



Soil properties influence bacterial abundance and diversity under different land-use regimes in semi-arid environments

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ABSTRACT

Bacterial diversity is known to be a driver of soil ecosystem services and it is influenced by the natural integrity of the environments. Samples from garden soil, saline soil and sludge-impacted soil were studied to evaluate the influence of soil properties on bacterial abundance and diversity for improved management. Soil samples were collected from 0 to 15 cm depth and analyzed for selected physico-chemical properties using routine laboratory procedures. 16S rRNA gene-based metagenomics analysis was used to identify and quantify bacteria from the samples. Garden soil had pH (H₂O) of 5.8, electrical conductivity (EC) of 0.3 dS m⁻¹, organic matter (OM) content of 0.66%, total phosphorus (P) and cation exchange capacity (CEC) of 132 ppm and 2.40 Cmol kg⁻¹, respectively, and 8.39% clay; sludge impacted soil had a pH (H₂O) of 5.8, EC of 5.7 dS m⁻¹, OM content of 57.5%, 2641 ppm P, CEC content of 8.62 Cmol kg⁻¹ and 18.23% clay; while salt affected (saline) soil had a pH (H₂O) of 7.6, EC of 4.4 dS m⁻¹, OM of 0.5%, P content of 24.9 ppm, CEC of 4.05 Cmol kg⁻¹ and 13.07% of clay contents. The distribution of bacteria phyla identified in the soils is as follows: sludge-impacted soil (15), garden (13) and bare saline (10). Next generation sequencing shows that five of the phyla in the order *Proteobacteria* > *Actinobacteria* > *Firmicutes* > *Bacteroidetes* > *Acidobacteria* were present and dominant in all the soils. Soils under the different land uses significantly varied in their properties. It is concluded that bacterial diversity was influenced by the degree of habitat disturbance caused by variation in land-use management practices which affected properties of the soils. This study opens up new frontiers in expanding metagenomics studies in the semi-arid environments of Botswana and contributes to the identification of soil bacteria which are useful to ecosystem functions.

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Introduction

The soil is considered a major reservoir of bacterial genetic diversity [35]. The abundance and diversity of biota beneath our foot far exceed the ones above ground [48]. Soil biota is the principal component of the decomposer subsystem regulating nutrient cycling, energy flow and ultimately plant and ecosystem productivity [49]. Most decomposition of crop residues and organic matter in soils is done by microbial communities including bacteria and fungi [16]. Bacteria abundance and

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Table 1

Selected physico-chemical properties of the soils in the agroecosystems.

Agroecosystem	Location	pH H ₂ O	EC dS m ⁻¹	OM %	P ppm	Ca -----cmol.kg ⁻¹	Mg -----cmol.kg ⁻¹	K -----cmol.kg ⁻¹	Na -----cmol.kg ⁻¹	CEC	Clay -----%----	Sand -----%----	Silt -----%----	TC*
Bare soil	22°29'33.83S 27°13'41.76"E	7.62	4.4	0.50	24.90	1.23	1.23	0.41	0.39	4.05	13.07	82.77	4.16	SL
Sludge-impacted soil	27°0'22.02"E 22°32'22.59"S	5.84	5.7	57.50	2641.3	6.31	0.87	1.26	0.18	8.62	18.23	78.91	2.82	SL
Garden soil	22°28'52.38"S 27°13'45.93"E	5.75	0.3	0.66	132.1	1.56	0.66	0.14	0.14	2.40	8.39	88.96	2.65	LS

LS: Loamy sand; SL: Sandy loam; pH: soil pH, EC: Electrical conductivity, OC: Organic carbon, P: Phosphorus, K: Potassium, Ca: calcium, Mg: Magnesium, Na: Sodium, CEC: Cation exchange capacity, TC: Textural class.

diversity as well as associated changes in the soil food web are biological properties of soils that have received great attention in recent times. The presence of bacteria and fungi wield different influences in an ecosystem. Soil environments dominated by bacteria is characterized by high degrees of disturbances, neutral to mildly acidic pH, a high nutrient availability, and low soil organic matter content because of elevated biological activity [46,50]. The major impact of the bacteria activities would be escalated rate of organic matter mineralization which is capable of depleting soil nutrient reserve in a short time.

Bacterial diversity is reflected as a driver of ecosystem services, to be specific, those soil ecosystem services connected to justifiable agricultural production including nutrient cycling, habitat for crop production and carbon sequestration. In most parts of the world, soil degradation, induced by changes to the physical and chemical properties of soils, is a major problem in agricultural lands [2,6,25]. Soil quality indicators such as biological properties, and their relationship to specific recognizable ecosystem services has been projected as an accepted approach to monitor and assess changes in soil functions and quality [3,41,54].

Arenosols constitute about 13% of sub-Saharan Africa in general and about 66% of Botswana landmass in particular. It is estimated that Arenosols make up about 23.1% of the total land area under cropland in Botswana [18] with open to sparse grassland and the occasional presence of shrubs. These are characterized predominantly by sandy soil texture (more than 70% sand and less than 15% clay), high permeability, low water-holding capacity, slow chemical weathering rates, low specific heat, and often minimal nutrient contents. Arenosols are unfavourable to support sustainable agriculture [47]. However, in many parts of southern Africa including Botswana, human population growth is leading to increasing use of Arenosols for crop production. In this geographic region, agriculture accounts for about 35% of gross domestic product and employs 70%–80% of the total labor force [1]. Therefore, the use of Arenosols for cultivation, calls for sustainable land use practices at all times.

At the turn of the century, early studies aimed at characterizing bacterial communities required the isolation of bacteria from soil sample by culture-dependant techniques. Diversities in bacterial biomass are usually identified by phenotypic classification of isolated strains [17]. However, the majority of soil bacteria cannot be cultured via classical laboratory methods and should be identified using culture-independent techniques. Metagenomics approach has proven useful in this regard [51], but has not been used extensively, to the best of our knowledge, in characterizing bacterial communities in Arenosols from Botswana.

Taking into account the poor physical and chemical properties of Arenosols and the influence of land-use change on soil properties, we predicted variations in bacterial diversity in terms of richness (total number of bacterial species) and abundance in three land-use regimes (bare saline, garden and sewage sludge-impacted soils). The objectives of the study were (1) to use 16S rRNA gene-based metagenomics approach to produce high-resolution characterization of bacterial abundance and diversity in Arenosols from the semi-arid Botswana, and (2) to investigate how the physico-chemical properties of soils under different land uses affect bacterial communities.

