LAND USE LEGACY REGULATES MICROBIAL COMMUNITY STRUCTURE AND FUNCTION IN TRANSPLANTED CHERNOZEMS

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By

Panchali Madubashini Katulanda

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ABSTRACT

Understanding long-term agricultural influences on soil nutrients and microbial dynamics is often complicated by the heterogeneity of soil environments as well as complex interactions between confounding factors of climate, topography and hydrology. A long-term soil transplant experiment located at the Agriculture and Agri-Food Canada (AAFC) Research Center in Lethbridge, AB provided a unique opportunity to study the long-term effects of agriculture management practices on various soil types, independent of these confounding effects. In 1990, a variety of Chernozemic soils were relocated to a common location and subsequently managed under common conditions. The experiment was a split plot design with soil type and rate of nitrogen (N) fertilizer (0 and 60 kg N ha^{-1}) as the main and subplot factors, respectively and crop residues were removed annually at harvest. A 2012 survey of soil microbial abundance and community structure revealed distinct communities among 10 different soil types, indicating a persistent legacy effect of previous land use, irrespective of the common climatic, topography and management. Next, DNA profiling of archived soils from 1990 and 2011 showed that the contemporary community structures differed from the original 1990 soils but that the degree of divergence was generally linked to the original soil fertility. Thus, although soil fertility of individual transplanted soils changed with common management, variability among soils persisted. Specific differences among transplanted soil microbial community structures were reflected in carbon (C) and N cycling bacteria. For example, nitrifying and denitrifying gene abundance and composition varied among transplanted soils. A ^{13}C stable isotope tracing experiment determined that community composition and function of active cellulose decomposers was different among soils indicating that the subset of the whole soil communities that was active also remained different between transplanted soils, despite common conditions. Overall, soil fertility integrates aspects of pedogenesis, land use and management history which resulted in a legacy effect on the microbial community abundance, composition and function which was more dominant than current conditions. These findings help to broaden our understanding of dominant drivers of agricultural soil ecosystem functioning and microbial communities which will be necessary for predicting the fate of future agricultural systems.

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LIST OF ABBREVIATIONS

AAFC	Agriculture and Agri-Food Canada
AOA	Ammonia oxidizing archaea
AOB	Ammonia oxidizing bacteria
AMF	Arbuscular mycorrhizal fungi
amoA	Gene encoding ammonia monooxygenase enzyme
BH	B Horizon
C	Carbon
C: N ratio	Carbon to nitrogen ratio
CC	Cereal cultivated
CH	C Horizon
d	Days
DGGE	Denaturing Gradient Gel Electrophoresis
DTF	Dryland tilled, fallowed
DW	Dryland wheat
HM	Heavy rate, manured
ITF	Irrigated tilled, fallowed
N	Nitrogen
NG	Native grassland
NH_4^+	Ammonia
nirK	Gene encoding copper containing subunit of nitrite reductase
nirS	Gene encoding cytochrome cd1 containing subunit of nitrite reductase
nosZ	Gene encoding catalytic subunit of nitrous oxide
NO_3^-	Nitrate
NOB	Nitrite oxidizing bacteria
OC	Organic, carbon
OM	Organic, matter
ON	Organic nitrogen
OTU	Operational taxonomic units
PE	Priming effect
PL	Pastureland

PCR	Polymerase chain reaction
qPCR	Quantitative Real Time Polymerase Chain Reaction
RM	Recommended rate, manured
SIP	Stable Isotope Probing
TC	Total carbon
TN	Total nitrogen
yr	Year

1.0. GENERAL INTRODUCTION

Agricultural systems provide food, fuel, forage, medicines, and pharmaceuticals for human well-being. Global human population is rapidly increasing. Simultaneously, the demand for agriculture products and services also is increasing. To fulfill the growing food and bioenergy demand, agriculture production needs to be doubled within next 50 years (yr). Currently, the productivity of agriculture ecosystems is maintained using inputs like fertilizer and pesticides (Bennett et al., 2001; Tilman et al., 2002). This must be done with minimal impact to the environment as climates are changing and improper land use may have a deleterious influence through land degradation, loss of biodiversity and water pollution. To feed the growing human population, the area used for agriculture needs to be expanded or the productivity of agricultural lands should be improved (Edgerton, 2009; Tilman et al., 2011). However, it is not feasible to expand the cultivated land area as the current human population already has exploited most of the arable lands, resulting in low productive soils. Therefore, it is vital that land/soil productivity increase through sustainable soil management (Fan et al., 2012). Improving crop productivity by preserving soil quality is one of the major challenges facing scientists today.

An ecosystem is a complex network of organisms interacting with one another and the physical environment, and the resulting biological and physical processes. Soil productivity is mainly governed by ecosystem functions such as nutrient recycling, organic matter (OM) decomposition, soil structural modification, food web interactions (Barrios, 2007), and detoxification of toxins (Sekercioglu, 2010), all of which are modulated by soil microorganisms. Soil microbes are the key players of soil OM decomposition and nutrient recycling (Powlson, 2001). As such, they are the key determinants of plant productivity and they can sustain plant growth in nutrient poor soils (Van Der Heijden et al., 2008). In terrestrial ecosystems, photosynthesis and soil respiration are the dominant processes maintaining the C balance between the soil and the atmosphere (Govind and Kumari, 2014). Annually 8% of C is

exchanged between the soil C pool and the atmosphere through net primary production and microbial decomposition (Gougoulias et al., 2014). Nitrogen-cycling is closely associated with the C-cycling within an ecosystem as N is intrinsically tied to C in protein structures and is an essential nutrient regulating primary production (Butterbach-Bahl and Gundersen, 2011). Ultimately, nutrient availability determines the functional stability of an ecosystem.

Soil microbes play a significant role in mediating processes such as biological N fixation, nitrification, and denitrification (Isobe and Ohte, 2014). These functions are not only important for soil productivity and ecosystem functions but also are essential for maintaining agriculture ecosystem sustainability (Barrios, 2007). Soil microbes and their functions are highly influenced by land management practices and climatic changes. Therefore, they can be used as sensitive soil quality indicators in agroecosystems (Doran and Zeiss, 2000). Although the importance of microbial process in ecosystem functions is well known, little is known about the main factors that control microbial abundance and diversity in agriculture ecosystems. The relationship between microbial community composition and microbial functions is one of the trending topics in current ecological studies (Konopka, 2009). There are many studies which have focused on the importance of understanding the connection between the microbial community structure and soil functions. However, it is hard to uncouple the main factors affecting soil microbial functions, as there are a variety of biotic and abiotic factors that are responsible for shaping microbial community structure.

Environmental factors and management practices influence microbial community structure and ecological functions (Drenovsky et al., 2010; Purahong et al., 2014; Cong et al., 2015; Ma et al., 2015). Although functional redundancy within a microbial community can alleviate, to varying degrees, the effect of microbial community structure changes on ecosystems functions, specific nutrient cycling rates may be particularly prone to changes due to changes in microbial community structures (Purahong et al., 2014).

Climatic factors have been shown to influence soil microbial dynamics. For example, a study conducted in forest ecosystems across a regional climatic gradient in Western Canada found distinct microbial community profiles in most of the forest ecosystems as a result of variation in soil moisture and OM content due to climatic conditions (Brockett et al., 2012). The varied

microbial community structures were observed at different elevations of forest ecosystems; however, this variation mainly occurred due to changing micro-climatic conditions as a result of seasonal moisture fluctuations (Swallow et al., 2009). Drenovsky et al. (2010) also identified soil moisture (based on the irrigation method) as a major factor influencing microbial composition in different land use types when they compared agriculturally managed (annual or perennial) and a natural ecosystem, and different methods of irrigation (drip or flood).

Agriculture land use type such as irrigation or tillage can alter the soil microbial community structure (Drenovsky et al., 2010). The top soil of long-term no till managed land had more fungal and bacterial biomass with fungi dominating the microbial community compared to an intensively tilled system (Helgason et al., 2009). Others have reported distinct microbial communities captured in a DGGE fingerprint analysis in soil microbial communities in rhizosphere soils of commercially cultivated maize, oat, barley and grass (Garbeva et al., 2008). However, it is difficult to link the temporal patterns of microbial community structure among different land use types even in the same climatic region (Lauber et al., 2013). Some found similar microbial community structures among the same land use types (Drenovsky et al., 2010; Ma et al., 2015). Many studies have reported that observed changes in microbial communities were tied to alterations in the soil fertility and ecosystem functions (Ye et al., 2009; Paula et al., 2014; Barančíková et al., 2016; Zhang et al., 2016).

The response of soil microbes towards agriculture management practices are highly complex and vary with biogeographical and soil factors. Some argue that soil parameters (bulk density, pH, cation exchange capacity, total C, C: N ratio, nitrate (NO_3^-), ammonia (NH_4^+), available phosphorus and potassium) significantly contribute to determining soil microbial communities (Peralta et al., 2010; Xue et al., 2013; Wu et al., 2015a) and account for 70% of the variability in microbial community structure (Xue et al., 2013). Additionally, diverse soil microbial community compositions and structures were observed in the forest, pastureland and agricultural soils indicating the impact of management practices on soil microbial community (Jangid et al., 2008). Hartmann et al. (2015) observed distinct soil microbiomes in conventional and organic farming systems demonstrating the quality of the fertilizer source in determining the microbial community structure where nutrient rich OM facilitated the growth of copiotrophs, thereby

increasing their abundance. Higher bacterial diversity was observed in poultry manure applied soils compared to inorganic fertilized soils where the abundance of specific operational taxonomic units (OTUs) increased; however it led to lower overall microbial diversity (Jangid et al., 2008).

Although many studies have investigated the impact of environmental and soil factors on microbial communities, there is uncertainty about the most significant factor that controls the biosphere microbial community composition and their functions, due in part to the difficulty in separating and isolating individual controlling factors. In most studies, microbial community structure and functions were analyzed by obtaining soil samples from different locations and correlating them with measured variables (e.g., soil moisture content, temperature, soil OM content, agriculture land use, etc.) (Lal, 1974; Brock et al., 2011). Although this approach provides valuable insight to soil microbial community composition and structure, these studies are influenced by the various confounding factors related to landscape, hydrology, and climate (Olson et al., 1996; Zvomuya et al., 2008). To fully elucidate the factors influencing microbial communities, confounding effects such as climate and topography needed to be considered (Zvoumya et al., 2008). For example, in an undulating landscape, higher moisture in depressional areas increases plant growth resulting in greater OM accumulation over time compared to upland areas (Olson et al., 1996) resulting in altered microbial communities (Swallow et al., 2009).

It is difficult to measure and compare soil quality parameters among different soil types where soil and climatic conditions are highly variable. A unique experimental site located at Agriculture and Agri-Food Canada (AAFC) Research Center in Lethbridge, AB, Canada, provided an invaluable opportunity to explore the influence of long-term agricultural practices on different soil types where soils from different donor sites with diverse cropping histories were transplanted to one location and managed under identical climatic and topographical conditions continuously for >21 yr. The top soils from 36 different donor sites were deposited on sub-surface soils of a flatland. These soils had diverse physical, chemical and biological properties at the time of transplanting (Olson et al., 1996). Zvoumya et al. (2008) sampled the site after 14 yr and reported a convergence in C and N mineralization of these soils.

There is a necessity to characterize changes in the microbial community structure with respect to long-term intensive agricultural practices in order to gain a better understanding of the microbial contribution to biogeochemical processes. The main goal of my research was to understand the impact of long-term agricultural management practices on soil microbial community structure and to observe the potential convergence of microbial phylogenetic and functional diversity in soils of different origin transplanted to a common location under identical climate and management conditions. Since there is a strong relationship between biogeochemical functions and soil microbes, the current research was conducted to determine the manner in which microbial functional groups involved in N (ammonia oxidizers and denitrifiers) and C cycling (decomposer) changed in the transplanted soils

1.1. ORGANIZATION OF DISSERTATION

The research presented in this thesis is organized in manuscript style. The dissertation consists of an introduction (Chapter 1), literature review (Chapter 2) and three studies presented in Chapters 3 to 5. The research chapters began by investigating how >21 yr of identical climate, topography and management conditions influenced microbial communities and fertility of diverse transplanted soils (Fig. 1.1). Additionally, the long-term influences of agriculture management practices on microbial communities were assessed using archived soils from 1990 and 2011. Finally, decomposer functional differences were evaluated using ^{13}C cellulose.

The main goal of Chapter 3 was to discover the microbial abundance, composition and community structures of different transplanted soils >21 yr under common conditions. An initial soil survey was conducted using phospholipid fatty acids (PLFA) analysis to observe how differences or similarities in microbial abundance and composition in the different transplanted soils. Based on the initial microbial community analysis, four soils were selected and analyzed for N-cycling gene abundance and their compositions using quantitative real-time polymerase chain reaction (qPCR) and denaturing gradient gel electrophoresis (DGGE) techniques, respectively.

Chapter 4 explored the microbial community divergence which occurred due to long-term agriculture land use. For this study, microbial community compositions of soils archived at

transplantation (1990) and 21 yr after transplantation (2011) were compared using Illumina Miseq sequencing platform.

Chapter 5 investigates how phylogenetic diversity of the microbial community in transplanted soils influenced functional diversity. This was done by linking ^{13}C cellulose decomposition patterns with microbial community structure. A stable isotope probing (SIP) study was conducted using ^{13}C cellulose as the carrier molecule. Soil microcosms with ^{13}C cellulose treatments were incubated for 78 d. Gas samples from the headspace of the microcosms were analyzed to quantify the microbial respiration (CO_2). Active ^{13}C assimilated microbial community (decomposer) was analyzed through ^{13}C incorporated PLFA biomarkers and ^{13}C DNA. Finally, Chapter 7 provides a summary and synthesis of the major findings of the research and suggests future work.

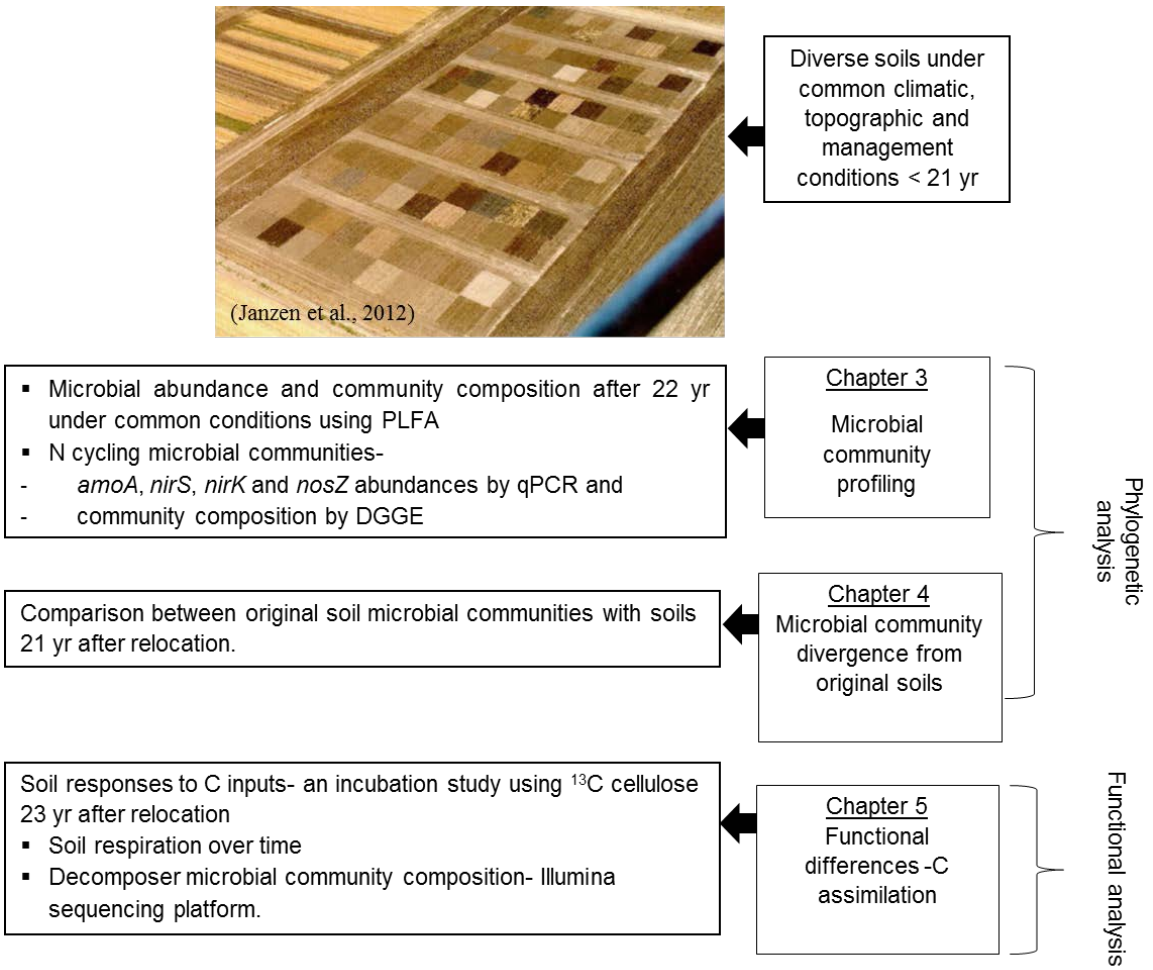


Fig. 1.1. Organization of dissertation.

2.0. LITERATURE REVIEW

2.1. THE ECOLOGY OF SOIL MICROBIAL COMMUNITIES

Soils are a highly diverse environment that hosts a variety of microorganisms that regulate key functions in ecosystems (Wagg et al., 2014; Graham et al., 2016). Soil factors and climate, and management practices are the main factors influencing soil microbial composition. A study conducted in eight different land use types across California found that soil microbial community composition was shaped by the soil environment (soil type, texture, pH, and climatic factors) and management regime (perennial and annual agriculture, irrigation, and tillage). Several studies have reported the effect of soil type on microbial composition (Schutter et al., 2001; Girvan et al., 2003). In the soil environment, local microhabitat conditions are reportedly dominant predictors of microbial community composition (Fierer and Jackson, 2006). Chau et al. (2011) reported that the soil physical environment combined with chemical and biological properties significantly determined microbial composition. Moreover, Girvan et al. (2003) reported that spatial and temporal heterogeneity arose from soil physical, chemical and biological characteristics subsequently creating diverse niches for microbial habitats.

Microbial communities are strongly influenced by soil characteristics. For example, according to Chau et al. (2011), a higher proportion of sand facilitates the development of isolated microhabitats that lower soil matric potential and increased bacterial richness in the soil. They observed that in coarse texture soils, low matric potential creates irregular water phases resulting in large numbers of isolated microhabitats for microorganisms. In contrast, Zhang et al. (2007) found that bacteria are closely associated with fine particle sizes (silt and clay) which provide physical protection. Others have reported that fine texture soils with higher amounts of soil OM are richer in microbial biomass than the coarse textured soils (Meliani et al., 2012). The fine fraction of soil typically consists of clay minerals and is able to retain large amounts of soil OC (Matus et al., 2007) as clay particles provide a higher surface area for binding of OC. In

addition to C, the number of macro and micronutrients, including N, were higher in fine textured soil than coarse textured soil (Reeve et al., 2010). Microbial communities of maize grown at 3 locations in different soil zones (Mollisols, Ultisols and Inceptisols) clustered based on the soil types. For example, Zhao et al. (2016) observed high fertility Mollisols contained larger amounts of C and higher total, bacterial and fungal biomass compared to other soil types with lower amounts of C.

Soil microbes regulate C- and nutrient-cycling along with many other ecological processes in soil ecosystems. Therefore, soil processes such as nitrification, denitrification, and biological N fixation have a close relationship with microbial composition (Reeve et al., 2010). Heterogeneity within soil environments results in a variety of niches which give rise to the diverse microbial population. These niches have varying soil fertility levels which is one of the main determinants of microbial community composition (Koorem et al., 2014). Girvan et al. (2003) indicated the importance of soil chemistry and structure in determining total bacterial community composition. Soil pH, OC, and total N content can be effective predictors of soil microbial community structures. For example, bacterial β diversity is strongly associated with soil OC content whereas fungal β diversity related with total N (TN) content (Hartmann et al., 2015). Furthermore, soil electrical conductivity, NH_4^+, Mn, B, and S had vast influence on microbial composition. Soil pH is also a stronger predictor of soil microbial community structure at the continental scale (Fierer and Jackson, 2006); specifically, the bacterial community structure was strongly correlated with soil pH at the phylum level. Others have reported when soil pH increased, *Acidobacteria* abundance increased while ∞ *Proteobacteria* abundance decreased resulting in a shift in soil bacterial community composition (Lauber et al., 2008). Additionally, a reduction in microbial diversity was observed in 4.5 to 8 pH range (Lauber et al., 2009). Rousk et al. (2010) reported that fungal community structure responded less to pH changes in the soil than bacteria.

Soil moisture and temperature are two factors that regulate the soil microbial community composition. These factors have the ability to shape microbial communities either directly or indirectly (Lauber et al., 2013). Soil moisture content determines the O_2 and nutrient availability for microbial activities. Microbial biomass is sensitive to abiotic changes and has been shown to increase more with precipitation than with soil OC (Serna-Chavez et al., 2013). Others have shown there was a drastic shift in relative abundance of fungal and bacterial community

compositions with rainfall intensity (Castro et al., 2010). Typically, the desiccation of soil reduces the microbial activity and facilitates fungal spore formation (Sylvia et al., 1999). However, Drenovsky et al. (2004) observed that at low moisture levels changes in soil microbial community composition were minor, indicating a greater adaptability under prolonged moisture deficit conditions than under excess moisture. Others have reported that gram negative (G^-) bacterial population was dominant in drier soils whereas gram positive (G^+) bacteria dominated in wet soils (Ma et al., 2015). The relative abundance of *Proteobacteria* was greater in wet conditions whereas *Acidobacteria* relative abundance was high in dry conditions (Castro et al., 2010). Sylvia et al. (1999) reported that flooded conditions with low levels of O_2 resulted in alterations in facultative and obligate anaerobes community abundances. Temperature also reportedly regulates soil moisture dynamics through evapotranspiration (Serna-Chavez et al., 2013), with fungal abundance more sensitive to increasing temperature than bacteria. This can be either due to the direct response of fungi or reduction in labile substrate concentrations as a result of plants response to an elevated temperature which indirectly facilitates fungal growth (Castro et al., 2010).

Soil ecosystems are directly affected by land use conditions (Chen et al., 2015). Land use changes are mainly determined by the interaction between human requirements and the biophysical environment. Frequently, natural ecosystems are converted into agroecosystems for intensified production. Ma et al. (2015) have suggested that land use disturbances and management practices are responsible for diversifying soil microbial profiles. Agriculture management and crop factors have significant effects on soil microbial communities. Changes in plant cover can modify the interactions between the plant and soil through nutrient acquisition and OM inputs. Under these influences, microbial community composition will gradually shift to most adapted species to the particular soil conditions (Miki et al., 2010). For example, distinct microbial community structures have been linked to forest, agriculture and native pastureland systems (Jangid et al., 2008). Girvan et al. (2003) concluded that long-term agriculture management practices have more significant impacts on soil bacterial community structure than on composition. Furthermore, the type of cropping system has enormous influences on soil functions and microbial biomass (Lalande et al., 2005).

In cropping systems, the bacterial community is strongly influenced by management practices while the fungal community is more strongly influenced by the type of crop (Lalande et al., 2005; Zak et al., 2014; Hartmann et al., 2015). Plant diversity and biomass are responsible for soil functions like C-cycling through OM decomposition and N-cycling, leading to increased availability of NH_4^+. Soil microbes are key regulators of soil nutrient cycling; thus, soil OM is the major source of available nutrients for plants and microbes.

Most agricultural management practices directly influence soil OM dynamics. For example, soils managed under a pasture system contained more OM than an annual cropping system (Blair et al., 2006). In soil ecosystems, crop residues are the major source of soil OM. Therefore, quality and quantity of residues govern soil OM dynamics. In order to maintain nutrient cycles, adequate amounts of residue should return to the soil (Arcand et al., 2016). Microbial processing of plant materials also varies with the degree to which organic residues are incorporated, with soil residue incorporation leading to more efficient nutrient cycling than surface applied residue (Helgason et al., 2014). Climatic conditions, which regulates soil moisture, similarly is a major factor influencing residue decomposition (Helgason et al., 2014).

Plant materials containing readily available components will easily degrade by the microorganisms while low-quality materials are less readily decomposed. For example, rye leaves with larger amounts of easily degradable substrates assimilated by soil microbes decomposed more quickly compared to poor quality wheat straw (Nicolardot et al., 2007). The quality of fresh OM often determines the diversity of decomposer community and during OM degradation, microbial community structure can shift in response to the available C resources (Bastian et al., 2009; Marschner et al., 2011). There is an immediate shift in microbial community structure after fresh OM addition due to an increase in the *r* strategist microbial population who prefer to live in nutrient rich environments (Pianka, 1970). At later stages of decomposition, *K* strategist microbial populations with slow growth rates will increase as they have a greater ability to effectively compete for resources in nutrient poor environments (Pianka, 1970).

A study conducted in different agriculture management systems including tillage, straw retention, and herbicide application identified soil OC, soluble C, microbial biomass C and soil

N as the factors that explained the variability in microbial community composition as measured using PLFA (García-Orenes et al., 2013). In cropping systems, zero or reduced tillage increases the microbial biomass compared to conventional tillage (Helgason et al., 2009; Jiang et al., 2011). In these studies, residue retention promoted greater microbial biomass than in soil with the residue removed. Tillage and residue retention practices have the ability to change microbial community structures by altering soil fertility (Fierer et al., 2007; Navarro-Noya et al., 2013). Chowdhury et al. (2015) reported changes in microbial biomass and structure altered soil functions where the microbial activity of the soil where residue was removed was lower than where residues had been retained. This indicated that the addition of residue releases limited nutrients for microbes and enhances the microbial activity. Interactions between C and N inputs can change the soil microbial community structure. For example, N fertilizer application improved soil OC by increasing root exudates and the amounts of crop residues returning to the soil (Christopher and Lal, 2007).

The influence of fertilizer application on microbial community dynamics can vary depending on the fertilizer source and application rate. Greater N availability facilitates copiotroph microbial community whereas oligotrophs become dominant under low N availability (Fierer et al., 2012). Others have reported that microbial abundance varies depending on the type of N fertilizer applied without any difference in application rate. For example, manure application increased the total PLFAs and bacterial biomass compared to inorganic N fertilizer application (Lazcano et al., 2013). Gram negative bacterial (Lazcano et al., 2013) and fungal abundances (Lalande et al., 2005) reportedly increased with manure application. Conversely, fungal biomass did not respond to N fertilizer type demonstrating other controlling factors on fungal abundance (Lazcano et al., 2013). Reeve et al. (2010) reported that organically managed systems promoted functional genes (nitrification, dinitrogen fixation, denitrification, urease, and dehydrogenase) when compared to conventional systems. Changes occurred in microbial community with biotic, abiotic and agriculture management having significant influences on ecosystem functions as microbes are the key players of soil nutrient cycling.

2.2. C-CYCLING IN TERRESTRIAL ECOSYSTEMS

2.2.1. Soil C cycle

The soil is the major reservoir of C in the terrestrial ecosystem and it contains 2000 Pg of C as soil OM (Janzen, 2004). Soil OM is the key component of ecosystem C and composed of a variety of organic components ranging from plant residues to highly decomposed humic compounds (Amundson, 2001). Net ecosystem production determines the balance between C entering into the soil and C leaving the soil to the atmosphere (Chapin et al., 2002). In natural ecosystems, soil C balance is mainly controlled by photosynthesis and soil respiration processes (Govind and Kumari, 2014). As primary producers, plants fixed atmospheric CO_2 through photosynthesis and convert atmospheric C into forms that can be utilized by living organisms. Likewise, algae and cyanobacteria are capable of fixing atmospheric C and they are the major primary producers in certain ecosystems (Aislabie et al., 2013). A large proportion of OC present in soil has a plant origin, much of which is from senescing plant parts and root exudates (Kuzyakov and Domanski, 2000). Soil OM formation occurs through decomposition and transformation of plant residues and root exudates by soil microorganisms. Soil microorganisms are the major players of soil C and N cycling (Doran, 1987). Heterotrophic bacteria and fungi are the major decomposers present in the soils. During decomposition, heterotrophic microorganisms respire and release C into the atmosphere (Chapin et al., 2002; Janzen, 2004).

Litter chemistry determines the quality of substrate received by the soil microorganisms and is the primary controller of residue decomposition (Wickings et al., 2012). Initial litter chemistry and decomposer community composition are responsible for chemical convergence in OM through decomposition process (Wickings et al., 2012). Plant biomass contributes to soil OM through litter decomposition. The formation of OM occurs by mineralization of organic materials released via microbial decomposition. In addition, OM forms by physically transferring litter particulates into the soil (Cotrufo et al., 2015). Agriculture exploitation of natural ecosystems is the main land use change responsible for the reduction of soil OM reservoirs (Janzen, 2004).

2.2.2. Influence of land use practices on terrestrial C cycling

Land use changes have a significant impact on soil C-cycling. The labile fraction of OM is highly susceptible to management-induced changes (Janzen et al., 1998). Changes in soil OC in response to the agriculture land use practices influence soil microbial abundance, composition and their activity (García-Orenes et al., 2013). The conversion of natural undisturbed land into agriculture land typically replaces the diverse plant community with monoculture cropping systems. Furthermore, in agricultural systems, much of the biomass produced is not returned to the soil but is instead harvested as a marketable product. Additionally, the practice of fallowing reduces the quantity of plant inputs into the soil OM (McLauchlan, 2006). Conservation agricultural practices such as crop rotation, minimal tillage, and retention of crop residues are known to improve soil quality and crop productivity (Ceja-Navarro et al., 2010). For example, increases in soil N and C contents, soil microbial biomass and microbial diversity are associated with fields subjected to zero tillage with residue retention (Ceja-Navarro et al., 2010). Increasing residue inputs increased total microbial and bacterial biomasses in no till systems (Wang et al., 2011; Hou et al., 2012). As a result, no till systems had greater microbial biomass C and TN compared to conventional tillage practices (Hou et al., 2012). The combination of no till practices with organic inputs increased the microbial biomass and their activity and this activity facilitated OC stabilization in the soil (Wang et al., 2011).

Retaining crop residues has been linked to increased crop yield (Wilhelm et al., 2004) and crop residue retention has the ability to restore soil productivity (Lal, 2004). Crop residues can provide 40 to 46% of C to the ecosystem following biomass decomposition. In addition, retaining crop residues can help control soil erosion (Janzen et al., 1998), and improve soil physical, chemical and biological properties (Blanco-Canqui and Lal, 2009). Soil water conservation is another benefit related to residue retention (Wilhelm, 2004). A major benefit of residue retention is the increase in soil OC content (Janzen et al., 1998). Crop residues are rich in OC compounds and greatly contribute to the soil OC pool. The main limiting factor for microbial growth is the availability of labile C (Bremer and Van Kessel., 1992). An increase in OM content in the soil improves aggregate stability and moisture availability which favors higher microbial activity (García-Orenes et al., 2013). For example, Le Guillou et al. (2012) found greater

microbial abundance and diversity in soil amended with wheat residue than miscanthus residue where wheat residues have higher amounts of labile C. A study conducted in a fluvo-aquic soil in China found that crop residue retention had positive affects on the soil C: N ratio and monounsaturated fatty acid/ saturated fatty acid ratio (Wang et al., 2012). When there is a larger amount of easily metabolized C compounds, the majority of microbes in the community are able to remain active (Paterson et al., 2011).

There is a positive correlation between microbial activity and soil labile and OC contents (Chowdhury et al., 2015). Long-term manure application enhances the soil C pool (Liu et al., 2013). They reported that application of farm yard manure or crop residues increased the particulate OC fraction and microbial biomass C to a greater extent than inorganic fertilizer application. In contrast, it has been suggested that crop residue retention may be responsible for the depletion of native C reserves in the soil through priming effect (PE), with the magnitude of the PE relating to soil nutrient availability (Chowdhury et al., 2014). Microorganisms need nutrients (N, P, K) to utilize C from the crop residues, thus limited available nutrients in residues may promote microbial dependency on native soil OM. The degree of soil quality and crop productivity enhancement can differ based on soil type, soil functions, cropping system and ecosystem services (Blanco-Canqui and Lal, 2009).

In Western Canada, the removal of crop residues for use in livestock production is a common practice (Zvomuya et al., 2008). Moreover, there is a growing trend towards producing paper products and fuel using crop residues (Malhi et al., 2006). The impact of crop residue removal for livestock and industrial purposes on soil quality is debatable. A study conducted using a Black Chernozemic soil revealed that relatively moderate amounts of residues can be removed from the field without any adverse effects on the overall soil C pool (Lemke et al., 2010). In contrast, when crop residues were removed over a 4 yr period, a substantial reduction in OC and nutrients in the top soil, was observed (Chowdhury et al., 2015). Different responses to crop residue removal may be related to other factors such as environmental conditions and management history. Crop residue removal reportedly has negative impacts on soil earthworm (Butt et al., 1999) and microbial (Blanco-Canqui and Lal, 2009) populations.

2.3. N-CYCLING IN TERRESTRIAL ECOSYSTEM: PROCESSES AND AGRICULTURAL IMPACT

2.3.1 Nitrogen inputs to ecosystem

Nitrogen (N) is a key nutritional element in living forms where it is a component of complex organic compounds such as amino acids, nucleic acids and proteins (Lambers et al. 1998; Canfield et al., 2010). In an ecosystem, N is a major factor that governs the net primary productivity of the ecosystem (Vitousek and Howarth, 2007). Nitrogen-cycling in a terrestrial ecosystem is a complex process, which is mediated through several microbial and plant processes (Galloway et al., 2003). During N-cycling, N may be either added or removed from the ecosystem.

In a natural ecosystem, biological N fixation is the main process by which N enters the biotic system. Nitrogen occupies 78% of the atmospheric volume (Lambers et al., 1998); however, plants and most living organisms are incapable of consuming inert atmospheric N_2, due to the high-energy requirement to break the triple bond between two N atoms (Canfield et al., 2010). Only certain groups of microorganisms that produce the nitrogenase enzyme are capable of converting N_2 into usable NH_4^+ (Cassman and Harwood, 1995). The main N-fixing strategy in an agricultural system is symbiotic fixation between legume plants and *Rhizobium* bacteria. In cropping systems, leguminous plants annually fix 18.5 Tg of N symbiotically (Herridge et al., 2008). In cereal cultivating systems, N fixation is mainly carried out by free-living microorganisms present in the soil. The amount of N fixed by free-living organisms is less than that which is fixed by symbiotic associations and depends on several biotic and abiotic factors. In non-legume crop lands (i.e., cereal crop lands), annual N fixation typically is less than 4 Tg (Herridge et al., 2008) and is not sufficient to sustain the crop growth. As a result, the N requirement is mainly fulfilled by applying N fertilizers.

Nitrogen fertilizer is often used in cropping systems to increase the plant yield, as N typically is the most limiting nutrient in the crop production (Malhi et al., 2006). In cereal plants, N accumulated in mature parts are transferred into the developing grain, an essential factor that determines the grain quality. It has been estimated that 67% of global N fertilizer used is annually lost from soils (Raun and Johnson, 1999) through leaching (Cui et al., 2010), volatilization (Ma et al., 2010; Zhang et al., 2014), immobilization and denitrification. These

losses can create serious environmental problems such as eutrophication, ground water contamination, greenhouse gas emissions and depletion of the ozone layer (Anbessa and Juskiw, 2012). At harvest, cereal grains typically capture less than half of the N fertilizer applied while the remaining N is incorporated into the soil OM through decomposition or is lost to adjacent environments (Raun and Jonhson, 1999).

Approximately 95% of total soil N exists in the soil OM (Schulten and Schnitzer, 1998). Litter produced through plants and animal debris is the major source for soil N (Butterbach-Bahl and Gundersen, 2011). Decomposition is a microbial mediated process that is mainly carried out to acquire energy either through the oxidation of N compounds or as an alternative electron acceptor under anaerobic condition (Butterbach-Bahl et al., 2011). During decomposition, the organic N present in the soil decomposes and releases N for plant and microbial uptake (Schulten and Schnitzer, 1998). During this process, complex structures of stored organic N break into bio-available monomers, which can be used by microbiota and plants. Soil microbes further break these monomers into simple compounds such as ammonium (NH_4^+) (Butterbach-Bahl and Gundersen, 2011). Both NH_4^+ and organic N can also be converted into nitrate through autotrophic and heterotrophic nitrification processes, respectively.

