



Nitrogen availability mediates soil organic carbon cycling in response to phosphorus supply: A global meta-analysis

Xianzhen Luo, Lingling Zhang, Yongbiao Lin, Dazhi Wen, Enqing Hou*

Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, 510650, China

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ABSTRACT

Soil organic carbon (SOC) is the largest carbon (C) pool in terrestrial ecosystems, and plays an important role in mitigating climate change. With increasing nitrogen (N) loading, phosphorus (P) becomes more limiting for plants and soil microbes, and consequently has a strong impact on ecosystem C sequestration. However, the response of SOC to altered P supply and its mechanisms have not been systematically investigated at the global scale. We conducted a meta-analysis of 536 paired observations from 232 published experiments to explore the dynamics of SOC stock in response to experimental P additions. We found that P additions significantly increased SOC stock across the globe, but the P addition effect on SOC strongly depended on soil N availability. This was confirmed by larger effect sizes in ecosystems dominated by N-fixing species compared with those dominated by non-N-fixing species, under high N availability (soil C:N ratio ≤ 16) compared with low N availability (soil C:N ratio > 16), and under N + P additions compared with P additions alone. Under high N availability, P additions significantly increased aboveground biomass and litterfall, but had no significant effect on soil microbial biomass and oxidase activity. Under low N availability, P additions not only increased aboveground biomass and litterfall but also increased soil microbial biomass and oxidase activity. However, the increases in aboveground biomass and litterfall did not induce a corresponding accumulation of C in the soil, possibly because P additions promoted microbial CO₂ release that offset the increasing plant C inputs. The reactive N input reduced P-addition-induced C loss from the soil to increase SOC sequestration, and the P addition effect on SOC was largely enhanced with increasing experimental duration, partly due to N retention in the soil after P additions. Collectively, the findings suggest that future changes in global exogenous P loads will have divergent impacts on SOC sequestration, depending on soil N availability.

1. Introduction

Phosphorus (P) is one of the two commonly limiting nutrients for plant growth (Elser et al., 2007; Vitousek et al., 2010; Hou et al., 2020; Wu et al., 2022). In recent decades, global P fertilizer consumption has increased to 17 million tons per year, which is a five-fold increase over the global atmospheric P deposition input to the soil (3.3 million tons year⁻¹) (Penuelas et al., 2020). At the same time, global atmospheric P deposition is rapidly increasing, but at a smaller magnitude than the global nitrogen (N) deposition (Penuelas et al., 2013). The increasing P fertilizer input and atmospheric P deposition may alleviate the P-limitation of plants and soil microbes, and thereby influence the dynamics of the soil organic carbon (SOC) stock (Don et al., 2017; Luo et al., 2022b). Moreover, global variation in soil P availability (total P concentration

ranges from 1.4 to 9630 mg kg⁻¹; He et al., 2021) may regulate the response of SOC to global change (e.g., N deposition and warming) (Wang et al., 2021). Thus, it is critical to understand the SOC response to altered P supply, and to accurately predict the SOC stock under global change (Clark et al., 2017).

A nutrient manipulation experiment is the most straightforward method suitable to study nutrient impacts on ecosystem properties (Eviner et al., 2000). Many P addition experiments have been established around the world (Armitage and Fourqrean, 2016; Houngnandan et al., 2020; Liu et al., 2021). However, most of the experiments have been performed over a short time (i.e., <5 years), with the focus on the impact of P additions on primary production, rather than on SOC, which is at least partly due to the slow response of SOC (Li et al., 2016; Yue et al., 2017; Jiang et al., 2021; Feng et al., 2022; Luo et al., 2022a;

* Corresponding author.

E-mail address: houeq@scbg.ac.cn (E. Hou).

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Lu et al., 2022; Wang et al., 2022; Wu et al., 2022). Phosphorus additions may affect soil carbon (C) dynamics via two main processes: plant C input via litter and exudates, and soil C losses via decomposition (Houghton, 2007; Steffens et al., 2015; Li et al., 2016). Phosphorus additions can increase P supply and alleviate the P limitation of plant growth, and thereafter increase litter and root C inputs into the soil (Yuan and Chen, 2012). In addition, P additions may increase the biomass of soil microbes and their demand for other nutrients (mainly N) through the mineralization of SOC, and thereby may reduce the SOC stock (Augusto et al., 2013; Zechmeister-Boltenstern et al., 2015; Li et al., 2016; Luo et al., 2022a). The impact of P additions on SOC may be mediated by experimental settings (e.g., experimental duration) and ecosystem properties other than soil properties (e.g., soil particle size), which all potentially affect the responses of plant productivity and soil microbial activities to P additions (Balesdent et al., 2018; Sokol and Bradford, 2019). Therefore, the net impact of P additions on SOC may be either positive (Houngnandan et al., 2020; Yuan et al., 2021), insignificant (Rebonatti et al., 2016), or negative (Armitage and Fourqurean, 2016) at the site level. These site-level findings motivate us to ask the following three questions: (1) What is the central tendency of P addition effects on SOC at the global scale? (2) What are the major mediators of the effect of P additions on SOC? (3) How do the major mediators regulate the effect of P additions on SOC sequestration?

