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Influence of *Acacia mangium* on Soil Fertility and Bacterial Community in *Eucalyptus* Plantations in the Congolese Coastal Plains

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Abstract: Productivity and sustainability of tropical forest plantations greatly rely on regulation of ecosystem functioning and nutrient cycling, i.e., the link between plant growth, nutrient availability, and the microbial community structure. So far, these interactions have never been evaluated in the *Acacia* and *Eucalyptus* forest planted on infertile soils in the Congolese coastal plains. In the present work, the soil bacterial community has been investigated by metabarcoding of the 16S rRNA bacterial gene in different stands of monoculture and mixed-species plantation to evaluate the potential of nitrogen-fixing trees on nutrient and bacterial structure. At the phylum level, the soil bacterial community was dominated by *Actinobacteria*, followed by *Proteobacteria*, *Firmicutes*, and *Acidobacteria*. A principal coordinate analysis revealed that bacterial communities from pure *Eucalyptus*, compared to those from plantations containing *Acacia* in pure and mixed-species stands, showed different community composition (beta-diversity). Regardless of the large variability of the studied soils, the prevalence of *Firmicutes* phylum, and lower bacterial richness and phylogenic diversity were reported in stands containing *Acacia* relative to the pure *Eucalyptus*. Distance-based redundancy analysis revealed a positive correlation of available phosphorus (P) and carbon/nitrogen (C/N) ratio with bacterial community structure. However, the Spearman correlation test revealed a broad correlation between the relative abundance of bacterial taxa and soil attributes, in particular with sulfur (S) and carbon (C), suggesting the important role of soil bacterial community in nutrient cycling in this type of forest management. Concerning mixed plantations, a shift in bacterial community structure was observed, probably linked to other changes, i.e., improvement in soil fertility (enhanced P and C dynamics in forest floor and soil, and increase in soil N status), and C sequestration in both soil and stand wood biomass with the great potential impact to mitigate climate change. Overall, our findings highlight the role of soil attributes, especially C, S, available P, and C/N ratio at a lesser extent, in driving the soil bacterial community in mixed-species plantations and its potential to improve soil fertility and to sustain *Eucalyptus* plantations established on the infertile and sandy soils of the Congolese coastal plains.

Keywords: nutrient-poor soil; soil phosphorus; soil fertility; *Acacia mangium*; *Eucalyptus*; soil bacterial community; microbial ecology; belowground biodiversity; ecosystem functions

1. Introduction

Introducing nitrogen-fixing trees (NFTs) such as *Acacia mangium* in *Eucalyptus* fast-growing plantations improves forest productivity [1–3], enhances C sequestration in both soil and biomass [4,5], and decreases N deficiency of inherently nutrient-poor soils previously beneath natural savannas in the Congolese coastal plains [5–7]. Soil phosphorus (P) status also improves through increased soil available P in the coarse fraction of particulate organic matter POM (cPOM; 4000–250 µm) of the plantation of *Acacia* or/and *Eucalyptus* compared to tropical savannas [6,8]. Even though the well-known high P demand of *A. mangium* as a NFT to sustain symbiotic root nodules and atmospheric N_2 fixation processes [9,10] involves a decrease in soil available P beneath stands containing *Acacia* relative to *Eucalyptus* [5,11], P cycling in these soils is dominated by biological processes, i.e., organic mineralization [12], while forest floor and mineral soil contain most of the extractable P in inorganic form, reaching up to 70% in the mineral soil P [13].

Forest plantation, i.e., afforestation or reforestation, is an important silviculture and forest management practice around the world [14]. Soil ecology of forest plantation plays an important role in several processes, e.g., improving nutrient cycling and soil fertility (N and P status) and forest productivity, and enhancing C sequestration, and has a potential impact in mitigating climate change [15–17]. Soil and rhizosphere microbial communities (known as microbiota) play an important role in sustaining the fitness, development, and productivity of trees [18,19]. Due to the long-living nature of perennial tree crops, the trophic interactions that occur between the host and its associated belowground microbiota could be assumed as more durable than that taking place in short-lived herbaceous plants [18]. Also, belowground microbial communities associated with perennial tree crops may be characterized by a well-adapted core microbiota that undergoes more persistent changes than those taking place in annual ones. It is well known that different factors such as land-use change or forest management [20–23], soil intrinsic properties like pH, soil organic matter (SOM), and texture [15], or physical disturbance [24] can affect the structure of soil microbial communities in any given tree crops. Changes in bacterial community composition were observed following the introduction of *Acacia* in the *Eucalyptus* plantations [22,25,26]. *A. mangium* regulates soil microbial communities and extracellular enzyme activities and gives rise to an increase in soil C storage and recalcitrant C composition in *Eucalyptus* plantations in subtropical China [25]. Changes in soil microbial indicators and increased C and N concentrations in SOM labile fractions have been observed in Brazil, the world's largest producer of *Eucalyptus* spp., after intercropping *Eucalyptus* forest plantations with *A. mangium* trees [23]. Mixed-species plantations of *Acacia* and *Eucalyptus* stimulated microbial and bacterial activities in litter and soil, which may sustain nutrient availability in the long term [21], and enhanced both leaf litter accumulation and plant growth [20].

A better understanding of the soil microbial community in mixed and pure plantations under different soil and climate conditions is crucial to understand how soil microorganisms contribute to regulating ecosystem functioning, SOM dynamics, and nutrient cycling (C, N, S, and P). Organic C content greatly influences and drives the abundance of microorganisms in tropical forests [27], whereas N addition may induce a decline in bacterial species richness and diversity and a shift of bacterial composition [28]. Despite its negative impact on soil acidification, soil buffering capacity, and vegetation diversity, sulfur (S) has a potential to stimulate growth and to enhance the biomass of *Actinobacteria*, gram-positive bacteria, and fungi [29], while available P is enhanced by arbuscular mycorrhizal fungi colonization, phosphatase activities, and organic acids liberated by plants and microorganisms [30]. Tree species diversity and richness also have a crucial impact on the structure of soil bacterial communities and lead to the change in soil pH, C/N ratio, N, and P availability due to litterfall and root exudates in a broad-leaved forest ecosystem in central Germany [31]. The authors also highlighted the strong influence of tree species, both monoculture and mixed-species on soil physicochemical properties, leading afterward to differences in bacterial community structure at both total and active community magnitude. This is of great importance for tree species used in forest plantation, as it facilitates the design of novel sustainable approaches for the benefit of these relevant

agro-ecosystems. So far, the link between bacterial community composition and soil fertility has never been studied in the plantations of *Acacia* and *Eucalyptus* established in the Congolese coastal plains. Enhanced activity of edaphic macro arthropod communities and litter quality have been reported, i.e., cockroaches were predominant in *Acacia* litter, while ants were predominant in *Eucalyptus* [1], while lignin accumulates beneath *Eucalyptus* relative to *Acacia* stands [32].

In the current study, soil bacterial community has been investigated in pure and mixed-species plantations of *Acacia* and *Eucalyptus* established on natural tropical savannas in the Congolese coastal plains. Previous studies revealed the higher amounts of N and C in the stands containing *Acacia* at the end of the 7th year of the first rotation and at 2 years into the second rotation [5,6]. They also highlighted that P is represented at 70% in inorganic form, mainly orthophosphate, while mixed-species stands immobilized higher P in organic forms [32] and its cycle is dominated by biological processes [12]. Therefore, the main objective of this study is to characterize the bacterial community and its link to nutrient cycling (N, C, C/N, S, and P status) in the soil. The soil bacterial community was investigated by sequencing of the 16S rRNA gene in different stands of the plantation to evidence the effects of nitrogen-fixing trees (NFTs) on soil bacterial structure and its link to nutrient dynamics. Our research study will permit to answer two main questions: (i) Does the bacterial community of stands containing *Acacia*, i.e., pure *Acacia*, and mixed-species differ from that of pure *Eucalyptus* due to their higher nutrient inputs (litter fall and biomass)? (ii) Is there any link between bacterial community and vegetation cover, nutrient cycling, and other parameters (N, C, C/N ratio, P availability, and pH)?

