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The Effect of Tillage System and Crop Rotation on Soil Microbial Diversity and Composition in a Subtropical Acrisol

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Abstract: Agricultural management alters physical and chemical soil properties, which directly affects microbial life strategies and community composition. The microbial community drives important nutrient cycling processes that can influence soil quality, cropping productivity and environmental sustainability. In this research, a long-term agricultural experiment in a subtropical Acrisol was studied in south Brazil. The plots at this site represent two tillage systems, two nitrogen fertilization regimes and three crop rotation systems. Using Illumina high-throughput sequencing of the 16S rRNA gene, the archaeal and bacterial composition was determined from phylum to species level in the different plot treatments. The relative abundance of these taxes was correlated with measured soil properties. The P, Mg, total organic carbon, total N and mineral N were significantly higher in the no-tillage system. The microbial diversity was higher in the no-tillage system at order, family, genus and species level. In addition, overall microbial composition changed significantly between conventional tillage and no-tillage systems. Anaerobic bacteria, such as clostridia, dominate in no-tilled soil as well as anaerobic methanogenic archaea, which were detected only in the no-tillage system. Microbial diversity was higher in plots in which only cereals (oat and maize) were grown. Soil management influenced soil biodiversity on Acrisol by change of composition and abundance of individual species.

1. Introduction

Agricultural management affects soil chemical, physical and biological features. Tillage typically involves complete removal of vegetation followed by plantings often designed to completely switch the crop cover from the previous year and this process increases soil erosion and degradation, which triggers many changes in the soil community composition [1–5]. Tillage also results in nitrous oxide (N_2O) and carbon dioxide (CO_2) emission to the atmosphere [6,7]. When the soil is not tilled, these gases remain stored within soil pores, which significantly reduce field emissions of these greenhouse gases [8].

No-tillage is a sustainable cropping management system that protects soil, water, air, and biodiversity [5,9,10], and authors have shown that even a reduction in tillage leads to increased microbial activity and biomass in contrast to surface soil under conventional tillage [11,12]. Agricultural areas under no-tillage systems are increasing worldwide largely as result of improved soil conservation efforts. In recent years, the United States, Brazil, and Argentina have converted about 26, 25 and 20 million hectares, respectively, into no-tillage cropping systems [13]. The residues left on soil after harvest increase soil organic matter (SOM), conserve soil moisture, and reduce soil temperature in summer [14]. Feng [12] found that a no-till management system led to significantly higher soil organic carbon (SOC) and microbial biomass in the soil surface compared to the conventional tillage treatment. Wang [14] found significant increases in soil N, organic C and SOM fraction with no-tillage system, while conventional tillage had deleterious impact on soil microbial biomass and also reduced SOC.

In conventional tillage and no-tillage systems, nitrogen (N) fertilization and crop rotations are also factors that can modify soil quality and fertility thereby affecting microbial community composition. Recent studies have demonstrated that microbial community composition changes with N fertilizer. Fierer [4] showed that N addition can result in a shift of microbial composition from oligotrophic to copiotrophic bacteria. Lu [15] showed that all nitrogen cycle processes increased with N addition. The crops rotation where plants predominate that are succulent and rich in proteins and sugars such as legumes release nutrients more quickly, whereas plants that are more fibrous, such as grasses and cereals, release nutrients more slowly, but promote more stable organic matter [16]. Systems that increase inputs of C and N through the inclusion of legumes or fibrous rooted crops in the crop rotation, may increase microbial populations and activities. After harvest, the cover crop residues that are left on the soil improve soil fertility, since they release nutrients to soil. Conventional tillage releases the nutrients more quickly because the residues are crushed and mechanically incorporated to soil, which stimulates microorganisms that degrade organic matter. In no-tillage conditions, the nutrient releasing is progressive, since the residues remain on the soil surface and therefore decomposition is slower. The crop rotation composition and the tillage management, together, determine the soil C:N ratio, which has significant effect on microbial composition [17], crop residue decomposition, and crop nutrient cycling.

The aim of this study was to understand how microbial diversity and composition change with agricultural management, as well as, to find relationships between microorganism's taxa and soil features. High-throughput sequencing of the 16S rRNA gene was used to determine how microbial diversity and composition changes in response to tillage, crop rotation, and N fertilization. The sequencing data was used to measure the relative abundance of operational taxonomic units (OTUs) and to calculate the Shannon diversity index (SDI) of soil samples. These data were then correlated with several measurable properties of soil to reveal any relationships.

2. Results

In total, 1,364,311 reads were obtained from Illumina sequencing after trimming, with 609,664 from tillage and 754,647 from no-tillage plots. The Bacteria and Archaea discovered in this work were distributed among 30 phyla (classified reads = 66%), 45 classes (classified reads = 60%), 99 orders (classified reads = 55%), 241 families (classified reads = 53%), 986 genera (classified reads = 45%) and 4,622 species (classified reads = 25%).

The SDI of the samples was used to determine how microbial diversity changed with different treatments (Table 1), also considering clustered unclassified reads.

Table 1. Shannon diversity index to phylum, class, order, family, genus and species level, for each treatment. Numbers followed by the same letters on the columns, are not different by Tukey test, under *p*-value ≤ 0.01. The numbers in bold, indicate the highest significant SDI values from each taxonomy level.

	Plylum		Class		Order		Family		Genus		Species	
Tillage, no N (oat+vetch/maize+cowpea)	0.16	abc	3.48	b	3.59	abc	3.68	abc	5.29	bcd	5.29	bcd
Tillage, 180 kg N/ha (oat+vetch/maize+cowpea)	0.08	cd	3.43	b	3.45	c	3.47	bc	5.20	cd	5.20	cd
Tillage, no N (oat/maize)	0.21	ab	**3.87**	**a**	3.93	ab	3.93	ab	5.73	ab	5.73	ab
Tillage, 180 kg N/ha (oat/maize)	**0.23**	**a**	3.68	ab	3.73	abc	3.76	abc	5.53	abc	5.53	abc
Tillage, no N (vetch/maize)	0.17	abc	3.64	ab	3.62	abc	3.67	abc	5.33	abcd	5.33	abcd
Tillage,180 kg N/ha (vetch/maize)	0.16	abc	3.53	ab	3.58	bc	3.62	abc	5.21	bcd	5.21	bcd
No tillage, no N (oat+vetch/maize+cowpea)	0.02	d	3.43	b	3.42	c	3.54	abc	4.89	d	4.89	d
No tillage, 180 kg N/ha (oat+vetch/maize+cowpea)	0.14	abc	3.54	ab	3.57	bc	3.64	abc	5.29	bcd	5.29	bcd
No tillage, no N (oat/maize)	0.13	bc	**3.88**	**a**	**3.96**	**a**	3.94	ab	**5.85**	**a**	**5.85**	**a**
No tillage, 180 kg N/ha (oat/maize)	0.18	abc	3.77	ab	3.89	ab	**3.98**	**a**	5.59	abc	5.59	abc
No tillage, no N (vetch/maize)	0.19	ab	3.74	ab	3.73	abc	3.72	abc	5.45	abc	5.45	abc
No tillage, 180 kg N/ha (vetch/maize)	0.16	abc	3.43	b	3.40	c	3.42	c	5.11	cd	5.11	cd

At the phylum level, the SDI was highest in the samples that experienced conventional tillage fertilized with 180 kg N ha^{-1}, and cultivated with cereal only (oats/maize). Although one sample had undergone conventional tillage and another sample no tillage, at the class level, the SDI was highest in the two samples in which no N was added and only cereals were cultivated. At the order, genus and species levels, SDI was highest in the sample from a no-tillage system, without N, and only cereal

cultivation. At the family level, the SDI was highest in a sample from a plot with no-tillage, 180 kg N ha^{-1} and only cereal cultivation.

