Long-term fertilization modifies the mineralization of soil organic matter in response to added substrate

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Abstract

The turnover of SOC in soils is strongly influenced by the availability of substrate and nutrients, especially nitrogen (N) and phosphorus (P). According to the theory of stoichiometry, the balance between C and nutrient inputs is likely to play a key role in SOM mineralization, but there are marked differences in SOM turnover in response to nutrients additions among different experimental studies. Here, we assessed how long-term fertilization modified SOM mineralization in response to added substrate in a tropical forest. We carried out a 90-day incubation study in which we added two structurally similar compounds which differed in microbial metabolic availability: corn cellulose or corn starch to soils collected from a long-term (11 years) factorial N and P fertilization experiment site in a tropical forest in south China. We measured total soil mineralization rate (CO_2 efflux) to characterize SOM mineralization and using ^{13}C isotope signatures to determine the source of the CO_2 (original soil C or added substrate) and assessed changes in extracellular enzyme activities: acid phosphomonoesterase (AP), β-1,4-glucosidase (BG), β-1,4- N-acetaminophen glucosidase (NAG), phenol oxidase (PHO) and peroxidase (PER), and microbial biomarkers to determine whether nutrient stoichiometry and decomposer communities explain differences in SOM mineralization rates. Total C mineralization increased substantially with substrate addition, particularly cellulose (5.38, 7.13, 5.58 and 5.37 times for N, P, NP fertilization and CK, respectively) compared to no substrate addition, and original soil C mineralization was further enhanced in long-term N (3.40% and 5.18% for cellulose and starch addition, respectively) or NP (35.11% for cellulose addition) fertilized soils compared to control treatment. Enzyme activities were stimulated by the addition of both substrates but suppressed by P-fertilization. Addition of both substrates increased microbial investment in P-acquisition, but only starch addition promoted C investment in N-acquisition. Finally, fungal abundance increased with substrate addition to a greater extent than bacterial abundance, particularly in cellulose-amended soils, and the effect was amplified by long-term fertilization. Our findings indicate that SOM mineralization might be enhanced in N and P enrichment ecosystems, since the litter input can liberate microbes from C limitation and stimulate SOM mineralization if N and P are sufficient. Our study further demonstrates that structurally similar substrates can have distinct effects on SOM mineralization and the extent of SOM mineralization is strongly dependent on elemental stoichiometry, as well as the resource requirements of microbial decomposers.

Keywords: SOM mineralization, Tropical forest, Substrate addition, Long-term fertilization, Nitrogen, Phosphorus

Abbreviations

SOM: Soil Organic Matter, SOC: Soil Organic C, SWC: Soil Water Content

1. Introduction

Around 2,344 Gt of organic carbon (C) is contained in soils, making soils the largest organic C pool in terrestrial ecosystems (Guo and Gifford, 2010). Tropical forest soils play a particularly important role in global C cycling, because they account for one third of global soil organic C (SOC) storage (Phillips et al., 1998; Grace et al., 2001; Guo and Gifford, 2010) and the turnover of organic C is rapid under warm and humid tropical conditions (Ramankutty et al., 2002). Microbial decomposition of organic material is not only a vital ecosystem process underpinning the storage of C in soils (Hobbie and Vitousek, 2000; Chapin et al., 2002), but also almost half of CO_2 emissions are produced by microbial respiration during the decomposition of organic C (Bond-Lamberty et al., 2004). Hence, even a seemingly insignificant change in soil C stocks and dynamics in tropical forests could influence the global C cycle (Townsend et al., 1992). Numerous chemical and physical factors especially nitrogen (N), phosphorus (P) and C availability affect the microbe-mediated decomposition processes and understanding how they influence the turnover and storage of SOC is of crucial importance to predict changes in global C dynamics (Cleveland and Townsend, 2006).

The turnover and storage of SOC involve numerous interactions between extant soil organic matter (SOM) and fresh plant C inputs, which are being affected by global changes (Crow et al., 2010). Elevated CO_2, warming, drought and changes in plant nutrient status can enhance inputs of plant litter or root exudates to the soil (Sayer et al., 2019). However, there is mounting evidence that increased plant inputs do not necessarily entail greater SOC storage, because additional inputs of plant-derived C can stimulate the mineralization of SOM and the release of SOC as CO_2 (Lajtha et al., 2014; Pisani et al., 2016; Lajtha et al., 2018; Sayer et al., 2019), a phenomenon referred to as the 'priming effect' (Kuzyakov et al., 2000). Nutrient availability, especially nitrogen (N) and phosphorus (P), plays a key role in the mineralization of soil organic matter (SOM) because they regulate microbial activity (Mackenzie et al., 2002; Jing et al., 2017). As SOM represents an important source of C, N and P, two complementary theories illustrate the importance of nutrient availability in regulating SOM mineralization: The theory of microbial N-mining (Hessen et al. 2004) proposes that when N demand is high, some microbes use labile C to decompose recalcitrant SOM in order to acquire additional N (Berg and McClaugherty, 2003; Hessen et al., 2004; Fontaine and Barot, 2005). Hence, greater N availability is likely to lead to a net decrease in decomposition rates by balancing the supply and demand for both C and N (Moorhead and Sinsabaugh, 2006). The theory of stoichiometry (Moorhead and Sinsabaugh, 2006) extends this theory by proposing that decomposition is determined by the stoichiometric balance of C:N:P in substrates and the microbial demand for resources (Melillo et al., 1982; Hessen et al., 2004), whereby decomposition rates peak when supplies of C, N and P simultaneously meet microbial demands (Melillo et al. 1982, Sterner and Elser 2002). Hence, the balance between C and nutrient

inputs is likely to play a key role in SOM mineralization.

Research into the role of N deposition in decomposition processes demonstrates that conflicting results for N-mining of SOM may be explained by the degree of N limitation in an ecosystem (Bradford et al., 2008; Janssens et al., 2010). Numerous studies have demonstrated that the mineralization of SOM declined with N addition (Berg and Matzner, 1997; Magill and Aber, 1998; Neff et al., 2002; Hagedorn et al., 2003). However, in strongly N-limited ecosystems, greater N availability can stimulate SOM mineralization by increasing microbial biomass and activity, which also promote the production of SOM-decomposing enzymes (Manning et al., 2008). In lowland tropical forests where N is not limiting, increased N supply can create a greater demand for other nutrients (Hessen et al., 2004; Mo et al., 2015). Consequently, N deposition can retard SOM decomposition (Zhang et al., 2020) due to decreased microbial biomass (Chen et al., 2013; Li et al., 2014), reduced extracellular enzyme activities (Carreiro et al., 2000) and suppressed N-mining (Berg and Matzner, 1997; Magill and Aber, 1998; Neff et al., 2002; Hagedorn et al., 2003).

In tropical forests, the role of P availability in SOM decomposition is of considerable interest because lowland tropical forests are regarded as P-limited (Vitousek et al., 2010; Hou et al., 2020) and P addition thus has a greater effect on decomposition processes than N addition (Cleveland and Townsend, 2006; Poeplau et al., 2016; Hui et al., 2020). Enhanced SOM decomposition with P addition can occur via three related mechanisms: i) P addition can alter soil microbial community composition (Liu et al., 2012), which can favor the growth and activity of SOM-degrading microbes (Mori et al., 2018); ii) P addition can alleviate P deficiencies, which limit soil microbial growth and activity (Rousk and Baath, 2007; Cruz et al., 2009) and thus boost the decomposition of SOM (Hobbie and Vitousek, 2000; Li et al., 2014; Mori et al., 2018); iii) increased availability of both P and C may induce N-limitation of microbial growth, and thus stimulate microbes to decompose SOM for N acquisition (Mehnaz et al., 2019). Thus, C:N:P stoichiometry and N-mining can explain SOM decomposition in different ecosystems (Chen et al. 2013), but it is uncertain whether there exists an equivalent microbial “P-mining” mechanism, in which labile C substrates are used to mine SOM for P in soils with low availability of inorganic P (Craine et al., 2007; Mori et al., 2018).

Although many experiments have been carried out to investigate the relationships between nutrient availabilities and SOM mineralization, they have generally involved incubations in which fertilizers were added to the soil at the start of the experiment (Dimassia et al., 2014; Qiao et al., 2016; Fan et al., 2019b; Mehnaz et al., 2019; Tian et al., 2019). By contrast, studies using soil from long-term fertilization experiments to investigate the fertilization effect on SOM mineralization are scarce (Fan et al., 2019a; Wu et al., 2019). Long-term fertilization also changes soil properties such as pH, organic C content, microbial communities and enzyme activities (Mo et al., 2008; Li et al., 2014; Poeplau et al., 2016; Wang et al., 2020b). Therefore, changes in decomposition processes in

response to short-term or one-off nutrient inputs are unlikely to reflect the effects of long-term fertilization or nutrient deposition. Here, we took advantage of a tropical coastal site with 11 years of N and P fertilization history to investigate how long-term N and P addition influence the decomposition of SOM in response to added C substrates. We used cellulose and starch as substrates, as both are C-rich polysaccharides with the same molecular weight, but the simpler molecular structure of starch makes it a more readily available source of C to soil microbes than cellulose (Mizuta et al., 2015). We used natural abundance isotope techniques to differentiate substrate-derived CO_2 released during decomposition from original soil C-derived CO_2 released during the mineralization of SOM. Given that the tropical soils at our study site have low P concentrations (Wang et al., 2014), we expected that fertilization with N would exacerbate P-limitation and thus reduce C turnover, whereas fertilization with P would accelerate C turnover. Specifically, we aimed to test the following hypotheses:

(H1) Starch will decompose more rapidly and stimulate original soil C mineralization to a greater extent than cellulose;

(H2) N-fertilization will reduce C turnover by slowing the decomposition of added substrates and reducing their stimulatory effect on original soil C mineralization;

(H3) By contrast, P-fertilization will increase C turnover by enhancing the decomposition of added substrates and their stimulatory effect on original soil C mineralization.

