https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

Microbial carbon use for incorporating biomass phosphorus drives CO_2 emission in phosphorus-supplied subtropical forest soils

Jianghao Tan [1,3#], Muhammed Mustapha Ibrahim [1#], Huiying Lin [1,2], Zhaofeng Chang [1,2], Conghui Guo [1,2], Zhimin Li [1], Xianzhen Luo [1], Yongbiao Lin [1], Enqing Hou [1*]

[1] Guangdong Provincial Key Laboratory of Applied Botany & Key Laboratory of National Forestry and Grassland Administration on Plant Conservation and Utilization in Southern China, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

[2] University of Chinese Academy of Sciences, 100039, Beijing, China

[3] Shanxi Agricultural University, Jinzhong 030801, China

[#] These authors contributed equally to this work

* Corresponding author: houeq@scbg.ac.cn (E. Hou).

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

Abstract

Subtropical forests store significant amounts of soil organic carbon (SOC) and are important in the global C cycle. Current understandings based on controlled experiments indicate that phosphorus (P) availability promotes SOC decomposition by alleviating microbial P limitation or rendering SOC available for microbial decomposition. While no alternative mechanism is currently known, it is uncertain if this mechanism holds across soils or P supply levels at the field scale. We formulated an alternative mechanism for acidic subtropical forest soils where organic C (OC) is bound to iron (Fe). Our hypothesis proposed that P supply would promote Fe-bound P formation and desorption of OC previously bound to Fe, and the microbial utilization of the desorbed OC for P-cycling contributes significantly to CO_2 emission. We tested our hypotheses by utilizing a forest P addition platform to explore C-dynamics, its regulators, and utilization across four P supply levels: 0, 25, 50, and 100 kg P ha^{-1} yr^{-1} (Con, P1, P2, and P3, respectively) for one year. Phosphorus supply significantly increased the periodic and cumulative dissolved OC (DOC) concentration, especially in P3, and was associated with increased iron (Fe)-bound P formation. With increased DOC following P addition, microbial biomass P (MBP) significantly increased, while MBC remained unchanged. The significantly positive relationship between MBP:MBC ratio and DOC, significant increase in MBP and carbon dioxide (CO_2) emission with P addition, and the reduction in CO_2 emission with increasing MBC:MBP ratio (0-10cm) supports our results that the desorbed-C alleviated microbial C-limitation induced during P-cycling, particularly, MBP incorporation, to drive CO_2 emission. Structural equation modeling and multivariate analyses projected MBP as a critical factor inducing CO_2 emission. Besides, insignificant alterations in the relative abundance of C-degrading functional genes and reductions in P- and C-

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

degrading enzyme activity indicated the sufficiency of desorbed OC for microbial use without further SOC degradation. Our study provides an alternative mechanism of P's impact on soil C-cycling processes in acidic subtropical forest soils vital for constraining process-based C models.

Keywords: phosphorus limitation, carbon turnover, subtropical forest, global change, acidic soils

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

1. Introduction

Forest soils are vital for carbon (C) storage in terrestrial ecosystems and store a significant proportion of soil organic C (SOC) (Slessarev et al., 2023). The SOC contains about twice the amount of the atmospheric C pool and about three times that of the terrestrial vegetation C pool; hence, small changes in its concentration will have a significant impact on atmospheric carbon dioxide (CO_2) concentration and ecosystem C stock (Harris et al., 2021; Zheng et al., 2022). On the other hand, phosphorus (P) is an essential mineral nutrient for plant growth and regulates terrestrial vegetation productivity, soil microbial activity, biodiversity, and the C storage potential of soils (Hou et al., 2021). Recent studies have shown that soil total P concentration is highly spatially heterogeneous globally, ranging from 1.4 to 9636.0 mg kg^{-1} (He et al., 2021), and is significantly affected by soil and climatic factors (Hou et al., 2021). However, it is still unclear how the spatial heterogeneity in P affects SOC pools and their responses to global change, thus limiting our ability to predict SOC dynamics in the context of global change.

(Sub)tropical forests account for 61% of global tree cover by area (Wri, 2023) and, thus, play a central role in global C sequestration. The soils of these forests are highly weathered, have low pH, and are rich in reactive iron (Fe) phases. The chemical binding of reactive Fe to organic C in these soils is a key chemical mechanism for stabilizing SOC pools (Chen et al., 2022). Under acidic conditions, organic C is bound to Fe (Fe-OC) by adsorption and co-precipitation, making up about 37.8% of the total SOC in acidic forest soils (Zhao et al., 2016; Chen et al., 2020). Under these acidic soil conditions, P and organic C have a high affinity for Fe, resulting in competition for binding sites on Fe, and could be an obstacle to the formation of Fe-organic C complexes (Du et al., 2022), but reports on its influence on

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

Fe-organic C complexation remain under-explored in subtropical forests. Factors inducing the desorption of organic C would increase the soil dissolved organic C (DOC) pool, which constitutes a small fraction of the SOC pool but is the most active and bioavailable C source used to maintain the growth and metabolic activities of soil microorganisms (Liu et al., 2021). Hence, changes in soil DOC concentration can significantly affect the stability of SOC pools by regulating key soil processes such as soil microbial metabolism, nutrient turnover, and C mineralization (Tiwari et al., 2022). Given the importance of DOC in soil processes and microbial respiration, exploring the mechanisms regulating the effect of P supply on DOC and the underlying mechanisms is critical to accurately assess the stability of SOC in (sub)tropical forest soils.

Increased CO_2 emission and microbial biomass formation have been reported following P addition (Fisk et al., 2015; Liu et al., 2012), primarily associated with alleviating P limitation in microbes. Alternative mechanisms underlying this process remain unexplored. The potential desorption of organic compounds in soils following P addition (Du et al., 2022; Neff et al., 2000; Spohn et al., 2022) could provide more labile C and promote CO_2 emission. However, it is still unclear how P addition would impact SOC in conditions of higher Fe/Al oxides characterized by limited microbial activity, making it challenging to understand the dynamic of SOC in acidic conditions (Wordofa et al., 2019). Overall, considerable debate exists on how P supplies affect SOC pools across diverse ecosystems.

While many studies on the impacts of P on SOC in forest systems have been limited to the bulk SOC (Xia et al., 2024; Fang et al., 2019), other studies evaluated non-forest soils or used incubation studies without recourse to P supply levels or the impact of soil acidity (Spohn et al., 2022; Spohn and Schleuss, 2019). Changes in the

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

bulk SOC or short-term changes in organic C dynamics in non-forest soils provide limited information on the realistic impact of P supplies on forest SOC. Forest SOC is exposed to various biogeochemical alterations (e.g., atmospheric nitrogen deposition), with different turnover rates of its active pools (Trumbore, 2006). Besides, available studies did not evaluate the dynamic turnover of DOC by exploring the interactions between P, active Fe, and Fe-bound organic C in acidic subtropical forest soils. Therefore, the impact of P supply on SOC dynamics and DOC turnover and the underlying mechanisms remain unclear. Hence, a clearer insight into how P supplies alter active pools of SOC, especially DOC, in acidic forest soils and its mechanisms is important to improve SOC estimates of terrestrial ecosystem process-based models and combating climate change.

This study is based on the hypothesis that (i). P addition will compete with organic C for adsorption of active Fe, thereby promoting Fe-bound P and inducing the desorption of organic C previously bound to Fe in acidic subtropical forest soils (ii). Unlike the widely reported effect of P on promoting microbial C mineralization by relieving their P limitation, we hypothesized that the increased DOC induced by P addition in acidic soils would provide more labile C that drives microbial cycling of P to induce CO_2 emission. The key objectives of this study were to explore the response of organic C dynamics to soil P availability in acidic subtropical forest soils and to explore alternative mechanisms inducing increased CO_2 emission following P supplies.

Because the covariations in nitrogen (N), potassium (K), and other soil and climatic properties make it impossible to disentangle the impacts of P supply on SOC dynamics from the effects of other factors using natural P supply gradients, P addition

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

controlled experiments are vital to subdue these covariations to ensure accurate estimation of the impacts of P supplies on SOC dynamics.

2. Methods

2.1. The study site

The study was conducted at the Heshan Hilly Comprehensive Open Experiment Station of the Chinese Academy of Sciences. The station is located in the central part of Guangdong Province (112°54′E, 22°41′N) (Fig. S1) and is situated along the transition zone between tropical and subtropical climate. It has an average annual temperature and precipitation of 21.7 °C and 1700 mm, respectively, and the soil type is classified as latosol red soil (equivalent to Ultisol in the USDA soil classification system). The project site is an evergreen, broad-leaved mixed forest of about 0.5 hectares planted in 1984. The main tree species are *Cinus glauca, Mucuna pruriens*, and *Schima superba*.