P content in samples from no tillage system were two-fold higher than in conventional tillage (Table 2). Also Mg, TOC, total N and mineral N were significantly higher in no tillage, indicating an increase in soil fertility within this system. Considering the crop rotations, pH and P content was higher where cereals were grown all year (oat and maize). TOC, total N and mineral N were significantly higher where cereals and legumes were grown together in the summer and winter successively. P, Ca, and Mg were significantly higher where no N fertilization was added, and Al and mineral N was higher where 180 kg N ha^{-1} were added.

Table 2. Soil features measured on samples from the different treatments. Asterisk indicates significant difference between the correspondent treatments on the line, by qui-square test. Numbers followed by the same letter are not statistically different by Tukey test under *p*-value ≤ 0.01.

|	Soil Tillage System		Crop System			N Fertilization Level	
	Tillage	**No Tillage**	**O+V/M+C**	**O/M**	**M/O**	**0 kg N ha^{-1}**	**180 kg N ha^{-1}**
pH	5.2	5.2	5.0 b	5.6 a	4.9 b	5.4	5.0
P (mg dm^{-3})	13.7	29.1*	21.1 b	27.8 a	17.6 b	25.7*	19.4
K(mg dm^{-3})	161	156	157	166	150	162	155
Al (cmol$_c$ dm^{-3})	0.5	0.5	0.6 b	0.1 a	0.9 b	0.3	0.7*
Ca (cmol$_c$ dm^{-3})	2.3	2.6	2.5	2.9	2.1	2.9*	2.2
Mg (cmol$_c$ dm^{-3})	1.2	2.6*	1.4	1.5	1.2	1.5*	1.2
TOC (%)	1.17	1.52*	1.54 a	1.20 b	1.37 b	1.32	1.38
Total N (%)	0.096	0.127*	0.127 a	0.099 c	0.11 b	0.110	0.114
Mineral N (mg kg^{-1})	5.24	6.90*	8.66 a	2.48 c	7.29 b	5.12	6.58*

Among the phyla, *Proteobacteria* was the most abundant and was well represented in all treatments (Figure 1). *Verrucomicrobia, Firmicutes, Crenarchaeota, Chlamydiae, Euryarchaeota*, and *Chlorobi* were significantly more abundant in samples from no-tillage systems (Figure 1). On the other hand, *Gemmatimonadetes, Cyanobacteria,* and *Deinococcus-Thermus,* were significantly more abundant in the samples from tilled systems (Figure 1). Among the archaeal phyla detected, Crenarchaeota, Thaumarchaeota and Euryarchaeota presented low relative abundance (less than 0.7%), but were significantly more abundant in the no-tillage system.

The 20 most abundant genera for both treatments were analyzed and the differences were represented in a heatmap (Figure 2). The relative abundances of *Sphingomonas*, *Candidatus* Koribacter, *Flavisolibacter*, *Burkholderia*, *Phenylobacterium*, *Streptomyces, Mycobacterium and Mucilaginibacter* were significantly higher in conventional tillage system. On the other hand, *Clostridium, Geobacter*, *Rhodoplanes, Duganella and Sphingobacterium* were significant higher in no tillage samples. Also, a range of archaeal methanogens, such as *Methanobacterium, Methanolinea, Methanomethylovorans, Methanoregula* and *Methanosarcina* (both anaerobe, *Euryarchaeota*), were found in no-tillage samples that were not detected in samples from the tilled system (data not shown).

Among the rotation systems, *Clostridium, Bacillus, Burkholderia, Bradyrhizobium*, and *Phenylobacterium* were relatively more abundant in samples from those crop rotations that included a

mix of legumes and cereals during all the year (Figure 2). In the cereal/legume plots, *Sphingomonas, Ca.* Koribacter and *Ca.* Solibacter were significantly more abundant than in the other two crop rotation systems. *Geobacter* was less abundant in legume/cereal rotation.

Figure 1. Relative abundance of 16S rRNA gene (% of total reads) to phylum level, in an Acrisol under conventional tillage and no-tillage soil management. Asterisk expresses that the average differs statistically by Tukey test, at p-value ≤ 0,01. The archaeal phyla are underlined.
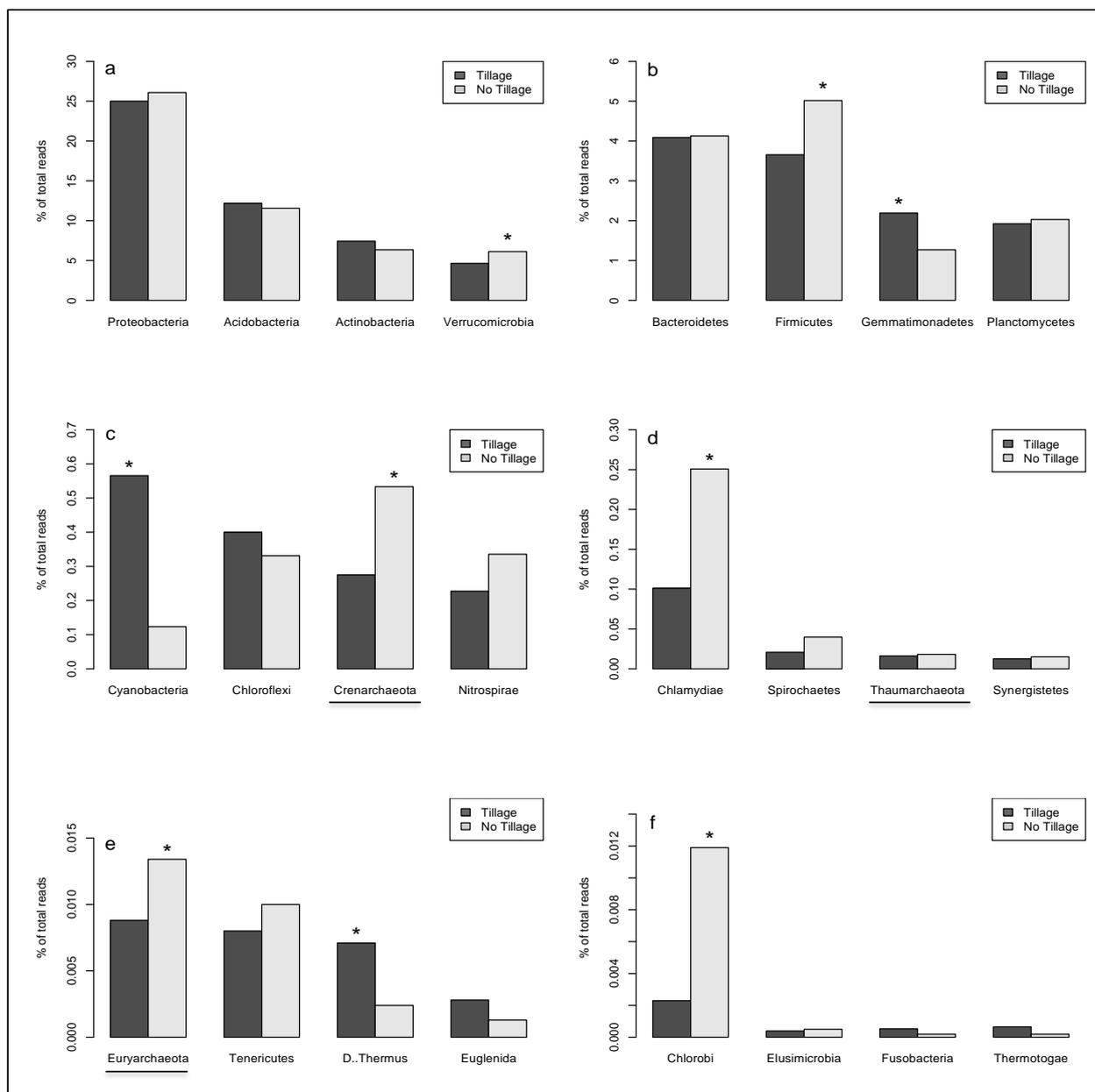
Clostridium, Bacillus, *Burkholderia, Bradyrhizobium* and *Paenibacillus* (nitrogen fixers) were significantly higher in plots with no added N, compared to those with added N. *Ca.* Koribacter, *Opitutus, Nitrospira* and *Mucilaginibacter* relative abundances were higher in those plots with added N (Figure 2).

