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Faster recovery of soil biodiversity in native species mixture than in *Eucalyptus* monoculture after 60 years afforestation in tropical degraded coastal terraces

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Abstract

Afforestation is an effective method to restore degraded land. Afforestation methods vary in their effects on ecosystem multifunctionality, but their effects on soil biodiversity have been largely overlooked. Here, we mapped the biodiversity and functioning of multiple soil organism groups resulting from diverse afforestation methods in tropical coastal terraces. Sixty years after afforestation from bare land (BL), plant species richness and the abundance of plant litter (398 ± 85 g m^{-2}) and plant biomass (179 ± 3.7 t ha^{-1}) in native tree species mixtures (MF) were restored to the level of native forests (NF; 287 ± 21 g m^{-2} and 243.0 ± 33 t ha^{-1}, respectively), while *Eucalyptus* monoculture (EP) only successfully restored the litter mass (388 ± 43 g m^{-2}) to the level of NF. Soil fertility in EP and MF was increased but remained lower than in NF. For example, soil nitrogen and phosphorus concentrations in MF (1.2 ± 0.2 g kg^{-1} and 408 ± 49 mg kg^{-1}, respectively; $p < 0.05$) were lower than in NF (1.8 ± 0.2 g kg^{-1} and 523 ± 24 mg kg^{-1}, respectively; $p < 0.05$). Soil biodiversity, abundance (except for nematodes), and community composition in MF were similar or greater than those in NF. In contrast, restoration with EP only enhanced the diversity of microbes and mites to the level of NF, but not for other soil biota. Together, afforestation with native species mixtures can end up restoring vegetation and most aspects of the taxonomic and functional biodiversity in soil whereas monoculture using fast-growing non-native species cannot. Native species mixtures show a greater potential to reach completely

similar levels of soil biodiversity in local natural forests if they are received some more decades of afforestation. Multifunctionality of soil biotic community should be considered to accelerate such processes in future restoration practices.

1 | INTRODUCTION

The importance of forests in the global carbon cycle that sustains life on Earth is undisputable (Malhi, 2012; Moomaw et al., 2020; Pugh et al., 2019; Stephens et al., 2007). The multifunctional roles of forests extend to many aspects such as moderating air temperature and supporting a vast array of biodiversity on Earth (Banerjee et al., 2020; IPCC, 2007; Makarieva & Gorshkov, 2007). Forests also provide economic (e.g., timber, food, and fiber) and social benefits (e.g., subsistence for local populations and cultures; Gren & Amuakwa-Mensah, 2020; Montagnini & Jordan, 2005). Among numerous forests, tropical forests sequester 0.4 Pg C year^{-1} (Baccini et al., 2017) and contain over half of the world's terrestrial biodiversity (Myers, 1979). However, global climate change and human activities such as farming, mining, infrastructure expansion, are causing deforestation and subsequent degradation of soil properties and functions (Durán et al., 2015; Lowman et al., 2013; Veldkamp et al., 2020; van der Werf et al., 2009). So far, more than half of the tropical forests have been destroyed (Poorter et al., 2016), with the rest being under threat (Esquivel-Muelbert et al., 2019; Taubert et al., 2018). Over decades, afforestation has been used as one of the main methods to restore degraded ecosystems. Afforestation has major positive direct or indirect effects on soil fertility and above- and belowground communities (Lagnière et al., 2010; Liu et al., 2017; Ren et al., 2016; Wang et al., 2013, 2017, 2019).

Aboveground community and land-use changes such as deforestation and afforestation can directly or indirectly impact belowground communities (Bezemer et al., 2010; Biederman et al., 2008; Farska et al., 2014; George et al., 2019; Szoboszlay et al., 2017). For example, communities of microorganisms, as the most abundant and diverse organisms on the planet, change dramatically after deforestation (Crowther et al., 2014); natural restoration progress also showed that soil biodiversity becomes more connected and the soil takes up more carbon (Morriën et al., 2017); afforestation with greater tree species richness can enhance soil heterogeneity and thus contribute to soil biodiversity (Dickie et al., 2002; Liu et al., 2018; Strukelj et al., 2021). Such effects on soil biota can subsequently affect ecosystem functioning as soil biodiversity is required for ecosystem multifunctionality and is considered important for ecosystem restoration (Bardgett & van der Putten, 2014; Guerra et al., 2021; Handa et al., 2014; Lefcheck et al., 2015; Soliveres et al., 2016). Soil biodiversity including connections in the soil food web declines in degraded ecosystems, leading to reduced functioning, with far-reaching consequences

for restoration efforts (Ekelund et al., 2009; Harris, 2009; Kardol & Wardle, 2010; Sánchez-Moreno & Ferris, 2007). Increasing evidence suggests that several parameters of soil biodiversity decline including species richness and functional diversity which results in loss of soil functions (Heemsbergen et al., 2004; Nielsen et al., 2011; Saleem et al., 2019; Wagg et al., 2014). The abundance of soil biota, especially those at higher trophic levels, is considered essential for the stability of the ecosystem (Allesina & Tang, 2012). Previous studies showed that afforestation positively affects the recovery of the biomass and diversity of soil biota (Gunina et al., 2017; Zhu et al., 2019). The B:F ratio, the abundance of arbuscular mycorrhizal fungi, the relative abundance of eukaryotic plant pathogens and plant parasite nematodes, and bacterivorous to fungivorous nematodes can indicate the functional characteristics of soil biota (Liu et al., 2019). However, afforestation effects on soil biodiversity and its functioning, especially integrated insights on multiple soil biodiversity groups at different trophic levels including abundance, diversity, community composition, and functional characteristics are largely unknown.

