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Bacterial Communities in the Rhizosphere of Biofuel Crops Grown on Marginal Lands as Evaluated by 16S rRNA Gene Pyrosequences

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Abstract Microbes are key components of the soil environment and are important contributors to the sustainability of agricultural systems, which is especially significant for biofuel crops growing on marginal lands. We studied bacterial communities in the rhizosphere of five biofuel crops cultivated in four locations in Michigan to determine which factors were correlated to changes in the structure of those communities. Three of these sites were marginal lands in that two were not suitable for conventional agriculture and one was regulated as a brownfield due to prior industrial pollution. Bacterial community composition and structure were assessed by 454 sequencing of the 16S rRNA gene. A total of 387,111 sequences were used for multivariate statistical analysis and to test for correlation between community structure and environmental variables such as plant species, soil attributes, and location. The most abundant bacterial phyla found in the rhizosphere of all crops were Acidobacteria, Proteobacteria, Actinobacteria, and Verrucomicrobia. Bacterial communities grouped by location rather than by crop and their structures were correlated to soil attributes, principally pH, organic matter, and nutrients. The effect of plant species was low but significant, and interactions between locations, plant species, and soil attributes account for most of the explained variation in the structure of bacterial communities, showing a complex relationship between bacterial populations and their environment. Bacterial diversity was higher in the agricultural sites compared to adjacent forest sites, indicating that the cultivation of those biofuel crops increased the rRNA diversity.

Keywords Rhizosphere · Community structure · Diversity · Biofuel crops

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Introduction

Biofuels have been presented as an alternative to petroleum-based fuels and to reduce net greenhouse gas emissions, but there are concerns regarding their economic feasibility, net energy efficiency, their environmental impact and their use on marginal lands [26]. Soil microorganisms are a potentially important resource for enhancing biofuel production at lower cost while maintaining ecosystem services. Their benefits can include nitrogen fixation, enhanced nutrient recycling and acquisition, production of growth stimulants, improved soil structure and water retention, and suppression of root diseases [30]. Any such benefits for biofuel crops on marginal lands would be especially important to sustainability. Hence, we sought to determine the microbial communities of biofuel crops using new high-capacity sequencing technology that provides much more extensive information than was possible from previous methods, such as clone libraries [25].

Cultivation is known to result in significant changes in the taxonomic composition and activity of microbial communities. Several variables are correlated to this effect such as plant species, land management (e.g., application of herbicides and fertilizers), changes in soil physical and chemical attributes, etc. [1, 8, 10, 17, 18, 20]. Differences in the composition of microbial communities are also linked to factors independent of the agricultural activity, such as soil type and geographic distance.

The objective of this study was to determine environmental factors which correlated to changes in the structure of bacterial communities in marginal lands cultivated with corn, switchgrass, soybean, canola, and sunflower as biofuel crops. Since we studied communities in the rhizosphere soil, we tested the hypothesis that plant species have a significant effect in determining the composition and structure of bacterial communities. We sampled biofuel crop rhizospheres from four different soil types and regions of Michigan, to determine the effect of soil and geographic region relative to the plant on the resulting microbial community.

Material and Methods

Site Description and Soil Sampling

Soil samples were collected in four different locations in the State of Michigan, USA in July and August 2008: Chatham (UP), at the MSU Upper Peninsula Experiment Station, Alger County; Lake City (LC), at the Lake City Experiment Station, Missaukee County; at a regulatory brownfield at Rose Township Dump Site (Rose) in Oakland County; and East Lansing (MSU), at the Michigan State University Crop and Soil Science Research Farm, Ingham County, which served as an experimental control (nonmarginal land) for crop yield and quality analyses (Table 1). Beginning in 2006 (UP, Rose, and MSU) and 2007 (LC), experiments were carried out at each of the sites to test the performance of soybean, canola, sunflower (not present at UP), corn, and switchgrass as biofuel crops. Three of the sites were less productive (UP and LC) or impaired (Rose—a regulatory brownfield, i.e., use restricted due to industrial pollutants) and are termed "marginal lands" [29]. All of the indicated annual crops were planted at each location in each year of the study and followed the rotational order of: canola–corn–soybean–sunflower, with sunflower omitted from the rotation at UP. The perennial crop, switchgrass, was planted and maintained in the same plot location over the duration of the experiment. The sampling occurred in late July or August of 2008 when the plants were in active growth prior to maturity. Soil samples were also taken at the same time from adjacent forests to represent the native soil–plant conditions at the respective sites.