Figure 2. Heatmap illustrating the 20 most abundant genera, in the three treatments. Different gradient color on the line represents significant difference (*p*-value ≤ 0.01) by Tukey test.
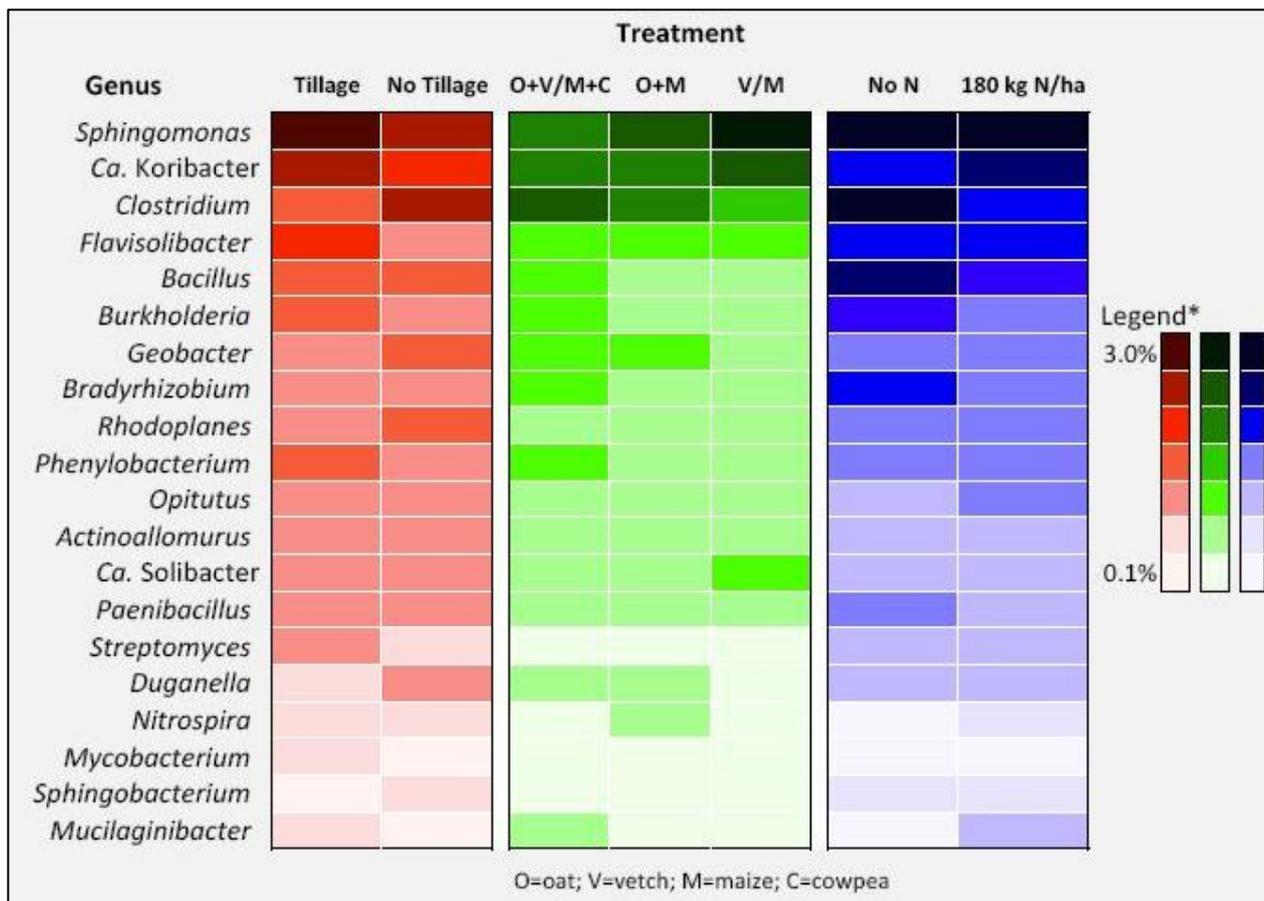
* Relative abundance of 16S rRNA gene (% of total reads).

The most abundant UTUs from each taxonomic level were correlated with tillage system and with chemical soil features (pH, TOC, Ca, Mg, P, K and Al) by Spearman correlation with a significance level of 0.1 (α < 0.001), and a correlation matrix was created. Only those taxa that represented at least 0.05% of total reads (using an average of 6 replicates from each OTU) were used to calculate Spearman correlation. Just those values with a significant rho above 0.5 are shown here (Tables 3 and 4). Using this approach, many taxa were found to be correlated with either the tilled or no-till systems. In the no tillage system, the relative abundance of Phylum Verrucomicrobia, the family *Verrucomicrobia* subdivision 3, as well as Proteobacteria, represented by families *Xanthomonadaceae* and *Hypomicrobiaceae (Rhodoplanes),* were strongly correlated with no-till (Table 3). *Chlamydiae*, and *Bacterioidetes* represented by *Terrimonas*, were also strongly correlated with no-tillage system.

The conventional tillage system was highly correlated with relative abundance *Streptomycetaceae*, *Geodermatophilaceae, Intrasporangiaceae, Kineosporiaceae* and *Micrococcaceae* families (Table 3). From *Bacteroidetes* phylum, the genera *Sphingomonas, Pedobacter* and *Flavisolibacter* were highly correlated with conventional tillage. Gemmatimonadetes phylum, and the genera *Herbaspirillum* and *Ramlibacter* also correlated with conventional tillage.