To investigate the afforestation effects on the recovery of belowground communities and their functioning, we studied the linkages between different afforestation practices and soil biodiversity in a tropical coastal forest in South China. The afforestation practices were initiated in the 1960s with no afforestation practices (bare land) and *Eucalyptus exserta* rotation monoculture. Later, in 1974, some *Eucalyptus* forests were clear-cut and reforested with native tree species. We tested whether afforestation with native tree mixtures would restore the community composition, diversity, and abundance of diverse soil biota (bacteria, fungi, actinomycetes, arbuscular mycorrhizal fungi, protists, nematodes, and mites) to levels of undisturbed native forests, while we expected these effects to be less in *Eucalyptus* rotation monoculture forests (Hypothesis 1). Furthermore, we tested if afforestation with native tree mixtures would increase functional characteristics of soil biota more than afforestation with *Eucalyptus* rotation monocultures (Hypothesis 2).

2 | MATERIALS AND METHODS

2.1 | Site description

The restoration study was initiated at the Xiaoliang Tropical Coastal Ecosystem Research Station, Chinese Academy of Sciences, in 1959. The site is located in Maoming City, Guangdong Province,

China (21°27′N; 110°54′E), a region influenced by tropical monsoons. The mean annual temperature is 23°C, the mean annual precipitation 1400–1700 mm, and the soil is laterite developed from granite (Yao et al., 1984). Until the 1850s, this area was covered by evergreen broad-leaved seasonal rainforest. By the 1950s, about 40,000 ha of tropical monsoon forests were cut (Ren et al., 2007), and the bare land was covered by coarse sand and mineral aggregates rich in iron oxides and manganese (Yu & Pi, 1985). Only a small patch of native forests was preserved. Native forests herein refer to tropical secondary forests, which were preserved for over 200 years. Recent vegetation surveys showed that the native forests were dominated by *Cinnamomum camphora*, *Sterculia lanceolata*, *Cryptocarya chinensis*, *Syzygium levinei*, *Syzygium hancei*, *Schefflera octophylla*, *Auquilaria sinensis* and exhibited a similar community composition as local climax vegetation (Cao & Yu, 1998). Therefore, the native forests in our study represent the natural forests in the region.

In 1959, a long-term restoration experiment was started by establishing one reference area (3.7 ha) of bare land (BL), and two sites with restoration treatments (3.9 and 3.8 ha, respectively) in three geographically similar catchments (Figure 1; Ren et al., 2007), which have a similar slope (<6°) and initial soil properties (Li et al., 1996). The initial soil organic matter concentration is 6 mg g^{-1} and total nitrogen concentration is 0.27 mg g^{-1} (Ren et al., 2007). Both restored catchments were initially restored with *Eucalyptus exserta* seedlings in the early 1960s. In 1974, one catchment was clear-cut and reforested with several species of native trees to become a mixed forest (MF), which now still has an average of 14.6 native tree species in 20 × 20 m sample plots. The dominant tree species in MF are *Aphanamixis polystachya*, *Schefflera octophylla*, *Carallia brachiate*, *Symplocos chunii*, *Acacia auriculaeformis*, *Photinia benthamiana*, and *Cinnamomum burmanni* according to a survey performed in 2015. The other catchment remained as a *Eucalyptus* rotation monoculture (EP) that is harvested every 5 to 8 years. Finally, BL, MF, EP, and preserved native forests (NF) were used as four study sites. Five replicated plots (20 × 20 m) at each site were designated for soil sampling during the wet season (June 2018). The native forest site is located about 2 km northeast of BL, EP, and MF, and shares the same climate, soil type, and topographic conditions. The plots at each study site were at least 100 m apart and located at an equal distance from the high tidal mark at each site. All plots were inundated and exposed to the same tide effects.

2.2 | Sample collections and analysis

Diversity and biomass of plant community were measured during the survey in 2015 according to Wen et al. (1999). Plant litter was collected by three 30 cm × 30 cm trappers, which were randomly distributed in each plot and then the collected litter was mixed into one composite sample. Litter samples were dried to constant weight at 60°C and weighted. Five random soil cores were collected in each plot by using a corer (5 cm in diameter, 20 cm in depth) and mixed to obtain one soil sample for each plot. Total organic carbon (TOC) and total nitrogen (TN) concentrations were analyzed by an elemental analyzer (Vario EL III, Elementar). Total soil phosphorus (TP) concentration was measured following H_2SO_4-$HClO_4$ digestion and using the molybdenum antimony colorimetric method (Lu, 2000). Soil P fraction extracting procedures were based on Hou et al. (2014). Dissolved organic carbon (DOC) was extracted by 0.5 M K_2SO_4 and measured by a TOC analyzer (TOC-VCSH, Shimadzu). NH_4^+-N and NO_3^--N were measured by a flow injection analyzer (AA3, Bran Luebbe). Soil pH was measured with a pH meter in a 1:2.5 (w/v) suspension. The activities (µmol g^{-1} dry soil h^{-1}) of β-1,4-glucosidase (BG), β-N-acetyl-glucosaminidase (NAG) and acid phosphatase (AP) were measured following Paz-Ferreiro et al. (2012) and the specific substrates were *p*-nitrophenyl-β-D-glucopyranoside, *p*-nitrophenyl-N-acetyl-β-D-glucosaminide and *p*-nitrophenyl-phosphate, respectively (Sigma).

