Impact of biochar application dose on soil microbial communities associated with rubber trees in North East Thailand

Laetitia Herrman, Didier Lesueur, Agnès Robin, Henri Robain, Wanpen Wiriyakitnateekul, Lambert Bräu

Impact of biochar application dose on soil microbial communities associated with rubber trees in North East Thailand

Laetitia Herrmann, Didier Lesueur, Agnès Robin, Henri Robain, Wanpen Wiriyakitnateekul, Lambert Bräu

PII: S0048-9697(19)33014-1
DOI: https://doi.org/10.1016/j.scitotenv.2019.06.441
Reference: STOTEN 33095

To appear in: *Science of the Total Environment*

Received date: 14 March 2019
Revised date: 26 June 2019
Accepted date: 26 June 2019

Please cite this article as: L. Herrmann, D. Lesueur, A. Robin, et al., Impact of biochar application dose on soil microbial communities associated with rubber trees in North East Thailand, Science of the Total Environment, https://doi.org/10.1016/j.scitotenv.2019.06.441

Impact of biochar application dose on soil microbial communities associated with rubber trees in North East Thailand

Laetitia Herrmann[a,b,1,*], Didier Lesueur[a,b,c,1,*], Agnès Robin[c,d], Henri Robain[e], Wanpen Wiriyakitnateekul[f] and Lambert Bräu[a]

[a] School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment - Deakin University, Melbourne, Australia

[b] CIRAD, UMR Eco&Sols, Land Development Department - Office of Science for Land Development, Paholyothin Road, Chatuchak, Bangkok 10900, Thailand

[c] Eco&Sols, Univ Montpellier, CIRAD, INRA, IRD, Montpellier SupAgro, Montpellier, France

[d] CIRAD, UMR Eco&Sols, F-34398 Montpellier, France

[e] IRD, UMR IEES, Land Development Department - Office of Science for Land Development, Paholyothin Road, Chatuchak, Bangkok 10900, Thailand

[f] Land Development Department - Office of Science for Land Development, Paholyothin Road, Chatuchak, Bangkok 10900, Thailand

[1] These authors contributed equally to this work

* Present address:

International Center for Tropical Agriculture (CIAT-Asia), Common Microbial Biotechnology Platform (CMBP), Hanoi, Vietnam

Corresponding author: laetitiaherrman@gmail.com; +84965693906

Abstract

Biochar is a potential tool to mitigate climate change by enhancing C sequestration in soils, but its use as a soil amendment to improve soil fertility and crop yields is still a contentious subject. In North East (NE) Thailand, biochar has been promoted to restore soil fertility in rubber tree plantations. Despite this, there is scarce information on the impact of biochar application on the soil biota, particularly on microbial communities associated with rubber trees. The effects of increasing doses of biochar on microbial communities were investigated in a rubber tree plantation in NE Thailand, 28 months after application. Biochar application resulted in increases of soil pH and nutrient contents and also had an impact on both bacterial and fungal communities. Changes in microbial composition and structure were observed although fungal communities were more markedly affected than bacterial communities. The nature and magnitude of the observed changes were strongly related to soil properties (pH, soil moisture and P content), while biochar dose (5, 10 or 20 tons/ha) effect was not significant. Our results highlight the need for additional research for a better understanding of the impact of biochar application on soil microbial communities and further cascading effects on ecosystem functions.

Keywords: Biochar, bacterial communities, fungal communities, rubber tree, North East Thailand

1. Introduction

Biochar is the product of the pyrolysis of C-based biomass under low or zero oxygen conditions. It is a fine-grained, porous, highly aromatic, and a very stable substance, with soil residence times estimated from hundreds to thousands of years. It has received increasing attention in recent years as an efficient tool to mitigate climate change by enhancing C sequestration after addition to soils (Lehmann et al., 2006; Sohi et al., 2010) while concurrently producing secondary agronomic benefits and improving soil function (Lehmann et al., 2011). However, biochar effectiveness as a soil amendment has been a contentious subject and both negative and positive results have been reported (Amini et al., 2016; Brassard et al., 2016; Ding et al., 2016; Sohi et al., 2010; Verheijen et al., 2009). A meta-analysis by Verheijen et al. (2009) showed that, globally, biochar application resulted in a small but significant positive effect on plant growth, soil nutrient status and crop productivity, although the intensity of this effect was dependent on the biochar type, the nature of the soils and the crop type, and the management practices. Improvement of physical properties of the soils, as well as changes in pH, soil organic matter (OM) and nutrient content have been reported to be responsible of significant changes in microbial communities' structure, diversity and functions, consequently leading to enhanced environmental performances (Biederman and Harpole, 2013; Bruun et al., 2014; Dai et al., 2017; Ding et al., 2016; Downie et al., 2009; Li et al., 2018a; Obia et al., 2016). Studies focusing on the changes in soil microbial communities following biochar application have recently emerged and have highlighted the roles of the complex interactions between biochar and soil characteristics (Imparato et al., 2016; Jenkins et al., 2017; Kolton et al., 2017; Yao et al., 2017a; Yao et al., 2017b; Zheng et al., 2016). However, the mechanisms involved and their ecological consequences have not been fully resolved. Moreover, these studies have mainly focused on

agricultural ecosystems over a short term (less than 1 year) and less research has been conducted on forest or perennial crops such as rubber tree.

The use of biochar is currently promoted as a tool for the improvement of soil quality and crop yields in Thailand, in particular in the North East (NE) provinces where soils are mainly sandy and very poor in nutrients (Herrmann et al., 2016; Le Guen et al., 2017). Thailand is the world leading producer of natural rubber, and rubber tree plantations are expanding in areas previously considered unsuitable, such as the NE regions. Biochar application to soils is being promoted as a means of enhancing rubber tree growth and productivity while reducing the negative impacts of extensive mineral fertilizer application. There is, however, scarce scientific evidence to support this, and the impact of biochar application on the soil microbial community associated with rubber trees in Thailand has only been recently investigated. A recent study by Le Guen et al. (2017) showed that fungal communities were more strongly affected by the application of biochar than soil bacterial communities, 18 months after application. However, these preliminary results were based on a DNA fingerprinting technique (DGGE) and more research is needed on the nature and intensity of these effects, particularly in relation with microbial communities' composition, structure and diversity.

Due to the variability of effects of biochar addition, which depend on factors including the biochar used, the soil type and the targeted crop — a case-by-case evaluation of each biochar type prior to its incorporation in a particular cropping system is mandatory. Interest in biochar research is still in its relative infancy and, as such, more data are required to draw robust conclusions regarding its effects across a range of soil, environmental and agronomic situations. Additionally, the irreversibility of biochar application and its long residence time in soils is crucial to consider before policies are developed and large scale usage is promoted. A better understanding of the interactions of biochar with rubber trees in poor soils is of

primary importance if it is to be considered for the development of effective alternatives for rubber tree plantation management and soil health restoration.

2. Material and methods

2.1. Site description, experimental design and sampling

The trial was set up in a 7 year-old rubber tree plantation located in Phu Wiang, Khon Kaen district, in NE Thailand (N16°38.544' and E102°16.864') in May 2013. The plantation was composed of 25 rows with a row spacing of 6.5-7.0 m. Tree spacing within a row was about 3 m, and all trees were of similar height. The average tree diameter (measured at 1.2 m height) was 27.8 ± 8.4 cm. The trial consisted of 4 doses of biochar: No biochar (T1), 5 tons/ha (T2), 10 tons/ha (T3) and 20 tons/ha (T4). Biochar was produced from bamboo biomass following a slow pyrolysis process (350 to 400°C for 8 h) and was applied in the top soil (0-30 cm) around each tree, 40 cm from the trunk (Le Guen et al., 2017). Biochar pH was 9.5 with an ash content of 6.4% and its carbon and nitrogen contents were 76.9% and 0.38%, respectively. Detailed biochar physicochemical characteristics are presented in Table S1.

An Electrical Resistivity Tomography (ERT) survey was carried out in July 2014 and indicated the presence of two main soil classes (C3 and C4) distributed along the four biochar dose treatments (see Le Guen et al. (2017) for details). In order to include the impact of soil classes on the effect of increasing doses of biochar on the microbial communities, trees belonging to these two soil classes were sampled and included for analysis. Six trees were sampled per treatment (dose-soil class combination) following the protocol described in Le Guen et al. (2017). Briefly, a total of 48 soil and root samples were collected in August 2016 (28 months after application) within the area where the biochar was applied, from 0-30 cm deep. Soils were sieved to pass a 2 mm mesh and stored at -20°C for molecular analyses or

dried at room temperature for chemical analyses. The finest rubber tree roots were collected from the sieve and dried at 45°C until analysis.

2.2. Soil analysis

Soil analyses were performed on 5 samples per treatment/soil class combination and included pH (in H_2O), organic matter (Walkley and Black, 1934), available P (Bray II) (Bray and Kurtz, 1945), total N (Bremner, 1965) and texture (Kilmer and Alexander, 1949). Soil water content was assessed after drying at 100°C for 48 hours.

