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Land use change alters carbon and nitrogen dynamics mediated by fungal functional guilds within soil aggregates

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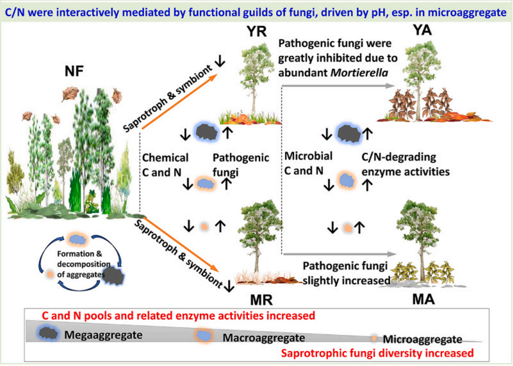
HIGHLIGHTS

- Forest conversion generally decreased C and N pools in all aggregates.
- Agroforestry systems improved C- and N-cycling enzyme activities in all aggregates.
- Young agroforestry systems inhibited pathogenic fungi in all aggregates.
- C and N pools, especially in micro-aggregates, were interactively mediated by fungal functional guilds.

GRAPHICAL ABSTRACT



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ABSTRACT

Land use change is one of the greatest threats to soil biodiversity and ecological functions; however, how such a transition affects soil carbon (C) and nitrogen (N) dynamics driven by fungal communities at the aggregate level remains unclear. Here, we explored the variation in soil C and N pools, specific enzyme activities and fungal communities and functional guilds within three aggregate sizes (megaaggregates, > 2 mm; macroaggregates, 0.25–2 mm; microaggregates, < 0.25 mm) in a natural forest, 12- and 24-year-old rubber monocultures and corresponding agroforestry systems in tropical China. Tropical forest conversion to rubber monocultures generally reduced C and N pools in all aggregates, while agroforestry systems decreased microbial biomass C and N. Carbon- and N-degrading enzyme activities responded differently to forest conversion and were enhanced in agroforestry systems. The levels of C and N pools and their related enzyme activities increased as the aggregate size decreased. Moreover, fungal compositional shifts in dominance from copiotrophic Ascomycota and Basidiomycota (r-strategists) into oligotrophic Zygomycota (K-strategists) were noted following forest conversion,

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resulting in more pathogenic fungi at the expense of saprotrophic and arbuscular mycorrhizal fungi. Pathogenic fungi were greatly inhibited due to abundant *Mortierella* after the establishment of 12-year-old agroforestry systems. The diversity of saprotrophic fungi was the highest in microaggregates. Regardless of land use type, aggregate-associated C and N pools, especially DOC, MBC, NO_3^--N and DON in microaggregates, were interactively mediated by functional guilds of fungi, which was primarily driven by soil pH. These results highlight the importance of fungal functional guilds in determining C and N dynamics at the aggregate level and provide insights into the sustainable management of cash tree plantations.

1. Introduction

Land use change, such as deforestation caused by tree plantation expansion, is a key driver of the ongoing global environmental change (Wang et al., 2021), especially in the tropics (Mueller et al., 2016). Tropical deforestation for the establishment of monoculture cash tree plantations poses the greatest threats to biodiversity. This has long-lasting effects on future ecosystem structure (e.g., plant community composition) and processes (e.g., soil microhabitat formation and nutrient cycling) (Singh et al., 2017; Berkelmann et al., 2020), thereby reducing ecological resistance and resilience to unpredictable climatic disturbances. Consequently, agroforestry as an integrated land use may be an alternative to mitigate biodiversity losses and land use legacies resulting from tropical deforestation and agricultural intensification worldwide; it can reassemble above- and belowground species interactions while maintaining acceptable productivity levels (Liu et al., 2018; Arevalo-Gardini et al., 2020; Ren et al., 2023). Unfortunately, it remains unclear how such stepwise land use transitions, which drastically alter vegetation structure, affect nutrient cycling driven by microorganisms at the soil microscale (Brinkmann et al., 2019).

Soil is often composed of a variety of aggregate fractions depending on the association between organic matter and mineral soil particles (Jiang et al., 2014). According to the hierarchical model, microaggregates are held by interactions of free primary particles with persistent binding agents, oxides and disordered aluminosilicates (Tisdall, 1994). Subsequently, microaggregates are bound together to form macroaggregates by transient binding agents (i.e., a network of roots and hyphae). Macroaggregates typically contain rich labile substrates derived primarily from plant residues and are easier to decompose, whereas microaggregates contain more recalcitrant organic matter with longer residence times (Six et al., 2000; Liao et al., 2020). Aggregates of different sizes and stability create heterogeneous microhabitats that differ in physicochemical properties for soil microorganisms (Trivedi et al., 2017; Upton et al., 2019), which affects their role in carbon (C) and nitrogen (N) cycling (Tiemann et al., 2015; Liao et al., 2018). To fully understand C and N dynamics within soil aggregates, it is necessary to separate them into distinct pools with different availability. Changes in land use affect the formation and maintenance of aggregates (Singh et al., 2017), the dynamics of organic C pools (Garcia-Franco et al., 2015; Trivedi et al., 2015) and the functional potential of microorganisms (Upton et al., 2019; Navas et al., 2020) within different aggregates. However, little is known about the linkage between soil microbial communities and C and N turnover at the aggregate level in response to land use conversion, especially natural forests (NF) conversion into tree plantations.

Fungi represent a key component of soil microbiota and contribute to multiple forest ecosystem processes by forming mutualistic symbioses with many tree species (Fontaine et al., 2011; Voříšková et al., 2014; Rodriguez-Ramos et al., 2021). They are also major agents responsible for the formation and stabilization of soil aggregates through secretion-based bonding, hydrophobicity modulation and hyphal entrapment. Greater fungal abundance and diversity have been found in microaggregates (Liao et al., 2018; Navas et al., 2020), attributing to increased enzymatic activities, particularly related to cellulose breakdown (Bach et al., 2018). By contrast, higher fungal network complexity has been reported in macroaggregates (Jiang et al., 2018; Li et al., 2019), consistent with the theory of microaggregtae–macroaggregate formation for improved C and N storage (Six et al., 2000). The differential distribution of fungal communities reflects their adaptability to aggregate-associated resource availability. Significant effects of tropical land use conversion on fungal communities across different soil layers have been revealed in some studies (Alele et al., 2014; Mueller et al., 2016; Brinkmann et al., 2019), but very little is known about variations in fungal communities within aggregates. Hence, it is essential to assess the impact of land use change on fungal structure and function across soil aggregates to understand their potential feedback on C and N cycling in tropical ecosystems.

Fungal guilds are taxa capable of different lifestyles based on differences in direct and indirect plant–soil associations (Nguyen et al., 2016). Each guild utilizes distinct nutrient acquisition strategies to fulfil ecological functions and can therefore be expected to be affected by varying abiotic and biotic environmental factors driven by land use changes (Ballauff et al., 2021; Deng et al., 2023). Increasing evidence suggests that interguild fungal interactions affect soil C and N dynamics through i) 'Gadgil effect', i.e., competition between mycorrhizal and saprotrophic fungi for limited resources, suppressing decomposition (Fernandez and Kennedy, 2016); and ii) 'priming effect', i.e., increased decomposition linked to intensive C inputs (Garcia-Franco et al., 2015), except for pathogenic fungi that exhibit indirect effect due to weak decomposition capacity (Shi et al., 2019; Carron et al., 2020). These ways usually cooccur rather than being mutually exclusive in the same ecosystem separated in time and space (Voříšková et al., 2014). Despite this knowledge, the mechanisms and relative contributions of different fungal guilds to C and N cycling in microhabitats facing tropical land use remain unclear.