2.2. Plot settings

The experimental platform was set up in June 2022 and comprises four levels of P and K additions (0, 25, 50, and 100 kg ha^{-1} yr^{-1}). For our study, we selected the plots with P addition from the research platform comprising four treatments: the control (no P) and different levels of P addition (P1: +25 kg P ha^{-1} yr^{-1}, P2: +50 kg P ha^{-1} yr^{-1}, and P3: +100 kg P ha^{-1} yr^{-1}). The basic nutrient concentration of the plots was obtained before establishing the experiment. Each plot is demarcated by PVC panels installed 30 cm above and below the ground, with a buffer zone of 2-5 m between plots (Fig. S1). Each treatment was set with three replicates, with a total of 12

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

quadrants of 10×10 m^2 (Fig. S1). Two static field-based chambers were installed per plot (Fig. S1) for the monthly measurement of CO_2 emissions following P addition.

2.2.1. Phosphorus addition

Analytical grade sodium dihydrogen phosphate (NaH_2PO_4) was used as the P source. It was dissolved in water to form a solution and applied according to the P rate specified for each treatment. The P addition rate was applied as a one-time, complete dose application evenly sprayed per plot in the form of understory spraying. Phosphorus was added using 20 L of P-dissolved water per quadrant with a back-mounted knapsack sprayer to ensure uniform distribution. The same amount of tap water was sprayed in the control treatment to eliminate possible differences between treatments due to the added water during P addition across the P addition treatments.

2.3. Samples collection and processing

In each quadrant, 0-10 cm and 10-20 cm mineral soil samples were collected (after removal of surface organic materials) after 2 weeks, 1, 2, 4, 6, 8, 10, and 12 months after P addition. Three cores per depth were collected in each plot and bulked to form composite samples. Soil sampling was limited to the top 20 cm mineral depth because it is the region with the most active biochemical reactions and microbial activity that can significantly impact our investigation. Litterfall (from understory and overstory plants) was collected monthly during the one year using two 1 m^2 meshes raised along the central region of each plot (Fig. S1). Forest floor litter was collected at 6-month intervals per plot, weighed, oven-dried, and analyzed for DOC concentration.

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

The fresh soil samples per depth (per plot) were well-mixed and passed through a 2 mm sieve to remove fine roots and stones. Some soil samples were stored at 4 °C for the analysis of soil microbial biomass and enzyme activity, while the other portion was stored at -80 °C for subsequent DNA extraction and next-generation sequencing of microbial functional genes. The remaining samples were air-dried and analyzed for soil Fe concentration and C and P components.

2.4. Soil CO_2 measurement

The static chambers shown in Fig. S1 were used to collect monthly gas samples, which were transferred into gas sampling bags. The gas samples were collected at 30-minute intervals from the chambers pre-installed on the field. The CO_2 concentration in each sample was analyzed using a gas chromatography (GC) system (Agilent 7820A, Santa Clara, CA, U.S.) (within 1-2 days after collection), and the values obtained were used to calculate the soil's CO_2 emission rate. Soil temperature and moisture content were measured simultaneously during gas sample collection using soil-installed thermo-hygrometer sensors.

2.5 Samples analyses

The soil pH value was measured using a water-to-soil ratio of 1:2.5 using a pH meter. The active Fe components in the soil were obtained by extracting free Fe-oxide with dithionite–citrate–bicarbonate solution, representing both crystalline and non-crystalline Fe oxides. The modified Hedley fractionation method (Hou et al., 2018) was used for the sequential extraction of P fractions into different groups with varying availability (resin Pi, $NaHCO_3$ Pi, $NaHCO_3$ Po, NaOH Pi, NaOH Po, HCl Pi, residual Pi, residual Po, and total P) to obtain the Fe-bound P represented by the NaOH Pi. The

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

NaOHPi was used to represent the Fe-bound P because, aside from Al, the Fe-bound P dominates the inorganic P extracted by NaOH (Hou et al., 2014). Soil available P concentration was determined using the malachite-green method (Ohno and Zibilske, 1991) and read on a spectrophotometer (UV-3802H, UNICO, Shanghai, China) at a wavelength of 640 nm. The DOC in soil and litter was measured using the cold water method extraction at a water:soil ratio of 1:4 and read on a TC/TN analyzer (Shimadzu TOC-V CPH, Japan). The amount of DOC extracted from the soil samples was used as a proxy for the C desorbed following P addition based on the description of Spohn and Schleuss (2019).

Enzymes that catalyze the degradation of organic carbon (β-1,4-glucosidase [BG]) and phosphorus (acid phosphatase [AP]) were quantified with fluorometric assays following the protocol developed by Bell et al. (Bell et al., 2013) using 2.75 g of fresh soil. Microbial biomass C (MBC) and microbial biomass P (MBP) were determined following soil fumigation with alcohol-free chloroform for 24 h (Vance et al., 1987). The extraction and measurement of soil MBC was done using 0.5 M K_2SO_4, while the MBP was extracted using the malachite green method described for the soil available P.

To explore the dynamics of functional genes mediating organic C degradation and utilization, soil genome-wide DNA extraction and next-generation high-throughput sequencing were carried out in fresh soils collected from the 0-10 cm soil depth. The Mag-Bind® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) was used for DNA extraction based on the manufacturer's protocols. The concentration and purity of extracted DNA were determined with TBS-380 and NanoDrop2000, respectively. The quality of the DNA extract was checked on 1% agarose gel. The extracted DNA was fragmented to an average size of about 400 bp using Covaris

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

M220 (Gene Company Limited, China) for paired-end library construction using NEXTFLEX Rapid DNA-Seq (Bioo Scientific, Austin, TX, USA). Paired-end sequencing was performed on Illumina NovaSeq (Illumina Inc., San Diego, CA, USA) at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) using the NovaSeq 6000 S4 Reagent Kit v1.5 (300 cycles) according to the manufacturer's instructions.

2.6 *Data processing and analyses*

Analysis of variance was used to statistically test the differences in the measured parameters among the treatments and sampling times. The relationship between DOC, CO_2 flux, and C and P components with P availability was established using structural equation modeling (SEM) with the *sem* package in R. Unlike the traditional correlation analysis, SEM allows for variables to serve as responses on one path and as predictors on another, making it useful for testing and quantifying indirect or cascading effects of P availability on soil C and P components and CO_2 emission. We further used the Mantel test to explore the spatial autocorrelation between soil properties, CO_2 emission, C and P components and their functional genes. All analyses were conducted using the R software (v4.3.2) and OriginPro (v2021).

The raw data obtained from high-throughput sequencing was processed using the free online platform of Majorbio Cloud Platform (www.majorbio.com). fastp (https://github.com/OpenGene/fastp, version 0.20.0) was utilized for adapter trimming and removing sequences with low quality (quality threshold value < 20 and length < 250 bp). Metagenomics data were assembled using MEGAHIT (version 1.2.9) (Li et al., 2015) with default k-mers. Contigs with lengths ≥ 300 bp were selected as the final assembly result and used for further gene prediction and annotation. The expression of the functional genes regulating C and P cycling was measured using

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

their relative abundance, which was normalized to transcript per million (TPM) based on the gene length and sequencing depth (based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthology-knockout (KO)) and quantified using Salmon v1.5.182). The alpha diversity of total bacterial functional genes was evaluated using the number of observed species (Sobs), Chao1, the Abundance-based Coverage Estimator (ACE), Shannon, and Simpson's indexes. Redundancy analysis (RDA) was conducted to establish the constrained relationship between soil P and C components and their influence on C and P cycling genes and CO_2 emission.

3. Results

3.1. Responses of soil phosphorus and carbon to phosphorus supplies

Over one year, a one-time supply of increasing P across the experimental plots consistently increased the cumulative concentration of available P (Figs. 1a, S2a) while increasing the P bound to Fe in the soil (Fig. 1b), particularly in the 0-10 cm depth. A similar pattern was observed in the 10-20 cm depth (Fig. S2b). While acid phosphatase decreased with P supply across both depths (Figs. 1c, S2c), microbial biomass P (MBP) significantly increased with P supplies, particularly at P2 and P3 (Figs. 1d, S2d).