2.3. DNA extraction and sequencing

DNA was extracted from 0.5 g of soils using the MP116004-500 Fast DNA Spin kit for soil, MP Biomedical, Santa Anna, CA) according to Tournier et al. (2015).

Fragments of the V4 variable region of the16S rRNA gene and of the ITS region were amplified by PCR using the primer pairs 341F (5'- CCTACGGGNGGCWGCAG -3')/785R (5'- GACTACHVGGGTATCTAATCC -3') and ITS1F (5'- CTTGGTCATTTAGAGGAAGTAA -3')/ITS2 (5'- GCTGCGTTCTTCATCGATGC -3') for the bacterial and fungal communities, respectively, and a barcode was included on the forward primers. Samples were pooled together in equal DNA concentrations and purified using calibrated Ampure XP beads. Purified DNA was used for the preparation of the DNA library by following Illumina TruSeq DNA library preparation protocol. Sequencing was performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq instrument following the manufacturer's guidelines. Raw sequence reads were submitted to the Sequence Read Archive (SRA) database (http://www.ncbi.nlm.nih.gov/sra) and are available under the BioProject accession number PRJNA525581.

2.4. Sequencing data processing

Sequences (5'-3' and 3'-5') were joined and barcodes were removed. Sequences were retained only if they carried the correct primer sequences and were >200 bp. Sequences with ambiguous bases and homopolymers exceeding 6 bp were also removed from the dataset. Operational taxonomic units (OTU) were defined by clustering at 97% similarity, and singletons and chimeras were eliminated (Dowd et al., 2008). Final OTUs were taxonomically assigned using BLASTn against a curated database derived from GreenGenes, RDPII and NCBI (www.ncbi.nlm.nih.gov, http://rdp.cme.msu.edu).

Sampling efficacy was assessed with rarefaction analysis using the functions rarefy from the R package Vegan (Oksanen et al., 2012). For subsequent analyses, sequencing depth per sample was standardized by retaining a random selection of reads in each sample, where the number retained corresponded to the minimum read count across all samples (52061 and 40784 reads for bacterial and fungal datasets, respectively).

2.5. AMF colonization assessment

AMF presence and colonization intensity were determined on fine roots (less than 1 mm in diameter) and AMF staining in root tissue was performed using the ink and vinegar technique (Vierheilig et al., 1998) with an additional tissue bleaching step as described by Koske and Gemma (1989). Fifteen roots fragments of 1 cm were observed per sample (x40) and the presence and the intensity (scored from 0 to 5) of colonization in each fragment were recorded to assess the frequency (F%) and the intensity (M%) of AMF colonization (Trouvelot, 1986).

2.6. Statistical analyses

Linear mixed-effects models (LME) were used to test for differences in richness and diversity indices (Shannon, Simpson and Evenness) in the different treatments (Pinheiro et al., 2013). The Shannon index (H') was exponentially transformed, resulting in a variable (expH') that, in contrast to the untransformed index, satisfies the replication principle and can be considered a linear representation of diversity (Jost, 2006).

Analysis of bacterial and fungal community structure was performed using quantitative data, where proportions of reads representing different species were used as a proxy for the relative abundance of the species in a sample (Moora et al., 2014). Differences in communities associated with different treatments were tested using a PERMANOVA, with the *adonis* function from R package Vegan. Two dimensional NMDS (function *metaMDS* from R package Vegan, 50 iterations) was used to explore variation in community composition. Ellipses representing standard deviations around group centroids were defined using the *ordiellipse* function from the R package Vegan.

Soil chemical and physical variables were ln-transformed before they were included in linear mixed-effects models (LME) to test for differences between the different doses and soil classes.

3. Results

3.1. Soil characteristics

General soil texture was similar across all the treatments, being mainly sandy (>80%), although some small differences were observed in the different biochar doses (Table 1). Soil chemical characteristics were significantly affected by the biochar application in the two soil classes. Soil pH tended to increase with the application of biochar regardless of the soil class,

but the increase was significant in C3 soils only (P = 0.019). Soil OM showed inconsistent results in the two soil classes: it tended to increase in C3 soils and to decrease in the C4 soils after biochar application. Soil N content significantly decreased after biochar application in C3 soils only, regardless of the dose (P < 0.0001). Biochar application resulted in an increase of soil P content in both C3 and C4 soils, but the results were significant in C4 soils only (P = 0.027) and depended on the biochar dose. Other nutrient levels (Ca, Mg, K) all showed a tendency to increase with the application of biochar in both soil classes (Table 1).

Soils from class C3 had a significantly higher water content than soils from class C4 (P < 0.0001), regardless of the biochar application treatment. Soil water content was affected by the biochar application in C3 soils (P = 0.082) but not in C4 soils (P = 0.632). Surprisingly, the soil water content was decreased after the addition of biochar, in particular for the smallest dose (T2, 5 ton/ha, P = 0.054, Table 1).

3.2. Bacterial and fungal sequencing data

A total of 3,401,226 and 4,490,456 quality filtered reads were retained for the bacterial and fungal datasets, respectively. Bacterial sequences were classified into 32,742 OTU, in 239 families and 29 phyla, while fungal sequences were classified into 10,709 OTU, in 562 families and 31 phyla.

3.2.1. Bacterial community composition, richness and diversity

Bacterial community was dominated by Proteobacteria (> 30%), followed by Acidobacteria (> 20%), Actinobacteria (> 14%) and Firmicutes (> 10%) in all biochar dose-soil class combinations. The most abundant class was Alphaproteobacteria, which represented about 17% of the sequences regardless of the treatment and soil class (Figure 1).

Most of the bacterial phyla and classes detected in the different samples (8 of the 9 phyla and 12 out of the 15 classes) were affected by the soil class, regardless of the biochar dose. However, when soil classes were studied separately, biochar application had a limited and variable effect on the abundance of the main phyla and classes (Figure 1). In C3 soils, Planctomycetes decreased with the application of biochar ($P = 0.027$), while an opposite trend was observed for Gemmatimonadetes ($P = 0.036$). In C4 soils, Acidobacteria were significantly decreased after application of biochar dose above 10 tons/ha ($P = 0.003$) while Chloroflexi and Verrumicrobia abundances increased and decreased after application of 5 tons/ha (T2), respectively (Figure 1). Proteobacteria classes were strongly affected by biochar application in C3 but not C4 soils. Alphaproteobacteria and Betaproteobacteria, respectively, increased and decreased after application of biochar, regardless of the dose ($P = 0.001$ and 0.020, respectively).

Species richness was affected by the soil class ($P = 0.002$) but not by the biochar dose ($P = 0.523$). Biochar application did not affect the diversity indices (expH' and 1/D), regardless of the dose and soil class (Table 2), however it is interesting to note that both richness and diversity were positively correlated with soil water content ($P < 0.001$ and $P = 0.021$, respectively) and pH ($P < 0.001$).

PERMANOVA indicated that both biochar dose and soil class significantly affected the bacterial communities, but the effect of the soil class was stronger than that of biochar dose ($P = 0.001$ and 0.015, respectively). Results of the ADONIS test showed that the effect of biochar application was only significant for C4 soils, but no significant difference was found between the different biochar rates. The NMDS analysis confirmed that the bacterial community composition was affected in C4 soils only; the samples from the control plots (T1) clustered separately suggesting a shift in the composition of the bacterial community after application of biochar in these soils, even at the smallest rate (5 tons/ha, Figure 2).

3.2.2. Fungal community composition, richness and diversity

Fungal communities were dominated by Ascomycota and Basiodiomycota (means of 50% and 42% respectively, across the treatments, Figure 3). The most abundant classes were Agaricomycetes and Dothideomycetes which represented more than 40% and 20% of the sequences from all treatment combinations, respectively.

Both Ascomycota and Basidiomycota were affected by the soil class (P = 0.005 and 0.008, respectively), but none of the detected fungal phyla were affected by the addition of biochar, regardless of the dose (Figure 3). At the class taxonomic level, however, fungal communities were differently affected by biochar application in C3 and C4 soils. Kickxellomycotina and Eurotiomycetes greatly increased in abundance after the application of a biochar dose of 5 tons/ha (T2) in C3 (P = 0.0002) and C4 soils (P = 0.017), respectively.

Richness and diversity indices of fungal communities were differently affected in C3 and C4 soils (Table 2). In C4 soils, biochar application tended to increase the fungal species richness (P = 0.191) and diversity (P = 0.058 for the 1/D index) in comparison to the controls although, surprisingly, there was no effect in the T3 treatment (10 tons/ha, Table 2). An opposite trend was observed in C3 soils: both richness and diversity indices tended to be negatively affected by the application of biochar, in particular for the highest dose of biochar (T4, 20 tons/ha, Table 2).

