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Running Title: SOIL BACTERIA COMMUNTIES UNDER SLASH AND BURN

Soil Bacteria Communities under Slash and Burn in Mozambique as Revealed by Metataxonomic Approach

ABSTRACT

The "slash and burn" system is a subsistence agronomical practice widely spread in tropical areas all over the world. This system has been deeply studied, especially for its impacts on agronomical aspects and soil physicochemical properties, while the knowledge on their microbial diversity is scarce. In the present study, for the first time the soil bacterial diversity of three locations from central Mozambique where "slash and burn" has been practiced with different duration of the forest fallow period (≈25, 35, and ≈50 years) has been elucidated through a metataxonomic approach. Bacterial communities were evaluated on genetic horizons of soils under charcoal kiln, crop field, and forest. The aim of this study was to examine the influence of spatial (location and land use), temporal (forest fallow period), and vertical (horizons) variations in selecting bacterial populations in relation to the physico-chemical properties of the soil. Metataxonomic analysis detected 25 different phyla whose distribution varied horizontally and vertically in relation to soil properties: pH, easily oxidizable organic carbon, total nitrogen, and available phosphorous, but also particle-size distribution and mineralogical composition. Such properties were strongly affected and altered by land use management; in particular, charcoal kilns showed better soil properties and the greatest differences in microbial community with respect to crop field and forest, which were quite similar. This might suggest the inability of a forest fallow period shorter than 50 years to improve soil fertility and induce changes in microbial community. The uncommon application of the pedologic approach for microbial evaluation has allowed detecting a clear separation in microbiota composition along the soil profile, with eutrophic bacteria mainly located in the A horizons, while oligotrophic bacteria abounded in the Bo horizons. Considering horizontal and vertical heterogeneity in the same study represent a novelty for bacteria metataxonomic analysis.

Key Words: 16S rRNA gene sequencing, agroforestry, land-use change, soil microbiota, soil physicochemical properties

INTRODUCTION

The agroforestry system known as "slash and burn" is largely practiced by smallholder farmers in tropical and subtropical regions (Mertz *et al.*, 2009a; FAO, 2015; Kukla *et al.*, 2018) and consists of occupying a piece of land, slashing, and burning vegetation in order to convert forest into agricultural fields (Gay-des-Combes, 2017b). During the conversion, some ephemeral charcoal kilns (3-6 per hectare) are arranged to produce charcoal for the family; thus, after 2-4 years of cultivation, the area is abandoned to natural reforestation until it will be slashed and burnt again after decades (Kabisa and Ncheengamwa, 2020). The forest fallow period is requested to allow soil fertility to recover before being further cultivated since no fertilizer is used in this farming system (Drexler, 2020). Once the fallow period lasted 50-100 years but, because of the demographic and economic changes over the last 4-5 decades, the cycle has been progressively shortened to one to a few decades (Jakovac *et al.*, 2016; Gay-des-Combes *et al.*, 2017a).

The slash and burn system often occurs on scarcely fertile soils (mainly Oxisols) and contributes accelerating their degradation (Styger *et al.*, 2007; Xu *et al.*, 2019). Therefore, slash and burn has been considered unsustainable since it favours deforestation, loss of biodiversity, soil depletion, and erosion (Kleinman *et al.*, 1995; Nath *et al.*, 2016; Gay-des-Combes *et al.*, 2017b). Soils subjected to this practice have been widely studied for their variable physicochemical properties and fertility levels (Juo and Manu, 1996; Thomaz *et al.*, 2014; Thomaz, 2018), whereas their microbial communities have been scarcely investigated (Nourou Sall *et al.*, 2006; Sul *et al.*, 2013; Saliou Sarr *et al.*, 2019). Microbial diversity and activity are very susceptible to ecosystem variations due to natural factors and/or anthropic activity, but the biotic functionality of the system is still hard to assess and understand (Nannipieri *et al.*, 2017). In detail, bacterial community diversity is strongly correlated with the nature of the parent material and soil physicochemical properties such as structure, texture, water holding capacity, nutrient availability, and organic matter content (Ulrich and Becker, 2006; Lauber *et al.*, 2008; Sofo *et al.*, 2019). Thus, to assess and understand the soil biotic functionality of the slash and burn system, it is mandatory to consider soil physicochemical properties and microbial diversity according to land use (spatial variation), duration of the forest fallow (temporal variation) and, within each soil, the nature of genetic horizons (vertical variation).

Based on these premises, we hypothesised that, notwithstanding centuries of slash and burn that could have homogenized all the system, bacterial community can differentiate horizontally (location and land use) or vertically (horizons). Therefore, the aims of the study were to evaluate the bacterial diversity through a metataxonomic approach in soils subjected to slash and burn and influenced by spatial (location and land use), temporal (forest fallow period), and vertical (horizons) variations correlated to the physicochemical properties of the soil. For testing this, we selected three locations of central Mozambique submitted to slash and burn where soil samples were collected under charcoal kiln, agricultural field, and forest (spatial variations). The locations were selected on the basis of the forests age, so to obtain a chronosequence driven by the duration of the forest fallow (temporal variation). The novelty of this research is that both horizontal and vertical heterogeneity was considered at once in the same study.