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

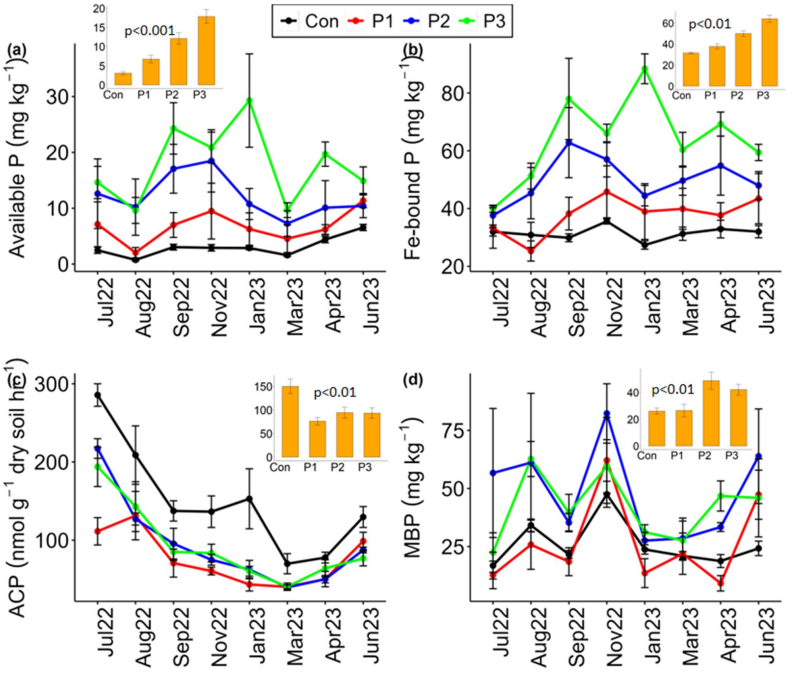


Fig. 1. Repeated measures of soil P dynamics over one year after phosphorus (P) additions in the 0-10 cm depth. a. available P concentration extracted by Bray-1 method, b. iron-bound P (NaOH Pi) concentration, c. acid phosphatase activity, and d. microbial biomass P concentration. Each line/bar represents the mean value of each treatment (n=3 (lines), n=24 (bars), $p < 0.05$). The error bars represent the standard error of the mean. MBP: microbial biomass P, ACP: acid phosphatase. Con: control, P1: 25 kg P ha^{-1}, P2: 50 kg P ha^{-1}, P3: 100 kg P ha^{-1}.

Across the different sampling times, there was a significant increase in the concentration of DOC with increasing P supply, particularly in the P3 treatment (Fig. 2a). Thus, after one year, there was a significant difference (p = 0.03) in the cumulative concentration of DOC among the treatments, with the highest value obtained in the P3 treatment. However, the increase in DOC with P supply was limited to the 0-10 cm depth as a significant reduction (p = 0.03) in DOC was recorded with P addition compared to the control (Fig. 2b). The cumulative surface litter DOC concentration did not significantly vary across the treatments (Fig. 2c).

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

Similarly, the cumulative total litterfall amount, which could contribute to DOC after decomposition did not vary with P addition levels (Fig. 2d).

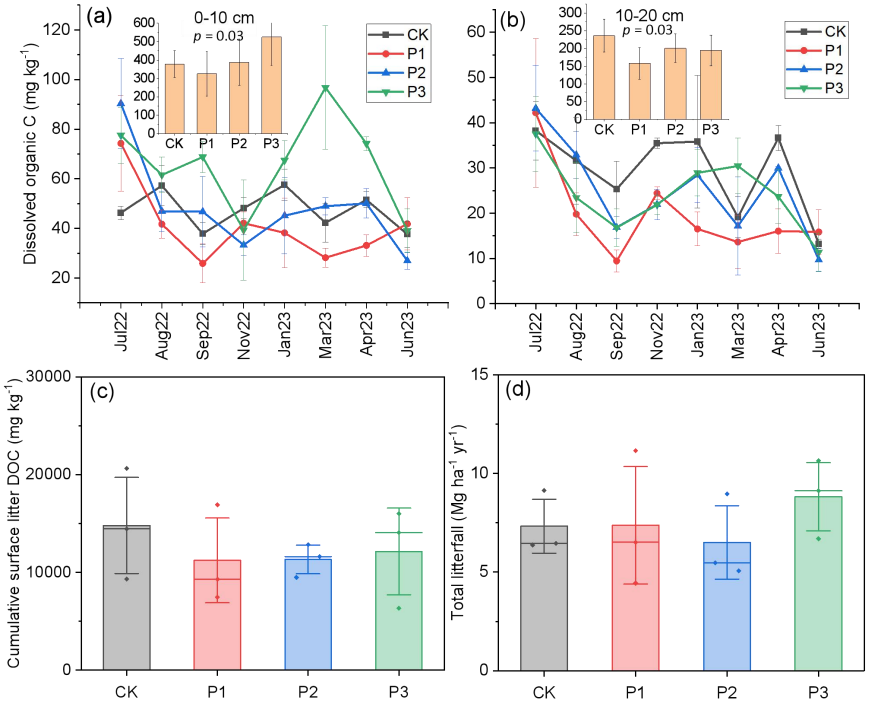


Fig. 2. Dynamics of dissolved organic carbon (C) in soil, surface litter, and litterfall quantity with increasing P supply. a. dissolved organic C in the 0-10 cm depth, b. dissolved organic C in the 10-20 cm depth, c. cumulative dissolved organic C in the surface litter, d. cumulative plant litter biomass. Each line/bar represents the mean value of each treatment (n=3 (lines), n=24 (bars), $p < 0.05$). The error bars represent the standard error of the mean, using Tukey's test (n=24, $p < 0.05$). CK: control, P1: 25 kg P ha^{-1}, P2: 50 kg P ha^{-1}, P3: 100 kg P ha^{-1}.

The beta-glucosidase activity, which is involved in the breakdown of organic C to release glucose for microbial use, was cumulatively significantly reduced (p = 0.04) after P addition compared to the control treatment, but without variation among the P addition rates in the 0-10 cm depth (Fig. 3a). Such a reducing trend, while not significant was observed in the lower 10-20 cm depth (Fig. 3b). The amount of C

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

stored in the microbial biomass did not significantly vary among the control and P addition both in the 0-10 and 10-20 cm soil depth (Fig. 3c and d). Across the 0-10 and 10-20 cm depth, the concentration of non-crystalline Fe in the soil showed an increasing trend with P addition across the sampling times and cumulatively over a year, with higher values obtained in P2 (Fig. S3a-d).

Monthly measurements of CO_2 emission reveal a consistent increase in CO_2 flux with higher P supplies, particularly in P2 and P3 (Fig. 3e). CO_2 flux was significantly lowest and did not vary among treatments during the cold periods (from December to March) (Fig. S4a) and during months with reduced precipitation (Fig. S4b). There was a significant (p = 0.02) difference in the cumulative CO_2 emission among the treatments, with the highest mean values recorded in P2 and P3 (40.7 and 43.9 µmol CO_2 m^{-2} s^{-1}, respectively), compared to the control (30.1 µmol CO_2 m^{-2} s^{-1}) and the P1 treatment (28.7 µmol CO_2 m^{-2} s^{-1}) (Fig. 3f).

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

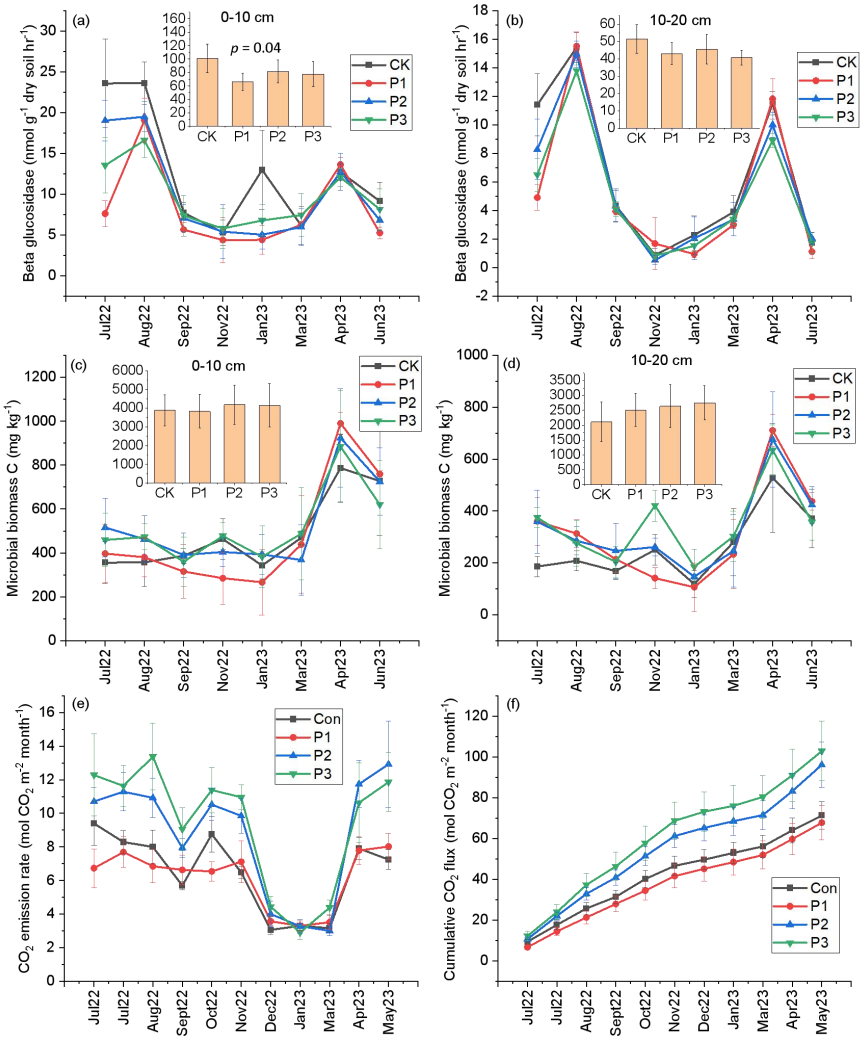


Fig. 3. Dynamics microbial carbon (C) cycling with increasing phosphorus supply. Beta-glucosidase activity in the a. 0-10 cm depth, b. 10-20 cm depth. c. microbial biomass C in the 0-10 cm depth, d. in the 10-20 cm depth, e. monthly CO_2 emission across sampling times, and f. cumulative CO_2 flux over one year. Each line/bar represents the mean value of each treatment (n = 3 (lines), n = 24 (bars), $p < 0.05$). The error bars represent the standard error of the mean, using Tukey's test. CK: control, P1: 25 kg P ha^{-1}, P2: 50 kg P ha^{-1}, P3: 100 kg P ha^{-1}.