The PERMANOVA results showed that both soil class and biochar application had a significant effect on the fungal community (P = 0.001 and 0.019, respectively). ADONIS results showed that the fungal communities were significantly affected by the rate of biochar, regardless of the soil class. Application of 10 tons/ha and 20 tons/ha had the strongest effect

as compared to the control in both C3 (P = 0.051 and 0.022 for T3 and T4, respectively) and C4 soils (P = 0.047 and 0.020 for T3 and T4, respectively).

The clustering of the different samples on the NMDS plot showed that the fungal communities associated to different soil classes differed strongly. Samples from T1 (controls, receiving no biochar) formed separate clusters from other biochar treatments, particularly in C3 soils. However, there was no clear differences between the communities after application of the different doses of biochar, although the biochar dose effect was more evident in C3 than in C4 soils (Figure 4).

3.3. AMF colonization

Roots collected in all treatments were highly colonized regardless of biochar dose and soil class (M ≥ 53%) and no root fragment was found without colonization (F = 100%). Biochar application tended to increase the intensity of mycorrhization in both soil classes (P = 0.160 and 0.122 in C3 and C4 soils, respectively), particularly for rates above 10 tons/ha (T3 and T4, Table 2).

4. Discussion

The effects of biochar application were strongly related to the nature of the soil class, both in terms of impact (positive or negative) and intensity. These findings support the preliminary results previously obtained 18 months after biochar application on the soil characteristics and microbial structure and confirms the importance of performing the analysis of C3 and C4 soil classes separately (Le Guen et al., 2017).

4.1. Biochar and soil characteristics

It is generally acknowledged that biochar application results in a decrease in soil bulk density, while increasing soil porosity, water holding capacity and aggregate stability (Burrell et al., 2016; Glab et al., 2016; Li et al., 2018a). In our study, biochar did not improve the water content in C4 soils which was quite low (< 7%) and surprisingly had a negative effect on the soil water content in C3 soils. A similar decrease was observed in a study conducted in a north temperate forest, 2 years after application of 5 tons/ha of biochar (Noyce et al., 2015). Neutral results on soil moisture characteristics have also been reported in agricultural soils (Domene et al., 2014). Studies reporting increases in water holding capacity often used higher rates of biochar (up to 200 tons/ha), and it is possible that the doses tested in this study were not sufficient to induce such an effect (Ameloot et al., 2014; Luo et al., 2017; Yao et al., 2017a; Zheng et al., 2016). Additionally, the responses of soil hydrological properties to biochar amendments have been shown to be both biochar- and soil-specific, and the associated mechanisms are yet to be fully understood, particularly in forest ecosystems (Li et al., 2018a).

Increases in soil pH following biochar application have been frequently reported, especially in acidic soils (Biederman and Harpole, 2013; Ding et al., 2016; Li et al., 2018a), and our results are in accordance with those findings. However, biochar application resulted in a limited pH increase, and the results were significant in C3 soils only. Limited and variable effects of biochar application on soil pH have been reported elsewhere and have been mainly attributed to differences in both biochar and soil characteristics (Dai et al., 2017; Noyce et al., 2015; Sackett et al., 2015). Other factors, including biochar dose and time since application were also shown to affect soil pH changes (Domene et al., 2014; Jones et al., 2012; Quilliam et al., 2012).

Nutrient content (apart from N) tended to increase with the biochar rate, regardless of the soil class. Biochar-induced changes in soil nutrient content (including P, N, K, Ca, Mg and Na) have previously been reported (Biederman and Harpole, 2013; Jones et al., 2012; Noyce et al., 2015; Wang et al., 2014) but neutral effects were also found (Ding et al., 2016; Noyce et al., 2015). Various mechanisms may explain the observed changes. Nutrients may be introduced to the soils through labile organic compounds associated with biochar and become available as these compounds weather (Sohi et al., 2010), however, these effects are presumed to be short-lived (less than 1 year) and, thus, are unlikely to explain the soil nutrient increase observed in our study (Ameloot et al., 2014; Biederman and Harpole, 2013; Zheng et al., 2016). Long-term effects of biochar addition may be related to changes in soil pH. Increase of soil pH can change the form of some nutrients (and of P in particular) and make them more available for plants or microorganisms (Ding et al., 2016). In low pH soils, P can be adsorbed onto iron oxides thus reducing plant availability. Biochar liming effects reduce the concentration of iron and aluminum in soils, releasing P which then becomes available to plants or microorganisms (Biederman and Harpole, 2013). Reduction of nutrient leaching due to biochar's physicochemical properties (porous structure, large surface area, and negative surface charge) may affect soil nutrients long-term through an increased adsorption to biochar's surface (Biederman and Harpole, 2013; Laird et al., 2010). In contrast to the other nutrients, N content decreased with the application of biochar, in both soil classes. It has been suggested that N compounds could be retained on biochar due to its sorption properties, thereby affecting soil N content (Brassard et al., 2016). The effects of soil type, biochar's characteristics, as well as biochar's age have all been shown to have impacts on soil nutrient availability (Brassard et al., 2016). For example, the aging of biochar changes its physiochemical properties, and these changes may have significant consequences for the

bioavailability and transport of nutrients, although the underlying mechanisms are still poorly understood (Mia et al., 2017).

Soil OM content tended to increase and decrease in C3 and C4 soils, respectively. Biochar has been shown to interact with soil OM and both increases and decreases in mineralization of native soil OM have been reported (Brassard et al., 2016; Gul et al., 2015; Maestrini et al., 2014; Zimmerman et al., 2011; Zimmermann et al., 2012). Priming direction (C mineralization stimulation or suppression) and magnitude are related to soil type and biochar characteristics (Zimmerman et al., 2011). One of the most cited hypotheses for the negative priming effect is that OM is absorbed on the biochar surface and pores (Zimmermann et al., 2012). However, changes in soil OM may also be directly and indirectly associated to changes in microbial communities. Li et al. (2018b) found that biochar application resulted in a decrease in heterotrophic soil respiration (which originates from microbial decomposition of soil OM) through changes in soil microbial community composition and activity of C-degrading enzymes. In a 3 year experiment in a forest ecosystem, Mitchell et al. (2016) found that the composition of the soil OM pool was altered by biochar application. These authors proposed that the observed compositional shifts were related to changes in microbial communities, and to stimulated fungal activity in particular. Similarly, increases in pH after biochar application may also affect the soil OM dynamics through an impact on microbial enzyme activities and microbial biomass (Brassard et al., 2016; Maestrini et al., 2014). Our results showed that pH and microbial communities were differently affected by biochar application in the two soil classes, and this could have affected the soil OM in the two soil classes differently.

4.2. Impact of biochar on soil bacterial and fungal community composition

Many studies have reported effects of biochar on microbial biomass, respiration or community structure using molecular tools such as qPCR, DGGE or T-RFLP (Ding et al., 2016; Domene et al., 2014; Noyce et al., 2015; Prayogo et al., 2014). Through sequencing of the PCR products thus generated it is possible to obtain data on community composition and diversity but this generally provides a low sequencing depth as compared to high throughput sequencing techniques. To date, studies focusing on perennial plantations in tropical regions are scarce and most recent studies showing the impact of biochar on microbial community composition and diversity using high throughput sequencing methods have focused on agricultural soils (Chen et al., 2015; Imparato et al., 2016; Kolton et al., 2017; Nielsen et al., 2014; Yao et al., 2017a; Yao et al., 2017b; Zheng et al., 2016).

In the present study, the bacterial community was dominated by Proteobacteria (Alphaproteobacteria in particular) followed by Acidobacteria, Actinobacteria and Firmicutes, regardless of the treatment and soil class. However, differences in community composition were found and abundances of phyla and classes were differently affected in C3 and C4 soils. Grossman et al. (2010) showed that the structure of the bacterial community in Brazilian anthrosol soils (naturally containing a high proportion of biochar) differed from that of adjacent soils containing no biochar but having similar mineralogy. Proteobacteria were negatively affected by the presence of biochar while Verrucomicrobia were more specifically found in the anthrosol soils. Similar differences in community composition were found in other experiments with soils amended with biochar, and differences were related to various phyla including Proteobacteria, Planctomycetes, Verrucomicrobia, Bacteroidetes and Acidobacteria (Chen et al., 2013; Imparato et al., 2016; Jenkins et al., 2017; Kolton et al., 2017; Luo et al., 2016; Nielsen et al., 2014; Noyce et al., 2015; Su et al., 2017; Yao et al., 2017b). This is in accordance with our results since we found that biochar addition mainly

affected the abundance of several Proteobacteria classes (Alphaproteobacteria, Betaproteobacteria and Deltaproteobacteria) and Planctomycetes in C3 soils and Acidobacteria and Verrucomicrobia in C4 soils. Interestingly, Nielsen et al. (2014) highlighted the occurrence of associations between members of the phyla Acidobacteria and Verrucomicrobia in the presence of biochar.