2. Materials and Methods

2.1. Site Description, Experimental Design, and Sampling

2.1.1. Location, Soil Classification, Climate, and Previous Vegetation Cover

The study site is located in the Republic of the Congo, precisely at plateau close to Tchissoko village (4°44′41′′ S and 12°01′51′′ E, 100 m above sea level (a.s.l), 35 km from Pointe-Noire. These soils are deep Ferralic Arenosols [33] with a low Cation-Exchange Capacity (CEC) (<0.5 $cmol_c$ kg^{-1}), more than 90% sand, and 6 and 2% of clay and silt content, respectively. Soils contain less than 1.5% of iron oxides content [34], and their pH values (<5 in the surface layers) as well as C (<1.50%) and N (<0.065%) contents [5] are low. The studied area is characterized by a subequatorial climate with 85% of mean annual air humidity, 25 °C of air temperature, and between 2% and 5 °C of seasonal variation. The mean annual precipitation is 1200 mm with a dry season of 4 months (June to September). The experimental site has been afforested in 1984 with *Eucalyptus* hybrids in replacement of the previous native tropical savanna dominated by the C_4 *Poaceae Loudetia arundinacea* (Hochst.) Steud.

2.1.2. Experimental Design and History

In May 2004, a complete randomized block design of 4375 ha with five blocks and a density of 800 trees ha^{-1} was set up (see Figure 1) [6]. Each block was composed of three stands: a monoculture stand of *A. mangium* (100 A), a monoculture stand of *Eucalyptus urophylla* × *E. grandis* (100 E), and a mixed-species stand of 50% of *Acacia* and 50% of *Eucalyptus* (50 A 50 E). Each stand contained 100 trees (10 × 10) with two buffer rows and an inner part of 36 trees on an area of 1250 m^2 [4]. This first rotation was harvested in January 2012 at the age of 7 years. Two months later in March 2012, a second rotation was planted with the same design using *E. urophylla* × *E. grandis* hybrid (18–147) and *A. mangium* [7,11]. The soils were fertilized with 150 kg ha^{-1} of KCl three months after planting to avoid K^+ depletion common to highly weathered tropical soils [35].

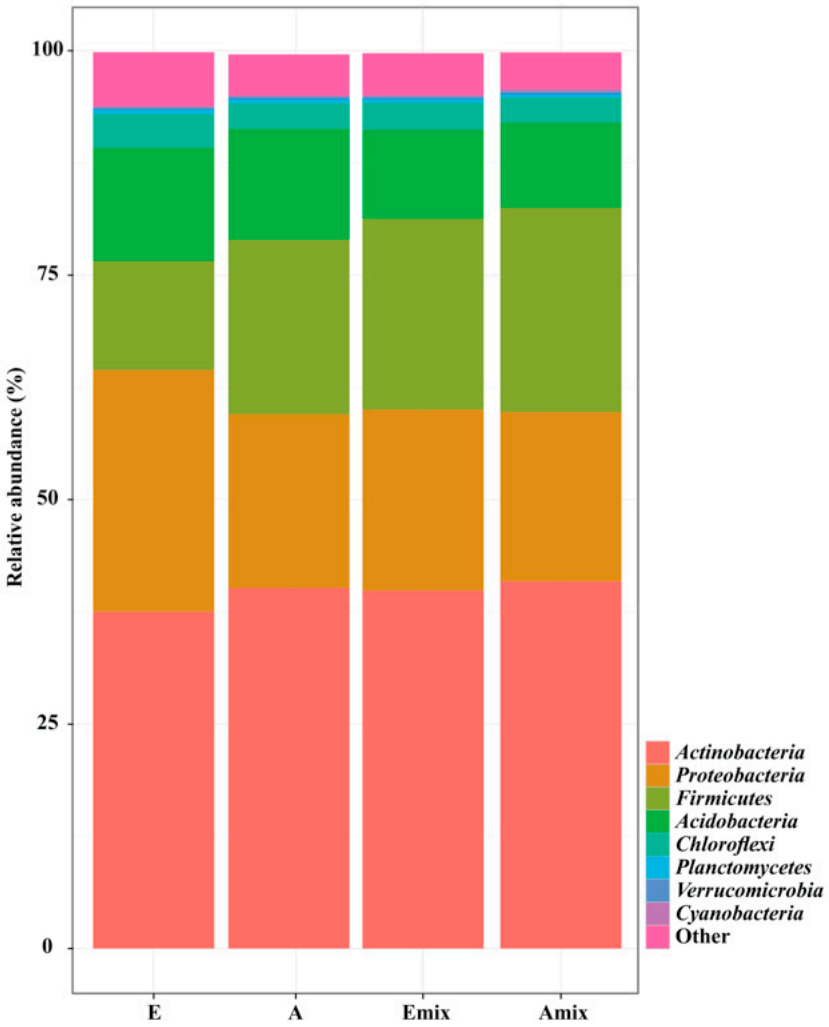

Figure 1. Taxa plot showing the relative abundance of the major phyla in stands of pure *Eucalyptus* (E) (n = 9) and pure *Acacia* (A) (n = 9), and mixed-species stands (Emix, 50% *Acacia* and 50% *Eucalyptus* with soil sampled near *Eucalyptus* trees, n = 9; Amix, 50% *Acacia* and 50% *Eucalyptus* with soil sampled near *Acacia* trees, n = 9): the relative abundance was calculated as the percentage of sequences belonging to a particular lineage of all 16S rRNA gene sequences recovered from a given plantation system. Other: unclassified taxa and other bacterial phyla with low operational taxonomic units (OTU) abundance.

2.1.3. Soil Sampling

Due to the higher SOM contents [36] and mesofauna density and richness [37] in the upper layer of the studied soil type, soil samples were collected with an auger at 0–0.05 m at 5 years into the second rotation (March 2017), as previously described [13]. In particular, the soil was sampled in 9 replicates by stand beneath monoculture of *A. mangium* (100 A) and *E. urophylla* × *E. grandis* (100 E) and 18 replicates mixed-species of *Acacia* and *Eucalyptus* (50 A 50 E) in 3 out of the 5 blocks. There were 27 (9 × 3 blocks) sampled points in monoculture stands (100 A and 100 E) against 54 in the mixed-species stands (18, 9 nearby *Eucalyptus* × 3 blocks and 9 nearby *Acacia* × 3 blocks) in 50 A 50 E (see Figure 1 in Koutika et al. [6]). They were collected along a transect from the base of a tree to the center of the area delimited by four trees within the inner part of each stand. Each transect contained three sampling cores separated by 0.7 m from each other. There were three transects in monoculture stands and six in mixed-species stands. For this study, a composite sample has been made from three samples in each stand. Three composite samples of pure *Acacia* (100 A) and *Eucalyptus* (100 E) and 6 of mixed-species

(50 A 50 E) stands were obtained by block, i.e., 12 samples per block and a total of 36 samples for 3 studied blocks.

2.2. Soil Carbon, Nitrogen, Sulfur Concentration, and Available Phosphorus Analyses

Macro VARIO Cube Elemental Analyzer (Elementar-Straße 1, D-63505 Langenselbold, Germany) was used to evaluate N, C, and S concentrations of 36 collected composite soil samples in 3 technical replicates by sample. Resin available P was determined using two anion exchange resins strips (BDH#551642S, 20 mm × 60 mm). Two resin strips were shaken for 16 h (100 revs min^1) with 0.5 g of dried and sieved soil in 30 mL distilled water. To recover adsorbed phosphate from resin, the strips were removed from the suspension and thoroughly rinsed with water before being eluted with 30 mL of 0.5 M HCl. Phosphate was determined according to the method of Tiessen and Moir [38].