The genus *Anaeromyxobacter* (order *Myxococcales,* Class Deltaproteobacteria), was highly positively correlated with pH (Table 4). In contrast, *Acidobacteria,* represented by *Acidobacteriaceae* and *Solibacteraceae* families, were negatively correlated with pH indicating that these microorganisms may grow in low pH.

Phenylobacterium zucineum was highly positively correlated with Al concentration (Table 4). Also, Actinobacteria and Acidobacteria (*Acidobacteriaceae* and *Solibacteraceae*) were correlated positively with Al concentration on soil. The genus *Anaeromyxobacter* was negatively correlated with Al.

Firmicutes phylum relative abundance, represented by *Clostridium beijerinckii* from the *Clostridiales* order, correlated positively with Ca and Mg and also with the no tillage system (Table 4). These divalent cations were present at higher levels in a no-tillage system. No organisms were negatively correlated with these two elements in soil.

Phyla Verrucomicrobia, and Firmicutes represented by *Clostridium* genus, correlated positively with soil P concentration (Table 4). *Clostridium* was also higher in no-tillage (Table 1). No-tillage plots had more than twice the level of P than conventional tillage plots (Table 2) supporting the relationship between P, soil management practices and *Clostridium* abundance. Also, *Geobacter* sp. (from Proteobacteria) correlated positively with P. *Desulforomonadales* from *Deltaproteobacteria* and *Rhodocyclales* from *Betaproteobacteria* correlated positively with P. *Actinobacteria* representatives, such as *Intrasporangiaceae*, *Micrococcaceae* and *Geodermatophilaceae*, as well as *Sphingomonas* from *Alphaproteobacteria* and *Mucilaginobacter* from *Bacteroidetes* correlated negatively with P and also with tillage.

Table 3. Spearman correlations (*p*-value ≤ 0.01) of the most correlated organisms with conventional tillage and no-tillage. The correlated microorganisms are within the colorful boxes. The names in gray color represent the correlated microorganism's related taxa.

Phylum	Class	Order	Family	Genus	Species	Tillage	No tillage
Chlamydiae						**−0.87**	**0.87**
Chlamydiae	*Chlamidiia*	*Chlamydiales*				**−0.82**	**0.82**
Verrucomicrobia						**−0.82**	**0.82**
Verrucomicrobia	*Verrucomicrobiae*					**−0.82**	**0.82**
Verrucomicrobia	*Verrucomicrobiae*	*Verrucomicrobiales*				**−0.82**	**0.82**
Verrucomicrobia	*Verrucomicrobiae*	*Verrucomicrobiales*	*Verrucomicrobia sub 3*	unclassified	*Ellin5121*	−0.72	**0.72**
Verrucomicrobia	*Verrucomicrobiae*	*Verrucomicrobiales*	*Verrucomicrobia sub 3*	unclassified	*Ellin515*	−0.72	**0.72**
Verrucomicrobia	*Verrucomicrobiae*	*Verrucomicrobiales*	*Verrucomicrobia sub 3*	unclassified	*Ellin518*	−0.72	**0.72**
Verrucomicrobia	*Verrucomicrobiae*	*Verrucomicrobiales*	*Verrucomicrobia sub 3*	unclassified	*Ellin5102*	**−0.77**	**0.77**
Proteobacteria	*Gamaproteobacteria*	*Xanthomonadales*	*Xanthomonadaceae*			**−0.82**	**0.82**
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*	*Hyphomicrobiaceae*			**−0.82**	**0.82**
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*	*Hyphomicrobiaceae*	*Rhodoplanes*		**−0.87**	**0.87**
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*	*Hyphomicrobiaceae*	*Rhodoplanes*	*Rhodoplanes sp.*	**−0.77**	**0.77**
Bacteroidetes	*Sphingobacteriia*	*Sphingobacteriales*	*Chitinophagaceae*	*Terrimonas*		**−0.77**	**0.77**
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Streptomycetaceae*			**0.82**	**−0.82**
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Streptomycetaceae*	*Streptomyces*		**0.87**	**−0.87**
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Streptomycetaceae*	*Kitasatospora*		**0.72**	−0.72
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Pseudonocardiaceae*	*Amycolatopsis*		**0.87**	**−0.87**
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Geodermatophilaceae*			**0.87**	**−0.87**
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Geodermatophilaceae*	*Modestobacter*	*Modestobacter sp.*	**0.87**	**−0.87**
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Intrasporangiaceae*			**0.87**	**−0.87**
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Kineosporiaceae*			**0.87**	**−0.87**
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Micrococcaceae*			**0.87**	**−0.87**

Table 3. *Cont.*

Phylum	Class	Order	Family	Genus	Species	Tillage	No tillage
Bacteroidetes	*Sphingobacteriia*	*Sphingomonadales*				**0.77**	**−0.77**
Bacteroidetes	*Sphingobacteriia*	*Sphingomonadales*	*Sphingomonadaceae*			**0.77**	**−0.77**
Bacteroidetes	*Sphingobacteriia*	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*		**0.77**	**−0.77**
Bacteroidetes	*Sphingobacteriia*	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*	*Sphingomonas sp.*	**0.77**	**−0.77**
Bacteroidetes	*Sphingobacteriia*	*Sphingobacteriales*	*Sphingobacteriaceae*	*Mucilaginibacter*	*M. ximonensis*	**0.82**	**−0.82**
Bacteroidetes	*Sphingobacteriia*	*Sphingobacteriales*	*Sphingobacteriaceae*	*Pedobacter*		**0.72**	−0.72
Bacteroidetes	*Sphingobacteriia*	*Sphingobacteriales*	*Chitinophagaceae*	*Flavisolibacter*		**0.87**	**−0.87**
Gemmatimonadetes						**0.77**	**−0.77**
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Oxalobacteraceae*	*Herbaspirillum*		**0.77**	**−0.77**
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Oxalobacteraceae*	*Herbaspirillum*	*Herbaspirillum sp.*	**0.87**	**−0.87**
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Ramlibacter*		**0.72**	−0.72
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Ramlibacter*	*Ramlibacter sp.*	**0.72**	−0.72

Table 4. Spearman correlations (p ≤ 0.01) of the most correlated organisms with phosphorus (P), potassium (K), aluminum (Al), calcium (Ca) and magnesium (Mg). The correlated microorganisms are within the colorful boxes. The names in gray color represent the correlated microorganism's related taxa.

