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Soil bacteria and archaea found in long-term corn (*Zea mays* L.) agroecosystems in Quebec, Canada

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[1]*Department of Natural Resource Sciences, Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, Québec, Canada H9X 3V9;* [2]*National Research Council, Biological Research Institute, 6100 Royalmount Avenue, Montreal, Quebec, Canada; and* [3]*Department of Bioresource Engineering, Macdonald Campus, McGill University, 21,111 Lakeshore Road, Ste-Anne-de-Bellevue, Quebec, Canada.*

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Sheibani, S., Yanni, S. F., Wilhelm, R., Whalen, J. K., Whyte, L. G., Greer, C. W. and Madramootoo, C. A. 2013. **Soil bacteria and archaea found in long-term corn (*Zea mays* L.) agroecosystems in Quebec, Canada**. Can. J. Soil Sci. **93**: 45–57. The soil microbial community controls all biological processes in soils and is considered a good indicator of general soil health. Assessment of the microbial community in intensively cropped soils that are under reduced tillage management is especially important because the microbes are the primary decomposers of the high residue input in such systems. We investigated the microbial biomass and diversity of bacteria and archaea in a sandy-loam Dystric Gleysol from a long-term (15 yr) corn (*Zea mays* L.) agroecosystem in Quebec, Canada, under conventional (CT), reduced tillage (RT), and no tillage (NT) and two residue inputs (high level: +R and low level: −R). Analysis included microbial biomass C and N (MBC, MBN), catalyzed reporter deposition-fluorescence in situ hybridization (CARD-FISH) and 5-(4, 6-dichlorotriazinyl) amino fluorescein hydrochloride (DTAF) cell counts, 16S rRNA polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and an archaeal clone library. The PCR-DGGE analysis identified Proteobacteria, Actinobacteria and Firmicutes as dominant groups in all tillage and residue management treatments. The archaeal group was diverse, with most individuals identified as belonging to the Crenarchaeota phylum. We also detected soil archaea belonging to the newly proposed phylum Thaumarchaeota, the chemolithoautotrophic ammonia-oxidizing archaeota, in a corn agroecosystem in Quebec, Canada. Microbial biomass increased in the +R treatment according to MBC concentration and direct cell counts. Considering results from the CARD-FISH counts (bacterial and archaeal cell counts without fungal cells) and from MBC results (all microbial biomass including fungi) we concluded the likelihood of greater fungal biomass in the NT plots.

Key words: Archaeal phylogeny, conservation tillage, corn agroecosystem, residue management, soil microbial biomass

Sheibani, S., Yanni, S. F., Wilhelm, R., Whalen, J. K., Whyte, L. G., Greer, C. W. et Madramootoo, C. A. 2013. **Bactéries et archaebactéries du sol dans les écosystèmes agricoles associés à la culture prolongée du maïs (*Zea mays* L.) au Québec (Canada)**. Can. J. Soil Sci. **93**: 45–57. La microflore du sol contrôle les processus biologiques dans le sol et on estime qu'elle donne une bonne idée de la santé générale du sol. Il est particulièrement important d'évaluer la microflore des sols exploités de manière intensive, mais peu travaillés, car les microorganismes sont ceux qui décomposent le plus les résidus abondants qu'on trouve dans les systèmes de ce genre. Les auteurs ont examiné la biomasse microbienne ainsi que la diversité des bactéries et des archaebactéries dans un écosystème agricole constitué d'un gleysol dystrique de type loam sablonneux utilisé pour la culture à long terme (15 ans) du maïs (*Zea mays* L.), au Québec (Canada). Le sol avait été travaillé, travaillé de façon réduite ou non travaillé pour deux apports de résidus (élevé : +R et faible : −R). Pour les analyses, on a recouru au dosage du C et du N de la biomasse microbienne (BMC, BMN), à la numération des cellules par dépôt de rapporteur catalysé-hybridation *in situ* en fluorescence (CARD-FISH) et avec le 5-(4, 6-dichlorotriazinyl) amino fluorescein hydrochloride (DTAF), à l'application de la réaction en chaîne de la polymérase-électrophorèse en gradient de dénaturation (PCR-DGGE) à l'ARNr 16S et à une bibliothèque de clones d'archaebactéries. L'analyse par PCR-DGGE a permis d'établir que les protéobactéries, les actinobactéries et les firmicutes sont les groupes dominants dans tous les cas où il y a travail du sol et gestion des résidus. Les archaebactéries sont diversifiées, la plupart des spécimens identifiés appartenant au phylum Crenarchaeota. Les auteurs ont également dépisté des archaebactéries telluriques appartenant au nouveau phylum Thaumarchaeota, les archaeota chimiolithoautotrophes qui oxydent l'ammoniaque, dans un écosystème agricole du maïs au Québec (Canada). Le traitement +R augmente la biomasse microbienne, si l'on se fie à la

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Abbreviations: **CARD-FISH**, catalyzed reporter deposition-fluorescence in situ hybridization; **CT**, conventional tillage; **DTAF**, 5-(4, 6-dichlorotriazinyl) amino fluorescein hydrochloride; **MBC**, microbial biomass carbon; **MBN**, microbial biomass nitrogen; **NT**, no tillage; **PCR-DGGE**, polymerase chain reaction-denaturing gradient gel electrophoresis; **−R**, residue removed **+R**, residue retained; **RT**, reduced tillage; **TOC**, total organic carbon

concentration de BMC et à la numération directe des cellules. Compte tenu des résultats obtenus avec la technique CARD-FISH (numération de bactéries et d'archaebactéries sans les cellules fongiques) et de la teneur en BMC (biomasse microbienne totale, avec les cellules fongiques), les auteurs pensent que la biomasse fongique est plus importante dans les parcelles au sol non travaillé.

Mots clés: Phylogénie des archaebactéries, travail de conservation du sol, écosystème agricole associé au maïs, gestion des résidus, biomasse microbienne du sol

The soil microbial community controls all the biological processes that define soil health and quality, soil organic matter content and quality, nutrient availability and cycling, greenhouse gas emissions, and stabilization of carbon (C) in the soil. Soil microorganisms can be useful indicators of soil health and sustainability (Doran and Zeiss 2000) and can be assessed by parameters such as microbial abundance and diversity in a given soil. Being the primary decomposers of organic matter and plant litter, their function is expected to be more important in low impact management systems that promote soil sustainability such as reduced tillage and increased residue input. In such cases, the decomposition function of the microbial community largely determines the availability of nutrients for the crop. The assessment of the microbial community in intensively cropped soils is pertinent, especially with the increase in the adoption of reduced tillage management systems in recent years. Corn (*Zea mays* L.) for grain and fodder, produced on about 416 000 ha in Quebec, Canada (Statistics Canada 2011a, b), constitutes about 20% of crop land in the province. Despite this, there is a paucity of information on the microbial community in corn fields, particularly of the archaeal community that likely contributes to ammonia oxidation in the soil nitrogen (N) cycle (Martens-Habbena et al. 2009).

