Research article

Linking rhizosphere bacterial diversity and soil fertility in tobacco plants under different soil types and cropping pattern in Tanzania: A pilot study

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ARTICLE INFO

ABSTRACT

Tobacco (*Nicotiana tabacum* L.), one of the major crop plants in Tanzania, cropping affects the level of soil fertility, but the reason has not been known. Plant rhizosphere plays an important role in affecting soil fertility through changing microbial composition. We planned a pilot study to understand the changes in microbial composition and soil nutrients in the rhizosphere soils of tobacco in three agro-ecological zone, namely Sikonge, Tabora and Urambo in Tanzania. This study assessed bacteriota composition using 16S rRNA sequencing and soil fertility in the rhizosphere of tobacco plants. The results showed that bacterial diversity in tobacco rhizosphere soils belonged to *Proteobacteria* phyla, associated significantly ($p < 0.05$) with solubilization of insoluble P, K and S. The solubilization of P, K and S in soils facilitates the availability of these nutrients to the tobacco plants (a heavy feeder crop) allows low levels of these nutrients in the soils for the subsequent crop. The *Proteobacteria* phyla also associated with an increase in soil N content through fixation. Therefore, bacteria diversity in tobacco rhizosphere influence solubilities of macronutrients (P, K, S) and quickly up taken by the tobacco plant and reduces their levels in soils, some bacteria involved in fixing N and increases total N in the soil.

1. Introduction

Tobacco (*Nicotiana tabacum* L.) is the major cash crop cultivated mainly in major three continents, namely Asia, America and Africa (Lisuma et al., 2019). Tobacco crop is mostly rotated with maize crop and considered to be the most soil nutrients heavy feeder crop (Sauer and Abdallah, 2007; Bastani et al., 2018; Moula et al., 2018). Crop rotation reported influencing soil fertility and rhizosphere microbes (Hauchhum and Tripathi, 2019; Manpoong et al., 2020). Rhizosphere's narrow zone around the plant roots, is characterized by the higher bacterial activity and soil nutrients than the bulk soil. Changes in the rhizosphere soils nutrients and microbial diversity are faster than the changes in the bulk soil due to management practices, and rhizosphere soils indicate the fertility of the soil in the range ecosystems. Tobacco rhizosphere is a complex and usually associated with bacteria diverse community involved in stimulating growth through nutrient acquisition, and hence, the nutrients reported to be depleted heavily from the soil by this crop

are; P, K and S (Xue et al., 2008; Farooq et al., 2014; Moula et al., 2018). However, some studies reported the tobacco crop to be associated with the increasing of Ca, Fe, N, Zn in the rhizosphere (López-Lefebre et al., 2001, 2002; Farooq et al., 2014; Zou et al., 2018).

Effects of cultivating tobacco crop on either increase or decrease of some nutrients in the rhizosphere have not been linked with diversity of soil bacteria and their roles in the rhizosphere (Lisuma et al., 2019). The bacterial diversity in the rhizosphere are unnumbered, and their ecosystem roles are associated on improving rhizosphere fertility, however, yet still, their roles have not been studied to the great depth (Camenzind et al., 2018). Bacterial phyla belonging to *Proteobacteria*, *Firmicutes*, *Actinomycetes*, *Cyanobacteria* and *Bacteroidetes* are known to be beneficial as PGPR – 'plant growth promotion rhizosphere' (Kyselková et al., 2009; Kim et al., 2013; Basharat et al., 2018). Soil bacteria play significant roles in the rhizosphere through solubilization and mineralization of nutrients/organic materials (Masood and Bano, 2016; Bhowmik et al., 2017). Through this process, soil bacteria contribute a large part in

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nutrient retention, recycling, availability of nutrients for plant growth (Bhowmik et al., 2017).

Soil fertility is among a critical factor for crop productivity. Primary macronutrients play a significant role in plant growth, development, yield and quality increase (Tripathi et al., 2014). Nitrogen (N) is one of the limiting nutrients in crops and is available in the soil through biological fixation and atmospheric deposition (Dalling et al., 2016; Dynarski et al., 2019). Phosphorous (P) is the second essential nutrient after N which is released slowly from the parent rocks and depleted over time through occlusion and run-off (Dalling et al., 2016; Teodoro et al., 2019). A third essential nutrient in crops is potassium (K) which limits crop production due to its large portion existing as insoluble forms despite its reserve being large, and hence little K becomes available to the plants (Ahmad et al., 2016; Masood and Bano, 2016). Secondary macronutrients such as Ca and Mg are released in the soil at a steady rate (Vitousek and Sanford, 1986) and hence always blended concurrently
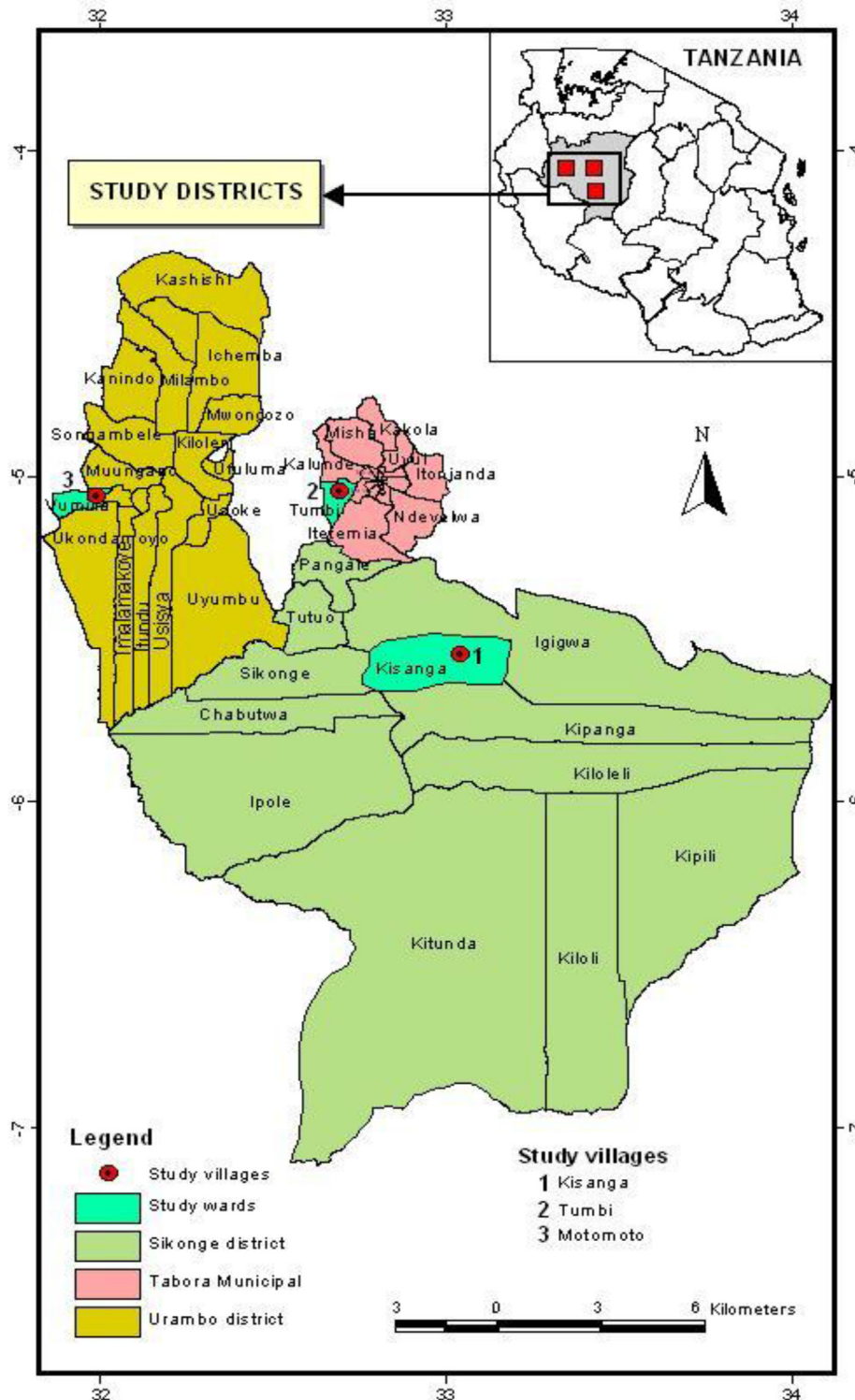