Fungal communities were affected by the biochar addition in both soil classes, although the composition was not strongly affected at the higher taxonomic levels (phyla and classes). Similar results were obtained by Yao et al. (2017a) and Su et al. (2017) who showed that fungal communities were strongly dominated by Ascomycota and Basidiomycota regardless of the treatment. It could be argued that rare OTU are more affected than abundant ones, thus resulting in community composition changes while maintaining the general abundance of particular phylum or class. Fungal community abundance, richness and diversity have been shown to be negatively affected by biochar addition in many studies (Chen et al., 2013; Gul et al., 2015; Jenkins et al., 2017; Li et al., 2018b; Mitchell et al., 2015; Zheng et al., 2016) and our results are in accordance with these findings. Arbuscular mycorrhizal fungi (AMF) colonization was not strongly affected by the biochar application, although it tended to increase with the biochar dose, regardless of the soil class. Similar positive results of biochar application on AMF were reported elsewhere (Biederman and Harpole, 2013; Luo et al., 2017; Warnock et al., 2007).

4.3. Role of biochar characteristics on microbial communities

Several mechanisms have been proposed to explain the effect of biochar on microbial communities and include i) a better protection against predators or competitors by exploring the pores of biochar, thus creating an expanded niche for both bacteria and fungi, ii) the initial addition of soluble nutrients contained in the biochar and an initial pulse in C and

mineralization of the labile fraction of biochar itself, associated with the release of numerous volatile and biologically active compounds, iii) reduced nutrient leaching and adsorption of toxic compounds and iv) the improvement of the water status of the soils through changes in soil aggregation and porosity (Biederman and Harpole, 2013; Ding et al., 2016; Domene et al., 2014; Gul and Whalen, 2016; Laird et al., 2010; Sohi et al., 2010). The initial status of the soils may affect the direction and the magnitude of each of the mechanisms (Gul et al., 2015; Jenkins et al., 2017; Li et al., 2018a), which may partly explain the different observations in the two soil classes identified in our study site.

Biochar pores may offer a protective habitat for microorganisms. Because of their larger size, fungi are mainly able to colonize biochar macropores while bacteria can access smaller pores and therefore may be better protected from grazing (Gul et al., 2015). However, Quilliam et al. (2013) argued that this is unlikely to occur in the short or medium term (less than 3 years after biochar application), and suggested that changes in soil physicochemical properties are more likely to alter the microbial communities, both directly and indirectly. Our results support this hypothesis. In particular, changes in soil pH and nutrient availability are likely to dictate major changes in microbial communities. pH is widely recognized to cause changes in microbial community composition and the increases in pH observed in our study may have positively affected bacteria while reducing growth of fungi as found elsewhere (Domene et al., 2014; Noyce et al., 2015; Prayogo et al., 2014; Rousk et al., 2010). Bacteria classified as Acidobacteria usually dominate oligotrophic and low pH soils and the decrease in their abundance is probably a response to the liming effect of biochar (Jenkins et al., 2017; Zheng et al., 2016).

As previously mentioned, modification of nutrient availability, and of P and N in particular, may be related to the retention effects of biochar and may enhance or limit growth and activity of specific groups of microorganisms, such as P-solubilizing bacteria, AMF or

bacteria involved in the N cycle process such as nitrification or denitrification process (Biederman and Harpole, 2013; Brassard et al., 2016; Ding et al., 2016). Consequently, the changes in the activity of soil organisms can potentially translate to changes in nutrient availability and cause further cascading effects on other microbial groups and trophic relationships.

4.4. Response to time since application and biochar dose

It is important to note that time since application may have a significant role in the nature and magnitude of any observed effects. The residence time of biochar in soils ranges from hundreds to thousands of years, thus the biochar aging effect should not be ignored. A majority of studies were conducted over a short-term period of time (≤ 1 year) and only a few studies reported on the medium to long term impacts of biochar application (Qin et al., 2016; Yao et al., 2017a; Yao et al., 2017b). The properties of biochar, and as consequence its effects, change with time (Gul et al., 2015; Mia et al., 2017; Yao et al., 2017a; Yao et al., 2017b). Sackett et al. (2015) found that most nutrient effects observed within 2 to 6 weeks after addition were no longer present by the end of the first year, and suggested that biochar may still impact the ecosystem several years after application although the effects may not be similar to that of the short term. Jenkins et al. (2017) reported that the effect of biochar on microbial communities changed over the first year and hypothesized that physical weathering may result in recalcitrant portions of the biochar becoming available, thus favoring copiotrophic groups of microorganisms over more specialized oligotrophic communities. In an experiment conducted on rice paddy soils, persistent changes in soil characteristics were still found 4 years after a single application, indirectly affecting microbial community structure and functioning (Zheng et al., 2016). As found in our study, several authors have reported that fungal communities were more severely affected than bacterial communities,

several years after biochar amendment (Luo et al., 2017; Yao et al., 2017a; Zheng et al., 2016). This supports the hypothesis that bacteria usually react quickly to environmental changes while fungi may struggle to adapt as rapidly. Moreover, changes in bacterial community composition, structure or functions may be transient due to the higher resilience of bacteria. As a result, short-term shifts in bacteria, followed by longer term shifts in fungi, may be expected (Gul et al., 2015; Noyce et al., 2015).

While changes were observed in both bacterial and fungal communities after biochar application, they did not seem to be correlated to the applied biochar dose. As previously mentioned, one of the possible reasons for the lack of dose effect may be that the amount of biochar application was less than that of other studies. The recommended rate for biochar application in rubber tree plantations in Thailand is currently of 8 tons/ha, and this is why the doses tested in our experiment ranged from 5 to 20 tons/ha. Other studies reported that the magnitude of the effect was related to the biochar dose, but the rates ranged from 20 to 200 tons/ha in these (Ameloot et al., 2014; Luo et al., 2017; Yao et al., 2017a; Zheng et al., 2016). Lower application rates (3 to 12 tons/ha) had a limited impact on soil parameters including pH (Domene et al., 2014). Similarly, a meta-analysis conducted on 371 biochar studies highlighted the absence of an obvious threshold or trend with increasing application rates (Biederman and Harpole, 2013).

5. Conclusions

This field study showed that biochar had a significant effect on soil characteristics and on bacterial and fungal communities associated with the rubber trees, 28 months after application. Fungal communities were more strongly affected than bacterial communities, even at the lowest applied dose. The effect of the soil class was generally stronger than the effect of the biochar dose. This highlights the importance of soil variability interacting with

the underlying mechanisms of biochar that are still poorly understood, especially over the long-term. Given the key roles of microorganisms in all biogeochemical cycles, complex interactions between biochar, soil biota and soil physical and chemical properties may have strong implications for soil functions and ecosystem services. More research is critical to assess the long-term effect of biochar application on perennial plantations, including its potential impact on ecosystem functions and plant productivity.

Acknowledgements

Deakin University, CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Dévelopement) and the UMR Eco & Sols financially supported this work. It was part of the TICA (Thailand International Cooperation Agency) project entitled "Towards an improvement of soil water quality in the context of land use and climate changes in Thailand" and the two NRCT (National Research Council of Thailand) projects entitled "Assessment of biochar applications on both soil fertility and soil microbial component in rubber tree plantation in North East Thailand" and "Impact of biochar applications on soil biology of a rubber plantation in North-Eastern Thailand: relationship between soil physic and soil microbiology". The authors are grateful to Khun Tum and Khun Kasem for assistance with the fieldwork.

References

Ameloot, N., Sleutel, S., Case, S.D.C., Alberti, G., McNamara, N.P., Zavalloni, C., Vervisch, B., Vedove, G., De Neve, S. 2014. C mineralization and microbial activity in four biochar field experiments several years after incorporation. Soil Biol Biochem, 78, 195-203.

Amini, S., Ghadiri, H., Chen, C., Marschner, P. 2016. Salt-affected soils, reclamation, carbon dynamics, and biochar: a review. J Soils Sediments, 16, 939-953.

Biederman, L.A., Harpole, W.S. 2013. Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. GCB Bioenergy, 5, 202-214.

Brassard, P., Godbout, S., Raghavan, V. 2016. Soil biochar amendment as a climate change mitigation tool: Key parameters and mechanisms involved. J Environ Manag, 181, 484-497.

Bray, R.H., Kurtz, L.T. 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Sci, 59, 39-45.

Bremner, J.M., 1965. Total Nitrogen. In: Black, C.A. (ed), Methods of Soil Analysis Vol. 2. American Society of Agronomy, Madison, pp. 1149-1178.

Bruun, E.W., Petersen, C.T., Hansen, E., Holm, J.K., Hauggaard-Nielsen, H. 2014. Biochar amendment to coarse sandy subsoil improves root growth and increases water retention. Soil Use Manage, 30, 109-118.

Burrell, L.D., Zehetner, F., Rampazzo, N., Wimmer, B., Soja, G. 2016. Long-term effects of biochar on soil physical properties. Geoderma, 282, 96-102.

