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Understory ferns alter soil carbon chemistry and increase carbon storage during reforestation with native pine on previously degraded sites

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ABSTRACT

Reforestation with native species and resulting understory succession can exert important influences on soil organic matter (SOM) storage and chemistry, but a mechanistic understanding of these effects is lacking. We studied different aged Masson pine (*Pinus massoniana* L.) plantations with and without the understory fern, *Dicranopteris dichotoma* (Thunb.) Berhn., in subtropical China to assess how SOM over a 30 year sequence of pine growth and fern expansion. To do this, we measured total SOM, lignin-derived phenols, soil carbon (total C and ^{13}C), soil nitrogen (total N and ^{15}N), and soil microbial community composition via phospholipid fatty acid (PLFA) analyses. We found that the accumulation of newly-formed SOM outweighed decomposition of old SOM, with the majority of this increase being derived from fern detrital inputs. Where ferns were present, ferns contributed 54–61% of total soil C storage in surface (0–10 cm depth) soils, which was 62–91% higher than pre-reforestation soil C storage. We found that the abundance of lignin-derived compounds was lower in fern dominated soils, perhaps because soils under ferns supported more soil fungi, the primary decomposers of the lignin in soil. Fern soils also showed higher ratios of syringyls to vanillyls and decreased $\delta^{13}C$ values, an indicator that ferns altered the composition of SOM at the molecular level while contributing significantly to SOM accumulation. Reforestation especially when accompanied by fern expansion also improved soil N and phosphorus (P) status, with observed declines in soil $\delta^{15}N$ in fern dominated soils aligning with increased nutrient retention and observed increases in soil C storage. Our study highlights the potentially important role of understory ferns in mediating SOM chemistry and soil C storage during ecosystem recovery.

1. Introduction

Deforestation and forest recovery exert large but opposing effects on terrestrial carbon (C) balance (Dixon et al., 1994; Achard et al., 2002; Le Quéré et al., 2009). Where deforestation leads to ecosystem degradation, soil C storage is typically reduced (Bai et al., 2008; Borrelli et al., 2017), but degraded sites often retain strong potential for soil C recovery (Xie et al., 2013; Kurganova et al., 2014, 2015). Forest plantation establishment is one approach to restoring degraded agricultural sites (Richter et al., 1999), with forest plantations supporting both ecosystem recovery and wood production goals. Globally, the total land area in plantation forests has increased strongly over the past half century (Binkley and Fisher, 2012), with plantation forestry being especially important in China, which supports the world's largest area in planted forests (Lal, 2002; Payn et al., 2015). In 2009, the gross timber volume of Chinese plantations was $19.6 \times 10^9\ m^3$ (Piao et al., 2009; Liu et al., 2012), with most of this volume occurring in single-species plantations.

Species selections can regulate C inputs and losses because of species-based variation in C production, decomposition, retention, and

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harvest-based export (Binkley and Giardina, 1998; Binkley and Fisher, 2012), but tests have been equivocal. For example, a global meta-analysis showed that soil C declined by 10% and 13% as a result of land use change from pasture and natural forests to plantation forests, respectively, whereas the reverse process increased soil C (Guo and Gifford, 2002). Similarly, in warm temperate grasslands of the southern USA, invasion by trees decreased belowground C pools (Jackson et al., 2002; Strickland et al., 2010; Tamura and Tharayil, 2014). In contrast, in the northern Great Lakes region of the USA, naturally regenerating mixed hardwood forests stored more total C than adjacent pine plantations after 60 years of stand development following agriculture; notably, both forest types showed similar increases in soil C compared to adjacent pasture despite very large differences in litter quality and total inputs to soil (Gahagan et al., 2015). This diversity of soil C responses to a shift from non-woody to woody vegetation indicates that additional studies are needed to understand the mechanisms of SOC response to species selections and afforestation.

Understory plants are nearly ubiquitous in forests, and the expansion of understory vegetation into newly planted forests can represent a management problem to forestry operations seeking to minimize competition for soil resources. Understory expansion rates can be rapid where composition of the colonizing vegetation includes non-native invasive species or where changing climate enhances understory plant growth and or alters canopy mortality (Parmesan and Yohe, 2003; Sardans et al., 2017). Further, understory vegetation can enhance forest biodiversity, and affect multiple ecosystem functions and services (Eldridge et al., 2013). In contrast, the effects of understory vegetation on ecosystem processes remain poorly understood and limited results have been dubious. For example, while it is well appreciated that understory vegetation can alter the quantity and quality of plant inputs to soil, how variation in understory species composition alters rates of SOC stabilization and SOC storage are poorly quantified (Craig et al., 2015; Figueiredo et al., 2017; Tamura et al., 2017). For example, while understory plant expansion into *Pinus* forests increased or decreased soil C storage (Tamura and Tharayil, 2014), understory invasions in tropical dry forest increased soil C fluxes but had no apparent effect on soil C storage (Litton et al., 2008). Overall, few studies have examined the independent and combined effects of afforestation and naturally colonizing understory plants on soil C processes.

Proposed mechanisms for species effects on soils have focused on litter quality. Invasive understory plants with high quality litter (e.g. low C/N) have been shown to increase soil C storage (Tamura and Tharayil, 2014), while those with low quality litter (e.g. high C/N) decrease soil C storage (Strickland et al., 2010; Tamura and Tharayil, 2014). These results follow a standard model for soil organic matter (SOM) formation that is most often captured in terrestrial ecosystem models (Parton et al., 1994), but decades of research in north temperate forests has shown that over time, decomposition can homogenize diverse materials into fairly uniform detrital inputs, what Melillo et al. (1989) called the decay filter hypothesis, perhaps partially explaining the lack of differences between pine SOC and mixed hardwood forest SOC studied by Gahagan et al. (2015). Interestingly, in sub-alpine forests of Colorado, USA, high quality aspen litter was actually retained more completely in soil compared with low quality pine litter (Giardina et al., 2001), perhaps due to higher microbial use efficiency and enhanced stabilization of aspen derived SOC (Giardina et al., 2001; Craig et al., 2015). Clearly, the linkages between the quantity and quality of species detrital inputs to soil, and actual effects on soil C storage are quite complex.

The degraded red soil lands of southern China have historically experienced high rates of erosion (Zhang, 1990; Cao et al., 2009; Gao et al., 2011), resulting in a degraded, N-poor condition. A common colonizer of eroded and degraded soils in this landscape, *Dicranopteris dichotoma* is a widely distributed tropical fern (Amatangelo and Vitousek, 2009; Zhao et al., 2012) that can play an important role in forest nutrient cycling (Dearden and Wardle, 2008) and stand development (Walker et al., 2010). We explored how reforestation with and without fern expansion affects SOC quality and SOC quantity in degraded lands of southern China. These systems offer an ideal study system to test basic hypotheses about afforestation and understory expansion effects on soils.

We identified a matrix of landscape conditions that included 30 year old Masson pine forest, 12 year old Masson pine forest, and open degraded badlands, each with and without fern cover (Xie et al., 2013). We used this landscape to examine the effects of active ecological restoration and natural native understory fern expansion on soil properties, SOM chemistry, SOC accumulation, and microbial community composition and function and test the following two hypotheses: (H1) SOC formation and stabilization are higher under fern cover than in adjacent fern free areas because ferns ameliorate soil microenvironment, increase total inputs, reduce quality of inputs, increase retention of detrital C and nutrients, reduce soil N losses (Zhao et al., 2012; Chen et al., 2016); and (H2) inputs of low quality fern litter alter soil microbial community composition, by increasing fungal abundance, which reduces the abundance of lignin-derived products.

2. Materials and methods

2.1. Site description

Our study was conducted at Changting Research Station for Erosion (309,720 ha) (25°33′N, 116°18′E), located at Hetian town of Changting county in Fujian Province, southeastern China (Fig. 1). The region has a subtropical monsoonal climate, a growing season that averages 260 days, a mean annual temperature of 18.4 °C, and a mean annual rainfall of 1716 mm (Xie et al., 2013). Soils are classified as easily eroded red soils (Humic Planosols, World Reference Base for Soil Resources, 2014) derived from medium to coarse crystalline granite. Native evergreen broadleaf forests across the region were clear-felled for agricultural uses early in the 20th century, leading to severe soil erosion and site degradation, especially in areas that were less suitable for agriculture (Wang et al., 2016). Our research site is broadly representative of the region, which is characterized by similarly long-term land use, degraded soils, and very slow rates of natural forest succession (Zhang, 1990, Cao, 2011).

Prior to 1980, our research site and the surrounding landscape were characterized by extensive areas of bare “badland” soils and sparse (< 20%) vegetation cover (Fig. 1 and Fig. S1). The degraded soils of the area have low C content and nutrient availability. To reverse land degradation, mitigate erosion, and increase the ecological and economic value of these lands, county governments restricted agricultural uses on these lands, and in 1981 created incentives for reforestation of private lands, 90% of which were owned by individual farmers (Cao et al., 2009). As a result, forest cover expanded rapidly in the region of this study (Fig. 1), with positive impacts on soil erosion, which declined by 14.2% between 1983 and 2003 (Xie et al., 2013; Fig. S1).

