Bacterial community dynamics and functional profiling of soils from conventional and organic cropping systems

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HIGHLIGHTS

- Organic and conventional cropping systems have similar α-diversity but different β-diversity.
- Conventional cropping systems were dominated by nitrogen cycling genera while the organic was dominated by carbon cycling.
- Organic cropping systems have higher antibiotic and antifungal producing genera.
- Organic cropping systems had higher carbohydrate and amino acid metabolism.

ABSTRACT

Soil microbiomes play an integral role in agricultural production systems. Understanding of the complex microbial community structure and responses to conventional compared to organic cropping systems is crucial for sustainable production and ecosystems health. This study investigated soil microbial community structure responses based on a four year long field experiment. Bacterial communities characterizing conventional and organic cropping systems were evaluated using Illumina MiSeq high-throughput sequencing targeting the V4-V5 variable region of the 16S rRNA gene. Soil bacterial community structure showed a cropping system dependant distribution, with nitrogen

cycling taxa (*Bacillus*, *Niastella*, *Kribbella*, and *Beijerinckia*) dominant in conventional cropping systems, while, carbon cycling taxa (*Dokdonella*, *Caulobacte*r, *Mathylibium*, *Pedobacter*, *Cellulomonas* and *Chthoniobacter* and *Sorangium*) were abundant in organic cropping systems. Functional prediction of the bacterial biomes showed conventional cropping systems to harbour a community adapted to carbon-limited environments, with organic cropping systems dominated by those involved in the degradation of complex organic compounds. These findings suggest the existence of niche specific communities and functional specialization between cropping systems with potential use in soil management through selective promotion of organisms beneficial to soil health.

Keywords: Bacterial communities; Functional potential*;* Niche specific community, Soil health

1. Introduction

Intensive agriculture is one of the main characterizing features of modern-day farming practices, to meet food demands from the ever-growing world population (Tilman et al., 2011). This, however, has resulted in adverse environmental impacts including increased soil erosion, nutrient leaching from intensive fertilizer application and declining soil microbial diversity (Tsvetkov et al., 2018; Han et al., 2016; Biswas et al., 2014). Bacteria and fungi as part of soil biota play key roles in nutrient cycling through organic matter decomposition and nutrient transformation and fixation (Rashid et al., 2016). Microbiomes are an integral part of almost all soil processes, with agricultural

management practices such as crop rotation directly impacting on plant/soil-associated microbial communities (van Bueren et al., 2002; Affaires et al., 2014). However, shifts in the resulting microbial communities due to variations in cropping systems are unclear, and may explain yield differences as well as provide new knowledge for future yield improvements.

The effects of farming systems on soil microbial communities are very complex and poorly described yet, understanding them is essential for the effective and sustainable management of agricultural ecosystems (Buckley and Schmidt, 2001). A thorough understanding of the potential role and impact of microorganisms on agricultural ecosystem is integral to understanding how management systems can improve or deteriorate soil health and productivity over extended periods of time (Ishaq, 2017). Soil microbial communities are the engines driving nutrient transformation and release, as well as being directly and indirectly involved in ecosystem services (Rillig et al., 2006, Lynch et al., 1985) such as climate regulation (Saccá et al., 2017), pest and disease control (Garbeva et al., 2004), and biodegradation of organic waste and xenobiotics (Paul et al., 2006). As such, a healthy, sustainable and productive soil is characterized by a diverse compliment of soil microbes and a balance of essential nutrient components in particular soil carbon and nitrogen (Mäder et al., 2002).

Maintaining the biodiversity of soil microbes is widely acknowledged as crucial to soil health (Rao, 2007). In previous research, the soil microbial community profile of organic cropping systems specific to the use of organic manure have been reported to shift microbial composition towards a more stable and fast-growing structure (Lupatini et al., 2017). This has further been suggested to have the potential to increase diversity as

well as promote specific taxa involved in maintaining plant health (Gonthier et al., 2014). Soils however, have direct impact on microbial community structure and function through natural perturbations or human activities (Upchurch et al., 2008; Weller et al., 2002; Tilak et al., 2005). While there are some agro-ecosystem studies that compare the effect of organic farming in its various forms to conventional systems on microbial community composition (Hartmann et al., 2015; Lori et al., 2017; Lupatini et al., 2017; Liao et al., 2018; Amalytė et al., 2019), only a few have focused on assessing the effect of organic cropping systems (the use of organic manure and chemical-free disease control methods) on bacterial community composition. Moreover, to the best of our knowledge, no study has reported on the effect of the organic cropping system and the associated soil health treatments (monocrop or rotation) and on the functional potential in the soil to date. As such, the primary aim of this study was to obtain a snapshot of bacterial community structure characterising organic and conventional cropping systems. The study further attempts to predict functional potential of different cropping systems and soil health treatments to determine effective soil health markers related to nutrient cycling. In this context, we hypothesized that bacterial composition and functional potential of soil differs between organic and conventional cropping systems, with organic system surpassing that in conventional farming.

2. Materials and Methods

2.1. Site description and management

The field site was located at the Nelson Mandela Metropolitan University, George campus at Saasveld, in the Western Cape Province of South Africa (22° 32’ 6.546” E; 33°

57’ 49.289” S). The area is approximately 160 m above sea level, with a characteristic Mediterranean climate. Monthly average temperatures range from 12 °C to 21 °C with an annual average rainfall of 827 mm. For over 20 years, the site area lay undisturbed, and was naturally habited by Kikuyu grass (*Pennisetum clandestinum*) as the dominant species, until it was cleared for the trial. In 2014, the site covering approximately 1500 m^2 of land was divided into three blocks, with each block consisting of nine 30 m^2 (5m x 6m) plots. Plots were randomly allocated for either organic or conventional cropping system under monocrop or crop rotation as well as the untreated control. The following nine soil health treatments (crop rotation sequences are given in Table 1) belonging to three cropping systems that were tested in the current study: 1) Untreated control cabbage – CC; 2) Conventional soil health treatments: (i) Conventional monocrop cabbage- CMC, (ii) Conventional rotation cabbage- CRC, (iii) Conventional rotation cowpea- CRCP, (iv) Conventional rotation sweet potato- CRSP and 3) Organic soil health treatments (i) Organic monocrop cabbage- OMC , (ii) Organic rotation cabbage- ORC, (iii) Organic rotation cowpea- ORCP, (iv) Organic rotation sweet potato- ORSP. Trials were conducted under rain fed conditions, between late October and March over a four-year period. The pH on all plots was amended to a suitable pH with Dolomitic lime which was applied at a rate of 1 ton ha^{-1} for three consecutive years (2014-2016) before each planting, except in 2017.

Table 1. Treatment designation and crop sequence of the Saasveld Comparative Trial, Western Cape, South Africa.

