Conversion to agroforestry and monoculture plantation is detrimental to the soil carbon and nitrogen cycles and microbial communities of a rainforest

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1 **Conversion to agroforestry and monoculture plantations is detrimental to the soil carbon and nitrogen**
2 **cycles and microbial communities of a rainforest**
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23 Running title: Land use affects soil carbon and nitrogen cycles

24   Abstract

25   The conversion of rainforests to plantations leads to about 50% loss in the organic carbon (C) content

26   of the soil and strongly influences nitrogen (N) cycling, potentially increasing greenhouse gas

27   emissions. However, the effect of land-use change on the link between C and N pools and microbial

28   communities in soil remains poorly understood. This study quantified C and N fractions of soil organic

29   matter in a tropical forest, rubber agroforestry system, 5- and 15-year-old rubber plantations. The

30   community structure and abundance of fungi and bacteria were studied using high-throughput

31   sequencing and q-PCR. Results showed that forest conversion substantially altered community

32   structure and abundance of microbial communities. Rainforest conversion to plantation enhanced

33   bacterial diversity and reduced soil C mineralization rate. In addition, land-use change also enhanced

34   the soil N mineralization rate in 5-year-old rubber plantations and agroforestry system. A structural

35   equation modelling suggested that soil microbial communities played more dominant roles in driving

36   the shift in C and N cycles caused by land-use change than soil C and N pools. These results provide

37   new mechanistic insights into the differential control of soil fungal and bacterial communities over C

38   and N mineralization, with clear implications for future land-use changes and management practices in

39   tropical ecosystems.

40   Keywords: "Rubber plantation"; "High-throughput sequencing"; "Soil organic matter"; "enzyme

41   activities".

Introduction

Tropical forests are critical reservoirs of global soil carbon (C) storage (van Straaten et al., 2015).

However, increased global food demand in the past two decades has resulted in a sharp increase in the

conversion of tropical rainforest into food plantations (van Straaten et al., 2015). Conversion of

rainforests to rubber plantations in tropical ecosystems has led to approximately 50% of stored C

being release as CO_2 into the atmosphere, causing a significant increase in greenhouse gas

emissions (van Straaten et al., 2015). However, the mechanisms that underpin the loss of C are poorly

understood. Soil microorganisms, being the principal decomposers of soil organic matter, can modify

nutrient availability and influence soil organic matter decomposition rate (Freedman et al., 2017).

Land-use changes are known to affect soil microorganisms (Bossio et al., 2005; Kuramae et al., 2012;

Rodrigues et al., 2013; Krashevska et al., 2015; Wang et al., 2017; Brinkmann et al., 2019; Song et al.,

2019) and the pools of N and C in the soil (Liu et al., 2018a). However, these studies do not

demonstrate direct links between soil C and N availability, that underpin process level differences, and

microbial communities. This is a critical knowledge gap which hinders the prediction of soil organic

matter dynamics under projected land use changes.

Forest conversion is known to reduce soil organic matter and alter its composition (Xu et al.,

2018). Generally, soil organic matter can be divided into labile and recalcitrant C and N fractions based

on chemical characteristics and turnover rates (Belay-Tedla et al., 2009; Xu et al., 2015; Liu et al.,

2018a). Labile fractions such as dissolved organic C and N, which are bio-reactive but represent a

small pool, are central to nutrient cycling and microbial growth (Belay-Tedla et al., 2009; Schmidt et

al., 2011; Xu et al., 2018) and control terrestrial C and N budgets (Belay-Tedla et al., 2009; Deng et al.,

2016). In contrast, recalcitrant fractions, which have a slow turnover and a large pool, dominate

64   long-term C and N stability (Belay-Tedla et al., 2009). Forest conversion can affect the type, rate, and

65   spatial allocation of plant C inputs, inducing a shift from recalcitrant pools to more labile pools (Li et

66   al., 2011; Xu et al., 2018). However, the relative contributions of the quantity and quality of soil C and

67   N pools to overall changes in soil organic matter due to land-use change have not been quantified under

68   field conditions.

69   Soil microorganisms play key roles in decomposing soil organic matter and mineralizing nutrients

70   in ecosystems (Singh et al., 2009; Li et al., 2018; Chen et al., 2019b). Alterations in the composition

71   and abundance of soil microbial community can shift the ratio of fungi:bacteria in tropical regions

72   (Wang et al., 2017), leading to changes in soil organic matter stability (Cusack et al., 2011; Tardy et al.,

73   2015; Dove et al., 2019). Fungal-dominated communities are considered to have a higher capability to

74   decompose complex and recalcitrant organic C than bacterial-dominated communities because fungi

75   produce a broader range of soil oxidative enzymes (Li et al., 2018; Nannipieri et al., 2018; Xu et al.,

76   2018). The effects of rainforest conversion on soil microbial communities (Bossio et al., 2005;

77   Krashevska et al., 2015; Wang et al., 2017; Brinkmann et al., 2019; Song et al., 2019) and soil enzyme

78   activities (Tischer et al., 2014; Lin et al., 2018) have been well studied, but the relative contributions of

79   fungal and bacterial communities and their abundances on changes in soil organic matter

80   decomposition induced by the land-use change have not been directly evaluated in tropical ecosystems.

81   In addition, a mechanistic understanding remains unclear, in particular whether shifts in soil microbial

82   community have an impact on soil enzyme activities or organic matter fractions, leading to increased C

83   and N released from soil organic matter (Li et al., 2011; Krashevska et al., 2015).

84   The main aim of this study was to identify the mechanisms which underpin the land-use change

85   induced loss in soil C and alterations in N cycling. To achieve this, we examined the effects of

86   conversion from a tropical rainforest in Hainan Island (China) to pure young rubber (*Hevea brasiliensis*)

87   (5 years old) and mature rubber (15 years old) plantations as well as rubber agroforestry (mature rubber

88   and black cardamom (*Alpinia oxyphylla* Miq) mixture) on soil labile and recalcitrant C and N fractions,

89   and microbial communities. We further assessed soil enzyme activity, and C and N mineralization rates.

90   Our hypotheses were that (1) the conversion of forests into agroforestry and monoculture plantations

91   has a detrimental impact on C and N cycles due to the relevant impact of altered land-use on soil

92   microbial communities and C and N pools, and (2) these effects decrease with tree age and the

93   introduction of cover crop in the rubber plantations.

2. Materials and Methods

2.1. Experimental sites

The study was performed in Baisha County in Hainan Province. The average annual temperature is 22.7 °C with an average rainfall of 1940 mm, with several precipitation peaks from May to October. The experimental design consisted of four land-use types: primarily natural old growth (> 100 years) forest with minimal human influence, 5-year-old rubber plantation, 15-year-old rubber plantation and rubber agroforestry system. These rubber-based plantations were established after clearing natural forest. The dominant species in rainforest were *Fagaceae spp.*, *Theaceaae spp.*, and *Juglandaceae spp.*, with a 75% overall canopy cover. The main shrub species were *Psychotria rubra*, *Melastoma condidum*, *Euodia lepta,* with a ground cover of 85%. In these plantations, rubber trees were planted at a density of 4 m × 6 m and thus the stocking density was 450 stems ha^{-1}. The age of rubber trees was 5- and 15-year-old rubber plantations. In agroforestry, 15-year-old rubber trees were intercropped with black cardamom (*A. oxyphylla*) with a density of 2 m × 1.5 m in inter-row between the two rows of rubber trees. Soil texture consisted of ~60% sand, ~19% silt and ~11% clay. The soil type was laterites (Oxisols) developed from granite and sandstone. No external fertilizer was used in these selected rubber plantations. Selected soil chemical and physical properties including pH, exchangeable potassium, total phosphorous, available phosphorous, and soil moisture content of the surface soil (0-20 cm) were obtained (Table S1) (Wang et al., 2017).

2.2. Soil samples

For each of the four land-use types, three replicates of 10×10 m plots, separated by 10 m, were randomly established in September 2014. As a result, there were 12 study plots in total (4 land-use types × 3 replicates). Soil samples were obtained at a depth of 0-20 cm at 3.5 cm increments. Five

116   soil cores were obtained from the middle and four corners of each plot and then combined to a

117   composite soil sample. Each sample was passed through a 2 mm mesh and immediately frozen at

118   -20 °C. Samples were assessed for microbial communities and soil chemical contents.

