Understory ferns removal downregulates microbial carbon use efficiency and carbon accrual in previously degraded lands

Cui Deng [a], Maokui Lyu [a b], Xiaoling Xiong [a], Josep Peñuelas [c d], Jordi Sardans [c d], Xiaojie Li [a], Weisheng Lin [a b], Yusheng Yang [a b], Jinsheng Xie [a b]

[a] Key Laboratory for Humid Subtropical Eco-Geographical Processes of the Ministry of Education, Fujian Normal University, Fuzhou 350007, China
[b] Sanming Forest Ecosystem National Observation and Research Station, Fujian Normal University, Fuzhou, 350007, China
[c] Global Ecology Unit CREAF-CSIC-UAB, CSIC, 08913 Cerdanyola del Vallès, Catalonia, Spain
[d] CREAF, 08913 Cerdanyola del Vallès, Catalonia, Spain

* Corresponding authors: 228lmk@163.com (M.L); jshxie@163.com (J.X)

Abstract

When reforesting degraded lands, understory ferns can take root and cover the forest floor, which can change the quality and quantity of litter added to soils. However, it is unclear how ferns and the ongoing succession of trees impact soil organic carbon (SOC) dynamics. Here, we established experimental sites in Masson pine (Pinus massoniana) stands for understanding the underlying mechanisms of how ferns affect SOC stock and soil respiration (Rs) in subtropical China. The established three pine stands covered with ferns (Dicranopteris dichotoma (Thunb.) Berhn.) were used to represent a 37-year restoration sequence: two restored sites with vegetation cover (VC) established in 1984 and 2002 (referred to VC-1984 and VC-2002), and an unrestored site (VC-0). In August 2014, two treatments were set i.e., ferns were retained in half area of the sites ("control"), and continuously removed ferns from the other half ("fern removal") for seven years. Our results showed that fern removal significantly decreased Rs by 25.8% in VC-2002 compared to the control over a three-year period from 2015 to 2018, but did not significantly affect Rs in VC-0 and VC-1984. We found that fern removal decreased fine root biomass by 50.4% in VC-0, 31.4% in VC-2002, and 54.7% in VC-1984 in 2019, with seven-year averaged SOC stocks decreased by 37.2%, 13.9%, and 22.7% in VC-0, VC-2002, and VC-1984, respectively. Moreover, fern removal significantly increased carbon- and nitrogen-degrading enzyme activities and decreased microbial carbon use efficiency, suggesting that fern removal may increase SOC decomposition. Taken together, we concluded that fern removal may increase SOC-derived CO2 efflux counteracting the reduced root respiration in VC-0 and VC-1984 but not in VC-2002, leading to a divergent response of Rs to understory fern removal. Our study emphasizes

the necessity of incorporating understory plants regulates SOC decomposition and accumulation in assessing forest recovery effects on soil carbon cycling

***Keywords*:** Vegetation cover, Degraded ecosystems; Soil respiration; Understory ferns; Soil carbon sequestration

1. Introduction

Preserving and restoring the subtropics and tropics' rich forest C stocks has been increasingly considered as a valuable and efficiency way to rapidly remove CO_2 from the atmosphere (Griscom et al., 2017; Griscom et al., 2020; Koch and Kaplan, 2022). In the past decades, deforestation lead to extensive land degradation, loss of biodiversity and soil carbon (C) Ekblad & Bastviken, 2019; Drake et al., 2019; Pearson et al., 2017). In contrast, afforestation is of great significance to reduce atmospheric carbon dioxide by increasing soil C (Xie et al., 2013; Huang et al., 2018; Lyu et al., 2019a), and it is necessary to implement on degraded soils and thereby benefiting the recovery of degraded ecosystems (Lamb et al., 2005; Bai et al., 2008; Cao et al., 2009; Coban et al., 2022). It has been widely consisted that planting trees can improve ecosystems productivity, increase timber production and in future carbon sequestration strategies on a large scale all over the world (China Forestry Administration, 2013; Hartsell and Conner, 2013; Johnsen et al., 2001). For instance, there are 65% of China's carbon sink in southern China has been a reason larger from reforestation (Piao et al., 2009). Single tree species (pure plantations) as the main way of plantation is widely distributed in the world (Richards et al., 2010; Johnsen et al., 2001; Wang et al.,2021). However, it is not clear