To test our hypotheses, we measured and partitioned soil respiration (CO_2 efflux) during an incubation experiment with substrate additions to fertilized soils, and we assessed changes in extracellular enzyme activities and in microbial community composition to interpret our findings.

2. Materials and Methods

2.1 Soil collection

The soils were collected from a long-term fertilization experiment at the Xiaoliang Research Station of Tropical Coastal Ecosystems, the Chinese Academy of Sciences (21°270'N, 110°540'E), Southwestern Guangdong Province, China. Detailed information on the site and experimental design is given in Wang et al. (2014). Briefly, the soil is a ferralsol (according to the FAO soil classification system) with a pH of *c*. 4 and low availability of P. In August 2009, a factorial fertilization experiment for N and P was established in a completely randomized block design. Within five field replicate blocks, N fertilization (+N), P fertilization (+P), N and P fertilization (+NP), and control treatments (CK) were randomly assigned to four 10 m × 10 m plots (Zhao et al., 2014). From September 2009, the plots were fertilized to give a total application of 100 kg N or P ha^{-1} $year^{-1}$. Specifically, every two months 476.6g NH_4NO_3 (equal to 166.6 g N) and/or 808 g NaH_2PO_4 (equal to 166.6 g P) was dissolved in 30 L groundwater and applied to

the corresponding blocks using a backpack sprayer, applying the solution as close as possible to the soil surface. In the control plots, 30 L groundwater was applied. The amounts of N and P added are comparable to other N-fertilization (Lu et al., 2010) and P-fertilization (Liu et al., 2012) experiments in this region. In March 2020, we took five soil cores to 10 cm depth at random in each plot and homogenized soils from each treatment into one soil sample. The soils were sieved through 2-mm mesh to remove stones and roots and stored at -20℃ for a month before use. The physiochemical properties of the soils are listed in Table 1.

2.2 Incubation experiment

To assess how long-term fertilization influences soil C dynamics and SOM mineralization, we conducted an incubation experiment in which substrate addition treatments were nested within long-term fertilization treatments (+N, +P, +NP, and unfertilized controls). We choose two structurally similar compounds which differed in microbial metabolic availabilities: corn starch and corn cellulose. The substrate addition treatments were comprised of corn cellulose (henceforth 'cellulose'; Shaanxi Hengling Natural Biological Products Co. LTD), corn starch (henceforth 'starch'; TOMIZ Co. LTD), and controls with no substrate addition. The ^{13}C isotopic abundance of the corn cellulose and corn starch was -14.462‰ and -12.759‰, respectively, whereas the ^{13}C isotopic abundance of soils from our site was *c.* -28‰ (Table S2). Each treatment in the incubation was replicated six times, giving a total of 72 incubations (four fertilization treatments × three substrate-addition treatments × six replicates). The soil was pre-incubated for 14 days in the dark at 25 ℃. For each incubation, 80 g dry-weight equivalent of fresh soil was placed in a 250 ml glass flask. Corn cellulose or corn starch were mixed into the soil at a rate of 4.4 mg C g^{-1} dry soil; soils without C addition were also mixed to ensure that all treatments received the same amount of disturbance. The flasks were sealed with Parafilm and incubated at 25℃ in the dark for 90 days. Soil water content (SWC) was maintained at 20% (average SWC at our field site) by regularly weighing the flasks and adding water as necessary.

2.3 Gas analyses and soil sampling

To determine microbial mineralization of substrates and SOM, gas samples (300ml) were taken from each flask on day 1, 3, 5, 8, 11, 16, 23, 30, 45, 60, 90 after substrate addition. At each time point, the gas in the flasks was evacuated with CO_2-free air and resealed for half an hour before sampling. After 30 mins, 130 ml gas in the flask was collected and diluted with 170 ml CO_2-free air, resulting in 300 ml gas samples for analysis of CO_2 concentrations using a Cavity Ring-down Spectrometer (G2201-i Isotopic CO_2/CH_4, Picarro, Santa Clara, CA, USA). We destructively sampled soils from three replicate flasks on day 45 and at the end of the incubation on day 90 to analyze extracellular enzyme activities and microbial community composition.

2.4 Enzyme analysis

We quantified the activities of enzymes involved in the decomposition of cellulose (β-1,4-glucosidase; BG), the acquisition of N (β-1,4- N-acetaminophen glucosidase; NAG) and P (acid phosphomonoesterase; AP), and the degradation of lignin (phenol oxidase; PHO; and peroxidase; PER (Freeman et al., 2004; Ghafoor et al., 2017; Nannipieri et al., 2018). Assays for BG, NAG and AP were measured by adding the substrates 4-nitrophenyl-β-D-glucopyranoside, p-nitrophenyl-N-acetyl-β-D-glucosaminidine and *p*-nitrophenyl-phosphate tetrahydrate, respectively, bound to the chromogen *p*-nitrophenol (*p*NP; Tabatabai and Bremner, 1969), and incubating the samples at 37℃ for 1 h for BG and NAG, and 0.5 h for AP. After incubation, the optical density of *p*NP released at 405 nm was measured on a spectrophotometer (Multiskan^{TM} FC, ThermoFisher, Massachusetts, USA). Oxidative assays of PHO and PER activities were measured spectrophotometrically using L-3,4-dihydroxyphenylala- nine (DOPA) as the substrate, according to Saiya-Cork et al. (2002). To assess microbial investment in the acquisition of nutrients, we calculated the ratios of C, N and P acquiring enzyme activities based on Sinsabaugh et al. (2008). Thus, we used C_{enzyme}/NAG and C_{enzyme}/AP to represent C investment in N or P acquisition, respectively, and NAG/AP to represent N investment in P acquisition, where C_{enzyme} is the sum of BG, PHO and PER (Sinsabaugh et al., 2009).

2.5 Phospholipid fatty acids (PLFAs)

To determine soil microbial community structure and microbial biomass, we performed phospholipid fatty acid (PLFA) analysis according to Bossio and Scow (1998). Peaks were determined by comparison to a 19:0 internal standard using gas chromatography (GC7890, Agilent, USA) and assigned following standard nomenclature (Tunlid et al., 1989). The biomass of single fatty acid was expressed as nM g^{-1} soil dry weight and calculated based on the concentration of the 19:0 internal standard. Fungi was represented by the PLFAs 18:2ω6c and 18:3ω3c (Frostegård and Bååth, 1996). Gram-positive (G+) bacteria were represented by PLFAs i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0 and Gram-negative (G-) bacteria was represented by the PLFAs 16:1ω7c, 16:1ω5c, 18:1ω9c, 18:1ω7c (Bossio et al., 2006). Bacterial biomass calculated from the total biomass of G+ and G- bacteria, and the PLFAs 15:0 and 17:0. The total PLFA biomass of the soil microbial community was calculated as the sum of fungi, bacteria and PLFAs 14:0, 16:0, 16:1ω5c, 17:1ω8c. The ratio of fungi to bacteria (F/B) was calculated to represent changes in community structure (Bardgett et al., 1996; Frostegård and Bååth, 1996).

2.6 Data analysis

The respired C derived from original soil C or added substrate was calculated from the differences in ^{13}C of the soil and substrates using a two-pool mixing model (Zhu et al., 2014):

$$C_{original} = C_{total} \times \frac{atom\%^{13}C_{substrate} - atom\%^{13}C_{total}}{atom\%^{13}C_{substrate} - atom\%^{13}C_{original}}$$

$$C_{substrate} = C_{total} - C_{original}$$

Where C_{total} is total respired C, $C_{original}$ and $C_{substrate}$ are respired C derived from original soil C or added substrate, respectively, $atom\%^{13}C_{total}$, $atom\%^{13}C_{original}$ and $atom\%^{13}C_{substrate}$ are atom%^{13}C values of C_{total}, $C_{original}$ and $C_{substrate}$, respectively.

Cumulative total soil mineralization, substrate-derived or original soil C -derived mineralization were calculated by piecewise linear interpolation using MATLAB (R2018a, MathWorks, USA). We used cumulative substrate-derived CO_2-C to represent decomposition of cellulose or starch, and cumulative original soil C derived CO_2-C to represent original soil C mineralization. All subsequent statistical analyses were carried out in R.4.0.5 for Mac (R Core Team, 2021).

We used one-way ANOVAs to evaluate the effect of long-term fertilization on soil physicochemical properties, microbial communities and enzyme activities before the incubation (n = 5 field plots per treatment). Where the effects were significant, post hoc tests (Tukey's HSD) were conducted to compare individual treatments.

The effects of long-term fertilization treatments and substrate types on the decomposition of original soil C-derived and substrate-derived C were investigated using linear mixed-effects models (*lmer* function in the lme4 package (Bates et al., 2015), with substrate additions nested within fertilization treatments to account for the study design. In the models for cumulative soil C and substrate-derived respiration, substrate type was nested within the long-term fertilization treatments as a fixed effect, and replicate incubation blocks and long-term fertilization were included as random effects. To test the influence of fertilization and substrate type on total C, original soil C and substrate C mineralization rates during the incubation, gas sampling time was included as an additional random effect. Full models were compared to null models to determine the model fit and the Chi-square values and p-values of the model fit are given (Table. S2). The significance of each term was determined using likelihood ratio tests and Satterthwaites approximation in the lmerTest package (Alexandra et al., 2017). Where the interactive effect of fertilization and substrate addition was significant, we used the individual model to analyze the differences among substrate addition treatments within each long-term fertilization treatment.