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

3.2. Relationships between carbon fractions, enzymes, and soil properties

Linear regression analysis revealed a positive increase in DOC with available P concentrations (p<0.001) (Fig. S5a). There was also an increase in DOC as Fe-bound P increased (p<0.001) (Fig. S5b). The concentration of DOC had a positive significant relationship (p<0.001) with the concentration of acid phosphatase (Fig. S5c), MBP (Fig. S4d), and beta-glucosidase activity (Fig. S5e). Similarly, DOC increased with MBC (p=0.001) (Fig. S5f) but significantly decreased (p<0.001) with the ratio of MBC:MBP (Fig. S5g). However, the concentration of DOC was not significantly altered by the amount of crystalline Fe in the soil (Fig. S5h). DOC concentration was significantly induced by an increase in soil temperature (p<0.001) (Fig. S5i) but not soil water content (p=0.16) (Fig. S5j), soil pH (p=0.59) or amount of Fe in the soil (p=0.29) (Fig. S5k).

Due to the distinct variation in soil microbial processes in the top (0-10 cm) and sub-soil (10-20 cm), we modeled the relationships between CO_2 emission and soil properties among the two soil depths using bivariate linear modeling. Unlike the concentration of DOC, the increase in CO_2 emission with increased available P and acid phosphatase activity was only significant in the topsoil (p=0.04 and p<0.01, respectively) (Fig. 4a and c). However, CO_2 emission significantly increased with increasing concentration of microbial biomass P in both soil depths (p<0.001) (Fig. 4d). The amount of CO_2 emitted was not significantly related to the DOC concentration (p=0.78) (Fig. 4e), while significantly related to beta-glucosidase activity and MBC (p<0.001) (Fig. 4f and g). Moreover, the amount of CO_2 emitted reveals a nonsignificantly decreasing trend with increasing MBC:MBP ratio in the topsoil and assumed the opposite direction in the subsoil (Fig. 4h). CO_2 emission significantly increased with soil temperature and moisture (p=<0.001) (Fig. 4i and j)

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

but was insignificantly altered soil pH and crystalline Fe concentration (p=0.86, and p=0.82, respectively) (Fig. 4k and l).

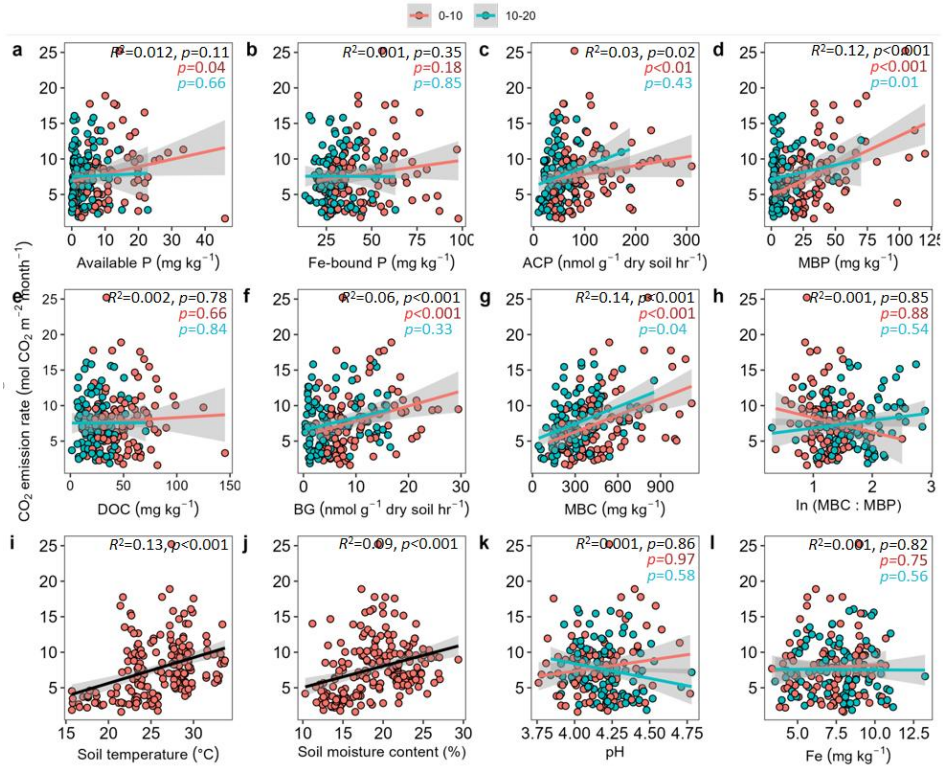


Fig. 4. Linear regression modeling showing the relationships between CO_2 emission rate and soil phosphorus (P) and C fractions across the 0-10 and 10-20cm soil depth. ACP: acid phosphatase, BG: beta-glucosidase, DOC: dissolved organic C, MBC: microbial biomass C, MBP: microbial biomass P, Fe: iron.

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

To establish the key regulators of DOC and CO_2 flux following P addition, our SEM analysis indicated a positive relationship between available P and DOC (r=0.45), MBP (r=0.38), and MBC (r=0.23) (Fig. 5). Notably, a negative association between available P and beta-glucosidase activity (r=-0.19) and acid phosphatase (r=-0.22) was obtained (Fig. 5). On the other hand, positive relationships between DOC and MBC (r=0.10), MBP (r=0.14), bete-glucosidase activity (r=0.51), and acid phosphatase (r=0.62) were observed. The CO_2 emission rate was mainly positively influenced by MBP (r=0.31), MBC (r=0.27), beta-glucosidase activity (r=0.12), and acid phosphatase activity (r=0.02).

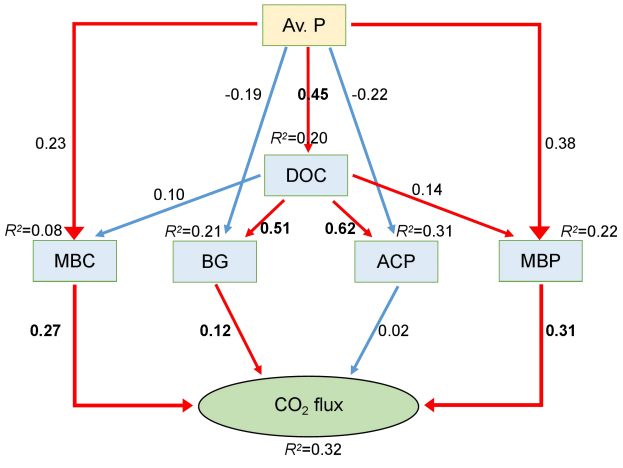


Fig. 5. Structural equation modeling showing the cascading effect of P availability on carbon and phosphorus components regulating CO_2 flux. The red lines indicate significantly positive interaction ($p < 0.05$). Av. P: available phosphorus, Fe-P: iron-bound phosphorus, ACP: acid phosphatase, MBP: microbial biomass phosphorus, MBC: microbial biomass carbon, BG: beta-glucosidase.

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

3.3. Responses of microbial functional genes to increasing phosphorus supplies

Our study shows that P addition did not alter the alpha diversity indices of the total functional genes knockout (KO) read numbers. The species richness of functional genes KO number represented by the Observed species (Sobs), Chao1, and Abundance-based Coverage Estimator (ACE) did not significantly vary across the P addition treatments (Fig. S6a). Also, the Shannon index, which is more sensitive to rare species, and the Simpson index, which is more sensitive to dominant species, did not significantly vary with P supplies compared to the control (p=0.75 and 0.99, respectively) (Fig. S6b).

Three functional gene KOs with high relative abundance (transcript per million) among the vast pools of C-cycling genes were selected and evaluated. The functional genes KOs representing labile C degradation (alpha-amylase, glucoamylase, and D-threo-aldose 1-dehydrogenase) did not significantly vary due to P addition (Fig. 6a-c). Similarly, those involved in recalcitrant C degradation (alpha-glucosidase, arabin and endo-1,4-betaxylanase) did not significantly differ among the treatments (Fig. 6d-f). Also, three genes with high relative abundances among the functional genes regulating P starvation responses, uptake, transport, solubilization, and mineralization were selected based on their TPM from the pools of genes for evaluation. The functional genes KOs representing P starvation response did not significantly vary due to P addition (Fig. S7a-c). This pattern also applied to the P uptake and transport genes (Fig. S7d-f) and the P solubilization and mineralization genes (Fig. S7g-i). The Mantel test used to explore the spatial autocorrelation between soil properties, CO_2 emission and C and P components and their functional genes indicated a significant positive relationship between CO_2 emission and MBP (r=0.73, **p < 0.01), AP (r=0.71, *p < 0.05), and Fe-bound P (r=0.80, **p < 0.01) (Fig. 6g).