59 which forest management or species composition would better promote soil C accumulation
60 in reforestation process (Tong et al., 2020; Morecroft et al., 2019; Huang et al., 2018)
61 Understory vegetation is widely observed in forests and is an important component of
62 forests. However, due to its small biomass they have been often overlooked (Nilsson and
63 Wardle, 2005; Zhao et al., 2012; Wardle et al., 2005; Averill et al., 2014). In fact, we are far
64 from understanding the ecological functions and roles of understorey vegetation. Previous
65 research demonstrated that most understory vegetation play a positively role in ecosystem
66 function like maintaining biodiversity, increasing biomass, forest regeneration, microclimate
67 regulation, soil and water conservation, nutrient cycling, and soil C sequestration in forest
68 ecosystems (Nilsson and Wardle, 2005; Negishi et al., 2006; Lyu et al., 2019a; Zhao et al.,
69 2012; Chen et al., 2016), while simultaneously, which have shown that a thick dense layers
70 forming, such as fern or mosses, hinders the growth of tree species, forest succession, and soil
71 organic matter decomposition and soil nutrients mineralization (Nilsson and Wardle, 2005;
72 Royo and Carson, 2006; Young et al., 2010). This suggests that the ecological functions and
73 roles performed by different understory vegetation types can be distinct in different regions
74 and forest types. It is well recognized that the understory ferns (Pteridophyta), a photophilous
75 plant which often occurs after canopy disturbance, fires and insect outbreaks, are widespread
76 throughout the world (Zhao et al., 2012; Pteridophyte Phylogeny Group, 2016; De et al.,
77 2002; Royo and Carson, 2006),. The genus of the family *Dicranopteris* (Gleicheniaceae)
78 accounts for about 96% of the global distribution in the tropics and subtropics (gbif.org), and
79 these ferns species generally form single-species dominant community widely distributed in
80 pure forests (Royo and Carson, 2006; Zhao et al., 2012; Yang et al., 2021), particularly the

low canopy conifer forests like pine plantations (De et al., 2002; Lyu et al., 2019a; Chen et al., 2016). Although many researches being longer focused on the fern over the last few decades, which appears to be negative in its ecological function during succession with improved conditions (Royo and Carson, 2006; Young et al., 2010), yet it can survive in environments with poor soils (Lyu et al., 2019a; Chen et al., 2016), the implications of understory fern are not clear.

Vegetation changes are often associated with changes in environmental factors and vegetation community (the input of litter quantity and quality) which will affect soil respiration and eventually soil carbon sequestration (Raich and Tufekcioglu 2000; Ward et al., 2015; Suseela et al., 2012; Metcalfe et al., 2011; Raich et al., 2002). The expansion of understory vegetation into newly planted forests has been considered to be a potentially C sink at regional and global scales due to the accumulation of C in both biomass and soils (Dean et al., 2015; Lyu et al., 2019a). However, at the ecosystem level, the effects of understory expansion on SOC stocks are often different, with some studies showing that understory vegetation expansion reduce SOC stocks (Strickland et al., 2010; Tamura and Tharayil, 2014). Because the expansion of understory plants equals to adding more litter to soils which in turns targeting more microbial respiration via a phenomenon called priming effect – microbes broke down native SOM to acquire nutrients (Lyu et al., 2018; Lyu et al., 2019b; Feng et al., 2021). Reasons for these inconsistencies among studies are still quite complex and not full understood, but may be related to changes in site nutrients and litter quality and quantity after understory plants expansion (Lyu et al., 2019a, 2019b). For instance, adding litter with contrast quality would prime decomposition of SOM and thus

103 release much CO_2 via microbial respiration (Lyu et al., 2019b), no influences (Lyu et al.,
104 2019c) and suppress decomposition of SOM (Chen et al., 2018b; Wild et al., 2019).
105 Therefore, the direction and magnitude of SOC decomposition response to expansion of
106 understory plants remain largely unknown, study of microbial decomposition is crucial for
107 understanding SOC dynamics and persistence in these newly recovered forest ecosystems and
108 adequate management.
109 In prior work, we found that reforestation associated understory fern expansion increased
110 SOC accumulation, with understory ferns contributed 54-61% of the total SOC stocks,
111 suggesting a profound role for understory ferns in soil C stocks of degraded ecosystems (Lyu
112 et al., 2019a). Possible mechanisms are due to understory ferns can change soil
113 microenvironment, increasing litterfall and root inputs and changing the quality of added litter
114 to soils (Liu et al., 2012). Therefore, ferns expansion can improve soil nutrients and change
115 the structure of soil microbial communities (Busse et al., 1996; Wu et al., 2011; Wu et al.,
116 2014; Lyu et al., 2019a) and thus affects soil C dynamics and other ecosystem services
117 (Coban et al., 2022).
118 In general, pine litter is considered as a low-quality litter with high C/N and lignin/N
119 whereas understory ferns have low C/N and thus been considered as high-quality litter (Lyu et
120 al., 2019a). Consequently, the expansion of understory ferns will make results been much
121 complicated since both litter quantity and quality been changed in previously degraded lands.
122 This raise a question that how the pine and ferns derived litter mixed will affect SOC
123 decomposition? It is increasing known that the high-quality litter can increase microbial
124 decomposition (Castellano et al., 2015; Angst et al., 2021), whereas this part of carbon source

of microbial decomposition (microbial-derived carbon) which in turns benefits long-term soil organic matter accumulation (Angst et al., 2021). However, the single low-quality litter (pine litter) input may lead to prime decomposition of the native SOC via priming effect, helping to explain why pine plantation without understory fern accumulate low density of SOC in previously degraded lands (Lyu et al., 2019a), but this hypothesis has not been tested yet.

