at our study site (unpublished data). Thus, one important question that requires answering is how the dominance of different factors changes geographically across the forests of China. Thus, the database of the national field survey campaign reported by Zhou et al. (2019) requires further evaluation at smaller scales.

The SEM analysis showed that soil pH and litter N:P were the main factors driving spatial variation to soil TN concentration (Fig. 4b, e). Soil pH and litter N:P significantly decreased soil TN at both 0–20 and 40–60 cm soil depths. Litter N facilitated the accumulation of TN in the upper soil layer, with a standardized path coefficient of 0.13 ($P < 0.05$; Fig. 4b). This strong correlation between litter N and soil N supported a previous study in the natural forests of northwestern China (Zhang et al., 2017). Topographical factors (convexity and slope), biotic factors (litter N:P), and soil factors (pH) all caused a significant decrease in TP in the top soil layer, based on the SEM results (Fig. 4c). Even though biotic factors (litter N:P) and soil factors (pH) significantly affected soil P in the deep soil layer, the effects of topographical factors (convexity and slope) became weak on soil TP (Fig. 4f). Although topography is recognized as an important factor in affecting SOC storage (Kreznor et al., 1989; Moore et al., 1993), the current study showed that topography only weakly influenced soil P storage in the subtropical evergreen forest.

4.2. Different factors controlling soil C:N, N:P, and C:P ratios across soil depths

This study demonstrated that C:N, N:P, and C:P ratios declined from the surface soil to deeper soil layers (Fig. 2d, e, f). In the SEM of soil C:N (Fig. 5a), pH, convexity, and slope were the main factors influencing soil C:N in the top soil layers. Soil pH significantly decreased soil C:N by negatively affecting both SOC and TN (Fig. 4a, b). Soil pH, convexity, and slope also significantly influenced soil C:N in the deep soil layer (Fig. 5d). Besides, litter quantity also caused soil C:N to decline in the deep soil layers due to its negative effect on SOC (Fig. 5d, 4d). The lower N:P and C:P ratios in deep soil layers supported the results of previous studies in typical terrestrial ecosystems of China based on data from both the second Chinese soil survey and the long-term monitoring experimental plots at the field stations of CERN (www.cerndata.ac.cn) (Chai et al., 2015; Tian et al., 2010). Soil pH, litter N:P, and convexity were the main factors influencing soil N:P in the top soil layer (Fig. 5b). Convexity facilitated an increase in soil N:P by negatively affecting soil TP (Fig. 4c). Soil pH significantly decreased soil N:P by decreasing both soil TN and TP at the same time (Fig. 4b, c). Convexity and litter N:P significantly increased soil N:P in the deep soil layer, while soil pH did not significantly affect soil N:P in the deep soil layer (Fig. 5e). The SEM analysis of soil C:P indicated that convexity and pH significantly affected soil C:P in both the top and deep soil layers (Fig. 5c, f), while slope only significantly affected soil C:P in the top soil layer.

