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A transition from arbuscular to ectomycorrhizal forests halts soil carbon sequestration during subtropical forest rewilding

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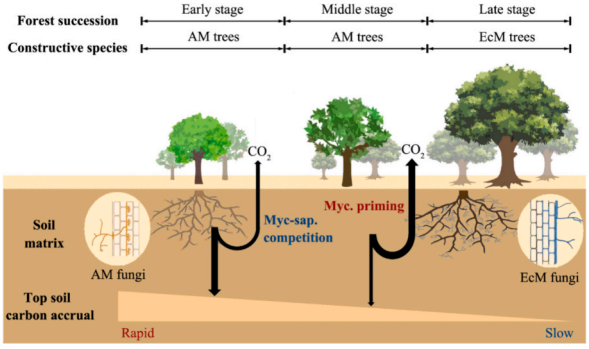
HIGHLIGHTS

- Ectomycorrhizal fungi diversity increases along forest succession.
- Ectomycorrhizal hyphae promote soil C decomposition and nutrient cycle.
- A transition from AM to EcM forests halts soil C sequestration across succession.

GRAPHICAL ABSTRACT



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ABSTRACT

Ecological succession and restoration rapidly promote multiple dimensions of ecosystem functions and mitigate global climate change. However, the factors governing the limited capacity to sequester soil organic carbon (SOC) in old forests are poorly understood. Ecological theory predicts that plants and microorganisms jointly evolve into a more mutualistic relationship to accelerate detritus decomposition and nutrient regeneration in old than young forests, likely explaining the changes in C sinks across forest succession or rewilding. To test this hypothesis, we conducted a field experiment of root-mycorrhizal exclusion in successional subtropical forests to investigate plant-decomposer interactions and their effects on SOC sequestration. Our results showed that SOC accrual rate at the 0–10 cm soil layer was 1.26 mg g^{-1} yr^{-1} in early-successional arbuscular mycorrhizal (AM) forests, which was higher than that in the late-successional ectomycorrhizal (EcM) forests with non-significant change. A transition from early-successional AM to late-successional EcM forests increase fungal diversity, especially EcM fungi. In the late-successional forests, the presence of ectomycorrhizal hyphae promotes SOC

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decomposition and nutrient cycle by increasing soil nitrogen and phosphorus degrading enzyme activity as well as saprotrophic microbial richness. Across early- to late-successional forests, mycorrhizal priming effects on SOC decomposition explain a slow-down in the capacity of older forests to sequester soil C. Our findings suggest that a transition from AM to EcM forests supporting greater C decomposition can halt the capacity of forests to provide nature-based global climate change solutions.

1. Introduction

Forests can support large carbon (C) stocks and play key roles in regulating climate change (Pan et al., 2011). Forest succession and restoration have the potential to rapidly promote multiple dimensions of ecosystem functions such as C sequestration, plant biomass, soil fertility, and microbial diversity (Bongers et al., 2015; Poorter et al., 2021; Zhou et al., 2022). In particular, a large amount of forest C is sequestered as organic matter in soils, and accumulate substantially during natural succession (Liu et al., 2022). We also know that, as forests age, their capacity of sequestering C is reduced to become C neutral (Odum, 1969), or even losing C through a process named forest retrogression (Yang et al., 2011). Little is known, however, about the factors controlling this limited capacity of forests to sequester soil organic C (SOC) in old forests. This knowledge gap highly limits our ability to predict long-term dynamics of forest C pool and guide the restoration of forests worldwide.

Soil organic C dynamics are determined by the long-term balance between plant-derived C inputs and microbial C consumption (Tang et al., 2009; Cheng et al., 2010; Zhou et al., 2012; Tao et al., 2023). Carbon input from plant residues is traditionally considered to be balanced by microbial decomposition in old-aged forests (Zhou et al., 2006). Annual plant C inputs usually increase with forest succession, which alleviates the C limitation of microbial growth (Yang et al., 2011). Accordingly, microbial population and activity increase and then stimulate SOC decomposition (i.e., priming effect; He et al., 2020). Successional theory predicts that plant-microbial interaction becomes more mutualistic to accelerate detritus decomposition and nutrient regeneration, and provide the positive feedback that contributes to forest stability in the older system (Odum, 1969). However, we are still far from understanding how plants and microorganisms interactively influence SOC sequestration during forest succession or rewilding.

Root-mycorrhizal interaction is one of the most important associations between plants and microbes (Verbruggen et al., 2016; Zheng et al., 2019; Zhang et al., 2022). Mycorrhizal hyphae can influence SOC storage through their positive or negative interactions with free-living saprotrophic microbes (Verbruggen et al., 2016; Lang et al., 2021). These mycelial hyphae can have facilitative interactions by releasing low molecular-weight organic compounds that enhance the decomposition of soil organic matter (SOM, called priming effect) and desorb mineral-associated organic matter (Adamczyk et al., 2019). On the other hand, competitive interactions can occur when mycorrhizal hyphae strive with free-living saprotrophs for soil resources such as water and nutrients. This can lead to a decrease in SOM decomposition, known as the "Gadgil effect" (Fernandez and Kennedy, 2016; Gadgil and Gadgil, 1971). Thus, changes in mycorrhizal fungi and in the capacity of soils to decompose organic matter may result in substantial changes in SOC sequestration. Yet, there are still large uncertainties on how mycorrhizal priming effects and microbial interguild competition influence SOC sequestration during forest rewilding.

Subtropical forests are major terrestrial C sink of anthropogenic-derived atmospheric CO_2 with high primary productivity and C sequestration capacity (Yu et al., 2014). Plants in subtropical forests are usually associated with mycorrhizal fungi to forage the limited nutrients in soils to meet the demand for their rapid growth (Jiang et al., 2022). However, the impact of mycorrhizal fungi on SOC decomposition and accrual is poorly understood (Liu et al., 2021). Here, we conducted a root-mycorrhizal exclusion experiment in three successional subtropical forests, transitioning from AM to EcM vegetation, to test the hypotheses that i) the presence of EcM fungi increases soil N- and P-degrading enzyme and saprotrophic activity to promote SOC decomposition and nutrient cycle at the late-successional stage; and ii) mycorrhizal priming effects slow SOC accrual in the old-aged forests.

2. Methods

2.1. Site description and experimental design

The experiment was conducted in Tiantong National Forest Ecosystem Observation and Research Station (29°48′N, 121°47′E) in Zhejiang province, China. The climax monsoon evergreen broad-leaved community has been protected from clear cutting in the center of the station (Wang et al., 2019). Outside this area, virtually all the vegetation is secondary evergreen broad-leaved forests (EBLFs), such as shrubs, coniferous forests, and sub-climax EBLFs. Experimental blocks were composed of three representative forests (early-successional forests: 25 years, young mixed woody community; middle-successional forests: 55 years, sub-climax *Schima superba* community (i.e., arbuscular mycorrhizal forests); and late-successional forests: 120 years, climax *Castanopsis fargesii* community (i.e., ectomycorrhizal forests; Liu et al., 2019, 2021; Soudzilovskaia et al., 2020)). More information about the experimental sites, such as plant community characteristics, could be found in Table S6.