2.3.2. Soil nitrification

Nitrification is the biological oxidation of ammonia (NH_4^+) into nitrate (NO_3^-) (Ward, 2013). This is an aerobic process that is carried out by three main microbial groups; autotrophic ammonia oxidizers, autotrophic nitrite oxidizers and heterotrophic nitrifiers (Prosser, 2005). During the nitrification process an ammonium or ammonia (NH_3) molecule is converted into nitrate (NO_3^-) (Ferguson et al. 2007). Each step is catalyzed by two functionally different groups of microorganisms. They are ammonia oxidizers and nitrite oxidizing bacteria (NOB) (Warrington, 1878; Kowalchuk and Stephen, 2001). The main source for reduced forms of N in the soil is decomposed organic N derived from plants and animals as well as applied ammonia-based fertilizer (Processor, 2005). Heterotrophic bacteria and fungi are capable of consuming organic N and they excrete NH_4^+ through cell lysis which also is a N source for nitrification (Butterbach-Bahl and Gundersen, 2011).

$NH_3 + 2H^+ + O_2 + 2e^- \rightarrow NH_2OH + H_2O$ [Eq. 2.1]

$NH_2OH + H_2O \rightarrow NO_2^- + 5H^+ + 4e$ [Eq. 2.2]

$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e$ [Eq. 2.3]

(adapted from Heil et al., 2016).

In ammonia oxidation, NH_3 converts into hydroxylamine (NH_2OH) that is catalyzed by the enzyme ammonia monooxygenase which is bound to a multi subunit plasma membrane (Eq. 2.1) (Kowalchuck and Stephen, 2001). Generated hydroxylamine converts into nitrite (NO_2^-) and this process is facilitated by the hydroxylamine oxidoreductase enzyme which is found in the periplasm (Eq. 2.2) (Processor, 2005). At the end of the process, NO_2^- is further oxidized into NO_3^- by NOB using nitrite oxidoreductase enzyme (Eq. 2.3) (Abeliovich, 2006).

2.3.3. Ammonia oxidizer community and factors affecting nitrification

Bacteria were once considered to be the sole group of ammonia oxidizers present in the soil (Auguet et al., 2010). However, recent studies have reported that archaea also are heavily involved in ammonia oxidation in various environments such as marine, terrestrial and hot water ecosystems. Soils normally contain few groups of *Crenarchaeota* that include members involved in nitrification (Leininger et al., 2006; Auguet et al., 2010) and their relative contribution to the nitrification process remains uncertain (Jia and Conrad, 2009; Banning et al., 2015). It is argued; however, that archaeal ammonia oxidizers (AOA) can play a vital role in the nitrification process (Leininger et al., 2006).

In agriculture systems, ammonia oxidizing bacteria (AOB) and AOA communities follow different biogeographical patterns. For example, in nutrient rich environments with high C and N contents, the AOB community tends to be more dominant than the AOA community (Wessen et al., 2011), while the AOA communities dominate under low nutrient and acidic soil conditions (Erguder et al., 2009). Di et al. (2009) reported AOB as the major drivers of nitrification in grassland soils. In agriculture systems in the semi-arid area, AOB abundance was higher in the soil surface, whereas AOA abundance increased along the soil depth. Simultaneously, the nitrification rate also decreased along with the soil depth indicating AOB as the main nitrification group in the semi-arid soils (Banning et al., 2015). A study conducted in agricultural soils across Australia reported that the distribution of AOA and AOB, and the AOA: AOB ratio

were strongly correlated with climate and seasonal factors (O' Sullivan et al., 2013). In their study, the AOA community was more dominant at dry and high temperature conditions, while AOB community was dominant at wet and low temperature conditions.

Fertilizer N application can influence the soil nitrifying community. For example, following inorganic N fertilizer application, soil gross nitrification increased rapidly, indicating the use of added N by soil nitrifiers (Inselsbacher et al., 2010). The AOB and AOA communities have different preferences towards soil N levels, in which AOB has a better adaptability towards changing environmental conditions with a preference towards nutrient rich environment (Di et al., 2010). In contrast, AOA are more abundant in low pH and poor nutrient status soils with oligotrophic condition (Valentine, 2007; Erguder et al., 2009; Di et al., 2010; Prosser and Nicol, 2012). Application of N into the topsoil increased the AOB abundance, indicating a greater contribution of AOB to ammonia oxidation compared AOA at topsoil (Di et al., 2010). It has been argued that nitrification in soils without N application is mainly governed by AOA population (Sterngren et al., 2015). Long-term N fertilizer application (inorganic and organic) reduced the AOA abundance in the agricultural soil, while it increased AOB abundance (Sterngren et al., 2015). Earlier it was believed that there was no single organism able to convert ammonia directly into nitrate (NO_3^-) because of energy limitations (Costa et al., 2006). However, a recent study conducted in a low productivity mangrove area found that a group of nitrate reducing bacteria are capable of converting ammonia into nitrate by themselves (Balk et al., 2015).

2.3.4 Soil denitrification

Denitrification is a respiratory process of microbiota, which reduces NO_3^- or NO_2^- to nitric oxide (NO), nitrous oxide (N_2O) or nitrogen gas (N_2). This is the main biological process that recycles fixed N back to the atmosphere (Shaw et al., 2006; Giles et al., 2012), and is also responsible for 70% of global N_2O emissions (Conrad, 1996). Subclasses of *Proteobacteria* are the major group contributing to soil denitrification processes. Additionally, few archaeal species such as *Pyrobaculum aerophilum* and *Haloferax denitrificans* (Hayatsu et al., 2008) and a few fungal groups (Zumft, 1997; Shoun et al., 2012) have also been reported to perform denitrification. For example, the reduction of NO_2^- and release of N_2O was observed in cultures

of *Fusarium oxysporum* and *Fusarium solanai* under low O_2 concentrations (Bollag and Tung, 1972).

A broad physiological group of organisms is involved in the denitrification process is initiated by reducing NO_3^- into NO_2^-. This process is performed using a molybdenum containing a membrane-bound enzyme, called nitrate reductase that is encoded by the Nas, Nar, and nap genes (Moreno-vivián et al., 1999). Nitrate is also reduced to NO or N_2O by the nitrite reductase enzyme that is encoded by *nirK* (Cu containing) and *nirS* (cytochrome cd1) genes (Levy-Booth et al., 2014). These two genes are commonly used markers in molecular studies and not commonly possessed by a single organism. Wolsing and Priemé (2004) reported that the *nirK* community was more sensitive to seasonal changes and N fertilizer sources than *nirS*, resulting in distinct community profiles with response to N fertilizer type (inorganic or organic). Nitric oxide reductase encoded by cytochrome bc is used in the transformation of NO to N_2O (Zumft, 1997. Reduction of N_2O to N_2 is facilitated by the nitrous oxide reductase enzyme that is encoded by the *nosZ* gene. The abundance of the *nosZ* gene is a strong parameter for assessing the N_2O to N_2 process or N_2O releasing potential of an ecosystem to the atmosphere (Philippot et al., 2009).

2.3.5 Factors affecting denitrification

The denitrification process contributes to a significant loss of N from agricultural systems. The key main factors influencing soil denitrification are climate, soil heterogeneity and agricultural management practices (Philippot et al., 2007). Emission of N_2O through denitrification is strongly correlated to soil moisture content, and in particular, water filled pore space (Signor et al., 2013), with high soil moisture content enhancing N_2O emissions. Optimum N_2O emission occurs at 70-80% water filled spore space. However, at soil saturation, the main product is N_2 (Davidson and Verchot, 2000). Additionally, frequent drying and wetting cycles typically favor N_2O emissions (Brentrup et al., 2000). Although bacterial denitrification occurs under anaerobic conditions, there are certain organisms that are capable of carrying the process in the presence of O_2. For example, *Burkholderia cepacia* is a species that can carry out denitrification under oxic conditions (Matsuzaka et al., 2003; Hayatsu et al., 2008). Additionally, increasing environmental temperature typically increases the denitrification process to an

optimum (Butterbach-Bahl et al., 2013). Furthermore, temperature induced respiration reduces the O_2 in the soil environment creating anaerobic conditions (Butterbach-Bahl et al., 2013). Soil pH was recognized as one of the major predictors of denitrification and its end product (N_2/N_2O ratio) where neutral soil conditions in grasslands had higher denitrification rates and high N_2/N_2O ratio whereas N_2O (lower N_2/N_2O) was the end product of denitrification in acidic pH condition (Sun et al., 2012).

2.3.6. Influence of N fertilizer application on soil microorganisms

Soil fertility plays a vital role in maintaining agricultural production. However, in a continuous cropping system, available nutrients supplied through nutrient cycling are not sufficient to meet maximum crop yields. Application of N fertilizer is one of the important practices that increases crop productivity. Soil microbial abundance and diversity are two main factors that govern sustainability and productivity of an ecosystem (Schimel, 1995). They mediate soil OM decomposition and nutrient cycling (Schimel and Schaeffer, 2012). Stability of the microbial community composition is an important aspect of maintaining soil functions, such as rate of denitrification, nitrification and N fixation (Hsu and Buckley, 2009). Soil microorganisms respond rapidly to environmental changes (fertilizer application, tillage, crop diversification, herbicide application), which also lead to dynamic changes in the microbial abundance, activity, and diversity (Bending et al., 2004).

Plants and soil microbes are the two main competitors for applied N fertilizer. The highest competition for N acquisition by the plants and microbes were reportedly observed in the first 4 hr following N application and gradually declined after 24 hr (Inselsbacher et al., 2009). Others have observed a significant influence of N fertilizer application on the soil microbial abundance, community structure and their activity (Chu et al., 2007; Zhang et al., 2008; Harter et al., 2013; Sun et al., 2015). Microbial community composition and activity can vary depending on the N source and the application of N fertilizer typically is associated with increased basal respiration rates (Enwall et al., 2005). For example, higher respiration rates were observed in organic N amended systems than inorganic N fertilizer (Enwall et al., 2005). Furthermore, organic fertilizer applications typically are associated with an increase in soil microbial biomass (Toyota and Kuninaga, 2006) and functional diversity (Li et al., 2015), whereas microbial biomass and the

diversity are frequently shown to decrease under conventionally managed systems relative to organically managed (farmyard manure) counterparts (Sarathchandra et al., 2001; Toyota and Kuninaga, 2006). Long-term N fertilizer application also has been shown to alter the bacterial and fungal community composition (Belay et al., 2002). Gram negative bacteria are present in a high abundance in organic systems due to the microbial community composition of the manure or growth promoting effects on *Bacillus spp* (Chu et al., 2007).

2.4. TOOLS TO SOIL STUDY MICROBIAL COMMUNITIES

There are several methods for microbial community analysis. The introduction of culture independent molecular techniques allowed researchers to uncover previously unknown details about the microbial community structure and functions (Smalla, 1999).

Determination of phospholipid fatty acids in soil is one of the common and popular methods used to study microbial biomass and community composition. Phospholipid fatty acids analysis (PLFA) quantifies fatty acids biomolecules present in the microbial cell walls and total PLFA give an idea about viable microbial biomass as phospholipids are deteriorated quickly after microbial death (Kaur et al., 2005). The PLFA technique is sensitive in detecting variations in the microbial community composition compared to molecular techniques (Frostegård et al., 2011). However, PLFA has restricted ability in detecting specific microbial functional groups in soils, as the resolution of the communities can vary due to overlapping PLFA biomarkers and variations in PLFA patterns in response to the environmental factors (Ramsey et al., 2006). Combining stable isotope probing (SIP) techniques with PLFA can be used to detect metabolically active communities in soils (Boschker and Middelburg, 2002). There are drawbacks associated with PLFA analysis. Specifically, PLFA analysis is not able to uncover microbial community details at the species level nor is it useful for detecting variations in the archaeal microbial community (Kaur et al., 2005).

A large number of microbial species has not been cultured under laboratory conditions, as they require specific conditions that are difficult to provide at the laboratory scale (Smalla and The, 1999). Analysis of uncultured microbial communities is carried out by analyzing biomolecules (proteins, lipids, and nucleic acids) found on their systems using culture independent approaches. Therefore, the sequencing of the microbial DNA and RNA facilitates

scientific investigations on whole microbial community based on specific marker genes (16S *rRNA* gene for bacteria and archaea and 18S *rRNA* gene for fungi) under laboratory conditions (Rastogi and Sani, 2011).

Quantitative polymerase chain reaction (qPCR) techniques are widely used in microbial studies to measure the abundance and expression of specific functional genes in environmental samples. These methods can be effectively used to capture the variations in functional genes under changing conditions (spatially and temporally). In qPCR techniques, a fluorescence dye or probe is used to measure the PCR product formation and gene quantifications are done at the exponential phase (Smith and Osborn, 2009). Numbers of amplicon detected during analysis are proportional to the quantity of the specific gene that was used in the starting template (Rastogi and Sani, 2011). A number of primers have been identified and designed to be used effectively in the qPCR analysis to quantify bacteria (16S *rRNA* genes) and fungal microbial abundances (Fierer et al., 2005). In the current study, a qPCR method was used to quantify the abundance of genes (bacteria and archaeal *amoA*, *nirK*, *nirS* and *nosZ*) involved in N-cycling of the transplanted soils. Further, the composition of nitrifier and denitrifier microbial communities were investigated by denaturing gradient gel electrophoresis technique (DGGE). Culture independent molecular approaches consist of low-throughput and high throughput approaches. DGGE, TGGE, TRFLP and Sanger sequencing are low-throughput methods, while next generation sequencing (454-sequencing and Illumina sequencing) are high-throughput sequencing methods by which a large number of parallel samples with added barcodes can be processed (Rincon-Florez et al., 2013).

Genetic diversity of microbial communities can be determined using the DGGE approach. For example, Uitierlinden (1993) amplified taxonomic targets from environmental DNA extractions using a PCR technique. Dissimilarities between the sequences of a specific gene were determined through gel electrophoresis. Separation of DNA fragments of equal length with different sequences exposed the DGGE fingerprints of the particular targets. During the gel electrophoresis, sequence length determines the migrating position in the gel. Identification of phylogenetic structure of specific microbial communities can be done by excising bands from the gel, reamplifying and sequencing for genetic identities (Rastogi and Sani, 2011). The main limitation of the DGGE technique is the inability to capture total microbial diversity.

As the cost of DNA sequencing decreases, researchers have started to heavily use next generation sequencing approaches to profile microbial diversity in environmental samples. High throughput sequencing techniques can capture advance information about phylogenetic and functional diversity in the microbial communities (Wu et al., 2015). Although there are numerous high throughput next generation sequencing techniques, the Illumina platform has become popular due to its low cost, quick analysis and greater precision relative to other techniques (Bartram et al., 2011; Caporaso et al., 2011, 2012). All the molecular analysis tools provide details regarding microbial identification; however, it is a great challenge in microbial ecology to identify regulators of ecosystems processes.

The stable isotope probing (SIP) approach is the main approach used to identify metabolically active microbial communities (Boschker and Middelburg, 2002). Using this stable isotope probing technique, commercially available stable isotope enriched substrates (typically ^{13}C) are added to an environmental sample and the microbial biomarkers enriched with the stable isotope are subsequently analyzed (Dumont and Murrell, 2005). After exposing the environmental sample to a labelled substrate, microbiota assimilates and incorporate these substrates into their biomass. By analyzing the PLFA and DNA enriched with isotope molecules, the microbial community diversity related with ecosystem functions can be more clearly understood (Neufeld et al., 2007).

3.0. A SURVEY OF MICROBIAL ABUNDANCE AND DIVERSITY IN TRANSPLANTED CHERNOZEMS MANAGED UNDER COMMON CLIMATE AND TOPOGRAPHIC CONDITIONS FOR 21 YEARS

3.1. PREFACE

Soil microbial community composition and diversity are important to ecosystem functions, including nutrient cycling. Therefore, identifying and understanding the dominant factors influencing microbial community composition and abundance is beneficial for sustainable agricultural management. To date, numerous studies have been conducted to elaborate how particular soil responds to management in a given location. However, different soils respond differently to the same management condition and the difference in response across soils is confounded by the climate and topography. The influence of confounding factors may mask the true determinants of microbial dynamics. The long-term transplanted soil experiment at AAFC, Lethbridge provides an opportunity to study diverse soil communities by uncoupling these confounding effects. The goal of this chapter is to determine how microbial community abundance and community structures changed across diverse soils maintained for >21 yr under common climatic, topographic and management conditions following management and land use changes.

3.2. ABSTRACT

Soil microbial community abundance and diversity are major determinants of biogeochemical cycling. A long-term soil quality experiment was established in 1990 at the Agriculture and Agri-Food Canada (AAFC) research station in Lethbridge, AB to examine the effects of agricultural management practices on different transplanted Chernozems managed under common climatic and topographic conditions. Transplanted soils were continuously cropped to wheat with residues removed at harvest and with nitrogen (N) fertilizer applied either at 0 or 60 kg N ha^{-1}. A survey of microbial community composition and nitrifier and denitrifier community structures was conducted in 2012, >21 yr after the field trial was established. Phospholipid fatty acid (PLFA) profiling, quantitative real-time Polymerase Chain Reaction (qPCR) and denaturing gradient gel electrophoresis (DGGE) analysis of N-cycling functional genes revealed that soil origin and land use history had a dominant long-term impact on microbial abundance and community composition. Furthermore, long-term N fertilizer application did not influence total microbial biomass, bacterial and fungal abundance. The highest microbial biomass (26.7 and 25.2 nmol g^{-1} soil) were observed in soils with a history of cereal cultivation (CC) and those which had received manure (RM) at a rate of 30 t ha^{-1}, respectively, whereas transplanted sub-surface soils from B and C horizons (BH and CH, respectively) had the lowest microbial biomass. Greater 16S *rRNA* gene abundance (log 10.7 gene copies g^{-1} dry soil) was observed in high fertility cereal cultivated (CC) soil. Bacterial *amoA* and *nirS* community compositions were significantly influenced by N application; however, the degree of influence was mainly regulated by soil origin. The bacterial *amoA* community structure of high fertility soils (CC and PL) converged following long-term N fertilization. Soil fertility was responsible for the greatest overall microbial community differences in transplanted soils. Soil origin reflected soil genesis and land use history and was the major basis of fertility differences even after >21 yr of common conditions. These findings suggest that the legacy of soil fertility is a more important determinant of microbial community composition than current conditions.

3.3. INTRODUCTION

Soil microorganisms are important for many agroecosystem functions and sustainable land use. They contribute to soil organic matter (OM) decomposition, carbon (C) dynamics and thus changes that occur in microbial communities consequently affect soil processes (Cavigelli and Robertson, 2001). Currently, there is a growing interest in determining the best predictors of microbial community abundance and composition which related to nutrient cycling in terrestrial ecosystems. Soil microbial abundance and community structure follow different patterns at various ecological scales; however, the key drivers behind those patterns are not fully understood. Understanding the cause of these patterns is the foundation for sustainable ecosystems management (Levin, 1992).

Microbial community composition and structure in agricultural soils are highly complex and are governed by various biotic and abiotic factors such as soil physical and chemical properties, soil management and environmental conditions. Several studies have shown the influence of agricultural and land use changes on the presence of soil organisms or specific functional groups (Buckley and Schmidt, 2001; Allison et al., 2005; Ding et al., 2013; Cederlund et al., 2014; Hartmann et al., 2015) while other studies found that soil environmental factors largely impacted microbial dynamics (Buckley and Schmidt, 2003; Fierer and Jackson, 2006). Furthermore, soil chemical properties such as pH, OC and TN content also affect soil microbial community structure (Girvan et al., 2003; Hartmann et al., 2015). These findings are useful in understanding the microbial functions and community composition under specific environmental conditions associated with a particular geographic location (Wieland et al., 2001; Buckley and Schmidt, 2003; Drenovsky et al., 2010; Marschner et al., 2011; Philippot et al., 2013). However, much of this work has been unable to decouple the effect of soil characteristics from confounding factors such as climatic, topographic and hydrology (Zvomuya et al., 2008; Olson et al., 1996).

A long-term field experiment in which a variety of soils were transplanted at a single site located at Lethbridge, AB, Canada on AAFC research station, provided a unique opportunity to assess the effects of soil quality parameters on microbial community abundance, composition and functions by decoupling the influence of confounding factors such as climate, topography and hydrology that normally complicates inter-site comparisons of different soils (Olson et al.,

1996; Zvomuya et al., 2008; Janzen et al., 2012). Long-term research sites serve as "test beds" which provide the opportunity to reveal dynamics of ecosystem processes and patterns which may develop slowly over many years (Hobbie et al., 2003). In 1990, when establishing the long-term soil quality experiment, researchers transplanted the Ap/Ah and two sub-surface horizons from 36 different Chernozems having distinct physical, chemical (variable fertility levels) and biological characteristics (Zvomuya et al., 2008; Janzen et al., 2012). Two levels of N fertilizer (0 and 60 kg N ha^{-1}) were applied to the soil treatments using a split plot design. The field has been continuously cropped with wheat and crop residue was removed annually (Olson et al., 1996). After 14 years of transplantation, some convergence in C and N mineralization was observed by Zvomuya et al. (2008). Since there is known to be a significant relationship between soil nutrient cycling and microbial dynamics, the current research hypothesized that identical agricultural management, climatic and topographic conditions would cause convergence of the abundance and diversity of microbial communities and N-cycling functional groups (nitrifiers and denitrifiers) in different transplanted soil types.

The main objectives were to survey the microbial abundance, composition and community structure of different Chernozems and to observe whether there was a convergence of microbial phylogenetic and functional diversity in soils of different origin, managed under common conditions for >21 yr. The specific objectives of this study were to compare the current microbial composition and community structure of the transplanted soils and to determine the impacts of N fertilization (+/-) on overall microbial abundance and community structure in nitrifier and denitrifier communities of transplanted soils.

3.4. MATERIALS AND METHODS

3.4.1. Long-term soil quality experiment design and implementation

The experimental site is located at the Agriculture and Agri-Food Canada Research Station at Lethbridge, AB, Canada (49°42′ N, 112°50′ W) and has been described by others (Olson et al., 1996; Zvomuya et al., 2008). The field site had level topography and uniform soil characteristics prior to soil transplantation. The original soil characteristics of the field were classified as an Orthic Dark Brown Chernozem (Typic Boroll) with a 20 cm Ap horizon, a thin B horizon (≤ 10

cm) and a calcareous C horizon and the site was annually cropped (Olson et al., 1996; Zvomuya et al., 2008; Janzen et al., 2012).

Donor sites included 36 different Chernozems from southern Alberta with different chemical, physical and biological characteristics. Most of the soils were obtained from sites located within the Lethbridge Research Centre and surrounding area; however, they had diverse chemical, physical and biological properties. Soil selection was carried out based on soil OM content, soil texture, and management history. Most of the transplanted soils included top soils (Ap/Ah horizons) from the research station; however, two transplanted soils were two sub-surface soils (B and C horizons) from adjacent locations that had experienced erosion. The remaining soils were selected from sites located within a 100 km radius of the experimental site (Olson et al., 1996).

In 1990, the original top soil (Ap) at the site was stripped with minimum compaction to expose the subsoil. Top soils were excavated from the three blocks by an excavator with a 2.4 m wide shovel (Olson et al., 1996). The average depth of excavation was 23 cm. After top soil removal, the exposed subsoil was mainly C horizon with some thin B horizon remaining in some locations. The subsoil was lightly tilled (~ 2 cm) using a disk harrow to roughen the interphase of original subsoil and transplanted soils (Zvomuya et al., 2008). One truck-load of donor soil (8 Mg dry weight) was used for each replicate of a particular soil. Three replicates of soil were collected separately from each donor site (Janzen et al., 2012). The soils were deposited in 5 m by 6 m plots that were delineated by temporary wooden frames, which were removed after the soils from adjacent plots had been deposited. The mean depth of deposition after two years of transplanting was 19 cm, with a range of 15 to 24 cm (Zvomuya et al., 2008). Visual differences in the transplanted soil types can be seen from an aerial photo taken four weeks after the site was established (Fig. 3.1).

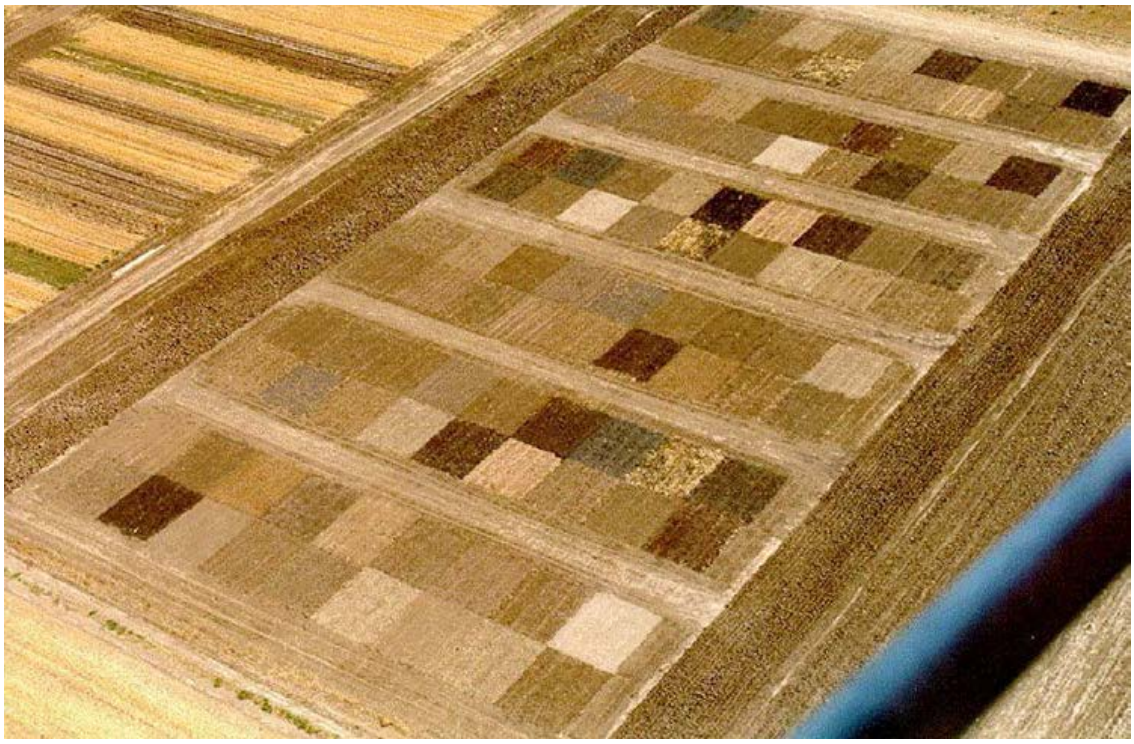


Fig. 3.1. Aerial view of the soil quality experiment field taken four weeks after initial deposition in 1990 (adapted from Janzen et al., 2012).

The experimental design was a split plot where the main plots were arranged in a randomized complete block design (n=3). Transplanted soil type was used as the main plot factor with the rate of N fertilizer as the subplot factor (Zvomuya et al., 2008). Each main plot was subdivided into two and treated with N fertilizer rates of 0 and 60 kg N ha^{-1} with subplots randomly allocated within each main plot. The N fertilizer was applied in 6 m by 3 m (0 kg N ha^{-1}) and 6 m by 2 m (60 kg N ha^{-1}) size plots and N was broadcasted as NH_4NO_3 prior seeding each year (Zvomuya et al., 2008).

In 1991, the site was seeded to spring wheat (*Triticum aestivum* L.) after N fertilizer was broadcasted. Phosphorous (P) fertilizer was applied as triple superphosphate in the seed row at a rate based on soil test P for each transplanted soil (13 to 20 kg P ha^{-1}). Beginning in 1991, the field has been continuously cropped to wheat and zero tillage has been practiced preventing soil mixing between the plots. Further, seeding and harvesting also have been conducted with minimal disturbances. After harvesting, remaining crop residues were swathed, baled and removed for livestock bedding (Zvomuya et al., 2008). This is a typical practice in the region

where the concentration of intensive beef feedlot operations is high, requiring straw as the bedding material.

3.4.2. Microbial community profiling

Starting from the establishment of the transplanted experiment, every seven years the soil has been sampled for routine chemical and physical analyses. However, to date, there were only limited microbial analyses carried out in these soils. In the current study, it was hypothesized that identical management, climatic and topographic conditions would result in the convergence over time of the diverse soil biogeochemical functions, microbial abundance and community structures of these transplanted soils. A survey was conducted to observe the potential changes in soil microbial properties after 21 yr of identical climatic, topographic and management conditions. To investigate the microbial properties, 10 transplanted soils with diverse soil properties at the time of transplanting, including both the N fertilized and unfertilized counterpart (i.e., 10 soil types by two N fertilizer treatments by three replicates) were selected. A summary of the history of land use and soil characteristics of the selected transplanted soils for the current study is presented in Table 3.1. Both management history and soil organic C (OC) and total N (TN) contents were used to select the soils that ultimately were further studied. Soil OC and TN contents measured in the original transplanted soils (1990) and 21 yr (2011) after transplantation were used to get a general idea of soil fertility, and a range of soils with different management and fertility were chosen for comparison (Figs. 3.2 and 3.3).

Table 3.1. Description and background information of selected transplanted soils.

Soil origin	Soil #	Soil zone	Soil texture	Soil characteristics
Native grassland (NG)	2	Dark Brown	Sandy clay loam	Previously uncultivated soil, native grassland Ah horizon
Dryland wheat (DW)	4	Dark Brown	Sandy clay loam	Continuous dryland wheat since 1911
Cereal cultivated (CC)	8	Black	Clay loam	Highest soil inherent fertility, land was a native grassland until 1982 and continuous cereal production
Pastureland (PL)	9	Black	Clay loam	Highest inherent fertility, native pastureland
Dryland tilled fallowed (DTF)	11	Dark Brown	Clay loam	Low inherent fertility, continuous dryland tilled and summer fallowed since 1911 (pathway to an experiment field)
Irrigated tilled fallowed (ITF)	16	Dark Brown	Sandy clay loam	Irrigated tilled summer fallowed since 1911 (pathway to an experiment field)
Manured (30 t ha^{-1}) (RM)	22	Dark Brown	Clay loam	Wet manure was applied 30 tons ha^{-1} since 1973 and continuous dryland barley cultivation
Manured (90 t ha^{-1}) (HM)	23	Dark Brown	Clay loam	Wet manure was applied 90 tons ha^{-1} continuously since 1973, continuous dryland barley cultivation
B horizon (BH)	26	Dark Brown	Clay loam	Sub-surface soil from an eroded native grassland
C horizon (CH)	27	Dark Brown	Loam	Sub-surface soil from an eroded native grassland

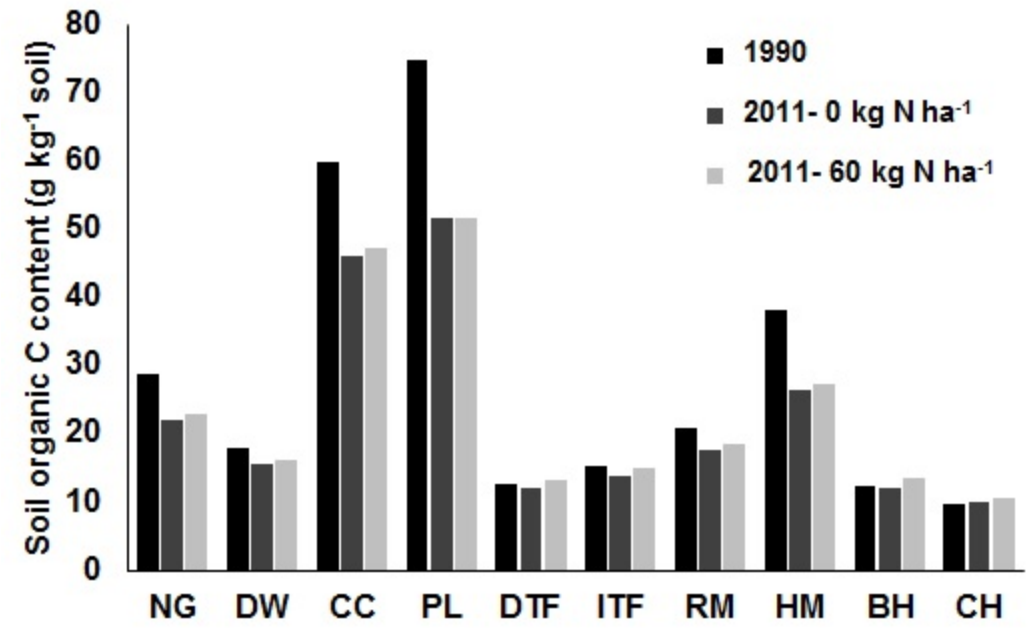


Fig. 3.2. Soil organic C content in 1990 and 21 yr (2011) after transplanting. (reproduced with permission from Janzen and Ellert, unpublished data 1999). Black to grey colors represent the fertility gradient from high to low. NG- native grassland, DW- dryland wheat, CC- cereal cultivated, PL- pastureland, DTF- dryland tilled fallow, ITF- irrigated TF, RM- manured 30 t ha^{-1}, HM- manured 90 t ha^{-1}, BH- B horizon, CH- C horizon.

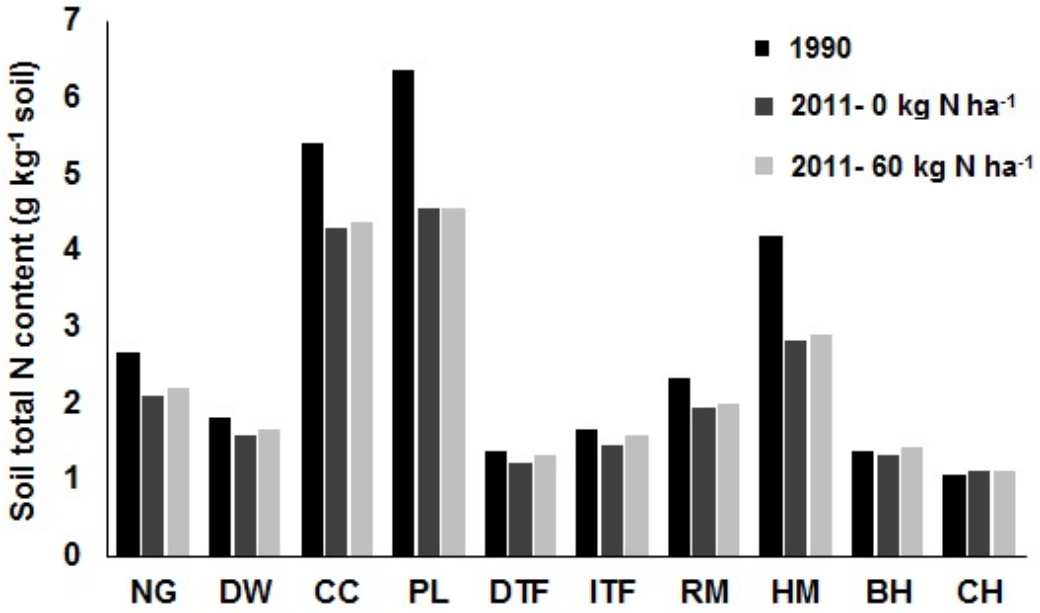


Fig. 3.3. Soil total N content in 1990 and 21 yr (2011) after transplanting**.** (reproduced with permission from Janzen and Ellert, unpublished data 1999). Black to grey colors represent the fertility gradient from high to low. NG- native grassland, DW- dryland wheat, CC- cereal cultivated, PL- pastureland, DTF- dryland tilled fallow, ITF- irrigated TF, RM- manured 30 t ha^{-1}, HM- manured 90 t ha^{-1}, BH- B horizon, CH- C horizon.

3.4.3. Soil sampling and processing

Soil sampling was carried out in September 2012 using an E.G. truck-mounted Giddings soil sampler, equipped with a 3.175 cm diameter sampling probe. Four soil cores were obtained from each replicate to a depth of 0 to 10 cm. The number of samples obtained from each plot was limited to avoid soil compaction due to the truck movement and to preserve the integrity of the experiment. Moreover, obtaining four soil cores has proven to be sufficient for long-term agro ecosystem studies across Canada to observe a significant difference in soil OC amounts (VandenBygaart and Angers, 2006). Soil samples were immediately placed in portable coolers in the field and later stored in a cooler (4ºC) at AAFC within 2 hr of sampling. Samples were transported from Lethbridge to Saskatoon in portable coolers.

The soil samples were processed by sieving through a 4 mm mesh size sieve and were subsequently stored at -80ºC for DNA analyses, -20ºC for PLFA analyses and 4ºC for soil chemical analyses. Soil gravimetric moisture contents were determined by placing 10 g of fresh soil in a conventional oven for 24 hr at 105ºC.

3.4.4. Microbial abundance and community composition analysis

Determination of phospholipid fatty acids (PLFA) in soil is one of the common methods used to study microbial abundance and community structure. Phospholipid fatty acid profiling is a rapid and economical method for detecting shifts in microbial community composition compared to molecular techniques (Frostegård et al., 2011). However, PLFA profiling is restricted in its ability to detect specific microbial functional groups in soils as the resolution of the communities can vary due to overlapping PLFA biomarkers and variation in PLFA patterns in response to environmental factors (Ramsey et al., 2006).