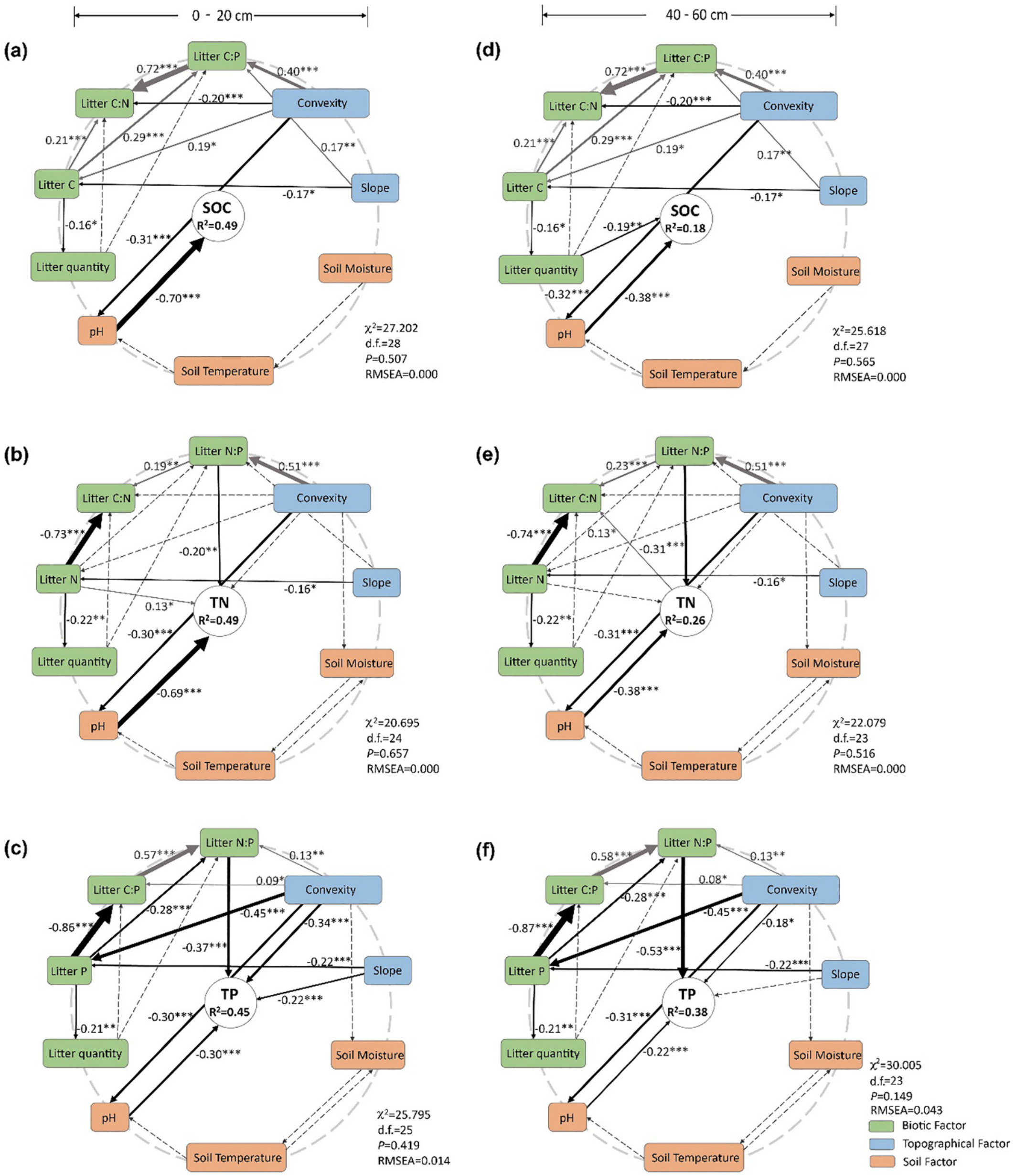

Fig. 4. Best-supported SEMs illustrating the influence of biotic factors (litter quantity, litter C concentration [litter C], litter N concentration [litter N], litter P concentration [litter P], litter C:N, litter N:P, litter C:P), topographical factors (convexity, slope), and soil factors (pH, soil temperature, soil moisture) on the spatial variation of SOC (a,d), TN (b,e), and TP (c,f) at different soil depths. Numbers near arrows are path coefficients and *P* values. *, ** and *** indicate $P < 0.05$, < 0.01 and 0.001, respectively.

4.3. Factors controlling C-N-P coupling

The strength of the C-N correlation is much stronger than that of the C-P and N-P correlations (Fig. 3). The spatial covariation between C and N has been widely reported in most ecosystems (Tian et al., 2010, Zhou et al., 2018). In this study, the SEM analyses show that SOC and TN were significantly influenced by pH in both the top and deep soil layers, whereas TP was influenced by a combination of topographical, biotic, and soil factors (Fig. 4). Thus, similar factors drive the spatial patterns of C and N, both of which differed to soil P.