2.3. DNA Extraction and Quality Assessment

The extraction of genomic DNA from around 400 mg of each 36 composite samples was performed using the QIAGEN's new DNeasy PowerSoil Pro Kit according to the manufacturer's instruction (QIAGEN Group, Hilden, Germany). DNA was quantified using both Thermo Scientific™ NanoDrop 2000 spectrophotometer and Qubit 2.0 fluorometer (Invitrogen, Life technologies). Integrity and purity of extracted DNA were checked through 1.5% agarose gel electrophoresis with 1X Tris Acetate EDTA buffer (Sigma-Aldrich), subsequently stained with GelRed (0.5 µL mL^{-1}), visualized, and photo-documented under ultraviolet light on Bio Red Molecular Imager ChemiDoc TM XRS+, US. The quality of metagenomic DNA was assessed by PCR amplification of the 16S rRNA gene using universal forward primer 314F (5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNBGCASCAG-3′) and reverse primer 805R (5′GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACNVGGGTATCTAATCC -3′) [39]. PCR was performed for all samples using a 25-µL reaction mixture, and including 0.5 µL of each primer (10 µm), 0.5 µL of dNTPs mix (10 mm), 0.2 µL of Taq, 3 µL of genomic DNA, and 2 µL of DNA. Each sample was performed in 3 replicates (one per block), and positive and negative control (free of DNA) were also included. Master cycler, Eppendorf (Hamburg, Germany), was adopted to carry out PCR under optimum conditions: (1) initial denaturation at 94 °C for 1 min; (2) 25 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s; (3) final extension of 72 °C for 7 min; and (4) hold at 4 °C.

2.4. Illumina 16S Library Construction and Sequencing

After checking the quality of the PCR product through 1.5% agarose gel electrophoresis, Illumina 16S library construction and metagenomic sequencing were performed using Illumina MiSeq platform and 300 PairedEnds strategy at BMR Genomics srl (Padua, Italy) (https://www.bmr-genomics.it). Briefly, the V3-V4 regions of 16S rRNA gene were amplified adopting the following primers: Pro341F, 5′-CCTACGGGNBGCASCAG-3′, and Pro805R, 5′-GACTACNVGGGTATCTAATCC-3′ [39]. Primers were modified with forward and reverse overhangs (5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-(locus-specific sequence)3′ and 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-(locus-specific sequence)-3′, respectively) necessary for dual index library preparation. Sequencing was performed on Illumina MiSeq using 300PE v3 chemistry strategy. Raw sequence data reported in this study have been deposited in the National Center for Biotechnology Information (NCBI) "Sequence Read Archive" (SRA) of the National Center for Biotechnology Information (NCBI), under project accession number PRJNA649230.

2.5. Bioinformatic and Statistical Analysis

The analysis of 16S rRNA gene amplicon sequences was performed using the Qiime (Quantitative Insights Into Microbial Ecology) software package [40], following the instructions for Illumina 16S rRNA analyses available at Qiime website (www.qiime.org). Raw reads were joined through the paired-end pipeline. Also, the reads were filtered by quality, and chimeric sequences were removed. We binned the

filtered files into operational taxonomic units (OTU) using the Sumaclust algorithm at 97% identity [41]. Each OTU was taxonomically classified based on SILVA's ribosomal database-132 [42]. Singleton and *Archaea* sequences were removed. Differences in species complexity among the samples were evaluated by β-diversity analysis through weighted and unweighted UniFrac using the core_diversity.py script in Qiime. A Principal Coordinates Analysis (PCoA) based on the UniFrac distance matrixes was performed to visualize the variations in bacterial community structure [43–45]. Also, richness (OTU number), faith's phylogenetic, and Shannon diversity indexes were obtained. The statistical significance of factors affecting the composition of soil bacterial community was evaluated using nonparametric permutational multivariate analysis of variance (PerMANOVA) that was applied to identify differences in bacterial community structure among treatments [44]. A distance-based redundancy analysis (db-RDA) to visualize the correlation between soil chemical attributes and soil bacterial community structure was applied [46]. Also, we applied a Spearman ranking correlation test to compare the relative abundance of all bacterial members belonging to each taxonomic level (i.e., phylum to genus) with soil chemical attributes. The diversity of species within community samples was analyzed by using α-diversity analysis based on the *observed_otus* metrics and Shannon index by using the Qiime software package. Phylogenetic diversity of the bacterial communities for each treatment was estimated in Qiime using Faith's phylogenetic diversity metric [47]. One-way analysis of variance (ANOVA) was used to compare the differences in chemical parameters among different soil plantations, in alfa-diversity indices among the four studied soils, and in the log-transformed taxon abundance percentages for different taxonomic ranks (phylum, class, order, family, and genus) (R, Graph-pad PRISM, version 8.0).

3. Results

3.1. Soil Nitrogen, Carbon, Sulfur Concentrations, and Available Phosphorus

In stands containing *Acacia* relative to *Eucalyptus*, a decrease in soil pH values, N and C concentrations, and available P in bulk soil along rotations was observed (Tables 1 and 2).

Monoculture *Acacia* (100 A) stands exhibited the lowest N concentration (0.11%), whereas the mixed-species nearby *Acacia* (50 A 50 E_{Ac}) had the highest (0.18%) (Table 2). Pure *Acacia* contained a lower C concentration value (1.5%) compared with other samples, and the mixed-species nearby *Eucalyptus* (50 A 50 E_{Eu}) had the highest (1.7%) C concentration. When considering the carbon to nitrogen ratio (C/N), the highest value was noticed in pure *Acacia* (14.2) and the lowest one was in mixed-species nearby *Acacia* (50 A 50 E_{Ac}) (9.8). The *Acacia* monoculture had the highest sulfur concentration value (0.15%), whereas the mixed-species nearby *Eucalyptus* (50 A 50 E_{Eu}) showed the lowest value (0.06%).

3.2. Sequencing Data and Overall Composition of Bacterial Community along with the Field Sites

Soil samples were analyzed by amplicon sequencing of the V3–V4 hypervariable region of the 16S rRNA gene. A total of 2,103,938 raw reads with an average of 58,442 ± 23,313 sequences detected per sample were generated from soil samples. We rarefied the OTU table at 16,958 sequences/sample depth. Overall, the rarefied bacterial OTUs were assigned to 28 phyla, 81 classes, 207 orders, 440 families, and 1097 genera. The four predominant phyla *Actinobacteria*, *Proteobacteria*, *Firmicutes*, and *Acidobacteria* covered more than 90% of the total bacterial community. Unclassified OTUs and other members with low relative abundance were grouped as "Other".

Table 1. Soil pH, N and C concentrations, and available P at different stages of rotation from the end of year 7 of the first rotation (R1Y7), year 2 of the second rotation (R2Y2), and year 5 of the second rotation (R2Y5).

	R1Y7			R2Y2			R2Y5		
	100 A	**50 A 50 E**	**100 E**	**100 A**	**50 A 50 E**	**100 E**	**100 A**	**50 A 50 E**	**100 E**
pH-H_2O	4.2 ± 0.03 c	4.4 ± 0.02 b	4.5 ± 0.04 a	4.4 ± 0.02 a	4.3 ± 0.03 b	4.4 ± 0.03 a	3.9 ± 0.05 b	4.0 ± 0.03 b	4.2 ± 0.04 a
pH-KCl	3.5 ± 0.02 a	3.5 ± 0.02 a	3.5 ± 0.03 a	3.3 ± 0.02 a	3.2 ± 0.02 b	3.3 ± 0.03 a	3.5 ± 0.02 a	3.5 ± 0.02 a	3.5 ± 0.04 a
ΔpH	0.8 ± 0.03 c	0.90 ± 0.01 b	1.0 ± 0.02 a	1.1 ± 0.02 a	1.1 ± 0.02 b	1.1 ± 0.03 a	0.4 ± 0.06 ab	0.5 ± 0.03 b	0.6 ± 0.05 a
N (%)	0.058 ± 0.003 ab	0.064 ± 0.003 b	0.050 ± 0.004 a	0.050 ± 0.002 a	0.065 ± 0.011 b	0.061 ± 0.016 ab	0.150 ± 0.015 a	0.168 ± 0.011a	0.164 ± 0.016a
C (%)	0.99 ± 0.074 ab	1.18 ± 0.078 b	0.87 ± 0.091 a	1.01 ± 0.055 a	1.50 ± 0.088 b	1.41 ± 0.149 ab	1.42 ± 0.091 a	1.49 ± 0.086 a	1.36 ± 0.140 a
Available P (mg kg^{-1})	8.07 ± 0.63 a	6.94 ± 0.45 b	8.46 ± 0.79 a	8.46 ± 0.42 c	9.34 ± 0.44 b	10.65 ± 1.05 a	1.47 ± 0.01 a	1.46 ± 0.01 a	1.46 ± 0.01 a

100 A and 100 E = monoculture stands of *Acacia* and *Eucalyptus*, respectively. 50 A 50 E = mixed-species (50% *Acacia* and 50% *Eucalyptus*) stands. cPOM = coarse POM (4000–250 μm). fPOM = fine POM (250–50 μm). OMF = organic-mineral fractions (<50 μm). Data display the mean values ± standard error. Different letters indicate that means are significantly different between stands ($p < 0.05$). pH data (R1Y7 and R2Y2) were adapted from [5,6]; pH data (R2Y5), and N and C concentrations from Koutika et al. [8].