It has previously been shown that tropical forest conversion to long-term intensive tree cash plantations, particularly monoculture rubber plantations, has inconsistent effects on soil fungal communities; a positive potential is noted when constructing diversified agroforestry systems, which is most likely related to individual pedoclimatic conditions and management regimes (Kerfahi et al., 2016; Berkelmann et al., 2020; Lan et al., 2020, 2021; Wang et al., 2020). These studies have mainly focused on whole fungal community or only on arbuscular mycorrhizal (AM) fungi characterized by phospholipid fatty acids (PLFA) and have disregarded other guilds with which they interact and synergize for ecosystem functioning (Krashevska et al., 2018; Monkai et al., 2018; Susanti et al., 2019). Moreover, to our knowledge, none has been conducted at the soil microenvironment scale (i.e., aggregate-sized fractions) (Brinkmann et al., 2019; Song et al., 2019; Ballauff et al., 2021). Therefore, to explore whether common or different functional mechanisms act across aggregates, it is imperative to compare how these typical land transitions affect aggregate-dwelling distinct guilds to regulate nutrient cycling.

Here, we assessed the impact of tropical land use change on soil fungal functional guilds and the effects on soil C and N cycling processes at the aggregate level. To this end, we determined C and N fractions, fungal communities, enzymatic activities and chemical properties within soil aggregates in Xishuangbanna, Southwestern China. We hypothesized that i) the conversion of NF to rubber monocultures would reduce C and N pools and associated degrading-enzyme activities across aggregates and then improve them after agroforestry establishment, primarily due to litter inputs; ii) land use change would have a strong influence on the structure and diversity of fungal guilds in macroaggregates depending on the availability of soil labile substrates linked to C and N turnover; and iii) the relationship between C and N dynamics and fungal guilds would vary across aggregates in response to land use change.

2. Materials and methods

2.1. Site description and experimental design

The study site located in Mengla County, Xishuangbanna in Southwestern China (21°33′N, 101°28′E, 880–950 m asl). It has a typical tropical monsoon climate, with distinct rainy season (May–October) and dry season (November–April). The mean annual temperature is 21.9 °C, with the highest temperature being 25.5 °C in June and the lowest being 16.5 °C in January. The mean annual precipitation is 1528 mm, with ~82 % occurring during the rainy season. The soil type is classified as acidic Oxisol according to the USDA soil taxonomy.

During the 1990s, a large area of tropical forest at the base of the mountain in this region was subjected to indiscriminate slash-and-burn and then converted to sugarcane plantations with scattered crop cultivation. Monoculture rubber was subsequently planted with a conventional spacing of 2.5 m × 8.0 m by replacing sugarcane plantations in 1994 and 2006. In June 2010, rubber-based agroforestry systems were established by intercropping with leguminous species–*Flemingia macrophylla* (0.8 m × 1.0 m, seven lines) in some monoculture stands (Liu et al., 2021). *F. macrophylla*, a fast-growing undershrub, is well adapted to infertile and extremely acidic soils and can produce much biomass residue for green manure. All stands were managed under local agricultural practices, with the exception of *F. macrophylla*, which was clipped ~30 cm above the ground and then in situ mulched at the end of each year. In March 2016, four different rubber stands were designed: young and mature monocultures (YR: 12 years old and MR: 24 years old, respectively) and their respective agroforestry systems (YA and MA, respectively). The adjacent tropical NF ~1 km away from rubber stand was chosen as a reference site. Each stand type had three 20 m × 25 m independent replicate plots.

2.2. Field sampling and aggregate fractionation

Soil samples from nine evenly distributed soil cores (20 cm depth, 5 cm diameter) in each plot were collected in mid-May 2018 and stored in a cooler until further processing. Field-fresh soils were passed through an 8-mm mesh sieve by gently breaking the soils along natural fracture planes, placed in open sterilized containers and dried at 4 °C to 10 %–14 % gravimetric water content, which is the optimal soil moisture content (i.e., maximum brittle failure) for dry sieving (Bach and Hofmockel, 2014; Tiemann et al., 2015). Dry sieving was performed to maintain the link between aggregates and their indigenous biota and nutrients, as a substantial proportion of soil microbial biomass is thought to live on or near the aggregate surface (Jiang et al., 2014; Trivedi et al., 2017). Using a rotary sieve shaker (XY-100; Xiangyu Inc., Beijing, China), the soils were separated into three classes: megaaggregates ($>$ 2 mm), macroaggregates (0.25–2 mm) and microaggregates ($<$ 0.25 mm) (Jiang et al., 2017). Each soil aggregate fraction was divided into three subsamples for biochemical and molecular analyses. We also measured litter biomass and nutrients in each plot (Fig. S1).

2.3. Soil biochemical analysis

Soil pH was measured in a 1:2.5 suspension of soil to water (*w/v*) using a pH meter (FE28; Mettler Toledo, USA). Cation exchange capacity (CEC) was assessed using the leaching method with 1 M ammonium acetate at pH 7.0. Exchangeable acidity (H^+ and Al^{3+}) was measured using 1 M KCl extracts by titrating with NaOH. These basic chemical properties are presented in Fig. S2. Recalcitrant C (RC) and recalcitrant N (RN) contents were measured using a two-step acid hydrolysis procedure (Rovira and Vallejo, 2007). Total C (TC), total N (TN), RC and RN contents were measured using a CN analyzer (Vario MAX CN; Elementar, Germany). Soil organic C (SOC) content was assumed to be equal to the TC as inorganic C content is negligible in acidic soil (Zhang et al., 2013). Labile C (LC) and labile N (LN) contents were calculated based on the difference between SOC and RC and between TN and RN, respectively. Recalcitrance index for C (RIC) and recalcitrance index for N (RIN) were calculated as described by Rovira and Vallejo (2007). Microbial biomass C (MBC) and microbial biomass N (MBN) were estimated by 0.5 M K_2SO_4 extraction after standardized chloroform fumigation using a TOC analyzer (Vario TOC cube; Elementar, Germany). Samples without fumigation were considered a measure of dissolved organic C (DOC) and dissolved organic N (DON) contents (Murugan et al., 2019). Nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N) contents were measured by 2 M KCl extraction using a segmented flow analyzer (Auto Analyzer 3; SEAL Analytical GmbH, Germany).

The activities of soil enzymes related to C and N cycling, namely α- and β-glucosidase (AG and BG, respectively), cellobiohydrolase (CB), β-xylosidase (XYL), *N*-acetyl-β-glucosaminidase (NAG) and leucine aminopeptidase (LAP), were assayed using a modified fluorescence-linked 4-methylumbelliferyl substrate (German et al., 2012). Specific enzyme activity was expressed per unit of MBC or MBN, which reveals more evidently soil responses to land use changes than the absolute enzyme activity (Raiesi and Beheshti, 2014).