MATERIALS AND METHODS

Study areas

Agro-ecological and vegetation characterization

The zone selected for the study is part of the Manica Province, central Mozambique (Fig. S1, see Supplementary Material for Figure S1). Here, we selected three locations with high agricultural potential where slash and burn is very common and going on for centuries: Vanduzi, Sussundenga, and Macate (Fig. S1). Based on climatic conditions, soil type, elevation, and farming system, these districts are located in the Agro-Ecological Zone R4, which includes lands between 200 and 1000 m above sea level (Maria and Yost, 2006). The mean annual rainfall of the zone ranges from 1000 to 1200 mm, while the mean annual air temperature is ≈21 °C, with February as the warmest month (24.2 °C) and July as the coldest one (16.0 °C) (Climate-Data, 2019). The soil moisture regime is *aridic,* and the soil temperature regime is *thermic* (Soil Survey Staff, 2014). Following the Köppen-

Geiger updated climate classification, the climate of the zone is humid subtropical with a cool to mild season from April to September and a hot and humid season from October to March (Kottek *et al.*, 2006; Belda *et al.*, 2014). The geology of the zone is dominated by metamorphic rocks of the Mesoproterozoic Southern Irumide Belt (950--1060 Ma) litho-tectonic unit (Chaúque *et al.*, 2019). The predominant soil type of the zone belongs to the order of Oxisols, with low fertility and a strong erosion due to the topography of the terrain (Maria and Yost, 2006). The main food crops are maize (*Zea mais* L.), sorghum (*Sorghum vulgare* Pers), millet (*Panicum miliaceum* L.), and beans. At the three locations, the forest conditions were generally poor in terms of plant biodiversity. As witnessed by the presence of several charcoal kiln rests (even more than 20 per hectare), the forests have been growing up on abandoned crop fields forming the so called *miombo* biome. This latter is typical of tropical woodland (open forest) comprising savannas and shrublands made of sparse trees with a more or less thick grass understorey (Sitoe, 2004). The *miombo* was made of an upper stratum mainly composed of the leguminous trees *Brachystegia spiciformis* Benth., *Brachystegia tamarindoides* Benth., and *Julbernardia globiflora* (Benth.) Troupin, with an understorey composed of herbaceous species like *Themeda triandra* Forssk., *Panicum maximum* Jacq., *Hyparrhenia filipendula* (Hochst.) Stapf, and *Andropogon gayanus* Kunth. At Vanduzi there were also a few old mango trees (*Mangifera indica* L.), remainders of an abandoned mango orchard. After abandonment of the fields, a slight exploitation of the reforesting ranges was maintained because they represent the source of subsistence goods like timber, poles, firewood, foods, medicines, grazing, leaf litter, and game (Chidumayo *et al.*, 1996; Dewees *et al.*, 2011).

The studied slash and burn systems

- Vanduzi

Information on Vanduzi was obtained by interviewing local leaders and field owners. According to them, the charcoal kiln had been arranged four years before the survey The crop field was settled one year before the survey with an intercropping system of different varieties of banana tree (*Musa paradisiaca* L.), horse radish tree (*Moriga oleifera* Lam.), and sorghum. On the basis of the information gathered, the forest was ≈25 years old.

- Sussundenga

Information about Sussundenga was also obtained by interviewing the landowner. The charcoal kiln had been used in the year of survey, while the crop field had been cultivated with maize for two years. Detailed information about the age of the actual forests was obtained from the Sussundenga Research Station at the *Instituto de Investigação Agrária de Moçambique* (IIAM/CZC). Here, documents attest the field-adjacent forest was cut in 1982, consequently in 2017 it was 35 years old and a portion of this forest was cut again in February 2017 to be cultivated.

- Macate

Information about Macate was also obtained by interviewing local leaders and field owners. The charcoal kiln was 16 years old; the crop field had been consecutively cultivated with maize for 16 years, and the field-adjacent forest was ≈50 years old.

To resume, the land use chronosequence followed the order: at Vanduzi the field was 1-year old and the forest was ≈25 years old; at Sussundenga, the field was 2 years old and the forest was 35 years old; at Macate, the field was 16 years old and the forest was ≈50 years old. For charcoal kilns it was not possible to obtain an increasing order of age for the same sequence of locations being the kiln 4 years old at Vanduzi, less than 1 year at Sussundenga, and 16 years at Macate. To prove the age of the forests, being useless the counting of tree rings, we ascertained that the average tree diameters of the ubiquitous *Brachystegia spiciformis* trees of Macate (33 cm) was higher compared with that of Sussundenga (26 cm) and Vanduzi (16 cm) trees.

