Rubber plantation ageing controls soil biodiversity after land conversion from cassava

Monrawee Peerawat[1,10], Aimeric Blaud[2], Jean Trap[3, 11], Tiphaine Chevallier[3, 11], Pascal Alonso[4], Frederic Gay[5, 11], Philippe Thaler[5, 11, 12], Ayme Spor[6], David Sebag[7,8], Choosai Chutinan[9], Nopmanee Suvannang[1], Kannika Sajjaphan[10, 12] * & Alain Brauman[3,4, 11]

[1] Land Development Department, LMI LUSES, Bangkok Thailand,

[2] Sustainable Agriculture Sciences Department, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK.

[3] IRD, UMR ECO&SOLS, 34000 Montpellier, France

[4] IRD, UMR ECO&SOLS, LMI LUSES, Bangkok Thailand

[5] CIRAD, ECO&SOLS, 34000 Montpellier, France

[6] INRA, UMR 1347 AGROECOLOGIE, 21065 Dijon, France

[7] Normandie University, UNIV ROUEN, UNICAEN, CNRS, M2C, 76000 Rouen, France

[8] Institute of Earth Surface Dynamics, Geopolis, University of Lausanne, Lausanne, Switzerland

[9] Khon Kaen University, Khon Kaen, LMI LUSES, Thailand

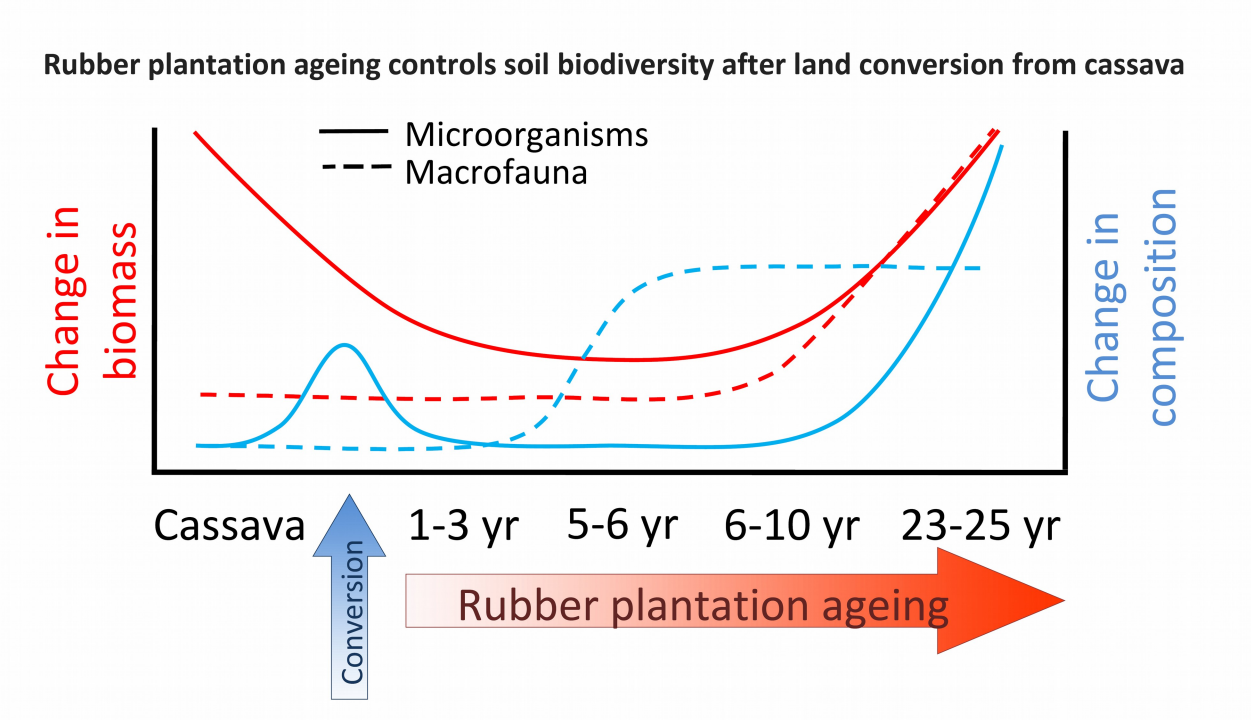
[10] Department of Soil Science, Center for Advanced Studies in Agriculture and Food, Kasetsart University, Bangkok, Thailand

[11] ECO&SOLS, University Montpellier, CIRAD, IRD, INRA, M-SUPAGRO, 34000 Montpellier, France

[12] Hevea Research Platform in Partnership, Kasetsart University, Centre of Thai-French Cooperation on Higher Education and Research, 10900 Bangkok, Thailand

*** Corresponding author:** Dr Kanika Sajaphan, E-mail: agrkks@ku.ac.th, Tel: (66) 892277188

Graphical abstract



Abstract

The rapid expansion of perennial crops is a major threat to biodiversity in Southeast Asia. The biodiversity losses related to the conversion of forest lands to oil palm or rubber plantations (RP) are well documented by recent studies. However, the impact of the conversion from intensively managed annual crops to perennial crops on soil biodiversity has not yet been addressed. This study aims at assessing the impact on soil biodiversity of a) the short-term effect of land use conversion from cassava crop to RP, and b) the long-term effect of RP ageing. Soil biodiversity (bacterial, fungal and macrofaunal), microbial activities and pedoclimatic characteristics were measured over a chronosequence of 1 to 25 years old of RP compared to cassava fields, the former crop, in Thailand. The conversion from cassava to young RP (1-3 yr) had a significant effect on microbial biomass and activities and fungal composition, but did not impact the bacterial and macrofaunal diversity. This effect of land use conversion could be explained by the change in land management due to the cultivation of pineapple in the inter-row of the young RP. Canopy closure appeared to be the main driver of soil biota shifts, as most of the biotic parameters, composition, abundance and activities were significantly modified after 7 years of RP. The changes of composition in older rubber plantations originated from the dominance of *Trichoderma* (fungi), Firmicutes (bacteria), and earthworms. Old rubber plantations (23-25 yr) harboured the highest microbial and macrofaunal biomass; however, they were also characterised by a significant decrease in bacterial richness. The change in pedoclimatic conditions across the rubber chronosequence, i.e. increase in soil moisture, litter and organic carbon content, was a stronger driver of soil biota evolution than land use conversion. The macrofaunal composition was more resistant to land use conversion than the bacterial composition, whereas the microbial biomass was sensitive to land use conversion, but showed

resilience after 20 years. However, bacterial, fungal and macrofaunal diversity, macrofaunal and microbial biomass and microbial activities were all sensitive to RP ageing.

Keywords: bacterial diversity, fungal diversity, soil macrofauna, perennial chronosequences

1. Introduction

Rubber plantations (RP) have expanded faster than all other tree crops in South East Asia (Fox and Castella, 2013), with a 1.8 fold increase in surface area over the last three decades. South East Asia represents more than 83% of the World rubber area (FAO, 2012). In Thailand, the first natural rubber producer (IRSG, 2015), this expansion has lasted for more than a century. In 2015, rubber plantations covered more than 3.5 million ha in Thailand, and represented the second largest area of rubber in the world (IRSG, 2015). Originally, the expansion of RP in Thailand replaced natural vegetation such as forest. However, today RP have replaced many subsistence agriculture or intensive annual cash crops such as sugarcane or cassava, instead of replacing natural vegetation due to forest depletion and protection (Chambon et al., 2016).

The ability of rubber trees to grow on a wide range of soil types and pedoclimatic conditions, from optimal tropical lowland to suboptimal environments such as low-fertility areas with distinct dry seasons or steep slope (Blagodatsky et al., 2016), partly explains the rapidity and success of RP expansion. However, according to Saengruksawong et al. (2012), only 102,000 ha in Thailand have suitable soil characteristics for RP. Thus, the majority of rubber expansion has taken place on poor soil with low fertility (Chambon et al., 2016). This

highlights the need to better determine the environmental impact of RP on the soil compartment. Like any other form of land use conversion, the development of a tree plantation leads to changes in ecosystem characteristics and fluxes (Njar et al., 2011). If RP are considered as one of the top four best land uses in South East Asia for carbon (C) stock (Ziegler et al., 2012), their C balance rarely considers the soil compartment (Blagodatsky et al., 2016). Globally, the effect of rubber trees on soil C balance and nutrient cycle first depends on the previous land use, being positive after conversion of arable land to RP (Njar et al., 2011; Yasin et al., 2010; L. Herrmann et al., 2016a, 2016b) and negative when forest was converted to RP (Li et al., 212; M. de Blécourt et al., 2013; S. Blagodatsky et al., 2016). However, studies investigating rubber tree plantations impact on soil have focused mainly on the physico-chemical parameters.