Phylum	Class	Order	Family	Genus	Species	pH	P	K	Al	Ca	Mg
Proteobacteria	*Deltaproteobacteria*	*Myxococcales*				**0.73**	0.36	0.39	**−0.82**	0.70	0.62
Proteobacteria	*Deltaproteobacteria*	*Myxococcales*	*Myxococcaceae*			**0.83**	0.60	0.29	**−0.94**	**0.82**	**0.75**
Proteobacteria	*Deltaproteobacteria*	*Myxococcales*	*Myxococcaceae*	*Anaeromyxobacter*		**0.79**	0.51	0.31	**−0.90**	**0.75**	0.66
Proteobacteria	*Betaproteobacteria*					**0.74**	0.46	0.57	**−0.80**	**0.77**	0.68

Table 4. *Cont.*

Phylum	Class	Order	Family	Genus	Species	pH	P	K	Al	Ca	Mg
Acidobacteria	*Acidobacteriia*	*Acidobacteriales*	*Acidobacteriaceae*	unclassified	*Ellin5017*	**−0.77**	−0.48	−0.46	**0.85**	**−0.91**	**−0.86**
Acidobacteria	*Solibacteres*	*Solibacterales*	*Solibacteraceae*	*Ca.* Solibacter		**−0.75**	−0.53	−0.19	**0.85**	**−0.75**	−0.70
Proteobacteria	*Betaproteobacteria*					**0.74**	0.46	0.57	**−0.80**	0.77	0.68
Proteobacteria	*Deltaproteobacteria*	*Desulfuromonadales*				0.62	**0.74**	0.11	**−0.76**	0.71	**0.73**
Proteobacteria	*Deltaproteobacteria*	*Myxococcales*				**0.73**	0.36	0.39	**−0.82**	0.70	0.62
Proteobacteria	*Deltaproteobacteria*	*Myxococcales*	*Myxococcaceae*			**0.83**	0.60	0.29	**−0.94**	**0.82**	**0.75**
Proteobacteria	*Deltaproteobacteria*	*Myxococcales*	*Myxococcaceae*	*Anaeromyxobacter*		**0.79**	0.51	0.31	**−0.90**	0.75	0.66
Proteobacteria	*Alphaproteobacteria*	*Caulobacterales*	*Caulobacteraceae*	*Phenylobacterium*	*P zucineum*	−0.67	−0.62	−0.18	**0.79**	**−0.81**	**−0.79**
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Catenulisporaceae*	*Catenulispora*		−0.63	−0.63	−0.22	**0.78**	**−0.82**	**−0.82**
Acidobacteria	*Acidobacteriia*	*Acidobacteriales*				−0.70	−0.33	−0.19	**0.76**	−0.69	−0.61
Acidobacteria	*Acidobacteriia*	*Acidobacteriales*	*Acidobacteriaceae*			−0.70	−0.33	−0.19	**0.76**	−0.69	−0.61
Acidobacteria	*Acidobacteriia*	*Acidobacteriales*	*Acidobacteriaceae*	unclassified	*Ellin5017*	−0.77	−0.48	−0.46	**0.85**	−0.91	−0.86
Acidobacteria	*Acidobacteriia*	*Acidobacteriales*	*Acidobacteriaceae*	unclassified	*Ellin5056*	−0.70	−0.66	−0.15	**0.80**	−0.67	−0.63
Acidobacteria	*Acidobacteriia*	*Acidobacteriales*	*Acidobacteriaceae*	unclassified	*Ellin5237*	−0.71	−0.38	−0.50	**0.77**	−0.80	−0.76
Acidobacteria	*Acidobacteriia*	*Solibacterales*				−0.68	−0.57	−0.13	**0.80**	**−0.74**	−0.71
Acidobacteria	*Acidobacteriia*	*Solibacterales*	*Solibacteraceae*			−0.68	−0.57	−0.13	**0.80**	**−0.74**	−0.71
Acidobacteria	*Acidobacteriia*	*Solibacterales*	*Solibacteraceae*	*Ca.* Solibacter		**−0.75**	−0.53	−0.19	**0.85**	**−0.75**	−0.70
Acidobacteria	unclassified	unclassified	unclassified	*Ca.* Koribacter	*Ca.* K. versatilis	−0.58	−0.69	−0.23	**0.73**	**−0.77**	**−0.83**
Firmicutes						0.50	**0.73**	0.31	−0.56	**0.77**	**0.85**
Firmicutes	*Clostridia*	*Clostridiales*				0.57	0.70	0.40	−0.61	**0.75**	**0.81**
Firmicutes	*Clostridia*	*Clostridiales*	*Clostridiaceae*			0.54	**0.73**	0.37	−0.59	0.72	**0.79**
Firmicutes	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium*		0.54	**0.73**	0.37	−0.59	0.72	**0.79**
Firmicutes	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium*	*C. beijerinckii*	0.59	0.53	0.42	−0.61	**0.77**	**0.78**
Firmicutes	*Bacilli*	*Bacillales*	*Paenibacillaceae*			0.52	0.31	0.49	−0.53	**0.77**	**0.77**
Proteobacteria	*Betaproteobacteria*					**0.74**	0.46	0.57	**−0.80**	0.77	0.68
Proteobacteria	*Betaproteobacteria*	Rhodocyclales				**0.57**	0.73	0.27	**−0.73**	0.70	0.76
Proteobacteria	*Betaproteobacteria*	Rhodocyclales	Rhodocyclaceae			**0.57**	0.73	0.27	**−0.73**	0.70	0.76
Proteobacteria	*Deltaproteobacteria*	Desulfuromonadales				**0.62**	0.74	0.11	**−0.76**	0.71	0.73

Table 4. *Cont.*

Phylum	Class	Order	Family	Genus	Species	pH	P	K	Al	Ca	Mg
Proteobacteria	*Deltaproteobacteria*	*Myxococcales*	*Myxococcaceae*			**0.83**	0.60	0.29	**−0.94**	**0.82**	**0.75**
Proteobacteria	*Deltaproteobacteria*	*Myxococcales*	*Myxococcaceae*	*Anaeromyxobacter*		**0.79**	0.51	0.31	**−0.90**	**0.75**	0.66
Verrucomicrobia						0.17	**0.78**	0.16	−0.28	0.49	0.66
Verrucomicrobia	*Verrucomicrobiae*					−0.38	**0.81**	0.01	0.54	−0.56	−0.67
Proteobacteria	*Deltaproteobacteria*	*Desulfuromonadales*				0.62	**0.74**	0.11	**−0.76**	0.71	**0.73**
Proteobacteria	*Deltaproteobacteria*	*Desulfuromonadales*	*Geobacteraceae*	*Geobacter*	*G. bemidjiensis*	0.51	**0.78**	0.03	−0.68	0.67	0.72
Proteobacteria	*Deltaproteobacteria*	*Desulfuromonadales*	*Geobacteraceae*	*Geobacter*	*Geobacter sp.*	0.43	**0.76**	−0.10	−0.61	0.59	0.64
Proteobacteria	*Alphaproteobacteria*	*Caulobacterales*	*Caulobacteraceae*	*Asticcacaulis*		−0.57	**−0.81**	−0.18	0.70	**−0.81**	**−0.88**
Proteobacteria	*Alphaproteobacteria*	*Sphingomonadales*				−0.19	**−0.75**	0.16	0.25	−0.26	−0.37
Proteobacteria	*Alphaproteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*			−0.21	**−0.76**	0.11	0.27	−0.27	−0.38
Proteobacteria	*Alphaproteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*		−0.19	**−0.75**	0.16	0.25	−0.26	−0.37
Proteobacteria	*Alphaproteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*	*Sphingomonas sp.*	−0.19	**−0.75**	0.16	0.25	−0.26	−0.37
Proteobacteria	*Betaproteobacteria*	*Rhodocyclales*				0.57	**0.73**	0.27	−0.73	0.70	**0.76**
Proteobacteria	*Betaproteobacteria*	*Rhodocyclales*	*Rhodocyclaceae*			0.57	**0.73**	0.27	−0.73	0.70	**0.76**
Firmicutes						0.50	**0.73**	0.31	−0.56	**0.77**	**0.85**
Firmicutes	*Clostridia*	*Clostridiales*	*Clostridiaceae*			0.54	**0.73**	0.37	−0.59	0.72	**0.79**
Firmicutes	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium*		0.54	**0.73**	0.37	−0.59	0.72	**0.79**
Firmicutes	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium*	*C. magnum*	−0.50	**−0.76**	−0.22	0.63	**−0.76**	**−0.87**
Bacteroidetes	*Sphingobacteriia*	*Sphingobacteriales*	*Sphingobacteriaceae*	*Mucilaginibacter*	*M. ximonensis*	−0.27	**−0.83**	−0.23	0.31	−0.51	−0.68
Actinobacteria						−0.36	**−0.75**	−0.11	0.51	−0.62	−0.72
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Intrasporangiaceae*			−0.05	**−0.76**	−0.18	0.15	−0.29	−0.48
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Micrococcaceae*			−0.26	**−0.85**	−0.09	0.36	−0.42	−0.57
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Geodermatophilaceae*			−0.12	**−0.81**	−0.24	0.25	−0.41	−0.60
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Streptomycetaceae*	*Streptomyces*		−0.03	**−0.76**	0.27	0.15	−0.22	−0.39
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Pseudonocardiaceae*	*Amycolatopsis*		−0.07	**−0.75**	0.11	0.20	−0.30	−0.49