2.4. DNA extraction, sequencing and bioinformatic analysis

Total DNA was extracted from each subsample using the PowerSoil Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA), according to the manufacturer's instructions. Fungal ITS1 region was amplified by two-step PCR using the universal primers ITS1F/ITS2 (Rodriguez-Ramos et al., 2021). Reads were demultiplexed by their unique barcode and screened from the dataset if they did not contain the 10-bp multiplex identifier, had more than one error in the barcode, had more than two errors in the primer, or were shorter than 200 bp or longer than 580 bp. Sequences were clustered into operational taxonomic units (OTUs) at a 97 % similarity threshold, following which reference-based chimera checking was performed. Taxonomic annotations were assigned using the UNITE database. The generated taxonomy was used to assign OTUs to putative functional guilds (i.e., saprotrophs, pathogens and symbionts) based on nutrient-acquisition strategies using the FUNGuild database (Nguyen et al., 2016). Moreover, symbionts were parsed into AM and ectomycorrhizal (EcM) fungi and other symbionts, including lichenized fungi, endophytes and other mycorrhizas. Of the total 5328 OTUs, 1467 were assigned to a functional guild using any available assignment, with confidence rankings of 'highly probable' for 146 OTUs, 'probable' for 788 and 'possible' for 533. An average of 15.5 % were saprotrophs, 10.3 % were pathogens, 1.2 % were AM fungi, 0.3 % were EcM fungi, 0.2 % were other symbionts and 72.5 % belonged to the community that was not assigned any functional group. EcM fungi were not analyzed due to very low OTU abundance, as specific host plants were lacking in rubber-dominated stands (Kerfahi et al., 2016; Song et al., 2019). Sequence data were deposited into the NCBI database (BioProject ID: PRJNA776113).

2.5. Statistical analyses

All statistical analyses were conducted using R v. 4.0.5 (R Core Team, 2021). Data were checked for normality using Shapiro–Wilk's test and log-transformed if necessary before statistical analysis. Differences in litter layer, soil properties, fungal diversity and relative abundance between land use types based on aggregate size were analyzed using analysis of variance (ANOVA), followed by the least significant difference (LSD) post hoc test. Differences in treatment effect across whole fungal community composition and individual guilds were assessed by permutational multivariate ANOVA (perMANOVA) with 999 permutations based on Bray–Curtis similarity matrices using the *adonis* function in the *vegan* package. Data were then visualized using the nonmetric multidimensional scaling (NMDS) ordination plot in the *vegan* package. Linear discriminant analysis effect size (LEfSe) was used to reveal taxon differences from phylum to genus level based on land use type and aggregate fraction. Associations of fungal guild communities with each plant, soil physiochemical variables, enzyme activities and fungal taxa were evaluated by Mantel test using the *mantel* function. Correlations among different plants, soil values and fungal taxa were evaluated using Spearman's R coefficient, and associated P values were determined using the *cor.test* function. The direct and indirect effects of various factors, including soil factors and fungal guilds, on C and N pools were examined by structural equation modeling (SEM) using the *piecewiseSEM* package. The goodness-of-fit of the overall model was assessed using Fisher's C statistic, with $P > 0.05$ indicating a good fit.

3. Results

3.1. C and N pools in soil aggregates

Soil C pools and RIC values were significantly affected by land use type and aggregate size ($P < 0.05$), with the exception of aggregate size effect on DOC ($P = 0.829$) and RIC ($P = 0.161$; Table S1). SOC, LC and RC contents were generally higher in NF than in rubber monocultures and agroforestry systems for all aggregates (Table 1). Their levels were slightly lower in MA than in MR and were higher in YA than in YR. Except for MBC in MA, DOC and MBC contents in NF were lower than those in mature rubber plantations (MR and MA) but greater than those in young rubber plantations (YR and YA). No significant differences in DOC and MBC contents were noted between young rubber plantations. By contrast, DOC and MBC contents were lower in MA than in MR. RIC was lower in mature rubber plantations than in others. Across land use types, SOC, LC and RC contents were obviously higher in microaggregates and MBC content was higher in mega- and macroaggregates.

Soil N pools were notably affected by land use type and aggregate size ($P < 0.001$), with the exception of land use effect on NH_4^+-N ($P = 0.180$) and aggregate size on LN ($P = 0.386$) and NO_3^--N ($P = 0.137$; Table S2). In all aggregates, higher TN, LN, RN and NO_3^--N contents were observed in NF than in four rubber-based plantations (Table 2). NO_3^--N content was higher in rubber agroforestry systems than in respective monocultures. DON content in NF was lower than that in mature rubber plantations but greater than that in young rubber plantations. MBN content was the highest in YR and lowest in MA in mega- and macroaggregates. Regardless of land use type, RN and NH_4^+-N contents were significantly higher in microaggregates, while MBN presented an opposite trend.

3.2. Specific enzyme activities in soil aggregates

Soil C-degrading enzyme activities were significantly affected by land use type, aggregate size and their interactions ($P < 0.05$), except for the interactive effect on XYL activity ($P = 0.086$; Fig. 1). The activities of AG, BG and CB were much lower in NF than in young rubber plantations in all aggregates, despite a lack of consistent patterns compared with those noted in mature rubber plantations (Fig. 1a–c). Their activities were higher in MA than in MR in macro- and microaggregates. Moreover, AG activity was higher in YA than in YR in all aggregates. The activity of XYL in NF was higher than that in MR in macroaggregates and lower than that in YA in microaggregates (Fig. 1d). Across land use types, microaggregates had the highest C-degrading enzyme activities.

Soil N-hydrolyzing enzyme activities were individually and interactively affected by land use type and aggregate size ($P < 0.05$), with the exception of aggregate size effect on NAG activity ($P = 0.093$; Fig. 2). In mega- and macroaggregates, NAG and LAP activities were lower in NF than in mature rubber plantations, except for MR in macroaggregates, where no difference was noted between NF and young rubber plantations. In the case of larger aggregates, their activities were higher in MA than in MR. No significant treatment effects were noted on N-hydrolyzing enzyme activities in microaggregates. Regardless of land use type, NAG and LAP activities increased as aggregate size decreased.

3.3. Fungal community characteristics in soil aggregates

The overall fungal community was dominated by Ascomycota (48.9 %), Zygomycota (21.6 %) and Basidiomycota (17.2 %), with the most abundant genera in these phyla being *Fusarium*, *Mortierella* and *Cryptococcus*, respectively (Figs. 3a and S3). Fungal taxa were significantly affected by land use type, with the lower relative abundances of Ascomycota and Basidiomycota and higher abundance of Zygomycota in NF in all aggregates. Similar trends were noted when comparing the abundances of these phyla between YA and YR. Aggregate size had little effect on fungal phyla, except for a noticeable decrease in the relative abundance of Zygomycota with decreassing aggregate size. However, aggregate size had a specific impact on taxonomic composition at lower classification levels, with a greater proportion of taxa preferring to inhabit microaggregates (Fig. S4).

Saprotrophs, pathogens and symbionts accounted for 32.0 %, 23.1 % and 0.3 % of fungal functional guilds, respectively, on average (Fig. 3b). Specifically, saprotrophs primarily consisted of Zygomycota (67.5 %) and pathogens mainly consisted of Basidiomycota (58.8 %). Moreover, 46.4 % of symbionts were primarily Glomeromycota (i.e., 100 % of AM

Table 1

One-way ANOVA analyses for the effects of land use on soil C pools by aggregate size. Values are means ± SE across treatments for each aggregate size and results of post hoc analyses of land use treatment effects. Different letter indicates significant differences ($P < 0.05$) among aggregate sizes. Significance of treatment effect: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; marginal $^{\dagger}P < 0.10$. NF: natural forest; MR: mature rubber monoculture; MA: mature rubber-based agroforestry system; YR: young rubber monoculture; YA: young rubber-based agroforestry system. SOC: soil organic carbon; LC: labile carbon pool; RC: recalcitrant carbon pool; DOC: dissolved organic carbon; MBC: microbial biomass carbon; RIC: recalcitrant carbon index.