Concerns about the impact of RP on soil biodiversity has been growing due to rapid rubber tree expansion in tropical regions associated with high level of biodiversity (Mumme et al., 2015; Warren-Thomas et al., 2015; Xu et al., 2014). Conversion of primary or secondary forest to rubber monoculture results in a severe decrease in species richness of aboveground diversity (Warren-Thomas et al., 2015), mainly in insect and fruit eating species (Aratrakorn et al., 2006) such as birds and bats (19-76%). Belowground diversity has so far been mostly investigated considering the conversion from forest to rubber. This conversion seems to reduce soil macrofauna diversity (Gilot et al., 1995; Lavelle et al., 2014), soil nematodes (Xiao et al., 2014) and soil microbial activities (Gilot et al., 1995; Abraham and Chudek, 2007). Moreover, this conversion modifies the soil microbial biomass and structure (Krashevska et al., 2015; Schneider et al., 2015) and may even increase soil prokaryotic richness (Schneider et al., 2015). The impact of annual crop conversion to rubber on soil biodiversity has only been addressed in one study,

focussed on arbuscular mycorrhizal fungi communities (Herrmann et al., 2016b). This study showed that a modification of the arbuscular mycorrhizal fungi communities composition was due to a change in soil texture and nutrient contents in RP after cassava cultivation. A range of different methods with different resolutions were used to determine the microbial composition or diversity (such as PLFA (Krashevskoa et al., 2015) or pyrosequencing (Schneider et al., 2015; Herrmann et al., 2016b)) and the macrofaunal community (using morphological techniques (Gilot et al., 1995; Lavelle et al., 2014)). However, these methods were not used simultaneously in the same sites and plots and were mostly focused on the effect of deforestation. Assessing the impact of land use and management changes on soil functioning should focus on a set of soil organisms playing major roles (Lavelle et al., 2006, 2014). Soil microbiota as decomposers and nutrients transformers and soil macrofauna as ecosystem engineers contribute to key functions such as carbon transformation, soil structure maintenance and nutrient cycling (Lavelle et al., 2006), which might be directly affected by land use conversion or RP ageing. Therefore, it is important to address the consequences of such specific land use conversion on the soil biota.

Beyond the land use conversion, the temporal dynamics of RP, i.e. canopy closure and ageing of the trees, may also play a critical role on soil biodiversity (Walker et al., 2010). The effect of RP ageing was mainly studied on soil properties, such as C stock (de Blécourt et al., 2013), nutrients concentrations (Aweto, 1987; Gilot et al., 1995), and microclimatic conditions (Gilot et al., 1995; Herrmann et al., 2016a) but not yet on the soil biodiversity. To address the temporal dynamics of soil biodiversity in a long term plant succession, chronosequences are recognised to be an efficient and necessary tool (de Blécourt et al, 2013). Thus, the aim of this study was to assess the effect of land use conversion from cassava and ageing of RP on the soil biodiversity (i.e. bacteria, fungi and macrofauna) and microbial activity. A chronosequence

design was used with three replications (blocks) including four age-classes (ranging from 1 to 25 years) and was compared with cassava fields, the former crop systems in the area.

2. Materials and methods

2.1. Study sites, plot setting and rubber management practices

The study was carried out in the rubber growing area of Chachoengsao Province in eastern Thailand (13°41'N, 101°04'E). The site is characterised by a tropical monsoon climate, with a strict dry season between November and April and a rainy season between May and October. The mean annual precipitation and temperature is 1,328 mm and 28.1°C, respectively (source Thai Meteorological Department). The soils in the plots belong to the Kabin Buri series, with 50% sand, 15% silt and 35% clay. Soil depth is limited to 1-1.5m by a compact layer of ferralitic concretions that strongly limits root growth. These pedoclimatic conditions are considered as a marginal area for rubber cultivation (Webster & Baulkwill.,1989). The soil is classified as isohyperthermic Vertic Endoaquepts soil based on soil taxonomy classification (USDA, 2014). The study was conducted in cassava fields and RP which belong to local farmers. We selected fifteen plots managed under local agricultural practices (Table 1). There were 5 treatments, including four RP age-classes and one cassava plantation (Table 1, Fig. S1). Twelve rubber (*Hevea brasiliensis,* clone RRIM 600) plantations were chosen to represent four classes of stand-age: 1-3, 5-6, 6-10 and 23-25 years. Three cassava plantations (C) were selected as references since cassava is the main annual crop cultivated in this region previously to RP. In cassava plantations, the soil was ploughed every year and plant materials (tuber and sometimes leaves) were exported. With a distance of 7 m between the rows and 2.5 m between trees, the rubber tree planting density varied from 444 to 667 trees per ha. The first RP age class (1-3 y) represented

147 the beginning of the rubber cycle. The soil was left bare and under direct light exposure. Young
148 pineapples were planted as inter-culture (~4 m, 8 lines of pineapples, 28.000 feet per ha) between
149 rubber rows (inter-row). During this phase, neither rubber nor pineapple were harvested. During
150 the second RP stand-age (5-6 y), pineapple fruits were collected while rubber trees were not yet
151 tapped. Rubber trees started to be tapped for latex harvesting at the beginning of the third RP
152 stand-age (6-10 y) after the canopy closure. The last RP stand-age (old, 23-25 y) represented the
153 end of the rubber culture cycle. In Thailand, rubber trees are usually cut after 25 y. Each
154 treatment was distributed within three blocks (A, B and C), these blocks were approximately 1-
155 1.5 km from each other (Fig S1).
156
157 **2.2 Soil physico-chemical analyses**
158 In each plot, eight soil samples were taken, four in tree rows and four at mid-distance
159 between two rows (inter-rows) at a depth of 0-5 cm along a 70 m transects, traced in the centre of
160 the plantation to avoid edge impact (Fig. S1). A total of 120 soil cores were sampled in
161 November 2012 using 100 cm^{-3}cylinders. Fresh soil samples were sieved at 2 mm and dried at
162 105°C over 24 h to measure soil moisture. All analyses were performed by the soil laboratory of
163 the Office of Science for Land Development in Land Development Department in Bangkok. The
164 air-dried soil was weighed without coarse particles >2 mm. The bulk density (g cm^{-3}) was
165 calculated as the ratio of the dry mass of fine soil (<2 mm) to the cylinder volume. Soil texture
166 was determined by the Bouyoucos Hydrometer method adapted from Gee and Bauder (1986).
167 Available phosphorus was determined using the Bray II method (Bray and Kurtz, 1945). The pH
168 was determined in distilled water (1:1 soil-water ratio). The cation exchange capacity (CEC) was
169 determined in distilled water (1:5 soil:water ratio). Potassium, Ca and Mg in the soil solution

were extracted by neutral 1 N ammonium acetate (Chapman, 1965) and analysed by flame photometer (Sherwood model 420) for K and Ca and by Atomic Absorption spectrophotometer (Shimadzu AA 6200) for Mg. Carbon content was analysed using a Rock-Eval 6 pyrolyzer (Vinci Technologies) at Lausanne University (Disnar et al., 2003; Sebag et al., 2016). Analyses were carried out with 30 to 70 mg of powder samples. In absence of carbonate minerals, the TOC (Total Organic Carbon) was considered as equal to the Total Carbon (TC). For each variable, the average of the four rows and four inter-rows samples taken in each plot were used for subsequent data analysis to match the number of samples used for sequencing (see below).

2.3 Microorganisms diversity and activities

The soil physico-chemical parameters, microbial biomass and metabolic profiles assessed by the MicroResp™ method (see below) did not show any significant difference between row and inter-row samples. Thus, the eight samples per plot were mixed into a composite sample to reduce the number of samples to analyse (total 15 soil samples). The soil samples were either air-dried (~ 50 g) at room temperature prior to pre-incubation (40% of the water-holding capacity, for one week at 23 ± 2°C) for community-level physiological profiling analysis or kept at -20°C prior to molecular analyses.