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

These results support the observation in our SEM analysis on the importance of P-cycling in CO_2 emission (Fig. 5). Besides, the thickness of the lines connecting the nodes of the P cycling related genes and DOC ($p < 0.05$) was an indication of the magnitude of the correlation between P cycling and the utilization of DOC. Similarly, the direction of ordination in the RDA revealed that MBP, Fe-P, and AP significantly influenced CO_2 emission compared to other variables (Fig. 6h). These relationships were strongest around the P2 and P3 treatments characterized by higher P components, and particularly related to the genes involved in P uptake and transport.

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

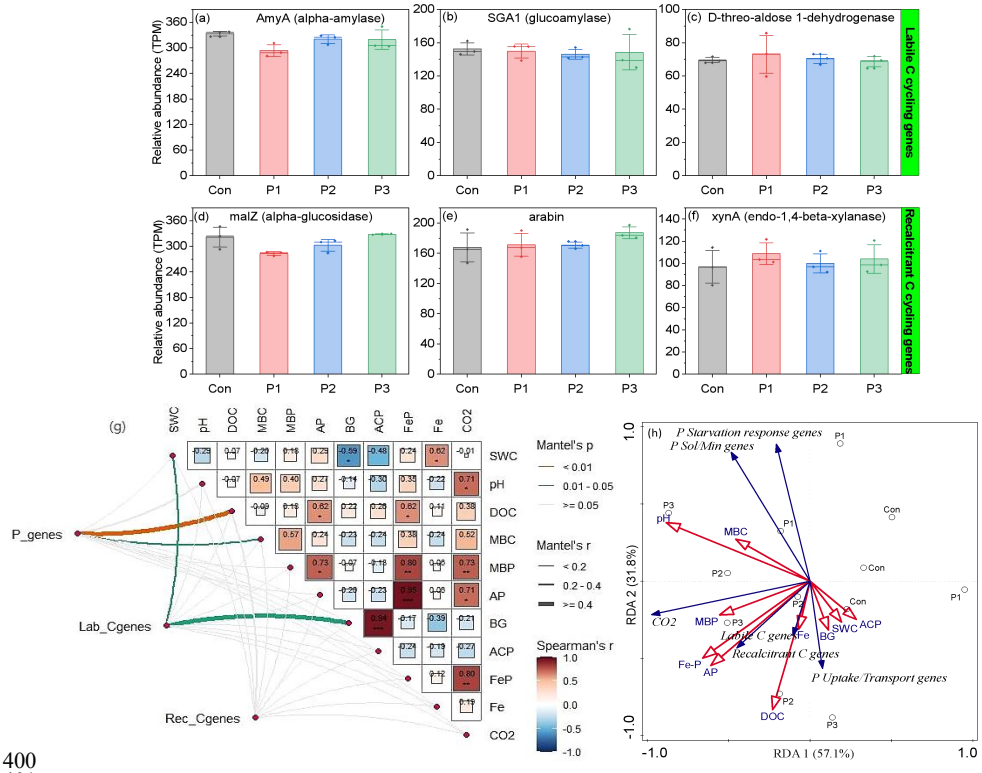


Fig. 6. The relative abundance of carbon (C) cycling functional gene KEGG-orthology and their multivariate relationship with soil properties across P addition at 0-10 cm depth. a-c. relative abundance of labile C-cycling genes, d-f. relative abundance of recalcitrant C-cycling genes, g. Mantel test showing the spatial autocorrelation between soil properties, CO_2 emission and C and P components and their functional genes, h. redundancy analysis showing how soil C and P components accounts for the variation in CO_2 emission and C and P cycling genes. TPM: transcripts per million, ACP: acid phosphatase, BG: beta-glucosidase, DOC: dissolved organic C, MBC: microbial biomass C, MBP: microbial biomass P, Fe: iron, Fe-P: Fe-bound P, SWC: soil water content. P Sol/Min genes: P solubilization/Mineralization genes, P_genes: P-cycling genes, Lab_C genes: labile C-cycling genes, Rec_Cgenes: recalcitrant C-cycling genes. CK: control, P1: 25 kg P ha^{-1}, P2: 50 kg P ha^{-1}, P3: 100 kg P ha^{-1}.

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

4. Discussion

4.1. Responses of soil phosphorus and carbon components to phosphorus supply

The increase in available P across the soils with increasing application rate is relevant for exploring its effect on the dynamics of organic C. Our observed increase in the Fe-bound inorganic P with increasing P concentration agrees with our first hypothesis that in acidic conditions, increasing P availability increases Fe-bound P concentrations following the potential desorption of the organic C previously associated with Fe. This is confirmed by the consistent increasing trend in the concentration of non-crystalline Fe, potentially released following organic C desorption with increasing P addition levels (Fig. S3a-d). The binding of organic C to Fe is a key SOC stabilization pathway in acidic soils (Zhao et al., 2016; Chen et al., 2020). Under acid-to-neutral conditions, phosphate anions in soil sorbs to positively charged surfaces, such as oxides and hydroxides of Fe and Al, and positively charged binding sites on organic matter and at the edges of phyllosilicates (Hinsinger, 2001). The phosphate ions in soil possess a higher affinity for binding sites than several organic C compounds (Guppy et al., 2005; Ruttenberg and Sulak, 2011; Du et al., 2022) and could undergo anion exchange with the organic C sorbed to the soil solid phase. This high affinity of P for Fe becomes an obstacle to the formation and stability of organic C complexes (Du et al., 2022). Therefore, the increase in DOC in the topsoil across sampling times and cumulatively following P addition, particularly at higher P rates, could be associated with increasing desorption of organic C. It was previously demonstrated that only one hour after adding inorganic P, an increase of 1.6-3.5 factors of DOC concentration was recorded across four soil horizons (Spohn and Schleuss, 2019). The long-term addition of inorganic P increased the concentration of DOC in tropical soils (Neff et al., 2000) and induced the dissolution

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

of organic acids (Afif et al., 1995). Also, adding P to temperate forest soils prevented the sorption of DOC to the soil solid phase (Schneider et al., 2010) and in different soil types (Spohn et al., 2022). To verify that the increase in DOC concentration was not influenced by factors such as litterfall amount or surface litter DOC concentration, our measurements showed an insignificant variation of litterfall amount and surface litter DOC among the treatments (Fig. 2c, d). This observation indicates that the interaction of Fe-OC-P mainly induced the increase in DOC with P addition via the competitive adsorption mechanism earlier hypothesized. Therefore, the amount of DOC introduced by the surface litter across the treatments did not influence the dynamic concentration of DOC with P supply in the soil system.

Our study showed that soil microbes did not invest in enzymatic activity that further degraded SOC following P addition. With the increase in DOC following P addition, a significant reduction in beta-glucosidase activity was involved in the breakdown of organic C to release glucose for microbial use. This observation is further supported by the fact that there was no significant change in the activity of C-degrading functional genes after P-addition. Previous studies have similarly reported that when adequate inorganic P is supplied, it could inhibit SOC decomposition in tropical forest soils (Schulze et al., 2000; Zhang et al., 2022) because soil microbes will decrease the investment in enzymes and, thus, reduce the decomposition of organic matter (Zheng et al., 2015). Furthermore, the insignificant effect of the P addition rate on soil MBC across the 0-10 and 10-20 cm depths indicates microbial utilization of most of the excess DOC for other metabolic processes at the expense of its incorporation as MBC. However, MBP increased with increasing DOC concentration, indicating greater allocation of the released labile C into building MBP, a process that requires an enormous amount of C. Similar observations of the

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

insignificant effect of inorganic P addition on soil MBC have been previously documented (Spohn and Schleuss, 2019; Spohn et al., 2022), even across different soil profiles (Heuck et al., 2015). In contrast, some studies reported increased soil MBC from the long-term addition of P to grasslands (Griffiths et al., 2012; Chen et al., 2014) and in mature tropical forests in the subtropical region of China (Liu et al., 2012). Similarly, soil MBC increased after long-term P addition in a lowland tropical forest (Turner and Joseph Wright, 2014). While these authors posit that the increase in MBC following P addition was due to the alleviation of P limitation in microbial biomass, the results from our study, however, provide an alternative mechanism that the desorbed organic C was utilized as C source for incorporating the added P into microbial biomass at the expense of MBC; hence, the insignificant change in MBC recorded in our study.