Figure 1. Map of pilot study areas: Sikonge (1), Tabora (2) and Urambo (3).

with NPK for crop production. Supplementation of the secondary macronutrients such as calcium (Ca), magnesium (Mg), sulphur (S) and micronutrients such as boron (B), copper (Cu), iron (Fe) and zinc (Zn) to primary macronutrients reported to increase significantly crop yields (Kihara et al., 2017).

Although the role of soil bacteria on solubilization of nutrients is well-documented (Kyselková et al., 2009; Kim et al., 2013; Masood and Bano, 2016; Bhowmik et al., 2017; Basharat et al., 2018), in our understanding, linkage of tobacco cultivation with the diversity of soil bacteria on soil fertility has not been studied. Therefore, a pilot study was conducted to investigate soil nutrients, and bacterial composition based on the 16S rRNA V3–V4 regions from tobacco rhizosphere of the three sites varying with soil texture and cropping systems in Tanzania. This pilot study involved linking of rhizosphere bacterial composition and soil nutrients to provide clear insights on the role of soil bacteria in tobacco cultivation in association with the soil fertility depletion or improvement before planting a subsequent maize crop after tobacco cultivation.

2. Materials and methods

2.1. Study site, soil sample collection and processing

Three experimental field sites of Sikonge, Tabora and Urambo, Tabora region, Tanzania with different soil types of loamy sand, sand and sandy loam, respectively, were selected during the 2018/19 cropping season (Figure 1). Sikonge site is located at 05° 31′ 47.4″ S, 032° 50′ 03.2″ E; 1,191 m a.s.l. with annual mean rainfall and air temperature of 1050 mm and 29 °C, respectively. Tabora site is located at 05° 03′ 44.4″ S, 032° 40′ 07.4″ E; 1,160 m a.s.l. with annual mean rainfall and air temperature of 950 mm and 27 °C, respectively. Urambo site is located at 05° 04′ 33.5″ S, 032° 00′ 09.8″ E; 1,108 m a.s.l. with annual mean rainfall and air temperature of 890 mm and 25 °C, respectively.

Plot size was 6 m × 6 m, 1.2 m between ridges and 0.50 m between plants planted for tobacco (K326 variety) and maize (DKC8053 variety). The fallow plot as a control had no any crop. A total of 54 soil samples were sampled using soil core randomly at 2 m away from each replicated tobacco plot to a depth of 20 cm. Soil adhered firmly to roots was categorized as rhizosphere soil. With the help of forceps, rhizosphere soil firmly adhered to fine roots dislodged, then further sieved through a 2 mm sieve and part of it used for bacteria DNA extraction and the determination of soil pH, organic carbon (OC), total N, available P, exchangeable K and Ca, extractable S, extractable Fe, Mn, Cu, and Zn analysis (Moberg, 2000). Since the tobacco root tip region of fine laterals is known to be the major site of nicotine synthesis, a second portion of the rhizosphere soil was further sieved through 1 mm and 0.5 mm in order to remove fine root tips for the nicotine determination (Figueiredo et al., 2009). Similar procedures were used to collect rhizosphere soil samples in maize and fallow plots for the bacteria DNA extraction.

2.2. Bacterial DNA extraction from soil samples

Extraction of bacterial DNA from the rhizosphere soil collected from the three experimental sites, as stated in section 2.1 was conducted at the Molecular Biology laboratory of the Nelson Mandela African Institution of Science and Technology (NM-AIST), Arusha Tanzania. About 0.25 g of each rhizosphere soil sample was used to extract DNA using DNeasy® PowerSoil® Kit (Qiagen, Hilden, Germany) as per the manufacturer's instructions. The extracted DNA was quantified using Qubit™ 3.0 Fluorometer (Thermo Fisher Scientific, Grand Island, NY). To ensure purified DNA was of high-quality, DNA was visualized through 1.0 % agarose gel electrophoresis. Extracted DNA was kept frozen at -20 and -80 °C.

2.3. Soil bacteriota composition using 16S rRNA sequencing

The purified DNA transported on dry ice to Inqaba Biotec™, a commercial sequencing service provider located in Pretoria, South Africa for

the bacteriota analysis through a run number 190708. The V3–V4 hypervariable regions of the 16S rRNA gene were amplified from the DNA extracts during the first PCR step using the universal primer pair 341F forward primer (5′-CCTACGGGNGGCWGCAG-3′) and uniquely barcoded 785R reverse primer (5′-GACTACHVGGGTATCTA ATCC-3′) for each sample. Resulting amplicons were gel purified, end-paired and Illumina TrueSeq adapters were ligated to each amplicon. Then samples were individually indexed, and another bead-based purification step was performed. Following quantification and equimolar pooling, amplicons were then sequenced on Illumina's MiSeq platform, using a MiSeq v3 600 cycles kit. 20Mb of data (2 × 300bp long paired-end reads) were produced for each sample. The length of the obtained sequences averaged 231 bp.