Materials and methods

Study area

Geographically, the sampled area lies approximately (22°33'0"S and 27°8'0"E) (Table 1). It is located in Palapye, central Botswana, with a semi-arid climate receiving average annual rainfall amount of 250 mm in the south-west to 650 mm in the northwest. It has a characteristic seasonal rainfall, which is unreliable and varies yearly. The study is in a hardveld area with dominant savannah vegetation including predominantly tree species such as *Colophospermum mopane* (Mophane) and *Acacia tortilis* (Umbrella thorn). The soils of the study area are classified as Ferralic Arenosols (FAO-WRB) or Ustic Quartzipsamments (Soil Taxonomy) [22].

Site description and soil sampling

Samples were collected from three land-use regimes described as follows; bare land (saline soil), sewage-impacted soil (sludge soil) and arable land (garden soil). The saline soil is currently bare, with no vegetation cover and not in use de-

spite its close proximity (< 50 m) to the Dikabeya dam; the sewage soil was collected from a sewage sludge dump site, this site continuously receives sewage sludge from the Palapye Wastewater Treatment Plant (PWTP), and the garden soil was collected from a horticultural arable land at the Dikabeya farm located approximately 8 km from PWTP. The sampling locations are approximately 2 km apart. Samples were collected in triplicates from each site using soil auger T-sampler at a depth of about 15 cm. All the instruments were disinfected with 70% ethanol and rinsed with sterile water before the next soil sample collection. Samples from the different locations were placed in separately labelled sterilized zip lock bags and taken to the laboratory in a cooler box with ice packs for analysis.

Laboratory analyzes

Soil analyzes

Samples for the physico-chemical analysis were air dried, gently ground and passed through a 2 mm sieve prior to laboratory analysis. Particle size distribution followed the modified Bouyoucos method [44]. The electrical conductivity (EC) and pH in H₂O were determined with an electrical conductivity meter and pH glass electrode meter in a 1:2 (soil to liquid) ratio, respectively. Soil organic carbon (SOC) was determined by the modified Walkley and Black wet digestion method [31]. Cation exchange capacity (CEC) was estimated as the sum of cations (calculated, CECsum) based on the quantities of Ca²⁺, Mg²⁺, Na⁺, and K⁺ extracted [23]. The exchangeable cations were extracted using a mechanical vacuum extractor and measured using atomic absorption spectrophotometer (AAS). Modified Bray II method was used to determine the extractable total phosphorus [52]. All readings were obtained in triplicates.

16S rRNA-gene sequencing of metagenomic DNA

DNA was extracted from the samples collected from each of the three land use regimes. The triplicate samples from each habitat were bulked together, homogenized and DNA was extracted in triplicate. One gram of the soil sample was subjected to DNA extraction procedures using a Power soil DNA extraction kit (MO BIO Laboratories, Carlsbad, CA, USA) following manufactures instructions. DNA samples were checked for quality and purity using a Nanodrop spectrophotometer (Lasec, Jenway Genova nano).

The 16S rRNA gene sequencing was conducted using the Ion Torrent 16S Metagenomics kit following manufacturer's recommendations [36]. Briefly, two primer pools (V2-4-8, V3-6-7-9) were simultaneously used to amplify 7 of the hyper variable regions of the 16S rRNA gene in bacteria. Afterward, the Ion Plus™ Fragment Library Kit was used to ligate barcoded adapters and synthesize libraries. Barcoded libraries from the sample were pooled and templated on the OneTouch2™ system followed by 400 bp sequencing on the Ion PGM sequencer. Automated Bioinformatics analysis of next-generation sequence data, annotation, taxonomic assignment and Krona charts visualization of diversity and relative abundance were generated using the Ion Reporter™ Software pipeline (version 5.2) hosted by ThermoFisher Scientific (<https://ionreporter.thermofisher.com/ir/>). Ion Reporter leverages QIIME's open-source bioinformatics pipeline to produce diversity analyzes and visualizations. Alpha diversity results describe the diversity within a single sample at the Species, Genus, and Family levels. Beta diversity results describe the diversity between multiple samples at the Species, Genus, and Family levels.

Statistical analysis of bacteria community data

Simpson Index (D) which is generally used in ecology for biodiversity analysis was used to assess bacterial biodiversity in this study. It is calculated from:

$$\text{Simpson Index of Dominance (D)} = \sum [n_i(n_i - 1) / N(N - 1)]$$

Where n_i is the spore numbers of a species and N is the total number of identified spore samples. For bacteria communities' similarities assessment, Sorenson's coefficient (CC) was used.

$$\text{Sorenson's Coefficient (CC)} = 2C / (S1 + S2)$$

Where C is the number of species any two habitats have in common, $S1$ is the total number of species found in habitat 1, and $S2$ is the total number of species found in habitat 2.

A one-factor analysis of variance (ANOVA) was conducted to evaluate the null hypothesis that there is no difference in the selected physicochemical properties of the soils under different landuse. STATISTICA version 7.0 by StatSoft Inc. [43] was used to obtain the least significant different (LSD at 5%) for landuse type - the independent variable and establishment of interactions among soil properties in different landuse scenarios. Worthy of note is that all data were log transformed to obtain a normal distribution before analysis.

Table 2

Relative abundance of bacteria phyla in soils under the three landuse types.

Phyla	Sludge-impacted soil ------(%)-----	Garden soil	Saline soil
<i>Proteobacteria</i>	84.00	65.00	63.00
<i>Actinobacteria</i>	2.00	19.00	23.00
<i>Firmicutes</i>	4.00	12.00	11.00
<i>Bacteroidetes</i>	6.00	1.00	0.70
<i>Acidobacteria</i>	2.00	1.00	0.10
<i>Nitrospinae</i>	0.80	0.00	0.00
<i>Gemmatimonadetes</i>	0.60	0.50	0.00
<i>Cyanobacteria</i>	0.00	0.70	0.00
<i>Planctomycetes</i>	0.50	0.20	0.00
<i>Chloroflexi</i>	0.50	0.40	0.40
<i>Nitrospirae</i>	0.30	0.07	0.10
<i>Synergistetes</i>	0.40	0.00	0.00
<i>Tenericutes</i>	0.05	0.40	0.00
<i>Crenarchaeota</i>	0.00	0.40	0.70
<i>Verrucomicrobia</i>	0.30	0.20	0.00
<i>Aquificae</i>	0.00	0.00	0.40
<i>Ignavibacteriae</i>	0.00	0.00	0.40
<i>Spirochaetes</i>	0.08	0.00	0.00
<i>Chlamydiae</i>	0.08	0.00	0.00

Results

Physico-chemical properties of the soils

Selected physical and chemical properties of the soils are summarised in Table 1. Sludge impacted soil had the highest electrical conductivity (5.7 dS m^{-1}), organic matter (58%), total phosphorus (2641 ppm), CEC ($8.62 \text{ Cmol kg}^{-1}$) and clay content (18%). Bare salt affected soil had the highest pH (H_2O) (7.62), least organic matter (0.5%), and total phosphorus (24.90 ppm). Garden soils had the highest sand content (89%) and least electrical conductivity (0.3 dS m^{-1}). The soils are generally arenic (sandy) in with sludge impacted soil and bare saline soil have sandy loam texture and garden soil having loamy sand textural class.