Study sites and soil sampling

In March 2017, in each area a geomorphological and soil survey was run in order to select the sampling sites. At each area we selected a rather flat area (plateau) with gentle slope (2-4%), with mostly Oxisols developed from similar metamorphic parent rocks: granitoid rock (possibly gneissic-granite) at Vanduzi and Sussundenga (Chaúque *et al.*, 2019; Wijnhoud, 1997), and a migmatitic paragneiss at Macate (Chaúque *et al.*, 2019). In all cases, each soil was characterized by two master horizons: a brownish A horizon (umbric) and a reddish Bo (oxic) horizon (Table S1, see Supplementary Material for Table S1). In each area, for any land use (charcoal kiln, agricultural field, and forest) we selected two representative sites with similar micro-topography and, for the forest, vegetation. Since Oxisols are very weathered soils and the mean temperature of the area slightly differ among seasons, to evaluate eventual differences in terms of bacterial community along the year, we chose to run two sampling campaigns following the most different agricultural seasons: crop end in March 2017 (Autumn) and field preparation for seeding in November 2017 (Spring). In the charcoal kilns the profiles were opened in the middle of their extension, while those in the agricultural fields were opened at ≈25 m from the border with forest. In this latter, profiles were opened at ≈1 m from the trunk of one of the biggest trees of *Brachystegia spiciformis*. The maximum distance among sampling sites was about 30 m at Sussundenga and Macate, while at Vanduzi forest and field sites were about 700 m distant. For each sampling campaign, the position where to dig the soil profiles was selected after opening several manual mini-pits and auger holes. Once excavated, each profile was described according to Schoeneberger *et al.* (2012) and sampled by genetic horizons (A and Bo). A large amount of sample (about 4 kg) was collected from each horizon. The amount of profiles excavated was 9 (3 land uses x 3 locations) in March and 9 in November, for a total of 18 profiles and 36 horizon samples.

Samples were collected in sterilized polyethylene bags and stored at ≈4 °C inside a portable fridge during the field operations. Once in the laboratory, the samples were air-dried and then passed through a sieve (2 mm mesh) to remove the skeletal particles and coarse vegetal residues.

Physicochemical and mineralogical analyses

The pH was determined potentiometrically in H_2O after one night of solid:liquid contact, using a combined glass-calomel electrode immersed into the suspension (1:2.5 solid:liquid ratio). Particle-size distribution was determined after dissolution of organic cements by NaClO at pH 9 (Lavkulich and Wiens, 1970). Sand (2-0.05 mm) was recovered by wet sieving, while silt was separated from clay by sedimentation maintaining the columns at 19-20 °C. The amount of easily oxidizable organic carbon (EOOC) was estimated by the Walkley-Black method by K-dichromate digestion without heating (Nelson and Sommers, 1996). The total nitrogen (N) content was determined by the semi-micro Kjeldahl method and potentially plant-available phosphorous (P) was determined according to Olsen *et al.* (1954). The mineralogical assemblage was assessed by X-ray diffractometry on manually compressed powdered samples by using a Philips PW 1830 diffractometer (Fe-filtered Co Kα1 radiation, 35 kV and 25 mA). Minerals were identified on the basis of their characteristic peaks (Brindley and Brown, 1980; Dixon and Schulze, 2002), while a semi-quantitative mineralogical composition was obtained by estimating the area of the diagnostic peaks by multiplying the peak height by its width at half-height.

Microbial DNA extraction and sequencing

Total microbial DNA was extracted from 100 mg of each soil sample using the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, Inc., Georgia, USA) following the manufacturer's instruction. DNA-based analysis was preferred to mRNA analysis because in complex matrices like soil, RNA can be rapidly degraded by RNAases, with a consequent less reliability of the soil microbial composition (Nannipieri *et al.*, 2020). The extracted DNA was quantified by using the Qubit dsHS kit (Thermo Fisher, Milan, Italy) and standardized at 25 ng μL^{-1}. One µl of each DNA suspension was used as template for PCR amplification by using primers 16SF (5’-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3’) and 16SR (5’-

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3’) spanning the V3-V4 region of the 16S rRNA gene following the procedure described by Klindworth *et al.* (2013), and a negative control was included in the PCR reactions by replacing the DNA solution with water. The PCR amplicons were purified according to the Illumina metagenomic pipeline instructions. Briefly amplicons were cleaned using the Agencourt AMPure kit (Beckman coulter, Brea, USA) according to the manufacturer’s instructions; subsequently, DNA concentrations of the amplicons were determined using the Quant-iT PicoGreen dsDNA kit (Invitrogen Life Technology) following the manufacturer’s instructions. In order to ensure the absence of primer dimers and to assay the purity, the quality of generated amplicon libraries was evaluated by a Bioanalyzer 2100 (Agilent, Palo Alto, CA, USA) using the High Sensitivity DNA Kit (Agilent). Following the quantitation, cleaned amplicons were mixed and combined in equimolar ratios. Paired-end sequencing (2x250 bp) using the Illumina MiSeq system (Illumina, San Diego, USA) were carried out at the Sequencing Platforms of the Fondazione Edmund Mach (FEM, San Michele a/Adige, Italy).

Bioinformatics analysis

After sequencing, raw reads were merged using Flash software (Magoc and Salzberg, 2011) and analyzed with QIIME 1.9.0 software (Caporaso *et al*., 2010); the detailed pipeline was described by Ferrocino *et al*. (2017). The USEARCH version 11 software (Edgar *et al.*, 2011) was adopted for chimera filtering, against the 16S reference databases. Centroid sequences of each operational taxonomic unit (OTU) cluster (at 97 % of similarity) by using UCLUST (Edgar, 2010) were mapped against Greengenes 16S rRNA gene database by means of the RDP Classifier, with a minimum confidence score of 0.80 (Wang *et al.*, 2007).