In this study, we explore the soil CO_2 input process under a matrix of landscape sites (restored pine plantation in 1984, restored pine plantation in 2002, and open un-restored degraded badlands) to examine the effects of understory plants on SOC decomposition (soil respiration) and accumulation across the restoration sequence on previously degraded lands. Specifically, through analysis of soil abiotic and biotic properties across this matrix of vegetation conditions, and then examined the relative importance of source of plant biomass, soil properties and microbial properties in regulating the variation of soil respiration. We aimed to address two questions: i) How does understory ferns affect soil respiration in the process of vegetation restoration; ii) whether ferns removal changed soil respiration during the ecological restoration process. By using a seven-year understory ferns removal and fern retention experimental site, we test the following hypotheses: (H1) understory ferns removal reduces aboveground and underground C input, total soil respiration (root and soil derived CO_2) would be decreased or constant; and (H2) ferns removal would promote SOC decomposition because single pine-derived low-quality litter input would target a phenomenon called priming effect – stimulating native SOC decomposition by increasing microbial activities to mineralize nutrients and decreasing microbial C use efficiency, which increases CO_2 production and supplements the reduced fern root-derived CO_2. Therefore, we

147 expect that ferns removal would reduce the SOC stocks in comparison to the ferns retention
148 plots.

149 **2. Material and methods**

150 **2.1. Site description**

151 The study was conducted at Hetian Research Station for Erosion (116° 18′ E, 25° 33′ N),
152 which is located at Hetian town of Changting county in western Fujian Province of southern
153 China (Fig. 1a). This region is characterized by a subtropical monsoon climate with warm and
154 humid characteristics. The mean annual precipitation is 1701.4 mm and mean annual
155 temperature is 18.4°C from 1951 to 2006. Soils are classified as easily eroded red soils
156 (Humic Planosols, World Reference Base for Soil Resources, 2014) derived from medium to
157 coarse crystalline granite (Lyu et al., 2019a). Native evergreen broad-leaved forests had been
158 clear-felled in the last one hundred years, which led to severe soil erosion and long-term site
159 degradation. Changing County gradually became one typical region of soil erosion in
160 southern China in past decades. To mitigate land degradation, since the 1980's, the county's
161 government has established plantations on bare land and closed access to hillsides to allow
162 natural regeneration (Cao et al., 2009). Now the dominant vegetation cover (VC) is *Pinus*
163 *massoniana* plantation (Xie et al., 2013; Lyu et al., 2019a).

164 **2.2. Experimental design**

165 We distinguished three vegetation cover conditions in our study area: vegetation cover
166 (VC) type closely approximates the pre-restoration condition (VC-0) and is characterized as
167 degraded shrubland with scattered natural recruits of *P. massoniana*; VC-2002 (established in

2002) and VC-1984 (established in 1984) of *P. massoniana* plantations, respectively (Fig. 1b). The VC-2002 and VC-1984 stands had similar background conditions to that of the VC-0 stand before the implementation of ecological restoration. We have provided solid and clear evidences to demonstrate the similar background of the three stands before restoration in our previous studies (Lyu et al., 2019a). Active restoration leading to VC-2002 and VC-1984 stands included planting pine at a density of 600 trees ha^{-1} followed by a broadcast application of 900 kg ha^{-1} of plant residues (produced during commercial oil extraction of *Camellia oleifera* seed) with the goal of providing some nutrients and improving soil organic matter content. While variable in composition, this amendment provided an estimated average input of 24 kg of N and 4 kg of P per ha to both VC-2002 and VC-1984 stands. Finally, 1050 kg ha^{-1} of calcium magnesium phosphate fertilizer was applied to plantations after planting to meet the nutritional needs of pine seedlings. The VC-0 stand did not receive either the organic matter or fertilizer treatments. Active restoration promotes pine growth and leading to the tree height and diameter breast height increased with restoration years, but ferns did not affect three growth during our seven years treatment period across the three stands (Table S1). The litter C/N of *D. Dichotoma* was 54.06*,* 46.46 and 56.53, for VC-0, VC-2002 and VC-1984 stands respectively. The litter C/N of *P. massoniana* was 158.93, 104.06 and 94.69 for VC-0, VC-2002 and VC-1984 stands, respectively (Fig. S2; Lyu et al., 2019a).