2.3 | Phospholipid fatty acids analysis

Soil microbial communities were characterized by the phospholipid fatty acid (PLFA) method according to Bligh and Dyer (1959). An internal standard of 19:0 methyl ester was added to the extract before derivatization of PLFAs. Fatty acids were analyzed using a gas chromatograph (6890N, Agilent Technologies) with MIDI peak identification software (MIDI, Inc.). A number of fatty acids, i15:0, a15:0, i16:0, i17:0, and a17:0, were used as biomarkers for Gram-positive bacteria (GP);

FIGURE 1 Degraded coastal land and vegetation restoration in the study area

Coastal forests   **Degraded coastal land**   **Vegetation restoration**
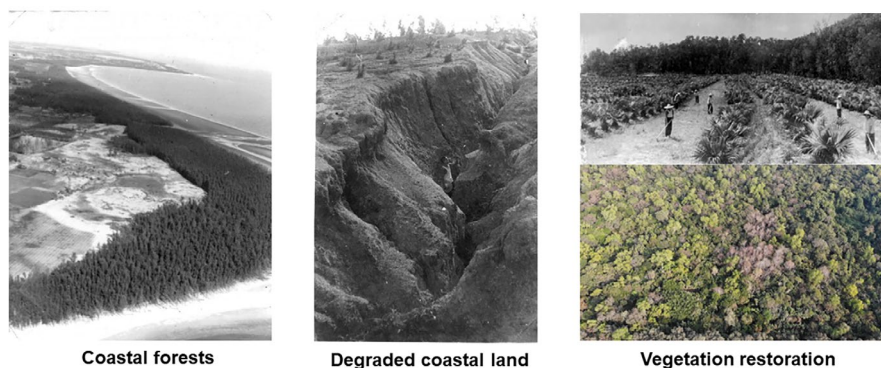
16:1ω7c, 16:1ω9c, 18:1ω7c, cy17:0, and cy19:0 were used as biomarkers for Gram-negative bacteria (GN). Saprophytic fungal biomarkers were 18:2ω6c and 18:3ω6c. The arbuscular mycorrhizal fungi (AMF) biomarker was 16:1ω5c, and actinomycete biomarkers were 10Me16:0, 10Me17:0, and 10Me18:0. The total microbial PLFA concentration was also calculated. All PLFA concentrations were expressed as nmol g^{-1} dry soil. "Microbe" in our study specifically refers to the soil microbial communities, which include fungi, bacteria, AMF, and actinomycetes.

2.4 | DNA extraction and high-throughput sequencing of nematodes and protists

Soil samples for high-throughput sequencing were stored at −80°C before processing. Total soil DNA was extracted from 0.25 g soil using the PowerSoil DNA kit (MOBIO). The isolated DNA was stored at −80°C until the PCR stage. Illumina Hiseq sequencing was used to assess the protist and nematode communities through massively parallel sequencing of 18S rDNA gene. The 18S rDNA gene of protists was amplified with the primer pair 1380F (3′-CCCTGCCHTTTGTACACAC-5′) and 1510R (3′-CCTTCYGCAGGTTCACCTAC-5′; Amaral-Zettler et al., 2009). The primer pair used to amplify nematodes was NF1 (3′-GGTGGTGCATGGCCGTTCTTAGTT-5′) and 18Sr2b (3′-TACAAAGGGCAGGGACGTAAT-5′; Porazinska et al., 2009). PCR amplification and sequencing were made by MAGIGENE. The OTUs of protists were grouped into four feeding groups: phagotrophs, plant pathogens, parasites, and phototrophs (Xiong et al., 2018), and those of nematodes were grouped into four feeding groups: bacterivores (BF), fungivores (FF), plant parasites (PP), and omnivores/predators (OP; Yeates et al., 1993).

2.5 | Extraction and taxonomic identifications of mites and nematodes

To obtain abundance and feeding group composition data of nematodes, morphological method was used also. Molecular information on mesofauna is in its infancy, and therefore we only performed standardized morphological information. Mites were extracted from the soil and litter samples and visually identified at the genus level using an inverted microscope (Nikon) at South China Agricultural University. Individual numbers of fungivorous and omnivorous/predatory mites (Laliberté et al., 2017) were counted, respectively. Nematodes were extracted from 100 g fresh soil by Baermann funnel (Whitehead & Hemming, 1965). The first 100 nematodes were identified using a reversed light microscope (Nikon) and individual numbers of four feeding groups (Yeates et al., 1993) were counted. If there were less than 100 nematodes, then all nematodes were identified and counted. Individual numbers of mites and nematodes per 100 g dry soil were calculated based on the soil moisture data. Nematode channel ratio (NCR) was calculated as BF/(BF + FF), where BF and FF are the relative abundance of bacterial-feeding and fungal-feeding nematodes, respectively (Yeates, 2003).