2.3.1 DNA Extraction, PCR amplification and barcoded pyrosequencing of 16S rRNA gene and fungal ITS region.

Total DNA was extracted from 0.5 g of each frozen soil sample using the commercial extraction kit FastDNA® SPIN Kit for Soil (MP Biomedicals). For each soil sample 500 mg aliquots were placed in MP Fast DNA® Spin kit lysing matrix E tubes and stored at -20°C

193 overnight. After this step at -20°C, the DNA extraction was processed following manufacturer's
194 instructions except for the washing DNA step. After adding the DNA Binding matrix solution,
195 500 µl of guanidine thiocyanate at 5.5 mol l^{-1} were added to the pellet to clean the DNA. DNA
196 was eluted in 100 µl FastDNA elution buffer and stored at -20 °C. The quality (A_{260}/A_{280}) and the
197 concentration of the extracted DNA were determined using a NanoDrop 1000 spectrometer
198 (Thermo Scientific). The integrity of the genomic DNA was checked by agarose gel
199 electrophoresis. DNA extracts were cleaned and concentrated by Genoscreen with the
200 commercial kit NucleoSpin® gDNA Clean-up (Mascherey-Nagel) following manufacturer's
201 instructions. Amplicons for barcoded sequencing was generated by PCR using 16S rRNA
202 specific primers 16SF (TACGGRAGGCAGCAG) / 16SR (GGACTACCAGGGTATCTAAT)
203 (Héry et al., 2014) targeting the V4 region of the bacterial 16S rRNA gene. For the fungal
204 biodiversity analysis, we used the first internal transcribed spacer (ITS1) region of the rRNA
205 operon, using the ITS1/ITS2 primer pair (Orgiazzi et al., 2012). Unidirectional sequencing of the
206 amplicon libraries was performed from the forward primer at Genoscreen Inc. (Lille, France)
207 using the 454-GS-FLX Titanium system on 15 DNA samples. Sequences were deposited into
208 SRA database under accession number SRP071714.

209

210 **2.3.2 Processing pyrosequencing data**

211 All sequence processing was done using the QIIME pipeline software (Caporaso et al.,
212 2010). Poor quality sequences (score <10 on a 50 bp sliding window) or sequences shorter than
213 300 base pairs for bacteria and 230 for fungi were discarded. *De novo* and reference-based
214 chimera detection, as well as clustering in OTUs were performed using USEARCH (Edgar,
215 2010) and the greengenes database (V. 05/2013) for 16S rRNA and UNITE database for ITS. The

identity thresholds were set at 97% for both 16S and ITS data. For 16S and ITS data, representative sequences for each OTU were aligned using PyNAST (Caporaso et al., 2010) and a 16S phylogenetic tree was constructed using FasTree (Price et al., 2009). Taxonomy was assigned using UCLUST (Edgar, 2010) and greengenes database (McDonald et al., 2012) for 16S and RDP Taxon Assigner (Wang et al., 2007) for ITS.

2.3.3 Community level physiological profiles

The MicroResp™ method was used to determine the basal respiration (BR) and the ability of the soil microbial community to metabolise a wide range of carbon sources, *i.e.* community-level physiological profiles (CLPP) (Campbell et al., 2003). The soil was air-dried, sieved at 2 mm and stored at room temperature before analyses. The MicroRespTM system consists in a 96-deep-well microplate (1.2 ml volume) filled with soil and addition of water only (BR) or aqueous carbon substrates, sealed individually to a colorimetric CO_2-trap microplate, and incubated in the dark at 23 ± 2 °C for 6 hrs. The dried soil samples were first distributed into a 96-deepwell microplates (0.5 g) and pre-incubated at 40% of the water-holding capacity (WHC) for one week at 23 ± 2°C in dark conditions (Bérard et al., 2012). Each well received separate organic substrates: carbohydrates (D-glucose, cellobiose, cellulose), carboxylic acids (oxalic acid, malic acid), phenolic acid (ferrulic acid, vanilic acid, catechol), amino acid (glycine, glutamine, glucosamine), N-rich compounds (urea, casein), and organic P-rich compound (phytate). These carbon sources are ecologically relevant as components typically found in or added to soils such as plant residues, root exudates, and as sources of mineralized nutrients (Campbell et al., 1997). The catabolic profiling was carried out with 4 replicates per substrate and per soil sample. The CO_2-trap absorbance was measured at 570 nm with a Victor3 multilabel counter (PerkinElmer) immediately before and after the 6h-incubation (Diakhaté et al., 2016). A calibration curve of

absorbance versus headspace equilibrium CO_2 concentration was fitted to regression model. Values were expressed in mg-C-CO_2.g.soil^{-1}.h^{-1} based on the average of the measurements from rows and inter-rows samples. Glucose-induced respiration was assumed to be proportional to total microbial biomass using the conversion factor of 40 (Anderson and Domsch, 1978).

2.4 Soil macrofauna

Sampling of soil fauna was performed in December 2012 along the same two transects used to collect soil cores. The "Tropical Soil Biology and Fertility" (TSBF) method proposed by Anderson & Ingham (1993) was used. Six monoliths (25×25×20 cm-depth) were extracted on each plot (corresponding to 1/16 m^2), at the same positions as the previous samples. Soil layers 0-10 cm and 10-20 cm were collected. Samples were individually hand-sorted and all visible organisms were collected and stored in 70° alcohol. Species-likes were identified, enumerated and classified along taxonomic groups. Fauna were further air-dried in the laboratory during 2 h and a total macro-invertebrate weight was assigned to each sample (i.e. each horizon of each sampling point). Macro-invertebrate weight, densities and relative abundance have been calculated for each plantation by taking the average of the two soil layers, row and inter-row samples.

2.5 Statistical analyses

Significance differences in richness, Pielou's evenness index, biomass and soil physico-chemical properties along the chronosequence were tested using Linear Mixed-Effect Models. The models were constructed with plantation (i.e. cassava, and different RP age-classes) as fixed effect and block design as random effect. The normality of the model residuals and the

homoscedasticity of the variances were checked. When one or both conditions were not met, the data were log transformed. When a significant ($P < 0.05$) effect of plantations was found, Tukey HSD multiple comparisons of means (post-hoc test) with a Bonferroni correction were performed to reveal differences among age-classes. In the few cases where the conditions could not be reached, a Kruskal-Wallis was performed coupled with Non-parametric relative contrast effects with Tukey contrast.

In order to analyse the overall effect of land use conversion and RP ageing on the soil microbial and macrofaunal communities, Between Class Analyses (BCA) were performed on Correspondence Analyses. Prior to BCA, the bacterial and fungal OTU relative abundances, and macrofaunal taxons relative abundance were log transformed. The BCA was also used on SIR relative abundance based on Principal Component Analysis. To test for a significant effect of plantations on bacterial, fungal and macrofauna, permutation Monte-Carlo tests (between-groups inertia; n = 1000) were performed. Finally, to identify potential drivers of the bacterial, fungal and macrofaunal relative abundance, Spearman's rank correlation coefficients ρ ($-1 \leq \rho \leq 1$) were calculated between the soil biota and the soil physico-chemical properties. To display the correlations, heatmaps were constructed; to facilitate the reading of the heatmap, cluster analyses were performed using Euclidean distance and group average method to cluster similar Spearman rank coefficient. All the statistical analyses were performed using R v3.1.0 (R Development Core Team, 2015), and the packages "vegan", "ad4", "nlme", "gplots", "multcomp" and "nparcomp".

3. Results

3.1. Soil physico-chemical properties

The soil in all treatments had the same physical properties in terms of texture and exchange

capacities (Table 2). The land use conversion from cassava to rubber 1-3 y did not significantly change the soil physico-chemical properties (Table 2). In contrast, some key chemical properties were affected by the RP age classes. Indeed, old RP (23-25 y) showed the most differences in terms of soil chemical properties in comparison to all the other classes (Table 2). The soil organic carbon (SOC) content was higher in old RP than in the young RP but was only significantly different from the young age classes (1-3 and 5-6 y). Litter quantity, together with moisture, increased at the end of the immature stage (5-6 y) and were significantly higher between age classes > 6 y compared to cassava and 1-3 y. With the exception of potassium (K), cations content (Ca, Mg) did not show any specific trend along the chronosequence.