Treatment	ID	Main factor	Sub-plot factor	Year			
				2014	2015	2016	2017
				Initial crop	Crop 2	Crop 3	Crop 4
1	OMC	Organic system	Monocrop	Cabbage	Cabbage	Cabbage	Cabbage
2	ORSP	Organic system	Rotation	Sweet potato	Cowpea	Cabbage	Sweet potato
3	ORCP	Organic system	Rotation	Cowpea	Cabbage	Sweet potato	Cowpea
4	ORC	Organic system	Rotation	Cabbage	Sweet potato	Cowpea	Cabbage
5	CMC	Conventional system	Monocrop	Cabbage	Cabbage	Cabbage	Cabbage
6	CRSP	Conventional system	Rotation	Sweet potato	Cowpea	Cabbage	Sweet potato
7	CRCP	Conventional system	Rotation	Cowpea	Cabbage	Sweet potato	Cowpea
8	CRC	Conventional system	Rotation	Cabbage	Sweet potato	Cowpea	Cabbage
9	CC	Control	Monocrop	Cabbage	Cabbage	Cabbage	Cabbage

Manure was applied on all organic plots at a rate of 22.7 tons ha^{-1} before the first planting (2013/2014) but was there after only applied on organic monocrop cabbage and whenever cabbage was planted during organic rotation at a reduced rate of 5 tons ha^{-1}

for the remaining three plantings. Manure was prepared by composting of green grass, crop leaf residues, and horse manure-straw mixture at a ratio of 1:1:1. Moreover, Calfos^{TM} (calcium phosphonate) was applied at a rate of 900 kg ha^{-1} on all organic soil health treatments to increase soil phosphorus from 12 to 30 mg kg^{-1}, before the beginning of 2016/2017 season.

In the first season (2014/2015), all conventional soil health treatments received synthetic (NPK 2:3:2 (30) + 0.5% zinc) fertilizer (Gromor (PTY), LTD, South Africa) at a rate of 400 kg ha^{-1} and a top dressing of 200 kg ha^{-1} of LAN (27). For all seasons, synthetic fertilizer (Gromor) was applied at a rate of 200 kg ha^{-1} and a top dressing of 100 kg ha^{-1} of LAN (27). The untreated control consisted of cabbage grown without fertilizer application for the entire duration of the experiment.

2.2. Crop selection criterion

Three crops representing different families commonly grown by commercial and subsistence farmers in South Africa were selected for the crop rotation system. These included cabbage (*Brassica oleracea* var. capitata), a heavy feeder, sweet potato (*Ipomoea batatas*), a light feeder and cowpea (*Vigna unguiculata*), which apart from fixing nitrogen, is not known to require fertilizer application. The crop planting sequence is shown in Table 1.

2.3. Soil sampling, DNA isolation and 16S rRNA gene amplification

Soil samples were collected once, at the end of the four-year experiment period in May 2018. A total of eight soil core (top-layer, 0-15 cm) subsamples from each replicate

plot in each of the three blocks was sampled with a clean auger (washed and disinfected with 70% ethanol between sampling). These eight replicate subsamples were then pooled to make a single heterogeneous composite sample, providing 27 independent samples. Soil samples were placed inside marked zip lock bags and transported to the laboratory in cooler boxes for storage at 4 °C and processed within two days to minimize the development of commensals. Total community DNA was extracted from 0.25 g of soil using the MoBio PowerSoil™ DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. The DNA in each sample was quantified using the Nanodrop ND-2000 UV-VIS Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) prior to further analysis.

2.4. Amplification, sequencing and sequence processing

The bacterial microbiomes were pair-end sequenced using the Illumina MiSeq platform (Caporaso et al., 2012) using the primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 909R (5'-CCCCGYCAATTCMTTTRAGT-3') (Wang and Qian, 2009), targeting the V4-V5 variable region in the 16S rRNA gene. Sequencing was performed at Molecular Research DNA (MR DNA, Shallowater, TX, USA) on a MiSeq Sequencer according to the manufacturer's guidelines. Paired-end sequences were merged and preprocessed to remove barcodes and primers by MR DNA using their in-house pipeline and freeware. Raw sequence data are available on NCBI-SRA under the BioProject accession number: PRJNA626528.

The bioinformatics package Quantitative Insights Into Microbial Ecology 2 (QIIME2) was used for the initial data processing (Bolyen et al., 2019). Demultiplexing of

sequences was performed with the q2-demux plugin (https://github.com/qiime2/q2-demux). Reads were trimmed at 260bp before quality filtering and de-replication using the q2-dada2 plugin (Callahan et al., 2016). This process simultaneously removes chimeras and produce sequence variants (SVs), using nucleotide quality scores. Taxonomic classification of SVs was assigned using the q2-feature-classifier which uses Naive Bayes machine-learning classifiers to assign taxonomies based on sequence k-mer frequencies (https://github.com/qiime2/q2-feature-classifier) and Greengenes 16S rRNA gene database (http://greengenes.lbl.gov) (Bokulich et al., 2017). The classification default assumption being that each species in the reference taxonomy is equally likely to be observed and has room to allow for prior probabilities to be set for each species (Bokulich et al., 2018).

2.5. Statistical analysis

The α- and β-diversity of bacterial communities was analyzed with MicrobiomeAnalyst, online pipeline (https://www.microbiomeanalyst.ca/) (Dhariwal et al., 2017; Chong et al., 2020). The α-diversity indices relative to the samples were represented by box plots and one-way analysis of variance (ANOVA) was used to compare the distribution of each bacterial community among the cropping systems and associated soil health treatments, and Tukey HSD mean comparisons were utilized to produce pairwise comparison of the different cropping systems and soil health treatments. Data were imported using the pipeline using default parameters including low count filter (minimum count of four with 20% prevalence in samples), low variance filter at 10% based on inter-quantile range and data scaling with total sum scaling. The effect of cropping systems and soil health

treatments on bacteria community structure was assessed using permutational multivariate analysis of variance (PERMANOVA) and principal coordinates analysis (Anderson, 2017). Homogeneity of multivariate dispersions was checked with the permutational multivariate dispersions (PERMDISP) test using the Bray-Curtis similarity matrix (Anderson, 2006). Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2; https://github.com/picrust/picrust2/) was used to predict potential functional gene abundances of the bacterial communities (Langille et al., 2013) based on the OTU table generated from 16S rRNA gene region sequences and taxonomy in QIIME2. This is accomplished in a two-step process whereby; gene content is precomputed for each organism in a reference phylogenetic tree and reconstructing a table of predicted gene family abundances. The subsequent metagenome inference step then combines the resulting gene content predictions for all microbial taxa with the relative abundance of 16S rRNA genes (OTUs) in one or more microbial community samples, corrected for expected 16S rRNA gene copy number, to generate the expected abundances of gene families in the entire community (Langille et al., 2013). The prediction of Kyoto Encyclopedia of Genes and Genomes (KEGG) Ortholog abundances was carried out with Hidden-state prediction (Zaneveld and Thurber, 2014) and the KEGG Orthologs (KOs) were collapsed into functional pathways, modules, and categories.