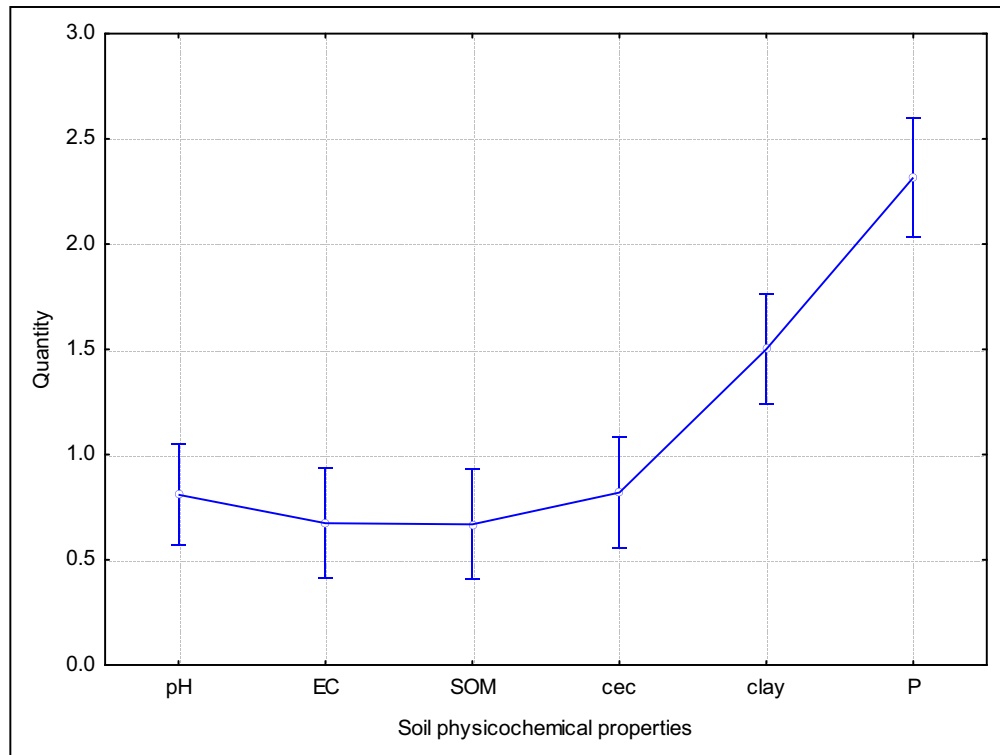
There were significant variations in the physico-chemical properties (clay, pH, EC, SOM, CEC and P) of the soils [F (5, 37) = 23.646, $p = 0.0000$], at the $p < 0.05$ level for the bare saline, sludge-affected and garden soils (Fig. 1A). Results of the interactions among the landuse types and the soil physicochemical properties showed a significant differences between the landuse types [F (10, 37) = 3.433, $p = 0.0029$], and this may explain the difference the bacteria phyla reported in this study (Fig. 1B). The pairwise differences between bare soil (BS), sludge-affected soil and garden soil in physicochemical properties are all statistically significant at $p < 0.05$ with the exception of soil organic matter for bare and garden soils (Fig. 1B).

Metagenomics analysis of bacterial community

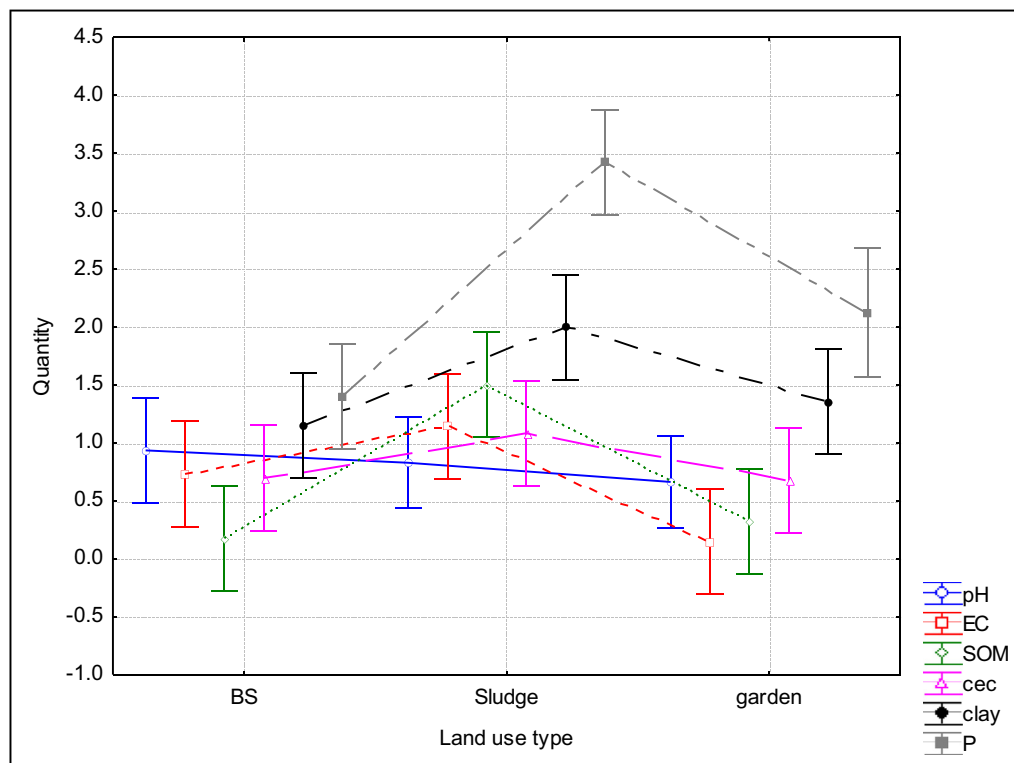
Quality DNA sequences were obtained from sewage sludge (33,008 reads), garden (20,251 reads) and saline (11,043 reads) soils following high throughput metagenomic DNA sequencing. For each soil type over 96% of the sequence reads were mapped and classified at phylum, class, order, family and genus levels, only 1% was unmapped for sludge, 3% and 4% were unmapped for garden and saline soils, respectively, (Table 2; Fig. 2A–C). Averaged across the samples, the most abundant bacterial phyla in the three soils were *Proteobacteria* which ranged from 63% to 84% and these phyla consistently had the highest relative abundances across all samples. *Actinobacteria* were present in garden and saline soils with relative abundance ranging from 18% to 23% respectively, and only 2% represented in sludge. *Firmicutes* phyla were well represented across all sites, relatively higher in saline (11%) and garden (12%) compared to sludge (4%) soil. *Bacteroidetes* were found in a higher percentage in sewage sludge (6%) compared to and garden (1%) and saline (0.7%) soils. Sludge soil reveals higher phylum diversity that is not present in saline and garden soils although at lower abundance of less than 1%. Other phyla represented in the samples in lower quantities include *Acidobacteria* at 2%, 1% and 0.1% in sludge (Fig. 4A), garden (Fig. 4B) and saline (Fig. 4C), respectively. *Nitrospinae* (0.8%), *Synergistetes* (0.4%), *Spirochaetes* (0.08%) and *Chlamydiae* (0.08%) were found only in the sludge-impacted soil. *Gemmatimonadetes*, *Planctomycetes*, *Verrucomicrobia*, *Tenericutes*, and *Cyanobacteria* were represented only in sludge and garden at lower percentages ranging from 0.05% to 0.6%. *Nitrospirae* and *Chloroflexi* were present in all samples also at lower abundance ranging from 0.07% to 0.5%, while *Crenarchaeota* (0.7%) *Aquificae* (0.4%), and *Ignavibacteriae* (0.4%) were present only in saline soil (Table 2; Fig. 2C).