A root-mycorrhizal exclusion experiment was conducted in August 2016 to investigate mycorrhizal effects on soil organic carbon (SOC) decomposition and saprotrophic activities (Fig. S1). Six replicate plots (10 m × 10 m) were established in each successional stands (18 plots in total) with 50–300 m distance. Plots of three successional forests were >1000 m apart from one another. Six replicate plots of each successional stands were located on similar slope position and elevation, and at least 100 m apart from forest edge. In each plot, four 0.7 m × 0.7 m subplots were established in the center of tree interspaces, with >5 m buffer zone between two subplots. We set 24 sub-plots as a total in each successional forests. Three following treatments was randomly assigned to one subplot in each plot (Fig. S1): (1) *Myc*, where one subplot was trenched to a depth of 0.8–1.0 m and lined with 50-μm nylon mesh to exclude roots only and allow the entry of mycorrhizal fungi; (2) *Soil*, where one subplot was trenched to a depth of 0.8–1.0 m and lined 1-μm nylon mesh to exclude both roots and mycorrhizal fungi. (3) *Root* + *Myc*, where two subplots kept as natural control. The trenches were refilled to minimize the trenching disturbance. Our prior study found that root and mycorrhizal growth (*Root* + *Myc* and *Myc* vs. *Soil* treatments) increased soil fungal and arbuscular fungal abundance, and decreased soil bacteria: fungi ratio, but did not change soil moisture and temperature (*Myc* vs. *Soil* treatments) (Liu et al., 2021). As such, we attributed the main effect of treatment to root-mycorrhizal exclusion rather than to potential environmental disturbance.

2.2. Soil respiration measurement

A PVC collar (20 cm in diameter and 10 cm in height) was installed 5 cm into the ground in each subplot after all the trenches were refilled. CO_2 flux was measured for all PVC collars three months after collar installation. R_{root} was measured as the difference in CO_2 flux between paired "*Root* + *Myc*" and "*Myc*" treatments in each plot. R_H was measured as CO_2 flux in "*Soil*" subplots. We defined the difference in CO_2 fluxes between "*Myc*" and "*Soil*" subplots (named *R_Myc*) as

mycorrhizal-induced changes in respiratory C loss (or C decomposition). Such changes may arise from respiration by mycorrhizal mycelium, respiration of mycelial inputs by free living microbes and respiration of organic matter owing to mycorrhizal inputs.

In our study, soil CO_2 flux was measured in each subplot from January to December 2018. Accordingly, if monthly mean of soil CO_2 fluxes in *Myc* subplot was lower than that in *Soil* subplot, we detected a suppressive (Gadgil) effect of mycorrhizal growth on SOC decomposition. On the contrary, if CO_2 flux in *Myc* subplot was higher than that in *Soil* subplot, we ascribed the increased CO_2 flux to stimulated SOC decomposition or/and mycorrhizal metabolic respiration. To eliminate above-ground plant respiration, we clipped all small living plants (seedlings and herbs) inside the subplots at the soil surface one day ahead of the measurements. Soil CO_2 flux in each subplot were measured once a month from January to December 2018, between 8:00 and 12:00 (local time), using a LI-COR 8100 portable soil CO_2 flux system (LI-COR. Inc., Lincoln, NE, USA). Over the course of the morning, soil CO_2 flux among different treatments in each successional forest was measured randomly.

2.3. Soil carbon mineralization

Soils in the top soil layer (0–10 cm) were sampled with a cylindrical core (diameter: 3.5 cm, length: 10 cm) at four random points per subplot in August 2020. These soil samples were gently sieved through a 2-mm mesh, and then mixed as a composite sample. A part of fresh subsample was stored at 4 °C for analyzing microbial biomass C、N and P concentration, enzyme activity and soil C mineralization. Another part of subsample was stored at −20 °C for analyzing microbial genomic DNA. Soil C mineralization was measured according to the method of He et al. (2023). Briefly, 20 g of field moist soil was placed into a 150 mL polyethylene plastic bottles and incubated for 24 h to activate the microbes. The CO_2 emission rate from soil was measured using soil flux system (PRI-8800; Pre-Eco, Beijing, China). Sample bottles were placed in a 16-hole water bath and the temperature was controlled at 25 °C. The CO_2 concentration in each bottle was measured by a CO_2 analyzer (Li-7000, LI-COR, Lincoln, NE, USA) every second, and the total measuring time for each soil sample was 180 s. Soil C mineralization was calculated by the following equation (He et al., 2023):

$$SCM = \frac{C \times V \times \alpha \times \beta}{22.4 \times m} \quad (1)$$

where SCM is the rate of soil C mineralization (μg C-CO_2 g^{-1} day^{-1}); C is the slope of CO_2 concentration; V is the volume of the gas tube and bottle (ml); m is the dry soil weight (g); α is the conversion coefficient for CO_2 mass (from CO_2 to C); and β is the conversion coefficient for time (from seconds to days).

2.4. Microbial biomass carbon and nitrogen and phosphorus

Chloroform-fumigation extraction method was used to determine microbial biomass C (MBC), nitrogen (MBN), and phosphorus (MBP) contents in fresh soil samples (Brookes et al., 1982, 1985; Liu et al., 2021). MBC and MBN were determined by subtracting the total dissolved C and N (DOC and DON) of nonfumigated subsamples from that of the fumigated subsamples with a conversion factor of 0.45 and 0.38, respectively (Brookes et al., 1985). MBP was determined by subtracting the extractable P of nonfumigated subsamples from that of the fumigated subsamples. A sample of field moist soils was split into three aliquots of 5 g fresh mass. One aliquot remained untreated; the second aliquot was fumigated for 24 h at 25 °C with ethanol-free $CHCl_3$; and the third aliquot was mixed with 250 mg P l^{-1} KH_2PO_4 solution (25 μg P g^{-1} soil). Extracts were prepared by mixing 50 g field moist soil with 20 ml $NaHCO_3$ (0.5 M). MBP was calculated as $E_P/k_{EP}/R$, where E_P = (PO_4-P extracted from fumigated sample) - (PO_4-P extracted from non-fumigated sample) and k_{EP} = 0.40 (Brookes et al., 1982). To correct for P adsorption when extracting soils from different successional stages, this parameter was divided by the recovery rate (*R*) of added P (25 mg PO_4-P g^{-1} soil): R = ((PO_4-P extracted from P-added sample) - (PO_4-P extracted from non-fumigated sample))/25 (Zederer et al., 2017).