3.2. Microbial diversity

Sequencing and quality filtering resulted in 44,481 high quality sequences for the 16S rRNA gene and 37,969 sequences for ITS with an average of 2965 and 2531 sequences per sample for the bacteria and fungi, respectively. The dataset comprised 1756 bacterial OTUs and 1338 fungal OTUs at 97% genetic identity. Whatever the treatment, the bacterial diversity was distributed among 4 dominant bacterial phyla (Fig 1A), which represented 94% of the overall bacterial diversity; Firmicutes (37%), Proteobacteria (25%), Actinobacteria (18%) and Acidobacteria (13%). For the fungal community (Fig. 1b), the number of representative phyla was low, as only two phyla represented 72% of the overall fungal biodiversity, namely Ascomycete (60%) and Basidiomycetes (12%), but 26% of fungal reads were unclassified at the phyla level (Fig. 1B).

The land use conversion from cassava to rubber 1-3 y did not affect significantly OTU richness or Pielou's evenness index of the soil bacterial and fungal community (Fig. 2A, 1B, 1D, 1E). The BCA revealed that the conversion from cassava to rubber 1-3 y slightly modified the

bacterial community structure at the OTU level along the second axis (25.7%; Fig. 1a). The conversion had a stronger effect on the fungal community structure at the OTU level but only on the second axis that explained 24.4% of the total inertia (Fig. 1B). The effect of conversion was not present at the phylum level but started at the family level, and was explained by strong decline of low relative abundance families (< 2.5%, Fig. S4) such as Ceratobasidiaceae, Chaetomiaceae and Phaeosphaeriaceae.

The old RP (> 23-25 y) clustered separately from the other age-classes on the first axis of the BCA at the OTU level (38.9% of the total inertia). The old RP host a specific bacterial community characterised by a lower diversity and Pielou's evenness index (Fig. 2A, 2D) and a higher relative abundance of Firmicutes (Fig. 1A). Firmicute abundance was highly positively correlated to change in soil moisture and litter amount (Fig. 3). In contrast, some phyla decreased with plantation age, including Actinobacteria, Chloroflexi, and Gemmatimonadetes, which were strongly negatively correlated to soil moisture and litter amount (Fig. 3; S2). Other phyla such as Acidobacteria decreased only in older RP (> 23 y) and their relative abundance was strongly negatively correlated with organic C and Mg and Ca contents (Fig.3; S2). Similar to the bacterial community structure, the BCA of the fungi community showed that RP 23-25 y clustered separately from the other age-classes along the first and second axis (35.7% and 24.4% of the total inertia, respectively; Fig. 1B). This separate clustering was explained by the significant increase in the relative abundance of the fungal families Agaricaceae, Chaetosphariaceae, Hypocreaceae, Halosphaeriaceae, and Lophiostomataceae. These increases were strongly positively correlated to soil moisture, litter amount and in some cases, to organic C content (Fig. 3, S3, S4). The ageing of the RP also decreased the relative abundance of the families Nectriaceae, and Herpotrichiellaceae specifically in the rubber 23-25 y, and were negatively correlated to some nutrients (Ca, K) and organic C content and soil moisture (Fig. 3, S3, S4). The

Hypocreaceae and Nectriaceae were the two dominant (> 2.5%, Fig. S3) families affected by ageing, mainly explained by a significant increase in the genus *Trichoderma* with RP age and a marginally significant (P = 0.06) decrease in *Fusarium,* respectively. The fungal richness increased with age-class to reach its maximum at RP 6-10 y and then decreased again, while Pielou's evenness index was not significantly affected (Fig. 2B, 2E).

3.3. Microbial biomass and activity

The microbial biomass, calculated from SIR data on Glucose, showed a significant decrease between cassava and all the rubber age-classes between 1 and 10 y, but then increased in rubber 23-25 y to reach similar level than in cassava (Fig. 2H). The respiration of each substrate decreased significantly between cassava and rubber 1-3 y, except for water, glutamine, phytate and phytate+glucose (Fig. S7). The respiration was stabilised for rubber 5-6 y or kept decreasing significantly in comparison to cassava for water and glutamine. Then, the respiration tended to increase in rubber 6-10 y. Finally, the respiration for rubber 23-25 y was always significantly higher than for cassava and other rubber age-classes, except for glucose and oxalic acid (Fig. S7).

The BCA of the community level physiological profile (CLPP) and Monte-Carlo test (Fig. 6) showed significant (P < 0.001) differences between cassava and young RP (from 1 to 6 y) and older RP (> 6 y). Cassava clustered together with rubber 1-3 and 5-6 y, indicating no effect of land conversion from cassava to rubber until 5-6 y (Fig. 6). On the first axis of the BCA (41.5% of total inertia) rubber 6-10 y clustered separately from the other age-classes. This clustering was mainly explained by the variance of glutamine, urea, oxalic acid, catechol, vanillic acid, malic acid and ferrulic acid respirations. On the second axis (37.2% of total inertia), rubber 23-25 y

clustered separately from the other age-classes, which was mainly explained by glucosamine, casein, phytate+glucose, phytate, glycine and cellulose substrate respirations.

3.4. Macrofauna density and diversity

In total, 43,120 macro-invertebrate individuals were identified across 183 species-like belonging to 17 taxa groups along the rubber chronosequence: *Annelida, Arachnida, Chilipoda, Coleoptera, Dermaptera, Diplopoda, Diptera, Embioptera, Formicidae, Hemiptera, Homoptera, Isopoda, Lepidoptera, Molusca, Orthoptera, Psocoptera, and Termitidae.* The most abundant groups were *Formicidae* (ants, 44%), *Termitidae* (termites, 21%), and *Annelida* (earthworms, 17%).

Land use conversion did not affect significantly the macrofauna community in terms of richness, Pielou's evenness index, biomass and community structure (Fig. 2C, 2F, 2I, 1C). In contrast, ageing of RP had strong effects on macrofauna biomass and community structure. The biomass of the macrofauna significantly increased by 4.5 times after the canopy closure (> 6 y) compared to cassava and younger RP (Fig. 2I). The increase in macrofauna biomass was mainly due to the significant increase in *Anelida* density (Fig. S6) in RP 6-10 and 23-25 y. The macrofauna community structure at the taxa level was significantly ($P = 0.001$) different between the RP age-classes (Fig. 1C), with the RP age-classes > 6 y clustering separately from each other along the first axis (39.1% of the total inertia) and from cassava and RP 1-3 y along the second axis (35.2% of the total inertia) (Fig. 1C). This clustering was explained by the significant increase in the relative abundance of *Anelida, Chilipoda* and to a lesser extent to *Isopoda* positively correlated to litter amount, and also to CEC and soil moisture for *Anelida* (Fig. 6, S5). In contrast, the relative abundance of *Formicidae* and *Hemiptera* decreased significantly and

were negatively correlated to different soil properties such as soil moisture, litter amount, clay and organic C content (Fig. 6, S5).

4. Discussion

This study is the first attempt to characterise the effect of land use conversion from cassava to RP and ageing of RP on soil biodiversity and microbial respiratory activities. The chronosequence approach included plots in each replicate, located within a block where soil physico-chemical characteristics and land use history are similar. Hence, the differences in biodiversity between the previous land use (cassava) and the converted land use type (rubber) can be attributed to recent land use conversion and not to site specificity.