Class and order level analysis

The most abundant bacteria at class level were *Alphaproteobacteria* percentages ranged from 22% to 32%, *Gammaproteobacteria* from 17% to 29% and *Betaproteobacteria* ranging from 12% to 21%, and were represented across the three samples



A



B

Fig. 1. Statistical analysis: A. ANOVA of the Means of the physico-chemical properties; B. Interactions of soil physicochemical properties under different landuse.

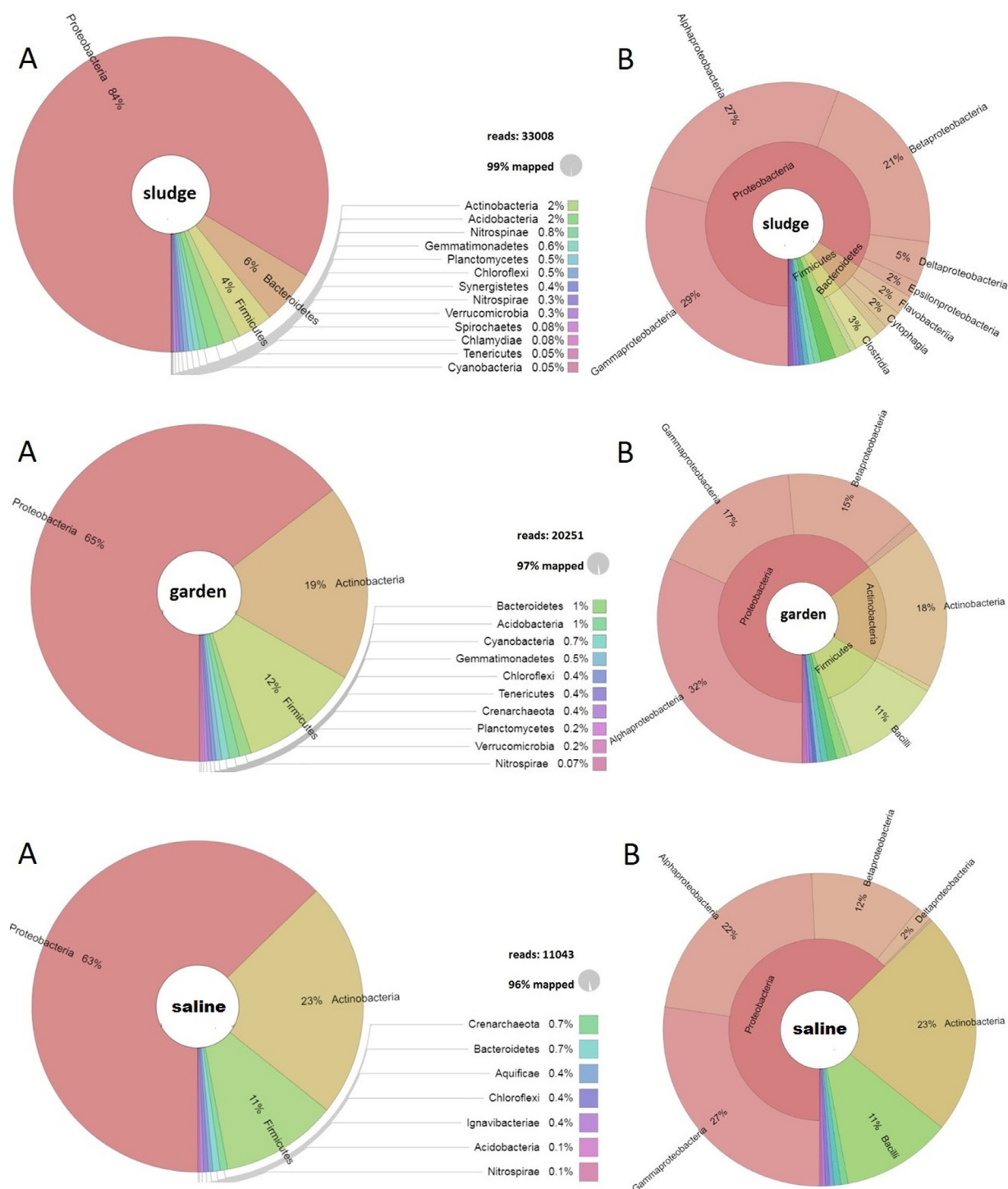


Fig. 2. Krona charts showing the phylum-level and Class-level abundance, diversity and distribution of bacterial populations in A. sludge impacted soil, B. garden, and C. saline soils.

(Fig. 2B,C). *Deltaproteobacteria* was present in sludge-impacted (5%) and saline (2%) soils, whereas *Epsilonproteobacteria* (2%), *Flavobacteria* (2%), *Clostridia* (2%) and *Cytophagia* (2%) were only present in sludge-impacted soil (Fig. 3B). *Bacilli* were consistent in both Garden (Fig. 2A) and Saline soils at 11% (Fig. 2C).