To address these questions, we compiled a global database of 789 paired observations under P additions alone, and N + P additions (hereafter N + P additions) from 220 articles published between 1978 and 2022. With this global database, we first quantified the global effect of P additions on SOC, then explored the major mediators of the effect, and finally examined the biological mechanisms underlying the impacts of P additions on SOC. We hypothesized that (1) globally, P additions would increase SOC stock, given their global positive effect on above-ground plant production in natural ecosystems (Hou et al., 2020). (2) The effect of P additions on SOC stock would be mediated by N availability, with a larger effect under high N supply. This is predicted from the microbial nutrient mining hypothesis, which states that microbial acquisition of N under low N availability would reduce SOC stock (Treseder, 2008; Zechmeister-Boltenstern et al., 2015). (3) The mediation by N supply would be explained by the interactive effects of N and P supplies on plant C inputs via plant production and litterfall, and soil C losses via soil microbial activities (Yue et al., 2017; Wang et al., 2021). Testing these hypotheses will improve our mechanistic and predictive understanding of nutrient regulations on SOC sequestration, which has important implications for predicting SOC stock under global change.

2. Materials and methods

2.1. Data collection

We compiled the dataset by searching the China Knowledge Resource Integrated Database (<https://www.cnki.net/>), Web of Science (<http://apps.webofknowledge.com/>), and Google Scholar (<http://scholar.google.com/>) for relevant studies. The collected articles were published prior to October 30, 2022 and reported the effects of P additions on SOC and its related variables. The related variables included plant-derived C inputs (e.g., aboveground biomass [AGB], belowground biomass [BGB], and litterfall), soil properties (e.g., soil pH and soil moisture content), soil microbial activities (e.g., enzyme activities, bacterial and fungal biomass), and soil C losses (soil respiration and its components). We assembled the dataset using various keyword combinations, including “phosph*addition”, “phosph*enrich*”, “phosph*supp*”, “phosph*fertili*”, “phosph*input”, “phosph*plant”, “phosph*carbon”, “phosph*litter”, “phosph*micro”, “phosph*respiration”, and “phosph*enzyme”. In addition, we selected the articles cited in previous meta-analyses (Li et al., 2016; Yue et al., 2017; Jiang et al., 2021; Feng et al., 2022; Lu et al., 2022; Wang et al., 2022; Wu et al., 2022). The detail procedure used for study selection is represented by a

PRISMA flow diagram (Fig. S1).

For inclusion in the database, studies were required to meet the following three criteria: (1) the experiment in the reported study was conducted in the field and contained both control and P additions, or both control and N + P addition treatments; (2) no other fertilization or manure was applied in all treatments during the experiment; and (3) SOC and at least one of its related variables were measured, and the mean, standard deviation or standard error, and sample size were reported for all treatments. When a study included several experiments at different sites or different P-supply quantities or different ecosystem types, we considered them to be different observations. The latest sampling was used if more than one measurement at different temporal scales was available for the same sampling site. In total, we generated a meta-dataset of 789 paired observations (536 paired observations for P additions and control; 253 paired observations for N + P additions and control) reported in 232 published experiments from 220 published papers (see supplemental information for the list of references). In addition to estimating the effects of P or N + P additions on SOC and its related variables, the database also included fertilization regimes and site characteristics. The fertilization regimes included fertilizer form, experimental duration, and P-addition quantity. The site characteristics incorporated the site location (i.e., latitude and longitude), topography (elevation), climate (i.e., mean annual precipitation [MAP] and temperature [MAT]), soil physicochemical properties, soil depth, and soil orders from the control or treatments before the start of the experiment (Table S1). The geographical distribution and site characteristics of the P addition experiments were summarized in Fig. S2 and Table S1.

To directly obtain data from figures and tables in the text and supplemental materials of the articles, we extracted data using the digitizer tool in Origin 2015 (OriginLab Corporation, Northampton, MA, US). In cases where the published articles did not report the latitude or longitude, the approximate latitude and longitude were derived by geocoding the name of the location site in Google Earth 7.0 (the free version). Missing MATs and MAPs were derived from WorldClim 2.1 (<https://www.worldclim.org/>), and missing elevations were derived from OpenLandMap, using the site geographic location (i.e., latitude and longitude). Missing soil sand content, soil pH, soil organic C, total N, and total P values were derived from OpenLandMap (Table S2). Soil orders are classified in accordance with the United States Department of Agriculture Soil Classification System and comprise 12 soil orders, namely, Entisols, Inceptisols, Histosols, Andisols, Gelisols, Alfisols, Mollisols, Aridisols, Vertisols, Oxisols, Ultisols, and Spodosols (Soil Survey Staff, 1999).

To compare the effect of P additions on SOC, experiments in the database were grouped using different approaches. First, experiments were grouped based on the ecosystem type, namely, cropland, forest, grassland, and wetland, which examined whether the response of SOC to P additions depended on ecosystem type. Second, experiments were grouped according to the initial soil C:N ratio (an indicator of soil N availability; Terrer et al., 2019): soil C:N ratio >16 and soil C:N ratio ≤16, based on the significantly negative regression between the response ratio of SOC and initial soil C:N ratio (Fig. 1c). The C:N ratio of soil organic matter is a proxy for soil N availability because it is associated with stoichiometric limitations of microbial processes in the soil (Cleveland and Liptzin, 2007; Manzoni et al., 2008). Third, experiments were grouped according to the N-fixing type of dominant species in the ecosystem: N-fixing species and non-N-fixing species. Fourth, experiments were grouped based on the experimental duration: experimental duration >5 years and experimental duration ≤5 years.

