Evaluation of Bacterial and Archaeal communities under different agricultural land management practices in southern Québec soils

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Abstract

This thesis represented the first attempt to investigate the effect of different tillage and crop residue management regimes on the genetic and functional diversity of soil Bacteria and Archaea in a corn agroecosystems in southern Québec. Soils were collected from a long-term (>15 year old) agricultural experiment with three tillage treatments- no-till, reduced tillage, and conventional tillage (mouldboard plowing) and two levels of residue input- with residues (corn roots, stems, and leaves) versus without residues (corn roots and little above ground residues). PCR-DGGE analysis of soil DNA extracts indicated that there was no significant difference of Bacterial and Archaeal communities in the different soil treatments. The potential for atrazine degradation was determine using a soil microcosm mineralization assay. The results of this experiment indicated that all the treatments had almost the same effect on atrazine mineralization. Functional gene microarray analysis of soil microorganisms affected by different treatments showed no clear difference among the different treatments. Microscopic analysis (CARD-FISH and DTAF) indicated that biomass and numbers of Bacterial and Archaeal were not significantly changed as a consequence of different treatments on agricultural soils in southern Québec. In conclusion, this study indicated that the different tillage practices (no-tillage, reduced tillage and conventional tillage) and crop residue managements (with residue and without residue) did not change soil microbial genetic/functional diversity, atrazine degradation, and microbial biomass.

Résumé

La présente thèse constituait la première opportunité d'étudier l'effet de différentes techniques de labour et de gestion de résidus de plantes sur la diversité génétique et fonctionnelle bactérienne et archaenne des terres agricoles du sud du Québec consacrées à la culture du maïs. Des analyses PCR-DGGE d'extraits d'ADN du sol ont indiqué qu'il n'y avait aucune différence majeure entre les communautés microbiennes de bactéries et d'archaea, peu importe les différents traitements des sols. Des analyses de microréseaux de gènes fonctionnels des communautés de microorganismes de ces sols n'ont aussi montré aucune différence significative entre les différentes techniques d'entretien des sols utilisées. La capacité de dégradation de l'atrazine a été déterminée par analyse de minéralisation via un microcosme de sol. Les résultats de ce test ont indiqué que toutes les techniques de gestion des sols ont à peu près le même effet sur la minéralisation d'atrazine. Des analyses microscopiques (CARD-FISH et DTAF) ont indiqué que la biomasse du sol, ainsi que le nombre de bactéries et d'archaeas, ne changeaient pas de façon significative malgré les différentes techniques d'entretien des sols agricoles dans le sud du Québec. Pour conclure, cette étude a démontré que les différentes pratiques de labour (sans labour, pratique aératoire antiérosive et travail du sol classique) et de gestion de résidus de plantes (avec ou sans résidus) n'ont pas changé la diversité génétique/fonctionnelle microbienne, la dégradation de l'atrazine ainsi que la biomasse du sol.

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Abbreviations

CT:	Conventional tillage
RT:	Reduced tillage
NT:	No-tillage
+R:	With residues
-R:	Without residues
N:	Nitrogen
P:	Phosphorous
S:	Sulphur
K:	Potassium
DNA:	Deoxyribonucleic acid
dNTP:	deoxynucleotide triphosphate
PLFA:	Phospholipid ester-linked fatty acid
T-RFLP:	Terminal restriction fragment length polymorphism
CLPPs:	Community-level-physiological-profiles
DGGE:	Denaturing gradient gel electrophoresis
TGGE:	Temperature gradient gel electrophoresis
ARDRA:	Amplified ribosomal DNA restriction analysis
AODC:	Acridine orange direct *count*
DAPI:	4', 6-diamidino-2-phenylindole
MPN:	Most probable number
FAME:	Fatty acid methyl ester
rRNA:	Gene for 16S small subunit rRNA
SSCP:	Single strand conformation polymorphism
RISA:	Ribosomal intergenic spacer analysis
LH-PCR:	Length heterogenecity PCR
PCR:	Polymerase chain reaction
PGMA:	Phylogenetic microarray
FGMA:	Functional gene microarray
CGA:	Community genome array
DTAF:	5-[4,6–Dichlorotriazinyl] aminofluorescein
FISH:	Fluorescence *in situ* hybridization
CARD-FISH:	Catalyzed reporter deposition fluorescence *in situ* hybridization
BLASTn:	A database DNA alignment and ranking tool provided by NCBI
NCBI:	National centre for biotechnology information
NRC-B.R.I:	National research council of canada biotechnology research institute
OTU:	Operational taxonomic unit
DOTUR:	Distance based OTU and richness determination
RDP:	Ribosomal database project
SSC:	Sodium chloride - sodium citrate buffer
SDS:	Sodium dodecyl sulfate buffer
BSA:	Bovin serum albumin
Dpm:	Disintegrations per minute
EDTA:	Ethylenediaminetetraacetic acid

g:	Force of gravity
GC:	Guanine and Cytosine
H':	Shannon's diversity index measurement
LB:	Luria-Bertani
TAE:	Tris acetate ethylene diamine tetraacetic acid
SB:	Sodium borate
U:	Unite
UV:	Ultraviolet
v/v:	volume/volume
w/v:	weight/volume
X-Gal:	5-bromo-4 chloro-3-indolyl-BD-galactopyranoside

Chapter 1: Introduction

1.1 Overview of literature review

Soil is the basis of agriculture and maintaining its health and quality is a moral responsibility of mankind. Healthy soil is a key to producing high-yielding harvests of crops and minimizing expenses such as fertilizers and pest control. Due to natural events or human activities, soil quality and health change over time. They are impaired by decisions that focus only on a single function, such as crop productivity, and are improved by management and land use decisions that consider the multiple functions of soil. Although soils have an inherent quality as related to their physical, chemical, and biological properties, land management has a great influence on soil quality and health. Tillage, crop residue management, and pesticide application are among the most important agriculture practices affecting soil quality [1-3]. This chapter will focus on these agricultural practices, which are relevant to this study. Later on, the concepts of soil health and quality will be discussed. The effects of agricultural practices on soil microbial communities and the selected methodologies for monitoring these effects will be reviewed. The research objectives and the hypotheses of this study will be presented at the end.

1.2 Tillage

For many years, people considered agriculture and tillage to be synonymous. However, this is not the case anymore. There are two main types of tillage systems: conventional tillage and conservation tillage. Conventional tillage is a system that uses chisel plows or moldboard plows with sweeps, followed by other secondary tillage operations such as disking and

harrowing to incorporate residues. This helps to prepare a seedbed and control weeds [4]. There are advantages and disadvantages associated with tillage. Advantages of tillage include mixing fertilizer throughout the rooting depth of the crop, creating a seedbed, promoting water infiltration, disrupting the life cycle of harmful pests, breaking up surface soil crusts, and reducing surface compaction. On the other side, some disadvantages of tillage consist of disrupting aggregates and reducing soil structure, increasing soil exposure to rainfall and promoting soil erosion, compaction and tillage pan formation, and disruption in the life cycles of beneficial organisms such as earthworms [5]. In conservation tillage, which is radically different from the conventional tillage, soil generally has a layer of crop residue at the surface that protects the soil from rain, wind, and extreme temperatures. The residue also provides a source of nutrients that are slow in release of nutrients into the soil during decomposition. Conservation tillage systems include reduced tillage and zero tillage. Reduced tillage systems involve the removal of one or more tillage operations to increase residue cover on the soil surface. In zero tillage or no-till, crops are planted into undisturbed soil by opening a narrow slot that is wide and deep enough to obtain proper seed coverage [4]. Some concerns exist about the effects of conservation tillage practices. These systems generally have higher water retention than tilled soils, and this may contribute to the invasion of opportunistic fungal pathogens. The level of weeds might be increased as well, and therefore more chemical herbicides might need to be applied. Conservation tillage may also reduce early season plant growth and may retard seed germination and seedling [6]. In recent years, the popularity of conservation tillage has grown steadily in Canada and elsewhere. In the province of Québec alone, reduced tillage increased by 28% and no-till by 65% between 1996 and 2006, with the crop land area under conventional tillage declining by about 9% [7]. Tillage or lack of tillage is one of the farming variables that

impacts our environment. It can alter the soil physical, chemical, and biological properties and consequently changes crop productivity [8]. Crops can be grown with or without tillage, so knowledgeable producers can select tillage systems that solve problems without creating others that are unacceptable [5].

1.3 Crop residues

Crop residues are the parts of plants left in the field after the crops have been harvested [9]. The presence of residue on the soil surface significantly impacts evaporation, soil water content, soil temperature, soil freezing, infiltration as well as runoff and erosion [10]. Beneficial effects of crop residues on soil physical, chemical and biological properties are not debatable when managed properly [11]. Crop residues are good sources of plant nutrients, are the primary source of organic material added to the soil, and are important components for the stability of agroecosystems. About 25% of nitrogen (N) and phosphorus (P), 50% of sulphur (S), and 75% of potassium (K) uptake by cereal crops are retained in crop residues, making them valuable nutrient sources. Crop residues have a great influence in the reduction of both wind and water erosion. Standing stubble also aids in the protection of winter crops from low soil temperatures, provides a favorable microclimate for emerging seedlings, and enhances the infiltration of water [12]. There are no known major disadvantages of cover crops. However, concern has been raised over potential harmful effects of cover crops to the succeeding crop [13]. Decomposition of crop residues may create N deficiency for the next crop which reduces the yield. This mainly happens because microorganisms use the free inorganic nitrogen in the soil in order to break down the organic materials. Sometimes early season deficiency could occur even when N

fertilizer was added [14-15]. Corn produces high amounts of residues which contain polysaccharides, simple sugars, amino acids, proteins, phenols, waxes, and etc. [9]. Corn residues reported to have about 29% soluble organic compounds, 27% hemicellulose, 28% cellulose, 6% lignin, 9% ash, and 10% nitrogen [16]. The C/N ratio and total plant N are the most used parameters in predicting the relative N mineralization potential of organic materials added to soils. The critical C/N ratio determining whether residue N is mineralized or immobilized is thought to equal 20 [17]

1.4 Pesticide application

Pesticides are added to agroecosystems to improve crop production [18]. However if not used wisely, these agrochemicals can reduce the quality of soil, water, and air. Atrazine [2-chloro-4-(ethylamino)-6 (isopropylamino)-s-triazine] is one of the most widely used herbicides in Canada to control pre- and post-emergence broadleaf and grassy weeds in major crops such as corn (*Zea mays*). In the province of Québec, atrazine represented 27% of all pesticide sales [19]. Products of atrazine decomposition are relatively persistent in soil. This pesticide has a solubility of 30 mg l^{-1} approximately and a half-life in soil of between 15 and 100 days [20]. The main concern is its relative persistence and mobility in some types of soils, resulting in contamination of surface and ground waters. It is often the most frequently detected herbicide in different environments including ground water and soil [21]. Both animal and human studies have suggested that atrazine is possibly carcinogenic. Atrazine was found to cause menstruation problems in human females. It has also been associated with low sperm quality and birth defects in humans. Both chemical and biological processes are involved in the degradation of atrazine in soil; however, microbial degradation is probably the main mechanism [22-23]. Various soil

microorganisms can degrade atrazine partially or totally, due to the fact that they use atrazine as a source of carbon and nitrogen [24]. In most cases, microbial isolates are capable of limited degradation of the atrazine molecule and usually remove the side chains by N dealkylation and subsequently, use the ethyl and isopropyl carbons via oxidative phosphorylation. A number of atrazine degrading Bacterial strains belong to different genera have been isolated and characterized for research and bioremediation purposes [25-27]. These include strains of *Agrobacterium* [28], *Nocardia* [29], *Rhodococcus* [28], *Pseudomonas* [30], *Rhizobium* [31], *Pseudaminobacter* [25], *Chelatobacter*, *Aminobacter* and *Stenotrophomonas* [26], *Ralstonia* [32], *Sinorhizobium* and *Polaromonas* [33], *Arthrobacter* [34], *Acinetobacter* [35] and *Alcaligens* [36]. Only a few microorganisms can enzymatically break down the *s*-triazine ring, leading to the complete mineralization of atrazine. *Pseudomonas* sp. strain ADP is the best studied atrazine degrading Bacterium in this regards as its atrazine degradation pathway, consisting three enzymatic steps, are well defined [37]. The first enzyme, AtzA, catalyzes the hydrolytic dechlorination of atrazine, yielding hydroxyatrazine. The second enzyme, AtzB, catalyzes hydroxyatrazine deamidation, yielding *N*-isopropylammelide. The third enzyme, AtzC, transforms *N*-isopropylammelide to cyanuric acid and isopropylamine. Cyanuric acid is the catabolized to carbon dioxide and ammonia. The complete genome sequence of pADP-1 showed that this strain contains the genes for the complete catabolism of cyanuric acid to CO_2 and NH_3, namely, *atzD*, *atzE*, and *atzF* [38-39]. Some studies have indicated that the major abiotic degradative pathway for atrazine in soils is via atrazine hydrolysis to hydroxyatrazine (2-ethylamino-4-hydroxy-6-isopropylamino-s-triazine), which is less mobile than atrazine [40-41].

1.5 Assessment of soil health

The concepts of resiliency and biodiversity are two fundamental aspects of soil health [42]. Healthy soils maintain a diverse community of soil organisms that help to control plant diseases, recycle plant nutrients, and improve crop production. Until recently, only the physical and chemical properties of soil were considered important. The role of soil microbial communities in maintaining soil quality and health are now well recognized [43-44]. Soil health and quality refer to the physical, chemical, and biological features of soil that are essential to long-term, sustainable agricultural productivity with minimal environmental impact. Thus, soil health provides an overall picture of soil functionality [44]. Soil resilience is defined as the ability of soil to recover after disturbance. There are two more or less conflicting hypotheses concerning the effect of disturbance on ecosystem stability [45]. The first predicts that stressed communities are less stable as the organisms have to spend energy to deal with the undesirable effects of stress. Therefore, they are less capable of surviving subsequent stress events. The alternative hypothesis is that stress enhances ecosystem stability, since the first stress has selected for relatively stable populations. In fact, stress enhances genetic adaptation which produces phenotypes that are capable of surviving in various stresses [46]. Anthropogenic activities, including agricultural practices, directly and indirectly affect soil environments and thus may alter the activity and diversity of soil microbial communities [47-48]. Therefore, assessing the effect of soil management practices on microbial community structure, diversity and activity is critical to advancing the understanding of the functionality, stability, and resilience of managed ecosystems [49-51]. Functional diversity is an aspect of the overall microbial diversity in soil and refers to the range of roles that organisms have in communities and ecosystems. It might thus also be a tool for predicting the functional consequences of biotic

change caused by humans [52]. The relationship between microbial diversity and function in soil is largely unknown, but biodiversity has been assumed to enhance ecosystem stability, productivity, and resilience towards stress and disturbance. Microbial diversity includes genetic variability within taxons (species), the number (richness) and relative abundance (evenness) of taxons, and functional groups in communities [53].

1.6 The effects of agricultural practices on soil Bacterial diversity

Of the microbial groups, *Bacteria* are the most diverse and abundant group of soil microorganisms with an estimated 10^3 to 10^7 Bacterial species per gram of soil sample [54]. Changes in Bacterial populations and activities may serve as excellent indicators of changes in soil health [27]. Different studies have been conducted to investigate the influences of agricultural practices on soil microbial communities. For example, the effects of conventional tillage and no-till in cropping systems with and without cover crops on Bacterial community structure were investigated by Peixoto et al. (2006). They employed polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) using 16S rRNA and *rpoB* genes. They observed different populations in response to cultivation, tillage, and depth, but not due to cover crops [55]. Cookson et al. (2008) studied the effect of diverse tillage practices on microbial community composition using the PCR-DGGE approach. In general, microbial community composition differed between all tillage methods and soil depths, and these differences were correlated with soil texture and soil pH [56]. Phospholipid ester-linked fatty acid (PLFA) profiles were used to evaluate soil microbial community composition for 9 land use types in two coastal valleys in California. These included never-tilled perennial grasslands, non-native annual grasslands and relict, irrigated and non-irrigated agricultural sites. The soil texture was loam or

sandy loam. Their results revealed that land use history and management practices produced a unique soil environment, for which a microbial community with specific environmental requirements was selected [57]. Combining terminal restriction fragment length polymorphisms (T-RFLP) with DGGE and BIOLOG methods, Wu et al. (2008) compared soil Bacterial communities under a number of geographically distant agricultural sites with two different soil series. Their results indicated that soil series was the key factor determining Bacterial community composition in these soils [47]. Similarly, microbial compositions in soils under vegetable production systems in Oregon were influenced more by soil type and field properties than by farm management [58]. The impact of two tillage systems, no-tillage and conventional tillage, on the fate of atrazine and microbial activity was studied in the 0-5 cm soil layer and revealed that a no-tillage system could reduce pesticide mobility in comparison with conventional tillage. Also, higher microbial populations and activity were observed in no-tillage soil [59]. In Southwestern Ontario, atrazine lose in surface runoff was measured in two conservation tillage treatments, reduced tillage and zero tillage, and compared with conventional tillage during three years. Averaged over years, atrazine concentration in surface runoff and tile discharge was, 1.3 to 1.8 times greater from conservation tillage than conventional tillage. Hence, conservation tillage with residue management was the most effective way to reduce soil deterioration [60]. A greenhouse experiment was conducted to compare atrazine mineralization in bulk soil and the corn rhizosphere at different development stages (4, 8 and, 12 weeks). Atrazine mineralization was lower in bulk soil than in planted soil during this period. Also, mineralization was stimulated to a greater extent after atrazine application in the greenhouse but, again, the presence of plants had a favorable effect [61]. A semi-arid soil treated with different atrazine concentrations was studied for bacterial diversity with several methods including

DGGE, CLPPs (community level physiological profile) and BIOLOG plates. Different banding patterns (DGGE), compared to no or low atrazine concentrations was observed and microbial community at high atrazine levels showed less capacity to use different carbon sources (CLPPs). This suggested that high atrazine concentration, affect bacterial diversity and may produce less functional diversity [27].