119   2.3 Carbon and nitrogen pool analysis

120   Soil C and N contents were determined using Belay-Tedla et al. (2009) method. In brief, 500 mg dry

121   soil was hydrolysed with 20 ml of 5 N H_2SO_4 for 30 min at 105 °C. Residues were rinsed in deionized

122   water and added to extracted hydrolysate (defined as labile pool I C or labile pool I N). Residues that

123   remained were hydrolysed in 13 mol L^{-1} H_2SO_4 for ≥10 h. After that, the residues were hydrolysed in 1

124   mol L^{-1} H_2SO_4 for 3 h at 105 °C, centrifuged (4500 g min^{-1}) and sieved through 0.45 μm filter paper.

125   Secondary hydrolysates were defined as labile pool II-C and labile pool II-N, and the remaining

126   residues were washed and oven-dried (65 °C). Acid-resistant C (recalcitrant pool-C) and N (recalcitrant

127   pool-N) were assessed on a C-N analyser. Dissolved organic C and N were also measured. Samples

128   that had been dried and sieved (~10 g) were then extracted in 0.5 M K_2SO_4, and analysed for dissolved

129   organic C and N using a Multi 3100 N/C TOC analyser.

130   2.4 Microbial biomass C and N assays

131   Soil microbial biomass carbon (SMB-C) and nitrogen (SMB-N) were assessed using standardized

132   fumigation-based extraction (Vance et al., 1987). Organic C and N were assessed before and after

133   extraction on a C/N analyser in both fumigated and non-fumigated samples to assess the SMB-C

134   (conversion factor: 0.45) and SMB-N (conversion factor: 0.64) (Vance et al., 1987).

135   2.5. Soil extracellular enzyme activity

136   Samples were added to 8 % sucrose solution (15 mL) in phosphate buffer at 37 °C for 24 h. Invertase

137   measurements were performed through the assessment of reduced sugars (e.g. glucose) using

138 colorimetry ($\sim$ 578 nm) via 3, 5-dinitrosalicylic acid (activity: mg of glucose g^{-1} soil 24 h^{-1}, as

139 described by Schinner and Von Mersi, 1990). Catalase (CAT) activity was measured through the

140 back-titration of the remaining H_2O_2 with $KMnO_4$ (Roberge, 1978). Urease and polyphenoloxidase

141 activity were assayed in 10 % urea and citric acid buffer (pH 6.7, 24 h at 37 °C). The indophenol

142 method was employed to measure the released NH_4^+ (urease activity: mg NH_4^+-N g^{-1} soil 24 h^{-1}, as

143 described by Kandeler and Gerber, 1988). L-3, 4-dihydroxyphenylalanine substrate was used to obtain

144 phenol oxidase levels in a 96-well plate format in the dark for 20 h at 25 °C (mmol h^{-1} g^{-1}) (Keeler et al.,

145 2009). For acid phosphatase activity (APA) measurements, 1 g of air-dried soil (< 2 mm) was mixed

146 with di-sodium phenyl phosphate solution at 37 °C in pH 5.0 acetate buffer for 1 h (APA activity: μmol

147 pNPP kg^{-1} soil h^{-1}) (Tabatabai, 1994).

148 **2.6. Mineralization of soil C and N**

149 Aerobic incubations were used to assess mineralization rates (Evans et al., 2001). Briefly, soil samples

150 ($\sim$ 20 g) were stored in 250 ml lidded jars at 25 °C for 21 d in the dark. The CO_2 released was then

151 determined at 1, 2, 3, 7, 10, 15, and 21 d post-incubation by gas chromatography (Agilent HP 5890

152 SERIES II, USA). Carbon mineralization rates were calculated as the average CO_2 released during the

153 entire incubation period. NH_4^+-N and NO_3^--N were extracted from the soil prior to and after mixing

154 with 2 mol L^{-1} KCl, and their contents were determined on a 2300 Kjeltec Analyser. Mineralization

155 rates were defined as the change in total N from the start to end of the experiments.

156 **2.7 DNA extraction, PCR amplifications and purification**

157 FastDNA Spin Kits were used for DNA soil extractions (Bio 101). We then amplified the bacterial

158 V3-V4 regions of 16S rRNA gene using the 338F and 806R primers (Xu et al., 2016). Fungal ITS were

159 amplified using the ITS1F and ITS4 primers (Gomez-Montano et al., 2013). PCRs: 5 μl 10 × PCR

160   buffer, 4 µl dNTP, 2 µl cDNA (2.5 ng µl^{-1}), 4 µl primers (10 mM µl^{-1}), and 0.25 µl of Pyrobest DNA

161   polymerase (5 U µl^{-1}) up to 50 µl in ddH$_2$O. PCR parameters: 95 °C for 5 min; 25 x 95 °C for 30 s;

162   55 °C for fungi (52 °C for bacteria) for 30 s and 72 °C for 40 s; and 72 °C for 7 min. Successful

163   reactions were confirmed by visualising PCR bands on 1% agarose gel. Amplicons were then

164   combined and paired-end sequenced on an Illumina MiSeq platform. All gene sequences were

165   deposited in GenBank (PRJNA563991 and PRJNA564366).

166   **2.8 Quantitative PCR**

167   The ABI 3700 Real Time PCR System was employed to quantify the abundance of bacterial and fungal

168   communities. Using ITS1F/ITS4 and 338F/806R primers, standard curves were constructed from

169   10-fold plasmid dilutions of *Escherichia coli* 16S rRNA gene or *Trichoderma* spp. ITS. Each 25 µl

170   reaction contained 12.5 µl RealMasterMix with 20 SYBR solution (5 Prime, Inc., Gaithersburg, MD,

171   USA), 0.5 µl BSA (0.01%), 1 µl primers (20 mM), 3.0 µl template DNA (2.5 ng ml^{-1}) and 8 µl sterile,

172   DNA-free water. PCR parameters: 95 °C for 10 min; 40 x 95 °C for 30 s; 55 °C for 30 s (fungi); 52 °C

173   for 30 s (bacteria); 72 °C for 30 s. Each standard, control and sample were prepared in triplicate.

174   Melting curves and gel electrophoresis were used to confirm specificity.

175   **2.9 Data analysis**

176   The fungal and bacterial sequences were processed using Mothur software (Schloss et al., 2009).

177   QIIME v. 1.3.0 (Caporaso et al., 2010) was employed for quality filtering. Sequences < 200 bp with

178   average quality scores < 25 were excluded. Homopolymers > 10 bp with ≥ 1 ambiguous base calls (N)

179   or errors were also removed. The remaining sequences were clustered into operational taxonomic units

180   (OTUs) at a dissimilarity of 0.03 for ITS and 16S rRNA using UCLUST. For fungi, the taxonomic

181   identity was determined based on the top-ranked BLAST matches. For bacteria, OTUs were identified

182   using the RDP classifier.

183   All OTUs were used to generate rarefaction curves, and to calculate the richness (chao 1),

184   diversity and evenness indices using R software (R Development Core Team, 2016), including

185   functions from the vegan package. Data were compared through a one-way analysis of variance (n=3).

186   LSD tests at a 0.05 significance level were used to assess differences. Data were compared using SPSS

187   13.0 software. The relative abundances of different phyla (or other taxonomic categories) in each

188   cropping cycle were calculated.

189   A one-way analysis of variance (ANOVA) was used to identify the differences in soil C and N

190   pools, diversity and abundance of bacterial and fungal communities, soil biomass C and N, soil

191   extracellular enzyme activities, and soil C and N mineralization ratio among different land using SPSS

192   13.0 software. Differences between means were considered to be statistically significant at $P < 0.05$

193   using the scheffe test. A generalized linear model was used to analyse the relationships between

194   microbial abundance and environmental variables as explanatory variables. Community composition

195   analysis was conducted using multivariate analyses of all non-singleton OTUs. We used non-metric

196   multidimensional scaling (NMDS) via the Bray-Curtis distance to evaluate community composition

197   differences because NMDS ordination was independent of a normal distribution of the species data

198   (McCune and Grace 2002) and could better reflect ecological data where many microbial OTU do not

199   occur in most soil samples (McCune and Mefford 1999). Differences in microbial community between

200   forest types were further tested by analysis of similarity (ANOSIM). Furthermore, envfit (vegan) was

201   used to elucidate the relationships among selected soil variables, soil C and N pools and microbial

202   community. Vectors for significant predictors ($p < 0.05$) were fitted to the NMDS plot. Analyses were

203   performed using the vegan package in R software.