2.2. Experimental design

We distinguished three land cover conditions in the study area (Fig. 1): degraded shrubland with scattered natural recruits of *Pinus massoniana* L. that closely approximates the pre-restoration (untreated = 0-yr) vegetation cover type (VC-0); and VC-12 and VC-30 cover types, which are 12-yr old (established in 2002) and 30-yr old (established in 1984) *P. massoniana* plantations. We assumed pre-planting conditions for the VC-12 stand (pre-2002) and the VC-30 stand (pre-1984) were indistinguishable from the 0-yr stand. This assumption is reasonable because: 1) all three stands had experienced nearly a century of severe soil erosion prior to 1981 (Fig. S2; Zhang, 1990); 2) soil organic C, N, P and potassium (K) content in the VC-0 stand did not vary over time (Fig. S1); 3) soil texture is similar across stands and across fern and no fern conditions (Table 1); and 4) the C, N and P

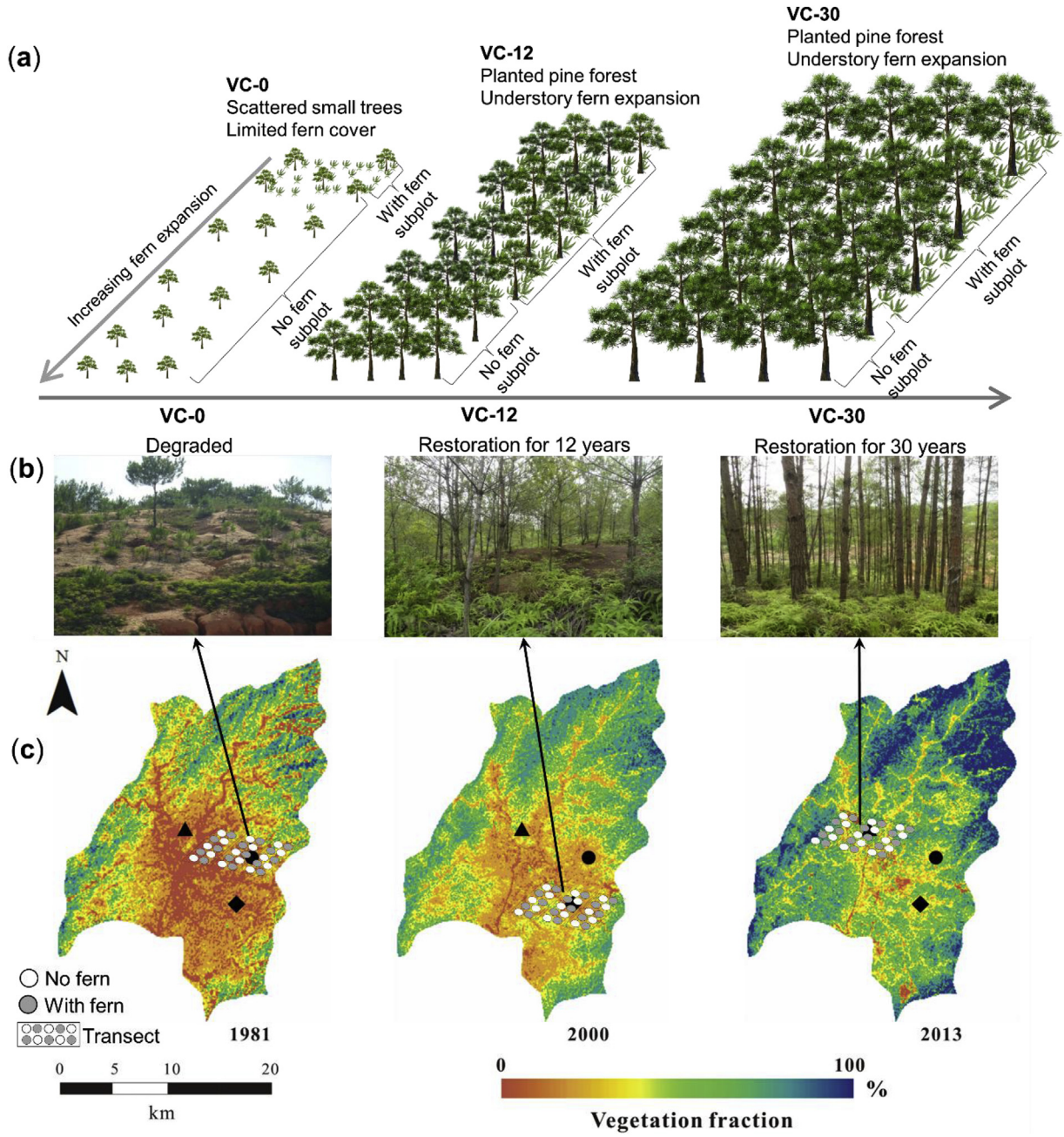

Fig. 1. Data from VC-0, VC-12 and VC-30 stands in Fujian Province, China, showing: (**a**) conceptual representation of the understory fern expansion and trees growth during ecological restoration; (**b**) three long-term stands (degraded land (0 year), artificial restoration for 12 years and 30 years), and (**c**) the remote sensing image from the Landsat data of the study sites in year 1981, 2000 and 2013; the increases in vegetation fraction (%) is evident across most of the region (Changting country) in which the study took place; five matched sampling plots for no-fern (NF) and with-fern (WF) were located in each transect (insert on remote sensing image). Each stand have three transects. Understory fern cover increased into previously bare land for each vegetation cover (VC) type.

content of deep soil (50–100 cm), a depth that is unlikely to be affected by short-term vegetation change, showed no significant differences across stands or across fern and no fern conditions (Table S1). This assumption allowed us to calculate change in soil parameters over the 12 or 30 years of stand development (see below) and across fern and no fern plots.

Active restoration leading to VC-12 and VC-30 stands included planting pine at a target 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, which was intended to provide a source of nutrients and to improve 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-12 and VC-30 stands. Finally, 1050 kg ha^{-1} of calcium magnesium phosphate fertilizer ($Ca_3(PO_4)_2$, $CaSiO_3$, $MgSiO_3$) was applied to plantations after planting to meet the nutritional needs of pine seedlings (Xie et al., 2013). This fertilizer provided an estimated average input of 338 kg of Ca, 76 kg of Mg and 116 kg of P per ha. The VC-0 received neither organic matter nor fertilizer treatments.

In the study area, improved microclimate and increased fertility of the plantations was accompanied by the expansion of the dominant understory fern (*Dicranopteris dichotoma* (Thunb.) Berhn.) into these plantations; coverage was 15%, 90% and 85% in the VC-0, VC-12 and VC-30 stands, respectively (Fig. 1a and b). Prior to active restoration of VC-12 and VC-30 stands, fern coverage across all stands was similarly low and patchy (Fig. 1b; Zhang, 1990; Cao, 2011). In the VC-0 stand,

Table 1
Characteristics of trees, litter input and C:N in the foliar and root in three different aged stands. The data are means of three replicates in each treatment with standard errors in parentheses.

Properties	No fern plots (NF)			With fern plots (WF)		
	VC-0	VC-12	VC-30	VC-0	VC-12	VC-30
Clay (%)	37.9 (0.1)	34.1 (0.1)	29.8 (0.8)	33.9 (0.1)	31.3 (0.3)	29.8 (0.4)
Silt (%)	18.9 (0.2)	24.5 (0.5)	27.1 (0.6)	22.9 (0.1)	21.7 (0.5)	21.0 (0.2)
Sand (%)	43.2 (0.1)	41.2 (0.7)	43.1 (0.3)	43.3 (0.2)	46.9 (0.2)	48.0 (0.2)
DBH[a] (cm)	3.6 (1.1)	8.4 (1.1)	12.9 (1.1)	4.9 (0.7)	9.4 (0.4)	14.3 (1.1)
Mean tree height (m)	1.1 (0.2)	8.5 (0.6)	13.2 (1.8)	1.7 (0.2)	9.0 (0.3)	14.4 (0.9)
Tree density (trees ha^{-1})	123 (18.5)	319 (41)	157 (11)	153 (24)	293 (23)	187 (42)
Pine aboveground litter biomass (g m^{-2})	62 (7)	311 (36)	286 (31)	69 (3)	305 (38)	422 (54)
Fern aboveground litter biomass (g m^{-2})				26 (7.1)	99 (2.2)	87 (9.6)
Pine root biomass (g m^{-2})	4.5 (0.1)	207 (12)	241 (23)	17.6 (0.9)	287 (15)	291 (23)
Fern root biomass (g m^{-2})				132 (14)	1254 (62)	520 (35)
Pine foliar C:N	190 (4)	145 (21)	206 (10)	204 (23)	199 (13)	192 (11)
Fern foliar C:N				85 (11)	126 (22)	49 (9)
Pine fine root C:N	184 (22)	144 (21)	138 (18)	301 (20)	166 (18)	164 (10)
Fern fine root C:N				292 (42)	159 (17)	144 (7)

Notes: Litterfall data are summed from May 2015 through April 2016. DBH = Mean diameter breast height, the DBH for VC-0 site are ground diameter. The fine root biomass was evaluated at depth of 0–10 cm.

fern coverage continues to be low, and occurs in isolated patches, while in VC-12 and VC-30 stands, areas without fern occur as open patches within a matrix of fern. Across the site, understory vegetation in non-fern areas is made up primarily of *Arundinella setosa* Trin., which provides limited cover in these bare soil areas.