2.4. Bioinformatics for the bacteriota composition

Due to very low-quality scores of the reverse-end reads, bacteriota analyses were performed using only forward-end reads. The low-quality scores can be contributed by the reverse primer linked to barcodes which help in demultiplexing of the samples in case several samples were pooled during sequencing. Likewise, the primers, dNTPs, and other reagents are almost exhausted in the reaction mixture towards the end of sequencing runs. Analysis of demultiplexed forward-end 16S rRNA gene reads was performed based on DADA2 (ver. 1.14.0) (Callahan et al., 2016) in R software (ver. 3.6.2) (R Core Team, 2019). DADA2 pipeline includes trimming and filtering of the quality reads, dereplicates sequences, learns error rates, generates amplicon sequence variants (ASV) abundance table, removes chimeric sequences using "bimera *denovo*" method, taxonomic assignment and classification of the ASVs using the SILVA reference (ver. 132) database (Quast et al., 2013). About 427,218 forward-end FASTQ reads generated from 9 samples were pre-processed in DADA2 pipeline by removing low-quality reads using the truncated length set at 220 bp and adapters trimmed at less than 10 bp. Reads were further filtered to remove reads with ambiguous base by setting maxN = 0 and maximum expected errors greater than two were discarded by setting the quality filtering measure (maxEE = 2). DADA2 pipeline detected 5.8% of the relative abundance in all reads as chimeric and removed from the datasets. The resulting ASV abundance table contained 375,429 high-quality non-chimeric reads from 9 samples.

2.5. Statistical analyses

Statistical analyses (two-factors: sites; Sikonge, Tabora, Urambo, and tobacco) for the soil nutrients was done using analysis of variance (ANOVA). The significant means were compared using Fisher Least Significance difference at $p < 0.05$. The correlation, and multiple regression analyses at $p < 0.05$ among soil biochemical properties, and bacterial diversity in tobacco plots were performed in STATISTICA 8th Ed. (StatSoft, Inc., Tulsa, OK, USA).

For the bacteriota phyla composition from tobacco, maize and fallow plots, downstream analyses included data inspection, normalization, abundance visualization, alpha and beta-diversity (observed and Shannon) analyses, and heatmaps were generated in R software (ver. 3.6.2) (R Core Team, 2019). After filtering and normalization of the sequence reads, 90 % rarefaction depth of the minimum sample depth in the dataset were used to simulate even number of reads per sample. Results show that 68 OTUs removed because they were not present in any sample after random sub-sampling. The alpha-diversity indexes (species richness) for the study sites and different experimental treatments (fallow/control, maize and tobacco plots) at the phylum level were calculated using the Observed and Shannon Diversity Indexes in *phloseq* (McMurdie and Holmes, 2013) package in R. Moreover, the beta-diversity indexes for the study sites and experimental treatments of the samples, PCoA with weighted Unifrac at phylum level performed using *phyloseq* (McMurdie and Holmes, 2013) package in R software (ver. 3.6.2). Statistical analyses between the groups for the alpha-diversity and

Table 1. Measured soil parameters before and after tobacco cropping.

	pH	OC (%)	N (%)	Ca (cmol (+) kg⁻¹)	K (cmol (+) kg⁻¹)	P (mg kg⁻¹)	S (mg kg⁻¹)	Cu (mg kg⁻¹)	Fe (mg kg⁻¹)	Mn (mg kg⁻¹)	Zn (mg kg⁻¹)	Nicotine (mg kg⁻¹)
Site:												
Sikonge	5.66 ± 0.12b	0.33 ± 0.01a	0.06 ± 0.00a	1.42 ± 0.08a	0.52 ± 0.00a	33.41 ± 4.91a	5.83 ± 1.47a	0.23 ± 0.01b	19.38 ± 2.18a	27.98 ± 1.66b	0.47 ± 0.00a	2.34 ± 1.05a
Tabora	5.47 ± 0.01c	0.14 ± 0.01c	0.04 ± 0.01c	0.59 ± 0.00b	0.24 ± 0.00b	39.14 ± 1.95a	4.57 ± 1.57c	0.25 ± 0.05a	18.97 ± 2.69a	16.75 ± 2.17c	0.23 ± 0.05c	1.15 ± 0.51b
Urambo	5.84 ± 0.02a	0.25 ± 0.00b	0.04 ± 0.00b	0.99 ± 0.00b	0.24 ± 0.00b	33.80 ± 8.78b	4.67 ± 1.57b	0.26 ± 0.01a	18.99 ± 2.53a	29.26 ± 2.32a	0.36 ± 0.00b	0.61 ± 0.26c
Treatment:												
Soil before tobacco	5.75 ± 0.06a	0.25 ± 0.02a	0.04 ± 0.00b	0.59 ± 0.04a	0.35 ± 0.04a	47.09 ± 1.57a	8.46 ± 0.16a	0.20 ± 0.02b	13.60 ± 0.24b	20.09 ± 2.04b	0.31 ± 0.05b	0.01 ± 0.00b
Soil after tobacco	5.57 ± 0.07b	0.23 ± 0.02b	0.05 ± 0.00a	1.40 ± 0.08a	0.32 ± 0.05b	23.81 ± 3.02b	1.58 ± 0.23b	0.29 ± 0.02a	24.62 ± 0.24a	29.24 ± 1.96a	0.40 ± 0.02b	2.71 ± 0.52a
2-Way ANOVA F Statistic												
Site (S)	16.39***	559.56***	45.50***	110.84***	1227.26***	46.14***	2091.50***	17.35***	1.13ns	1213.25***	968.47***	68.86***
Treatment (T)	11.22***	39.06***	12.50***	320.34***	47.35***	1832.08***	152097.10***	306.78***	1911.66***	1604.71***	330.88***	482.39***
S × T	6.86*	15.44***	6.50*	38.39***	22.04***	263.47***	73.4***	174.65***	7.67*	16.46***	307.76***	69.53***

a' is very highly significant: 'b' is highly signifcant: 'c' is significant: ***p-value < 0.05, is significant: *p-value < 0.05, is significant: ***p-value < 0.001 is very highly significant.

beta-diversity indexes were performed using the pairwise-wilcoxon test and the permutational ANOVA (PERMANOVA) analysis using *vegan* (Oksanen, 2011) package in R, respectively.