Principle components analysis (PCA) was also done to identify relationships among the 30 most abundant families and genera with soil parameters and experimental treatments (Figure 3). The tilled system was clustered with *Geodermatophilaceae, Sphingomonadaceae* and *Streptomycetaceae* (Figure 3 a). No-till samples clustered with *Clostridiaceae*, *Micromonosporaceae, Rhodospirillaceae, Verrucomicrobia* and *Hypomicrobiaceae*. Sites with low N or with no N fertilization were positively correlated with *Comonadaceae* and *Paenibacillaceae*. *Sphingobacteriaceae, Acetobacteraceae, Caulobacteraceae* and *Thermomonosporaceae* clustered with 180 kg N ha^{-1} treatment.

Figure 3. Principal component analysis (correlation-based PCA), between the 30 most abundant families (a), and genera (b). The treatments were used as supplementary variables.
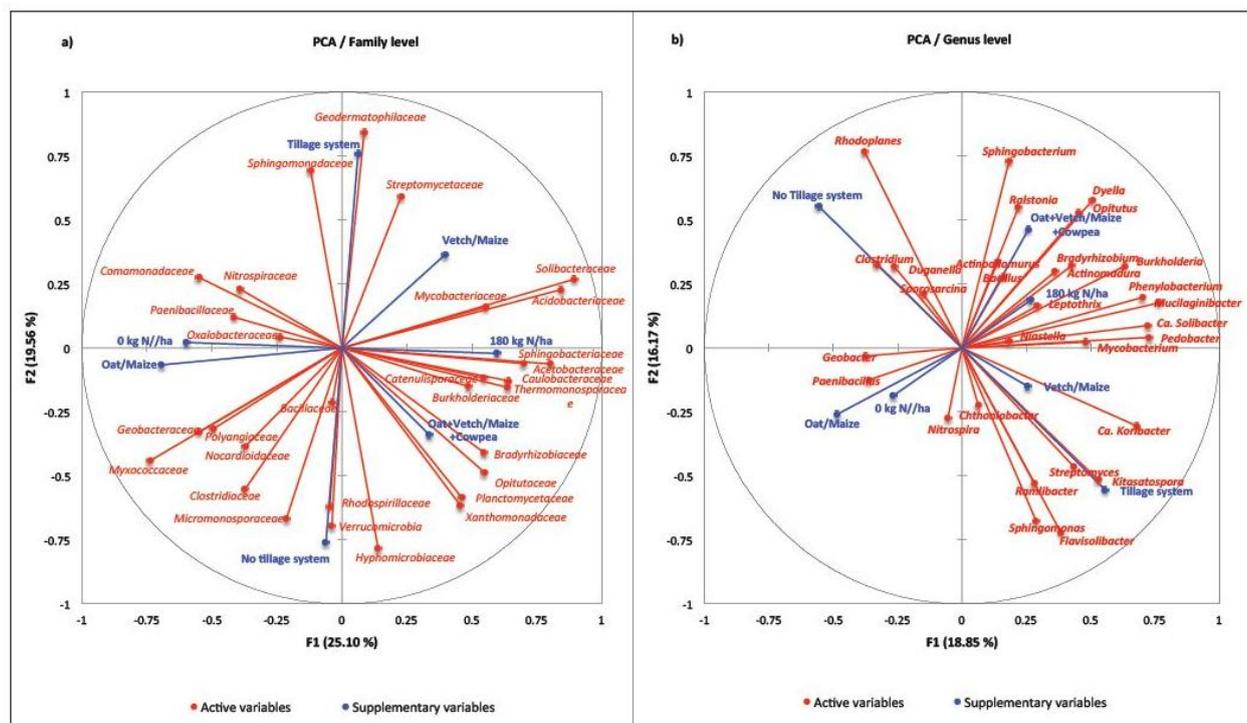
Among the 30 most abundant genera, the tilled system, in some way, clustered with *Kisatospora, Streptomyces, Ramlibacter, Sphingomonas* and *Flavisolibacter* while the no-tillage system clustered with *Rhodoplanes* and *Clostridium* abundance (Figure 3 b). The cropping system that included cereals and legumes in winter and in summer, clustered with *Dyella, Opitutus, Sphingobacterium, Ralstonia, Bradyrhizobium* and *Actinomadura*.

The genera *Actinoallamurus, Paenibacillus, Nitrospira, Chthoniobacter, Sporosarcina* and *Niastella* have shown weak contributions on the PCA (see short arrows on Figure 3 b) suggesting that they may be more ubiquitous than other organisms and adapt well in a range of environments and do not require specific conditions to grow.

3. Discussion

This work used 16S rRNA barcoded Illumina sequencing to describe soil microbial diversity and composition from phylum to species level, as well as correlating the microbial composition with the soil features. The soil was sampled from plots located on a Brazilian agricultural long-term research

experimental site, and this research is the first sequence-based approach of the local microbial community.

The microbial diversity, measured by the Shannon diversity index (SDI), was significantly higher in samples from no-tillage system plots in four taxonomic levels (order, family, genus and species), which agree with Ceja-Navaro [18], who found that soils under no-tillage had the highest levels of microbial diversity compared to the conventional tillage system. Also, at all taxonomic levels, SDI was significantly higher when oat and maize were cultivated all year without legumes, indicating that the crop system based on cereals, with higher C:N ratio, stimulates microbial diversity on the soil. Vargas [19] studying this same long-term experiment site, identified greater microbial biomass in no-tillage systems and determined the increased biomass was related to the C:N ratio. A high C:N ratio stimulates the microbial community to degrade organic substrate thereby increasing determined microbial population. C:N ratio is higher in cereals straw substrates [20,21], which provides more substrate to microorganisms explaining the highest SDI found in this work on plots where uniquely cereals (oat and maize) were grown throughout the year. Besides crops and C:N ratio, root exudates are also key factors that influence microbial communities, as they provide a carbon source to soil microorganisms [22,23]. Through the exudation of a wide variety of compounds, roots may regulate the soil microbial community in their immediate vicinity, stimulate beneficial symbioses, change the chemical and physical properties of the soil, and inhibit the growth of competing plant species [24], which may also determine the microbial diversity around rhizosphere.