4.1 Impacts of land use conversion from cassava to young rubber on soil biota diversity

The effects of land use conversion from cassava to young RP affected the microbial activities and biomass, the soil fungal community structure, and to a lesser extent, the soil macrofauna density. However, this conversion had limited effects on bacterial and macrofauna diversity due to the absence of changes in soil physico-chemical properties (Table 2), which are recognised as the main drivers of the soil biodiversity (Birkhofer et al., 2015; Ruiz et al., 2011; Thomson et al., 2015). This is in contrast to the conversion from natural ecosystems (e.g. rainforest, Indonesia) to RP which significantly changes bacterial community structure, highlighting the importance of the initial ecosystem for land use conversion studies (Schneider et al. 2015). The significant decrease of microbial activity and biomass may originate from a change in intensity of agricultural practices, which is a key driver of microbial activities (Creamer et al., 2016). Land use conversion from cassava to rubber has led to an increase of agricultural practice intensities (Table 1), especially tillage (1 and 3 tillage per year for cassava and 1-3 y RP,

403 respectively; Table 1). This increase in practices results from a switch between two annual crops,
404 cassava and pineapple, which is used as an intercrop, rather than a change in land cover from an
405 annual to a perennial crop. These changes in agricultural practices affected the fungal community
406 structure, with a steep decrease of some specific fungal families (Ceratobasidiaceae,
407 Chaetomiaceae and Phaeosphaeriaceae), which could be directly related to the cassava crop.
408 Thus, the bacterial and macrofaunal diversity seems more resistant to land use conversion than
409 the fungal diversity.
410
411 **4.2 Impact of rubber plantation age on soil biota**
412 The ageing of the RP had stronger and wider effects on the bacterial, fungal and
413 macrofaunal diversity and microbial activities than the land use conversion. These effects were
414 maximal in the oldest RP (23-25 y). After 6-7 years, RP ecosystems are mainly characterised by
415 (i) an increase in the canopy closure with positive consequences for soil biota in terms of litter
416 availability and soil moisture (Ogunkunle and Awotoye, 2011), and (ii) a decrease in soil
417 perturbations due to the end of inter-cropping and tillage. This is a general trend when tree
418 plantations are established on soils that have been previously used for continuous annual
419 cropping (Paul et al., 2002; de Blécourt et al., 2013). Furthermore, the litter of rubber,
420 characterised by a poor biochemical quality (Abraham and Chudek, 2008), favoured slow
421 decomposition processes, which increase soil carbon storage (~100kg C $ha^{-1}.an^{-1}$ based on Table 2
422 data).
423 The bacterial community shifted from being dominated by Acidobacteria and
424 Actinobacteria in young RP to Firmicutes in older RP. The temporal dynamic of the microbial
425 structure was governed by the changes in soil properties, which affected the availability of
426 resources. The young RP, characterised by a low organic status, harboured a more oligotrophic

community (ability to grow under low substrate concentrations) such as Acidobacteria (Cleveland et al., 2007; Fierer et al., 2007), and Actinobacteria, which are also able to degrade more recalcitrant compounds (Ho et al., 2017). Moreover, the ability to degrade recalcitrant compounds such as aromatic substrates seems to constitute a physiological property of the soil microbial community of the cassava and young RP (Fig. 6). In contrast, the ecological significance of Firmicutes which dominates in older RP is not yet well understood, but they could be related to copiotrophs microorganisms (Ho et al., 2017). Firmicutes are also found in stable habitats such as tropical rain forest (Cleveland et al., 2007) and has also been shown to be sensitive to the decrease in agricultural practices intensity (Jangid et al., 2008).

One of the most unexpected and original results was the steep decrease in the bacterial richness (and to a lesser extent, bacterial evenness) along the rubber chronosequence. This trend was specific to the bacterial community (Fig. 2A, 2B). Schneider et al. (2015) showed that bacterial richness increased in managed rubber land compared to rainforest. This trend supports recent studies showing that bacterial diversity is greater at intermediate levels of land use intensity (Tardy et al., 2015; Bouchez et al., 2016). Thus, a more stable and less disturbed soil environment, such as in older RP (with no tillage or plant harvesting), can harbour a specific but less diverse soil bacterial community in a similar manner to forests.

As for the bacterial community, the evolution of the pedoclimatic context along the chronosequence also drove the fungal community changes (dominated by the family Nectriaceae in young RP to Hypocreaceae in old RP). The dominance in young RP of the family Nectriaceae could be due to a soil legacy effect, as the genius *Fusarium* (which belong to Nectriaceae) is recognised as a pathogen of cassava crops (Bandyopadhyay et al., 2006). Meanwhile, the increase in Hypocreaceae in old RP was specific to rubber trees, since *Trichoderma* (i.e. *T.* TR057; Geraldine et al., 2013), an endophytic genius belonging to this family, is often isolated from rubber

trees leaf and sapwood (Gazis and Chaverri, 2010), and can be a beneficial fungi against RP pathogens such as white root disease (Ikediugwu and Ubugo, 2012; Mohammed et al., 2014).

Like microbial and macrofaunal biomass, the microbial activity pattern (2 fold increase between young and old RP) was also governed by the soil nutrient status (SOC and litter content) known as key parameters governing soil respiration (Abraham and Chudek, 2008). These results showed that in contrast to the claim of previous studies done in a similar tropical context (Abraham and Chudek, 2008; Nurulita et al., 2015), the decline in soil microbial biomass and activity in RP did not seem a specific property of RP but rather a characteristic related to land history (Gilot et al., 1995; Abraham and Chudek, 2008; Zhang et al., 2007). Overall, this study clearly shows that the microbial activity exhibited high sensitivity to land use conversion and the resilience of microbial activities has to be assessed in the long-term, as microbial activity took more than a decade to recover. Thus, in adverse pedoclimatic conditions such as this study, RP offers more stable conditions over time to sustain biotic activity such as microbial one.

The increase in density of earthworms in older rubber age-classes is likely due to the absence of tillage in plantations > 6 y, which is known to be a key driver of earthworm abundance (Karlen, 2004). Thus, the absence of tillage combined with the increase in soil moisture and litter amount are key factors in restoring earthworm density. In contrast, the decline of ants in older plantations was related to the increase in soil moisture, but could also be explained by the fact that ants usually prefer open ecosystems for their foraging activities (Lassau and Hochuli, 2004). This pattern differed from the only comparative study done on soil fauna in a rubber chronosequence in Ivory Coast, where the total biomass and density of the soil macrofauna decreased along the RP chonosequence (from 5 to 30 y; Gilot et al. 1995). However, the differences could be again explained by the soil legacy effect (forest in Ivory Coast) and substrate availability (wood in Ivory Coast) which explain the termite dominance. Increases in

earthworms at the expense of ants could be seen as positive for soil fertility, considering their key roles in soil functioning (Jouquet et al., 2014; Lavelle et al., 2006). The earthworm population was dominated by *Pontoscolex corethrurus* species (Thibaud Decaens, personal communication), an endogenic and exotic species which can constitute 72% of earthworms density in RP (Chaudhuri et al., 2008). The presence of this species is not surprising as *P. corethrurus* is the most widely distributed earthworm in the world (Gates, 1972). However, this species dominates in tropical disturbed ecosystem (Hendrix and Bohlen, 2002), suggesting that if RP harboured an abundant soil fauna, then the structure of this soil fauna was still far from natural ecosystems, such as forest.

Conclusion

This study showed that in an adverse pedoclimatic context (low organic matter content, sandy soil, long dry season), the replacement of cassava crop by a RP increased soil organic matter content and the soil biological density and activities after two decades. The soil macrofauna was, in old RP, dominated by soil engineers such as earthworms and termites, known to have a positive role on soil functioning. Thus, in such adverse contexts, the replacement of an annual cash crop by a perennial one could be one way to restore degraded soil. However, we also showed that in the younger phase, inter-cropping had a strong adverse effect on soil biota biomass and the restoration took more than 20 years to occur. The setting of more sustainable practices, such as legumes inter-cropping associated with less intensive practices (i.e. reduced use of tillage, herbicides etc.) could be a way to speed up this restoration effect. Even if the soil biota density was higher in old RP compared to cassava, we also showed that these old plantations harboured more specific and less diverse soil biota characterised by the dominance of the bacterial phyla Firmicutes, fungal genus *Trichoderma*, and one species of endogeics earthworm

(*P. Corethreus*). This decrease in soil biological richness challenged the long-term effect of RP on soil biodiversity, since rubber could be replanted 3 to 4 times leading to a continuous mono-cropping over more than 75 years on the same land.