C pool	Megaaggregate (> 2 mm)		Macroaggregate (0.25–2 mm)		Microaggregate (< 0.25 mm)	
	Mean ± SE	Treatment effects	Mean ± SE	Treatment effects	Mean ± SE	Treatment effects
SOC (mg g^{-1})	13.7 ± 0.6 b	NF > YA, YR, MA; MR > YR, MA**	14.6 ± 0.7 ab	NF > MR, YA, MA, YR**	16.6 ± 1.1 a	NF > YA, MR, MA, YR**
LC (mg g^{-1})	5.7 ± 0.3 b	NF > MA, YR; MR > YA, MA, YR**	5.9 ± 0.4 ab	NF > YA, YR†	6.9 ± 0.4 a	NF > MA, YR†
RC (mg g^{-1})	7.9 ± 0.4 b	NF > YA, MR, YR, MA*	8.8 ± 0.4 ab	NF > YA, MR, YR, MA; YA > YR, MA***	9.7 ± 0.7 a	NF > YA, MR, YR, MA**
DOC (mg kg^{-1})	259.7 ± 32.3	MR, MA > NF > YA, YR***	262.3 ± 32.8	MR > MA > NF > YR; MA > NF, YA***	267.0 ± 35.6	MR > MA, NF > YA, YR***
MBC (mg kg^{-1})	583.2 ± 29.9 a	MR > MA, YR, YA; NF > YR, YA**	521.3 ± 30.7 a	MR > NF > YA, YR, MA**	398.0 ± 27.7 b	MR > YR, MA; NF > YA > MA **
RIC	0.58 ± 0.01	YR > MR; NF, YA > MR*	0.60 ± 0.01	YA > MA, MR*	0.58 ± 0.01	NF, YA, YR > MA, MR***

Table 2

One-way ANOVA analyses for the effects of land use on soil N pools by aggregate size. Values are means ± SE across treatments for each aggregate size and results of post hoc analyses of land use treatment effects. Different letter indicates significant differences ($P < 0.05$) among aggregate sizes. Significance of treatment effect: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; marginal †$P < 0.10$. TN: total nitrogen; LN: labile nitrogen pool; RN: recalcitrant nitrogen pool; NO_3^--N: nitrate N; NH_4^+-N: ammonium N; DOC: dissolved organic nitrogen; MBC: microbial biomass nitrogen; RIC: recalcitrant nitrogen index. See land use abbreviations in Table 1.

N pool	Megaaggregate (> 2 mm)		Macroaggregate (0.25–2 mm)		Microaggregate (< 0.25 mm)	
	Mean ± SE	Treatment effects	Mean ± SE	Treatment effects	Mean ± SE	Treatment effects
TN (mg g^{-1})	1.5 ± 0.0	NF > YA, YR, MA*	1.6 ± 0.1	NF > MR, YA, MA, YR*	1.7 ± 0.1	NF > YA, MR, MA, YR*
LN (mg g^{-1})	0.9 ± 0.0	NF > YA, MA, YR†	0.9 ± 0.1	NF > YA, MA, YR†	1.0 ± 0.1	NF > MR, YA, MA, YR†
RN (mg g^{-1})	0.6 ± 0.0 b	NF > MR, YA, MA†	0.7 ± 0.0 ab	NF > YA, YR, MR; YA > MA***	0.8 ± 0.0 a	NF > YA, YR, MR; YA > MA***
NO_3^--N (mg kg^{-1})	3.6 ± 0.6	NF, YA > MA, YR, MR***	4.6 ± 0.9	NF > YA > MA, YR, MR***	4.0 ± 0.8	NF > YA > MA, YR, MR***
NH_4^+-N (mg kg^{-1})	13.9 ± 0.4 c		18.2 ± 0.8 b		24.2 ± 0.8 a	
DON (mg kg^{-1})	40.5 ± 3.3	MR > NF > YA > YR; MA > YA > YR***	45.9 ± 3.5	MR > MA, NF > YR, YA ***	49.8 ± 4.5	MR > MA > YR, YA; NF > YR, YA***
MBN (mg kg^{-1})	32.3 ± 3.6 a	YR > NF > YA, MR > MA***	23.8 ± 2.6 b	YR, MR, NF > MA*	21.2 ± 1.6 b	
RIN	0.42 ± 0.01		0.43 ± 0.01		0.44 ± 0.01	