Tillage systems have a direct effect on soil structure and soil organic matter dynamics (Vyn and Raimbault 1993; Paustian et al. 1997), which are expected to impact the soil microbial biomass, community structure and diversity. Low-impact systems include reduced tillage (RT) that is cultivated by a harrow to a shallower depth and less frequently than the conventional system (CT), and the no-till (NT) system that is minimally disturbed with the residue left intact on the soil surface. These tillage systems provide a good opportunity to investigate the soil microbial community because of their contrasting habitats that might support certain types of organisms more than others. Microbial biomass and diversity are reported to be greater in RT and NT systems compared with CT (Granatstein et al. 1987; Lupwayi et al. 1998; Govaerts et al. 2007; Van Groenigen et al. 2010; Lupwayi et al. 2012) as a result of less disturbance and more residue left after harvesting. Moreover, the reported differences between these systems are usually detected in the top 0–5 cm layer of soil (Lupwayi et al. 2004) where the difference in residue availability between CT and conservation tillage (i.e., RT and NT systems) is most pronounced.

The objective of this study was to investigate the abundance of soil microorganisms and identify the dominant bacteria and archaea in a corn agroecosystem with long-term tillage and residue management treatments in an intensively cropped corn field in Quebec. The effects of tillage and residue management on the microbial abundance were also assessed. The dominant bacteria and archaea were detected with 16S rRNA PCR-DGGE and an archaeal clone library while microbial abundance was assessed with CARD-FISH, direct microscopic cell counts, and microbial biomass by chloroform fumigation–direct extraction.

MATERIALS AND METHODS

Site Description

The experimental site was located at the Macdonald Research Farm of McGill University in Ste-Anne de Bellevue, Quebec, Canada (lat. 45°30′N, long. 73°35′W, elevation 35.7 m). Mean annual temperature is 6.2°C with 979 mm of precipitation, based on climate data from the nearby Pierre Elliott Trudeau Airport in Dorval, Quebec, from 1971 to 2000 (Environment Canada 2010). The soil was a Dystric Gleysol of the St-Amable and Courval series with a sandy-loam texture (815 g kg^{-1} sand, 89 g kg^{-1} silt, and 96 g kg^{-1} clay) and 19.9 g organic-C kg^{-1} in the top 20 cm. Additional site description was provided by Burgess et al. (1996) and Dam et al. (2005). The site where the soil was sampled was designed as a factorial experiment with three types of tillage (NT, RT and CT) and two levels of residue input [low: −R, <15 cm of corn stalk plus roots; and high: +R, received corn stover (leaves, stalks) and roots, which accounted for an extra 8.2 to 9.8 Mg ha^{-1} of residue (dry matter basis) in 2006 and 11.3 to 12.2 Mg ha^{-1} of residue (dry matter basis) in 2007; Halpern et al. 2010]. There were three replicates of each treatment, giving 18 experimental plots in total. The site was plowed in the spring of 1991 when the agronomic study was established and planted with continuous corn; details of all cultivation, fertilization and herbicide applications is detailed in Burgess et al. (1996), Dam et al. (2005) and Halpern et al. (2010).

Community DNA Extraction and Amplification of 16S rRNA Genes and DGGE Procedures

The composition of the soil bacterial and archaeal communities was investigated with 16S rRNA gene-targeted PCR-DGGE on soil samples (0–5 cm depth) collected in October 2006, May 2007, August 2007 and

October 2007. Soils were kept at −20°C until analysis. The PCR primers, mixtures (also see Whyte and Greer 2005) and operating conditions are summarized in Table 1. Soil DNA was extracted from 1 g of field-moist, frozen soil using the UltraClean™ Soil DNA Kit (Mobio Laboratories, Solana Beach, CA) according to manufacturer instructions. The extracted DNA was diluted 10-fold, and 5 μL of 1:10 DNA dilution containing 50–90 ng DNA (quantified with a NanoDrop ND–1000 spectrophotometer, NanoDrop Technologies, Wilmington, DE) was used as a template DNA for bacterial and archaeal 16S rRNA genes amplification. Primers used in this study were purchased from MWG-Biotech (Huntsville, AL) and prepared as 10 μM solutions in PCR water. All PCR reactions were achieved in 50 μL volumes by adding PCR water to the PCR mixture.

For DGGE analysis, 800 ng of bacterial and archaeal amplicons were applied to an 8% (wt/vol) acrylamide gel containing a 35 to 65% (bacteria and archaea) or 40 to 60% (bacterial group specific) denaturing gradient, as described by Perreault et al. (2007). All DGGE analyses were performed in two replicates to ensure reproducibility of results. Comparisons of DGGE banding patterns were made with GelCompar software (www.applied-maths.com) by constructing dendrograms with the UPGMA method for grouping and the Jaccard coefficient of similarity.

Selected DGGE bands (120 in total, 67 sent for sequencing) were excised from the bacterial and archaeal gels with a cutting tip (DiaMed Laboratory, Mississauga, ON) and eluted in 50 μL of water at 4°C overnight and re-amplified with the appropriate corresponding bacteria and archaea primers (Table 1) without the guanine-cytosine (GC) clamps to optimize the PCR reaction. The PCR products from bacterial DGGE were purified with a Qiagen gel purification kit (Mississauga, ON) to remove salts, dNTPs, primers, and enzymes following the manufacturer's protocol, cloned with the pGEM-T Easy vector system (Promega, Madison, WI) following Steven et al. (2007) and sent for sequencing at the Laval University Bioinformatics Centre. No bands were excised from α- Proteobacteria and β-Proteobacteria DGGE gels for sequencing. The archaeal 16S rRNA fragments were sequenced directly without cloning at the Laval University Bioinformatics Centre.

The 16S rRNA gene fragments in isolated DGGE bands and clones were identified by comparing to known DNA sequences in the NCBI, GenBank database using the BLASTn algorithm (Altschul et al. 1990). Taxonomic affiliations of sequences were also determined using the RDP classifier function of the Ribosomal Database Project-II release 9 with a confidence threshold of 80% (http://rdp.cme.msu.edu/classifier). Nucleotide sequences from this study were submitted to the GenBank database under accession numbers JF303758-JF303854.

Phylogenetic Analysis of Archaeal 16S rRNA Clone Library

An archaeal clone library was constructed to help identify individual populations in the community, which was expected to complement and confirm the identification obtained from DGGE sequences. An RT+R soil sample collected during the growing season (August 2007) was selected because the DGGE profile showed that this sample harbored high archaeal diversity, relative to all other samples. The library was constructed as described by Steven et al. (2007), using the 751F and 1406R primers (Patricia et al. 2006). For restriction fragment length polymorphism (RFLP), a double digestion of PCR amplified clone DNA was performed using the 4-mer restriction endonucleases *Rsa*I and *Hha*I (Invitrogen, Burlington, ON). Digestion reactions were composed of 7.6 μL of PCR water, 0.3 μL of each of 10 U μL^{-1} *Rsa*I and *Hha*I, 1.8 μL of 10 × REact I buffer (50 mM Tris-HCl (pH 8.0) and 10 mM $MgCl_2$) (Invitrogen, Carlsbad, CA), and 8 μL of PCR product. Reactions were incubated at 37°C for 3 h. To inactivate the enzymes, reactions incubated further at 65°C for 20 min. Electrophoresis was carried out using a Sub-Cell Model 96 Agarose Gel Electrophoresis System (Bio-Rad Laboratories, Hercules, CA) for 3 h at 60 V in 1 × TAE buffer. Gels were stained by adding 1 μL of ethidium bromide (EtdBr) to the gel directly. Gels were viewed under UV light (Bio-Rad Laboratories, Hercules, CA) and images were captured with the associated GeneSnap software (Synege, Fredrick, MD). A Neighbor-Joining tree was constructed from a ClustalW alignment (across 251 bp of shared sequence) using MEGA software (version 5.05) (Tamura et al. 2011), included bootstrap analysis with 10 000 replications. Rarefaction analysis, library coverage and diversity indices were performed using the DOTUR program (computer algorithms for defining operational taxonomic units and estimating species richness) at 95% sequence similarity (Schloss and Handelsman 2005).