This study revealed significant microbial community changes between tillage systems, such as predominance of anaerobes in no tillage, as well as, presence of resilient microorganisms in tillage system, that are usually is found in extreme environments. In a conventional tillage system, annual soil tilling increases total aeration capacity up to 20 cm deeper compared with no-tillage system [25]. This process is expected to stimulate the relative abundance of aerobic microorganisms. The results in this study confirmed this expectation, as aerobic microorganisms from the phyla Deinococcus-Thermus, Gemmatimonadetes, and Cyanobacteria, were significantly more abundant on conventional tillage. Deinococcus-Thermus were two-fold more abundant in tillage than in no-tillage. This phylum includes many species that are resistant to environmental hazards, to ionizing radiation, ultraviolet light (UV) and desiccation [26], and this phylum has been observed in soil based solely on their branching patterns in 16S rRNA and other phylogenetic trees [27,28]. Pagaling [29] found this phylum in a hot spring boiling pool with temperatures of 60-65 °C and a pH of 8.5 in China. McLaughlin [30] detected Deinococcus-Thermus on 2.6% of 16S rRNA gene clone library in fecal samples. Pukall [28] isolated a Deinococcus strain resistant to radiation in excess of 10 kGy from an Arizona desert. Changes in soil structure that occur in a tillage system exposes the soil to solar radiation, since there is no residues in the soil surface to protect the soil, and it may explain the dominance of potentially radiation and heat resistant Deinococcus-Thermus representatives in bare and tilled soils. Opposed to this, in the no-tillage system, the harvest residues are left on the soil surface, acting as a "roof", which protects the soil against solar radiation, among other benefits. Gemmatimonadetes were detected in about 2.2% of reads in the tillage system compared to 1.2% in the no-tillage system. Janssen [31] identified Gemmatimonadetes as one of the top nine phyla found in soils comprising 2% of soil bacterial communities. Cyanobacteria were five-fold more abundant in tillage than in no tillage in this study

(Figure 1). Members of this phylum are able to fix nitrogen gas into ammonia (NH_3), nitrite (NO_2) or nitrate (NO_3), and reduce carbon aerobically [32].

The archaeal phylum Thaumarchaeota was evenly detected in both management systems. Thaumarchaeota comprises the soil ammonia-oxidizing-archaea genus *Candidatus* Nitrososphaera, which oxidizes ammonia by using ammonia monooxygenase (AMO) enzyme [33].

In no-tillage systems, the soil is not disturbed physically, and because of this, there is less aeration than in tilled soils. This feature may stimulate the anaerobes microorganisms. In no-tillage, at phylum level, Euryarchaeota, Verrucomicrobia, Firmicutes, Crenarchaeota, Chlamydia and Chlorobi were significantly more abundant than in the tilled system. The archaeal phylum Euryarchaeota was almost twice more abundant without tillage. Euryarchaeota comprises diverse species including extreme halophiles, thermoacidophiles, sulfate reducing microbes and a quite diverse group of anaerobic methanogens [34–37]. The Euryarchaeota genera *Methanobacterium, Methanolinea, Methanomethy-lovorans, Methanoregula* and *Methanosarcina* were detected only in the no-tillage system. The phylum Crenarchaeota was twice more abundant in no-tillage. This group is metabolic less diverse than Euryarchaeota, and comprises many extreme thermophiles and species that can metabolize sulfur [36]. The bacterial Chlorobi phylum, was four-fold more abundant without tillage. These microorganisms are obligate photolithotrophs and carry out anaerobic photosynthesis withdrawing electrons from hydrogen sulfide [38]. Collectively, these data support the notion that the no-tillage system supports a greater proportion of anaerobic microorganisms, which can be explained by a higher number of anaerobic microsites available as a result of reduced soil aeration compared to conventional tillage. Also, in no-tillage soil, the higher content of organic matter provides increased water storage capacity providing higher humidity and anaerobic sites that stimulate the expansion of anaerobes microorganisms.

The 20 most abundant genera from all treatments, represented by the heatmap, demonstrated that composition varied significantly among the three evaluated treatments (tillage, N fertilization and crop rotation). Clostridia were more abundant in the no-tillage system in the oat/maize rotation, without added N. These bacteria could be as high as 2.5% of total reads in some samples. Soil clostridia are often free-living diazotrophs under anaerobic conditions. Nitrogenase is inhibited by external N supply. This result is consistent with past results showing the association of free-living diazotrophic bacteria associated with grasses [39,40]. Verrucomicrobia were also correlated with the no-tillage system from the phylum to species levels. Studying undisturbed soils from four continents, Bergmann [41] found that 23% of bacterial sequences analyzed belonged to the Verrucomicrobia, which also was often the dominant phylum in these soils with under-recognized dominance in soil. Here in this work, Verrucomicrobia was found in the no-tillage system, nearly undisturbed soil, between 4–8% of total reads.

P is an important nutrient for plants and microorganisms, and is usually high correlated with pH. It is very well known that P turns soluble in the soil solution, when pH is higher than 6.0 [42,43]. In tropical areas, such as the studied site, the soil is naturally acidic and to grow plants in these soils, the farmers have to add lime in order to increase the pH, which creates chemical conditions to P (and also micronutrients) be solubilized. If some soil has high P content but pH is acidic, this P will be adsorbed in the soil matrix, and will be available neither to the microorganisms, nor to the plants. In this work, we found higher number of OTUs correlated whit P, than OTUs correlated with pH. A close relationship of microorganisms and P in the studied soil, suggests that P is an essential nutrient for the

found taxa, whose may be involved on phosphate solubilizing processes. Correlation of abundance of microorganisms with P, as well as between P and pH, confirms that pH regulates P availability for microorganisms in soil.

PCA analysis summarized the analyses at family and genus level, and placed tillage and no tillage systems in totally opposite sides on the quadrants, as well as, the crop rotations clustered with different group of bacteria, showing that the tillage system and cropping system significantly influenced microbial composition; N fertilization influenced microbial composition to a lesser degree. The oat/maize rotationm clustered with *Geobacillus* and *Paenibacillus,* suggested their preference for low nitrogen environments. *Chitinobacter, Nitrospira* and *Niastella* appear to be ubiquitous as they were detected in all samples with no significant changes in their relative abundance. Microbial diversity and composition in agricultural lands does not depend solely on soil management, but also on soil features and type. Together, these parameters determine the availability of nutrients, and consequently the relative abundance of oligotrophs or copiotrophs [44]. Kuramae [45] found high correlations between bacterial groups and soil chemistry as opposed to correlations with vegetation type and land-use practices. In this work, the tillage system had a greater impact than soil chemistry on microbial abundance. Fierer [46] observed that in environments where microorganisms are exposed to environmental stress, particularly where the stress stems from low resource concentrations, oligotrophs predominate over copiotrophs.

