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Impact of Land Use on Bacterial Diversity and Community Structure in Temperate Pine and Indigenous Forest Soils

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Abstract: Soil microbial communities are an important part of ecosystems that possess the capability to improve ecosystem services; however, several aspects of the ecology of forest soil bacterial communities are still unknown. Here, we investigated the impact of land-use change on soil bacterial communities and the soil characteristics. High-throughput sequencing was used to ascertain the bacterial diversity and canonical correspondence analysis was used to determine relationships between the bacterial communities and environmental variables. Our results show spatial heterogeneity in the distribution of the microbial communities and significant relationships between the microbes and soil characteristics (axis 1 of the canonical correspondence analysis (CCA) plot explained 64.55% of the total variance while axis 2 described 24.49%). Knowledge of this is essential as it has direct consequences for the functioning of the soil ecosystem.

Keywords: 16S rRNA amplicon sequencing; forest management; metabolic potentials; microbial ecology; ribosomal data project; South Africa

1. Introduction

Forests are ecosystems that are extremely productive, representing approximately 30% of the whole global land area, and display great spatial heterogeneity because of their multi-layered vegetation [1]. Forests frequently act as carbon sinks with huge quantities of recalcitrant organic matter in their soils [2]. Abundance of trees in forests is a notable feature that distinguishes them from other biomes [3]. The composition of soil microbial communities plays pivotal roles in the productivity of forests and in soil functioning [4–6]. Soil microbial communities perform important roles in the biogeochemical cycles, thereby contributing to the fertility and functioning of soil ecosystems. The loss of diversity in soil ecosystems has functional consequences [7,8]. Bacterial activities in forest soils are extremely important because they facilitate majority of the biogeochemical processes and eventually regulate the accessibility of mineral nutrients in the soils [9]. The composition of bacterial communities can be influenced by the modification of the physical, biological and chemical properties of soils by land management practices [10,11]. Crucial ecosystem functions such as carbon sequestration, the control of climate and water purification are closely associated to microbial activities and influenced by the conversion of land-use [12]. Intensive anthropogenic perturbations like the transformation of natural lands into managed lands have been reported to intensely alter soil microbiota. Approximately 40% of lands worldwide have been transformed into controlled systems and about 85% of them are affected by anthropogenic activities in one way or the other [13–15]. Humans have increased the immensity of land-use and land-use change is the most direct indication of the influences of human actions on the natural environment. [16]. A considerable fraction of forests has been used up for timber mining and agricultural development as a result of the growing demand for human security [17–19].

The diversity of terrestrial biomes is diminished by the substitution of native vegetation with a few crops and this causes the degradation of land, loss of biodiversity and depletion of nutrient in the soil, among other concerns. Through the provision of functional diversity and redundancy, a high microbial diversity is thought to be essential for the maintenance of ecosystem stability [20]. Even though soil bacteria have been investigated for a long time, most of its diversity is still not explained because this domain is among the most copious and diverse set of organisms on Earth [21]. Forest soil bacterial communities perform significant functions; however, most studies have focused on fungal communities in this environment. While the knowledge of forest soil bacterial ecology has progressed considerably in recent years, it is still partial [1]. A thorough ecological comprehension of soil bacterial species will enhance the capacity to actively control these microbes for the advancement of their functional proficiencies [21].

The role of soil bacteria in ecosystem functioning can be affected by the same factors that influence their community composition [11]. An understanding of the response of soil bacteria to land-use change would therefore enable predictions of their influence on the soil ecosystem functioning. Thus, we investigated the influence of land-use change in forest ecosystems on bacterial diversity using the high-throughput 16S rRNA amplicon sequencing approach. Culture-independent 16S ribosomal RNA sequencing has been extensively used in recent times to examine bacterial diversity from numerous environments since sequencing of PCR-amplified 16S rRNA overcomes the drawbacks of culture-based bacterial detection [22]. We hypothesized that the different vegetation types and the environmental conditions of the sampling sites would affect the diversity of the bacterial communities inhabiting the soils. We also proposed that the indigenous forests that are natural would have more diverse bacterial communities relative to the managed pine forests.