The experimental design at each site was a randomized block design, with six replicates per crop at UP, LC, and Rose and four replicates at MSU. Composite samples were taken from three replicates of each crop. Each composite sample was formed by the rhizosphere soil of three randomly chosen plants dug to a depth of 20 cm. Roots and associated soil were transported to the laboratory on ice and then processed to obtain the rhizosphere soil. The roots were shaken to remove the loose soil, and the tightly attached soil including small aggregates (<0.5 cm) was used for DNA extraction. Three composite samples were taken randomly in the adjacent forest as we did for the crop sites, except that three soil cores instead of rhizosphere soil composed those samples. Samples were homogenized and stored at −20°C until processing. Soil chemical analysis was carried out for each sample by the MSU Soil Testing Laboratory (Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI, USA).

DNA Extraction and 16S rRNA Gene Amplification

Five hundred milligrams of each well-mixed soil sample was used for DNA extraction with the Power Soil DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA), according to the manufacturer's instructions. The DNA was quantified with a Nanodrop ND-1000 spectrophotometer (Nanodrop Technology) and stored at −20°C until use. Primers targeting a fragment of 270–300 bp in the V4 region [3, 31] of the 16S rRNA gene were used for the polymerase chain reaction (PCR). The 454 primers constituted of an adapter sequence, added as recommended by Roche; barcodes of 8 bp (added to forward primers only) used to distinguish samples results from the same sequenc-

Table 1 Soil classification, soil texture, geographic coordinates, temperature, precipitation, soil nutrient concentrations, and organic matter content of the studied sites

Site*	Soil class	Soil texture	Location	Max. annual average temperature (°C)	Min. annual average temperature (°C)	Annual rainfall (mm)	pH**	P** (ppm)	K** (ppm)	NH_4^+** (ppm)	OM** (%)
UP	Frigid Hapludolls	Sand-skeletal	46° 20′ N 86° 54′ W	10.2	–0.2	874.5	7.3 (7.1)	55.2 (7.0)	113.3 (51.7)	2.5 (6.3)	3.4 (19.7)
LC	Frigid Glossoboralfs and Alfic Upidsamments	Coarse-loamy	44° 18′ N 85° 12′ W	12.2	-0.1	777.8	5.6 (4.7)	79.6 (28)	65.1 (19.7)	1.1 (2.6)	1.3 (2.9)
Rose	Mesic Glossudalfs	Fine-loamy	42° 43′ N 83° 37′ W	14.5	3.6	767.9	7.4 (5.3)	40.8 (27)	78.6 (65.3)	2.0 (5.3)	2.7 (3.1)
MSU	Aeric Ochraqualfs	Fine-loamy	42° 41′ N 89° 29′ W	13.3	2.5	751.9	6.4 (4.7)	56.9 (25)	149.6 (32.3)	1.8 (11.5)	1.6 (3.9)

UP Chatham, *LC* Lake City, *Rose* Rose Township, *MSU* East Lansing

**Numbers outside parenthesis refer to averages in the agricultural sites and those into parenthesis to the averages in the forest sites