In August of 2014, we sampled vegetation and soils within each of the three vegetation conditions (VC-0, VC-12 and VC-30). To do this, we established three parallel transects (= plot) within each stand type, and then established five pairs of fern-covered (WF) and no fern (NF) subplots within each transect, organized in a matched pairs design (Fig. 1c). Each transect was separated by at least 40 m and the 10 subplots were organized along each transect to capture natural fern gradients. Specifically, WF subplots were located at least 6 m inside a WF area that was at least 400 m^2 while NF subplots were located at least 3 m from the edge of a WF areas, with the NF area also at least at 400 m^2. Pairing samples from matched subplots (15 WF and 15 NF per stand type; Fig. 1c) is common in studies of invasion ecology (Jackson et al., 2002; Strickland et al., 2010; Tamura and Tharayil, 2014), because vegetation effects on soils can be examined while reducing the typically high variation encountered in purely random designs.

2.3. Soil, foliage and fine root sampling

We sampled soil, foliage and fine roots from all subplots during summer 2014. Fern foliage was sampled once in three randomly placed 1 m × 1 m quadrats within each subplot; fine roots were sampled once in six randomly located soil profiles (0.2 m × 0.2 m × 0.1 m) within each subplot. Samples were taken to the lab to separate roots from soil by floatation. Soil samples (0–10 cm) were collected from a subset of plots described above and included 5 subplots located at the top (2), middle (1) and bottom (2) of each transect. We used an auger (diameter 3.5 cm) to collect soils from 5 subplots of WF and 5 subplots of NF subplots along each of three transects for each of the three aged stands (VC-0, VC-12 and VC-30) for a total of 90 subplots, with samples of WF and NF soils within each transect (referred to plot) being composited into a single sample for WF and NF, respectively (Fig. 1c). Mineral soil samples were sampled after removing forest floor material. In addition to the soil C samples, samples for soil bulk density estimates (oven dried mass per unit core volume) were collected from four randomly selected locations in each subplot. From May 2015 to April 2016, litterfall was collected monthly in each subplot with one litter traps (80 cm × 80 cm), i.e., five litter traps for each WF and NF transect, respectively. Live foliar samples were collected from the new and older green needles of four pine trees within each transect. Samples were pooled by needle age into one composite sample for two different foliage age groups. All foliar samples were ground for determination of N content and $\delta^{15}N$ measurement after oven drying at 60 °C to constant mass (see below).

2.4. Analysis of soil properties and microbial communities

Total C and total N content in soil were determined with a Vario MAX CN elemental analyzer (Elementar Vario EL III, Germany). Total P was measured on a SKALAR San^{++} Analyzer (Skalar, Breda, The Netherlands) after digestion with perchloric acid and sulphuric acid (after Hedley et al., 1982 with modifications in Fan et al., 2018). Soil pH was measured with a pH meter in a 1:2.5 mass: volume suspension in deionized water. Potential net N mineralization was estimated as the net change in the concentration of mineral N (NH_4^+–N and NO_3^-–N) after a 28-day aerobic incubation with soil moisture kept at 60% of water holding capacity and 25 °C (Huang et al., 2013). We incubated 10 g (air-dry basis) of each soil sample, and collected half of the sample after a 7-day incubation period, which was then extracted with 2 M KCl to determine the concentrations of NH_4^+–N and NO_3^-–N, using a SKALAR San^{++} Analyzer (Skalar, Breda, The Netherlands). Because of the lack of difference in initial conditions across stands, we used total soil C, N, P and K values (2.60, 0.12, 0.14 and 4.65 g kg^{-1} respectively) in the VC-0 stand as a baseline for calculating change in these elements in the VC-12 and VC-30 stands.

Phospholipid fatty acids (PLFA) profiles were used to determine the abundance and structure of soil microbial community following White et al. (1979) and Högberg et al. (2014). We extracted phospholipids from 16 g of soil; this is a large soil sample, but soils in the VC-12, VC-30 and especially VC-0 stands generally had very low SOM concentrations and low microbial biomass. Samples were analyzed with gas chromatography and the lipid compounds were identified with the Sherlock Microbial Identification System (MIDI Inc., Newark, DE). Detailed methods and indicators of specific microbial groups that used in this experiment are similar to those described in Lü et al. (2015). We used the PLFA 18:2ω6,9 and 18:1ω9 as biomarker for ectomycorrhizal fungi (EMF) and saprophytic fungi (SF), respectively, as has been done in previous studies (Högberg et al., 2011, 2014; Näsholm et al., 2013).

2.5. Analyses of $\delta^{13}C$ and $\delta^{15}N$ signatures

Stable C and N isotope analyses were performed on a Finnigan MAT-253 Mass Spectrometer (Thermo Electron, Germany), coupled to an automatic, online elemental analyzer (Flash EA 1112, Thermo Electron,

Germany) using oven dried (60 °C to constant weight) soil, foliage and fine roots samples, which were folded into tin capsules. Stable isotope ratios are expressed in δ notation relative to Vienna PeeDee Belemnite for C and the atmosphere for N (Coplen, 2011). Comparisons with previously analyzed internal laboratory standard and highly homogenized soil samples show that the precision of the on-line procedure was within ± 0.12‰ and ± 0.2‰ for C and N isotope ratios, respectively.

2.6. Biomarker analyses

Lignin-derived phenols of pine and fern foliage, roots, and WF and NF soil samples were analyzed at the State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing, China to partition SOC in WF plots into pine or fern derived C according to Otto et al. (2005) and Feng et al. (2008). To do this, dried and ground plant tissues (~100 mg) or soils (2 g) were mixed with 1 g CuO, 100 mg ammonium iron (II) sulfate hexahydrate [$Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$], and 20 mL of nitrogen-purged NaOH solution (2 M) in teflon-lined sealed metal containers. We used internal check standards to quantify the lignin-derived phenols with a Trace GC 1310 gas chromatograph coupled to an ISQ mass spectrometer (Thermo Fisher Scientific, USA) using a DB-5MS column (30 m × 0.25 mm i.d., film thickness, 0.25 μm) for separation. We used the external quantification standards to normalize the response factor for different lignin-derived phenols separately (Feng et al., 2016; Cai et al., 2017).

Lignin-derived phenols include vanillyls (V; vanillin, acetovanillone, and vanillic acid), syringyls (S; syringaldehyde, acetosyringone, and syringic acid) and cinnamyls (C; p-coumaric acid, and ferulic acid) (Hedges and Ertel, 1982). The ratios of vanillic acid to vanillin and of syringic acid to syringaldehyde were used to assess lignin degradation, both of which increase with lignin degradation (Opsahl and Benner, 1995; Otto and Simpson, 2006; Feng et al., 2016). We used the ratio of cinnamyls to vanillyls (C/V) and the ratio of syringyls to vanillyls (S/V) in soils and vegetation to run a simple mixing model that allowed us to qualitatively understand the source of lignin in soils (Otto and Simpson, 2006; Cai et al., 2017). We also used the totals for vanillyls, syringyls and cinnamyls to calculate total lignin (Hedges and Ertel, 1982; Feng et al., 2008).