204   A priori structural equation model (SEM) was established to reflect hypothetical relationships,

205 based on the assumption that land use alters soil variables, soil C and N pools, microbial community

206 and enzymes (C, N and P), which in turn affect mineralization of C and N (FigS1). First, only those

207 variables that were significantly affected by land use were selected in the initial model construction

208 according to the results of ANOVA (Eisenhauer et al., 2012). Second, we selected soil variables that

209 were significantly correlated with soil carbon and nitrogen mineralization. Third, we carried out a

210 Spearman correlation analysis between NMDS axes and the relative abundance of OTU level to

211 demonstrate that our axes are good representation of our microbial data (Table S2, S3). The

212 compositions of bacterial and fungal community were represented by the scores of the first dimensions

213 of the NMDS biplot for all variables (Liu et al. 2015; Eldridge et al. 2016). Fourth, number of variables

214 was minimized using PCA for soil C and N pools prior to SEM (Chen et al., 2019b). The first principal

215 component (PC1) explained 74% and 69% of the total variance in soil C and N pools, respectively.

216 Fifth, Geometric means were assessed using GMEA = (invertase×catalase×urease×phenol oxidase

217 ×acid phosphatase) $^{1/5}$ (García-Ruiz et al., 2008). All data were classified into (i) exchangeable

218 potassium (AK); (ii) pH; (iii) soil C pools (TOC, LPI-C, LPII-C, RP-C, DOC); (iv) soil N pools (TN,

219 LPI-N, LPII-N, RP-N, DON); (v) soil bacterial communities (first NMDS axis); (vi) soil fungal

220 communities (first NMDS axis), (vii) the fungi:bacteria (F/B) ratio, and (viii) soil enzyme activities.

221 The fit was measured using modelling approaches in Amos 22.0 (IBM SPSS Inc., Chicago, IL).

222 Maximum‖likelihood estimations were used to assess SEMs ($\chi 2$ tests for goodness-of-fit index (GFI),

223 Akaike information criterion (AIC), and root mean squares error of approximation (RMSEA). Model

224 concepts were revised accordingly. Non-significant $\chi 2$ tests were used to confirm adequate fits (p >

225 0.05) for high GFI (≥ 0.90), low RMSEA and AIC (≤ 0.05) values (Grace and Keeley, 2006).

226   **3. Results**

227   **3.1. Soil C and N**

228   Natural forest had the highest contents of labile pool I-C and recalcitrant-C, whereas agroforestry

229   system had the lowest labile pool I-C and recalcitrant-C contents (Table 1). Labile pool II-C was higher

230   in the 5-year-old rubber plantation than in the other three stands, while the agroforestry system did not

231   significantly differ from the natural forest. Soil labile pool I-N values were significantly higher in the

232   natural forest and the agroforest than the 5- and 15-year-old rubber plantations. The natural forest had

233   the highest levels of labile pool II-N. Following the conversion of the forest, labile pool II-N declined

234   in the 5-year-old rubber plantation with increasing rubber age (Table 1). The labile pool II-N in

235   agroforest soils was higher than that in 15-year-old rubber plantation soils. Recalcitrant-N showed peak

236   values in the natural forest, medium levels in the agroforest and the 5-year-old rubber plantation, and

237   the lowest levels in the 15-year-old rubber plantations (Table 1). The conversion of a rainforest into

238   rubber plantations significantly decreased labile (sum of labile pool I and II) and recalcitrant C and N

239   pools. The agroforest had lower labile and recalcitrant C pools but higher labile and recalcitrant N

240   pools than monoculture rubber plantations. The natural forest and the agroforest had the same content

241   of dissolved organic C, but had higher values than 5- and 15-year-old rubber plantations. The

242   15-year-old rubber plantation had the lowest values (Table 1). In addition, the natural forest had the

243   highest content of dissolved organic N, whereas the 15-year-old rubber plantation had the lowest values

244   (Table 1).

245   **3.2. Bacterial and fungal community composition and abundance**

246   Land-use change significantly altered bacterial and fungal community structures (ANOSIM, R=0.4537,

247   P=0.004 bacteria; R=0.6759, P=0.001 fungi) (Fig. S2 A, B). For soil bacteria, shifts in community

248   induced by the land-use change were related to a decline in relative abundance of *Actinobacteria* and

249   *Verrucomicrobia* and an increase in the relative abundance of *Acidobacteria* and *Chloroflexi* (Fig. 1 A).

250   For soil fungi, shifts in community structure induced by land-use change were correlated with a decline

251   in the relative abundance of *Ascomycota* and *Zygomycota* but increased relative abundance of

252   *Basidiomycota* (Fig. 1 B). Non-metric multidimensional scaling (NMDS) showed a correlation between

253   the bacterial community dissimilarities and pH, exchangeable potassium total N, dissolved organic N,

254   labile pool I-N and labile pool II-C. Similarly, significant correlations were also observed between the

255   fungal community dissimilarities and soil pH, exchangeable potassium, available phosphorous, total N,

256   dissolved organic N, labile pool I-N, labile pool II-N, labile pool I-C, labile pool II-C and recalcitrant C

257   (Fig. 1 A, B). We observed significantly lower bacterial richness (Chao1) in the natural forest with

258   respect to the 15-year-old rubber plantation and the agroforestry system (P < 0.05), but phylogenetic

259   diversity was not altered by land-use changes (Fig. S3 A). In contrast, land use did not influence the

260   richness of the fungal communities (Chao1) or phylogenetic diversity (Fig. S3 B).

261   Quantitative PCR (qPCR) data indicated that the bacterial abundance was highest in the natural

262   forest and declined in the following order: natural forest > agroforest > 15-year-old rubber plantation >

263   5-year-old rubber plantation (Fig. 2 A). The fungal abundance was comparable among all samples

264   except for the 5-year-old rubber plantation, which had a low abundance (Fig. 2 B). The F/B ratio was

265   highest in the 5-year-old rubber plantation. The agroforest and the 15-year-old rubber plantation had

266   the second largest and generally similar F/B ratios. The natural forest displayed the lowest F/B ratio

267   (Fig. 2 C).

268   **3.3. Soil microbial biomass C and soil microbial biomass N, enzyme activities and mineralization**

269   The land-use change influenced both soil microbial biomass C and N (Fig. 3A, B). Soil microbial

270   biomass C (SMB-C) was the higher in the natural forest than the 5-year-old rubber plantation, the

271   15-year-old rubber plantation and the agroforest. The highest concentration of soil microbial biomass N

272   (SMB-N) was obtained in the natural forest samples. Soil microbial biomass N was significantly

273   decreased in 5- and 15-year-old rubber plantations. However, the agroforest soil showed the lowest

274   values of soil microbial biomass N.

275   Urease and invertase activities showed the highest activities in the natural forest (Fig. 4 A, B). The

276   soil catalase activity was the highest in the 15-year-old rubber plantation, intermediate in the 5-year-old

277   rubber plantation and the agroforest system, with no significant difference between the two sites, and

278   the lowest in the natural forest (Fig. 4 C). Polyphenoloxidase activity was significantly higher in the

279   15-year-old rubber plantation, than in the agroforest (Fig. 4 D). Acid phosphatase activity was the

280   highest in the natural forest, intermediate in the 15-year-old rubber plantation and lowest in the

281   5-year-old rubber plantation, but the differences were not significant (Fig. 4 F). Urease, invertase, and

282   acid phosphatase activities were positively correlated with community dissimilarities of the bacteria

283   and fungi (Table 2). Catalase activity was negatively associated with community dissimilarities and

284   abundances of the bacteria and fungi. Moreover, urease and invertase activities were only positively

285   associated with fungal abundance (Table 2). Acid phosphatase activity was positively associated with

286   both bacterial and fungal abundances (Table 2).

287   The soil C mineralization rate was highest in the natural forest, intermediate in the agroforestry

288   system, and lowest in the 5- and the 15-year-old rubber plantations, with no significant differences

289   between the 5- and the 15-year-old rubber plantations (Fig. 5 A). The soil N mineralization rates

290   increased after the conversion of the natural forest into 5-year-old rubber plantation but decreased with

291   increasing rubber age. The presence of a cover crop increased the soil N mineralization rate in the

292 agroforest (Fig. 5 B).

293 3.4. Relationship between soil C and N, microbial communities and mineralization rates

294 Overall, SEM indicated that the forest conversion directly decreased soil C and N, pH and

295 exchangeable potassium (AK) (Fig. 6). In addition, the increased F/B ratio and the altered fungal

296 community structure was linked to the forest conversion. Furthermore, a large proportion in soil

297 enzyme variation was described by soil pH, bacterial community and the F/B ratio (Fig. 6).