2.2. Meta-analysis

We quantified the effects of P additions on SOC using an inverse-variance and random-effect model. To evaluate the responses of SOC to P additions, the effect of each observation was calculated as the natural logarithm-transformed (ln) response ratio (RR):

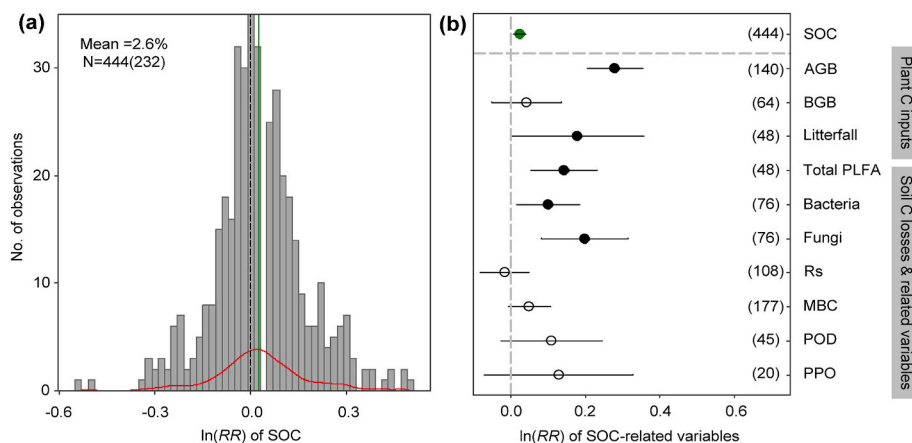


Fig. 1. Effect of phosphorus (P) additions on soil organic carbon (SOC) and related variables. **(a)** Frequency distribution of natural log-transformed response ratios (lnRRs) of SOC. **(b)** P addition effects on SOC-related variables. The dashed line, solid green line, and red curve in **(a)** represent $\ln\text{RR} = 0$, the weighted mean, and the density distribution curve, respectively. Values in **(b)** are the mean \pm 95% confidence intervals (CIs) of natural log response ratios with the number of observations in each category shown. AGB: aboveground biomass; BGB: belowground biomass; PLFA: phospholipid fatty acids; MBC: microbial biomass carbon; POD: polyphenol oxidase; PPO: peroxidase.

$$\ln(\text{RR}) = \ln \frac{\bar{X}_t}{\bar{X}_c} = \ln \bar{X}_t - \ln \bar{X}_c \quad (1)$$

where \bar{X}_t and \bar{X}_c are the means of SOC and its related variables under P additions or N + P additions relative to control, respectively. The variance (v_i^*) of $\ln(\text{RR})$ was calculated as follows:

$$v_i^* = \sqrt{\frac{S_t^2}{n_t \bar{X}_t^2} + \frac{S_c^2}{n_c \bar{X}_c^2} + T^2} \quad (2)$$

$$w_i^* = \frac{1}{v_i^*} \quad (3)$$

where n_t and n_c are the number of replicates for P additions or N + P additions relative to control, respectively, s_t and s_c are the corresponding sample standard deviations, and w_i^* is the weighting factor of the experiment in the group. The calculation of T^2 is described by Borenstein et al. (2010).

The weighted mean response ratio ($\ln(\text{RR}_{++})$) of P additions or N + P additions relative to control was calculated as follows:

$$\ln(\text{RR}_{++}) = \frac{\sum_{i=1}^m w_i^* \ln(\text{RR}_i)}{\sum_{i=1}^m w_i^*} \quad (4)$$

The 95% confidence interval (CI) for the $\ln(\text{RR}_{++})$ and effect size were also calculated.

$$95\% \text{CI} = \ln(\text{RR}_{++}) \pm 1.96s(\ln(\text{RR}_{++})) \quad (5)$$

$$\text{Effect size (\%)} = (\exp(\ln(\text{RR}_{++})) - 1) \times 100\% \quad (6)$$

A random-effect model calculates the mean effect size as the weighted mean of individual effect sizes, using the inverse of the sum of the within-study variance. This was performed using “*rma.mv*” function in the R package “*metafor*” (Viechtbauer, 2010). We included “site” as a random effect (assuming that different local sites, ecosystem types and P-addition quantities within one site are fully independent). The effects of P additions on SOC and its related variables were considered significant if the 95% confidence intervals (CIs) did not overlap with zero. We generated funnel plots to detect possible publication bias using the “*funnel*” function (Sterne and Egger, 2001) with $\ln(\text{RR})$ and v_i^* . Egger’s regression test for the “funnel” was conducted using the “*regtest*” function (Stanley and Doucouliagos, 2014), and Rosenthal’s fail-safe number was calculated using the “*fsn*” function (Orwin, 1983). We also performed an analysis using a random-effect model with potential variables as the only fixed effect to examine whether SOC and its related variables in response to P addition differed among ecosystem types, initial soil C:N ratios and experimental durations.

2.3. Quantifying the relative importance of moderators

To predict the effects of P additions on SOC, we conducted a MetaForest analysis to identify the most important predictor of SOC and its related variables in the dataset. MetaForest is a recently developed method that can be used to explore the various treatment effects in a meta-analysis using random forests. This method has several advantages over a traditional meta-analysis and regression analysis, in that it can handle many potential predictors and their interactions, and considers nonlinear relationships (Wilson, 2020). In the present analysis, we aimed to incorporate as many potential predictors as possible to better account for the variation in P-addition effect on SOC. This included variables that define climate (i.e., MAT and MAP), topography (i.e., elevation), fertilizer regimes (i.e., P-addition quantity, fertilizer type, and experimental duration), ecosystem properties, and soil properties (i.e., soil C:N ratio, soil total N, SOC, soil pH, and soil sand content).