Table 2. Nitrogen (N), carbon (C), and sulfur (S) concentrations and CN ratios in pure *Acacia* (100A), and *Eucalyptus* (100 E), and mixed-species (50 A 50 E) stands.

Stands	N (%)	C (%)	C/N (%)	S (%)
100 A	0.11 ± 0.03 a	1.5 ± 0.25 a	14.2 ± 2.39 a	0.15 ± 0.10 a
50 A 50 E (Ac)	0.18 ± 0.03 ab	1.57 ± 0.18 a	9.8 ± 2.31 a	0.07 ± 0.10 a
50 A 50 E (Eu)	0.14 ± 0.03 ab	1.7 ± 0.31 a	13.1 ± 2.39 a	0.06 ± 0.10 a
100 E	0.14 ± 0.03 ab	1.6 ± 0.32 a	12.1 ± 2.74 a	0.08 ± 0.10 a

100 A and 100 E = monoculture stands of *Acacia* and *Eucalyptus*, respectively. 50 A 50 E = mixed-species (50% *Acacia* and 50% *Eucalyptus*) stands. Data display the mean values ± standard error. Different letters indicate that means are significantly different between stands ($p < 0.05$).

As shown in Figure 1, at the phylum level, the structures of the microbial communities differed in terms of both the predominant phylum and the relative abundance of each phylum. *Actinobacteria* was the dominant phylum in all soil samples (mean value of 39.6% of total relative abundance), but no significant differences in the percentages of relative abundance between pure and mixed stands were observed ($p > 0.05$). *Proteobacteria* was the second abundant phylum with the highest percentage (27%) in stands containing *Eucalyptus*, followed by mixed-species plantations nearby *Eucalyptus* (20%) and lower percentages in pure *Acacia* stands (19%) and mixed-species plantations nearby *Acacia* (18.8%). Interestingly, the relative abundance of *Proteobacteria* was much higher in pure *Eucalyptus* than in mixed plantations ($p < 0.001$) and in pure *Acacia* ($p < 0.01$). *Firmicutes* was the third most abundant phylum. The percentage of *Firmicutes* was significantly higher in *Acacia* (19%) and in mixed-species nearby *Acacia* (23%) than *Eucalyptus* (12%) ($p < 0.05$ and $p < 0.001$, respectively) as well as higher in mixed-species nearby *Eucalyptus* (21%) than *Eucalyptus* (12%) ($p < 0.01$). As the fourth most prevalent phylum, *Acidobacteria* showed no statistically different values of relative abundance in the pure *Eucalyptus* and *Acacia* plantations compared to the mixed-species stands ($p > 0.05$). Four less abundant phyla were detected in the analyzed soil samples, i.e., *Chloroflexi*, *Planctomycetes*, *Verrucomicrobia*, and *Cyanobacteria*, with no significant differences among the stands ($p > 0.05$).

At class level, *Actinobacteria, Alphaproteobacteria,* and *Bacilli* were the dominant bacterial classes found across the site (Figure S1). In the *Actinobacteria* phylum, the class *Actinobacteria* was the dominant one, being present in the highest abundance in mixed-species (36%) and in the lowest one in the pure *Eucalyptus* (30%), but there was no significant difference among the stands ($p > 0.05$). *Alphaproteobacteria*, *Deltaproteobacteria*, and *Gammaproteobacteria* were the *Proteobacteria* classes detected in all the soil samples. The relative abundance of *Alphaproteobacteria* was significantly higher in the pure *Eucalyptus* (25%) than in pure *Acacia* and mixed-species nearby *Acacia* (17%) ($p < 0.01$) and in mixed-species nearby *Eucalyptus* (18%) ($p < 0.01$). The phylum *Firmicutes* was represented only by the *Bacilli* and *Clostridia* classes with the lowest percentages in the pure *Eucalyptus* (11 and 1%, respectively). The ANOVA test indicated that *Bacilli* were significantly more abundant in mixed-species (18%) than *Eucalyptus* alone ($p < 0.05$). Only the class *Acidobacteria* was detected within the phylum *Acidobacteria*, with similar percentages in pure plantations of *Eucalyptus* and *Acacia* (12%) and mixed-plantations nearby *Acacia* and *Eucalyptus* (9 and 10%, respectively) ($p > 0.05$).

Within the *Actinobacteria*, *Frankiales* was the dominant order with the highest abundance detected in mixed-species nearby *Eucalyptus* and *Acacia* (32 and 33% of the total sequences, respectively), showing no significant differences among samples ($p > 0.05$) (Figure S2). Within *Bacilli*, the relative abundance of *Bacillales* was higher in mixed plantations (18%) and pure *Acacia* stands (17%) compared to pure *Eucalyptus* (11%) ($p < 0.05$). The majority of *Alphaproteobacteria* belonged to the *Rhizobiales* order, which was present in high abundance in pure *Eucalyptus* (15%) compared to the other three soil samples (pure *Acacia*, mixed-plantations nearby *Acacia*, and nearby *Eucalyptus*, accounting each for 7% of the total sequences) ($p < 0.001$).

At the family level, sequences belonging to the *Acidothermaceae* family dominated in all soil samples, with no significant differences among samples ($p > 0.05$) (Figure S3). Higher percentages of sequences were found in mixed-plantations (32 and 33% for mixed-species nearby *Eucalyptus* and *Acacia*,

respectively) and pure *Acacia* samples (30%), compared to the pure *Eucalyptus* (27%). *Xanthobacteraceae* was the main family detected within the *Rhizobiales* order showing the highest relative abundance in pure *Eucalyptus* stands (13%) ($p < 0.001$).

The genus *Acidothermus* showed the highest percentages of relative abundance in mixed-plantations (32 and 33%, for mixed-species nearby *Eucalyptus* and *Acacia*, respectively) with no significant differences when compared with pure stands ($p > 0.05$) (Figure S4). The other genera detected showed a mean relative abundance lower than 7%. Among them, an uncultured candidatus genus within the *Xanthobacteraceae* family was detected with the highest percentage in pure *Eucalyptus* stands (11%, $p < 0.001$). An increased abundance of the *Paenibacillus* genus was found in pure *Acacia* and mixed plantations (mean relative abundance of 5%) than pure *Eucalyptus* ($p < 0.001$).

3.3. Bacterial Alpha and Beta Diversity

The alpha diversity indices, including observed species, Shannon index, and phylogenetic diversity (PD) whole tree, were calculated for each data set to gain further insights into the complexity of the soil bacterial communities. Alpha diversity was quantified by Richness (expressed as the number of observed OTUs) and Shannon diversity index, which reflects species number and evenness of species abundance, and was measured by the Phylogenetic diversity (PD_whole_tree) which reflects the sum of all branch lengths on the constructed phylogenetic tree from all taxa. Our results showed that the *Eucalyptus* stands (both pure and mixed) had overall higher alpha diversity than those of *Acacia* stands controls, although no significant difference was observed (Tukey's multiple comparisons, One-way ANOVA, $p > 0.05$) (Figure 2). The mean OTU value was more than 5000 in all stands. The pure *Eucalyptus* (100 E) has the highest value (7202 ± 1168,) followed by the mixed-species 50 A 50 E nearby *Eucalyptus* (6868 ± 932), pure *Acacia* (6497 ± 638), and 50 A 50 E nearby *Acacia* (5009 ± 591). The lower values were found in stands containing *Acacia*, both pure and mixed. Shannon Index was more than 7 for all stands. The highest value (7.8 ± 0.186) was found in pure *Eucalyptus*, and the lowest (7.2 ± 0.252) was in mixed-species nearby *Acacia*. Higher phylogenetic diversity whole tree values (>500) were found in pure *Eucalyptus* (516.9 ± 64.1) and mixed-species nearby *Eucalyptus* (509.5 ± 55.9). The stands containing *Acacia* had the lowest values, i.e., 488.9 ± 35.9 for pure *Acacia* and 402.2 ± 38.6 for the mixed-species nearby *Acacia*.