Centroids sequence were manually blasted to check the taxonomic identification. To avoid biases due to the different sequencing depths, OTU tables generated through QIIME were rarefied at the lowest number of reads and showed the highest taxonomy resolution that was reached. Alpha diversity index (Shannon and Chao1) were calculated by the QIIME alpha diversity script. The data generated by sequencing were deposited in the NCBI Sequence Read Archive (SRA) and are available under the Bioprojects Accession Number-PRJNA550507 for replicate 1 and PRJNA631872 (biosamples accession number from SAMN14895357 to SAMN14895411) for replicate 2.

Statistical treatment of the data

RStudio program (vv 1.3.1093) (RStudio Team, 2020) was used for statistical analysis. By ANOVA we assessed that the results obtained from the analyses of samples collected in the two sampling campaigns in terms of physicochemical properties (pH, particle-size distribution, EOOC, total N, and available P) did not differ (Table S2, see Supplementary Material for Table S2, $P > 0.05$). Because of this, the samples collected in the two sampling campaigns were considered as replicates and ANOVA was run to test significant differences for sampling locations (Vanduzi, Sussundenga, and Macate), land uses (charcoal kiln, crop field, and forest), and horizons (A and Bo) (Table S3, see Supplementary Material for Table S3, $P > 0.05$). To apply the ANOVA, we previously verified the normal distribution of the data and the equal variances. The improvement of the assumption to normality and homoscedasticity was verified on residuals by the Shapiro-Wilk statistical test (*stats* R package) (R Core team, 2013) and by Levene's test (*car* R package) (Fox and Weisberg, 2019), both at 5 % of significance level. When data were non-normally distributed, each numerical variable was transformed by the Box-Cox procedure (Meloun *et al*., 2005). If the transformed data were normally distributed, a post-hoc Tukey's Honest Significant Difference (HSD) test with $P \leq 0.05$ was used to compare the means. When normality was not respected, the Kruskal-Wallis test was applied to assess if the differences were significant. In case of heteroscedasticity, the Welch one-way ANOVA test was performed. ANOVA tests were deemed significant when $P \leq 0.05$. In case of heteroscedasticity and non-normality, we run the Friedman test (*rstatix* package) (Kassambara, 2020) combined with Kendall's W to measure the Friedman test effect size and pairwise Wilcoxon signed-rank tests. The arithmetic means and relative standard deviations for physicochemical properties (Tables I, II, and III) and OTUs were calculated for sampling locations (n=12), total land use (n=12), land use of each area (n=4), total horizons (n=18), and horizon of each site (n=6). In doing this, technical replicates were treated as experimental replicates, as it often occurs in ecosystem scale experiments (Osburn *et al*., 2019). Non-parametric pairwise Wilcoxon tests were used when appropriate to determine the significant differences of OTU abundance and alpha diversity. Spearman correlation analysis between OTUs and physicochemical properties was performed through the *psyc* package (Revelle, 2021) and plotted by using the function *corrplot* of RStudio program (vv 1.3.1093). The *P* values were adjusted for multiple testing using the Benjamini-Hochberg procedure, which assesses the false discovery rate (FDR).

RESULTS

Soil morphology

In all locations (Vanduzi, Sussundenga, and Macate), the soils were Oxisols due the presence of diagnostic Bo horizons (Soil Survey Staff, 2014) (Table S1, see Supplementary Material for Table S1). The A horizons under charcoal kiln showed a charcoal content always higher than 30 %, to become ≈1 % in the crop fields and to be absent under forests. The Bo horizons showed a reddish colour and, especially at Vanduzi, they displayed a relatively high content of Fe-Mn-oxides (≈5 %). In general, both A and Bo horizons presented a good degree of aggregation, with the presence of sub-angular and angular blocks generally coarser in the A than in the Bo horizons (Table S1, see Supplementary Material for Table S1). The good state of aggregation, the coarse texture (from loamy sand to sandy loam), and the absence of any redoximorphic feature indicated these soils are well-drained and, consequently, with low water-holding capacity (Agrawal, 1991; Suzuki *et al*., 2007).

Microbiota diversity

The relative abundances of bacterial taxa were examined at phylum rank to determine whether there were differences at the scale of location, land use, or horizon (Fig. 1). In total, 25 different phyla approximately totaled 96.5 % of the bacterial pool, with Actinobacteria (22 %), Proteobacteria (19

%), Chloroflexi (17 %), Firmicutes (15 %), Planctomycetes (10 %), Acidobacteria (5 %), Verrucomicrobia (3 %), Nitrospirae (2 %), and AD3 (1 %) as the most representative by considering the average relative abundance for all samples. Regarding the minor OTUs fraction, Bacteroidetes, Gemmatimonadetes, Armatimonadetes, Cyanobacteria, GAL15, Chlamydiae, TM7, OD1, and Crenarchaeota (relative abundance between 0.1 and 1 %) represented about 3.4 % of the total bacterial community. At Vanduzi and Macate, for the alpha diversity value we observed the highest number of OTUs and a higher richness (Chao1 and Shannon index) in the A horizons than in the Bo horizons (data not shown, FDR < 0.05), while no difference was observed among the land uses. Conversely, at Sussundenga, the alpha diversity value showed no difference between forest and crop field, which displayed a higher number of OTUs and a higher richness than the charcoal kiln (FDR < 0.05, data not shown); no difference was observed between the horizons.