With increasing P supplies, soil DOC increased and was associated with a consistent increase in CO_2 flux, particularly at higher P supply rates (P2 and P3) (Fig. 3e). This indicates the capacity of the desorbed organic C to provide sufficient labile C required for increasing microbial metabolism and respiration. Our finding of increased respiration following P addition agrees with previous studies reporting increased soil respiration due to inorganic P addition (Cleveland et al., 2002; Fisk et al., 2015; Liu et al., 2012; Spohn and Schleuss, 2019; Munevar and Wollum, 1977). The increase in respiration after P addition in these studies was attributed to the alleviation of microbial P limitation. However, our investigation revealed that C limitation was induced due to the high amount of C needed for incorporating the added P into microbial biomass. Thus, DOC was increasingly utilised as the primary microbial C source, further inducing respiration. The building of MBP requires significant utilization of enormous labile organic C to execute, thus substantially

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

increasing microbial respiration with increasing P availability. Generally, when there is an excess of available soil P, microbes can utilize this P more efficiently if there is an adequate supply of readily available organic C (Fanin et al., 2015). Thus, the desorbed C due to P supply provided the energy and the necessary building blocks for microbial growth and incorporation of excess P into biomass, a process that contributed to the increased CO_2 recorded. If the DOC availability is limited, the ability of microbes to utilize and incorporate the excess soil P into their biomass may be reduced. Microbial C limitation has been shown to induce a lag effect of P addition on soil microbial biomass in the initial years following P addition (Liu et al., 2015). Therefore, our study provides newer insights that the desorbed C alleviated microbial C limitation to drive the incorporation of P into microbial biomass to further induce CO_2 emission in P-amended soils.

4.2. Relationships between carbon and phosphorus components

The significant positive relationship between DOC, available P, and Fe-bound P concentrations in our linear regression model supports our hypothesis of the preferential sorption of free Fe desorbed from the Fe-OC interface to P (Xia et al., 2024; Spohn et al., 2022). Besides, the positive relationships between acid phosphatase and MBP with DOC concentration are consistent with the fact that the amount of DOC in soil determines the capacity of microbes to utilize and incorporate excess available P into microbial biomass effectively. However, we observed that higher CO_2 emission, particularly in the topsoil, only occurs when MBP incorporation exceeds MBC. This is because an enormous C is required for incorporating P into microbial biomass, which is the key regulator of CO_2 emission under P addition. Our reports agree with the conclusions of Heuck et al. (2015), who reported that the

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

516 microbial biomass possesses higher C limitation but is not limited by N or P when P
517 is available. Our linear regression model further shows significant positive increases
518 in β-glucosidase and MBC with DOC, indicating the capacity of microbes to utilize
519 and incorporate the excess labile C into the microbial biomass. However, a negative
520 relationship ensued between DOC and the MBC:MBP ratio, indicating that the labile
521 organic C was preferentially used for building MBP instead of MBC. However, the
522 concentration of DOC was not significantly related to the amount of crystalline Fe in
523 the soil (Fig. S4k), indicating that most of the free Fe in the soil was increasingly
524 associated with P after the desorption of Fe-bound organic C.

525 Our observed positive linear relationship between available P in the topsoil
526 and CO_2 emission agrees with the increased soil respiration due to inorganic P
527 addition previously reported (Cleveland et al., 2002; Fisk et al., 2015; Liu et al., 2012;
528 Spohn and Schleuss, 2019; Cleveland and Townsend, 2006). While the amount of
529 CO_2 emitted increased with MBC and MBP, it showed a decreasing trend with a
530 higher MBC:MBP ratio. This finding implies that higher incorporation of P into
531 microbial biomass relative to C drives CO_2 emission. Taken together, the increase in
532 CO_2 emission upon inorganic P addition is primarily a consequence of the
533 incorporation of P into microbial biomass, a process that requires enormous labile C.
534 Previous studies have often concluded that the stimulation of soil CO_2 efflux due to
535 the addition of inorganic P is due to the removal of microbial P limitation and the
536 stimulation of microbial activity (Cleveland et al., 2002; Fisk et al., 2015; Liu et al.,
537 2012; Spohn and Schleuss, 2019). However, our results provided an alternative
538 mechanism that C-limitation is induced during microbial incorporation of excess P
539 into biomass and that the desorbed organic C is quickly metabolized by soil
540 microorganisms, thus inducing CO_2 emission. CO_2 emission was further stimulated

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

during periods with higher soil temperature and a relative increase in soil moisture to support microbial activities. CO_2 emission increases with increasing soil temperature (Natali et al., 2015). While excess soil moisture could limit CO_2 emission (Ibrahim et al., 2022), increasing soil moisture to a sufficient level could promote microbial activities and drive respiration. Seasonal transition from wet to dry drove an 18% annual increase in CO_2 emission from the P-fertilized plots (Cleveland and Townsend, 2006), indicating the key role of soil moisture in regulating CO_2.

The SEM analysis revealed that the amount of DOC indirectly influenced CO_2 emission due to its role in sustaining the immediate labile C requirement for microbial activities. The significantly positive relationship between CO_2 emission, acid phosphatase, and MBP compared to beta-glucosidase and MBC indicated that microbial utilization of DOC as an energy source for P cycling and mobilization into biomass was the dominant regulator of CO_2 emission. Compared to beta-glucosidase activity, the stronger positive relationship between DOC and acid phosphatase buttresses our observation of its higher utilization for P cycling. Despite these observations, it was evident that C-cycling also significantly contributed to CO_2 emission.

4.3. Responses of microbial functional genes to increasing phosphorus supplies

Microbial gene KO read numbers are a powerful tool for studying gene function and exploring how specific genes in microorganisms regulate soil processes. We show no alteration of the alpha diversity indices of total functional genes KOs to P addition. We show that P supplies had insignificant effects on the relative abundances of the functional genes KOs representing labile and recalcitrant C degradation (Fig. 6a-f). This result suggests that the labile C needed for immediate

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

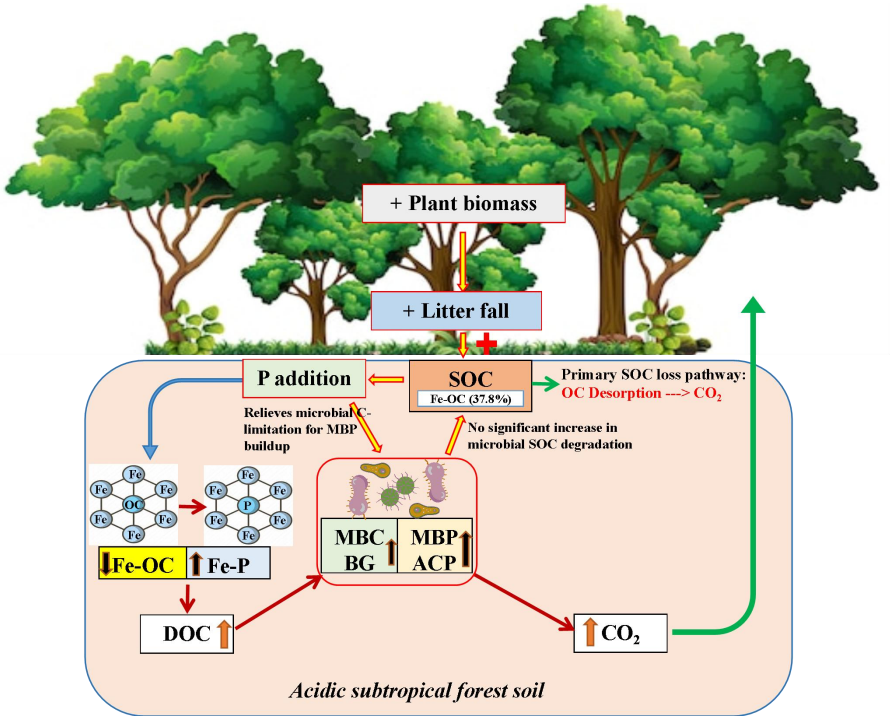
565 microbial use was not limiting, and the degradation of further SOC was not prioritized.
566 These results infer that the desorbed organic C due to P addition was available to
567 sustain microbial C limitation needed to drive microbial metabolic processes.
568 Previous reports have indicated that P addition promoted SOC decomposition by
569 stimulating C-cycling-related genes, with a greater effect on the abundance of
570 recalcitrant C-degrading genes than labile C-degrading genes in saline-sodic soils (Du
571 et al., 2023), and in alpine meadow (Ye et al., 2023).

572 Our hypothesis that P cycling significantly contributed to CO_2 emission was
573 further verified by multivariate analyses using the spatial autocorrelation of the
574 Mantel test (Fig. 6g). Similarly, the multivariate RDA supports our hypothesis by
575 indicating the proximity, the low acute angle, and the length of arrows of cluster
576 points of CO_2, MBP, Fe-bound P, available P, and DOC compared to other variables
577 (Fig 6g). P-cycling requires enormous C to meet microbial energy needs (Heuck et al.,
578 2015). Besides, the strong autocorrelation between P-cycling genes and DOC in the
579 Mantel test was further clarified in the RDA, where an acute angle between CO_2
580 emission rate and microbial genes involved in P uptake and transport ensued. These
581 results further support our earlier observation that the incorporation of MBP and the
582 roles of genes involved in P uptake and transport significantly drive CO_2 emission.
583 Even though P addition results in the degradation of SOC (Ye et al., 2023; Du et al.,
584 2023), the microbial C need for further P-cycling could have stimulated this process.
585 Therefore, we alternatively propose that the desorbed organic C following P addition
586 in acidic forest soil was sufficient to meet microbial P requirement without
587 significantly degradation SOC for their C needs.