2.6 | Bioinformatics and statistical analyses

The sequences were demultiplexed and trimmed with a read quality score above 30 using FastQC (Andrews, 2012). Sequences with lengths less than 150 bp and rare sequences were removed. Putative chimeric sequences were detected and removed using Mothur (Schloss et al., 2009). High-quality sequences were assigned to OTUs at ≥97% similarity level. Nematode and protist OTUs were taxonomically assigned at the genus level by blasting against SILVA v.132 (https://www.arb-silva.de) using the classify command in Mothur. Streptophyta, Fungi, Metazoa, and ambiguous taxa in eukaryotes were removed from the OTU table. Diversity indices at the OTU level were calculated.

Alpha diversity of microbial groups was calculated based on the data of PLFA biomarkers (de Vries et al., 2012), and the diversity of various soil faunal groups was calculated based on OTU or morphology data. Statistical analyses were done in SPSS v.23 (SPSS). One-way ANOVAs were used to analyze differences in soil properties, PLFA concentrations, diversity, abundance of soil biota among sample sites with statistical differences considered significant at $p < 0.05$. The least-significant difference (LSD) test was used to assess treatment differences in one-way ANOVA analyses. Graphs were created using GraphPad Prism 7 (GraphPad Software). The PCoA analysis of soil microbial and animal communities based on OTUs or genera data was performed using R (version 3.6.1, R Development Core Team, 2016) with the "vegan", "ggplot2", and "plyr" package.

The PLFA data of soil bacteria, fungi, actinomycetes, and AMF, the abundance data of soil nematodes and mites at the genus level, and the OTU numbers of the 50 most-abundant protists (Table S6) were used for the co-occurrence network analysis using R version 3.6.1 (R Development Core Team, 2016). The visualization of the correlation matrix was created, and network properties were inferred by Gephi version 0.9.2 (https://gephi.org). Significant correlations ($p < 0.05$) including both positive and negative correlations with values greater than 0.9 were visualized.

Structural equation models (SEMs) were constructed by AMOS (SPSS) to analyze the effects of plant resource input by afforestation on soil fertility, the diversity, and abundance of soil biota, and further on soil biodiversity functioning. We defined soil biodiversity functioning by the combination of the B:F ratio, the abundance of AMF, BF:FF nematode ratio, the relative abundance of plant pathogenic protists and PP nematodes (Liu et al., 2019). Positive effects on these represent an increase of these variables which means a stronger bacterial energy channel and greater relative abundance of plant pathogens, while negative effects represent a decrease of these variables which means a stronger fungal energy channel and lower relative abundance of plant pathogens. Detailed information about the variables used for SEM analysis is listed in Table S1. Data of BL were not used for SEM as they cannot be compared with forests. Outliers found in litter biomass data (one in EP and one in MF) were winsorized (90%) as they would cause spurious correlations. Maximum likelihood estimation was used to fit the covariance matrix to the model. Chi-square value, associated p-value ($p > 0.05$), goodness-of-fit-index (GFI > 0.90), and root mean square error of

approximation (RMSEA < 0.05) were measured to ensure the model adequately fitted.