ing; and the primer sequence itself, which was AYTGGGY DTAAAGNG for the forward primer and TACCRGGGT HTCTAATCC, TACCAGAGTATCTAATTC, CTACDSRG GTMTCTAATC, and TACNVGGGTATCTAATCC for the reverse primers (http://pyro.cme.msu.edu/). A mixture of the four reverse primers was used. The PCR was performed in triplicate for each sample, and the PCR products were mixed [11]. The reactions contained 1× reaction buffer, 1.8 mM $MgCl_2$, 0.2 mM of each dNTP, 1 μM of each primer (Integrated DNA Technology, Coralville, IA, USA), 1.5× BSA, 1 ng μl^{-1} of template, and 1 U of FastStart High-Fidelity PCR System enzyme blend (Roche Applied Sciences, Indianapolis, IN, USA). The following conditions were chosen for amplification after optimization: initial denaturation for 3 min at 95°C; 30 cycles of 45 s at 95°C, 45 s at 57°C, and 1 min at 72°C; and final extension for 4 min at 72°C. After analyzing the PCR products on gels, fragments with the expected size (270–300 bp) were excised and extracted with the Qiagen gel extraction kit (Qiagen, Valencia, CA, USA). PCR products were further purified with the Qiagen PCR purification kit (Qiagen, Valencia, CA, USA). Sequencing was performed with a GS FLX sequencer (454 Life Sciences, Branford, CT, USA) at the Michigan State University Research Technology Support Facility.

Data Analysis

All sequences that passed the quality controls of the GS FLX software were uploaded on the Ribosome Database Project (RDP) high-throughput pipeline [3] and analyzed by using the RDP tools (release 10). Raw sequences were sorted by barcode; the 16S rRNA gene primers were trimmed and filtered to remove sequences of low quality. Alignments were obtained with the software INFERNAL [19] and used to calculate distance matrices and to generate a matrix containing information about the operational taxonomic units (OTUs) for use with EstimateS and R. OTUs were defined at the level of 3% dissimilarity, approximately species level.

Redundancy analysis was performed with Hellinger-transformed data as advised elsewhere [15]. The contribution of soil attributes, location, and crop species, as well as interactions between them, was estimated by variation partition analysis, with Hellinger-transformed data [23]. Renyi diversity profiles were built to rank communities according to diversity [13]. Taxonomic assignments were performed by using the RDP classifier tool, set at the 50% confidence threshold [31]. Relative abundance data of phyla were regressed against the ordination scores in order to determine significant correlations with the ordination scores. All statistical analyses were made with the packages Vegan [22] and BiodiversityR [13] in the program R [24].

Results

We obtained 387,111 sequences with an average of 6,049± 3,141 sequences per sample and an average read length of 207.3 bp. These samples were aligned and clustered by the complete linkage method resulting in 19,430 clusters at the distance of 3%. Among those clusters, 8,762 are formed by singletons. Sample coverage varied between 72.4% and 92.7%, with an average coverage of 86.1±4.5%.

There was a significant correlation between community structure and soil attributes ($P<0.001$) as shown by redundancy analysis (RDA) and the first and second axes of the RDA explain, respectively, 17.5% and 6.35% of the total variation in the data. The graphic displays that bacterial communities from the same sites formed separate groups, with forest soil communities grouping separately from biofuel crop communities along the second axis (Fig. 1). Rose and UP biofuel communities were more alike and related to higher pH, Ca, and salt concentrations, as shown by their close grouping and by the vectors. On the other hand, East Lansing and LC biofuel soil communities formed a separate group associated with higher P and K concentrations and lower pH, Ca, and salt concentrations. Forest communities were correlated to higher NH_4^+ concentration and organic matter content. Among those communities, UP communities were associated with higher pH, Ca, and salt concentrations, and other forest communities are related to lower values of those variables.