Because MetaForest models require complete moderator values for predictors, we applied single imputation using the missForest algorithm (Stekhoven and Buhlmann, 2011). Missing values for elevation (validation $R^2 = 0.98$), MAT ($R^2 = 0.96$), MAP (validation $R^2 = 0.68$), soil orders (49% accuracy), and soil pH (validation $R^2 = 0.57$) were derived from global maps (Fig. S3). Given the low validation ($R^2 = 0.09$ – 0.45 ; Fig. S3) of soil properties (soil sand content, SOC, and total N and P concentrations), missing values for these indicators were, therefore, filled using the multivariate imputation (Fig. S4) using the chained equations with random forests method in the “*micranger*” package (Wilson, 2020).

We performed the MetaForest analyses using the R package “*metaforest*” (Van Lissa, 2020). For this purpose, we used the “*glmulti*” function (Calcagno and de Mazancourt, 2010) and “*metafor*” function (Viechtbauer, 2010) to automate the fitting of all possible models containing the important predictors and their interactions. We repeated the MetaForest analyses five times and used the averages. A cut-off of 0.8 was set to differentiate between important and redundant predictors. We selected the model with the smallest root mean square error (RMSE) among all candidate models as the final model based on five-fold clustered cross-validation and with relative importance near or less than 0.5 considered unimportant. We evaluated the fitness of the model by examining the RMSE, the retrodictive R^2 , and the predictive R^2 obtained by cross-validation (R_{cv}^2) and obtained during out-of-bag tests (R_{oob}^2). Finally, we used a linear regression model to examine the correlation between response ratios of SOC and its related variables. All statistical analyses were conducted in R 4.1.2 (R Development Core Team, 2019).

3. Results

3.1. Response of SOC and its related variables to P addition

Across the individual experiments, P additions significantly increased SOC concentration by an average of 2.6% at the global scale (Fig. 1a). Since soil bulk density did not change after P additions, SOC stock increased similarly (by 2.6%) after P additions relative to control (Figs. S6a and b). Given the minor difference in response ratio between SOC concentration and SOC stock, our following analyses were all based on SOC concentration.

The effect of P addition on SOC increased with decreasing initial soil C:N ratio and increasing experimental duration (Fig. S6). Phosphorus additions significantly increased AGB, litterfall, and biomass of total phospholipid fatty acids (PLFA), bacteria, and fungi, but had no significant effect on soil respiration, microbial biomass carbon (MBC), and the activities of peroxidase (POD) and polyphenol oxidase (PPO) (Fig. 1b). P addition significantly increased soil total P and available P, and decreased soil C:P and N:P ratio, but did not significantly affect bacterial and fungal residual C (Fig. S7).

3.2. Soil N availability mediates P-addition effects on SOC

The final Meta-Forest model explained 83% of the variation in P addition effect on SOC (Fig. 2a, Table S3). Among the selected mediators, the initial soil C:N ratio was the most important mediator followed by experimental duration (Fig. 2a). Specifically, the response ratio of SOC was negatively correlated with initial soil C:N ratio (Fig. 2b and S6c), but positively correlated with experimental duration (Figs. S6d and S11a). The effect size of P addition on SOC was significantly larger in ecosystems dominated by N-fixing species than in ecosystems dominated by non-N-fixing species (Fig. S8). Across ecosystems, the effect size of P-addition effect on SOC in cropland was larger than that in forest, grassland, and wetland, and the proportion of experiments associated with N-fixing species in cropland was larger than that in forest, grassland and wetland (Fig. S8). Moreover, the effect size of N + P additions on SOC (7.7%) was significantly larger than the effect of P addition alone (2.6%) (Fig. 3 and Table S4).

The effect size of P additions on SOC and AGB were larger, whereas total PLFA was lower, under high N availability (soil C:N ratio ≤ 16) than that under low N availability (soil C:N ratio > 16) (Fig. 3). The response ratio of SOC, AGB, BGB, and litterfall were larger, whereas that of total PLFA, bacterial, and fungal biomass, POD, MBC, and soil respiration (Rs) were lower, under N + P additions than that under P additions alone (Fig. 3 and Table S4). The effect size of P addition on SOC and AGB was larger, whereas the effect size of P addition on total PLFA, bacterial and fungal biomass was lower, under long-term experimental duration than

that under short-term experimental duration, and the response ratio of SOC was positively correlated with increasing experimental duration (Fig. S11).

The response ratios of total N, NO_3^- -N and NH_4^+ -N were larger under high N availability than that under low N availability, larger under N + P addition than that under P addition alone, and larger under long-term experimental duration (> 5 years) than that under short-term experimental duration (≤ 5 years) (Fig. S9). The response ratio of SOC was positively correlated with the response ratio of NO_3^- -N and β -glucosidase activity, but negatively correlated with the response ratio of soil pH and PPO activity (Fig. 4). The response ratio of total PLFA was positively correlated with NO_3^- -N, and the response ratio of MBC was positively correlated with the response ratio of soil pH (Fig. 4). The response ratio of soil respiration was positively correlated with the response ratio of MBC and total PLFA (Fig. S12).