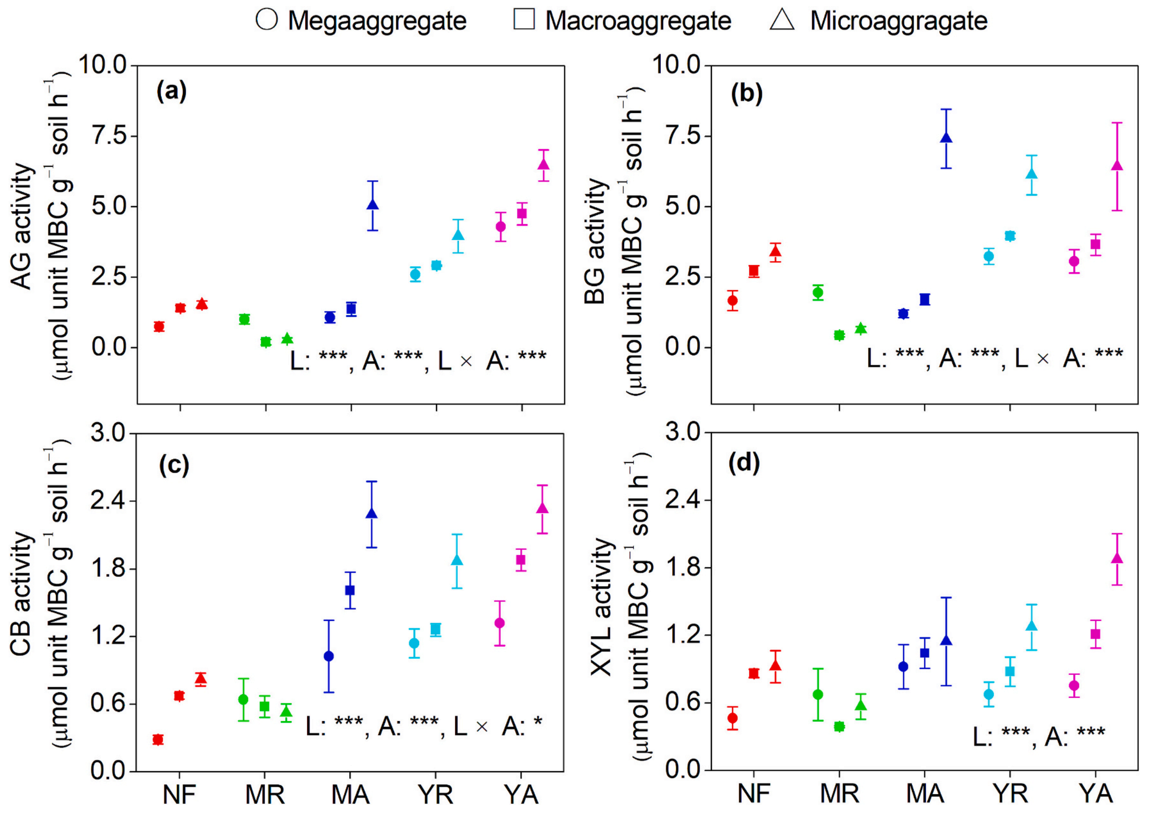

Fig. 1. Specific C-cycling enzyme activities per unit of microbial biomass C (MBC) among different aggregate fractions under five land uses. Points and bars represent means ± SE. L, land use effect; A, aggregate size effect; L × A, interactive effect of land use and aggregate size. *, $P < 0.05$; ***, $P < 0.001$. AG: α-glucosidase; BG: β-glucosidase; CB: cellobiohydrolase, XYL: β-xylosidase. See land use abbreviations in Table 1.

fungi). In addition, Glomeraceae and Acaulosporaceae were the predominant AM families, and *Mortierella* and *Fusarium* were the predominant saprotrophic and pathogenic genera, respectively (Fig. S3). Fungal guilds varied with land use type, with the higher relative abundances of saprotrophs and symbionts and lower pathogens in NF in all aggregates. The abundance of AM fungi was higher in NF than in mature rubber plantations, and no differences were noted between NF and young rubber plantations, except for YA in megaaggregates. Similar patterns were noted when comparing the abundances of these guilds between YA and YR. Only saprotrophs were affected by aggregate size, with greater abundances in megaaggregates.

The NMDS plots revealed that the soils from different land use types harbored distinct compositions of fungal communities (perMANOVA, $R^2 = 0.56$, $P < 0.001$) and functional guilds (saprotrophs: $R^2 = 0.58$, $P < 0.001$; pathogens: $R^2 = 0.44$, $P < 0.001$; symbionts: $R^2 = 0.34$, $P < 0.001$; and AM fungi: $R^2 = 0.38$, $P < 0.001$) in each aggregate size; however, no significant effects of aggregate size were noted at each land use type (Figs. 4, S5 and S6). Furthermore, α diversity of whole fungal communities and functional guilds (except for AM fungi) was significantly affected by land use type and aggregate size ($P < 0.05$), with the exception of the effect of land use on symbiotic Shannon diversity ($P = 0.750$) and that of aggregate size on Shannon diversity and evenness of pathogens ($P = 0.194$ and $P = 0.842$, respectively) and symbionts ($P = 0.141$ and $P = 0.157$, respectively; Figs. S7 and S8). Generally, richness of fungal communities was higher in NF than in four rubber plantations. Moreover, it was higher in rubber agroforestry systems than in the

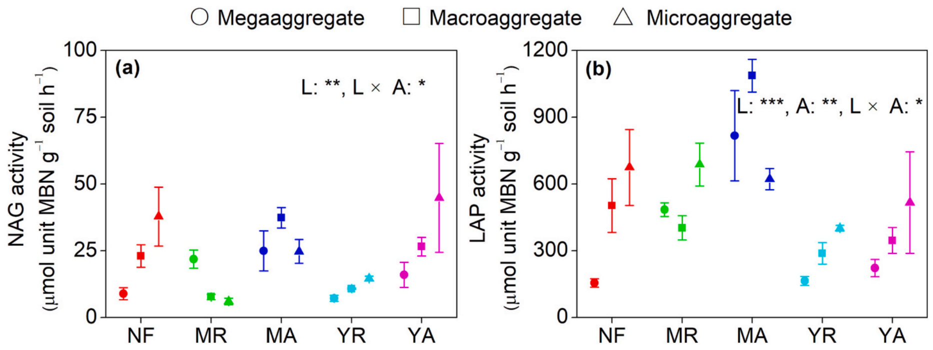

Fig. 2. Specific N-cycling enzyme activities per unit of microbial biomass N (MBN) among different aggregate fractions under five land uses. Points and bars represent means ± SE. L, land use effect; A, aggregate size effect; L × A, interactive effect of land use and aggregate size. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. NAG: *N*-acetyl-β-glucosaminidase; LAP: leucine aminopeptidase. See land use abbreviations in Table 1.

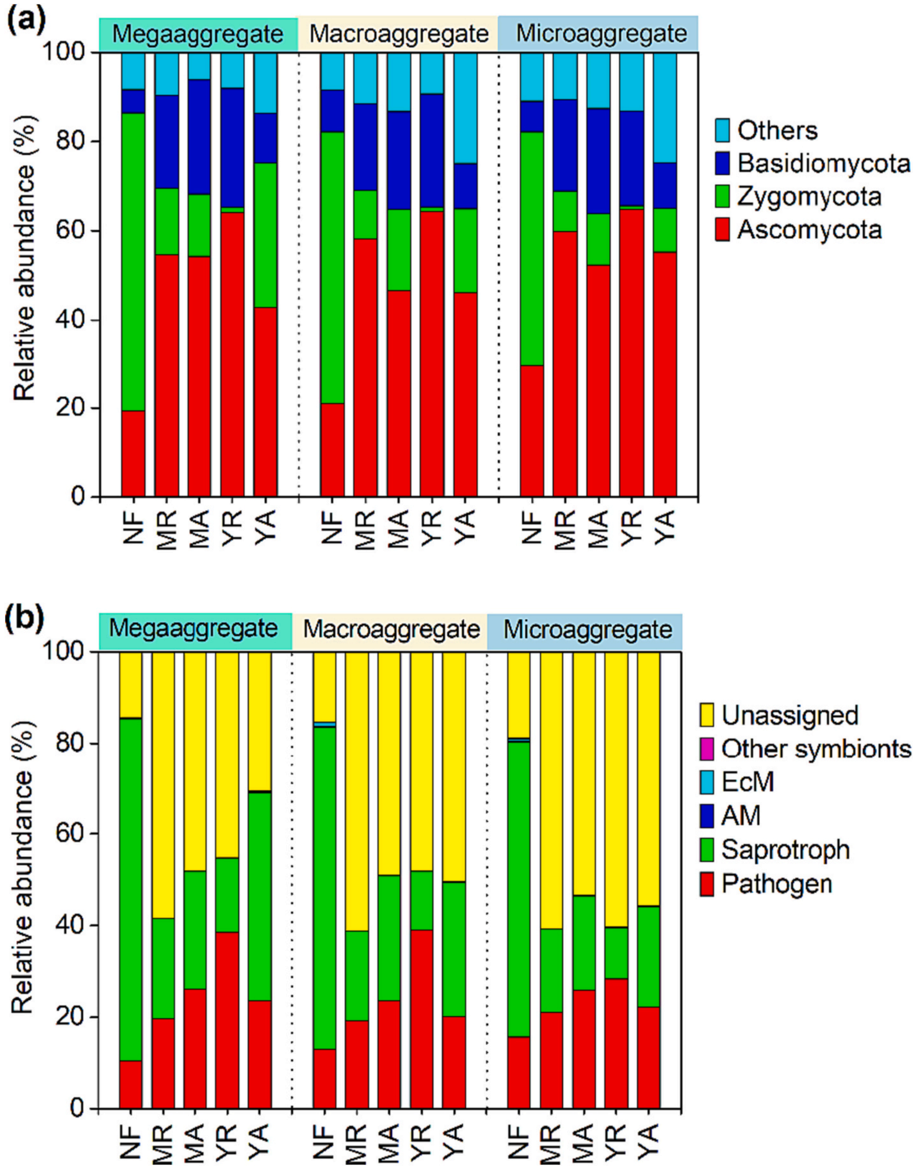

Fig. 3. Relative abundance of dominant fungal phyla (>1 %, a) and functional guilds (b) among different aggregate fractions under five land uses. Taxa in addition to Glomeromycota (mean 0.12 %) in the "Others" are predominantly "Unclassified", but also include Blastocladiomycota, Chytridiomycota and Rozellomycota. AM, arbuscular mycorrhizal; EcM, ectomycorrhizal. See land use abbreviations in Table 1.