Soil available P (VP) was determined as the extractable P of non-fumigated subsamples. Soil inorganic N (SIN, the sum of NH_4^+-N and NO_3^--N) was extracted from fresh soil samples by shaking with 1 mol KCl at soil to solution ratio of 1:10 in weight for 30 min. The extracted supernatant was then filtered through Whatman 42 filter paper. SIN was determined in the supernatant using a Holland Skalar San ~ (++) continuous flow analyzer (Quik Chem from method 10–107- 064- D for NH_4^+ and 10,107- 04- 1- H for NO_3^-, Germany).

2.5. Microbial community and network analysis

Genomic DNA was extracted from 0.5 g frozen soil samples (−80 °C) using PowerSoil ® DNA Isolation Kits (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instruction (Su et al., 2020). Internal Transcribed Spacer (ITS) ribosomal RNA and 16S were analyzed on the Illumina platform after polymerase chain reaction (PCR) amplification and purification. Microbial raw sequences (>300 bp length, quality score > 30) were examined using the Quantitative Insights into Microbial Ecology (QIIME 2) pipeline (Caporaso et al., 2010). Operational taxonomic units (OTUs) were clustered at 97 % sequence similarity using UPARSE-OTU algorithm and then through the Ribosomal Database Project (RDP) classifier (Wang et al., 2007; Edgar et al., 2011).

Bacterial and fungi classification were determined according to the RDP 16S rRNA and UNITE dataset (Nilsson et al., 2019), with a 50 % confidence threshold (Su et al., 2020). Fungal functional groups, including soil decomposers (saprotrophic fungi), arbuscular and ectomycorrhizal fungi, were identified using fungal zOTU tables and FungalTraits database (Põlme et al., 2020). The diversity of bacterial and fungal functional groups was determined using MiSeq platform (2 × 300 PE) (Illumina, San Diego, California, United States). Co-occurrence networks of bacterial and fungal communities were constructed using the microbial OTUs tables. Based on spearman's (r > 0.7) rank correlation between every two OTUs, we constructed microbial co-occurrence networks in which nodes represented OTUs, and edges represented significant correlations between two nodes (p < 0.05). The network-level topological characteristics were quantified for a set of parameters concluding average degree (Tables S1–S3).

2.6. Soil enzyme activity and microbial C use efficiency

Enzyme activities involved in C, N and P cycling included soil β-glycosidases, βG; peroxidase, PER; β-1, 4-*N*-acetylglucosaminidase, NAG; and acid phosphatase, AP, which were measured according to the method of Saiya-Cork et al. (2002) and Su et al. (2020). Enzyme measurements began within 48 h after soil samples were collected. The data and method description of C degrading enzyme is similar to our prior study (Fig. S4; Liu et al., 2021), we thus only exhibit the data of N and P degrading enzyme in the main results to explore mycorrhizal effects on soil nutrient cycle in the older forests.

Ecoenzymatic vector length (Vlength, relative C: nutrient acquiring ratio) and angle (Vangle, relative P: N acquiring ratio) were calculated following the methods of Feng et al. (2021) to illustrate the strategy of microbial resource acquisition. Higher eco-enzymatic Vlength suggested higher microbial C vs. nutrient acquisition strategies, and higher eco-enzymatic Vangle indicated higher microbial P vs. N acquisition efforts. Eco-enzymatic Vlength and Vangle were calculated following Eqs. (2) and (3), respectively:

$$\text{Vlength} = \sqrt{x^2 + y^2} \quad (2)$$

$$\text{Vangle (degree)} = \text{degrees (atan2(x, y))} \quad (3)$$

where atan2 represents the four-quadrant inverse tangent, x and y represent the relative C:P and C:N acquiring enzyme ratios, respectively.

Microbial C use efficiency (CUE) was calculated indirectly through the biogeochemical equilibrium model (Eqs. (4)–(6), Feng et al., 2021).

$$CUE = CUE_{max} \times \sqrt{\frac{S_{C:N} \times S_{C:P}}{(K_{C:N} + S_{C:N}) \times (K_{C:P} + S_{C:P})}} \quad (4)$$

$$S_{C:N} = M_{C:N}/L_{C:N}1/EEA_{C:N} \quad (5)$$

$$S_{C:P} = M_{C:P}/L_{C:P}1/EEA_{C:P} \quad (6)$$

where $EEA_{C:N}$ was calculated as ln (βG)/ln(NAG), and $EEA_{C:P}$ was calculated as ln (βG)/ln(AP). Molar C:X ratios of labile substrates were used to estimate $L_{C:N}$ and $L_{C:P}$. Labile C, N and P were DOC, inorganic N (the sum of NH_4^+-N and NO_3^--N) and available P, respectively. $M_{C:N}$ and $M_{C:P}$ were the molar ratios of microbial biomass C:N and C:P, respectively. CUE_{max} was maximum microbial growth efficiency (fixed to 0.60) based on thermodynamic constraints and saturating Michaelis-Menten formulation. $K_{C:X}$ was the half-saturation constant and fixed to 0.50.

2.7. Plant community structure and diversity

Plant community structure and diversity was investigated in August 2015. Species richness was estimated by counting the total number of species of trees and shrubs in each 10 × 10 m plot. We also classify tree species into arbuscular and ectomycorrhizal ones, and calculate the ratio of basal area of AM to EcM trees (AM/EcM.basal.area).

2.8. Soil organic carbon accrual rate

Five soil samples at the depth of 0–10 cm were taken with PVC cylinders (diameter: 10 cm, length: 20 cm) per subplot during the growing season (August), 2004, 2014, and 2018. Plant residues and screes were removed from soil samples using tweezers in the laboratory. Five soil samples in each subplot were mixed to obtain one composite sample and then naturally dried at room temperature. Next, 50 g of air-dried soils were taken from each composite sample and sieved to 0.149 mm. Soil organic C was then measured using the Walkley–Black wet digestion method. SOC accrual rates at different successional stages were then calculated by subtracting SOC concentration in 2018 from those in 2004 dividing by the sampling interval time of 14 years.

2.9. Data analysis

Linear mixed-effects models (LMMs) analysis was used to test the effects of forest succession and root-mycorrhizae exclusion treatments on soil CO_2 efflux, C mineralization, nutrient concentrations, soil microbial community and richness, microbial C: N: P stoichiometry and soil enzyme activities, with plot replicate as the random factor, using the '*nlme*' package in *R* software (Tables S4 and S5; Pinheiro et al., 2012). Pairwise comparisons were tested using the lsmeans function in '*lsmeans*' package with a Tukey's adjustment of *p*-values (Lenth, 2016). We also used one-way analysis of variance (ANOVA) to test successional effects on SOC accrual rates, soil respiration components, EcM richness and AM richness, followed by a LSD (Least Significant Difference) test, which was used to compare variables among three successional stages.