Phospholipid fatty acids analysis was performed to assess total microbial abundance and community structure (i.e., bacteria, fungal and AMF). Phospholipids fatty acids analysis was conducted according to the method adopted by Helgason et al. (2009) from White et al. (1979) based on the original method of Bligh and Dyer (1959). In preparation for PLFA, the soils were freeze-dried for 48 hr and stored at -80ºC until use. Prior to use, the freeze-dried soil samples were ground using a mortar and pestle. Briefly, fatty acids were extracted from 4 g of dried

lyophilized sample using 15 mL of phosphate buffered extractant solution (CH_3OH: $CHCl_3$: Phosphate buffer at a ratio of 2:1:0.8). The fatty acids were then separated on a solid phase extraction column (0.50 g Si; Varian Inc. Mississauga, ON). Fatty acids were methylated and identified using gas chromatography (Hewlett Packard 5890 Series II, Hewlett Packard Scientific Instruments, Palo Alto, CA) and peaks were identified using fatty acid standard and MIDI software (MIDI Inc., Newark, DE). Methyl nonadecanoate (19:0) was used as an internal standard. Total microbial biomass was calculated by summing all identified PLFA peaks. Specific biomarkers were used to assess the relative abundance of microbial functional groups (Table 3.2). Physiological stress biomarkers for stress 1 and 2 were evaluated by ratios of cy17:0 to 16:1ω7c and cy19:0 to 18:1ω7c, respectively (Grogan and Cronan, 1997).

Table 3.2. Biomarkers used to calculate the abundance of specific functional groups.

Functional group	Biomarkers	References
Bacteria	i14:0, i15:0, a15:0, i16:0, 16:1ω7c, 10Me16:0, i17:0, a17:0, cy17:0, 10Me17:0, 18:1ω7, 10Me18:0, cy19:0	(Helgason et al., 2010a) (Bååth and Anderson, 2003)
G^+ bacteria	i14:0, i15:0, a15:0, i16:0, i17:0, a17:0	(Helgason et al., 2010b) (Hedrick et al., 2005)
G^- bacteria	16:1ω7t, 16:1ω9c, 16:1ω7c, 18:1ω7c, 18:1ω9c, cy17:0, cy19:0	(Helgason et al., 2010b) (Macdonald et al., 2004)
Fungal	18:2ω6,9	(Bååth and Anderson, 2003)
AMF[†]	16:1ω5c	(Helgason et al., 2010)

[†]Arbuscular mycorrhizal fungi

3.4.5. Extraction of DNA, microbial abundance and community structure analysis

DNA was extracted from 0.25 g of fresh soil stored in -80ºC using a PowerSoil DNA isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA) per the manufacturer's instructions. Concentrations of DNA were measured using a Qubit® 2.0 Fluorometer with Qubit® dsDNA HS assay kit (Thermo Fisher Scientific, Waltham, MA USA). Extracted DNA was stored at -80ºC prior to further analysis.

Quantitative real-time Polymerase Chain Reaction (qPCR) was conducted to quantify the number of gene copies per unit of soil for key target genes including the 16S *rRNA* gene, bacterial *amoA* (AOB), archaeal *amoA* (AOA), *nirS*, *nirK* and *nosZ* genes. The community

structure of microbial groups involved in key aspects of N turnover (bacterial and archaeal *amoA*, *nirK*, *nirS* and *nosZ*) also were analyzed using a Denaturing Gradient Gel Electrophoresis (DGGE) technique.

3.4.6. Estimation of microbial population size using qPCR

Standard preparation was performed prior to qPCR analysis and the primer pairs for the desired targets were selected based on the literature indicated in Table 3.3. The PCR amplified template was loaded onto a 1% agarose gel and excised. Gel purification was performed using a Qiagen QIAquick gel extraction kit (Qiagen, Mississauga, Ontario, Canada). The target insert was ligated into the vector using an Invitrogen TOPO TA Cloning® Kit with One Shot® MAX Efficiency™ DH5α-T1R *E. coli* (Life Technologies, Burlington, ON). Colonies successively cloned with the insert (white colour) were cultured in liquid broths following the protocol in the TOPO TA Cloning® Kit and incubated at 37ºC for 16 hr. DNA (plasmid with insert) was extracted from the cultures using Qiagen QIAprep Spin Miniprep kit (Qiagen, Mississauga, Ontario, Canada). The DNA was sent for Sanger sequencing (Macrogen, Seoul, Korea) to verify the presence of target sequences. Until further use, DNA was stored at -80ºC. Prior to qPCR analysis, linearization of plasmids with insert was done using Hind III restriction enzyme (Life Technologies, Burlington, ON) and run on a 1% agarose gel. The bands with linearized products were excised and gel purified. DNA was quantified using a Qubit® 2.0 Fluorometer with Qubit® dsDNA HS assay kit (Thermo Fisher Scientific, Waltham, MA USA). The detailed description of the standards used for the qPCR analysis is indicated in Table 3.3.

Prior to qPCR analysis, genomic DNA template concentrations of the samples were measured using a Qubit® 2.0 Fluorometer with Qubit® dsDNA HS assay kit (Thermo Fisher Scientific, Waltham, MA USA) and the concentrations were adjusted to 10 ng μL^{-1}. Template DNA was further diluted to remove inhibition where required, based on trial and error method (up to 100-fold dilutions). Gene quantification of each sample was performed in triplicate in 96 well plates and quantified using a ABI StepOnePlus Real-time PCR system (Life Technologies, Burlington, ON).

The 16S *rRNA* (Fierer et al., 2005), bacterial *amoA* (Rotthauwe et al., 1997; Stephen et al., 1999), archaeal *amoA* (Tourna et al., 2008), *nirS* and *nirK* (Throbäck et al., 2004) and *nosZ* (Henry et al., 2006; Clark et al., 2012) gene copy numbers were quantified using the primer pairs mentioned in the Table 3.3. Detailed descriptions of reaction mixtures and conditions can be found in appendix A.

Table 3.3. Description of qPCR standards and primers.

Gene	Source	Product size (bp)	Primers	References
16S *rRNA*	*Pseudomonas stutzei*	200	EUB338 EUB518	(Fierer et al, 2005)
Bacterial amoA	*Nitrosomonas europa*	~450	amoA1F amoA2R	(Rotthauwe et al., 1997; Stephen et al., 1999)
Archaeal amoA	*Fosmid 54d9*	624	Crenamo23F Crenamo616R	(Tourna et al., 2008)
nirS	*Pseudomonas stutzei*	410	Cd3aF R3cd-R	(Throbäck et al., 2004)
nirK	*Sinorhizobium meliloti*	473	nirKH1F nirK1R/R3Cu	(Dandie et al., 2011)
nosZ	*Pseudomonas stutzei*	268	nosZ2F nosZ2R	(Henry et al., 2006)

3.4.7. Assessing nitrifier and denitrifier community structure

Denaturing gradient gel electrophoresis was conducted for bacterial *amoA*, archaeal *amoA*, *nirS, nirK* and *nosZ* genes on selected transplanted soils based on qPCR results and PLFA analysis. Based on PLFA and qPCR data, soils with the highest (i.e., CC, PL, RM) and moderate levels (i.e., DTF) of total microbial biomass and 16S *rRNA* gene copy numbers were used for DGGE analysis. In addition, these soils had different historical backgrounds in terms of origin and land use which gave rise to variable fertility levels. The detailed description of the primers used for the DGGE is indicated in Table 3.4

Except for AOA, each set of primers contained a GC clamp on the 5' end of either forward (bacterial *amoA*, and *nosZ*) or reverse primer (*nirS* and *nirK*). A nested PCR approach was used for the bacterial *amoA* gene (Muyzer et al., 1993; Kowalchuck et al., 1997) while a touchdown PCR approach was used for the *nirK* and *nirS* genes (Smith et al., 2010). The PCR reaction mixture and conditions were followed according to the Table 3.5. The PCRs were performed with a Bio-Rad T100™ Thermal Cycler (Bio-Rad Mississauga, Ontario, Canada). The PCR products were subjected to gel electrophoresis on 1% agarose gel stained with SYBR Safe (Life Technologies, Burlington, Ontario, Canada) and visualized under UV light to confirm the correct product size. The PCR products were purified from the agarose gel using QlAquick PCR purification kit (Qiagen, Mississauga, Ontario, Canada). The purified PCR products were quantified using a Qubit® 2.0 Fluorometer with Qubit® dsDNA HS assay kit (Thermo Fisher Scientific, Waltham, MA USA).

Denaturing gradient gel electrophoresis was performed with a BioRad DCode system (Bio-Rad, Mississauga, Ontario, Canada). Stock solutions of 80% (30 mL of 40% Acrylamide Bis, 2 ml of 50 × TAE buffer, 32 mL formamide, 33.6 g urea and deionized H_2O) and 0% (25 mL of 40% Acrylamide Bis and deionized H_2O) denaturants were prepared and stored at 4^oC.

Table 3.4. Description of primers used in DGGE analysis.

Gene	Primers[‡]	References
Bacterial *amoA*[†]	**CTO189F** GGAGRAAAGCAGGGGATCG	Kowalchuck et al., 1997
	CTO654R CTA GCY TTG TAG TTT CAAACGC	Muyzer et al., 1993
	P3(357F-GC) gc-CCTCGGGAGGCAGCAG	
	P2(518R) ATTACCGCGGCTGCTGG	
Archaeal *amoA*	**CrenamoA23F** ATGGTCTGGCTWAGACG	Tourna et al., 2008
	CrenamoA616R GCCATCCABCKRTANGTCCA	Offre et al., 2009
nirS[††]	**Cd3aF** GTSAACGTSAAGGARACSGG	Thröback et al. 2004
	R3cd gc-GASTTCGGRTGSGTCTTGA	Smith et al. 2010
nirK	**F1aCu** ATCATGGTSCTGCCGCG	Thröback et al. 2004
	R3Cu gc-GCCTCGATCAGRTTGTGGTT	Smith et al. 2010
nosZ[††]	**nosZ-F1181** gc-CGCTGTTCITCGACAGYCAG	Ma et al., 2008
	nosZ-R1180 ATGTGCAKIGCRTGGCAGAA	

[†]Nested PCR approach

[‡]Primers were purchased from Sigma Aldrich, Oakville, Ontario, Canada

[††]Touchdown PCR approach

Table 3.5. Polymerase chain reaction (PCR) amplification conditions for DGGE.

Target	Primer	Reaction mixture	PCR conditions
Bacterial *amoA*	CTO654R CTO189F	- PCR master mix† 10 µl -Primers (25 µM) 0.5 µl -Bovine serum albumin (10 mg μl^{-1}) 0.625 µl -Rnase free H_2O 6.37 µl -Template DNA 2 µl	95°C, 5 min 1 cycle followed by10 cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 1 min Followed by 25 cycles of 92°C for 30 sec, 55°C for 30 sec, 72°C for 1 min and 1 cycle of 72°C for 10 min
	P2(518R) P3(357F-gc clamp)	-PCR master mix 10 µl -Primer (25 µM) 0.5 µl -Bovine serum albumin (10 mg μl^{-1}) 0.625 µl -Rnase free H_2O 7.8 µl -Template DNA 4 µl (<200 ng)	95°C, 5 min 1 cycle followed by10 cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 1min Followed by 25 cycles of 92°C for 30 sec, 55°C for 30 sec, 72°C for 1 min and 1 cycle of 72°C for 10 min
Archaeal *amoA*	Crenamo23F Crenamo616R	-PCR master mix 10 µl -Primers (25 µM) 0.75 µl -Bovine serum albumin (10 mg μl^{-1}) 0.625 µl -Rnase free H_2O 3.87 µl -Template DNA 4 µl	95°C, 5 min 1 cycle followed by10 cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 1min Followed by 25 cycles of 92°C for 30 sec, 55°C for 30 sec, 72°C for 1 min and 1 cycle of 72°C for 10 min
nirS	Cd3aF R3cd-R-gc clamp	-PCR master mix 10 µl -Primers (25 µM) 0.75 µl -Bovine serum albumin (10 mg μl^{-1}) 0.625 µl -Rnase free H_2O 6.37 µl -Template DNA 2 µl	95°C for 5 min 1 cycle followed by 7 cycles of 94°C for 45 sec, 61°C for 60 sec (-1°C per cycle), 72°C for 90 sec, followed by 30 cycles of 94 °C for 45 sec, 59 °C for 60 sec and 72 °C for 90 sec and 1 cycle of 72 °C for 15 min
nirK	nirKH1F nirK1R/R3Cu	-PCR master mix 10 µl -Primers (25 µM) 0.75 µl -Bovine serum albumin (10 mg μl^{-1}) 0.625 µl -Rnase free H_2O 6.37 µl -Template DNA 2 µl	95°C for 5 min 1 cycle followed by 7 cycles of 94°C for 45 sec, 61°C for 60 sec (-1°C per cycle), 72°C for 90 sec, followed by 30 cycles of 94 °C for 45 sec,59 °C for 60 sec and 72 °C for 90 sec and 1 cycle of 72 °C for 15 min
nosZ	nosZ2F nosZ2R	-PCR master mix 12.5 µl -Primers (25 µM) 0.75 µl -Bovine serum albumin (10 mg μl^{-1}) 0.78 µl -Rnase free H_2O 12.22 µl -Template DNA 3 µl	95°C for 5 min 1 cycle, followed by 35cycles of 94°C, 40 sec 56°C, 40 sec, 72 °C, 1 min and 1 cycle of 72 °C, 5 min

†HotStarTaq Master Mix Kit (Qigen, Toronto, Ontario, Canada), Bovine serum albumin (Amersham Biosciences, Mississauga, ON, Canada), primers (Sigma Aldrich, Oakville, Ontario, Canada.

Denaturing gradient gel electrophoresis gels containing 8% acrylamide were used to obtain the gel gradients. For each replicate sample (n=3), approximately 600 ng of amplified product was loaded into each lane (30 µL). The gel gradient, voltage, and duration for gel electrophoresis were followed according to Table 3.6. At the end of electrophoresis, the resulting gel was stained with SYBR Safe (Life Technologies, Burlington, Ontario, Canada) in TAE buffer for 30 min and visualized using a gel imaging unit (BioRad, Mississauga, Ontario, Canada). Selected bands (i.e., those that were either unique or very abundant) were excised from the gel using a sterilized scalpel and DNA was eluted in sterilized deionized water by incubating for 30 min at 37ºC. Eluted DNA was re-amplified according to the protocols mentioned in Muyzer et al. (1996) and Kowalchuk et al. (1997) for bacterial *amoA*, Offre et al. (2009) for archaeal *amoA*, Smith et al. (2010) for *nirS* and *nirK* and Ma et al. (2008) for *nosZ*. The resulting PCR products were purified using QlAquick PCR purification kit (Qiagen, Mississauga, Ontario, Canada) and sent for Sanger sequencing (Macrogen, Seoul, Korea). Microbial identifications were conducted by comparing DNA sequences with GenBank databases using the BLAST algorithm (Altschul et al., 1997).

Denaturing gradient gel electrophoresis gels were analyzed using Bionumerics v.5.1 software (Applied Maths, Austin, TX). DGGE bands were selected using minimum profiling of 5%, a position tolerance of 1.5% and an optimization of 2%. Cluster analysis was performed using Pearson correlation coefficients created by densitometric curves and the Ward linkage method. Band matching and binary presence-absence matrix were obtained using densiometric curves.

Table 3.6. DGGE gel electrophoresis conditions for bacterial and archaeal *amoA*, *nirS, nirK* and *nosZ.*

Target	Gradient	Voltage (mv)	Time (hr)	Reference
Bacterial *amoA*	35-55%	75	17	(Muyzer et al., 1993; Kowalchuk et al., 1997)
Archaeal *amoA*[†]	20-50%	100	15	(Offre et al., 2009)
nirS	30-70%	75	17	(Smith et al., 2010)
nirK	30-70%	75	17	(Smith et al., 2010)
nosZ	40-60%	40	16	(Ma et al., 2008)

[†]The linear gradient was optimized to 20 to 50%

3.4.8. Soil chemical analysis

Analysis of available NO_3^-, SO_4^{2-}, PO_4^{3-} and K^+ were performed by a commercial soil analysis laboratory (ALS Environmental, Saskatoon, SK). Available NO_3^- and NO_2^- were extracted from soil using a diluted $CaCl_2$ solution. The extracted NO_3^- was reduced to NO_2^- by passing through a reducing Cd column. The color development was done by adding diazotizing with sulfanilamide followed by N-(1-naphthyl) ethylenediamine dihydrochloride. The absorbance of resulting magenta color solution was measured at 520 nm using a spectrophotometer (Alberta, Soils, and Animal Nutrition Laboratory, 1988).

Plant available phosphorous (PO_4^{3-}) and potassium (K^+) were extracted using a modified Kelowna solution (Qian et al., 1994). The P concentration was measured colorimetrically at 880 nm using a spectrophotometer. The K^+ was determined by flame emission spectrophotometer at 770 nm. The plant available SO_4-S was extracted using a weak $CaCl_2$ solution. The SO_4-S in the extractant was measured using an ICP Optical Emission Spectrometer.

The analysis of soil OC was done in the Department of Soil Science, University of Saskatchewan, SK. The HCl acid pretreatment was performed to remove the inorganic C (Harris et al., 2001). For this 0.25 g of finely ground soil was placed in a silver boat liner and moistened by adding distilled water. The samples were placed in a vacuum desiccator along with beakers containing concentrated HCl (12 M) for 24 hr. The samples were then dried in a conventional oven for 4 hr at 60°C. The OC in dried samples were analyzed by an automated C632 LECO analyzer (Dhillon et al., 2015).

3.4.9. Statistical analysis

The significance of difference in measured variables (abundance of PLFA biomarkers, qPCR gene copy numbers, and soil chemical properties) between the different transplanted soils and the N fertilizer treatments were analyzed by ANOVA using the PROC MIXED procedure in SAS 9.3 (SAS Institute Inc, NC, USA). The block and the interaction between block and transplanted soil type were considered as a random effect and soil type with N fertilizer rate as a fixed effect. The statistically significant criterion was a Type III error rate of $P = 0.05$ with 95% confidence

interval. When the interaction effects were significant, the multiple mean comparison method LS means (least Square Means) was used. If the main treatment effects were significant, LSD (Least square difference) was computed.

The ordination of PLFA biomarkers as well as DGGE community banding patterns generated by binary presence and absence matrix were performed by non-metric multidimensional scaling (NMDS) analysis using PCOrd software version 6 (Gleneden Beach, OR 97388, USA). The Sorensen distance measure was applied with Autopilot slow and thorough selections. The statistical significance of the final solutions was determined by comparing the final stress values among the best solution for each axis using the Monte Carlo test. The final stress value implies the consistency of the final ordination in relation to the dissimilarities within the dataset. Lower (5 to 10) final stress values indicate better ordination of the data. If the stress value is greater than 20, misinterpretation of data is possible. Multi-response permutation procedure (MRPP) test was conducted to identify group differences among the transplanted soils and N fertilizer levels (priori groups). Chance-corrected within-group agreement (A) value becomes one when all the samples within the group are identical. A value closer to zero indicates more heterogeneity within the samples. PERMANOVA analysis was conducted to observe interaction effects between soil origin and N fertilizer rate.

Correlation analysis between the total microbial biomass, bacterial, fungal and AMF abundances with initial soil TN, TC, OC, light fraction C, light fraction N, C: N ratio and changes occurring in TN, TC, OC, light fraction C, light fraction N in the last 21 yr were computed. Correlation analysis also was performed with 16S *rRNA* gene, bacterial *amoA*, archaeal *amoA*, *nirS*, *nirK* and *nosZ* genes abundances to the above-mentioned soil properties.

3.5. RESULTS

This study evaluated the microbial abundance and community composition of transplanted Chernozemic soils after >21 yr under identical climatic, topographic and management conditions. The selected soils had diverse physical, chemical and biological properties at the time of transplanting (1990) which likely arose due to inherent differences in the soil and various land use and management practices (Zvomuya et al., 2008). Soil sampling was performed in the fall of 2012 (after harvesting). Phospholipid fatty acid analysis was used to estimate microbial abundance and evaluate community composition of the transplanted soils. Further, the abundance of N cycling genes (bacterial and archaeal *amoA*, *nirS*, *nirK* and *nosZ*) was assessed using a quantitative real-time polymerase chain reaction (qPCR) which expanded the investigation towards the potential functioning of the transplanted soils. The community structures of ammonia oxidizers and denitrifiers were evaluated using DGGE fingerprinting technique. These microbial properties were correlated with the original soil chemical characteristics at the time of transplanting (1990) and after >21 yr to observe the influence of soil characteristics on soil microbial dynamics.

3.5.1 Soil chemical properties 21 years after transplantation

After >21 yr of identical climatic and topographic conditions, soil chemical properties such as soil available NO_3^-, SO_4^{2-}, PO_4^{3-}, K^+ and OC content differed and were related to soil origin (Table 3.7). Only the available NO_3^- and K^+ were significantly affected by N fertilizer application. Even after >21 yr of continuous cultivation and crop residue removal, the originally high fertility PL and CC soils had greater NO_3^- and soil OC content than the other soils of varying origin. The soil with a history of exceedingly high levels of manure application (HM) (90 t ha^{-1}) prior to transplanting was more enriched with available SO_4^{2-}, PO_4^{3-}, K^+ and OC content than RM whereas former sub-surface soils (BH and CH) from B and C horizons had lower available nutrients and soil OC content. Nitrogen fertilizer application increased the soil available NO_3^- content for most of the soils except soils from the DW and CH. However, the impact of N fertilizer application was more prominent in high fertility CC and PL soils (Fig. 3.4A). The available K^+ content in soils from NG, DW and high fertility CC and PL soils increased with N fertilizer application ($P<0.001$) (Fig. 3.4B).

Table 3.7. Soil available NO_3^-, SO_4^{2-}, PO_4^{3-}, K^+ and organic C contents >21 yr after transplanting.

Soil origin†	NO_3^-	SO_4^{2-}	PO_4^{3-}	K^+	Organic C
			mg kg^{-1} soil		
NG	4.9dc‡	9.8ab	69.8ce	308.2d	24.2bc
DW	3.9dc	6.2c	58.9de	357.0c	15.6de
CC	16.6a	11.3a	100.4c	408.3b	51.3a
PL	14.8a	10.9a	99.0c	203.8e	49.0a
DTF	3.5dc	5.1c	85.9c	354.8c	13.4de
ITF	7.0bc	7.7bc	179.5b	408.8b	17.7cd
RM	3.6dc	4.8c	52.3de	242.5e	13.0de
HM	10.7bc	11.4a	479.3a	787.3a	23.1b
BH	2.8d	6.1c	40.6e	123.8f	10.6e
CH	3.7dc	5.1c	54.4de	233.2e	14.2de
Soil origin	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
N rate	0.01	0.96	0.08	<0.0001	0.46
Soil origin × N rate	0.19	0.26	0.91	0.90	0.82

†NG- native grassland, DW- dryland wheat, CC- cereal cultivated, PL- pastureland, DTF- dryland tilled fallow, ITF- irrigated TF, RM- manured 30 t ha^{-1}, HM- manured 90 t ha^{-1}, BH- B horizon, CH- C horizon.
‡Means with same letters in one column are not significantly different at P<0.0001.

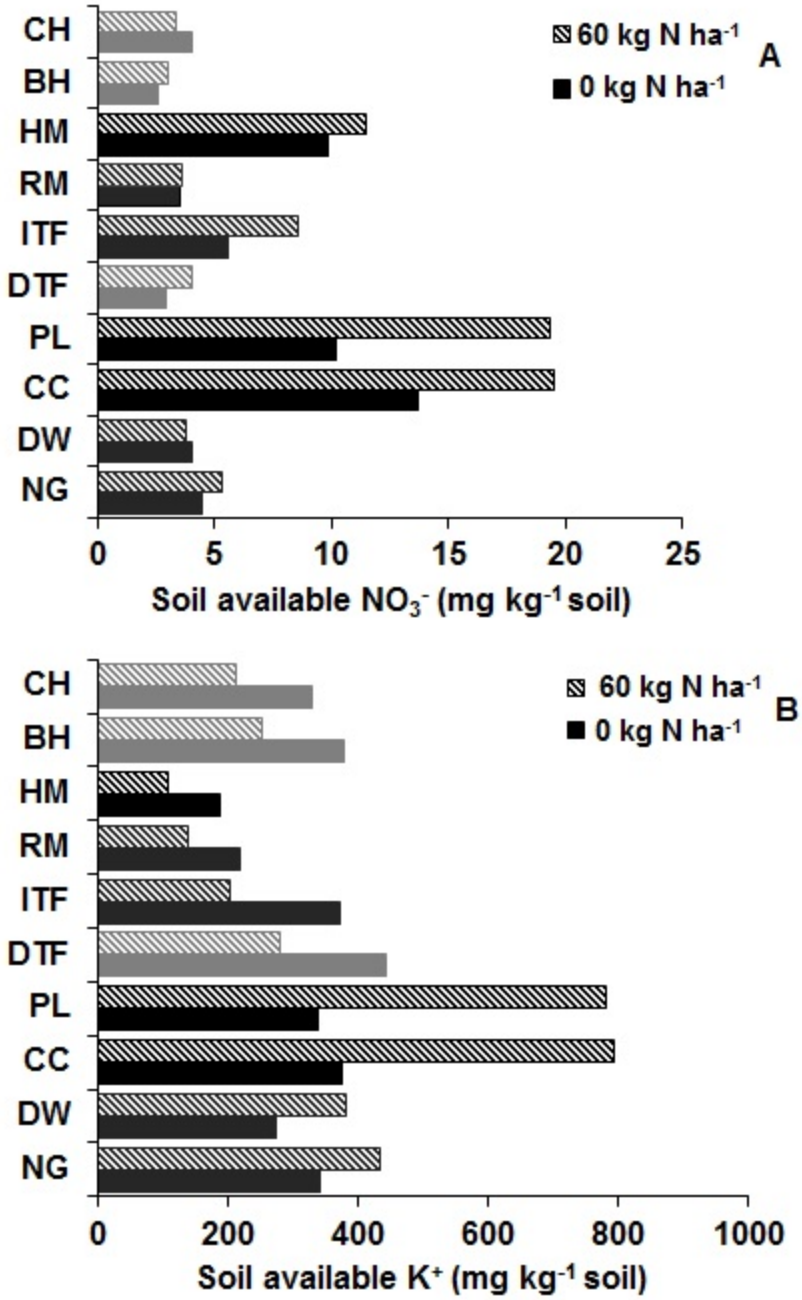


Fig. 3.4. Soil available A) NO_3^- and B) K^+ at 0 (N^-) and 60 (N^+) kg ha^{-1} N levels. Means with same letters are not significantly different at $P<0.01$. Black to grey colors represent the fertility gradient from high to low. NG- native grassland, DW- dryland wheat, CC- cereal cultivated, PL- pastureland, DTF- dryland tilled fallow, ITF- irrigated tilled fallow, RM- manured 30 t ha^{-1}, HM- manured 90 t ha^{-1}, BH- B horizon, CH- C horizon.

3.5.2. Microbial abundance and community composition

Even after >21 yr under identical climatic, topographic and management conditions abundance of total PLFAs, bacterial, fungal and AMF biomarkers were greatly influenced by initial soil characteristics at the time of transplanting. Further, inorganic N fertilizer application was not the main driving force of microbial community composition and abundance of the transplanted soils. Although the absolute abundance of fungal biomarker was significantly affected by the initial soil characteristics (i.e., soil origin, previous management practices) relative abundance was not (Table 3.8). However, the fungal: bacterial ratios of the transplanted soils were not influenced by soil origin (P>0.19) or long-term N fertilizer (P>0.14) application indicating the proportional distribution of fungi and bacteria in the transplanted soils. Stress 1 (cy17:0 / 16:1ω7c) and stress 2 (cy19:0 / 18:1ω7c) biomarkers were also influenced by initial soil characteristics (Table 3.8).

Table 3.8. Analysis of variance (ANOVA) for total PLFAs, bacteria, fungi, AMF abundances and fungal: bacteria ratio, stress 1 and stress 2 biomarkers >21 yr after transplanting

Variables	Unit	Soil origin	N rate	Soil origin × N rate
		P value		
Total PLFAs	nmol g^{-1} soil	0.0002	0.60	0.49
Bacteria	nmol g^{-1} soil	0.0001	0.55	0.29
	mol%	0.04	0.96	0.40
Fungi	nmol g^{-1} soil	0.049	0.17	0.08
	mol%	0.83	0.07	0.87
AMF	nmol g^{-1} soil	0.006	0.47	0.81
	mol%	0.0001	0.09	0.94
Fungi: Bacteria		0.19	0.14	0.47
Stress 1		0.0006	0.09	0.40
Stress 2		0.0001	0.57	0.10

Total PLFA was greater in the high (CC and PL) and medium (ITF and RM) fertility soils and reduced in low fertility sub-surface soils (BH and CH), as well as DTF and HM (Fig. 3.5). The effect of initial soil characteristics on bacterial PLFA followed a pattern similar to that of total PLFAs. Gram positive (G^+) (P<0.01) and Gram negative (G^-) (P<0.001) bacterial biomarkers were significantly different among soil types and followed a similar trend to that of

total PLFA (Fig. 3.6). Except for G^+ abundance in NG soil, total bacterial and G^+ abundances were high and comparable to the CC soil (Fig. 3.6). The RM soil was enriched with fungal biomarkers while other transplanted soils including low fertility soils (DTF, BH, and CH) had low fungal abundance of similar levels (Fig. 3.7A).

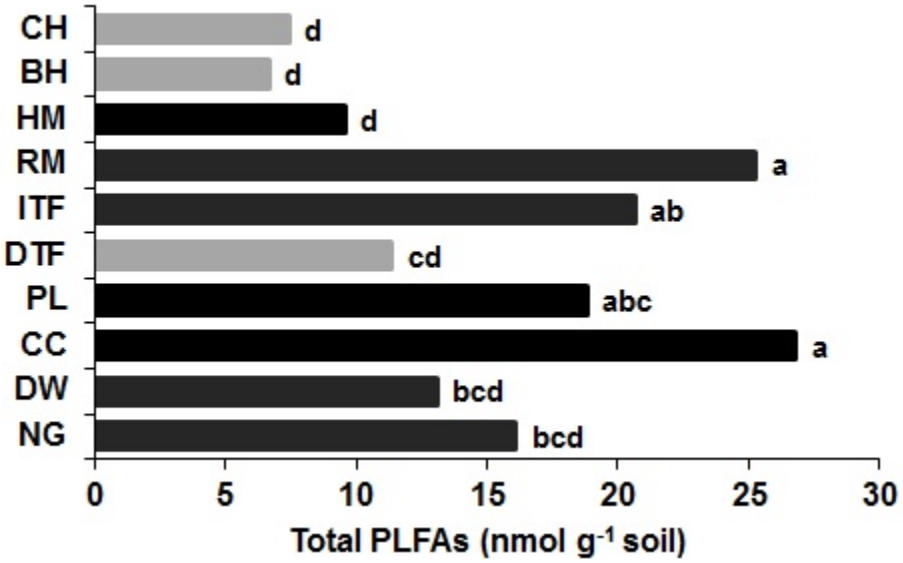


Fig. 3.5. Total PLFA of the transplanted soils 21 yr after transplanting. Means with same letters are not significantly different at $P<0.001$. Black to grey colors represent the fertility gradient from high to low. NG- native grassland, DW- dryland wheat, CC- cereal cultivated, PL- pastureland, DTF- dryland tilled fallow, ITF- irrigated tilled fallow, RM- manured 30 t ha^{-1}, HM- manured 90 t ha^{-1}, BH- B horizon, CH- C horizon.

The AMF abundance was higher in CC, ITF and RM soils than the other soils (Fig. 3.7B). In contrast, the high fertility PL soil had lower AMF abundance despite being consistently higher in total microbial and bacterial abundance, compared to other high fertility CC soil. Like the total and bacterial PLFA abundance, the AMF abundance was lower in sub-surface soils (BH and CH). The stress 1 biomarker was significantly higher in PL soil compared to other soils while the NG soil had lower stress 1 biomarker (Fig. 3.8). Among high fertility soils with the exception of the HM soil, the stress 2 biomarkers were highest for CC and PL soils (Fig. 3.8).

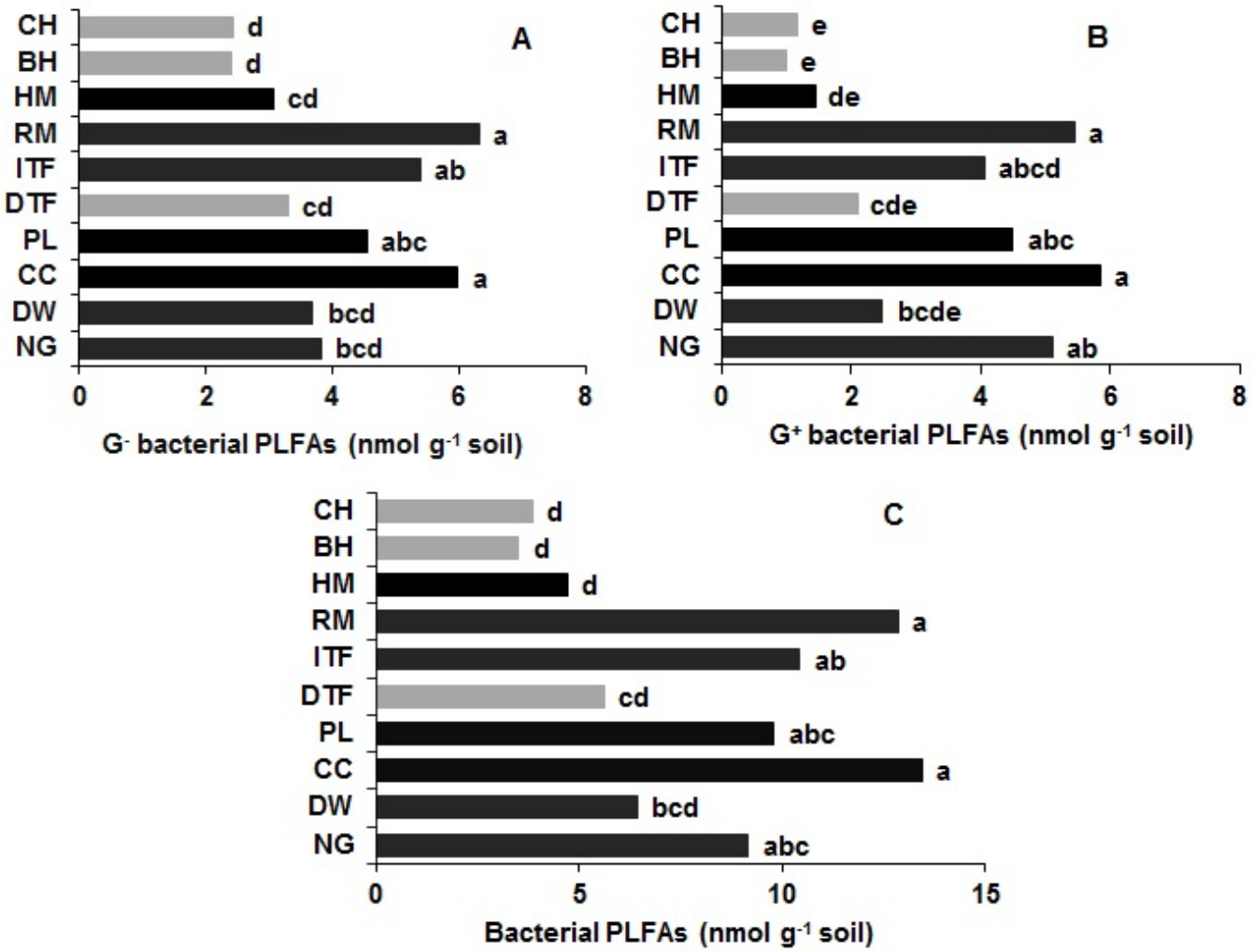


Fig. 3.6. The abundance of A) gram negative (G^-), B) gram positive (G^+) and C) total bacterial PLFA biomarkers >21 yr after transplanting. Means with same letters are not significantly different at $P<0.01$. Black to grey colors represent the fertility gradient from high to low. NG- native grassland, DW- dryland wheat, CC- cereal cultivated, PL- pastureland, DTF- dryland tilled fallow, ITF- irrigated tilled fallow, RM- manured 30 t ha^{-1}, HM- manured 90 t ha^{-1}, BH- B horizon, CH- C horizon.