Chen, J., Liu, X., Zheng, J., Zhang, B., Lu, H., Chi, Z., Pan, G., Li, L., Zheng, J., Zhang, X., Wang, J., Yu, X. 2013. Biochar soil amendment increased bacterial but decreased fungal gene abundance with shifts in community structure in a slightly acid rice paddy from Southwest China. Appl Soil Ecol, 71, 33-44.

Chen, J., Liu, X., Li, L., Zheng, J., Qu, J., Zheng, J., Zhang, X., Pan, G. 2015. Consistent increase in abundance and diversity but variable change in community composition of bacteria in topsoil of rice paddy under short term biochar treatment across three sites from South China. Appl Soil Ecol, 91, 68-79.

Dai, Z., Zhang, X., Tang, C., Muhammad, N., Wu, J., Brookes, P.C., Xu, J. 2017. Potential role of biochars in decreasing soil acidification - A critical review. Sci Tot Environ, 581-582, 601-611.

Ding, Y., Liu, Y., Liu, S., Li, Z., Tan, X., Huang, X., Zeng, G., Zhou, L., Zheng, B. 2016. Biochar to improve soil fertility. A review. Agron Sustain Dev, 36, 1-18.

Domene, X., Mattana, S., Hanley, K., Enders, A., Lehmann, J. 2014. Medium-term effects of corn biochar addition on soil biota activities and functions in a temperate soil cropped to corn. Soil Biol Biochem, 72, 152-162.

Dowd, S.E., Callaway, T.R., Wolcott, R.D., Sun, Y., McKeehan, T., Hagevoort, R.G., Edrington, T.S. 2008. Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). BMC Microbiology, 8, 125-

Downie, A., Crosky, A., Munroe, P., 2009. Physical properties of biochar. In: Lehmann, J.,Joseph S. (eds), Biochar for environmental management: Science and technology. Earthscan, London, pp. 13-32.

Glab, T., Palmowska, J., Zaleski, T., Gondek, K. 2016. Effect of biochar application on soil hydrological properties and physical quality of sandy soil. Geoderma, 281, 11-20.

Grossman, J.M., O'Neill, B.E., Tsai, S.M., Liang, B., Neves, E., Lehmann, J., Thies, J.E. 2010. Amazonian anthrosols support similar microbial communities that differ distinctly from those extant in adjacent, unmodified soils of the same mineralogy. Microb Ecol, 60, 192-205.

Gul, S., Whalen, J.K., Thomas, B.W., Sachdeva, V., Deng, H. 2015. Physico-chemical properties and microbial responses in biochar-amended soils: Mechanisms and future directions. Agric Ecosyst Environ, 206, 46-59.

Gul, S., Whalen, J.K. 2016. Biochemical cycling of nitrogen and phosphorus in biochar-amended soils. Soil Biol Biochem, 103, 1-15.

Herrmann, L., Lesueur, D., Bräu, L., Davison, J., Jairus, T., Robain, H., Robin, A., Vasar, M., Wiriyakitnateekul, W., Öpik, M. 2016. Diversity of root-associated arbuscular mycorrhizal fungal communities in a rubber tree plantation chronosequence in Northeast Thailand. Mycorrhiza, 26, 863-877.

Imparato, V., Hansen, V., Santos, S.S., Nielsen, T.K., Giagnoni, L., Hauggaard-Nielsen, H., Johansen, A., Renella, G., Winding, A. 2016. Gasification biochar has limited effects on functional and structural diversity of soil microbial communities in a temperate agroecosystem. Soil Biol Biochem, 99, 128-136.

Jenkins, J.R., Viger, M., Arnold, E.C., Harris, Z.M., Ventura, M., Miglietta, F., Girardin, C., Edwards, R.J., Rumpel, C., Fornasier, F., Zavalloni, C., Tonon, G., Alberti, G., Taylor, G. 2017. Biochar alters the soil microbiome and soil function: results of next-generation amplicon sequencing across Europe. GCB Bioenergy, 9, 591-612.

Jones, D.L., Rousk, J., Edwards-Jones, G., DeLuca, T.H., Murphy, D.V. 2012. Biochar-mediated changes in soil quality and plant growth in a three year field trial. Soil Biol Biochem, 45, 113-124.

Jost, L. 2006. Entropy and diversity. Oikos, 113, 363-375.

Kilmer, V.J., Alexander, L.T. 1949. Methods of making mechanical analyses of soils. Soil Sci, 68, 15-24.

Kolton, M., Graber, E.R., Tsehansky, L., Elad, Y., Cytryn, E. 2017. Biochar-stimulated plant performance is strongly linked to microbial diversity and metabolic potential in the rhizosphere. New Phytol, 213, 1393-1404.

Koske, R.E., Gemma, J.N. 1989. A modified procedure for staining roots to detect VA mycorrhizas. Mycol Res, 92, 486-488.

Laird, D.A., Fleming, P., Davis, D.D., Horton, R., Wang, B., Karlen, D.L. 2010. Impact of biochar amendments on the quality of a typical Midwestern agricultural soil. Geoderma, 158, 443-449.

Le Guen, M.E., Herrmann, L., Robain, H., Wiriyakitnateekul, W., de Oliveira, T., Robin, A., Srimawong, P., Bräu, L., Lesueur, D. 2017. Relevance of taking into account the fine scale soil variability to assess the effects of agricultural inputs on soil characteristics and soil microbial communities: A case study of biochar application in a rubber plantation in North East Thailand. Geoderma, 305, 21-29.

Lehmann, J., Gaunt, J., Rondon, M. 2006. Bio-char sequestration in terrestrial ecosystems - a review. Mitigation and Adaptation Strategies for Global Change, 11, 403-427.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D. 2011. Biochar effects on soil biota - a review. Soil Biol Biochem, 43, 1812-1836.

Li, Y., Hu, S., Chen, J., Müller, K., Li, Y., Fu, W., Lin, Z., Wang, H. 2018a. Effects of biochar application in forest ecosystems on soil properties and greenhouse gas emissions: a review. J Soils Sediments, 18, 546-563.

Li, Y., Li, Y., Chang, S.X., Yang, Y., Fu, S., Jiang, P., Luo, Y., Yang, M., Chen, Z., Hu, S., Zhao, M., Liang, X., Xu, Q., Zhou, G., Zhou, J. 2018b. Biochar reduces soil heterotrophic respiration in a subtropical plantation through increasing soil organic carbon recalcitrancy and decreasing carbon-degrading microbial activity. Soil Biol Biochem, 122, 173-185.

Luo, S., Wang, S., Tian, L., Li, S., Li, X., Shen, Y., Tian, C. 2017. Long-term biochar application influences soil microbial community and its potential roles in semiarid farmland. Appl Soil Ecol, 117-118, 10-15.

Luo, Y., Yu, Z., Zhang, K., Xu, J., Brookes, P.C. 2016. The properties and functions of biochars in forest ecosystems. J Soils Sediments, 16, 2005-2020.

Maestrini, B., Herrmann, A.M., Nannipieri, P., Schmidt, M.W.I., Abiven, S. 2014. Ryegrass-derived pyrogenic organic matter changes organic carbon and nitrogen mineralization in a temperate forest soil. Soil Biol Biochem, 69, 291-301.

Mia, S., Dijkstra, F.A., Singh, B., 2017. Long-term aging of biochar: a molecular understanding with agricultural and environmental implications. In: Sparks, D.L. (ed), Advances in Agronomy. Academic Press, pp. 1-51.

Mitchell, P.J., Simpson, A.J., Soong, R., Simpson, M.J. 2015. Shifts in microbial community and water-extractable organic matter composition with biochar amendment in a temperate forest soil. Soil Biol Biochem, 81, 244-254.

Mitchell, P.J., Simpson, A.J., Soong, R., Schurman, J.S., Thomas, S.C., Simpson, M.J. 2016. Biochar amendment and phosphorus fertilization altered forest soil microbial community and native soil organic matter molecular composition. Biogeochemistry, 130, 227-245.

Moora, M., Davison, J., Öpik, M., Metsis, M., Saks, Ü., Jairus, T., Vasar, M., Zobel, M. 2014. Anthropogenic land use shapes the composition and phylogenetic structure of soil arbuscular mycorrhizal fungal communities. FEMS Microbiol Ecol, 90, 609-621.

Nielsen, S., Minchin, T., Kimber, S., Van Zwieten, L., Gilbert, J., Munroe, P., Joseph, S., Thomas, T. 2014. Comparative analysis of the microbial communities in agricultural soil amended with enhanced biochars or traditional fertilisers. Agric Ecosyst Environ, 191, 73-82.

Noyce, G.L., Basiliko, N., Fulthorpe, R., Sackett, T.E., Thomas, S.C. 2015. Soil microbial responses over 2 years following biochar addition to a north temperate forest. Biol Fertil Soils, 51, 649-659.