1.7 The effects of agricultural practices on soil Archaeal diversity

While the importance of *Bacteria* in soil ecosystem function has long been recognized, it has only recently become evident that *Archaea* are also ubiquitous and abundant organisms within temperate soils [62-64]. *Archaea* have long been thought to be primarily extremophiles. They usually divided into two major phyla, *Euryarchaea* and *Crenarchaea*. Two additional phyla, *Korarchaea* and *Nanoarchaea*, have also been proposed [65]. The majority of Archaeal rRNA sequences indentified from oxic soils belong to Crenarchaeota [66-68]; within this phylum, uncultured representatives were found to be widespread in a variety of non-extremophiles terrestrial environments [62-63, 66, 69-70]. Environmental factors and human activities can affect the diversity, abundance, and community structure of soil *Crenarchaea* [65, 71]. Euryarchaeota are often found within anoxic environment such as rice paddy soils; however sequences with affinities to the euryarchaeal order *Thermoplasmatales* have been infrequently detected in oxic soils [69, 72-73]. Korarchaeota [74-75] and Nanoarchaeota remain unrecorded in soil, possibly because studies conducted to date used PCR primers that do not amplify known representatives of these groups [69, 76-77].

There are few reports available regarding the effect of agricultural treatments on soil Archaeal diversity, and most of the available literature concentrated on the role of methanogenic

Archaea in flooded soils. For example, the change of the methanogenic Archaeal community and activity with soil particle size fractions under different fertilizer applications was studied by Zhang et al., (2007). Their results showed that fertilizer application did not change the methanogenic Archaeal community; however different Archaeal communities and activities within soil particle size was observed [78]. Studying the population dynamics of *Archaea* after flooding of an Italian rice field soil over 17 days using T-RFLP analysis revealed that the structure of *Archaea* remained constant over time after flooding [79]. Long-term application of N, P, and K fertilizers caused different behavior in two Chinese soils; in acidic soils, both Archaeal and Bacterial *amoA* genes were detected, but only the Archaeal community structure was changed by the fertilizer regime. In an alkaline soil, Archaeal *amoA* genes were more abundant, but only the Bacterial ammonia oxidizer community structure changed due to the fertilizer regime [80-81]. The influence of soil pH on ammonia oxidizers at different soil pH values (ranging from 3.7 to 6) revealed that the Archaeal *amoA* gene was more abundant than the Bacterial *amoA* gene, implying greater potential activity [82].

Soil Crenarchaeotes could be also nitrifiers [83]. The expression of Archaeal *amoA* genes in soil was first reported by Treusch et al. (2005) [84]. Leininger et al. (2006) subsequently quantified *amoA* gene transcripts in three different soils and compared Bacterial and Archaeal gene transcription. Their findings indicated a greater abundance of Archaeal over Bacterial ammonia oxidizers [85-86]. Another study also indicated a higher transcriptional activity of Archaeal over Bacterial ammonia oxidizers in soils [87]. The discovery of Archaeal ammonia oxidation provides a further potential explanation for nitrification in acid soils [82].

It has been demonstrated that nonthermophilic crenarchaeotes colonized tomato roots in high frequencies [88]. In a separate study it has been shown that Crenarcheotes associated with roots

of maize [89]. In a study extending across divergent terrestrial plant groups showed significant differences were found in the Crenarchaeotes in the rhizosphere compared with those in bulk soil, suggesting that Crenarchaeotes are able to form associations with phylogenetically diverse plants in native environments [90].

1.8 Methods for studying soil microbial communities

Various methodologies to characterize microbial communities from environmental samples can be generally classified as microbiological-based, biochemical-based, and nucleic acid-based techniques. A detailed review of these methods has been published by Spiegelman et al. (2005) [91].

Microbiological methods comprises the most varied techniques. Generally, they rely on traditional tools and provide virtually no information on specific phylogenetic groups in a complex microbial community. They include direct cell counting techniques such as acridine orange direct count *(*AODC) [92], 4, 6-diamidino-1-phenylindole (DAPI) [93], morphological cell counting [94], metabolic assays including community level physiological profiles (CLPP) [95], as well as the widely used indirect Most Probable Number (MPN) technique [96]. Some of the microbiological methods are inexpensive and can rapidly create a community profile, which can be used to compare two or more entire consortia. Microbiological methods are useful only to screen for the presence of a few key predetermined species, whose growth conditions and morphological characteristics are well defined and reproducible. These techniques (cell counting, selective growth, and microscopic examination) do not offer much information without the complementary use of other more specialized techniques that involve molecular methods.

The second group includes biochemical-based methods and consist of DNA reassociation kinetics [97] and total community DNA hybridization analysis [98]. In addition, assays based on differences in the lipid/phospholipid composition of microbial cells, such as quinine profiling [99], phospholipids fatty acids (PLFA) [100], and fatty acid methyl esters (FAME) [101] analyses, have been used to characterize microbial communities. Although quinine and PLFA methods are useful methods, their major drawback is that there are limited databases of community profiles that exist from which to compare data sets. Many biochemical-based techniques only produce a profile that is characteristic of the microbial community as a whole, and provide no information about individual members of the community.

The effects of agricultural land management practices on soil microbial communities have been widely studied using culture-based techniques and biochemical methods [47, 102-111]. However, these methods are limited by their ability to detect only 0.1 to 10% of the total Bacterial population in soil [47, 112-113]. The vast diversity of the uncultured microorganisms in soil has stimulated the development of culture-independent methods.

The third group of techniques, nucleic acid-based methods, is the largest in scope. They are principally, but not exclusively, based on taxonomic markers such as the genes encoding the small subunit (SSU) of the ribosomal RNA (rRNA). Many different techniques make use of sequence variations and permit the characterization of complex microbial communities based on these differences [114]. The major techniques used are as follows: amplified ribosomal DNA restriction analysis (ARDRA) [115-118], randomly amplified polymorphic DNA (RAPD) [119-122], denaturing gradient gel electrophoresis (DGGE) [123-128], temperature gradient gel electrophoresis (TGGE) [129-133], single-strand conformation polymorphism (SSCP) [134-137], terminal restriction fragment length polymorphism (T-RFLP) [107, 135, 138-140], length

heterogeneity polymerase chain reaction (LH-PCR) [47, 104, 141-142] and ribosomal intergenic spacer analysis (RISA) [117, 139, 143]. Molecular techniques greatly expanded the view of microbial diversity. The advantages of PCR-sequencing approaches lie in their reproducibility, in the quality of their data, and in the information obtained [144].

Microarray technology is also widely used in molecular biology and is useful in addressing specific environmental processes [145]. Most of the methods either provide information on a single species or a small number of species however, microarrays have a great potential for generating a large amount of quantitative data. Compared to conventional, membrane-based hybridization, microarrays offer the additional advantages of rapid detection, automation, and low background level [146]. The specificity, sensitivity, and quantitative capabilities of microarray technology for environmental applications are still at early stages of development and evaluation [147]. Although faced with a great number of obstacles, microarrays for environmental studies hold promise for analyzing complex communities. Table 1 briefly describes the different types of microarrays used in microbial ecology studies. Microarrays can be divided into three classes: phylogenetic microarrays (PGMAs), functional gene microarrays (FGMAs), and community genome arrays (CGAs). Here, I will describe the techniques which have been selected for this study.

1.8.1 Denaturing Gradient Gel Electrophoresis (DGGE)

DGGE is one of the most commonly used techniques for microbial community characterization [91]. DNA fragments of about the same length are produced by amplification of a target gene by PCR. Then a gel composed of a linear gradient of denaturant is used to separate PCR products base on their GC content. To prevent complete strand separation, a high GC

sequence (GC clamp) is added to the end of one PCR primer. The resulting genetic profiles or fingerprints represent the community structure. Following the staining of the gel, the banding patterns can be used to compare different communities or the same community following a perturbation [148-150]. The number of populations is approximately represented by each band and their relative abundance is represented by band intensity within the amplified community [151]. Properly calibrated DGGE is sensitive enough to detect even single base-pair differences between amplicons [152]. DGGE have the advantages of being reliable, reproducible, and relatively rapid. Multiple samples can also be analyzed at the same time, making it possible to follow changes in microbial populations [153]. There are some problems associated with DGGE. The brightest bands in a DGGE profile are often assumed to represent the dominant members of the community. However, the biases associated with PCR could cause relative under- or over-representation of a given taxon in the DGGE profile [154]. DNA fragments of different sequences may have similar mobility or co-migrate in the gel. Therefore, one band may not necessarily represent one species [155-156]. This technique is limited to DNA fragments typically below 500 bp in size. For effective resolution, DGGE requires a large quantities of PCR product (approximately 500 ng for each sample) [148].

1.8.2 Bacterial and Archaeal 16S rRNA gene clone library

16S rRNA genes clone library is one of the widely used approaches to examine Bacterial and Archaeal diversity. This technique is based on the cloning of PCR-amplified 16S rRNA genes into a suitable vector and transforming this cloned product into a suitable host, usually *E.coli*. Each *E.coli* cell that has been transformed with a clone product contains a single 16S

rRNA gene product amplified from the environment of interest. Amplification and sequencing of the cloned sequence can therefore provide insight into the identity of individual populations in the community. This method has proven useful in microbial ecology as it allows for the identification of cultivable and non-cultivable members within a community [157]. The successful characterization of individual clones from a clone library largely depends on the screening technique used. It is important to realize that libraries of PCR-amplified 16S rRNA genes may not represent a complete or accurate picture of the actual compositions of *in situ* communities. That is mainly because the species diversity is so high [158-160] and libraries of <400 cloned sequences must represent only an incomplete sampling. In addition, there may be biases in each step of this method including sample collection, cell lysis, nucleic acid extraction, PCR amplification, and cloning [161-163]. However, it is assumed that probable biases are less severe than those introduced when a complex Bacterial community is analyzed by culture conditions [164]. The efficiencies of nucleic acid extraction may be different for different *Bacteria*, the number of copies of 16S rRNA genes per cell varies, and there may be preferential amplification of some sequence types relative to others by PCR [165]. Some but not all, of these biases may be overcome as metagenomic data sets accumulate [166-167]. For the time being, the available libraries of 16S rRNA genes allow a survey of the global soil Bacterial and Archaeal community structure.

1.8.3 Phylogenetic Microarrays (PGMAs)

Environmental phylogenetic microarrays are used to study the diversity, type, and abundance of organisms and how microbes interact with each other and the environment. They

contain short oligonucleotide probes, targeting phylogenetic marker genes. The most widely used target is the small-subunit ribosomal RNA (SSU rRNA) and its gene [168-169]. Alternative probe targets with a species-level resolution include the large-subunit ribosomal RNA (LSU rRNA) [170], the SSU–LSU rRNA intergenic spacer region [171] and various house-keeping genes such as *rpoB* [172-173], *gyrA* [174], *gyrB* [175], *recA* [176], *tuf* [177], *groEL* [178], *atpD* [177], *ompA*, *gapA*, and *pgi* [179]. The geographic distribution of microorganisms in Antarctica was recently studied using a PGMA. In order to determine which environmental variables had the greatest affect on community structure, the study compared numerous sites along a north-south transect finding a clear decrease in diversity moving towards the pole. In addition, the distributions of specific phyla were correlated to higher or lower latitudes. The same study demonstrated the feasibility of combining PGMA with functional gene arrays to observe the relationships between taxonomic structure and community function. The results demonstrated that communities with closely related taxonomic composition also possessed closely related functional genes; significant correlations were found between abundances of certain taxa and functional genes related to methane, nitrogen and carbon cycling [180]. One of the advantages of PGMA technology is the ability to simultaneously probe the environment for thousands of species [181]. The insufficient sequencing information of microbial diversity creates gaps in PGMA data, where probes have no sequence to be designed from, resulting in false-negatives and uncertainty in the data.

1.8.4 Functional Gene Microarrays (FGMAs)

Functional gene microarrays have been mainly developed to understand microbial ecology and biogeochemistry within specific environments such as soil. FGMA are particularly useful in addressing specific environmental processes and may be applied in order to reveal the community structure of microorganisms involved in these processes, as well as addressing their activities. They contain DNA probes targeting genes conferring a specific function such as nitrification, denitrification, nitrogen fixation, methane oxidation, sulfite reduction, etc. [182-184]. In fact, a range of genes can be targeted on a single array. The applied probes in FGMA might be short (typically 15-30-mer) [185-186] and long (typically 40-70-mer) [182, 187-188] oligonucleotides as well as PCR-amplified gene fragments [183, 189-190]. Recently, some studies successfully applied functional gene microarray for different types of soils including agricultural soils and soils from the Canadian high Arctic [180, 182, 186, 191]. Rhee et al. (2004) developed an array that consisted of 1662 group specific probes, targeting most of the genes and pathways known (at that time) to be involved in biodegradation and metal resistance. The applicability of this array was demonstrated in naphthalene amended enrichment cultures as well as in microcosm experiments with soil containing polyaromatic hydrocarbons [192]. For the purpose of studying nitrogen cycle, a 70-mer long oligonucleotide FGMA, containing nirS, nirK, nifH and amoA probes, was developed. The specificity threshold of 87% enabled perfect differentiation of major lineages within the different gene families. Results showed significant changes in the denitrifier community along a gradient of salinity, dissolved organic carbon and inorganic nitrogen [182]. A similar microarray, contained 50-mer probes targeting genes involved in nitrogen and sulphur cycling was developed and gene sequences with less than 86–90% similarity were discriminated [188]. A microarray consisting of 64 gene probes, targeting

catabolic genes was developed and tested for expression analysis of these genes in microbial communities. Induction of two of five 2,4-dichlorophenoxy acetic acid (2,4-D) catabolic genes (*tfdA* and *tfdC*) from populations of a degrading inoculant strain as low as 10^5 cells ml^{-1} was clearly detected against a background of 10^8 cells ml^{-1} [190]. Yergeau et al. (2009) used microarray platform, targeting the functional genes involved in hydrocarbon degradation to compare two distinct bioremediation sites in the Canadian high Arctic. Their results demonstrated the utility of microarrays as a tool to rapidly monitor *Bacteria* and their functions in polar environments during bioremediation. In our study, we used the same FGMA targeting the same genes. The only difference related to the part that we used oligonucleotide microarray, whereas Yergeau et al. used a PCR product microarray [180].

1.8.5 Community Genome Arrays (CGAs)

Community genome arrays are based on whole-genome, isolated from cultured microorganisms. CGAs may potentially be made from metagenomic environmental libraries. A clear advantage of CGA is that, there is no need for PCR amplification of the target [145]. Compared with the traditional DNA–DNA reassociation approach, CGAs have several advantages for determining species relatedness. As many Bacterial genomes can be deposited on microarray slides, the laborious pair-wise hybridization is not needed with this technique. In contrast to the traditional DNA–DNA reassociation approach, which generally requires about 100 μg DNA, CGA requires only about 2 μg of genomic DNA. This is important for determining the relationships between bacterial species that grow very slowly. A major drawback of the CGA approach is that only cultivated *Bacteria* can be used to generate probes.

Novel metagenomic techniques generating large genome fragments from uncultivated microbes [193] may help in overcoming this limitation. The results of Zhou and Thompson (2002) showed that DNA–DNA hybridization on CGAs in the presence of 50% (vol/vol) formamide at 55°C could discriminate between microbial genomes of different species within a genus whereas, in many cases, genomes could not be clearly distinguished at the subspecies level. By rising the hybridization temperature to 65–75°C, CGA discrimination between closely related Bacterial strains could be improved. Their work suggested that CGA hybridization is potentially a quantitative tool for the detection and identification of microorganisms in environmental samples. However, when using CGAs for detecting Bacterial populations in a mixed microbial community, strict hybridization conditions should be used to minimize any potential cross-hybridization among closely related species and strains [194].

1.8.6 (Catalyzed Reporter Deposition)-Fluorescent *in situ* Hybridization (CARD)-FISH

Fluorescence *in situ* hybridization (FISH) has been one of the most powerful techniques that enable cultivation independent characterization of microorganisms and measurement of biomass. In this technique, oligonucleotide probes are designed based on signature nucleotide positions in the Bacterial/Archaeal 16S rRNA and may be used to target either a narrow or broad group of organisms. With the FISH, it may be possible to detect the active Bacterial populations in the soil directly and to determine the responses of the *Bacteria* to different soil conditions. FISH can be used in combination with DAPI (4′,6′-diamidino-2-phenylindole), INT (2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride), or CTC (5-cyano-2,3-ditolyl tetrazolium chloride) [195-196] to determine the contribution made by the populations of interest

to total abundance or active cell count. For a number of different reasons, FISH can fail to detect microbes in many ecosystems. One common problem arises when targeted organisms have a low cellular rRNA content, which produces weak fluorescence under the microscope [197]. For such situations, different methods have been developed to improve the sensitivity of FISH. CARD-FISH is one of the techniques that help to overcome some of the problems associated with FISH [198-202]. By using a novel tyramide substrate for the generation of fluorescent signal by an oligonucleotide-linked catalytic enzyme, researchers have been able to enhance fluorescence intensities and signal-to-noise ratios, increase the detection rates of particular taxa, and in some cases to detect taxonomic groups undetectable by standard FISH [203-205]. In spite of these improvements, the minimal numbers of rRNA target molecules required to obtain a visible fluorescence signal after CARD-FISH (with rRNA-targeted probes) have not been determined yet [206].