2. Materials and Methods

2.1. Site Description and Soil Sampling

Soil samples were collected from four sites: The Tweefontein indigenous forest (TI), the adjacent Tweefontein commercial forest (TC), the Witklip indigenous forest (WI) and the adjacent Witklip commercial forest (WC) (Figure 1). Tweefontein plantation is situated in Graskop and is typified by biodiversity. Lush grasslands on the pinnacle of the western mountain range to extensive areas of indigenous forest comprising the kranses fashioned by quartzite bands. Diverse and spectacular waterfalls also characterize this plantation. Sampling was done in the commercial and indigenous forest plantations, which are illustrative of temperate climatic conditions. The dominating tree species in the commercial plantation is the *Pinus patula*, while *Acacia xanthophloea* and *Celtis africana* dominate the indigenous forest. The commercial plantation covers an area of 5965.84 ha while the indigenous forest covers 10,484.09 ha. The mean annual precipitation of the area is 1012 mm and a mean annual temperature of 16.2 °C. In the Witklip plantation, the commercial plantation covers 5616.62 ha while the indigenous forest covers 4693.44 ha. *Pinus patula* also inhabit the commercial forest. The two plantation forests are currently on second rotation (one rotation = 30 years) and they practice sustainable forest management. These plantations have been FSC certified for the past 20 years.

Soil samples were collected from the two indigenous and commercial forests in July 2016. Ten soil cores (2 cm in diameter and 10 cm in depth) were collected within multiple tree rows at various points within the four sampling sites. These cores were then pooled together and homogenized into a composite sample per site. After sampling, the soil samples were preserved temporarily in cooler boxes filled with ice and conveyed to the laboratory where they were stored in a fridge at a temperature of 4 °C for 2 weeks before further analysis.

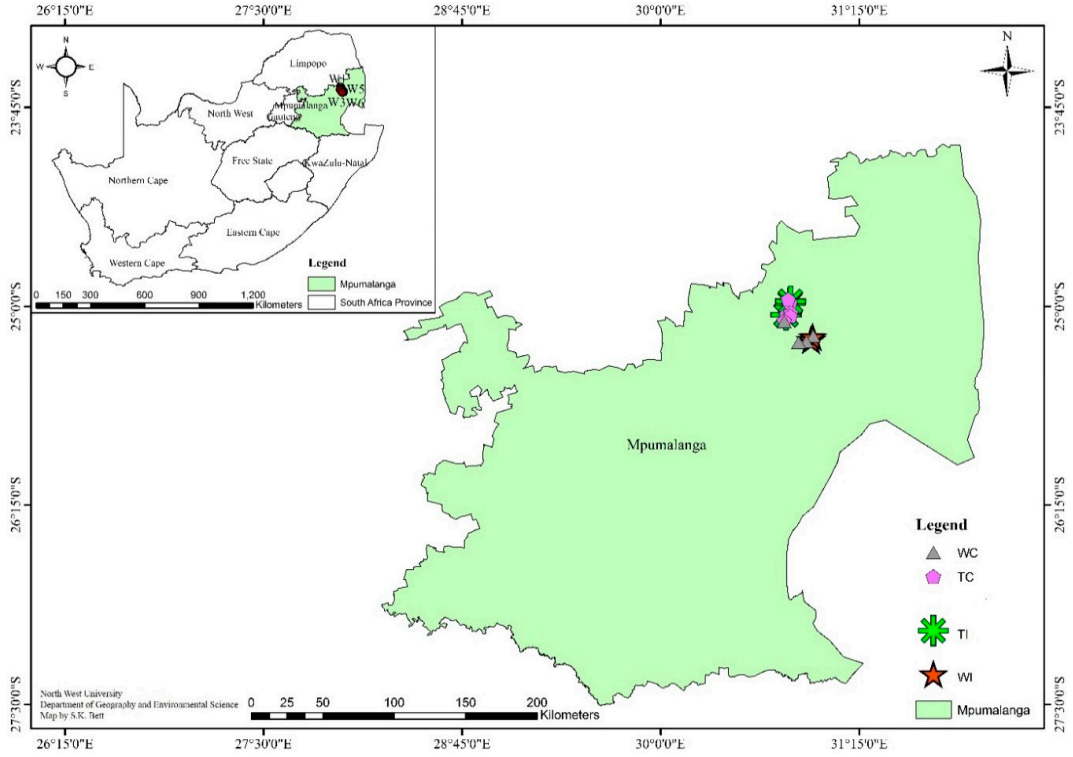

Figure 1. Distribution of the sampling sites in Tweefontein and Witklip forests.

2.2. Analyses of Soil Properties

Using a 2 mm mesh, the samples were sieved to remove debris. The soil pH was measured by mixing 2 g of fresh soil in 10 mL deionized water using a Jenway 3520 pH-meter (Cole-Parmer Instruments, Staffordshire, UK). The size of the soil particles was classified according to the method used by Enagbonma, et al. [23]. Total carbon and nitrogen were measured using the dry combustion method as described by Santi, et al. [24]. Soil nitrate was determined by KCl extraction method. Organic carbon in the soil was determined by the Walkley Black method [25]. Soil calcium, sodium, magnesium and potassium were examined after extraction using 1 M ammonium acetate at pH 7.0. Thereafter, magnesium, sodium and calcium in the extracts were measured using an atomic absorption spectrophotometer while potassium was measured through a flame photometer [26]. Phosphorus was measured with a spectrophotometer [27].