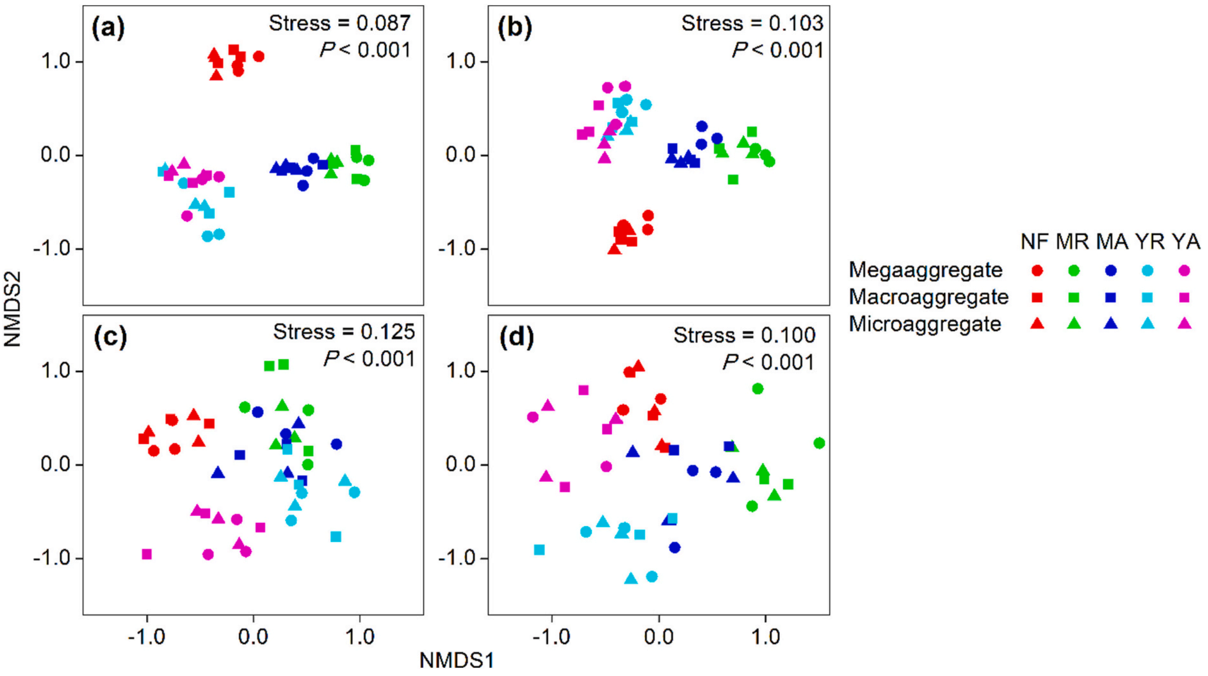

Fig. 4. Nonmetric multidimensional scaling (NMDS) plots of the effect of land use on saprotrophic (a), pathogenic (b), symbiotic (c) and arbuscular mycorrhizal (d, AM) fungal community composition in three aggregate fractions. Significance from land use is tested using perMANOVA at 0.05 level. See land use abbreviations in Table 1.

respective monocultures. Microaggregates exhibited the highest richness across land uses.

3.4. Correlations among soil C and N pools, fungal communities and other attributes across soil aggregates

Overall, DOC, MBC, DON and NO_3^--N contents were more strongly related to both fungal communities and functional guilds levels; however, almost no significant correlations were noted among other C and N pools in all aggregates (Fig. 5, Table S3). pH and CEC largely contributed to whole fungal communities and functional guilds, especially in microaggregates, but they exhibited varying correlations with enzyme activities in all aggregates. Complex correlations were noted between C and N pools and other soil biochemical properties (Fig. 5). The abundance of Ascomycota and that of Zygomycota were negatively correlated with each other and exhibited opposite relationships in all soil variables.

SEM revealed that soil water content (SWC) had a direct positive impact on C pools in mega- and macroaggregates (Fig. S9a and b). Moreover, pH and CEC exhibited indirect impacts on C pools by affecting saprotrophic, AM and symbiotic fungi in microaggregates, respectively (Fig. S9c). SWC had a direct positive effect on N pools in mega- and macroaggregates, and pH had a direct negative effect on N pools in macroaggregates (Fig. S9d and e). Moreover, pH and CEC indirectly impacted N pools by affecting AM and symbiotic fungi in microaggregates, respectively (Fig. S9f).

4. Discussion

4.1. Land use influences C and N dynamics in soil aggregates

The conversion of NF to YR and MR gradually decreased aggregate-associated SOC and TN pools (i.e., labile and recalcitrant fractions), especially in microaggregates (Tables 1 and 2). This is mainly induced by severe soil erosion resulting from understory vegetation loss and reduced physical protection resulting from aggregation for purposive soil disturbance. A higher LC with a lower RIC in MR than in YR was attributed to the efficient use of high litter production in the former to meet the system nutrient requirements through heterotrophic microbes-mediated acceleration of decomposition (Liu et al., 2019). However, RC increased with increasing rubber age, probably due to the high tannin and waxy compounds from more leaf litter inputs (Nath et al., 2018). By contrast, no difference in TN pool was observed between MR and YR, thereby achieving a steady state of N input and loss. Wang et al. (2020) reported that lower LC after deforestation reduced further with rubber development, indicating a temporary increase in the proportion of active C driven by residues left at the early forest clearance in non-tapping rubber plantations. However, LN increased accompanied by an opposite RN, suggesting that mineral N was slowly released. As expected, compared with YR, SOC pool was slightly increased in YA due to an improvement in C status with a large input of cover crop residue; however, a declining trend was noted in MA. This result indicates a positive microbial priming effect of cover crops. However, agroforestry systems did not alter TN fractions due to the stimulation of nitrification and associated N_2O emissions (Rao et al., 2021). Thus, SOC and TN turnover and sequestration are relatively dynamic processes that respond to land conversion and will eventually reach their equilibrium and act as long-term sinks.