Location effect

The soils from Vanduzi showed the highest pH and the highest content of available P, while EOOC and total N were the greatest at Macate (Table I). Particle-size distribution was always dominated by the sand fraction and mineralogically by quartz, with minor contents of clay minerals and plagioclases. Regarding OTUs association, Vanduzi displayed the highest abundance of Actinobacteria, Firmicutes, Nitrospirae, and WS3; Sussundenga the highest for Firmicutes, Cyanobacteria, and WS4; Macate showed the highest presence of Chloroflexi, Planctomycetes, Verrucomicrobia, and WS3 (Fig. S2, see Supplementary Material for Figure S2, FDR < 0.05).

Distributions at a low taxonomical rank were presented in Fig. S3 (see Supplementary Material for Figure S3) (FDR < 0.05), with the highest abundances summarized in Appendix 1. The results of bacteria diversity at phylum rank among locations were schematically synthetized in Fig. 2.

Land use effect

The soils under charcoal kiln had the highest pH, while both charcoal kilns and forests displayed the largest available P content (Table II). In all land uses, sand was the most represented separate and the mineralogy was dominated by quartz. In the different locations, the highest pH values were observed for the charcoal kilns of Vanduzi and Sussundenga (Table II). The largest available P content occurred at Macate for charcoal kilns, while at Sussundenga and Vanduzi there was a scarce dotation of this nutrient (Table II). Charcoal kilns showed the highest abundance of Gemmatimonadetes and OD1 and the lowest abundance of Armatimonadetes and Tenericutes. An opposite trend was observed for the same taxa in soils under crop field and forest (Fig. S4, see Supplementary Material for Figure S4, FDR < 0.05). Looking at each location, soils under charcoal kiln at Vanduzi abounded in Firmicutes, while at Sussundenga showed the lowest abundance of Planctomycetes (Fig. S5, see Supplementary Material for Figure S5, FDR < 0.05). At Macate, the soils under charcoal kiln showed the lowest abundance of Armatimonadetes, while those under forest showed the lowest abundance of Chlamydiae (Fig. S5, FDR < 0.05).

At low taxonomical rank, differences of bacterial distribution were displayed in Fig. S3 (FDR < 0.05), and briefly reported in Appendix 1, while differences at phylum level among land uses, and among land uses within location were synthetized in Figs. 2 and 3, respectively.

Horizon effect

As a whole, the pH and the contents of EOOC, total N, and available P were higher in the A compared with the Bo horizons, while, as expected for Oxisols, the clay content was much larger in the Bo than in the A horizons (Table III, $P < 0.05$). In all the locations, EOOC and total N were the highest in the A horizons. At Sussundenga and Macate the sand content was the highest in the A horizon, while the clay abounded in the Bo horizon. Only at Sussundenga the available P abounded in the A horizons (Table III, $P < 0.05$). Mineralogical assemblage was similar in all situations, with quartz as the most abundant mineral, always higher in the A than in the Bo horizons, while clay minerals were always higher in the Bo than in the A horizons (Table III). With respect to soil use, in the charcoal kilns only the pH values were higher in the A than in the Bo horizons. In the crop fields, EOOC and available P showed the highest contents in the A horizons, while in the forests EOOC and total N were the largest in the A horizons.

By comparing the OTUs composition, the A horizons displayed the largest quantities of Actinobacteria, Planctomycetes, Verrucomicrobia, Bacteroidetes, Gemmatimonadetes, and TM7, whereas the Bo horizons displayed the highest abundance of AD3, GAL15, Thermi, and WPS−2 (Fig. S6, see Supplementary Material for Figure S6, FDR < 0.05). At Vanduzi, Verrucomicrobia and TM7 were found to be the most abundant taxa in the A horizons, while Proteobacteria, Nitrospirae, AD3, and GAL15 were mainly associated with the Bo horizons (Fig. S7, see Supplementary Material for Figure S7, FDR < 0.05). At Sussundenga, Actinobacteria and Verrucomicrobia were predominant in the A horizons, while AD3 and GAL15 abounded in the Bo horizons (Fig. S8, see Supplementary Material for Figure S8, FDR < 0.05). At Macate, Actinobacteria and WS3 abounded in the A horizons, while AD3 and GAL15 predominated in the Bo horizons (Fig. S9, see Supplementary Material for Figure S9, FDR < 0.05). Considering the soil horizons under different land use, the Bo horizons under charcoal kiln was dominated by AD3 and GAL15 (Fig. S10, see Supplementary Material for Figure S10, FDR < 0.05). Under crop field, the A horizons were characterized by Actinobacteria, Bacteroidetes, Gemmatimonadetes, and TM7, while the Bo horizons were dominated by AD3 and GAL15 (Fig. S11, see Supplementary Material for Figure S11, FDR < 0.05). Under forest, the A horizons showed the highest abundance of Planctomycetes and Verrucomicrobia, with the Bo horizons dominated by AD3, Cyanobacteria, GAL15, and Thermi (Fig. S12, see Supplementary Material for Figure S12, FDR < 0.05).