3. Results

3.1. Effects of tobacco cultivation on selected soil parameters and bacterial diversity

Soil pH was lowered as a result of tobacco cultivation, and soil OC decreased significantly ($p < 0.001$) following the cultivation of tobacco crop. The nicotine released in the soil increased significantly across the sites. Total soil N, Ca, Cu^{2+}, Fe^{2+}, Mn^{2+}, and Zn^{2+} increased significantly ($p < 0.001$) under tobacco cultivation. While available P, K and S decreased significantly ($p < 0.001$) under tobacco cultivation (Table 1). There were significant interactions between sites under tobacco cultivation on measured parameters soil pH, OC, nicotine, Cu, Fe, Mn, Zn, N, P, K, Ca and S.

Soil measured parameters were correlated with bacterial diversity (Table 2). Across the site, soil pH was significantly positively correlated with bacterial diversity (Chao1 alpha-diversity index) to tobacco plots ($r = 0.57, p < 0.05$). Macronutrient S, P, N and K were positively correlated with bacterial diversity at $p < 0.05$; however, N and K showed to have a low correlation. Calcium and Mn were negatively correlated with bacterial diversity. Other micronutrients such as Cu, Fe and Zn were negatively correlated with bacterial diversity, but Zn had the lowest correlation. The nicotine released in soils by the tobacco plant showed to be negatively significantly correlated with bacterial diversity.

Association of bacterial diversity and soil parameters was performed using multiple regression analysis (Table 3). Results showed that bacterial diversity was positively correlated ($R^2 = 56.57\%$) with soil pH, which is a primary determinant of nutrients available to the plants. Soil pH, along with OC regressed with bacterial diversity, improved R^2 value to 65.40%. However, soil pH along with N, S, P, Ca, K and soil pH along with Cu, Fe, Mn and Zn significantly improved the R^2 values from 94.88% to 96.05%, respectively, in predicting bacterial diversity.

3.2. General distribution of soil bacteria phylum in tobacco, maize and fallow plots

The 375,429 classifiable sequences in this study, were correlated with 12 relative abundance bacterial phyla from each experimental site (Sikonge, Tabora and Urambo) covering all crops (Figure 2). To all cropping systems, the dominant phyla spotted as *Actinobacteria* and *Proteobacteria*, accounting for >60% of all the phyla. Other phyla in decreasing order were *Chloroflexi*, *Acidobacteria*, *Planctomycetes*, *Gemmatimonadetes*, *Firmicutes* and *Bacteroidetes*. Bacterial phyla with <1% abundance were excluded, and not considered as dominant. The bacterial distribution at the phylum level varied in the different cropping systems and relative abundance.

3.3. Comparison of soil bacteria phylum diversity from different soil types and crops

In tobacco rhizosphere, the following phyla ranked in their abundance levels were in order: *Proteobacteria* (37.7%), *Actinobacteria* (27.7%), *Acidobacteria* (9.0%), *Chloroflexi* (7.0%), *Firmicutes* (5.7%), *Planctomycetes* (5.3%), *Bacteroidetes* (4.3%) and *Gemmatimonadetes* (4.0%) (Figure 3).

Proteobacteria and *Firmicutes* for tobacco crop soil were abundant in Tabora sand soil by 5.36 and 0.92%, respectively; 3.38 and 0.43% in Urambo sandy loam soil, respectively; 3.15 and 0.42% in Sikonge loamy sand soil, respectively. *Actinobacteria*, *Acidobacteria*, *Chloroflexi* and *Planctomycetes* in tobacco plots were most dominant by 3.64, 1.28, 1.03 and 0.70% in loamy sand soil of Sikonge, respectively; followed by sandy loam soil in Urambo by 3.59, 1.27, 0.92 and 0.66%, respectively, and

Table 2. Correlation between soil parameters and bacterial diversity indices ($p < 0.05$).

	Chao1	SDI	pH	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})	N (%)	OC (%)	S (mg kg^{-1})	P (mg kg^{-1})	Ca (cmol (+) kg^{-1})	K (cmol (+) kg^{-1})	Nicotine (mg kg^{-1})
1. Chao1	1													
2. SDI	0.44	1												
3. pH	0.57	0.28	1											
4. Cu (mg kg^{-1})	-0.80	-0.23	-0.1	1										
5. Zn (mg kg^{-1})	-0.09	-0.48	0.28	0.42	1									
6. Mn (mg kg^{-1})	0.02	0.14	0.27	0.39	0.75	1								
7. Fe (mg kg^{-1})	-0.59	0.01	-0.34	0.69	0.39	0.64	1							
8. N (%)	0.06	-0.44	-0.25	-0.10	0.61	0.49	0.31	1						
9. OC (%)	0.58	-0.21	0.55	-0.26	0.73	0.54	-0.13	0.61	1					
10. S (mg kg^{-1})	0.63	-0.14	0.39	-0.70	-0.22	-0.56	-0.98	-0.16	0.31	1				
11. P (mg kg^{-1})	0.67	-0.02	0.16	-0.89	-0.35	-0.52	-0.89	0.04	0.26	0.89	1			
12. Ca (cmol (+) kg^{-1})	-0.10	-0.08	0.09	0.41	0.76	0.85	0.75	0.55	0.50	-0.62	-0.63	1		
13. K (cmol (+) kg^{-1})	0.38	-0.53	0.02	-0.39	0.57	0.22	-0.11	0.81	0.80	0.30	0.37	0.39	1	
14. Nicotine (mg kg^{-1})	-0.56	-0.4	-0.55	0.40	0.46	0.49	0.77	0.70	0.05	-0.70	-0.48	0.58	0.30	1

SDI = Shannon diversity index.

Tabora sandy soil by 1.75, 0.43, 0.29 and 0.33%, respectively. *Bacterioidetes* were 1.23% in abundance for Tabora sand soil and 0.17% in Urambo sandy loam soil, while *Gemmatimonadetes* were abundance in loamy sand soil of Sikonge and sandy loam soil of Urambo by 0.64 and 0.66%, respectively.

In maize rhizosphere, the abundant phyla were in order: *Actinobacteria* (11.74%), *Proteobacteria* (7.71%), *Chloroflexi* (3.71%), *Acidobacteria* (3.32%), *Planctomycetes* (1.99%), *Gemmatimonadetes* (1.90%), and *Firmicutes* (1.86%). In maize plots, the distribution of phyla abundances was as follows; *Actinobacteria* were 4.77% in loamy sand soil (Sikonge), 3.99% in sandy loam soil (Urambo), and 2.98% in the sandy

soil (Tabora). *Proteobacteria* was 3.24% in Tabora sandy soil, 2.43% in sandy loam soil of Urambo and 2.04% in Sikonge loamy sand soil. *Acidobacteria, Chloroflexi* and *Planctomycetes* were mostly abundant by 1.44, 1.39 and 0.80% in Urambo sandy loam soil, respectively; 1.21, 1.27 and 0.60% in Sikonge loamy sand soil, respectively; 0.66, 1.04 and 0.59% in Tabora sandy soil, respectively. *Firmicutes* and *Gemmatimonadetes* were 1.04 and 0.84% in Tabora sandy soil, respectively; 0.43 and 0.61% in Sikonge loamy sand soil, respectively, and 0.40, 0.45% in Urambo sandy loam soil, respectively.