Across all aggregates, DOC and DON initially decreased in YR and subsequently increased in MR after forest conversion, as determined by the amount of dissolved organic matter, such as litter residues, root exudates and microbial degradation (de Brito et al., 2019). This was also noted in the case of the MBC but not MBN, suggesting that a significant change in N cycling-related microbial composition, as confirmed by the greater abundance of N bacteria in YR (Liu et al., 2019). Moreover, striking losses of NO_3^--N leaching occurred in rubber monocultures with lower soil buffering capacity (i.e., CEC, Fig. S2). Although a higher aboveground litter input after agroforestry establishment (Fig. S1), available organic nutrients and microbial biomass were constant in YA and depleted in MA, primarily due to plant growth and yield latex. Lower root biomass in MA may be another reason for microbial nutrient limitation (Liu et al., 2018). This phenomenon can be compensated by NO_3^--N enhancement due to high nitrification rate (Rao et al., 2021). These results indicate that the introduction of leguminous cover crops increases the potential competition for nutrients between plants and

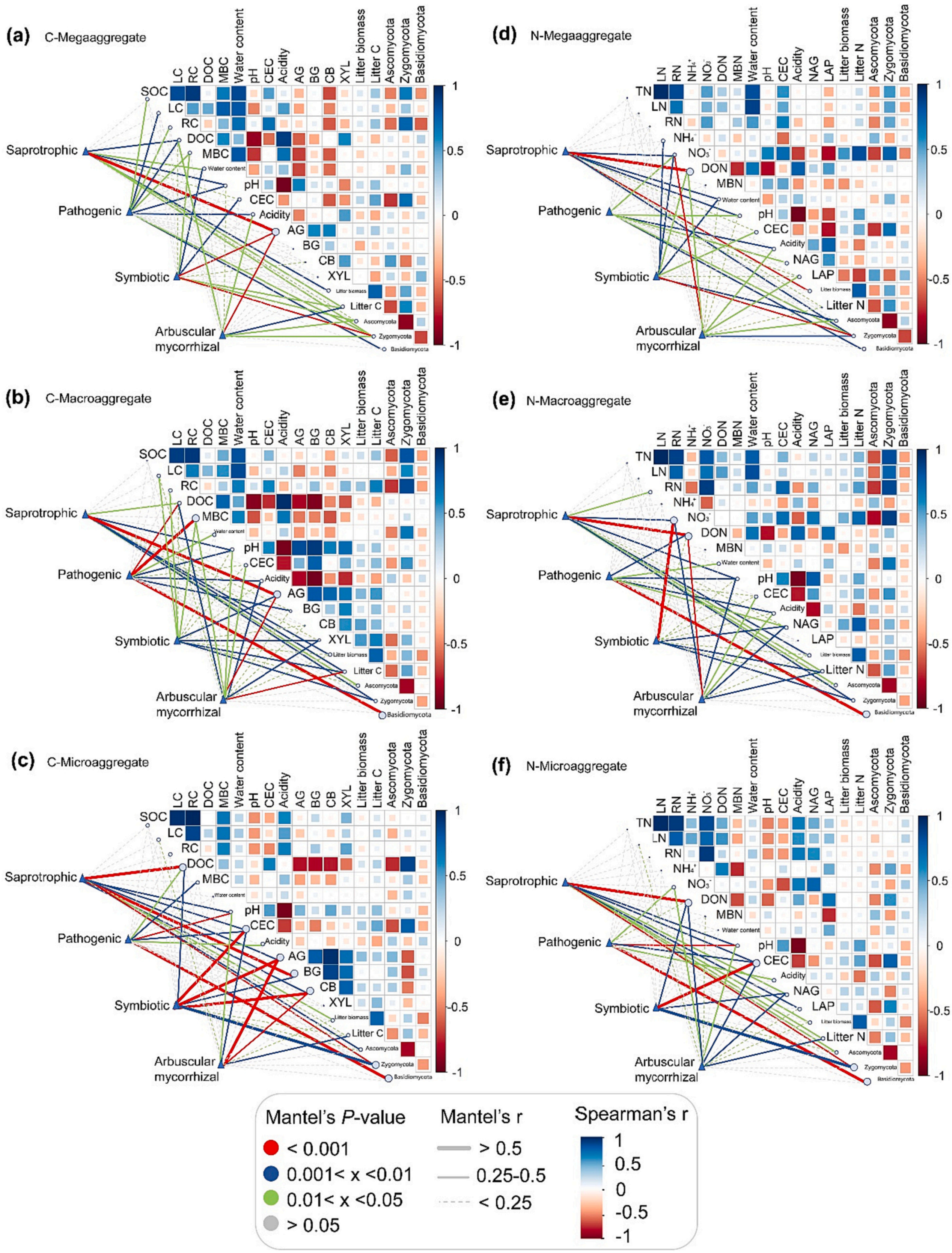

Fig. 5. Correlations among soil C (a, b, c) and N (d, e, f) pools, basic physiochemical traits, abundant fungal taxa (phylum level) and functional guilds composition in three aggregate fractions. Pairwise comparisons of soil physiochemical traits and abundant fungal taxa are shown, with a colour gradient denoting Spearman's correlation coefficient. Fungal functional community compositions were related to each plant parameter, soil physiochemical factor and fungal taxon by a Mantel test. Edge width corresponds to the Mantel's r statistic for the corresponding distance correlations, and edge colour denotes the statistical significance based on 999 permutations. See soil parameter abbreviations in Tables 1 and 2.

microbes, highlighting the necessity for accelerating residue decomposition.

Regardless of land use effect, SOC pools and RN increased with decreasing aggregate size, mainly because microaggregates contain stable humus, while macroaggregates contain more easily available substrates (Six et al., 2000). These higher contents in microaggregates can enable better control of organic matter conservation and nutrient loss due to slower turnover rates (Jiang et al., 2018). Similarly, the differential distribution of chemical forms of C and N has been reported in different-sized aggregates (Tiemann et al., 2015; Trivedi et al., 2017; Somasundaram et al., 2018). By contrast, the greater biological fractions (i.e., MBC and MBN) retained in mega- and macroaggregates indicate high microbial activity (e.g., saprotrophic fungi) that can accelerate crop residue decomposition and lead to nutrient mineralization, because they are very sensitive to environmental alterations related to land conversion as a result of their rapid cycling and short residence time in the soil. A previous study reported that organic matter input appears to be the primary driver in the formation of macroaggregates that are generally stable for longer periods when oversaturated with organic matter (Murugan et al., 2019). Thus, microbial C- and N-use efficiencies tended to increase in large aggregates, which is further confirmed by the higher MBC/LC and MBN/LN ratios. Moreover, microaggregates exhibited a strong NH_4^+-N adsorption capacity due to their high specific surface area, which may reduce the accessibility of microorganisms and enzymes.

4.2. Land use changes specific enzyme activities in soil aggregates

Specific enzyme activities related to C- and N-cycling varied with land use type and aggregate size (Figs. 1 and 2). They represent the metabolic status of microbial community, indicating fluctuations in stabilized enzyme activities and correlate with microbial community composition (Raiesi and Beheshti, 2014). The increased C-hydrolyzing enzyme activities in all aggregates following the conversion of NF to YR could depend on the relatively low MBC (Table 1) and high enzyme levels, suggesting a fast SOC turnover rate driven by more metabolically active microbes (Zhang et al., 2019). Conversely, lower N-hydrolyzing enzyme activities were observed in YR, primarily due to increased N assimilation by heterotrophic microorganisms combined with low enzyme activities (Table 2). By contrast, the conversion of NF to MR lead to similar C-degrading enzyme activities, whereas N-hydrolyzing enzyme activities responded differently to different aggregates. This indicates that the changes in soil enzyme activities can occur independently of changes in microbial biomass pools in different aggregates (Raiesi and Salek-Gilani, 2018). The establishment of agroforestry systems improved C- and N-cycling enzyme activities to a large extent, leading to enhanced nutrient mineralization. This can be attributed to multiple factors, such as large residue inputs of cover crops, soil microclimate and properties (Figs. S1 and S2), in addition to the tight relationships among specific enzyme activities and C and N fractions as well as pH (Fig. 5).