Results at order level show that within the class *Alphaproteobacteria*; *Sphingomonadales* was the most abundant in the garden (42%) and saline (22%) soils compared to sludge (5%) (Fig. 3A–C). *Rhizobiales* and *Rhodospirillales* ranged from 3%

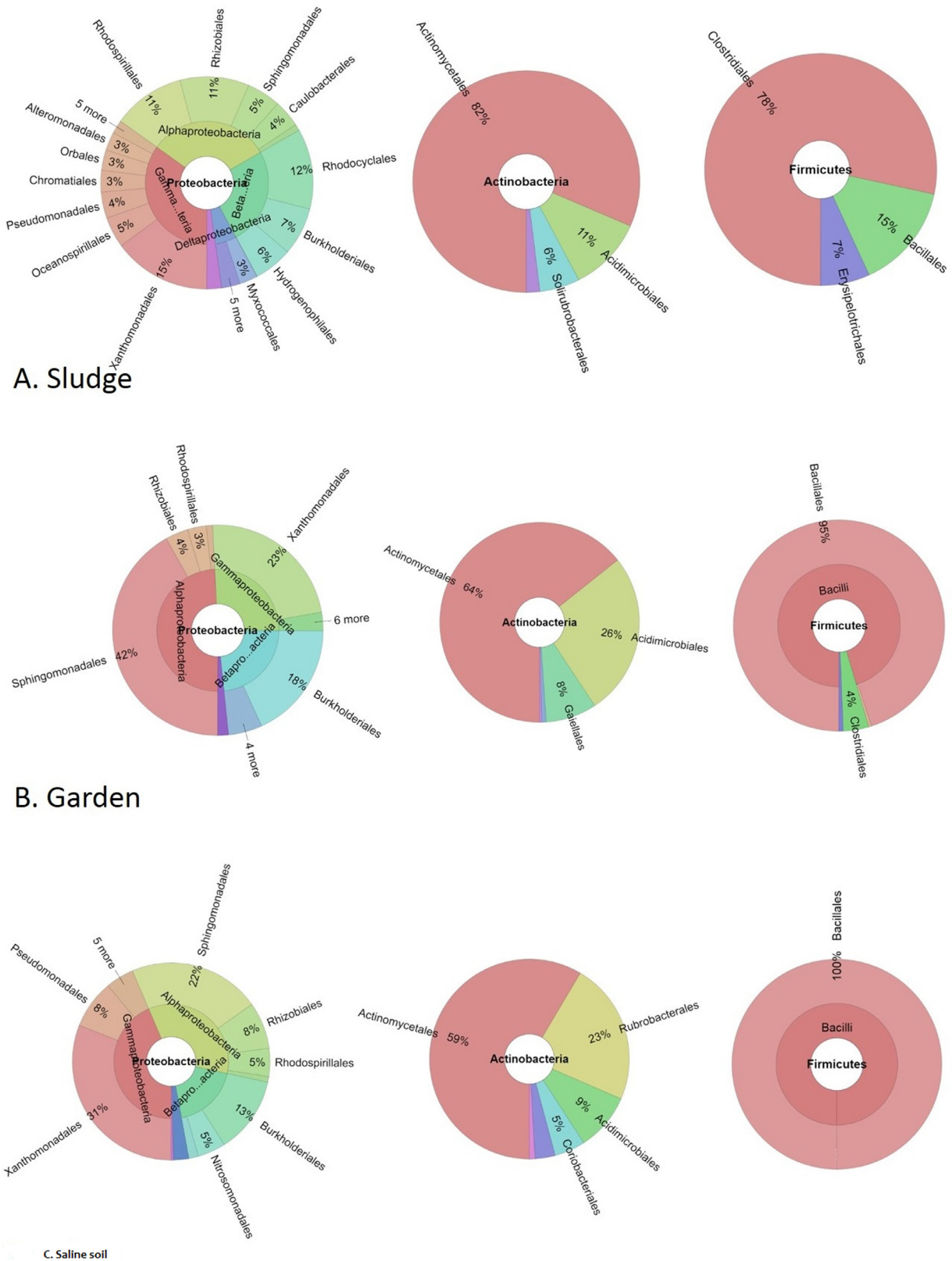


Fig. 3. Krona charts showing the order-level, bacteria abundance, diversity and distribution of bacterial populations in A. sludge, B. garden, and C. saline soils.

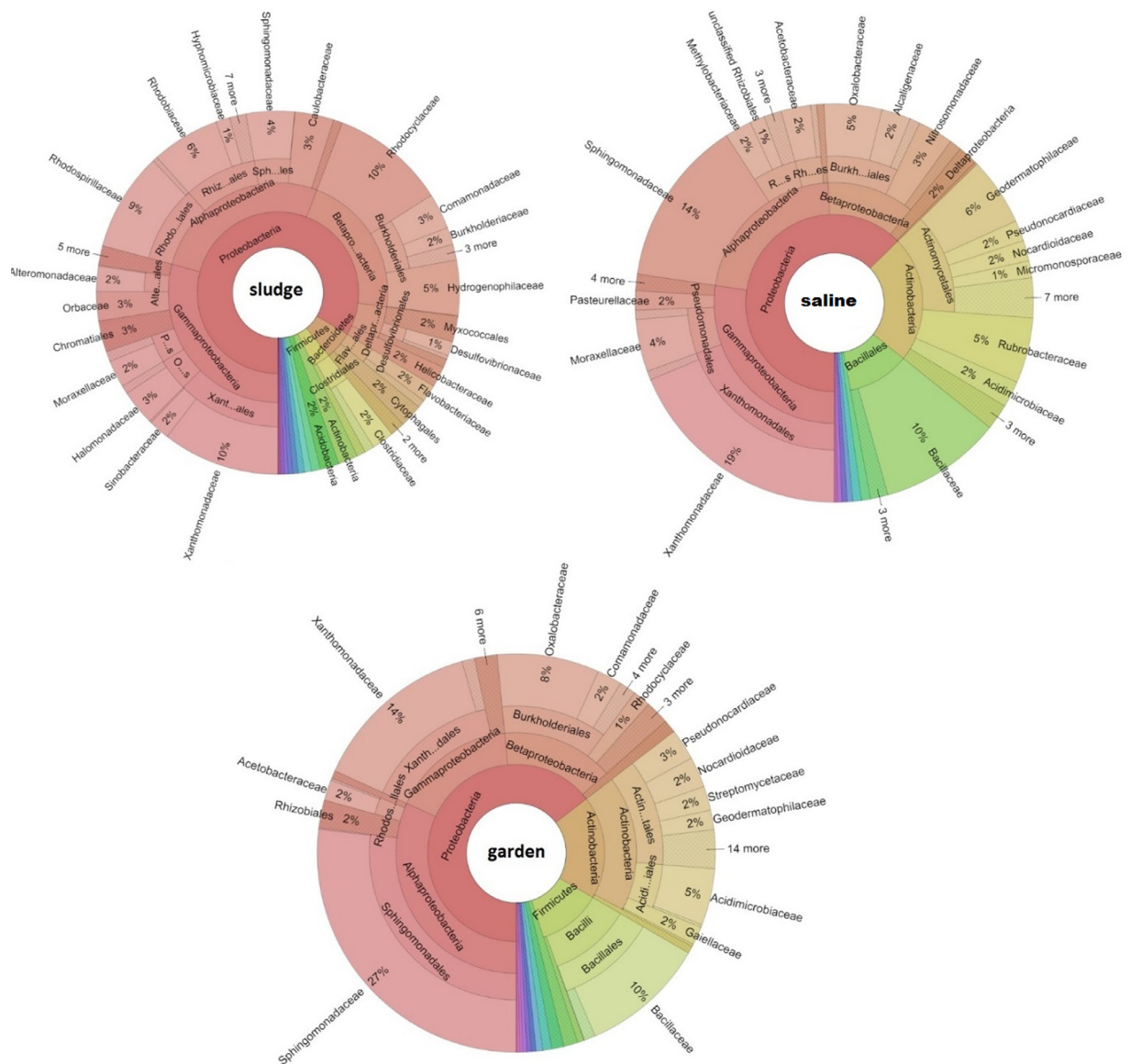


Fig. 4. Krona charts showing family-level abundance, diversity and distribution of bacterial populations in sludge, garden, and saline soil.