Because V phenols (vanillin + acetovanillon + vanillic acid) are relatively stable in soil (Opsahl and Benner, 1995; Otto and Simpson, 2006), and the ratio of 3,5-dihydroxy benzoic acid (DHA) to V phenols ($\frac{DHA}{V}$) of pine tissues is higher than of fern tissues (see Results), we used the $\frac{DHA}{V}$ to calculate the contribution of fern versus pine derived C to total SOC. The use of phenols to partition the contributions of fern versus pine lignin to SOC is a novel application. The ratios for fern foliage and root samples were 0.08 ± 0.01 and 0.08 ± 0.02, respectively, while the ratios for pine foliage and roots were 0.03 ± 0.01 and 0.01 ± 0.00, respectively. The ratios for soils in the NF plots are 0.03 ± 0.01, 0.50 ± 0.15 and 0.22 ± 0.08 for VC-0, VC-12 and VC-30 stands, respectively; the ratios of soils in the WF plots were 0.48 ± 0.17, 0.87 ± 0.13 and 0.62 ± 0.06 for VC-0, VC-12 and VC-30 stands, respectively (Fig. S3). The $\frac{DHA}{V}$ in the WF plots are significantly higher than that in the NF plots. As with end member mixing analyses (EMMA) using the contrasting $\delta^{13}C$ of two plant end members to identify sources of SOC (Balesdent et al., 1987), we used $\frac{DHA}{V}$ as end members in an EMMA to partition SOC into pine derived and fern derived SOC. Specifically we used the following binary EMMA model:

$$C_{F(0)} = C_{FS(0)} - C_{S(0)} \quad (1)$$

$$C_{F(n)} = C_{F(0)} + [C_{FS(n)} - C_{FS(0)}] \times \frac{\left[\left(\frac{DHA}{V}\right)_{FS(n)} - \left(\frac{DHA}{V}\right)_{FS(0)}\right] - \left[\left(\frac{DHA}{V}\right)_{S(n)} - \left(\frac{DHA}{V}\right)_{S(0)}\right]}{\left(\frac{DHA}{V}\right)_{F(n)} - \left[\left(\frac{DHA}{V}\right)_{S(n)} - \left(\frac{DHA}{V}\right)_{S(0)}\right]} \quad (2)$$

$C_{F(0)}$ and $C_{F(n)}$ are the amount of fern-derived C in soil in the VC-0 stand and restored (VC-*n*) stands, respectively; $C_{FS(0)}$ and $C_{FS(n)}$ are the amounts of total soil C in the plots with fern in unrestored (0 year) and restored (VC-*n*) sites, respectively; $\left(\frac{DHA}{V}\right)_{FS(0)}$ and $\left(\frac{DHA}{V}\right)_{FS(n)}$ are the $\frac{DHA}{V}$ of soil within fern plots in unrestored (0 year) and restored (VC-*n*) sites, respectively; $\left(\frac{DHA}{V}\right)_{S(0)}$ and $\left(\frac{DHA}{V}\right)_{S(n)}$ are the $\frac{DHA}{V}$ of soil in plots without fern in unrestored (0 year) and restored (VC-*n*) sites, respectively; $\left(\frac{DHA}{V}\right)_{F(n)}$ is the $\frac{DHA}{V}$ of fern litter and roots.

In developing this phenol tracer approach, we identified two important considerations. First, in order to move from plant tissue ratios of 0.03–0.08 to soil ratios of 0.5–0.8, the proportion of DHA to V must increase strongly, which can only happen if DHA is significantly more stable than V during decomposition or in soil. If V is stable in the soils (Opsahl and Benner, 1995; Otto and Simpson, 2006), then DHA must be very stable. Alternatively, there may be larger than appreciated variation in the stability V during decomposition and in soils, with the pine and fern V of our site being not very stable. Second, our approach relies on important assumptions: (1) the lands being compared had the same initial conditions prior to changes in plant cover and management associated with reforestation; (2) the chemistry of pine and fern derived lignin does not vary between NF and WF plots, and the $\frac{DHA}{V}$ of VC-0 plots serves as an accurate baseline for examining change in VC-12 and VC-30 plots; and (3) all plots experienced similar breakdown rates for DHA and V across stands and plots. These are reasonable assumptions based on the following information. For our first assumption, remote sensing data suggest that sites were relatively uniform prior to tree planting. For assumption 2, because the sites were relatively uniform, all pine trees came from the same planting stock, and a single species dominated the understory across WF plots, we do not envision that pine or fern chemistry varied across WF and for pine across NF plots, in part confirmed by our chemical analyses of pine and fern material sampled from all stands. For our final assumption, while microclimate conditions would have varied across plot and stand types, we expect that within WF plots under pine, climate conditions would have been fairly uniform, with the largest differences occurring between VC-0 and the other stand types and between WF and NF plot types. These microclimate variations could affect litter decomposition rates and so the input of various compounds into soil, as well the persistence of these compounds in soil – which we consider in our interpretation of the results.

2.7. Calculation of soil organic carbon stock

Soil organic carbon stock (Mg ha^{-1}) for the 0–10 cm depth was calculated as SOC content (%) × bulk density (g cm^{-3}) × soil depth (cm). In our calculations, we estimate values based on an equivalent soil mass basis (Ellert and Bettany, 1995), to account for likely modifications in soil bulk density resulting from ecological restoration. A maximum soil mass across all sites of 1500 Mg ha^{-1} was used to compare the effect of ecological restoration and fern on SOC variables.

2.8. Statistical analysis

All data were collected from 5 subplots of WF and 5 subplots of NF subplots along each of three transects for each of the three aged stands (VC-0, VC-12 and VC-30) for a total of 90 subplots. Our sampling of these 90 subplots constitutes simple spatial pseudoreplication. While our study system is well suited for asking novel questions about

biogeochemical effects of understory colonization by ferns over three decades of restoration, we recognize the statistical limitations of this design. We suggest that the relative homogeneity of the three stands prior to sampling support interpretation of any observed differences across plots as being caused by planted or colonizing vegetation. We also used generalized linear mixed models (GLMM) where sampling unit can be included as a random effect in addition to the variables of interest (fixed effects) (e.g. Millar and Anderson, 2004; Wilcox et al., 2016; Prendergast-Miller et al., 2017). And so GLMM was used to assess differences in the soil organic C and N, $\delta^{13}C$, $\delta^{15}N$, nutrients, lignin-derived phenols, and microbial community composition across stand types (continuous variable) and fern expansion (factor with two levels: NF or WF). In the fitted GLMM, stand (three levels of age), treatment (WF and NF) and their interaction terms were modeled as fixed effects, transect was modeled as a random block effect, and a Type II Wald Chi-square test was used to evaluate the significance of tested effects. We estimated 95% confidence intervals for model parameters; parameter estimation was achieved through bootstrapping using 500 simulations for each model, which included Fern × Stand interaction terms in order to account for initial differences between NF and WF plots. The GLMM analysis was carried out by using the *lmer* function in the lme4 package (Bates et al., 2013) and the ANOVA function in the car package (Fox and Weisberg, 2011) in the statistical platform R 3.0.2 (R Development Core Team, 2013).

We used indicator PLFAs and principal components analysis (PCA) to compare soil microbial community structure across the three stand types. We also performed redundancy analysis (RDA) to determine which environmental factors were related to soil microbial community structure using the CANOCO software (version 5.0, Microcomputer Power, Inc., Ithaca, NY). We used Spearman linear correlation analysis to examine the relationship between the $\delta^{13}C$ values of soil and the $\delta^{13}C$ values of litter and root. Where $p \leq 0.05$, we considered effects to be significant. Unless specified, all statistical tests were performed using SPSS 17.0 (version 17.0; IBM, Armonk, New York, USA).

3. Results

3.1. Soil C stocks, nutrient availability, above- and belowground litter quality and quantity

In VC-12 and VC-30 stands, 90 and 85% of stand ground area, respectively, were covered by fern, which represents substantially more fern cover than in the VC-0 stand (Fig. 1). The storage of SOC was highest in VC-30 and lowest in VC-0, with intermediate levels in VC-12 (Fig. 2; $p < 0.001$). Within VC stand types, we found that mineral soils in WF plots consistently stored more SOC than soils in NF plots (Fig. 2; $p < 0.01$). These WF versus NF differences in SOC storage were largest in VC-0 (NF: 2.3 Mg C ha^{-1}; WF: 9.1 Mg C ha^{-1}; $p = 0.001$), smallest in VC-12 (NF: 9.0 Mg C ha^{-1}; WF: 14.9 Mg C ha^{-1}; $p = 0.002$) and intermediate in VC-30 (NF: 7.7 Mg C ha^{-1}; WF: 17.6 Mg C ha^{-1}; $p < 0.001$). Compared with NF plots, soil from WF plots had higher average total N (0.61 versus 0.38 g N kg^{-1}), P (99.1versus 68.6 mg P kg^{-1}), NH_4^+ (8.18 versus 4.27 mg N kg^{-1}) and potential N mineralization (28.9 versus −68.4 μg N kg^{-1} d^{-1}), but lower soil pH (4.7 versus 4.9) and extractable NO_3^- (0.16 versus 0.46 mg N kg^{-1}) (Fig. 3).

Fern aboveground litter biomass and harvested belowground fine root biomass in WF plots ranged across stands from 26 to 99 g m^{-2} and 132–1254 g m^{-2}, respectively, and was highest in the VC-12 stand and lowest in the VC-0 stand (Table 1). Pine aboveground litterfall (collected monthly) in the WF plots ranged 69 g m^{-2} in the VC-0 stand, 305 g m^{-2} in the VC-12 stand, and 422 g m^{-2} in the VC-30 stand, while in the NF plots pine litterfall biomass was 62 g m^{-2} in the VC-0 stand, 311 g m^{-2} in the VC-12 stand, and 286 g m^{-2} in the VC-30 stand. Pine harvested belowground fine root biomass in WF and NF plots ranged across stands from 18 to 291 g m^{-2} and 5–241 g m^{-2}, respectively (Table 1). Fern roots greatly affected belowground C content (SOC + fine roots) in all WF plots (Table 1; Fig. 2), where fern fine root biomass was 132 g m^{-2} in the VC-0 stand, 1254 g m^{-2} in the VC-12 stand, and 520 g m^{-2} in the VC-30 stand.