Differences in the relative abundance of taxonomic groups between cropping systems as well as between soil health treatments were performed in R version 3.4.3 (R Core Team, 2017) with the Kruskal-Wallis test across cropping systems and individual treatments followed by pairwise comparisons using the Wilcoxon rank sum test. Test results with $p < 0.05$ were considered statistically significant. The linear discriminant

analysis (LDA) effect size (LEfSe) algorithm (Segata et al., 2011) was employed to identify biomarker genera and KOs from the top 100 matches. Samples were classified by conventional and organic cropping systems using an alpha value for the factorial Kruskal-Wallis test of 0.05, and a threshold on the logarithmic LDA score for discriminative features of 2.0.

3. Results

3.1. Bacterial community abundance, composition and variability

A total of 1 818 951 (67 370 ± 21 451 per sample) $16S_{V4\text{-}V5}$ bacteria sequences were recovered from 27 soil samples after paired-end alignments, quality filtering, and deletion of chimeric sequences. These were subsequently assigned to a total of 6 425 (2 107 ± 483 per sample) sequence variants for all the samples. Taxonomic compositions of bacteria from organic and conventional farming systems investigated in this study are shown in Fig 1. Overall, a total of 28 phyla, 90 classes, 132 orders, 133 families and 145 bacterial genera were detected for all the samples. Proteobacteria was the dominant phylum (37.5%-41.4%), followed by Acidobacteria (15.3%-18.7%), Actinobacteria (13.2%-17.2%), Bacteroidetes (5-9%), Verrucomicrobia (4.2%-5.4%), Gemmatimonadetes (5.0%-5.1%), Chloroflexi (3.6%-3.9%), Planctomycetes (1.8%-2.7%) and Firmicutes (0.6-1.0%) (Fig. 1A).

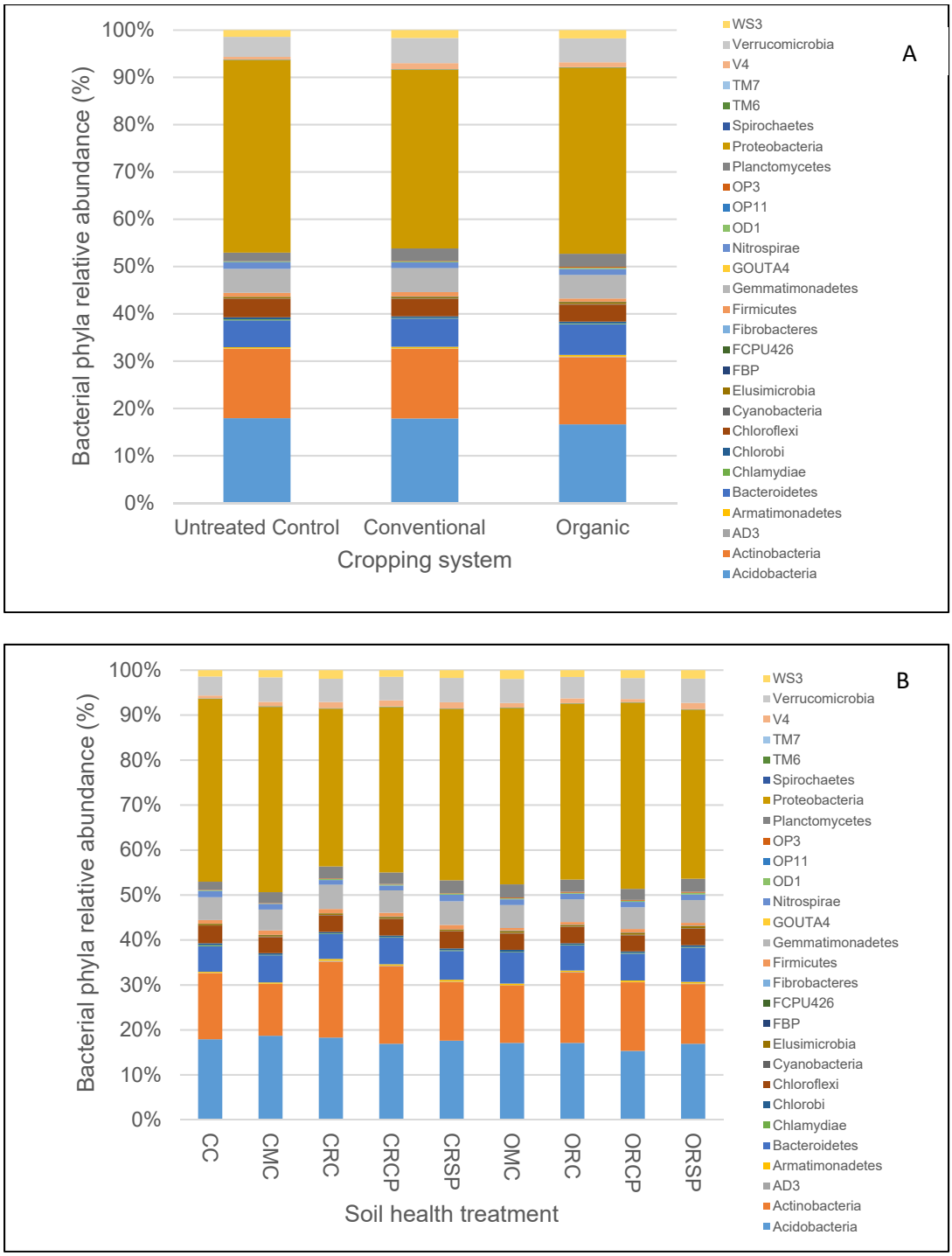

Fig. 1. Taxonomic abundance of major bacterial phyla in (A) different cropping systems and (B) individual soil health treatments. CC, untreated control cabbage; CMC, conventional monocrop cabbage; CRC, conventional rotation cabbage; CRCP, conventional rotation cowpea; CRSP, conventional rotation sweet potato; OMC, organic monocrop cabbage; ORC, organic rotation cabbage; ORCP, organic rotation cowpea; ORSP, organic rotation sweet potato.