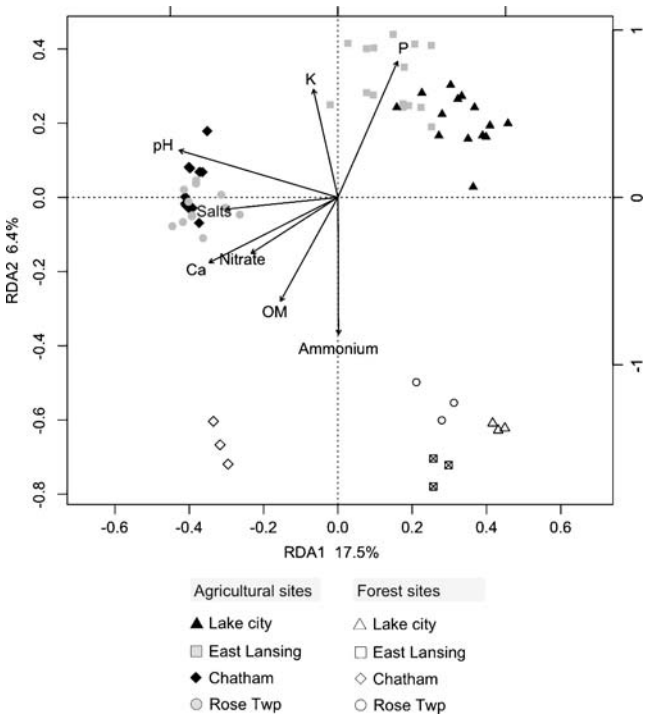

Fig. 1 Redundancy analysis for soil bacterial communities in the rhizosphere of biofuel crops in different locations in Michigan, USA. The distance of 3% was used to define operational taxonomic units. Each vector points to the direction of increase for a given variable, and its length indicates the strength of the correlation between this variable and the ordination scores

Variation partitioning analysis was used to determine the contribution of location and crop species, besides soil attributes. The variation in bacterial community structure was partitioned among soil attributes, crop, and location, as well as interactions between them; 27% of the variation was explained by those three factors, and small portions of the explained variation were explained by each of those factors alone (Fig. 2). Location, crops, and soil accounted for 3.5% ($P=0.01$), 2.1% ($P=0.01$), and 0.7% ($P=0.15$), respectively. The variation was mostly explained by interactions between location and soil attributes and crops and soil attributes, which accounted for 21.5% and 12.8%. The interaction between crop and location accounted only for 2.9% of the variation.

Twenty-four phyla were identified when all samples were considered. Among them, Acidobacteria had the highest relative abundance in rhizospheres of plants from all locations, followed by Proteobacteria, Verrucomicrobia, and Actinobacteria (Fig. 3). We estimated that between 8% and 36% of the sequences could not be assigned to any phyla by the RDP classifier. Inasmuch as the main differences were associated with location rather than crops, we determined the relative abundance of bacteria in each location, regardless of crop species. Clear differences in relative abundance among locations can be seen in Acidobacteria, Gemmatimonadetes, Chloroflexi, and WS3. Acidobacteria was more abundant in Rose and UP biofuel crop sites and in the UP forest. Gemmatimonadetes was more abundant in MSU and in LC sites and followed by Chloroflexi being more abundant in the UP and Rose sites.

Renyi diversity profiles were used to rank the sites according to diversity (Fig. 4). When using these profiles, two communities can be ranked only when their curves do not intersect. Since location and soil attributes played a greater role in defining community structure, we calculated the Renyi profiles for each location, regardless of plant species. In general, agricultural sites present higher richness, Shannon index, Simpson index, and evenness (indicated, respectively, by the alpha values of 0, 1, 2, and infinity). However, the MSU and LC sites cannot be ranked as indicated by the crossing of curves or overlapping of confidence intervals. Forest sites, however, in these two locations show greater dominance within communities than do the agricultural sites. There is no correlation between richness and diversity, as measured by the Chao and Shannon indices, respectively, and soil attributes, except for a low but significant positive correlation between diversity and pH.

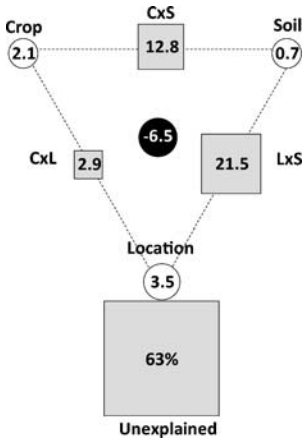

Fig. 2 Partitioning analysis of the variation in bacterial community structure into soil attributes (*S*), location (*L*), and crops (*C*) components. *Circles* on the *edges* of the *triangle* show the percentage of variation explained by each factor alone. The percentage of variation explained by interactions between two or three of the factors is shown as *squares* on the sides and as a *circle* in the *center* of the *triangle*. The unexplained variation is depicted in the *square* on the *bottom*. The *size* of *circles* and *squares* is proportional to the variance explained

Discussion

We studied bacterial communities in the rhizosphere of biofuel crops in order to assess the factors that are correlated with changes in the structure of these communities. Bacterial communities clearly differed between biofuel and forest soils. We cannot exclude that the contrast between bulk soil and rhizosphere soil is contributing to the differences observed between the forest and agricultural sites, but we still can affirm that the differentiation is due to the conversion to nonforest uses.