588 A key implication of the study is that C loss as CO_2 could be potentially
589 higher in acidic subtropical forests, soil rich in Fe, and regions of higher P availability.

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

590 Therefore, a trade-off exists between SOC accumulation from the potential increase in
591 plant biomass (resulting from higher litterfall and SOC accumulation) in regions of
592 higher P and the amount lost via increased CO_2 emission. This could undermine the
593 overall subtropical forest C storage potential if the amount of litter-derived organic C
594 does not exceed the amount of desorbed organic C because we revealed that the
595 excess DOC was not leached down the soil for deep storage. Following our results,
596 we reconstructed the framework for the processes occurring in acidic subtropical
597 forest soil following P supplies in Figure 7.



598

599 **Fig. 7.** A framework of the effect of phosphorus (P) addition on soil organic carbon
600 (SOC) stability from the study. P addition induces the desorption of Fe-bound organic
601 C to increase DOC. The DOC serves as the key microbial C source for enzymatic
602 activities and incorporating P and C into microbial biomass. The enormous C required
603 for MBP incorporation drives CO_2 emission at a faster rate than the C required for
604 MBC.

605

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

While our study provided an innovative and alternative mechanism on how P supplies influence C cycling and CO_2 emission from subtropical forest soils, some limitations still exist in our explorations. First, our study was limited to one forest type, and samplings were carried out periodically over one year. Thus, how the mechanisms reported here fluctuate in the long-term following continuous P addition remains uncertain. Second, our study focused on the one-time application of P rates per year and measuring the impulse response pattern of SOC, unlike the continuous replenishment strategy used in some instances. Besides, our findings and conclusions are limited to acidic subtropical forest soil rich in Fe and capable of binding anions. Thus, these findings would require validation before they can be extrapolated to other soil ecosystems. Despite these limitations, we have provided important alternative mechanisms for understanding and predicting the alterations in SOC subtropical forest soils. Future research should consider integrating the roles of climate, soil type, and structure, forest types, tree species diversity, and soil nutrient interactions (and their spatial distribution) on the alteration of SOC under acidic conditions.

5. Conclusion

Our study supports our hypothesis that the availability of inorganic P in acidic subtropical forest soil increases soil DOC by promoting the formation of Fe-bound P following the desorbing organic C previously bound to Fe. Increased microbial respiration was stimulated following P addition because the desorbed organic C was mainly utilized to relieve microbial C limitation, which was induced following the incorporation of P into the microbial biomass. Thus, CO_2 emission was significantly stimulated, especially with increasing MBP. The microbial utilization of desorbed C for MBP build-up was the most significant factor in regulating CO_2 emission. Moreover, the non-significant change in microbial C-degrading enzymes and the

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

relative abundance of C-degrading functional genes indicates that the microbial C source was primarily derived from freshly desorbed C rather than further microbial degradation of SOC. Our study highlights newer and alternative mechanisms into how P addition influences CO_2 emission and SOC dynamics in acidic soils, which is vital for understanding the fate of SOC across P concentration gradients in subtropical forests.

CRediT authorship contribution statement

JT: Investigation, Methodology, Software, Writing - review & editing. MMI: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Funding acquisition, Writing - original draft, Writing - review & editing. HL: Conceptualization, Investigation, Methodology. ZC: Investigation, Methodology. CG: Investigation, Methodology. ZL: Investigation, Methodology. XL: Investigation, Methodology. YL: Investigation, Methodology. EH: Conceptualization, Investigation, Methodology, Data curation, Funding acquisition, Supervision, Validation, Visualization, Writing- review & editing, Project administration, Resources.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (W2433082; 32322054, 32271644), China Postdoctoral Science Foundation (2023M743544), and the Guangdong Basic and Applied Basic Research Foundation (2022B1515020014).

Data availability

The data supporting the findings of this study are openly available in "Figshare" at https://doi.org/10.6084/m9.figshare.28122539.

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

Conflict of interest statement

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 article.

References

Afif, E., Barrón, V., and Torrent, J.: Organic matter delays but does not prevent phosphate sorption by Cerrado soils from Brazil, Soil Science, 159, 207-211, 1995.

Bell, C. W., Fricks, B. E., Rocca, J. D., Steinweg, J. M., McMahon, S. K., and Wallenstein, M. D.: High-throughput fluorometric measurement of potential soil extracellular enzyme activities, JoVE (Journal of Visualized Experiments), e50961, 2013.

Chen, C., Hall, S. J., Coward, E., and Thompson, A.: Iron-mediated organic matter decomposition in humid soils can counteract protection, Nature Communications, 11, 2255, 2020.

Chen, J., Hu, Y., Hall, S. J., Hui, D., Li, J., Chen, G., Sun, L., Zhang, D., and Deng, Q.: Increased interactions between iron oxides and organic carbon under acid deposition drive large increases in soil organic carbon in a tropical forest in southern China, Biogeochemistry, 158, 287-301, 2022.

Chen, X., Daniell, T. J., Neilson, R., O'Flaherty, V., and Griffiths, B. S.: Microbial and microfaunal communities in phosphorus limited, grazed grassland change composition but maintain homeostatic nutrient stoichiometry, Soil Biology and Biochemistry, 75, 94-101, 2014.

Cleveland, C. C. and Townsend, A. R.: Nutrient additions to a tropical rain forest drive substantial soil carbon dioxide losses to the atmosphere, Proceedings of the National Academy of Sciences, 103, 10316-10321, 2006.

Cleveland, C. C., Townsend, A. R., and Schmidt, S. K.: Phosphorus limitation of microbial processes in moist tropical forests: evidence from short-term laboratory incubations and field studies, Ecosystems, 5, 0680-0691, 2002.

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

683 Du, J., Liu, K., Huang, J., Han, T., Zhang, L., Anthonio, C. K., Shah, A., Khan, M. N.,
684 Qaswar, M., and Abbas, M.: Organic carbon distribution and soil aggregate
685 stability in response to long-term phosphorus addition in different land-use
686 types, Soil and Tillage Research, 215, 105195, 2022.
687 Du, X., Ge, Y., Zhang, Y., Hu, H., Zhang, Y., Yang, Z., Ren, X., Hu, S., Feng, H.,
688 and Song, Y.: Responses of soil carbon cycling microbial functional genes to
689 nitrogen and phosphorus addition in saline-sodic soils, Plant and Soil, 490,
690 261-277, 10.1007/s11104-023-06070-y, 2023.
691 Fang, X.-M., Zhang, X.-L., Chen, F.-S., Zong, Y.-Y., Bu, W.-S., Wan, S.-Z., Luo, Y.,
692 and Wang, H.: Phosphorus addition alters the response of soil organic carbon
693 decomposition to nitrogen deposition in a subtropical forest, Soil Biology and
694 Biochemistry, 133, 119-128, 2019.
695 Fanin, N., Hättenschwiler, S., Schimann, H., and Fromin, N.: Interactive effects of C,
696 N and P fertilization on soil microbial community structure and function in an
697 A mazonian rain forest, Functional Ecology, 29, 140-150, 2015.
698 Fisk, M., Santangelo, S., and Minick, K.: Carbon mineralization is promoted by
699 phosphorus and reduced by nitrogen addition in the organic horizon of
700 northern hardwood forests, Soil Biology and Biochemistry, 81, 212-218, 2015.
701 Griffiths, B. S., Spilles, A., and Bonkowski, M.: C: N: P stoichiometry and nutrient
702 limitation of the soil microbial biomass in a grazed grassland site under
703 experimental P limitation or excess, Ecological Processes, 1, 1-11, 2012.
704 Guppy, C. N., Menzies, N. W., Moody, P. W., and Blamey, F. P. C.: Competitive
705 sorption reactions between phosphorus and organic matter in soil: a review,
706 Soil Research, 43, 189-202, 2005.
707 Harris, N. L., Gibbs, D. A., Baccini, A., Birdsey, R. A., De Bruin, S., Farina, M.,
708 Fatoyinbo, L., Hansen, M. C., Herold, M., and Houghton, R. A.: Global maps
709 of twenty-first century forest carbon fluxes, Nature Climate Change, 11, 234-
710 240, 2021.
711 He, X., Augusto, L., Goll, D. S., Ringeval, B., Wang, Y., Helfenstein, J., Huang, Y.,
712 Yu, K., Wang, Z., and Yang, Y.: Global patterns and drivers of soil total
713 phosphorus concentration, Earth System Science Data Discussions, 2021, 1-21,
714 2021.