No significant differences were observed between cropping systems at the phylum level with respect to the relative abundance of Proteobacteria (Kruskal-Wallis test: χ^2 = 3.55, P = 0.17), Acidobacteria (Kruskal-Wallis test: χ^2 = 3.84, P = 0.15), Actinobacteria (Kruskal-Wallis test: χ^2 = 0.39, P = 0.82), Bacteroidetes (Kruskal-Wallis test: χ^2 = 2.33, P = 0.31), Verrucomicrobia (Kruskal-Wallis test: χ^2 = 3.91, P = 0.14) and Gemmatimonadetes (Kruskal-Wallis test: χ^2 = 0.11, P = 0.95) across all the treatments. The Kruskal-Wallis test revealed significant differences in the relative abundance of Planctomycetes (χ^2 = 7.39, P = 0.02) and Firmicutes (χ^2 = 17.10, P = 0.0002) between the two cropping systems. Further evaluation of Planctomycetes relative abundances showed higher proportions in conventional (2.6%) and organic (2.8%) cropping systems, than in the untreated control cabbage (1.8%).

Among individual soil health treatments associated with the two cropping systems, significant differences were observed in the relative abundances of Proteobacteria (χ^2 = 17.63, P = 0.02), Actinobacteria (χ^2 = 15.70, P = 0.04), Planctomycetes (χ^2 = 10.89, P = 0.021) and Firmicutes (χ^2 = 20.90, P = 0.01) using the Kruskal-Wallis test. The highest relative abundance of Proteobacteria was observed in the organic rotation cowpea (ORCP) (41.4%), conventional monocrop cabbage (CMC) (41.2%), untreated control cabbage (CC) (40.6%) and organic monocrop cabbage (OMC) (39.2%) soil health treatments and the lowest were observed in the conventional rotation cowpea (CRCP) (36.8%) and conventional rotation cabbage (CRC) (35.1%) soil health treatments (Fig. 1B). The relative abundance of Actinobacteria was observed to be significantly (χ^2 = 15.70, P = 0.04) higher (>16.9%) in the two conventional soil health treatments, CRCP and CRC as well as in ORCP and untreated control treatments. The organic rotation

sweet potato (ORSP) and OMC treatments had the highest relative abundance of Planctomycetes (2.9%) while the CMC and CC treatments demonstrated the lowest (2.4% and 1.8%, respectively). The Firmicutes phylum was generally present in very low relative abundance (<1.5%) in all soil health treatments. No significant differences were observed in the relative abundances of Acidobacteria ($\chi^2 = 7.70$, $P = 0.46$), Bacteroidetes ($\chi^2 = 10.62$, $P = 0.22$) and Verrucomicrobia ($\chi^2 = 6.43$, $P = 0.59$) across individual soil health treatments.

LEfSe analysis was performed to identify microbes specifically enriched at the genus level in the different cropping systems. The organic and conventional cropping systems had each, nine differential taxa with an LDA score > 2.0, while the untreated control treatment had two (Fig. 2). At genus level, DA101 was the most differential taxon in the conventional cropping systems (LDA score > 4) followed by A17, *Flavisolibacter* (Bacteriodetes), *Bacillus* (Firmicutes), *Niastella* (Bacteriodetes), *Kribbella* (Actinobacteria), *Alicyclobacillus* (Firmicutes), *Beijerinckia* (Proteobacteria) and *Kibdelosporangi*. The genomic features in organic systems identified the genera *Dokdonella* (Proteobacteria), *Caulobacter* (Proteobacteria), *Mathylibium* (Proteobacteria), *Pedobacter* (Bacteriodetes), *Ramlibacter* (Proteobacteria), *Cellulomonas* (Actinobacteria), *Chthoniobacter* (Verrucomicrobia), *Sorangium* (Proteobacteria) and *Planctomycete* (Planctomycetes) as important taxonomic contributors. The preferential taxa in the untreated control treatment were the genera *Themomonas* (Proteobacteria) and *Iamia* (Actinobacteria). It is worth noting that no distinct taxonomic differences (LDA > 2.0) were observed between individual soil health treatments.