At a low taxonomical rank, differences were displayed in Fig. S3 (FDR < 0.05), and details about the highest abundances between A and Bo horizons within locations were summarized in Appendix 2. The bacteria diversity at phylum rank between A and Bo horizons, and between horizons within location was synthetized in Figs. 2 and 3, respectively.

Correlation between microbiota and physicochemical properties

By plotting the correlation between OTUs of the most represented phyla and the soil physicochemical properties (Fig. S13, see Supplementary Material for Figure S13, FDR < 0.05), we observed that the presence of Actinobacteria was positively associated with available P, while Chloroflexi was directly associated with clay and inversely with sand, available P, and pH. Firmicutes were positively associated with pH and sand but inversely correlated with total N. Planctomycetes was negatively associated with pH and, together with Verrucomicrobia, they were positively correlated with EOOC, total N, and silt. Armatimonadetes and AD3 resulted negatively correlated with available P and sand, but positively correlated with clay. Bacteroidetes, Gemmatimonadetes, and TM7 were directly associated with pH and available P. GAL15 displayed the highest negative correlation with pH, EOOC, available P, and sand, and were positively correlated with clay, while OD1 displayed the opposite correlations (FDR < 0.05).

DISCUSSION

Location effect

The three locations differed in microbial community abundances for several taxa. In detail, Actinobacteria phylum (among which *Rubrobacteraceae*, *Streptomycetaceae*, and *Streptosporangiaceae* were the most abundant families and Micrococcales the most abundant order) was the dominant in the soils of Vanduzi. Actinobacteria phylum has been widely reported for soils under various environmental conditions, including Antarctica and Sahara (e.g., Saker *et al*., 2015; Tytgat *et al*., 2016); it is probably the wide adaptability of the species belonging to this phylum the reason of its abundance in the soils of Vanduzi. Araujo *et al*. (2020) found that some Actinobacteria taxa abounded in soils near to neutral pH, including *Rubrobacter* genus belonging to *Rubrobacteraceae* family. Instead, Koyama *et al*. (2014) reported a reduction of Actinobateria in soils enriched of N, while Prada Salcedo *et al.* (2014) found that some Actinobacteria strains can solubilize both calcium phosphate and Al-phosphate in acid soils, making P available in solution. Correlation plot of Fig. S13 showed that Actinobacteria was positively correlated with the available P but, as in the case of Vanduzi, also with the highest pH values and the lowest total N contents. At Vanduzi there was also the highest presence of Nitrospirae, specifically of the Nitrospirales order. Vipindas *et al.* (2020) described Nitrospirae as chemolithoautotrophic bacteria mainly involved in N mineralization, in particular in the oxidation of nitrite to nitrate. In fact, Wang *et al*. (2018) reported that the nitrate addition to soil resulted in the decline of Nitrospirae and of the nitrification activity. In addition, Zhou *et al*. (2015) associated a high presence of Nitrospirae to soils with neutral pH and not fertilized with N and P. It is therefore conceivable that bacteria of the Nitrospirae group abound in scarcely fertile soils where they play an important role producing nitrate by nitrite oxidation.

Sussundenga soils were characterized by the dominance of Cyanobacteria and WS4. Cyanobacteria abounded in the Sussundenga soils, where there was the largest quartz content, but they were scarce at Macate, where quartz was in the lowest quantity. The fact that the different distribution of quartz may influence Cyanobacteria abundances was ascribed to the adaptation of these bacteria to arid conditions (Lacap-Bugler *et al*., 2017), which are well-expressed at the surface of grain quartz, one of the less hydrophilic silicates in soil because of its lack of isomorphic substitutions (Tarasevich *et al*., 2002).

At Macate, soils showed the highest presence of Chloroflexi, Verrucomicrobia (among which the family *Chthoniobacteraceae*) and Planctomycetes (with the family *Gemmataceae*). Various studies have reported that Chloroflexi are involved in the organic matter decomposition and, consequently in the C and N cycling (e.g., Hug *et al*., 2013; Ibrahim *et al*., 2020). Chloroflexi abounded at Macate, where there were the highest amounts of EOOC and total N, even though this correlation was not statistically significant. Instead, at Macate, Verrucomicrobia were positively correlated with the contents of EOOC, total N, and silt, and the correlations were statistically significant. Similar results were reported by Buckley and Schmidt (2001), who found a positive correlation between

Verrucomicrobia and soil organic carbon, total N, and soil moisture. Also, Planctomycetes are directly correlated with EOOC, total N and silt, but inversely with pH. Zhao *et al*. (2018) also observed a significant correlation between soil organic carbon and Planctomycetes abundance. Firmicutes, represented in large amount by *Paenibacillaceae* and *Bacillaceae* families, abounded at Vanduzi and Sussundenga and showed a positive correlation with pH and sand content, but negative with total N. Vos *et al*. (2011) described *Paenibacillaceae* as mesophilic and termophilic, but also as neutrophilic and alkaliphilic bacteria. Since the soils at Vanduzi and Sussundenga displayed pH values closed to neutrality and the prevalence of sand particles that favour high temperatures transmission at depth in case of heat flow (Abu-Hamdeh and Reeder, 2000), we may suppose that Firmicutes proliferated in these soils because of these physicochemical properties.