Obia, A., Mulder, J., Martinsen, V., Cornelissen, G., Børresen, T. 2016. In situ effects of biochar on aggregation, water retention and porosity in light-textured tropical soils. Soil Tillage Res, 155, 35-44.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.N., Wagner, H. 2012. Package 'vegan' : Community Ecology Package, version 2.0-4.

Pinheiro, J., Bates, D., Saikat, D., Sarkar, D., The R Development Core Team 2013. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-113.

Prayogo, C., Jones, J.E., Baeyens, J., Bending, G.D. 2014. Impact of biochar on mineralisation of C and N from soil and willow litter and its relationship with microbial community biomass and structure. Biol Fertil Soils, 50, 695-702.

Qin, X., Li, Y., Wang, H., Liu, C., Li, J., Wan, Y., Gao, Q., Fan, F., Liao, Y. 2016. Long-term effect of biochar application on yield-scaled greenhouse gas emissions in a rice paddy cropping system: A four-year case study in south China. Sci Tot Environ, 569-570, 1390-1401.

Quilliam, R.S., Marsden, K.A., Gertler, C., Rousk, J., DeLuca, T.H., Jones, D.L. 2012. Nutrient dynamics, microbial growth and weed emergence in biochar amended soil are influenced by time since application and reapplication rate. Agric Ecosyst Environ, 158, 192-199.

Quilliam, R.S., Glanville, H.C., Wade, S.C., Jones, D.L. 2013. Life in the "charosphere" - Does biochar in agricultural soil provide a significant habitat for microorganisms? Soil Biol Biochem, 65, 287-293.

Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., Fierer, N. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J, 4, 1340-1351.

Sackett, T.E., Basiliko, N., Noyce, G.L., Winsborough, C., Schurman, J., Ikeda, C., Thomas, S.C. 2015. Soil and greenhouse gas responses to biochar additions in a temperate hardwood forest. GCB Bioenergy, 7, 1062-1074.

Sohi, S.P., Krull, E., Lopez-Capel, E., Bol, R. 2010. A Review of biochar and its use and function in soil. Adv Agron, 105, 47-82.

Su, P., Lou, J., Brookes, P.C., Luo, Y., He, Y., Xu, J. 2017. Taxon-specific responses of soil microbial communities to different soil priming effects induced by addition of plant residues and their biochars. J Soils Sediments, 17, 674-684.

Tournier, E., Amenc, L., Pablo, A.L., Legname, E., Blanchart, E., Plassard, C., Robin, A., Bernard, L. 2015. Modification of a commercial DNA extraction kit for safe and rapid recovery of DNA and RNA simultaneously from soil, without the use of harmful solvents. MethodsX, 2, 182-191.

Trouvelot, A. 1986. Mesure du taux de mycorhization VA d'un systeme radiculaire. Recherche de methodes d'estimation ayant une significantion fonctionnelle. Mycorrhizae: physiology and genetics (First european symposium on mycorrhizae), 217-221.

Verheijen, F., Jeffery, S., Bastos, A.C., van der Velde, M., Diafas, I., 2009. Biochar application to soils - a critical scientific review of effects on soil properties, processes and functions . In: EUR 24099 EN report

Vierheilig, H., Coughlan, A.P., Wyss, U., Piché, Y. 1998. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Appl Environ Microbiol, 64, 5004-5007.

Walkley, A., Black, I.A. 1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid in soil analysis. Soil Sci, 37, 29-38.

Wang, Y., Yin, R., Liu, R. 2014. Characterization of biochar from fast pyrolysis and its effect on chemical properties of the tea garden soil. J Anal Appl Pyrolysis, 110, 375-381.

Warnock, D.D., Lehmann, J., Kuyper, T.W., Rillig, M.C. 2007. Mycorrhizal responses to biochar in soil - concepts and mechanisms. Plant Soil, 300, 9-20.

Yao, Q., Liu, J., Yu, Z., Li, Y., Jin, J., Liu, X., Wang, G. 2017a. Three years of biochar amendment alters soil physiochemical properties and fungal community composition in a black soil of northeast China. Soil Biol Biochem, 110, 56-67.

Yao, Q., Liu, J., Yu, Z., Li, Y., Jin, J., Liu, X., Wang, G. 2017b. Changes of bacterial community compositions after three years of biochar application in a black soil of northeast China. Appl Soil Ecol, 113, 11-21.

Zheng, J., Chen, J., Pan, G., Liu, X., Zhang, X., Li, L., Bian, R., Cheng, K., Jinwei, Z. 2016. Biochar decreased microbial metabolic quotient and shifted community composition four years after a single incorporation in a slightly acid rice paddy from southwest China. Sci Tot Environ, 571, 206-217.

Zimmerman, A.R., Gao, B., Ahn, M.Y. 2011. Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. Soil Biol Biochem, 43, 1169-1179.

Zimmermann, M., Bird, M.I., Wurster, C., Saiz, G., Goodrick, I., Barta, J., Capek, P., Santruckova, H., Smernik, R. 2012. Rapid degradation of pyrogenic carbon. Global Change Biol, 18, 3306-3316.

Table 1. Soil characteristics for the different biochar rates in the two soil classes.

Soil class	Biochar dose	Soil texture			Chemical characteristics							
		Sand (%)	Silt (%)	Clay (%)	pH (H_2O)	OM (%)	P (bray II) (mg/kg)	N (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	K (mg/kg)	Water content (%)
C3	T1	83.4 ± 0.91a	13.8 ± 1.36b	2.8 ± 0.57a	4.62 ± 0.21b	0.50 ± 0.07a	6.62 ± 5.46a	0.121 ± 0.042a	43.12 ± 16.28a	14.28 ± 2.83a	24.40 ± 5.30a	9.67 ± 0.36a
	T2	83.4 ± 1.77a	13.9 ± 1.75b	2.7 ± 0.57a	4.66 ± 0.28b	0.55 ± 0.15a	8.45 ± 5.89a	0.032 ± 0.014b	68.95 ± 20.91a	14.18 ± 4.22a	19.42 ± 7.42a	7.68 ± 1.23b
	T3	83.0 ± 1.98a	14.4 ± 1.50b	2.6 ± 0.65a	5.11 ± 0.29a	0.40 ± 0.12a	5.22 ± 4.09a	0.023 ± 0.011b	94.15 ± 66.80a	20.35 ± 7.10a	24.70 ± 6.51a	8.43 ± 1.48ab
	T4	79.7 ± 1.15b	17.7 ± 0.46a	2.6 ± 0.82a	4.81 ± 0.15ab	0.63 ± 0.04a	10.31 ± 4.06a	0.040 ± 0.011b	103.56 ± 53.12a	24.02 ± 10.74a	44.64 ± 29.71a	8.66 ± 2.19ab
C4	T1	78.9 ± 3.26b	18.5 ± 2.85a	2.62 ± 0.86a	4.48 ± 0.30a	0.51 ± 0.06a	9.74 ± 3.28ab	0.087 ± 0.068a	52.66 ± 21.46a	11.33 ± 2.93a	10.22 ± 3.66b	6.65 ± 2.01a
	T2	83.9 ± 2.40a	14.0 ± 2.05b	2.1 ± 0.42ab	4.57 ± 0.13a	0.45 ± 0.07a	19.89 ± 11.28a	0.121 ±0.198a	42.88 ± 6.17a	10.63 ± 1.15a	13.02 ± 4.70ab	6.08 ± 1.28a
	T3	83.2 ± 1.79a	14.9 ± 1.70b	1.9 ± 0.65ab	4.63 ± 0.26a	0.40 ± 0.18a	6.84 ± 2.46b	0.041 ± 0.0244a	57.03 ± 23.59a	12.34 ± 3.57a	12.84 ± 4.91ab	6.87 ± 0.67a
	T4	84.6 ± 1.96a	14.5 ± 1.43b	0.9 ± 0.65b	4.69 ± 0.18a	0.40 ± 0.12a	14.00 ± 4.06ab	0.054 ± 0.034a	56.47 ± 13.03a	13.49 ± 2.77a	18.80 ± 4.76a	6.70 ± 0.59a

C3 and C4: soil classes, T1=0 t of biochar/ha, T2=5 t of biochar/ha, T3=10 t of biochar/ha, T4=20 t of biochar/ha;

Means accompanied by the same letter do not differ significantly $P \leq 0.05$ (pairwise comparisons using the Tukey (HSD) test).

Table 2. Richness and diversity indices for bacterial and fungal communities after application of different rates of biochar in two soil classes.