4. Discussion

Our meta-analysis showed that P additions significantly increased SOC concentration at the global scale, which supported our first hypothesis. Our estimate is robust, given the large sample size based on 536 observations included in 232 P-addition experiments, and no publication bias in the dataset was detected (Fig. S5).

As expected, the positive effect of P additions on SOC resulted from increased plant C inputs (e.g., increased AGB and litterfall) after P additions. This hypothesis was also supported by the lack of response of soil respiration to P additions. These results suggest that SOC sequestration would be enhanced with increased plant inputs in response to state change, such as an increase in P supply (Steffens et al., 2015; Wang et al., 2021). Given sufficient time, the effect of P additions on SOC may be larger, as indicated by the increase in SOC response ratio with experimental period (Fig. S11). Our meta-analysis showed that P addition increased total PLFA, bacterial, and fungal biomass. The enhanced soil microbial biomass not only increased the contribution of microbial necromass C (e.g., increased fungal residual C by 0.8%; Fig. S7) to SOC sequestration, but also increased soil POD and PPO activities, which accelerated soil microbial C mineralization for their growth (Chen et al., 2014; Feng et al., 2022; Luo et al., 2022a). The mineralization of SOC partly counteracted the contribution of plant C inputs and microbial necromass C to SOC sequestration under P additions. Consequently, P additions slightly increased SOC accumulation, unlike that N additions greatly increased SOC accumulation (Treseder, 2008; Xu et al., 2020).

Among the selected mediators, the initial soil C:N ratio (an indicator of N availability; Cleveland and Liptzin, 2007; Manzoni et al., 2008) was the most important mediator regulating the response ratio of SOC (Fig. 1b). Consistent with our second hypothesis, P-addition effect on SOC was negatively correlated with initial soil C:N ratio, and was

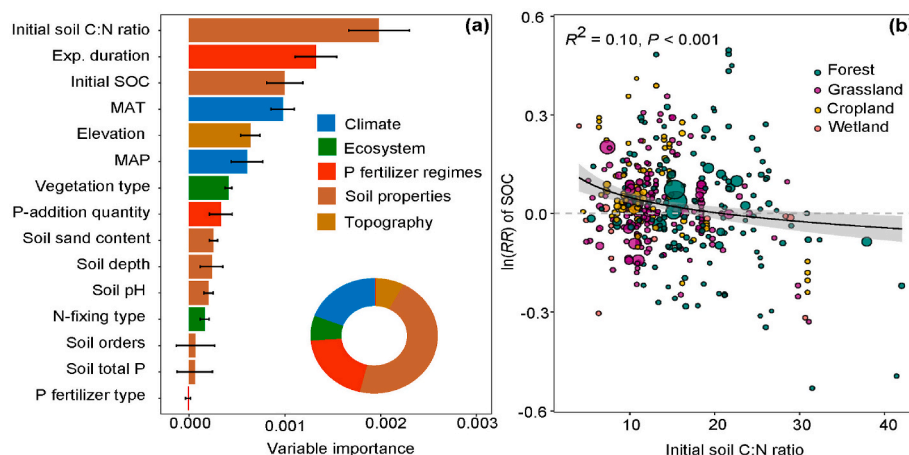


Fig. 2. Effect of phosphorus (P) additions on soil organic carbon (SOC) in relation to its moderators. (a) Importance of variables in determining SOC accumulation under P additions. (b) Effect of P additions on SOC relation to the initial soil C:N ratio (an indicator of nitrogen availability). The variable importance in (a) is quantified based on the final Meta-Forest model with five replicates. The data in (b) represent initial soil C:N ratio responses with bootstrapped 95% confidence intervals indicated by gray shading. MAT: mean annual temperature; MAP: mean annual precipitation.