The spatial correlations of both C-P and N-P were enhanced from the surface layer to the deep soil (Fig. 3). The SEM analyses show that litter N:P had more similar effects on TN and TP concentration in the deep soils (Fig. 4e, f) compared to the top soils (Fig. 4b, c). The negative effects of soil pH on TN and TP concentration were more convergent at 40–60 cm soil depth (Fig. 4d, f) compared to 0–20 cm soil depth (Fig. 4a, c). Similarly, the negative effects of soil pH on SOC and TP concentration were comparable at 40–60 cm soil depth (Fig. 4d, f) but not at the depth of 0–20 cm (Fig. 4a, c). There are more similar influence patterns of P with C and N in the deeper soil layers, which enhance P cycle with the C and N cycles in the deep soil layer. Our results are inconsistent with a previous study in a subtropical plains region, which has detected a strong spatial correlation between C and P in the topsoil, and a decreasing correlation in C-P with increasing soil depth (Zhou et al., 2018). This discrepancy might be associated with N_2-fixing honey mesquite trees being grown in the subtropical plain, which has high P concentrations in the leaves and fine root tissues as the P input, especially in the topsoil (Zhou et al., 2018).

4.4. Implications for soil biogeochemistry in subtropical forests

Soil biogeochemistry plays a pivotal role in regulating terrestrial

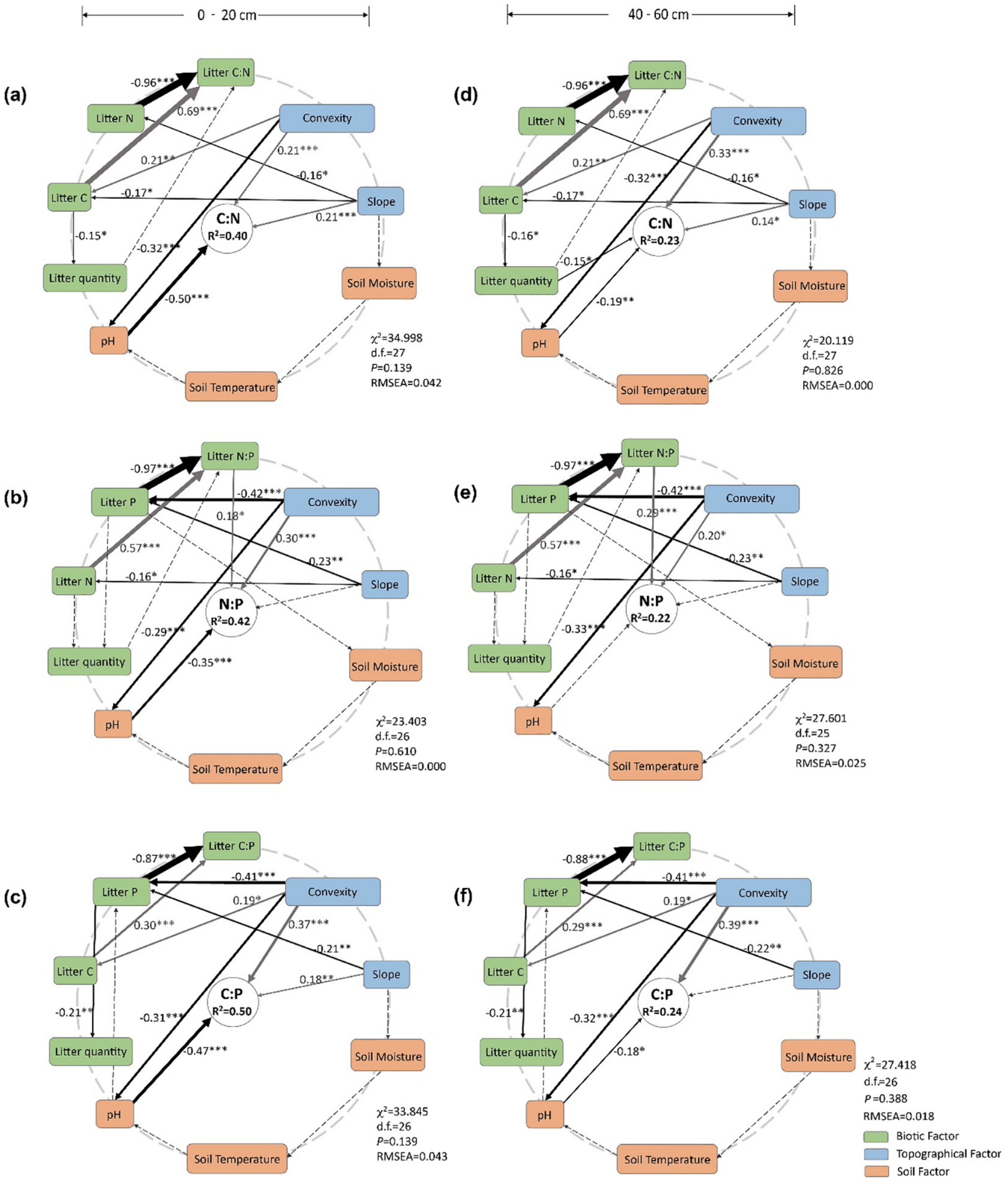

Fig. 5. Best-supported SEMs illustrating the influence of biotic factors (litter quantity, litter C concentration [litter C], litter N concentration [litter N], litter P concentration [litter P], litter C:N, litter N:P, litter C:P), topographical factors (convexity, slope), and soil factors (pH, soil temperature, soil moisture) on the spatial variation of soil C:N (a,d), N:P (b,e), and C:P (c,f) at different soil depths. Numbers near arrows are path coefficients and *P* values. *, ** and *** indicate $P < 0.05, < 0.01$ and 0.001, respectively.