3 | RESULTS

3.1 | Afforestation effects on soil properties and aboveground community

Soil fertility, as indicated by the concentrations of TOC, DOC, TN, TP, NH_4^+-N, and NO_3^--N was greatly enhanced by afforestation, but DOC, TP, TN, and NO_3^--N concentrations were still lower than those in NF ($p < 0.05$; Figure 2; Table S2). TOC concentration in MF (19 ± 3.1 g kg^{-1}) was restored to the level of NF (24 ± 2.0 g kg^{-1}), but not in EP (5.8 ± 0.3 g kg^{-1}). DOC concentration in both EP (137 ± 6.0 g kg^{-1}) and MF (271 ± 18 g kg^{-1}) were increased but not to the level of NF (552 ± 13 g kg^{-1}; $p < 0.05$). TN and TP concentration in MF were both increased compared with BL and EP (1.2 ± 0.2 g kg^{-1} and 408 ± 49 mg kg^{-1}, respectively; $p < 0.05$), but were lower than in NF (1.8 ± 0.2 g kg^{-1} and 523 ± 24 mg kg^{-1}, respectively; $p < 0.05$). The NO_3^--N concentration in both EP (0.1 ± 0.0 mg kg^{-1}) and MF (2.5 ± 1.0 mg kg^{-1}) was not yet restored to the level of NF (8.1 ± 0.9 mg kg^{-1}; $p < 0.05$), while the NH_4^+-N concentration in EP (1.2 ± 0.1 mg kg^{-1}) was restored to the level of NF (0.8 ± 0.3 mg kg^{-1}) and it was even higher in MF (1.8 ± 0.3 mg kg^{-1}; $p < 0.05$). The soil C:N ratio increased to the same level in EP (15.8 ± 0.4) and MF (15.5 ± 0.4) and was lower in NF (13.0 ± 0.2; $p < 0.05$). Soluble P was low in EP compared with all other sample sites (0.6 ± 0.0 mg kg^{-1}; $p < 0.05$), while available P, residual P, and BG did not show significant differences (Table S2) and the other variables referring to carbon, nitrogen, phosphorus, and enzyme activity were all increased by afforestation (Table S2). Soil moisture content (MC) in MF (30.2 ± 0.8%) was significantly increased to the level of NF (29.5 ± 0.5%), and pH was significantly decreased in MF (4.2 ± 0.0) to the level of NF (4.1 ± 0.1; Figure 1; Table S2). Plant biomass (including above- and belowground biomass) in MF (179 ± 3.7 t ha^{-1}) was significantly improved to the level of NF (243 ± 33.0 t ha^{-1}). Litter biomass in EP (388 ± 43 g m^{-2})
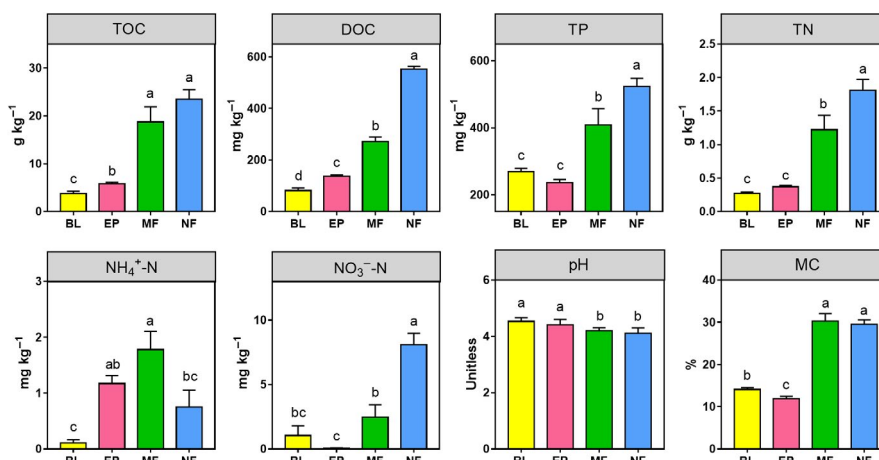
and MF (398 ± 85 g m^{-2}) was the same as in NF (287 ± 21 g m^{-2}). Tree species richness in MF (14.6 ± 0.9) was also similar as in NF (12.6 ± 0.4; Figure 3).

3.2 | Afforestation effects on belowground communities

Following afforestation, the microbial Shannon–Wiener Index in MF (2.53 ± 0.01) was higher than in BL (2.47 ± 0.00) and even higher than in NF (2.49 ± 0.00; $p < 0.05$; Figure 3). The total microbial PLFA concentrations in MF (22.4 ± 3.1 nmol g^{-1}) were significantly increased to the level of NF (36.2 ± 2.9 nmol g^{-1}), but not in EP (10.6 ± 0.4 nmol g^{-1}; Figure 3). Neither the monoculture nor the mixture had significant effects on the diversity of protists and their relative abundance among all eukaryote reads (Figure 3). The abundance of nematodes (per 100 g dry soil) was increased by afforestation, but MF (20.6 ± 3.7) was not yet restored to the level of NF (60.0 ± 15.0; Figure 3). The diversity of nematodes in EP (0.6 ± 0.3) was significantly lower than that in MF (1.6 ± 0.1) and NF (1.7 ± 0.4; Figure 3). Mite diversity increased in MF (2.0 ± 0.2) which was even higher than that in NF (1.4 ± 0.2; $p < 0.05$), but its abundance showed no significant variation (Figure 3). The activities of BG (0.4 ± 0.1 μmol g^{-1} dry soil h^{-1}), NAG (0.2 ± 0.0 μmol g^{-1} dry soil h^{-1}), and AP (4.9 ± 0.4 μmol g^{-1} dry soil h^{-1}) in MF were increased to the level of NF (0.6 ± 0.1 μmol g^{-1} dry soil h^{-1}, 0.2 ± 0.0 μmol g^{-1} dry soil h^{-1} and 4.2 ± 0.2 μmol g^{-1} dry soil h^{-1}, respectively; $p < 0.05$) and the activity of AP in MF was higher than in EP ($p < 0.05$; Table S2). More detailed information of the changes of belowground communities is shown in Tables S3–S5. Detailed information of the high-throughput sequencing results and identifications of the belowground communities are shown in supplemental results (Supplemental file S1) and Table S7.