Land use effect

As expected, charcoal kilns represented a unique ecosystem, with peculiar microbial community if compared to crop field and forest like, for example, a higher abundance of OD1 and Gemmatimonadetes. Following the report of Coomes *et al*. (2017), who also found Gemmatimonadetes in soils under charcoal kiln, and the correlations reported in Fig. S13, we ascribed the presence of these bacteria in our charcoal kiln soils to the relatively large content of available P and relatively high pH values. A similar distribution is valid for OD1, which were largely abundant in charcoal kiln soils and resulted positively correlated with pH, available P, and sand, but negatively with clay (Fig. S13). Since pH showed the most significant variations between charcoal kiln soils and crop field/forest soils, we suggest OD1 bacteria are mainly influenced by soil reaction rather than the other correlated properties. On the contrary, Armatimonadetes were more abundant in crop field and forest soils than in charcoal kilns and showed a positive correlation with clay but a negative correlation with available P and sand. These results suggested a predilection of Armatimonadetes for soils scarce in available P. Moreover, Armatimonadetes have been found to be negatively correlated with pH but positively correlated with moisture (Tytgat *et al*., 2016), indicating that soils under charcoal kiln are less preferred by the species of this phylum because of the large content of charcoal, which commonly supplies soluble P to soil (Rafael *et al.*, 2020) and reduce soil moisture due to the overheating consequent to the dark colour. Tenericutes mainly abounded in forest soil, with no significant correlation to physicochemical properties. Lanc *et al.* (2013) reported that Tenericutes were particularly abundant in soils from Brazilian semi-arid forests during the rainy season. Although more investigation on this phylum is needed, we suppose Tenericutes proliferation is favoured by the presence of relatively high soil organic matter content and moisture, conditions that occurred in our forest soils (Scott and Kleb, 1996).

A few microbial differences among land uses were restricted to some locations. For example, at Vanduzi, Firmicutes abounded in the charcoal kiln area possibly because of i) the high pH values due to the alkalinising effect of ash and biochar (Fidel *et al.*, 2017) and ii) the sand content that favours the penetration of high temperatures in soil during charcoal production. As a support of this, Firmicutes belonging to the Bacillales order abound in soils after wildfire and burning treatments (Smith *et al.*, 2008; Sul *et al.*, 2013), while bacteria of the *Bacillaceae* family include spore-forming species able to resist the extremely high temperature (Battistuzzi and Hedges, 2009; Galperin, 2013). At Sussundenga, Planctomycetes showed the lowest abundance in the charcoal kiln soil. Yang *et al.* (2020) and Jenkins *et al.* (2017) observed a decrease of Planctomycetes when soil pH increased following fire or biochar addition. As a demonstration of this, Navarrete *et al.* (2015) reported a higher abundance of Planctomycetes in forest soils with low pH. Our results agreed with the above-mentioned studies, being the soil pH at Sussundenga the highest in the charcoal kiln soils and the relation between Planctomycetes and pH negative (Fig. S13). At Macate, differences were detected for Armatimonadetes, the least abundant phylum in charcoal kiln soils, and Chlamydiae, the least abundant in forest soils. We ascribed Chlamydiae distribution to the behaviour of some Chlamydiae bacteria as pathogens of arthropods (Horn *et al.*, 2004; Wagner and Horn, 2006), including soil isopods like woodlouse (Collingro *et al.*, 2020). Specifically, soil isopods are Chlamydiae’s soil dwelling that generally feed of decaying organic matter (Saska, 2008) including corn litter (Johnson *et al.*, 2012), which was the major remainders of cultivation in the Macate fields.

Horizon effect

The horizon effect has marked a clear separation of the physicochemical properties and microbiota. The higher abundance of Actinobacteria in the A than in the Bo horizons appeared correlated with the highest contents of available P, EOOC, and total N at Sussundenga and Macate and in the crop fields. Although Actinobacteria have been associated to soils with low organic carbon content (Sul *et al.*, 2013; Fu *et al.*, 2019), other studies demonstrated that their optimum growth substrate is represented by soils rich in organic matter and N, with neutral pH, good soil aeration, and moderate temperature (e.g., Tang *et al.*, 2016; Liu *et al.*, 2017; Dai *et al.*, 2018), conditions that mainly attained in the A horizons (Table III). In the soils at Vanduzi, Proteobacteria were the most abundant in the Bo horizons, probably because these horizons are particularly rich of Fe-Mn nodules (≈5%), and this property could have favoured bacteria of this phylum being Proteobacteria able to catalyse the Fe-oxidation reactions (Hedrich *et al.*, 2011). Planctomycetes (among which the *Phycisphaerae* family) abounded in the A horizons under forest, probably because species belonging to this phylum are involved in carbon and N turnover (Fuerst and Sagulenko, 2011). Like Planctomycetes, Verrucomicrobia (in detail *Chthoniobacteraceae* family and Pedosphaerales order) abounded in the A horizons especially at Vanduzi and Sussundenga, and under forest. In our case, Verrucomicrobia were largely present concomitant with the highest quantities of EOOC, total N, and available P. At this regard, Sangwan *et al.* (2004) and O'Brien *et al.* (2016) recognized *Chthoniobacteraceae* as utilizers of saccharides derived from plant biomass or engaged in symbiosis with soil nematodes. Instead, Pedosphaerales were found by Bach *et al.* (2018) to abound in large macroaggregates rather than in microaggregates. Thus, the large abundance of Verrucomicrobia in the A horizons was ascribed to their relatively higher organic matter content, which fairly includes sugars, and the generalized coarser structure.