The biofuel sites were grouped mainly according to their location, rather than by crop species, and the correlations with several soil attributes were significant, indicating that soil type and land management were key factors in defining community structure. Even though the crop species alone was significant in determining the microbial community, its contribution was smaller if compared with interactions among locations, plant species, and the soil environment. Those interactions accounted for most of the explained variation in the studied communities, showing a complex picture of the interaction between bacterial communities and their environment. The variation that cannot be explained by the variation partitioning analysis may be accounted for by factors not measured, such as previous land use history, preexisting microbial populations, and noise.

The effect of the plant species in determining the composition of bacterial communities in the rhizosphere is documented by several authors, but there are also studies in which a strong effect of the plant species on the structure of rhizosphere communities was not observed. Our results can be included in the second case since the soil environment seems to be a more significant factor. Smalla et al. [28] describe a significant effect of plant species, in which communities in the rhizosphere of rapeseed, strawberry, and potato could be differentiated, and Marschner et al. [17] found that both plant species and soil were highly significant. On the other hand, soil type rather than plant species was the major determinant of community structure in the rhizosphere of the grasses *Lolium perenne*, *Anthoxanthum odoratum*, and *Agrostis capillaries* [27]. Nunan et al. [21] observed that topography and other uncharacterized factors were important in driving changes in the composition of bacterial communities in grassland soils. And similar to what we found, Buyer et al. [1] observed a very strong soil effect but little plant effect between corn and soybean rhizosphere communities by fatty acid analysis, indicating that the overall microbial community structure

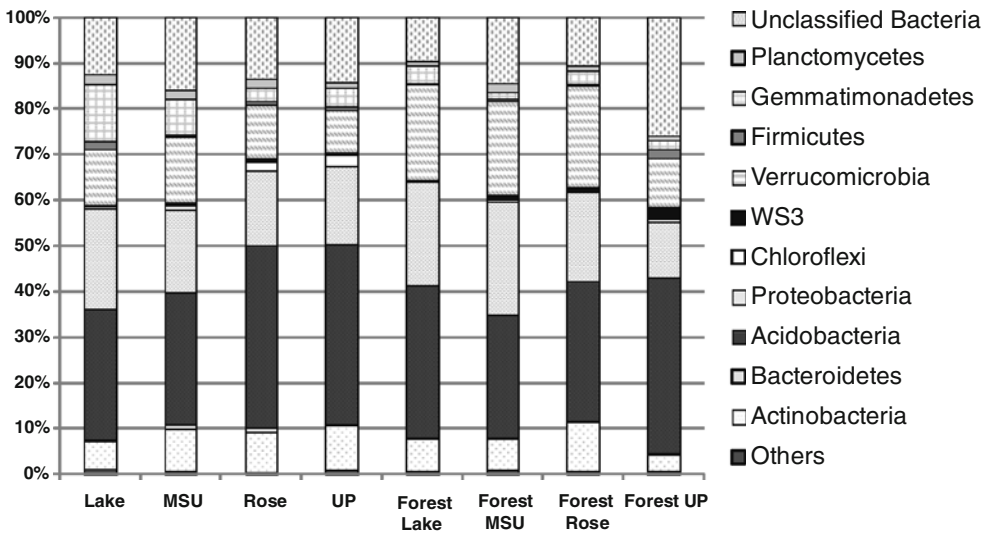

Fig. 3 Relative abundance of the most abundant bacterial phyla present in the rhizosphere of biofuel crops in different locations in Michigan. The order of the phyla in the bars and in the legend, from top to bottom is the same