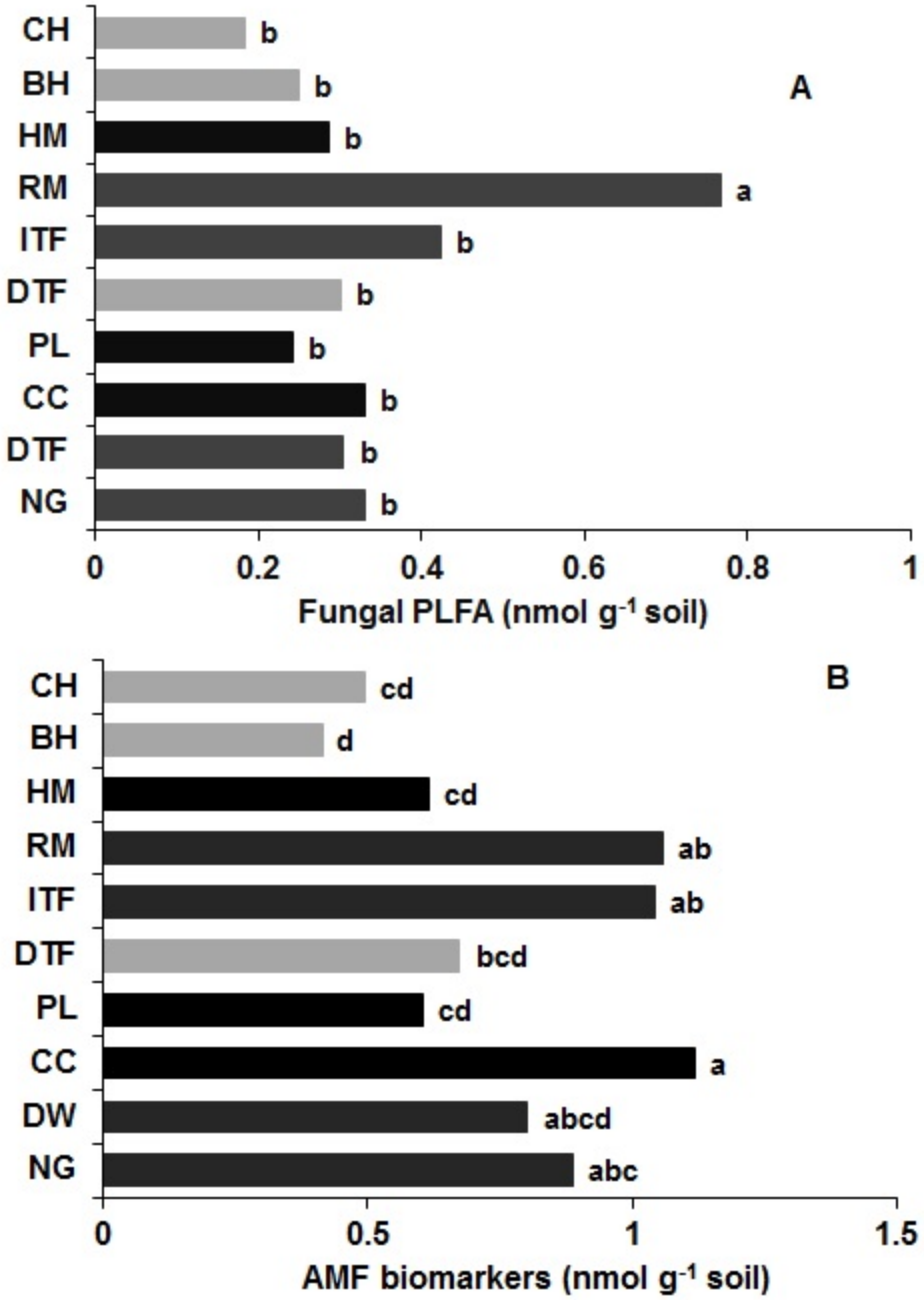


Fig. 3.7. The abundance of A) fungal and B) Arbuscular mycorrhizal fungal (AMF) (16:1ω5c) biomarkers >21 yr after transplanting. Means with same letters are not significantly different at $P<0.05$. Black to grey colors represent the fertility gradient from high to low. NG- native grassland, DW- dryland wheat, CC- cereal cultivated, PL- pastureland, DTF- dryland tilled fallow, ITF- irrigated tilled fallow, RM- manured 30 t ha^{-1}, HM- manured 90 t ha^{-1}, BH- B horizon, CH- C horizon.

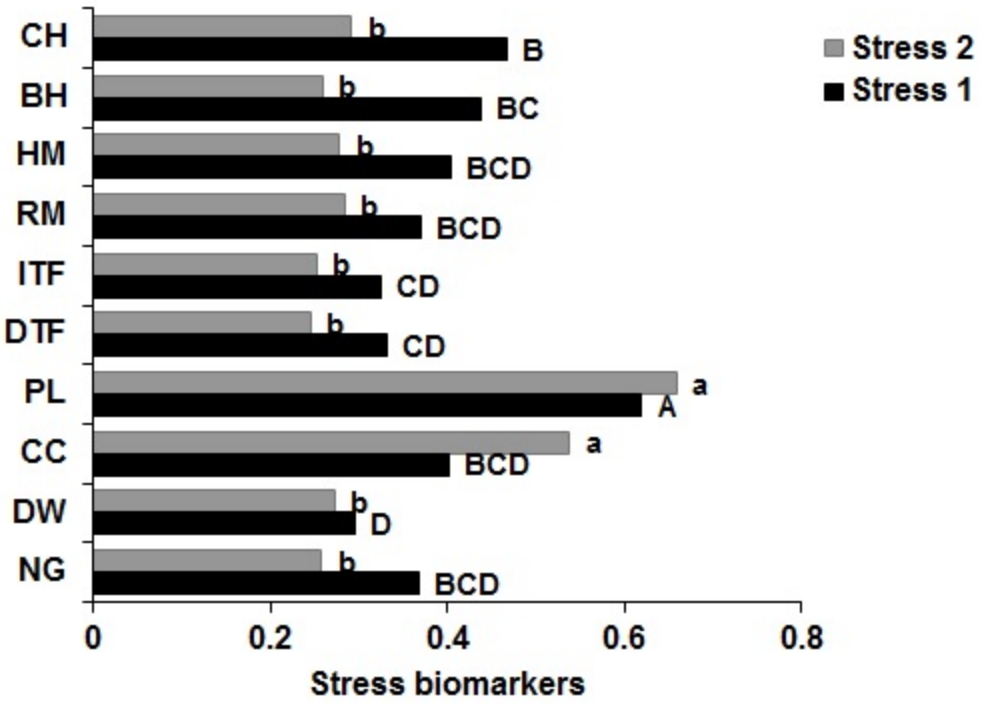


Fig.3.8. Stress 1 (cy17:0 / 16:1ω7c) and stress 2 (cy19:0 / 18:1ω7c) PLFA biomarkers >21 yr after transplanting. Means with same letters are not significantly different at $P<0.001$. Black to grey colors represent the fertility gradient from high to low. NG- native grassland, DW- dryland wheat, CC- cereal cultivated, PL- pastureland, DTF- dryland tilled fallow, ITF- irrigated tilled fallow, RM- manured 30 t ha^{-1}, HM- manured 90 t ha^{-1}, BH- B horizon, CH- C horizon.

Non-metric multidimensional scaling (NMDS) and MRPP analysis of PLFA profiling (mol %) indicated heterogeneity among microbial communities of transplanted soils (Fig. 3.9). However, N fertilizer application did not have a significant effect on microbial community structure. Microbial community profiles of diverse transplanted soils were mainly differentiated along axis 1, which explained 76% of the variability in the NMDS solution. The CC, PL and low rate of manure applied (30 t ha^{-1}) soils which had higher total microbial bacterial biomarker abundance clearly separated from microbial communities of remaining transplanted soils. Soils with high abundance of total PLFAs and bacterial biomarkers were also positively correlated with soil OC content.

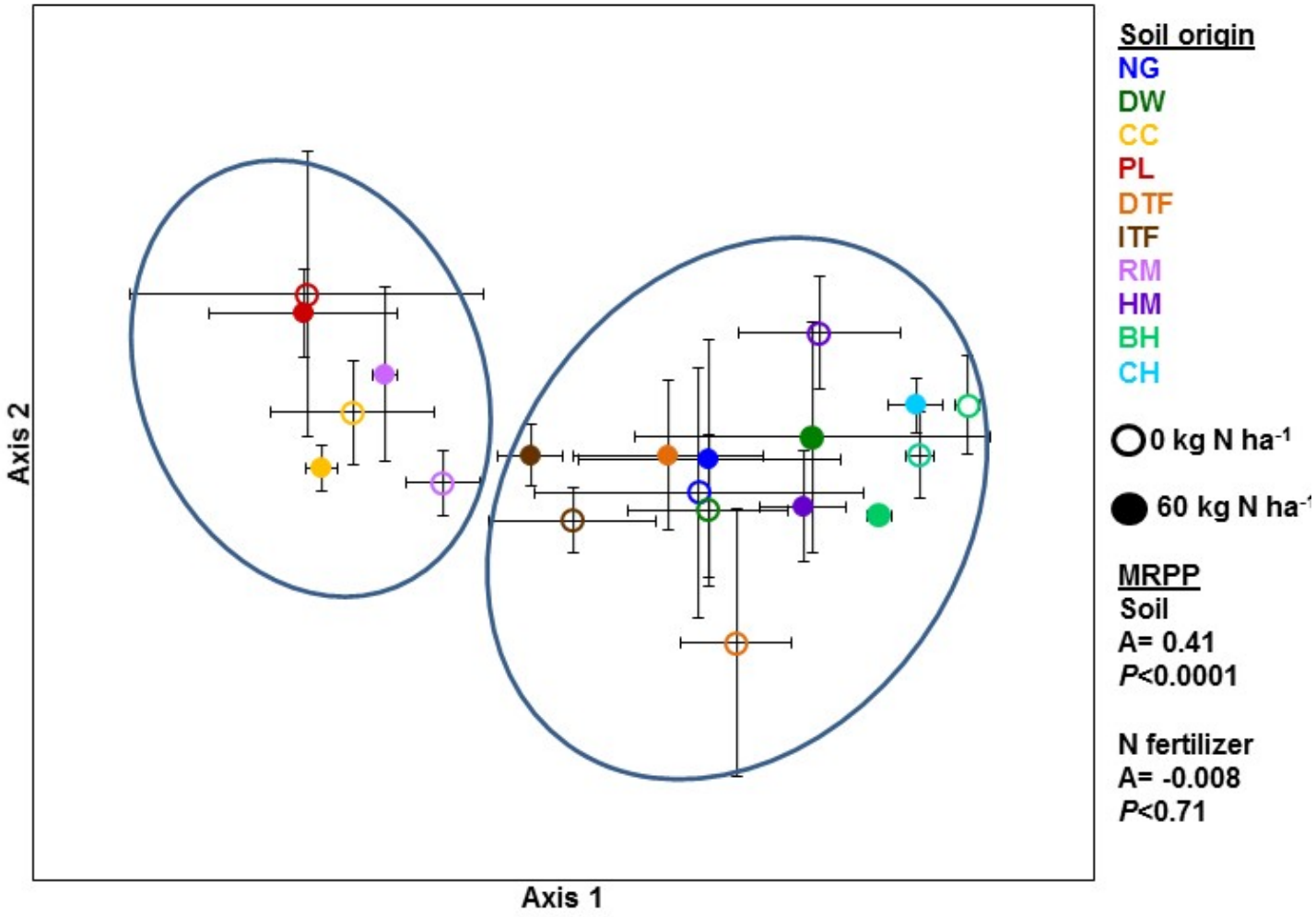


Fig. 3.9. Non-metric multidimensional scaling (NMDS) and multi-response permutation procedure (MRPP) analysis of soil origin and N fertilizer application effects on microbial community structure (mol% PLFA) >21 yr after transplantation. Final stress 13.46%. NG- native grassland, DW- dryland wheat, CC- cereal cultivated, PL- pastureland, DTF- dryland tilled fallow, ITF- irrigated tilled fallow, RM- manured 30 t ha^{-1}, HM- manured 90 t ha^{-1}, BH- B horizon, CH- C horizon.

3.5.3. Abundance of genes involved in nitrification and denitrification

The abundance of 16S *rRNA* gene and bacterial *amoA* gene copy numbers were significantly influenced by soil origin, even after 21 yr of transplanting (Table 3.9). Higher 16S *rRNA* gene abundance was observed in the high fertility CC soil. The PL and NG soils transplanted from uncultivated ecosystems also had higher 16S *rRNA* gene abundance compared to other soils. The soil with a history of excessive manure application (90 t ha^{-1}) contained lesser 16S *rRNA* gene copies compared to other soils (Fig. 3.10).

Table 3.9. Analysis of variance for 16S *rRNA*, bacterial (AOB) and archaeal (AOA) *amoA, nirS*, *nirK* and *nosZ* genes copy numbers >21 yr after transplanting.

Variables	Units	Soil origin	N rate	Soil origin × N rate
			P value	
16S *rRNA*	copies g^{-1} soil	0.0001	0.76	0.76
AOB	copies g^{-1} soil	0.0003	<0.0001	0.65
	AOB: 16S *rRNA*†	0.26	0.21	0.36
AOA	copies g^{-1} soil	<0.0001	0.01	<0.0001
	AOA: 16S *rRNA*	<0.0001	0.01	<0.0001
nirS	copies g^{-1} soil	<0.0001	0.01	0.0014
	nirS: 16S *rRNA*	<0.0001	0.82	0.98
nirK	copies g^{-1} soil	0.05	0.17	0.08
	nirK: 16S *rRNA*	0.22	0.59	0.13
nosZ	copies g^{-1} soil	<0.0001	0.16	0.34
	nosZ: 16S *rRNA*	<0.0001	0.75	0.06

† %ratio of functional gene abundance (AOB, AOA, *nirS*, *nirK* or *nosZ*) to 16S *rRNA* gene abundance.

Both soil origin and N fertilizer application significantly affected the abundance of bacterial *amoA* gene (Table 3.9). The bacterial *amoA* gene was most abundant in the BH and HM (90 t ha^{-1}) soils compared to other soils. Nitrogen fertilizer application had varying effects on bacterial *amoA* abundance in transplanted soils. Statistical data analysis of the impact of N fertilizer for individual soils confirmed that there was a significant increase in bacterial *amoA* genes in NG, DTF, ITF, RM (30 t ha^{-1}) and CH soils with N application (Table A.1). However, bacterial *amoA* gene abundance was not affected by N fertilization in high fertility CC and PL soils (Fig. 3.11A).

Except for sub-surface (BH and CH) and HM (90 t ha^{-1}) the rest of the soils had higher AOA abundance compared to AOB.

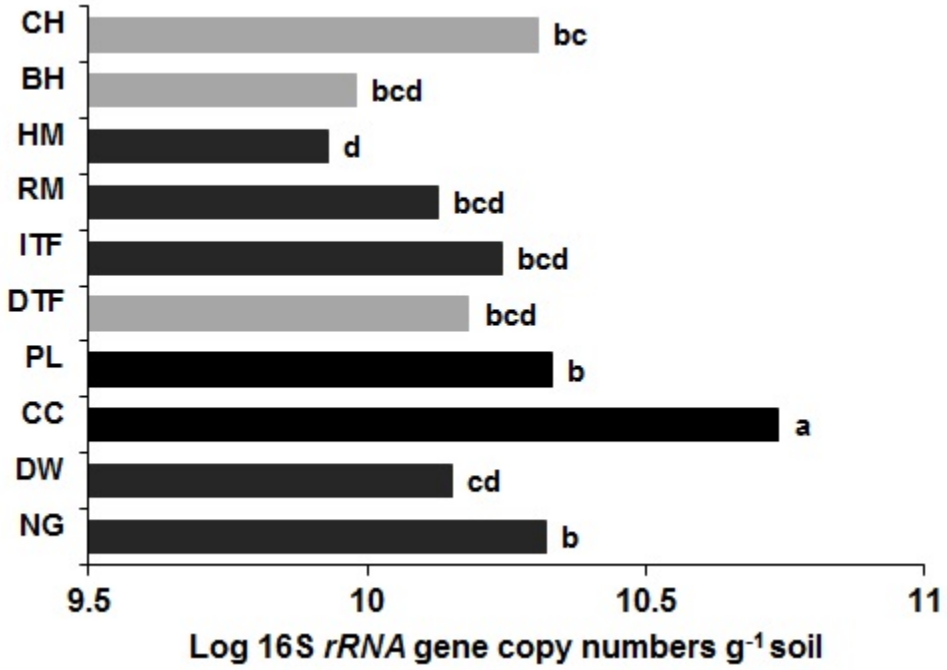


Fig. 3.10. The abundance of 16S *rRNA* gene >21 yr after transplanting. Means with same letters are not significantly different at $P<0.0001$. Black to grey colors represent the fertility gradient from high to low. NG- native grassland, DW- dryland wheat, CC- cereal cultivated, PL- pastureland, DTF- dryland tilled fallow, ITF- irrigated tilled fallow, RM- manured 30 t ha^{-1}, HM- manured 90 t ha^{-1}, BH- B horizon, CH- C horizon.

There was a significant interaction effect of soil origin and long-term N fertilizer on the abundance of archaeal *amoA* and *nirS* genes (Table 3.9). However, the impact of N fertilizer application was only observed for AOA and *nirS* in DTF and PL soils, respectively. Among the soils, the AOA gene was predominantly higher in the unfertilized DTF soil (Figure 3.11B); however, this difference was negated by N fertilizer application. *NirS* gene abundance in PL soil increased with N application (Fig. 3.12A). Higher *nirS* abundance was observed in sub-surface (BH and CH) and RM soils whereas higher fertility soils (CC and PL) had significantly lower *nirS* abundance. The abundance of *nirK* and *nosZ* genes were mainly governed by the soil origin; N fertilizer application did not influence gene abundance (Table 3.9). An opposite trend to *nirS* was observed for *nirK* abundance which was higher in high fertility (CC and PL) and ITF soils. The RM soil had the lowest *nirK* gene copy number among the transplanted soils (Fig. 3.12B).

The ITF, RM and CH soils had more abundant *nosZ* genes while high fertility soils (CC and PL) showed comparatively low *nosZ* gene abundance (Fig. 3.13).

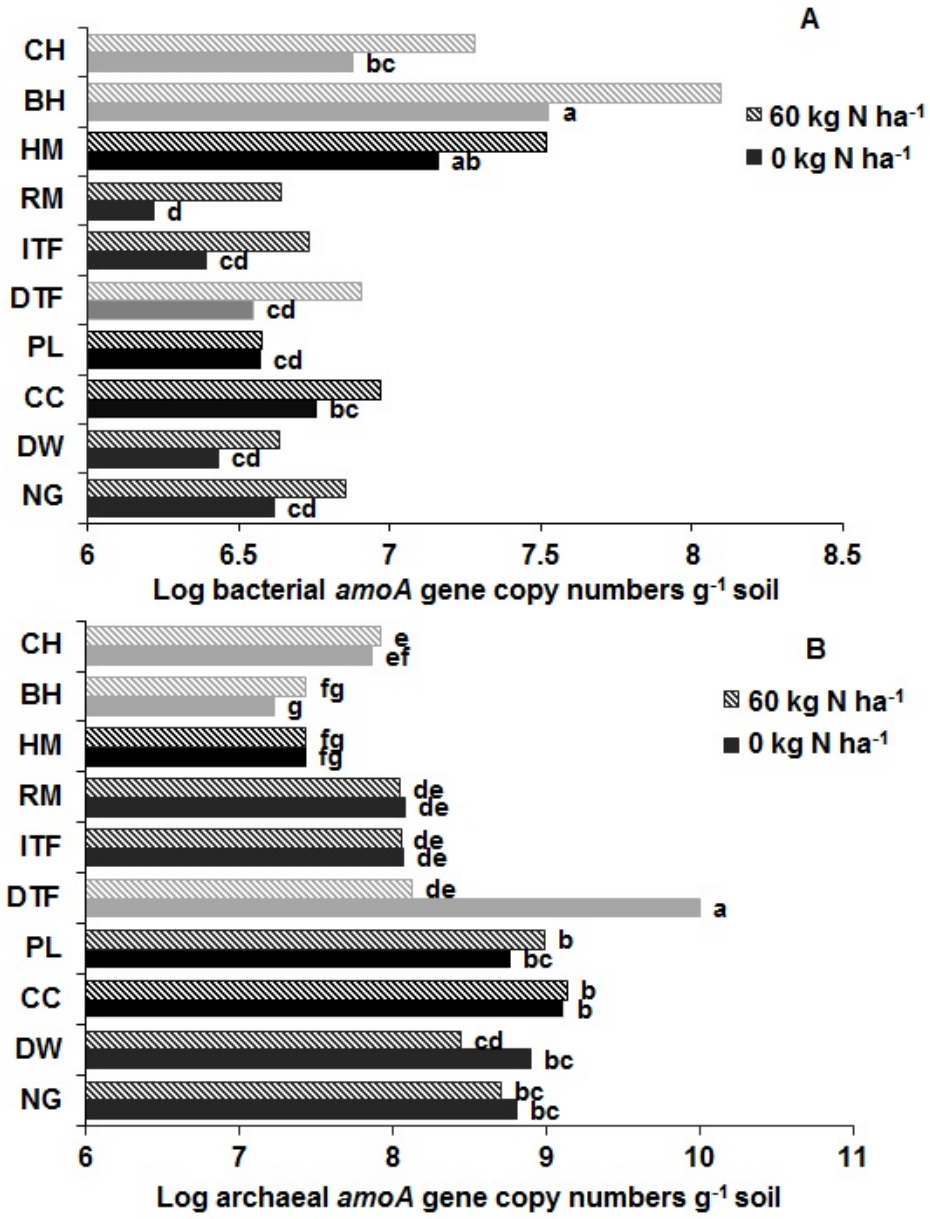


Fig. 3.11. The abundance of A) bacterial *amoA* and B) archaeal *amoA* genes >21 yr after transplanting. Means with same letters are not significantly different at $P<0.001$. Black to grey colors represent the fertility gradient from high to low. NG- native grassland, DW- dryland wheat, CC- cereal cultivated, PL- pastureland, DTF- dryland tilled fallow, ITF- irrigated tilled fallow, RM- manured 30 t ha^{-1}, HM- manured 90 t ha^{-1}, BH- B horizon, CH- C horizon.

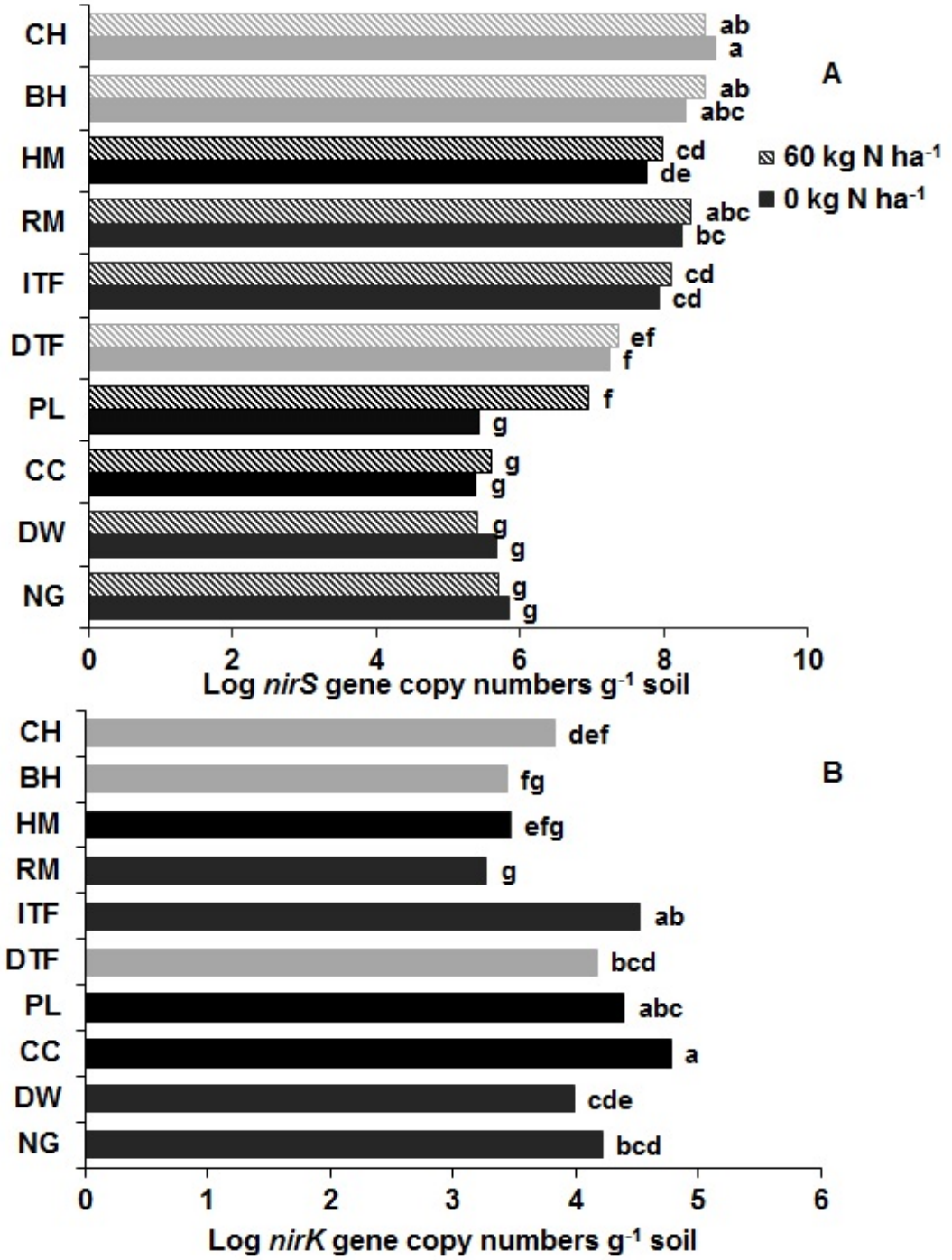


Fig. 3.12. The abundance of A) *nirS* and B) *nirK* genes >21 yr after transplanting**.** Means with same letters are not significantly different at $P<0.05$. Black to grey colors represent the fertility gradient from high to low. NG- native grassland, DW- dryland wheat, CC- cereal cultivated, PL- pastureland, DTF- dryland tilled fallow, ITF- irrigated tilled fallow, RM- manured 30 t ha^{-1}, HM- manured 90 t ha^{-1}, BH- B horizon, CH- C horizon.

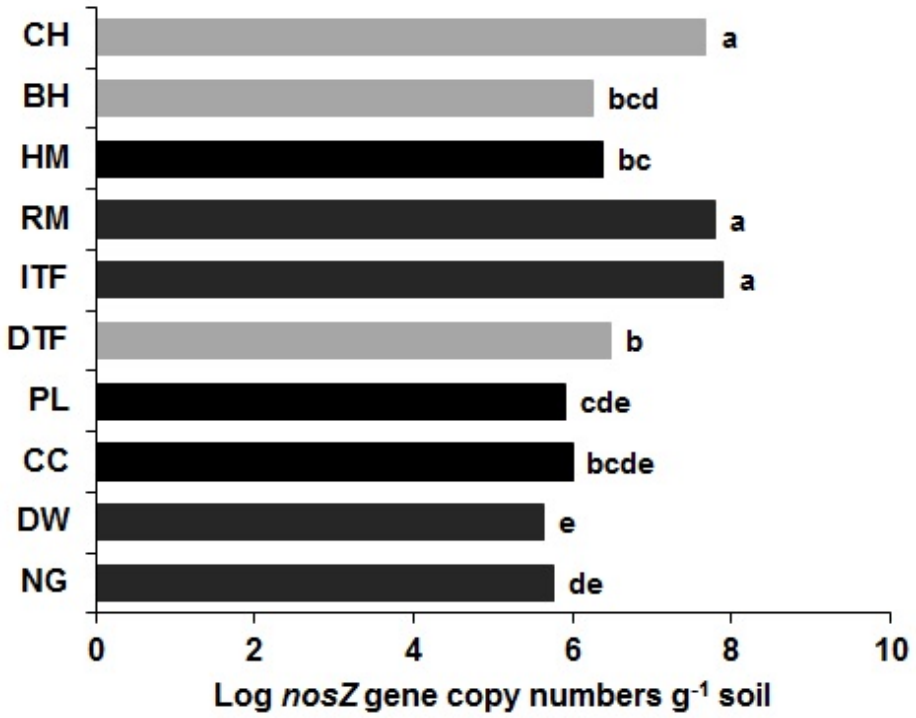


Fig. 3.13. The abundance of *nosZ* gene >21 yr after transplanting. Means with same letters are not significantly different at $P<0.001$. Black to grey colors represent the fertility gradient from high to low. NG- native grassland, DW- dryland wheat, CC- cereal cultivated, PL- pastureland, DTF- dryland tilled fallow, ITF- irrigated tilled fallow, RM- manured 30 t ha^{-1}, HM- manured 90 t ha^{-1}, BH- B horizon, CH- C horizon**.**

3.5.4. Ammonia oxidizer and denitrifier community structures of the transplanted soils

Ordination and MRPP analysis for DGGE finger prints of the AOB community indicated that there was a significant impact of soil origin and N fertilizer application on AOB communities of the transplanted soils (Fig. 3.14). However, soil origin had a greater influence on AOB community structure along axis 1 compared to N fertilizer application which affected the soil in a way that made these AOB communities of high fertility soils (CC and PL) similar. Bacterial *amoA* community structures of high fertility soils (CC and PL) were positively correlated with soil OC content, available NO_3^- and SO_4^{2-}. Further, the AOB community of N unfertilized DTF soil had a positive correlation with available K^+ content. Ammonia oxidizing archaeal community fingerprints yielded a three-dimensional solution which accounted for 95.5% variability, with a final stress of 5.58 (Fig. 3.15). There was a clear separation of AOA profiles as a function of soil type (A= 0.525) along axis 1 while N fertilizer application did not have a

significant influence on AOA communities of the transplanted soils. This was supported by PERMANOVA which indicated a significant effect of soil type (P<0.001) but not N fertilizer (P<0.26) on AOA community structure. Visual assessment of the MDS ordination indicated some separation of N fertilized and unfertilized AOA communities in the DTF soil.

Non-metric multidimensional scaling analysis of *nirK* community structure revealed a significant influence of soil origin on *nirK* communities of the transplanted soils (Fig 3.16). Axis 1 explained 64% of the variability on *nirK* community profiles while axis 2 described another 29% of the variability. However, PERMANOVA analysis indicated a significant interaction effect of soil origin and N fertilizer (P<0.01) on *nirK* communities. Long-term N application gave rise to some separation of *nirK* community profiles in CC, PL and DTF soils compared to soils that did not receive N fertilizer. The impact of N fertilizer on *nirK* communities was opposite for CC soil compared to other soils. However, N fertilization did not have a significant effect on the *nirK* community of RM.

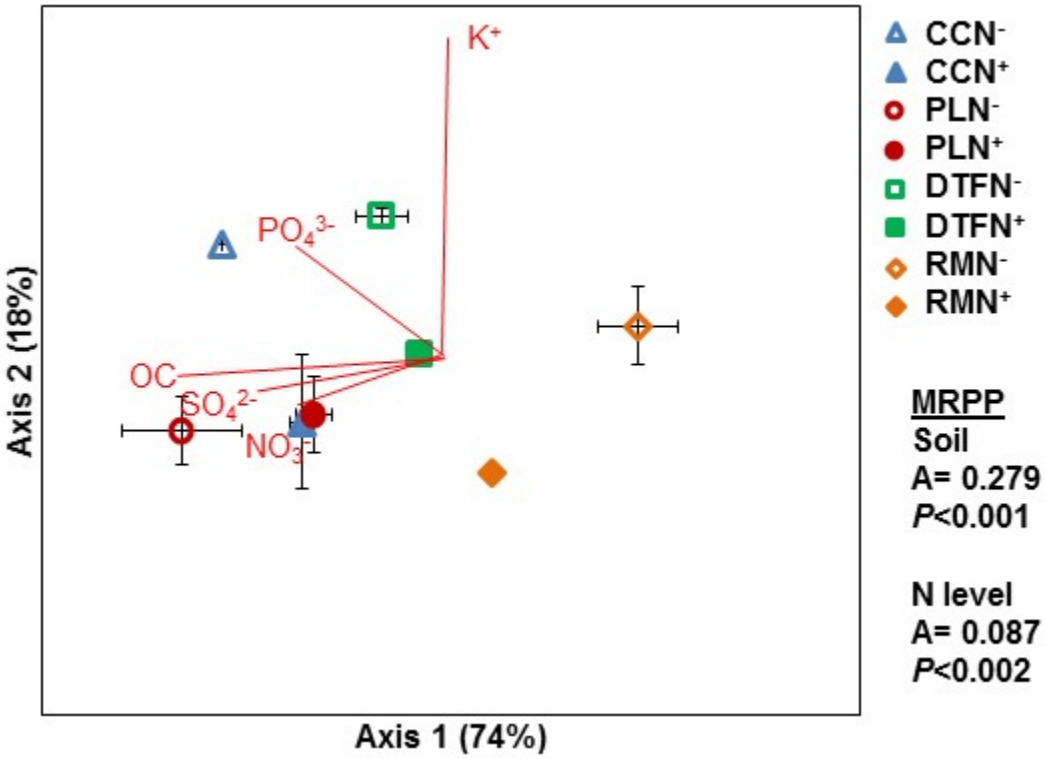


Fig. 3.14. Non-metric multidimensional scaling (NMDS) for DGGE banding patterns of bacterial ammonia oxidizer communities >21 yr after transplanting. Final stress of 8.3. CC- cereal cultivated, PL- pastureland, DTF- dryland tilled fallow, RM- manured 30 t ha^{-1}.

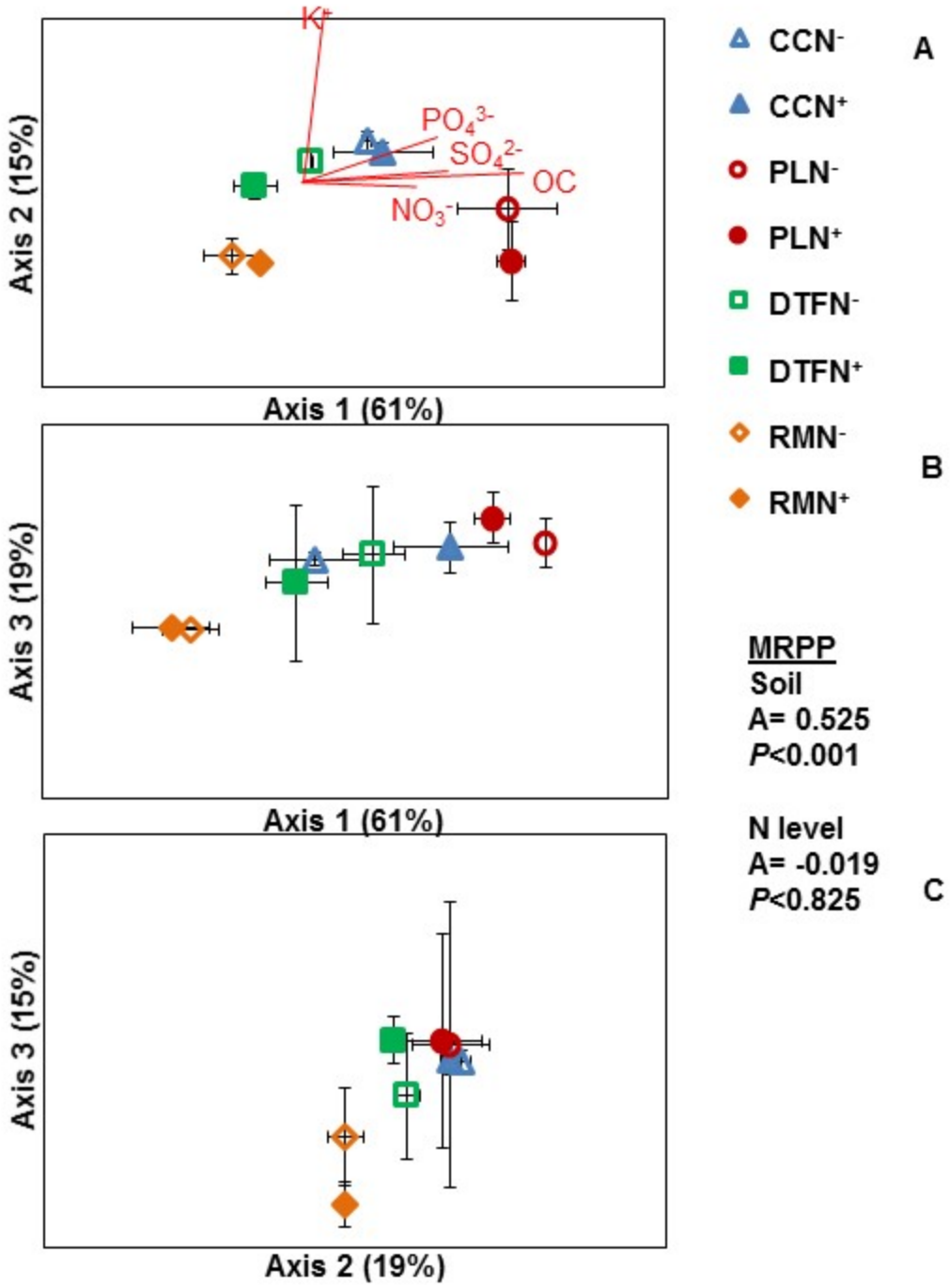


Fig. 3.15. Non-metric multidimensional scaling (NMDS) for DGGE banding patterns of archaeal ammonia oxidizer communities >21 yr after transplanting A) axis 1 and 2, B) axis 1 and 3, C) axis 2 and 3. Final stress of 5.58. CC- cereal cultivated, PL- pastureland, DTF- dryland tilled fallow, RM- manured 30 t ha^{-1}.