Using FISH, native *Bacteria*, *Pseudomonas* and filamentous *Bacteria* were quantified and localized on wheat roots grown in the field. Across all wheat roots, *Bacteria* averaged 15.4×10^5 cells per mm^3 rhizosphere, and of these, *Pseudomonas* and filaments comprised 10% and 4%, respectively, with minor effects of sample time, and no effect of plant age [207]. *Bacteria* were quantified in the rhizosphere of wheat grown in the field, using fluorescence in situ hybridization (FISH). Across all samples, collected at different seasons (spring, early and late winter) *Bacteria* ranged widely from 0 to 85.9×10^5 cells per mm^3 rhizosphere [208]. FISH were also used to determine the abundance of distinct methanotroph groups in a *Sphagnum* peat sample of pH 4.2. Results revealed that the acidophilic methanotroph *Methylocella palustris* was present at a relatively high abundance (1.2×10^6 cells per g of wet peat) [209]. In another study,

the application of FISH and CARD-FISH for the quantification of *Dehalococcoides* spp. both in a trichloroethene (TCE) dechlorinating culture and in environmental samples collected from a chlorinated ethenes and ethanes-contaminated site was compared. *Dehalococcoides* spp. ranged between 2% and 6% of the total *Bacteria* when estimated by CARD-FISH on site samples, whereas FISH failed, most likely due to the low ribosome content. The differences between the numbers of *Dehalococcoides* cells detected by FISH and the numbers of total cells visualized by CARD-FISH (including the low active/inactive cells) could become a useful gross parameter for a preliminary evaluation of the actual bioremediation capacity of a contaminated site [210]. In a study, FISH was applied to the samples from forest soil and the results indicated that soil *Archaea* account for 0.21% ± 0.65% of the detectable cells in this soil [211].

1.9 Research objectives

Many studies have been conducted to investigate the influences of agricultural practices on soil microbial communities, but because of the differences in land management, soil types, climates and methodologies, it is difficult to generalize the influence of agriculture practices from the experimental results obtained to date. There is no universal or fixed relationship between tillage system, residue management, and soil microbial genetic diversity and activity. The relationships between these factors vary due to climate, soil type, and cultivated crop. The debate over different tillage practices and residue management is ongoing and there is no single solution for all circumstances. The objectives of this study were to determine the effects of the interaction of tillage and residue management on:

1. Soil Bacterial diversity, using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE);

2. Soil Archaeal diversity, using PCR-DGGE and 16S rRNA gene clone library analyses;
3. Activity of atrazine degrading microorganisms, using a C-14 atrazine mineralization microcosm assay;
4. Functional diversity of soil microorganisms (pollutant and pesticide degradation, especially atrazine), using an oligonucleotide functional gene microarray (FGMA);
5. Soil biomass, and number of *Bacteria* and *Archaea* using catalyzed reporter deposition-fluorescent *in situ* hybridization (CARD-FISH) and 5-(4, 6-dichlorotriazinyl) amino fluorescein hydrochloride (DTAF).

All the above analyses were performed on soils cultivated for corn production. Corn was selected because for the past 35 years, it has been the second most widely grown crop in Québec province and occupies 24% of the cropped land. For Bacterial and Archaeal diversity, PCR-DGGE approach was applied, since it is a reliable technique to monitor changes in microbial communities in response to environmental parameters. As explained previously, the assessment of soil management practices on soil Bacterial and Archaeal diversity has the potential to provide useful insights into soil health. Application of Archaeal 16S clone library provided insight into the Archaeal diversity of the most agricultural soils, especially Southern Québec agricultural soil to build foundational information for future research. To our best of knowledge, there is no information regarding Archaeal diversity, not only in southern Québec agricultural soil but also, in all the province of Québec. The activity of atrazine degrading microorganisms was evaluated using radiorespiratory assays. This herbicide is widely used to control pre- and post-emergence broadleaf weeds in corn. In Québec, atrazine represented 27% of all pesticide sales [19].

Functional diversity of soil microorganisms was also investigated, which provided information relevant to the influences of the applied treatments. Application of CARD-FISH and DTAF offered useful information regarding soil biomass and the Bacterial and Archaeal abundance under different land management practices.

We hypothesized that the interaction of three level of tillage practices and two crop residues treatments may change soil Bacterial and Archaeal genetic/functional diversity. The applied treatments may also alter the activity of soil microorganisms involved in atrazine degradation and consequently soil functional diversity. We also hypothesized that microbial biomass and the abundance of soil *Bacteria* and *Achaea* may change as a consequence of application of different agricultural treatments. Overall, we hypothesized that land management practices may produce unique soil environments, resulting in differences in microbial community composition and diversity and potentially affecting soil health.

Chapter 2: Materials and Methods

2.1 Experimental site

The experimental field plots used in this study were located at the Macdonald Research Farm of McGill University, Ste. Anne de Bellevue, QC, Canada (45°28' N, 73°45' W, elevation 35.7 m). The soil is a Humic Gleysol, classified as a St. Amable loamy sand and shallow loamy sand with pockets of Courval sandy loam, overlying clay at a mean depth of 0.46 m. Soils in the 0 to 0.2 m depth contain on average, 82% sand, 9% silt, and 10% clay, with 15 g organic C kg^{-1} and a pH of 6.1. The mean slope at the site is less than 1%. The experiment consisted of a factorial combination of three tillage treatments (conventional tillage (CT), reduced tillage (RT), and no-tillage (NT)), and two crop residue managements (without (-R) and with residues (+R)). The six treatment combinations were laid out in three replicated blocks, resulting in 18 plots. Each plot had dimensions of 18.5 m × 80 m. Each plot had a centrally located subsurface drain installed at a mean depth of 1.2 m. Two meter buffer strips separated the plots, while 3 to 4 m wide buffer strips separated the blocks. The layout of the field experiment plots is illustrated in Figure 1. Conventional tillage (CT) consisted of mouldboard ploughing after harvest (October-November) to a depth of 0.20 m and tandem disking to a depth of 0.10 m in May, before planting. Reduced tillage (RT) consisted of offset disking to 0.15 m after harvest and tandem disking to a depth of 0.10 m before planting. No-till (NT) was not tilled at any time. Crop residue is defined as the non-edible plant parts which are left in the field after harvest [212]. The treatment without residues (-R) consisted of corn harvested as silage corn, where only stubble (0.15 m of stalk) remained, resulting in a smaller amount of residue coverage. The treatment with residues (+R) consisted of harvesting only the kernels as grain corn. The cobs, leaves, and stalks were chopped by combine and returned to the field. The residues remaining on the soil

surface in no-till were partially incorporated in reduced tillage and completely incorporated in conventional tillage. The same tillage and residue treatments were imposed in 1991, and have been continued annually to the present. Since 1991, the site had been under continuous monocropped corn (*Zea mays* L.).

2.2 Soil sample collection

Soil samples were collected during the first and the second cycle of the experiment. The first cycle of the experiment started in November 2006 and ended in October 2007. In the first cycle of the experiment and for the purpose of detection, quantification and community analyses of *Bacteria* and *Archaea*, soil sampling was performed before atrazine application (May, 2007), during the growing season (August, 2007), and after harvesting (November, 2006/October, 2007). In order to assess the functional diversity and the potential for pollutant and pesticide degradation in the first cycle of the experiment, sampling was also performed before applying atrazine (May, 2007), one and two weeks after atrazine application (July, 2007). The second cycle of the experiment started from after harvesting in October 2007 until after harvesting in November 2008. In this cycle, soil samples were collected two weeks after atrazine application (June, 2008) to evaluate the functional diversity of the soil microbial community and also the potential for pollutant and pesticide degradation. Sampling was also carried out after harvesting in November 2008 to detect and enumerate the Bacterial and Archaeal communities. All the soil samples in the first and the second cycle of the experiment were collected from five different locations in each plot (replicates) at 2 depths (0-5 and 5-20 cm) and mixed to yield one composite sample per depth per plot. The soil samples were taken from the planted rows and not

from the space between the rows. The samples were then combined to form a single composition per treatment and depth, yielding a total of 12 composite samples. After each sampling, soil samples were stored on ice in a cooler and quickly transported to the Macdonald Campus of McGill University, where they were stored at +4^{o}C and -20^{o}C.

2.3 Commonly used procedures and methods

2.3.1 DNA extraction and Polymerase Chain Reaction (PCR) preparations

All community DNA extractions from 1 g of soil were performed using UltraClean^{TM} Soil DNA Kit (Mobio laboratories, Solana Beach, CA) according to the manufacture's instructions. The extracted DNA was then subjected to the PCR. PCR water was prepared by 30 min UV treatment of sterile, purified deionized water (Millipore Corporation, Billerica, MA). PCR reagents including 10× buffer without $MgCl_2$, 10× buffer containing 15 mM $MgCl_2$, $MgCl_2$ (50 mM), *Taq* DNA polymerase (5U μl^{-1}), and HotStar *Taq* DNA polymerase (5U μl^{-1}) were supplied by Qiagen (Mississauga, ON) and Invitrogen (Burlington, ON). An aliquot of 10 mM of each deoxyribonucleotide triphosphate (dNTP) was prepared in PCR water from 100 mM dATP, dCTP, dGTP, and dTTP stocks (Invitrogen, Burlington, ON). 10 mg ml^{-1} bovine serum albumin (BSA) was prepared by dissolving 10 mg of BSA (TCN Biochemical, Cleveland, OH) in 10 ml of PCR water. All primers used in this study were purchased from MWG-Biotech (Huntsville, AL) and are listed in Table 2. Primers were prepared as a 10 µM solutions in PCR water from supplied stocks. PCR reagents were stored at -20^{o}C and thawed on ice prior to preparation of the reactions. All PCR reactions were prepared in thin walled 12-tube strips (DiaMed Laboratory, Mississauga, ON). For the purpose of sterilization all tips, pipettes, tubes, and PCR water, were

UV treated for 15 min prior to use. All PCR reactions were carried out with a negative control, in which PCR water replaced by template DNA in the reaction. In order to confirm that proper reaction conditions were achieved, positive control was used with a known source of DNA. All PCR reactions were carried out in a TC-312 Thermocycler (TECHNE, Burlington, NJ), or a Touchgene Gradient Thermocycler (TECHNE, Burlington, NJ). PCR conditions for various experimental procedures are explained in detail in the related sections.

2.3.2 Gel Electrophoresis

In order to assess the success of DNA extraction and PCR amplification, gel electrophoresis was performed on extracted DNA and PCR products. 30 ml 0.8% (w/v) agarose gels were prepared either in 1× TAE (40 mM Tris-acetate and 1 mM ethylenediamine tetraacetic acid (EDTA) (pH 8.0)) or 1× SB (sodium borate). Gels were loaded with 5 µl of sample after adding 2 µl of 5× loading buffer (0.3 M Tris-HCl, 0.05% bromophenol blue, 10% SDS, and 50% glycerol). 1× TAE gels were run at 80 V for 45 min and 1× SB gels were run at 160 V for 6 min using a 100 bp ladder (Invitrogen, Carlsbad, CA) as a molecular weight marker. DNA quantification was also determined using a NanoDrop ND-1000 spectrophotometer (Nanodrop Technologies). For restriction fragment length polymorphism (RFLP), 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). Denaturing gradient gel electrophoresis (DGGE) gels was described separately in section 2.3.5.

2.3.3 Bacterial PCR

The composition of the soil Bacterial and Archaeal communities was investigated based on 16S rRNA gene-targeted PCR-DGGE. This technique was performed for the samples collected before atrazine application (May, 2007), during the growing season (August, 2007) and after harvesting (November, 2006/October, 2007). In addition, PCR-DGGE using α-Proteobacteria and β-Proteobacteria 16S rRNA gene primers was performed for the samples collected after harvesting in October 2007. The application of the group-specific primers provided a higher resolution genetic fingerprinting approach than existing primer sets. In order to have the proper amount of DNA for PCR reaction, all DNA extractions were diluted tenfold and Bacterial 16S rRNA genes were amplified by PCR. Three types of PCR were performed using 16S rRNA universal primers [213], α-Proteobacteria 16S rRNA and β-Proteobacteria 16S rRNA group specific primers [214]. For Bacterial 16S rRNA gene amplification, PCR mixture was prepared as 50 µl reactions containing 33.30 µl PCR water, 5 µl PCR 10× buffer with 1.5 mM $MgCl_2$, 0.5 µl BSA 10%, 1µl of 10mM dNTPs mix, 2.5 µl of each primer (341F+GC/758R) (10 µM), and 0.2 µl of HotStar *Taq* DNA Polymerase (5U μl^{-1}). At the end, 5 µl of 1:10 DNA dilution (50-90 ng) was added to this mixture. The PCR program consisted of 15 min at 95^{o}C, followed by 10 touchdown cycles (65-55^{o}C) and further 25 cycles at 55^{o}C of 30 s, followed by 72^{o}C for 1 min and 30 s, and a final extension of 10 min at 72^{o}C. For each sample, a total of three to five reactions were pooled to accumulate enough PCR products for DGGE analyses. For α-Proteobacteria 16S rRNA gene amplification, a nested PCR approach was employed. The first PCR reaction mixture contained 36.30 µl PCR water, 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 primer (Alf28F/Alf684R) (10 µM), 0.2 µl of HotStar *Taq* DNA Polymerase (5U μl^{-1}), and 5 µl of template DNA. Samples were

amplified under the following conditions: initial denaturation of 15 min at 95^{o}C, 35 cycles of 95^{o}C for 1 min, annealing temperature of 65^{o}C for 1 min, 72^{o}C for 1 min, followed by a final extension at 72^{o}C for 10 min. 5 µl of group specific PCR products (dilution 1:100) were used as template in a re-PCR with the Bacterial 16S rRNA genes primers. PCR conditions and cycle protocol for the second PCR were the same as those used for first α-Proteobacteria PCR, except for the primers (341F+GC/518R) and annealing temperature (55^{o}C). For β-Proteobacteria, a semi-nested PCR was used. The first PCR reaction contained 36.30 µl PCR water, 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 primer (Beta359F/Beta682R) (10 µM), 0.2 µl of HotStar *Taq* DNA Polymerase (5U μl^{-1}), and 5 µl of template DNA. PCR cycling was performed at 95°C for 15 min, followed by 35 cycles at 95°C for 1 min, annealing temperature of 60^{o}C for 1 min, and extension at 72°C for 1 min. The final cycle was followed by a 10 minute extension at 72°C. The second PCR was performed under the same conditions as the first PCR. Aliquots of the first PCR (5 µl of 1:100 dilution) were reamplified using the primer pair (518F+GC/Beta682R). The cycling protocol was the same as described for the first PCR, except for the annealing temperature (57^{o}C).

2.3.4 Archaeal PCR

For Archaeal 16S rRNA gene sequences, a nested PCR approach was applied [215]. Reaction mixtures were prepared in a total volume of 50 µl and contained 33 µl PCR water, 5 µl PCR buffer 10× with 1.5 mM $MgCl_2$, 0.5 µl BSA 10%, 1 µl of 10 mM dNTPs, 1 µl of each primer (109F/934R) (10 µM) and 0.5 µl of HotStar *Taq* (5 U/µl). At the end, 5 µl of tenfold DNA dilution was added to this. PCR was performed using the following conditions: 95°C for

15 min, followed by 35 cycles of 30 s at 95°C, 45 s at 60°C, 1 min and 30 s at 72°C and a final extension at 72°C for 10 min. The PCR products generated from the first PCR were diluted 1:100 with PCR water and used as template for a second PCR. The second amplification carried out the same as the first one, except for the primer (344F+GC/934R). Reaction conducted with the following condition: initial denaturation of 15 min at 95^{o}C, 30 cycles of 95^{o}C for 1 min, annealing temperature of 57^{o}C for 1 min, 72^{o}C for 1 min, followed by a final extension at 72^{o}C for 10 min.

2.3.5 Bacterial and Archaeal DGGE analyses

DGGE for the PCR products of the *Bacteria* and *Archaea* was performed using a DCode Universal Mutation Detection System as described in the manufacturer's instructions (Bio-Rad, Hercules, CA). An 8% acrylamide gel with a gradient of urea and formamide from 35%- 65% was prepared using a Bio-Rad Model 385 Gradient Former and a casting comb was inserted into the gel. The gel was allowed to solidify for 2 h. Each lane was loaded with ~ 800 ng of PCR product in a final volume of 50 µl after adding of DGGE loading buffer (0.05% bromophenol blue, 0.05% xylene cyanol, and 70% glycerol in deionized water). Samples were run for 16 hours at 80 V in 1× TAE buffer at 60^{o}C. Gels were stained for 20 min in a solution of 5% (v/v) ethidium bromide prepared in 1× TAE buffer and destained for 10 min in deionized water. Gels were imaged on Bio-Rad molecular imager (Hercules, CA). DGGE of the PCR products form group specific primers was performed in the same manner except for the denaturing gradients varied between 40% and 60%. 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. The Shannon index (*H*) was also used to estimate soil Bacterial and Archaeal diversity based on the intensity and number of bands.

2.3.6 16S rRNA DGGE fragment isolation and sequencing

Bands were excised from DGGE gels with a cutting tip (DiaMed Laboratory, Mississauga, ON) and were eluted at 4^{o}C overnight in 50 µl of PCR water. The eluted DNA samples were PCR amplified using the same conditions described before (section 2.3.3 and 2.3.4). Following PCR, re-amplified DGGE DNA fragments from Archaeal DGGE gels were sent directly for sequencing to the Laval University Bioinformatics Centre. Due to the difficulty of direct sequencing DGGE fragments of Bacterial 16S rRNA gene, a cloning approach using the pGEM-T Easy Vector (Promega, Madison, WI) was employed. The reamplified bands from Bacterial DGGE gels were purified using a Qiagen gel purification kit (Mississauga, ON) to remove salts, dNTPs, primers, and enzymes following the manufacture's protocol. After the purification, cloning was performed and clones were sent to the same center for sequencing. No bands were excised from α-Proteobacteria and β-Proteobacteria DGGE gels.

2.3.7 Media preparation for cloning, ligation, and transformation

All components used in media preparation were obtained from Fisher Scientific (Whitby, ON). Luria-Bertani (LB) broth contained: 10 g tryptone, 5 g yeast extract, and 5 g NaCl (pH 7.0) per liter. LB agar had the same composition as LB broth but also contained 15 g l^{-1} of agar.