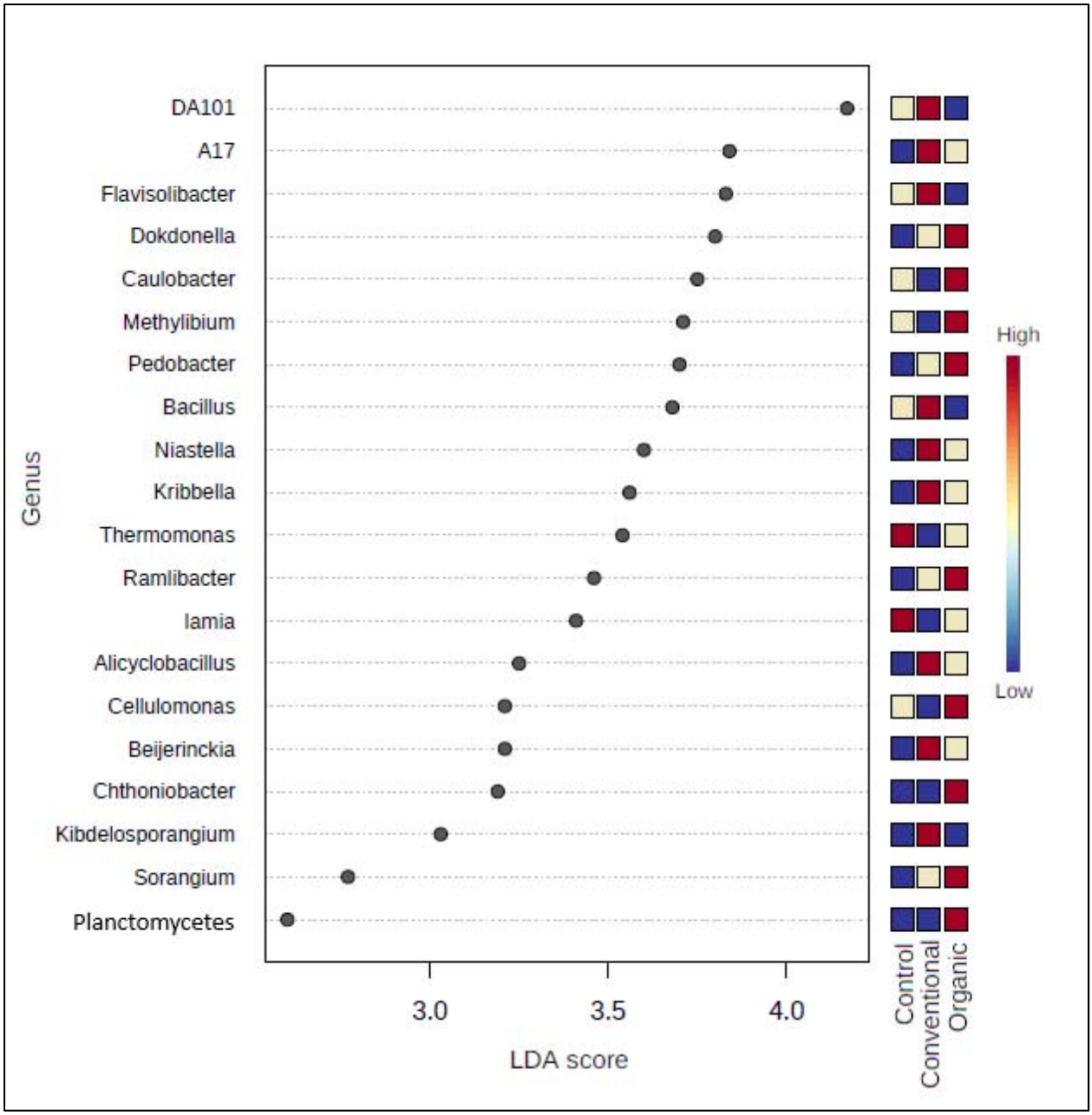

Fig. 2. Linear discriminate analysis (LDA) of effect size (LEfSe) to identify differential taxa at the genus level in each cropping system. Control, untreated control; Conventional, conventional cropping systems; Organic, organic cropping systems.

3.2. Effect of cropping system on microbial diversity

Evaluation of alpha diversity indices (Fig. 3A-B) among the three cropping systems showed significant differences (P = 0.017334; [ANOVA] F = 4.8244), although pairwise

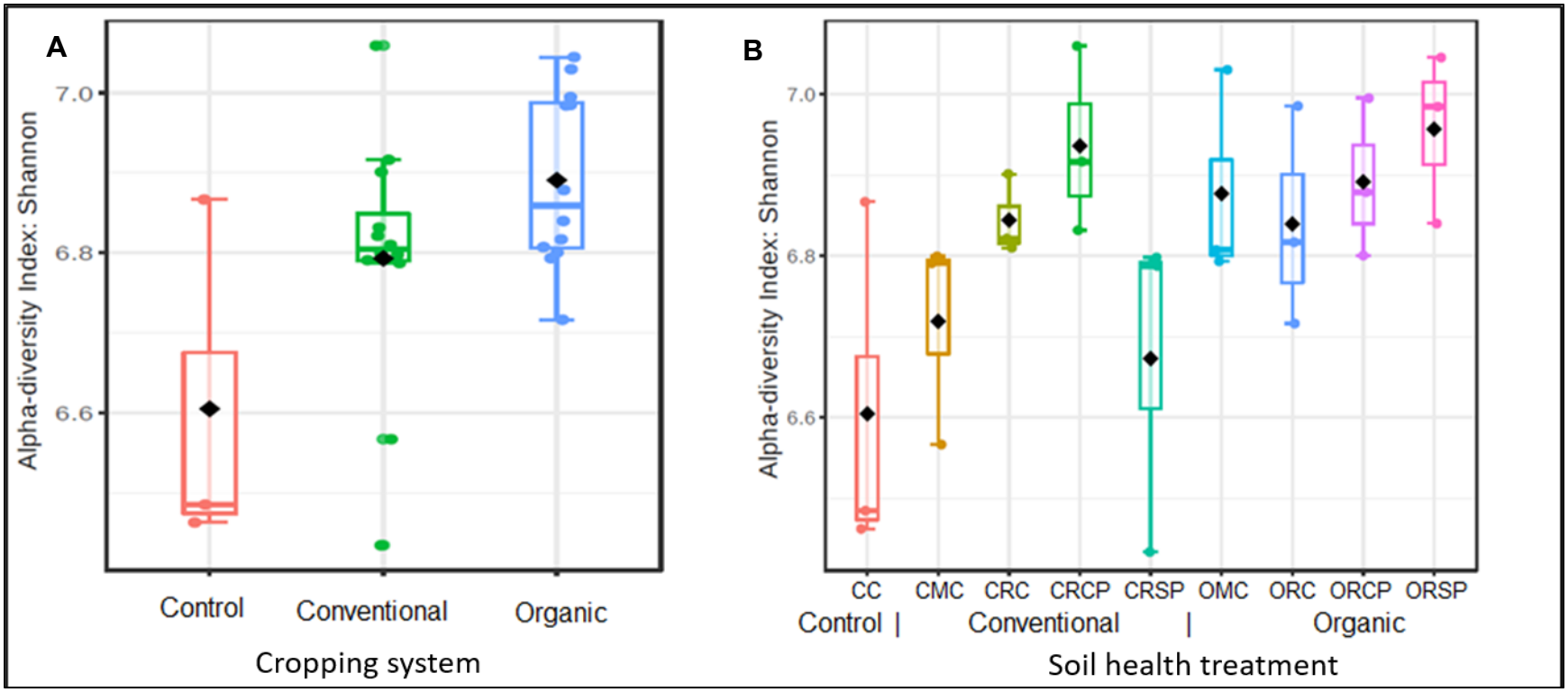

Fig. 3. Effect of cropping system on α-diversity based on Shannon index in cropping systems that used organic fertilizer (compost), conventional (inorganic fertilizer) and the control (no fertilizer) (A), and diversity of individual soil health treatments and associated crops in the respective cropping systems (B), after a four year period. The ends of the whiskers represent the minimum and maximum, the bottom and top of the box are the first and third quartiles, and the line inside the box is the median. The values for each diversity index are shown on the y-axis and cropping system or soil health treatment on x-axis. No significant differences were observed for the pairwise comparisons based on two-way ANOVA on α-diversity and is depicted by a black diamond (♦). CC, untreated control cabbage; CMC, conventional monocrop cabbage; CRC, conventional rotation cabbage; CRCP, conventional rotation cowpea; CRSP, conventional rotation sweet potato; OMC, organic monocrop cabbage; ORC, organic rotation cabbage; ORCP, organic rotation cowpea; ORSP, organic rotation sweet potato.

comparisons between the cropping systems including organic and conventional (P = 0.19868; [T-test] statistic = -1.3284), untreated control and organic (P = 0.17129; [T-test] statistic = -1.9302) and untreated control and conventional (P = 0.26918; [T-test] statistic = -1.4614) were all not significant. However, cropping systems were shown to be significant drivers of bacterial β-diversity with the control, conventional and organic cropping systems forming distinct clusters in PCoA (Fig. 4). The significant effect of cropping systems was further confirmed by a PERMANOVA test (F = 3.218; R^2 = 0.211; P = 0.001), with samples having homogenous dispersions across systems (PERMDISP test: F = 0.177; P = 0.8389). PERMDISP test of pairwise comparison showed no significant difference in dispersions between organic and conventional (F = 0.0001; P = 0.992) cropping systems, and organic and untreated control (F = 0.426; P = 0.525) cropping systems. Evaluation of the impact of crop sequences with PERMANOVA test showed no significant impact on observed community structure in crop rotation for both conventional (F = 1.618; R^2 = 0.378; P = 0.064) and organic (F-value: 1.5426; R^2 = 0.339; P = 0.113) cropping systems. Similar observations were made for monocrop and crop rotation in the organic soil health treatments (F = 1.296; R^2 = 0.11473; P = 0.198) except in the conventional soil health treatments where significant differences (F = 1.901; R^2 = 0.128; P = 0.04) were observed.