Three experimental plots (20 × 20 m) were established in each stand in August 2014. Each plot was divided into four subplots (10 × 10 m) for treatments. We randomly selected two subplots from each plot as the control (fern retention) and fern removal treatments, each subplot was therefore considered as a replicate. The randomized block design had two levels

for each of the two factors: control and understory fern removal. A 40-cm deep trench was created around each subplot to eliminate the intrusion of roots from the other subplots. understory fern plants in fern removal plots were manually removed (remained the below-ground component) with a machete and any regrowth was removed monthly. Ferns removal did affect soil bulk density whereas increased soil pH values in VC-2002 and VC-1984 stands (Table S1).

2.3. Soil respiration, litter collection and soil incubation

Soil respiration was measured monthly from April 2015 to March 2018 between 9:00 a.m. and 12:00 a.m. with an LI-8100 automated soil CO_2 flux system (LI-COR Inc. Lincoln, NE, USA). To measure soil respiration, three PVC collars (20 cm diameter and 10 cm height) were placed at a depth of 5 cm in each subplot, and small living plants in the soil collars were removed by hand. PVC collars remained fixed throughout the experiment. Soil temperature at 5cm depth was measured by a digital thermometer (SK-250WP, Sato Keir-Yoki, Kanda, Japan). The volumetric soil moisture at 12cm depth were measured by probes (TDR) (Model TDR300, Spectrum Company, Aurora, USA) including three replicates when the respiration was recorded. We measured aboveground litterfall monthly from April 2015 to March 2019 by collecting fallen fine litter of pine in five permanently installed litter traps (0.64 m^2) per subplot. The fern litter collected monthly from five 1 m × 1 m quadrats within each subplot.

Because it is hard to exclude the roots of understory ferns if we using the trench way to measure mineral soil decomposition. Instead, we conducted a field soil incubation experiment by litter bag approach (Fig. S3), to test the effects of ferns removal on mineral soil C decomposition. In detail, we sampled the soil from a nearby secondary forest (coniferous and

broad-leaved mixed forest) then take back and sieved (< 2 mm). Twenty grams sieved soil was placed into each nylon mesh bag (< 1 um) then they were replaced into the soil. All bags were inserted into the previously excavated holes to a depth of 10 cm, after which we applied the litter treatments within each of the three stands. After 6 months, all bags were taken back to the lab for short-term soil C mineralization incubation. This experiment can indirectly show the potential capacity of mineral soil C decomposition in control and ferns removal subplots within each stand.

2.4. Field sampling and laboratory analysis

Soil samples were collected from 0 to 10 cm depth (A horizon) from 2014 to 2021. Ten soil cores (2.5 cm diameter) were randomly taken and then combined into one composite sample for each subplot. Soil samples were sieved (< 2 mm) and stored at 4 °C. A sub-sample of soil was air-dried before being analyzed for soil pH, soil organic C SOC) and soil total N (TN). Soil pH was measured in a soil suspension with deionized water at a ratio of 10 mL to 4 g soil. SOC and TN concentrations were determined using a Vario MAX CN elemental analyzer (Elementar Vario EL III, Germany).

Soil dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were extracted from 10 g of soil (<2 mm) was mixed with 40 ml of de-ionized water at 20 °C, shaken for 1 h and the then centrifuged at 3500 rpm for 20 min. The suspension filtered through a 0.45 μm filter membrane and they were determined using an Elementar TOC analyzer (Liqui TOCC II, Germany).

Fine roots were randomly sampled in soil coring method (5cm diameter, 10cm depth), six soil cores combined as one composite sample. All sample carefully sieved (< 2 mm) to

234 separate the fine roots (< 2 mm). The root samples were oven-dried at 65 °C for 72 h and
235 weighed. The ring-knife method was used to measure the soil bulk density at 0–10 cm of soil
236 depth.
237 To explore the role of microorganisms in regulating total soil respiration and soil
238 mineralisation potential, soil microbial properties were further analyzed, including microbial
239 biomass (MBC, MBN), and enzyme activity (βG, CBH, NAG). The MBC and MBN were
240 measured by the method of chloroform fumigation (Vance et al. 1987). We investigated soil
241 enzymes determined in our study for the mineralization of C, N nutrients in soils, including β-
242 glucosidase (βG, EC3.2.1.21), Cellobiohydrolase (CBH, EC3.2.1.91), Nacetyl
243 glucosaminidase (NAG, EC 3.1.6.1) (Saiya-Cork et al. 2002). The activities of βG, CBH and
244 NAG were assayed using fluorometric techniques. The details are as follows: Sample
245 suspension was prepared by adding 1 g soil to 125 ml of 50 mm acetate buffer with pH 5.0
246 and homogenize it by continuous stirring with magnetic stirrer for 10 min. Take 200 µl of its
247 suspension with a pipette and transfer it to a 96 well microplate. Each sample has 16
248 repetitions for each test. Eight reference standards (200 µl acetate buffer solution plus 50 µl
249 substrate solution), eight blank wells (200 µl Sample suspensions plus 50 µl buffer solution)
250 and eight quench standards (200 µl Sample suspensions plus 50 µl standard solution) were
251 used as controls and corrections for each assay (the standard solution was 10 µM 4-
252 methylumbelliferone (MUB)). The plate was incubated in the dark at 20°C for 3–6.5 h. The
253 reaction was stopped by adding 10 µl of 1.0 M NaOH to each well, depending on the assay
254 requirements. Fluorescence was measured using a microplate fluorophotometer equipped with
255 a 365 nm excitation light and 450 nm emission light filter.