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

715 Heuck, C., Weig, A., and Spohn, M.: Soil microbial biomass C: N: P stoichiometry
716 and microbial use of organic phosphorus, Soil Biology and Biochemistry, 85,
717 119-129, 2015.
718 Hinsinger, P.: Bioavailability of soil inorganic P in the rhizosphere as affected by
719 root-induced chemical changes: a review, Plant and soil, 237, 173-195, 2001.
720 Hou, E., Chen, C., Wen, D., and Liu, X.: Relationships of phosphorus fractions to
721 organic carbon content in surface soils in mature subtropical forests,
722 Dinghushan, China, Soil Research, 52, 55-63, 2014.
723 Hou, E., Tang, S., Chen, C., Kuang, Y., Lu, X., Heenan, M., and Wen, D.: Solubility
724 of phosphorus in subtropical forest soils as influenced by low-molecular
725 organic acids and key soil properties, Geoderma, 313, 172-180, 2018.
726 Hou, E., Wen, D., Jiang, L., Luo, X., Kuang, Y., Lu, X., Chen, C., Allen, K. T., He,
727 X., and Huang, X.: Latitudinal patterns of terrestrial phosphorus limitation
728 over the globe, Ecology Letters, 24, 1420-1431, 2021.
729 Ibrahim, M. M., Guo, L., Wu, F., Liu, D., Zhang, H., Zou, S., Xing, S., and Mao, Y.:
730 Field-applied biochar-based MgO and sepiolite composites possess CO2
731 capture potential and alter organic C mineralization and C-cycling bacterial
732 structure in fertilized soils, Science of the Total Environment, 813, 152495,
733 2022.
734 Li, D., Liu, C. M., Luo, R., Sadakane, K., and Lam, T. W.: MEGAHIT: an ultra-fast
735 single-node solution for large and complex metagenomics assembly via
736 succinct de Bruijn graph, Bioinformatics (Oxford, England), 31, 1674-1676,
737 10.1093/bioinformatics/btv033, 2015.
738 Liu, F., Wang, D., Zhang, B., and Huang, J.: Concentration and biodegradability of
739 dissolved organic carbon derived from soils: A global perspective, Science of
740 the Total Environment, 754, 142378, 2021.
741 Liu, L., Gundersen, P., Zhang, T., and Mo, J.: Effects of phosphorus addition on soil
742 microbial biomass and community composition in three forest types in tropical
743 China, Soil Biology and Biochemistry, 44, 31-38, 2012.
744 Liu, L., Gundersen, P., Zhang, W., Zhang, T., Chen, H., and Mo, J.: Effects of
745 nitrogen and phosphorus additions on soil microbial biomass and community
746 structure in two reforested tropical forests, Scientific Reports, 5, 14378,
747 10.1038/srep14378, 2015.

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

748 Munevar, F. and Wollum, A. G.: Effects of the addition of phosphorus and inorganic
749 nitrogen on carbon and nitrogen mineralization in Andepts from Colombia,
750 Soil Science Society of America Journal, 41, 540-545, 1977.
751 Natali, S. M., Schuur, E. A. G., Mauritz, M., Schade, J. D., Celis, G., Crummer, K. G.,
752 Johnston, C., Krapek, J., Pegoraro, E., and Salmon, V. G.: Permafrost thaw
753 and soil moisture driving CO2 and CH4 release from upland tundra, Journal of
754 Geophysical Research: Biogeosciences, 120, 525-537, 2015.
755 Neff, J. C., Hobbie, S. E., and Vitousek, P. M.: Nutrient and mineralogical control on
756 dissolved organic C, N and P fluxes and stoichiometry in Hawaiian soils,
757 Biogeochemistry, 51, 283-302, 2000.
758 Ohno, T. and Zibilske, L. M.: Determination of Low Concentrations of Phosphorus in
759 Soil Extracts Using Malachite Green, Soil Science Society of America Journal,
760 55, 892-895, 1991.
761 Ruttenberg, K. C. and Sulak, D. J.: Sorption and desorption of dissolved organic
762 phosphorus onto iron (oxyhydr) oxides in seawater, Geochimica et
763 Cosmochimica Acta, 75, 4095-4112, 2011.
764 Schneider, M. P. W., Scheel, T., Mikutta, R., Van Hees, P., Kaiser, K., and Kalbitz,
765 K.: Sorptive stabilization of organic matter by amorphous Al hydroxide,
766 Geochimica et Cosmochimica Acta, 74, 1606-1619, 2010.
767 Schulze, E. D., Högberg, P., Van Oene, H., Persson, T., Harrison, A. F., Read, D.,
768 Kjøller, A., and Matteucci, G.: Interactions between the carbon and nitrogen
769 cycles and the role of biodiversity: a synopsis of a study along a north-south
770 transect through Europe, in: Carbon and nitrogen cycling in European forest
771 ecosystems, Springer, 468-491, 2000.
772 Slessarev, E. W., Mayer, A., Kelly, C., Georgiou, K., Pett-Ridge, J., and Nuccio, E. E.:
773 Initial soil organic carbon stocks govern changes in soil carbon: Reality or
774 artifact?, Global Change Biology, 29, 1239-1247, 2023.
775 Spohn, M. and Schleuss, P.-M.: Addition of inorganic phosphorus to soil leads to
776 desorption of organic compounds and thus to increased soil respiration, Soil
777 Biology and Biochemistry, 130, 220-226, 2019.
778 Spohn, M., Diáková, K., Aburto, F., Doetterl, S., and Borovec, J.: Sorption and
779 desorption of organic matter in soils as affected by phosphate, Geoderma, 405,
780 115377, 2022.

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

781 Tiwari, T., Sponseller, R. A., and Laudon, H.: The emerging role of drought as a
782 regulator of dissolved organic carbon in boreal landscapes, Nature
783 Communications, 13, 5125, 2022.
784 Trumbore, S.: Carbon respired by terrestrial ecosystems–recent progress and
785 challenges, Global Change Biology, 12, 141-153, 2006.
786 Turner, B. L. and Joseph Wright, S.: The response of microbial biomass and
787 hydrolytic enzymes to a decade of nitrogen, phosphorus, and potassium
788 addition in a lowland tropical rain forest, Biogeochemistry, 117, 115-130,
789 2014.
790 Vance, E. D., Brookes, P. C., and Jenkinson, D. S.: An extraction method for
791 measuring soil microbial biomass C, Soil biology and Biochemistry, 19, 703-
792 707, 1987.
793 Wordofa, D. N., Adhikari, D., Dunham-Cheatham, S. M., Zhao, Q., Poulson, S. R.,
794 Tang, Y., and Yang, Y.: Biogeochemical fate of ferrihydrite-model organic
795 compound complexes during anaerobic microbial reduction, Science of the
796 total environment, 668, 216-223, 2019.
797 WRI: World Resources Institute: Global Forest Review, forest-extent-indicators 2023.
798 Xia, Y., Peñuelas, J., Sardans, J., Zhong, X., Xu, L., Yang, Z., Yang, Y., Yang, L.,
799 Yue, K., and Fan, Y.: Phosphorus addition accelerates soil organic carbon
800 mineralization by desorbing organic carbon and increasing microbial activity
801 in subtropical forest soils, Applied Soil Ecology, 193, 105166, 2024.
802 Ye, L. F., Liu, H. Y., Dan Deng, H., Zheng, Y. P., Han, Y. W., Gao, X. T., Abbott, L.
803 K., Zhao, C. M., and Li, J. H.: Effects of decadal nitrogen and phosphorus
804 fertilization on microbial taxonomic and functional attributes associated with
805 soil organic carbon decomposition and concentration in an alpine meadow,
806 Ecological Indicators, 146, 109790, 2023.
807 Zhang, J., Zhou, J., Lambers, H., Li, Y., Li, Y., Qin, G., Wang, M., Wang, J., Li, Z.,
808 and Wang, F.: Nitrogen and phosphorus addition exerted different influences
809 on litter and soil carbon release in a tropical forest, Science of the Total
810 Environment, 832, 155049, 2022.
811 Zhao, Q., Poulson, S. R., Obrist, D., Sumaila, S., Dynes, J. J., McBeth, J. M., and
812 Yang, Y.: Iron-bound organic carbon in forest soils: quantification and
813 characterization, Biogeosciences, 13, 4777-4788, 2016.

https://doi.org/10.5194/egusphere-2025-310
Preprint. Discussion started: 4 February 2025

814 Zheng, M., Huang, J., Chen, H., Wang, H., and Mo, J.: Responses of soil acid
815 phosphatase and beta-glucosidase to nitrogen and phosphorus addition in two
816 subtropical forests in southern China, European Journal of Soil Biology, 68,
817 77-84, 2015.
818 Zheng, M., Zhang, T., Luo, Y., Liu, J., Lu, X., Ye, Q., Wang, S., Huang, J., Mao, Q.,
819 and Mo, J.: Temporal patterns of soil carbon emission in tropical forests under
820 long-term nitrogen deposition, Nature Geoscience, 15, 1002-1010, 2022.
821
822