Microbial Biomass and Direct Counts of Bacteria and Archaea

Composite soil samples (about 300 g subsamples, from five randomly selected locations within the plot) were taken, from the 0–5 cm soil layer, after corn harvest, before fall tillage operations in October 2006 and October 2007 for microbial biomass by chloroform fumigation–direct extraction (kept at +4°C and processed within 2–7 d; we assumed that microbial cell growth during storage was uniform across treatments). Samples for CARD-FISH and direct cell counts were taken in October 2006 and May, August, and October 2007 and transferred immediately to a freezer at −20°C until analysis.

The chloroform fumigation–direct extraction method (Voroney et al. 2008) was used to assess microbial biomass C and N concentrations. Paired subsamples were either directly extracted with 0.5 M K_2SO_4

Table 1. Description of PCR primers and condition used in this study

No.	PCR type	Primers	PCR mixture	PCR condition
1	Bacteria 16S rRNA: regular PCR	Universal primers: 341F+GC/758R (Steven et al. 2008)	5 μL PCR 10 × buffer with 1.5 mM $MgCl_2$, (Qiagen Canada), 0.5 μL BSA 10%, 1 μL of 10 mM dNTPs mixture, 2.5 μL of each 10 μM primer (341F+GC/758R), 0.2 μL of HotStar *Taq* DNA Polymerase (5U μL^{-1}) (Qiagen, Canada) and 5 μL of template DNA	Denaturation: 95°C (15 min), 10 touchdown cycles (65–55°C), 25 cycles at 55°C (30 s), 72°C (1 min and 30 s), final extension: 72°C (10 min)
2	Bacteria 16S rRNA: nested PCR	α- Proteobacteria primers: Alf28F/Alf684R (Muhling et al. 2008)	5 μL PCR 10 × buffer with 1.5 mM $MgCl_2$, 0.5 μL BSA 10%, 1 μL of 10 mM dNTPs mix, 1 μL of each 10 μM primer (Alf28F/Alf684R), 0.2 μL of HotStar *Taq* DNA Polymerase (5U μL^{-1}) and 5 μL of template DNA	First PCR: 95°C (15 min), 35 cycles at 95°C (1 min), annealing temperature of 65°C (1 min), 72°C (1 min), final extension: 72°C (10 min) Second PCR: 5 μL of group specific PCR products (dilution 1:100) were used as template in a re-PCR with 341F+GC/518R primers, under same PCR conditions (except annealing temperature was 55°C)
3	Bacteria 16S rRNA: semi-nested PCR	β-Proteobacteria primers: Beta359F/Beta682R (Muhling et al. 2008)	5 μL PCR 10 × buffer with 1.5 mM $MgCl_2$, 0.5 μL BSA 10%, 1 μL of 10 mM dNTPs mix, 1 μL of each 10 μM primer (Beta359F/Beta682R), 0.2 μL of HotStar *Taq* DNA Polymerase (5U μL^{-1}), and 5 μL of template DNA	First PCR: 95°C (15 min), 35 cycles at 95°C (1 min), annealing temperature of 60°C (1 min), 72°C (1 min), final extension: 72°C (10 min) Second PCR: 5 μL of group specific PCR products (dilution 1:100) were reamplified using the primer pair 518F+GC/Beta682R, under same PCR conditions (except annealing temperature was 57°C)
4	Archaea 16S rRNA: Semi-nested PCR	First PCR: 109F (Bottos et al. 2008) /934R (Perreault et al. 2007) Second PCR: 344F+GC/934R (Bottos et al. 2008)	First and second PCR as describe for No.1 except for the 0.5 μL of HotStar *Taq* DNA Polymerase (5U μL^{-1})	First PCR: denaturation: 95°C (15 min), 35 cycles at 95°C (1 min), annealing: 60°C (1 min), extention: 72°C (1 min), final extension: 72°C (10 min) Second PCR: as the first PCR except for annealing temperature at 57°C (1 min)

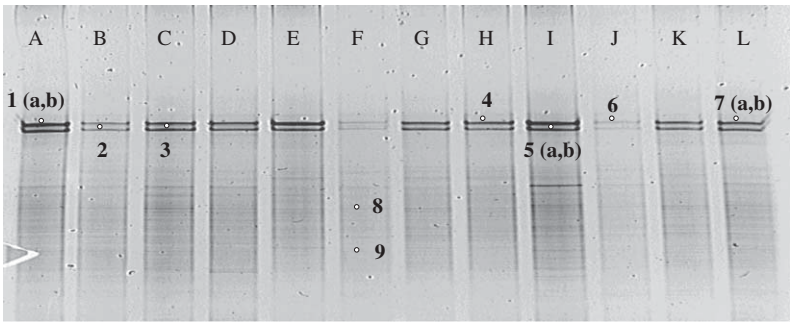

Fig. 1. A representative denaturing gradient gel electrophoresis (DGGE) analysis of Bacterial 16S rRNA genes amplified from the following samples: RT+R (0–5 cm): A, after harvest (Nov. 2006); B, in spring (May 2007); C, during growing season (Aug. 2007); D, after harvest (Oct. 2007). RT+R (5–20 cm): E, Nov. 2006; F, May 2007; G, Aug. 2007; H, Oct. 2007. NT–R (5–20 cm): I, Nov. 2006; J, May 2007; K, Aug. 2007; L, Oct. 2007. DGGE was run on an 8% acrylamide gel with a gradient of urea and formamide from 35 to 65%.

(1:4 soil:extractant) or kept at 20°C and fumigated with chloroform for 5 d before extraction. Analytical blanks (empty beakers without soil) were used to correct the baseline C and N concentrations. Microbial biomass C was the difference in total organic carbon (TOC, determined using a Shimadzu TOC-V analyzer, Shimadzu Corporation, Kyoto, Japan) concentration of fumigated and un-fumigated extracts divided by an efficiency factor $k_{EC} = 0.45$ (Joergensen 1996). For MBN, the fumigated and un-fumigated extracts underwent persulfate digestion (Cabrera and Beare 1993) and colorimetric analysis for NH_4-N and NO_3-N using a Lachat Quik Chem AE flow injection autoanalyzer (Lachat Instruments, Milwaukee, WI). Microbial biomass N was the difference in mineral N concentration between fumigated and un-fumigated extracts, divided by the efficiency factor $k_{EN} = 0.54$ (Joergensen and Mueller 1996).

The CARD-FISH was performed as described by Niederberger et al. (2010) using the probes EUB338 and ARCH915 (Dijk 2008) with the following modifications: we used 0.25 g (dry weight basis) of frozen, field-moist soil. Fixed samples were diluted a 1000 times using sodium phosphate buffer, dispersed by ultrasound at 300 J mL^{-1} for 20 s and then filtered. No proteinase K digestion was performed. The filter was viewed using a Nikon Eclipse E600 microscope at 1000 × magnification. Bacterial or archaeal cells, stained green against the red background, were counted in a minimum of 10 fields of view for each sample and their numbers calculated following the procedure of Bhupathiraju et al. (1999).