LB/amp agar refers to LB agar supplemented with filter sterilized 10 mg ml^{-1} ampicillin solution to a final concentration of 100 µg ml^{-1}. SOC broth was composed of the following ingredients per liter: 20 g tryptone, 5 g yeast extract, 10 ml of 1M NaCl, 2.5 ml of 1 M KCl, 10 ml of 2 M dextrose, and 10 ml of 2 M Mg^{+2} (prepared as 203.3 g l^{-1} $MgCl_2.6H_2O$ and 246.5 g l^{-1} $MgSO_4.7H_2O$) (pH 7.0).

Cloning of purified PCR products was completed in 8:1 and 3:1 ratios of inset (PCR product) to vector. A positive control reaction was performed in which a control insert provided in the kit was ligated into the vector in a 2:1 ratio by volume. A negative control was prepared by producing a ligation reaction with 1 µl of PCR product and no vector for each sample, in order to ensure no vector contamination of any solution or samples.

Transformation of vector into competent cells was carried out as described by the manufacture's protocol with minor modifications. The reagent volumes suggested by the original protocol were split in half, resulting in a total volume of 5 µl. The total volume of each ligation reaction was added to each DH5α competent cells (Invitrogen, Burlington, ON), mixed gently by flicking, and maintained on ice for 20 min. Cells were then heat shocked for 45 s in a 42^{o}C water bath and immediately placed back on ice for 2 min. Cells were removed from ice, mixed with 150 µl of SOC broth, and incubated at 37^{o}C, shaking at 115 rpm for 1 h. Aliquots of 100 µl of each transformation were plated on LB/amp plates spread with 100 µl of 40 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and 100 µl 2% (w/v) 5-bromo-4-chloro-3-indolyl-β-D-galactopyr- anoside (X-Gal) 30 min prior to inoculation. Plates were incubated overnight at 37^{o}C.

2.3.8 Clone analyses

Plates of transformation were analyzed by blue/white screening for recombinant in order to determine successful cloning. White colonies putatively contain vector with 16S rRNA gene insert. Colonies were picked randomly from plates with a sterile toothpick and incubated into the wells of a 96-well plate containing 50 µl of sterile UV treated water in each well. Boiling lysis was then performed. The 96-well plate was then heated to 96^{o}C for 10 min in a Touchgene Gradient Thermocycler and cooled to 4^{o}C. DNA extracted from clones by boiling lysis was used to amplify clones inserts from the pGEM-T Easy vector by PCR. The PCR mixture contained 35.50 µl PCR water, 5 µl PCR 10× buffer, 3 µl $MgCl_2$ (50 µM), 1 µl of 10 mM dNTPs mix, 2.5 µl of each primer (Sp6, T7) (10 µM) and 0.2 µl of *Taq* DNA Polymerase (5U μl^{-1}). 5 µl of supernatant from boiling lysis was then added to the PCR mixture. The PCR conditions consisted of 5 min at 95^{o}C, 30 cycles of 45 s at 95^{o}C, 30 s at 57^{o}C, and 1 min at 72^{o}C, and a final extension of 5 min at 72^{o}C. Isolated DGGE bands and clones were identified based on 16S rRNA gene sequencing. DNA sequences were compared to known sequences in the NCBI, GenBank database using the BLASTn algorithm [216]. 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).

2.3.9 Construct and analysis of a Archaeal 16S rRNA clone library

An Archaeal clone library was constructed from one of the soil samples (RT+R 0-5 cm) collected during the growing season. DGGE profile showed that the selected sample harbored high Archaeal diversity among the samples collected in 2007. The cloning procedure was

described before (section 2.3.7) using the 751F and 1406R primers. The PCR products were screened for similarity by RFLP analysis. 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 were incubated further at 65°C for 20 min. The RFLP pattern was viewed by gel electrophoresis as described before. Sequences with identical RFLP patterns were grouped together as similar sequences and representatives of each group were chosen for sequence analyses. Cloned insert sequences were compared to known sequences in the NCBI database using the BLASTn algorithm. All sequencing was undertaken by the Laval University Bioinformatics Centre. Sequences of the clone library were aligned using ClustalW software and neighbor-joining phylogenetic trees were produced with the MacVector 7.0 software package (Oxford Molecular Ltd., Oxford, UK) using Jukes-Cantor modeling with 1000 bootstrap re-samplings [217]. Rarefaction analysis was performed and diversity indices were calculated to characterize the Archaeal diversity of the soil sample. The rarefaction curves were constructed using Analytic Rarefaction 1.3 (http://www.uga.edu/~strata/software/index.html). The coverage of the library was calculated as defined by Perreault et al. (2007), with the following formula: $C = (1 - n1/N) \times 100$, where *n1* is the number of phylotypes appearing only once in a library and *N* is the library size. Shannon diversity indices were calculated via the DOTUR software [217].

2.3.10 Preparation of microcosm for atrazine mineralization

[U-ring-^{14}C] atrazine with the specific activity of 9.5 mCi mmol^{-1} was purchased from Sigma-Aldrich Chemical Company and had purity greater than 98%. Radiolabeled atrazine was first diluted in methanol 90% (v/v). Non-radioactive atrazine, AAtrex 480, (Syngenta, Guelph, ON) kindly was provided by Peter Kirby at soil science department. In the second cycle of the experiment, a respiration assay was performed with the samples collected in June 2008 from the depth of 0-5 cm. Radioactive CO_2 produced from the breakdown of ^{14}C labeled atrazine molecules was trapped in a solution of KOH. The amount of radioactivity present in the KOH was measured every five days for five weeks. Briefly, a quantity of moist soil corresponding to 20 g dry soil was dispensed into sterile 50 ml serum bottles (Fisher Scientific, Whitby, ON), using a sterile spatula. Soil moisture was set at 70% of the field capacity with the solution containing commercial atrazine to a final concentration of 0.015 mg active atrazine in each microcosm. Soils were then spiked uniformly with 20 µl aliquot of ^{14}C labeled atrazine solution with an activity of 0.5 µCi per microcosm. A sterile (8 × 40 mm) culture tube (Fisher Scientific, Whitby, ON) containing 1 ml of trap solution (1 M KOH) was placed inside the serum bottle. Microcosms were capped with a sterile butyl seal, stirred for 10 s. The rubber cap was replaced and a steel seal was crimped onto the top of the bottle to seal the microcosm. Microcosms were then incubated at 22^{o}C. In parallel to this experiment and in order to compare atrazine mineralization rate in agricultural soil with forest soil, another mineralization assay was also performed. Forest soil samples for this experiment were collected from the Morgan Arboretum of McGill University, within 2 km of the experimental plots. Negative controls were prepared by the same methods used for preparation of the experimental microcosms; however, prior to the addition of the radioactive atrazine, the serum bottles containing sample were autoclaved three

times, 24 h apart at 121^{o}C for 30 min. Microcosms were performed in triplicate for each sample [218-220].

2.3.11 Measurements of CO_2 evolution in microcosm

The evolution of CO_2 in the microcosms was measured every 5 days. Briefly, the top of the rubber plug was wiped with 70% ethanol to sterilize the top surface of each microcosm. A sterile steel needle attached to a 5 ml syringe was pushed through the rubber stopper to draw the trap solution into the syringe. The trap solution was deposited into a scintillation vial containing 18 ml of Scintiverse fluid (Fisher Scientific, Whitby, ON). 1 ml of fresh trap solution was dispensed through the needle into the trap tube, and rinsed up and down three times in order to remove residual radioactive solution. This 1 ml of trap solution was then deposited into the same scintillation vial. A fresh 1 ml aliquot solution was dispensed into the trap tube and the needle was removed from the microcosm. The mixture in the scintillation vial was capped and mixed briefly by shaking. The radioactivity within the scintillation vials was determined using a LS 6500 multipurpose scintillation counter and supplied software (Bechman Coulter, Fulletron, CA) [221].

2.3.12 Functional Gene Microarray (FGMA) analysis of agricultural soils

The oligonucleotide FGMA used in this study was developed by Dr. Greer's laboratory at the Biotechnology Research Institute of Montreal (BRI) and contains 100 gene probes derived from various Bacterial catabolic pathway for organic pollutants and biogeochemical cycles including gene targets relevant to this study: chlorinated organics, 3 genes for atrazine

degradation, 6 genes for 2,4-dichlorophenoxyacetic acid (2,4-D) degradation, 2 genes for haloalkane dehalogenation, and several other chloroorganic compound degradation (PCP, chlorocatechol) genes; denitrification genes, *nosZ* (nitrous oxide reductase), *norB* (nitric oxide reductase), *nirS*, *nirK* (nitrite reductase), and *narHI* (nitrate reductase beta subunite). The layout of the FGMA chips is illustrated in Figure 2. The description of the selected soil samples for FGMA is described in Table 3. The gene used as a negative control was *GFP*, encoding a green fluorescent protein first isolated in protein extracts from the luminescent hydrozoan jellyfish *Aequorea* [222]. This was considered a negative control because of the extremely low likelihood that this gene would appear in the sample microbial communities. The *luxA* gene was used in this study as a labeling control. This gene encodes the α-subunit of the light-emitting luciferase protein from Bacterial *Vibrio* symbionts of luminescent marine invertebrates [223]. Each probe labeling reactions was spiked with a constant amount of *luxA* amplicon. When data from more than one hybridization was pooled, the intensities of the *luxA* control spots were used to normalize the data.

2.3.13 Fluorescent DNA labeling

Fluorescent-labeled DNA samples were prepared using BioPrime DNA Labeling Kit (Invitrogen, Burlington, ON). 400 ng of DNA of the selected samples was used as template for labeling. 0.2 ng of *lux*A PCR amplicon (1:2000 of the template DNA) were added to each labeling reaction to be used as a labeling control for hybridization. In each 50 µl reaction, 20 µl of 2.5× random octomer primers solution was added to the template DNA and denatured in a PCR machine (TC-312 Thermocycler or Touchgene Gradient Thermocycler) for 5 min at 95^{o}C, followed by incubation on ice for 5 min. On ice, 5µl of 10× dCTP and 2 µl of 1 mM Cy5-dCTP

(GE Healthcare) were added and mixed briefly. Finally, 1 µl of Exo-Klenow polymerase (40 U μl^{-1}) was added, gently mixed and incubated at 37°C for 3 h. The reaction was stopped by adding 5 µl of stop buffer (0.5 M Na_2EDTA, pH 8.0). Each labeling reaction was purified using the PureLink PCR Purification Kit (Invitrogen, Whitby, ON) with some modifications. After binding of DNA to the column, two extra washes were performed with the supplied binding buffer (B2) to eliminate unreacted Cy5-dCTP nucleotides from the column. Using a NanoDrop ND-1000 Spectrophotometer DNA (Nanodrop Technologies) DNA concentration was determined by measuring the intensities at 260 and 280 nm and Cy5 incorporation was measured at 650 nm.

2.3.14 Microarray hybridization

All the LifertSlips coverslips (Erie Scientific) washed with 70% ethanol and then with 100% isopropanol prior to utilization. Microarray slides were covered using a LiferSlip (25 × 60 mm). 150 µl of preheated (50°C) prehybridization buffer (10 mg of BSA + 995 µl of 5× SSC buffer (0.75 M NaCl and 0.075 M sodium citrate) + 5 µl of 20% SDS (pH 7.0)) was loaded on the microarray slide using a pipette. The slides were placed in a Corning hybridization chamber (Corning) and were incubated at 50°C for 1 h. After pre-hybridization, microarrays were washed by dipping five times in 3 consecutive Falcon tubes (Fisher scientific, Whitby, ON) containing 0.1× SSC buffer, followed by a wash in Zenopure water and air dried for 2-3 minutes. Microarrays were covered using the LifterSlip coverslips (221 × 251 mm) prior to hybridization. Labeled DNA was concentrated in a DNA speed vac and then resuspended in 32 µl of DIG Easy hybridization buffer (Roche Applied Science), supplemented with 1 µl tRNA (Sigma) and 1 µl

salmon sperm (Invitrogen, Whitby, ON). Hybridization buffer was denatured at 95^{o}C for 2 min prior to loading onto microarrays. Microarrays slides were placed in a Corning hybridization chamber and incubated at 50^{o}C for 16 h. 10 µl of water placed in each well to maintain the moisture level during hybridization. The water bath lid was covered with aluminum foil to avoid photo bleaching. Each microarray slide was then transferred to a Falcon tube containing preheated 0.1× SSC-0.1% SDS (42^{o}C) and mixed in a Belly Dancer for 5 min. This step was repeated 2 more times. Washing buffer was removed by incubating slides 3 times (1 min each) with rinse buffer (0.1× SSC-0.1% SDS) and mixing by inversion. The slides were then dipped 2 times (1 s each) in 100% isopropanol to give the microarray a final rinse. Microarrays dried under a mild stream of compressed air.

2.3.15 Microarray scanning, image analysis and data normalization

Microarray chips were scanned with a ScanArray Lit Microarray Analysis System (PerkinElmer, Mississauga, Ontario). Scanning was performed at 10 µm resolution with 90% laser power variable photomultiplier tube (PMT) voltage setting to obtain maximal signal intensities with minimal background by excitation of Cy5 dye at 650 nm to generate raw fluoresce intensity values. Fluoresce images were saved as multilayer tiff images. Scan Array Express software (Perkin Elmer, Mississauga, Ontario) was used for image analysis such as hybridization spot finding and raw data collection for quantification. Spots were quantified using adaptive circle segmentation to define signal and background pixels, as this method calculates the area based on the shape of the spot and therefore works well when the spots are irregular in shape. Results were then transformed to Microsoft Excel spreadsheets for data

analysis. All raw intensities from microarray scans were screened for true positive signal intensities. Microarray data was normalized in two steps. The first step was applied to all quantitative data generated by the ScanArray Express software. Median pixel intensity, signal to noise ratios and average background signal for each microarray spot were calculated by the software. The average of all background values of each hybridization was subtracted from the median signal for each spot, to provide a hybridization signal intensity value corrected for background noise. Signal intensity values standardized by this technique are reported in the results with the designation "corrected". In the second step, the difference data from disparate hybridizations were normalized on the basis of *luxA* labeling control. The average intensity of all *luxA* control spots in all hybridizations was divided by the average intensity of all *luxA* spots in a single hybridization, generating relative a correction factor for each hybridization. All signal intensity values (corrected) were multiplied by this correction factor. Designation of a microarray spot as hybridization positive was based on the signal to noise value for that spot. A signal to noise ratio greater than or equal to 2 (SNR≥2) was considered to constitute a positive signal. Principal coordinate analyses (PCoA) was carried out as described by Yergeau et al. (2009) [180].

2.3.16 CARD-FISH sample fixation and permeabilization

In order to determine the relative amounts of *Bacteria* and *Archaea,* and estimate soil biomass, CARD-FISH was performed for the samples collected after harvesting from 0-5 cm in the first year and the second cycle of the experiment. Soil samples, 0.25 g (dry weight), were fixed with 1.5 ml formaldehyde (4% in phosphate saline buffer, PBS: 137 mM NaCl, 3 mM KCl,

8 mM Na_2HPO_4, and 1.5 mM KH_2PO_4) at 4^oC for the 16 h. Fixed sample were washed twice with 1× PBS, centrifuged at 12,000 xg for 5 min at 4^oC. 1 ml of a 1:1 PBS/ethanol was added to the sample and stored at -20^oC. 100 µl of the fixed sample was diluted with 900 µl dilution buffer (0.1% (w/v) $Na_2PO_7.10H_2O$ (pH 7.0)) and dispersed by ultrasound at minimum power for 20 s. Serial dilutions up to 10^{-3} were prepared by transferring 1 ml of the sonicated buffer to 9 ml of dilution buffer in a Falcon tube. The mixture was diluted two more times by adding 1 ml of mixture to 9 ml of dilution buffer. An aliquot of 1 ml of diluted and sonicated sample was filtered through a 0.2 µm pore size, white polycarbonate membrane filter (Poretics Corp., Livemore, CA), while a 0.45 µm pore size, white polycarbonate membrane filter was underneath (Poretics Corp., Livemore, CA) to improve distribution of cells. The filter was sealed in an autoclavable filter funnel (effective filtration area 4.90 cm^2). The tubes containing diluted and sonicated samples were rinsed three times with the dilution buffer in order to ensure capture of all cells on the filter.

To prevent cell loss during the permeabilization procedure, the cells have to be attached onto filters. The filters were covered with the low melt agarose (0.1%) (Invitrogen, Carlsbad, CA) at 40°C, (using a 1000 µl pipette) and dried at 37°C for 3 h. Cells were premiabilized with lysozyme (Thermo scientific, San Jose, CA). Filters were incubated in 20 ml of fresh lysozyme solution (10 mg ml^{-1} in 0.05 M EDTA (pH 8.0), 0.1 M Tris-HCl (pH 8.0)) for permiabilization for 60 min at 37°C in a water bath. Filters were washed in a petri plate containing 25 ml deionized water, followed by washing with absolute ethanol. Filters were dried on a microscope glass slide for 30 min (Fisher Scientific, Whitby, ON).

2.3.17 Hybridization and washing

A small section of filter was cut out of the whole filter and placed in 1.5 ml centrifuge tube (DiaMed Laboratory, Mississauga, ON), containing hybridization buffer (0.9 M NaCl, 20 mM Tris-HCl (pH 7), 10% (w/v) dextran sulfate, 0.02% (w/v) sodium dodecyl sulfate (SDS), 1% blocking reagent (Roche Applied Science), 55% (v/v) formamide (for EUB338 and ARCH915), and HRP (horseradish peroxidase) labelled oligonucleotide probe (300:1) purchased from MWG-Biotech (Huntsville, AL). Universal Bacterial and Archaeal probes used in this study were EUB338 (5′-GCTGCCTCCCGTAGGAGT-3′) and ARCH915 (5′-GTGCTCCCCCGC CAATTCCT-3′) [224]. Hybridization was performed overnight on a rotation shaker at 10 rpm and the temperature was adjusted at 35°C. The tubes were taped to the rotator directly. 1% blocking reagent was prepared in maleic acid buffer (100 mM maleic acid, 150 mM NaCl; pH 7.0). Hybridization buffer can be stored at -20°C for several months. Thereafter, filter sections were washed in 50 ml preheated (37°C) washing buffer (13 mM NaCl, 5 mM EDTA (pH 8), 20 mM Tris-HCl (pH 7), 0.01% (w/v) sodium dodecyl sulfate (SDS)) for 10 to 15 min.