Physical disturbance caused by tillage may be crucial factor in determining decreasing of soil species diversity. No tillage may create relatively stable environments, which results in more diverse decomposer communities and slower nutrient turnover. The relative abundance and distribution of the soil microorganisms are good indicators of the stability of the ecosystem, but more studies of monitoring the microbial communities from agricultural areas are essential to create a model of soil functioning and quality.

4. Experimental Section

4.1. Site Description

This study was based on soil samples collected in a 27 year old experiment, at the Agronomical Experimental Station from Federal University of Rio Grande do Sul, Southern Brazil. This site has a subtropical climate with the geographic coordinates 30°50'52"S, 51°38'08"W. The local annual mean temperature is 19.4 °C, and the average rainfall is 1440 mm. The soil is classified as Acrisol according to FAO classification system and as Typic Paleudult by US taxonomy, with 54% sand, 24% silt, and 22% clay. The clay fraction type is composed mainly of kaolinite (720 g kg^{-1}) and iron oxides (109 g Fe$_2$O$_3$ kg^{-1}) [47]. The experiment began in 1985, and before this, the soil had been degraded, eroded, and compacted due to 15 years under a conventional tillage system causing extensive soil disturbance and erosion [48].

4.2. Experimental Design and Treatments

The experiment was a randomized block design with split-plots for two regimes of soil tillage, three different rotation systems, and two mineral N rates, on three field replicates for a total of 36 plots. The

soil-tillage regimes were conventional tillage and no-tillage. In conventional tillage, total crop residue was incorporated into the soil by disk plow with two disks with a soil disturbance depth of about 20 cm. In the no-tillage system, crop residues were maintained on the soil surface since 1985. Three crop rotations were selected in order to obtain wide variation of quality crop residue addition to the soil, varying between cereals and legumes. These rotations were as follows: a) Oat (*Avena strigosa* Schreb) + common vetch (*Vicia sativa* L.) during winter / maize (*Zea mays* L.) + cowpea (*Vigna unguiculata* L.) during summer; b) Oat during winter/ maize during summer; c) Vetch during winter / maize during summer.

The two levels of N fertilization were without N and 180 Kg ha^{-1}. Every year 50 kg ha^{-1} of P_2O_5 (as single or triple superphosphate) and 50 kg ka^{-1} of K_2O (as KCl) were applied on the plots. Irrigation and phytosanitary treatments were applied when necessary.

4.3. Soil Sampling

Soil samples were collected from 1 to 10 cm layer, with sterile 30 mm wide cores in the middle of each plot. Three subsamples were collected from each plot. The samples were immediately frozen until further analyzes could be performed.

4.4. Chemical Analysis

Soil subsamples were room temperature dried and sieved (0.2-mm sieve). pH, P-Mehlich, Ca, Mg, Al were analyzed following methodology of Tedesco [49]. Total organic carbon (TOC) was analyzed using Walkley Black procedure [50].

4.5. DNA Extraction and PCR

For each sample, DNA was isolated from 0.5 g of soil using the MoBio PowerSoil™ DNA Isolation Kit (Carlsbad, CA, USA). Extractions were performed according to the manufacturer's protocol. All genomic DNA concentration and purity was determined by NanoDrop spectrophotometry (Thermo Scientific, Wilmington, DE, USA). PCR was performed at an initial denaturation temperature of 94 ℃ for 3 min, followed by 20 cycles of 94 ℃ for 45 s, 53 ℃ for 30 s, and 65 ℃ for 90 s. A final elongation step at 65 ℃ was run for 10 minutes. PCR products were purified using the Qiagen™ PCR purification kit following the manufacturer's protocol with the exception of eluting in sterile water (Qiagen, Valencia, CA, USA) and quantified in Qubit 2.0 Fluorometer (Invitrogen, NY, USA).

4.6. Illumina High-Throughput Sequencing of 16S rRNA Genes

Primers 515F and 806R [51] were used to target the V4 region of the 16S rRNA gene with the addition of a barcoded sequence and the required Illumina adapters. Sequencing was performed on an Illumina (Illumina, Inc., CA, USA) with two paired-end read cycles of 101 bases each. Sequence analysis and OTU identification was based on the methods of Giongo [52] and Fagen [53]. Reads were trimmed to remove low quality bases and to remove the first 11 bases corresponding to the primer region by a script based on Trim2 [54,55]), and then the reads were separated by barcode [56]). Paired reads were assembled using CLC Assembly Cell v3.0.2b to the reference Ribosomal Database Project

(RDP) [57] 16S SSU rRNA database. Full taxonomic descriptions based on the NCBI taxonomy database [58] were generated for the entries in the RDP database using TaxCollector [59]. Matches were filtered at 80% length fraction and classified at the 80% identity level for domain and phylum, 90% identify level for class, order and family, 95% identity level for genus, and 99% identity level for species. The total number of pairs matching 16S rRNA sequences in the database at each level of similarity created an OTU abundance matrix for each level of taxonomy across samples. Pairs that did not match to the same sequence in the RDP database were annotated according to their Last Common Ancestor (LCA), and pairs that did not have an LCA, or any match in the RDP database, were considered to be unclassified. To normalize for varying sequencing depths, the OTU abundance matrices for each sample were divided by the total number of pairs after trimming.

4.7. Statistical Analysis

T-test, Tukey test, PCA and Spearman correlations were done with XLSTAT-Pro 2011 (Addinsoft's core software). To integrate Spearman correlations analysis, it was used solely the OTUs with relative abundance equal or higher than 0.05% of total reads (cutoff). To perform correlation-based PCA ($p \leq 0.01$), the data was normalized in order to have the same (=1) variance of the samples along all species axes. For this, each genus or family's relative abundance value was subtracted by the mean count, and then it was divided by the standard deviation ((genus/family abundance-mean)/standard deviation). Taxonomic diversity levels per sample were estimated using the Shannon diversity index (SDI).

5. Conclusions

This study demonstrates that the no-tillage system significantly increase the soil microbial diversity in an Acrisol. The exchangeable P, Mg concentration, total organic carbon (TOC), total N and mineral N were significantly higher in a no-tillage system. The crop system based on cereals (grass) growing throughout the year, stimulates microbial diversity in the soil. Microbial composition changed significantly between conventional tillage and no-tillage systems. Verrucomicrobia, Firmicutes, Crenarchaeota, Chlamydiae, Euryarchaeota and Chlorobi representatives were predominant in a no-tillage system. Anaerobic groups dominate the community in no-tillage samples, and Clostridium was the most abundant genus detected without tillage. Gemmatimonadetes, Cyanobacteria and Deinococcus-Thermus representatives were predominant in the tilled system. Bacteria abundance and composition varies between tillage and no-tillage, but Archaea are most abundant at no tillage system. The radiation favorable environment on a tillage system stimulated radiation tolerant phylum such as Deinococcus-Thermus. Soil management influences soil microbial diversity in agricultural ecosystems through alteration of composition and abundance of individual microbial species.

Acknowledgments

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