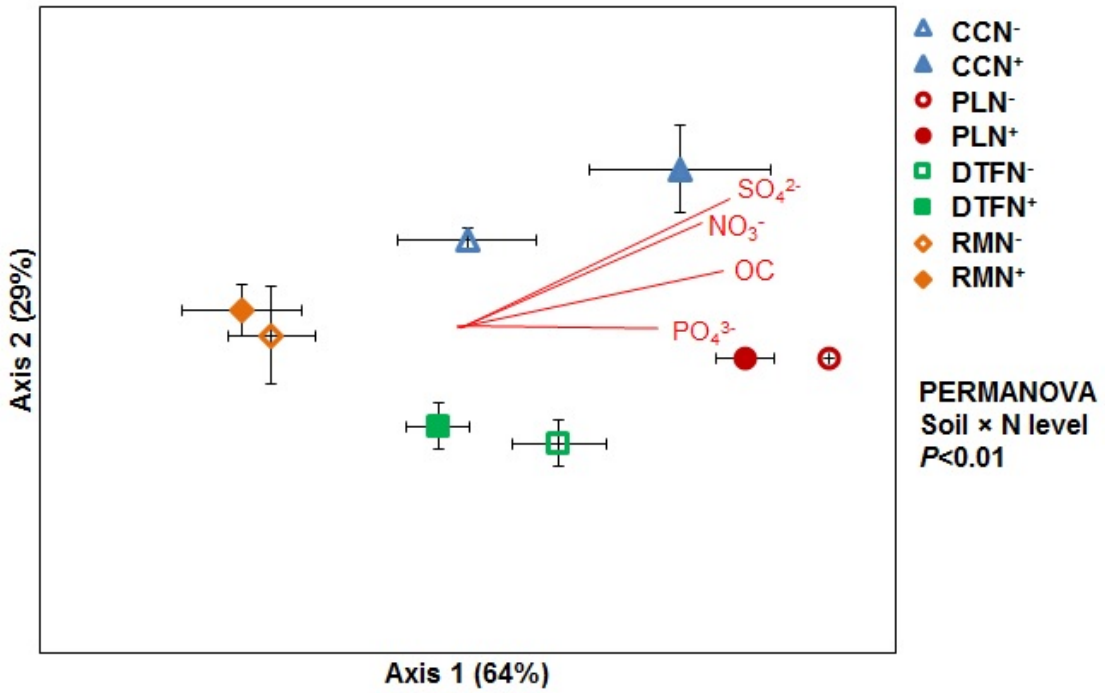


Fig. 3.16. Non-metric multidimensional scaling (NMDS) for DGGE banding patterns of *nirK* gene community structure >21 yr after transplanting. Final stress of 5.6. CC- cereal cultivated, PL- pastureland, DTF- dryland tilled fallow, RM- manured 30 t ha^{-1}.

Soil origin, but not N fertilizer affected *nir*S community composition (Fig. 3.17). The analysis suggested a two-dimensional solution with a final stress of 8.58. There was a clear separation in *nirS* communities of different transplanted soils along axis 1 which explained 82% of the variability. The PERMANOVA analysis indicated a significant interaction effect of soil type and N fertilizer application on *nirS* community profiles. Further, visual assessment of the ordination indicated N fertilizer application resulted in distinct *nirS* communities for the high fertility CC and the PL soils. Dendrogram analysis for AOB, AOA, *nirS* and *nirK* can be found in Fig.A1. Denaturing gradient gel electrophoresis fingerprinting of *nosZ* genes yielded a low diversity with a similar banding pattern for all of the transplanted soils in both unfertilized and fertilized treatments (Fig. A2).

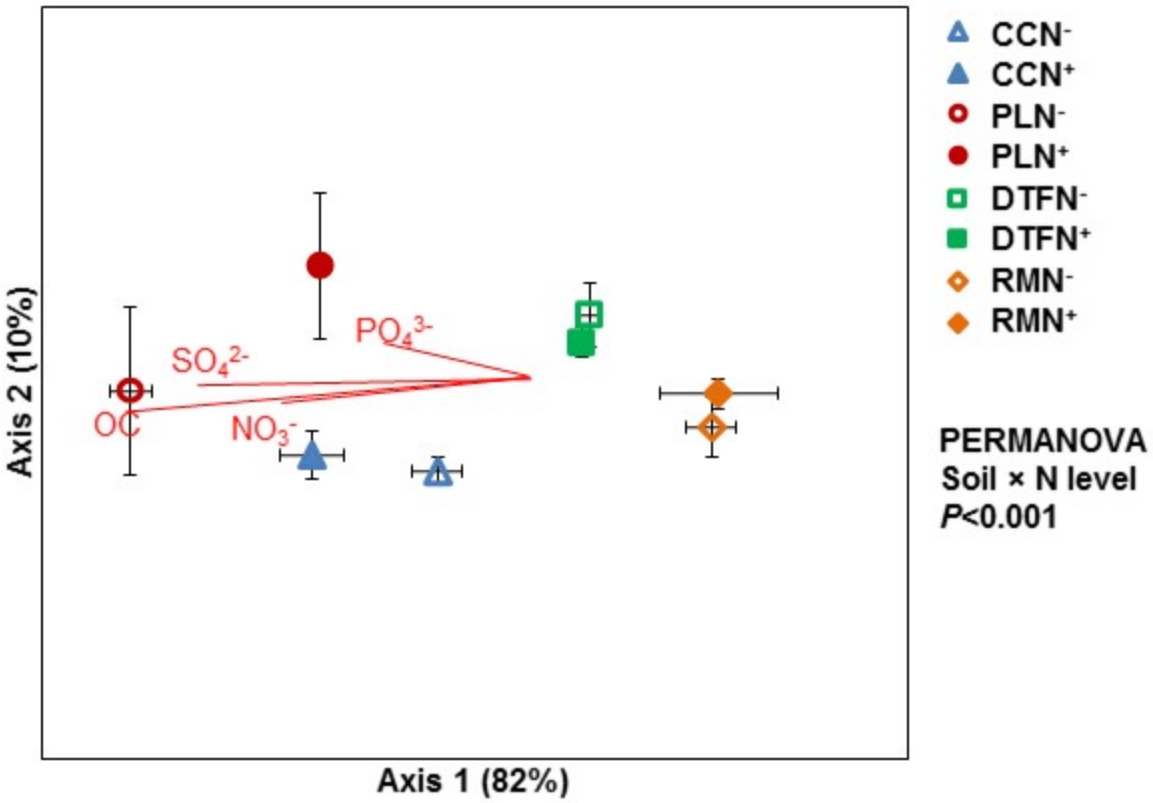


Fig. 3.17. Non-metric multidimensional scaling (NMDS) for DGGE banding patterns of *nirS* gene community structure >21 yr after transplanting. Final stress of 8.6. CC- cereal cultivated, PL- pastureland, DTF- dryland tilled fallow, RM- manured 30 t ha^{-1}.

3.5.5. Changes in soil C and N pools 21 years after transplantation

High fertility PL and CC soils had greater TC, TN, and OC at the time of transplanting (1990) and 21 yr later (2011). The manured (RM and HM) and NG soils had substantial amounts of TC, TN, and OC compared to other soils in 1990 and 2011. Low fertility DTF and BH soils had similar levels of TC, TN, and OC in both 1990 and 2011. The higher TC and lower OC contents in CH soil indicated that majority of the C pool was occupied by inorganic C from the parent materials in 1990 (Table 3.10).

For most of the soils, reductions in soil C and N pools were observed due to continuous cultivation with crop residue removal following transplantation (Table 3.11). The extent of the OC decrease was higher in CC and PL soils; however, N fertilizer application did not make any difference in soil N levels. The TN content of the sub-surface soils (BH and CH) that received N

fertilizer increased by three to four percent, respectively. All the low fertility soils (i.e., DTF, BH, and CH) that received N fertilizer had higher TC contents compared to the original soils from 1990. This was particularly evident in BH (9.6%) and DTF (5.6%) soils. The OC increased by nearly 8% in the BH and CH soils that received fertilizer N.

Table 3.10. The soil total N, total C, organic C contents in 1990 and 2011 (21 yr) after transplantation

Properties[1†]	TN (g kg^{-1} soil)			TC (g kg^{-1} soil)			OC (g kg^{-1} soil)		
Soil origin[‡]	1990	2011 N^-	2011 N^+	1990	2011 N^-	2011 N^+	1990	2011 N^-	2011 N^+
NG	2.7	2.1	2.2	29.2	23.3	23.9	28.8	22.0	22.8
DW	1.8	1.6	1.7	18.6	16.3	16.9	17.9	15.4	16.2
CC	5.4	4.3	4.4	60.1	46.4	47.6	59.8	46.1	47.4
PL	6.4	4.6	4.5	74.8	51.8	52.4	74.7	51.7	51.6
DTF	1.4	1.2	1.3	13.4	12.9	14.1	12.7	12.0	13.1
ITF	1.7	1.5	1.6	20.3	18.8	19.9	15.2	13.7	14.9
RM	2.3	1.9	2.0	30.9	27.3	27.7	20.9	17.6	18.5
HM	4.2	2.8	2.9	41.4	29.6	30.6	38.0	26.3	27.3
BH	1.4	1.3	1.4	13.3	13.9	14.6	12.3	12.2	13.4
CH	1.1	1.1	1.1	34.0	33.1	34.2	9.7	10.1	10.5

[†]TN- total N, TC-total C, OC- soil organic C

[‡]NG- native grassland, DW- dryland wheat, CC- cereal cultivated, PL- pastureland, DTF- dryland tilled fallow, ITF- irrigated TF, RM- manured 30 t ha^{-1}, HM- manured 90 t ha^{-1}, BH- B horizon, CH- C horizon.

[1] Data obtained from Soil Biochemistry research group, AAFC, Lethbridge (Appendix D).

Table 3.11. The changes in soil total N, total C and organic C 21 yr after transplantation.

Changes in properties[2†]	Δ TN%		Δ TC%		Δ OC%	
Soil origin[‡]	N^-	N^+	N^-	N^+	N^-	N^+
NG	-21.2	-18.0	-20.2	-18.2	-23.7	-20.7
DW	-13.5	-8.3	-12.5	-9.2	-13.9	-9.4
CC	-20.9	-19.2	-22.9	-20.8	-22.9	-20.8
PL	-28.1	-28.5	-30.7	-30.0	-30.8	-30.9
DTF	-11.9	-4.3	-3.4	**+5.6**	-5.3	**+3.8**
ITF	-12.2	-4.6	-7.1	-1.9	-10.0	-2.1
RM	-17.2	-14.5	-11.6	-10.2	-15.5	-11.3
HM	-32.1	-30.8	-28.4	-26.1	-30.8	-28.1
BH	-4.1	**+3.3**	**+4.1**	**+9.6**	-1.2	**+8.4**
CH	**+3.0**	**+4.4**	-2.7	**+0.6**	**+3.4**	**+7.9**

† % changes in total N (TN), total C (TC), soil organic C (OC)

‡NG- native grassland, DW- dryland wheat, CC- cereal cultivated, PL- pastureland, DTF dryland tilled fallow, ITF- irrigated TF, RM- manured 30 t ha^{-1}, HM- manured 90 t ha^{-1}, BH- B horizon, CH- C horizon.

[2] Data obtained from Soil Biochemistry research group, AAFC, Lethbridge (Appendix D).

3.5.6. Relationships of current microbial abundance and composition with original soil properties

Most of the current microbial analysis of transplanted soils revealed significant relationships with soil origin, implying an influence of soil conditions at the time of transplanting that was retained for many years. Spearman's rank correlation was computed to identify initial soil determinants which had a persistent influence on microbial community compositions of the transplanted soils. Further, changes that occurred in soil chemical composition from 1990 to 2011 were also used to predict the relationships with microbial composition observed in 2012.

Total N, TC, OC and the C: N ratio of the original soils (1990) were positively correlated with total microbial biomass and bacterial abundance and negatively correlated with the fungal: bacteria ratio (Table 3.12). Total C content at transplanting (1990) was positively correlated with stress 1, and both TN and TC were positively correlated with stress 2 21 yr after transplantation (Table 3.12).

Changes in soil characteristics that have occurred since soils were transplanted revealed that TN, TC, and OC were negatively correlated with total PLFA and bacterial biomarkers (Table 3.13). This observation indicates that total PLFA and bacterial biomarker abundance were mainly determined by initial soil TN and TC contents rather than the changes that occurred over 21 yr of cultivation. Further, the fungal biomarker was negatively correlated with the changes that arose in soil TC and OC content.

Total N, C, OC and the C: N ratio of the original transplanted soils were positively correlated with 16S *rRNA* gene abundance while AOA abundance was positively correlated with soil TN and OC content (Table 3.14). The abundance of *nirS* gene was negatively correlated with initial TN and OC content where as *nirK* gene abundance was largely controlled by initial TN, TC and OC content of the transplanted soils. The abundance of the *nosZ* gene was positively correlated with the C: N ratio. However, total 16S *rRNA* gene and N cycling genes did not have strong relationships with the changes that occurred in these general measures of soil fertility in the transplanted soils in the time since transplanting (Table 3.15).

Table 3.12. Spearman's correlation coefficient between total PLFA, bacterial, fungal, AMF biomarkers, fungal: bacteria ratio, stress 1 and stress 2 and initial soil total N and C, organic C and C: N ratio at 1990.

	TN[†]	TC	OC	C: N ratio
Total PLFAs	0.46***	0.33**	0.46***	0.38*
Bacterial	0.47***	0.33**	0.47***	0.38*
Fungal	0.12	-0.08	0.12	0.08
AMF	0.27	0.10	0.23	0.25
Fungal: Bacteria	-0.42*	-0.41**	-0.43**	-0.32*
Stress 1	0.19	0.44**	0.19	0.17
Stress 2	0.38*	0.47**	0.39*	0.21

[†]Total N (TN), total C (TC), soil organic C (OC)
Significant values represent *P*-value *, *P*<0.05; **, *P*<0.001; ***, *P*<0.0001

Table 3.13. Spearman's correlation coefficient between total PLFA, bacterial, fungal, AMF biomarkers, fungal: bacteria ratio, stress 1 and stress 2 and changes occurred in total N and C, organic C and C: N ratio during last 21 yr compared to the original soil.

	Δ TN[†]	Δ TC	Δ OC	Δ C: N ratio
Total PLFAs	-0.28*	-0.31*	-0.32*	-0.10
Bacterial	-0.29 *	-0.33*	-0.33*	-0.12
Fungal	-0.25	-0.29*	-0.29 *	-0.12
AMF	-0.13	-0.20	-0.19	-0.26*
Fungal: Bacteria	0.07	0.08	0.09	0.01
Stress 1	0.02	0.05	0.03	0.21
Stress 2	-0.16	-0.09	-0.14	0.19

[†]% changes in total N (TN), total C (TC), soil organic C (OC)
Significant values represent *P*-value *, *P*<0.05; **, *P*<0.001; ***, *P*<0.0001

Table 3.14. Spearman's correlation coefficient between 16S *rRNA*, *amoA*, *nirS*, *nirK* and *nosZ* gene abundance and initial soil total N and C, organic C and C: N ratio at 1990.

	TN†	TC	OC	C: N ratio
16S *rRNA*	0.32*	0.41*	0.31*	0.14*
AOB	-0.10	-0.01	-0.10	-0.22
AOA	0.38*	0.21	0.39*	0.08
nirS	-0.63***	-0.21	-0.63***	0.25
nirK	0.33*	0.27*	0.34*	0.12
nosZ	-0.46	-0.11	-0.46	0.40**

†Total N (TN), total C (TC), soil organic C (OC)
Significant values represent *P*-value *, *P*<0.05; **, *P*<0.001; ***, *P*<0.0001

Table 3.15. Spearman's correlation coefficient between 16S *rRNA*, *amoA*, *nirS*, *nirK* and *nosZ* gene abundance and changes occurred in total N and C, organic C and C: N ratio during last 21 yr compared to the original soil.

	Δ TN†	Δ TC	Δ OC	Δ C: N ratio
16S *rRNA*	-0.05	0.02	-0.01	0.18
AOB	0.04	0.06	0.11	0.10
AOA	-0.11	-0.04	-0.12	0.13
nirS	0.24	0.20	0.25	-0.04
nirK	0.05	0.10	0.02	0.14
nosZ	0.18	0.10	0.15	-0.18

†% changes in total N (TN), total C (TC), soil organic C (OC)
Significant values represent *P*-value *, *P*<0.05; **, *P*<0.001; ***, *P*<0.0001

3.6. DISCUSSION

Microbial diversity and abundance differed from one soil to another (Figs. 3.5 to 3.7 and Fig. 3.9). These differences were related to various biotic and abiotic factors. Major contributors of microbial diversity and abundance in arable soil are not fully understood. It was hypothesized that identical climatic, topographic and management conditions for >21 yr might result in a convergence of microbial properties of different transplanted soils over time. However, the current study revealed a significant lasting impact of soil origin and land use legacy on the soil microbial abundance and community composition. Similar to the current study, in a short term (1 yr) reciprocal transplanted study conducted in non vegetated glacier fore fields, no changes in relative abundance and dominant species present between transplanted and their original soils were observed (Lazzaro et al., 2011). In a similar transplanted soil study conducted in aspen and spruce forest floors, it was concluded that forest floor origin has a dominant effect on microbial community composition (Hannam et al., 2007). In contrast to the current study, Sun et al. (2014) reported that bacterial communities of 20 yr old transplanted soils converged over time and were ultimately similar to those of the new geographic location. However, 4.5 yr post transplantation, microbial communities of these transplanted soils grouped by the soil origin (Sun et al., 2013).

The transplanted soils used in this study were managed under identical climatic, and topographic conditions using equivalent management practices, yet differences in microbial abundance and community composition persisted for as long as >21 yr after transplanting (Table 3.8). The climate, topography and management practices may have influenced microbial community composition over past 21 yr; however, diverse abundance and community structures between the transplanted soils indicated there were other dominant factors shaping microbial communities of the studied soils. The transplanted soils had different fertility levels, as indicated by the chemical properties (Table 3.7). Soil chemical properties are known to influence microbial community composition (Girvan et al., 2003; Jangid et al., 2011; Hu et al., 2014). For example, Girvan et al. (2003) concluded that soil chemistry and structure were the primary factors determining the total bacterial composition. Others have suggested that soil pH is a key major determinant of microbial community composition (Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2010); however, in the current study, the impact of soil pH did not differ

between the soils. A greater variation was found for the impact of soil OC and available nutrient contents (NO_3^-, SO_4^{2-}, PO_4^{3-} and K^+) of transplanted soils on microbial communities. Nitrogen fertilizer application enhanced NO_3^- level for most of the soils except for DW and CH soils. Sub-surface soil from the C horizon (CH) had higher OC content at 2011 compared to 1990 and nitrogen fertilized soils retained more OC than N unfertilized soils. Building OC requires added fertilizer N, particularly in low fertility soils, resulting lower NO_3^- level in the fertilized soil. The current research revealed that high fertility (CC and PL) and RM soils had greater microbial biomass and bacterial abundance than low fertility soils (DTF, BH, and CH), reflecting the effect of soil fertility level on total and bacterial abundances (Figs. 3.5 and 3.6). The total microbial, bacterial biomass and 16S *rRNA* gene abundances were positively correlated with TN, TC and soil OC content, suggesting the importance of resource availability in determining the microbial abundance and community composition of the studied soils (Tables 3.12 and 3.14). There are studies that reported influence of soil fertility on abundance and spatial distribution of microorganisms (Franklin and Mills, 2009; Koorem et al., 2014).

Hu et al. (2014) argued that soil OC content and available N are the best predictors of total and bacterial PLFA abundance in the soil. Several studies have indicated soil C sources as the main ecological driver of microbial dynamics (Haichar et al., 2008; Djukic et al., 2010; de Vries et al., 2012). The PLFA biomarkers of transplanted soils did not respond to N fertilizer application suggesting that C, but not N, is the main factor influencing the observed microbial community dynamics (Table 3.8). Crop residue removal for use as a bedding material for livestock is a common practice in Western Canada (Campbell et al., 1991; Zvomuya et al., 2008), and this practice may influence soil microbial communities by altering the soil fertility. Soil microorganisms are mostly dominated by heterotrophs that consume soil OC as their energy source. In the current study, crop residues were removed from all soils post harvest for >21 yr and are considered to be under C limited conditions; consequently, microorganisms may be utilizing native soil OC (pre-transplanting), which is consistent with the observation of Hu et al. (2014) who found a predominant effect of soil OC on microbial abundance and composition under C limited conditions. As the transplanted soils have different origins, it is likely that the quality of the native soil OC varied, resulting in different forms of available substrates for microorganisms. The apparent response of the soils towards crop residue removal varied

depending on the soil fertility status which was determined by the soil origin. For most of the tested soils a reduction in soil OC content was observed over time; however, the differences between the soil OC levels persisted and followed a similar trend as the original soils (Table 3.11). Irrespective of N rate, the highest rate of C depletion occurred in high fertility PL and CC soils. Specifically, soil OC levels declined by 31% in the PL soil and by 21% in the CC soil relative to the original soil OC contents (Table 3.11). In contrast, the low fertility soils (DTF, BH, and CH) with long-term N fertilization gained soil OC and TN contents >21 yr after transplanting, irrespective of crop residue removal. However, previous studies conducted in the Canadian Prairies reported no influence on soil OC and soil organic N of a fallow-wheat-wheat cropping system with crop residue removal in Black Chernozems after 50 yr (Campbell et al., 1991; Lafond et al., 2009). The gain of soil OC in low fertility soils, even with residue removal, suggests the OM incorporation in transplanted field was higher than the site of origin. The DTF soil was obtained from a pathway to an experiment field which was frequently tilled and fallowed, and weeds were the only source of soil OM prior to transplantation. Furthermore, the BH and CH soil were obtained from sub-surface soils, which are not the primary storage horizons of soil OM. Variable responses of soil OC content confirmed the long-term effect of land use legacy on soil OC pool.

The contribution of root exudates, root biomass and the standing wheat stubble (ca. 20 cm in length) are important sources of C that likely influenced the C dynamics of the transplanted soils. It is expected that the N fertilizer application would enhance the OM mineralization resulting from the increased levels of soil OC from the plant biomass and the root exudates. Nearly 20 to 30% of photosynthetically assimilated C is transferred to the soil by cereal crops (Kuzyakov and Domanski, 2000). Approximately, 26% of root translocated assimilated C is released into the soil matrix (Kuzyakov and Domanski, 2000), which represents a considerable amount of C input to the soil. In addition to root exudates, the decaying roots also are a significant source of C to the soil. A study conducted using Dark Brown and Brown Chernozemic soils estimated that as much as 485 and 664 kg C ha^{-1}, respectively, was deposited within 0 to 10 cm of the soil surface in continuous wheat cropping systems (Comeau et al., 2013). A long-term study conducted in the Canadian Prairie region reported that under normal climatic conditions continuous wheat cultivation yielded 2880 kg ha^{-1} of dry matter per season (Gan et al., 2012). The wheat residue is

easily decomposed by microorganisms (Chowdhury et al., 2014) and C from residues become available in the soil (Berg, 2000). In the current study, 20 cm of wheat stubble was left standing in the transplanted soils following harvest, indicating that as much as 40% of the residue was returned to the soil (Boyden et al., 2001) which could contribute substantially to soil nutrient cycling.

The decomposition of OM follows different pathways depending on the properties of the OM, the labile C inputs (Qiao et al., 2016) and the microbial community structure (Haichar et al., 2008). The nature of the labile C inputs received was similar for all of the transplanted soils, that is, the cropping sequence was the same in all soils following transplantation. However, the quality of the OM received prior to transplanting and variable microbial community structures that existed at the beginning of the experiment may give rise to various C dynamic responses in the studied soils. The microbial community structure in the low fertility soils (DTF, BH, and CH) were distinct from those in high fertility soils (CC and PL) (Fig. 3.9). Furthermore, the microbial abundance was also lower in the low fertility soils compared to the high fertility soils. It has been argued that the presence of readily available substrates prevents microorganisms from utilizing protected soil OM. This mechanism is named as preferential substrate utilization (Cheng, 1999). The amount of N fertilizer and C inputs received by low fertility soils may be more than the amount required for microbial utilization. As a result, surplus C inputs were stored in the soil indicating a negative priming effect. In agreement, Blagodatskaya et al. (2007) reported a zero or negative priming effect with a high amount of available C and N in the soil. They found a negative priming effect by adding glucose and N ($KNO_{3\ (aq)}$) in an incubation study conducted using a Chernozemic soil. The high fertility soils (CC and PL) which had higher microbial abundance may require greater C inputs to fulfill the microbial demand for C. A similar phenomenon apparently occurred in most of the transplanted soils, with the exception of DTF, BH and CH soils. In most cases in soils where OC depletion occurred, depletion during the past 21 yr was greater in N unfertilized soils than fertilized. The quality of the fresh OM and microbial demand for C are two main factors influencing the decomposition of stabilized OM present in the soil (Wang et al., 2015). Soil OC changes in N unfertilized treatments indicated a greater depletion than fertilized soils. This suggests that under limited low-quality residue (mostly the wheat stubble with roots) return conditions, soil microbes must access existing OM

to fulfill the nutrient demand. Since N unfertilized soils lacked N inputs, microbes apparently mined the soil for nutrients resulting in a reduction in soil OM.

Exploration of specific functional groups involved in N cycling broaden our understanding of nitrifier and denitrifier communities in transplanted soils. Quantitative real time PCR and DGGE analysis revealed that abundance and community structure of *amoA, nirS, nirK* and *nosZ* genes involved in N-cycling varied distinctly between different transplanted soil types (Figs. 3.10 to 3.13 and Figs. 3.14 to 3.17). Although the influence of long-term N fertilizer application was not reflected in the PLFA profiles, microbial communities responsible for N cycling indicated different responses towards N fertilization. The N effects were predominantly captured in the N cycling community structures rather than in the abundance of different functional targets. In the current study, N fertilization occurred at the beginning of the growing season at a rate of 60 kg N ha^{-1}. In agricultural systems, the concentration of NH_4^+ decreases within a few days to weeks after incorporation of N fertilizer due to plant uptake and nitrification (Hou et al., 2010; Pelster et al., 2011). In the current study, soil sampling was carried out nearly six months after N fertilization occurred and it is presumed that added NH_4^+ had been nitrified into NO_3^-. Higher NO_3^- in the N fertilized soils relative to the unfertilized soils (Fig. 3.4A) is evidence that long-term N fertilization has the ability to enhance the N fertility resulting in more available N (Chu et al., 2007). However, the current study suggests that the changes captured in microbial abundance and community structure of N-cycling genes were due to persistent effects of long-term N application.

The ammonia oxidizer community abundances were positively correlated to initial TN and OC content, but did not correlate with changes in TN, TC and OC that occurred since the initiation of the experiment (Tables. 3.14 and 3.15). This validates the speculation that soil origin has a long-term effect on the abundance of ammonia oxidizing communities. High fertility soils (CC and PL) with higher soil OC had greater 16S *rRNA* gene abundance indicating the presence of a larger heterotrophic population. In addition to OM decomposition, heterotrophs also compete with ammonia oxidizers for available NH_4^+ in the soil (Schnürer et al., 1985; Shi and Norton, 2000; Burger and Jackson, 2003; Shen et al., 2013). The nitrifiers are poor competitors for N acquisition relative to heterotrophs (Verhagen et al., 1995) which might be the reason for lower AOB abundance in OM rich soils. Similar to these findings others have also found an

increase in AOB populations with N fertilizer application (Shen et al., 2008; Levičnik-Höfferle et al., 2012; Szukics et al., 2012) and AOA correspondingly either did not respond to, or reduced their abundance in response to long-term N application (Hai et al., 2009).

With the exception of sub-surface (BH and CH) and HM soils, the bacteria *amoA* gene copy numbers ranged from $\log_{10}$ 6.25 to 7 gene copies per g of dry soil, which are similar to values reported in other studies (Hermansson and Lindgren, 2001; He et al., 2007). The AOA abundance ranged between $\log_{10}$ 7.25 to 10 gene copies per g of dry soil. Most of the studies conducted in agricultural soil found AOA gene copy numbers ranged between $\log_{10}$ 7 to 8 which was narrower than the present observations (Kelly et al., 2011; Sterngren et al., 2015). Long-term strong responses towards N fertilizer application were indicated by increased the bacterial *amoA* gene abundance in soils receiving NH_4NO_3 and lower abundance in plots that did not receive NH_4NO_3 (Fig. 3.11A). These phenomena are consistent with those observed by Hai et al. (2009) in long-term urea fertilizer application study. In agreement with He et al. (2007), Shen et al. (2008) and Hai et al. (2009), reported that in most of the soils they studied there was higher AOA abundance than AOB. The AOB is favored in resource rich conditions (Webster et al., 2005) while AOA prefers low fertility or oligotrophic environments (Valentine, 2007; Prosser and Nicol, 2012). It has been found that AOA is functionally dominant in soils with continuous release of low rates of NH_4^+ through mineralization (Offre et al., 2009; Levičnik-Höfferle et al., 2012). The transplanted soils were limited with OM inputs resulting in lower amounts of NH_4^+. These conditions are preferable for AOA which are more adapted to lower energy environments than bacteria (Valentine, 2007). The presence of a higher AOA than the AOB abundance does not necessarily indicate the functional dominance of the AOA in agricultural soils (Jia and Conrad, 2009). However, Sterngren et al. (2015) found a larger contribution of AOA over AOB to soil nitrification at neutral soil pH which might be true for transplanted soils that had neutral pH conditions. A typical AOB cell carries two to three copies of *amoA* genes (Okano et al., 2004) and AOA has only one copy (Sterngren et al., 2015). When considering the number of genes per cell, the AOB community abundance is even less than the AOA in the studied soils. In sub-surface (BH and CH) and HM soils, AOB abundance was greater than the AOA abundance. However, N fertilizer application increased AOB abundance without changing the AOA abundance. It has been shown that AOA is less responsive towards environmental changes than

the AOB (Santoro et al., 2008). Distinct AOB and AOA community structures resulted for each of the four transplanted soils emphasizing the long-term impact of soil origin on ammonia oxidizer communities (Figs. 3.14 and 3.15). In the tested soils, significant changes occurred in AOB community structures in response to N fertilization. These changes may indicate that a part of the AOB population plays a dominant role in NH_3 oxidation in N fertilized systems. Similar to the current study, a 17 yr long-term N fertilized experiment has also revealed a shift of AOB community structure without any changes in AOA community (Shen et al., 2008). Specifically, in a long-term experiment conducted with different organic and inorganic fertilizer combinations in China, Chu et al. (2007) reported a shift in the AOB community structure with 16 yr of N fertilizer application, with more diversified AOB communities in inorganic N treatments than organic N treatments. The current study is in agreement with previous studies, suggesting that shifting of AOB community with long-term N fertilizer application. It has been shown that the AOA community is more stable than the AOB community in response to environmental changes (Jin et al., 2010). In high fertility CC and PL soils, the AOB community structures became similar to each other with long-term N application. Under resource rich conditions, there may be similar highly adapted AOB groups that respond to the N fertilizer application in high fertility soils.

Soil properties and land use are important factors affecting the diversity of denitrifier communities (Miller et al., 2008; Hallin et al., 2009). The abundance of key denitrifier functional groups and community composition of the studied soils were mainly dependent on the soil origin, but not on N addition. Sun et al. (2015) observed a larger contribution of the *amoA* gene under long-term N fertilizer (34 yr) management than denitrifiers (*nirS*, *nirK* and *nosZ*). In the current study, only the *nirS* population size increased in high fertility PL soil in response to N amendment among the studied denitrifier communities (i.e., *nirS*, *nirK* and *nosZ*) (Figs.3.12A, 3.12B and 3.13). The *nirS* denitrifier population is more sensitive to inorganic N fertilizer application than the *nirK* (Yin et al., 2014). The soil type has a significant impact on *nosZ* gene abundance (Gao et al., 2016) which is in agreement with the transplanted soils. The changes that occurred in *nirS* gene abundance in the PL soil is more likely due to the effect of land use changes and N fertilizer application on previously uncultivated soils. The denitrifier community abundances in the remaining soils may not be as sensitive to the 60 kg of N ha^{-1} rate. Further, in a

study conducted in canola fields in the Canadian prairies, Yang et al. (2015) observed significant increases of *nirS* and *nirK* genes in Brown, Dark Brown and Black Chernozems with N fertilizer application (60 and 120 kg N ha^{-1}) without any changes in *nosZ* abundance. This is similar to the current observations of *nosZ* abundance in which no influence of N fertilization was observed. In agreement to our findings, a reduction in *nosZ* gene abundance in OC and TN rich soils was observed in an incubation study conducted in 160 yr old long-term Broadbalk Wheat Experiment which was established to examine the long-term effect of fertilizer and manure application (Clark et al., 2012).

The correlation analysis of *nosZ* gene abundance with TN and OC did not indicate any relationships to initial TN and OC and their changes during the past 21 yr (Tables 3.14 and 3.15). In agreement with Girvan et al. (2003) distinct community profiles for *nirS* and *nirK* were observed in soils that did not receive N fertilizer (Figs. 3.16 and 3.17). Further, N fertilization may activate part of the *nirS* and *nirK* populations resulting in more diverse community profiles than the N unfertilized soils. *NirS* community structures of RM and low fertility DTF N fertilized and unfertilized soils were clustered together, indicating lesser sensitivity towards long-term N fertilization than soils with initially higher fertility (CC and PL). A similar trend in the *nirK* community composition also was found in DTF soils. In agreement with the above findings, it has been reported that the soil resource, and specifically soil fertility, has a significant impact on the denitrifier community structure (Bru et al., 2011). The community *nirK* and *nirS* compositions of the high fertility soils (PL and CC) indicated the influence of soil nutrient profiles (OC, NO_3^-, SO_4^{2-} and PO_4^{3-}). The *nirS* and *nirK* denitrifier communities of the high fertility soils (CC and PL) were closely arranged in the ordination confirming resource availability has somewhat of an influence on shaping the denitrifier community structure. Denaturing gradient gel electrophoresis analysis for *nosZ* genes yielded a similar band for all the transplanted soils, including the different N levels (Fig.A.2). Regardless of soil origin and N fertilizer application resulted in a single DGGE banding pattern indicating a simple *nosZ* gene carrying community in the studied soils. This also implied dominancy of a particular denitrifier group in the transplanted soils regardless of N fertilizer application. The sequence obtained for PCR amplified DGGE bands were not acceptable as two or more dissimilar sequences resulted

for each band. Similar phenomena were observed by Ma et al. (2008) for DGGE analysis of nitrifier and denitrifier communities in two different landscape types.

The linkage between microbial abundance and composition with respect to the soil origin was studied by providing identical climatic, topographic and management practices for >21 yr to a set of soils with diverse physical, chemical, and biological properties. The results of the current transplanted soil study revealed that soil properties related to soil origin was a major determinant of microbial properties >21 yr after transplanting. There have been many soil transplant experiments completed worldwide to more fully understand the effects of global changes and geographic location on both plant and soil microbial ecosystems (Balser and Firestone, 2005; Lazzaro et al., 2011; Vanhala et al., 2011; Sun et al., 2014; Zhao et al., 2014). Many have reported that changes in microbial community composition and their functions occurred when soil was transplanted (Vanhala et al., 2011; Sun et al., 2013; Zhao et al., 2014). Sun et al. (2014) reported a strong effect of geographic location (after transplantation) on bacterial communities after soil transplantation. Microbial community structure of the soil transplanted into warmer regions than their origin changed towards the community structure of the new geographic location (south) 2 yr after transplantation (Vanhala et al., 2011). Most of the aforementioned studies transplanted soils into sites located more than 1000 km from the original site. This distance created drastic climatic changes which had a significant influence on soil microbial communities. In the current study, the transplanted soils were obtained from sites within 100 km radius to the experimental field and did not experience substantial climate changes. Additionally, the soil origin and land use history may have altered or hindered the influence of climate and management conditions (after transplantation) on microbial communities of the transplanted soils resulting in diverse microbial composition even among specific functional (i.e., N-cycling) communities.