Aggregate size strongly affected specific enzymes regardless of land use type, with the highest enzymatic activities in microaggregates, reflecting the distribution of microbial communities as a result of different habitats (Bach et al., 2018). However, previous studies have reported that specific C- and N-degrading enzyme activities differed among aggregate sizes, although absolute levels were the highest in microaggregates (Tiemann et al., 2015; Trivedi et al., 2015, 2017). This inconsistency results from the potential limitation of enzyme assays that activate residual microbial enzymes and the significant differences in the basic amount of normalized enzyme activity (e.g., SOC, MBC or PLFA biomass) and the stability and forms of these enzymes among aggregates (Trivedi et al., 2017). It should be noted that specific enzyme activities in MR increased with increasing aggregate size. This finding may be explained by more enzymes resulting from plant root exudation (Liu et al., 2019) and microbial investment to mediate organic matter decomposition under severe resource limitation (Tiemann et al., 2015). These results suggest that the differential responses of specific enzyme activities within soil aggregates may be crucial for C and N cycling in response to tropical land conversion.

4.3. Land use alters whole fungal communities and their functional guilds within soil aggregates

The community structure and diversity of fungi and their functional guilds varied with land use type across all aggregates (Figs. 3 and 4). Forest conversion to rubber monocultures, especially YR, increased the relative abundances of Ascomycota and Basidiomycota at the expense of Zygomycota. This led to an increase in pathogenic fungi and a decrease in saprotrophic and symbiotic fungi in response to soil degradation. Compositional shifts from the dominance of copiotrophic (faster-growing r-strategists) into oligotrophic groups (slower-growing K-strategists) were also detected in other studies after forest conversion (Brinkmann et al., 2019; Lan et al., 2020). The establishment of YA favored the abundance of Zygomycota, which contains many wood-degrading decomposers, and inhibited pathogenic fungi due to abundant coarse woody debris (Fig. S1) and roots that exude specific flavonoids from cover crops (Liu et al., 2019). Hence, the abundance of *Mortierella* species belonging to Zygomycota, which can produce antibiotic compounds against various soil-borne diseases, was also higher in YA (Fig. S3). YA promoted the growth of AM fungi by dense roots, especially in megaaggregates, as AM fungi are closely related to the formation and stability of large aggregates via secretion of glomalin-related soil proteins (Zhang et al., 2013). By contrast, MA establishment had little effect on fungal communities due to a negative cover crop effect (Liu et al., 2021). In addition, the diversity of fungal communities and functional guilds exhibited different responses to forest conversion (Figs. S7 and S8), such as increasing (Berkelmann et al., 2020; Lan et al., 2021) or being relatively steady (Kerfahi et al., 2016; Song et al., 2019). Subsequently, the establishment of agroforestry systems enhanced diversity, especially in the case of YR in microaggregates. These differences can be attributed to changes in plant species, litter residues and soil chemical properties (Ballauff et al., 2021).

Megaaggregates had the highest relative abundance of saprotrophic fungi resulting from high abundance of Zygomycota regardless of land use type. This is because saprotrophic fungi prefer highly porous and aerobic environments, and thus facilitate the decomposition of fresh plant residues that first input into megaaggregates (Jiang et al., 2018). A high abundance of saprotrophic fungi can also stabilize large aggregates (Tisdall, 1994). Conflicting results have been reported in agricultural soils treated with fertilizers (Li et al., 2019; Liao et al., 2020). These differences are mainly attributed to changes in soil texture involved in fine tillage management. However, fungal taxa at lower classification levels preferred to inhabit microaggregates (Fig. S4), resulting in high diversity (Figs. S7 and S8), which can be attributed to the richness of soil nutrients and availability in such sizes. In accordance with this finding, Bach et al. (2018) found that microaggregates supported highly diverse and distinct fungal communities. These results suggest that fungal diversity is more strongly differentiated by aggregate size than community composition.

4.4. Fungal guilds affect C and N pools in soil aggregates

Fungal functional guilds determined soil C and N cycling depending on aggregate size (Figs. 5 and S9). In megaaggregates, pathogenic fungi had a significant negative effect on soil N pools, which could be indirectly mediated by pH and CEC. The reduction of pathogenic fungi (in which *Fusarium* was the most abundant genera) has the potential to suppress soil-borne diseases and increase root biomass and extracellular enzyme activity (Shi et al., 2019), thereby accelerating N transformation, especially after legume intercropping (Arevalo-Gardini et al., 2020). This is supported by the fact that pathogenic fungi are correlated with inorganic N (Detheridge et al., 2016). In microaggregates, saprotrophic fungi negatively affected C and N pools, primarily driven by the abundant Ascomycota, which are known for opportunism and reliance on available substances. Additionally, symbiotic fungi synthesized numerous specific enzymes to decompose refractory organic matter (Carron et al., 2020) and exhibited a negative correlation with C and N pools in microaggregates. However, AM fungi facilitated C and N storage associated with microaggregates through mycelial production and hyphal regrowth. These results suggest that high diversity of fungal guilds plays an essential role in the internal cycling of C and N in microaggregates. Specifically, the fungal functional guilds structured by soil pH levels within different aggregates significantly affected DOC, MBC, NO_3^--N and DON, indicating that both cooperative and competitive interactions are successively activated and increasingly contribute to C and N turnover.

5. Conclusions

We found that the varying effects of land use change on soil C and N dynamics mediated by fungal functional guilds across aggregate sizes. In all aggregates, C and N pools generally decreased in response to soil degradation after forest conversion to rubber monocultures. Microbial C and N continuously decreased when agroforestry systems were established. Meanwhile, C- and N-hydrolyzing enzyme activities responded differently to forest conversion and were improved in agroforestry systems. The levels of C and N pools and their enzyme activities increased with decreasing aggregate size. Moreover, forest conversion resulted in an increase in pathogenic fungi at the expense of saprotrophic and symbiotic fungi. The establishment of YA favored the abundance of Zygomycota (e.g., *Mortierella* species) and suppressed pathogenic fungi (e.g., *Fusarium* species). Fungal diversity rather than composition was affected by aggregate size, with the highest diversity in microaggregates. Regardless of land use type, C and N pools within aggregates were variedly affected by fungal functional guilds, mainly driven by soil pH. Overall, these differences are attributed to changes in species diversity, soil properties and involved agroforestry management. Our study strengthens the understanding of how fungal functional guilds interactively regulate C and N dynamics at the aggregate level. It also provides an important insight into the early establishment of agroforestry systems in tropical cash tree plantations due to the greater advantages in nutrient cycling and pathogen control. Future work should explore the role of complex microbial networks (e.g., microbial food web) in nutrient cycling at the aggregate level.

CrediT authorship contribution statement

Chenggang Liu, Yanqiang Jin and Fangmei Lin contributed to the conception and design of the study, as well as data collection. Data analysis was performed by Chenggang Liu and Yanqiang Jin. The first draft of the manuscript was mainly written by Chenggang Liu, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.166080.

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