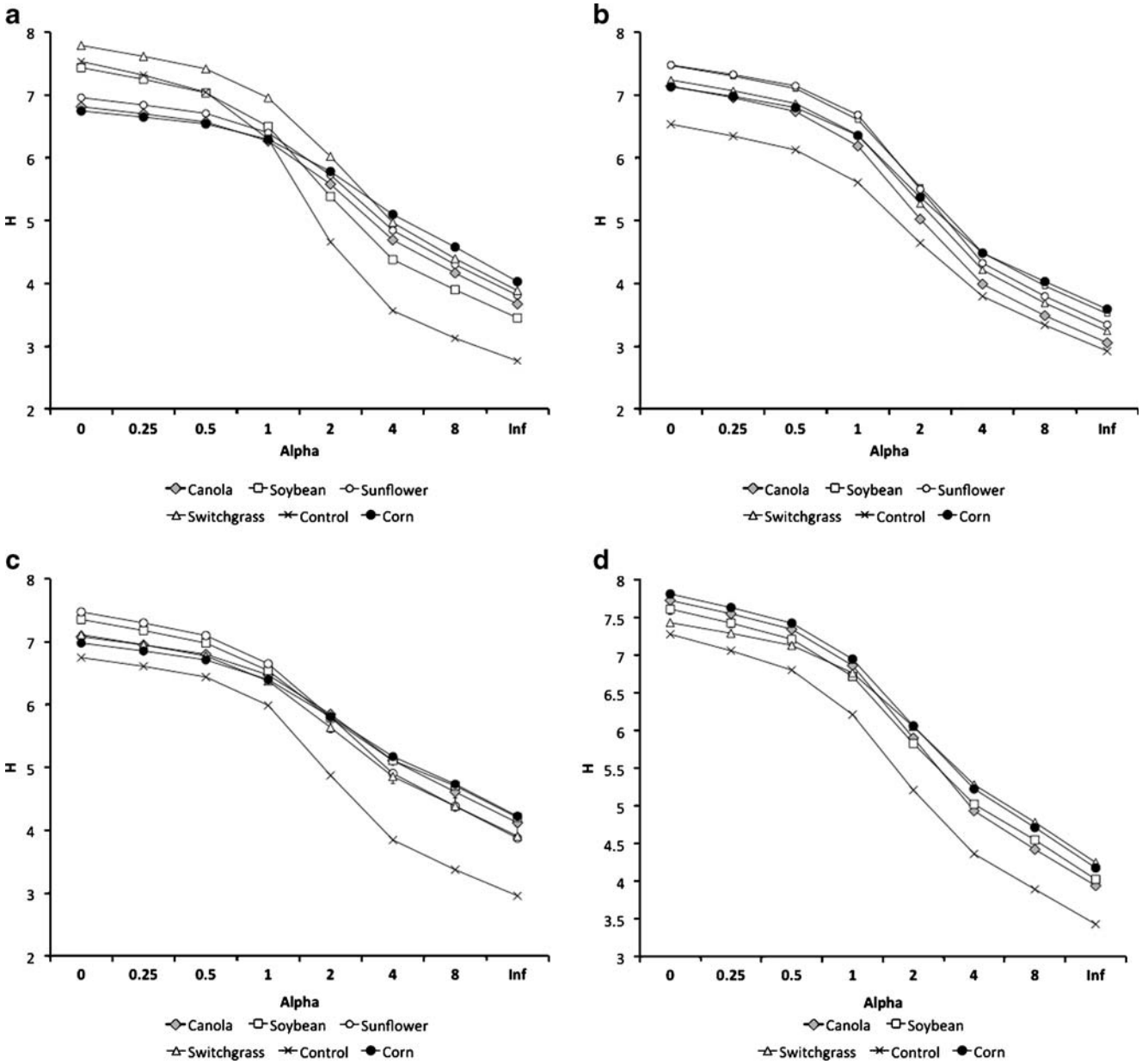

Fig. 4 Renyi diversity profiles of soil bacterial communities in **a** East Lansing, **b** Lake City, **c** Rose Township, and **d** Chatham (UP). The *x*- and *y*-axis show, respectively, the alpha value of the Renyi's formula and their associated Renyi diversity profile values (H_α). Renyi profile values at the scales of 0, 1, 2, and infinite are related to species richness, Shannon diversity index, Simpson diversity index, and Berger-Parker index, respectively

was not affected by these particular rhizospheres. The difference in these results is difficult to explain due to differences in the experimental conditions, methodologies, plant species, etc. However, they do indicate that the influence of the different factors can vary with plant growth stage, region of the roots sampled, soil type, and fertilization among others [17, 18, 28]. Besides, while our results are based in short sequences of a conserved gene, it could be that the effect of plants might be more apparent if less conserved genes were analyzed.