feedbacks to climate change in the Earth system (Wieder et al., 2015; Luo et al., 2016; Wang et al., 2019b). In the current framework of Earth system models, the soil is commonly conceptualized as a few homogeneous pools, with it being assumed that C:N:P ratios are vertically invariant (Thomas et al., 2013; Luo et al., 2016). However, the current study reveals the depth-dependent C-N-P stoichiometry in an old-growth subtropical forest. This finding had some important implications on how soil biogeochemistry is modeled in subtropical forest regions. First, the vertical dynamics of soil biogeochemistry have been subject to extensive theoretical analyses (Bosatta and Ågren, 1996) and site-level modeling (Jenkinson and Coleman, 2008), but must also be incorporated in Earth system models. For example, a model with a vertically solved soil profile could better simulate the soil C pool at high latitudes where old C is usually miss-represented in current models (Koven et al., 2013; Wang et al., 2019a). Second, the subtropical forests of China have one of the highest levels of N deposition globally (Penuelas et al., 2013). Although the soil N availability is temporally stable in China (Wei et al., 2019), the large amount of N deposition in this subtropical area could enhance soil carbon accumulation, but decrease TP concentrations. This phenomenon might further increase the C:N ratio in surface soils, but reduce the C:N ratio in deep soils (Yu et al., 2018). Increased soil acidity under N deposition suppresses both litter decomposition and soil respiration, promoting the accumulation of soil carbon (Mo et al., 2008). N deposition also facilitates P uptake and plant growth (Deng et al., 2016; Li et al., 2016), causing soil P concentrations to decline. However, it remains unclear whether future Earth system models can reproduce the detected pattern of depth dependence in stoichiometric ratios using existing theoretical approaches, e.g., the continuous quality theory (Bosatta and Ågren, 1996). Third, most subtropical forests in China are currently young but are protected to allow them to grow to form old forests in the future. It remains unclear whether and how the dependence of C-N-P stoichiometry on soil depth differs between young and old forests. Therefore, we recommend that more effort should be placed on researching how C-N-P coupling changes with soil depth in forests of different ages to inform and improve models.