2.3. Determination of Relative Bacterial Diversity and Taxonomic Richness

Total genomic DNA was extracted from 0.25 g of soil using the PowerSoil® DNA isolation kit (MoBio Laboratory, Carlsbad, CA, USA) according to the manufacturer's instructions. The examination of the bacterial community was carried out using 16S amplicon sequencing. The 16S rRNA gene variable region V4 was sequenced using an Illumina MiSeq sequencer by the Next Generation Sequencing Service at Molecular Research LP (MR DNA, Shallowater, TX, USA). Employing the PCR primers 515F (5′-AATGATACGGCGACCACCGAGATCTACAC TATGGTAATT GT GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-CAAGCAGAAGACGGCATACGAGAT TCCCTTGTCTCC AGTCAGTCAG CC GGACTACHVGGGTWTCTAAT-3′), paired-ends reads of 312 bp were obtained [28]. Data analysis was carried out using the MR DNA analysis pipeline and MG-RAST. The paired ends were joined; barcodes, sequences < 150 bp and sequences with ambiguous base calls were removed. The sequences were then denoised and screened for the presence of chimeras. Analogous sequences were binned into operational taxonomic units (OTUs). OTUs were defined by clustering at 3% divergence (97% similarity). Final OTUs were taxonomically classified using BLASTn against a curated database derived from RDPII and NCBI [29]. Rarefaction was used to estimate species

richness while Shannon and evenness indices were used to depict the alpha diversity. The diversity indices were evaluated across sites using the Kruskal–Wallis test and all the analyses were carried out using PAST version 3.20 [30]. Taxonomic richness was expressed as an OTU number. Sequences used in this study have been deposited in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) under the bioproject numbers SRR8136388 (WC), SRR8136221 (WI), SRR8135323 (TC) and SRR8134476 (TI).

2.4. Statistical Analyses

The Shinyheatmap [31] was used to plot a graph of the relative abundance of bacterial communities at phylum level. The bacterial community composition (abundance data of relative OTU) was analyzed using principal coordinates analysis (PCoA) [32] based on a Bray-Curtis distance matrix using CANOCO 5 (Microcomputer Power, Ithacha, NY, USA). Permutational multivariate analysis of variance (PerMANOVA) was performed to evaluate the significance of land-use types [33] using the software R 3.3.3 (R Core Development Team 2017) [34]. The correlations between physicochemical parameters were determined by one-way ANOVA with Tukey's HSD test using the SPSS package (v25.0). $P < 0.05$ were considered statistically significant. CANOCO 5 was also used to carry out the canonical correspondence analysis (CCA), which was used to evaluate the likely connections between microbial communities and the measured physicochemical parameters. To find the environmental variables that best explained bacterial composition, the forward selection of environmental properties and the Monte Carlo permutation test was employed. For the significance test, 999 random permutations were used. The environmental variables enumerated in Table 1 were used as explanatory variables in the CCA analysis.

Table 1. Mean ± standard error values of the physical and chemical properties of the forest soils.

Sample	TC	TI	WC	WI
Organic C (%)	3.21 ± 0.06	4.08 ± 0.07	2.97 ± 0.05	2.67 ± 0.07
NO_3^- (mg/kg)	63.38 ± 0.16	65.13 ± 0.01	22.01 ± 0.19	5.91 ± 0.03
Total C (%)	3.97 ± 0.05	4.58 ± 0.02	3.04 ± 0.00	2.91 ± 0.03
Total N (%)	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.001 ± 0.00
pH	4.28 ± 0.01	4.78 ± 0.11	5.31 ± 0.03	5.10 ± 0.01
P (mg/kg)	3.16 ± 0.00	2.33 ± 0.02	3.21 ± 0.08	5.03 ± 0.01
Ca (mg/kg)	15.85 ± 0.05	234.50 ± 1.50	320.50 ± 1.50	164.50 ± 1.50
Mg (mg/kg)	52.35 ± 0.15	95.60 ± 0.10	117.00 ± 1.00	97.65 ± 0.45
K (mg/kg)	54.00 ± 0.10	70.30 ± 0.10	69.95 ± 0.35	92.20 ± 0.10
Na (mg/kg)	11.60 ± 0.30	15.35 ± 0.15	16.60 ± 0.30	15.50 ± 0.20
Sand (%)	23.50 ± 1.40	59.85 ± 3.25	55.15 ± 1.95	55.10 ± 0.60
Silt (%)	50.80 ± 1.60	35.20 ± 2.10	17.45 ± 0.75	16.95 ± 0.45
Clay (%)	19.00 ± 0.10	9.00 ± 0.60	23.45 ± 1.05	25.05 ± 0.75