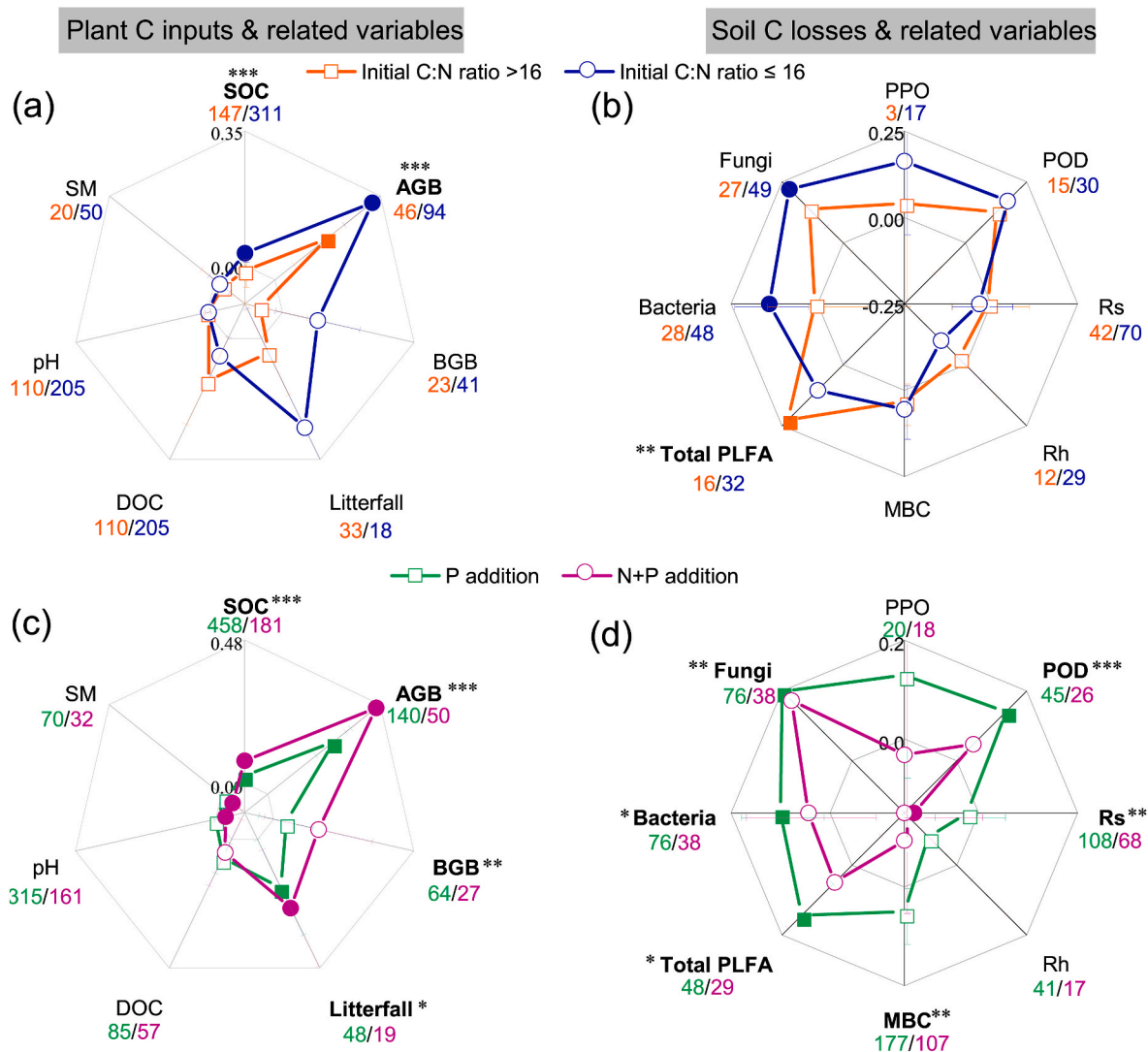


Fig. 3. P addition effects on soil organic carbon (SOC)-related variables depend on soil nitrogen (N) availability. Comparisons of the effects of SOC-related variables between initial soil C:N ratio >16 and initial soil C:N ratio ≤16 ((a) and (b)), and between P additions and N + P additions ((c) and (d)). Values are means ± 95% confidence intervals (CIs) of natural log-transformed response ratios with the number of observations in each category shown. The open and closed symbols indicate non-significant and significant effects, respectively. Significant comparisons between initial soil C:N ratio >16 and initial soil C:N ratio ≤16 or between P additions and N + P additions are identified in bold text. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. AGB: plant aboveground biomass; BGB: belowground biomass; SOC: soil organic carbon; DOC: dissolved carbon; SM: soil moisture content; MBC: microbial biomass carbon; PLFA: total phospholipid fatty acids; G+: Gram-positive bacteria; G-: Gram-negative bacteria; ACT: Actinomycete; PPO: polyphenol oxidase; POD: peroxidase; NAG: β -1,4-N-acetylglucosaminidase; ACP: acid phosphatase; Rs: soil respiration; Ra: autotrophic respiration; Rh: heterotrophic respiration.

significantly larger under a soil C:N ratio ≤16 (high N availability, 3.3%) than under a soil C:N ratio >16 (low N availability, -1.3%). Moreover, the effect size was significantly larger in ecosystems dominated by N-fixing species than in those dominated by non-N-fixing species (Fig. S8b), which is consistent with the findings of a recent meta-analysis (Feng et al., 2023), because of the high N availability in ecosystems dominated by N-fixing species (Augusto et al., 2013; Luo et al., 2019). The effect of N + P additions on SOC was significantly larger than the effect of P addition alone (Fig. 3, Table S4). These three lines of evidence consistently suggest that the effect size of P supply on SOC is greater with increasing N supply.

The greater P-addition effect on SOC under high N supply was associated with larger plant C inputs and lower soil C losses, in comparison to that under low N supply. On the one hand, we found that the effect of P additions on AGB was larger under high N availability than under low N availability, and the effects of N + P additions on plant productivity and litterfall were larger than the effect of P additions

alone. These results suggest that the contribution of plant C inputs to SOC accumulation after P additions is larger under high N supply because of the sufficient N available for plant growth (Fig. S9). The effect of N + P additions on BGB was larger than that of P additions alone, indicating that BGB still increases with increasing N availability despite the decrease in the root: shoot ratio (Penuelas et al., 2013), consequently forming stable soil organic matter efficiently to accelerate SOC accumulation (Fornara and Tilman, 2012; Yuan and Chen, 2012). This result was supported by the positive correlation between the response ratio of SOC and the response ratio of β -glucosidase (Fig. 4a). A possible reason is that the decomposition of litter and roots generally is mediated by hydrolytic enzymes, such as β -glucosidase (Allison and Vitousek, 2005). On the other hand, we found that the effect sizes of microbial biomass and soil respiration were lower under high N availability than under low N availability, and were also lower under N + P additions than under P additions alone (Fig. 3b, d). These results suggest that soil C losses are reduced with increasing N supply, consequently accelerating SOC