298 Furthermore, SEM showed that the most of the variation in the soil C mineralization rate was explained

299 by the bacterial community structure, AK, F/B ratio and soil enzyme activities (Fig. 6), whereas a

300 significant proportion of soil N mineralization could be explained by the fungal community structure,

301 N pools and soil enzymes (Fig. 6).

4. Discussion

Here, we report that the conversion of a rainforest into rubber plantations differentially influenced C and N pools and the microbial community depending on the age of the plantations and the presence of a cover crop. In consistence with previous studies of land-use type/changes in various ecosystems (van Straaten et al., 2015; Xu et al., 2018), forest conversion generally resulted in decreased soil organic matter and microbial abundance. Our SEM showed that these findings could be explained by the increased net N mineralization rate and decreased soil C mineralization rate in rubber plantations. Taken together, these results highlight the complexity of the linkages between soil C and N pools, microbial communities, and C and N mineralization, which provide new insights into the mechanisms of soil organic matter loss induced by land-use changes.

4.1. Land use influences C and N pools

The decline in soil organic C content as well as C fractions upon conversion of forest into 5- and 15-year-old rubber plantations are consistent with previous studies (van Straaten et al., 2015; Guo et al., 2016). The loss of soil organic C and all C fractions might have been triggered by the loss of vegetation cover, the reduction in physical protection through aggregates or an increase in soil erosion due to the felling of trees after forest clearing (Guo et al., 2016). When rubber trees grow older, a further decrease in the labile C pool was observed in the 15-year-old plantation compared to the 5-year-old plantation. However, Nath et al., (2018) found that soil organic matter inputs such as litter and root biomass were enhanced with increase in the age of rubber plantation. It is possible that the higher labile C pools in 5-year-old rubber plantation are driven by litter residues left after forest clearing. This argument is supported by a previous similar finding in Chinese fir plantations (Chen et al. 2016), suggesting that the forest conversion can temporarily increase soil labile C fractions in 5-year-old rubber plantation.

324   We revealed significantly lower soil organic C and all C fractions in the agroforestry systems than

325   in the 15-year-old rubber plantation, which could be explained by the positive priming effect of cover

326   crop. However, the conversion of monoculture rubber plantations into rubber–*Flemingia macrophylla*

327   plantations was shown to promote and maintain soil organic C levels (Wu et al., 2017). These

328   discrepancies can be explained via species-specific impacts on C storage. *F. macrophylla* is a

329   leguminous shrub, whereas black cardamom is a zingiberaceous plant. It is well known that plant

330   species have different abilities to impact C storage (Wu et al., 2017). Fixed N is required to enhance

331   soil organic matter and C storage (Wu et al., 2017). In contrast, non-legume plants act as competitors in

332   systems in which N availability is limited, ultimately promoting soil organic matter decomposition (Wu

333   et al., 2017). In addition, leguminous plants with high quality litters (low C/N) can improve microbial

334   C use efficiency by releasing higher DOC content, thereby generating more stable microbial-derived

335   soil organic matter and enhancing soil C storage (Castellano et al., 2015; Tamura and Tharayil, 2014;

336   Hu et al., 2019; Lyu et al., 2019). On the contrary, non-legume plants with low quality litter (high C/N)

337   could potentially increase soil C loss and reduce soil C accumulation (Tamura and Tharayil, 2014; Lyu

338   et al., 2019). This suggests that the black cardamom introduction may have caused net loss of soil C

339   which may explain the land-use change induced C loss in our agroforestry systems.

340   A marked decrease in labile N and recalcitrant N occurred as a result of the conversion of the

341   natural forest to the 5-year-old rubber plantation, which aligns with previous reports from a Chinese fir

342   plantation (Chen et al., 2016). When the rubber stands developed from the 5- to the 15-year-old

343   plantation, the labile N significantly increased, while recalcitrant -N declined, suggesting that mineral

344   N was gradually released. The improvement in above-ground productivity contributed to the enhanced

345   N release in mature stands due to the accumulation of degradable organic matters (Chen et al., 2016).

346   Additionally, the rubber agroforestry system showed higher labile N and recalcitrant N pools than the

347   5- and 15-year-old rubber plantations, indicating that cover crop introduction is important for

348   improving N content in the soil of the rubber plantations (Liu et al., 2018a). This finding can be

349   explained by three potential mechanisms. First, the higher labile and recalcitrant N levels could be

350   related to increased root exudates and litter input in the presence of cover crop (Belay-Tedla et al.,

351   2009). Second, the presence of cover crop could increase physical protection through aggregate

352   formation due to improvements in arbuscular mycorrhizal fungi biomass (Li et al., 2015; Wang et al.,

353   2017). Finally, the development of cover crop in agroforestry system may help minimize soil exposure

354   to erosion and nutrient leaching (Li et al., 2012), although it can enhance plant N uptake due to

355   relatively higher plant production. This suggests that agroforestry system undergo a rapid depletion of

356   organic C in the soil but show enhanced N retention compared with monoculture rubber plantations.

357   The conversion of the 5-year-old rubber plantation from the natural forest decreased dissolved

358   organic C and N. The lower microbial biomass and decrease in below-ground biomass and litter input

359   could explain this observation (Xu et al., 2015; Xu et al., 2018), as also supported by the decreased soil

360   microbial mass C and N in the 5-year-old rubber plantation. Previously, it was shown that 15-year-old

361   plantation could increase leaf litter input and may increase dissolved organic C and N in the mineral

362   soils (Zhou et al. 2015). In our study, however, dissolved organic C and N were higher in the

363   5-year-old rubber plantation than the 15-year-old rubber plantation. On one hand, roots and litter

364   decomposition following forest clearing had favourable effects on dissolved organic C and N in the

365   5-year-old rubber plantation (Chen et al., 2016). The low contents of dissolved organic C and N in the

366   15-year-old rubber plantation could be explained by the decrease in water fluxes because the canopy in

367   mature plantation could decrease throughfall (Zhang et al., 2019). In addition, the higher concentrations

368   of dissolved organic C and N in the agroforestry system than in the 15-year-old rubber plantation could

369   be explained by the fact that cover crop introduction may increase root exudation, photosynthesis, litter

370   deposition and the turnover of soil organic matter (Belay-Tedla et al., 2009; Xu et al., 2015). Taken

371   together, our results highlight that the production and turnover of labile C are slower following forest

372   conversion, but can be enhanced in the presence of cover crop in agroforestry system.

373   Upon the establishment of the rubber plantations, the decrease in soil microbial biomass C and N

374   was likely due to limited resource availability that prevented the growth and activity of soil organisms

375   (Xu et al., 2018). We also found that the introduction of a cover crop decreased the N in microbial

376   biomass but not C, indicating that the priming of soil organic matter in agroforestry systems was not

377   determined by the microbial demand for C and N. The reduction in soil microbial N biomass is

378   potentially driven by N competition between microbes and plants (Belay-Tedla et al., 2009). This

379   suggests that introduction of a cover crop can increase the competition for nitrogen between soil

380   microbes and plants in agroforestry system.

381   4.2 Role of soil C and N in shaping the microbial community

382   Land-use change increased the relative abundance of oligotrophic phyla (mainly *Acidobacteria*,

383   *Planctomycetes* and *Chloroflexi*) and decreased the abundance of copiotrophic taxa (mainly

384   *Actinobacteria* and *Proteobacteria*) (Lee-Cruz et al., 2013). The NMDS analysis showed that the shift

385   in the bacterial community was associated with pH, exchangeable potassium, total N, labile C and N

386   pools and dissolved organic N in the soils. The importance of soil pH and nutrients in the bacterial

387   community structure has been widely reported (Kuramae et al., 2012; Delgado-Baquerizo et al., 2017;

388   Wang et al., 2017; Yang et al., 2019; Tang et al., 2020). For example, the increase in relative

389   abundance of *Acidobacteria* and *Chloroflexi* was likely associated with a decline in pH (Liu et al., 2014;

390   Yang et al., 2019) and lower contents of labile C (Trivedi et al., 2013; Silva et al., 2015), whereas the

391   increase in relative abundance of *Proteobacteria* with a higher labile C content confirming that

392   different taxa had different responses to soil pH and nutrient (Liu et al., 2018b; Ramírez et al. 2020).

393   Our results revealed a transition from a copiotrophic to oligotrophic system due to the decrease in pH

394   and nutrient availability (Trivedi et al., 2013).