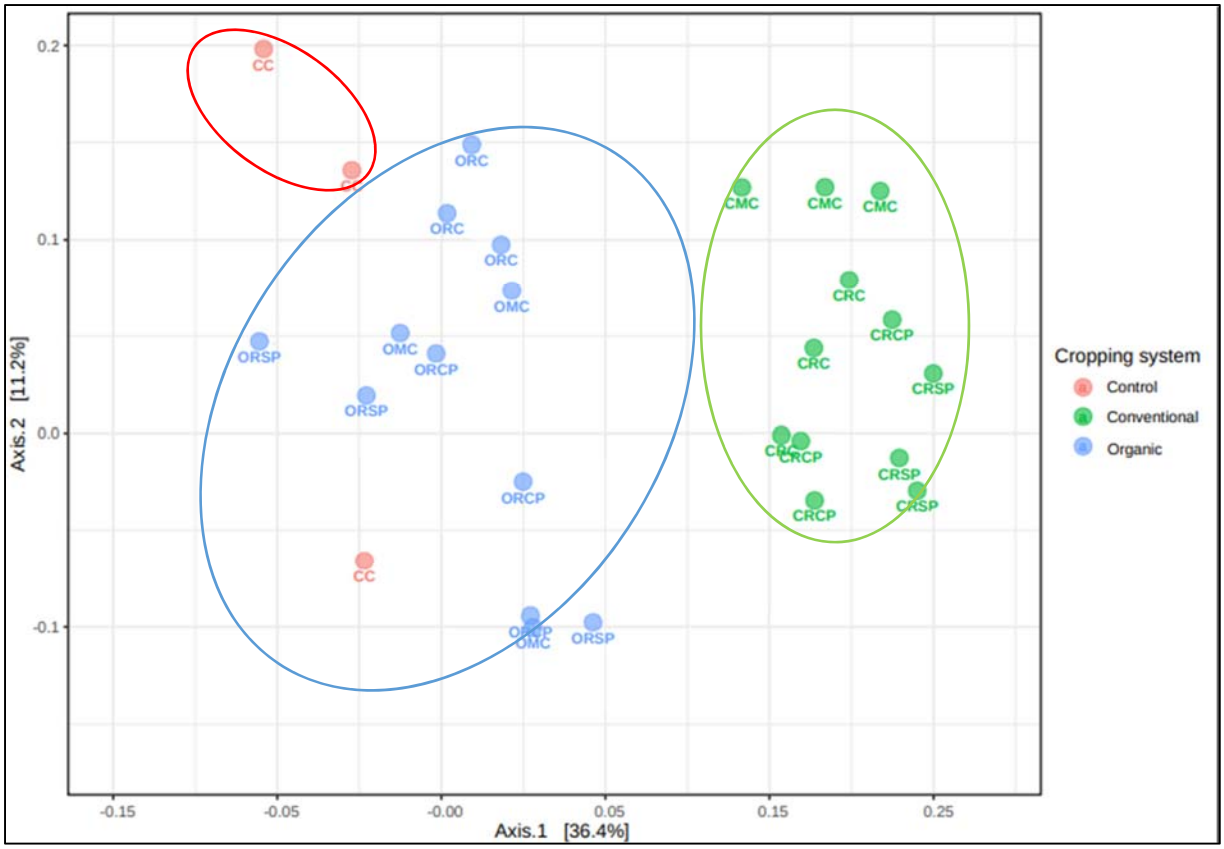

Fig. 4. Principle coordinates analysis (PCoA) of untreated control, conventional and organic soil cropping systems bacterial community compositions. Control, untreated control; Conventional, conventional cropping systems; Organic, organic cropping systems. CC, untreated control cabbage; CMC, conventional monocrop cabbage; CRC, conventional rotation cabbage; CRCP, conventional rotation cowpea; CRSP, conventional rotation sweet potato; OMC, organic monocrop cabbage; ORC, organic rotation cabbage; ORCP, organic rotation cowpea; ORSP, organic rotation sweet potato.

3.3. Predicted metabolic functions using PICRUSt

Bacterial community functions in conventional and organic cropping systems were predicted by PICRUSt. Functional predictions were generated from the KEGG database using the 16S metagenome data. In total, 7 674 KEGG orthologs (KOs) comprising 143 KEGG pathways, 204 KEGG modules and 22 KEGG categories were identified in the study. The five most dominant of KEGG functional categories consisted of amino acid transport and metabolism (10.5%), inorganic ion metabolism (6.2%) carbohydrate

transport and metabolism (5.1%), nucleotide transport and metabolism (4.3%) and lipid transport and metabolism (3.6%) (Fig. 5). Out of the 7 674 KOs obtained, 3 350 (43.7%) were assigned to functional categories, while 4 324 (56.3%) either did not have known functional roles or had uncharacterized functions and were filtered from the analysis. A total of 27 KOs were differentially abundant in the different cropping systems (Table 2). Of these, five KOs were significantly higher in conventional cropping systems, with one, mannose-6-phosphate-isomerase (K01809), being involved in two KEGG pathways (fructose and mannose metabolism, amino sugar and nucleotide metabolism) (Table 2). In organic cropping systems, 18 KOs were differentially expressed with five KOs involved in seven KEEG pathways, K01051 (starch and sucrose metabolism) and K01198 (starch and sucrose metabolism, amino sugar and nucleotide sugar metabolism), K00648 (fatty acid metabolism), K01051 (pentose and glucuronate interconversions) and K01424 (cyanoamino acid metabolism, alanine-aspartate-glutamate metabolism) (Table 2). The untreated control had four significantly higher KOs with two being involved in three pathways, K01052 (sulphur metabolism) and K04765 (pyrimide metabolism, purine metabolism). LEfSe was used to explore the differences in predicted functional characteristics of each microbiome at the level of modules, pathways and orthologs for conventional and organic cropping systems (Table 2). No KOs were differentially expressed in individual soil health treatments. There was no evidence of any predicted function (KEGG modules and pathways) being a significant biologically informative feature in the cropping system or soil health treatment.

Table 2. Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologs enriched among microbiomes in different cropping systems.* *p*-value lower than 0.05 means that KEGG orthologs are significantly different.