		Bacterial communities				Fungal communities				Arbuscular Mycorrhizal Fungi (AMF)
Soil class	Biochar dose	Richness	expH'	1/D	Evenness	Richness	expH'	1/D	Evenness	Intensity of colonization (M%)
C3	T1	1083	148.18	57.46	0.71	648	34.79	11.81	0.53	71.51
	T2	1047	129.61	48.45	0.70	609	24.65	8.62	0.45	81.04
	T3	1074	143.12	55.33	0.71	616	23.17	8.15	0.44	80.03
	T4	1087	139.64	50.40	0.70	584	14.74	5.05	0.40	83.18
C4	T1	1015	123.50	46.76	0.69	605	20.64	6.70	0.47	81.20
	T2	1011	116.83	43.26	0.69	635	30.38	10.97	0.52	74.97
	T3	1022	127.16	48.67	0.70	597	20.94	7.31	0.47	84.22
	T4	1037	133.90	53.20	0.70	654	26.78	7.61	0.50	86.14

C3 and C4: soil classes, T1=0 t of biochar/ha, T2=5 t of biochar/ha, T3=10 t of biochar/ha, T4=20 t of biochar/ha;

Pairwise comparisons of the means using the Tukey (HSD) test did not show any significant difference at $P \leq 0.05$

Fig. 1 Composition of the soil bacterial community at the phylum level (A) and class level (B) in the different site types

C3 and C4: soil classes, T1=0 t of biochar/ha, T2=5 t of biochar/ha, T3=10 t of biochar/ha, T4=20 t of biochar/ha

Within each soil class (C3 and C4), means accompanied by the same letter do not differ significantly at $P \leq 0.05$ (pairwise comparisons using the Tukey (HSD) test)

Fig. 2 Non-metric multi-dimensional scaling (NMDS) plots displaying bacterial communities detected in the soils associated with rubber trees after application of different rates of biochar in two soil classes

C3 and C4: soil classes, T1=0 t of biochar/ha, T2=5 t of biochar/ha, T3=10 t of biochar/ha, T4=20 t of biochar/ha

Ellipses indicate one standard deviation around the centroid position of each biochar dose-soil class combination

Fig. 3 Composition of the soil fungal community at the phylum level (A) and class level (B) in the different site types

C3 and C4: soil classes, T1=0 t of biochar/ha, T2=5 t of biochar/ha, T3=10 t of biochar/ha, T4=20 t of biochar/ha

Pairwise comparisons of the fungal phyla means using the Tukey (HSD) test did not show any significant difference at $P \leq 0.05$

Within each soil class (C3 and C4), fungal class means accompanied by the same letter do not differ significantly at $P \leq 0.05$ (pairwise comparisons using the Tukey (HSD) test)

Fig. 4 Non-metric multi-dimensional scaling (NMDS) plots displaying fungal communities detected in the soils associated with rubber trees after application of different rates of biochar in two soil classes

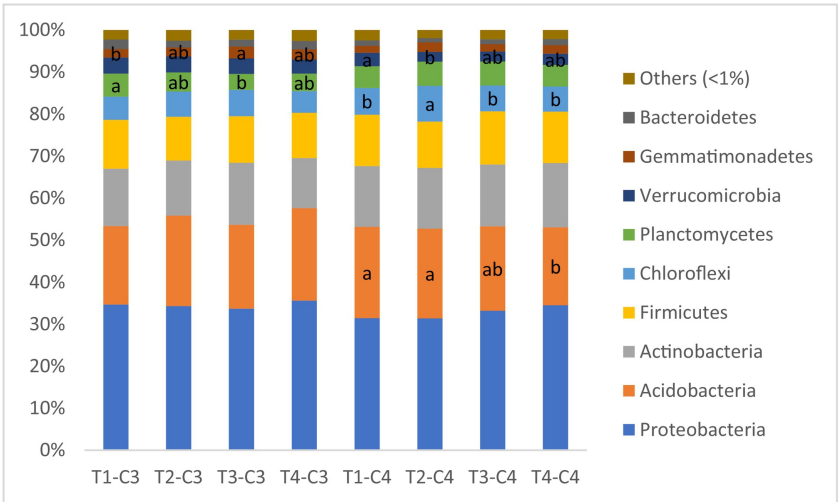
C3 and C4: soil classes, T1=0 t of biochar/ha, T2=5 t of biochar/ha, T3=10 t of biochar/ha, T4=20 t of biochar/ha

Ellipses indicate one standard deviation around the centroid position of each dose-soil class combination

Highlights

- Biochar addition affected soil microbial communities associated with rubber trees
- Biochar application resulted in higher soil pH and nutrient content
- Both community composition and structure were affected by biochar application
- Fungal communities were more severely affected than bacterial communities
- The effect of soil type was stronger than that of biochar dose on all parameters

(A)


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
T1-C3
T2-C3
T3-C3
T4-C3
T1-C4
T2-C4
T3-C4
T4-C4
Others (<1%)
Bacteroidetes
Gemmatimonadetes
Verrucomicrobia
Planctomycetes
Chloroflexi
Firmicutes
Actinobacteria
Acidobacteria
Proteobacteria

(B)

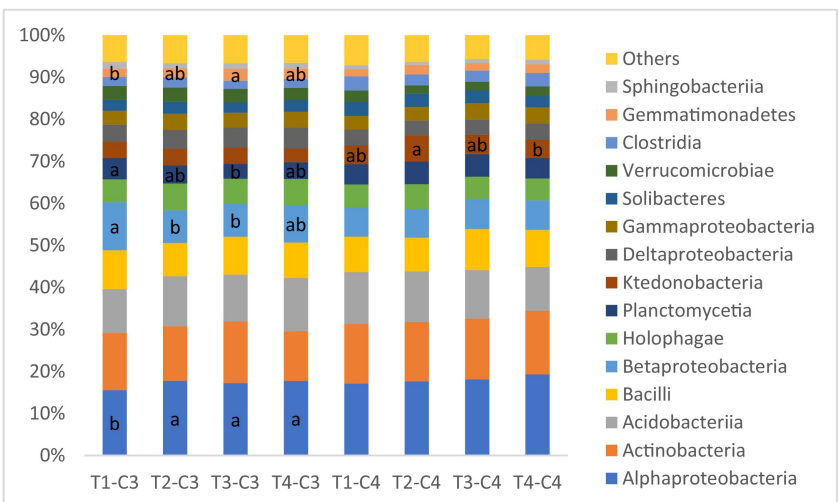

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
T1-C3
T2-C3
T3-C3
T4-C3
T1-C4
T2-C4
T3-C4
T4-C4
Others
Sphingobacteriia
Gemmatimonadetes
Clostridia
Verrucomicrobiae
Solibacteres
Gammaproteobacteria
Deltaproteobacteria
Ktedonobacteria
Planctomycetia
Holophagae
Betaproteobacteria
Bacilli
Acidobacteriia
Actinobacteria
Alphaproteobacteria