395   Forest conversion had also altered the structure of the soil fungal community by increasing the

396   relative abundance of lignin-degrading *Basidiomycota* at the expense of wood-decaying fungal taxa

397   (*Ascomycota* and *Zygomycota*) (Wang et al., 2019). The shift in soil fungal community structure was

398   correlated to a significant decrease in pH, exchangeable potassium, available phosphorus, the labile C

399   and N pools and dissolved organic C and N in the soil, highlighting the significant role of nutrient

400   quality and quantity in shaping the fungal community structure. For example, *Ascomycota* has a limited

401   ability to degrade recalcitrant C but rapidly responds to labile C substrates (Wang et al., 2019).

402   *Basidiomycota,* in contrast, synthesises numerous enzymes to breakdown recalcitrant C (including

403   lignin) (Chen et al., 2014), with a negative correlation to soil N and C (Wang et al., 2019). In addition,

404   higher F/B ratio observed after the rainforest conversation was linked to decreased labile C and N

405   fractions (Xu et al., 2018). Overall, microbial response to land-use change was correlated with

406   alternation of soil pH, exchangeable potassium, available phosphorus, and soil C and N pools, which

407   favoured oligotrophic bacteria and lignin-degrading fungi and reduced bacterial abundance.

408   4.3 Effects of land use on soil ecosystem functioning

409   The decline in soil C mineralization rate observed in this study following forest conversion was

410   consistent with a previous study (Lang et al., 2017). Structural equation modelling (SEM) identified the

411   main contributing factors influencing the different responses to soil C mineralization among

412 exchangeable potassium, pH, soil C and N pools, microbial activity and microbial communities.

413 Exchangeable potassium was significantly correlated with soil C mineralization and likely influenced C

414 mineralization through its effects on the soil bacterial community and microbial activity. Soil pH might

415 have an indirect effect on C mineralization by altering soil enzyme activity and increasing DOC or

416 availability of clay-bound SOM (Curtin et al., 1998; Malik et al., 2018). Our results are supported by

417 previous studies reporting that pH and exchangeable potassium have an important role in shaping the

418 soil microbial community (Wang et al. 2017; Tang et al., 2020). In addition, the quantity and quality of

419 soil C pools regulate soil organic C mineralization by altering the F/B ratio (Lin et al., 2018).

420   Our SEM results further supported the observation that the decline in carbon mineralization was

421 directly and positively correlated to the bacterial community structure and was negatively related to the

422 F/B ratio, indicating that the bacterial community structure and abundance play a more important role

423 in regulating C mineralization than the fungal community. Previous studies observed comparable shifts

424 in the soil microbial communities and the regulation of SOC mineralization (Lin et al., 2018; Chen et

425 al., 2019a). Our results further indicated that land use could suppress bacterial abundance and alter the

426 bacterial community from a copiotrophic to oligotrophic system and thus could change microbially

427 driven carbon mineralization processes. In addition, the effects of land-use changes on soil organic C

428 mineralization and the variation in bacterial communities and the F/B ratio suggest that the production

429 of extracellular enzymes by bacterial communities is widely distributed among different microbial

430 groups in terrestrial ecosystems and that soil enzymes play an important role in regulating C

431 mineralization (Trivedi et al., 2016). Taken together, the decline in soil organic C mineralization was

432 directly regulated by a decrease in exchangeable potassium, soil enzyme activity and a shift in the

433 bacterial community from a copiotrophic to oligotrophic system upon forest conversion.

434   The conversion of the forest to a young rubber plantation enhanced net N mineralization (Zhang

435   et al., 2019), as rapid N mineralization is driven by enhanced soil temperatures. This finding indicated

436   that there was a significant loss of labile soil N during that period, as the ground cover was low and

437   nutrient uptake was limited (Chen et al., 2016). However, the low net N mineralization rates in the

438   forest compared to the young rubber plantation did not reflect N transformation levels, with net rates

439   masked by enhanced N immobilization (Zhang et al., 2019). It can be explained by the fact that when

440   rubber trees attain their maximum growth rate, demands for nutrients increase. The higher net N

441   mineralization in the agroforestry system suggests that soil heterotrophs under plantations had less

442   access to nutrients, metabolized C less efficiently, and had to decompose more soil organic matter to

443   meet their nutrient demands.

444   Our SEM results suggested that the variation in the fungal community structure and enzymatic

445   activities could explain the effects of land use on net N mineralization. This was directly and negatively

446   regulated by the fungal community structure, while soil enzyme activities were positively related to net

447   N mineralization. It was previously shown that *Absidia cylindospora* (zygomycetal saprotroph) can

448   produce nitrate (Li et al. 2017) and contribute to nitrification in acidic forest soils (De Boer and

449   Kowalchuk, 2001). Soil bacteria are crucial to soil N cycling because they secrete exoenzymes that

450   lead to the depolymerization of N-containing compounds (Freedman et al., 2017; Chen et al., 2019a).

451   Our analysis showed that soil bacterial community was positiviely related to soil enzymatic activities

452   across land-use types, indicating that the shift in the bacterial community from copiotrophic to

453   oligotrophic enhanced N mineralization to meet microbial and/or plant demands for N. Moreover, the

454   F/B ratio was negatively related to soil enzymatic activities, indicating that the decrease in the bacterial

455   abundance observed in this study likely contributed to reduced enzymatic activities, which had a

456   detrimental effect on N mineralization.

457   **5. Conclusions**

458   Consistent with our first hypothesis, the conversion of a rainforest into rubber-based plantations

459   resulted in significant C and N losses, which was closely related to changes in the structure and

460   abundance of soil microbial communities. Land-use changes reduced C mineralization, primarily

461   because of shifts in the soil bacterial community structure from copiotrophic to oligotrophic and

462   reduced microbial enzymatic activities. However, the rubber-based agroforestry system promoted soil

463   C mineralization, indicating that the introduction of cover crops depleted soil C, which is contrary to

464   the second hypothesis. Moreover, soil N mineralization was regulated more by the soil fungal

465   community than by the bacterial community and soil N pools. These results provide a mechanistic

466   understanding of C and N losses from the land-use change and highlight the importance of microbial

467   communities in the decomposition of soil organic matter. Our findings have implication for the

468   development and management of agroforestry systems, and suggest that future works should examine

469   effective options (e.g. intercropped plant species, supply of organic fertilizers) to maintain the C

470   balance in agroforestry systems to ensure environmental sustainability.

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Table 1 Effects of rainforest conversion on soil organic carbon (C) and nitrogen (N) pools.

Soil C and N pools（mg kg^{-1}）	5-year-old rubber plantation	15-year-old rubber plantation	Agroforest	Natural forest
SOC	13300±834b	11810±284b	7490±335c	19310±1426a
TON	1373±77c	1367±106c	1625±46b	1915±76a
LP7-C	5370±780b	4230±150c	3200±370d	9370±320a
LP7-C	1140±260a	800±60b	460±70c	550±100c
RP-C	6790±1120b	6780±840b	3830±420c	9390±1140a
LP7-N	796±32c	848±12b	864±12b	996±13a
LP7-N	250±27d	300±24c	355±18b	430±66a
RP-N	327±11c	219±54d	404±10b	489±22a
DOC	48.78±1.48b	39.01±0.83c	63.72±3.31a	66.30±5.02a
DON	1.81±0.35b	0.57±0.31c	1.56±0.31b	11.32±0.88a

TOC, total organic carbon; TON, total organic nitrogen; LPI-C, labile carbon pool I; LPII-C, labile carbon pool II; RP-C, recalcitrant carbon pool; LPI-N, labile nitrogen pool I; LPII-N, labile nitrogen pool II; RP-N, recalcitrant nitrogen pool; DOC, dissolved organic C; DON, dissolved organic N. The values are means ± SE, n = 3. Values not sharing the same letter are significantly different (P < 0.05) according to scheffe test.

Table 2. The relationships between soil enzyme activities and soil microbial properties after forest conversion.

	Bacterial composition	Fungal composition	Bacterial abundance	Fungal abundance
Urease	**0.708**[**]	**0.956**[**]	0.535	**0.695**[*]
Invertase	**0.707**[*]	**0.890**[*]	0.495	**0.630**[*]
Acid phosphatase	**0.723**[**]	**0.962**[**]	**0.650**[*]	**0.739**[**]
Catalase	**-0.667**[*]	**-0.832**[**]	**-0.643***	**-0.759**[**]
Polyphenoloxidase	-0.134	-0.342	-0.471	-0.457

Significant values are shown in bold. * and ** indicates significant correlation at P< 0.05 and P < 0.01, respectively.