Fallow plots considered as control plots of which no any planted crops except weeds in order to study bacteria phyla abundances in their natural

Table 3. Multiple regression between bacterial diversity and soil parameters.

	Variables	R^2	R^2 adjusted	R^2 predicted
1.	BD: soil pH	56.57%	27.76%	32.00%
2.	BD: soil pH, OC	65.40%	35.14%	42.77%
3.	BD: soil pH, Ca, N, P, K, S	94.88%	83.03%	90.02%
4.	BD: soil pH, Cu, Fe, Mn, Zn	96.05%	89.04%	92.26%

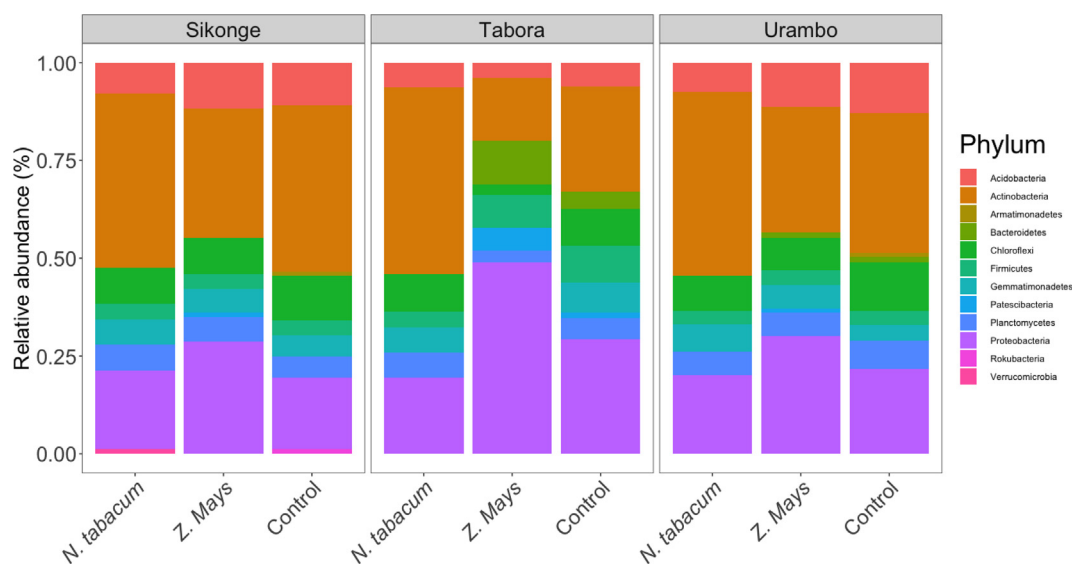BD = bacterial diversity.

Figure 2. Relative abundances (%) of phylum for each cropping system in Sikonge, Tabora and Urambo.
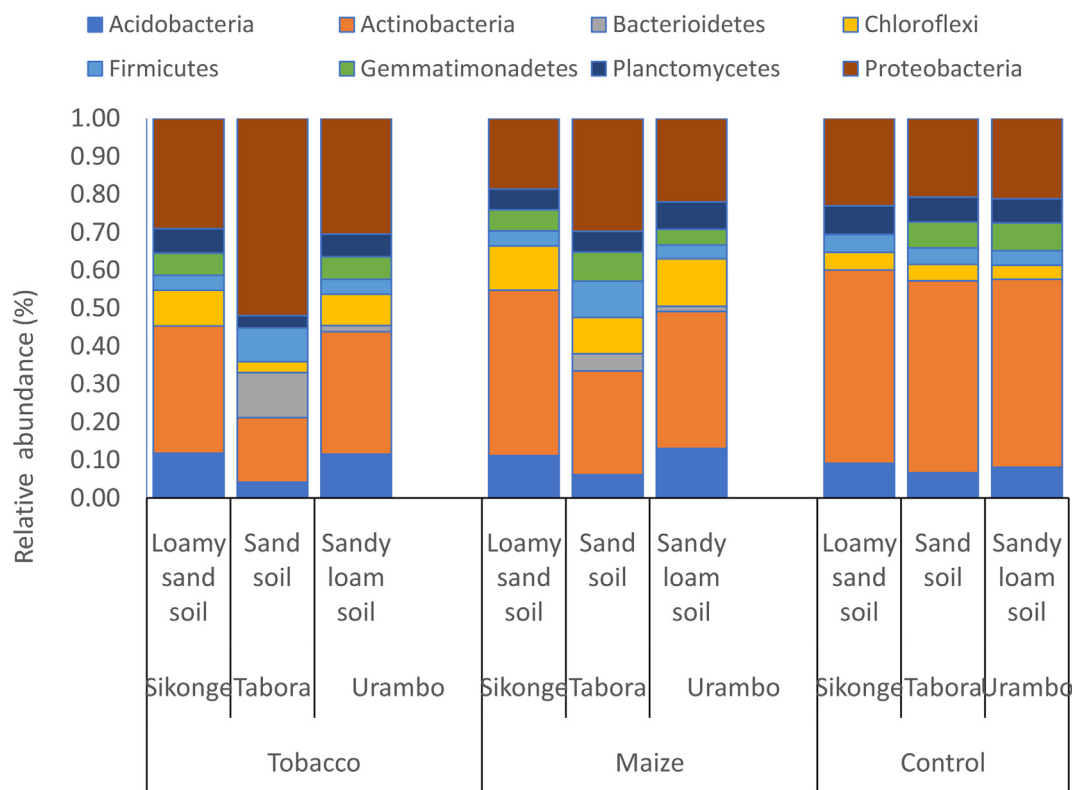
Figure 3. Relative abundancies of bacteria phyla in different soil types and cropping systems.

environment. The abundances of phyla in control/fallow plots were; *Actinobacteria* (15.48%), *Proteobacteria* (6.65%), *Acidobacteria* (3.09%), *Chloroflexi* (2.43%), *Gemmatimonadetes* (2.19%), *Planctomycetes* (2.10%), and *Firmicutes* (1.31%). In control plots the distribution of phyla abundances was as follows; *Actinobacteria* and *Gemmatimonadetes* abundance were dominant by 5.31 and 0.71% in Tabora sandy soil, respectively; 5.21 and 0.77% in Urambo sandy loam soil, respectively; 4.97 and 0.01% in Sikonge loamy sand soil, respectively. *Proteobacteria* and *Acidobacteria* abundance were 2.25 and 0.89% in Sikonge loamy sand soil, respectively; 2.23 and 0.84% in Urambo sandy loam soil, respectively; 2.17, 0.70% in Tabora sandy soil, respectively. *Chloroflexi* were 1.06% in Tabora sandy soil, 1.03% in Sikonge loamy sand soil and 1.00% in Urambo sandy loam soil. *Planctomycetes* and *Firmicutes* were 0.73 and 0.45% in Sikonge loamy sand soil, respectively; 0.69 and 0.45% in Tabora sandy soil, respectively; 0.67 and 0.40% in Urambo sandy loam soil, respectively.