The effects of long-term fertilization, substrate addition and their interactions on soil enzyme activities and microbial functional groups were also tested using linear mixed-effects models with substrate addition nested within fertilization treatments as a fixed effect, and replicate incubation blocks and fertilization as random effects. Full models were compared to null models to determine the model fit and the Chi-square values and p-values of the model fit were given (Table. S3 and S4). Where overall models were significant, we assessed treatment

contrasts using the *pairs* function in the emmeans package (Lenth, 2021). We present significant results at $P < 0.05$ and marginally significant trends at $P < 0.1$.

3. Results

3.1 Total C, substrate- C and Original soil C mineralization

Total C mineralization rates were significantly influenced by substrate addition and the magnitude of effects differed among long-term fertilization treatments ($P<0.05$, Table S2, Fig.1a and Fig. 2a). Total C mineralization rates increased rapidly during the first five days after substrate addition, and then declined gradually between five and 30 days, whereas the soils without substrate input showed a gradual decline in total C mineralization rates throughout the incubation period. Most soil C mineralization occurred during the first 30 days (Fig. S1a). Both N and P fertilization increased the total C mineralization in soils added with substrates ($P<0.05$, Table S2, Fig. 1). Soils amended with cellulose had the highest respiration rates regardless of fertilization treatment, while soils without substrate addition had the lowest respiration rates ($P<0.01$; Fig. 1). Total C mineralization rate was initially enhanced to a greater extent by the addition of cellulose than starch, and the total amount of mineralized C (cumulative total C mineralization) over the 90 days of incubation was also greater with cellulose addition than starch addition ($P<0.05$; Fig. 1a and Fig. 2a). In soils amended with cellulose, total C mineralization rate was significantly higher with P fertilization compared to the unfertilized controls ($P<0.01$; Fig. 1a), but in soils amended with starch, total C mineralization rate was higher with N fertilization compared to unfertilized controls ($P<0.01$; Fig. 1a). At the end of the incubation, the total amount of mineralized C was generally higher in P-fertilized soils than in N or NP-fertilized soils, except for soils amended with starch, where total C mineralization was lower with P fertilization ($P<0.05$; Fig. 2a). In soils without substrate inputs, the total amount of mineralized C was highest in the unfertilized controls, suggesting that long-term fertilization with N, P and NP significantly reduced C turnover ($P<0.01$; Fig. 1a, Table S2). Hence, total C mineralization and cumulative total C mineralization increased with substrate addition, but long-term fertilization influenced the magnitude of the response to different substrates.

Long-term P fertilization significantly accelerated the decomposition of cellulose, whereas starch decomposition was slower in P-fertilized soils compared to unfertilized controls ($P<0.01$, Fig. 1c, Table S2). Accordingly, the total amount of cellulose decomposed during the incubation was greater than the total amount of starch in P-fertilized and unfertilized soils ($P<0.01$, Fig. 2c). Overall, the total amount of cellulose and starch decomposed during the incubation did not differ among long-term fertilization treatments ($P>0.05$, Fig 2c, Table S2). Hence, long-term fertilization had a greater influence on the decomposition rate of cellulose than starch while had no significant

influence on the total amount of substrate decomposition.

The mineralization of original soil C showed a different pattern to the decomposition of substrate-C. The addition of both substrates enhanced the mineralization of original soil C (P<0,01, Table S2), but the effects of starch and cellulose differed between N and P fertilized soils. In soils amended with cellulose, total original soil C mineralization was enhanced by NP fertilization compared to unfertilized controls (*P*<0.01, Fig. 2b), whereas in soils amended with starch, total original soil C mineralization increased with N fertilization. Hence, starch stimulated original soil C mineralization to a greater extent than cellulose in N-fertilized soils, whereas cellulose stimulated original soil C mineralization to a greater extent in NP-fertilized soils. Surprisingly, P fertilization had no influence on original soil C mineralization in soils with added substrates. However, in soils without substrate addition, all long-term fertilization treatments suppressed total original soil C mineralization compared to unfertilized controls (*P*<0.01; Fig. 2b). Hence, substrate addition stimulated original soil C mineralization and the effect was enhanced by long-term N or NP fertilization.

3.2 Soil Enzyme Activities

Before the incubation started, there were no significant differences in the activities of BG, NAG, PHO and PER among fertilization treatments, but AP activity was lower in P and NP-fertilized soils compared to N-fertilized and unfertilized soils (*P*<0.01, Fig. S2). Microbial C investment in P acquisition (C_{enzyme}/AP) was significantly greater in the NP-fertilized soils compared to unfertilized soils (*P*<0.01, Fig. S3a), but there were no differences among treatments in NAG/AP or C_{enzyme}/NAG ratios.

After 45 days of incubation, the activities of AP, BG and NAG were higher and PHO activity was lower in cellulose-amended soils compared to soils amended with starch or without substrate addition (*P*<0.01, Fig. 3, Table S3). However, regardless of substrate addition, the activities of AP, BG and NAG and PER were higher in N-fertilized and unfertilized soils compared to P and NP fertilized soils (*P*<0.05, Fig. 3) but PHO activity was marginally higher in P-fertilized soils compared to N and NP fertilized soils (*P*=0.06, Fig. 3d). Hence, enzyme was generally stimulated by cellulose addition but suppressed by P-fertilization. Microbial investment of C in P-acquisition (C_{enzyme}/AP) was generally higher in soils amended with cellulose compared to soils without substrate addition, but the increase was only significant in P-fertilized and NP-fertilized soils (Fig.S4a). However, microbial investment of N in P acquisition (NAG/AP) was much higher in cellulose-amended soils than either starch-amended soils or soils without substrate addition, regardless of fertilization history (Fig.S4c). By contrast, microbial C investment in N acquisition (C_{enzyme}/NAG) was substantially higher in starch-amended soils than soils amended with cellulose or without substrate addition, but the increase was smaller in P-fertilized soils (*P*<0.05, Fig. S4b). Surprisingly, overall microbial C investment in P acquisition was significantly greater in all fertilized treatments compared to unfertilized

soil (P<0.05, Fig. S4a). Thus, cellulose promoted greater investment of resources in P-acquisition, but starch promoted microbial investment of C in N-acquisition.

After 90 days of incubation, the differences in enzyme activities among substrate addition treatments were more prominent (P<0.01, Fig. 4, Table S3). Both cellulose and starch addition significantly increased the activities of AP and BG compared to soils without substrate additions, regardless of fertilization history (P<0.05, Fig. 4a, b). The activity of NAG was significantly higher in soils amended with cellulose compared with soils amended with starch and soils without substrate addition (P<0.05, Fig. 4c), whereas PHO activity was highest in starch-amended soils (P<0.05, Fig. 4d). In contrast to the patterns of enzyme activities after 45 days, the higher activity of PER with N fertilization was greatest in unfertilized soils (P<0.05, Fig. 4e), and PHO activity was significantly higher in unfertilized compared to fertilized soils, regardless of substrate addition (P<0.05, Fig. 4d). Hence, substrate addition generally stimulated enzyme activity throughout the incubation, but the effects of fertilization declined over time. After 90 days, microbial investment of C in N-acquisition was still generally higher in soils amended with starch (P<0.05, Fig. S5b) and microbial investment of N in P acquisition was still greater in cellulose-amended soils (P<0.05, Fig. S5c). However, microbial C investment in P-acquisition was greater in P-fertilized and unfertilized soils amended with starch, compared to cellulose-amended soils and soils without substrate addition. Across all substrate treatments, C_{enzyme}/AP was highest in NP-fertilized soils. (P<0.05, Fig. S5a). Thus, substrate addition and fertilization generally increased microbial investment of resources in P-acquisition, even in P-fertilized soils, but only starch addition promoted C investment in N acquisition.

3.3 Soil Microbial Community Composition.

Before the incubation started, there were no significant differences in total PLFA biomass, the relative abundance of fungal PLFAs (henceforth: fungal abundance), Gram-positive bacterial PLFAs (henceforth: G+ bacterial abundance), Gram-negative bacterial PLFAs (henceforth: G- bacterial abundance), or the F/B ratio among soils from different fertilization treatments (Fig. S6a, c, d, f and e). However, the relative abundance of bacterial PLFAs (henceforth: bacterial abundance) was significantly lower in N-fertilized soils compared with soils from the P fertilization and unfertilized treatments (P<0.01, Fig. S6b).

After 45 days of incubation, substrate addition generally enhanced both fungal and bacterial (mainly G-) abundances, total PLFA biomass and the F/B ratio compared to soils without substrate addition, but the magnitude of the effects differed among fertilization treatments and substrates additions (P<0.01, Fig 5, Table S4). With cellulose addition, soils fertilized with N, P or NP had greater fungal abundance but lower bacterial abundance and thus a higher F/B ratio than unfertilized soils (P<0.05, Fig. 5). By contrast, with starch addition, only soils fertilized with N had higher fungal abundance, lower bacterial abundance, and thus a higher F/B ratio compared to soils

from the P and NP fertilization treatments or unfertilized controls (P<0.05, Fig. 5a, b, f). In soils without substrate addition, total PLFA biomass was lower in P-fertilized soil compared to unfertilized controls (P<0.05, Fig. 5e), and soils fertilized with NP had higher fungal abundance, lower bacterial abundance and thus a higher F/B ratio than soils fertilized with P alone (Fig. 5a, b, f). G+ bacterial abundance was lower in soils added with cellulose compared to the soils added with starch and without substrate addition (P<0.05, Fig. 5c), while G- bacterial abundance was higher in soils with substrate addition than without substrate addition (P<0.05, Fig. 5d). Thus, substrate addition promoted G- bacteria but after 45 days, fungal abundance had increased with substrate addition to a greater extent than bacterial abundance, particularly in cellulose-amended soils, and the effect was amplified by long-term fertilization.

