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Full Length Research Paper

Bacterial diversity as affected by tillage practice in a raised bed planting system

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Carbon and nitrogen dynamics are altered by tillage so it was hypothesized that the composition of the microbial population that control these processes was affected too. Soil was sampled from conventional beds (CB) tilled and reformed each year with crop residue incorporation and permanent raised beds (PB) with zero tillage seeding and residue retention on the top soil, while the bacterial community composition was investigated with a phylogenetic analysis of the 16S rDNA. Five phyla were detected in both treatments, that is, Acidobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes and Proteobacteria with Acidobacteria and Proteobacteria the most abundant. In PB, the GP6 class belonging to the Acidobacteria with a relative proportion of 42.0%, Sphingomonadales with 17.3% and the Xanthomonadales with 10.7% were the most abundant. In CB, Xanthomonadales with a relative proportion of 31.5%, GP6 with 24.5% and Sphingomonadales with 13.3% were the most abundant groups. Other groups found in both treatments were GP3, Caldilineales, Caulobacteriales, Gemmatimonadales, Pseudomonadales and Rhizobiales. Oceanospirillales, GP4, GP17 and GP22 were only found in the PB soil, whereas GP1, Actinomycetales, Anaerolineales, Myxococcales, Burkholderiales and Rhodospirillales in the CB soil. It was found that tillage did not change the most abundant bacterial groups, but it did affect their relative proportion.

Key words: Bacterial identification, conservation versus conventional agriculture, DGGE analysis, genomic libraries.

INTRODUCTION

Agronomic and soil management practices are known to affect characteristics and soil processes, which are essential to maintain soil fertility. Crop residue management and tillage can alter a soil profoundly. Tillage can deteriorate the soil structure aggravated by the use of heavy machinery (Bronick and Lal, 2004). Removal of crop residues can dramatically reduce the soil organic carbon (SOC) content within a short time and reduce the soil microbial biomass (Albaladejo et al., 1998). Soils under no-till with crop residues retention have higher organic matter contents in the surface layer than soils with conventional tillage, which results in increased water infiltration and a higher water holding capacity in the former (Calderon et al., 2000; Ussiri and Lal, 2009). Thus, no-till soils with surface crop residue retention do not only contain more organic material in the top soil, but they are also wetter and cooler with less fluctuation in water content and temperature (Shinners et al., 1993; Ussiri et al., 2009). There are also indications that the chemical composition and structure of soil

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organic matter is different under conventional and no-till with crop residue retention (Ding et al., 2002). Surface soils (0-5 cm) under no-till are high in light fraction material as well as particulate organic matter (Motta et al., 2001) and humic acids in no-tilled soil contained more reactive functional groups than in conventionally tilled soil (Peacock et al., 2001).

Crops have been cultivated on raised beds presumably since the beginning of intensive agriculture (Sayre, 2004). Cultivation on raised beds have been used by farmers mostly to manage water. Raised beds can often be found along rivers or nearby lakes to reduce the possible negative effects of excess water on crops. Examples are the 'Chinampas' used in the Valley of Mexico, the 'waru warus' near lake Titicaca in Peru and Bolivia, the 'deep ditch-high bed systems' found in the Pearl River basin in China, and the mound planting systems used to avoid problems with the shallow presence of petroplinthite in Africa, Asia, many Pacific Islands and the Americas (Thurston, 1992). In arid or semi-arid parts of the world, such as Central Asia, China and Zimbabwe, crops have been cultivated on raised beds to maximize irrigation efficiency. For instance, the technique has been used in the arid western United States for the production of row-planted crops, such as cotton (*Gossypium* sp.) (Sayre, 2004).

Before planting the next crop, intensive tillage operations are used to form the beds (Aquino, 1998; Sayre, 2004). However, making the raised beds 'permanent', and only reshaping them before planting without destroying or altering the top of the raised bed, would strongly reduce costs for the farmer. This agricultural system gave promising results under irrigated conditions. Therefore, a field experiment was started under rain fed conditions by the International Maize and Wheat Improvement Centre (CIMMYT) in the volcanic highlands of Central Mexico in 1999. Higher yields for both maize (*Zea mays* L.) and wheat (*Triticum* spp. L.) were obtained for permanent raised beds with full residue retention, compared to conventionally tilled raised beds (Sayre et al., 2005). Permanent raised beds with retention of crop residue resulted in higher soil organic matter content, aggregate stability and an increased protection of C and N in the micro-aggregates within the macro-aggregates compared to conventionally tilled raised beds. Emissions of N_2O and CO_2 were reduced, and production of NO_3^- increased in soil with permanent raised beds with crop residue retention, compared to soil under conventionally tilled raised beds. The C and N cycling was affected by tillage so it could be hypothesized that the bacterial communities were also affected. Soil was sampled from permanent raised beds and from beds remade each crop cycle (conventionally tilled raised beds), in both systems crop residue was retained in the field. Phylogenetic trees and dominant groups were determined in both soils. The objective of this study was to determine the effect of permanent raised beds (PB) and conventionally tilled raised beds (CB) on the composition of the microbial population when crop residue was retained. It was hypothesized that the different management practices would change the bacterial population in soil.