3. Results

3.1. Soil Properties (Physical and Chemical) of the Forest Soil Samples

The physicochemical properties of the soils were affected by land-use change and forest management practices (Table 1). Organic carbon ($F_{3,4} = 104.570$, $P = 0.000$), soil nitrate (NO_3^-) ($F_{3,4} = 56{,}900.188$, $P = 0.000$), total carbon ($F_{3,4} = 792.180$, $P = 0.000$), pH ($F_{3,4} = 63.115$, $P = 0.001$), phosphorus ($F_{3,4} = 751.937$, $P = 0.000$), soil calcium ($F_{3,4} = 9840.714$, $P = 0.000$), magnesium ($F_{3,4} = 2412.648$, $P = 0.000$), potassium ($F_{3,4} = 6457.109$, $P = 0.000$) and sodium ($F_{3,4} = 78.443$, $P = 0.001$) were significantly influenced by land-use practices. However, total nitrogen was not found to be significantly different across the four sites ($F_{3,4} = 2.434$, $P = 0.205$). The mineral soils in TC had the lowest share of the sand fraction (23.50%) and the highest share of silt (50.80%). Site WI had the highest clay fraction (25.05%) (Table 1). The sand particles ($F_{3,4} = 67.251$, $P = 0.001$), silt ($F_{3,4} = 135.737$, $P = 0.000$) and clay fractions ($F_{3,4} = 102.436$, $P = 0.000$) were significantly different across the forest soils.

3.2. Rarefaction Analysis

The richness of the bacterial diversity in the forest soil samples was assessed by rarefaction analysis (Figure 2). The results revealed that the rarefaction curves for indigenous forests (WI and TI) were higher than the commercial forests (WC and TC).

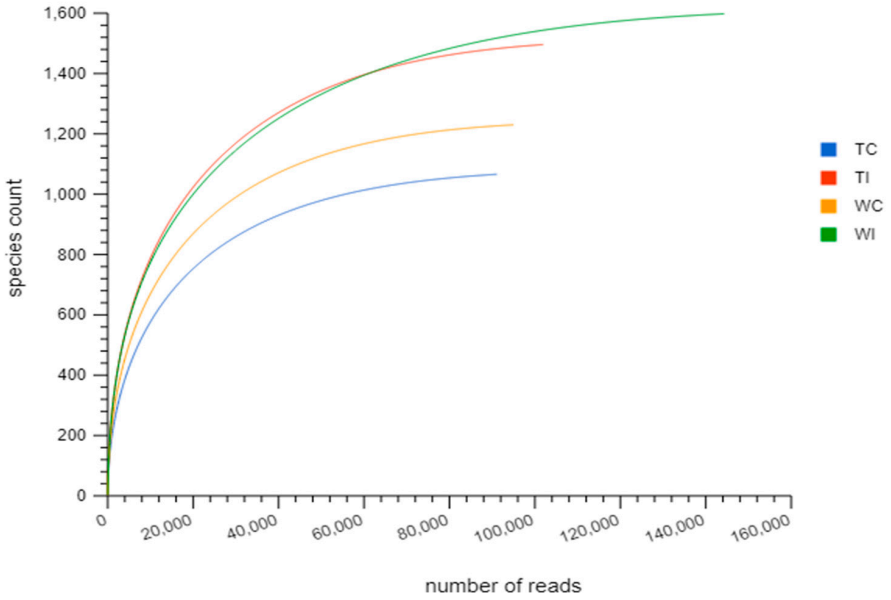

Figure 2. Rarefaction curves showing the estimated richness in the forest soils and sampling effort. The number of OTUs estimated after sampling is represented on the vertical axis while the number of sequences is presented on the horizontal axis.

3.3. Assessment of Diversity Indices

RDP classifier was employed for the assignment of sequence reads of the indigenous and commercial forest soils into OTUs with 3% nucleotide cut-off values. The Shannon and evenness indices are also estimated in Table 2.

Table 2. Analysis of sequencing data and diversity estimation of the amplicon metagenomes of the forest soil samples.