2.3.18 Catalyzed Reporter Deposition

The filter section was removed from the washing buffer and then incubated in a perti plate containing 20 ml of 1× PBS for 15 min at room temperature. In an aluminum foil covered eppendorf tube, 1 ml of amplification buffer ((10% (w/v) dextran sulfate, 2 M NaCl, 0.1% (w/v) blocking reagent, and 0.002% H_2O_2 in PBS)) and 4 µl of fluorescently labeled tyramid were mixed. The filter sections were incubated for 15 min in the dark at 46°C. Excess liquid was then removed by dabbing the filters onto blotting papers. Filter sections then were washed in 50 ml

deionized water followed by washing in absolute ethanol and then air-dried on a glass microscope slide. Filters were then mounted with 2 µl of Citifluor (Citifluor, London): Vectashield (Vector, Burlingame, CA) (4:1) and covered with a glass cover slip. Citifluor was used as anti-fading agent. In order to determine the total count of microbial cells, 1 µl of DAPI (4',6-diamidino-2-phenylindole) was also added to the Citifluor : Vectashield (1:1000). The filter was viewed using a Nikon Eclipse E600 microscope at 1000 × magnification through a Texas Red and DAPI filters. Microbial cells, stained green against the red background, were counted in a minimum of 10 fields of view for each sample. For calculating the number of cells per gram of soil (BC), the mean count of *Bacteria* per counting area (B), the microscope factor (area of the sample spot/area of counting field) (M), the dilution factor (D), and the weight of soil sample (W) were determined and calculated in the equation $BC = B \times M \times D \times W^{-1}$[225].

2.3.19 Non-selective staining of all cells (DTAF staining)

Direct microbial counts were also applied to the same soil samples on which CARD-FISH was performed, using 5-(4, 6-dichlorotriazinyl) amino fluorescein hydrochloride (DTAF) with the general methods described by Kepner & Pratt (1994) [226]. Serial dilution of soil samples and sonication were performed in the same manner explained previously for CARD-FISH. A fresh filter-sterilized DTAF solution was prepared by dissolving of 0.003 g of DTAF (Sigma) in 9 ml of phosphate buffer (0.05M Na_2HPO_4 with 0.85% NaCl, pH 9) [227] and protected from light by covering the tube in an aluminum foil. 100 µl of DTAF solution was then added to 1 ml of three-folds dilution of sonicated samples and stained for 1 h in the dark. The soil suspension was then filtered onto a 0.22 µm pore black polycarbonate filters (Poretics Corp.,

Livemore, CA). Cells retained on the filter surface were then washed 2-3 times with the dilution buffer to remove unbound DTAF. Following the final wash, all filters were air dried in the dark and immediately transferred to clean microscope slides. 1 drop of *Bac*Light mounting oil (Invitrogen, Eugene, OR) was applied to the filter surface and cover slips were laid on the mounted filter immediately and examined by epifluorescent microscopy at 1000 × magnification. Only cells that had a green fluorescence with common Bacterial morphologies were considered for counting. Counting and calculation were performed in the same manner explained previously for CARD-FISH.

Chapter 3: Results

This study represents the first comparison of the influence of different agricultural management practices on soil microbial genetic/functional diversity and activity in Southern Québec agricultural soils, using PCR-DGGE, FGMA, Archaeal 16S clone library analyses, and atrazine mineralization assays. CARD-FISH and DTAF microscopic techniques were also applied to quantify soil *Bacteria* and *Archaea* and soil microbial biomass under different agricultural land management practices.

3.1 DGGE analyses of the soil Bacterial community

DGGE analyses of Bacterial 16S rRNA genes were performed to compare the Bacterial structure/composition of the soil samples under different treatments collected over the different sampling times. The DGGE banding patterns were used to construct dendrograms. Bacterial universal primers, 341F-GC and 758R (Table 2), 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. Separation of the PCR fragments using DGGE produced a complex banding pattern for all the samples at two different depths (Figure 3). This pattern was repeated over the four sampling times 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 different treatments. Most of the observed bands seemed to be

shared between samples and no major bands were observed to appear or disappear (Figure 3). All the DGGE profiles were characterized by the presence of a limited number (2 bands) of strong bands residing in the low gradient region and a larger number of weaker bands (Figure 3). Dendrogram analysis was used to compare the banding patterns. The Bacterial community differentiated either into two or three clusters (Figure 5); cluster analyses for the samples collected after harvesting, in November 2006, and before atrazine application, May 2007, showed that DGGE banding patterns divided in 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 (Figure 5). For samples collected during the growing season in August 2007, and after harvesting in October 2007, three groups were observed with a low similarity index (S_{AB}: 17% and 14%, respectively). Table 4 summarizes the results of Shannon diversity index obtained from the DGGE banding pattern of different samples by statistical analyses. The major and minor DGGE bands were excised from the gel and phylogenetic information was determined by sequencing of these bands. Visualization limitations prevented all bands that could be viewed digitally from being physically isolated. Not all bands that appeared in gel photo were isolated and not all isolated bands returned reliable sequence information. The 16S rRNA gene sequences of 38 bands were submitted for comparison to the GenBank databases using the BLAST algorithm. A BLASTn similarity search in the GenBank database (Table 5) indicated that the majority (25 bands out of 38) of the derived sequences belonged to the phylum Proteobacteria. Within the Proteobacteria, the relative number of Alphaproteobacteria class was higher than Beta- and Gamma- and Deltaproteobacteria. The rest of the bands belonged to the phyla Actinobacteria (7 bands), Firmicutes (3 bands) and Acidobacter (2 bands). BLAST analyses also indicated that top

BLAST matches were from soil environments, such as agricultural soil, cropland, rhizosphere, and savanna soil. The obtained DGGE patterns of the α- and β-Proteobacteria were not as complex as DGGE obtained by Bacterial universal primer (data not shown). The application of the group specific primers provided similar results to the Bacterial universal primers regarding banding pattern. No major differences between DGGE profiles from all the analyzed samples, with respect to treatment and soil depth, were observed.

3.2 DGGE analyses of the soil Archaeal community

The effect of the applied treatments on soil Archaeal diversity was evaluated using Archaeal 16S rRNA PCR-DGGE. Following PCR with Archaeal universal primers, all soil samples gave the expected fragment size, except for the following treatments: no-tillage with residues, depth 0-5cm (NT+R (0-5cm)) and conventional tillage without residue, depth 5-20 cm (CT-R (5-20 cm)) from the samples collected before atrazine application in May 2007; no-tillage with residues, depth 0-5cm (NT+R (0-5cm)) and also no-tillage without residues, at two different depths (NT-R (0-5cm and 5-20 cm)) in August 2007. In comparison with Bacterial 16S rRNA DGGE, all Archaeal DGGE profiles were represented by limited numbers of bands. Among all the treatments, the lowest and the highest number of the detected bands were 6 and 15 respectively. The intensity of the bands was relatively uniform and similar banding patterns were observed for the samples collected after harvesting in 2006 and 2007 (data not shown). However, samples collected before atrazine application (data not shown) and during the growing season (Figure 4), showed unique banding patterns not similar to any other samples. Overall, it appeared that samples taken during the growing season harbored higher Archaeal diversity than

did the other samples collected before atrazine application and after harvesting. Cluster analysis of all the Archaeal DGGE profiles was performed and dendrograms were constructed. No clear trend in clustering of the different treatments among the diverse sampling time was observed. The dendrogram of the samples collected during the growing season is illustrated in Figure 6 as an example. Table 5 summarized the results of Shannon diversity index obtained from the DGGE banding pattern of different samples by statistical analyses. The sequencing results from excised DGGE bands are presented in Table 6. All of the sequenced bands grouped with the *Crenarchaea*. Due to a lack of cultivated representatives, the physiology and ecological function(s) of these organisms remains largely unknown. In all of the samples, the closest BLAST matches were isolated from agricultural soils, rhizosphere, and forest soils which are the same environment that we expected. The RDP classifier classified all the sequences in *Thermoprotei* class.

3.3 Archaeal 16S rRNA clone library analysis

An Archaeal clone library was constructed from one of the soil samples (RT+R 0-5 cm) collected during the growing season. DGGE profile showed that the selected sample harbored high Archaeal diversity among the samples collected in 2007. The application of the Archaeal 16S clone library provided insight into the Archaeal diversity of agricultural soil in Québec.

RFLP analysis of inserts from 59 clones revealed the dominance of three RFLPs (data not shown). Poor Archaeal primer specificity produced clones related to *Bacteria* determined by both BLASTn and RDP; of 59 Archaeal clones, 26 were identified as *Bacteria*. These sequences were not used in clone library analyses or in constructing the phylogenetic tree. Archaeal clone

sequences were assigned to operational taxonomic units (OTUs) at a level of 98% similarity for statistical analyses and 95% similarity for phylogenetic tree building. A total of 33 reliable sequences providing a total of 10 phylotypes were recovered for the Archaeal clone library. The rarefaction curve was produced by plotting the number of phylotypes observed against the number of clones sequenced and is illustrated in Figure 7. The coverage of Archaeal community with Good's percent coverage estimates of 84% which is a high coverage. In fact, clone coverage provides a quantitative estimate of how well the sample size reflects the apparent diversity within the clone library. However Shannon diversity index values of 1.40 indicated a notably low diversity of *Archaea*. The phylogenetic grouping of the sequences is illustrated in Figure 8. The Archaeal 16S rRNA clone library was dominated by clones most clearly related to the Crenarchaeota. The RDP classifier classified all the sequences in the *Thermoprotei* class. Clones classified as Crenarchaeota by RDP did not cluster with any cultured representatives and their top BLASTn matches were with temperate soil, agricultural soil, turf field, forest soil, and mesophilic soil. Bands sequenced from DGGE analysis were also entirely related to Crenarchaeota classified as *Thermoprotei*.

3.4 C-14 atrazine mineralization assays

In order to assess the potential for atrazine degradation, a mineralization assay was performed for the samples collected in June 2008 from the depth of 0-5 cm. In parallel to this experiment and in order to compare atrazine mineralization rate in agricultural soil with forest soil, another mineralization assay was also performed. Forest soils were used as a control as they had no previous history of atrazine application. The results of atrazine mineralization assays at 22°C are presented in Figure 9. It appeared that all of the samples had the potential to mineralize

atrazine regardless of the treatment. There were only small differences among the mineralization rates of agricultural soils. After 5 days of incubation, about 10% of the initially applied atrazine had been mineralized. After 10 days, atrazine mineralization increased to 40%. The greatest amount of mineralization occurred in all the treatments, where 60% of the added ^{14}C-atrazine had evolved as $^{14}CO_2$ after 20 days. The total evolved $^{14}CO_2$ after 30 days was around 60%. Mineralization was greater in agricultural soil than forest soil. The highest amount of mineralization for forest soil was ~20% of the initial applied atrazine. Mineralization of ^{14}C-atrazine was negligible (~1%) in the sterilized control soils.

3.5 Functional Gene Microarray (FGMA) analysis of agricultural soils

The potential for pesticide and the other soil pollutant degradation (i.e. hydrocarbons and heavy metals) was evaluated by the FGMA. We also examined how different genes associated with biogeochemical cycles responded to different agricultural land management practices. A total of eight soil samples collected at different times were studied. The description of the selected soil samples is illustrated in Table 3. From 100 genes a total of 32 genes were detected in all of the examined samples (Table 7). The highest number of the detected genes belonged to the RT+R (0-5 cm) before atrazine application with 27 genes and the lowest number related to the RT-R (0-5 cm) two weeks after atrazine application in July 2007 with 12 detected genes (Table 7). Atrazine degrading genes (*atzA*, *atzB*, and *atzC*) were not detected in any of the samples. Genes responsible for 2,4-D degradation (*tfdA*, *tfdB*, and *tfdC*) were not detected in all samples. *CymA* and *CadA*, genes involved in heavy metal (Arsenate and Cadmium) transformation, were detected in all of the treatments. From the genes that play role in the Nitrogen cycle (*Nir, Nos, Nor*, and *Ure*) only *Nir* was detected within the examined samples.

Regarding genes involved in hydrocarbons degradations all the samples were strongly associated with xylene, alkane, toluene, carbozole, and pyrene degradation genes and partly to naphthalene, styrene, catechol, and nitrotoluene degradation genes. The results of PCoA analyses are shown in Figure 10. The no-tillage soil samples with or without residues grouped together, however conventional tillage were closer to reduced tillage samples, regardless of soil depth and presence or absence of residues (data relayed to sampling time not shown). A FGMA scan image for the RT+R (5-20 cm) collected one week after atrazine application in 2007 is shown in Figure 11.

3.6 CARD-FISH and DTAF microscopic analyses

In order to determine the relative amounts of *Bacteria* and *Archaea*, to estimate soil biomass and also to investigate whether the applied treatments affected the number of *Bacteria* and *Archaea*, CARD-FISH and DTAF approaches were employed. These techniques were applied to the samples collected after harvesting from 0-5 cm in October 2007 (first cycle of the experiment) and November 2008 (second cycle of the experiment). The first cycle of the experiment started in November 2006 and ended in October 2007; the second cycle of experiment started from after harvesting in October 2007 until after harvesting in November 2008.

The results of cell numbers obtained by CARD-FISH and DTAF are shown in Figure 12 and 13, respectively. CARD-FISH results revealed that in the first cycle of experiment the highest number of Bacterial cell numbers belonged to RT-R treatment (9.5×10^8 cells/g dry soil) and the lowest average recorded for CT-R (6.1×10^8 cells/g dry soil). In the second cycle of experiment the highest Bacterial cell number per gram of dry soil belonged to the RT+R (9.8×10^8 cells/g dry soil). The lowest average number belonged to the NT-R (6.7×10^8 cells/g dry

soil). Generally, in the first and the second cycle of experiment the highest number of *Bacteria* was linked to reduced tillage, with or without residues. The results obtained by DTAF showed that, the average of total count in the first and the second cycle of experiment were similar except for the RT+R treatment. In the first and second cycle, the highest average number belonged to the RT+R (4.8×10^8 cells/g dry soil) and RT-R (4.4×10^8 cells/g dry soil) respectively, while the lowest number belonged to the CT-R (2.2×10^8 and 2.3×10^8 cells/g dry soil). Only slight differences among the treatments were found (Figure 12).

In the first cycle of experiment the lowest average number of *Archaea* belonged to the CT-R (5.1×10^7 cells/g dry soil) while the highest number was counted for the RT+T (8.8×10^7cells/g dry soil). In the second cycle, the number of *Archaea* ranged from 6.1×10^7cells/g dry soil for CT-R treatment, to 8.7×10^7cells/g dry soil for the RT+R. In general, the lowest number of *Achaea* in the first and second cycle of experiment belonged to the CT-R however; the highest number was related to reduced tillage (RT) either with residues or without residues. Overall, my results showed that *Bacteria* numbers were about four-fold greater than *Archaea* in most of the treatments. The contribution of Bacterial and Archaeal to the total DAPI stained cell was not detectable due to the high soil background. However, the DTAF technique was employed to determine the total microbial count, but since DTAF and CARD-FISH are two different techniques with different sensitivity, we were not be able to determine the contribution of *Bacteria* and *Archaea* to the total count.

Chapter 4: Discussion

The aim of this study was to investigate the effect of different tillage and plant residue managements on soil Bacterial and Archaeal genetic/functional diversity and activity in Southern Québec agricultural soils cultivated by corn. In this study PCR-DGGE, FGMA, Archaeal 16S clone library, mineralization analyses, and two microscopic techniques were employed.