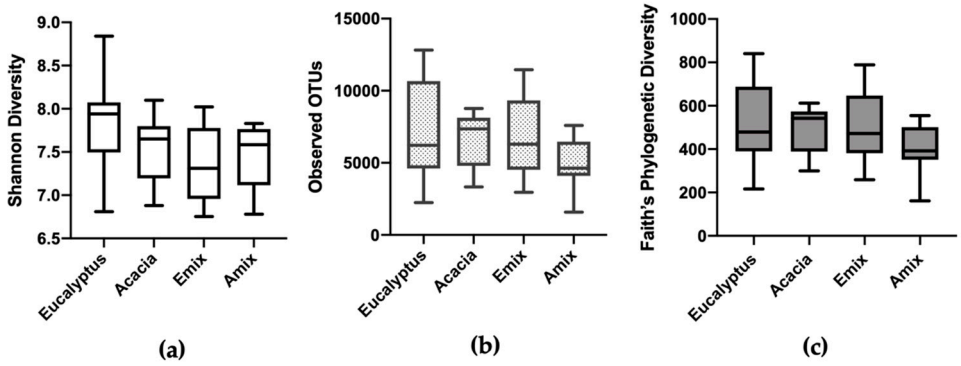

Figure 2. Box plots of the Shannon diversity index (**a**), observed OTUs (**b**) and phylogenetic diversity (**c**) in the four studied soils: the line inside the box represents the median, while the whiskers represent the lowest and highest values within the 1.5 interquartile range (IQR). The width of the distribution of points was proportionate to the number of points at that *Y* value. Statistical analysis showed no difference for each measurement (one-way ANOVA, $p > 0.05$).

The analysis of β-diversity by UniFrac metrics, coupled with standard multivariate statistical techniques including principal coordinates analysis (PCoA), permitted to investigate the within-habitat variation in soil bacterial community. The PCoA based on weighted UniFrac metric revealed a first separation (PCo 1 = 24.72), the secondary separation (PCo 2 = 19.30%), and the third one (PCo 3 = 9.80%)

(Figure 3A). The values even decreased when the unweighted UniFrac metric was used, i.e., the first separation was only 7.49, the secondary separation was 4.66, and the third was 4.05% (Figure 3B). The β-diversity analysis revealed that pure *Eucalyptus* stands were separated from others, i.e., stands beneath *Acacia*, the monoculture *Acacia* (100 A), the mixed-species nearby *Eucalyptus*, and nearby *Acacia* (PerMANOVA test = $p < 0.001$) (Figure 3A,B).

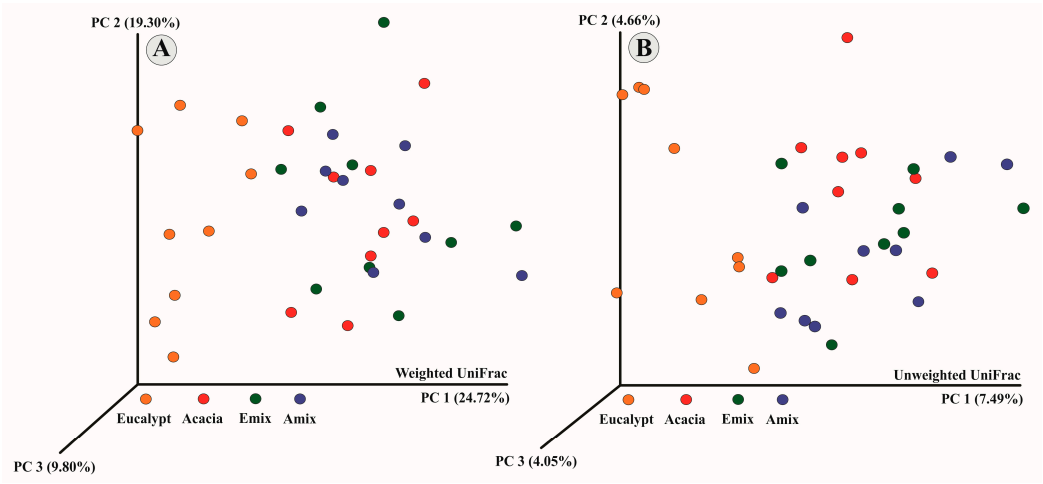

Figure 3. Principal Coordinate analysis (PCoA) of the bacterial community from soil samples (0–05 cm) based on (**A**) weighted and (**B**) unweighted UniFrac metrics: axes represent the percentage of data explained by each coordinate dimension. Treatments: pure *Eucalyptus*, pure *Acacia*, Emix (50% *Acacia* and 50% *Eucalyptus* with soil sampled near *Eucalyptus* trees), Amix (50% *Acacia* and 50% *Eucalyptus* with soil sampled near *Acacia* trees).

3.4. Relationship between Soil Microbiota and Soil Characteristics

The distance-based redundancy analysis (db-RDA) revealed that available P was the prevailing factor for driving the soil bacterial community composition in all soil samples ($R = 0.19$, $p = 0.0012$), followed by the carbon to nitrogen ratio (C/N) ($R = 0.15$, $p = 0.0511$), whereas no significant correlation between soil bacterial community structure and C, N, and S concentrations was found (Figure 4).

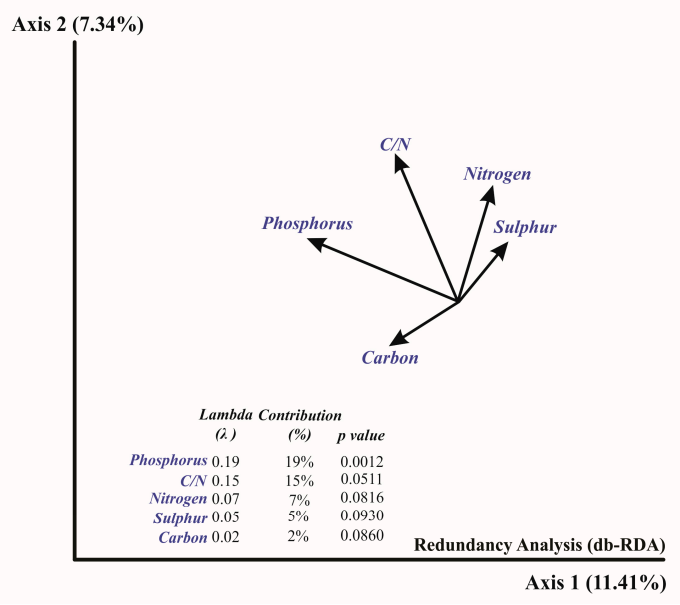

Figure 4. Correlation between soil chemical attributes (phosphorus, C/N, nitrogen, sulfur, and carbon) and soil bacterial community structure by distance-based Redundancy Analysis (db-RDA).

We also evaluated the correlations between soil bacterial communities at the phylum, class, order, family, and genus levels and soil properties (C, N, C/N, S, and P). As shown by the Spearman correlation heatmaps (Figure 5 and Figures S5–S8), soil bacterial communities were correlated to C and S at all taxonomic levels, to N at the class level, and to C/N ratio at the genus level, while they were positively correlated to P at the order, family, and genus levels. At the phylum level (Figure 5), the dominant phylum *Actinobacteria* presented a strong positive correlation with S ($R = 0.55$; $p = 0.0004$); on the contrary, *Proteobacteria*, *Firmicutes*, and *Acidobacteria* were not related to any of the soil chemical properties. Significative positive correlation was found between *Planctomycetes* and C ($R = 0.33$; $p = 0.0492$), while *Chloroflexi* was negatively correlated with C ($R = -0.36$; $p = 0.0289$) and S ($R = -0.39$; $p = 0.0192$).