Bioproject Number	**SRR8135323**	**SRR8134476**	**SRR8136388**	**SRR8136221**
Sampling site	TC	TI	WC	WI
		Uploading Information		
bp count	41,707,827	46,709,377	43,701,871	66,310,459
Sequences count	91,160	101,138	95,076	144,319
Mean sequence length (bp)	458 ± 14	458 ± 14	460 ± 15	459 ± 14
Mean GC content (%)	57 ± 2	57 ± 3	56 ± 3	56 ± 2
		Post QC Information		
bp count	5,310,782	6,752,690	6,060,310	7,440,367
Sequences count	11,570	14,666	13,134	16,140
Mean sequence length (bp)	459 ± 12	460 ± 12	461 ± 12	461 ± 11
Mean GC content (%)	57 ± 3	57 ± 3	56 ± 3	56 ± 3
		Processed Sequences		
Predicted protein features	93	85	65	79
Predicted rRNA features	14,918	22,226	16,029	26,074
		Aligned Sequences		
Identified protein features	28	27	19	19
Identified rRNA features	13,720	21,040	15,252	24,702
Shannon_H	1.95	1.98	1.88	1.95
Evenness_eˆH/S	1.31	1.31	1.36	1.62

3.4. Principal Coordinates Analysis

The PCoA plot showed no sample-type-specific clustering and resolution of individual sample datasets, which depicts significant difference (PerMANOVA, $P = 0.001$) in the bacterial composition between the indigenous and commercial forest soils (Figure 3). These data suggest that there are differences in the features of the individual datasets when compared to each other.

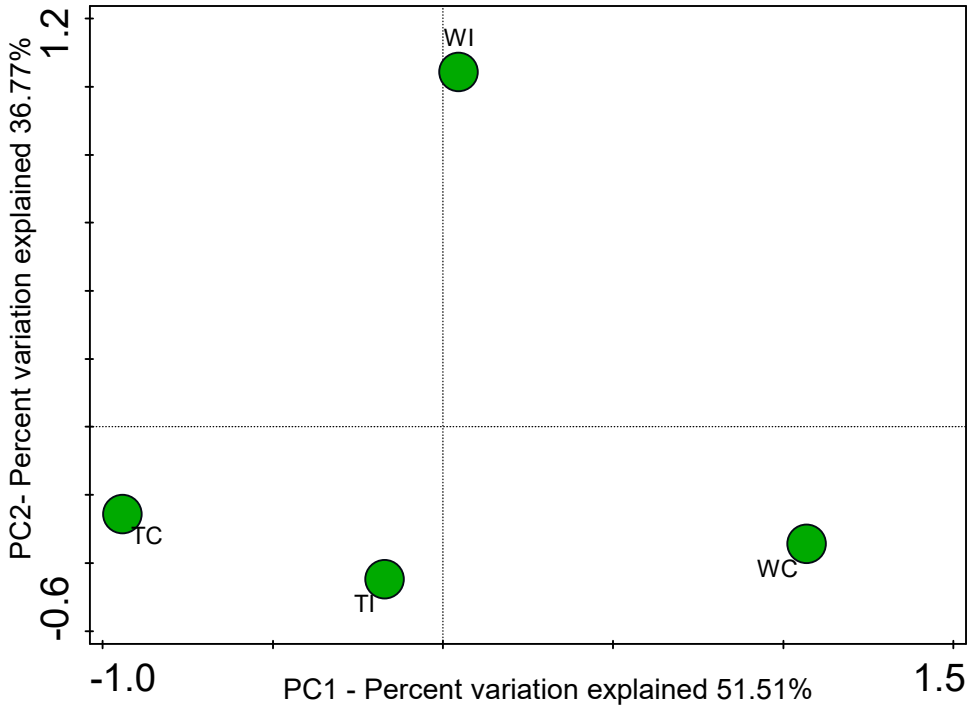

Figure 3. Principal coordinates analysis (PCoA) plot of bacterial community composition at operational taxonomic units (OTU) level.

3.5. Phylum and Genus Level Distributions of the Bacterial Communities

The metagenomic rapid annotations using subsystems technology (MG-RAST) at http://www.mg-rast.org [35] was used to assign sequence tags of indigenous and commercial forest samples into different taxa. Figure 4 sums up the relative abundance of bacterial phyla for each sample. The indigenous forests were predominated by the phyla *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, *Spirochaetes*, *Cyanobacteria*, *Dictyoglomi* and *Tenericutes*, whereas the commercial forests were predominated by *Planctomycetes* and *Nitrospirae* (Figure 4). Figure 5 shows the relative abundance of the bacterial genera for each site. In the indigenous forests, *Chthoniobacter*, *Candidatus Koribacter*, *Candidatus Solibacter* and *Ktedonobacter* were the predominant genera while the commercial forests were dominated by *Chthoniobacter*, *Candidatus Solibacter*, *Bradyrhizobium* and *Ktedonobacter*.