MATERIALS AND METHODS

Experimental site

The mean maximum and minimum temperature at the experimental site are 24 and 6°C, respectively (1991-2009) and the average annual rainfall is 625 mm y^{-1}, with approximately 545 mm falling between May and October. Short, intense rain showers followed by dry spells typify the summer rainy season and the total yearly potential evapotranspiration of 1550 mm exceeds annual rainfall. The El Batán experiment station has an average growing period of 132 days.

The soil at the experimental site is classified as a Haplic Phaeozem (Calyic) in the World Reference Base system (IUSS Working Group WRB, 2007) and as a fine, mixed, thermic Cumulic Haplustoll in the USDA Soil Taxonomy system (Soil Survey Staff, 2003). The particle size distribution was clay 409, silt 240 and sand 351 g kg^{-1}.

The rain-fed experiment was started in 1999. Each plot in the experiment measured 6 m × 20 m with 8 raised beds of 75 cm width. Four management practices were studied in 14 treatments with two replicates in a randomized complete block design. The first factor studied is residue management: 1) all above ground crop residues chopped and kept in the field whether incorporated for CB or retained on the soil surface for PB (K), 2) all crop residues removed by baling for fodder (R) and 3) crop residues partly removed (P), wheat residues cut by combine, and maize residues cut just below the ear. The second factor studied is tillage: 1) conventional tillage with raised beds formed after each crop (CB) 2) zero tillage with continued reuse of the existing raised beds (re-shaped if required) (PB). The third factor is both phases of the maize-wheat rotation, while tied-ridges (T) and open furrows (NT) between the raised beds are the fourth factor. Currently recommended crop cultivars were used with maize planted at 60,000 plants ha^{-1} in two rows on top of the 75 cm raised beds and wheat planted in 20 cm rows at 100 kg seed ha^{-1}. Urea was applied at 120 kg N ha^{-1} with all N applied to wheat at the 1st node growth stage (broadcast) and to maize at the 5-6 leaf stage (surface-banded). Herbicides were applied to control weed when necessary, but no disease or insect pest controls were utilized, except for seed treatments applied by commercial seed sources. Maize and wheat were planted between June 5 and 15 at the onset of summer rains.

Two treatments were considered in this study, that is, conventional beds tilled and reformed each year with crop residue incorporation (considered the CB treatment) and permanent raised beds with zero tillage seeding and residue retention on the top soil (considered the PB treatment). Beds were formed after each crop (CB) or the beds were reused each year and re-shaped if required without disturbing the top of the bed, only lightly retracing the furrows. The soil preparation in CB consisted of harrowing at 20 cm depth, with a disc harrow starting some days after harvest incorporating the crop residue and repeated when needed for weed control (at least once) during the dry season. To prepare the seed beds, a spike tooth harrow was used at least once after which the beds were freshly formed. Crop residue was chopped before being incorporated through tillage to a depth of 20 cm in the CB. Soil samples were collected from the 0-15 cm soil layer of plots cultivated with maize on 21st of July 2008. Composite soil samples of four random sub-samples per plot were collected with an auger

Table 1. Characteristics of soil cultivated with wheat under conventional tilled (CB) or permanent beds raised (PB) with crop residue retained.

Beds	WHC[a]	Organic C	Total N	SMB[b]	pH	EC[c]
	——— (g kg^{-1} soil) ———			(mg kg^{-1})		(mS m^{-1})
Conventional	728 (22) [d]	11.8 (0.4)	1.30 (0.01)	430 (7)	7.0 (0.02)	92 (4)
Permanent	784 (43)	13.2 (0.3)	1.36 (0.04)	473 (21)	7.0 (0.02)	72 (1)

[a]WHC: Water holding capacity; [b]SMB: soil microbial biomass; [c]EC: electrolytic conductivity; [d]values between parenthesis are standard errors of the estimates.

of 2 cm diameter, 5 mm sieved and characterized (Table 1). Crop residues large enough to remain on the sieve were discarded.