Figure 1

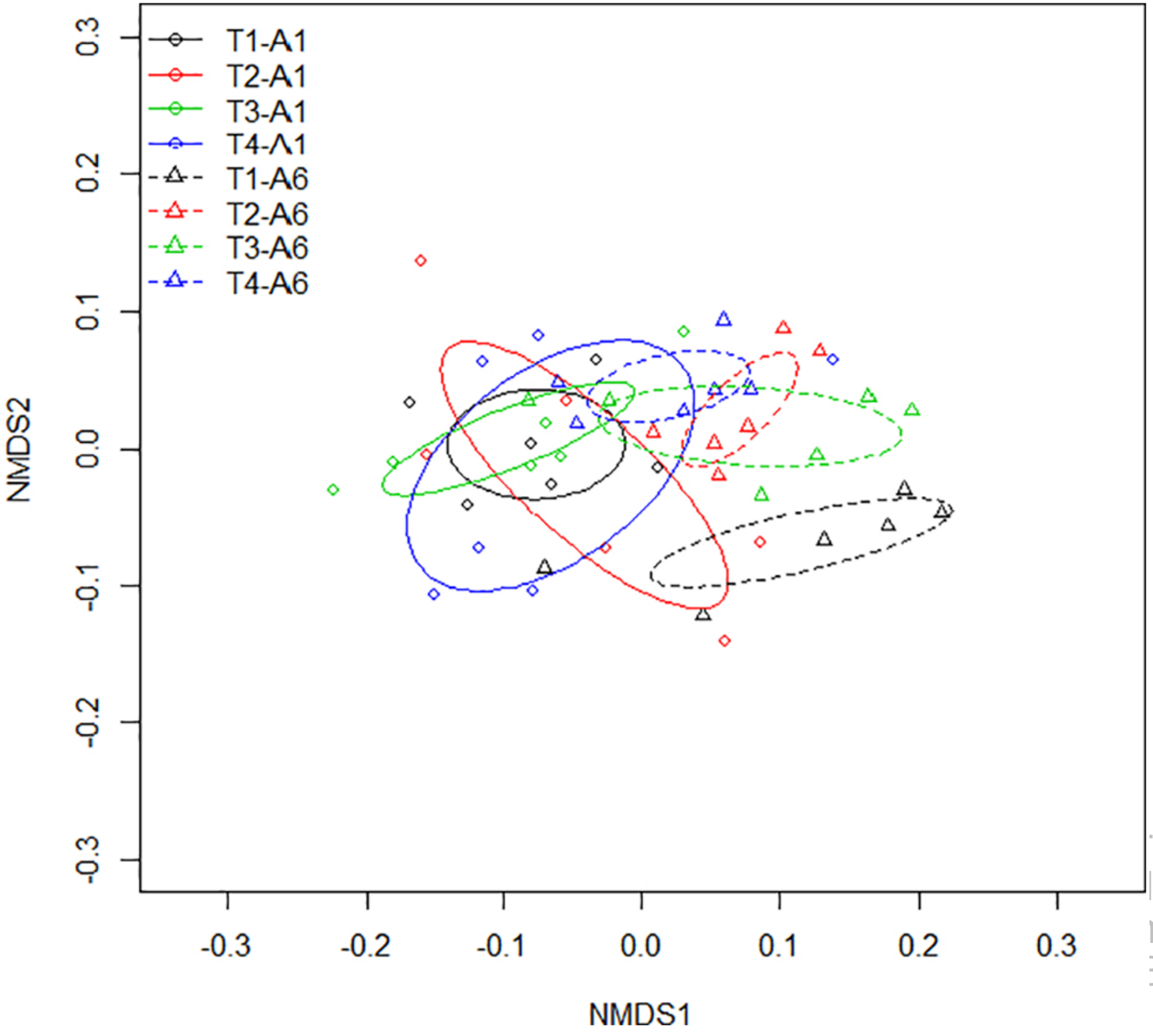
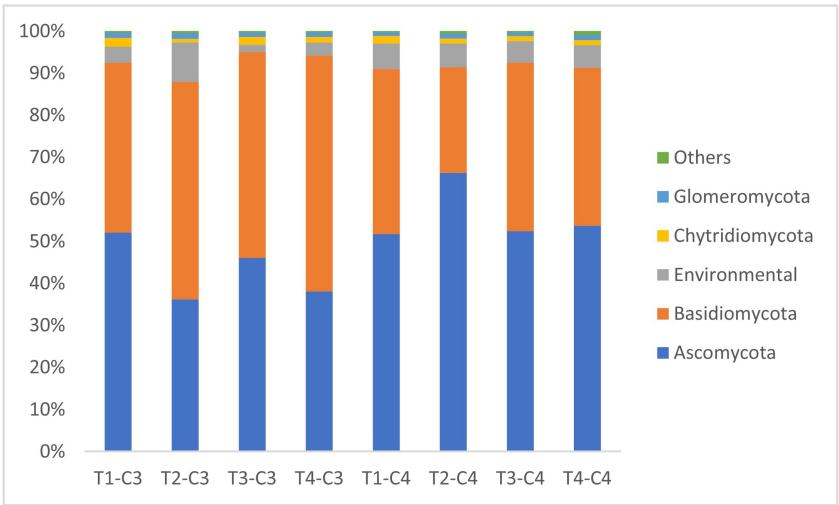

T1-A1
T2-A1
T3-A1
T4-A1
T1-A6
T2-A6
T3-A6
T4-A6
NMDS1
NMDS2
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3

Figure 2

(A)


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
T1-C3
T2-C3
T3-C3
T4-C3
T1-C4
T2-C4
T3-C4
T4-C4
Others
Glomeromycota
Chytridiomycota
Environmental
Basidiomycota
Ascomycota

(B)

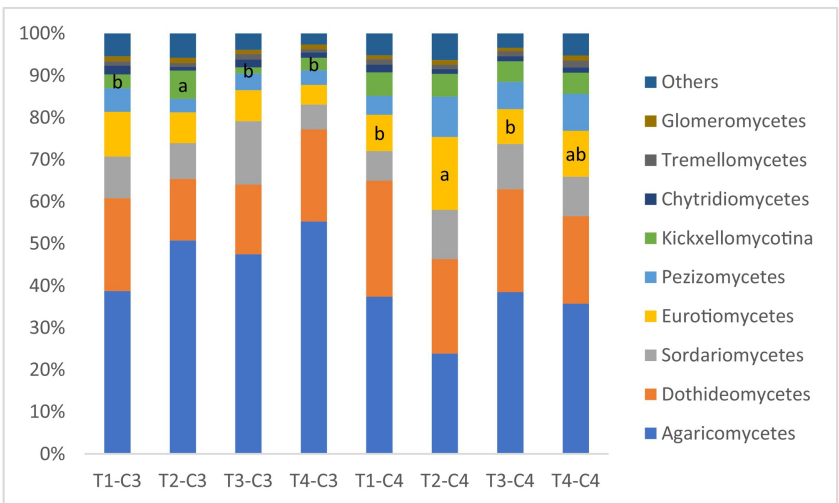

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
T1-C3
T2-C3
T3-C3
T4-C3
T1-C4
T2-C4
T3-C4
T4-C4
b
a
b
b
b
a
b
ab
Others
Glomeromycetes
Tremellomycetes
Chytridiomycetes
Kickxellomycotina
Pezizomycetes
Eurotiomycetes
Sordariomycetes
Dothideomycetes
Agaricomycetes

Figure 3

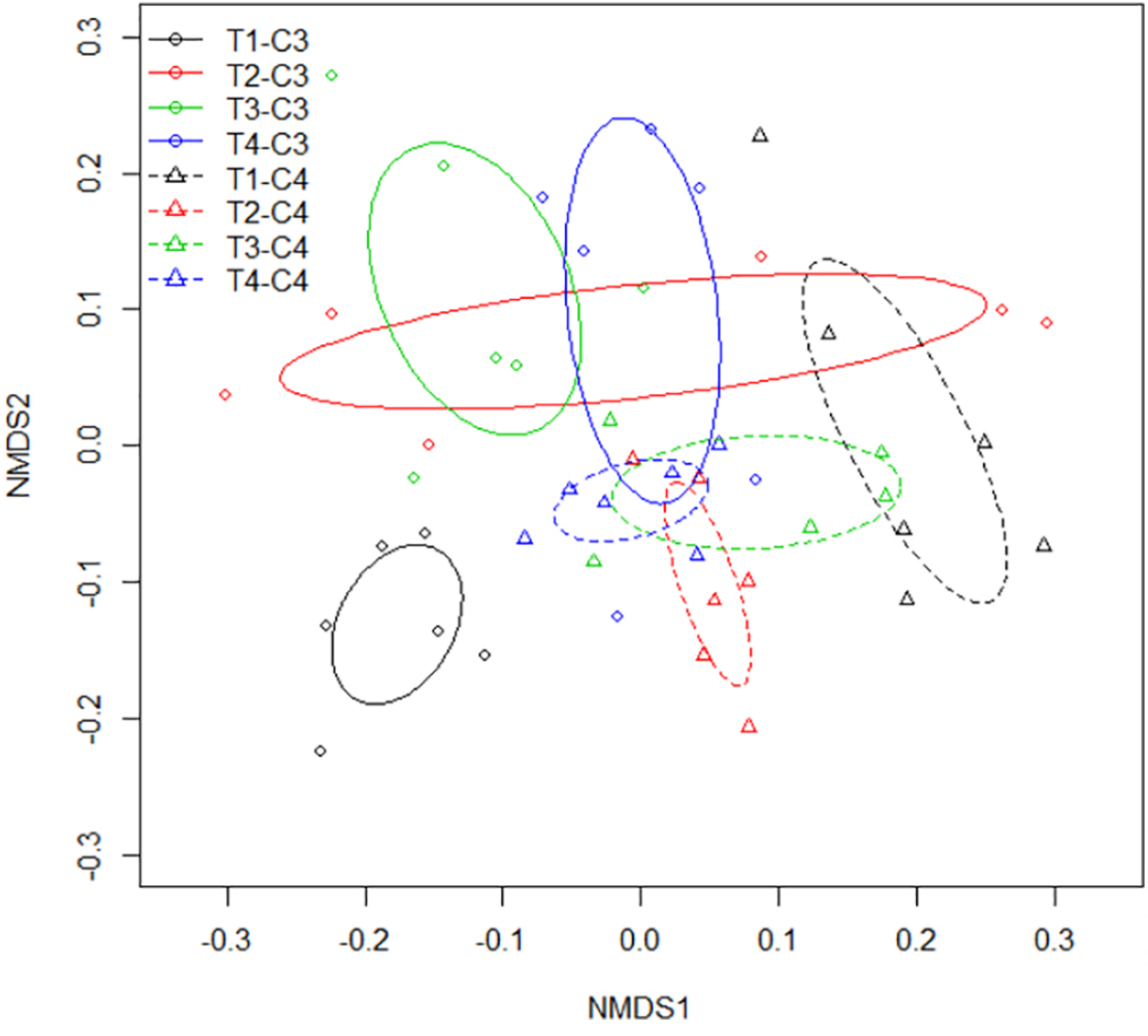

T1-C3
T2-C3
T3-C3
T4-C3
T1-C4
T2-C4
T3-C4
T4-C4
NMDS1
NMDS2
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3

Figure 4