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Tables

Table 1. Site locations, descriptions and main agricultural practices

Plant type	Replicate	Area (ha)	Sampling Easting	Location (average) Northing	Age (y)	Previous crop	Intercropping	Tillage (y^{-1})	Herbicide (y^{-1})	Fertilisation (y^{-1})
Cassava	1	4.68	765,164.8	1,503,337.2	-	Cassava	-	1	1	2
	2	0.78	767,780.3	1,502,739.8	-	Cassava/Pineapple	-	2	1	2
	3	0.46	766,831.2	1,504,018.7	-	Cassava	-	3/4	1	1
Rubber 1-3 y	1	2.82	765,084.5	1,503,317.1	1	Eucalyptus	Pineapple	3	3	2
	2	2.93	767,763.9	1,503,406.6	3	Cassava	Pineapple	4	1	2
	3	3.91	767,356.9	1,504,373.1	1	NA	Pineapple	NA	NA	NA
Rubber 5-6 y	1	3.30	765,245.1	1,503,574.7	6	Cassava	Papaya/pineapple	1	1	2
	2	3.04	768,039.5	1,503,434.1	6	Cassava/Eucalyptus	Pineapple	2	2	1
	3	3.62	766,991.8	1,504,292.7	5	Cassava/Pineapple	Pineapple	2	3	1
Rubber 6-10 y	1	1.49	765,341.8	1,503,198.4	8	Cassava	Papaya/pineapple	2	3	2
	2	2.07	768,019.4	1,503,286.1	10	Eucalyptus	-	0	0	2
	3	3.40	767,130.5	1,504,005.9	6	Cassava/Mango	-	0	0	1
Rubber 23-25 y	1	1.20	765,184.9	1,503,196.6	25	Fruit Tress	-	0	3	1
	2	3.88	767,948.2	1,503,110.7	23	Cassava	-	0	0	2
		1.64	766.964.4	1,504,184.9	25	Cassava	-	0	1	1

** Fertilization: for pineapple cycle: NPK (21-7-4) 370 kg ha^{-1}; for Rubber NPK (20-10-12) 120 kg ha^{-1}*

NA: information not available

Table 2. Soil properties along cassava and rubber plantation age classes. Mean values and standard errors are shown (n = 3). Different letters indicate significant differences ($P < 0.05$) between age classes for each soil property according to Tukey test. CEC: cationic exchange capacity. OM: organic matter.

	Cassava	Rubber 1-3 y	Rubber 5-6 y	Rubber 6-10 y	Rubber 23-25 y
pH	5.0 ± 0.33 a	5.0 ± 0.20 a	4.8 ± 0.15 a	4.5 ± 0.11 a	4.9 ± 0.37 a
P (mg kg^{-1})	20.1 ± 5.9 a	14.1 ± 6.26 a	14.9 ± 4.36 a	14.5 ± 1.67 a	14.8 ± 7.23 a
Ca (mg kg^{-1})	214 ± 69.8a	264 ± 33.3 a	189 ± 45.4 a	212 ± 8.7 a	341 ± 73.8 a
Mg (mg kg^{-1})	33.2 ± 7.45 a	51.3 ± 9.93 a	48.4± 9.75 a	51.5 ± 4.77 a	62.2 ± 18 a
K (mg kg^{-1})	34.9 ± 14.42 a	50.4 ± 1.55 ab	32.3 ± 1.74 a	38.7 ± 1.28 ab	59.2 ± 1.48 b
CEC (mmol kg^{-1})	6.6 ± 2.29 a	5.2 ± 0.81 a	5.4 ± 1.26 a	6.9 ± 1.67 a	9.8 ± 2.24 a
Sand (%)	60.7 ± 3.49 a	57.7 ± 3.19 a	58.3 ± 0.17 a	53.4 ± 4.31 a	59.3 ± 1.22 a
Silt (%)	20.1 ± 0.67 a	22.1 ± 1.17 a	20.0 ± 1.20 a	21.5 ± 0.86 a	24.5 ± 2.24 a
Clay (%)	19.1 ± 2.82 a	20.2 ± 3.95 a	21.7 ± 1.22 a	25.1 ± 4.53 a	16.2 ± 2.53 a
Organic C (%)	0.78 ± 0.11 a	0.91 ± 0.08 a	0.89 ± 0.08 a	0.98 ± 0.07 ab	1.33 ± 0.11 b
Litter (%)	43.0 ± 22.3 a	44.6 ± 11.2 a	139.3 ± 49.3 ab	160.8 ± 38.1 b	197.0 ± 48.4 b
Soil moisture (%)	12.3 ± 6.98 a	11.3 ± 0.87 a	16.4 ± 1.28 ab	20.2 ± 3.41 b	22.2 ± 2.17 b
Bulk density (g cm^{-3})	1.4 ± 0.02 ab	1.4 ± 0.01 ab	1.5 ± 0.07 b	1.4 ± 0.10 ab	1.3 ± 0.03 a

Figure captions:

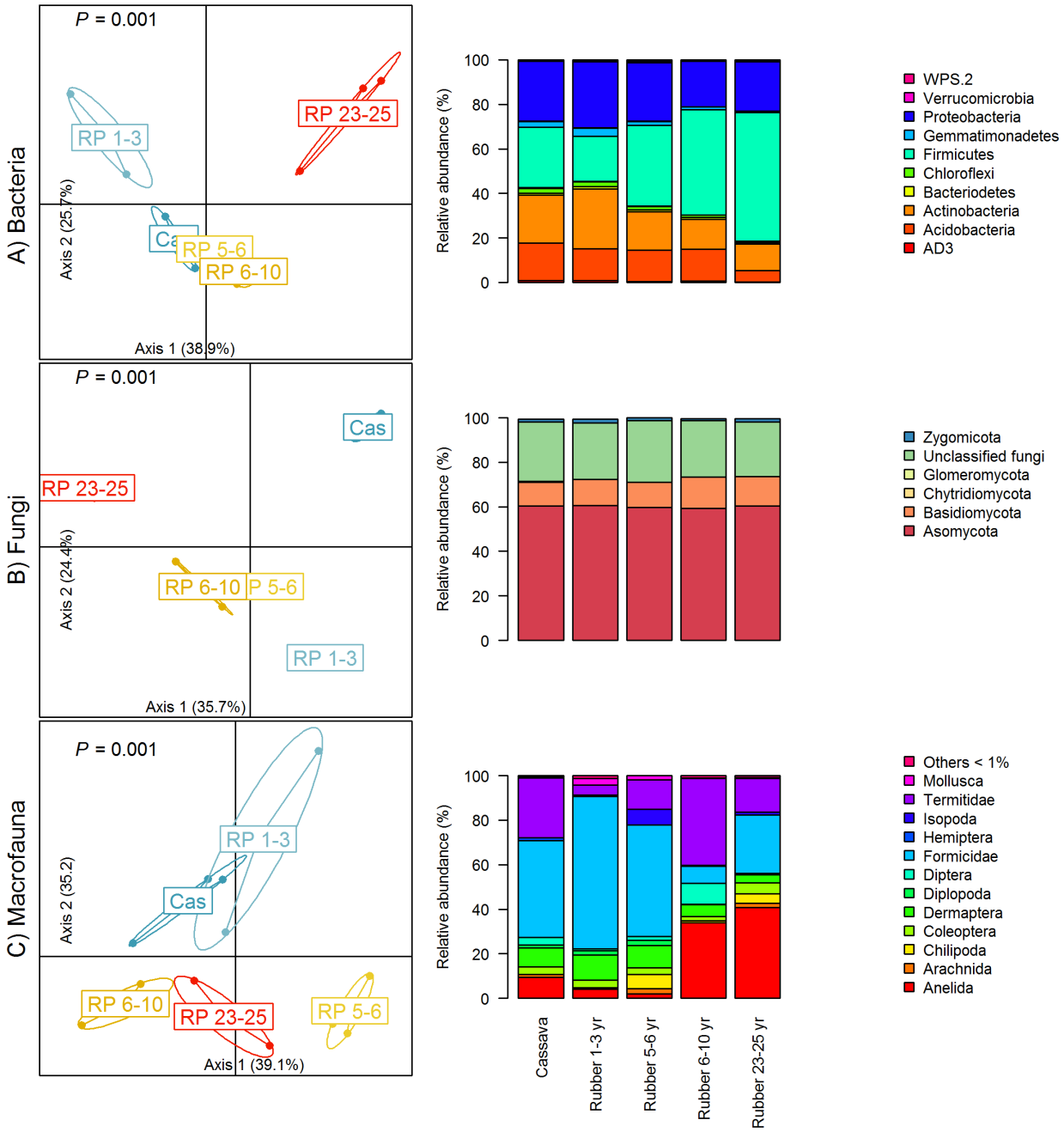
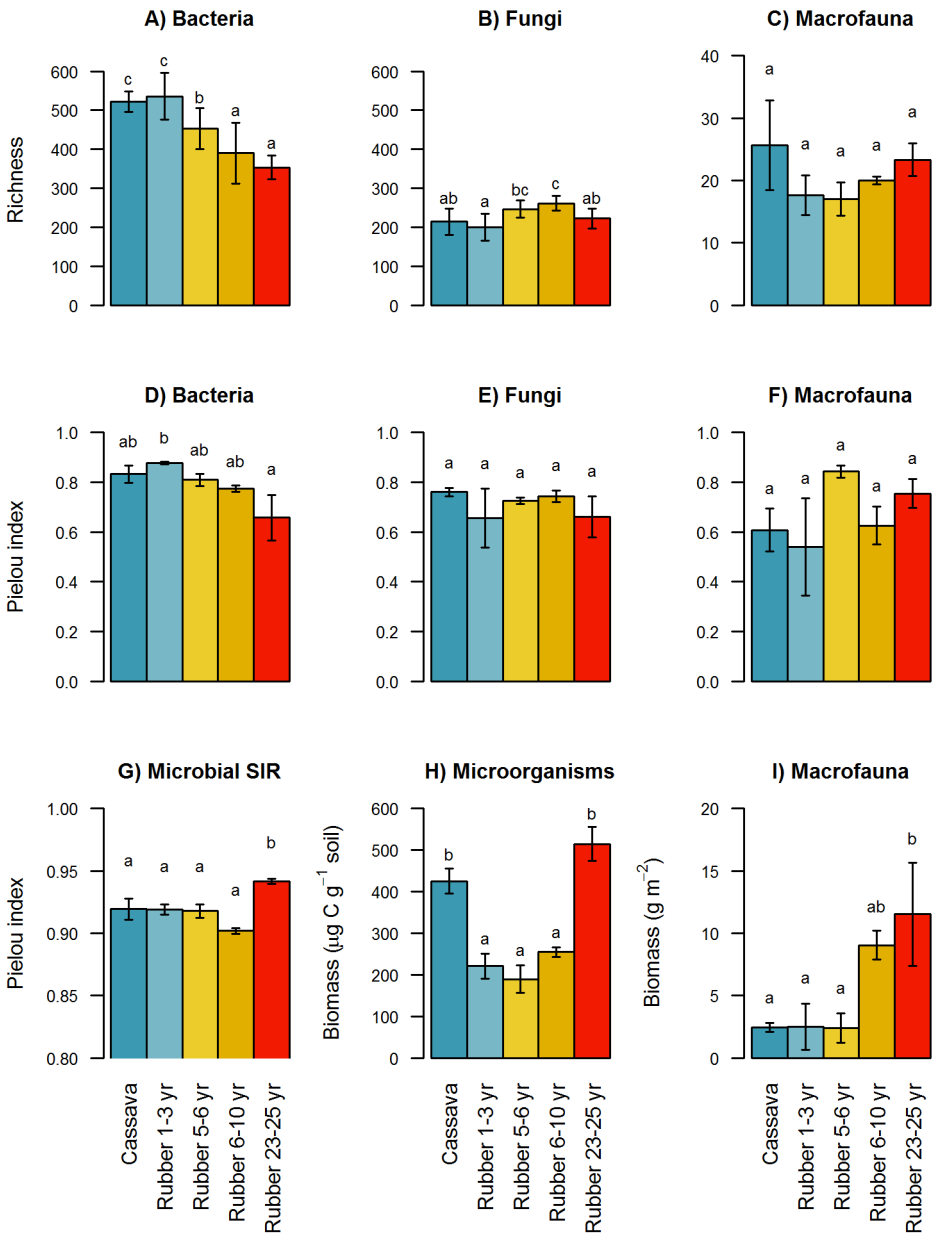