DNA extraction and purification

The DNA was extracted from soil with a technique based on direct cell lysis (Guo et al., 1997). A 5 g soil sample was added to a 50 ml Oak Ridge tube containing 5 ml 0.12 M sodium phosphate buffer (PB buffer pH 8.0), vortexed for 1 min, incubated at room temperature for 10 min and centrifuged at 3,000 *g* for 10 min. The supernatant was decanted and 5 ml PB buffer was added to the soil pellet. The procedure of vortexing, incubation and centrifugation was repeated once. The soil pellet was re-suspended in 5 ml lysis solution I (0.15 M NaCl, 0.1 M EDTA, pH 8.0, 10 mg lysozyme ml^{-1}), mixed and incubated at 37°C for 1 h with occasional gentle mixing. A 5 ml lysis solution II (0.1 M NaCl, 0.5 M Tris-HCl, pH 8.0, 12% SDS) was added. The soil suspension was passed through two cycles of freezing at -40°C for 20 min and thawing at 65°C for 20 min and then centrifuged at 7,700 *g* for 10 min. The supernatant was mixed with 2.7 ml 5 M NaCl and 2.1 ml 10% TRITON-X100 in 0.7 M NaCl and incubated for 10 min at 65°C. An equal volume of $CHCl_3$: isoamyl alcohol (24:1) was added, mixed and centrifuged for 5 min at 3,000 *g*. The supernatant was transferred to a clean tube. An equal volume of 13% PEG [polyethylene glycol (8,000 MW) dissolved in 1.6 M NaCl] was added to the supernatant, incubated on ice for 30 min and centrifuged at 12,000 *g* and 4°C for 10 min. The supernatant was decanted. The pellet was washed with 5 ml 70% cold ethanol and air-dried. The pellet of crude DNA extracts was re-suspended in 500 µl deionized H_2O. Two volumes of ethanol were added to the supernatant, mixed and centrifuged for 30 min at full speed at 4°C. The pellet was washed with 500 µl 70% cold ethanol and air-dried. The DNA was recovered in 500 µl of bi-distilled water. The DNA yield was quantified by the use of a UV Transilluminator 2000 (Gel Doc 2000, BIO-RAD Laboratories Inc., Carlsbad, CA) after 0.8% (w/v) agarose gel electrophoresis, and staining with ethidium bromide solution (0.5 µg ml^{-1}). The 1-kb DNA ladder (Invitrogen Life Technologies Carlsbad, CA) was used as molecular size marker and compared with DNA. The DNA extract was stored at -20ºC until required for PCR amplification.

PCR amplification, construction of 16S rDNA libraries and sequencing

Purified DNA was used as template for the amplification of bacterial 16S rDNA via PCR. The reaction mixture (25 µl) contained 100 ng of genomic DNA; the appropriate primers at 0.5 µM each; dATP, dCTP, dGTP and dTT, each at a concentration of 200 µM; 2.5 mM MgCl; and 1 U of Taq DNA polymerase in the PCR buffer provided by the manufacturer (Invitrogen Life Technologies, Sao Paulo, Brazil). A pair of universal primers 46F (5´GCCTAACACATGCAAGTC3´) and 1540R (5´GGTTACCTTGTTACGACTT3´) were used to amplify the V1-V9 highly variable regions of 16S rDNA, *ca.* 1500 bp (Edwards et al., 1989; Yu and Morrison, 2004). Amplification conditions included a denaturation step at 94ºC for 12 min followed by 25 cycles at 94ºC for 1 min, at 50ºC 1 min, at 72ºC 2 min and a final step at 72ºC for 7.5 min. The amplification was done with a Touchgene Gradient FTGRAD2D (TECHNE DUXFORT, Cambridge, UK).

The PCR products of 16S rDNA were cloned directly into the vector Topo® TA by using the TOPO TA Cloning Kit (Invitrogen Life Technologies Carlsbad, CA). The positive clones were detected by the appearance of white colonies in LB plates containing 40 µg ml^{-1} of X-Gal (Invitrogen Life Technologies Carlsbad, CA) and 50 µg ml^{-1} ampicillin. Recombinant plasmids were isolated from overnight cultures by alkaline lysis (Sambrook et al., 1989) and a restriction analysis with *Eco*RI was done to assure the insertion was successful. The 16S rDNA sequences were obtained with a 3730X DNA Analyzer (Applied Biosystems, Foster City, CA) using M13 primers.

Bacterial molecular identification and phylogenetic analysis

All sequences obtained in this work were checked for chimeras using the CHIMERA-CHECK online analysis program, of the RDP-II database (Cole et al., 2003). The partial sequences were then subjected to a BLAST search (Altschul et al., 1997) and the naïve Bayesian rRNA classifier was used to determine taxonomic hierarchy of the sequences (Wang et al., 2007). Multiple alignment analyses were performed with CLUSTAL X (Thompson et al., 1997) selecting related sequences from the NCBI Taxonomy Homepage (TaxBrowser) and Ribosomal Database Project-II databases. Only common 16S rDNA regions and parsimony informative sites were included in the analysis. All alignment gaps were treated as missing data. The transversion/transition weighting using the Tamura-Nei model (Tamura and Nei, 1993) and the number of base substitutions between each pair of sequences was estimated using program MEGA v. 2.1 (Kumar et al., 2001). The phylogenetic trees were constructed using the Neighbor-joining method and Tamura-Nei model of distance analysis and 500 bootstrap replications were assessed to support internal branches. Sequences that differed by less than 3% were considered to belong to the same phylotype (Stackebrandt and Goebel, 1994). Sequences with similitude percentages below 95% were assigned to the closest family (Rosselló-Mora and Amann, 2001).

Community richness and composition analysis

Rarefaction, richness, and diversity statistics were calculated using mothur (Schloss et al., 2009). The input files were in the form of distance matrices generated by using the program phylip dnadist (Felsenstein, 1989). Mothur used the furthest neighbor algorithm to collapse similar sequences into groups at arbitrary levels of

Table 2. Bacterial groups and taxonomic assignment of the clones obtained from the genomic libraries of the soil of permanent and conventional tilled beds (80% Confidence threshold[a]).