2.5. Data analysis

Microbial CUE was calculated as the following Eqs. (1) and (2):

$$CUE_{C:N} = CUE_{\max}\left[S_{C:N}/\left(S_{C:N}+K_N\right)\right] \quad (1)$$

$$S_{C:N} = \left(1/EEA_{C:N}\right)\left(B_{C:N}/L_{C:N}\right) \quad (2)$$

$S_{C:N}$ is a scalar that indicates the extent to which the allocation of enzyme activity offsets the disparity between the available microbial resource stoichiometry (DOC:DON ratio) and microbial biomass (Sinsabaugh et al. 2016; Chen et al. 2018). CUE_{max} is the upper limit of microbial growth efficiency (0.6) based on thermodynamic constraints. The value of K_N is set to 0.5 (Sinsabaugh et al. 2016). $B_{C:N}$ and $EEA_{C:N}$ is the ratio was calculated as microbial biomass C/N and enzymatic activity of (βG + CBH)/NAG respectively. $L_{C:N}$ is the C:N ratio of labile organic matter (Sinsabaugh et al. 2016; Chen et al. 2018).

Repeated measure ANOVA was used to examine the differences in soil respiration, soil temperature, soil moisture. Two-way ANOVA was used to compare the treatment difference for soil annual flux as well as biotic and abiotic variables. The statistical analyses were conducted using SPSS 22 (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1 Soil C stocks

Across the seven years of ferns removal, the stocks of SOC in control plots increased with years whereas which in Ferns removal plots showing decreased and then increased trends across the three stands (Fig. 2). Ferns removal decreased the SOC stocks by 37% in VC-0 (p=0.047), 14% in VC-2002 (p=0.459) and 23% in VC-1984 (p=0.072) stands on averaged across seven years of ferns removal (Fig. 2).

3.2 Plant aboveground and belowground biomass

For aboveground litterfall, ferns removal significantly decreased 29.2%, 36.9% and 42.0% of the total aboveground litterfall (pine + ferns litter) across the restoration sequence. For the belowground fine root, ferns removal significantly decreased 86%, 46% and 65% of the total belowground fine root biomass (pine + ferns roots) in VC-0, VC-2002, and VC-1984 stands, respectively (Fig. 3). The ferns litterfall accounted for 52%, 80%, and 51% of the pine litterfall in VC-0, VC-2002, and VC-1984 stands, respectively (Fig. 3).

3.3 Soil CO_2 efflux

The soil respiration (R_s) had similar seasonal patterns of Control and Ferns removal plots across three stands. Fern removal did not significant affect R_s in VC-0 and VC-1984 stands across the three years of observation (Fig. 4; Table S2). However, ferns removal had negative effect on R_s in VC-2002 stand ($p = 0.001$) with significant decreases in R_s by 32.8% in the fourth year after ferns removal ($p < 0.05$; Fig. 4d). The effects of ferns removal on R_s were pronounced during June to October in the third year in VC-2002 stand (Fig. 4c).

We measured the microbial C mineralization potential by using litter bag approach, our results showed that microbial C mineralization potential decreased with increasing stand ages both in control and ferns removal plots ($p < 0.001$; Fig. S5). The magnitude of ferns removal impacts on the microbial C mineralization potential increased with increasing stand ages with ferns effects being significantly and positively in VC-1984 stands ($p = 0.043$; Fig. S5). Overall, ferns removal positively and marginal significantly increased the microbial C mineralize potential when all three stands were considered ($p = 0.093$; Fig. S5).

3.4 Soil microbial properties

By measuring the hydrolytic enzymes related to C-acquisition (βG + CBH) and N-acquisition, results showed that ferns removal increased C- and N-degrading enzyme activities across all three stands, both of which showing decreased trends with stand ages within Control and Ferns removal plots (p<0.001; Figs 5a and b). Microbial C use efficiency (CUE) increased with stand ages within control and ferns removal plots (p<0.001; Fig. 5c). Ferns removal significantly decreased microbial CUE in VC-0 and VC-1984 stands but nonsignificant in VC-2002 stand (Fig. 5c). Overall, ferns removal significantly increased C-degrading (p<0.001) and N-degrading enzyme activities (p=0.008), and decreased microbial CUE (p=0.036) across the three stands (Fig. 5).