Bacteroidetes (among which the *Chitinophagaceae* family), Gemmatimonadetes, and TM7 abounded in the A horizons, particularly of the crop fields, and were positively correlated with pH values and available P (Fig. S13). As reported by Wolińska *et al.* (2017), Bacteroidetes are involved in the organic matter cycle and, joined with Gemmatimonadetes, they have been found associated with the degradation of complex organic polymers (Chaudhry *et al.*, 2012). In particular, *Chitinophagaceae* mainly colonize the rhizosphere rather than the bulk soil (Madhaiyan *et al.*, 2015) and have been found to be positively correlated with the C:N ratio (Dennis *et al.*, 2019). Furthermore, Zhou *et al.* (2015) reported of positive correlations between TM7 and the contents of total N, nitrates, ammonium, and soil organic matter. All this considering, the abundance of Bacteroidetes, Gemmatimonadetes, and TM7 in the A horizons was ascribed to a predilection for complex organic substrates with an incipient decaying of organic matter.

AD3 and GAL15 were more abundant in the Bo than in the A horizons. Looking at the correlation plot (Fig. S13), AD3 was directly correlated with clay and inversely correlated with available P and sand. This distribution was probably due to the general properties of Oxisols, which showed an increase of acidity and clay with increasing depth. As a support to this, Mesa *et al.* (2017) found abundant AD3 in biofilms and sediments of acid mine drainage. Also GAL15 resulted to be directly correlated with clay and inversely correlated with available P and sand, but also with pH and EOOC. Since the members of these taxa seemed to prefer oligotrophic habitats (e.g., Li *et al.*, 2020; Liu *et al.*, 2020), it is conceivable they diffused in the Bo rather than in the A horizons. Also the phyla Thermi and WPS-2 abounded in the Bo horizons. Since Thermi were found in hypolithic communities of Taklimakan Desert in China (Lacap-Bugler *et al.*, 2017) and WPS-2 were more abundant in unfertilized soils and in oil palm plantation than in primary and regenerated forests (Wood *et al.*, 2017), we hypothesized that the members of these phyla prefer oligotrophic soil conditions, and consequently mainly inhabit the Bo horizons.

Only at Vanduzi, Proteobacteria and Nitrospirae showed a large abundance in the Bo horizons, with no significant correlation with the soil physicochemical properties (Fig. S13). Similar conditions were found by Hedrich *et al*. (2011), who ascribed to Proteobacteria a high grade of adaptation and the peculiarity to survive with iron-oxidizing forms in presence of oxygen and preferably with neutral to acid pH. The diffusion of Nitrospirae in the Bo horizons fitted with their preference to colonize soil compartments with neutral pH and scarce N.

CONCLUSIONS

Oxisols submitted to slash and burn differed in terms of spatial and vertical changes for their bacterial diversity. Our study suggests that bacteria were affected by soil physicochemical properties reliant on both soil genesis and human activities. Actinobacteria, Nitrospirae, WS3, Chloroflexi, Verrucomicrobia, Planctomycetes, and Firmicutes varied among locations in conjunction with different pHs and nutrients availability, while Cyanobacteria abundance seemed to depend on quartz content. Also land use determined a strong selection of microbiota in particular under charcoal kilns, where soil physicochemical properties have been changed by temperature and addition of charcoal and ash. Gemmatimonadetes, OD1, Armatimonadetes, Firmicutes, and Planctomycetes were also affected by the presence of the charcoal kiln while, Tenericutes and Chlamydiae proliferated, respectively, in the soils under forest for the high organic matter content and moisture and in the soil under crop field at Macate because of mulching practices. Except for Tenericutes, no other significant difference in terms of taxa abundances and physicochemical properties were encountered between forests and crop fields, despite the forest fallow might let suppose a considerable soil fertility restoration – with consequent microbiota change – over time. Remarkable results were found along the soil profiles, confirming the importance of genetic horizons in determining microbiota composition. Actinobacteria, Planctomycetes, Verrucomicrobia, Bacteroidetes, Gemmatimonadetes, TM7, and WS3 were abundant in the A horizons, suggesting a predilection for eutrophic conditions, while AD3, GAL 15, Thermi, WPS-2, Proteobacteria, and Nitrospirae abounded in oligotrophic Bo horizons. These results allowed us recognizing two main groups of bacteria: those strongly affected by spatial, temporal, and vertical variations, and those homogeneously distributed in soil independently from the physicochemical variations among horizons.

Our findings contribute to improving the knowledge on spatial, temporal, and vertical soil bacteria diversity, and dependence of this latter from physicochemical properties in Oxisols. More studies are needed to better disclose the relationships between microbiota and soil properties.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article ca be found in the online version.

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