3.4. Composition of phyla community variation with crops and locations

Comparison of bacteria phyla community varying with treatments (fallow, maize, tobacco) performed across the locations. The significant abundant phyla across the locations along with fallow, maize and tobacco crops were *Actinobacteria*, *Acidobacteria*, *Proteobacteria*, *Chloroflexi*, *Planctomycetes*, *Firmicutes*, *Gemmatimonadetes* and *Bacterioidetes* (Figure 4).

3.5. Observed and shannon diversity index showing location and treatment phylum level diversity

Different crops observed to change bacteria phyla or their proportions in different locations (Figure 5). The alpha diversity for Tabora versus Sikonge had $p=0.54$; $p=1$ value for the observed and Shannon diversity index, respectively. Urambo versus Tabora had both $p=1$ values, while Urambo versus Sikonge had $p=0.35$; $p=1$ value for the observed and Shannon diversity index, respectively.

Concerning treatment crops, alpha diversity for tobacco versus control/fallow plots and maize versus tobacco had $p=1$; $p=0.8$ value for the observed and Shannon diversity index, respectively. Maize versus control/fallow plots had $p=1$; $p=0.3$ for observed and Shannon, respectively.

3.6. Principal component analysis (PCoA) of bacterial phyla based on crops and pilot locations

All crops had relative abundance distribution of bacteria phyla (Figure 3). Bacterial phyla under control plots distributed almost equally and their relative abundance were significantly higher than bacterial phyla in tobacco and maize crop. Total bacteria phyla were lowest in the fallow plots compared to tobacco and maize crops. The PCoA score revealed that the maize treatment clustered together and separated away from tobacco treatment with the 70.6% power of separation in the first principal component (Figure 6).

4. Discussion

4.1. Rhizosphere soil fertility changes along with bacterial diversity

Tabora site had acidic soil pH 5.47 and very low OC (0.14%) in comparison to Sikonge site with soil pH 5.66; OC of 0.33% and Urambo which had soil pH and OC of 5.84 and 0.25%, respectively (Table 1). Due to the high soil pH and low OC in Tabora site, resulted into slightly decreased bacteria diversity in tobacco plots with the exception of *Proteobacteria* (11.89%) (Figures 2 and 3). *Proteobacteria* phylum showed an increasing higher trends towards the coarse-textured (sandy) acidic soil in Tabora than other experimental sites. Next to *Proteobacteria* phylum in terms of abundance within tobacco plots was *Actinobacteria* (8.97%), these two phyla indicating their suitability and withstanding nicotine levels (Table 1) to the tobacco rhizosphere (Dey et al., 2012; Saleem et al., 2018). Other phyla were *Acidobacteria*, *Chloroflexi*, *Firmicutes*, *Planctomycetes, Bacteroidetes* and *Gemmatimonadetes* in 2.99, 2.24, 1.77,
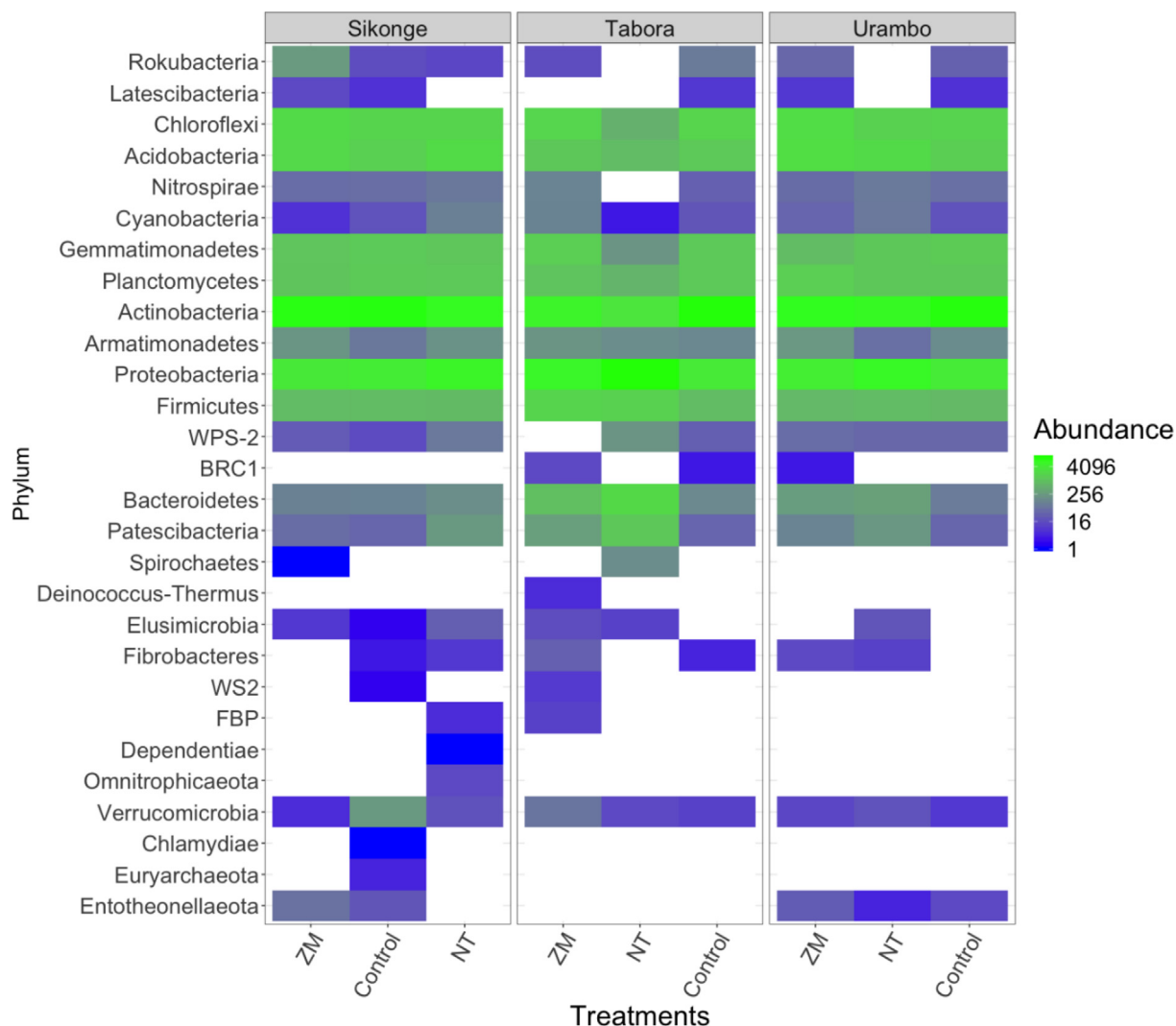
Figure 4. Heatmap indicating the phyla relative abundance in ZM (maize), NT (tobacco) and control.
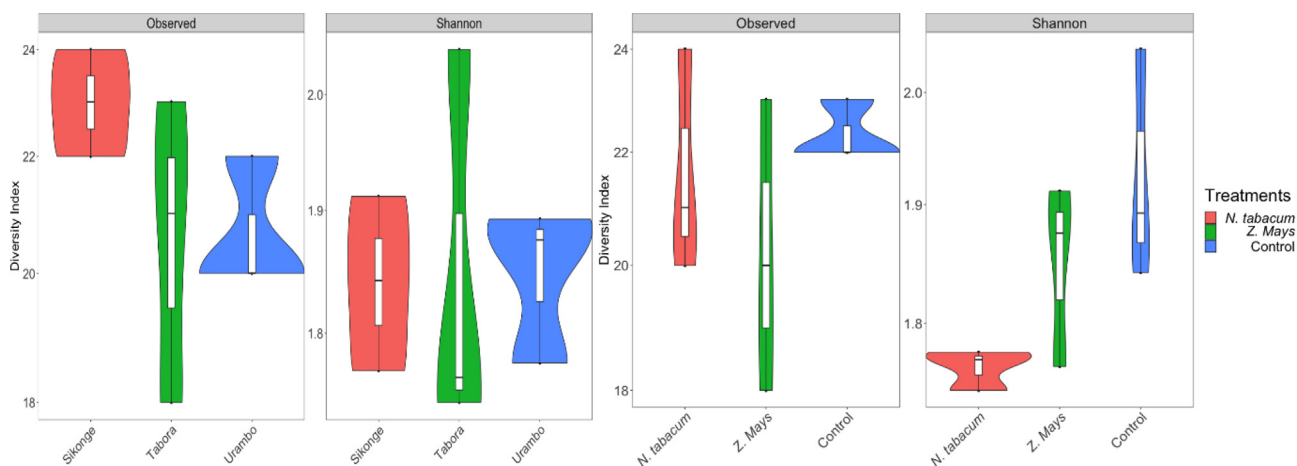