4.1 DGGE analyses of the soil Bacterial community

Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) is one of the most widely used methods in microbial ecology to study microbial communities in environmental samples. Despite the drawback of PCR-based rRNA analysis, DGGE profiling of soil microbial communities is a reliable culture independent method. As the DGGE profiles of microbial communities in soil are often very complex when analysed with universal Bacterial primers, several group specific PCR-DGGE primers have recently been developed [228-229]. These primers assist us to understand the structures of specific subgroups of the complex soil Bacterial community. In the present study, PCR-DGGE analysis with both universal and group specific primer systems showed high Bacterial diversity with little variation in banding pattern among the soils under different treatments. No major differences between DGGE profiles from all the analyzed samples with respect to treatment and soil depth were observed. It has been stated that on temperate agricultural soils, the soil type is the primary factor that determines the Bacterial community structure and smaller secondary influences are due to differing management practices [155, 230-231]. The obtained results showed that the applied treatments possibly could not influence the structure of soil Bacterial community. A possible explanation of

this observation is that soil microbial communities require long periods of time to respond to different treatments and they have the ability to persist despite unfavorable conditions [232]. It also suggests that none of the agricultural treatments induced a dramatic change in microbial habitats (e.g. tillage alters soil structure in macro-aggregates but does not disturb micro-aggregates, where *Bacteria* and *Archaea* live. Also, there is enough substrate from corn residues in the –R treatment to support a diverse community, the extra residues in the +R treatment could be metabolized by the organisms present, but do not support more kinds of organisms. Due to more food with +R treatment we may expect more biomass but not necessarily more types of organisms (diversity). Soil has been shown to have a vast capacity for microbial diversity and, therefore, a large buffering capacity before the results of management practices could affect the dominant members of the community. However, the longer-term impacts of these practices might be significant. Agricultural soils are relatively stable environments and when the competition among microbial organisms is not severe, species may persist throughout fluctuations in above-ground vegetation [231]. However, Bacterial communities whose overall structure appears similar by PCR-DGGE analyses may still possess ecologically significant differences in community composition. Strain or species level changes in community composition could be responsible for differences in the physiological capacity of microbial communities whose structure is very similar. An alternative explanation for little variation in soil Bacterial community structure might be due to the fact that PCR-DGGE was not sensitive enough to show the changes that may occur at the level of individual strains or even species. DGGE analyses characterize only differences in relative abundances of different phylotypes but do not allow an absolute quantification of each phylotype. It might be also possible that soils were so similar that the different treatments only had a minimal effect on soil microbial composition. In contrast

to our study, some other studies found changes in Bacterial community structure due to applications of agricultural treatments. Seghers et al. (2006), investigated the effect of 20 years of atrazine and metolachlor application on the community structure, abundance and function of Bacterial groups in the bulk soil of a maize monoculture using group-specific PCR-DGGE of 16S rRNA genes. Their results indicated that the long-term use of these herbicides resulted in an altered soil community structure, in particular for the methanotrophic *Bacteria* [233]. In a four year experiment, Garveba et al. (2006) highlighted the importance of agricultural management practices for soil microbial community structure and diversity as well as the level of soil suppressiveness. They investigated the microbial diversity of soil under different agricultural regimes; permanent grassland, grassland turned into arable land, long-term arable land, and arable land turned into grassland, using cultivation-based and cultivation-independent methods. Both types of methods revealed differences in the diversities of soil microbial communities between different treatments. Moreover, a positive correlation between restraint of Rhizoctonia solani AG3 and microbial diversity was observed [234]. Differences in the Bacterial communities of soils caused by disturbances and land management were also identified in rRNA gene libraries prepared from conventional tilled and no tilled cropland in Southern Georgia. For the clones that were classified by RDP, the most abundant phylum was Proteobacteria, which consisted of 32% of the total number of clones. Moreover, the α-Proteobacteria was the largest Proteobacterial group within all the libraries and included clones similar to many common soil *Bacteria*, such as nitrifying *Bacteria* and *Rhizobiaceae*. Their study also showed that the second abundant phylogenetic group was Actinobacteria, with about 7% of clones [235]. The presence of Actinobacteria in soil microbial community is not surprising. Following a survey of 43 environmental samples, Barns et al. (1995) concluded that members of the phylum

Actinobacteria are genetically and metabolically diverse, environmentally widespread, and are as ecologically important as Proteobacteria [236]. Dominance of Firmicutes in agroecosystems has been reported in agricultural soils as well [237-238]. In some published literature on the average abundance of 16S rDNA clones from the alpha, beta, and gamma Proteobacteria and Actinobacteria, originating from diverse soils from three different continents, samples showed remarkable similarities [239-241]. Our results also demonstrated that the most dominant Bacterial group was Proteobacteria, followed by Actinobacteria and Firmicutes. The Proteobacteria may thus be the principal microbial group responsible for the high similarity observed in the DGGE profiles. The obtained results of Shannon diversity index showed a high Bacterial diversity and were comparable to the similar studies on agricultural soils [102, 242-243] . However, on the species level, the Bacterial community in permafrost soils was found to be highly diverse with an overall Shannon index of 5.3 [244-245]. In conclusion, our results were generally in agreement with the fact that agricultural management does not control microbial community structure. Whether soil type is the major determinant of microbial community structure, as suggested by other researchers, still remains to be investigated in Québec.

4.2 DGGE analyses of the soil Archaeal community

DGGE analyses of 16S rRNA gene fragments provided a measure of the composition and structure of the Archaeal community. There were similarities in community structure between the samples collected after harvesting in November, 2006 and in October 2007. The fact that the DGGE patterns in these samples were more similar to each other than to the other samples may

suggest that sampling season was a more important factor in changing the Archaeal community structure than the applied treatments. Although the other DGGE profiles varied with regard to the treatment and sampling time, we were unable to relate this to a real difference in Archaeal community. The absence of a particular band from a DGGE profile does not necessarily indicate that the sequence is completely absent from the community; it may indicate that the level of the sequence is below the detection level. We should note that changes in community structure are not necessarily related with changes in community performance and it is still possible for these *Archaea* to maintain a similar function within the community. Overall, it appeared that samples from during the growing season harbored higher Archaeal diversity than before atrazine application and after harvesting. Roesch et al. (2007) showed that in an environment where fertilization is common, the number and diversity of *Archaea* was very high compared to forest soils [246]. The high diversity of *Archaea* may be also an indication of a more favorable condition found during the growing season. BLASTn analysis showed that the Archaeal community in this study was composed solely of members of the Crenarchaeota and no Euryarchaeota sequences were found. The findings therefore, suggested that Crenarchaeota may be the dominant soil *Archaea* in the agricultural soils.

Our results are in agreement with some studies showed the patchy distribution of soil Archaeal population. For example, Nicol et al. (2003) showed that, 0.1 and 1 g samples of soils had dissimilar Archaeal communities regardless of sampling distance, while the 10 g soil samples were similar even if taken several meters away from each other. In a study by Oline et al. (2006) the differences in the Archaeal populations could be observed only on a very small scale (sampling distance). Authors concluded that the soil *Archaea* exist in highly localised and clonal populations. They also observed that at a large scale there was no clear difference between

sites at different altitudes even though these had different soils and vegetation [247]. Heterogeneity in Archaeal population in an environment as diverse as soil is understandable [248]. In our results, the closest BLASTn matches were isolated from agricultural soils, rhizosphere, and forest soils which are the same environment that we expected. The RDP classifier classified all the sequences in *Thermoprotei* class which have generally been described as thermophilic organisms. Thermophilic *Archaea* also seem to be mesophile, and exist in temperate or even cold environments [249]. The origin of thermophilic organisms in psychrophilic and mesophilic environments is still unclear. Thummes et al. (2007) discussed the distribution of moderate thermophilic *Archaea* via air and fertilization to temperate soils [250]. *Thermoprotei* includes three orders; Thermoproteales, Desulfurococcales, and Sulfolobales. Thermoproteales are important organisms within complex food webs. They can function as primary producers and/or as consumers of organic material. As primary producers, they use oxygen, elemental sulfur, sulfate, thiosulfate, sulfite, and nitrate as electron acceptors for growth and molecular hydrogen as the electron donor in these energy-yielding reactions [251]. Desulfurococcales can function as primary producers and/or as consumers of organic material. For primary producers (e.g., *Ignicoccus*, *Pyrodictium* and *Pyrolobus*) elemental sulfur, thiosulfate, nitrate, oxygen and sulfite are suitable electron acceptors for growth, and molecular hydrogen serves as the electron donor. The end product of their metabolism is H_2S, H_2O or ammonium [252]. The order Sulfolobales mainly contains aerobic thermophilic organisms that oxidize sulfur as the energy substrate [251]. In comparison with Bacterial ecology and physiology, the community ecology and functional roles of *Archaea* in soil remain poorly characterized. This is at least partially due to the lack of soil *Archaea* in culture [67, 253-255]. The values obtained for Shannon index of diversity were higher in comparison with a similar

study. Andreas et al. (2006) reported the Shannon index of around 0.5 for *Archaea* in arable European soils treated with manure [256].

4.3 Archaeal 16S rRNA clone library

Our results of Archaeal clone library showed that members of Crenarchaeota represented a ubiquitous and significant component of our sample. Therefore, it is possible that Archaeal community in our soil have adopted a highly specialized ecological niche, or perhaps such low diversity is a consequence of the rather limiting factors which may allow a small subset of *Archaea* to thrive. However, the clone abundance in libraries does not necessarily reflect population abundance in environmental samples [257]. Clone coverage provides a quantitative estimate of how well the sample size reflects the apparent diversity within the clone library. The clone coverage estimate of 84% for the Archaeal library suggested that the number of unique sequence types sampled from this library approaches the total number of unique sequences within the library. We compared our results with few available reports on studying Archaeal diversity in agricultural soils using 16S rRNA clone library. Borneman et al. (1996) conducted an experiment on soil microbial diversity in a clover-grass pasture in southern Wisconsin by sequence analysis of a universal clone library of genes coding for small-subunit rRNA (rDNA). However, no sequences of the domain *Archaea* were found [239]. In a similar study, the microbial populations in no-till agricultural soil and casts of the earthworm *Lumbricus rubellus* were examined by constructing Archaeal clone libraries of the 16S rRNA genes. Their results of representative clones indicated that they constituted a single Archaeal lineage which had been detected previously in soil. *Archaea* in these soils appeared to have limited diversity; they

included a number of deep phylogenetic groups that had never been cultivated. We were not be able to fully compare the results obtained from Archaeal clone library with similar studies related to the agricultural soils, as to my knowledge the publications mostly focused on the construction of Archaeal clone library from anoxic soils or extreme environments [79, 258-259].

4.4 C-14 atrazine mineralization assays

Mineralization assays indicated that the atrazine degradation potential for the soils with different treatments was considerable. The pattern that we observed for mineralization in all the treatments was similar to finding of Martin-Laurent et al. (2004), who showed that in the majority of French soils, atrazine mineralization started without a lag, was rapid and after 20 days of incubation, reached a plateau corresponding to 60 to 80% of the initially added atrazine [260]. Some of the soils obtained from Canada also rapidly mineralized the herbicide and atrazine mineralization occurred after a lag phase of 5 days and reached a total of 55% of the initially applied atrazine by the end of the incubation [261]. The results obtained from our study demonstrated no significant difference among the treatments. Hang et al. (2003) also observed that no-tilled or tilled soils had the same behavior in atrazine degradation [262]. Our results indicated that atrazine mineralization in agricultural soils was significantly different from the forest sample. The results from this study correspond to a study by Barriuso and Houot (1996) where atrazine was only mineralized in soils with previous history of atrazine application and less than 4% mineralization was observed in the soils with no atrazine exposure [261]. However, there is still the evidence that forest soils harbored microorganisms capable of atrazine metabolism. In the soil samples with no history of atrazine application, mineralization is slow and by the end of the incubation only 20% or less of the initially applied atrazine was

mineralized [263]. The lower rate of atrazine mineralization in forest soil can be explained by a different microflora present in this soil. It may caused also by the absence of specific microorganisms able to use the atrazine as a growth substrate, rather than unfavorable conditions in the soil [264]. Chemical degradation is another way of atrazine degradation, however microbial degradation is the main mechanism. Radosevich et al. (1996) concluded that the absence of atrazine degraders was the reason for no atrazine mineralization in agricultural soils [265]. It has been reported that atrazine application every other year in a corn–soybean rotation was enough to develop an adapted microflora able to mineralize the triazine ring [262].

4.5 Functional Gene Microarray (FGMA) analysis of agricultural soils

The aim of using FGMA was to evaluate the feasibility of this technique to determine the functional capacity in the Québec soils and potentially detect the effect of different treatments. The microcosm studies showed that the soil microbial communities were capable of atrazine mineralization, though genes related to atrazine degradation were not found by FGMA in our samples. A possible explanation is that some microorganisms in our soil samples may contain atrazine degradation genes but our FGMA failed to detect them. There are three genes that encode enzymes, *atzA, atzB,* and *atzC,* responsible for breaking down atrazine to cyanuric acid. Alternatively, our soil samples may contain as yet undescribed genes responsible for atrazine degradation. Despite the fact the FGMA holds the potential to simultaneously follow functional of hundreds of microorganisms involved in key environmental processes, in order to obtain precise prediction of the presence of the genes related to atrazine degradation we may need to use alternative techniques. Regular PCR and reverse-transcriptase real-time PCR (RT-PCR)

methods for screening and quantifying *atzA*, *atzB*, and *atzC* genes coding for enzymes responsible for atrazine transformation could be effective. Detection of the genes related to degradation of the other pollutants (e.g. hydrocarbons) indicated a strong potential for degradation of this type of compounds; however the possibility of agricultural soil contamination with hydrocarbons and heavy metals is not very high. Other detected genes were associated with nitrogen cycle. Again, due to the lack of detection of all the genes which required completing a part of the nitrogen cycle is hard to make a conclusion regarding the effect of agricultural treatments on the nitrogen cycle. The presence of a gene does not necessarily mean that it is being actively transcribed, providing some limitations in our ability to draw conclusions about the active function of microbes from these analyses. Analysis of mRNA would allow more direct connections to be drawn. However, recent research on environmental samples using both mRNA and genomic DNA microarrays has shown that the dominant species identified by mRNA arrays are also the most abundant in terms of genomic DNA [266]. A similar FGMA was used to evaluate how soil bacteria in the Canadian High Arctic respond to hydrocarbon pollution and bioremediation treatments [180]. Their results demonstrated the utility of microarrays as tools to rapidly monitor bacteria and their functions.

4.6 CARD-FISH and DTAF microscopic analyses

In order to determine the relative amounts of *Bacteria* and *Archaea*, and estimate soil biomass, CARD-FISH and DTAF was performed for the samples collected after harvesting from 0-5 cm in the first year and second cycle of experiment. The number of *Bacteria* and *Archaea* showed a little variation among the treatments in the first and second cycle of experiment. These

might be due to the fact that the applied treatments had almost the same effect and different types of tillage and residue managements affected the soil Archaeal and Bacterial in the same way. Although our results indicated that the Bacterial populations were almost four times higher than Archaeal populations this may not be an indication of minor role of *Archaea* in agricultural soils. The relative numbers of Archaea were still high compared to the forest soils. A study in which FISH was used showed that, in forest soil *Archaea* accounted for $0.21\% \pm 0.65\%$ of the detectable cells [211]. Our results of the number of *Bacteria* were relatively higher than findings of Caracciolo et al. (2005) [208] and Watt et al. (2006) [207]. In a study conducted on three Danish soils, they observed Bacterial numbers in the profiles declined from about 20 to 1% from the top to the bottom. The decline in Bacterial number with increasing depth observed in this study agrees with that observed by Beloin et al. (1988), but is somewhat more pronounced than the decline in total Bacterial counts observed by Dodds et al. (1996) [267]. Since our study was only performed for the samples collected from 0-5 cm we were unable to compare the Bacterial and Archaeal numbers regarding to the soil depth. CARD-FISH technique is mostly applied to the sediment samples and to my knowledge this study is the first occasion of using this method for analyzing agricultural soil *Bacteria* and *Archaea*.

Chapter 5: Conclusions

The methodologies used in this research provided advantages over conventional analyses and improved our understanding of soil Bacterial and Archaeal communities under different agricultural treatments. This study was the first attempt to look at the soil Archaeal diversity in agricultural soils in Southern Québec. Functional gene microarray analyses were applied for the first time to investigate the functional diversity of these soils and the ability of bacterial and archaeal to degrade target substrates including atrazine. Management practices were found not to affect either microbial community composition or structure and activity significantly and there was little variation among the treatments. Future research could focus on determining whether this soil condition was correlated with improvements in soil health, and on identifying key functional microbial components that responded to differing management practices, at a micro scale. Overall, the results indicate that in southern Québec agricultural soils three tillage practices (No-tillage, reduced tillage and conventional tillage) and presence or absence of the plant residues had almost the same effect on microbial communities. Consequently, the decision to choose from these treatments should be made based on the other factors that may affect crop yield and influenced soil chemical and physical properties.

Table 1 Various types of microarrays used for environmental microbiology studies.

	1	**2**	**3**	**4**	**5**
Typical probe size	18–28 bp	18–28 bp	50–100 bp	200–1000 bp	entire genome, but fragmented
Nature of targeted genes	phylogenetic	functional	functional	functional	entire genome
Information on functional activity	no	yes	yes	yes	no
Targeted microorganisms	culturable and nonculturable	culturable and nonculturable	culturable and nonculturable	culturable and non culturable	culturable, potentially also non culturable
Number of different genes targeted	a few-maximum	a few-maximum	many	many	NA
Taxonomic resolution	species/strains	species/strains	genus/species	genus /species	genus/ species
Potential to discover novel bacteria	yes	yes	no	no	no
PCR amplification of targeted genes	required	required	not required	not required	not required
Construction of comprehensive arrays	*in silico* probe design	*in silico* probe design	*in silico* probe design	PCR amplification	whole genome preparation

1.Phylogenetic oligonucleotide arrays

2. Short oligonucleotide based functional gene microarrays

3. Long oligonucleotide based functional gene microarrays

4. PCR product based functional gene microarrays

5. Community genome arrays

Table 2 PCR primers used in this study.

Primer	Sequence 5' to 3'	Target	Reference
341F (GC)*	CTA CGG GAG GCA GCA GTG GG	Bacterial 16S rRNA gene	[213]
758R	CTA CCA GGG TAT CTA ATC C	Bacterial 16S rRNA gene	[213]
109F	CAC AAT GGC CTG TGA GGA G	Archaeal 16S rRNA gene	[215]
344F (GC)*	ACG GGG CGC AGC AGG CGC GA	Archaeal 16S rRNA gene	[215]
915R	GTG CTC CCC CGC CAA TTC CT	Archaeal 16S rRNA gene	[215]
Alf28F	AGC CGA ACG CTG GCG GCA	α-Proteobacteria 16S rRNA gene	[214]
Alf684R	TAC GAA TTT CTA CCT CTA CA	α-Proteobacteria 16S rRNA gene	[214]
518R	ATT ACC GCG GCT GCT GG	Bacterial 16S rRNA gene	[214]
Beta359F	GGG GAA TTT TGG ACA ATG GG	β-Proteobacteria 16S rRNA gene	[214]
Beta682R	ACG CAT TTC ACT GCT ACA CG	β-Proteobacteria 16S rRNA gene	[214]
518F(GC)*	CCA GCA GCC GCG GTA AT	Bacterial 16S rRNA gene	[214]
SP6	GAT TTA GGT GAC ACT ATA GG	Cloning vector	Promega**
T7	TAA TAC GAC TCA CTA TAG GG	Cloning vector	Promega**
751F	CCG ACG GTG AGR GRY GAA	Archaeal 16S rRNA gene	[268]
1406R	ACG GGC GGT GTG TAC	Archaeal 16S rRNA gene	[268]
LuxAb F	CCG ACT GCC CAT CCG GTT CGA CAA GC	*LuxA* gene	[269]
LuxAe R	CTC CGC GAC GAC ATA AAC AGG AGC ACC ACC	*LuxA* gene	[269]

*GC-clamp sequence GCG GGC GGG GCG GGG GCA CGC GGG GCG CGG CGG GCG attached on 5' end for primers denoted (GC) either in the table or text.
** Promega Corporation, Madison, WI.