After 90 days of incubation, the differences among substrate additions were more prevalent than the differences among fertilization treatments (P<0.01, Table S4). Substrate addition, especially cellulose, resulted in significantly higher fungal abundance, total PLFA biomass, and a higher F/B ratio compared to soils with no substrate input, irrespective of fertilization history (P<0.01, Fig. 6). However, both G+ and G-, and thus total, bacterial abundances were generally slightly higher in soils amended with starch and slightly lower in soils amended with cellulose (P<0.05, Fig. 6b,c,d). Starch-amended soils from N and P fertilization treatments had lower fungal abundance, higher bacterial abundance and thus a lower F/B ratio compared to NP-fertilized soils and unfertilized controls (P<0.05, Fig 6). Without substrate addition, P-fertilized soils had the highest bacterial abundance, the lowest fungal abundance and therefore the lowest F/B ratio but there were no differences in total PLFA biomass among soils from different long-term fertilization treatments (P>0.05, Fig. 6c). Thus, after 90 days, fungal abundance had increased in cellulose-amended soils, regardless of fertilization treatment, but changes in bacterial abundance varied with fertilization treatment in starch-amended soils and soils without substrate additions.

3.4 Correlations among C mineralization, enzyme activities and microbial community composition.

There were significant positive correlations among total C mineralization, BG activity, C_{enzyme}/NAG, Fungi, G- bacteria and F/B ratio (P<0.05, Fig. 7 and Table. S5). The substrate addition and fertilization enhanced the total C mineralization by increase the extracellular enzyme activities, fungal and bacterial abundance and total PLFAs biomass which was in line with the “stoichiometric decomposition theory” (graphic abstract a).

Similar with the total C mineralization, the positive correlations existed among original soil C mineralization and BG activities, C_{enzyme}/NAG, G- bacteria and F/B ratio, while original C mineralization was negatively correlated with NAG/AP (P<0.05, Fig. 7 and Table. S5). In soils added with cellulose and starch, P fertilization enhanced original soil C mineralization by increasing bacterial abundance. In soils added with cellulose, N fertilization enhanced original soil C mineralization by increasing bacterial abundance, while in soils added with starch, N fertilization also

enhanced original soil C mineralization through increasing the fungal abundance and F/B ratio. In soils without substrate addition, both N and P fertilization decreased the original soil C mineralization by decreasing the bacterial abundance before the incubation or decreasing the fungal abundance, respectively (graphic abstract b).

Different from total C and original C mineralization, significant negative correlations existed among substrate-C and BG, NAG activities and NAG/AP, while positive correlations showed up among substrate-C and PHO activities and C_{enzyme}/NAG (P<0.05, Fig. 7 and Table. S5). N fertilization decreased the cellulose decomposition and increased the starch decomposition through the decreased or increased fungal abundance and F/B ratio, respectively. P fertilization accelerated the cellulose decomposition through increasing bacterial abundance, while impeded the starch decomposition through the reduced fungal abundance (graphic abstract c).

4. Discussion

Our study provides insights into the role of stoichiometry in the mineralization of original soil C and the impacts of long-term fertilization on soil C dynamics. However, the interactions between substrate additions and fertilization treatments were more complex than anticipated, and we only found partial support for each of our three hypotheses. First, although cellulose unexpectedly decomposed faster than starch, the mineralization of original soil C was nonetheless stimulated to a greater extent by starch (H1). Second, overall C turnover and original soil C mineralization were reduced by long-term N fertilization, but original soil C mineralization was nonetheless greater in N-fertilized soils amended with starch (H2). Finally, original soil C mineralization was greater in NP-fertilized soils amended with cellulose, but long-term P fertilization suppressed original soil C mineralization in soils without substrate additions (H3). Enhanced original soil C turnover in long-term N- and P-fertilized soils after the addition of C-rich substrates supports the 'stoichiometry decomposition theory' (Melillo et al., 1982; Hessen et al., 2004), because the highest mineralization rates occurred when both C and nutrient needs of microbes were met. Here, we draw on the differences in enzyme activities and microbial communities to interpret our findings for substrate decomposition and original soil C mineralization in the light of the stoichiometric decomposition theory.

4.1 Substrate C mineralization

In contrast to our initial hypothesis (H1), cellulose decomposed faster than starch in all soils regardless of the long-term fertilization history, demonstrated by higher initial rates of cellulose-derived respiration across soils. Decay rates are usually inversely related to the complexity of the substrate (Berg and McClaugherty, 2003; Cornwell et al., 2008; Michael et al., 2009) and starch has a simpler chemical structure than cellulose (Mischnick and Momcilovic, 2010). We identified three reasons for the slower decay of starch in our study: First, starch from seeds

and storage organs (such as the corn starch used in this study) has a high proportion of amylopectin and thus decays more slowly than the starch contained leaf chloroplasts (Bailey and Macrae, 1973; Haslam, 2004). Second, decay rates are determined by the adaptation of soil microbial communities to plant inputs and thus, plant litter often decays faster in its place of origin (the home-field advantage;(Grégoire et al., 2012)). Whereas cellulose is one of the major structural components of plant material, comprising *c*. 40% of litter mass (Berg and McClaugherty, 2003), starch concentrations comprise less than 1% (Haettenschwiler et al., 2008; Leitner et al., 2012) and thus, many fewer soil microbial taxa in forests are able to utilize starch (German et al., 2011). Indeed, higher decomposition rates of starch in the later stages of the incubation (Fig. 2c) could indicate slower resource depletion due to lower abundances of starch-degrading microbes, rather than inherently lower decay rates. Finally, starch is primarily broken down by α-glucosidase and although we did not measure the activity of α-glucosidase in this study, it is generally much less prevalent in soils than cellulolytic and hemicellulolytic enzyme activities (German and Allison, 2015). However, the large initial increases in BG activity and fungal abundance with starch addition in N-fertilized soils are striking (Figs. 3b and 4a), because fungi play a key role in starch degradation (Wang et al., 2020a) and have a high N-requirement (Tudzynski, 2014).

Overall, the greater effect of cellulose additions on total C mineralization (Figs. 1a, 2a), hydrolytic enzyme activities (Fig. 3) and total PLFA biomass (Fig. 5e) likely reflects the abundance of cellulose in terrestrial ecosystems (Berg and McClaugherty, 2003; Mischnick and Momcilovic, 2010) and thus the widespread adaptation of microbial communities to utilize cellulose as a substrate. Fungi are the primary decomposers of cellulose (Aneja et al., 2006; Rousk and Baath, 2007; Kuramae et al., 2013; Talbot et al., 2013; Purahong et al., 2014), and much higher rates of NAG activity with cellulose addition likely reflect the greater fungal abundance and activity (Anderson and Cairney, 2004). Nonetheless, bacteria can also benefit from readily available substances formed during the degradation of complex macromolecules by fungal exoenzymes (de Boer et al., 2005; Romani et al., 2006), which would explain why total soil respiration and PLFA biomass increased markedly in response to cellulose addition. Higher cellulose-derived respiration rates in P-fertilized soils at the start of the incubations (Fig. 1b), and higher AP activity in both unfertilized and N-fertilized soils with cellulose amendment (Figs. 3a, 4a and 7) support the findings of other studies that decomposition in tropical soils may be P-limited (Cleveland and Townsend, 2006). Indeed, higher C_{enzyme} /AP and NAG/AP ratios in soils amended with cellulose indicate that microbes are investing more C and N to acquire P (Lyu et al., 2019). However, similar to our previous field study, we found no stimulatory effect of P on total decomposition during the 90-day incubation (Fig. 2a) and whereas previous work in the study region showed that N-addition slowed litter decomposition by creating greater demand for P (Fang et al., 2007; Mo et al., 2008; Zhou et al., 2017; Zhang et al., 2020), and we noticed the reduced decomposition of cellulose in NP-fertilized soils.

However, although there were no clear or consistent effects of long-term-fertilization on the decomposition of starch or cellulose, both substrates stimulated AP activity at 45 days and BG activity throughout the study (Figs. 3a,b, 4a,b and 7), and greater initial AP and BG activity and NAG/AP in response to substrate additions in N-fertilized soils suggests a greater investment of N in the acquisition of P and the mineralization of added C (Allison and Vitousek 2005), which supports the stoichiometric decomposition theory (Melillo et al., 1982; Hessen et al., 2004; Craine et al., 2007).