Direct microbial counts were also measured by fluorescence microscopy after staining with 5-(4, 6-dichlorotriazinyl) amino fluorescein hydrochloride (DTAF) following the procedure of Steven et al. (2007). Soil fixation, sonication and bacterial enumeration were performed as mentioned above for CARD-FISH analysis.

Statistical Analysis

As the field experiment was arranged in a completely randomized design with one replicate per block, the

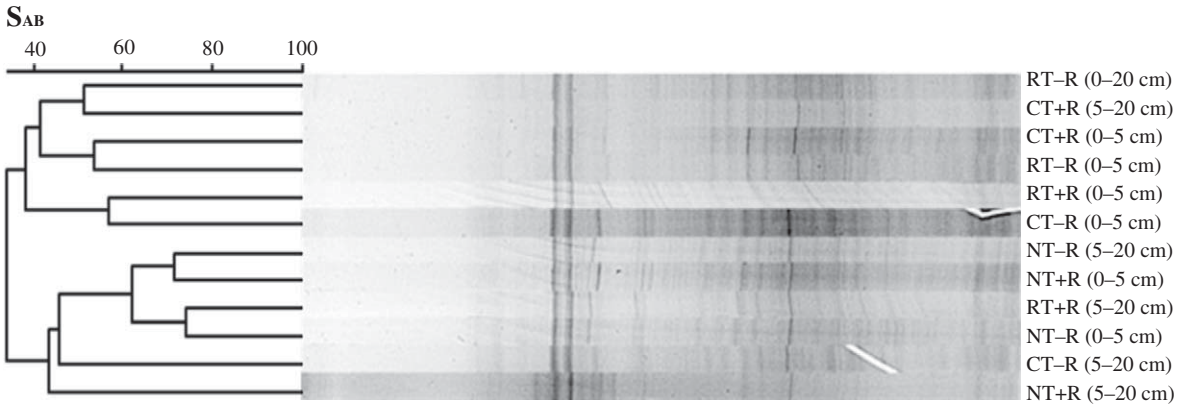

Fig. 2. Dendrogram of denaturing gradient gel electrophoresis (DGGE) of Bacterial 16S rRNA communities for the samples collected during the growing season (Aug. 2007). A gradient runs from left to right at 40–60% on 8% acrylamide for Bacterial gel. The scale of the dendrogram was given as percent of similarity.

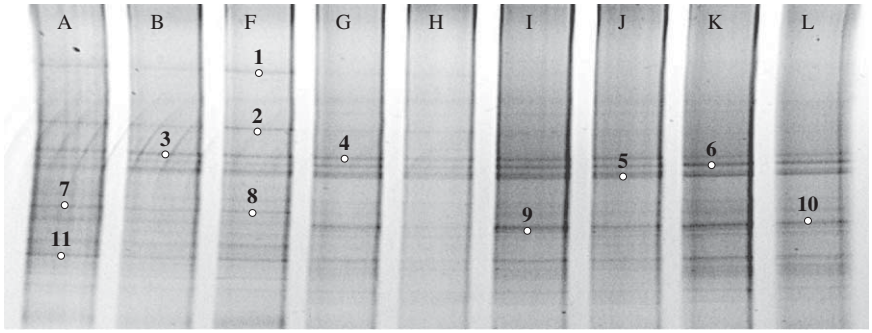

Fig. 3. A representative denaturing gradient gel electrophoresis (DGGE) analysis of Archaeal 16S rRNA genes amplified from samples collected during the growing season (Aug. 2007). A: RT + R (0–5 cm); B: RT + R (5–20 cm); F: NT + R (5–20 cm); G: CT + R (0–5 cm); H: CT + R (5–20 cm); I: RT–R (0–5 cm); J: RT–R (5–20 cm); K: CT–R (0–5 cm); L: CT–R (5–20 cm). DGGE was run on an 8% acrylamide gel with a gradient of urea and formamide from 35 to 65%.

main effects of the residue, tillage and block and the tillage by residue interaction, on microbial biomass C and N, CARD-FISH and direct cell counts were tested using the Proc GLM procedure on SAS statistical software (SAS Institute, Inc. 2009). All data were tested for normality prior to analysis of variance. Least square means with the Tukey adjustment were calculated and significance reported at the 95% confidence level ($P < 0.05$).

RESULTS AND DISCUSSION

Bacteria and Archaeal Analysis by PCR-DGGE

Bacterial universal primers, 341F-GC and 758R (Table 1), amplified a ~400 bp region of the Bacterial 16S rRNA gene, which included a ~40 bp GC clamp. Visual observation of DGGE profiles showed many similarities in banding pattern among all of the analyzed samples from two different depths (Fig. 1). This pattern was repeated over the four sampling dates with no noticeable changes. The banding patterns indicated that the structure of the bacterial community at different depths was fairly constant and it did not change significantly as a consequence of tillage and residue treatments. Most of the observed bands seemed to be shared between samples and no major bands were observed to appear or disappear (Fig. 1). All DGGE profiles were characterized by the presence of two strong bands residing in the low gradient region and a larger number of weaker bands (Fig. 1). Dendrogram analysis was used to compare the banding patterns The bacterial community differentiated either into two or three clusters (Fig. 2); cluster analyses for the samples collected after harvest (November 2006) and the following spring (May 2007) showed that DGGE banding patterns divided into two clusters based on their depth (0–5 cm and 5–20 cm). The similarity index (S_{AB}) value between the two depths of sampling was low (S_{AB}: 34% and 19.7% respectively), indicating distinctly different communities (Fig. 2). For samples collected during the growing season (August 2007) and after harvest in October 2007, three groups were observed with low similarity index (S_{AB}: 17% and 14%, respectively, Fig. 2). Group-specific primers (α- and β-Proteobacteria) provided a similar banding pattern, but fewer bands, than the bacterial universal primers (data not shown).

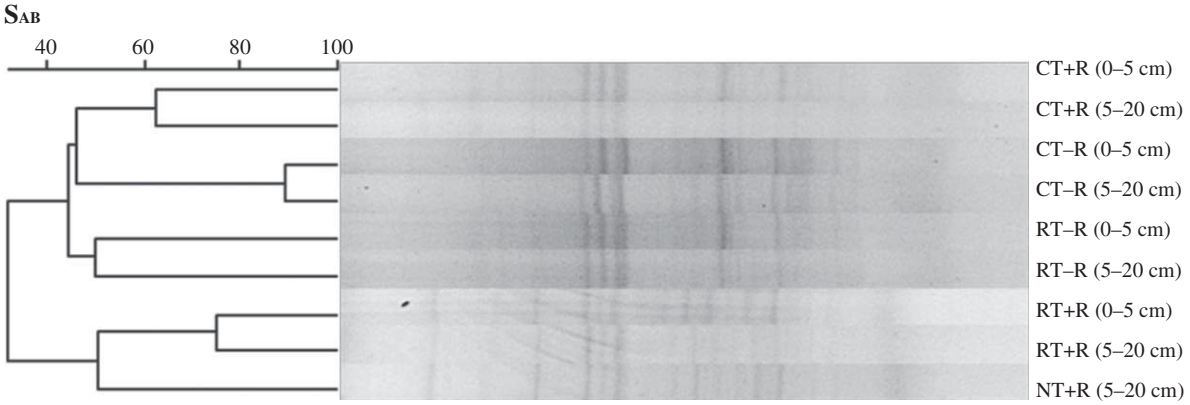

Fig. 4. Dendrogram of denaturing gradient gel electrophoresis (DGGE) of Archaeal 16S rRNA communities. A gradient runs from left to right at 40–60% on 8% acrylamide for Archaeal gel. The scale of the dendrogram was given as percent of similarity.