Treatment	Number of:					
	Clones	Phylum	Class	Order	Family	Genus
Permanent beds	150	5	10	8	11	23
Conventional beds	143	5	11	12	14	17
Total	293	5	14	14	15	29

[a]RDP Naive Bayesian rRNA Classifier Version 2.2, March 2010.

taxonomic similarity and then computed the Shannon, Chao1 and ACE statistics for that taxonomic level (Schloss et al., 2009). Furthermore, the Good's coverage of our libraries was calculated (Good, 1953). Operational taxonomic units (OTUs) for community analysis were defined at a 98% difference in nucleic acid sequences (Rosselló-Mora and Amann, 2001).

Two hundred ninety-three clones were used for the phylogenetic reconstruction (Table 2). The size of the 16S rDNA sequences was between 1480–1510 bp. Phylogenetic reconstructions were done by distance (data not shown) and maximum likelihood methods. Similar tree topologies were obtained for both techniques. Figures 1 and 2 shows the phylogenetic relationship between amplified 16S rDNA sequences from CB and PB with known 16S rDNA sequences from the GenBank.

Nucleotide sequence accession numbers

The sequences were deposited in the GenBank database and assigned the accession numbers HM447651-HM447960.

RESULTS

Five phyla were detected in both treatments, that is, Acidobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes and Proteobacteria, but 2.0% of the sequences remained unclassified in PB and 1.4% in CB (Table 3). After phylogenetic analysis of the PB sequences, 23 could be assigned to the level of genus, 11 to that of family, eight to that of order and 10 to a class (Table 2). After phylogenetic analysis of the sequences obtained from CB, 17 allowed characterization to the level genus, 14 to that of family, 12 to an order, 11 to the level of class and five to a phylum.

The most abundant groups in the PB treatment were the class GP6 belonging to the Acidobacteria with a relative proportion of 42%, 17.3% were Sphingomonadales and 10.7% Xanthomonadales (Table 3). In the CB soil, Xanthomonadales were the most abundant group with a relative proportion of 31.5%, while 24.5% of the sequences belonged to the class GP6 and 13.3% to the Sphingomonadales.

Other groups found in both treatments were GP3, Caldilineales, Caulobacteriales, Gemmatimonadales, Pseudomonadales and Rhizobiales. Bacteria belonging to GP4, GP17, GP22 and Oceanospirillales were exclusively found in the PB soil, whereas GP1, Anaerolineales, Actynomycetales, Burkholderiales, Myxococcales and Rhodospirillales in the CB soil.

A LIBSHUFF analysis indicated that the differences in the composition of the libraries were due to underlying differences in the communities from which they were derived, since both systems, that is, PB and CB, were significantly different ($P < 0.001$). Rarefaction analysis at the 3% cut-off showed that the libraries of each treatment showed little difference in diversity (Figure 3). A decline in the rate of OTUs at 10 and 20% cut-off indicated that only the most dominant bacterial phyla were detected (No data shown). Analysis with the mothur software showed that the diversity and richness were similar in PB and CB (Table 4).

DISCUSSION

It can be speculated that the agricultural system applied can have a profound effect on the soil microorganisms. For instance, the amount of crop residue retained in soil will determine the total amount of micro-organisms, that is, organic material serves as a C substrate, and the characteristics of residue left, for example, C-to-N ratio, lignin (hemi) cellulose content, will favour certain microorganisms. No-till enhances physical and biological soil quality parameters. The modification of the soil structure due to the agricultural practices, affects directly the oxygen concentration in the soil allowing different rates of microbial respiration (Müller et al., 2002). This respiration can create zones of low O_2 concentration and even anaerobic conditions. The diffusion of O_2 is highly dependent on soil water content; a reduced water content and better soil structure favors O_2 diffusion.

It is clear that the combination of the above mentioned factors could have a profound effect on the bacterial communities in soil, but not in this study (Wolf et al., 2005). As such, differences in tillage practices, that is, incorporated in CB and left on the soil surface in PB, and the response of the bacterial enzymatic machinery to the subsequent changes in environmental conditions, that is, water, oxygen, nutrients availability and plant activities, did not affect on the bacterial community structure (Fauci, 1994; Ha et al., 2008).

The three most dominant groups in both PB and CB were bacteria belonging to the class GP6 (Acidobacteria), Sphingomonadales (Alphaproteobacteria) and the Xanthomonadales (Gammaproteobacteria). The Acidobacteria have been reported as the dominant bacterial group in different types of soil (Felske et al., 2000; Upchurch et al., 2008). They are considered to have K-strategist features, such as degradation of soil organic matter and slow growth by focusing on extracellular enzyme production (Rui et al., 2009). They also possess r-strategist capabilities, such as adaptation to rapid growth depending on inputs of fresh organic

Table 3. Percentage of clones belonging different phyla, classes and orders based on the ribosomal data project in of the soil of permanent and conventional tilled beds (80% Confidence threshold[a]).