3.2. Soil lignin-derived phenols

Total content of lignin-derived phenols in soil (V *plus* S *plus* C) in the NF plots were 7.1, 207.4 and 84.6 mg g^{-1} soil for VC-0, VC-12 and VC-30 stands, respectively, but showed a pattern of increasing linearly with stand age in WF plots (86.1, 168.7 and 183.9 mg g^{-1} soil for VC-0, VC-12 and VC-30 stands, respectively) (Fig. 4a). The SOC-normalized content of lignin-derived phenols in the NF plots were highest for the VC-12 stand (4.7, 31.2 and 18.8 mg g^{-1} SOC for VC-12, VC-0 and VC-30 stands respectively), whereas lignin-derived phenols mass was constant across WF plots and stand age (Fig. 4b). Taken together, total soil lignin content in the WF plots was significantly higher than that in the NF plots ($F = 21.6$, $p < 0.001$) while an opposite trend was observed for the SOC-normalized content of lignin-derived phenols ($F = 4.6$, $p = 0.033$; Fig. 4a and b). For VC-12 and VC-30 stands, ratios of vanillic acid to vanillin were higher in soils of WF than NF plots

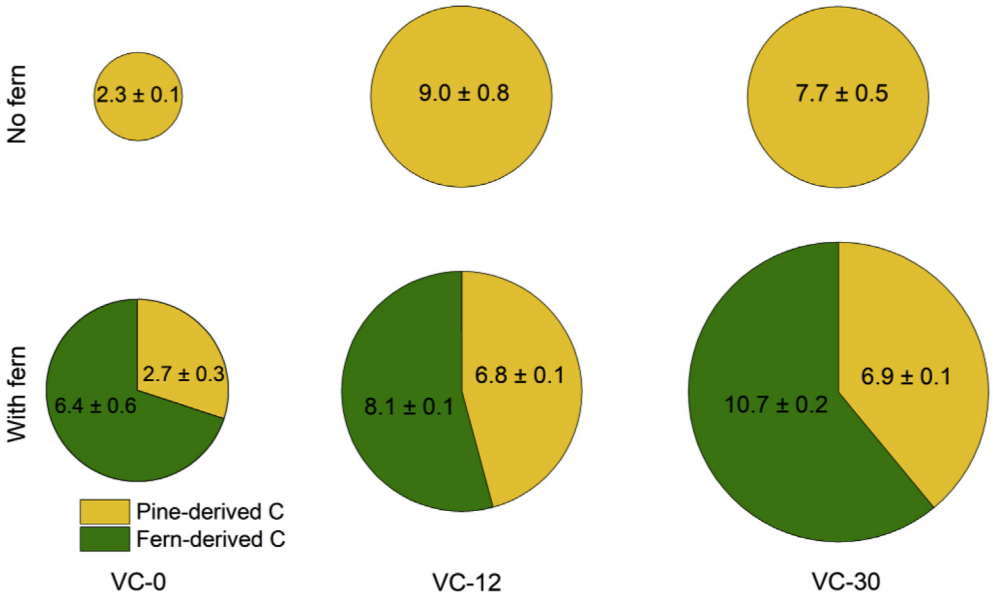

Fig. 2. Data from VC-0, VC-12 and VC-30 stands in Fujian Province, China, showing total surface soil (Mg ha^{-1} in 1500 Mg soil mass layer) C stock, which was calculated based on soil equivalent mass, and the contribution of fern-derived C during restoration of pine plantations in WF plots and soil C stocks in NF plots. The size of each circle represents soil C pool size.

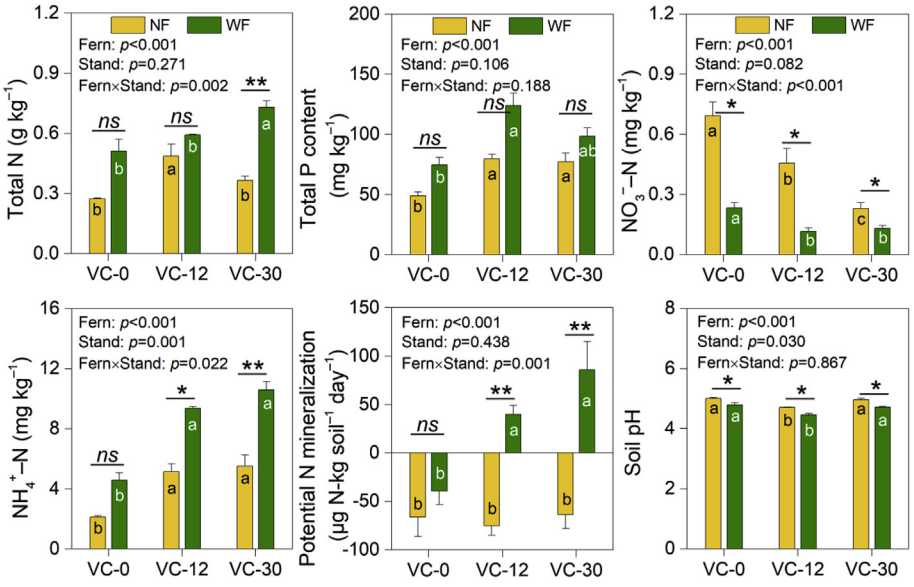

Fig. 3. Data from VC-0, VC-12 and VC-30 stands in Fujian Province, China, showing values for various soil fertility metrics measured in plots with fern (WF) and without fern (NF). Letters in the same treatment (NF or WF) represents significant differences ($p = 0.05$) among three stands. *, above bar indicates significant difference between NF and WF within same stand; ns, no significant difference; *, $p < 0.05$; **, $p < 0.01$. Data represent means ± SE ($n = 3$).

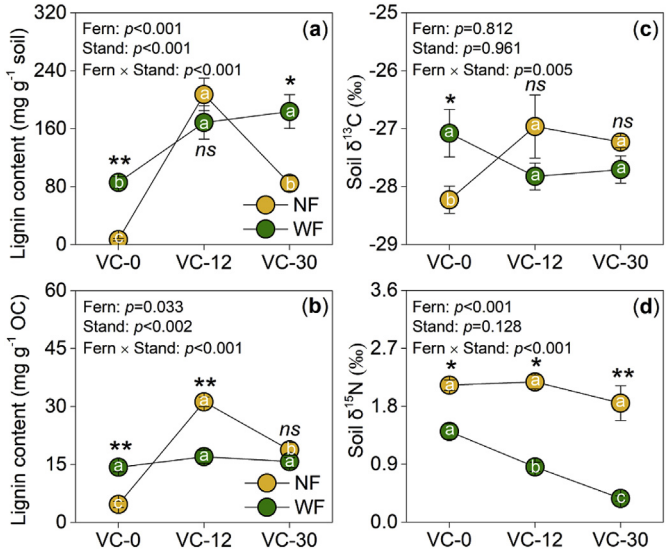

Fig. 4. Data from VC-0, VC-12 and VC-30 stands in Fujian Province, China, showing (**a**) total soil lignin content (mg g^{-1} soil), (**b**) SOC-normalized lignin content (mg g^{-1} SOC), (**c**) $\delta^{13}C$ and (**d**) $\delta^{15}N$ signature of soil in the no-fern (NF) and with fern (WF) plots across the three stands. Letters in the same treatment (NF or WF) represents significant differences ($p = 0.05$) among three stands. *, above bar indicates significant difference between NF and WF within same stand; ns, no significant difference; *, $p < 0.05$; **, $p < 0.01$. VC-0, VC-12 and VC-30 represented with non-restoration, artificial restoration for 12 years and 30 years, respectively.

($p = 0.001$; Fig. 5a). Compared to the NF plots, the soils of WF plots had higher ratios of syringyls to vanillyls (S/V) ($p < 0.001$; Fig. 5b).