4. Discussion

In support of the initial hypothesis, our 7-year continuous observations of SOC dynamics demonstrated that ferns removal consistently and rapidly reduced SOC across the restoration sequence; with ferns effect were strongest in VC-0 stand, intermediate in VC-1984, the weakest in VC-2002 stand (Fig. 2). The decreased SOC stock could result from either the decline of above and belowground litter mass inputs and/or increase in native SOC decomposition via priming effect by the increased input of low-quality litter (pine litter; Lyu et al., 2019b). However, there two processes would be accompanied by divergent changes in SOC stock. For the former interpretation, we may intuitively think that the ferns removal will reduce the input of aboveground litter however it may not in this case. Because there is about 50% of the overstory pine litterfall is being intercepted by the understory fern (Yang et al., 2014; Lyu et al., 2019a), then in Control plots, pine litter may not be reaching soils (Dearden

and Wardle, 2008) and therefore these 50% of pine litter will reach to soils when ferns were removed. Moreover, the annual litterfall of ferns were accounted for 51-80% of the pine litterfall which roughly equals to the mass of pine litterfall intercepted by the ferns (Fig 3 and 6a), suggesting the removed ferns litterfall can be compensated by the previously intercepted pine litterfall in Ferns removal plots. We therefore called this phenomenon as the "Filtering effect" for the pine litter interception from the fern forming dense thicket layers (George et al., 1999; Royo and Carson, 2006) – the aboveground litter of the understory ferns to replace the pine litter, and leading to nonsignificant differences in litterfall mass between the Control plots and Ferns removal plots (Fig. 6a). It is obviously that the quantity of aboveground litterfall may not the factor affect SOC stocks but the changed litter quality may do supports the later interpretation that ferns removal may increase SOC decomposition. Thus taking all together we can hypothetize that total aboveground litter production is higher with ferns presence.

Despite not conclussive results of litter quantity, the quality of litter has been changed due to the additional input of pine litter intercepted by fern and the removed high-quality fern litter. Because adding more low-quality pine litter are often led to microbial N limitation (Lyu et al., 2019b), thereby increasing microbial N mining and SOM decomposition via priming effect (Fig.6a; Lyu et al., 2018, 2019c; Tamura and Tharayil, 2014). This interpretation is strongly supported by the finding that ferns removal significantly increased C- and N-degrading enzyme activities (Fig. 5) suggest a more active microbial community, which should increase enzyme production to assure a sufficient nutrient relaeas from a more recalcitrant litter favoring C and N mineralization (Lyu et al., 2019b, 2019c).

Furthermore, we measured three years of soil respiration to further determine how ferns removal affect SOC decomposition. Consistent with our first hypothesis that ferns removal did not affect total soil respiration in VC-0 and VC-1984 stands but which been significantly decreased by 26% in VC-2002 stand (Fig. 4c, d). Interestingly, ferns removal significantly decreased 46% of the total belowground fine root biomass of VC-2002 stand whereas the percentage of fine root biomass reduction in Ferns removal plots were 86% in VC-0 and 65% in VC-1984 stands (Fig. 3). Logically, it is likely that ferns removal would reduce total soil respiration in VC-0 and VC-1984 stands but not in VC-2002 stand. Ferns removal induced decrease of total soil respiration in VC-2002 stand (13-16 years-old stand) may be related to the higher root respiration rate in comparison to the un-restored and mature stands (Wu et al., 2011). Moreover, ferns removal did not affect microbial CUE and mineralize potential capacity (Fig. S4), and with observed nonsignificant changes in SOC stock in VC-2002 stand albeit its increased C- and N-degrading enzyme activities (Fig. 5a, b). Our measurements suggest that ferns removal had minor impacts on native SOC decomposition in VC-2002 stand during our 7-year observation. Therefore, small changes in native SOC decomposition in VC-2002 stand would be unable to compensate the decreased root respiration thereby resulting decline in total soil respiration in response to ferns removal.