4.2 Original soil C mineralization

In soils without substrate additions, lower overall respiration rates and reduced original soil C mineralization in all long-term fertilization treatments (Figs. 2a and 2b) indicate that mining of SOM for both N and P (Craine et al., 2007; Nottingham et al., 2015) likely plays a key role in SOM turnover in our study forest. Indeed, higher AP and NAG activities (Figs. 3 and 4) in unfertilized soils compared with fertilized soils suggest that microbes are investing more resources in P and N acquisition (Wang et al., 2020b). Several studies have measured reduced respiration rates in long-term N fertilized soils (Mo et al., 2008; Janssens et al., 2010; Zhou et al., 2014) and N deposition also generally reduces soil respiration rates in forests (Janssens et al., 2010). Lower bacterial abundance in the N fertilization treatment in the field (Li et al., 2014) and in this study before the start of incubations (Fig. S6), likely contribute to reduced respiration and SOM turnover with N fertilization (Purahong et al., 2015; Urbanová et al., 2015; Johnston et al., 2016). Moreover, lower respiration rates with N-fertilization could indicate reduced microbial degradation of lignified material, which often involves phenol oxidases (Sinsabaugh, 2010). Studies have found that N can increase the decomposition of labile compounds while impede the decomposition of recalcitrant compounds (Janssens et al., 2010) and activity of PHO at the end of our incubations was lowest in N-fertilized soils, regardless of substrate addition can be explained by this preferential decomposition caused by N fertilization.

Greater P-limitation relative to N-limitation in tropical forests (Hou et al., 2020) explains the larger reduction in total soil respiration and original soil C mineralization in long-term P-fertilized soils in our study. Although our findings contradict with previous incubation studies demonstrating the enhanced soil respiration with P fertilization (Cleveland et al., 2002; Hui et al., 2020), these studies measured the mineralization of highly labile substrates (Cleveland et al., 2002) or responses to one-off short-term nutrient additions (Hui et al., 2020). Reduced original soil C mineralization rates with P-fertilization in our study might be explained by the lower fungal biomass and F/B ratio in long-term P fertilized soil at the end of the incubation (Fig. 6a) because fungi are generally thought the principal agents in the degradation of recalcitrant organic matter (Sinsabaugh, 2010). Thus, although there was some evidence to support our second hypothesis that N fertilization suppresses C turnover, we rejected our third hypothesis that P-fertilization would stimulate C turnover at our study site. In addition, neither hypothesis was

supported by our findings for substrate-amended soils, because starch stimulated original soil C mineralization to a greater extent than cellulose in unfertilized and N-fertilized soils, whereas cellulose addition stimulated original soil C mineralization to a greater extent in NP-fertilized soils. Instead, the changed enzymatic stoichiometry in soils added with substrates and fertilizers highlight the importance of C:N:P stoichiometry in decomposition processes (Manzoni et al., 2010) and provide further evidence to support the stoichiometric decomposition theory (Melillo et al., 1982; Hessen et al., 2004).

4.3 Original soil C mineralization in response to substrate addition

Both substrates greatly stimulated the mineralization of original soil C, with the largest increases in original soil C -derived respiration within a few days of substrate addition, which is consistent with 'priming effects' (Kuzyakov et al., 2000). Although the stimulatory effect of substrate addition on original soil C mineralization is well known, the underlying mechanisms are less clear (Kuzyakov et al., 2000; Fontaine et al., 2003), but there is some evidence that the availability of both N and P can play a role in SOM priming in tropical soils (Nottingham et al., 2015). Whereas many incubation experiments investigating priming effects used substrates that differ markedly in their decomposability, our study demonstrates that similar C-rich substrates with the same molecular weight can also stimulate original soil C mineralization to a different extent, depending on nutrient availability (Fig. 2b) and the nutrient requirements of decomposer organisms. It is worth noting that we did not measure substantial activation of oxidative enzymes (PER and PHO) that would explain the observed increases in original soil C mineralization. However, most original soil C mineralization occurred during the first 30 days (Fig. S1b), but we only sampled the soils from the incubations after 45 and 90 days, and thus many enzyme activities might have already returned to constitutive levels (Allison and Vitousek, 2005). The lagged measurement of enzyme activities and soil microbial community composition suggest that future experimental design should consider both dynamics of soil biochemical process and microbial activities to better interpret findings.

The interactions between substrate additions and long-term fertilization treatments indicate that C:N:P stoichiometry and microbial mining of SOM for N or P might explain the differences in original soil C mineralization in response to cellulose vs. starch. In cellulose-amended soils, higher original soil C mineralization in the NP-fertilized soils, might indicate that long-term enhanced nutrient availability in the +NP field plots has resulted in C limitation for soil microbial communities (Eberwein et al., 2015), and the added cellulose provided sufficient energy for microbes to invest in enzymes to degrade SOM for additional C (Allison and Vitousek, 2005). C limitation of microbial processes is also indicated in soils without substrate amendments by lower respiration rates, suppression of SOM mineralization by all fertilization treatments (Yoshitake et al., 2007) and lower investment of C in N or P-acquiring enzymes (Figs. S3 and S4). Increased G- bacterial abundance in soils with substrate addition

also suggests that microbial activity is limited by easily available sources of C because G- bacteria include many fast-growing, copiotrophic taxa that likely increased overall rates of respiration and enzyme activity (Andrews and Harris, 1986; Vangestel et al., 1993). By contrast, higher AP and substantially higher NAG activities in cellulose-amended soils without added P (Fig. 3c) suggest that SOM was being mined for both N and P to support cellulose decomposition (Turner and Wright, 2014; Yokoyama et al., 2017), which might also explain why the microbial investment of C in both N- and P-acquisition tended to be lower in cellulose-amended soils without added P. In starch-amended soils, it is conceivable that greater original soil C mineralization in N-fertilized soils indicates higher P requirements of microbes capable of degrading starch, especially as original soil C mineralization in response to added starch tended to be lower in soils with added P (Fig. 2b). Higher P requirements with starch addition is also indicated by greater initial investment of C in P acquisition in N and NP-fertilized soils (Fig. S3a). Thus, although original soil C mineralization appears to be largely controlled by C:N:P stoichiometry, the balance between C and nutrients is also determined by substrate type and decomposer communities, which could explain the many conflicting results among studies in different tropical forests (Cornwell et al., 2008; Allison et al., 2014; Fan et al., 2019a).

5. Conclusions

Our study demonstrates that SOM mineralization in response to fresh C substrates is largely determined by nutrient stoichiometry and the specific C and nutrient requirements of microbial decomposers. The distinct changes in respiration rates, microbial biomarkers, enzyme activities and microbial investment of resources in starch-amended vs. cellulose-amended soils suggest that even chemically similar substrates can differ markedly in their effects on original soil C mineralization, and thus the nutrient stoichiometry, activities and abundances of decomposer organisms must be taken into consideration to fully understand how different plant inputs might influence SOM turnover. Our findings further suggest that experiments using short-term fertilized soils or one-off nutrient additions may not accurately reflect processes in soils subjected to long-term multiple fertilization. In lowland tropical forest soils, the low availability of P is likely to be exacerbated by increasing atmospheric N deposition. Our findings suggested that the resulting imbalance between N and P availability could either enhance or reduce SOM mineralization, depending on complex interactions between plant C inputs and microbial decomposers. There is thus an urgent need for additional studies of SOM mineralization in long-term fertilization experiments at other tropical forest sites.

Declaration of interests

None declared.

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Table 1. Soil chemical characteristics (0–10 cm depth) in long-term fertilization treatments in a secondary tropical forest of south China, where +N is nitrogen (N) fertilization, +P is phosphorus (P) fertilization, +NP is fertilized with both N and P, and CK is unfertilized controls, TN is total N, TP is total P, TC is total carbon, NH_4^+-N is ammonium-N, NO_3^--N is nitrate N, and Inorganic N is the sum of NH_4^+-N and NO_3^--N; values are means ±SE for n = 5, different lowercase letters within a row indicate significant differences among treatments at $P < 0.05$ (after correction for multiple comparisons).

Treatment	TN (g/kg)	TP (g/kg)	TC (%)	NH_4^+-N (mg/kg)	NO_3^--N(mg/kg)	Available P (mg/kg)	Inorganic N/ available P	pH
+N	1.76±0.20	0.38±0.18	2.15±0.36	7.73±0.63	20.76±2.53a	4.00±0.73b	8.06±1.43a	4.17±0.07
+P	1.38±0.12	0.50±0.06	1.72±0.21	6.71±0.23	11.98±0.71b	25.24±0.46a	0.74±0.04b	4.61±0.12
+NP	1.58±0.19	0.47±0.04	2.03±0.20	6.80±0.24	11.23±1.30b	25.13±0.25a	0.72±0.06b	4.26±0.07
CK	1.72±0.16	0.21±0.01	2.28±0.33	6.84±0.70	12.87±2.14b	3.66±0.83b	6.02±0.90a	4.34±0.06