3.6. Influence of Environmental Factors on Bacterial Communities

The relationship between the measured soil physicochemical parameters and the relative abundances within bacterial phyla was investigated using the canonical correspondence analysis. Three parameters, including total C, pH, and P, were selected for CCA based on the significant test (Figure 6) and, as shown in Table 3, they are the environmental factors that best explained variation in bacterial composition.

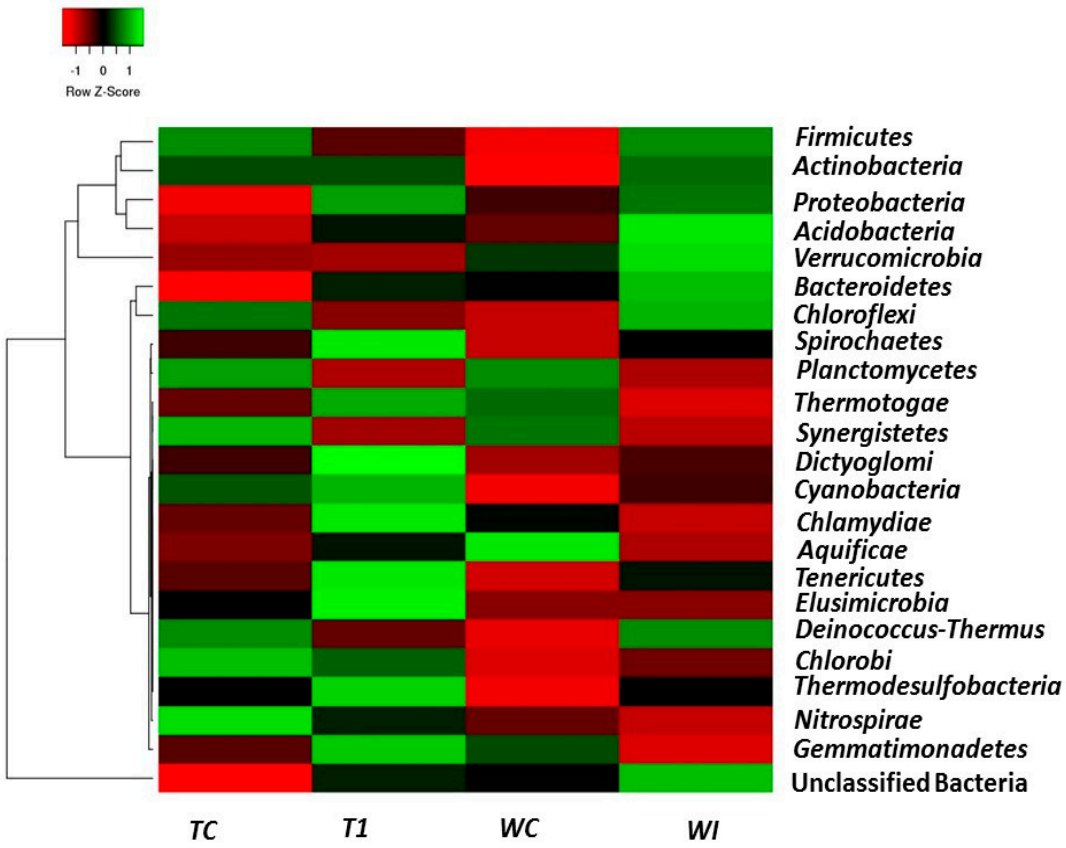

Figure 4. Taxonomic classification of the bacterial distribution at phylum level from the forest samples.