Figure captions

Figure. 1. Effect of conversion of rainforest into rubber plantation on dominant (a) soil bacterial and (b) soil fungal phyla. NF, natural forest; YR, 5-year-old rubber plantation; MR, 15-year-old rubber plantation; AF, agroforest system.

Figure. 2. Effect of conversion of rainforest into rubber plantation on soil bacterial (A) and fungal (B) abundance and F/B ratio (C). Values are means±standard errors (n = 3). Means with different letters are significantly different at P <0.05 based on scheffe test. NF, natural forest; YR, 5-year-old rubber plantation; MR, 15-year-old rubber plantation; AF, agroforestry system.

Figure 3. Effect of conversion of rainforest into rubber plantation on soil biomass C (A) and N (B).Values are means± standard errors (n = 3). Means with different letters are significantly different at P <0.05 based on scheffe test. NF, natural forest; YR, 5-year-old rubber plantation; MR, 15-year-old rubber plantation; AF, agroforestry system.

Figure 4. Activities of soil invertase (A), catalase (B), urease (C), phenol oxidase (D), acid phosphatase (E) at different study sites after forest conversion. Values are means± standard errors (n = 3). Means with different letters are significantly different at P <0.05 based on scheffe test. NF, natural forest ; YR, 5-year-old rubber plantation; MR, 15-year-old rubber plantation; AF, agroforestry system.

Figure 5. Effect of conversion of rainforest into rubber plantation on soil C (A) and N (B) mineralization ratio. Values are means+ standard errors (n = 3). Means with different letters are significantly different at P <0.05 based on scheffe test. NF, natural forest; YR, 5-year-old rubber plantation; MR, 15-year-old rubber plantation; AF, agroforestry system.

Figure. 6. Structural equation model (SEM) analysis of the effects of forest conversion on the soil microbial community and C (Cmin) and N (Nmin) mineralization rates. Results of model fitting: (a) $\chi2$=6.103, P=0.636, df=8, GFI=0.905; RMSEA=0.000; AIC=76.347. Red solid arrows indicate positively effects and blue dotted arrows represent negative effects. R^2 values associated with response variables indicate the proportion of variation explained by relationships with other variables. Values associated with solid arrows represent standardized path coefficients.
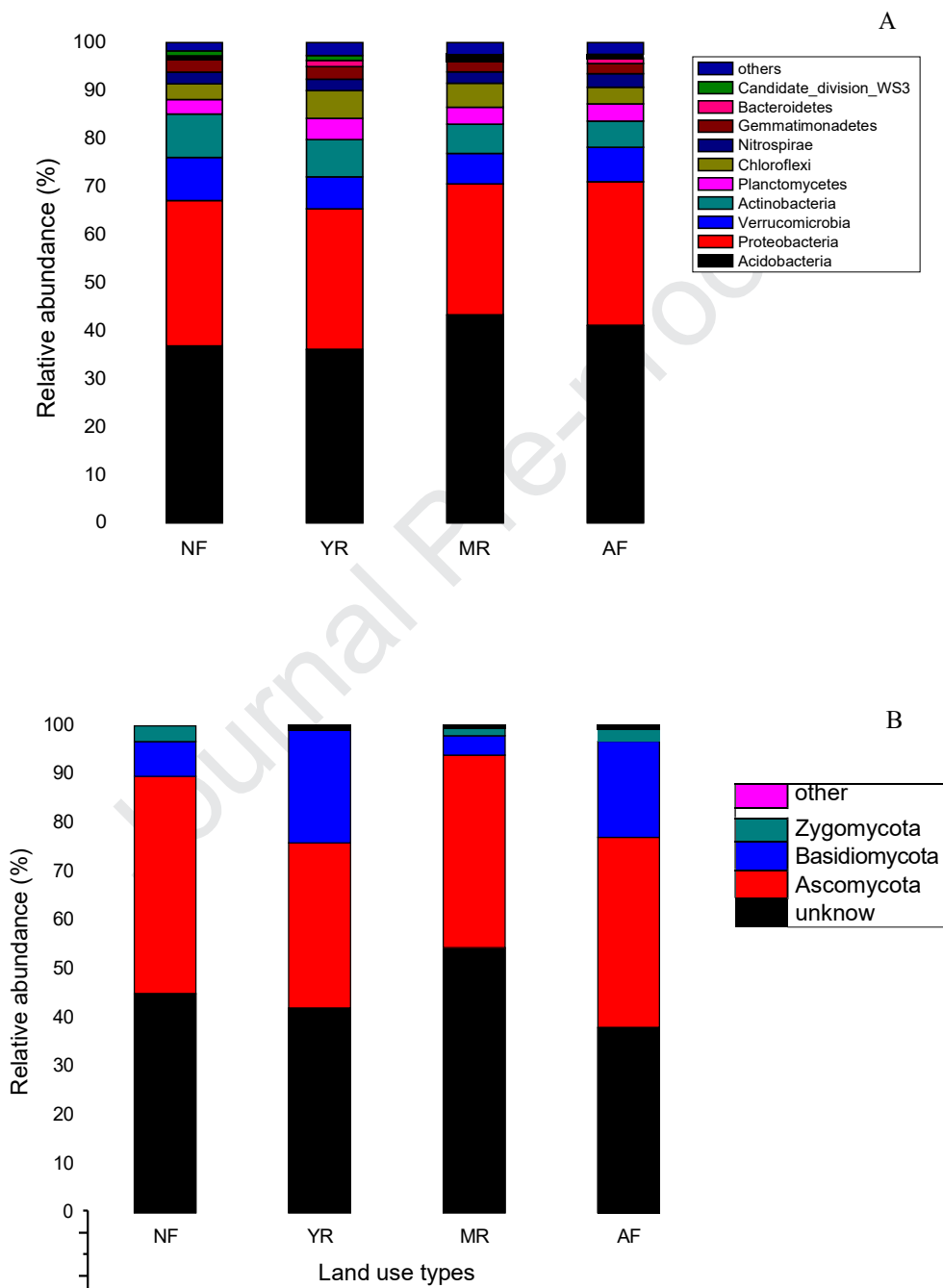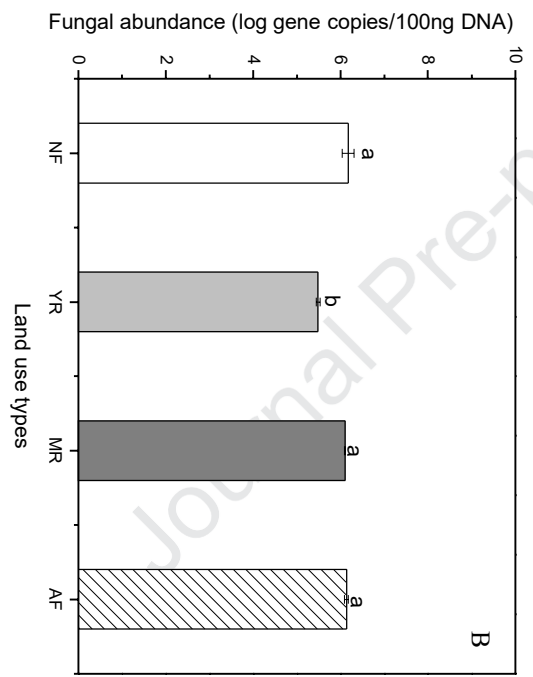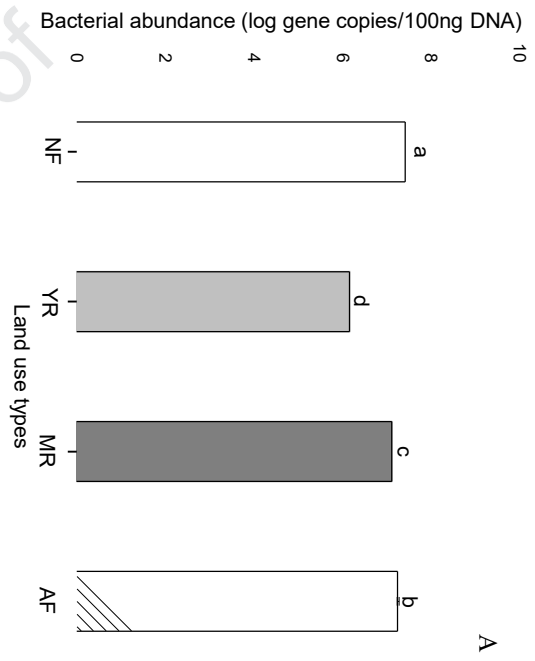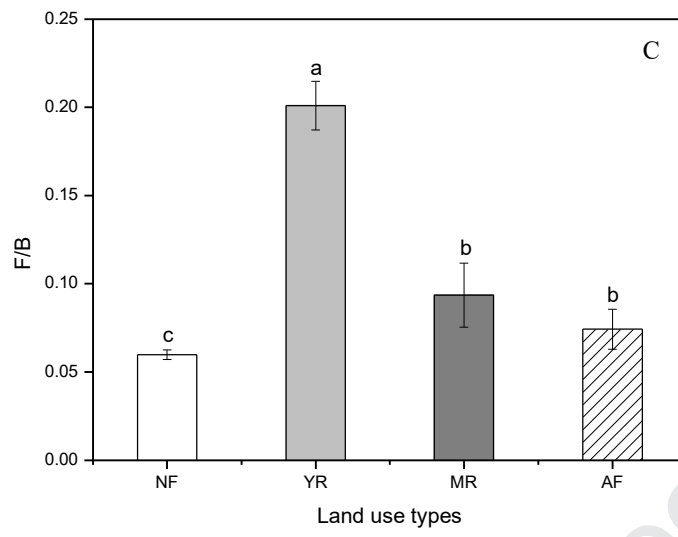
A