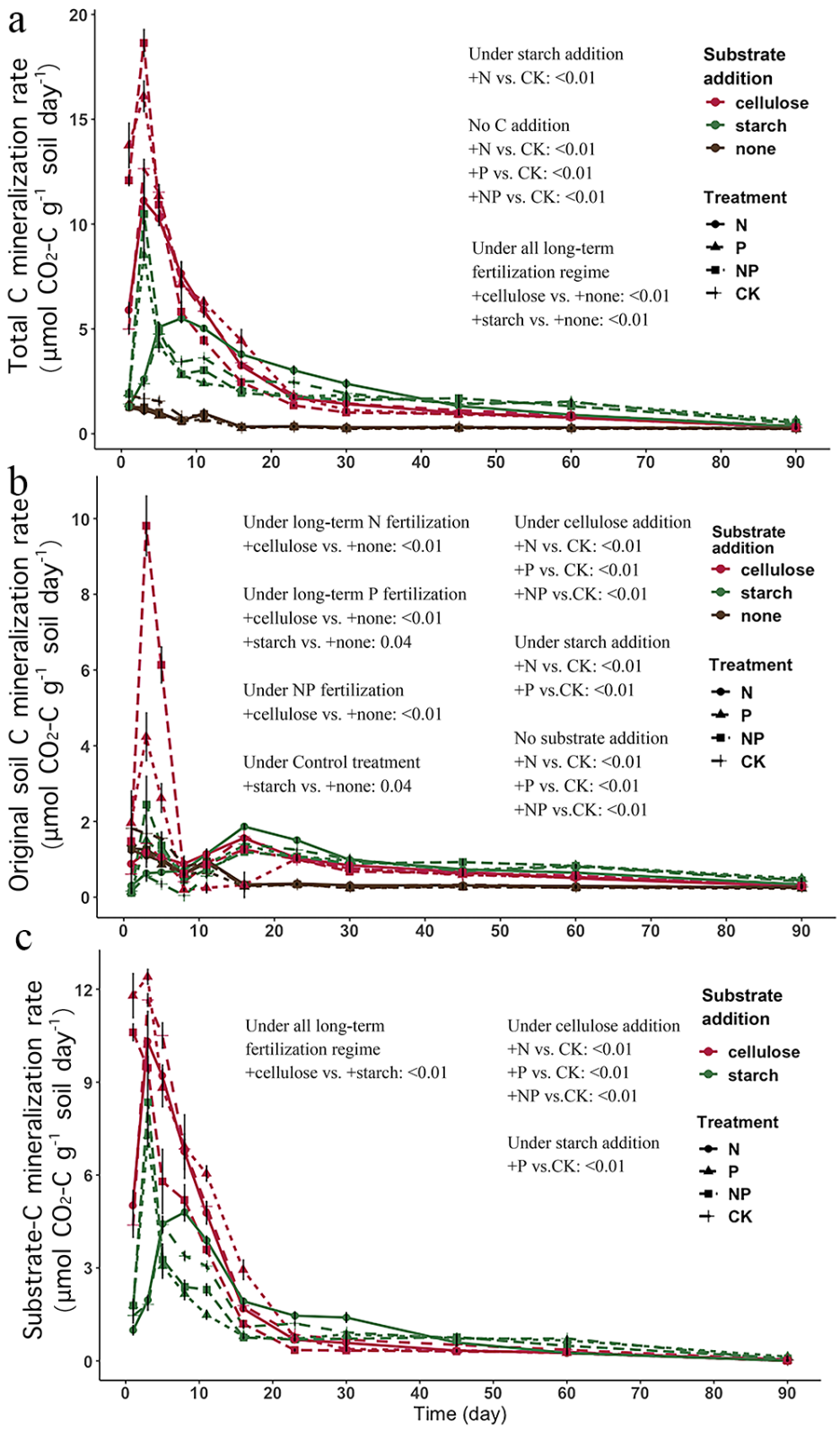

Figure 1. Carbon (C) mineralization rates in soils from long-term fertilization treatments in a secondary tropical forest of south China during a 90-day incubation experiment with substrate additions, showing **(a)** total C mineralization, **(b)** original soil C mineralization and **(c)** substrate-C mineralization; +N is nitrogen (N) fertilization, +P is phosphorus (P) fertilization, +NP is fertilized with both N and P, and CK is unfertilized controls. Symbols and error bars represent means and standard errors for *n* = 6 before and including day 45 and *n* = 3 after day 45, and *P*-values from LMEs for the effects of substrate addition and long-term fertilization are shown.

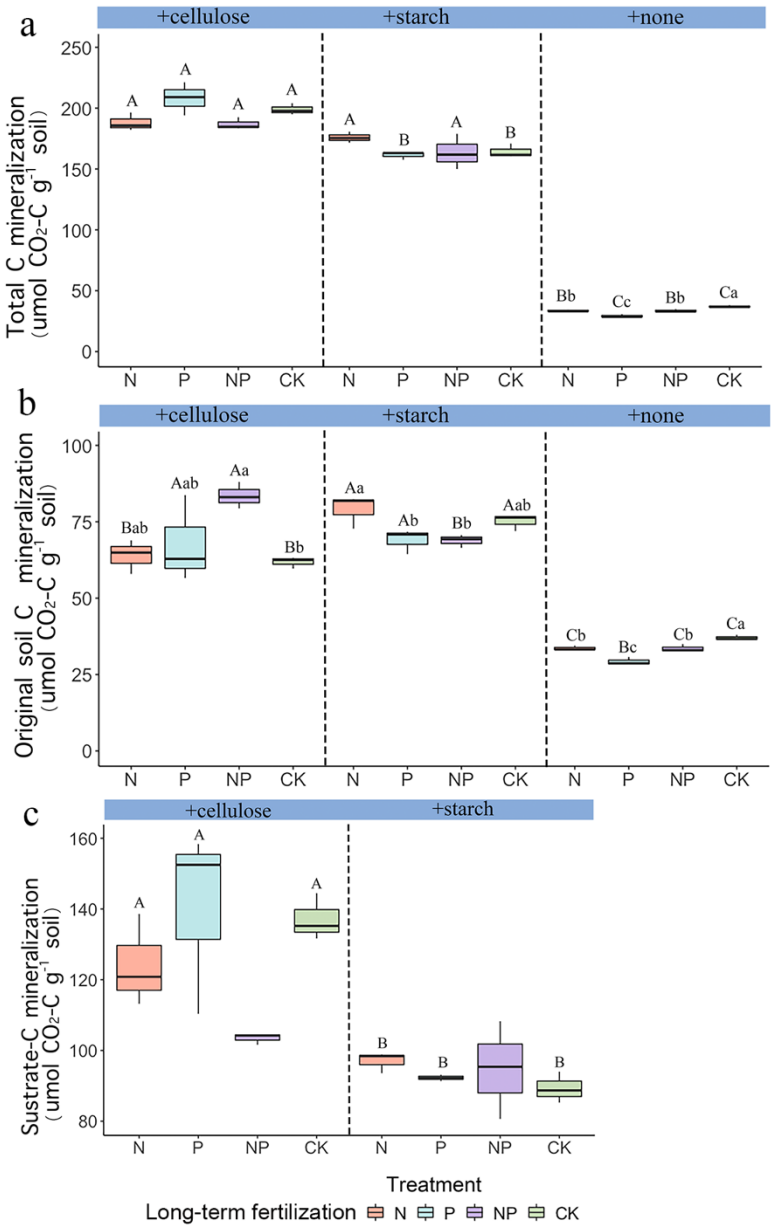

Figure 2. Cumulative total carbon (C) mineralization in soils from long-term fertilization treatments in a secondary tropical forest of south China during a 90-day incubation experiment with substrate additions, showing **(a)** total C mineralization, **(b)** original soil C mineralization and **(c)** substrate-C mineralization; +N is nitrogen (N) fertilization, +P is phosphorus (P) fertilization, +NP is fertilized with both N and P, and CK is unfertilized controls. Different capital letters indicate significant differences among substrate additions and different lowercase letters indicate significant differences among long-term fertilization treatments at $P < 0.05$ (after correction for multiple comparisons).