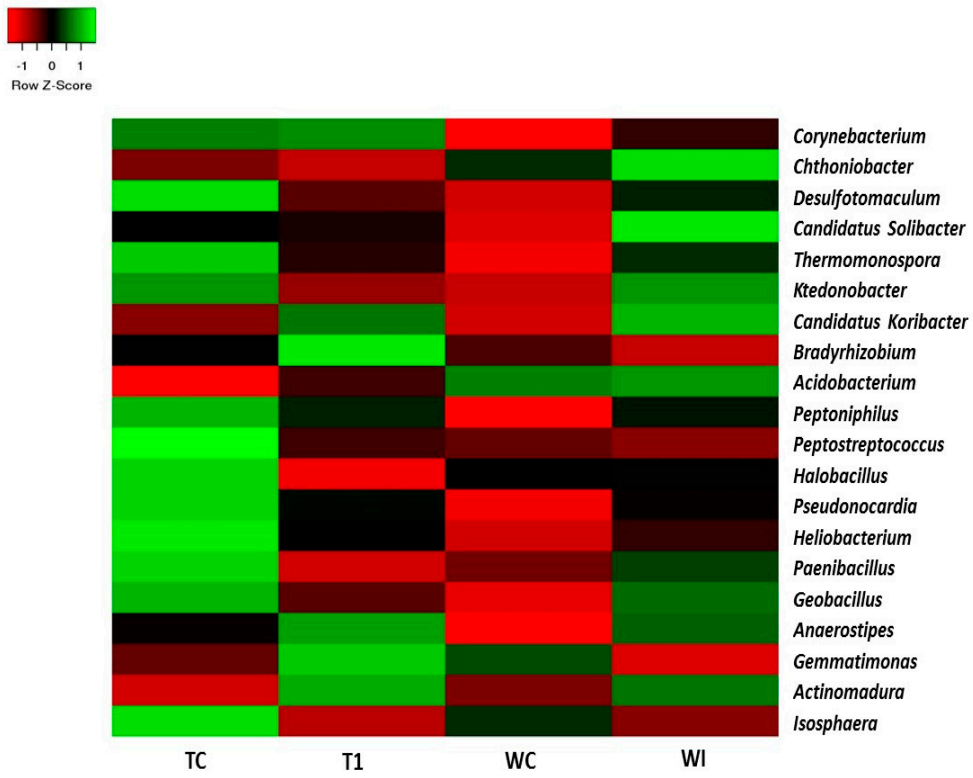

Figure 5. Taxonomic classification of the bacterial communities in the forest soils at genus level.

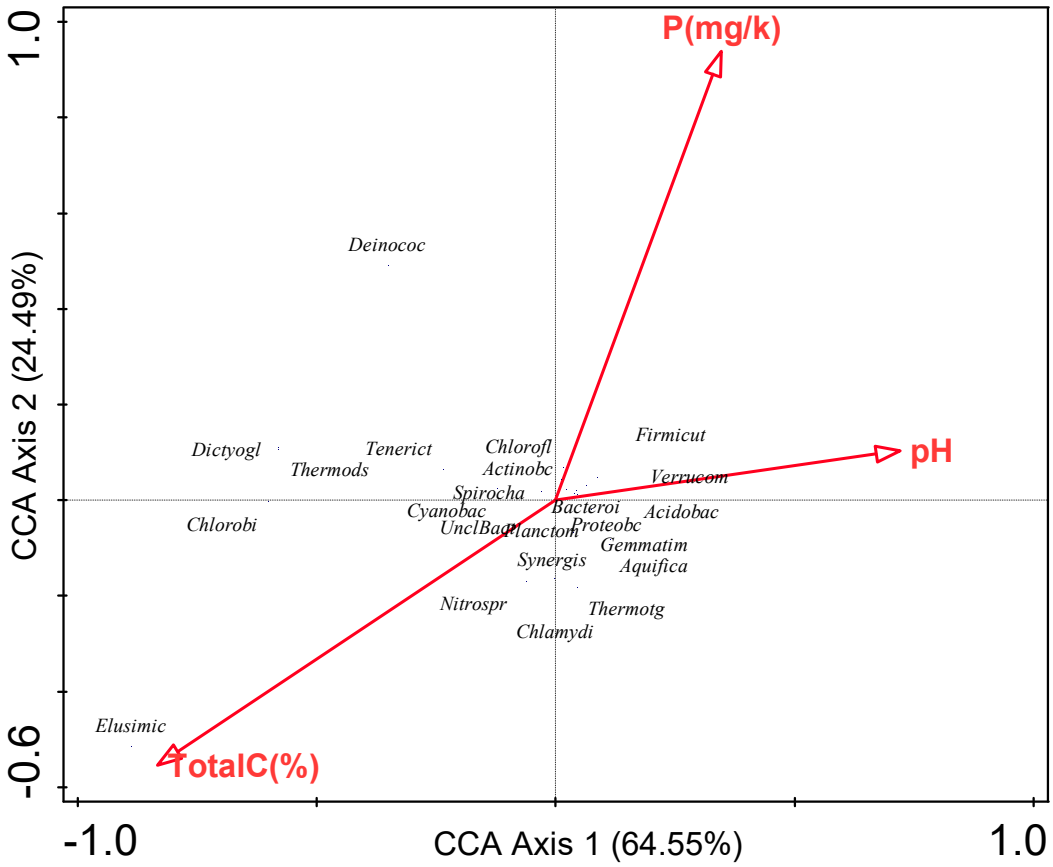

Figure 6. Canonical correspondence analysis (CCA) of relative abundances at the phylum level and major physicochemical parameters in the soils.