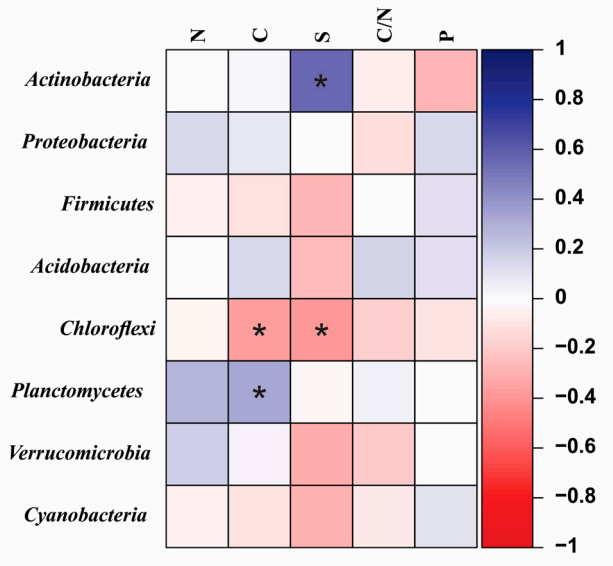

Figure 5. Heatmap of Spearman's rank correlation coefficients between major bacterial phyla with soil chemical attributes: the values of correlation coefficients are indicated according to the scale bar. Significant correlations ($p < 0.05$) are shown by an asterisk.

At the class level (Figure S5), all four classes of *Actinobacteria* phylum were positively associated to S, i.e., *Actinobacteria* ($R = 0.40$; $p = 0.0157$), *Thermoleophilia* ($R = 0.46$; $p = 0.0050$), and *Acidimicrobiia* ($R = 0.35$; $p = 0.0364$). Negative correlations between S and the classes *Ktedonobacteria* ($R = -0.45$; $p = 0.0062$) and *Verrucomicrobiae* ($R = -0.31$; $p = 0.0557$), between C and the classes *Ktedonobacteria* ($R = -0.32$; $p = 0.0589$) and *Acidimicrobiia* ($R = -0.31$; $p = 0.0543$), and between N and *Gammaproteobacteria* ($R = -0.31$; $p = 0.0539$) were found.

All four orders of *Actinobacteria* phylum were significantly correlated with S, i.e., *Frankiales* ($R = 0.40$; $p = 0.0166$), *Solirubrobacterales* ($R = 0.47$; $p = 0.0040$), *Corynebacteriales* ($R = 0.38$; $p = 0.0210$), and IMCC26256 ($R = 0.39$; $p = 0.0193$) (Figure S6). However, S was negatively correlated to *Pseudonocardiales* ($R = -0.35$; $p = 0.0382$) from *Actinobacteria*, *Acetobacterales* ($R = -0.50$; $p = 0.0019$), *Solibacterales* ($R = -0.39$; $p = 0.0185$), and *Ktedonobacterales* ($R = -0.45$; $p = 0.0065$) belonging to the *Proteobacteria*, *Acidobacteria*, and *Chloroflexi* phyla. C was positively related to *Acetobacterales* ($R = 0.44$; $p = 0.0071$) (*Proteobacteria*), and negative *Ktedonobacterales* ($R = -0.35$; $p = 0.0379$) and *IMCC26256* ($R = -0.36$; $p = 0.0319$) from *Chloroflexi* and *Actinobacteria*, respectively. P was positively linked to *Acidobacteriales* ($R = 0.32$, $p = 0.0560$), a member of *Acidobacteria*.

Only two families were linked to C, one positively, i.e., *Acetobacteraceae* ($R = 0.44$; $p = 0.0071$) (*Proteobacteria)*, and another negatively, *Ktedonobacteraceae* ($R = -0.35$; $p = 0.0353$) (*Chloroflexi*) (Figure S7). S was still the most correlated soil parameter, with bacterial groups showing positive links with 3 families of *Actinobacteria*, i.e., *Acidothermaceae* ($R = 0.40$; $p = 0.0167$), *Solirubrobacteraceae* ($R = 0.47$; $p = 0.0035$), and *Mycobacteriaceae* ($R = 0.38$; $p = 0.0231$), and negative ones with *Solibacteraceae* ($R = -0.39$;

$p = 0.0184$) (*Acidobacteria*), *Acetobacteraceae* ($R = -0.50$; $p = 0.0019$) (*Proteobacteria*), and *Ktedonobacteraceae* ($R = -0.47$; $p = 0.0038$) (*Chloroflexi*). P was found to be positively related to one uncultured member from the *Acidobacteria* phylum only ($R = 0.32$, $p = 0.0588$).

Acidisphaera, a genus in the phylum *Proteobacteria*, was positively linked to C ($R = 0.42$; $p = 0.0107$), while negative correlation was reported between C and *Conexibacter* (*Actinobacteria)* ($R = -0.28$; $p = 0.0978$) (Figure S8). The C/N ratio was found to be positively related to unassigned genera of the phylum *Proteobacteria* ($R = 0.30$; $p = 0.0536$). Four genera of the phylum *Actinobacteria* were positively linked to S, i.e., *Acidothermus* ($R = 0.40$; $p = 0.0167$), *Conexibacter* ($R = 0.49$; $p = 0.0021$), *Solirubrobacter* ($R = 0.36$; $p = 0.0307$), and *Mycobacterium* ($R = 0.38$; $p = 0.0231$), while negative associations were reported with four genera, i.e., *Solibacter* ($R = -0.34$; $p = 0.0407$), a genus in the phylum *Acidobacteria*, *Acidisphaera* ($R = -0.48$; $p = 0.0029$), uncultured_forest_soil_bacterium ($R = -0.38$; $p = 0.0202$) of the phylum *Proteobacteria*, and *Thermosporothrix* ($R = -0.34$; $p = 0.0426$) in the phylum *Chloroflexi*. One uncultured bacterium of the phylum *Proteobacteria* was positively associated to P ($R = 0.05$, $p = 0.2966$).

4. Discussion

Discovering the soil bacterial communities and investigation of their potential associations with nutrient cycling are crucial for understanding the ecosystem function of soil microbial communities in tropical forest plantations of the Congolese coastal plains. Soil microorganisms play an important role as regulators of major biogeochemical cycles and can significantly affect the functioning of tree crop ecosystems [18]. Disentangling the complexities of the soil microbiome, it has been found that the soil environment contains highly diverse microorganisms, dominated by *Acidobacteria*, *Verrucomicrobia*, *Bacteroidetes*, *Proteobacteria*, *Planctomycetes,* and *Actinobacteria* [48]. In the present study, the soil bacterial community in pure and mixed-species plantations of *Acacia* and *Eucalyptus* in the Congolese coastal plains was investigated. The prevalence of *Actinobacteria*, *Proteobacteria*, *Firmicutes*, and *Acidobacteria* accounting for more than 90% of the phylum composition (0–0.5 cm topsoil) in stands containing *Acacia* and 89% in pure *Eucalyptus* was revealed by sequencing of the 16S rRNA gene. This may suggest a shift in the bacterial community that could be due to both afforestation of natural savannas and introduction of NFTs, since microbial diversity increases with afforestation [49]; N inputs; and mineral N availability [50]. The most prevalent phylum in the studied soils, i.e., *Actinobacteria*, has a critical role in decomposing soil organic materials, such as cellulose and chitin [51]. *Actinobacteria* and *Proteobacteria* are common to acidic forest soils [22] and have potential to improve nutrient cycling [52]. In accordance with our findings, *Firmicutes*, *Proteobacteria*, *Actinobacteria*, and *Acidobacteria* were reported as the most abundant making over 85% of the total sequences in an agroforestry system of walnut (*Juglans regia L.*) and wheat (*Triticum aestivum L.*) in the southern part of Loess Plateau (China) [52].

Our results allow us to respond to the first question of this research study: (i) Does the bacterial community of stands containing *Acacia*, i.e., pure *Acacia* (100 A), and mixed-species (50% *Acacia* and 50% *Eucalyptus,* 50 A 50 E) differ from that of pure *Eucalyptus* (100 E) due to their higher nutrient inputs (litter fall and biomass)? Stands containing *Acacia* had higher percentages of the third most abundant genera, *Firmicutes*, i.e., 23% in mixed-species nearby *Acacia,* 21% nearby *Eucalyptus,* and 19% in the pure *Acacia* (100 A), against 12% in the pure *Eucalyptus* (100 E). This is probably due to their high soil N status [5,7] since N inputs, especially mineral N availability, change bacterial community structure [53,54] and microbial biomass [55]. Correlation analysis showed that N affected the change in the bacterial community by greatly driving the shift of *Firmicutes* [50] and significantly affected the diversity and abundance of the bacterial community in a boreal forest [54]. The prevalence of *Firmicutes* phylum in the stands beneath *Acacia* is probably linked to enhanced soil N cycling in *Acacia* stands [7] and to increased N content in coarse particulate organic matter at year 7 into the first rotation and at year 2 into the second rotation [6] compared to *Eucalyptus*.