others
Candidate_division_WS3
Bacteroidetes
Gemmatimonadetes
Nitrospirae
Chloroflexi
Planctomycetes
Actinobacteria
Verrucomicrobia
Proteobacteria
Acidobacteria

B

other
Zygomycota
Basidiomycota
Ascomycota
unknow

Land use types

Fig. 1.

Fungal abundance (log gene copies/100ng DNA)

Bacterial abundance (log gene copies/100ng DNA)

Fig. 2.

Fig. 3.

C

D

Fig. 4.

Acid phosphatase acitivity (umol p-NPP kg-1soil h-1)
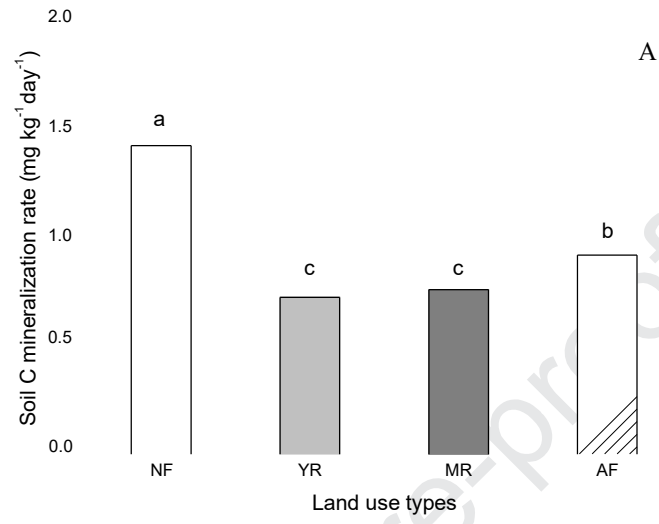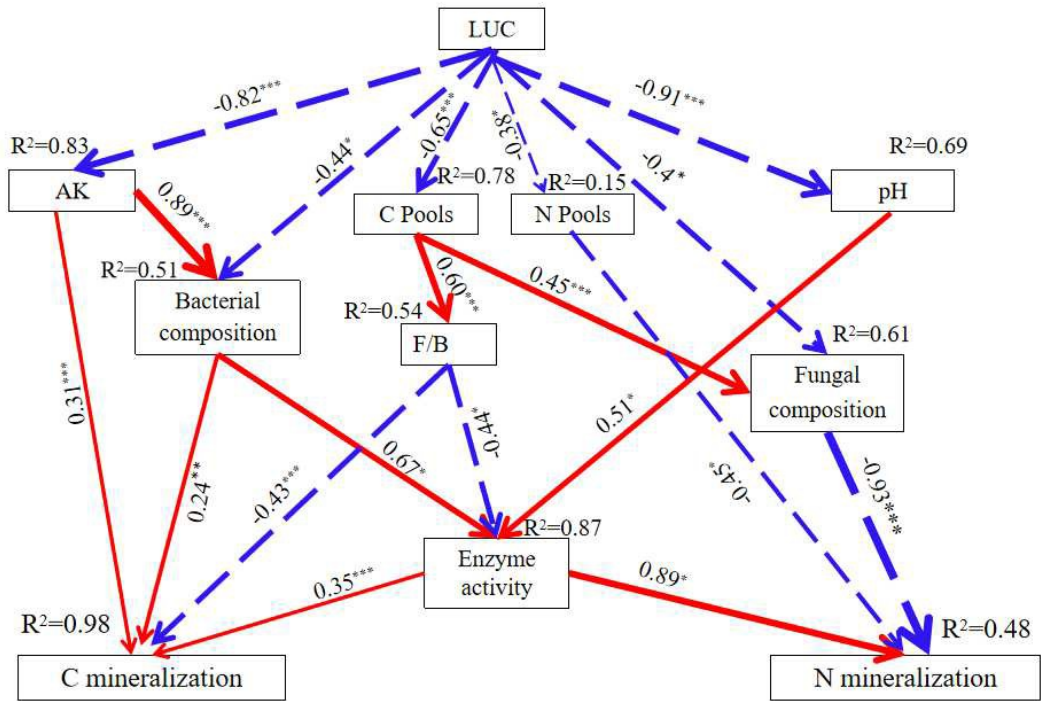
Land use types
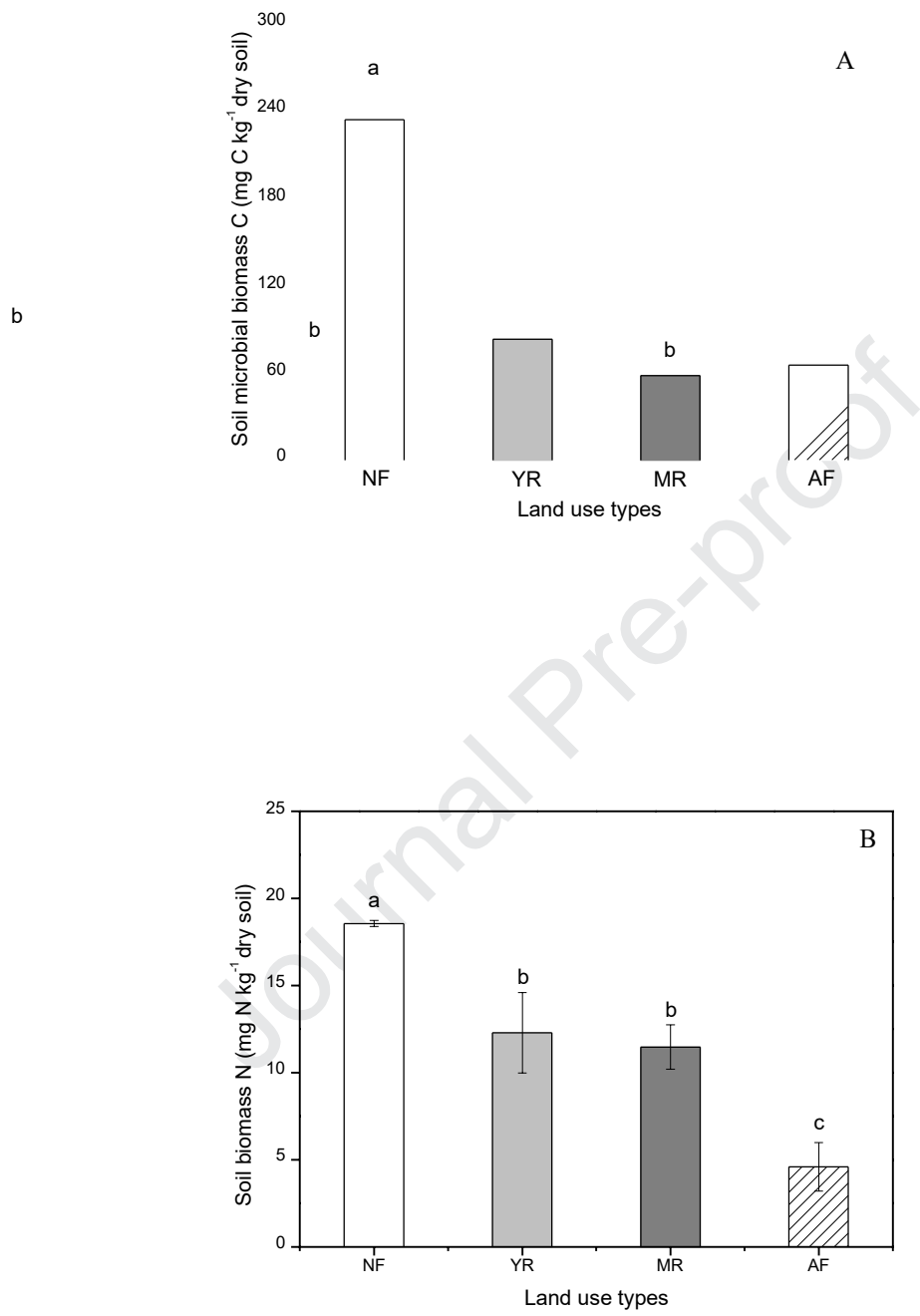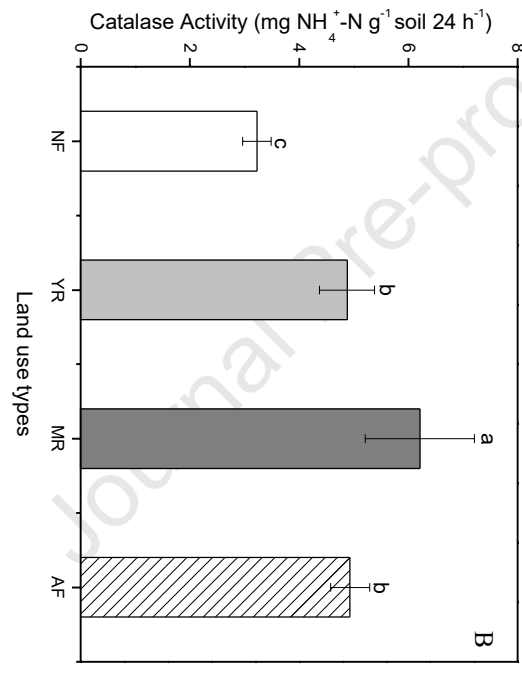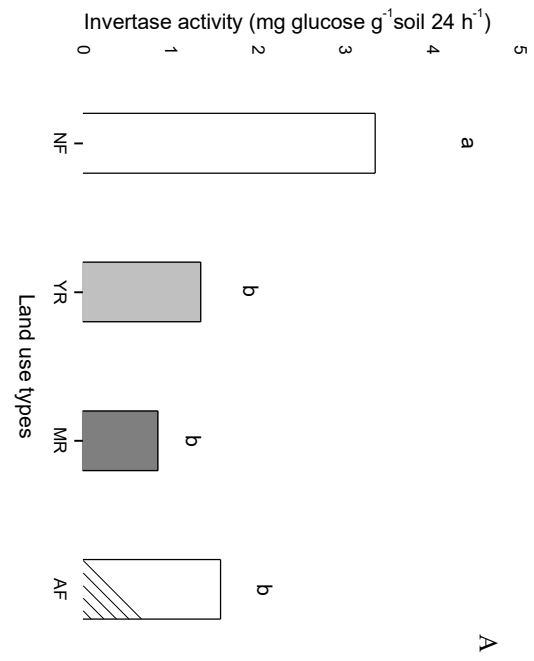