For the changes in feeding groups and community composition (Figures 3–5), the relative abundance of fungi and actinomycetes in both EP (11.7 ± 0.8% and 15.6 ± 0.6%, respectively) and MF (17.1 ± 0.9% and 16.2 ± 0.3%, respectively) were restored to the level of NF (13.3 ± 1.3% and 15.3 ± 0.2%, respectively). The GP:GN ratio also significantly decreased after afforestation (Table

FIGURE 2 Main variation referring to soil properties on bare land (BL), *Eucalyptus* rotation monoculture (EP), native tree species mixture (MF), and native forest (NF). Data are means ± SE ($n = 5$). Bars with different lowercase letters are significantly different ($p < 0.05$, LSD). DOC, Dissolved organic carbon; MC, Soil moisture content; TN, Total nitrogen; TOC, Total organic carbon; TP, Total phosphorus
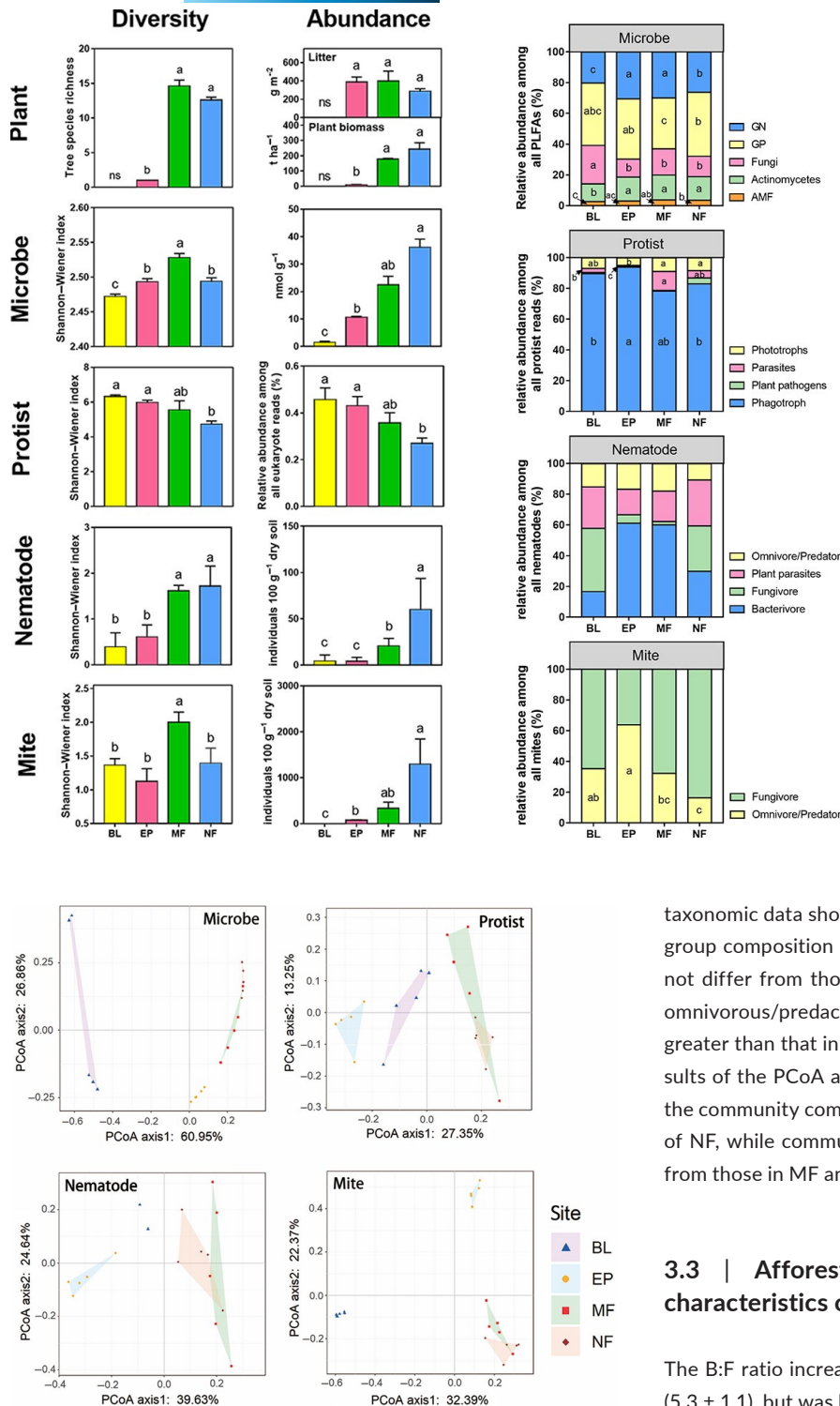
FIGURE 3 Diversity and abundance of soil biota and plant parameters, and relative abundance of different trophic groups on bare land (BL), a *Eucalyptus* monoculture (EP), a native tree species mixture (MF) and a native forest (NF). Data are means ± SE (*n* = 5). Bars with different lowercase letters are significantly different (*p* < 0.05, LSD), and only data with significant differences are marked with a lowercase letter. The word "Microbe" specifically refers to the soil microbial communities, which include fungi, bacteria, AMF, and actinomycetes. GN: Gram-negative bacteria; GP: Gram-positive bacteria

FIGURE 4 Results of PCoA analyses of different soil biota communities on bare land (BL), a *Eucalyptus* monoculture (EP), a native tree species mixture (MF), and a native forest (NF). "Microbe" specifically refers to the soil microbial communities, which include fungi, bacteria, AMF and actinomycetes

S3), and was lower in EP (1.3 ± 0.0) and MF (1.1 ± 0.0) than in NF (1.6 ± 0.0; *p* < 0.05). The relative abundance of phagotrophic protists decreased from 89.7 ± 0.7% in BL to 78.3 ± 6.1% in MF. The

taxonomic data showed no significant changes in nematode feeding group composition by afforestation (Table S5). The NCR values did not differ from those in NF (Table S4). The relative abundance of omnivorous/predacious mites in EP (63.9 ± 12.7%) was significantly greater than that in MF (32.3 ± 8.5%) and NF (16.3 ± 4.3%). The results of the PCoA analysis based on OTU/genera data showed that the community composition of soil biota in MF overlapped with that of NF, while community compositions in BL and EP were different from those in MF and NF.