3.7 CONCLUSION

Even after two decades of identical climatic, topographic and management conditions, microbial community composition and structure of transplanted Chernozems remained most strongly related to soil origin. This indicates there was a persistent influence of soil origin and land use history on soil resource availability, microbial abundance and community composition

even under C and N limited conditions. After >21 yr of common environmental conditions diverse soil fertility levels were observed among transplanted soils. Although, soil fertility changed due to current environmental conditions, the degree of change was determined by soil origin, land use and management history. These results suggested that soil fertility is a product of pedogenesis, land use and management history which creates a strong legacy effect on soil microbial community abundance and composition.

Regardless of N fertilizer application, a convergence in microbial abundance and soil fertility of transplanted soils was not strongly apparent. The long-term N fertilizer application did not have a strong influence on bacteria and fungi abundance in the studied soils. This suggested that the N is not the most limiting factor for the functioning of microbial communities of the transplanted soils and soil fertility legacy effect was stronger on microbial communities over long-term N fertilizer application.

Diverse and distinct nitrifier and denitrifier abundance and community structures indicated a dominant effect of soil origin on N-cycling communities. The AOA was the dominant nitrifier group present under C limited conditions. The N cycling functional genes had positive responses towards the long-term N application. However, nitrifier and denitrifier responses to N fertilizer application was pre-determined by the soil origin and land use history. Long-term N application resulted in an increase in bacterial *amoA* gene abundance suggesting a stimulation of AOB without any impact on AOA gene abundance. Long-term N fertilizer application resulted in a convergence in the AOB community structure of high productivity soils under common environmental conditions.

4.0. CHANGES IN MICROBIAL COMMUNITY STRUCTURE USING ARCHIVE SOILS OF TRANSPLANTED CHERNOZEMS

4.1. PREFACE

Chapter 3 surveyed the current status of the transplanted soil microbial communities after 21 yr of common conditions. The results indicated variable abundance, composition and community structures in total communities and N-cycling functional groups in the transplanted soils. Chapter 4 focusses on the identification of the microbial community responses to transplanted conditions in comparison to the original soil microbial communities. Archived soils from the 1990 (original) and 2011 (21 yr.) preserved collection were used for this investigation.

4.2. ABSTRACT

Agricultural soil ecosystems are influenced by the management practices that alter their capacity to fulfill essential ecological functions. To ensure these critical functions, it is important to understand how soil microbes respond to intensive agricultural management. An experiment was conducted using archived soils from a long-term transplanted soil quality field experiment at AAFC, Lethbridge, AB. Briefly, 21 yr ago, 36 different Chernozems were transplanted to a single field and since then have been managed under identical climatic and topographic conditions. Starting in 1991, the field was continuously cropped with wheat under two different N fertilizer rates (0 and 60 kg N ha^{-1}). DNA samples from soils sampled and archived in 1990 and 2011 were successfully extracted and the microbial community structures were investigated using the 16S *rRNA* gene profiling. Although some storage effects on the DNA signatures of the archived soils likely occurred, results suggest that factors other than those related to storage affected the community structures in these archived soils. In 1990, the differences in the bacterial community profile were mainly associated with soil origin and land use legacy but divergence from these original communities and among different transplanted soils were observed in 2011. Shifts in the relative abundance of different taxa of the soil bacterial community were observed. In some cases, some new members were observed while others were no longer detected. Changes in the microbial community structure were particularly prominent between different classes and orders belonging to *Proteobacteria*. These changes were more visible in initial high fertility (cereal cultivated (CC) and pastureland (PL)) soils and transplanted soil from undisturbed native land (NG). The reduced relative abundance of *Betaproteobacteria* and *Bacteroides* was consistent with a decline in soil fertility with wheat monoculture and crop residue removal. Further, changes with respect to long-term N fertilization were apparent in bacterial community profiles; however, species richness and evenness did not follow a particular pattern with either soil origin or N fertilization. Our results indicate that changes in bacterial community structure can be elucidated using archived soils to bring new information to light about the changes across experimental treatments over time. Archived soils can add value to the myriad of long-term soil experiments around the world by allowing us to peer back in time and compare treatments effects with powerful new molecular tools.

4.3. INTRODUCTION

A major challenge currently facing the agriculture sector is the need to double food production within next few decades to fulfill growing food demands and energy requirements (Blum, 2013). This must be accomplished with minimal environmental impacts to avoid land degradation, climate change and loss of biodiversity. Soil provides vital ecosystem services including organic matter (OM) decomposition and nutrient cycling. Most of the ecosystem functions are mediated by soil microbes (Schlesinger, 2013). For example, OM decomposition and C-cycling is mediated by the soil decomposer community (Schimel, 1995). Further, nitrification and denitrification processes of N-cycling are facilitated by soil microorganisms (Hayatsu et al., 2008). It has been estimated that nearly 10000 bacterial species are present in one gram of soil (Torsvik et al., 2002) and their diversity is important in maintaining soil functions. Regulation of nutrient cycling is essential for the integrity of the terrestrial ecosystem. Intensive agriculture management can result in ecosystem degradation and thereby can have a negative influence on soil processes. In some instances, ecosystem services normally provided by soil microorganisms have been replaced by agriculture inputs (Barrios, 2007). Moreover, agriculture management practices and inputs can alter the microbial community composition.

Significant effects of land use changes reportedly are reflected in changes in microbial community composition (Girvan et al., 2003; Ding et al., 2013). For example, long-term organic and conventionally managed cropping systems had distinct microbial structures corresponding to management practices (Hartmann et al., 2015). Nitrogen fertilizer application, tillage and crop residue removal have the ability to alter the total microbial composition and composition of N-cycling and decomposers communities (Jiménez-Bueno et al., 2016). Understanding the responses of soil microbial communities to the ecosystem and environmental changes are essential for managing soil functions (Griffiths and Philippot, 2013). There is no common approach to determining the effect of intensive agriculture management on microbial diversity because the microbial response depends on the magnitude of the cause and soil history (Griffiths and Philippot, 2013). Moreover, changes in microbial diversity in response to a particular management practice can vary over time. The aforementioned studies were conducted within a limited time frame which may not provide a complete picture of the overall consequences of

microbial diversity changes in response to agriculture implications. To address this issue a long-term investigation was needed to assess the microbial diversity within different time frames.

Long-term experiments can capture the different trends in ecosystem adaptations to answer fundamental questions about ecosystem functions (Janzen, 2009). The unique long-term transplanted experimental site was initially established in 1990 at AAFC, Lethbridge to investigate the relationship between soil quality and dryland wheat production in a semi-arid prairie region (Janzen et al., 2012). Further, the study provided opportunities to study the soil itself by decoupling the influences of the confounding factors (i.e., topography, climate and hydrology) from the original transplanted soils. At establishment, 36 different Chernozems were obtained for transplantation from donor sites with different management regimes. Following transplantation to the single site, soils were managed under identical climatic and topographic conditions for over two decades. In particular, continuous cultivation, N fertilizer application (0 and 60 kg N ha^{-1}) and crop residue removal were the common agriculture practices carried out during the regular management of this experiment.

Starting from 1991, soil samples were obtained once every seven years from the transplanted soils, air dried, sieved and stored as archived collections for future use. It is a common strategy to archive soil samples from long-term field experiments which can be used to address future research questions by linking with past unexplored facts (Dolfing and Feng, 2015). Archive soils are resources that provide researchers with opportunities to construct the missing pieces of soil ecology with respect agriculture management practices. Archived soils can be used to track the soil microbial diversity responses to management and climatic changes. Even 50 yr old archived samples have been used to investigate microbial community changes (Dolfing et al., 2004). The molecular tools used to study microbial ecology are rapidly changing and provide more details than previously. The presence of an archived soil collection gives the opportunity for deeper exploration of microbial profiles with advancing technology (Dolfing et al., 2004; Clark and Hirsch, 2008).

The soils from the archive collection of the transplanted soil quality experiment were previously used to track the changes that have occurring in the soil chemical properties. The changes occurred in microbial communities over time have not explored in transplanted soils

compared to the original soils in 1990. For this, a survey was conducted 21 yr post-transplantation to determine the effects of long-term cultivation and N fertilizer application on the microbial community structure compared to the original soils (1990). Archived soils from 1990 and 2011 collections were comprehensively surveyed through high-throughput DNA sequencing of 16S *rRNA* gene profiles.

The main goal of this study was to survey the microbial community profiles of the transplanted soil at the time of field establishment and after 21 yr of establishment using archived soils. The objectives were: 1) to track the changes that may have occurred in microbial community structure after 21 yr in the transplanted soils; and 2) to determine the impacts of +/- N fertilization, continuous wheat cultivation and crop residue removal on microbial community structure of transplanted soils.

4.4. MATERIALS AND METHODS

The long-term transplanted field trial located in AAFC, Lethbridge is an important study in addressing ecological issues relevant to soil factors and soil origin by decoupling the confounding effects associated with different sites (i.e., climate, topography, and management differences between sites). The current study was conducted using the original transplanted soils and soils archived in 2011 (21 yr after transplanting) to track the microbial community profile changes that have occurred over time.

4.4.1 Establishment of long-term transplanted plots

The long-term transplanted experiment site is located at the AAFC research station, Lethbridge, AB, Canada (49°42' N 112°50' W) and has been described by others (Zvomuya et al., 2008; Yanni et al., 2016)). Briefly, the original soil at the site was a Dark Brown Chernozem (Typic Boroll) with a 20 cm Ap horizon, a thin B horizon (0 to 10 cm) and a calcareous C horizon (Olson et al., 1996; Zvomuya et al., 2008). This site had been continuously cropped until 1988 and fallowed until the transplantation experiment was established in 1990 (Zvomuya et al., 2008).

Prior to transplanting, the top soil was removed from the field and the exposed sub-surface soil was tilled to roughen the interface between the existing and transplanted soils. Temporary wooden frames were used along the plot (5 × 6 m) boundaries to prevent soil mixing (Zvomuya et al., 2008). Thirty-six different Chernozems, from diversely managed donor sites, located within 100 km radius of the experimental field were selected for transplanting. Most of the selected donor sites were long-term experimental fields within the research station. Soils were collected from the Ap or Ah horizons using a Bobcat skid steer loader and transported to the experimental site by trucks (usually one truck load per plot); in two cases, sub-surface soils from the B and C horizons from an eroded grassland also were included. At the time of transplanting, these soils had diverse chemical, physical and biological properties that arose as a result of different origins (location and management practices) (Olson et al., 1996). Two years after transplanting (i.e., in 1992), the mean depth of the deposited soil layer was 19 cm (Zvomuya et al., 2008) (Fig. 4.1). The experimental units were arranged using a split plot design. Transplanted

soil type was the main plot factor (6 × 5 m) while the rate of N fertilizer was the subplot factor which consisted of 0 kg N ha^{-1} (6 × 3 m) and 60 kg N ha^{-1} (6 × 2 m) (Zvomuya et al., 2008).

Starting in 1991, the experiment field was continuously cropped with spring wheat (*Triticum aestivum* L.). Before seeding, N fertilizer was broadcasted onto the appropriate subplots. Phosphorous fertilizer was applied with the seed at a rate based on the soil test P levels for each transplanted soil (13 to 20 P kg ha^{-1}) (Zvomuya et al., 2008; Janzen et al., 2012). In 1992 and 1997 seed germination was poor due to severe drought; therefore, the field was chemically fallowed using glyphosate for the remainder of the growing season. Each season, harvesting was done manually from a selected area and the remaining plant materials were removed mechanically from the field (Zvomuya et al., 2008). All of the agronomic practices were conducted in such a way as to minimize soil disturbance, which included the use of no-till management (Olson et al., 1996; Janzen et al., 2012).

In 1990, at the time of establishment, soil samples from each plot were collected from the 10 cm depth and air-dried, ground and stored at room temperature as archived soils for future use (Zvomuya et al., 2008). Soil sampling for the field trial was carried out every seven years (1997, 2004 and 2011) for a standard suite of soil analyses, and sub-samples were preserved in the soil archive (Janzen et al., 2012).

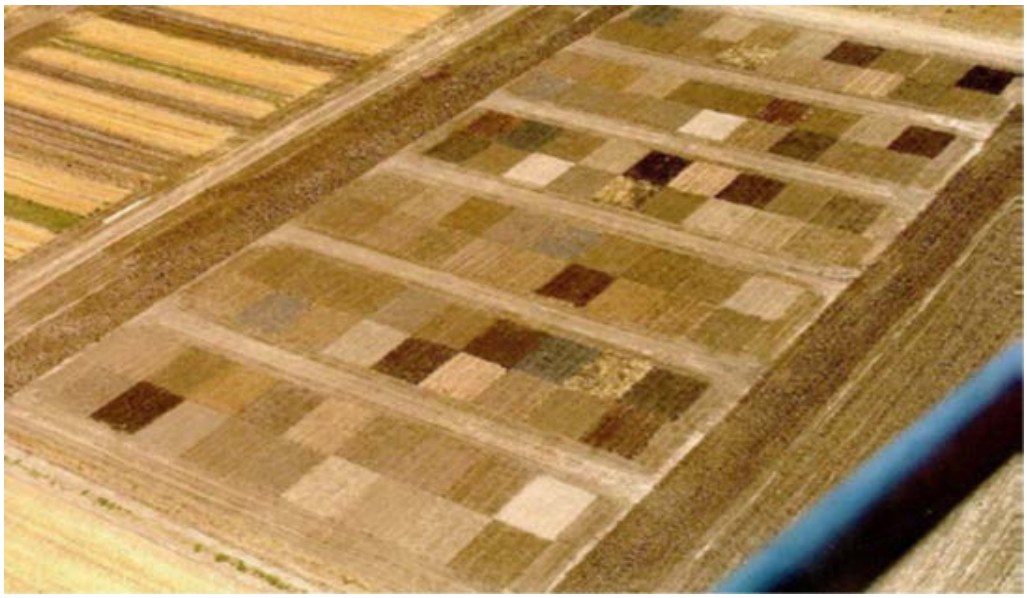


Fig. 4.1. Aerial view of transplanted field trial, after established in 1990 (adapted from Janzen et al., 2012).

4.4.2 Soil selection and sampling

For the current study, 10 archived soils and their +/- N sub-plots were selected. The selection was based on the soil origin and fertility levels (C and N content) to encompass soils with a broad range of fertility prior to transplanting. The summary of selected soil zones, textures and origins are listed in Chapter Three (Table 3.1) and included: previously uncultivated (native grassland - NG; pastureland - PL; dryland tilled fallowed (obtained from a pathway to an experiment field) - DTF; irrigated tilled fallowed - ITF; continuously cultivated and conventionally managed (cereal cultivated - CC; dryland wheat - DW); continuously cultivated and manure amended (RM (manured 30 t ha^{-1}); HM (manured 90 t ha^{-1})) and sub-surface soils from an eroded native grassland (B horizon - BH); C horizon - CH). The archived collection from 1990 had the original soils prior to imposing the N treatments (10 soil types × 3 replicates; n=30) while the 2011 archived collection had the corresponding soils with the addition of the N treatments (10 soil types × 2 N levels × 3 replicates; n=60).

Selected soils had different origins that resulted in diverse physical, chemical and biological properties at the time of transplanting. Total OC content was highest in PL followed by CC, whereas DTF, BH and CH soils had the lowest OC (Chapter 3, Fig. 3.2). A similar pattern was observed in the OC content of the archived soils collected 21 yr after transplantation (Chapter 3, Table 3.10). Total N content varied similar to the OC content for particular soil (Chapter 3, Fig. 3.3).

4.4.3 DNA extraction and Miseq Illumina sequencing of archived soils

Prior to DNA extraction, archived soil samples were rehydrated according to the method suggested by Clark and Hirsch (2008) to increase the quality and quantity of the DNA extracted. The gravimetric moisture content of each fresh field collected soils in 2012 were used as a guide to determine the amount of water needed for rehydration of archived soils. The moisture content of archived soil samples was adjusted to the wet weight (0.25 g) of fresh soil (2012) by adding UltraPure™ DNase/RNase-Free Distilled Water (ThermoFischer Scientific Life Technologies Inc. 5250 Mainway, Burlington, ON) and samples were incubated at 4ºC for 30 min.

Following rehydration, DNA was extracted from 0.25 g of rehydrated archived soil using a PowerSoil DNA isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA). For each sample,

DNA from two technical replicates was extracted. DNA concentrations were measured using a Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) with Qubit® dsDNA HS assay kit (Thermo Fisher Scientific, Waltham, MA USA). Variable DNA concentrations were observed for different soils at the two sampling times. Except for DW, CC, and PL soils, the rest of the transplanted soils had higher DNA concentration in 1990 archived collection than the 2011(Fig. 4.2). Extracted DNA was stored in -80ºC until further sequencing.

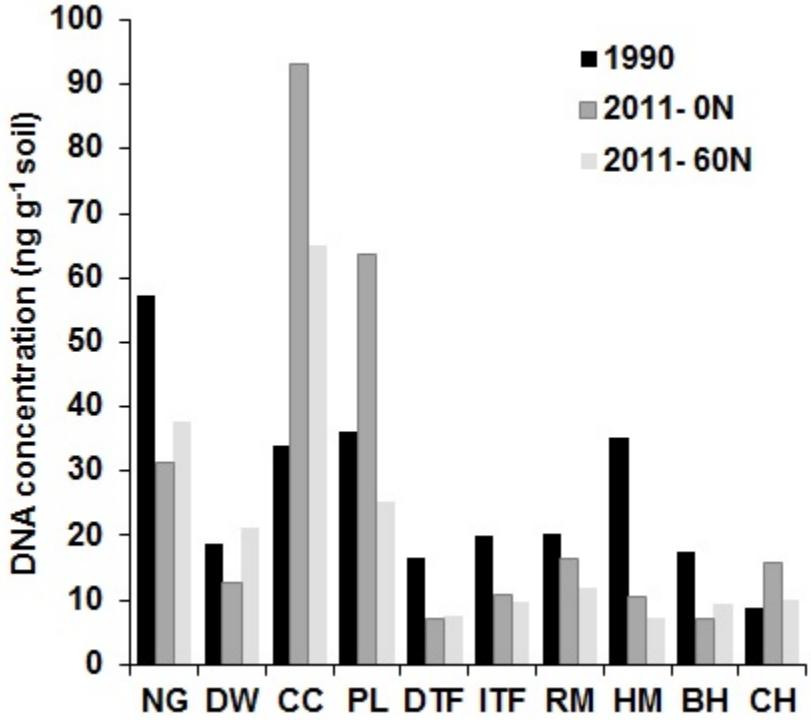


Fig. 4.2. DNA quantities yielded from 1990 and 2011 archived soils. NG- native grassland, DW- dryland wheat, CC- cereal cultivated, PL- pastureland, DTF- dryland tilled fallow, ITF- irrigated TF, RM- manured 30 t ha^{-1}, HM- manured 90 t ha^{-1}, BH- B horizon, CH- C horizon. N^- and N^+ are unfertilized and N fertilized (0 and 60 kg N ha^{-1}) for 2011 soils respectively.

To determine the diversity of bacterial and archaeal communities, 16S *rRNA* gene libraries were constructed using PCR amplification with the 515F (GTGCCAGCMGCCGCGGTAA)/ 806R (GGACTACHVGGGTWTCTAAT) primer pair which targets the V4 region of both bacteria and archaeal 16S *rRNA* genes (Caporaso et al., 2012; Wu et al., 2015). The PCR reagents included 13.0 µL PCR grade water, 10.0 µL Five Primer Hot Master Mix, 0.5 µL each of forward and reverse primers (10 µM), and 1 µL template DNA, for a total reaction volume of 25 µL. The PCR conditions (for a 96 well plate) were 94ºC for 3 min; 35 × (94ºC for 45 seconds, 50ºC for 60 seconds, 72ºC for 90 seconds); 72ºC for 10 min; 4ºC hold. Samples were amplified

in triplicates and pooled for further analysis. They were run on an agarose gel to confirm the product size (300 to 350 bp) (Caporaso et al., 2012). Amplicons were quantified using Picogreen and equal quantities (240 ng) from each sample were pooled for sequencing. Amplicons were purified and quantified for concentrations. An aliquot of purified products was sequenced by the Miseq Illumina sequencing technique (Caporaso et al., 2012).

4.4.4 16S *rRNA gene sequence data processing and statistical analysis*

The Mothur v. 1.36.0 software package (Kozich et al., 2013) and the pipeline required to analyze Illumina Miseq sequencing data were obtained from the Mothur website (http://www.mothur.org/wiki/MiSeq_SOP) on November 2015 (Schloss et al., 2009). First, the reads were joined together to prepare contigs. Ambiguous base calls with unusually long reads without any meaningful overlap within the sequences were omitted (Kozich et al., 2013). Following alignment of the sequence with Silva's bacteria reference database, sequences that did not align with the reference database were removed. Samples were further de-noised using a pre-clustering algorithm which split the samples into groups and arranged them according to the abundance (high to low). Chimeras were removed using the uchime algorithm. The taxonomic classification was done with a naive Bayesian classifier. Gene sequences that classified into the sequence of mitochondria and eukaryotes were removed. When assigning OTUs, first sequences were split into groups, then assigned to OTUs at a 0.03 dissimilarity level (97% similarity) (Kozich et al., 2013). Rarefaction curves for Illumina Sequencing data of 1990 and 2011 archived soils are presented in Fig. B.1.

PCOrd software version 6 (Gleneden Beach, OR 97388, USA) was used to perform Principal Coordinate Analysis (PCoA) to investigate the ordination of 16S *rRNA* gene community profiles of the original and 2011 transplanted archived soils at the species level. Non-metric multidimensional scaling (NMDS) was done at the order level for manured soils to observe the effect of transplantation and management practices on community profiles. Multi-Response Permutation Procedure (MRPP) was used to compute the group difference within the treatments. Bray-Curtis dissimilarity indices were used to calculate the distance matrix in the non-parametric multivariate analysis. Alpha diversity was assessed using common metrics; observed species richness (Sobs), Chao 1 and inverse Simpson diversity indexes were generated using R statistical

software (R i386 3.2.3) using the functions in the Vegan package (https://www.r-project.org/). DNA concentrations, the relative abundance of phyla and diversity indices were statistically analyzed to determine the significant effects of treatments using PROC MIXED procedure in SAS 9.6 (SAS Institute Inc, NC, USA).

4.5. RESULTS

Using high throughput sequencing, 821 931 qualitative sequences were detected for the V4 variable region of the 16S *rRNA* gene. After quality control and chimera removal, a total of 12 153 operational taxonomic units (OTU) were generated at 97% similarity level. Except for the PL soil, rarefaction curves of the soils reached a plateau indicating that the number of sequences used was sufficient to capture the microbial diversity within the archived transplanted soils (Fig. A2.1). Shifts in microbial community structures occurred 21 yr after transplantation to varying degrees, depending on the soil type. It is possible that these changes were either related to differences between original soils or changes that occurred in soils over time. However, the microbial community structure differences clearly existed at different taxonomic levels for particular soils.

4.5.1. Microbial community structure

Overall, 17 bacterial and two archaeal phyla were observed in soils across both years (1990 and 2011) and N levels (0 and 60 kg ha^{-1}). The *Actinobacteria*, *Proteobacteria*, and *Acidobacteria* phyla dominated the microbial communities of the transplanted Chernozems (Fig. 4.3). Nearly 50% of the total sequences were represented by the *Actinobacteria* phylum followed by *Proteobacteria* (21%). Prominent changes over time at the phyla level were observed for PL, CC and sub-surface (BH and CH) soils compared to the original soils. The abundance of *Actinobacteria* was significantly higher in the low fertility soils (BH, CH and ITF) (P<0.001) and comprised nearly 50% of the community profiles in 2011. Similar to the 2011 profiles, *Actinobacteria* abundance was high in 1990 profiles of sub-surface soils from B (59%) and C horizons (57%). Except for NG and sub-surface soils, the relative abundance of *Actinobacteria* increased in the remaining soils 21 yr after transplantation. A reduction in relative abundance of *Bacteroidetes* was found in 2011 for all soils. Further, the relative abundance of *Proteobacteria* decreased in high fertility soils over time relative to the original soil sampled in 1990 and this reduction was more distinct in the PL soil.

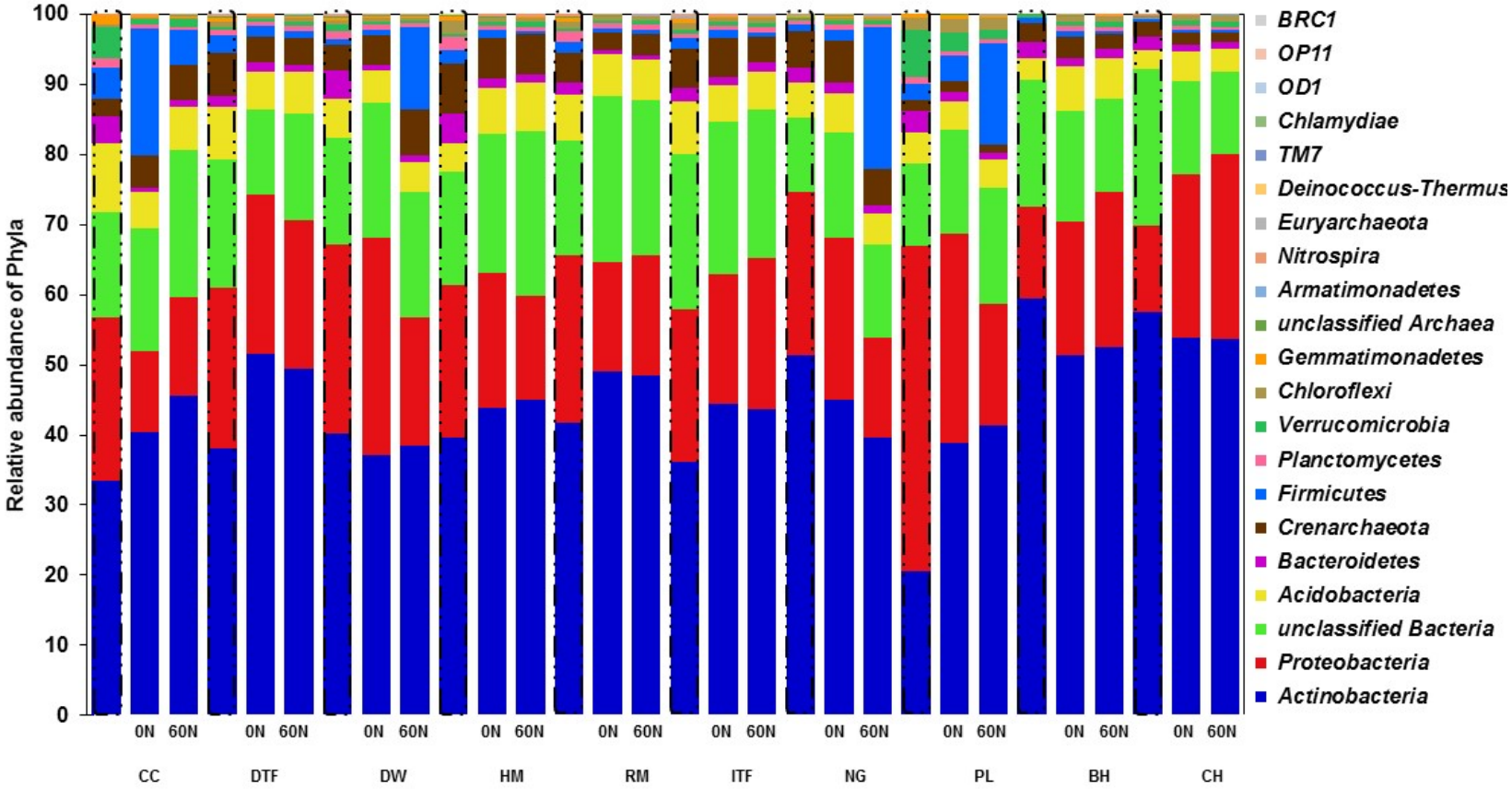


Fig. 4.3. Relative abundance of phyla observed from archived soils obtained at the beginning (1990) and 21 yr (2011) after transplantation. NG- native grassland, DW- dryland wheat, CC-cereal, DTF- dryland tilled fallowed, RM- manure 30 t h^{-1}, HM- manure 90 t ha^{-1}, ITF- irrigated tilled fallow, PL- pastureland, BH- B horizon, CH-C horizon. **Bars bordered by a dashed line represent the original soils for 1990; 0N and 60N are unfertilized and N fertilized (0 and 60 kg N ha^{-1}, respectively) for 2011 soils.

The *Proteobacteria* classes present in the transplanted soils exhibited different relative abundances in 2011 compared to the original soils (Fig. 4.4). These changes were more evident in CC, PL and NG soils. The *Betaproteobacteria* was the predominant *Proteobacteria* class found in original PL soils. After transplantation and continuous wheat cultivation, the relative abundance of *Betaproteobacteria* was reduced in both N fertilized and unfertilized PL soils. Similar trends were observed in sub-surface soils (BH and CH). In CC and NG soils *Alphaproteobacteria* was the dominant *Proteobacteria* class in the 1990 soils. Similar to the PL soil, a reduction in *Betaproteobacteria* abundance was observed in NG and CC soils. Further, in the 2011 unfertilized PL soil, the *Proteobacteria* community was dominated by the *Gammaproteobacteria* class while in the N fertilized soil *Alphaproteobacteria* dominated. Such changes were not observed in CC and NG soils.

The changes observed in *Proteobacteria* at the class level were also reflected at the order level. The relative abundance of orders *Rhodospirillales* and *Sphingomonadales* which belongs to the class *Alphaproteobacteria* increased in the 2011 PL soils following a change in land use to continuous wheat cultivation and residue removal (Fig. 4.5). This increase was prominent in the N fertilized PL soils. Bacteria belonging to the order *Burkholderaceae* (class *Betaproteobacteria*) occupied 65% of the total *Proteobacteria* community in the original PL soil but was reduced in 2011 where N fertilized and unfertilized soils only had 4% and 9%, respectively. Higher abundance of *Enterobacteriales* was found in N fertilized PL soil. *Alteromonadales* was identified in 2011 PL soils (but not in 1990 soils) with higher abundance in N unfertilized soil (Fig. 4.5). Changes in PL soil were also observed in NG and high fertility CC soil at the order level (Fig. 4.6). However, *Alteromonadales* was only detected in the N fertilized NG and CC soils.

The high relative abundance of the *Chloroflexi* phyla was observed for both 1990 and 2011 in the PL soil. Among transplanted soils, NG, DW and PL soils had *Firmicutes* in higher abundance. The *Clostridia* was the dominant *Firmicutes* class present in the original PL soil; however, *Bacilli* became dominant in 2011. In CC and NG soils *Bacilli* was the dominant *Firmicutes* class at all the times. A decrease in the relative abundance of *Verrucomicrobia* was also observed in PL and CC soils in 2011 (Fig. 4.7).

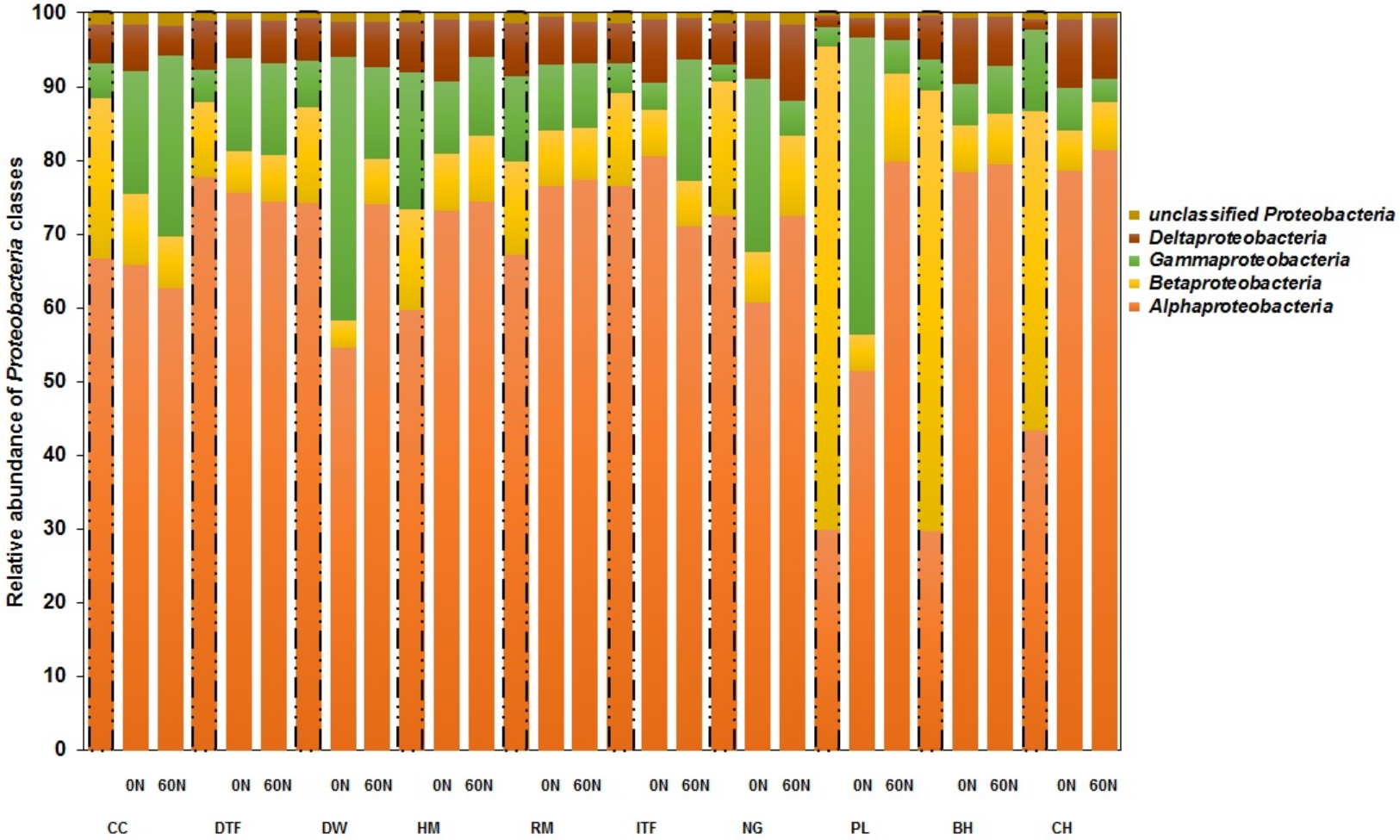


Fig. 4.4. Relative abundance of *Proteobacteria* classes observed from archived soils obtained at the beginning (1990) and 21 yr (2011) after transplantation. NG- native grassland, DW- dryland wheat, CC-cereal, DTF- dryland tilled fallowed, RM- manure 30 t ha^{-1}, HM- manure 90 t ha^{-1}, ITF- irrigated tilled fallow, PL- pastureland, BH- B horizon, CH-C horizon. **Bars bordered by a dashed line represent the original soils for 1990; 0N and 60N are unfertilized and N fertilized (0 and 60 kg N ha^{-1}, respectively) for 2011 soils.