In contrast, we believed that the removal of understory ferns may increase the mineralization of SOC in VC-0 and VC-1984 stands, and the increased SOC decomposition would roughly offset the reduced root produced CO_2. Because root produced CO_2 accounted for about 45% of the total soil CO_2 (Hanson et al., 2000; Bond-Lamberty et al., 2018), ferns removal decreased the fine root biomass by 86% in VC-0 and 65% in VC-1984 stands which

366 means a reduction of ~39 and 29% of total soil CO_2 (Fig. 3), suggesting that ferns removal
367 stimulated 39 and 29% of native SOC derived CO_2, thereby would be anticipated to induce
368 soil C loss to offset the reduced root produced CO_2 in un-restored and mature pine stands.
369 Surprisingly, our estimated percentage of increase in SOC decomposition due to ferns
370 removal (39 and 29%) was roughly equal to that of ferns removal induced SOC loss in VC-0
371 and VC-1984 (37% and 23%). Consequently, it is not surprising that ferns removal had non-
372 significant impacts on total soil respiration (Fig. 4). This interpretation is supported by the
373 finding that ferns removal significantly increased C- and N-degrading enzyme activities and
374 microbial mineralize potential capacity, and decreased microbial CUE in VC-0 and VC-1984
375 stands (Fig. 5 and S5). Because microbial growth in soils of degraded ecosystems is often
376 limited by N availability (Mo et al., 2016), when compared with the Control plots, the
377 additional 50% of pine litter adding to soils in Ferns removal plots will led to increased
378 microbial N mining of SOM, increased SOM decomposition via priming effect (Lyu et al.,
379 2019b, 2019c), thereby decreasing soil C stocks in VC-0 and VC-1984 stands. We therefore
380 conclude that any factors like artificial disturbance and natural degradation induced decline in
381 understory ferns would have pronounced and negative impacts on SOC stocks.
382 Consequently, our findings challenge the common views that biomass of understory
383 plants is quite small by contrast to overstory trees with its contribution to soil C storage are
384 often negligible (Nilsson and Wardle, 2005; Zhao et al., 2012; Wardle et al., 2005; Averill et
385 al., 2014). Instead, we show that understory ferns have positive effect on soil C accumulation
386 by altering the quality of litter inputs but not sure the quantity with ferns contribute to make
387 up 54-61% of the total soil C stocks (Lyu et al., 2019a). The strong positive effects of ferns on

388 soil C stocks were likely due to the “filtering effect” of ferns on litter inputs. In presence of
389 ferns, most overstory pine litter cannot reach to soil surface but directly been decomposed
390 over the ferns (Dearden and Wardle, 2008). The understory ferns acted as a “Ecological
391 Sieve” (Yang et al., 2021), leading to most of the pine litter-derived C been lost via CO_2 while
392 the nutrients would be leached to the soil for microbial and plants uptake which like the input
393 of high-quality litter to soil (Fig. 6a). It is clear that the input of high-quality litter are often
394 favoring microbial utilize and driving high microbial CUE and low priming effect, ultimately
395 resulting fast C accumulation in soils (Fig. 6a). By contrast, in absence of understory ferns,
396 there will be additional more than 50% of pine litter input to soil – doubling pine litter inputs,
397 which would intensify microbial nutrients demands, leading to low microbial CUE and high
398 priming effect thereby slowing C accumulation in soils. Therefore, we provided a new insight
399 into the understanding of how understory plants like ferns likely play a “small body with
400 strong power” role in mediating soil C dynamics and stocks during pine plantation
401 development. Because understory ferns could be like facilitators in driving microbial
402 community in restoration ecology (Harris, 2009) which is dependent on what is going on in
403 the above-ground community and can indicate the impact of restoration-management
404 practices.

405 While we emphasized the importance of understory ferns mediated the effect of quality
406 of aboveground litter inputs on soil C dynamics, it is not equivalent to deny the importance of
407 litter quantity. It is therefore crucial to note that what understory ferns brings to the disparity
408 at each successional stage. It is possible that the stoichiometric imbalances may be an
409 important factor affecting the carbon cycle during pine plantation development on previously

410 degraded lands (Vitousek et al., 1997; Zechmeister-Boltenstern et al., 2015). We anticipated
411 that the early stages (before ecological restoration for 13-19 years) could be soil C preferential
412 accumulation stage since the soil C stock of VC-2002 stand reaches a significant difference
413 compared to VC-0 stand (Fig. 3a and b). In addition, C-degrading enzymes decrease
414 significantly with the vegetation restoration (Fig. 5a). In contrast, after 13-19 years of
415 recovery, the rate of N accumulation was slower than that of C due to the preferential
416 accumulation of C in the early stages, leading to an imbalance of soil C and N (Miller et al.,
417 2019). Therefore, the microbes will turn to the N mining stage in order to adjust to the
418 disparity in their own C to N ratio (Hume et al., 2016; Deng et al., 2013; Bai et al., 2021;
419 Feng et al., 2021). This could be an explanation for the nonsignificant differences in soil C
420 stocks between young (VC-2002) and mature (VC-1984) pine stands (Fig. 2), indicating that
421 degraded lands restoration with single pine may be unable to making SOC stock reach a high
422 level. An alternative explanation for this involves the decline of understory ferns coverage
423 related changes in low inputs of higher litter quality and soil nutrient availability since the
424 coverage of ferns in VC-1984 stand was lower than VC-2002 stand (Lyu et al., 2019a). Our
425 research has also shown that ensuring a critical mass of high-quality tree species is important
426 for soil carbon accumulation during forest succession stages.
427 It was also worth mentioning that we cannot provide direct evidence to address the
428 impacts of climate variables on the permanence of restored soil C, we saw flat or rapidly
429 increasing SOC stocks with increasing ferns removal years across all stands (Fig. 2). One
430 possible explanation for this discrepancy relates to the impacts of interannual variation in
431 rainfall on forest productivity then above- and belowground C inputs (Lyu et al., 2021). Here,