Structural equation model was used to analyze the direct and/or indirect relationships among the main factors in explaining the variation in SOC accrual rate across succession (the piecewiseSEM package in *R*, Lefcheck, 2016). Principal component analysis (PCA) was first used to reduce the number of variables associated with microbial enzyme activity, including C-, N- and P-degrading enzyme. We then used the data of the first principal component (PC1) for the structural equation model analysis to represent microbial enzyme activity. The goodness of SEM fit was evaluated using Fisher's C statistic, Akaike information criterion (AIC), and the whole-model *P* value. Relative importance analysis was used to explore the main factors that controlled soil CO_2 efflux (the *rfPermute R* package, Jiao et al., 2018), and the ranking method was based on mean square error (%IncMSE).

We also conducted principal component analysis of stand characteristics and microbial traits. Stand characteristics include plant richness, the ratio of basal area of AM to EcM trees (AM/EcM.basal.area), SOC, and inorganic nitrogen concentration (SIN). Microbial traits include microbial biomass carbon (MBC), microbial C use efficiency (CUE), specific microbial respiration (qCO_2), arbuscular mycorrhizal fungal PLFAs (AMF.PLFAs) and richness (AM.richness), ectomycorrhizal richness (EcM.richness), saprotrophic fungal richness (sapro.fungal.richness), microbial respiration (Microbial.resp), and EcM and saprotrophic fungal PLFAs (EcM.and.sapro.PLFAs), β-glycosidases (βG), B-*N*-acetylglucosaminidase (NAG), peroxidase, and acid phosphatase (AP). qCO_2 was calculated as the ratio of soil C mineralization to MBC stock. Data of AMF.PLFAs and EcM.and.sapro.PLFAs was derived from Liu et al. (2021). Statistical analyses were conducted in *R* software (*R* core team, version 4.1.1) and SPASS. Figures were drawn with *R* software (*R* Core Team, 2019).

3. Results

3.1. Changes in C stocks and mycorrhizal communities across succession

Soil organic carbon (SOC) accrual rate from 2004 to 2018 at the depth of 0–10 cm was determined in three successional forests by their SOC difference divided by 14 years. SOC accrual rate was 1.26 mg g^{-1} yr^{-1} at the early-successional stage, which was higher than that at the mid-successional (0.29 mg g^{-1} yr^{-1}) and late-successional stage (non-significant change, Fig. 1a). Along succession with vegetation changes from arbuscular mycorrhizal (AM)- to ectomycorrhizal (EcM)- related forests, EcM fungal richness increased, but AM fungal richness decreased (Fig. 1b, e). SOC accrual rate was negatively correlated with EcM fungal richness, but positively correlated with AM fungal richness (Fig. 1c, f).

3.2. Soil respiration and C mineralization

Mycorrhizae–induced changes in soil CO_2 efflux were calculated by subtracting respiration rates in both root and mycorrhizal hyphae exclusion treatment (only soil saprotrophic growth, *Soil*) from those in the root exclusion treatment (mycorrhizal fungal and saprotrophic growth, *Myc*), which differed at three successional stages. Mycorrhizal growth suppressed soil CO_2 efflux by 16.8 % at the early-successional stage, but accelerated it by 15.4 % and 27.7 %, respectively, at the mid- and late-successional stages (Fig. 2a–c). Similarly, mycorrhizal growth suppressed soil C mineralization by 26.9 % at the early-successional stage, but accelerated it by 10.1 % and 21.4 %, respectively, at the mid- and late-successional stages (Fig. 3a). Respiration rates from saprotrophic microbes (R_{soil}) and soil C mineralization ($C\text{-}min_{soil}$) in the *Soil* plots decreased along forest succession, while the sum of mycorrhizal and saprotrophic respiration (R_{myc}) and soil C mineralization ($C\text{-}min_{myc}$) in the *Myc* plots increased (Figs. 2d, e and 3a). R_{myc} and $C\text{-}min_{myc}$ was positively correlated with EcM fungal richness, but negatively with AM fungal richness (Fig. 2f–i, $P < 0.05$). Over forest succession, SOC accrual rate was positive correlated with $C\text{-}min_{soil}$ (Fig. 3b), but negatively correlated with R_{myc} and $C\text{-}min_{myc}$ (Figs. 2f and 3c).

3.3. Soil decomposers, enzyme activity and microbial carbon use efficiency

Forest succession and root-mycorrhizal exclusion treatments significantly affected soil microbial composition along forest succession (Fig. 4a, d). Specifically, soil bacterial and fungal richness in the *Root* + *Myc* plots (i.e., natural control) was lower in the early-successional

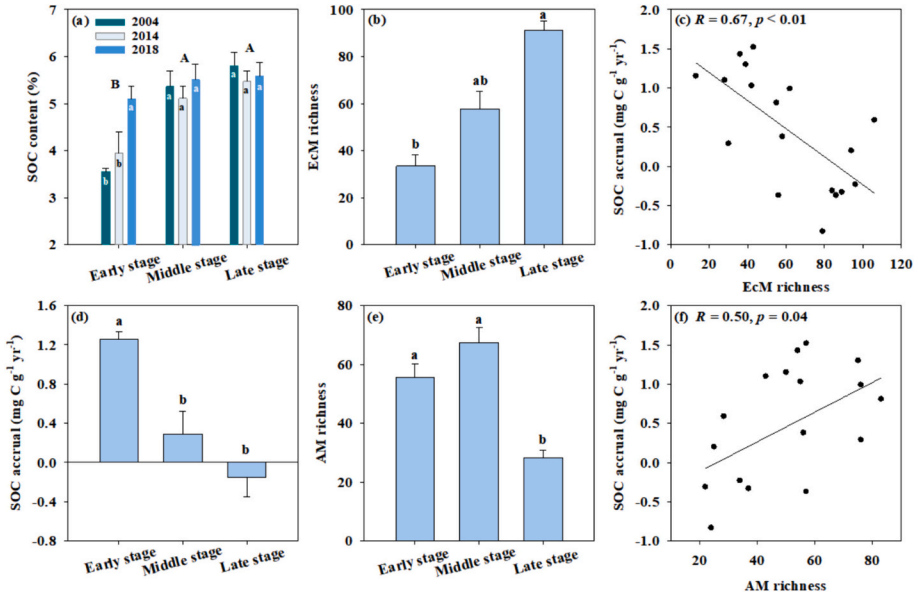

Fig. 1. Long-term changes in soil C pool at different successional stage and their correlation with mycorrhizal diversity. In subfigure (a), bars with different color represent soil organic carbon concentrations in different years. Different lowercase and capital letters above bars show significant effects of sampling year and forest succession, respectively, $P < 0.05$. In subfigure (e) and (f), EcM richness: ectomycorrhizal fungal richness, AM richness: arbuscular mycorrhizal fungal richness.