to 11% across all the samples. In the Gammaproteobacteria, order *Xanthomonadales* was most abundant in saline (31%) and garden (23%) soils compared to sludge-impacted soil (15%). *Pseudomonadales* ranged from 4% to 8% in sludge and saline soil, respectively. Sludge-impacted showed more diversity consisting of orders not present in garden and saline soils; *Oceanospirales* (5%), *Chromatiales* (3%), *Orbales* (3%) and *Alteromonadales* (3%). Within the Betaproteobacteria the order *Burkholderiales* was found in all the samples with abundance higher in saline (13%) and garden (18%) soils relative to sludge-impacted soil (7%). *Nitrosomonadales* were present at 5% respectively, in saline soil (Fig. 2). More order diversity within the *Proteobacteria* found only in the sludge-impacted soil consists of *Hydrogenophila* (6%), *Rhodocyclales* (12%), *Caulobacteriales* (4%) and *Myxococcales* (3%). More similarity is observed between garden and saline soils as both had representations of order *Actinomycetales* at the abundance of 64% and 59% respectively, compared to 82% in sludge-impacted soil. *Acidimicrobiales* were present in saline (9%), garden (26%) soils and sludge-impacted soil (11%). *Rubrobacteriales* (23%) *Coriobacteriales* (5%) were found only in saline soil, while *Gaillales* (8%) and *Solirubrobacteriales* (6%) were present only in garden soil and sludge-impacted soil, respectively. Garden and saline soils also have a higher abundance of *Bacillales* at 11% respectively. Orders *Clostridiales* of the *Firmicutes* were most frequently found in sludge (78%) compared to garden (4%) Saline soil was completely dominated by *Bacillales* (100%), compared to garden soil (95%) and sludge-impacted soil (15%). *Erysipelotrichales* (7%) was present only in sludge-impacted soil (Fig. 5A).

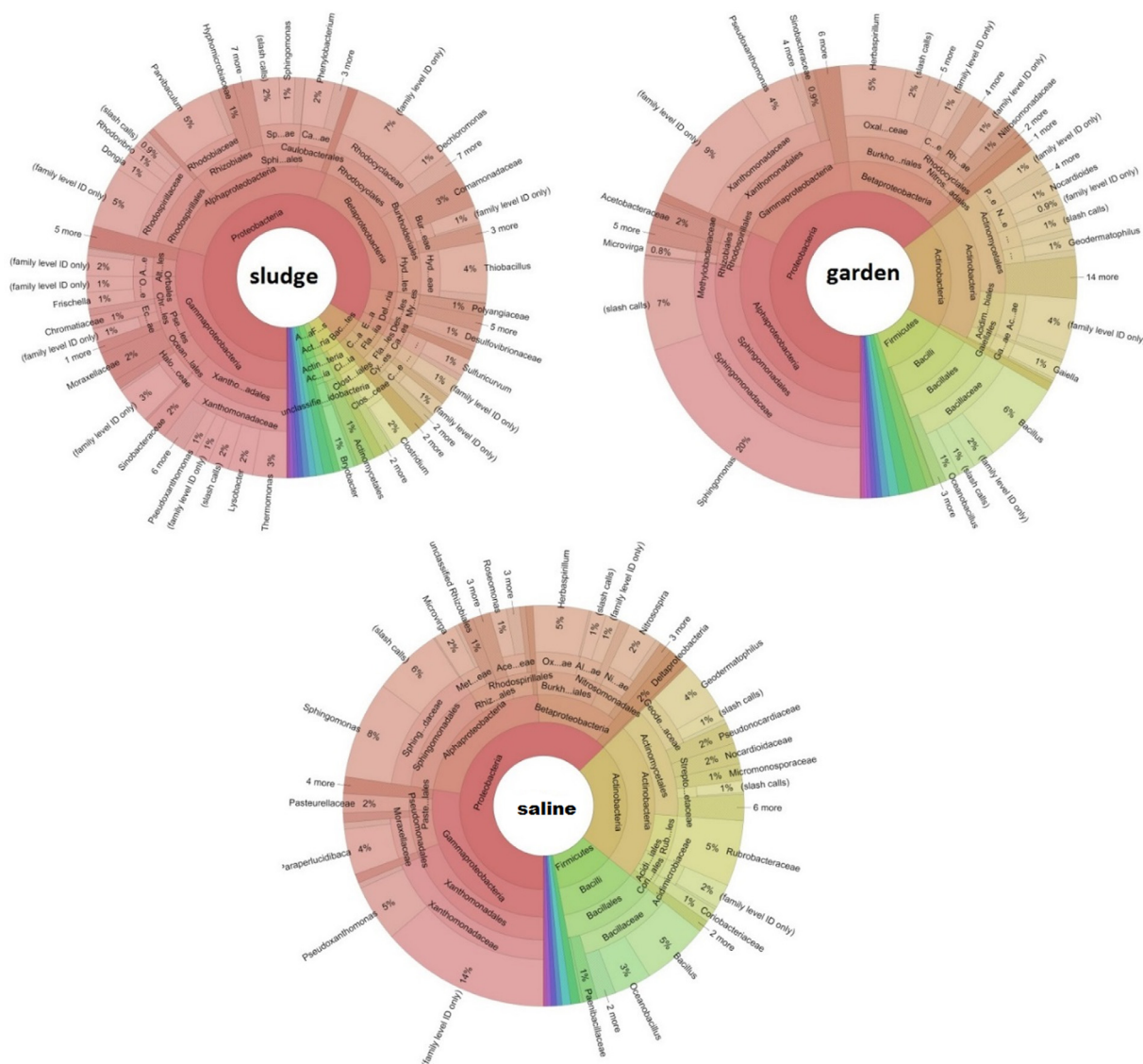


Fig. 5. Krona charts showing genus-level abundance, diversity and distribution of bacterial populations in sludge, garden, and saline soil.