Table 3 Samples selected for FGMA analyses.

Treatment	Depth (cm)	Sampling Time
RT+R	0-5	May 2007, before atrazine application
NT+R	0-5	May 2007, before atrazine application
RT+R	5-20	July 2007, one week after atrazine application
CT+R	0-5	July 2007, one week after atrazine application
RT-R	0-5	July 2007, two weeks after atrazine application
CT+R	5-20	July 2007, two weeks after atrazine application
NT-R	0-5	June 2008, two weeks after atrazine application
CT-R	0-5	June 2008, two weeks after atrazine application

Table 4 Shannon index (*H*) of soil Bacterial and Archaeal community obtained from the DGGE banding pattern of the soils collected during the four sampling time.

	Nov. 06	May 07	Aug. 07	Oct.07
Bacteria	3.2	3.00	3.4	3.4
Archaea	1.9	1.5	1.9	1.7

Table 5 16S rRNA gene analyses of isolated bands from *Bacteria* DGGE. Sequences were compared to 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.

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)	Betaproteobacteria100%(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)

Band	Closest BLAST Match	Origin of BLAST Match	Similarity to BLAST Match	RDP Grouping
12	Ramlibacter tataouinensis 153-3 (AJ871240)	Biological soil crusts	96%(388/401)	Comamonadaceae 92%(family)
13	Alphaproteobacterium OS-C02 (EF612398)	Soil	99%(385/381)	Sphingomonadaceae98%(family)
14	Agricultural soil bacterium isolate SI-15 (AJ252582)	Agricultural soil bacterium	98%(401/408)	Sphingomonadaceae100%(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)	Sphingomonadaceae98%(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)

Band	Closest BLAST Match	Origin of BLAST Match	Similarity to BLAST Match	RDP Grouping
28	Actinobacterium TH1-94 (AM690885)	Environmental sample	98%(404/413)	Actinomycetales 99%(order)
29	Caulobacterales Plot29-H11 16S (EU202838)	Agricultural soil	97%(326/333)	Sphingosinicella100%(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)	Sphingomonadaceae98%(family)
35	Bradyrhizobium sp. JS 15-10 (EU529843)	Forest soil	91%(334/367)	Rhizobiales 91% (order)

Table 6 16S rRNA gene analyses of the isolated bands from *Archaea* DGGE. Sequences were compared to 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)

Band	Closest BLAST Match	Origin of BLAST Match	Similarity to BLAST Match	RDP Grouping
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)

Table 7 The total number and the description of the detected genes in samples analyzed by FGMA. The values of "0" and "1" indicated the absence and presence of the indicated genes.

Gene	Samples							
	A	B	C	D	E	F	G	H
nirK	1	1	1	1	1	1	1	1
ntdA	1	1	1	1	1	1	1	1
antA	1	1	1	1	1	1	1	1
tfdC	1	1	1	1	1	1	1	1
xylK	1	1	1	1	1	1	1	1
nahG	1	0	1	1	1	1	1	1
paaK	1	1	1	1	1	0	1	1
cadA	1	1	1	1	1	1	0	1
tfdC	1	1	1	1	1	1	0	1
todF	1	1	1	1	1	1	0	1
xylE	1	1	1	1	1	1	0	1
pcpC	1	1	1	1	1	1	1	0
cymA	1	1	1	1	1	1	1	0
alkB1	1	1	1	1	1	0	0	1
nosZ	1	1	1	0	1	1	0	0
tfdB	1	0	1	1	0	1	0	1
todD	1	0	1	1	0	1	0	1
dmpN	1	0	1	0	0	1	1	1
styE	1	1	1	1	1	0	0	0
tfdB	1	0	1	0	0	1	1	0
dmpE	1	0	1	0	0	1	1	0
katG	1	0	1	0	0	0	0	1
alkB2	0	0	0	1	0	0	0	1
cymA	0	0	1	0	0	0	0	1
nahG	1	0	0	0	1	0	0	0
tfdB	0	0	0	0	1	0	0	0
tfdA	0	0	1	0	0	0	0	0
nahF	1	0	0	0	0	0	0	0
paaK	1	0	0	0	0	0	0	0
nirS	0	0	1	0	0	0	0	0
catA	1	0	0	0	0	0	0	0
clcA	1	0	0	0	0	0	0	0

A: RT+R (0-5 cm)- May 2007- before atrazine application
B: NT+R (0-5 cm)- May 2007- before atrazine application
C: RT+R (5-20 cm)- July 2007- one week after atrazine application
D: CT+R (0-5 cm)- July 2007- one week after atrazine application
E: NT-R (0-5 cm)- June 2008- two weeks after atrazine application
F: CT-R (0-5 cm)- June 2008- two weeks after atrazine application
G: RT-R (0-5)- July 07-two weeks after atrazine application
H: CT+R (5-20)- July 07- Two weeks after atrazine application

Block 1	CT +R	NT -R	NT +R	CT -R	RT +R	RT -R
Block 2	NT -R	RT +R	CT -R	RT -R	NT +R	CT +R
Block 3	CT -R	RT -R	CT +R	NT +R	NT-R	RT +R

NT+R: no-till with residue (grain corn)
RT+R: reduced till with residue (grain corn)
CT+R: conventional till with residue (grain corn)
NT-R: no-till without residue (silage corn)
RT-R: reduced till without residue (silage corn)
CT-R: conventional till without residue (grain corn)

Figure 1 Layout of the experimental field plots.

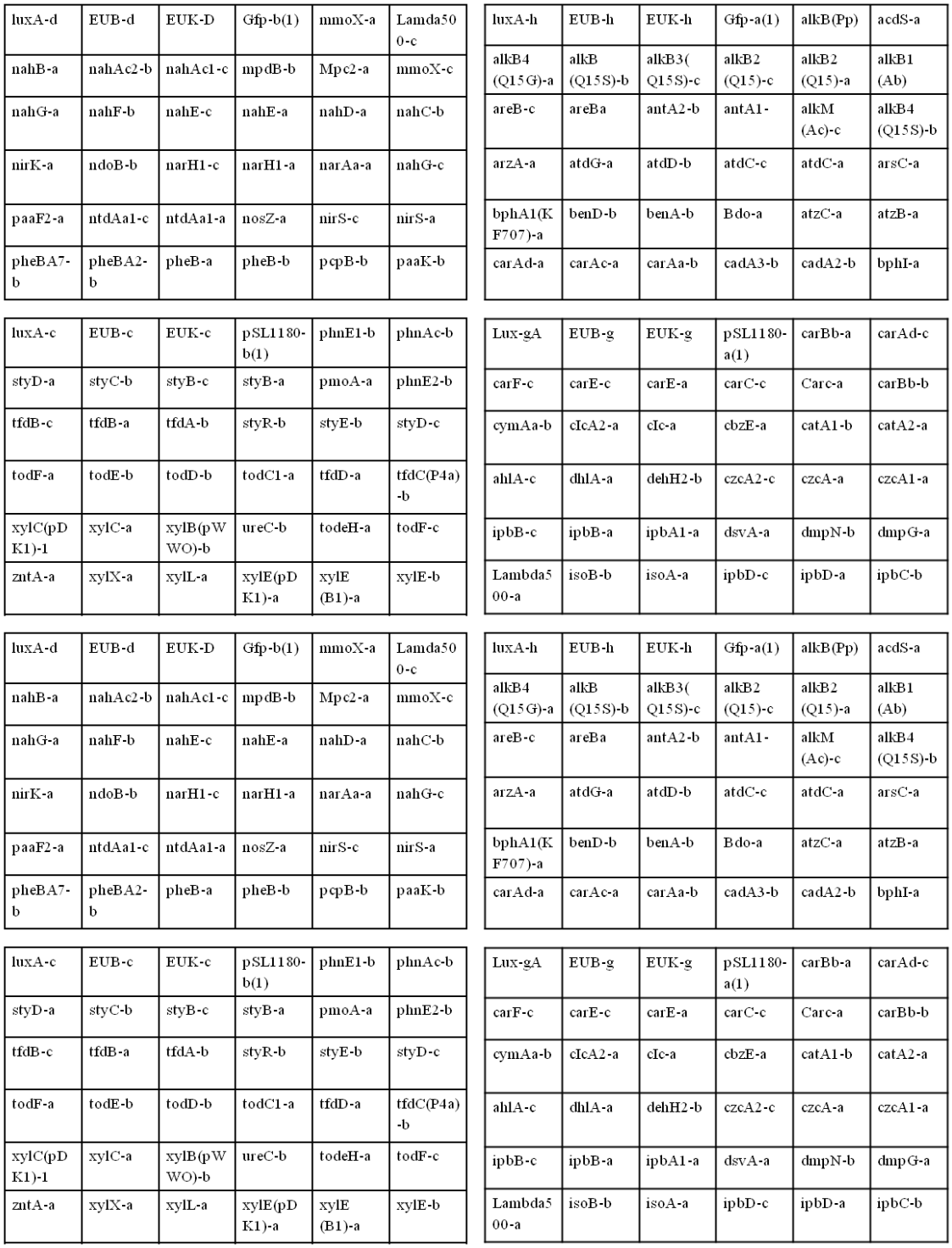

Figure 2 The Layout of the oligonucleotide FGMA chip**.** The full description, usage and analysis of the functional gene microarray is given in the NCBI Gene Expression Omnibus (GEO) under platform accession number GPL8960 (http://www.ncbi.nlm.nih.gov /geo/query /acc.cgi? acc= GPL8960).

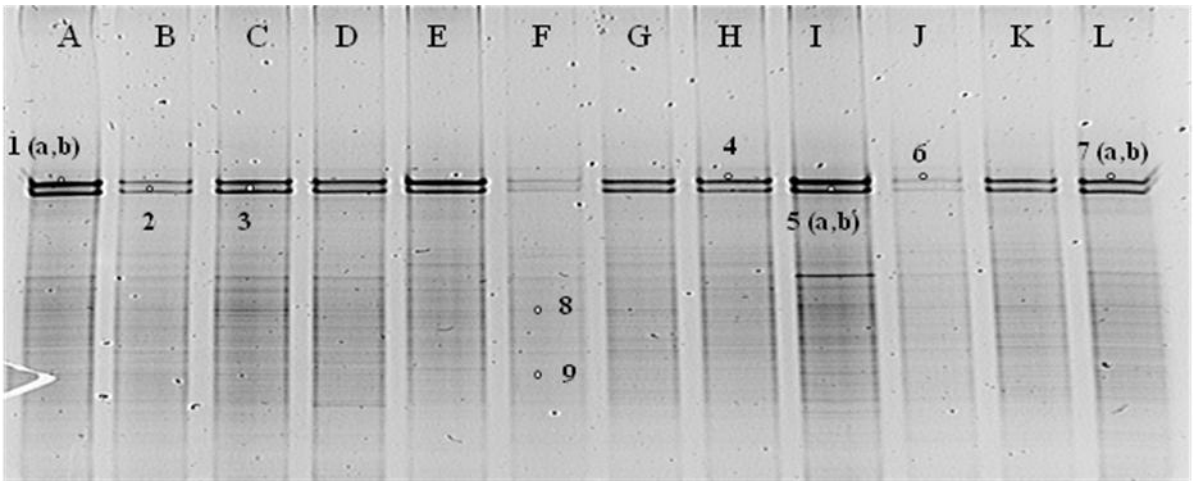

Figure 3 A representative denaturing gradient gel electrophoresis (DGGE) analysis of Bacterial 16S rRNA genes amplified from the following samples: RT+R (0-5 cm): A (after harvesting, Nov. 2006), B (before atrazine application, May 2007), C (during growing season, Aug. 2007) D (after harvesting Oct. 2007). RT+R (5-20 cm): E (after harvesting, Nov. 2006), F (before atrazine application, May 2007), G (during growing season, Aug. 2007) H (after harvesting Oct. 2007). NT-R (5-20 cm): I (Nov. 2006), J (before atrazine application, May 2007), K (during growing season, Aug. 2007) L (after harvesting. Oct. 2007). DGGE was run on an 8% acrylamide gel with a gradient of urea and formamide from 35% to 65%.

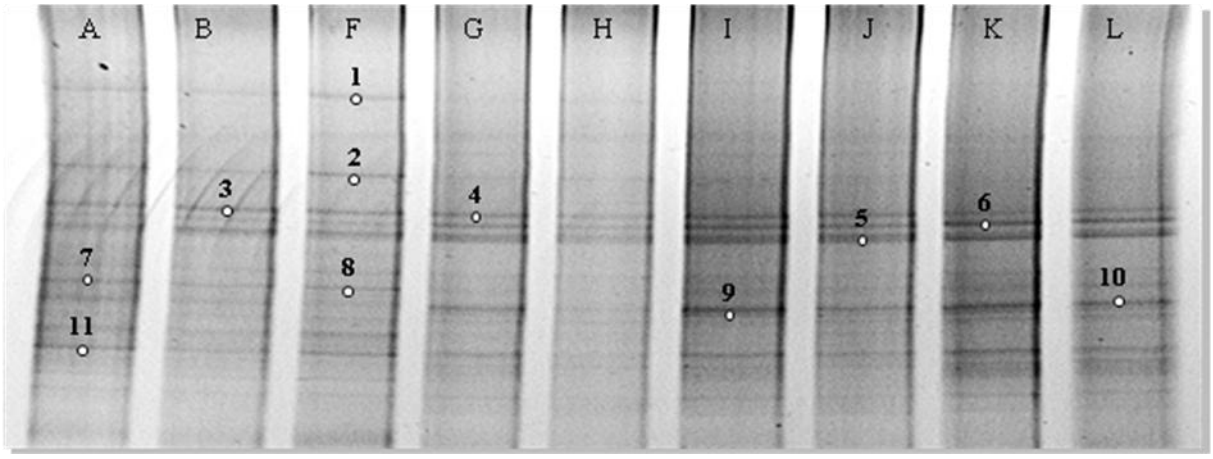

Figure 4 A representative denaturing gradient gel electrophoresis (DGGE) analysis of Archaeal 16S rRNA genes amplified from samples collected during the growing season in August 2007. A: RT+R (0-5 cm), B: RT+R (5-20 cm), F: NT+R (5-20 cm), G: CT+R (0-5 cm), H: CT+R (5-20 cm), I: RT-R (0-5 cm), J: RT-R (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%.

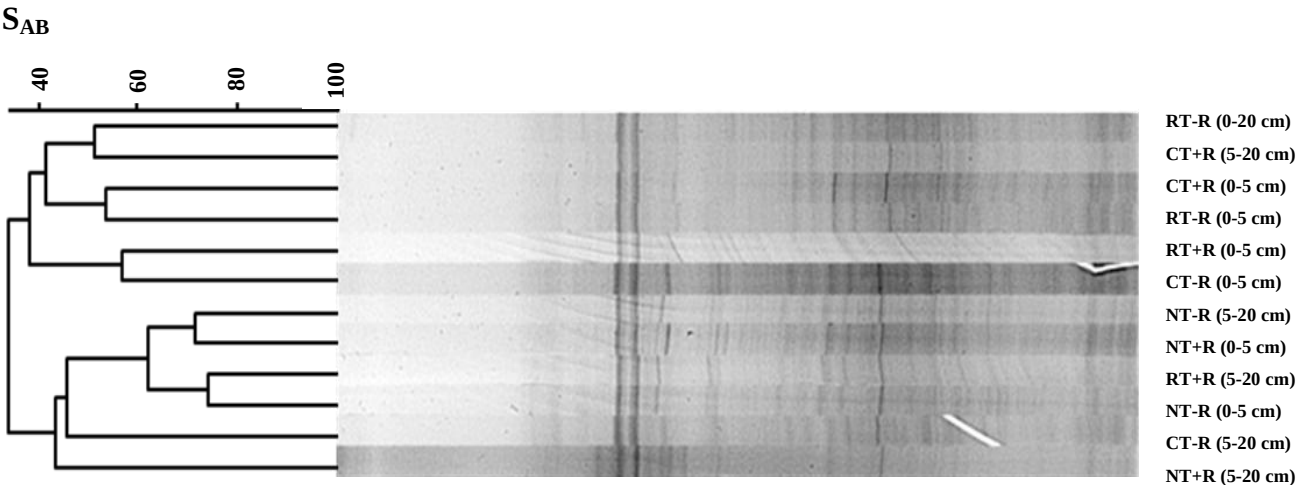

Figure 5 Dendrogram of denaturing gradient gel electrophoresis (DGGE) of Bacterial 16S rRNA communities for the samples collected during the growing season in August 2007. A gradient runs from left to right at 40- 60% on 8% acrylamide for Bacterial gel. The scale of the dendrogram was given as percent of similarity.