3.3 | Afforestation effects on functional characteristics of soil biota

The B:F ratio increased in EP (6.1 ± 1.0) to the same level as in NF (5.3 ± 1.1), but was lower in MF (3.7 ± 0.6; *p* < 0.05). The abundance of AMF in both EP (0.32 ± 0.01 nmol g^{-1}) and MF (0.84 ± 0.16 nmol g^{-1}; *p* < 0.05) were increased, but it was still lower than that in NF (1.30 ± 0.10 nmol g^{-1}; *p* < 0.05). The relative abundance of plant pathogenic protists and plant parasite nematodes did not differ from that in NF (Table S5). The BF:FF nematode ratio in MF (11 ± 0.0) increased and was higher than that in NF (2.3 ± 0.8).

The results of the co-occurrence network and SEM (Figures S1–S3) should be taken with caution due to the low number of replicates, but we wanted to explore the entire soil food web characteristics through these results. However, no clear patterns of potentially

FIGURE 5 Variables of functional characteristics of soil biota on bare land (BL), a *Eucalyptus* monoculture (EP), a native tree species mixture (MF), and a native forest (NF). Data are means ± SE (*n* = 5). Bars with different lowercase letters are significantly different (*p* < 0.05, LSD), and only data with significant differences are marked with a lowercase letter. BF, bacterivores; FF, fungivores; PP, plant parasites
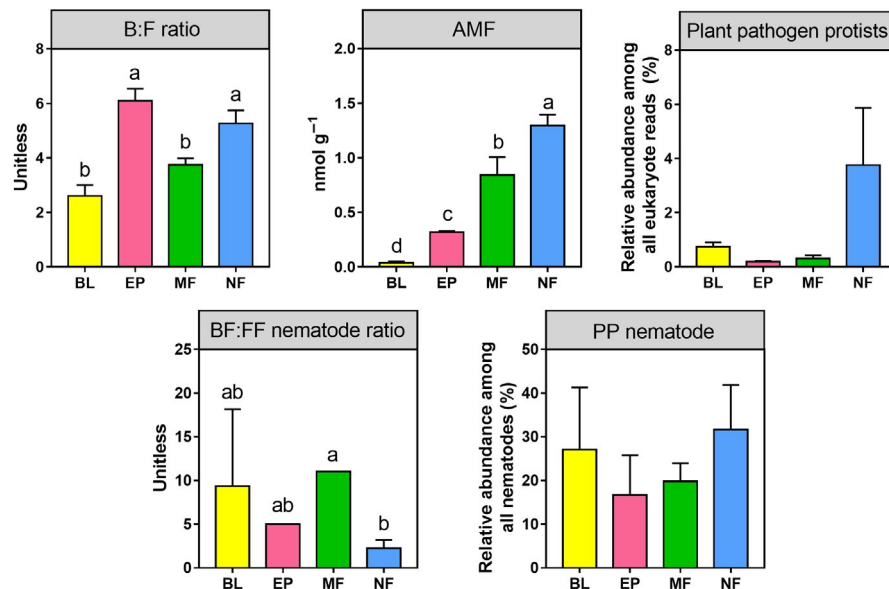
increased network complexity in mixed and natural forests were found and no significant effects among the diversity, abundance, and soil biodiversity functioning were found.

4 | DISCUSSION

We provide partial support for our first hypothesis. MF, indeed, more successfully restored the belowground biodiversity than EP, but not always to the level of NF. For example, the abundance of nematodes and soil fertility in MF was higher than that in EP, but it was not yet restored to the level of NF. Community compositions of all soil biota in MF resembled that of NF, but fundamentally differed in EP and BL. Underlying drivers of these patterns could have been plant resource input and plant species richness as carbon pumps into soils, as these were greater in MF than in EP, and obviously in BL (Eissfeller et al., 2013; Schneider et al., 2012). Furthermore, plant species richness affects soil biota not only through litter input, but also through other resources such as root exudates (Eisenhauer et al., 2010; Preece & Peñuelas, 2016; Srivastava et al., 2009). Mixtures of native tree species might restore soil biodiversity by providing more niches for soil biota, thereby increasing species richness. The resulting soil biodiversity might be more like that in native forests which might be adapted to the local soil biodiversity (Dassen et al., 2017; Dawud et al., 2017; Lang & Polle, 2011; Leff et al., 2018; Orwin et al., 2006). The significantly decreased pH value and increased soil moisture in MF likely further influenced soil biodiversity, such as shown for bacteria, protists, and nematodes (Bates et al., 2013; Delgado-Baquerizo et al., 2018; Hu & Qi, 2010; Rousk et al., 2010). This emphasizes the positive role of a diverse mixture of trees in restoration efforts to increase ecosystem multifunctionality (Di Sacco et al., 2021). The profound difference between MF and EP including reduced biodiversity in EP can also be explained by constant rotation every 5–8 years in EP that led to the temporary loss of input of plant resources which might further disturb soil biodiversity