Phylum / Class / Order	Beds		
	Permanent	**Conventional**	**Total**
Acidobacteria	44.7	27.3	36.2
Gp1	ND	1.4	0.7
Gp3	0.7	1.4	1.0
Gp4	0.7	ND	0.3
Gp6	42.0	24.5	33.4
Gp17	0.7	ND	0.3
Gp22	0.7	ND	0.3
Actinobacteria	2.7	2.1	2.0
Actinobacteria			
Actinomycetales	ND	0.7	0.3
Unclassified Actinobacteria	2.7	1.4	1.7
Chloroflexi	0.7	2.8	1.7
Anaerolineae			
Anaerolineales	ND	1.4	0.7
Caldilinae			
Caldilineales	0.7	0.7	0.7
Unclassified Chloroflexi	ND	0.7	0.3
Gemmatimonadetes			
Gemmatimonadetes			
Gemmatimonadales	5.3	4.2	4.8
Proteobacteria	44.7	62.2	53.2
Alphaproteobacteria	26.0	21.7	23.9
Caulobacterales	3.3	0.7	2.5
Rhizobiales	5.3	4.2	4.8
Rhodospirillales	ND	3.5	1.7
Sphingomonadales	17.3	13.3	15.4
Betaproteobacteria	ND	1.4	0.7
Burkholderiales	ND	1.4	0.7
Deltaproteobacteria	0.7	3.5	1.7
Myxococcales	ND	1.4	0.7
Unclassified Deltaproteobacteria	0.7	2.1	1.0
Gammaproteobacteria	18.0	35.7	26.6
Oceanospirillales	1.3	ND	0.7
Pseudomonadales	2.0	3.5	2.7
Xanthomonadales	10.7	31.5	20.8
Unclassified Gammaproteobacteria	4.0	0.7	2.4
Unclassified	2.0	1.4	2.0

[a]RDP Naive Bayesian rRNA Classifier Version 2.2, March 2010.

Table 4. Comparisons of microbial richness and diversity of 16S rDNA libraries of the soil of permanent and conventional tilled beds as calculated with mothur (Schloss et al., 2009).

Treatment	Number of DNA sequences	No difference OTUs[a]	Richness estimator		Diversity index	
			Ace[b]	Chao1[c]	H[d]	D[e]
Conventional tilled beds	143	94	253 (179, 390)[f]	259 (178, 418)	4.24 (4.06, 4.42)	0.0194 (0.0069, 0.0319)
Permanent beds	150	93	175 (137, 246)	154 (124, 213)	4.37 (4.24, 4.50)	0.0085 (0.0053, 0.0117)

[a]No difference OTUs defined by using the furthest-neighbor algorithm in mothur at 97% similarity, [b]Ace, abundance based coverage estimator, [c]Chao1, bias corrected Chao1, [d]H, Shannon-Weaver diversity index, [e]D, Simpson diversity index, [f]Confidence intervals (95%) are given between parenthesis.

material (Beare et al., 1997). The genus *Sphingomonas* is often found in association with plants and different strains have been isolated from the rhizosphere. They might play different roles in the rhizosphere as some have been identified as pathogens, for example, *Sphingomonas suberifaciens* causing corky root disease of lettuce (Van Bruggen et al., 1990; Kim et al., 1998), while other bacteria belonging to the order of the Sphingomonadales have shown antagonism against the phytopathogenic fungus *Verticillium dahliae* (Berg and Ballin, 1994). The Xanthomonadales are well known bacterial phytopathogens, which can affect plants in different ways (Alfano and Collmer, 1996).

Other bacterial groups were found also in both treatments, that is, GP3, Caldilineales, Caulobacteriales, Gemmatimonadales, Pseudomonadales and Rhizobiales. Pseudomonadales have been isolated from different soils, indicating that these bacteria easily adapt to different conditions (Latour et al., 1996). An isolated strain of *Pseudomonas* sp. has the capacity to colonize plants when temperature and water content are favorable, thereby affecting their growth and development (De Weger et al., 1995). There are reports that suggest that disease suppressiveness in plants might be caused partly by the enzymatic complex of *Pseudomonas* sp. that inhibits pathogen growth (O´Sullivan and O´Gara, 1992; Khalid et al., 2004). The genus *Pseudomonas* might affect plant growth by producing antibiotics, siderophores, and/or plant growth promoters, such as auxin (Arshad and Frankenberger, 1998; Herman et al., 2008). The presence of Pseudomonadales in both CB and PB might indicate that soil in both systems are not degraded, while bacteria related to the genus *Burkholderia* found in CB, but not in PB, might indicate a more deteriorated soil in CB than in the PB. Rhizobiales contain a large number of genera that have been studied widely, such as the symbiotic nitrogen-fixing bacteria that usually develop symbiotic association with leguminous plants (Young, 1996). The importance of the interaction is based on the capacity of *Rhizobium* strains to form nodules and fix atmospheric nitrogen (Miethling et al., 2000). Normally these microorganisms are ubiquitous in soil and form symbiosis in 80% of terrestrial plants (Jeffries et al., 2003). Although the Pseudomonadales and Rhizobiales have been studied extensively, little is known about other groups, such as the Gemmatimonadales. The phylum Gemmatimonadetes was only recently described by Zhang et al. (2003). Based on surveys of 16S rDNA, this group appears to be abundant in a number of soils (Axelrood et al., 2002; Furlong et al., 2002).