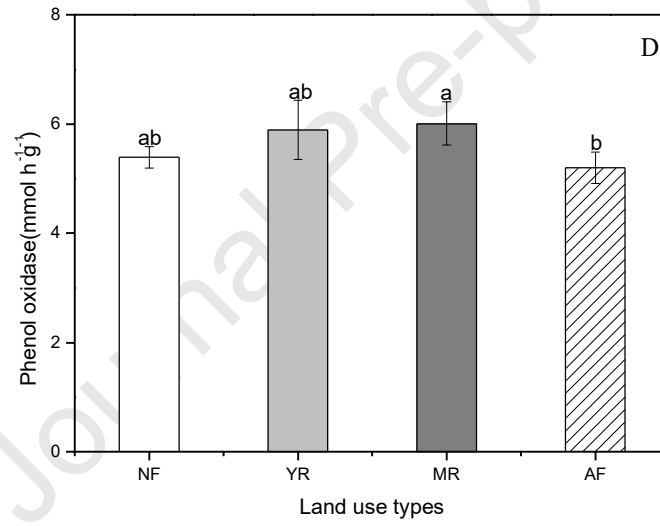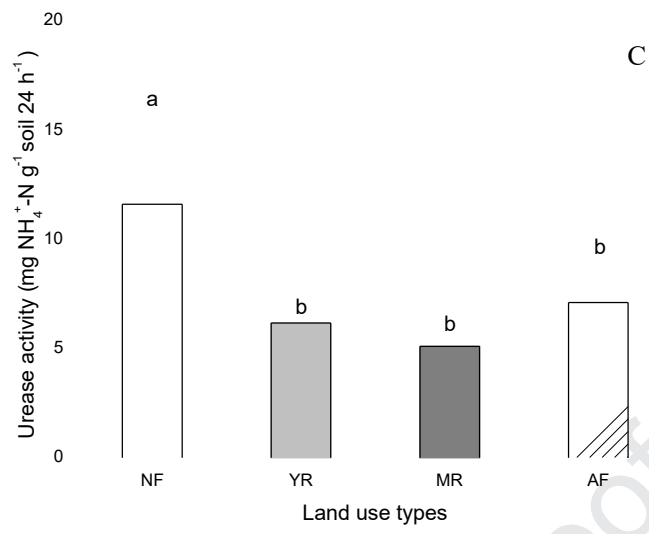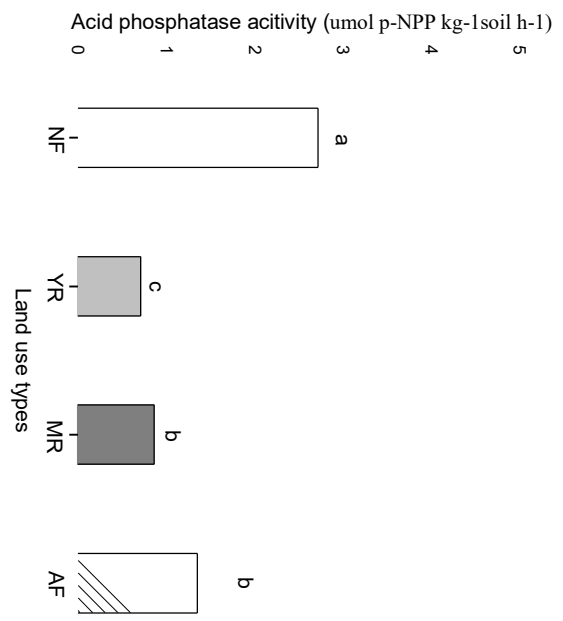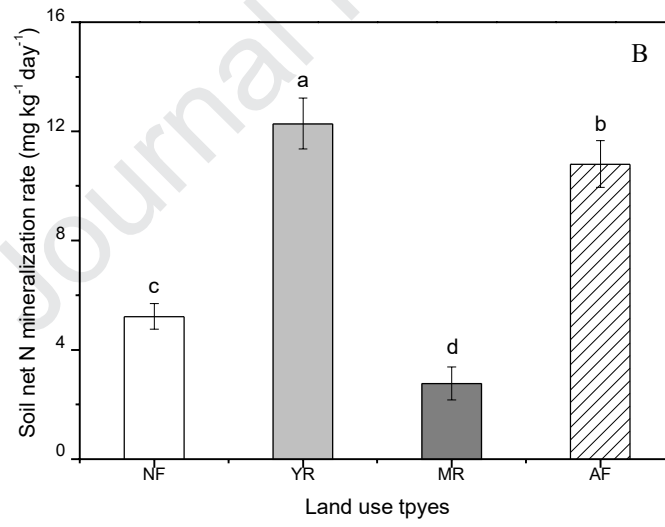
Fig. 5.

Fig. 6.

Highlights

- Rainforest conversion into rubber-based plantations decreased soil C and N pools.

- Bacterial abundance was reduced but oligotrophic bacteria are less affected by land-use change.

- Land-use change increased relative abundance of fungi.

- Shifts in the soil bacterial and fungal communities explained ecosystem functions.

Declaration of Interest Statement

We declare that we have no conflicts of interest to this work.