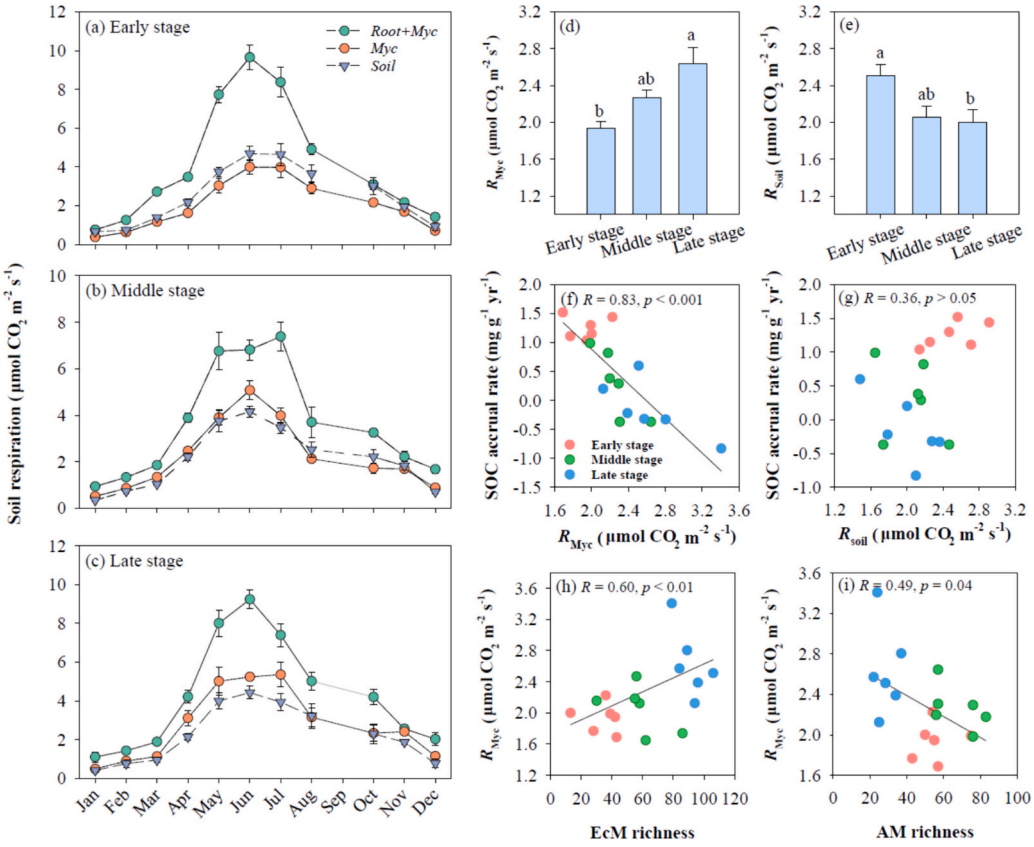

Fig. 2. Soil respiration components (a–c, h and i) and their correlation with soil C accrual rate (d and e) and mycorrhizal richness (f and g) across succession. *Root* + *Myc* treatment: natural control, allow the entry of roots and mycorrhizal fungi; *Myc* treatment: exclude roots and allow the entry of mycorrhizal fungi; *Soil* treatment: the exclusion of roots and mycorrhizal fungi. In subfigures a–c, R_{myc} represents the soil CO_2 efflux in *Myc* plots, R_{Soil} the soil CO_2 efflux in *Soil* plots.

forests than those in the mid- and late-successional ones (Fig. 4a, d; $P < 0.05$). Root exclusion (*Myc* vs. *Root* + *Myc* treatments) significantly decreased soil fungal richness and saprotrophic fungal richness by 31.6 % and 22.3 %, but mycorrhizal exclusion (*Soil* vs. *Myc* treatments) increased them by 52.3 % and 32.6 %, respectively, in the early-successional forests (Figs. 4d and S2; $P < 0.05$). Root and mycorrhizal exclusion decreased fungal richness and saprotrophic fungal richness in both mid- and late-successional stages (Figs. 4d and S2), but did not

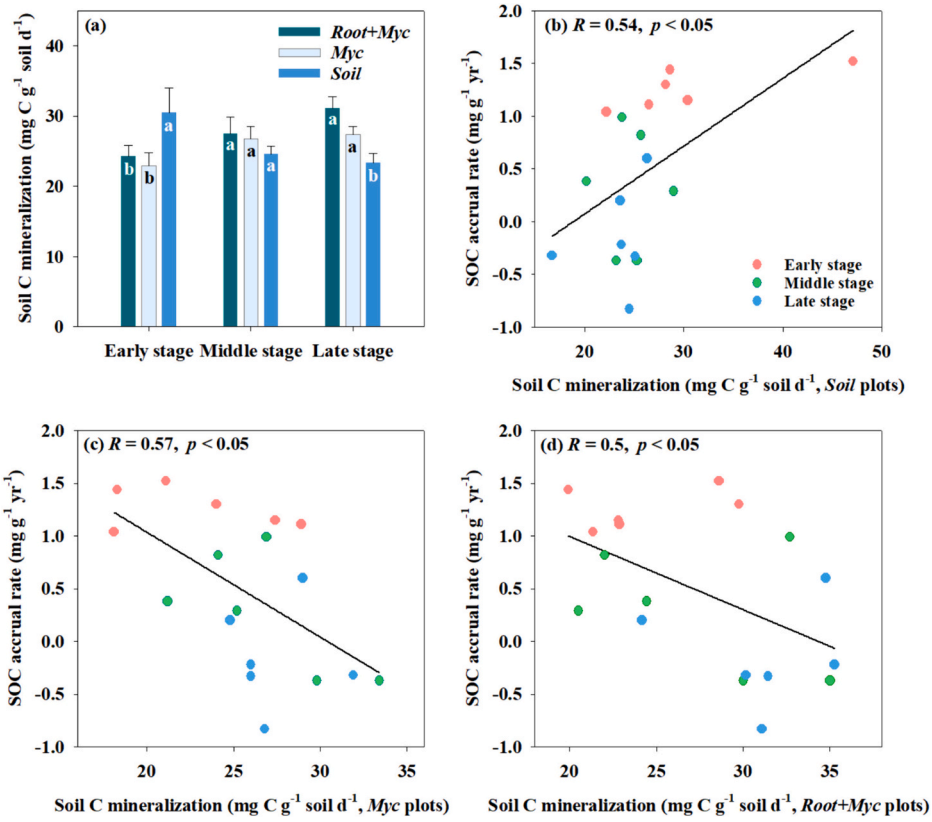

Fig. 3. Effects of plant root and mycorrhizal exclusion on soil C mineralization (a) and their correlation with soil C accrual rate (b–d). *Root* + *Myc* treatment: natural control, allow the entry of roots mycorrhizal fungi; *Myc* treatment: exclude roots and allow the entry of mycorrhizal fungi; *Soil* treatment: the exclusion of roots and mycorrhizal fungi. Different lowercase and capital letters above bars show significant effects of treatments and forest succession, respectively, $P < 0.05$.