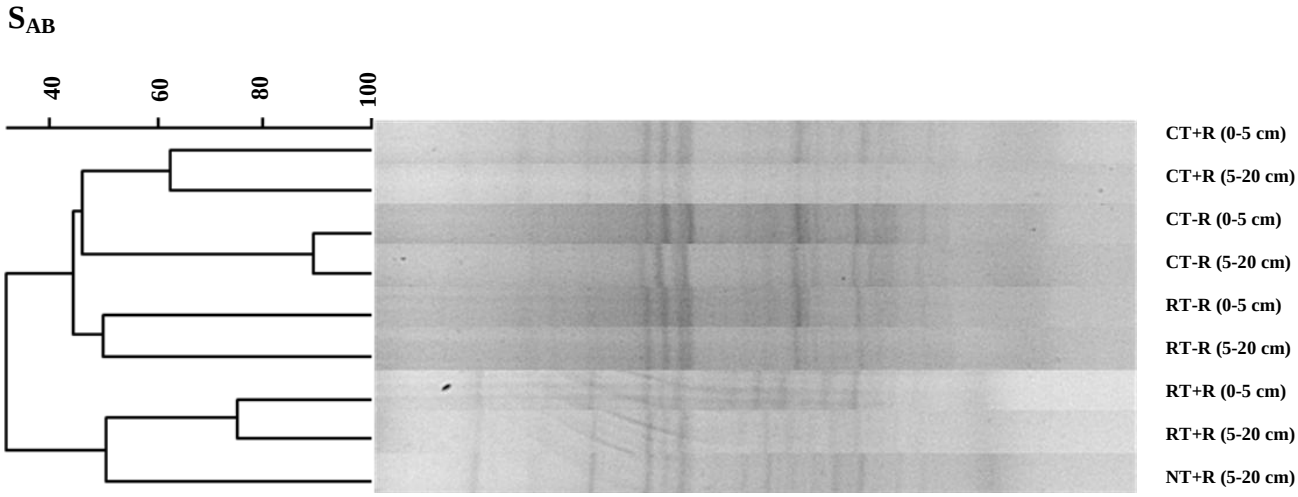

Figure 6 Dendrogram of denaturing gradient gel electrophoresis (DGGE) of Archaeal 16S rRNA communities. A gradient runs from left to right at 40- 60% on 8% acrylamide for Archaeal gel. The scale of the dendrogram was given as percent of similarity.

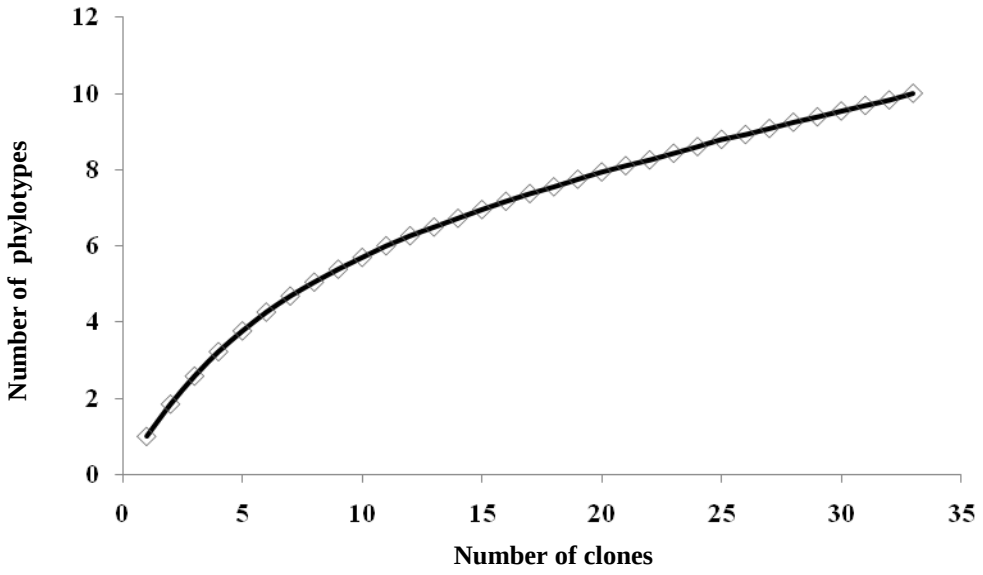

Figure 7 Rarefaction curves generated for 16S rRNA genes in the clone library from RT+R (0-5 cm) treatment collected during the growing season in August 2007. Clones were grouped into phylotypes based on sequence similarity of ≥98%.

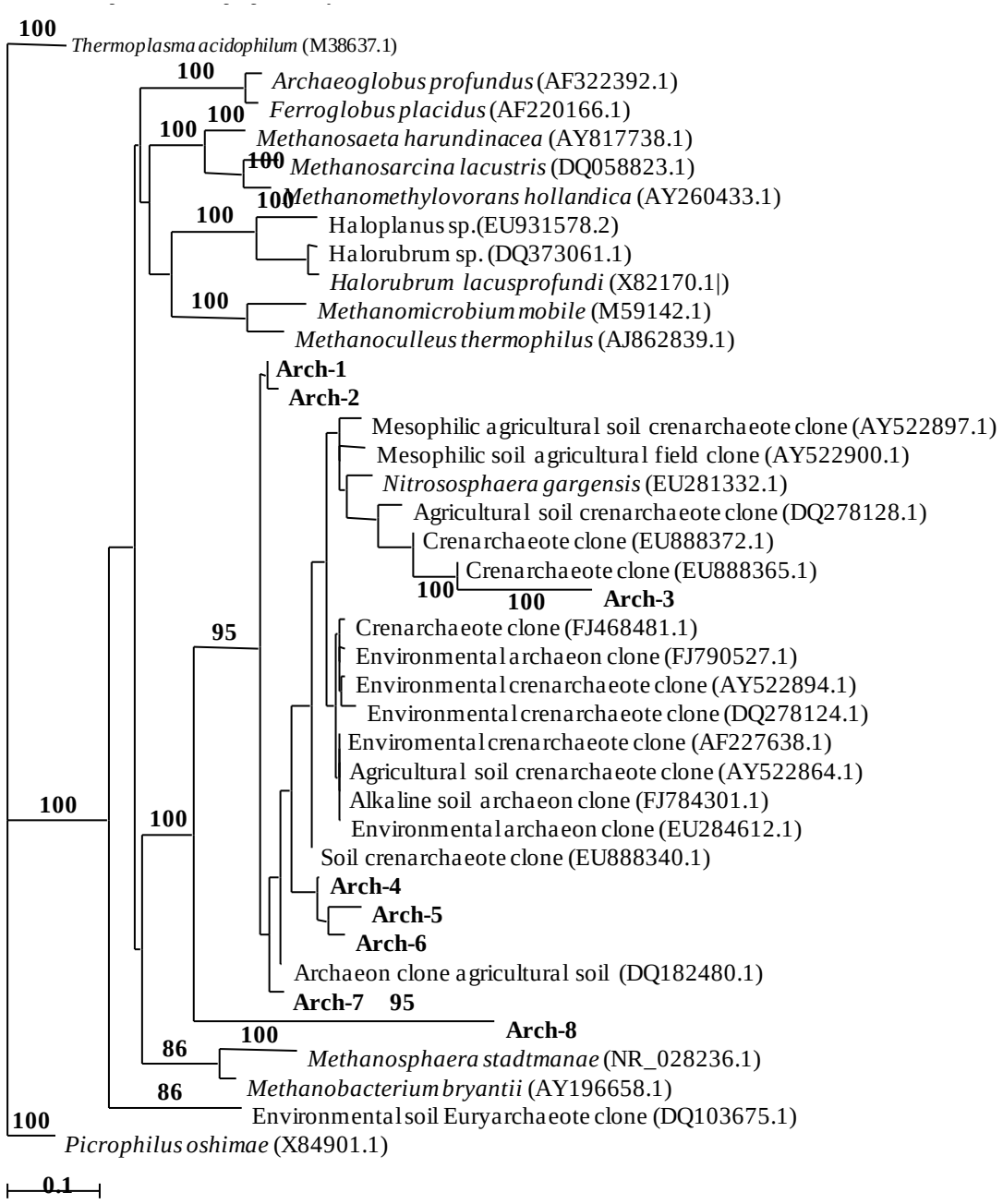

Figure 8 Archaeal 16S rRNA distance based phylogenetic tree including boot strap values for the RT+R (0-5 cm) sample collected during the growing season in August 2007. Clones from this study are bolded; Top BLASTn matches are in plain text and cultured representatives italicized**.**

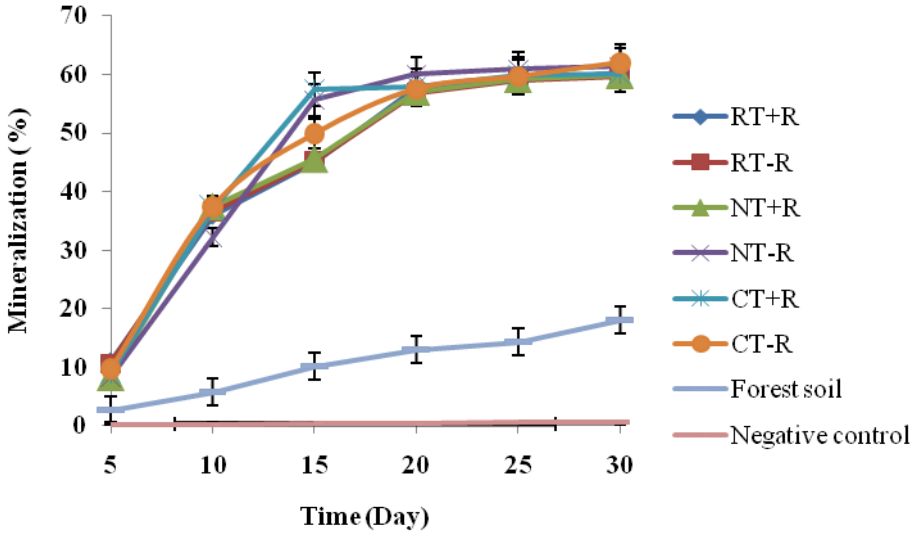

Figure 9 C-14 atrazine mineralization assay for the samples collected in the second year of experiment. Each point represents cumulative mineralization (% $^{14}CO_2$ recovered) from triplicate assays including sterile control. Error Bars indicate standard deviation of the mean.

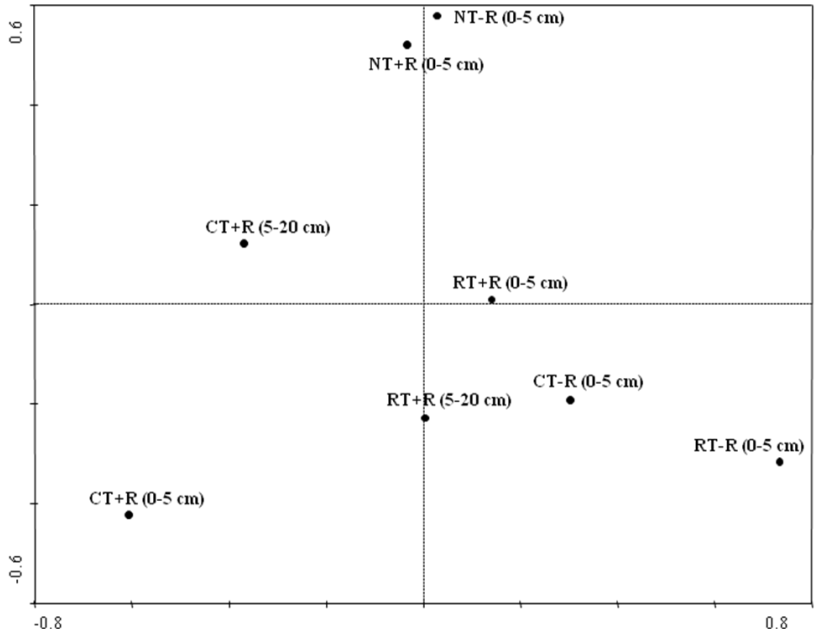

Figure 10 Principle coordinate analysis based on Jaccard's similarity based on the presence or absence of detections on the FGMA for the eight selected samples

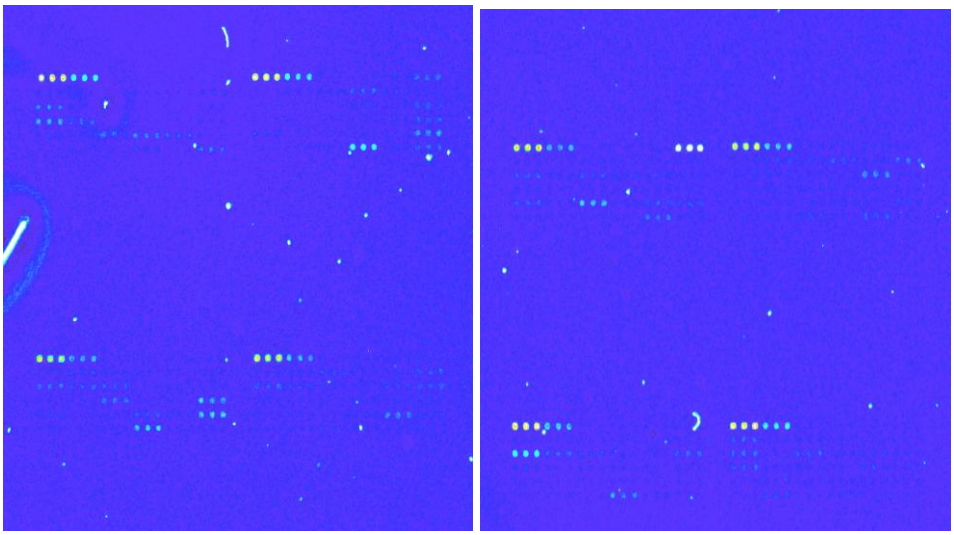

Figure 11 A representative FGMA scan image for the RT+R (5-20 cm) collected one week after atrazine application in July 2007.

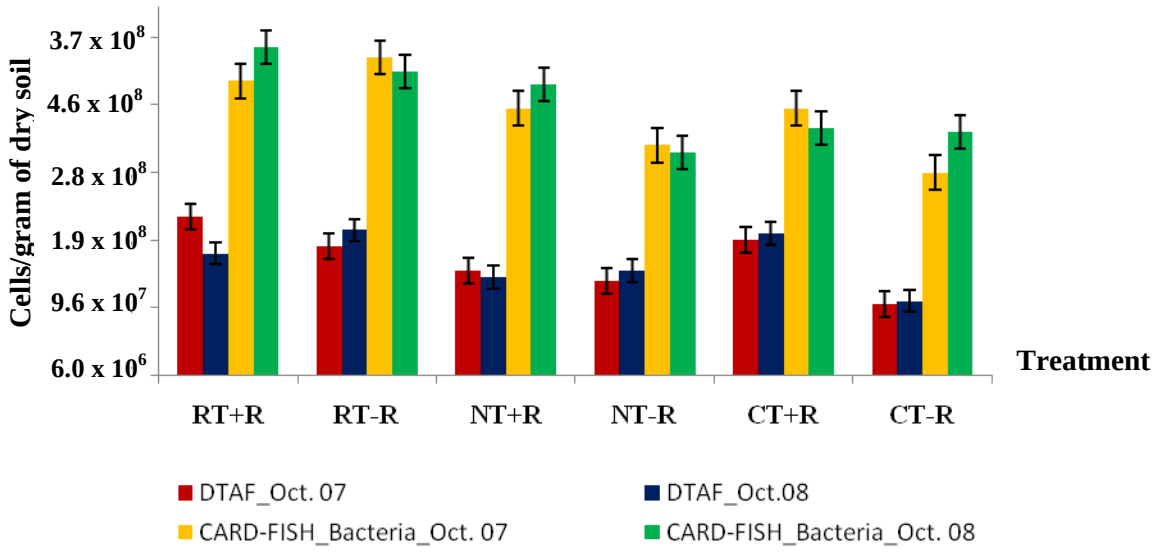

Figure 12 Bacterial cell numbers per gram of dry soil in the first and second year of experiment numerated with DTAF and CARD-FISH. Bars are standard deviation of the mean.

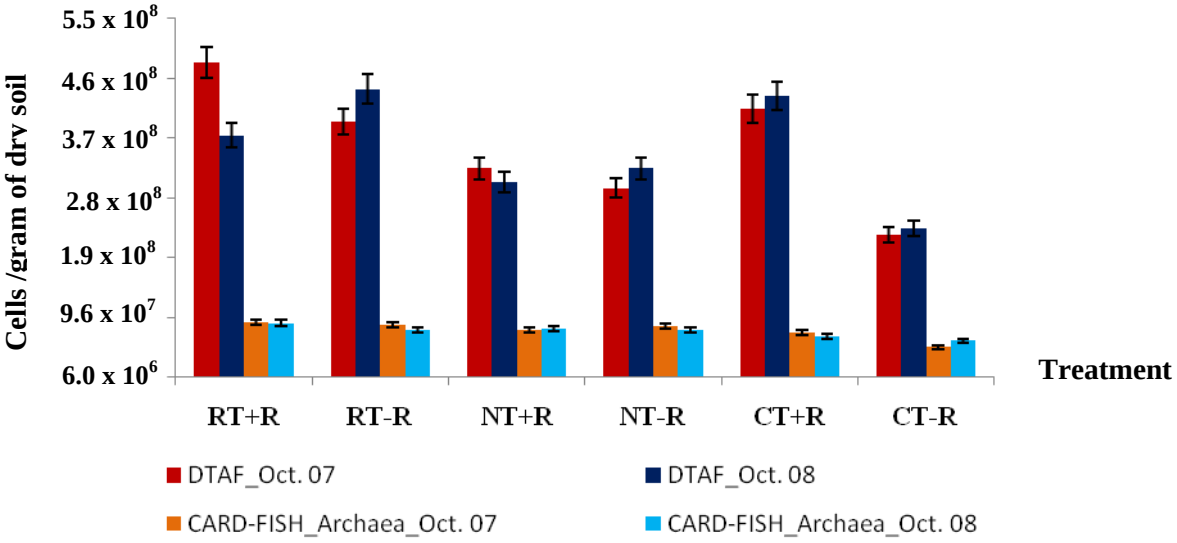

Figure 13 Archaeal cell numbers per gram of dry soil in the first and second cycle of experiment numerated with DTAF and CARD-FISH. Bars are standard deviation of the mean.

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