Table 2. Microbial biomass C and N concentrations, CARD-FISH bacterial and archaeal cell counts, and DTAF total counts, in the 0–5 cm depth of plots under continuous corn production with tillage and residue input treatments. Values are the mean ± standard error ($n = 6$ for tillage effect and $n = 9$ for residue effect)

Treatment	MBC (mg C kg^{-1})	MBN (mg N kg^{-1})	MBC (mg C kg^{-1})	MBN (mg N kg^{-1})	Bacterial CARD-FISH (cells g^{-1} soil)	Archaeal CARD-FISH (cells g^{-1} soil)	DTAF total cell counts (cells g^{-1} soil)
	2006				*2007*		
NT	342 ± 43.2*a*	58 ± 9.7	351 ± 36.7*a*	63.4 ± 22.6	$7.50 \times 10^8 \pm 3.74 \times 10^7 a$	$7.97 \times 10^7 \pm 2.94 \times 10^6 a$	$3.08 \times 10^8 \pm 1.65 \times 10^7 a$
RT	244 ± 66.1*ab*	32 ± 10	290 ± 47.3*ab*	43.3 ± 10.2	$9.15 \times 10^8 \pm 4.02 \times 10^7 b$	$8.61 \times 10^7 \pm 4.78 \times 10^6 a$	$4.26 \times 10^8 \pm 1.93 \times 10^7 b$
CT	130 ± 5.63*b*	36 ± 3.0	201 ± 16.3*b*	20.4 ± 2.80	$7.06 \times 10^8 \pm 5.14 \times 10^7 a$	$6.23 \times 10^7 \pm 5.77 \times 10^6 b$	$3.20 \times 10^8 \pm 4.23 \times 10^7 a$
−R	175 ± 31.1*a*	34 ± 7.3	232 ± 27.2*a*	24.2 ± 2.95	$7.51 \times 10^8 \pm 5.34 \times 10^7$	$7.29 \times 10^7 \pm 6.19 \times 10^6$	$3.06 \times 10^8 \pm 2.67 \times 10^7 a$
+R	302 ± 65.2*b*	50 ± 10	330 ± 34.9*b*	60.5 ± 15.7	$8.29 \times 10^8 \pm 3.38 \times 10^7$	$7.92 \times 10^7 \pm 3.39 \times 10^6$	$3.97 \times 10^8 \pm 2.19 \times 10^7 b$
					Treatment effects		
Tillage	$P = 0.0042$**	NS	$P = 0.0080$**	NS	$P = 0.0063$**	$P = 0.0074$**	$P = 0.0005$**
Residue	$P = 0.0085$**	NS	$P = 0.0096$**	NS	NS	NS	$P = 0.0040$**
Tillage × Residue	NS	NS	NS	NS	NS	NS	$P = 0.0114$*

a, *b* Within a column, values with the same letter within a treatment (tillage, residue input) did not differ significantly at $P < 0.05$ (Tukey test).
*, ** Treatment effects significant at $P < 0.05$ and $P < 0.01$, respectively; NS, non-significant.

The PCR-DGGE analysis of Archaeal 16S rRNA PCR-DGGE revealed 6 to 15 bands of relatively uniform intensity and with similar banding patterns (Fig. 3). There were more bands during the growing season (August 2007) than in spring (May 2007) or after harvest (November 2006 and October 2007), but cluster analysis did not reveal clear trends with respect to 16S rRNA sequences in the various treatments or at different sampling dates (Fig. 4).

Bacteria and Archaeal Diversity

Visualization limitations prevented the physical isolation of all bands in the DGGE gels, but bacterial 16S rRNA gene sequences of 38 bands were compared with known sequences in GenBank using the BLAST algorithm. This analysis showed that bands belonged to the phyla Proteobacteria (25 bands), Actinobacteria (7 bands), Firmicutes (3 bands) and Acidobacteria (2 bands), which are common in agricultural soils (Table 3). Proteobacteria and actinobacteria are usually reported to be amongst the most abundant phyla in soils (e.g., Amann et al. 1995; Buckley and Schmidt 2001; Valinsky et al. 2002; Upchurch et al. 2008). Proteobacteria are a major phylum in soil, with high morphological, physiological and metabolic diversity that permits them to participate in global carbon, nitrogen and sulfur cycling; this group includes N_2 fixing and ammonia oxidizing bacteria (Kersters et al. 2006). Soil actinobacteria, mostly aerobic, are known to be important decomposers, particularly of cellulose, the most abundant material in plant litter (Upchurch et al. 2008). Some members of the Actinobacteria form branching filaments similar to fungal mycelia, which in theory may make them susceptible to disturbance by tillage, similar to fungi (Upchurch et al. 2008). The Firmicutes are also abundant in soils, with aerobic and anaerobic free-living or pathogenic members; some are decomposers of organic matter and many are resilient because of their ability to survive extreme dry conditions by endospore formation (Cleveland et al. 2007; Ludwig et al. 2009). The functions of the newly established phylum, the Acidobacteria, are not yet fully clear but they are diverse, ubiquitous and abundant in soils and therefore are thought to have important ecological functions (Barns et al. 1999; Quaiser et al. 2003).

There were no major differences between the profiles with respect to tillage and residue management treatments at the four sampling dates in this study, which is consistent with some reports that showed no effect of tillage and residue management on microbial diversity (e.g., Buckley and Schmidt 2001; Helgason et al. 2010) but is in contrast with others such as Peixoto et al. (2006), Garbeva et al. (2006) and Upchurch et al. (2008), which showed bacterial diversity to be affected by tillage and residue inputs. Since diverse bacterial phyla were detected across tillage and residue treatments, it suggests that bacteria were either minimally affected by tillage and residue inputs, or had the ability to recover quickly

Table 3. 16S rRNA gene analyses of isolated bands from bacteria DGGE. Sequences were compared with known sequences in GenBank using the BLAST algorithm. Phylogenetic classification of sequences was determined using the RDP classifier function of the Ribosomal Database Project-II release 9