5. Conclusions

To the best of our knowledge, this study provides the first proof of depth-dependent changes of C-N-P stoichiometry in a mature subtropical broadleaf forest. The concentrations of SOC and TN are strongly correlated across space, and slightly decrease through the soil profile. However, the spatial correlations of both C-P and N-P are enhanced with increasing soil depth. These observed vertical patterns of soil C-N-P stoichiometry in this study provide a useful benchmark for C-N-P coupled global land models, which are implementing multiple soil layers. Overall, this study calls for an improved understanding of depth-dependent changes in soil biogeochemistry in subtropical forests.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

J.X. designed the study. Y.Q. and J.W. performed the field sampling. H.L. and Q.Y. maintained the plots for sampling and provided information on these plots. Y.Q. analyzed the data. Y.Q. and J.X. wrote the manuscript. K.H., R.L., L.Y., X.W., and J.X. provided critical suggestions for the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2020.114357.

References

Afsar, M., Hoque, S., Osman, K., 2012. Phosphate desorption characteristics of some representative soils of Bangladesh: effect of exchangeable anions, water molecules and solution to soil ratios. Open J. Soil Sci. 2 (3), 234–241.

Alberti, G., Vicca, S., Inglima, I., et al., 2015. Soil C: N stoichiometry controls carbon sink partitioning between above-ground tree biomass and soil organic matter in high fertility forests. Iforest. https://doi.org/10.3832/ifor1196-008.

Bao, S.D., 2010. Soil and Agriculture Chemistry Analysis. China Agriculture Press, Beijing.

Batterman, S.A., Hedin, L.O., Breugel, M.V., Ransijn, J., Craven, D.J., Hall, J.S., 2013. Key role of symbiotic dinitrogen fixation in tropical forest secondary succession. Nature 502, 224–227.

Barrow, N.J., 1984. Modeling the effects of pH on phosphate sorption by soils. J. Soil Sci. 35 (2), 283–297.

Bosatta, E., Ågren, G.I., 1996. Theoretical analyses of carbon and nutrient dynamics in soil profiles. Soil Biol. Biochem. 28, 1523–1531.

Chadwick, O.A., Derry, L.A., Vitousek, P.M., Huebert, B.J., Hedin, L.O., 1999. Changing sources of nutrients during four million years of ecosystem development. Nature 397, 491–497.

Chai, H., Yu, G., He, N., Wen, D., Li, J., Fang, J., 2015. Vertical distribution of soil carbon, nitrogen, and phosphorus in typical Chinese terrestrial ecosystems. Chin. Geographical Sci. 25, 549–560.

Chen, L., Li, P., Yang, Y., 2016. Dynamic patterns of nitrogen: phosphorus ratios in forest soils of China under changing environment. J. Geophys. Res. Biogeosci. 121, 2410–2421.

Crews, T.E., Kitayama, K., Fownes, J.H., Riley, R.H., Herbert, D.A., Mueller-Dombois, D., Vitousek, P.M., 1995. Changes in soil phosphorus fractions and ecosystem dynamics across a long chronosequence in Hawaii. Ecology 76 (5), 1407–1424.

Cui, E., Huang, K., Arain, M.A., Fisher, J.B., Huntzinger, D.N., Ito, A., et al., 2019. Vegetation functional properties determine uncertainty of simulated ecosystem productivity: a traceability analysis in the East Asian monsoon region. Global Biogeochem. Cycles 33, 668–689.

Delgado-Baquerizo, M., Maestre, F.T., Gallardo, A., et al., 2013. Decoupling of soil nutrient cycles as a function of aridity in global drylands. Nature 502, 672.

Deng, M., Liu, L., Sun, Z., et al., 2016. Increased phosphate uptake but not resorption alleviates phosphorus deficiency induced by nitrogen deposition in temperate *Larix principis-rupprechtii* plantations. New Phytol. 212, 1019–1029.

Fontaine, S., Barot, S., Barre, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450, 277–280.