Family and genus level analysis

The family represented with the highest relative abundance was *Sphingomonadaceae*; 27% present in the garden soil and 14% in saline soil. *Xanthomonadaceae* was also of relatively high abundance across all the samples; saline (19%), garden (14%) and sludge (10%). Saline and garden soils also revealed a higher abundance of *Bacillaceae* both at 10%. *Rhodospirillaceae* (9%) and *Rhodobiaceae* (6%) were present in the sludge-impacted soil, whereas *Oxobacteraceae* (8%) showed up relatively higher in the garden soil compared to the saline soil (5%). *Rubrobacteraceae* is another family (Fig. 4). Metagenomics 16S rRNA gene sequencing also revealed some bacterial population at the genus level. At least 22 different bacteria were revealed at the genus level with varying relative abundance across all the sites (Fig. 5). Sludge-impacted soil had the highest diversity comprising of 13 bacteria genera: *Rhodovibrio* (1%), *Dongio* (1%), *Sphingomonas* (1%), *Bryobacter* (1%), *Pseudoxanthomonas* (1%), *Frischella* (2%), *Dechloromonas* (1%), *Phenylbacterium* (2%), *Clostridium* (2%), *Lysobacter* (2%) *Thermomonas* (3%), *Thiobacillus* (4%) and *Parvibaculum* (5%). Garden and saline soils have eight and nine different bacteria genus, respectively, seven are common to both soil types. Garden soil had the highest abundance of *Sphingomonas* (20%) compared to saline soil (8%) and sludge-impacted soil (1%). *Bacillus* was present in both garden and saline soils at the relative abundance of 6% and 5% respectively. *Oceanobacillus* was higher in saline soil (3%) compared to garden soil (1%). *Herbaspirillum* was detected at equal abundances (5%), while *Pseudoxanthomonas* and *Geodermatophilus* were slightly higher in saline soil (5%) compared to garden

Table 3

Bacteria phyla diversity in the garden, saline and sludge impacted soils.

Land use	Simpson index (D)	Sorenson's Coefficient (CC)
Garden	0.47	Saline = 0.70, Sludge = 0.71
Bare saline	0.46	Garden = 0.70, Sludge = 0.56
Sludge impacted	0.71	Garden = 0.71, Saline = 0.56

soil (4%), *Pseudoxanthomonas* was also detected in the sludge-impacted soil at relatively lower percentage (1%). *Nitrosospira* (2%) and *Roseomonas* (1%) were present only in saline soil.

Bacteria phylum diversity relationship with soil properties across the land use regimes

The biodiversity results of the bacteria phyla obtained from Simpson's index and Sorenson's coefficient is presented in Table 3. The Simpson index varied from 0.45 to 0.71 showing that the sludge-impacted soil had the highest diversity followed by garden and saline soils. Sorenson's coefficient showed that garden soil had more overlapping or similar diversity with both sludge and saline soils at (0.70 and 0.71), respectively, while sludge-impacted soil had less overlapping diversity to saline at 0.56.

Discussion

Bacterial community dynamics in the different land-use regimes

Climate change, intermittent disturbances such as tillage, use of pesticides and application of inorganic fertilizers are some of the factors very pronounced in agroecosystems, and they are capable of impeding natural selection [21]. Each of these has specific impacts on soil bacterial abundance and diversity. Arenosols in southern Africa is characterized by low fertility status orchestrated by low organic matter and clay contents, also strongly influenced by human activities and climatic conditions. Prevailing climate conditions such as rainfall intensity and distribution, fluctuation in temperature and physico-chemical properties of soils combine to pave way for the diversity and abundance of bacteria species in soils. The climate of central Botswana is for the most part uniformly semi-arid, so the nature and properties of the soils are the strongest factors that affected bacteria diversity in the soil environments. Bacterial abundance and diversity observed from this experiment was as a result of changes in the physicochemical properties of the soils brought about by differences in land-use. The sludge amended soil had more organic matter than garden and bare saline soils and this could account for the source of nutrients needed for microbial survival and multiplication. The reverse was the case for bare saline soil which had the least organic matter content.

The most dominant bacterial group belongs to the phylum *Proteobacteria* which is consistent with reports by several other studies related to agricultural ecosystems [5,40,45]. *Proteobacteria* are a phylum of Gram-negative bacteria, very common in soil environments and are related to a wide range of functions involved in carbon, nitrogen, and sulphur cycling [42]. The present study results are in agreement to those previously reported for other soil types such as crops, forests, and grasslands [19,51,53]. Members belonging to the *Proteobacteria* phylum occupied the highest richness in all soil samples. *Proteobacteria* members are prevalent in many soil ecosystems, including the rhizospheres, saline soils and semiarid soils [42] as observed in the study. Their relative richness which increases with high organic carbon availability in soils is in line with findings from previous studies such as Fierer et al. [13] and Eilers et al. [11]. The *Alphaproteobacteria* was one of the most abundant classes across all samples, comprising orders *Sphingomonadales* and *Rhizobiales*, which play roles in degradation of inorganic compounds and nitrogen fixation, respectively [28]. The *Betaproteobacteria* consist of several groups of facultative anaerobic bacteria that are also versatile in their degrading capabilities. Examples include genus *Nitrosomonas*, which was detected in both saline and garden soil, this genus play an important role in nitrogen fixation. The *Rhodocyclales* genus within the *Betaproteobacteria* is also important in degrading inorganic compounds, and its high abundance in sludge-impacted compared to the other soils is not surprising. *Deltaproteobacteria*, although detected at relatively low numbers compared to other classes of *Proteobacteria*, has an important ecosystem function. An example is the *Myxococcales*, comprising of soil dwelling organic degrading and sulphur reducing bacteria.

Participating in carbon cycling and producing secondary metabolites, *Actinobacteria* were dominant in saline and garden soils compared to sludge-impacted soil. This finding is also established in a study by Jenkins et al. [20]. *Actinobacteria* are a phylum that consist of many Gram-positive bacteria that play a vital role in the cycling of organic compounds and have been linked with soil organic matter production, including manufacturing of the black pigments called melanin, which is associated with soil humic acid [39].

Members belonging to the *Firmicutes* phylum show a clear inconsistency among the samples, particularly in saline and garden soils, where their percentages are higher than sludge-impacted soil. This is a rather interesting finding when considering the general role of the members of the *Firmicutes*, most associated with diseases particularly in humans. One would hypothesize that members of this group particularly genus *Bacillus* will be predominant in the sludge, however, their persistence and ultimately their abundance could be associated with their ability to resist desiccation [10]. The higher abundance

of *Bacteroidetes* phylum in sludge-impacted soil relative to saline and garden soils is not surprising because of their common occurrence in the gut of animals. However, members belonging to the *Bacteroidetes* phylum have been found to be widespread in several different ecosystems, where they can function as degraders of polymeric organic matter [13]. They have been detected to be the major phyla living under anaerobic conditions existing in the rhizosphere and nearby surface soil ecosystems [29].