Oceanospirillales were exclusively found in the PB soil. The clones analyzed were related to an isolated strain of *Halomonas* sp. and this genus can reduce NO^-_3 to NO^-_2 under anaerobic conditions (Llamas et al., 2006). Bacteria belonging to the class GP1, Rhodospirillales, Burkholderiales, Myxococcales and Actinomycetales, were found in the CB soil. The bacteria related to the genus *Burkholderia* are found in deteriorated soils (Giller et al., 1997). In these systems the biodiversity declines, inducing a loss in soil functions resulting in a reduction in the ability of these agricultural systems to withstand further disturbances or unexpected periods of stress. Salles et al. (2006) reported that agricultural practices, such as fertilization and tillage, have an impact on the Burkholderiales community structure. Species belonging to the genus *Burkholderia* have been identified as biocontrol agents of many plant-pathogenic fungi (Li et al., 2002).

The ability to suppress plant disease was observed in different crops, such as corn, sweet corn, cotton, pea, tomato and pepper. Xanthomonadales and Pseudomonadales, common pathogens in agricultural soil, were found in both treatments, so the antagonistic effect of *Burkholderia* was not absolute. Other studies have shown that Beta- and Gammaproteobacteria are important copiotrophic taxa in soil and their relative abundance might be related to the supply of labile C substrates in soil (Eilers et al., 2010).

The normal habitat of Myxococcales, found in CB only,

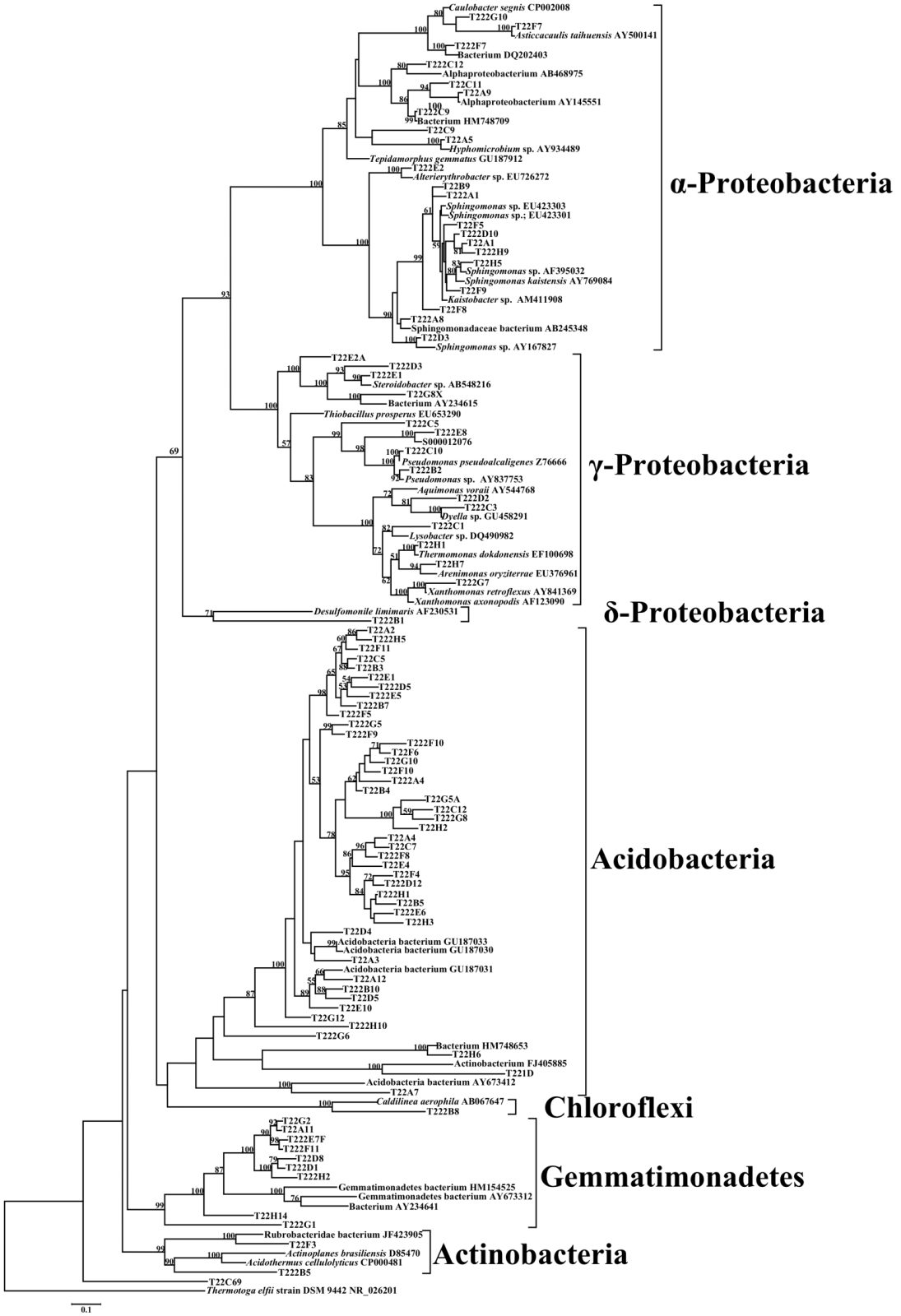

Figure 1. Phylogenetic relationship between the 16S rDNA sequences obtained from agricultural soil with permanent raised beds (PB). The tree was constructed with related sequences obtained from NCBI database (accession number in parenthesis) by using the likelihood method. Bootstrap percentages less than 50% are not shown. *Thermotoga elfii* (NR_026201) was used as the outgroup. The scale bar represents 10% sequence divergence.