Band	Closest BLAST match	Origin of BLAST match	Similarity to BLAST match	RDP grouping
1a	Bacterium KMS200711 068 (EU881327)	Maize cropland soil	96% (358/372)	Polyangiaceae 100% (family)
1b	Soil bacterium 4M1-E07 (EU052014)	Savanna soil	96% (391/408)	Bacteria 100% (domain)
2	Bacterium FFCH8343 (EU133494)	Undisturbed soil	99% (406/411)	Rhizobiales 99% (order)
3	Soil bacterium 2_G8 (EU589321)	Rice paddy field soil	92% (399/431)	Betaproteobacteria 100% (phylum)
4	Firmicutes Raunefjorden 04 (AM706659)	Environmental sample	97% (398/411)	Lactobacillales 96% (order)
5a	Bacterium FFCH15545 (EU132916)	Undisturbed soil	92% (372/405)	Actinomycetales 99% (order)
5b	Firmicutes Raunefjorden11 (AM706663)	Environmental samples	99% (401/400)	Lactobacillales 95% (order)
6	Idobacteria GASP-WB1W1_E08 (EF073599)	pasture	98% (386/390)	Acidobacteriaceae 95% (family)
7a	Firmicutes Raunefjorden 11 (AM706663)	Environmental sample	95% (371/390)	Lactobacillales 97% (order)
7b	Soil bacterium clone 2_G9 (EU589322)	Rice paddy field soil	94% (384/408)	Proteobacteria 96% (phylum)
8	Actinomycetales TLI226 (EU699684)	Soil	99% (405/410)	Actinomycetales 100% (order)
9	Micromonospora sp. HBUM49436 (EU119220)	Soil sample	94% (374/397)	Actinomycetales 99% (order)
10	Betaproteobacterium GASP-MA2S2 H05 (EF662904)	Cropland	89% (315/355)	Proteobacteria 97% (phylum)
11	Ramlibacter sp. P-8 (AM411936)	Rice paddy soil	97% (393/404)	Comamonadaceae 96% (family)
12	Ramlibacter tataouinensis 153-3 (AJ871240)	Biological soil crusts	96% (388/401)	Comamonadaceae 92% (family)
13	Alphaproteobacterium OS-C02 (EF612398)	Soil	99% (385/381)	Sphingomonadaceae 98% (family)
14	Agricultural soil bacterium isolate SI-15 (AJ252582)	Agricultural soil bacterium	98% (401/408)	Sphingomonadaceae 100% (family)
15	Alphaproteobacterium 5kpl2aC11 (EF092525)	Environmental sample	99% (398/394)	Rhodobacteraceae 98% (family)
16	Deltaproteobacterium g65 (EU979074)	Rhizosphere of faba bean	92% (375/408)	Polyangiaceae 100% (family)
17	Pseudomonase sp.G-229-23 (EF102852)	Rhizosphere of tobacco	98% (402/411)	Pseudomonas 83% (genus)
18	Prophyrobacter sp. AUVE_14G05 (EF651683)	Cropland	94% (361/382)	Sphingomonadaceae 98% (family)
19	Bacterium 4PS16S. (AY365088)	Agricultural soil	98% (387/393)	Pseudomonas 91% (genus)
20	Bacterium 7PS16S. (AY365091)	Agricultural soil	97% (398/410)	Pseudomonas 91% (genus)
21	Acidobacteria GASP-WB1W1_E08 (EF073599)	pasture	98% (386/390)	Gp3 99% (genus)
22	Rhizobiales bacterium AhedenP3 (FJ475499)	Forest soil	97% (326/333)	Rhizobiales 100% (order)
23	Actinomycetales bacterium TLI213 (EU699671)	Soil	98% (390/398)	Streptomycetaceae 98% (family)
24	Gammaproteobacterium GASPMA2S3_D1 (EF663052)	Cropland	97% (401/412)	Proteobacteria 96% (phylum)
25	Hyphomicrobiaceae GASP-KC1S3_A04. (EU299238)	Restored grassland	90% (230/255)	Alphaproteobacteria 100% (class)
26	Hyphomicrobium sp. AUVE_04B07 (EF651116)	Cropland	93% (297/319)	Alphaproteobacteria 92% (class)
27	Soil bacterium W4Ba49 (DQ643713)	Agricultural soil	97% (358/366)	Alphaproteobacteria 98% (class)
28	Actinobacterium TH1-94 (AM690885)	Environmental sample	98% (404/413)	Actinomycetales 99% (order)
29	Caulobacterales Plot29-H11 16S (EU202838)	Agricultural soil	97% (326/333)	Sphingosinicella 100% (genus)
30	Kaistobacter sp. Plot03-H09 (EU276575)	Agricultural soil	97% (373/381)	Sphingosinicella 98% (genus)
31	Bacterium FFCH15545 (EU132916)	Undisturbed soil mixed grass	93% (369/396)	Actinomycetales 100% (order)
32	Streptomyces sp. 33D (EF585406)	Soil	97% (395/407)	Streptomycetaceae 98% (family)
33	Bacterium 7PS (AY365091)	Agricultural soil	98% (415/421)	Pseudomonadaceae 83% (family)
34	Sphingomonas sp.GASPMA1W1_C03 (EF662616)	Cropland	99% (316/319)	Sphingomonadaceae 98% (family)

from disturbance. Tillage breaks up larger soil aggregates (>250 μm), fragments and incorporates surface residue, and eliminates some of the larger soil fauna (Paustian et al. 1997), but there is no evidence that it induces a fundamental change the microstructure at the scale of bacterial habitats (within micropores, 10 μm or less); this needs to be evaluated more closely in future studies. The +R plots received between 8 and 12 Mg ha^{-1} yr^{-1} of extra corn residues, but it was the same type of residue as in the –R plots, so this leads to the expectation of larger bacterial populations (Metting 1993), but not necessarily a shift in diversity because the food quality was the same across the field. We also acknowledge a bias with PCR-based techniques such as DGGE, in that short subunits of rRNA are amplified according to a competitive enzymatic reaction so that the most abundant microbial populations yield the most amplicons, and organisms that are present at 1% or less of the total community might not even appear on the gels, even if their populations exceed 10^4 to 10^5 cells per gram of soil (Forney et al. 2004). It is possible that bacterial species sensitive to the tillage and residue treatments were not detected due to limitations of the PCR-DGGE method.

Comparing archaeal 16S rRNA sequences to those in GenBank revealed matches with the phylum Crenarchaeote in isolates from agricultural, rhizosphere and forest soils, which were all classified as Thermoprotei (Table 4). Orders of the class Thermoprotei includes the Thermoproteales and Desulfurococcales, which can be primary producers using oxygen, elemental sulfur, sulfate, thiosulfate, sulfite, and nitrate as electron acceptors, and the Sulfolobales, which oxidize sulfur as an energy substrate and are mainly aerobic thermophilic archaea (Huber et al. 2006; Huber and Stetter 2006).