Frizano, J., Johnson, A.H., Vann, D.R., Scatena, F.N., 2002. Soil P fractionation during forest development on landslide scars in the Luquillo Mountains, Puerto Rico. Biotropica 34, 17–26.

Goulding, K.W.T., Bailey, N.J., Bradbury, N.J., Hargreaves, P., Howe, M., Murphy, D.V., Poulton, P.R., Willison, T.W., 1998. Nitrogen deposition and its contribution to nitrogen cycling and associated soil processes. New Phytol. 139, 49–58.

Houlton, B.Z., Morford, S.L., Dahlgren, R.A., 2018. Convergent evidence for widespread rock nitrogen sources in Earth's surface environment. Science 360, 58–62.

Huang, Z., Liu, B., Davis, M., Sardans, J., Penuelas, J., Billings, S., 2015. Long-term nitrogen deposition linked to reduced water use efficiency in forests with low phosphorus availability. New Phytol. 210, 431–442.

Jenkinson, D.S., Coleman, K., 2008. The turnover of organic carbon in subsoils. Part 2. Modelling carbon turnover. Eur. J. Soil Sci. 59, 400–413.

Jobbágy, E.G., Jackson, R.B., 2001. The distribution of soil nutrients with depth: Global patterns and the imprint of plants. Biogeochemistry 53, 51–77.

Kelly, E.F., Chadwick, O.A., Hilinski, T.E., 1998. The effect of plants on mineral weathering. Biogeochemistry 42, 21–53.

Kou, D., Ma, W., Ding, J., Zhang, B., Fang, K., Hu, H., Yu, J., Wang, T., Qin, S., Zhao, X., Fang, J., Yang, Y., 2018. Dryland soils in northern China sequester carbon during the early 2000s warming hiatus period. Funct. Ecol. 32, 1620–1630.

Koven, C.D., Riley, W.J., Subin, Z.M., Tang, J.Y., Torn, M.S., Collins, W.D., Bonan, G.B., Lawrence, D.M., Swenson, S.C., 2013. The effect of vertically resolved soil biogeochemistry and alternate soil C and N models on C dynamics of CLM4. Biogeosciences Discussions. 10 (4), 7201–7256.

Kreznor, W. R., Olson, K. R., Banwart, W; L., & Johnson, D. L. 1989. Soil, landscape, and erosion relationships in a northwest Illinois watershed. Soil Sci. Soc. Am. J., 53, 1763–1771.

Lal, R., 2009. Sequestering carbon in soils of arid ecosystems. Land Degrad. Dev. 20, 441–454.

Leclerc, M.L., Nolin, M.C., Cluis, D., Simard, R.R., 2001. Grouping soils of the Montreal Lowlands (Quebec) according to fertility and P sorption and desorption characteristics. Can. J. Soil Sci. 81 (1), 71–83.

Li, Y., Niu, S., Yu, G., 2016. Aggravated phosphorus limitation on biomass production under increasing nitrogen loading: a meta-analysis. Glob. Change Biol. 22, 934–943.

Liu, G., 1996. Soil Physical and Chemical Analysis, Description of Soil Profiles. Standard Press of China, Beijing.

Luo, Y., Ahlström, A., Allison, S.D., Batjes, N.H., Brovkin, V., et al., 2016. Toward more realistic projections of soil carbon dynamics by Earth system models. Global Biogeochem. Cycles 30, 40–56.

Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H., 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions – a review. Eur. J. Soil Sci. 57, 426–445.

Manzoni, S., Jackson, R.B., Trofymow, J.A., Porporato, A., 2008. The global stoichiometry of litter nitrogen mineralization. Science 321, 684.

Mo, J., Zhang, W., Zhu, W., Gundersen, P.E.R., Fang, Y., Li, D., et al., 2008. Nitrogen addition reduces soil respiration in a mature tropical forest in southern China. Glob. Change Biol. 14, 403–412.

Moore, I.D., Gessler, P., Nielsen, Ga. E., Peterson, G. 1993. Soil attribute prediction using terrain analysis. Soil Sci. Soc. Am. J., 57(2), 443–452.