Figure 5. Observed and Shannon Diversity Index showing location and treatment Phylum level diversity. Locations: Sikonge, Tabora and Urambo did not differ significantly. Treatments: Crops differed significantly on phylum levels. A horizontal line across inside the graphics represents the median.

1.69, 1.40 and 1.31% proportions, respectively. These bacteria phyla were identified in the studied tobacco areas for the first time. The trends showed to increase in abundances towards the fine-textured soils for the *Acidobacteria, Chloroflexi, Bacteroidetes* and *Gemmatimonadetes. Firmicutes*

and *Proteobacteria* showed an increasing trend of their abundances towards coarse-textured soils. The phyla identified in our study areas to the tobacco plots were almost similar to the phyla reported by Wu et al. (2016) in tobacco fields.
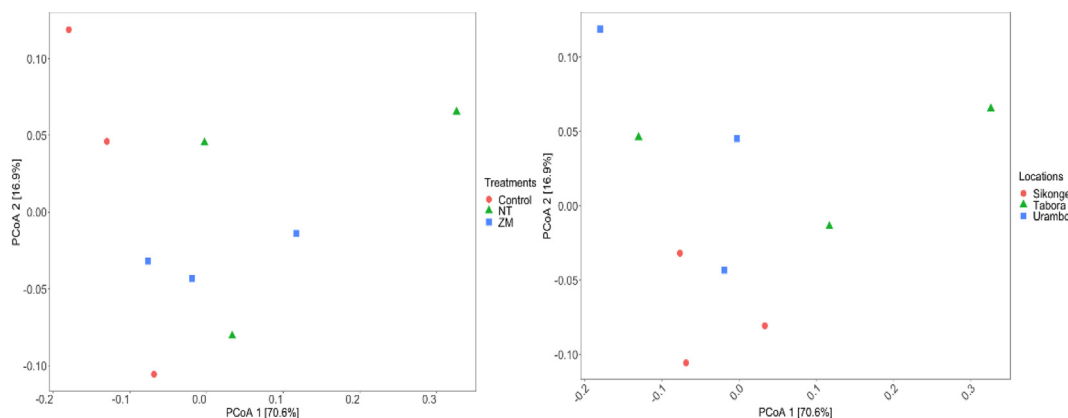
Figure 6. PCoA of the crop treatments and pilot locations based on bacteria phyla abundance.

Proteobacteria and *Actinobacteria* were high in abundance in Tabora site which had high availability of P and linked to be involved in the solubilization of P and make this nutrient available to the tobacco plant (Manpoong et al., 2020). Unavailability of P in soils has been reported limiting bacteria diversity and abundance (Marschner et al., 2004; Leff et al., 2015; Jing et al., 2017; Camenzind et al., 2018). Initial available P was 33.41, 33.80 and 39.14 mg kg^{-1} for Sikonge, Urambo, and Tabora, respectively, depicted the increasing trend of *Proteobacteria* (Marschner et al., 2004). Similar results of increasing P levels in soils associated with an increase in bacteria population was reported by Camenzind et al. (2018).

The Bacteria under *Proteobacteria, Actinobacteria* and *Firmicutes* phyla have been reported to solubilize K, P, S, and increases uptakes of these nutrients (Alain et al., 2002; Chakraborty et al., 2010; Zeng et al., 2012; Zhang and Kong, 2014; Subhashini, 2015; Sungthong and Nakaew, 2015; Saha et al., 2018). Since tobacco is a dense nutrients feeder crop, more uptakes of P, K and S result in decreasing levels of these nutrients in the soil (Table 1). On the other hand, bacteria species under *Proteobacteria* have also been reported to fix N in the soils and hence increasing soil total N after tobacco (Table 1; Balsanelli et al., 2015; Trovero et al., 2018; Zúñiga-Feest et al., 2018). *Chloroflexi,* another dominant phylum identified in tobacco growing areas, reported to catalase Mn and also involved in the nitrification process in soils (Sorokin et al., 2012; Baginski and Sommerhalter, 2017).