A total of 59 clones were screened by RFLP and grouped by identical restriction pattern (phylotypes). Analysis of the inserts showed three restriction patterns, but sequencing revealed that 26 clones were related to bacteria, due to poor primer specificity for archaea in

Table 4. 16S rRNA gene analyses of the isolated bands from archaea DGGE. Sequences were compared with known sequence in GenBank using the BLAST algorithm. Phylogenetic classification of sequences was determined using the RDP classifier function of the Ribosomal Database Project-II release 9

Band	Closest BLAST match	Origin of BLAST match	Similarity to BLAST match	RDP grouping
1	Archaeon clone CAP128RC (EU223281)	Corn rhizosphere soil	98% (441/449)	Thermoprotei 82% (class)
2	Archaeon clone Elev_16S_arch_974 (EF023083)	Rhizosphere	99% (396/400)	Thermoprotei 87% (class)
3	Crenarchaeote clone MBS11 (AY522889)	Mesophilic soil, forest	99% (398/399)	Thermoprotei 81% (class)
4	Crenarchaeote clone MWS36 (AY522861)	Mesophilic soil, turf field	98% (401/409)	Thermoprotei 91% (class)
5	Crenarchaeote clone NRP-M (AB243804)	Rice paddy soil	99% (426/428)	Thermoprotei 85% (class)
6	Crenarchaeote clone A364I-21 (AM292013)	Acidic forest soil	98% (424/432)	Thermoprotei 87% (class)
7	Crenarchaeote clone A364I-08 (AM292000)	Acidic forest soil,	98% (400/407)	Thermoprotei 86% (class)
8	Crenarchaeote clone CBS16S2-2-8 (EF450809)	Agricultural soil	96% (370/384)	Thermoprotei 94% (class)
9	Crenarchaeote clone CBS16S2-2-5 (EF450808)	Agricultural soil	99% (284/286)	Thermoprotei 82% (class)
10	Archaeon clone Elev_16S_arch_999 (EF023106)	Rhizosphere	97% (324/333)	Thermoprotei 85% (class)
11	Crenarchaeote clone MWS36 (AY522861)	Mesophilic soil, turf field	99% (495/499)	Thermoprotei 95% (class)
12	Archaeon clone Elev_16S_arch_945 (EF023055)	Rhizosphere	99% (496/499)	Thermoprotei 96% (class)
13	Crenarchaeote clone A364I-08 (AM292000)	Acidic forest soil	98% (492/498)	Thermoprotei 97% (class)
14	Crenarchaeote clone A364I-08 (AM292000)	Acidic forest soil	97% (481/493)	Thermoprotei 91% (class)
15	Crenarchaeote clone A109-18 (AM291988)	Acidic forest soil	96% (463/479)	Thermoprotei 95% (class)
16	Crenarchaeote clone OdenB-100b (DQ278124)	Soil	98% (481/487)	Thermoprotei 95% (class)
17	Archaeon clone Elev_16_arch_539 (EF022693)	Rhizosphere	99% (493/494)	Thermoprotei 99% (class)
18	Archaeon clone 1-I-12 (EU223277)	Corn rhizosphere soil	98% (489/495)	Thermoprotei 98% (class)
19	Archaeon clone pTN-23 (AB182772)	Rice paddy soil	99% (489/495)	Thermoprotei 96% (class)
20	Archaeon clone pTN-FC-16m (AB182772)	Rice paddy soil	98% (489/495)	Thermoprotei 97% (class)
21	Crenarchaeote clone MBS11 (AY522889)	Mesophilic soil, forest	98% (489/494)	Thermoprotei 92% (class)
22	Crenarchaeote clone CBS16S1-1-2 (EF450802)	Agricultural soil	98% (440/447)	Thermoprotei 81% (class)
23	Archaeon clone Elev_16S_ arch_974 (EF023083)	Rhizosphere	98% (487/493)	Thermoprotei 98% (class)
24	Crenarchaeote clone A364I-08 (AM292000)	Acidic forest soil	98% (490/496)	Thermoprotei 93% (class)
25	Crenarchaeote clone MBS11 (AY522889)	Mesophilic soil, forest	99% (494/496)	Thermoprotei 96% (class)
26	Crenarchaeote clone TREC89-24 (AY487106)	Tomato rhizosphere	99% (488/492)	Thermoprotei 98% (class)
27	Crenarchaeote clone MBS11 (AY522889)	Mesophilic forest soil	99% (398/399)	Thermoprotei 81% (class)

agricultural soils. The primer sets selected (Table 1) were developed to detect archaea in the Canadian arctic (Perreault et al. 2007; Bottos et al. 2008) and at the time of this study were the only ones available for Canadian soils. We recommend that researchers select archaea primers developed for temperate agricultural soils to avoid overestimating archaea presence in such soils. Thirty-three clones were reliably identified as archaea from the sequence data, and these were grouped into eight phylotypes. Archaeal clone sequences were assigned to operational taxonomic units at a level of 95% similarity for phylogenetic tree building. Clone coverage with Good's percent coverage, which provides a quantitative estimate of how well the sample size reflects the apparent diversity within the clone library, was 84%. The phylogenetic grouping of the sequences is illustrated in Fig. 1. Archaeal clone library results showed that members of Crenarchaeota were dominant, with one sequence (SS-30) closely related to Thaumarchaeota. Clones classified as Crenarchaeota by RDP did not cluster with any cultured representatives and their top BLASTn matches were with isolates and clones from temperate soil.

The Thaumarchaeota is a newly proposed phylum that was initially classified as Crenarchaeota. In this group are ammonia oxidizing archaea that fix carbon dioxide and oxidize ammonia to nitrite, important functions in the C and N cycles (Leininger et al. 2006; Spang et al. 2010; Tourna et al. 2011). Leininger et al. (2006) reported that NH_4-oxidizing archaea in soils may be more abundant than NH_4-oxidizing bacteria. The related NH_4-oxidizing marine archaea, *Nitrososphaera maritimus,* and warm water spring archaea *Nitrososphaera gargensis* have a chemolithoautotrophic metabolism, but Tourna et al. (2011) reported that *N. viennensis*, the only NH_4-oxidizing archaea isolated from soil to date, showed enhanced growth with the addition of an organic substrate. The detection of close relatives to this NH_4-oxidizing archaea in the corn-cropped soil indicates that archaeal groups are likely contributing to nitrification, which produces NO_3^- for corn uptake, leaching and denitrification in this soil. Ammonia oxiders generally catalyze two reactions, converting ammonia to hydroxylamine and then to nitrite, which also produces nitrous oxide (N_2O) and accounts for its emission in the nitrification process (Whalen and Sampedro 2010). Archaeal ammonia oxides may compete for substrates with bacterial ammonia oxidizers, or perhaps each thrives in differences niches that are suitable to their particular needs. This possibility requires further investigation.

Reports of archaea in upland (oxic) soils are few. Borneman et al. (1996) reported no sequences of archaea in a study on soil microbial diversity in a clover–grass pasture in southern Wisconsin, although they did not clone and sequence their small subunit rDNA