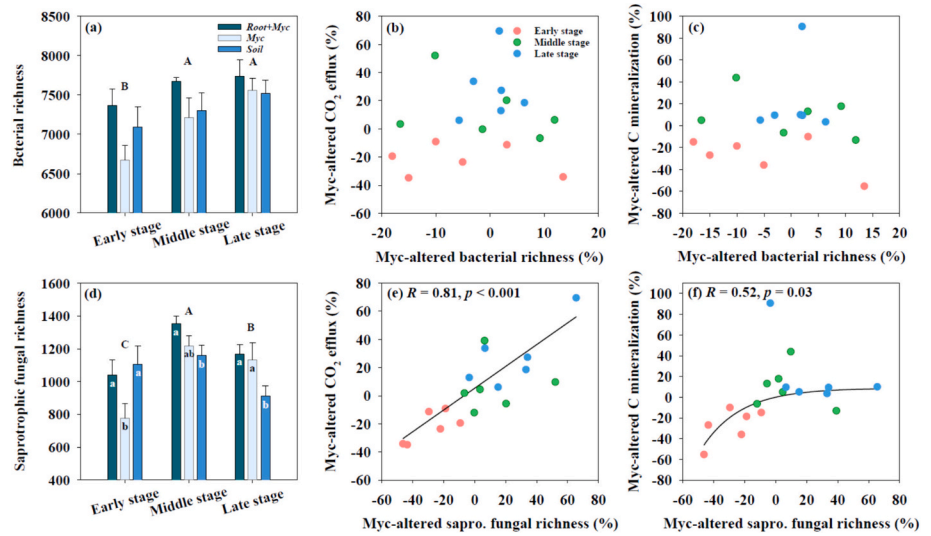

Fig. 4. Effects of plant root and mycorrhizal exclusion on bacterial and saprotrophic fungal richness (a and d) and their correlation with mycorrhizae altered CO_2 efflux (b and e) and C mineralization (c and f) across succession. *Root* + *Myc* treatment: natural control, allow the entry of roots mycorrhizal fungi; *Myc* treatment: exclude roots and allow the entry of mycorrhizal fungi; *Soil* treatment: the exclusion of roots and mycorrhizal fungi. *Myc*-altered bacterial richness and sapro.fungal richess indicate mycorrhizal growth induced changes in soil bacterial richness and saprotrophic fungal richness. Different lowercase and capital letters above bars show significant effects of treatments and forest succession, respectively, $P < 0.05$. Vertical bars represent the standard error.

change bacterial richness across forest succession (Fig. 4a; $P > 0.1$). Mycorrhizal effects on soil CO_2 efflux and C mineralization were tightly correlated with mycorrhizal growth-induced changes in soil saprotrophic fungal richness, but not with the changes in soil bacterial richness (Fig. 4).

Both root and mycorrhizal exclusion treatments (*Soil* vs. *Root* + *Myc*) significantly decreased acid phosphatase (AP), β-1, 4-*N*-acetylglucosaminidase (NAG), and enzyme N:P ratio in the late-successional forests (Fig. 5a–c, $P < 0.05$). Microbial C use efficiency (CUE), which was calculated based on enzyme activity, decreased along forest succession

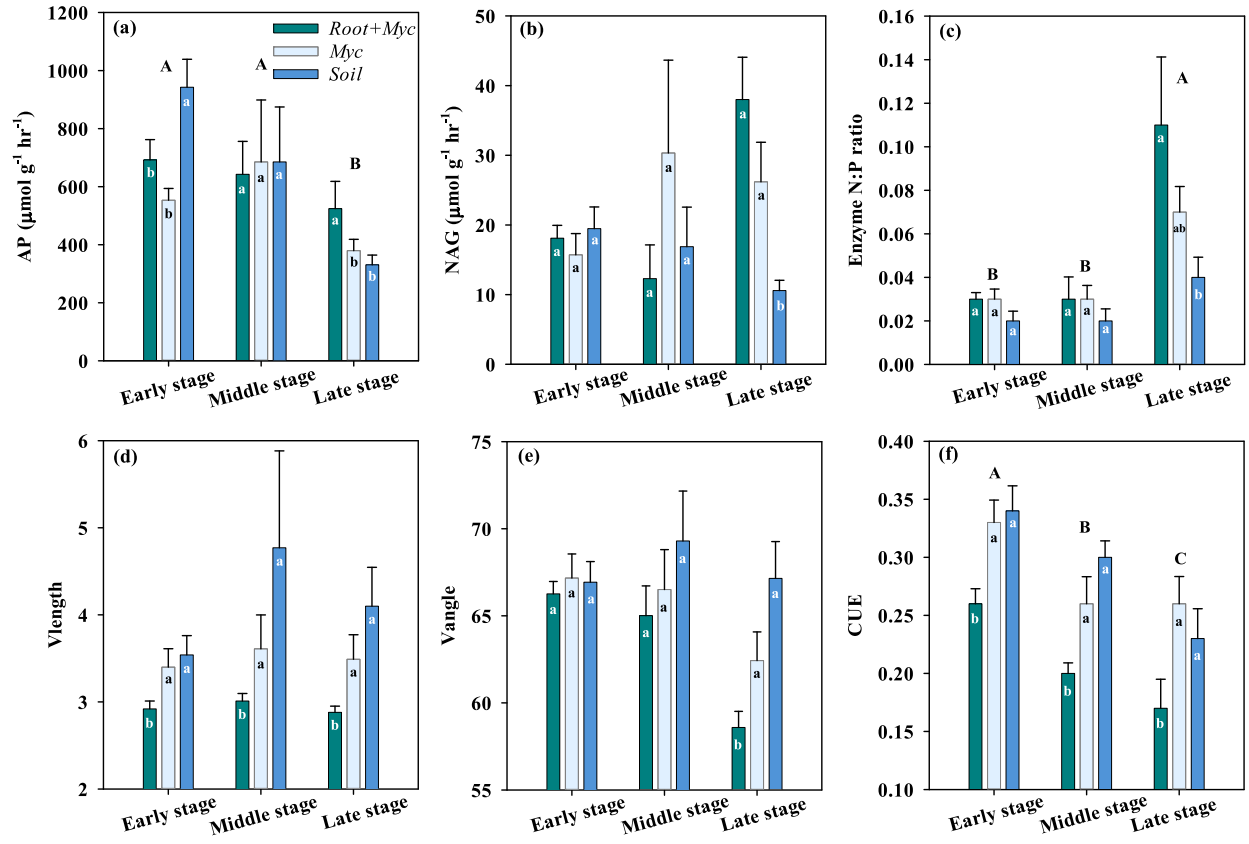

Fig. 5. Effects of plant root and mycorrhizal fungi on nitrogen and phosphorus degrading enzyme activity (a–b), enzyme N: P ratio (c), Vlength (d), Vangle (e) and CUE (f) across succession. *Root* + *Myc* treatment: natural control, allow the entry of roots mycorrhizal fungi; *Myc* treatment: exclude roots and allow the entry of mycorrhizal fungi; *Soil* treatment: the exclusion of roots and mycorrhizal fungi. AP: acid phosphatase, NAG: β-1, 4-*N*-acetylglucosaminidase. Different lowercase and capital letters above bars show significant effects of treatments and forest succession, respectively, $P < 0.05$. Vertical bars represent the standard error.