Fig. 1 Between-Class Analyses (BCA) and relative abundance of bacterial (A), fungal (B) and macrofaunal (C) communities along a cassava-pure rubber plantation chronosequence (n = 3). The BCA for bacterial and fungal communities were performed at the OTU level, and at taxon level for macrofauna. P-values for BCA indicate significant total inertia clustering among age-classes based on permutation test (Monte-Carlo test between-groups inertia; 1000 permutations). The relative abundance shows the percentage of bacterial and fungal phylum, and macrofaunal taxons. Cas: cassava plantation; RP 1-3: rubber plantation 1-3 y; RP 5-6: rubber plantation 5-6 y; RP 6-10: rubber plantation 6-10 y; RP 23-25: rubber plantation 23-25 y. Means values for relative abundance are shown (n = 3).



748
749 **Fig. 2** Richness (A, B and C), Pielou's (D, E and F) indexes and biomass (H and I) of bacteria,
750 fungi and macrofauna, respectively, and Pielou's index for microbial SIR (G) along a cassava-
751 rubber plantation chronosequence. Different letters indicate significant differences ($P < 0.05$)
752 between age-classes. Mean values and standard errors are shown ($n = 3$).

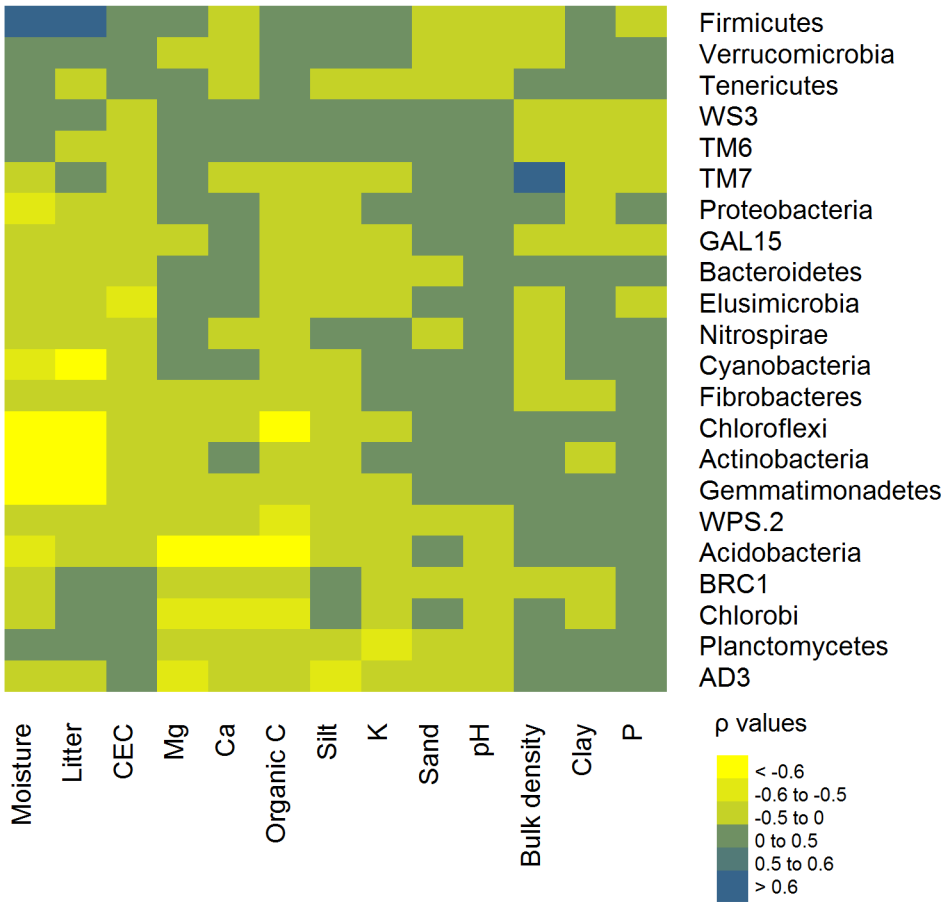


Fig. 3 Heatmap of Spearman's rank correlation coefficients between relative abundance of bacterial phyla and soil properties along the cassava and rubber plantation chronosequence. Colours represent the ρ values of Spearman correlations, i.e. the strength of the correlations between bacteria and soil properties. The ρ values > 0.5 and < -0.5 have a significant $P < 0.05$. To facilitate the reading of the heatmap, cluster analyses were performed using Euclidean distance and group average method to cluster bacterial phyla and soil properties with similar Spearman rank coefficients.