we took advantage of one drought year (2014) followed by 2 wet years (2015-2016) then drought (2017) and normal rainfall (2018-2020) to explain the flat or rapidly increasing SOC stocks with years. We speculate that a dry year followed by two wet years in our study will stimulate forest productivity and then litterfall inputs which in line with the findings that moisture and rainfall positively related to plant productivity (Chu et al., 2016; Taylor et al., 2017). The increased productivity will turn to litterfall when it comes to the dry year (2017), producing large amount of litterfall input to soil, which supported by our observation of increasing litterfall during 2017-2019 (Zhang et al., 2021). The increased litterfall will be the part of labile SOC like particulate organic matter in short-term (Xie et al., 2013); Concurrently, soil microbial biomass has a positive response to the change of precipitation (Ren et al., 2017) – soil microorganisms may show a downward trend after 2016, while the increased particulate organic matter accumulates more due to the decrease of soil microbial activity, ultimately leading to rapidly increases in SOC stocks during 2018-2021 (Fig. 2). Consequently, we do note that interannual variation in climate variables may indirectly affect SOC dynamic despite we cannot directly address the ongoing and changing climate may threaten the permanence of C stored through restoration.

In addition, we suggest that understory ferns can low the risk of C loss and facilitating soil C accumulation in response to climate change like rising temperature. For example, in un-restored VC-0 stand, where scrubby pine with sparsely distributed, the soil temperature can reach over 30 to 40℃ in July and August, which is of great significance for the accelerated old SOM decomposition in understory ferns removal plots may be accelerated in summer due to higher temperatures (Negishi et al., 2006; Curiel et al., 2010) which partly supported by our

findings that understory ferns removal slightly increased total soil respiration in the un-restored stand.

Overall, firstly, we believe understory ferns is very powerful for the restoration in such degraded ecosystems, especially in the early stages of the lack of vegetation cover, and is beneficial for mitigating soil carbon decomposition due to changing precipitation and rising temperature, which is beneficial for mitigating future climate change. Previously, in the middle and late stages of succession in ameliorated soil conditions, higher species diversity and stronger plant competition for light, and this fern weakened its function in such an environment because shading out light to reduce temperature was likely to affect the budding of the tree species (George et al., 1999; Royo and Carson, 2006). In contrast, in poor soils, at the early of restoration, widespread species with a single, while the expansion of the fern in large numbers lowered the soil temperature, maintained water and nutrients. It has been suggested that the presence of ferns acts as an ecological filter during succession, and determination of which plant species to be established in the next successional stage (Yang et al., 2021). Second, our study highlights the presence of fern as a high-quality litter (low C/N or lignin/N) at the beginning of succession and it improves N retention and microbial carbon use efficiency, increased soil C pools (Shao et al., 2019; Xiong et al., 2020). Simultaneously, it avoids the soil carbon loss by the priming effect of low-quality pine litter for its indirect interception of dense thicket layers (Fig. 6a). Third, we depict changes in soil C accumulation and decomposition with succession following ferns removal. In the early stages (VC-0) of the lack of overstory vegetation cover (large areas bare land), fern removal resulted in significant primed soil CO_2, accompanied by a reduction in the soil C stock and C accumulate rate

annually, while soil carbon primed minimize from fern removal in middle stage (VC-2002) as stand conditions improved. In the late stages of understory fern degradation (undisturbed), however, primed CO_2 and C loss occurred again, along with a reduction in the soil C accumulation rate (Fig. 6b). Therefore, it is urgently to find the most promising ways to rapidly and continuously sequestrating C in these restored forests like introducing tree species with high-quality litter (i.e., high nutrient availability) during the medium restoration stage. By doing so, it is possible to protecting the permanence of restored soil C defensing the threat the ongoing and future climate change because tropical and subtropical forest restoration play an irreplaceable role in reverse climate change (Koch and Kaplan, 2022).

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Author contributions

C. D., M. L. and J. X. wrote and improved the manuscript; M. L. and J. X. designed and supervised the experiment; C. D., X. X. and W. L. carried out the measurements following the field incubation, collected and analyzed data; M. L., X. L. and J. X. supervised the experiment and reviewed the manuscript. All authors discussed the data and contributed to the final draft.

Competing interests

The authors declare no competing interests.

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