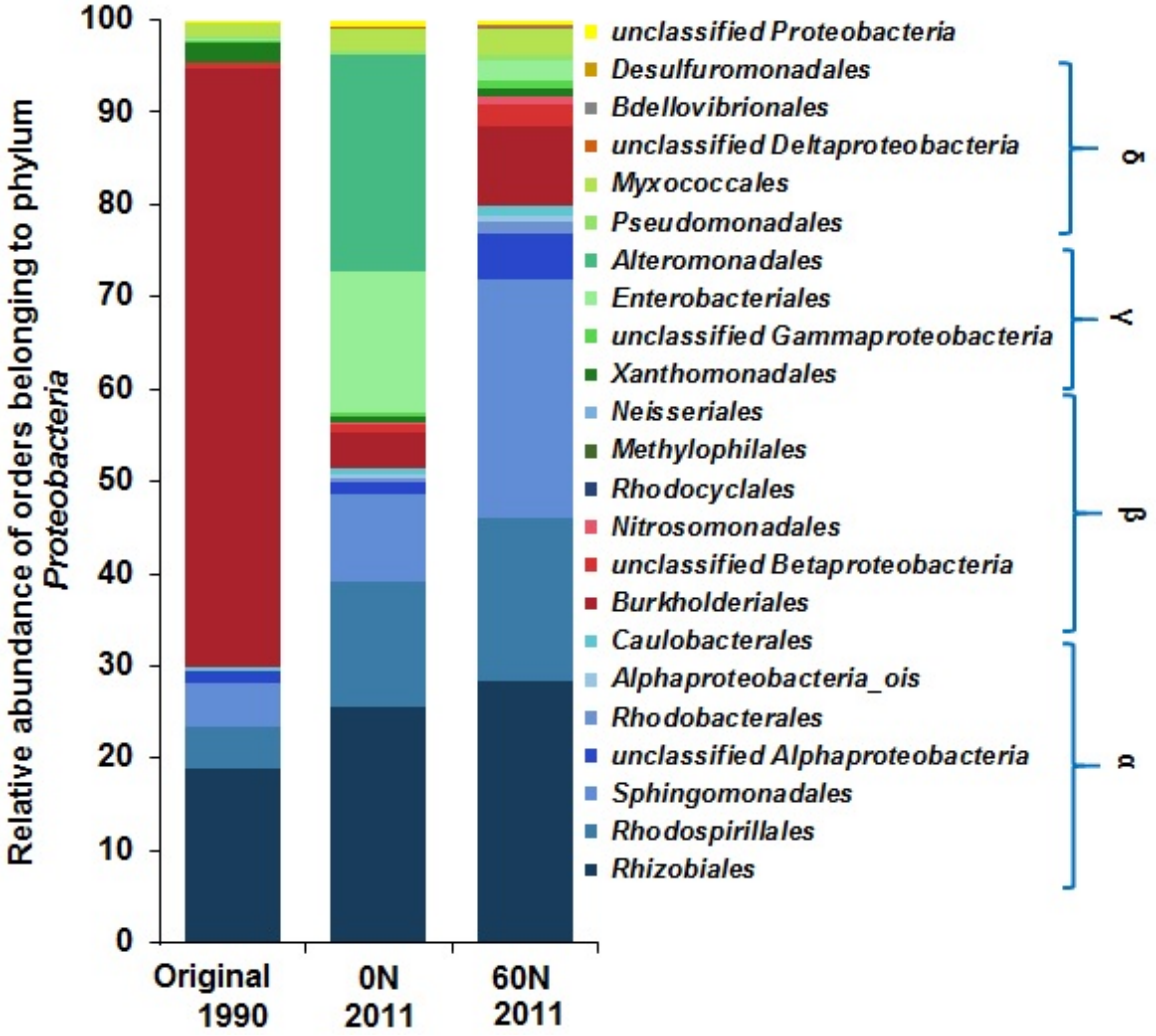


Fig. 4.5. Phylogenetic comparison of phylum *Proteobacteria* at order level for archived pastureland (PL) soils. 0N and 60N are N rates of 0 and 60 kg N ha^{-1} for 2011 soils.

Changes in microbial community structure following transplantation differed in low fertility soils (DTF, BH, and CH) which were compared to high fertility soils (CC and PL). For example, distinct changes in the *Proteobacterial* community were not apparent in the DTF soil at either class or order levels compared to the original archived soils. In sub-surface soils, a decrease in the *Betaproteobacteria* and an increase in *Alphaproteobacteria* occurred between the 1990 and 2011 soils (Fig. 4.8). Similar to PL, NG and CC soils, the relative abundance of *Burkholderiales* decreased and *Rhodospirillales* increased in sub-surface soils after transplantation. A reduction in relative abundance of *Pseudomonadales* and *Caulobactrales* was seen in 2011 sub-surface soils (BH and CH) (Fig. 4.9). Changes occurred in phylum *Chloroflexi* in sub-surface soils from

1990 to 2011. *Ktedonobacteria* class found in BH soils in 1990 were undetected in 2011 soils (Fig. 4.10). In contrast, *Ktedonobacteria* class was found in 2011 unfertilized CH soils but was not present in the original CH soil. The presence of the *Chloroflexi* class in both sub-surface soils was observed in 2011 (Fig. 4.10). The presence of *Subdivision3 Verrrucomicrobia* class was observed in 2011 soils compared to 1990 (Fig. 4.11).

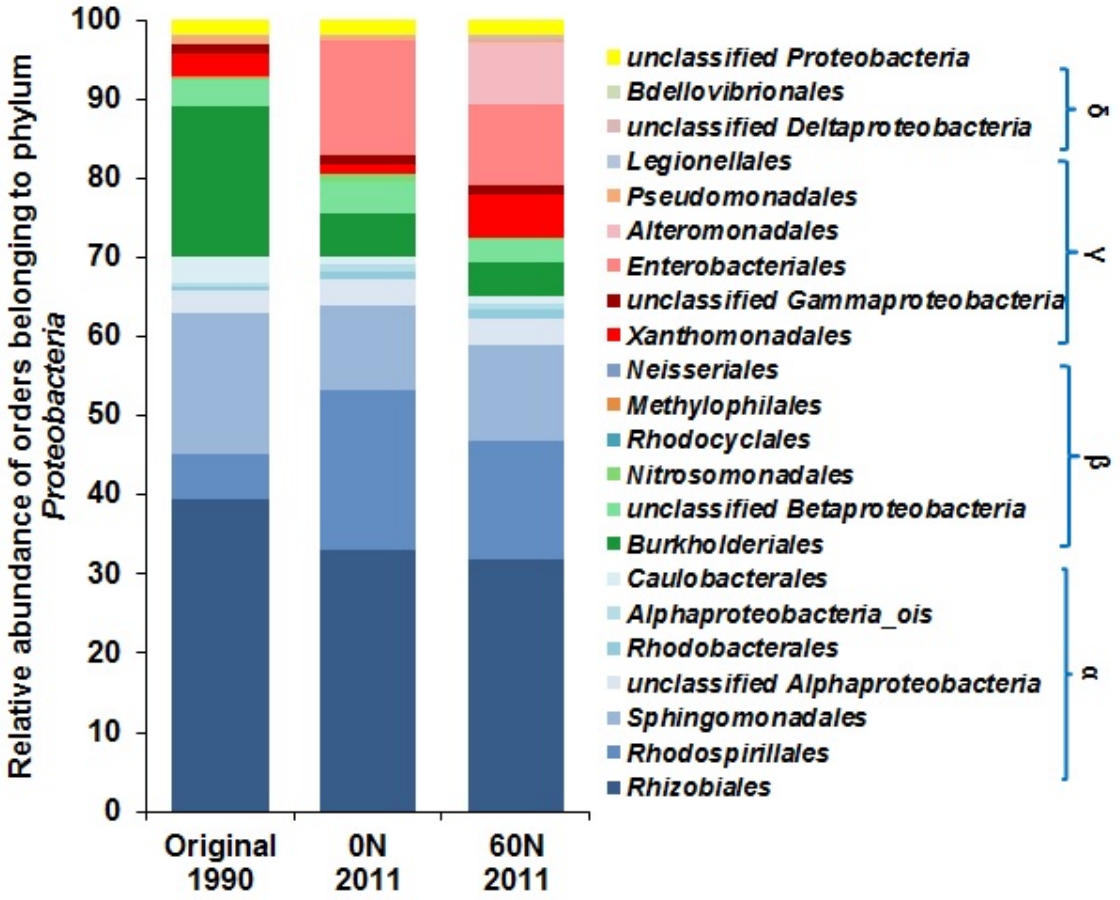


Fig. 4.6. Phylogenetic comparison of phylum *Proteobacteria* at order level for archived cereal cultivated (CC) soils. 0N and 60N are N rates of 0 and 60 kg N ha^{-1} for 2011 soils.

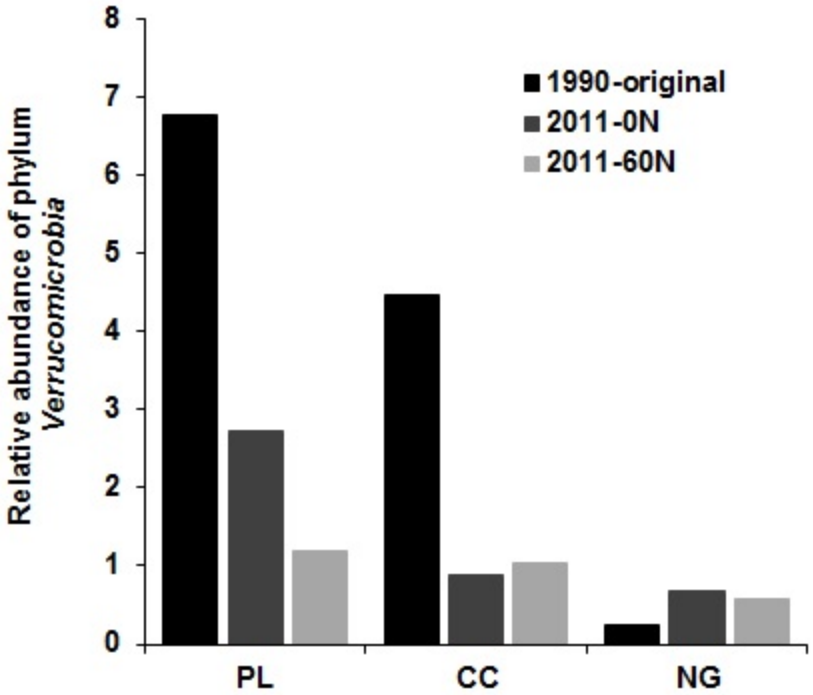


Fig. 4.7. Relative abundance of phylum *Verrucomicrobia* for archived pastureland (PL), cereal cultivated (CC) and native grassland (NG) soils. 0N and 60N are N rates of 0 and 60 kg N ha^{-1} for 2011 soils.

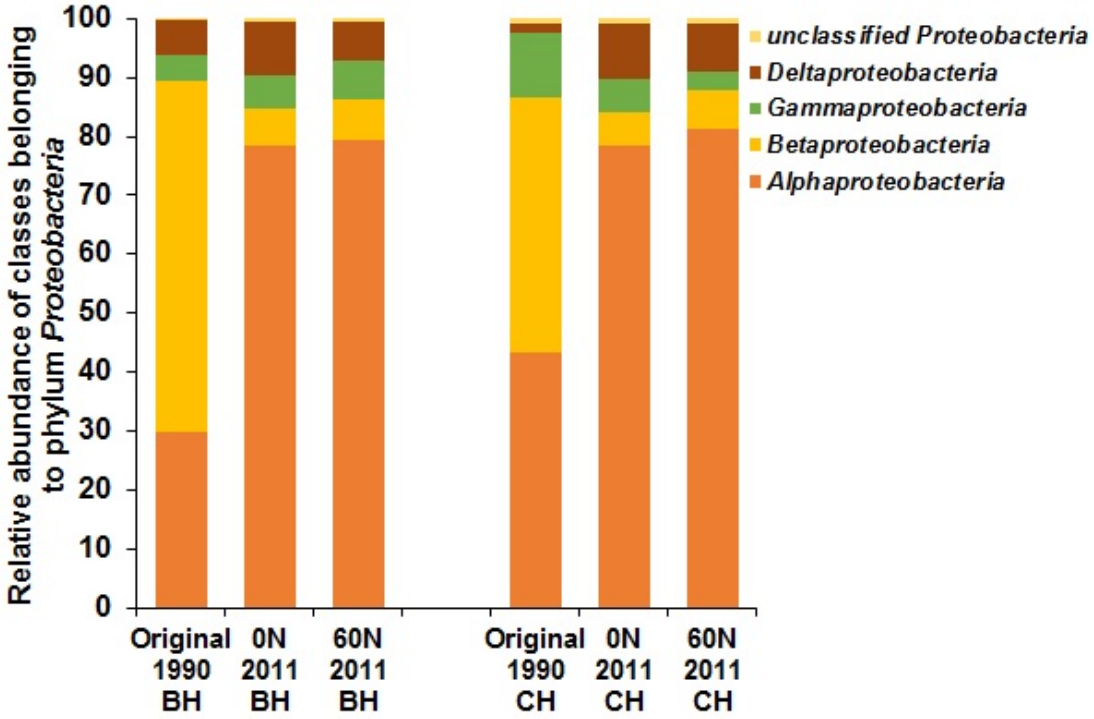


Fig. 4.8. Distribution of *Proteobacteria* classes in sub-surface soils. (BH- B horizon, CH- C horizon). 0N and 60N are N rates of 0 and 60 kg N ha^{-1} for 2011 soils.

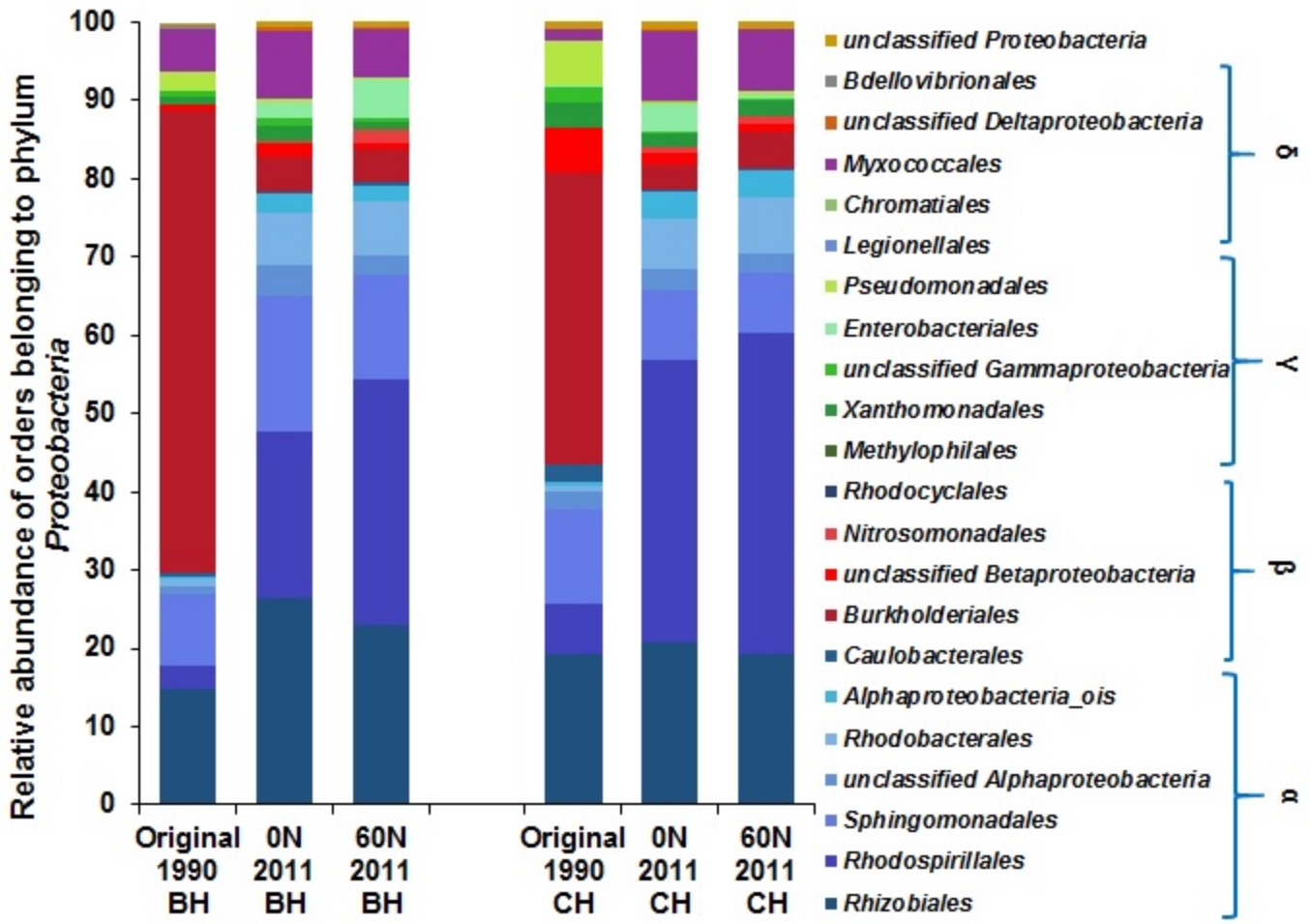


Fig. 4.9. Distribution of *Proteobacteria* orders in sub-surface soils. (BH- B horizon, CH- C horizon). 0N and 60N are N rates of 0 and 60 kg N ha^{-1} for 2011 soils.

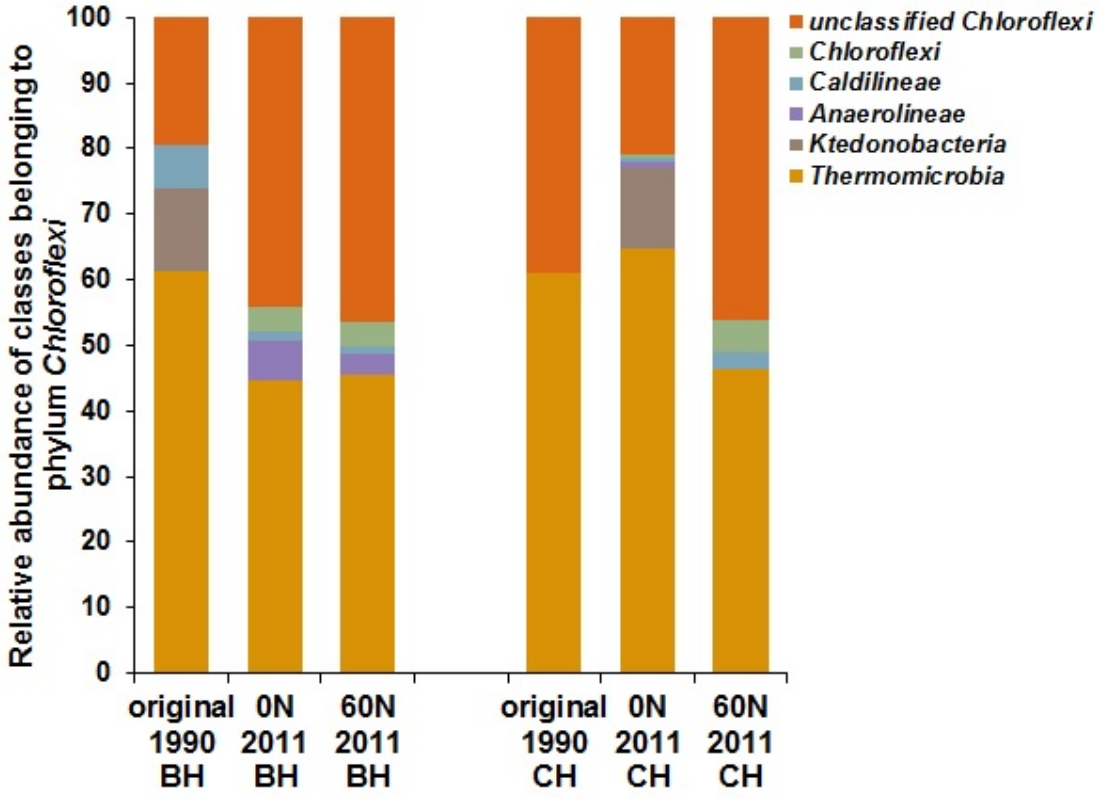


Fig. 4.10. Distribution of *Chloroflexi* orders in sub-surface soils (BH- B horizon, CH- C horizon). 0N and 60N are N rates of 0 and 60 kg N ha^{-1} for 2011 soils.

Significant differences were not detected in long-term manured soils (RM, HM) at the phyla and class levels. However, ordination analysis using NMDS analysis conducted at the order level indicated a shift between fertilized and unfertilized 2011 RM and HM microbial community profiles as compared to the original soils (Fig. 4.12). The N fertilized RM community structure shifted closer to the HM. Further, 2011 HM soil had lower *Chloroflexi* abundance than the original soil.

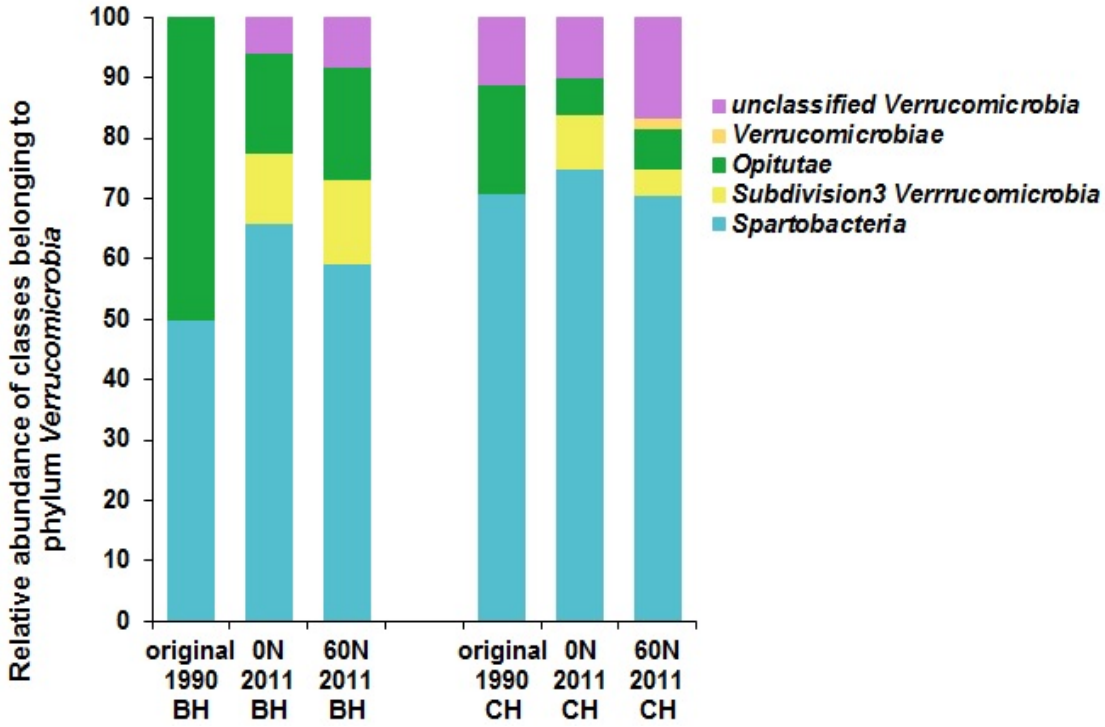


Fig. 4.11. Distribution of *Verrucomicrobia* classes in sub-surface soils (BH- B horizon, CH- C horizon). 0N and 60N are N rates of 0 and 60 kg N ha^{-1} for 2011 soils.

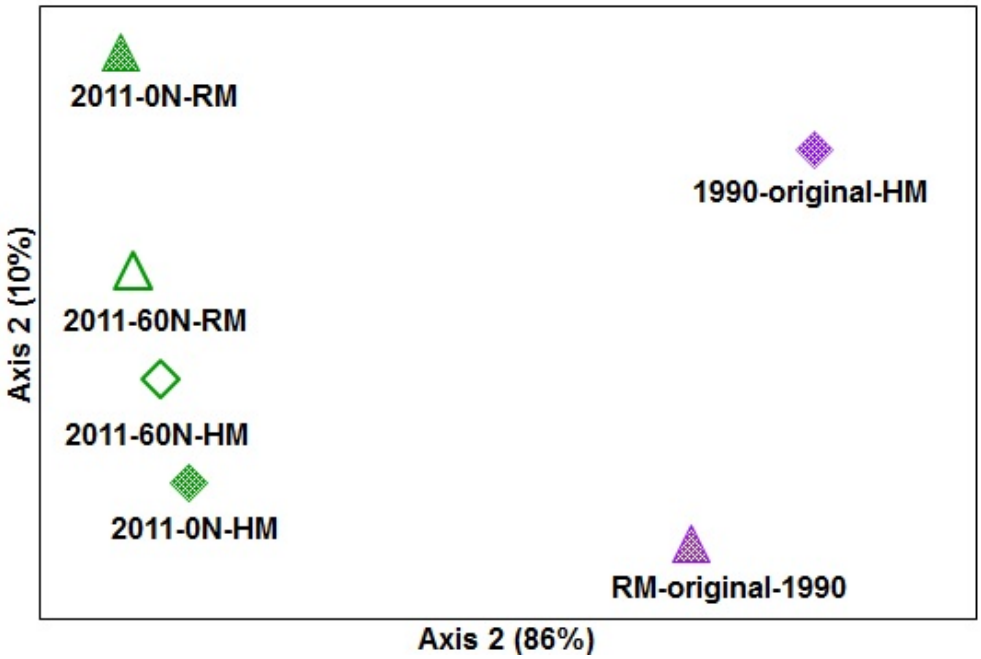


Fig. 4.12. Non-metric multidimensional scaling (NMDS) analysis for microbial communities of the manured archive soils at order level. RM- manure 30 t ha^{-1}, HM- manure 90 t ha^{-1}, N^- and N^+ are unfertilized and N fertilized (0 and 60 kg N ha^{-1}) for 2011 soils.

Two decades after transplantation, the microbial community composition had shifted to varying degrees depending on the soil origin (Fig. 4.13). Different microbial communities were found in PL, BH, CH, NG, CC, RM and HM soils in 2011 compared to the initial soil. The microbial community profile of the PL soil that was obtained from a native pastureland had changed substantially with continuous wheat cultivation and crop residue removal. The soil transplanted from native grassland (NG) and continuous cereal cultivation (CC) also indicated a shift in the microbial community structure over time. Convergence in the structure of the sub-surface soil (BH and CH) microbial communities towards a structure more similar to the top soils were also observed 21 yr after transplantation; however, N fertilization was not the dominant driver of the changes. At the beginning of the transplantation, manured soils (HM and RM) had different microbial community structures depending on whether a high or recommended rate of wet manure was applied prior to transplantation; however, clustering of community profiles of RM and HM soils in 2011 emphasized that continuous cultivation and N fertilizer application had minimized the initial differences with respect to manure rate. The effect of N fertilizer application varied depending on the origin of the particular transplanted soil. For example, N fertilized soils from the BH, CH, CC, HM, DTF, DW, and ITF did not have distinct community profile changes compared to the unfertilized soil. However, the N fertilizer application resulted in different microbial communities in PL and NG soils that were originally from native ecosystems and had not previously received inorganic N fertilizer. Total C, N and OC contents were positively correlated with PL and CC microbial communities. Further, microbial communities in high fertility and sub-surface soils were positively correlated with *Verrucomicrobia* and *Actinobacteria*, respectively, at the OTU level (Fig. 4.13).

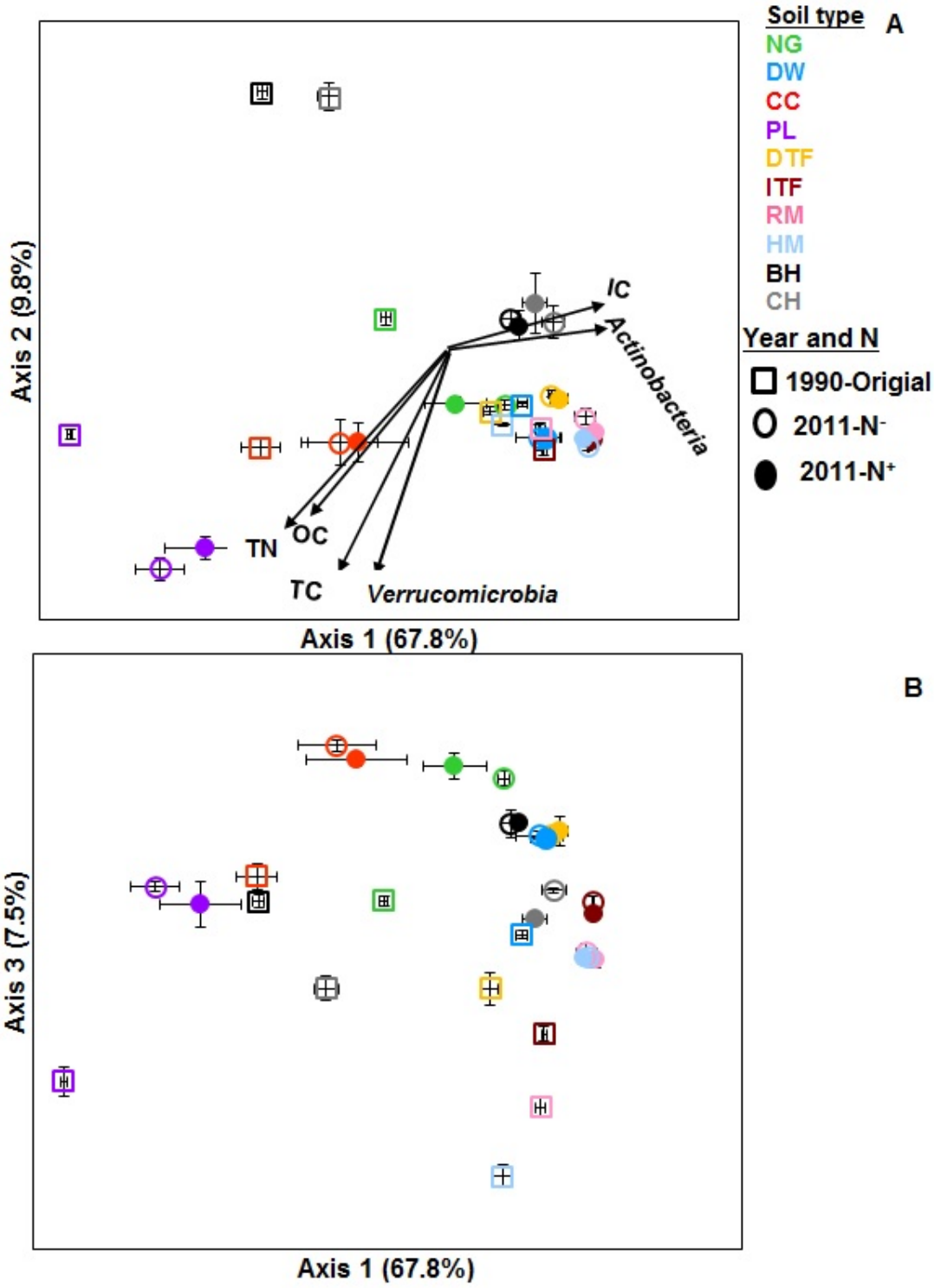


Fig. 4.13. Principal coordinate (PCoA) analysis for OTUs observed in microbial communities of the transplanted soils from 1990 and 2011 archive collections. A) Axis 1 and 2, B) Axis 1 and 3. NG- native grassland, DW- dryland wheat, CC-cereal, DTF- dryland tilled fallowed, RM- manure 30 t ha^{-1}, HM- manure 90 t ha^{-1}, ITF- irrigated tilled fallow, PL- pastureland, BH- B horizon, CH-C horizon.

4.5.2 Diversity indices

The species richness of the transplanted soils with respect to the sampling year and N level was measured using alpha diversity indices Chao 1 (Chao, 1984) and observed species richness (Sobs) (Table 4.1). Higher Sobs was found in 1990 high fertility (PL, CC) and DTF, ITF, RM and HM soils than the 2011 archived soils. Original PL soil had some community members in higher abundance than the others (low species evenness) and converting to annual cropping increased the evenness as a result of the change in land use. In the CC, DTF and manured (HM and RM) soils reduction of Sobs, Chao 1 and inverse Simpson for 2011 implied a reduction of both species richness and evenness. Depending on the N rate, no difference was observed in species abundance of CC soil. Although Sobs and Chao 1 increased in 2011 for NG and DW soils, lower inverse Simpson values indicated a reduction in the evenness. In sub-surface soils (BH and CH), the species richness and evenness increased after transplantation. For the BH soil, increases in the inverse Simpson index in both 0 and 60 kg of N ha^{-1} indicated closer relative abundance levels among the community members than observed in the original soil. However, the above-mentioned trend was only observed for the CH soil that did not receive N fertilizer.

Table 4.1. Richness and diversity estimates for transplanted soils from 1990 and 2011 (N^+/N^-) rarefied to minimum read number.

Soil[†]	Sobs			Chao 1			Inverse Simpson (1/λ)		
	1990 Original	2011 (0 kg N ha^{-1})	2011 (60 kg N ha^{-1})	1990 Original	2011 (0 kg N ha^{-1})	2011 (60 kg N ha^{-1})	1990 Original	2011 (0 kg N ha^{-1})	2011 (60 kg N ha^{-1})
NG	731±120	829±12	773±93	773±129	889±15	830±81	82±10	54±27	35±20
DW	601±351	737±72	709±57	667±350	795±66	768±62	84±22	52±41	46±31
CC	916±83	799±95	798±63	953±92	847±76	839±46	103±8	38±24	79±7
PL	693±66	619±124	570±102	638±119	604±43	720±68	12±4	37±26	53±24
DTF	828±31	587±245	681±58	876±33	658±212	725±71	82±8	70±10	78±2
ITF	840±92	778±87	816±95	895±86	831±86	866±96	76±6	71±5	68±14
RM	930±38	511±300	731±27	968±38	559±283	783±31	91±6	65±10	70±3
HM	992±64	798±12	762 ±78	1017±68	856±9	810±66	96±2	73±1	68±4
BH	573±66	796±87	739±103	593±72	847±84	778±119	43±8	90±5	80±7
CH	585±71	608±271	720±62	603±80	649±271	751±68	57±5	80±8	64±6

[†]NG- native grassland, DW- dryland wheat, CC- cereal, PL- pastureland, DTF- dryland tilled fallow, ITF- irrigated tilled fallow, RM- manured 30 t ha^{-1}, HM- manured 90 t ha^{-1}, BH- B horizon, CH- C horizon

4.6. DISCUSSION

The current study explored how changing management practices influence the microbial community structures of the transplanted Chernozems of different origin, managed under identical climatic and topographic conditions for 21 yr. DNA was successfully extracted from the 1990 and 2011 archived soils of the soil quality experiment (Fig. 4.2). Previously, Clark and Hirsch (2008) were able to extract DNA successfully even from samples archived in the 19th century. These researchers reported that rehydration of dried soil samples increased the quality and quantity of extracted DNA. Most of the archived soils from 1990 resulted in larger DNA concentrations than 2011 archived soils which were processed by sieving and drying. Prolonged drying removed moisture from the soil and may have disintegrated bonds between DNA and OM, resulting greater DNA quantities in 1990 archived soils than 2011. Variable amounts of DNA were extracted from the transplanted soils. It has been found that the microbial biomass in higher OM soils has a greater chance of survival under long-term storage (up to 103 yr) (De Nobili et al., 2006). This was not completely in agreement with the current study as high fertility soils (i.e., high OM and nutrients) such as CC and PL did not have the highest extractable DNA (Fig. 4.2). Tzeneva et al. (2009) reported that 18% and 21% of the variability in microbial profiles was attributed to drying and storage in two different long-term grassland soils. Significant reductions in species richness and diversity in dried and stored samples were observed compared to fresh soils. The soil drying can alter certain chemical properties. For example, desiccation increases soluble nutrients such as P (Turner and Haygarth, 2001) and OC (Wu and Brookes, 2005). The water extractable C content increases during storage (De Nobili et al., 2006). However, Blake et al. (2000) reported no major changes in the TC and TN with long-term storage (8 to 69 yr). They also observed a small change in soil pH and exchangeable cation content by storing air dried soils for over 27 yr. Most of the transplanted soils from 1990 yielded a substantially greater quantity of DNA than soils from 2011 indicating a minimum effect of prolonged drying and storage conditions on survival of soil DNA (Fig. 4.2). Even in dried and stored soils, the effect of environmental variables (pH and N fertilizer) including agricultural practices were reflected clearly in microbial profiles (Tzeneva et al., 2009). This also implied that there are other factors influencing the DNA quantity of the archived soils other than drying

and storing and changes in soil chemical properties. Higher DNA quantities in older archive soils (1990) might be due to the influence of soil origin at the time of transplanting. Perhaps, the 21 yr effect of agricultural practices and climate change may be the reason behind lower DNA quantities in the soil archived in 2011. Not only the DNA quantities but also the microbial profile variations between archive soil collections were evident over time. The observed species richness was higher for CC, PL, DTF, ITF, RM and HM soils obtained in 1990 emphasizing the ability of various bacterial and archaeal species to survive under prolonged storage conditions (Table 4.1). The greater observed species richness in 21 yr old (1990) samples than one-year old (2011) samples further confirm that drying and storage were not the only drivers influencing the microbial profiles. The observed differences in transplanted soils between 1990 and 2011 could be due differential changes that have occurred as a result of interactions between the soil origin and management factors after transplanting. Since the transplanted soils were obtained from various donor sites with different management regimes, there could be a prolonged influence of soil origin on the microbial profiles. Likewise, 21 yr of continuous cultivation, N fertilizer application and crop residue removal after transplantation are likely responsible for microbial community differences from 1990 and 2011.

The dominant effect of soil origin was evident as different soils had variable microbial community structures even under identical climatic, topographic and management conditions for 21 yr (Fig. 4.13). The effect of soil origin on microbial community structure is in agreement with many studies conducted using transplanted and reciprocal transfer experiments (Hannam et al., 2007; Sun et al., 2013). Other than the soil origin, the history of management is a significant factor that has a lasting effect on microbial community structure (Buckley and Schmidt, 2001; Steenwerth et al., 2002). Greater similarities were observed in community profiles of cultivated land that had been abandoned for seven years as compared to adjacent cultivated land, than similar comparisons with previously uncultivated land (Buckley and Schmidt, 2001). Likewise, various management practices (i.e., uncultivated ecosystem, continuous cultivation, OM application…. etc.) prior to transplanting can be the major reason for variability between the community profiles of transplanted soils. Based on the PCoA analysis, the differences in microbial community structure between the original (1990) and the transplanted soils from 2011 may be a combination of soil origin, land use history and current land use (Fig. 4.13). Variable

community structures were distinguished for each soil type with different origins at both sampling points. This was confirmed by Steenwerth et al. (2002) who observed different microbial community profiles between intensively managed agriculture, grassland and previously cultivated grassland sites in California. Soil characteristics inherent from soil origin and history of land use may produce unique environmental responses to current land management practices which are favorable for certain microbial groups leading to different community composition at 2011.