Identifier	KEGG ortholog description	Effect size (LDA score)	*P*-value	Abundance		
				Conventional	Organic	Untreated control
K00648	3-oxoacyl-[acyl carrier protein] synthase	2.41	0.000272	9825	10340	9903.8
K13893	microcin C transport system substrate-binding protein	2.39	0.00218	2139.3	1650.4	1801.1
K03286	mpA-OmpF porin, OOP family	2.36	0.002086	2269.9	2723.3	2420.7
K02078	acyl carrier protein	2.35	0.000423	10959	11405	11186
K03929	para-nitrobenzyl esterase	2.35	0.002086	1795.6	2221.6	1780.6
K15726	obalt-zinc-cadmium resistance protein	2.29	0.001027	5963.2	6350.5	5994.6
K06959	protein Tex	2.24	0.001392	3281.2	3501.4	3153.3
K01809	mannose-6-phosphate isomerase	2.21	0.000923	3427.9	3185.9	3107.3
K07107	acyl-CoA thioester hydrolase	2.21	0.001212	12643	12968	12788
K07001	NTE family protein	2.20	0.003108	11688	11881	12007
K13924	two-component system, chemotaxis family	2.19	0.000376	1369.2	1643.9	1339.1
K04765	nucleoside triphosphate diphosphatase	2.19	0.001688	2924.9	2804.7	3112.9
K01524	exopolyphosphatase	2.18	6.47E-05	8469.8	8742.6	8767.6
K03772	FKBP-type peptidyl-prolyl cis-trans isomerase	2.16	0.000685	2461.6	2746.9	2514.7
K15725	outer membrane protein, cobalt-zinc-cadmium efflux system	2.15	0.001021	3460.2	3741.9	3480.2
K07267	porin	2.12	0.000376	925.09	682.26	662.93
K01082	3'(2'), 5'-bisphosphate nucleotidase	2.12	0.001162	2172.9	2281.7	2433.4
K01424	L-asparaginase	2.11	0.001774	2916.5	3096.5	2839.7
K06910	phosphatidylethanolamine-binding protein	2.10	0.003108	2963.7	3214.2	3097.8
K17763	rsbT co-antagonist protein	2.08	0.000353	818.81	1058.3	918.13
K07120	uncharacterized protein	2.06	0.002965	1579.7	1354.5	1487.7
K02346	DNA polymerase IV	2.03	0.001742	7515.5	7728.8	7530.6
K08151	MFS transporter, DHA1 family, tetracycline resistance protein	2.03	0.002842	1047.6	1238.9	1028.3
K05367	penicillin-binding protein 1C	2.02	0.000531	1651.7	1858.1	1706.5
K01198	xylan 1,4-beta-xylosidase	2.02	0.001074	454.72	660.66	510.21
K01051	pectinesterase	2.02	0.003145	388.87	594.82	458.24
K16089	outer membrane receptor for ferrienterochelin and colicins	2.01	0.001475	1207.2	1407.8	1226.6

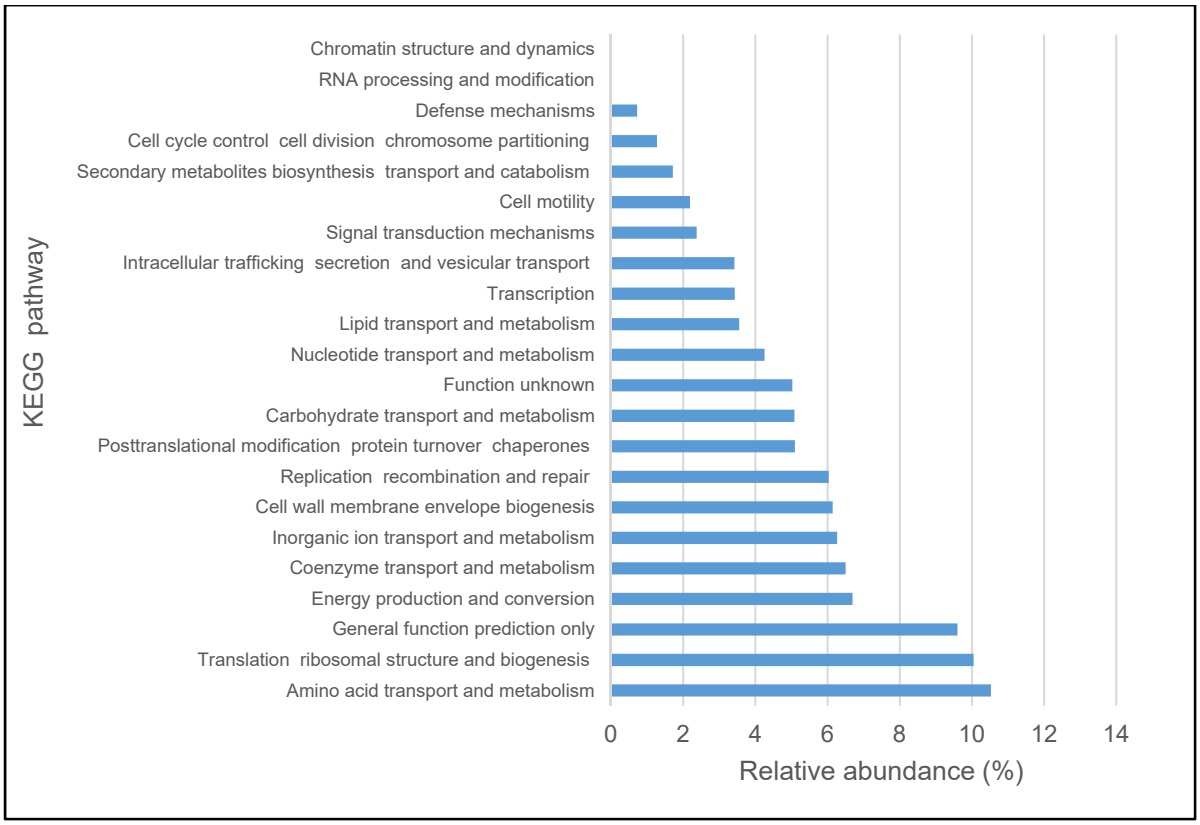

Fig. 5. The relative abundance of Kyoto Encyclopedia of Genes and Genomes (KEGG) functional categories identified via Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) predictions.

4. Discussion

Soil microbial diversity and abundance are important to the stability of soil ecosystems. To date, most microbial ecology studies of soil bacterial communities in organic cropping systems, specifically on the use of organic manure, have focused on diversity and composition (Hartmann et al., 2015; Lori et al., 2017; Lupatini et al., 2017; Liao et al., 2018; Amalytė et al., 2019), with little attention on the functional potential which remains largely unknown. The current study, a four-year long trial on the impact of different cropping systems and soil health treatments could not establish significant variation on alpha diversity similar to previous findings (Buckley and Schmidt, 2003).