Miller, R.H., Keeney, D.R., 1982. Methods of soil analysis. Part 2: Chemical and microbiological properties, 2nd edn. American Society of Agronomy, Soil Science Society of America, Madison.

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36.

Neufeldt, H., da Silva, J.E., Ayarza, M.A., Zech, W., 2000. Land-use effects on phosphorus fractions in Cerrado oxisols. Biol. Fertil. Soils 31, 30–37.

Novais, R.F., Smyth, T.J., 1999. Fósforo em solo e planta em condições tropicais. Universidade Federal de Viçosa, Viçosa, MG, pp. 399.

Panda, D., Sen, H.S., Patnaik, S., 1988. Spatial and temporal distribution of nitrogen in a puddled rice soil following application of urea-based fertilizers by different methods. Biol. Fertil. Soils 6 (1), 89–92.

Penuelas, J., Poulter, B., Sardans, J., Ciais, P., Van Der Velde, M., Bopp, L., Boucher, O., Godderis, Y., Hinsinger, P., Llusia, J., Nardin, E., Vicca, S., Obersteiner, M., Janssens, I.A., 2013. Human-induced nitrogen–phosphorus imbalances alter natural and managed ecosystems across the globe. Nat. Commun. 4, 2934.

Schermelleh-Engel, K., Moosbrugger, H., Müller, H., 2003. Evaluating the fit of structural equation models: tests of significance and descriptive goodness-of-fit measures. Methods of Psychological Research Online 8 (2), 23–74.

Song, Y., Wang, X., 1995. Vegetation and Flora of Tiantong National Forest Park. Shanghai Scientific Documentary Press, Zhejiang Province.

Sterner, R.W., Elser, J.J., 2002. Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere. Princeton University Press.

Stone, M.M., Plante, A.F., 2014. Changes in phosphatase kinetics with soil depth across a variable tropical landscape. Soil Biol. Biochem. 71, 61–67.

Tashi, S., Singh, B., Keitel, C., Adams, M., 2016. Soil carbon and nitrogen stocks in forests along an altitudinal gradient in the eastern Himalayas and a meta-analysis of global data. Glob. Change Biol. 22, 2255–2268.

Thomas, R.Q., Zaehle, S., Templer, P.H., Goodale, C.L., 2013. Global patterns of nitrogen limitation: confronting two global biogeochemical models with observations. Glob. Change Biol. 19, 2986–2998.

Tian, H., Chen, G., Zhang, C., Melillo, J.M., Hall, C.A.S., 2010. Pattern and variation of C:N: P ratios in China's soils: a synthesis of observational data. Biogeochemistry 98, 139–151.

Toor, G.S., Bahl, G.S., Vig, A.C., 1997. Pattern of P availability in different soils as assessed by the adsorption equations. J. Indian Soc. Soil Sci. 45 (4), 719–723.

Trudgill, S.T., 1988. Soil and Vegetation Systems. Oxford University Press, New York, US.

Valencia, R., Foster, R.B., Villa, G., et al., 2004. Tree species distributions and local habitat variation in the Amazon: large forest plot in eastern Ecuador. J. Ecol. 92, 214–229.

Vitousek, P.M., Porder, S., Houlton, B.Z., Chadwick, O.A., 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. Ecol. Appl. 20, 5–15.

Walker, T.W., Adams, A.F.R., 1956. Studies on soil organic matter: I. Influence of phosphorus content of parent materials on accumulations of carbon, nitrogen, sulfur, and organic phosphorus in grassland soils. Soil Sci. 85, 307–318.

Walker, T.W., Syers, J.K., 1976. The fate of phosphorus during pedogenesis. Geoderma 15, 1–19.

Wang, J., Yang, Q., Qiao, Y., Zhai, D., Jiang, L., Liang, G., Sun, X., Wei, N., Wang, X., Xia, J., 2019a. Relative contributions of biotic and abiotic factors to the spatial variation of litter stock in a mature subtropical forest. J. Plant Ecol. 12 (4), 769–780.