3.3. Partitioning soil organic carbon contributions

In WF plots, the ratios of 3,5-dihydroxy benzoic acid (DHA) to V (vanillin + acetovanillon + vanillic acid) phenols and our EMMA analyses indicate that 54–61% of total soil C in 0–10 cm depth soils was derived from fern inputs (Fig. 2). In the VC-12- and VC-30 stands, total SOC stock in soil equivalent mass (1500 Mg soil ha^{-1}) of WF soil was 14.9 and 17.6 Mg C ha^{-1}, respectively, compared to 9.1 Mg C ha^{-1} in the VC-0 stand (Fig. 2). Fern-derived SOC in WF plots under VC-12- and VC-30 stands were 8.1 and 10.7 Mg C ha^{-1}, respectively (Fig. 2). For

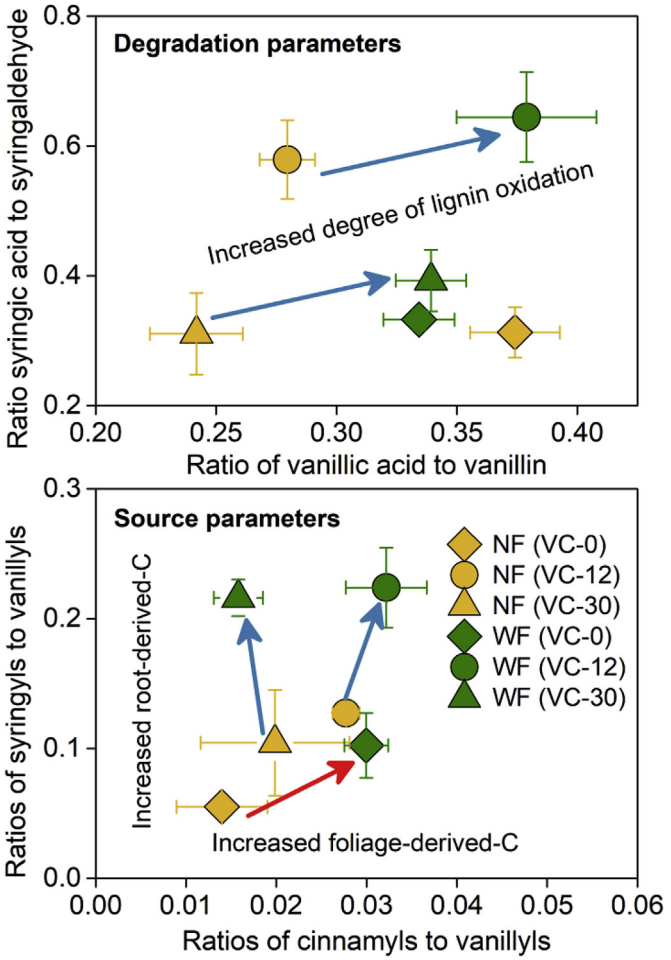

Fig. 5. Data from VC-0, VC-12 and VC-30 stands in Fujian Province, China, showing (**a**) differences in lignin degradation and (**b**) SOM formation derived from pine no-fern (NF) plots and from pine and fern with fern (WF) plots, before and after restoration. Arrows indicate the comparison of NF and WF for each stand.

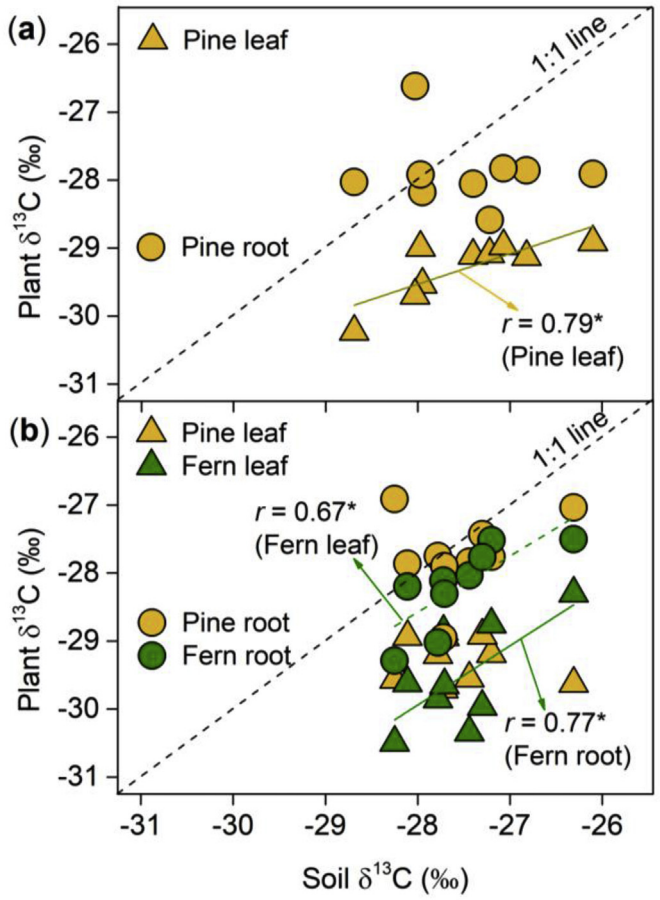

Fig. 6. Data from VC-0, VC-12 and VC-30 stands in Fujian Province, China, showing relationships between $\delta^{13}C$ in foliage, fine root and soil in (**a**) no-fern and (**b**) with fern plots under the restoration sequence. Only significant regression lines are shown (*, $p < 0.05$).

the NF plots, pine-derived SOC amounted to 9.0 and 7.7 Mg C ha^{-1} for VC-12 and VC-30 stands, respectively – much higher than the 2.3 Mg C ha^{-1} in the NF plots of the VC-0 stand. The pine-derived soil C in WF plots was significantly lower (VC-12: 6.8 Mg C ha^{-1}, VC-30: 6.9 Mg C ha^{-1}) compared with pine-derived C in NF plots (VC-12: 9.0 Mg C ha^{-1}, VC-30: 7.7 Mg C ha^{-1}). Percentage wise, in WF plots, the contribution of fern-derived C to total soil C storage accounted for 54 and 61% in VC-12 and VC-30 stands, respectively (Fig. 2).

3.4. Stable isotope ratios for soil and plant C and N

The $\delta^{13}C$ values for SOM were higher in NF than WF plots in both the VC-12 and VC-30 stands, with smaller differences in VC-0 stand (Fig. 4c), resulting in a Fern × Stand interaction effect on soil $\delta^{13}C$ ($F = 10.6$, $p = 0.005$). Soil $\delta^{15}N$ values decreased linearly with stand age in the WF plots ($p < 0.001$), but were constant in the NF plots (Fig. 4d). The $\delta^{15}N$ values of soil in the WF plots were lower than those in the NF plots ($F = 344$, $p < 0.001$; Fig. 4d). In the NF plots, we found a significant positive relationship between $\delta^{13}C$ of soil and pine foliage ($r = 0.79$, $p < 0.05$; Fig. 6a). In the WF plots, soil $\delta^{13}C$ was correlated with the $\delta^{13}C$ of both fern foliage ($r = 0.67$, $p < 0.05$) and fern roots ($r = 0.77$, $p < 0.05$) but not with foliage or roots of pine (Fig. 6b).

3.5. Soil C dynamics and the associated shift in microbial community structure

WF soils had higher abundances of saprotrophic fungi ($F = 17.5$, $p < 0.001$; Fig. 7c), ectomycorrhizal fungi ($F = 32.2$, $p < 0.001$; Fig. 7d), and arbuscular mycorrhizal fungi ($F = 13.2$, $p < 0.01$; Fig. 7e), but lower abundances of gram-positive bacteria ($F = 37.2$, $p < 0.001$; Fig. 7a) and actinomycetes ($F = 41.2$, $p < 0.001$; Fig. 7f). Compared with NF soils, WF soils had higher fungal to bacterial biomass ratios ($F = 29.5$, $p < 0.001$; Fig. 7h).

Redundancy analysis (RDA) showed that the composition of soil microbial community in the NF plots was related to both soil C to N ratio, explaining 40.9% of the variance in composition ($p = 0.002$), and to foliar $\delta^{13}C$ of pine, which explained 16.6% of the variance in composition ($p = 0.034$; Fig. 8a; Table S3). The selected plant and soil properties explained 67% of the variation in the microbial community composition in the NF plots (Fig. 8a). The composition of soil microbial community in the WF plots was related to: foliar $\delta^{13}C$ of fern, which explained 57.4% of the variance ($p = 0.002$); and soil $\delta^{15}N$, which explained 26.7% of the variance ($p = 0.004$). Together, these selected environmental variables explained 91% of the variations in the microbial community composition in the WF plots (Fig. 8b; Table S3).

4. Discussion

4.1. Biogeochemical effects of restoration and ferns

There was a strong influence of understory fern presence on soil C measures with an estimated 54–61% of total soil C in pine restored stands being derived from fern litter and roots in WF plots (Fig. 2). The effect of ferns on soils is likely related to the fact that in WF plots, ferns contributed a significant fraction of total inputs (Table 1). In a synthesis of global forest ecosystems, litterfall represents approximately 10% of net primary production (Litton et al., 2007), while total belowground inputs represent another 22–50% of net primary production (Giardina et al., 2004; McCormack et al., 2015). Because these degraded lands can rapidly accumulate large amount of SOC, particularly during early stages of stand development (Xie et al., 2013), it is reasonable that a large percentage of the net increase in SOC in WF plots relates to high inputs of fern litterfall and senesced roots to soils – in line with our first hypothesis.