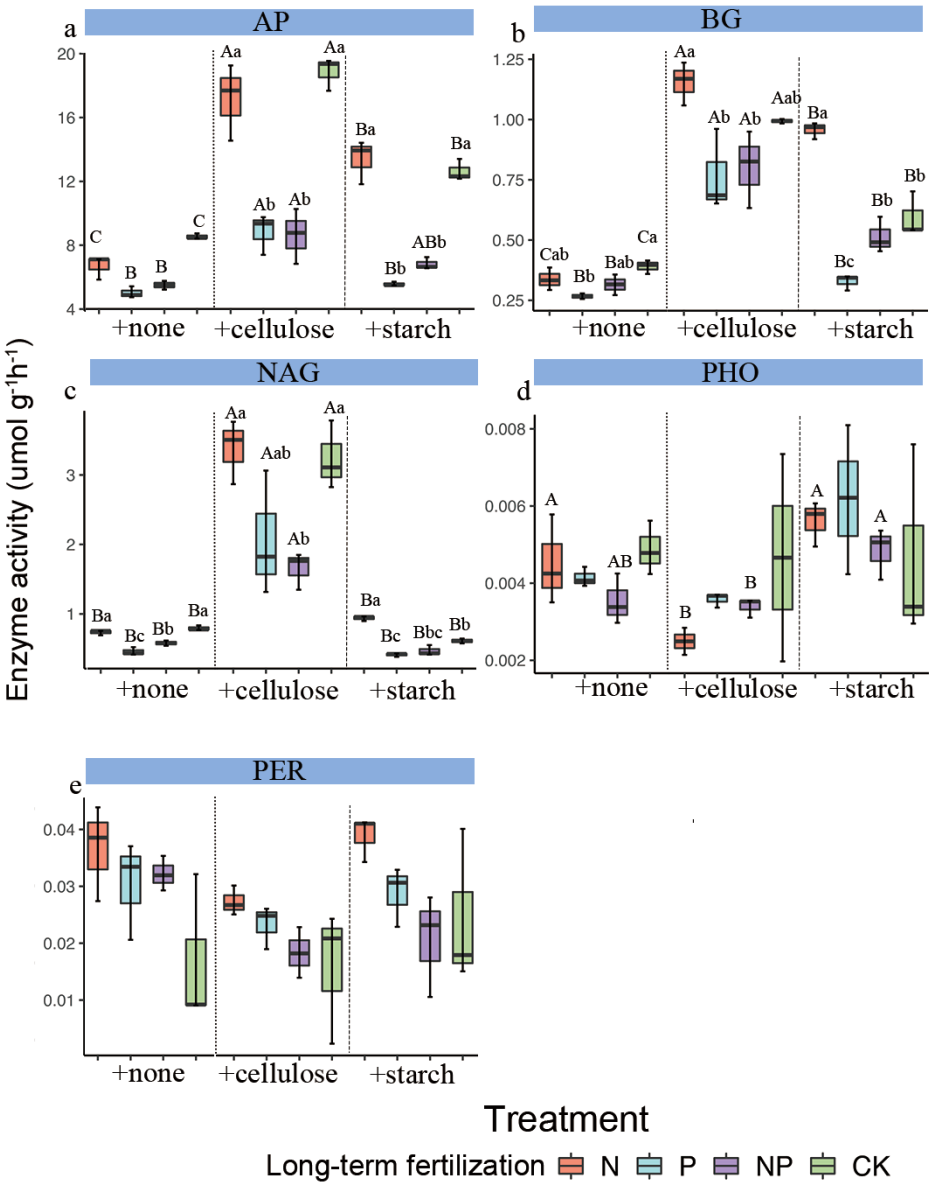

Figure 3. Extracellular enzyme activities in soils from long-term fertilization treatments in a secondary tropical forest of south China after 45 days of a 90-day incubation experiment with substrate additions, showing: **(a)** acid phosphomonoesterase (AP), **(b)** β-1,4-glucosidase (BG), **(c)** β-1,4- N-acetaminophen glucosidase (NAG), **(d)** phenol oxidase (PHO) and **(e)** peroxidase (PER), where +N (orange) is nitrogen (N) fertilization, +P (turquoise) is phosphorus (P) fertilization, +NP (purple) is fertilized with both N and P, and CK (green) is unfertilized controls. Different capital letters indicate significant differences among substrate additions and different lowercase letters indicate significant differences among long-term fertilization treatments at $P < 0.05$ (after correction for multiple comparisons).

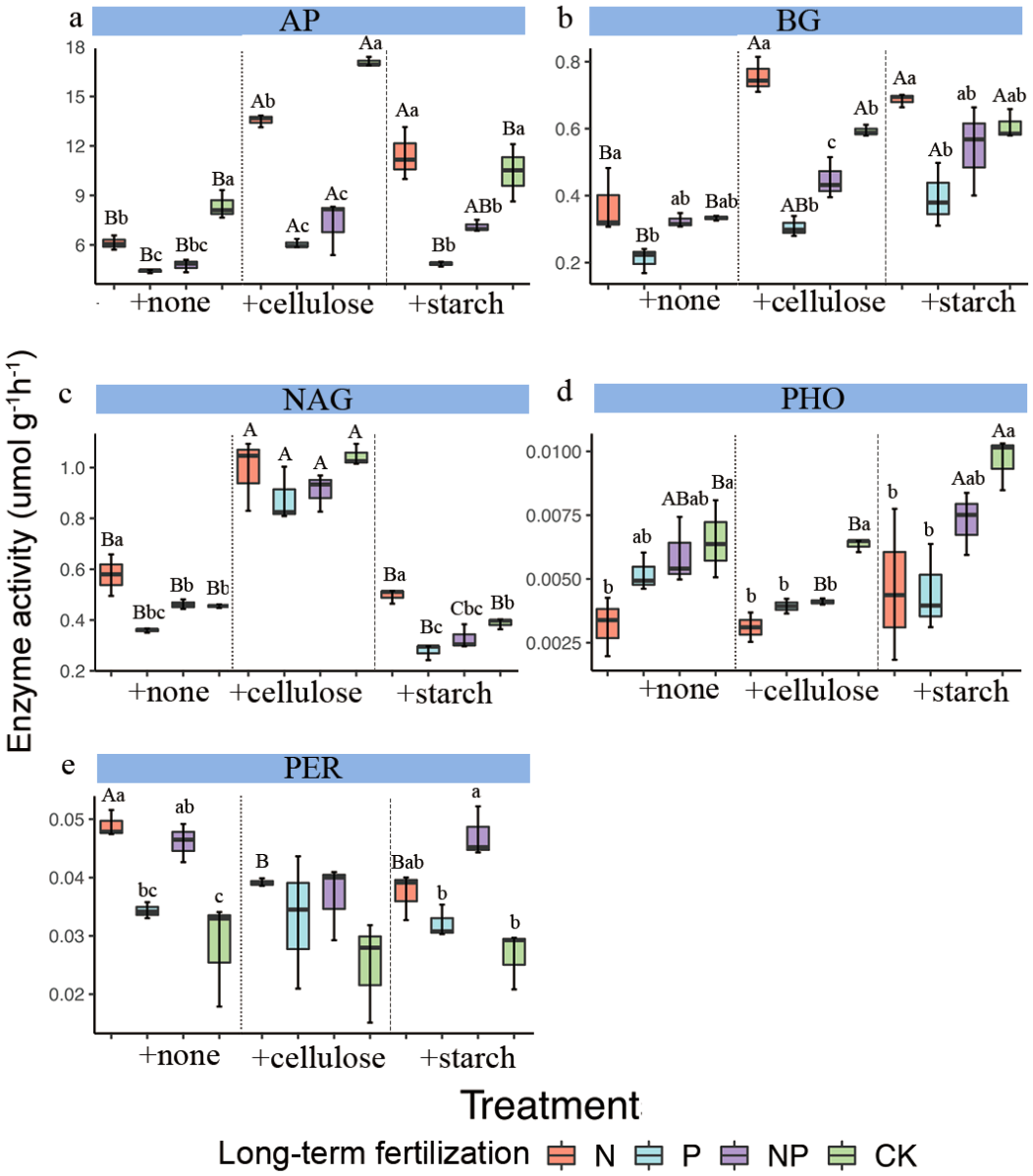

Figure 4. Extracellular enzyme activities in soils from long-term fertilization treatments in a secondary tropical forest of south China at the end of a 90-day incubation experiment with substrate additions, showing: **(a)** acid phosphomonoesterase (AP), **(b)** β-1,4-glucosidase (BG), **(c)** β-1,4- N-acetaminophen glucosidase (NAG), **(d)** phenol oxidase (PHO) and **(e)** peroxidase (PER), where +N (orange) is nitrogen (N) fertilization, +P (turquoise) is phosphorus (P) fertilization, +NP (purple) is fertilized with both N and P, and CK (green) is unfertilized controls. Different capital letters indicate significant differences among substrate additions and different lowercase letters indicate significant differences among long-term fertilization treatments at $P < 0.05$ (after correction for multiple comparisons).