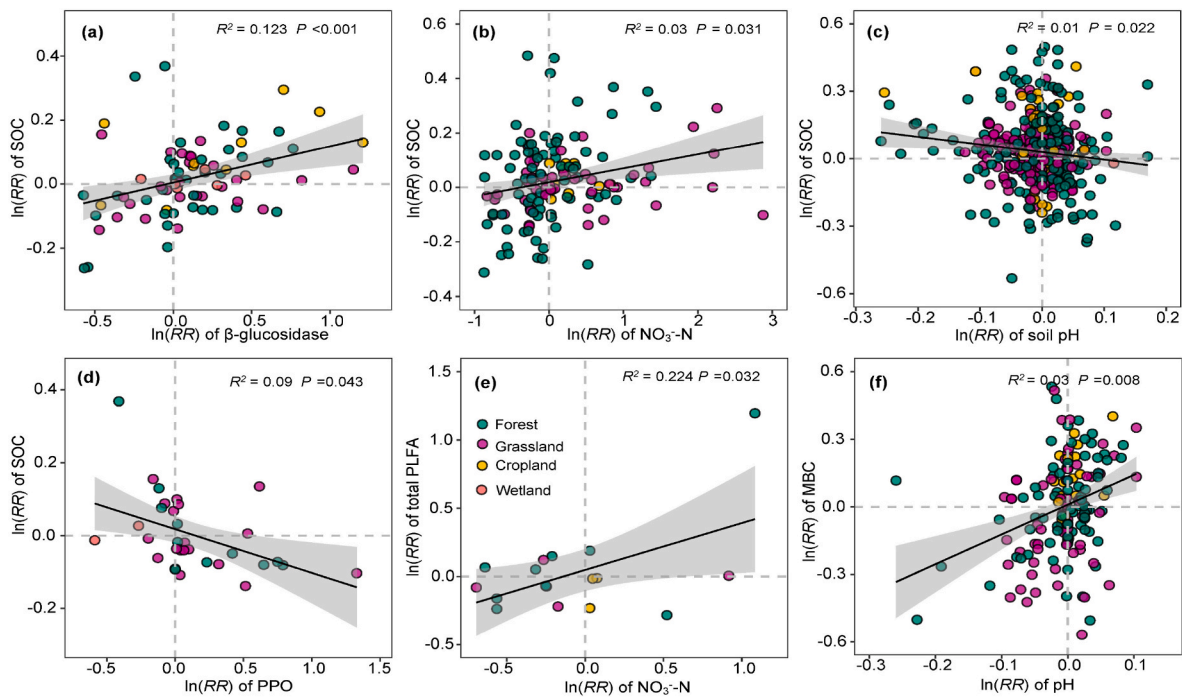


Fig. 4. Relationships between the response ratios (lnRRs) of soil organic carbon and its related variables under phosphorus (P) additions. The dashed lines indicate the absence of changes. The error bands representing bootstrapped 95% confidence intervals are indicated by gray shading. The R^2 and P values were calculated from two-tailed tests. SOC: soil organic carbon; MBC: microbial biomass carbon; PLFA: phospholipid fatty acid; PPO: polyphenol oxidase; BG: β -glucosidase.

accumulation after P supply.

In support of our third hypothesis, soil C losses after P additions were lower under high N availability than those under low N availability. Such a pattern might be explained by the following two aspects. First, soil pH is an important factor in determining soil microbial activities (Janssens et al., 2010). In the present analysis, the lower soil pH under high N availability inhibited soil microbial growth and activity, ultimately decreasing the mineralization of SOC to reduce soil C losses. This result was confirmed by the positive correlation between the response ratio of MBC and the response ratio of soil pH, as well as the negative correlation between the response ratio of SOC and the response ratio of soil pH (Fig. 4b, d). Moreover, the effect size of soil pH, soil microbial biomass and soil respiration were lower under N + P additions than that under P additions alone, and the response ratio of MBC or total PLFA were positively correlated with the response ratio of soil respiration (Fig. S3). These results suggest that lower soil pH inhibits soil microbial activities, and thus reduces soil C losses under high N availability.

Second, previous studies have reported that N deficiency can constrain the positive effects of P additions on the growth of plants and microorganisms (Li et al., 2016; Prescott et al., 2020; Luo et al., 2022a). In N-deficient soil, plants allocate more photosynthetic C belowground, triggering a “priming effect” and promoting the microbial mineralization of SOC as energy to acquire N (Zechmeister-Boltenstern et al., 2015). This was confirmed by the significant increase in soil microbial biomass under P additions and the positive correlation between the response ratio of total PLFA and the response ratio of NO_3^- -N (Fig. 4e). The mineralization of SOC counteracted the positive effect on plant C inputs, and thus P additions slightly increased SOC sequestration. In contrast, SOC accumulation in response to P additions was larger under high N availability, where soil N is more readily available (Zhang et al., 2016; Prescott et al., 2020). The lower soil microbial biomass (e.g., total PLFA and bacterial biomass), activities of oxidases (POD and PPO), and soil respiration under N + P additions than those under P additions alone would potentially decrease microbial C mineralization to reduce soil C losses. This result was supported by the negative correlation between the response ratio of PPO activity and the response ratio of SOC, and the

positive correlation between the response ratio of SOC and the response ratio of NO_3^- -N (Fig. 4b, d). Collectively, high P-addition effects on SOC under high N availability can be attributed to decreases in soil microbial biomass and soil respiration via the decreased soil pH and “priming effect”.