We hypothesized that increases in understory fern cover would reduce nutrient losses. The lower soil $\delta^{15}N$ values (Fig. 4d), and the lower soil NO_3^-–N and higher NH_4^+–N availability in WF plots (Fig. 3) compared with adjacent NF plots show that ferns either enriched soils with N, thereby elevating N cycling rates and N retention capacity, or they preferentially colonized microsites with higher N, capitalizing on spatial patterns in soil N availability. Clearly, both drivers of soil C and N dynamics may be operating, where small differences in soil nutrient status drive preferential colonization by ferns, which in turn enhances nutrient cycling, C process rates, and N and C storage (Døckersmith et al., 1999).

The former interpretation is supported by the finding that soils in NF plots were more enriched in ^{15}N, indicating a more open N cycle and greater rates of N loss than in WF plots (Hobbie and Ouimette, 2009). Compared with NF plots, the less-enriched soil $\delta^{15}N$ under WF plots could be attributed to lower rates of N transformation or enhanced N uptake, both of which could limit nitrate leaching or N loss to soil erosion. Further, over time, the presence of ferns linearly reduced soil $\delta^{15}N$ whereas NF plots showed no pattern with time (Fig. 4d). Finally, an elevated N supply would be anticipated to enhance soil microbial populations and use efficiency of plant litter, thereby causing increases in the transfer of plant litter to SOM (Kirkby et al., 2013; Rodríguez et al., 2014; Tamura et al., 2017). Because microbial growth in soils of degraded ecosystems is often limited by N and P availability (Mo et al., 2016), it is not surprising that elevated soil N availability due to fern expansion led to reduced microbial N mining of SOM, reduced SOM mineralization, and enhanced soil C stock. The fact that total soil N but especially P were generally higher in WF compared with NF plots supports the later interpretation that ferns colonized higher nutrient sites within this degraded landscape (Fig. 3), which is not surprising.

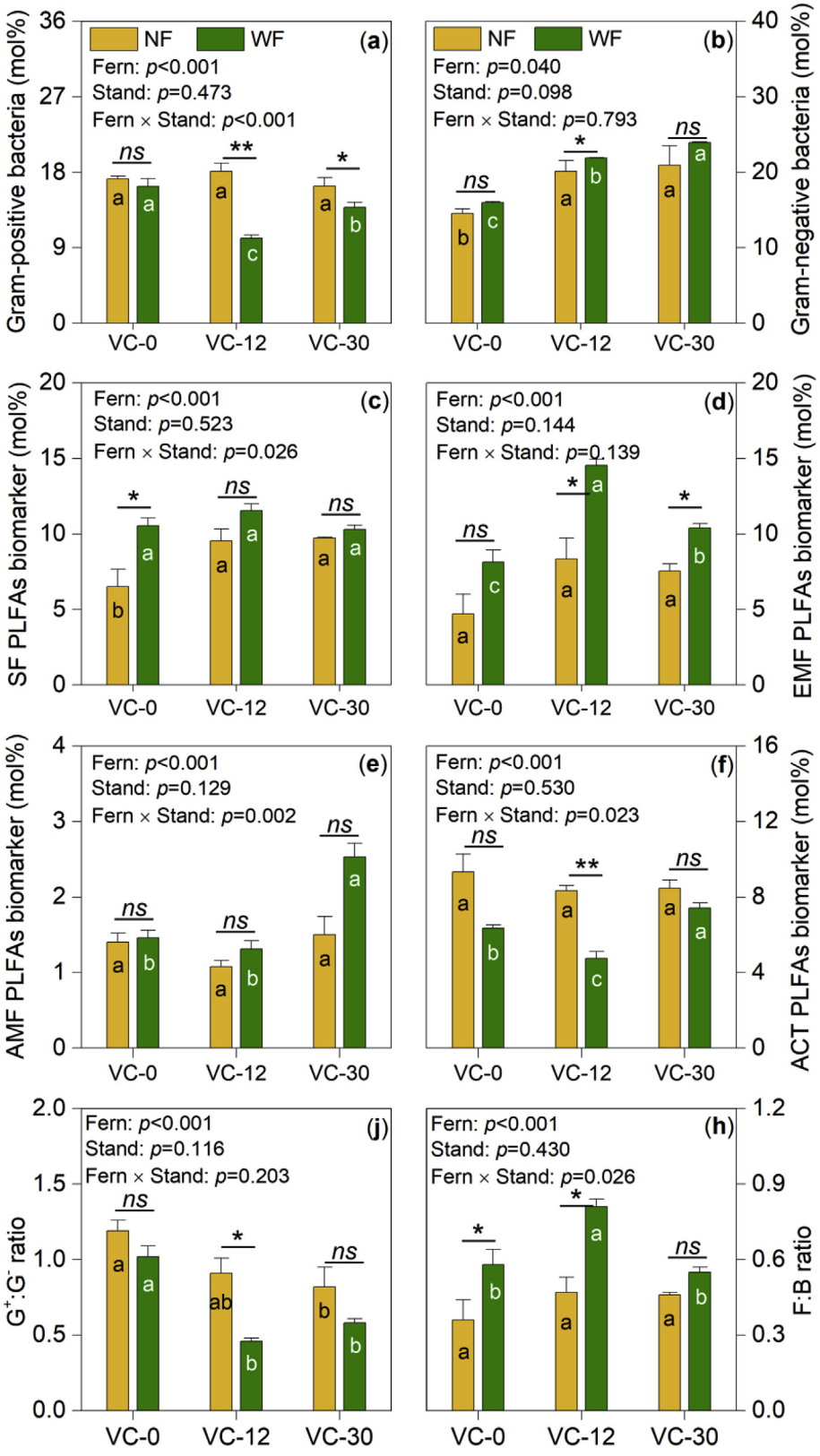

Fig. 7. Data from VC-0, VC-12 and VC-30 stands in Fujian Province, China, showing selected soil microbial PLFA groups at 0–10 cm soil layer sampled in plots with and without understory fern in the three pine stands. NF, no fern plots; WF, with fern plots. Letters in the same treatment (NF or WF) represent significant differences ($p = 0.05$) among the three stands. *, indicates significant difference between NF and WF within the same stand; ns, no significant difference; *, $p < 0.05$; **, $p < 0.01$. Error bars show standard errors ($n = 3$). Arbuscular mycorrhizal fungi (AMF) 16:1ω5c. Saprotrophic fungi (SF) 18:1ω9c. Ectomycorrhizal fungi (EMF) 18:2ω6,9c. Actinomycetes (ACT). G^+: G^-, ratio of gram-positive to gram-negative bacteria; F: B, ratio of fungal to bacterial biomass.

Given the large influence of ferns on soil chemistry, it is likely that both interpretations contribute to the patterns observed here. Taken together, these results support our first hypothesis that fern expansion would reduce soil N losses, ameliorate the soil microenvironment, and increase nutrient retention of pine and fern litter inputs, all of which should drive increases in soil C formation.

4.2. Microbial effects of restoration and ferns

The presence of ferns in all stands was associated with significant increases in soil C (Fig. 2) but also altered SOM composition, which is consistent with results from previous studies showing that plant encroachment can alter C distribution and stability in relation to soil

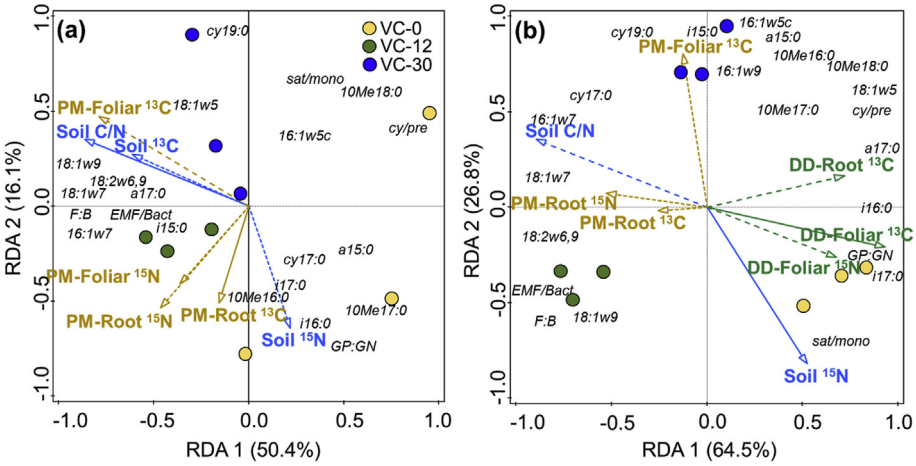

Fig. 8. Redundancy analysis (RDA) for the community structure of PLFAs data in the sub plots without (NF) fern (**a**) and with (WF) fern (**b**) in VC-0, VC-12 and VC-30 stands in Fujian Province, China. Solid lines indicate significance at $p < 0.05$; dashed lines show non-significant relationships. DD-fern, PM-pine.

mineral and aggregate fractions (Briggs et al., 2005; Tamura and Tharayil, 2014; Tamura et al., 2017). Our results show that the abundance of lignin-derived compounds are different between NF and WF plots. The observed decline in lignin-derived compounds in the VC-12 stand might indicate either accelerated decomposition of lignin-derived compounds originating primarily from pine (See 4.3 section), or a more pronounced dilution effect in VC-12 compared with VC-30 stand where the incorporation of large amounts of fern-derived litter would dilute the overall lignin signature of the WF plots. In VC-0 stand, low C and lignin content of soils in the NF plots, highlights that fern litter inputs in the WF plots can drive an accumulation of soil C without accelerating lignin decomposition or causing a dilution effect.