Less copious phyla (*Acidobacteria*, *Chloroflexi*, *Chlamydiae*, *Crenarchaeota*, *Planctomycetes*, *Verrucomicrobata*, *Spirochaetes*, *Cyanobacteria*, *Nitrospirae*, *Synergistetes*, *Tenericutes*, *Aquificae*, and *Ignavibacteriae*) were also identified in this study. *Nitrospirae* which comprise the genus *Nitrospira* are known to contribute to soil nitrification [4]. The presence of members belonging to the *Cyanobacteria* phylum was surprising considering their aquatic nature. However, a study found they can be present in soil crust in arid ecosystems and are documented as primary producers, with ability to fix nitrogen [33]. Associates of the *Gemmatimonadetes* phylum were also determined by the 16S rRNA- based metagenomics approach, and are important to note because they are difficult to cultivate using traditional culture approach [24]. However, they have been noted in different soil ecosystems, particularly dry soils, and at low abundance [9]. Members belonging to *Acidobacteria* phylum were also present at very low numbers and it is very important to highlight this phylum which is often affected by soil physico-chemical properties.

At the phylum level, less diversity of the most dominant phylum is observed; therefore it does not appear to be sufficient for evaluating differences in soil bacterial communities. Consequently, differences in diversity across the samples are better appreciated when evaluating classifications at lower levels. At the genus level, bacterial communities of the selected agroecosystems show an important variation in terms of diversity and abundance. The sludge soil had more diversity as many bacterial communities present in it were absent in the garden and saline soils. The following genera *Thiobacillus*, *Clostridium*, *Parvibaculum*, *Thermomonas*, *Lysobacter*, *Bryobacter*, *Phenylobacterium*, *Rhodovibrio*, *Dougio*, *Frischella*, and *Dechloromonas* were only found in varying abundance in the sludge-impacted soil and not in the garden and saline soils. The results at genus level also reveal a higher abundance of *Sphingomonas* (member of the phylum *Proteobacteria*) in the garden soil when compared to the lower abundance in saline and sludge. In the previous studies of Lepleux et al. [26], members of the *Bacillus* genera (showing higher abundance in garden and saline soils relative to sludge soil), were reported as important bio-indicators of weathering and also indicative of the presence of complex minerals (e.g., silicate). We can better appreciate the variations in diversity and abundance at the level of family, genus and species (not indicated here) from each sampling site, which could also indicate that the differences are mainly due to different soil management factors at each site.

Soil type has remained a crucial factor influencing bacterial species [7]. The most abundant groups often observed are members belonging to the *Actinobacteria*, *Proteobacteria*, and *Bacteroidetes* phyla while, in lower proportions, members belonging to the *Firmicutes*, *Acidobacteria*, *Gemmatimonadetes* and *Cyanobacteria* phyla [15],[51]. Furthermore, it has been described that *Bacteroidetes* were more predominant in agricultural systems than in the same soil under non-disturbed conditions, whereas the opposite movement was found for *Actinobacteria* [37]. Bacteria of the genera *Rhizobium* were also abundant in soils, where they form symbiotic associations with legume roots for improved soil nutrition.

Influence of soil physicochemical properties on the bacteria community and implication for management of rain-fed agriculture

Chemical properties are known to be good indicators of soil quality and the health of microorganisms [14]. There is an observed trend that long-term applications of organic manure are beneficial for the accumulation of soil organic matter and thus improve different aspects of soil fertility such as bacterial diversity [27], and in this study, it was demonstrated by the higher bacterial diversity in the sewage sludge impacted soil. The organic carbon contents of the soils were generally low except in sludge-impacted soil. This could be attributed to the high rate of organic matter mineralization in soils [8], especially Arenosols. The high amount of organic carbon in sludge impacted soil corresponded to a high bacterial population, which could be partly explained by the fact that soil organic carbon is a strong indicator of soil fertility status. It releases nutrients for plant growth, promotes the structure, biological and physical health of soil, and it is a buffer against harmful substances. Soils with large quantities of negative charge are more fertile because they retain more cations [30]. The clay fraction has a more diverse bacterial community than silt or sand fractions [38]. This is evident in sludge-impacted soil because it yielded more bacterial diversity ascribed to high clay fraction. Agricultural practices can impact the bacterial population richness and diversity of soils, through alterations in soil physical and chemical properties [12,32].

Biodiversity, when viewed in relation to land-use, is not only a matter of a high number of species but is also concerned with the life strategy of the constituent phyla. The sewage sludge-impacted soil had the highest organic matter, cation exchange capacity, and clay contents, and these, in turn, influenced soil bacteria the most as it recorded the highest bacterial occurrence and diversity. It is also evident from the results of Simpson's index that the diversity of bacteria community is more evenly distributed in garden and saline soils than in sewage sludge where the diversity of the bacteria gravitated more towards the *Proteobacteria*. In these indices, the bigger the numbers; the larger the diversity. A few rare phyla with only a few representatives will not affect the diversity. According to Sorenson's coefficient, the bacteria communities in the saline, garden and sewage sludge soils have similarities. These similarities are mostly pronounced (0.71) between sewage sludge-impacted and garden soils, whereas it is least (0.56) between saline and sewage sludge-impacted soil.

Agricultural practices including the addition of compost manure from time to time would prove invaluable in promoting microbial activities in Arenosols. Sewage sludge enriched the soil with organic matter; therefore, application of same to

bare saline soils could help boost their biological activities. However, care should be taken in application of sewage sludge as it has also been reported to house antimicrobial resistance genes in the soil environments [34]. Total phosphorus and cation exchange capacity of the soils also had effects on microbial community distribution. In view of this, agricultural practices including bush burning and application of lime, which are capable of altering soil pH should always be thoroughly considered.

Conclusion

This study assessed bacterial abundance and diversity in soils under different land-use in a semi-arid environment of Botswana. The garden soil, bare soil and sludge-impacted soil varied significantly in selected physicochemical properties including particle size distribution, pH, organic matter content, electrical conductivity and cation exchange capacity. NGS result shows that the dominant phyla in the three landuse regimes are *Proteobacteria*, *Actinobacteria*, *Firmicutes*, and *Bacteroidetes*. Differences in the land-use pattern are most likely responsible for the variation in the soils' properties and bacteria diversity. This study adds empirical evidence that soil bacterial communities are impacted considerably by land-use. In view of the important roles played by bacteria in our ecosystem, practices which encourage the elevation of P and OC levels in agricultural soils should be adopted. Some of these practices include the non-removal of crop residues from farmlands, the addition of organic matter and the application of the right amount of inorganic phosphate fertilizers and phosphate rocks, where available. This study opens up new frontiers in expanding metagenomics studies in this region and contributes to the identification of soil bacteria which are useful to humans and ecosystem functions.

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