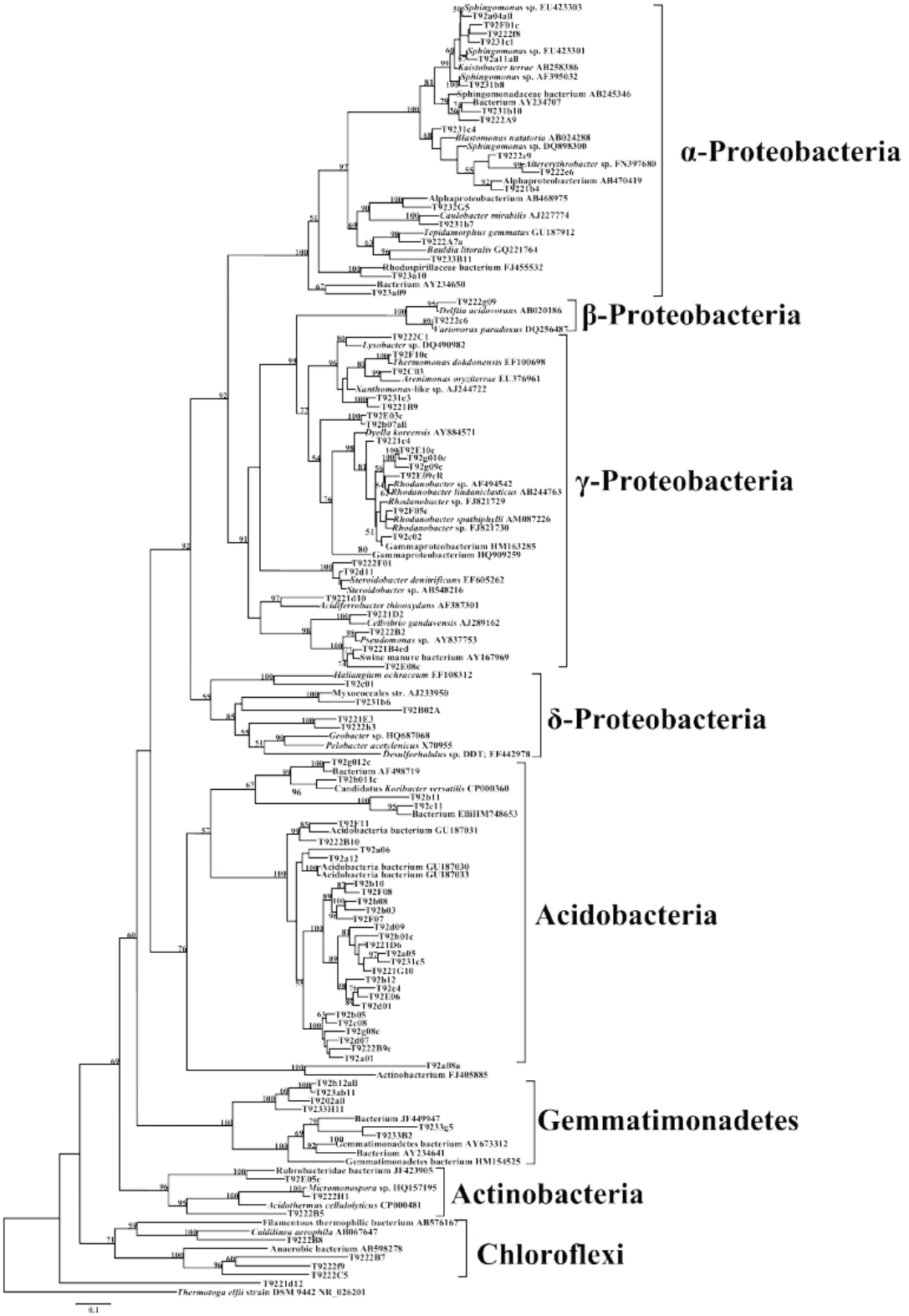

Figure 2. Phylogenetic relationship between the 16S rDNA sequences obtained from agricultural soil with conventional tilled beds (CB). The tree was constructed with related sequences obtained from NCBI database (accession number in parenthesis) by using the likelihood method. Bootstrap percentages less than 50% are not shown. *Thermotoga elfii* (NR_026201) was used as the outgroup. The scale bar represents 10% sequence divergence.