The prevalence of *Firmicutes* in stands containing *Acacia* relative to *Eucalyptus* may also be explained by their lower bacterial richness and phylogenetic diversity. Peerawat et al. [56] reported

more specific and less diverse soil biota characterized by the dominance of the bacterial phyla *Firmicutes* in old rubber plantations in Thailand. Stands containing *Acacia* may also have favored the prevalence of the *Firmicutes* phylum in the older stage of forest plantation, i.e., 5 years into the second 7-year rotation probably to develop the potential to fight drought. Lower soil water content down to 15 cm beneath the pure *Acacia* revealed its lower potential to tolerate drought relative to *Eucalyptus* at the younger stage, i.e., before 2 years into the second rotation [57]. Acosta-Martínez et al. [58] reported a prevalence of *Firmicutes* in the soil with lower moisture content, and their survival ability under stressful conditions, such as warming and desiccation, were highlighted by Battistuzzi et al. [59].

Being the fourth most prevalent phylum, *Acidobacteria* was more represented in the pure *Eucalyptus* (13%) stands followed by pure *Acacia* (12%), mixed-species nearby *Eucalyptus* (10%), and nearby *Acacia* (9%). Even though N and C inputs do undeniably change bacterial community structure [50–53], N inputs could reduce *Acidobacteria* abundance by 26.5% in fir plantations in China [53]. The difference between stand types in the predominance of phylum groups, bacterial richness, and phylogenetic diversity highlights the effects of introducing NFTs in *Eucalyptus* plantation on soil properties and environment [9,20–23,60].

Pereira et al. [22] reported a predominance of *Proteobacteria* and *Acidobacteria* bacterial groups more frequently in samples between 0 and 300 cm, while *Firmicutes* and *Proteobacteria* were the more predominant bacterial groups in pure *Eucalyptus* stands on a Ferralsol at Itatinga, Brazil. The authors also found that matter *Acidobacteria* phylum predominated by 19.94% in the surface layer (0–100 cm) of the stands and *Proteobacteria* by 27.34% in the subsurface (100–300 cm). The predominance of *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, and *Bacteroidetes* phyla have been often attributed to the acidic nature of forest soils in the temperate and tropical areas [22,61–63]. Soils of the Congolese coastal plains are acidic, and three out of four prevalent phyla are common in forest acidic soils, i.e., *Actinobacteria*, *Proteobacteria,* and *Acidobacteria* [22]. Besides the soil depths, i.e., topsoil (0–0.5 cm) at Tchissoko (Congo) and 0–800 cm at Itatinga (Sao Paulo State, Brazil), the difference in the phylum composition beneath *Acacia* and *Eucalyptus* plantations in the two sites may also be due to other factors such as climate, forest management, soil intrinsic properties (pH, SOM, texture, etc.), physical disturbance, and environment [15,20,21,24]. Even though soils in both locations contained more than 80% of sand, soils in Brazil (Itatinga, Sao Paulo state) are Ferralsols with 13% of clay and 3% of silt, while those in the Republic of the Congo (Tchissoko) are Ferralic Arenosols with only 3% of clay and 6% of silt [4]. Other differences between the two sites established on a similar experimental design have been reported in other studies, e.g., stand wood biomass and forest productivity [2–4]; C and N concentrations and storage [5,7,64]; P cycling dominated by physicochemical processes at Itatinga, biological processes at Tchissoko [12]; and bacterial community composition even though soil depth, sampling, and preparation do not allow any comparison [22].

There are several other effects such as increased stand wood biomass and forest productivity [2–4], and shifted forest floor composition [20,49]. This creates heterogeneous ecosystems with a different composition of the soil bacterial community [65] since forest trees select specific groups of microorganisms [66] and monoculture stands preferentially select homogeneous bacterial communities [67,68]. Furthermore, heterogeneous ecosystems contain more bio-diverse sources of microbes which boost their efficiency in the rhizosphere [22,69,70]. Our findings respond to the first question of this research study. It is highlighted by (i) higher percentages of *Proteobacteria* in pure *Eucalyptus* relative to stands containing *Acacia*, while *Firmicutes* were abundant in stands containing *Acacia* vs. pure *Eucalyptus*; (ii) clear separation of the bacterial structure (PCoA) of pure *Eucalyptus* from the stands containing *Acacia*; and (iii) lower bacterial richness and polygenetic diversity whole-tree. The phyla composition is linked to the specific stand type such as lower percentages of *Proteobacteria* in stands containing *Acacia* relative to pure *Eucalyptus* and *Firmicutes* prevalence in stands containing *Acacia* vs. pure *Eucalyptus*, while PCoA shows that the bacterial structure of the pure *Eucalyptus* clearly separated from the stands containing *Acacia*. Our study also highlights the specificity of mixed-species stands. Mixed-species plantations nearby *Acacia*, when compared with pure *Acacia*, showed higher

N and C content and lower value of C/N ratio and S content. It is well reported that low C/N ratios, high available N and P, as well as high pH, as observed in mixed-species plantations nearby *Acacia*, promoted tree productivity [71]. Despite the highest N content and the lowest C/N ratio, the lowest microbial richness and diversity indices were found in mixed-species plantations nearby *Acacia* even though there were no significant differences among stands. Li et al. [28] reported a significant decline in bacterial species richness and diversity and a substantial shift of bacterial community composition after N addition in a subtropical deciduous oak mixed forest in China. We suggest that the loss of one or more species does not dramatically affect the functioning of the ecosystem, probably due to the high functional redundancy of soil microorganisms [72]. As suggested by Dukunde et al. [31], although soil characteristics have been frequently reported as strong drivers of microbial diversity [73], tree species have been shown to exhibit a stronger impact on community structure than the soil environment. Due to the long-term *Acacia* and *Eucalyptus* rotation, repeated soil sampling over forest development will permit a more in-depth investigation of changes in soil parameters and microbial diversity in the Congolese coastal plains.

To respond to the second question of our study: (ii) "Is there any link between bacterial community and vegetation cover, nutrient cycling, and other parameters (N, C, C/N ratio, P availability, and pH)?", several biogeochemical processes such as C decomposition and fixation, N cycling and fixation, P utilization, methane metabolism, and sulfur cycling are regulated and linked to microbial communities [74]. The impact of S and, to a lesser extent, C on the bacterial community of the studied soils was greater, as shown by the Spearman test. It affects our findings owing to its impacts on the bacterial community structure. In the *Pinus massoniana* plantation established on Ultisol in Subtropical China, Xu et al. [29] reported the growth of *Actinobacteria*, gram-positive bacteria, and fungi via an increase in their biomass following S amendments. This is in accordance with our findings reporting a positive correlation between S and the most prevalent phylum, i.e., *Actinobacteria,* with a very high statistical significance ($R = 0.55$; $p = 0.0004$). This strictly positive connection was detected at all levels of *Actinobacteria* taxonomy, e.g., for the three classes *Actinobacteria, Thermoleophilia* and *Acidimicrobiia* and for the four orders *Frankiales*, *Solirubrobacterales*, *Corynebacteriales,* and IMCC26256, with the only exception of the class *Pseudonocardiales*. Dong et al. [75] reported that the phylum *Actinobacteria* was dominant in the areas with important concentrations of H_2S. This may explain the strong correlation between S and *Actinobacteria* in the forest plantations of the Congolese coastal plains, where H_2S may have been deposited following oil exploration in the last 4 decades (L.-S. Koutika, *personal communication*). This must be prospected and confirmed in the future by analysis of soil beneath the natural savanna of the area in comparison to the savanna from another region. Being associated at all levels to the bacterial community, even though at the lesser extent than S, C is revealed to be a matter factor driving biogeochemical processes such C and N mineralization and cycling of the studied soils and confirms other studies highlighting its importance in forest ecosystems [27,31,74]. Correlation between soil N and C/N ratio and bacterial community has been detected only at the class and genus levels, respectively. However, both parameters are crucial for ecosystem functioning processes linking soil, plant, and environment. Accretion in N storage previously observed [5] may have led to a decrease not only in microbial richness and diversity indices [28] but also in lighting its link to the bacterial community of the studied soils.