Wang, J., Xia, J., Zhou, X., Huang, K., Zhou, J., et al., 2019b. Evaluating the simulated mean soil carbon transit times by Earth system models using observations. Biogeosciences 16, 917–926.

Ward, S.E., Smart, S.M., Quirk, H., Tallowin, J.R.B., Mortimer, S.R., Shiel, R.S., Wilby, A., Bardgett, R.D., 2016. Legacy effects of grassland management on soil carbon to depth. Glob. Change Biol. 22, 2929–2938.

Wei, N., Cui, E., Huang, K., Du, Z., Xu, X., Wang, J., Yan, L., Xia, J., 2019. Decadal stabilization of soil inorganic nitrogen as a benchmark for global land models. J. Adv. Model. Earth Syst. 11, 1088–1099.

Wieder, W.R., Allison, S.D., Davidson, E.A., Georgiou, K., Hararuk, O., et al., 2015. Explicitly representing soil microbial processes in Earth system models. Global Biogeochem. Cycles 29, 1782–1800.

Xia, S., Chen, J., Schaefer, D., Detto, M., 2015. Scale-dependent soil macronutrient heterogeneity reveals effects of litterfall in a tropical rainforest. Plant Soil. 391, 51–61.

Yang, Q., Ma, Z., Xie, Y., Zhang, Z., Wang, Z., Liu, H., Li, P., Zhang, N., Wang, D., Yang, H., Fang, X., Yan, E., Wang, X., 2011. Community structure and species composition of an evergreen broad-leaved forest in Tiantong's 20 ha dynamic plot, Zhejiang Province, eastern China. Biodiversity Sci. 19, 215–223.

Yang, Y., Fang, J., Guo, D., Ji, C., Ma, W.H., 2010. Vertical patterns of soil carbon, nitrogen and carbon: nitrogen stoichiometry in Tibetan grasslands. Biogeosciences Discussion 7, 1–24.

Yang, Z., Baoyin, T., Minggagud, H., Sun, H., Li, F.Y., 2017. Recovery succession drives the convergence, and grazing versus fencing drives the divergence of plant and soil N/P stoichiometry in a semiarid steppe of Inner Mongolia. Plant Soil 420, 303–314.

Yu, G., Chen, Z., Piao, S., Peng, C., Ciais, P., Wang, Q., et al., 2014. High carbon dioxide uptake by subtropical forest ecosystems in the East Asian monsoon region. Proc. Natl. Acad. Sci. U.S.A. 111, 4910–4915.

Yu, Z., Wang, M., Huang, Z., Lin, T., Vadeboncoeur, M.A., Searle, E.B., Chen, H., 2018. Temporal changes in soil C-N-P stoichiometry over the past 60 years across subtropical China. Glob. Change Biol. 24, 1308–1320.

Yue, K., Fornara, D.A., Yang, W., Peng, Y., Li, Z., Wu, F., Peng, C., 2017. Effects of three global change drivers on terrestrial C:N: P stoichiometry: a global synthesis. Glob. Change Biol. 23, 2450–2463.

Zhang, G., Zhang, P., Peng, S., Chen, Y., Cao, Y., 2017. The coupling of leaf, litter, and soil nutrients in warm temperate forests in northwestern China. Sci. Rep. 7, 11754.

Zhang, S., Yan, L., Huang, J., Mu, L., Huang, Y., Zhang, X., Sun, Y., 2016. Spatial heterogeneity of soil C: N ratio in a mollisol watershed of northeast China. Land Degrad. Dev. 27, 295–304.

Zhou, G., Xu, S., Ciais, P., et al., 2019. Climate and litter C/N ratio constrain soil organic carbon accumulation. Natl. Sci. Rev. 6, 746–757.

Zhou, Y., Boutton, T.W., Wu, X., 2018. Soil phosphorus does not keep pace with soil carbon and nitrogen accumulation following woody encroachment. Glob. Change Biol. 24, 1992–2007.