Additionally, phosphorus additions significantly increased SOC concentration with increasing experimental duration (Fig. S11a), owing to long-term SOC accumulation and soil N retention (Table S5). The AGB and litterfall continuously increased from short-term (experimental duration ≤ 5 years) to long-term (experimental duration > 5 years) P additions, which can directly contribute to SOC sequestration. This result was supported by the increase in dissolved organic carbon (DOC) with increasing experimental duration under P additions (Fig. S11b). Moreover, the increased soil N availability (i.e., soil N retention) from short-term to long-term P additions (Fig. S9c) can indirectly increase SOC accumulation by increasing plant C inputs and decreasing SOC losses. The increased soil N availability over time would inhibit soil microbial activities and decrease soil respiration (Fig. S11c) to reduce SOC losses via the decreases in soil pH and “priming effect” (Balesdent et al., 2018; Wang et al., 2021; Wu et al., 2022; Luo et al., 2023). Given that most previous research on P-addition effect on SOC is in of short-term duration (≤ 5 years), the impacts of P addition on SOC may be underestimated.

The present findings clearly illustrated the fundamental role of soil N availability in regulating P-addition effects on SOC storage (Fig. 5). Global atmospheric N deposition expected to increase by as much as $\sim 200 \text{ Tg N year}^{-1}$ by 2050 (Galloway et al., 2008) and the global SOC stock is approximately 2300 Pg in the topmost 1 m (Georgiou et al., 2022). It will be an enormous challenge to estimate SOC stock in the face of the global variation in soil P availability and the increasing global exogenous P loads, especially in the surface soil, because it is sensitive to environmental disturbance (Balesdent et al., 2018). Moreover, few terrestrial C-cycle models consider the interactive effect of N and P on SOC, which causes a large uncertainty in the prediction of further terrestrial C cycling and sequestration. These results imply that future estimation of the terrestrial C stock under various global soil P

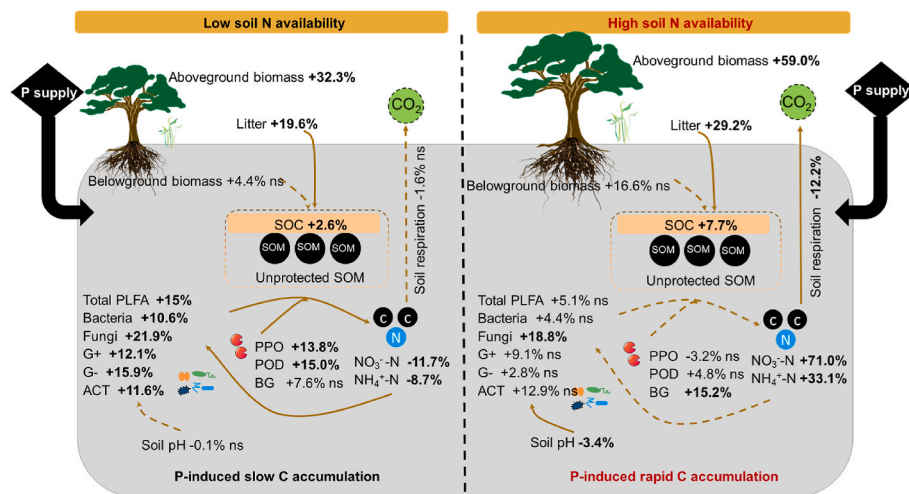


Fig. 5. Schematic illustration of our main findings of phosphorus (P) supply effects on soil carbon (C) accumulation. The values next to the parameters under low soil N availability were derived from P addition experiments and those under high soil N availability were derived from N + P addition experiments. The dashed and solid lines represent a non-significant difference and a significant difference, respectively. Ns indicates non-significant difference; SOC: soil organic carbon; SOM: soil organic matter; PLFA: total phospholipid fatty acid; G⁺: Gram-positive bacteria; G⁻: Gram-negative bacteria; ACT: Actinomycete; PPO: polyphenol oxidase; POD: peroxidase; BG: β -glucosidase.

availabilities and exogenous P loads should take the terrestrial N stock into consideration. Furthermore, several factors might influence the positive effect of P supply on SOC, including climate change and the capacity for soil C sequestration (Feng et al., 2022). For example, many studies have reported that warming stimulates soil respiration (Yue et al., 2017; Wu et al., 2022), which would increase SOC losses. Whether the negative effects of warming influences the response of SOC to P supply remains uncertain. Therefore, further research on the SOC cycle in response to global variation in soil P availability under a changing climate are essential for an improved understanding of the feedback of terrestrial C to climate change.

5. Conclusion

Our analyses suggest that, while increase in P supply would enhance SOC sequestration, the impact essentially depends on soil N availability. The P-addition-induced SOC stock was larger under high N availability (soil C:N ratio ≤ 16) than under low N availability (soil C:N ratio > 16), and was amplified from short- to long-term P additions. The increase in SOC after P additions resulted from stimulation of plant C inputs via increased AGB and litterfall, and reduction of soil C losses via reduced soil microbial biomass and respiration. Moreover, SOC accumulation in the current analysis highlighted that the present estimate of SOC sequestration in response to P supply underestimated the effects of long-term P supply on terrestrial ecosystems. Our findings suggest that exogenous P supply could increase SOC sequestration over time in terrestrial ecosystems, especially in ecosystems with high soil N availability in our analysis and play an important role in mitigating global warming in the future.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2023.109158>.

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