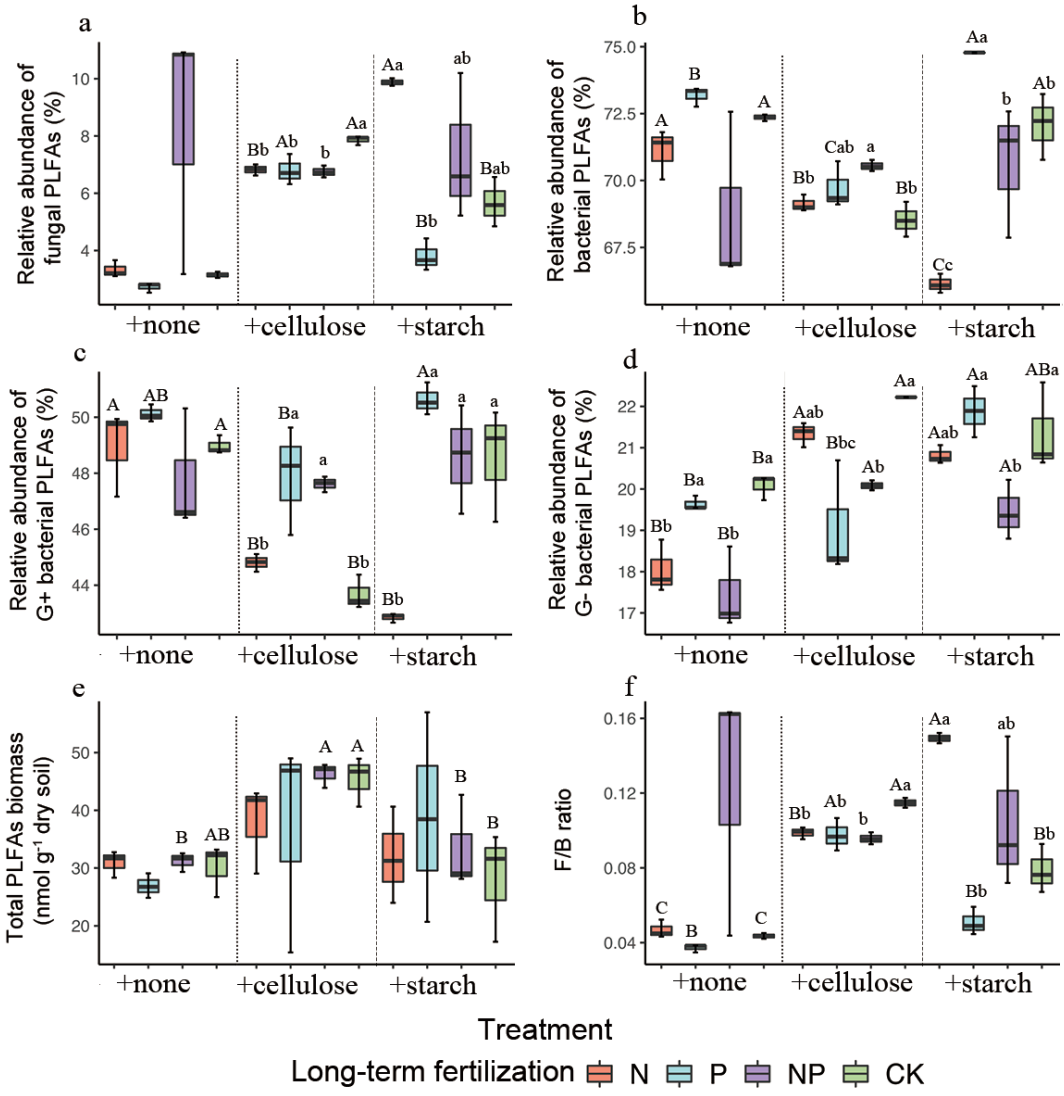

Figure 5. Phospholipid fatty acid (PLFA) biomarkers representing microbial community composition in soils from long-term fertilization treatments in a secondary tropical forest of south China after 45 days of a 90-day incubation experiment with substrate additions, showing the relative abundances of **(a)** fungal PLFAs, **(b)** bacterial PLFAs, **(c)** Gram-positive bacterial PLFAs, **(d)** Gram-negative bacterial PLFAs, **(e)** total PLFA biomass, and **(f)** the ratio of fungi to bacteria (F/B ratio), where +N (orange) is nitrogen (N) fertilization, +P (turquoise) is phosphorus (P) fertilization, +NP (purple) is fertilized with both N and P, and CK (green) is unfertilized controls. Different capital letters indicate significant differences among substrate additions and different lowercase letters indicate significant differences among long-term fertilization treatments at $P < 0.05$ (after correction for multiple comparisons)

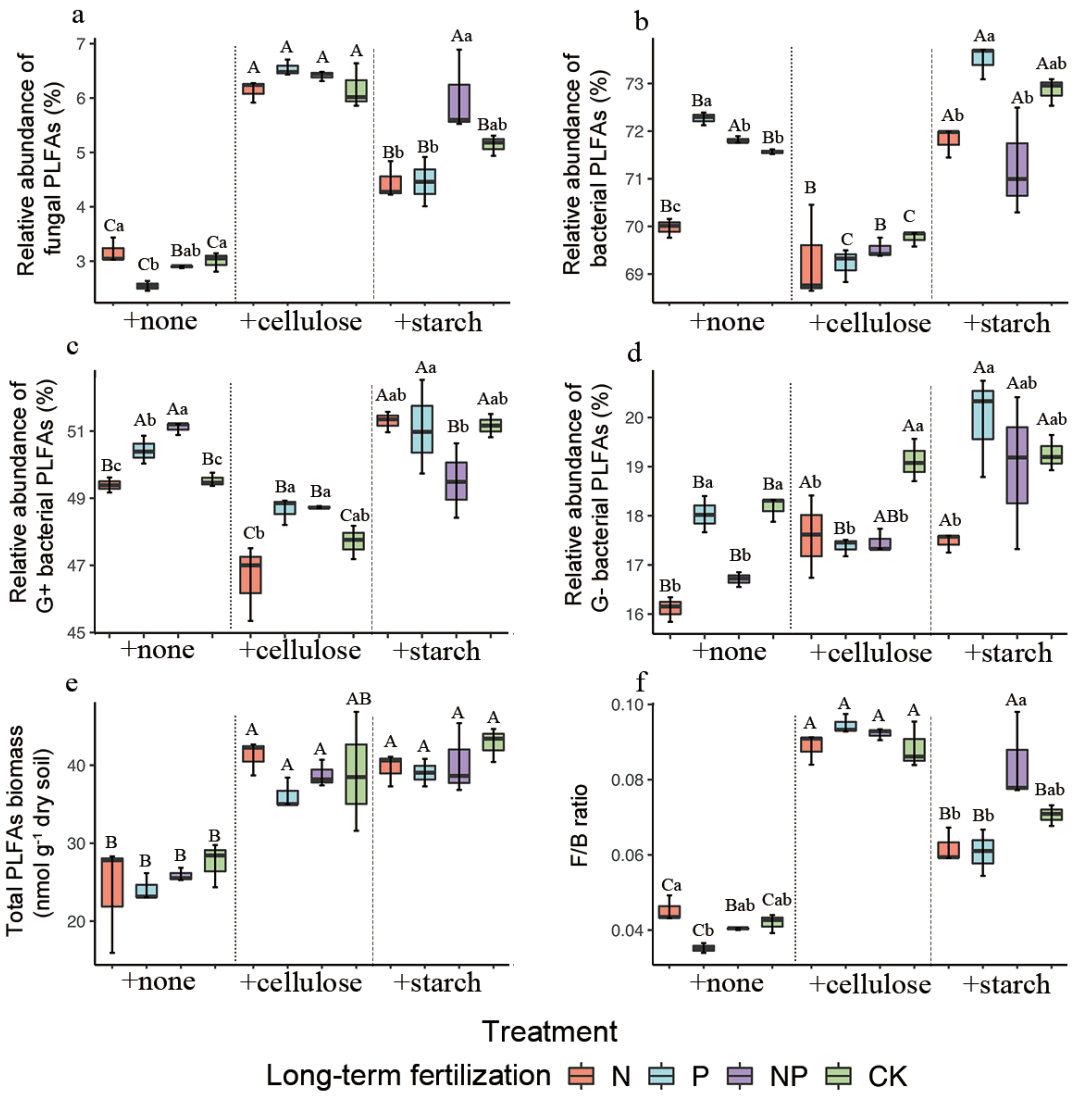

Figure 6. Phospholipid fatty acid (PLFA) biomarkers representing microbial community composition in soils from long-term fertilization treatments in a secondary tropical forest of south China at the end of a 90-day incubation experiment with substrate additions, showing the relative abundances of **(a)** fungal PLFAs, **(b)** bacterial PLFAs, **(c)** Gram-positive bacterial PLFAs, **(d)** Gram-negative bacterial PLFAs, **(e)** total PLFA biomass, and **(f)** the ratio of fungi to bacteria (F/B ratio), where +N (orange) is nitrogen (N) fertilization, +P (turquoise) is phosphorus (P) fertilization, +NP (purple) is fertilized with both N and P, and CK (green) is unfertilized controls. Different capital letters indicate significant differences among substrate additions and different lowercase letters indicate significant differences among long-term fertilization treatments at $P < 0.05$ (after correction for multiple comparisons).

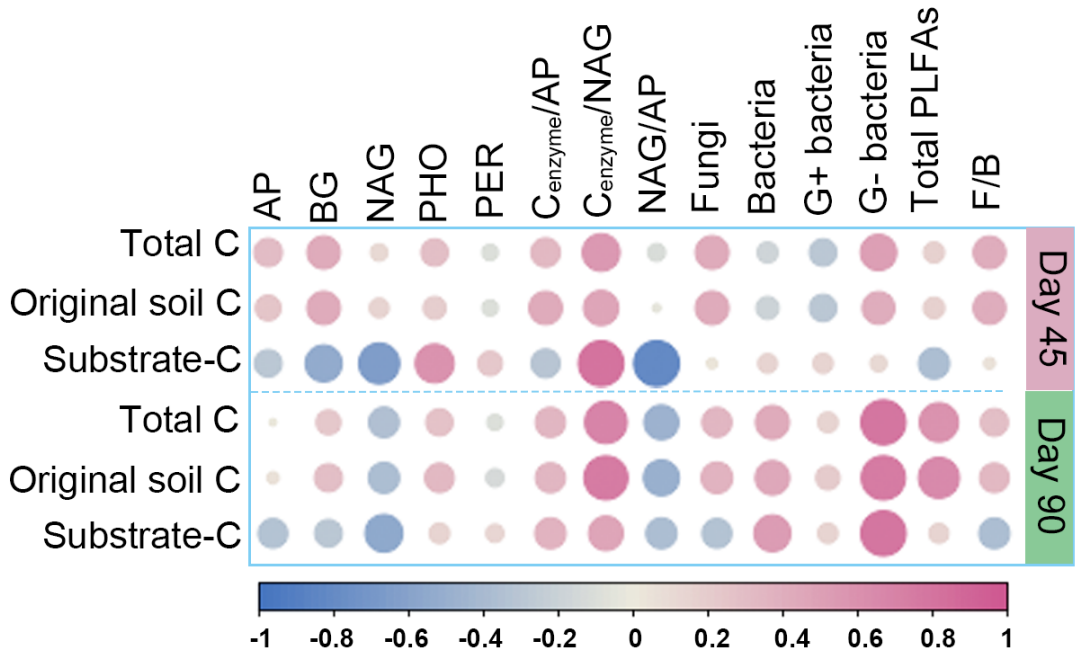

Figure 7. Pearson rank correlation between soil C mineralization rate, enzyme activities, microbial commuity composition in soils from long-term fertilization treatments in a secondary tropical forest of south China after each sampling time. Circles with red and blue colors indicate positive and negative relationships, respectively; circle size indicates the P-values, where total C, original soil C and subsrate-C is the minerlaizaition rate of total C, original soil C and substrate soil C respectively; AP is acid phosphomonoesterase, BG is β-1,4-glucosidase, NAG is β-1,4- N-acetaminophen glucosidase (NAG) < PHO is phenol oxidase and PER is peroxidase, Cenzyme is the sum of BG, PHO and PER, G+ is Gram-positive, G- is Gram-negative, and F/B ratio is the ratio of fungal to bacterial biomarkers, and total PLFA is total phospholipid fatty acids.