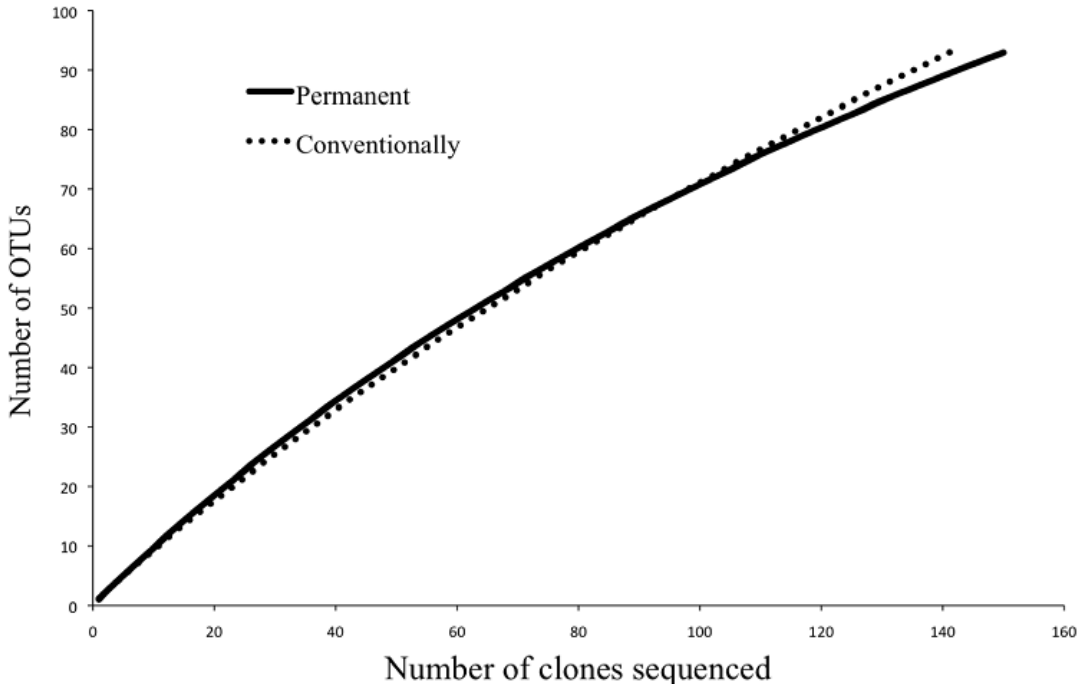

Figure 3. Rarefaction analysis for samples of permanent raised beds and conventional beds based on pairwise distance. Rarefaction is shown for OTUs with differences that do not exceed 3%.

is the soil as long as the pH is near neutral, that is, ranging between 5 and 8. There is still little information about the population density of myxobacteria in soil. They are generally considered mesophilic, strictly aerobic organotrophic soil microbes with a temperature optimum of 30ºC. Two main groups are considered depending on their ability to utilize N-lacking compounds. The first group can degrade N-lacking compounds, such as cellulose and glucose. The other group, representing the majority of the myxobacterial species, is unable to use cellulose. They depend upon amino acid containing growth substrates as N providers that are supplied by enzymatic degradation of proteins, such as oligopeptides or single amino acids. Under natural conditions, these myxobacteria feed on other organisms, such as eubacteria or yeasts by bacteriolysis or cellular lysis. Secreted exoenzymes (proteases, nucleases, lipases, glucanases) destroy intact living cells. The nutrient lysate formed in this way is the nutritional basis of these myxobacteria and they have therefore been called micropredators (Reichenbach and Dworkin, 1991). The Actinomycetales are abundant in acidic environments and important cellulolytic bacteria found when cellulose is abundant in soil (Ulrich and Wirth, 1999). They are known to fix nitrogen in different types of soils (O´Neill, 1994) and have been described as antagonist to fungal root pathogens (Scott et al., 2008).

Although, current molecular techniques have substantially improved our knowledge of bacterial diversity, the study of environmental microbial diversity is still a largely unexplored field (Sogin et al., 2006). This work, like most of the published studies on environmental microbial diversity, is limited to the identification of dominant groups that mediate biogeochemical processes. While this offers an important characterization of the soil microbial communities, complete characterization of its biological diversity will require the development of techniques that allow the identification of members of microbial populations with low abundance.

It was found that tillage did not affect the bacterial groups that were most abundant in the PB and CB soil, that is, GP6, Sphingomonadales and Xanthomonadales, but their relative proportion changed. The presence of Pseudomonadales in both CB and PB might indicate that soil in both systems are not degraded, while bacteria related to the genus *Burkholderia* found in CB, but not in PB, might indicate a more deteriorated soil in the first than in the latter. The biodiversity had declined in the CB soil compared to PB soil, which will result in a reduction in the ability of these agricultural systems to withstand further disturbances or unexpected periods of stress. Additionally, reducing tillage has a positive effect on microbial population and appears thereby to reduce disease pressure by increasing biological control. It can be speculated that a higher diversity in PB results in a system more resilient to changing environmental conditions. Further research, however, will be required to link changes in the microbial population with changes in

soil processes. As such, the role and importance of each bacterial group will have to be investigated and the effect of the different management practices on them determined. Although more functional information is needed, the results already support the importance of adopting PB with residue retention as increased diversity supporting resilience.

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