Acidobacteria, Proteobacteria, Verrucomicrobia, and Actinobacteria were the most abundant phyla in the rhizosphere of all plants. These phyla are frequently found in soils and are among the most dominant bacterial phyla in rhizospheres [5]. Proteobacteria and Actinobacteria have several cultivated representatives isolated from the rhizosphere, while no strains of Acidobacteria and Verrucomicrobia have yet been isolated from the rhizosphere of plants, so all the information about their presence in this environment comes from culture-independent studies [5].

Acidobacteria were the most abundant and metabolically active bacteria in the rhizosphere of chestnut tree (*Castanea crenata*), followed by Proteobacteria, suggesting that these bacteria may play an important role in this environment [14]. Singh et al. [27] also found that Acidobacteria was the most abundant phylum in the rhizosphere of the grasses *L. perenne* and *A. odoratum*, followed by Proteobacteria as the second-most abundant phylum. However, Kielak et al. [12] found that the percentage of Acidobacteria was higher in the bulk soil than in the rhizosphere. These authors attribute this finding to the assumption that Acidobacteria are oligotrophic and that the rhizosphere environment would favor fast growers due to greater C availability. Nevertheless, similar characteristics are broadly distributed among rhizosphere bacteria [5]. Also, other studies found Proteobacteria as the most abundant phyla in the rhizosphere of maize [2] and grasslands [16], which are the type of the plants we studied. One hypothesis to explain the similarity between rhizosphere and bulk soil communities, a relationship also noted by de Ridder-Duine et al. [7], is that the latter provides a massive inoculum for the former, hence causing domination of the rhizosphere community.

The differentiation between biofuel and forest sites was found also when communities are ranked according to their diversity, for which agricultural sites tend to have higher bacterial diversity, even though their plant diversity is lower. Recent results in the literature indicate that plant diversity may not be directly related to diversity in soil bacterial communities [4, 6, 9] and that soil variables may play a more important role [4, 9]. Thus, a possible explanation for the results that we observed may be that changes in the soil environment, such as the increase in pH in the agricultural sites, favor an increase in bacterial diversity.

We can conclude that interactions among soil type, soil chemistry, and crops are correlated to the changes in the structure of the studied communities and that the soil environment is one of the main factors affecting those communities. Agricultural sites have increased diversity when compared to the paired forest sites.