Our study observed that bacterial diversity was highly correlated with soil fertility in tobacco plots (Table 2). Chao1 and Shannon diversity index showed bacterial diversity positively influenced soil fertility on P, S, OC, N, K, and Mn which are mostly required (with exception to Mn) by tobacco plants. Furthermore, Chao1 and Shannon diversity index showed bacterial diversity to have a negative correlation with micronutrient Cu, Fe, Zn, and macronutrient Ca, indicating that micronutrients are required in trace amount by the tobacco plant. Hence, their levels in soils increased after tobacco (Table 1). Our study performed soil nutrients characterization on microbial diversity using multiple regression analysis (Table 3) in order to associate on how bacterial diversity are linked to soil fertility in tobacco rhizosphere. Results showed that microbial diversity was positively correlated with soil pH and OC (R^2 = 56.57 and 65.40%), respectively. Further correlation with soil pH, a most critical parameter in soils along with Ca, K, Mg, N, P, S and micronutrients Cu, Fe, Mn and Zn, significantly improved the R^2 values (94.88–96.05%) in predicting microbial diversity fertility. Thus, our study revealed that an increase or a decrease of soil nutrients in tobacco plots is not caused by the nicotine released in soils, but rather by the bacterial diversity in the rhizosphere with a role of solubilizing nutrients.

4.2. Proportion of bacteria diversity to maize, tobacco and fallow plots

This study revealed a higher proportion of *Actinobacteria,* and *Proteobacteria* phyla with the abundance of 11.74 and 7.71% respectively, in

maize plots. Next to these phyla were *Chloroflexi, Acidobacteria, Planctomycetes, Gemmatimonadetes* and *Firmicutes* in the proportional abundance of 3.71, 3.32, 1.99, 1.90 and 1.86%, respectively. With exception to *Proteobacteria* phylum, the rest of the phyla increased in their proportion abundance when compared to the tobacco phyla proportions (Figures 3 and 5). *Actinobacteria* and *Chloroflexi* phyla increased significantly in the maize plots than tobacco plots by 2.77 and 1.47%, respectively. The small increase in *Acidobacteria, Planctomycetes, Gemmatimonadetes* and *Firmicutes* phyla were by 0.33, 0.30, 0.59 and 0.09% in comparison from the tobacco phyla proportions.

These results indicate maize crop to be a hotspot of bacterial infestation than tobacco crop (Li et al., 2014) and thus maize roots could exudate metabolites different from tobacco nicotine (Table 1) and influence an increase of bacteria proportions (Dey et al., 2012; Li et al., 2014). Maize rhizosphere, as similar to tobacco rhizosphere, showed a trend of *Actinobacteria, Acidobacteria, Chloroflexi,* and *Planctomycetes* increasing towards fine-textured soils (from sandy soil, sandy loam to loamy sand soils). On the other side, *Bacteroidetes* (not reported in this study had 0.64% abundance, less than 1%), *Firmicutes* and *Proteobacteria* increased in abundance from loamy sand, sandy loam to sandy (coarse textured) soil. The most abundant phyla reported in this study were similar with other studies that reported dominant phyla in maize rhizosphere to be *Proteobacteria, Bacteroidetes, Actinobacteria* and *Firmicutes* (Pereira et al., 2011; Li et al., 2014; Verma et al., 2017).

In the fallow plots (no-till land) which were control in this study, the bacteria phyla belonging to *Actinobacteria, Gemmatimonadetes* and *Planctomycetes,* their proportions were highest than tobacco and maize crops by reaching 15.48, 2.19 and 2.10%, respectively. Other phyla proportions were at 6.65, 3.09, 2.43 and 1.31% for *Proteobacteria, Chloroflexi, Acidobacteria,* and *Firmicutes* respectively. Abundances of these phyla in general, were in low proportions, indicating that crop rhizosphere influences large proportions and diversity of bacteria. *Acidobacteria, Actinobacteria, Proteobacteria* and *Bacteroidetes* have also been observed to be abundant in no-till land (Yin et al., 2010; Figuerola et al., 2012; Aslam et al., 2013; Dong et al., 2017a, 2017b). In our studies, we observed similar phyla results, in addition to that *Chloroflexi, Gemmatimonadetes, Planctomycetes,* and *Firmicutes* were found in the control plots. These phyla have also been recently observed in no-till land (Dong et al., 2017b; Yin et al., 2017). Bacteria reported to be more abundant at the crop rhizosphere than in bulk soils, the soil types on the other hand also were found to be the significant parameter affecting bacterial diversity in soils (Grzadziel and Galazka, 2018; Khan et al., 2018). However, Helgason et al. (2009), in their study, indicated that bacteria phylum was not consistent in no-till soils.

Different crops (maize, and tobacco) revealed to have influences on the soil chemical properties and exudates of metabolites. Bacteria phyla proportions and diversity in maize crop were higher than in tobacco crop across the experimental locations (Figure 5). Thus, different crops may have variance in their proportions in different locations. Based on the

Shannon diversity indices (Figure 5), tobacco had bacterial diversity dominated by *Proteobacteria* (Figures 2, 3, and 4), while maize crop was observed to have a significant proportion of *Actinobacteria* (Figures 2, 3, and 4) in a wide range. Fallow plots were observed to have bacterial diversities in equal proportions (Figures 3, 4, and 5). Besides, fallow and maize plots had bacteria phyla which did not separate widely based on PCoA results (Figure 6).

5. Conclusion

Proteobacteria and *Actinobacteria* phyla based on the pilot study indicated to be the most dominant in tobacco, maize and fallow plots, but with different proportions. Bacterial diversity correlated with soil fertility improvements. The bacterial diversity in tobacco plots significantly positively correlated macronutrients, but micronutrients were significantly negatively correlated by the bacterial diversity in tobacco plots. However, multiple regression analysis revealed that bacterial diversity significantly favoured soil fertility to all sites. Therefore, this pilot study recommends further research that will include a collection of more soil samples for detailed information regarding soil bacteriota composition and their roles for conclusive results.

Declarations

Author contribution statement

Jacob Bulenga Lisuma: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Zavuga Zuberi: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Patrick Alois Ndakidemi: Performed the experiments; Wrote the paper.

Ernest Rashid Mbega: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Competing interest statement

The authors declare no conflict of interest.

Additional information

Data associated with this study has been deposited at the National Center for Biotechnology Information (NCBI) under the BioProject ID PRJNA643246, BioSample SAMN15407342, SAMN15407343, and SAMN15407344 with accessions from SRR12127562 to SRR12127570.

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