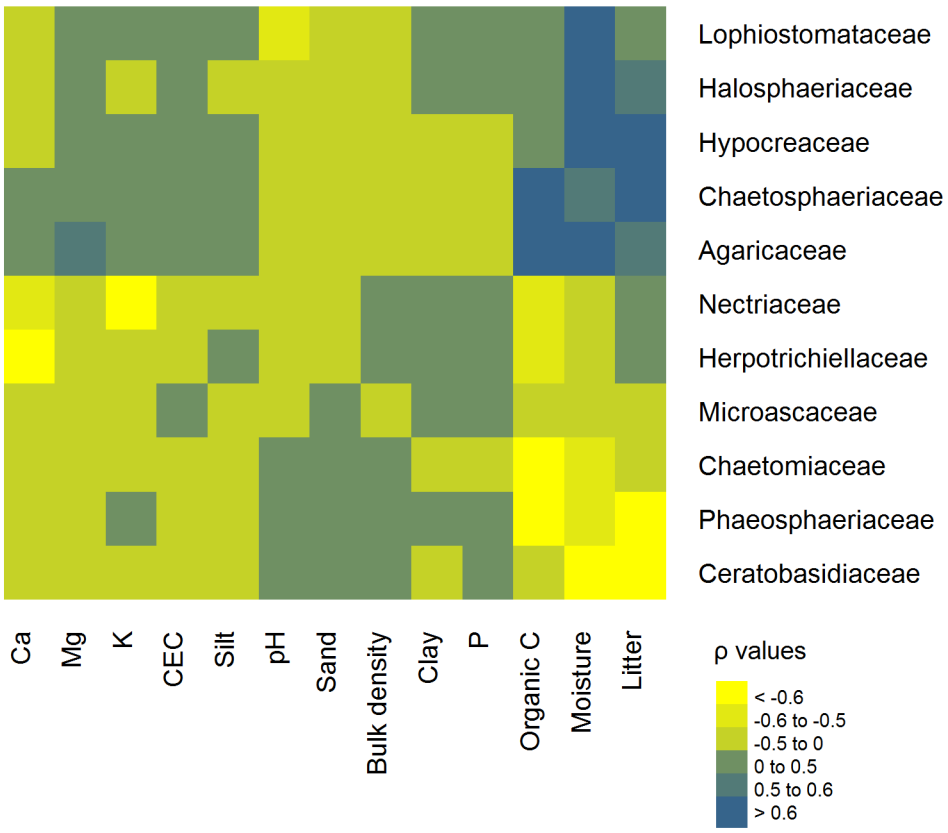


Fig. 4 Heatmap of Spearman's rank correlation coefficients between relative abundance of fungal families and soil properties along the cassava and rubber plantation chronosequence. Only fungal families that were significantly affected by the chronosequence are shown. Colours represent the ρ values of Spearman correlations, i.e. the strength of the correlations between fungi and soil properties. The ρ values > 0.5 and < -0.5 have a significant $P < 0.05$. To facilitate the reading of the heatmap, cluster analyses were performed using Euclidean distance and group average method to cluster fungal families and soil properties with similar Spearman rank coefficients.

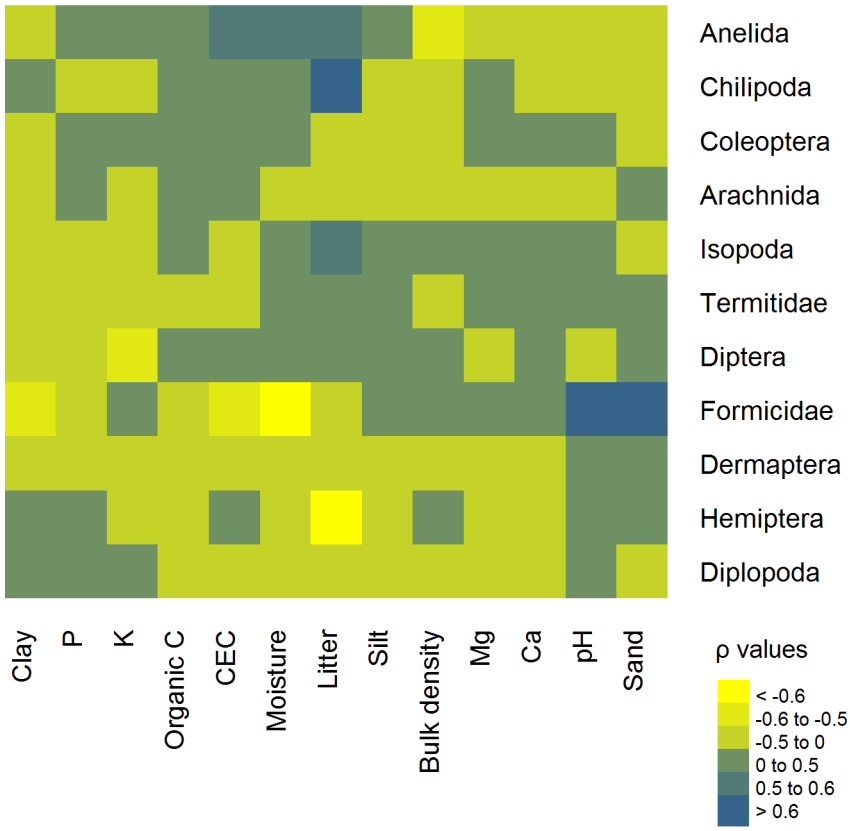
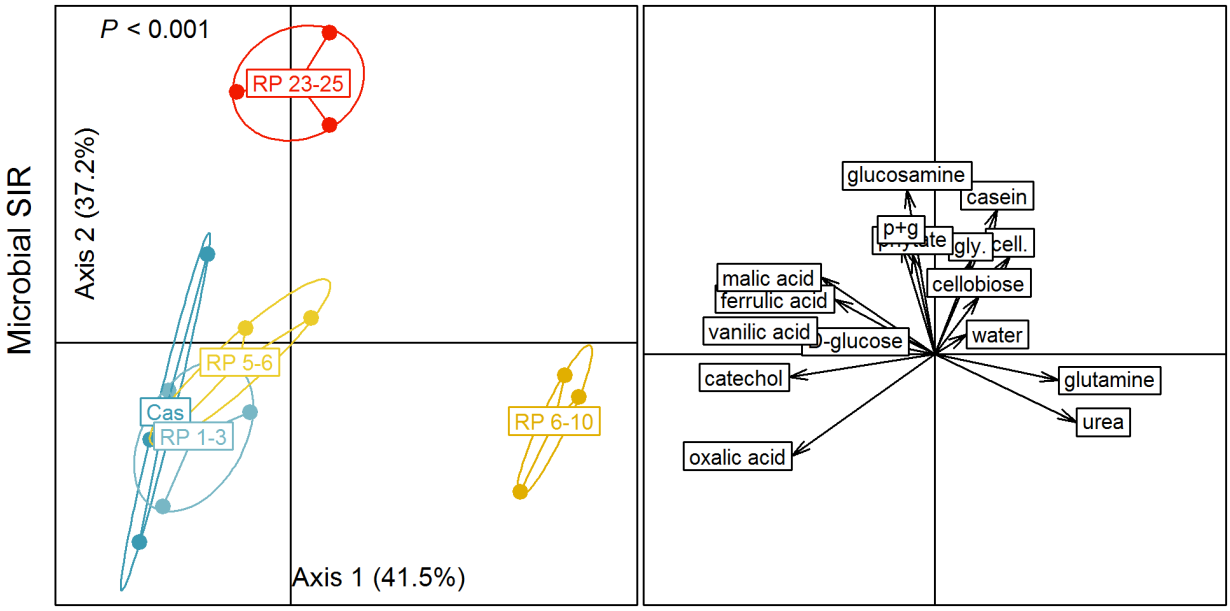


Fig. 5 Heatmap of Spearman's rank correlation coefficients between relative abundance of macrofauna taxon and soil properties along the cassava and rubber plantation chronosequence. Colours represent the ρ values of Spearman correlations, i.e. the strength of the correlations between macrofauna and soil properties. The ρ values > 0.5 and < -0.5 have a significant $P < 0.05$. To facilitate the reading of the heatmap, cluster analyses were performed using Euclidean distance and group average method to cluster macrofaunal taxon and soil properties with similar Spearman rank coefficients.



780 **Fig. 6** Between Class Analysis performed on the microbial SIR data (µg $C\text{-}CO_2$ g^{-1} soil h^{-1})
781 obtained along a cassava-pure rubber plantation chronosequence. *P*-values for BCA indicate
782 significant total inertia clustering among age-classes based on permutation tests (Monte-Carlo
783 test between-groups inertia; 1000 permutations). Cas: cassava plantation; RP 1-3: rubber
784 plantation 1-3 y; RP 5-6: rubber plantation 5-6 y; RP 6-10: rubber plantation 6-10 y; RP 23-25:
785 rubber plantation 23-25 y; p+g: phytate+glucose; gly.: glycine; cell.: cellulose.