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References

1. Buyer JS, Roberts DP, Russek-Cohen E (2002) Soil and plant effects on microbial community structure. Can J Microbiol 48(11):955–964
2. Chelius MK, Triplett EW (2001) The diversity of archaea and bacteria in association with the roots of *Zea mays* L. Microb Ecol 41(3):252–263
3. Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, Kulam-Syed-Mohideen AS, McGarrell DM, Marsh TL, Garrity GM, Tiedje JM (2009) The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res 37:D141–D145. doi:10.1093/nar/gkn879
4. da Jesus EC, Marsh TL, Tiedje JM, Moreira FMS (2009) Changes in land use alter the structure of bacterial communities in Western Amazon Soils. ISME J 3:1004–1011
5. da Rocha UN, van Overbeek L, van Elsas JD (2009) Exploration of hitherto-uncultured bacteria from the rhizosphere. FEMS Microbiol Ecol 69(3):313–328
6. da Silveira EL et al (2006) Bacterial diversity of soil under eucalyptus assessed by 16S rDNA sequencing analysis. Pesq Agropec Bras 41(10):1507–1516
7. de Ridder-Duine AS, Kowalchuk GA, Gunnewiek PJAK, Smant W, van Veen JA, de Boer W (2005) Rhizosphere bacterial community composition in natural stands of *Carex arenaria* (sand sedge) is determined by bulk soil community composition. Soil Biol Biochem 37(2):349–357
8. Duineveld BM, Rosado AS, van Elsas JD, van Veen JA (1998) Analysis of the dynamics of bacterial communities in the rhizosphere of the chrysanthemum via denaturing gradient gel electrophoresis and substrate utilization patterns. Appl Environ Microbiol 64(12):4950–4957
9. Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci U S A 103(3):626–631
10. Gomes NCM, Heuer H, Schönfeld J, Costa R, Mendonça-Hagler L, Smalla K (2001) Bacterial diversity of the rhizosphere of maize (*Zea mays*) grown in tropical soil studied by temperature gradient gel electrophoresis. Plant Soil 232(1–2):167–180
11. Kanagawa (2003) Bias and artifacts in multitemplate polymerase chain reactions (PCR). J Biosci Bioeng 96:317–323
12. Kielak A, Pijl AS, van Veen JA, Kowalchuk GA (2009) Phylogenetic diversity of Acidobacteria in a former agricultural soil. ISME J 3(3):378–382
13. Kindt R, Coe R (2005) Tree diversity analysis. A manual and software for common statistical methods and biodiversity studies. World Agroforestry Centre (ICRAF), Nairobi
14. Lee S-H, Ka JO, Cho J-C (2008) Members of the phylum *Acidobacteria* are dominant and metabolically active in rhizosphere soil. FEMS Microbiol Lett 285(2):263–269
15. Legendre P, Gallagher ED (2001) Ecologically meaningful transformations for ordination of species data. Oecologia 129:271–280
16. MacCaig A, Glover LA, Prosser JI (1999) Molecular analysis of bacterial community structure and diversity in unimproved and improved upland grass pastures. Appl Environ Microbiol 65 (4):1721–1730
17. Marschner P, Crowley D, Yang CH (2004) Development of specific rhizosphere bacterial communities in relation to plant species, nutrition and soil type. Plant Soil 261(1–2):199–208
18. Marschner P, Yang CH, Lieberei R, Crowley DE (2001) Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biol Bioch 33:1437–1445
19. Nawrocki EP, Eddy SR (2007) Query-dependent banding (QDB) for faster RNA similarity searches. PLoS Comput Biol 3:e56
20. Nazih N, Finlay-More O, Hartel PG, Fuhrmann JJ (2001) Whole soil fatty acid methyl ester (FAME) profiles of early soybean rhizosphere as affected by temperature and matric water potential. Soil Biol Biochem 33(4–5):693–696
21. Nunan N, Daniell TJ, Singh BK, Papert A, McNicol JW, Prosser JI (2005) Links between plant and rhizoplane bacterial communities in grassland soils, characterized using molecular techniques. Appl Environ Microbiol 71(11):6784–6792
22. Oksanen J, Kindt R, Legendre P, O'Hara RB (2007) Vegan: community ecology package version 1.8-6. http://cran.r-project.org/

23. Peres-Neto PR, Legendre P, Dray S, Borcard D (2006) Variation partitioning of species data matrices: estimation and comparison of fractions. Ecology 87:2614–2625
24. R Development Core Team (2007) R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2007. http://www.R-project.org
25. Rothberg JM, Leamon JH (2008) The development and impact of 454 sequencing. Nat Biotechnol 26(10):1117–1124
26. Schmer MR, Vogel KP, Mitchell RB, Perrin RK (2008) Net energy of cellulosic ethanol from switchgrass. Proc Natl Acad Sci U S A 105(2):464–469
27. Singh BK, Munro S, Potts JM, Millard P (2001) Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. Appl Soil Ecol 36:147–155
28. Smalla K, Wieland G, Buchner A, Zock A, Parzy J, Kaiser S et al (2001) Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis-plant-dependent enrichment and seasonal shifts revealed. Appl Environ Microbiol 67 (10):4742–4751
29. Smith SL (2009) Bioenergy cropping systems on marginal land. Thesis, Michigan State University
30. Swift MJ, Izac A-MN, van Noordwijk M (2004) Biodiversity and ecosystem services in agricultural landscapes—are we asking the right questions? Agricult Ecosys Environ 104:113–134
31. Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73 (16):5261–5267