Legacy effects of cropping systems or soil health treatment occurs in specific microbial groups and cannot be resolved by determining the diversity of the entire microbial community as shift in some groups might be compensated by shifts in others (Lupatini et al., 2017). Contrary to the observations on alpha diversity, microbial community composition (beta diversity) of the two cropping systems and their associated soil health treatments were distinct. The more dispersed communities observed in organic soil health treatments have been previously attributed to heterogenous habitat niches associated with the use of cattle farmyard manure-based compost and biological practices in organic cropping systems (Lupatini et al., 2017).

The present study further explored the impact of cropping system and soil health treatments on bacterial community composition and functional potential under field conditions. Soil microbes have been reported to sensitively respond to changes in the soil environment (Liu et al., 2019), hence, exploring distinct microbial taxa under different cropping systems and soil health treatments may reveal the ecological importance of predominant taxa. The higher abundance of Proteobacteria in conventional and Planctomycetes in organic cropping systems is consistent with previous reports (Lupatini et al., 2017). Inorganic fertilizer application creates a copiotrophic environment, which increases plant growth and carbon availability, favouring the growth of the Proteobacteria, while the addition of manure promotes Planctomycetes (Lupatini et al., 2017). Copiotrophic groups such as Proteobacteria have a fast growth rate and are likely to increase in nutrient rich conditions following nitrogen and phosphorous fertilizer application (Wang et al., 2018) as observed in the present study. On the other hand, Planctomycetes is a kind of anaerobic ammonium oxidizing bacteria which participates in

the soil carbon (C) and nitrogen (N) cycle, hence the higher relative abundance in organic cropping systems (Chen et al., 2017).

The presence of several differentially abundant taxa between cropping system provides ecological information on soil microbe responses to different agricultural management systems (Liao et al., 2019). The significant enrichment of genera DA101 (*Canditatus Udaebacter* copiosus), A17 and *Flavisolibacter* in conventional cropping systems was contrary to our expectation. These genera are mostly found in soils receiving elevated amounts of labile carbon inputs (Lian et al., 2017; Brewer et al., 2016). The application of inorganic N fertilizer in conventional cropping systems has been reported to indirectly enhance soil organic C storage through increase in plant biomass, which may promote copiotrophic taxa common in soils with high labile carbon pools (Tian et al., 2015) and associated mineralization rates (Yao et al., 2017).

Evaluation of the roles played by differentially expressed genera in organic cropping systems showed most to be associated with complex C metabolism including cellulose (*Cellulomonas* sp.) (Margulis and Chapman, 2009) and hemicellulose (*Methylibium* sp.) (Leung et al., 2016; Xia et al., 2019). While *Caulobacter* (Wilhelm, 2018), *Chthoniobacter* (Kant et al., 2011) and *Planctomycete* (Chen et al., 2016), are associated with general organic matter decomposition. In compost manure treated soils, the genus *Dokdonell* has been reported as the most dominant taxa similar to our findings (Chen et al., 2017). The dominance of pathogen controlling genera including *Sorangium*, a prolific producer of secondary antifungal or antibacterial by-products (Pradella et al., 2002), *Pedobacter*, a potential biocontrol agent (De Boer et al., 2007) may confer

significant advantages in the suppression of soil-borne bacterial and fungal pathogens in organic cropping systems (Song et al., 2017).

In conventional cropping systems, *Bacillus,* one of the significantly abundant genera, is a well characterized group, known to produce a broad range of antibiotics and is reportedly linked to pathogen suppression (Bais et al., 2004; Radhakrishnan et al., 2017). The dominance of *Bacillus* in conventional cropping systems together with other genera involved in N cycling including *Niastella* (N_2-generating denitrifier)*, Kribbella* (nitrate reduction), *Beijerinckia* (N fixation) appears to be greatly influenced by inorganic nitrogen fertilizer application (Hamamoto et al., 2018; Becking, 1961; Pitombo et al., 2016). The abundance of *Kribbella genus* has previously been reported to decrease with inorganic nitrogen fertilizers applications contrary to findings in this study (Kihara, 2017). In agreement to our findings, increased relative abundance of *Kribella* in the rhizosphere soil after nitrogen application which is useful to the growth of the bacteria was previously reported by Shang and Yi (2015).

Evaluation of expressed functional potential in conventional and organic cropping system including those associated with metabolic capabilities showed 27 differentially expressed KOs. No KOs were differentially expressed in soil health treatments belonging to the two cropping systems. Both conventional and organic cropping system had non niche specific capabilities (K01809- conventional and K01198- organic) to metabolize sugars, which are a primary soil metabolite, mostly availed from root exudates (Yurgel et al., 2019). Organic cropping systems were, moreover, characterized by high abundance of KOs involved in the starch and sucrose metabolism pathway which are important in global carbon cycling (Salam, 2018; Berlemont and Martiny, 2015) where it generates a

range of sugars as metabolites to fuel plant growth and synthesis of essential compounds such as cellulose (Ruan, 2014).

In this study KOs involved in alanine-aspartate-glutamate metabolism were observed in higher abundance in organic cropping systems. The predominance of alanine-aspartate-glutamate metabolism pathway in soils that received organic manure was previously reported by Tank et al. (2017) in cilantro and eggplant cultivation. The alanine-aspartate-glutamate metabolism pathway has been shown to be less abundant in roots under N stress (Sheflin et al., 2019). This pathway is vital in the metabolism of glutamate and glutamine, which are the first organic nitrogen compounds derived from the assimilation of nitrate and ammonium in plants (Kan et al., 2017). Glutamate is a functional amino acid that plays important roles in plant nutrition, metabolism, and signal transduction (Kan et al., 2017) and occupies a central position in the amino acid metabolism. Thus, the KOs involved in alanine-aspartate-glutamate metabolism pathway, have the potential of being used as biomarkers for early warning of soil glutamate levels depletion.

In conclusion, this study showed that although bacterial communities in conventional cropping systems may be as diverse as those in organic systems, they have significantly different taxa abundances associated with carbon and nitrogen cycling. Bacterial genera involved in nitrogen cycling were higher in conventional cropping systems while the organic cropping systems were dominated by genera involved in carbon cycling. The increased abundance of predicted metabolic functions (KOs) involved in carbohydrate and amino acid metabolism in the organic cropping systems over conventional cropping systems, indicated niche-specific functions, providing a better

understanding of the overall crop soil microbiome metabolism and its functional specialization in the two production systems. Future studies, should therefore, focus on establishing whether the observed differences in the two cropping systems may be translated into extended capacity to adapt to climate change over a long period, both in small and commercial farming systems.

Author Contributions

Authors were involved in different stages of the experiment. M.B did the soil sampling and DNA extraction. Statistical analysis, data analysis, structuring and interpretation of the paper was done by M.B and L.C. Further interpretation and editing was done by J.G. and L.K. while N.L. supervised the project and corrected the final version of the manuscript. All authors read and approved the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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