Table 3. Forward selection of environmental variables which best explained disparity in bacterial community composition between samples.

Environmental variable	Explains %	Contribution %	Pseudo-F	*P*
Total C	52.30	52.30	2.20	0.18
P	36.80	36.80	3.40	0.22
pH	11.00	11.00	<0.1	1.00

4. Discussion

Our results showed high bacterial diversity in the pine and indigenous forests. The community structure of bacteria varied in the investigated forests. This study provides evidence that land-use practices affected the composition of bacterial communities in the sites investigated. Bacterial communities from both natural forests (TI and WI) were distinct from the communities inhabiting the managed forests along the major axis (Figure 3). *Proteobacteria* accounted for the highest abundances and was the most dominant phylum across the indigenous forests (Figure 4). *Proteobacteria* have been reported to be dominant at the expense of other phyla in various environments including natural woodlands, forest plantations [8] and mine tailings. Members of the phylum *Proteobacteria* have been described to possess versatile metabolic capabilities (Xiao et al., 2016). The abundance of the phyla *Verrucomicrobia*, *Acidobacteria*, *Actinobacteria* and *Chloroflexi* was also relatively high as has been reported by other studies of forest soils [8,36]. Bacterial communities in TI and WI seemed very similar suggesting that a change in the land-use has a strong impact on the bacterial communities. Several studies have reported that changing native forests into monoculture plantations alter the microbial community composition of forest soils [37,38]. Our results showed higher bacterial richness and

diversity in the indigenous forests relative to the commercial forests. The community structure of bacteria varied significantly (PerMANOVA, $P = 0.001$) in the investigated forests as revealed by the PCoA plot (Figure 3). This study provides evidence that land-use practices affected the composition of bacterial communities in the sites investigated.

All the parameters considered were vital in determining the structure of the microbial communities. Total C explained 52.30% of the total variation. Previous studies have stated that total carbon is a key factor influencing the structure of soil microbial communities. Total carbon has been reported to have substantial direct influence on soil bacterial biomass and it could afford bacteria the benefit of competition with fungal communities for resources [39–41]. Phosphorus also affected the structure of the bacterial communities in this study. Soil P influences the absolute abundances of soil microbes [42] and the incessant anthropogenic additions of P are vital in the enhancement of primary production and decomposition in forest ecosystems [43]. Considering the length of the vector of pH, it was a strong determinant in the shaping of the microbial communities. The pH of the soils ranged from 4.28 ± 0.01 to 5.31 ± 0.03 and was significantly different across the sites. The structure and function of soil microbes have been reported to be driven by soil pH. The reaction of microbes to soil pH changes differs. Soil pH has great effects on the biodiversity patterns and activities of microbes in contrast to land-use [44–46]. Environmental variables are key factors in the structure and function of bacterial communities in soils. Shifts in bacterial communities in response to environmental properties have been published [47,48]. Our results confirmed that nutrients affected the structure of bacterial communities in the present study. Studies that have examined the influence of edaphic factors on soil bacterial communities reported that these communities were shaped by the accessibility of nutrients [49,50].

5. Conclusions

The examination of belowground communities in temperate forests presented evidence that environmental variables are crucial drivers of soil bacterial diversity, abundance and community composition. This has explicit consequences for the functioning of the soil ecosystem. These results provide further evidence that the patterns of distribution of soil bacteria and their structure undergo spatial heterogeneity. The characterization of abiotic properties of soils provides perception on the factors that influence the diversity of soil bacterial communities and how these communities are altered. Additional evidence from manipulative and extensive experiments is essential.

The structure of soil microbial communities is mainly affected by land-use change. Even though forest plantations are being recommended as avenues for the sequestration of the increased atmospheric CO_2, they can alter soil microbial community structure as substantiated by this study. The replacement of indigenous forests with plantations should therefore be prevented completely, if possible. This is necessary for ecosystem sustainability.

Author Contributions: A.E.A. and O.O.B. designed the study; A.E.A. conducted the field and laboratory components; A.E.A. and O.O.B. made substantial contributions to the analysis and interpretation of data. A.E.A. wrote the first draft of the manuscript and both authors contributed critically to drafts. Both authors approved the article for publication.

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Conflicts of Interest: The authors declare no conflict of interest.

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