(Fig. 5f). Eco-enzymatic Vlenth (i.e., relative C: nutrient acquiring ratio) and Vangle (relative P: N acquiring ratio) showed no significant difference among three successional stages (Fig. 5d, e). Across forest succession, root exclusion (*Myc* vs. *Root* + *Myc* treatment) significantly increased Vlength and CUE (Fig. 5d, f). Root exclusion (*Myc* vs. *Root* + *Myc* treatment) also increased Vangle in the late-successional forests,

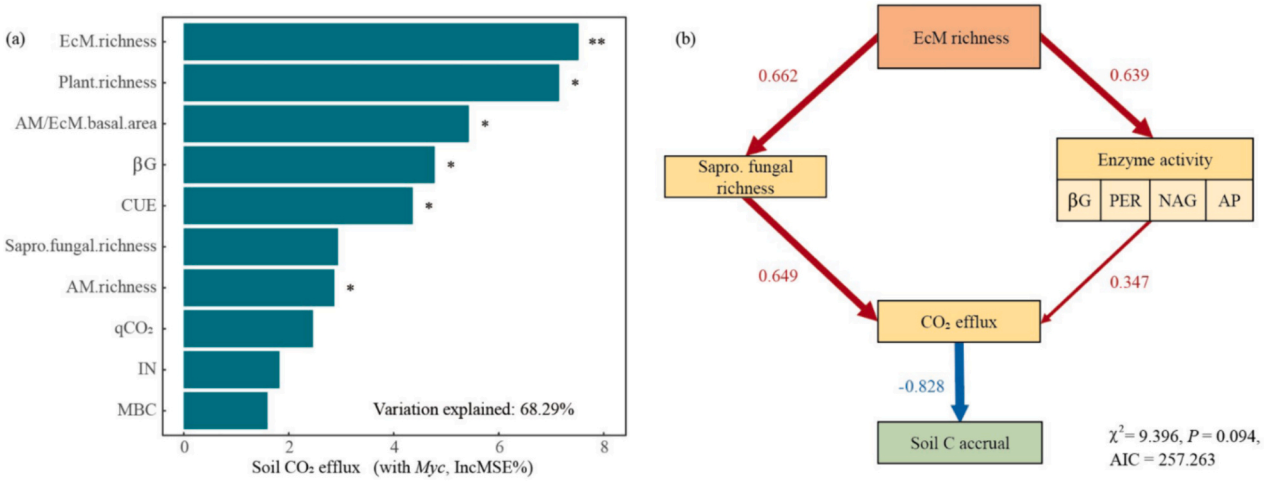

Fig. 6. Relative contribution of stand characteristics and microbial traits to the variation of soil CO_2 efflux (a) and structural equation model explaining the contribution of different factors to the variation of soil C accrual across succession (b). Stand characteristics include plant richness, the ratio of basal area of AM to EcM trees (AM/EcM.basal.area), soil organic carbon concentration (SOC), and inorganic nitrogen concentration (IN). Microbial traits include microbial biomass carbon (MBC), microbial C use efficiency (CUE), specific microbial respiration (qCO_2), arbuscular mycorrhizal fungal PLFAs (AMF.PLFAs) and richness (AM.richness), ectomycorrhizal richness (EcM.richness), saprotrophic fungal richness (sapro.fungal.richness), β-glycosidases (βG), B-*N*-acetylglucosaminidase (NAG), peroxidase, and acid phosphatase (AP). In subfigure (b), sapro.fungal richness is the data of mycorrhizal induced changes in saprotrophic fungal richness, CO_2 efflux indicated the respiration in the *Myc* plots. The red line indicated a positive correlation between two variables, while the blue ones indicated a negative correlation. The thickness of the lines represent the size of the correlation.

but did not change it in the early- and mid-successional ones (Fig. 5e). Three soil enzyme activities (βG, peroxidase, and NAG activity) and CUE were significantly correlated with EcM fungal richness, but not with AM fungal richness (Fig. S8).

3.4. Factors affecting soil C efflux and SOC accrual

Using structural equation model, we found that SOC accrual rates were directly affected by soil CO_2 efflux, and indirectly by mycorrhizal richness and decomposer activity and enzyme activity across forest succession (Fig. 6b). Effects of mycorrhizal richness on soil CO_2 efflux were mainly dependent on the extent to which mycorrhizae altered saprotrophic fungal richness and enzyme activity (Fig. 6b). In addition, principle component analysis (PCA) showed that respiration rates in the *Myc* plots (R_{myc}) was positively correlated with plant richness, βG and NAG activities, microbial biomass C (MBC), and soil available nitrogen (SIN), and negatively with AMF PLFAs, EcM and saprotrophic fungal PLFAs, and AM: EcM basal area (Fig. S6). Moreover, random forest analysis revealed that among these factors, EcM fungal richness was the most important driver for soil CO_2 efflux from saprotrophic and mycorrhizal microbes across forest succession (Fig. 6a).

4. Discussion

Understanding how mycorrhizal community varies across forest succession and potentially affects soil C dynamics is crucial for better predicting long-term C sequestration (Clemmensen et al., 2013, 2015). In this study, we found that a transition of mycorrhizal community from early-successional AM to late-successional EcM taxa elucidates the changes in SOC accrual rate during forest rewilding. Ectomycorrhizal fungi increase saprotrophic fungal richness and SOC decomposition, leading to a slow-down in the capacity of old-aged forests to sequester soil C (Figs. 3a and 4d). These findings highlight the importance of mycorrhizal community in regulating old-aged forest C cycling, which should be considered to apply in the future forest management.

As dominant tree species shift from AM to EcM ones during forest succession, soil EcM fungal richness increased but AM fungal richness decreased (Fig. 1b, e). Our results revealed that EcM fungal richness was the best predictor of soil CO_2 efflux from saprotrophic and mycorrhizal microbes during subtropical forest rewilding (Fig. 6). In the late-successional forests, the presence of ectomycorrhizal hyphae promotes soil CO_2 efflux by 27.7 % and C mineralization by 21.4 %, respectively (Figs. 2 and 3). More importantly, across forest succession, SOC accrual rate was negatively correlated with soil CO_2 efflux and C mineralization in the *Root* + *Myc* and *Myc* subplots, but not or positively correlated with those in the *Soil* subplots (Figs. 2f,g and 3b–d). These results suggest that mycorrhizal-induced priming effects on SOC decomposition might halt SOC accrual in the older EcM stands. Notably, the changes in C input across succession could strongly affect SOC accrual. However, in our previous study (Liu et al., 2022), we found that C input from litterfall increased with forest succession, and it is hard to explain the slowdown in soil organic carbon accrual in the late-successional forests. Therefore, we attributed the changes in SOC accrual rate to the decomposition.