One possible interpretation of this result is that 12 year old Mason Pine stands are temporally closer than 30 year old stands to when the stand was more open (pre-canopy closure). Given a higher light understory environment and access to elevated nutrient supply in disturbed soils of our study site, plus nutrients from decomposing plant residue and fertilizer additions, ferns likely expanded rapidly in the first 6–8 years of stand development. As the canopy started to close, and the young trees begin to shade out the ferns, we speculate that between year 8 and 10, ferns began to respond to increasing shade, as well as increased competition for a reduced nutrient supply, by senescing and releasing detritus to the soil. This could explain the lack of difference in lignin compounds between VC-12 and VC-30 plots; over this time span, the large pulse of senesced fern biomass would slowly decompose and the system would adjust to the new lower level of fern biomass inputs.

Fern expansion increased lignin content of the soil across the three stands under WF plots, whereas lignin content in the NF plots increased between VC-0 and VC-12 plots and then declined to an intermediate level in VC-30 plots (Fig. 4a) appearing to match universal age related productivity patterns for single aged tropical forests (Ryan et al., 1997, 2004). And so in plots without ferns, age related declines in productivity following canopy closure may have resulted in declining lignin content in NF but not in WF plots where declining pine productivity was possibly offset by fern productivity. Notably, while post-canopy closure canopies intercept more light than more open young stands, there typically is a decline in leaf area following canopy closure that can explain age related declines in productivity (Ryan et al., 2004). The SOC-normalized content of lignin-derived compounds after active restoration showed more muted patterns across the three aged stands (Fig. 4b), likely because SOC content is more stable than lignin content (Fig. 2). Interestingly, the increases in pine derived SOC were larger between VC-0 and VC-12 than between VC-12 and VC-30, in line with expected productivity patterns in even aged subtropical plantations (Ryan et al., 2004), and matching SOC patterns for NF plots (Fig. 2).

In contrast, increases in fern derived C were larger in the latter half of the chronosequence. Part of the slower increase in pine derived SOC in the second half of the chronosequence could be due to fern and pine litter mixing, which could accelerate decomposition of the pine litter including native lignin decomposition. In the process, fern-derived lignin compounds accumulated, along with a reduced level of pine derived lignin. Hence, newly formed fern-derived SOC offset slower increases pine derived lignin compounds.

The contrasting pattern of soil $\delta^{13}C$ between the WF and NF plots could be explained by C-mixing, whereby $\delta^{13}C$ values of SOM are influenced by the mixing of new litter-derived C inputs with older soil C (Billings and Richter, 2006). The link between soil $\delta^{13}C$ and foliar $\delta^{13}C$ provides insights into these effects. Pine litter $\delta^{13}C$ and soil $\delta^{13}C$ are correlated in the NF plots, but this correlation disappears in the WF plots, where correlations with fern foliage and roots, indicates that fern detritus dominated inputs (Fig. 6). It could be that in the absence of ferns, aboveground pine litter is deposited on the ground where products of decomposition become incorporated into SOM. When ferns are present, this litter may become suspended in fern foliage where it decomposes more slowly (Dearden and Wardle, 2008; Yang et al., 2014), and which would reduce pine litter inputs to soil – aligning with apparent declines in the accumulation rate of pine derived SOC (Fig. 2). Moreover, in WF plots, the slope of the relationship between $\delta^{13}C$ of plant tissues and soil closely track the 1:1 line (Fig. 6b), supporting the idea that fern detritus dominated litter inputs to WF soils.

To identify the above- and belowground origin of SOM in WF and NF plots, we calculated the ratio of cinnamyls to vanillyls (C/V), an indicator of foliage derived C, and the ratio syringyls to vanillyls (S/V; Otto and Simpson, 2006), an indicator of root derived C (Feng et al., 2008). Our results indicate that S to V ratios were significantly higher in WF plots compared with NF plots, also pointing to a larger contribution of fern derived litter to soil C pools. However, vanillyl phenols are reported to be more stable than cinnamyl phenols in sediments and soils (Opsahl and Benner, 1995), and the increased S/V ratio may have resulted from an increased input of syringyls phenols, which mainly come from fern root tissues, rather than a selective degradation of vanillyl phenols. This interpretation would suggest that increased S to V ratio of SOM at VC-12 and VC-30 stands in the WF plots (Fig. 5b) resulted from increased incorporation of fern root-derived C – again supporting our hypothesis about fern impacts on soils.

Changes to the quality and amount of litter inputs to soil are often associated with shifts in microbial substrate utilization (Kulmatiski et al., 2016), with resulting changes to microbial biomass, community composition and function (Collins et al., 2016). Fern presence was associated with a strong shift in soil microbial composition and biomass

across the three stand types (Fig. 8b), which is consistent with prior research showing that shrub expansion altered community composition including increasing bacterial and archaeal richness and diversity (Collins et al., 2016). The RDA analyses indicate that the soil microbial community in NF plots was significantly related to $\delta^{13}C$ values of pine root and soil C/N ratios (Fig. 8a), while in WF plots the soil microbial community was significantly related to fern foliar $\delta^{13}C$ and soil $\delta^{15}N$ values (Fig. 8b). Again, if 20–80% of the overstory pine litterfall biomass is being intercepted by the understory fern (Yang et al., 2014), then in WF plots, pine litter may not be reaching soils (Dearden and Wardle, 2008). Such a shift in how pine foliar litter enters soils could alter substrate use by microbes, including changes in response to greater inputs from fern foliar and root litter sources in WF plots.

4.3. Consequences

Fern induced changes in soil lignin composition might be caused by some level of priming. Studies have demonstrated that increasing fresh litter inputs or plant invasion can prime decomposition of native recalcitrant C (such as lignin) due to increases in fungal biomass and activity (Fontaine et al., 2004, 2011; Lü et al., 2015; Tamura and Tharayil, 2014; Tamura et al., 2017). Increases in the ratios of common lignin oxidation products (*i.e.*, vanillic acid to vanillin and syringic acid to syringaldehyde), indicate greater lignin oxidation (Opsahl and Benner, 1995; Otto and Simpson, 2006; Feng et al., 2008). The ratios of vanillic acid to vanillin were significantly higher in WF plots compared to NF plots under both VC-12 and VC-30 stands (Fig. 5a), this indicates that ferns may be stimulating lignin oxidation. To this end, the lower levels of pine-derived C in WF plots compared to NF plots (Fig. 2) may suggest that ferns are accelerating the rate at which pine-derived detrital C in WF plots is being decomposed, perhaps through a priming mechanism. We found that fern expansion increased the abundance of saprotrophic and ectomycorrhizal fungal PLFA in soil compared to soils in NF plots, supporting the interpretation that fern inputs could prime decomposition of pine-derived SOM (Fig. 7; Lyu et al., 2018). Specifically, increased abundance of fungi may have promoted lignolytic (lignin-degrading) enzyme activity (Finzi et al., 2006; Feng et al., 2008), which led to enhanced lignin oxidation (Fig. 5a). These findings support our second hypothesis that the expansion of ferns into previously fern free areas stimulates the activity of oxidative enzymes involved in the degradation of lignin. Alternatively, pine detrital inputs could decompose more completely when mixed with fern inputs, and so less pine detritus is stabilized as SOC. Either accelerated decomposition or more complete decomposition of pine litter could giving the appearance of priming, pointing to the need for longer-term mixed versus pure litter decomposition study in this kind of study system. Critically in VC-0 stands, pine derived SOC was higher in WF plots than in NF plots, and differences were small in VC-12 and VC-30 plots indicating that neither of these mechanisms are having a large effect on the outcome of pine litter decomposition on the storage pine derived SOC.

Overall, research on SOM chemistry, storage and dynamics in degraded ecosystems is important to understanding the role that restoration can play in mitigating atmospheric CO_2. This study reports several novel findings with implications for restoration, plant and microbial community ecology, and carbon cycling science. First, ecological restoration and understory fern expansion can exert independent effects on C and N transformations and retention, including driving a shift from an open to a closed N cycle. Second, fern-derived C can make up a large fraction of total SOC (range 54–61%), suggesting an important role for ferns in soil C stock of some degraded ecosystems. Third, by analyzing lignin oxidation at the molecular level, as well as source-specific compounds and stable isotopic composition, it is possible to quantify how ferns influence soil lignin composition, including molecular-level processes for increased fern-derived but microbially mediated C sequestration. These results highlight that overstory management and understory responses to ecological restoration can play important role in enhancing SOM formation and stability.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2019.02.004.

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