(Pawson et al., 2013). A peculiar finding was the contrasting pattern observed for protists: unlike most other soil biota, protists showed a slightly decreased trend in both diversity and relative abundance among all eukaryotes following afforestation. This pattern might be caused by the nature of the data as sequence data are relative (Geisen et al., 2018; Geisen & Bonkowski, 2018). We have shown a profound total increase of fungal biomass which might be greater than that of protists, meaning that protists might indeed increase in absolute terms. The exact meaning of the relative decrease of protists needs further examination. We assume that protists feed more on fast-growing bacteria and yeasts, and therefore increase hyphae-forming fungi (Geisen et al., 2016), which are representative for later-successional plant communities such as diverse and mature forests (Hannula et al., 2017). As such, the ratio of fungi to protists may be a meaningful and easy-to-apply measure to trace successful restoration.

We confirm our second hypothesis that afforestation with native tree mixtures increased functional characteristics of soil biota more than that of a *Eucalyptus* rotation monoculture. The lower B:F ratio in MF than in EP indicates a more fully restored status in MF, because the B:F ratio decreases during secondary succession at a global level (Fierer et al., 2009; Zhou et al., 2017), and forest degradation consistently decreases the abundance of fungi (Zhou et al., 2018). It also suggests that the microbial community in MF was more stable and more resistant to a lower quality or availability of resources than those in EP. A possible explanation is that NH_4^+-N and NO_3^--N concentrations in EP were lower than those in MF, although soil C:N ratio did not differ in EP and MF. Fungi require less nitrogen for growth than bacteria (Bardgett & McAlister, 1999; De Deyn et al., 2008) which makes them less dependent on high nitrogen availability in the soil (Fierer et al., 2009). Higher activities of the microbial enzymes in MF than in EP suggest a more active microbial community, which could promote the cycling of C, N, and P (Stott, 2019). The increased abundance of AMF in MF suggests more favorable functional characteristics because AMF are crucial for the deposition of slow-cycling

organic compounds (Wilson et al., 2009), formation of soil aggregates that protect organic matter from decomposition (Rillig et al., 2002), phosphorus uptake, cycling of nitrogen, and protection of plants against rhizosphere pathogens (Feng et al., 2002; Govindarajulu et al., 2005). High soil biodiversity supposedly enhances the functioning of soil biota which might accelerate restoration (Bardgett & van der Putten, 2014; Kardol et al., 2006; Lefcheck et al., 2015; Liu et al., 2019; Soliveres et al., 2016). However, even though the diversity and biomass of aboveground community and the diversity of soil biota in MF was restored to the level of NF, soil fertility in MF was still lower than that in NF, whereas the abundance of soil biota showed a similar restoration trend as soil fertility. Among soil biodiversity, the abundance of higher trophic level organisms is of major importance for soil food web functions and soil health (Ferris & Bongers, 2006; Ferris et al., 2004; Sánchez-Moreno & Ferris, 2007) and is closely related to the increased abundance of microbes and plant resources (Bardgett & Wardle, 2010; Scherber et al., 2010). Also, intense predator–prey interactions likely increase ecosystem stability (Allesina & Tang, 2012). However, the abundance of nematodes and mites, which contain consumers and predators at higher trophic levels in MF were still lower than those in NF. This may lead to reduced predator–prey interactions and cause a further reduction of the transfer of energy, carbon, and nitrogen, etc., that released from aboveground community transferred into and sequestered by the soil food web (Bartley et al., 2019; Coleman, 2008; Zhang et al., 2021). Thus, insufficient soil fertility in restored sites may be explained by incomplete restoration outcomes of soil biodiversity. The results of the co-occurrence network and SEM should be interpreted with caution because of the low number of replicates, which may explain why no clear patterns of potentially increased network complexity in natural and mixed forests were found and no significant effects among diversity, abundance, and functioning of soil biota.

5 | CONCLUSIONS

Our study provides detailed results on afforestation effects on the restoration of belowground biodiversity and its functioning. We conclude that afforestation with native tree species mixtures restores soil biodiversity more effectively including its functioning. Although 60 years afforestation with native species did not reach the full level of soil fertility, abundance, and functional characteristics of belowground community of the more than 200-year-old native forest, certain traits of diversity of soil biota reached similar levels of the native forest. This indicates that the overall habitat restoration may take several decades, with likely consequences for ecosystem functioning. However, the afforestation with native species can end up reaching similar levels of diversity of native forests whereas plantations using fast-growing non-native species cannot.

CONFLICT OF INTEREST

The authors declare to have no conflict of interest.

DATA AVAILABILITY STATEMENT

All data that support the findings of this study are available in Zenodo (https://doi.org/10.5281/zenodo.4769247). The raw data and relative information of high-throughput sequencing are available in the US National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov) under BioProject PRJNA730783.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.