The extent to which mycorrhizal hyphae affect soil C decomposition largely depend on the altered saprotrophic fungal richness and enzyme activity (Fig. 6b). By root and mycorrhizal exclusion experiments, we found that EcM fungi increased saprotrophic fungal richness by 5 % ~ 24 % at the mid- and late-successional stages, but decreased it by 32.6 % at the early stage (Fig. 4d). The changes in saprotrophic fungal richness were tightly correlated with mycorrhizal respiration and mycorrhiae-altered C mineralization (Fig. 4e, f). We further found that ectomycorrhizal fungi promoted the assembly and collaboration of saprotrophic fungi (indicated by increased positive edges in microbial network analysis), suggesting mycorrhizae-saprotroph interaction in the older stands (Table S2). Moreover, EcM hyphae promote soil nutrient cycle by increasing N- and P-degrading enzyme activity at the later stage of forest development. We also observed positive relationships of EcM fungal richness with the change in the extracellular enzymes βG, PER and NAG (Fig. S8). Greater saprotrophic and enzyme activity by EcM hyphea promote SOC decomposition in the older stands. These results were consistent with other tropical/subtropical studies (Gui et al., 2017; Wang et al., 2016), but opposite with negative effects of EcM fungi on SOC decomposition in temperate and boreal forests (Leifheit et al., 2015; Verbruggen et al., 2016). Several studies pointed out that the direction and extent to which mycorrhizal fungi significantly affected SOC decomposition depends on soil N condition (Fernandez and Kennedy, 2016). High levels of N in our low-latitude subtropical ecosystems may weaken EcM fungi-saprotroph competition and increase SOC decomposition, while the limited N condition in high-latitude ecosystem might lead to the opposite results (Averill et al., 2016).

In the early-successional AM dominant stands, mycorrhizal fungal growth has a negative impact on soil microbial activity and C decomposition (Figs. 2a, 3a and S3a), which might be attributed to AMF-saprotrophic competition for the limited soil nutrients (Averill et al., 2014, 2016; Sulman et al., 2017). In fact, our prior study found that the limited N in soil drove the mycorrhizae-saprotroph competition at the early stages of forest succession (Liu et al., 2021). However, mycorrhizae growth decreased soil acid phosphatase (AP) activity in these stands (Fig. 5a), suggesting that the phosphorus (P) competition may be also occurring. Mycorrhizal fungi negatively affected microbial diversity at the early stages of forest succession, with stronger effects on fungal diversity than bacterial ones (Figs. 4 and S2). These changes were probably attributed to the following two reasons. First, the population of soil saprotrophic fungi are often limited by soil nutrients, especially at the nutrient-limited early stage of forest succession (Liu et al., 2021). In contrast, bacterial community is highly limited by soil C availability (Dorado-García et al., 2015; He et al., 2020). Mycorrhizal forage of soil available nutrients usually aggravates the nutrient limitation of saprotrophic fungi, while the C inputs by mycorrhizal fungi might stimulate the growth of soil bacterial community (Averill et al., 2016; Qin et al., 2016). Second, the niche overlap of mycorrhizal with saprophytic guilds was greater than those with bacterial community, likely causing strong mycorrhizae-saprotroph competition (Fernandez and Kennedy, 2016).

Interestingly, plant roots likely dampen the effects of mycorrhizal fungi on soil microbial population and diversity, but slightly influence soil enzyme activities at the early-successional stage (Figs. 4, 5, S3 and S4). Roots can release various energy-enriched compounds through exudation to increase rhizosphere microbial population and activity (Chaparro et al., 2014; Finzi et al., 2015), while the individual roles of plant roots and mycorrhizal fungi are rarely quantified (Meier et al., 2015). Moreover, we observed that the C inputs from plant roots decreased microbial C use efficiency (CUE, Fig. 5f) across forest succession. High-energy C compounds released from roots would promote the metabolic activity of microorganism, resulting in higher distribution of respiration C by soil microorganism (Chen et al., 2020; Tang et al., 2021). In the rhizosphere, microbes also produce more nutrient-degrading enzyme than C-degrading ones, causing the decrease in vector length of enzyme activity (Vlength, Fig. 5d). Based on the random forest analysis, we found that variables related to soil N and P supply (soil inorganic N, VP, MBC: MBP ratio and MBN: MBP) were the primary abiotic factors governing soil CUE (Fig. S5), suggesting the potential N and P limitation for soil microorganisms in subtropical forests.

In summary, our results showed that ectomycorrhizal fungi promote SOC decomposition and halt soil C sequestration in old-aged subtropical forests, suggesting that mycorrhizal community and diversity play vital roles in explaining soil C dynamics across subtropical forest succession. However, previous studies have pointed out that soil C decomposition might be regulated by certain species of ectomycorrhizal fungi such as *Cortinarius acutus* (Lindahl et al., 2021). In this study, some ectomycorrhizal fungi, including genus *Cortinarius* and *Cenococcum*, increased with forest succession (Fig. S7), but it is difficult to quantify their effects on decomposition and SOC accrual. Future studies that use the

techniques of ^{13}C isotope tracer and DNA sequencing are urgently needed to explore the underlying mechanism regulating individual ectomycorrhizal effects on microbial C consumption. Moreover, we found that plant roots dampen the effects of mycorrhizal fungi on soil saprotrophic population and diversity in the early-succession forests with low nutrient condition, but interact with mycorrhizal fungi to increase saprotrophic fungal richness in the late-successional forests with high nutrient condition (Figs. 4 and 5). These findings suggest that next generation Earth systems models should explicitly incorporate the influence of interactions among roots, mycorrhizas and saprotrophic microbial communities on ecosystem biochemical cycle to predict future C-climate change feedback.

5. Conclusion

A transition of mycorrhizal community from early-successional arbuscular mycorrhizal (AM) to late-successional ectomycorrhizal (EcM) taxa elucidates the changes in SOC accrual rate during forest rewilding. Soil organic C at the top soil layer accumulate rapidly in early-successional AM forests, but change slightly than that in the late-successional EcM forests. The presence of ectomycorrhizal hyphae promotes SOC decomposition and nutrient cycle by increasing soil nitrogen and phosphorus degrading enzyme activity as well as saprotrophic microbial richness in the late-successional forests. Ectomycorrhizal priming effects on SOC decomposition explain the slow-down in the capacity of old-aged forests to sequester soil C.

CRediT authorship contribution statement

Ruiqiang Liu: Writing – original draft, Methodology, Investigation. **Xuhui Zhou:** Supervision, Funding acquisition, Conceptualization. **Yanghui He:** Investigation. **Zhenggang Du:** Investigation. **Hongyang Chen:** Investigation. **Yuling Fu:** Supervision. **Liqi Guo:** Investigation. **Guiyao Zhou:** Investigation. **Lingyan Zhou:** Investigation, Formal analysis. **Jie Li:** Methodology, Investigation. **Hua Chai:** Investigation. **Changjiang Huang:** Methodology, Investigation. **Manuel Delgado-Baquerizo:** Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.174330.

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