Available P is required for N_2 atmospheric symbiotic fixation by NFTs and is also crucial in forest tropical ecosystems in general and in the studied forest plantations dominated by biological processes in particular [53]. P availability exhibited a positive correlation with bacterial structure ($R = 0.19$, $p = 0.0012$) followed by the C/N ratio ($R = 0.15$, $p = 0.0511$), while Spearman correlation test reflected a positive correlation between P and bacterial community from order to genus. Therefore, both redundancy analyses and Spearman's coefficients highlighted the correlation between P and bacterial communities in the studied soils. Our results are in accordance with [31], that demonstrated a positive significant link between changed soil attributes (C/N ratio, pH, and P) due to the effects of litterfall and root exudates, and bacterial communities in a broad-leaved forest ecosystem in

central Germany. Even though P availability appeared to be no limiting factor affecting bacterial diversity in Chinese fir plantation [53], it is a very important element in sustaining tropical forest plantations [30,76,77]. In previous studies, a decrease in soil available P has been reported in stands containing *Acacia* relative to *Eucalyptus* established in the Congolese coastal plains [5,11] because of the well-known requirement of NFTs to sustain symbiotic root nodules and atmospheric N_2 fixation processes [9,10,67]. However, its status improved in all planted stands with *Eucalyptus* and/or *Acacia* compared to savannas [6]. Its significance is also shown through the great amount of extractable P in the forest floor of the *Acacia* stands [13] resulting from important inputs of P in organic residues and litterfall [11] relative to *Eucalyptus*, probably due to *Acacia* ability to phosphorous retranslocation [10]. The dynamics of P in the studied soils is also explained by the fact that most of the mineral soil P is in inorganic (70%) form with orthophosphate as the prevalent P form in floor forest and mineral soil (0–5cm) [13] and P cycling is dominated by biological process [12]. Other findings outlining increased available soil P as a crucial factor that reinforce the link between plant diversity, soil attributes, and ecosystem function which ensure soil P bioavailability and alleviate soil P limitations [78]. This is probably due to fungal community activities by the exudation of phosphatases [78], displaying a large potential to accumulate mineral or organic P from the soil and even absorb inorganic P from the soil solution [30] or precisely to arbuscular mycorrhizal fungi colonization and phosphatase activities which commonly boost soil P cycling in the pure or mixed *A. mangium* with *Eucalyptus* plantations [77].

The positive correlation between soil attributes (C, S, available P, and C/N ratio) and soil bacterial community in the mixed *Acacia* in *Eucalyptus* plantations indicated improved soil fertility with the potential to sustain forest productivity and ecosystems. Nutrient cycling (C, N, S, and P), i.e., its link to microbiota, exerts an important role in processes such as arbuscular mycorrhizal fungi colonization, phosphatase activities, atmospheric N fixation, and C sequestration [9,10,27,74,77], especially in the nutrient-poor soils such as those of the Congolese coastal plains. In fact, in the less infertile soils, no correlation was reported between soil attributes and bacterial community beneath *Acacia* and *Eucalyptus* plantations at Itatinga (Brazil) in a young forest (27 and 39 months) [23]. The response to the second question has been given: soil microbiota of studied soil samples is linked to vegetation cover, nutrient cycling, and parameters such as C, N, C/N, S, and P. Our study revealed the ecological roles of the bacterial community associated to pure and mixed-species plantations of *Acacia* and *Eucalyptus* in the Congolese coastal plains in community maintaining and soil nutrient cycling.

5. Conclusions

Our results revealed that the sustainability of mixed-species forest plantations established in the nutrient-poor soils in the Congolese coastal plains relies on the link between tree species, soil nutrient availability, and the bacterial community. The phyla composition, bacterial richness, and phylogenetic diversity beneath mixed-species plantations are undeniably linked to forest management (afforestation of savannas with *Eucalyptus* and introduction of *Acacia*), soil intrinsic properties (nutrient cycling, pH, texture, etc.), and environment (climate, relief). A shift in the bacterial community in stands containing *Acacia* mixed-species relative to *Eucalyptus* was evidenced by the dominance of *Proteobacteria* in pure *Eucalyptus* against *Firmicutes* in the former. Although no differences in the percentage of relative abundance of sequences belonging to *Actinobacteria* have been detected among stands, a strong correlation to S at all levels of its taxonomy was observed that needs further studies. Changes in bacterial community structure are found to be linked to other shifts occurring in these ecosystems, i.e., enhanced P dynamics in forest floor and soil and change in SOM status through C, N, and C/N ratio and through S cycling, as evidenced by the positive correlations between the bacterial community composition and soil parameters. In conclusion, our work revealed the strong reliance of Congolese coastal plains ecosystem sustainability on the interaction between soil attributes, plant, bacterial communities, and environment, confirming the benefits of the NFTs in improving soil fertility and sustaining *Eucalyptus* plantations established on the Ferralic Arenosols in the coastal plains of the Republic of Congo.

Supplementary Materials: The following are available online at http://www.mdpi.com/2071-1050/12/21/8763/s1. **Figure S1:** Taxa plot showing the relative abundance of bacterial classes in stands of pure *Eucalyptus* (E) ($n = 9$) and pure *Acacia* (A) ($n = 9$), and mixed-species stands (Emix, 50% *Acacia* and 50% *Eucalyptus* with soil sampled near *Eucalyptus* trees, $n = 9$; Amix, 50% *Acacia* and 50% *Eucalyptus* with soil sampled near *Acacia* trees, $n = 9$). Other: unclassified taxa and other bacterial phyla with low OTU abundance. **Figure S2:** Taxa plot showing the relative abundance of bacterial orders in stands of pure *Eucalyptus* (E) ($n = 9$) and pure *Acacia* (A) ($n = 9$), and mixed-species stands (Emix, 50% *Acacia* and 50% *Eucalyptus* with soil sampled near *Eucalyptus* trees, $n = 9$; Amix, 50% *Acacia* and 50% *Eucalyptus* with soil sampled near *Acacia* trees, $n = 9$). Other: unclassified taxa and other bacterial phyla with low OTU abundance. **Figure S3:** Taxa plot showing the relative abundance of bacterial families in stands of pure *Eucalyptus* (E) ($n = 9$) and pure *Acacia* (A) ($n = 9$), and mixed-species stands (Emix, 50% *Acacia* and 50% *Eucalyptus* with soil sampled near *Eucalyptus* trees, $n = 9$; Amix, 50% *Acacia* and 50% *Eucalyptus* with soil sampled near *Acacia* trees, $n = 9$). Other: unclassified taxa and other bacterial phyla with low OTU abundance. **Figure S4:** Taxa plot showing the relative abundance of bacterial genera in stands of pure *Eucalyptus* (E) ($n = 9$) and pure *Acacia* (A) ($n = 9$), and mixed-species stands (Emix, 50% *Acacia* and 50% *Eucalyptus* with soil sampled near *Eucalyptus* trees, $n = 9$; Amix, 50% *Acacia* and 50% *Eucalyptus* with soil sampled near *Acacia* trees, $n = 9$). The relative abundance was calculated as the percentage of sequences belonging to a particular lineage of all 16S rRNA gene sequences recovered from a given plantation system. Other: unclassified taxa and other bacterial phyla with low OTU abundance. **Figure S5:** Heatmap of Spearman's rank correlation coefficients between major bacterial classes with soil chemical attributes: the values of correlation coefficients are indicated according to the scale bar. Significant correlations (p-value < 0.05) are shown by an asterisk. **Figure S6:** Heatmap of Spearman's rank correlation coefficients between major bacterial orders with soil chemical attributes: the values of correlation coefficients are indicated according to the scale bar. Significant correlations (p-value < 0.05) are shown by an asterisk. **Figure S7:** Heatmap of Spearman's rank correlation coefficients between major bacterial families with soil chemical attributes: the values of correlation coefficients are indicated according to the scale bar. Significant correlations (p-value < 0.05) are shown by an asterisk. **Figure S8:** Heatmap of Spearman's rank correlation coefficients between major bacterial genera with soil chemical attributes: the values of correlation coefficients are indicated according to the scale bar. Significant correlations (p-value < 0.05) are shown by an asterisk.

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