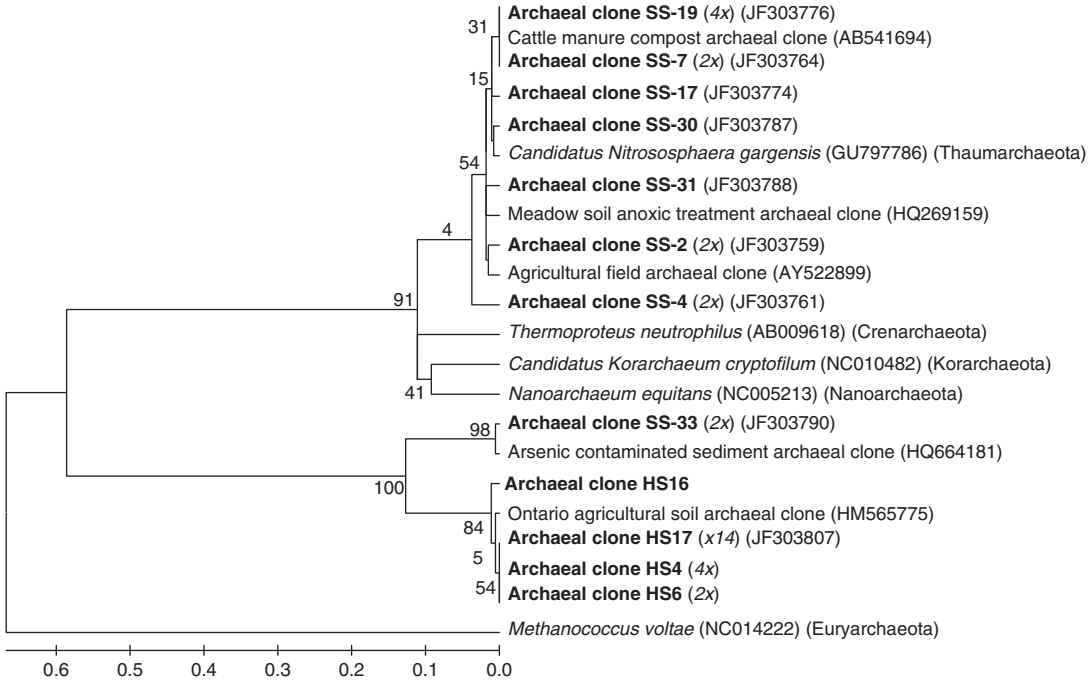

Fig. 5. Archaeal 16S rRNA distance based (Neighbor-Joining) phylogenetic tree including bootstrap values for the RT + R (0–5 cm) sample collected during the growing season in Aug. 2007. Clones with greater than 95% sequence similarity were grouped together with the number of each operational taxonomic and show the number of clones represented by each branch. Top BLASTn matches are in plain text and cultured representatives from major archaeal clades in italics.

amplification products to confirm the presence or absence of archaea. Yet, Furlong et al. (2002) reported that five patterns constituting a single archaeal lineage were detected in soil and earthworm casts from NT plots at the Horseshoe Bend Long Term Environmental Research site in Georgia, USA. As well, Bintrim et al. (1997) reported a cluster of archaea affiliated with the Crenarchaeota in soils sampled at the West Madison Agricultural Research Station in Wisconsin, USA. Their analysis also showed that the full-length sequences of 10 cloned small subunit ribosomal rRNA genes had phylogenetic affinity to members of the planktonic division of Crenarchaeota.

Microbial Biomass and Cell Counts

The MBC and MBN concentrations and CARD-FISH and DTAF counts are given in Table 2. The MBC concentration was significantly greater in the NT plots than CT plots and in the + R plots than the − R plots in 2006 and 2007. DTAF cell counts also showed a greater count in the + R plots compared with the − R plots. The MBN trend was similar to MBC but differences were not statistically significant. There was 119% more MBC in the NT plots compared with the CT plots similar to results of Spedding et al. (2004), Granatstein et al. (1987) and Feng et al. (2003), who reported a range between 24 and 140% greater MBC in NT compared with CT. In contrast, the cell counting methods (CARD-FISH and DTAF) showed a somewhat different trend indicating that RT plots contained more microbial biomass (based on bacterial and archaeal counts) than CT plots. An interaction effect (Table 2) was detected by the DTAF count such that the RT + R plots had greater counts than the NT + R, NT − R, and CT–R plots.

The cell count results suggest that conditions in the RT plots (moderate soil disturbance, some fragmentation and incorporation of crop residues) were more favorable for the development of bacteria and archaea populations than conditions in the CT or NT plots. These methods do not count fungal cells, which are included in MBC and MBN assessments, so cannot be compared directly to the chloroform fumigation–direct extraction method. Taken together, the MBC, CARD-FISH and DTAF results seem to suggest a change in microbial communities leading to greater fungal biomass in NT plots than other tillage systems and larger bacteria and archaea populations in RT than CT plots. Fungal biomass was not directly measured and the fungal to bacterial ratio needs further investigation to verify the abundance of fungal populations in NT systems. Acidic soil conditions are considered to promote fungal populations (Whalen and Sampedro 2010), but soils from NT plots were significantly ($P < 0.05$) less acidic (pH = 6.9 ± 0.1) than soils from the CT (pH = 6.1 ± 0.1) and RT (pH = 6.2 ± 0.1) plots. Our results suggested that the higher MBC concentrations in NT plots were due to more fungal biomass, probably not

related to soil pH conditions but likely related more to the lack of tillage disturbance in NT. We also note that archaea cell counts by CARD-FISH were affected by tillage (NT, RT > CT), which indicates an important presence of archaea in less-disturbed soils plots as well.

The +R plots also exhibited an increase in total bacteria cells with the DTAF method, but there was no difference in the bacteria and archaea populations due to residue management according to the CARD-FISH method. The minimal number of rRNA target molecules required to obtain a visible fluorescence signal after CARD-FISH (with rRNA-targeted probes) is not yet determined and we had difficulty to observe cells with CARD-FISH in soil due to high background fluorescence from the soil itself. This may be a limitation to broader application of the CARD-FISH method to census soil bacteria and archaea populations.

CONCLUSIONS

This is one of the first reports of archaeal groups in corn agroecosystem soils in Quebec (Canada), as far as we know. One of our sequences was also closely related to Thaumarchaeota, order Nitrososphaerales. Thaumarchaeota has been proposed as a third archaeal phylum. Members of this phyla are ammonia oxidizers and may have different features from those of hyperthermophilic crenarchaeota (Brochier-Armanet et al. 2008). Thaumarchaeota have significant roles in the global C and N cycles and might be the most abundant ammonia oxidizers in soil (Tourna et al. 2011). The extent of the archaeal contribution to microbial functions in this oxic soil is not yet known, but their large population size and known role as ammonia oxidizers (Tourna et al. 2011) in soil suggests that they contribute to the production of plant-available N (e.g., NO_3) and gaseous emissions (e.g., N_2O as a byproduct of ammonia oxidation) in our agricultural soils.

Tillage and residue input affected microbial biomass – more microbial biomass in NT plots than CT plots, and greater microbial biomass with higher residue inputs, as indicated by the MBC and supported by the MBN trends. Based on evidence from the chloroform fumigation method (MBC) and the microscopic CARD-FISH and DTAF analysis taken together we propose that tillage affects the microbial community as follows: NT plots are suggested to have a dominant fungal population, with an appreciable archaeal population, RT plots are dominated by bacteria and archaea, and fewer fungi, and the CT plots have the smallest microbial biomass/populations. Soil bacterial diversity was difficult to assess due to few dominant groups (Proteobacteria, Actinobacteria and Firmicutes). Changes in bacterial diversity due to tillage and residue management were not detected by the DGGE method. Microbial community diversity in this study was limited to bacteria and archaeal groups, and it is important also to consider the diversity of fungi, which are more sensitive to tillage disturbance than bacteria (Paustian et al. 1997). High-throughput DNA sequencing techniques like pyrosequencing prove helpful to assess soil microbial diversity (Liu et al. 2007; Roesch et al. 2007; Jones et al. 2009) and the effect of tillage and crop rotation on the soil microbial community (Yin et al. 2010). Integration of massively parallel pyrosequencing with quantitative real-time PCR (qPCR) holds promise for assessing microbial community structure and functions (Zhang et al. 2011) and could better describe soil microbial responses to long-term agricultural practices like tillage and residue management.

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