The influence of current land use on the microbial community profile of a particular soil was apparent through the divergence of community structure as compared to the original soil. Ding et al. (2013) found a significant influence of land use changes on the bacterial community in an alfalfa cultivated field as compared to natural scrubland. The intensity of microbial structural changes due to current land use was related to previous management history (Ding et al., 2013). Similarly, the community profiles of the 2011 transplanted soils varied from the original soil without convergence in community structures in different soils (Figs. 4.3, 4.4 and 4.13). Others have reported that agriculture management practices that influence soil OM content significantly modify the original microbial community structure (Peacock et al., 2001; Enwall et al., 2007; Navarro-Noya et al., 2013). In the current study, long-term crop residue removal was one of the major factors influencing soil OM availability in the transplanted soils after 21 yr (2011). Likewise, N fertilizer application has the ability to alter the microbial community composition (Cederlund et al., 2014). However, soil N was not the most limiting factor for soil microbial communities in CC, DW, ITF, HM, and BH soils. With the exception of the BH soil, all soils had a history of agricultural use (N fertilizer application) which included N fertilizer application prior to transplanting. The changes that occurred in NG, PL, DTF, RM, and CH soils were apparent when the microbial profiles were explored at different taxonomic levels.

Similar to an analysis conducted using fresh soil (Janssen, 2006), the *Actinobacteri*a, *Proteobacteria,* and *Acidobacteria* were the dominant phyla observed in archived soils (Fig. 4.3). Divergence in microbial community structure was observed when comparing the 2011 and 1990 soils (Fig. 4.13). The changes that occurred in the microbial profiles were associated with shifting of the relative abundance of specific microbial phyla at the class and group levels, detection of new DNA signatures from the community and loss of certain DNA signatures that

were present in the original soils (Figs. 4.5 and 4.6). Shifting of the microbial community structure was more obvious in the high fertility soils and soils from native ecosystems, such as NG and PL. The shifts in the microbial community compositions in these soils were mostly associated with the land use changes where soils were subjected to diverse agricultural management practices and different land cover (wheat cultivation) after transplantation. A significant effect of land use on bacterial community structure was apparent between alfalfa cultivation and adjacent scrubland through DGGE analysis in Mexico (Ding et al., 2013). Several studies have emphasized the effect of land use changes on soil nutrient concentrations (Jesus et al., 2009; Groppo et al., 2015; Zhang et al., 2016). In the current study, soil analysis conducted using the fresh soils collected in 2011 indicated that variations in nutrient contents (TC, OC, and TN) during past two decades might be associated with the land use changes such as continuous wheat cultivation, long-term N fertilization and crop residue removal. Except for sub-surface (BH and CH) and DTF soils, the remaining soils indicated depletion in TC, OC, and TN over 21 yr. In addition to the changes that occurred in soil nutrient dynamics, plant community changes with continuous wheat mono-cropping might also account for shifts in the microbial communities in transplanted soils. Land cover type and below-ground inputs are strongly linked with microbial functions (Kardol and Wardle, 2010). Following soil transplantation, a shift in soil C inputs may lead to divergence in the soil microbial composition from the original soil.

Continuous cultivation and residue removal for 21 yr reduced the soil OM and nutrients inputs in high fertility soils, possibly resulting in oligotrophic conditions in most of the soils. Reduction in soil OC and TN from the initiation of the study to 2011 were detected and reported by Yanni et al. (2016). For example, prior to transplantation high fertility soils were established with high C inputs. Subsequent relocation and management of soils that had received low C inputs due to crop residue removal led to low nutrient profiles in PL, NG, and CC soils. Oligotrophic bacteria are common in soils which have low nutrient availability (Fierer et al., 2007). The reduction in soil nutrient status was consistent with an increase in the relative abundance of *Actinobacteria* in most of the soils (except DW, NG and BH, CH) as they are sensitive to soil C: N ratio changes (de Menezes et al., 2015) (Fig. 4.3). The *Actinobacteria* are also responsible for cycling of physically protected OM in the soil (Miyadoh, 1997). Their community is sensitive to pH changes (Jiménez-Bueno et al., 2016). Further, high pH has been

associated with increases in the abundance of the *Actinobacteria* community (Fierer et al., 2012b). The pH did not significantly change from 1990 to 2011 in transplanted soils. However, in air dried soil, $CaCO_3$ tends to increase resulting in an increase of pH which is favorable for *Actinobacteria* (Oskay, 2009). An overall reduction in abundance of copiotroph taxa of *Proteobacteria* in the high fertility (CC and PL) and sub-surface (BH and CH) soils corresponds with nutrient depletion from current agriculture managements. Furthermore, a lower relative abundance of *Bacteroides* assemblages in all the 2011 transplanted soils was another indicator of fertility reduction as the *Bacteroides* are copiotroph that can be found in nutrient rich environments. In particular, they tend to live in C rich soils (Fierer et al., 2007). In high fertility soils, a reduction in relative abundance of *Chloroflexi* (Fig. 4.3) after 21 yr supports a microbial adaption to nutrient depletion (Will et al., 2010). However, the overall community shift was prominent in the *Proteobacteria* at both class and order levels (Figs. 4.5 and 4.6).

Shifts in *Proteobacteria* composition at class and order levels were identified from 1990 to 2011 for PL, CC, NG and sub-surface soils (Figs. 4.5 and 4.6). The *Proteobacteria* consists of morphologically, physiologically and metabolically diverse members involved in C, N and sulfur cycling (Kersters et al., 2006). In the high fertility PL and CC soils, the *Proteobacteria* community was dominated by Beta and Alpha classes, respectively, prior to relocation (Figs. 4.5 and 4.6). Similar to CC, the NG soil was dominated by *Alphaproteobacteria* in 1990 (Fig. B 2). The *Alphaproteobacteria* and *Betaproteobacteria* prefer copiotroph environments and are sensitive to soil C availability (Fierer et al., 2007) as indicated by higher relative abundances in C rich soils (Smit et al., 2001; Fierer et al., 2007). The *Proteobacteria* community structure of N fertilized soils had changed from unfertilized and original soil communities. The higher abundance of *Alphaproteobacteria* and *Gammaproteobacteria* were found in CC and PL soils respectively. Increases in *Alphaproteobacteria* and *Gammaproteobacteria* in N fertilized soils also was reported by Fierer et al. (2012b) and Campbell et al. (2010). Long-term N fertilizer application resulted in the emergence of copiotrohic bacterial communities as N fertilizer application increased the OC availability in the soil by increasing plant productivity (Zvomuya et al., 2008; Fierer et al., 2012a; Yanni et al., 2016). This was applicable to the PL and NG soils which had been transplanted from native ecosystems. Transplantations from native ecosystems indicated higher abundances of *Alphaproteobacteria* and *Betaproteobacteria* in N fertilized soils

while the *Gammaproteobacteria* abundance increased in N unfertilized soils (Fig. 4.4). In agreement with current findings, Nemergut et al. (2010) reported a strong correlation between soil N availability and *Alphaproteobacterial* abundance. In contrast to PL and NG soils, the *Proteobacterial* community of the CC soil, that had been cultivated and received N fertilizer before transplanting, displayed minor changes with N fertilizer application (Figs. 4.4 and 4.6).

Other than shifts in the microbial community structures, invasion, and extinction of certain microbial members were detected in transplanted soils at the order level. The microbial community structure is a historical product of diversification, immigration, and extinction (Fukami et al., 2007; Nemergut et al., 2013). Since these soils are exposed to a dynamic environment where research was conducted with other soil types, foreign microorganisms may have entered and invaded the transplanted soils. However, unless the conditions were favorable not all of the incoming microbes survived. For example, the order *Alteromonadales* in N unfertilized PL and CC soils (Figs. 4.5 and 4.6), and the *subdivision 3 Verrucomicrobia* in sub-surface soils (Fig. 4.11) were first detected in 2011, presumably because the conditions were favourable for supporting their growth. The relative abundance of the order *Alteromonadales* increased with labile organic substrate availability (Goldfarb et al., 2011). The PL and CC had higher C availability among the soils favoring the survival of the order *Alteromonadales*. In 2011, class *Ktedonobacteria* had disappeared from the BH soil (Fig. 4.10). Based on the community profile analysis, it was confirmed that convergence of soil microbial community structure of sub-surface soils resulted from continuous wheat cultivation practices. The long-term N fertilizer application may not be the major driving force of community convergence as similar degrees of change were observed in both N fertilized and unfertilized sub-surface soils (Fig. 4.13). This was further confirmed by increased species richness and evenness in the 2011 BH and CH soils as a result of agriculture management practices (Table 4.1). Before transplanting, the sub-surface soils had lower soil OC content because of soil erosion. After transplantation, even under continuous wheat cultivation and crop residue removal conditions, these soils had gained substantial amounts of OC over time. The N fertilizer application further enhanced soil OM increase. The gain in soil OC amounts in sub-surface soils was reported for the same experiment after 21 yr (Yanni et al., 2016).

Species richness and evenness did not follow a particular pattern with either soil fertility level or N fertilization (Table 4.1). Others have observed site specific bacterial diversity response towards N fertilizer application (Ramirez et al., 2010; Fierer et al., 2012a). Alpha diversity followed robust patterns among the studied soils and there were predictable trends based on the land use history. Exposure of NG and PL soils to agriculture practices resulted in different responses. The PL soil obtained from a native pastureland which previously had been used for cattle production likely received high levels of manure and urine in isolated spots within the field creating nutrient rich patches where copiotrophic microbial communities with lower evenness became dominant. Subsequently, it was continuous wheat cultivation, crop residue removal and N fertilizer application which drove increases in evenness of microbial community assemblages. Long-term N fertilizer application had variable impacts on microbial evenness in the high fertility soils. The evenness decreased in CC soil with N fertilization while the opposite trend was observed for PL soil. This response seems to depend on the history of management as the CC soil, which was previously exposed to N fertilization, may have increased the abundance of N responding community members, as the conditions were more favorable for them. Manure application reportedly alters microbial diversity by increasing richness and decreasing evenness (Hartmann et al., 2015). Clear dilution of species richness and evenness was evident in RM and HM soils where the effect of the added manure diminished over time. Current nutrient depletion conditions may reduce the richness, which can facilitate the slow growing oligotrophic community. However, it is possible that of microbial diversity was underestimated in these archived soils due the influence of drying and storage factors. The shift in sub-surface soil microbial communities towards top soil is an indication of microbial community convergence under common field and climatic conditions for 21 yr. Although low fertility sub-surface soils able to converge within 21 yr high fertility soils may take a longer period of time (more than 100 yr) for microbial communities to converge.

4.7. CONCLUSION

Archived soil can be effectively used to capture microbial community changes that occurred over time as a result of land use changes and agricultural management. The current findings

suggest that archived soils can be use effectively to bring new information about microbial community structure changes across experimental treatments overtime.

The variability of microbial community profiles is a product of soil origin that resulted from intrinsic soil characteristics, different land use histories, as well as current land use practices. The soils were obtained from different donor sites with variable land use histories. However, the identical climatic, topographic and management conditions after transplantation did not result in highly similar soil microbial communities (i.e., convergence). Microbial communities of transplanted soils diverged from the original soils (1990) and from each other by 2011, even with common field and climatic conditions. The alpha diversity followed variable patterns of species richness and evenness with soil origin and N fertilization. Soil origin and history of management modified the microbial community profiles together with current management practices. Soil fertility level was a major factor influencing microbial profiles associated with DNA profiles in the archived soils. This confirmed that microbial community responses to environmental changes is mainly governed by soil fertility legacy. Other than the soil formation, land use and management history play an important role in future soil fertility which has predominant effect on microbial communities.

Soil erosion exposed sub-surface soils to the soil surface and agriculture management practices which deposited most of the crop residues (straw and roots) resulted in convergence in microbial profiles similar to topsoil. The microbial community conversion in sub-surface soil was mainly driven by continuous cultivation and was less influenced by N fertilization. The community changes with respect to current management practices are evident through shifts in microbial communities, invasion of new species and disappearance of existing species in the transplanted soils. Each soil had its own pattern in community changes indicating variable responses to current agriculture land use. However, most dominant shifts occurred in high fertility and previously uncultivated soils and reflected nutrient depletion due to continuous cultivation and crop residue removal. The shifts in the *Bacteroides* and *Chloroflexi* phyla reflected the oligotrophic status of the soil. The phylum *Proteobacteria* is a better indicator of microbial community shifts in the transplanted soils. Long-term N fertilizer application shifted *Alphaproteobacteria* and *Betaproteobacteria* classes to varying degrees, depending on the soil origin. Distinct community compositions among transplanted soils indicated that community

responses to current management practices were governed by past land use. Different directions of microbial community changes in transplanted soils were related to soil origin and land use history.

5.0. SOIL ORIGIN REGULATES THE DECOMPOSITION DYNAMICS OF TRANSPLANTED CHERNOZEMS

5.1. PREFACE

The previous two chapters described distinct differences in microbial community abundances and structures between soils which had been transplanted to a common site such that the soils were managed similarly under identical climatic and topographic conditions over more than two decades. A microbial community comparison study of original (1990) and 2011 archived soils suggested a divergence in microbial communities in each of the soils from the time of transplanting to sampling >21 yr later. Further, soil analysis indicated loss of TC, TN, and OC in most soils after transplantation in response to the imposed land use practices (continuous wheat cultivation, crop residue removal, and N fertilizer application). Thus, it was deemed important to further study nutrient dynamics of transplanted soils that ultimately contributed to the TC, TN, and OC losses. For this, OM decomposition processes were selected for further investigation as they are the main process releasing available nutrients for plant and microbes. A microcosm study was conducted coupled with a stable isotope probing (SIP) technique to explore transplanted soil community responses to an added C source. ^{13}C cellulose was used as the substrate, from which ^{13}C was tracked in the microbial phospholipid fatty acids (PLFA) and DNA to identify the active decomposer communities. Despite the established differences in overall microbial community structure, the use of ^{13}C-SIP allowed me to discern whether the shared conditions imposed on different soils after transplanting had selected for a common subset of the total community that responded to the C addition.

5.2. ABSTRACT

Organic matter (OM) decomposition contributes to soil fertility and shifts in microbial community composition can change soil C-cycling, including OM decomposition. Microbial response to land use change is governed by several factors; however, the main determining factors that control the microbial decomposer community in different soils remain poorly understood. Several studies have been conducted to examine factors influencing microbial community structure, typically by comparing soils from diverse ecosystems with the goal of linking soil microbial community dynamics to OM decomposition patterns. However, these studies were necessarily influenced by confounding factors such as different climate, topography, and hydrology at the experimental sites. A stable isotope probing (SIP) study was conducted using ^{13}C labelled cellulose to investigate C-cycling in four Chernozems of different origin and management history (CC-cereal cultivated, PL- pastureland, DTF- dryland tilled fallowed and RM- manured) obtained from a long-term (23 yr) soil quality transplant experiment at AAFC, Lethbridge, Canada. Soil microcosms were prepared by adding ^{13}C cellulose and soil respiration was measured over 78 d. The active decomposer community at 30 and 78 d was analyzed using ^{13}C-PLFA and sequencing of ^{13}C labelled 16S *rRNA* genes. Mineralization responses were mainly governed by the most limiting nutrient for microbial metabolism, which differed among transplanted soils. For example, application of cellulose to high fertility CC and PL soils that had received N fertilizer for years increased the C use efficiency by 5 and 23% respectively, resulting in low CO_2 emissions; the low fertility soil (DTF) followed an inverse trend. Long-term N fertilizer application reduced *Actinobacterial* and *Proteobacterial* diversity in PL and RM soils, whereas opposite trends were observed for the CC and DTF soils. Although *Proteobacteria* and *Actinobacteria* were the most abundant taxa in all soils, distinct active decomposer communities were associated with each transplanted soil, indicating a prolonged influence of soil origin and land use legacy through soil fertility on decomposer microbial dynamics. Preferential utilization of added cellulose over soil OM signified the important role of fresh OM quality on the fate of added C inputs in the soil. However, cellulose activated a different subset of microbial groups unique to each soil indicating a prolonged influence of soil origin, land use history and C and N fertility legacy on the decomposer community. These results suggest a persistent legacy effect of soil fertility on decomposer community structure and their

functions in soil C turnover. Because the dynamics of C and N availability in the transplanted soils resulted in different flows of freshly added C through the microbial community, differences in soil OM composition and thus soil fertility are likely to persist despite decades under common conditions.

5.3. INTRODUCTION

Understanding C-cycling is important in sustainable cropping systems. Soil C-cycling contributes to the global C cycle (Fontaine et al., 2007) as the soil is the most dynamic C reservoir in terrestrial ecosystems (Janzen, 2004). In terrestrial ecosystems, the main reservoir of C is soil OM (Schimel, 1995) and changes in C-cycling have a major impact on soil productivity. Soil C-cycling regulates the atmospheric CO_2 levels by storing CO_2 assimilated by plants as soil OM and releasing CO_2 to the atmosphere through OM decomposition. Decomposition of soil OM releases nutrients for plant and microbial uptake (Johnston et al., 2009) and these processes are mediated by soil microorganisms (Aislabie et al., 2013). The rate of soil OM decomposition is governed by soil microbial community composition and environmental conditions (Baumann et al., 2012). Soil microorganisms not only facilitate decomposition but also directly contribute to the C-cycle as microbial products are important constituents of soil OM (Kleber, 2010). Environmental and management modifications can influence soil C-cycling by shifting microbial community structure and their metabolic activities. Therefore, understanding decomposer microbial community dynamics is pertinent in maintaining ecosystem stability.

Crop residues contain valuable nutrients (N, P, K) in complex forms, which may not be readily available for plant uptake. The decomposition process breaks these complex organic compounds into readily available forms for plants and microbes (Gonzalez-Quiñones et al., 2011; Gleixner, 2013). This process is driven by the interactions between quantity and quality of the plant residues (Chowdhury et al., 2015; Wang et al., 2015), soil properties (Comeau et al., 2013), agriculture management practices (Moran et al., 2005; Chowdhury et al., 2015) and climate (Davidson and Janssens, 2006). The C: N ratio of the residue material has a significant impact on decomposition where materials with high C: N ratio are only partially assimilated by soil microorganisms resulting in longer residence time in the soil, whereas materials with low C:N ratio are readily available for microbial uptake and decompose rapidly (Kirkby et al., 2013).

In Western Canada, crop residue removal is a common practice carried out to provide bedding materials for livestock (Campbell et al., 1991). Crop residue removal decreases both OC and available nutrients in the top soil compared to systems where residues remain in the field.

Reduced microbial biomass and soil OC are two main features of soils from which crop residues have been removed (Chowdhury et al., 2015). Under N limited conditions, soil microorganisms scavenge for N present in the soil OM pool (Fontaine et al., 2011). Nitrogen fertilizer application is a common practice conducted in agriculture fields to facilitate crop growth (Zhang et al., 2008). Addition of N fertilizer reduces the overall C: N ratio of the soil. In addition to enhancing plant biomass production, N fertilizer also enhances the conversion of residue C inputs into stable soil OM (Moran et al., 2005). Several soil physical and chemical properties have been reported to influence OM decomposition (Enriquez et al., 1993; Giardina et al., 2001; Krull et al., 2001; Xu et al., 2016). For example, soil texture, water holding capacity (Giardina et al., 2001), pore size distribution (Krull et al., 2001) and the C: N ratio (Enriquez et al., 1993) have various influences on OM decomposition in soil (Xu et al., 2016).

There is a complex relationship between soil OM composition, microbial community composition and soil nutrient dynamics (Ng et al., 2014). Microbial diversity and community structure affect soil OM turnover (Baumann et al., 2012). Researchers have reported changes in microbial composition at the early stages of wheat decomposition (Marschner et al., 2011). Therefore, understanding major driving forces governing soil microbes involved in C-cycling will help facilitate sustainable management in agricultural ecosystems. Thus, understanding decomposer community dynamics enhance the fundamental knowledge of soil C-cycling.

A number of studies have been conducted to determine the relationship between soil microbial diversity and composition and soil OM decomposition in different ecosystems (Bernard et al., 2007; Denef et al., 2007; Moore-Kucera and Dick, 2008; Baumann et al., 2012; Sun et al., 2013; Ng et al., 2014; Xu et al., 2014; Zhou et al., 2016). The major challenge is identifying the main factor shaping diversity of the decomposer community at a global scale as microbial community composition has complex relationships with ecosystem properties. It is hard to decouple microbial compositional variability associated with soil factors from confounding factors, like topography and climate. Therefore, a long-term soil transplant experiment provides a unique resource for studying microbial community diversity, composition and functions of different soils under identical climate, topography and agronomic practices. The experimental field was established by transplanting 36 different Chernozemic soils (top soil) with diverse physical, chemical and biological properties to a single location. After

transplantation, the soils were continuously cultivated to wheat (*Triticum aestivum)* under two levels of N fertilizer (0 and 60 kg N ha^{-1}) for 23 yr. Crop residue removal was a routine practice conducted in the transplanted field. Therefore, 23 yr of limited residue retention may have created C limited condition in the soils. In addition to residue removal, long-term continuous wheat cultivation may have depleted soil available nutrients for plant and microbial utilization exacerbated in the soils managed with 0 kg of N fertilizer. Despite difference in abundance and community composition of the soil microbial communities measured by PLFA and DNA sequencing after 22 yr post transplantation, I hypothesized that there was an active subset of the broader community that dominated C-cycling that was more similar across different soils in response to common environmental conditions and management. This hypothesis was tested by conducting a SIP study. ^{13}C labelled cellulose was used as the carrier molecule and ^{13}C tracer molecules were tracked in PLFA and microbial DNA to gain a better understanding of active C assimilation populations present in the transplanted soils. Investigations in ^{13}C assimilated PLFA and DNA allowed the identification of metabolically active microbial decomposer communities in different transplanted Chernozemic soils. The objectives of this study were to: (1) observe the fate of soil incorporated ^{13}C labeled cellulose and characterize active microbial population during incubation and (2) observe the variations in ^{13}C labeled cellulose decomposition rates in N fertilized soils and unfertilized soils.

5.4. MATERIALS AND METHODS

5.4.1. Soils for the study

The soils for the SIP study were obtained from the long-term soil quality experiment located at AAFC, Lethbridge, AB, Canada. A detailed description of the establishment of the transplanted field experiment can be found in Chapter 3 and 4. Briefly, top soils of 36 different Chernozems obtained from various donor sites were deposited on the experimental field from which the Ap and B horizons had previously been removed. These transplanted soils had variable physical, chemical and biological properties related with soil origin and land use legacy. After transplantation, soils were maintained under identical climatic, topographic and agriculture management conditions. From this experiment, four soils were selected including their +/- N fertilizer treatments based on the 2012 field experiment analysis. Specifically, the cereal

cultivated (CC) and pastureland (PL) soils with inherently high fertility, a soil that had a history of long-term manure application at a recommended rate of 30 t ha^{-1} (RM), and dryland soil from a tilled bare fallowed (DTF) field with low fertility were chosen for the study. A detailed description of the selected soils can be found in Table 5.1. According to the 2012 PLFA analysis, soils with the highest inherent fertility (CC and PL) displayed higher total, bacterial and fungal abundances compared to other soils but had different microbial community structure profiles. In the four soils selected, N fertilizer application did not significantly affect microbial abundance. The CC and PL soils were selected as they had comparatively similar fertility levels and microbial biomass. The total C (TC) content of RM soils was lower than high fertility soils (CC and PL) and higher than the DTF soil. Further, the DTF soil with low TC, which was originally obtained from a bare fallow land that had been subjected to dryland tillage, had the lowest microbial biomass among the selected soils (please refer to Chapter 3 for microbial analysis). A preliminary study (60 d) was conducted using the 2012 soil samples to determine the rate of C needed and appropriate length of incubation, determined by the respiration response to cellulose amendment.

Soil samples (0- to 10-cm depth) were collected in Fall 2013 from replicate (n=3) CC, PL, DTF and RM plots, including both N fertilized and unfertilized split-plot treatments. Six sub samples were obtained from each replicate plot, bulked and sieved (< 2 mm). Before applying the cellulose treatments, soil samples were pre-incubated for seven days at 20ºC to achieve a biological steady state by mineralizing readily available C. Nine replicates from each N fertilizer level of a particular soil were prepared for the incubation (4 types of soil× 2 levels of N× 3 field replicates× 3 destructive sampling dates (0, 30 and 78 d)). For this, soils (15 g (d.w.) for each microcosm) were incubated in the dark at 70% (w/w) field capacity for a week.

5.4.2 Application of ^{13}C labelled cellulose

^{13}C labeled cellulose was used as the C source. The cellulose substrate was obtained as a ground powder, extracted from the potato plant (*Solanum tuberosum*) and was uniformly labelled (>97 atom%) (IsoLife Company, Wageningen, NL). Half of the soil samples were treated with ^{13}C labelled cellulose at a rate of 0.5 g cellulose kg^{-1} soil which is equivalent to 0.22 g of C kg^{-1} of soil. Each soil sample (15 g) was amended with ^{13}C cellulose which provides 3.3 mg of C and

mixed thoroughly. Control microcosms were prepared without cellulose. Descriptions of the treatments are illustrated in Fig. 5.1.

5.4.3. Incubation conditions

^{13}C cellulose treated and control soils were packed as microcosms in 100 mL beakers with equal density and placed in a 1 L Mason jars containing rubber septa on the lid for gas sampling and flushing. The jars were flushed with ultra-zero air (CO_2 free) (Praxair Specialty Gases, Edmonton, AB) for 7 min at a rate of 1 L min^{-1} after sealing the jar containing a microcosm. Flushed jars containing microcosms were incubated for 78 d (Adaptis a1000, Conviron, Winnipeg, MB). During incubation**,** microcosms were held at a constant temperature (20ºC) and two Tinytag ULTRA 2 data loggers (Gemini Data Loggers Ltd., Chichester, UK) were used to monitor the temperature. The moisture content of the microcosms was maintained at 70% field capacity (w/w) and monitored and adjusted by weight at the time of each gas sampling.

Table 5.1. Description and background information of selected transplanted soils.

Soil origin	Soil#	Soil zone	Soil texture	Soil characteristics
Cereal cultivated (CC)	8	Black	Clay loam	Highest soil inherent fertility, land was a native grassland until 1982 and continuous cereal production
Pastureland (PL)	9	Black	Clay loam	Highest inherent fertility, native pastureland
Dryland tilled fallowed (DTF)	11	Dark Brown	Clay loam	Low inherent fertility, continuous dryland tilled and summer fallowed since 1911, obtained from a pathway to an experimental field
Manured (30 t ha^{-1}) (RM)	22	Dark Brown	Clay loam	Wet manure was applied 30 tons ha^{-1} since 1973 and continuous dryland barley cultivation

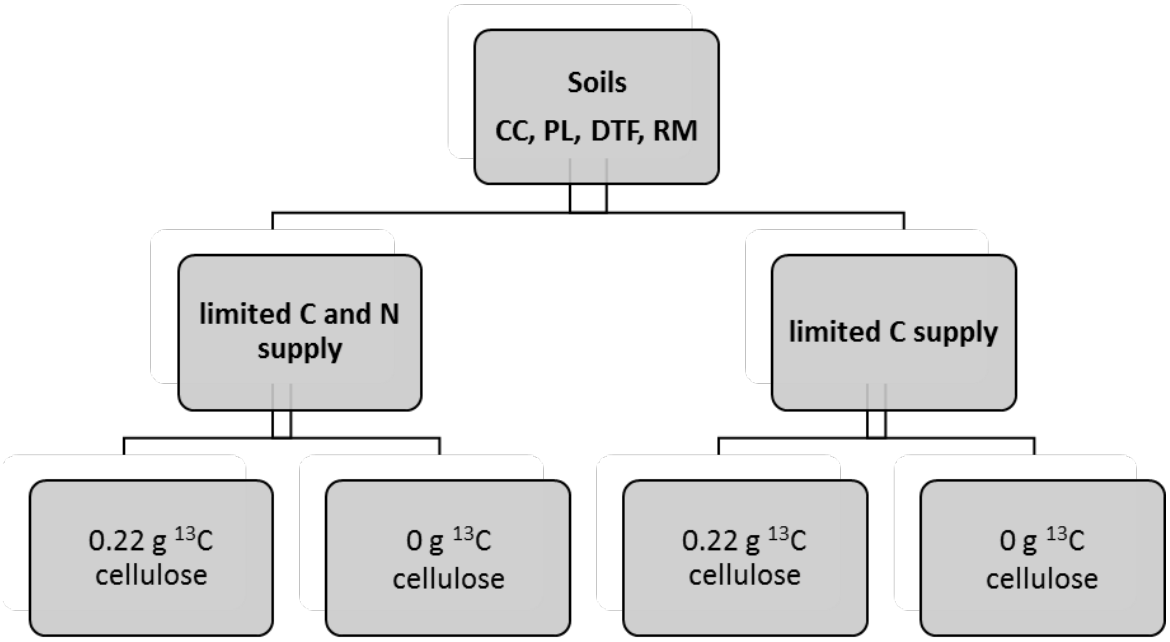


Fig. 5.1. Illustration of treatment allocation of the ^{13}C SIP study. CC (cereal cultivated) PL (pastureland), DTF (dryland tilled fallowed) and RM (30 t ha^{-1} manured).

5.4.4. Measurements of CO_2 concentration and ^{13}C cellulose mineralization

Organic matter and ^{13}C cellulose mineralization were assessed by measuring $^{12}CO_2$ and $^{13}CO_2$ in the headspace of the microcosm at various times during incubation. Thirty-five milliliter gas samples were obtained from the jar's head space using a syringe (35 mL), compressed to 25 mL and transferred to a 12 mL air evacuated Exetainer® (Labco Inc, Lampeter, UK) (evacuated to 0.007 kPa). Immediately following ^{13}C cellulose addition, the head space of the jar was frequently flushed with ultra zero air to prevent anaerobic conditions from developing and gas sampling was done prior to each flushing. Gas samples were obtained at 1, 2, 4, 7 and, 10 d, and once a week thereafter until 78 d. Samples were stored at room temperature until analysis.

Gas samples were analyzed for total CO_2 ($^{12}CO_2$ and $^{13}CO_2$) concentration using a gas chromatograph (GC-FID) (Varian Canada Inc. Mississauga, ON). To determine the isotopic ratio ($^{12}C/^{13}C$), gas samples were analyzed using a PICARRO CRDS analyzer (G2201-*I*, Santa Clara, CA). Prior to analysis, samples were diluted using ultra zero air accordingly to acquire the

sensitive measuring range of PICARRO CRDS analyzer. To obtain CO_2 concentration, headspace volume was calculated by subtracting soil and beaker's volume from jar volume. Total OM mineralization rate was calculated by calculating total CO_2 emitted at each sampling time and calculating cumulative emissions through the entire incubation period. Carbon dioxide concentration was calculated per soil mass and then converted into the amount of C evolved per gram soil. The amount of $^{13}CO_2$ released from the cellulose between 1 and 9 d, 16 and 37 d and 41 through 78 d periods was estimated using the isotopic ratio measured on days 9, 37 and 78 respectively, which were applied to total CO_2 concentrations based on linear extrapolation of the isotopic ratio between the two measured dates. The priming effect (PE) was computed using the method described by Guenet et al. (2011) as follows,

$$PE = (1 - \alpha)\, CO_{2\ sample} \qquad \text{[Eq. 5.1]}$$

$$\alpha = \frac{A13C\ cellulose - Asample}{A13C\ cellulose - Acontrol} \qquad \text{[Eq. 5.2]}$$

$CO_{2\ sample}$ – mg CO_2 kg^{-1} soil

Where $A_{13c\ cellulose}$ is the isotopic abundance of ^{13}C cellulose, A_{sample} is the CO_2 in the sample and $A_{control}$ is the CO_2 in the control atmosphere.

5.4.5. Determination of active decomposer microbial community

Soil microcosms were destructively sampled at 0, 30 and 78 d after ^{13}C cellulose addition. Sub samples from the microcosms were stored at -80ºC for DNA extraction. The remaining soils were stored at -20ºC, freeze dried and ground for PLFA analysis. Total microbial communities were explored using PLFA and 16S *rRNA* gene sequencing at 30 and 78 d. Microorganisms containing ^{13}C cellulose were considered as the active decomposer community. ^{13}C found in active microbial biomass was derived either through direct assimilation of added ^{13}C cellulose or predation on previously ^{13}C cellulose labelled microbes. Tracer ^{13}C molecules were tracked in microbial fatty acids at 30 and 78 d.

5.4.5.1 PLFA

Phospholipid fatty acid analysis was carried out to assess the microbial abundance and community composition of ^{13}C amended and unamended soils at 30 and 78 d. The PLFA extraction method outlined in Section 3.4.3 was followed here. Briefly, the soils were freeze-dried and ground before the lipid extraction. The lipids were extracted from 4 g of freeze-dried soils using chloroform, methanol and phosphate buffer (CH_3OH: $CHCl_3$: Phosphate buffer at a ratio of 2:1:0.8) and PLFA were isolated using a solid phase extraction column (0.50 g Si; Varian Inc., Mississauga, ON). Total fatty acids (^{12}C+^{13}C) were methylated and identified using a GC-FID (Hewlett Packard 5890 Series II, Hewlett Packard Scientific Instruments, Palo Alto, CA) and peaks were identified using fatty acid standard and MIDI software (MIDI Inc., Newark, DE). Methyl nonadecanoate (19:0) was used as the internal standard to quantify the peaks.

The total PLFA was determined by summing all the detected peak areas. Total bacterial abundance was quantified by summing the following biomarkers: i14:0, i15:0, a15:0, i16:0, 16:1ω7c, 10Me16:0, i17:0, a17:0, cy17:0, 10Me17:0, 18:1ω7 and 10Me18:0, cy19:0 (Bååth and Anderson, 2003; Helgason et al., 2010). Gram positive (G^+) and G^- bacterial group abundances were assessed using i14:0, i15:0, a15:0, i16:0, i17:0, a17:0 (Hedrick et al., 2005; Helgason et al., 2010) and 16:1ω7t, 16:1ω9c, 16:1ω7c, 18:1ω7c, 18:1ω9c, cy17:0, cy19:0 (Macdonald et al., 2004; Helgason et al., 2010) biomarkers, respectively. Fungal abundance was determined using 18:2ω6,9 biomarker (Bååth and Anderson, 2003; Helgason et al., 2010). Physiological stress indicators were obtained from ratios of cy17:0 to 16:1ω7c (stress 1) and cy19:0 to 18:1ω7c (stress 2) (Grogan and Cronan, 1997).

To determine active decomposer community composition (^{13}C PLFA) at 30 and 78 d, fatty acids were quantified by their C isotope ratio using a Delta ^{PLUS} isotope mass ratio spectrometer (Thermo Fisher Scientific, Waltham, MA) fixed to a Hewlett Packard 6890 Series IIGC (Agilent, Palo Alto, CA) via a GC Combustion-III interface (Thermo Fisher Scientific, Waltham, MA). Prior to analysis of every sample, a CO_2 sample with known isotopic composition was injected as the reference. Further, a mixture of eight fatty acids with known isotopic composition was

used as a standard with every batch of samples. Isotopic enrichment of ^{13}C was adjusted for the C atoms derived from methanol during the methylation process (Arcand et al., 2016). The quantification of ^{13}C enrichment in individual fatty acids was calculated according to the method described by Helgason et al. (2014) using the following equations:

$$^{13}Cen = (F_{t30} - F_{t0}) \times PLFA_{(total)t30} \quad \text{[Eq. 5.3]}$$

where $PLFA_{(total)t30}$ is the concentration of ^{12}C and ^{13}C of an individual fatty acid in the ^{13}C cellulose added soils to obtained from GC-FID/MIDI at 30 d (similar for 78 d). The Ft_0 fraction was from control sample (natural abundance) and F_{t30} was for ^{13}C cellulose treated sample and computed as:

$$F = R/(R+1) \quad \text{[Eq. 5.4]}$$

The carbon isotopic ratio was obtained from GC-C-IRMS analysis relative to the VPDB standard as follows:

$$R = (\delta\ ^{13}C/1000+1) \times R_{VPDB} \quad \text{[Eq. 5.5]}$$

The percentage of ^{13}C incorporated into individual fatty acid or functional group was calculated by dividing $^{13}C_{en}$ in individual fatty acid (µg g^{-1} soil) or the functional group (e.g. G^+ bacteria) by the total enrichment (total $^{13}C_{en}$) in all fatty acids (µg g^{-1} soil).

5.4.5.2 Isopycnic centrifugation and DNA fractionation

To determine the active microbial community, microcosms from the 30 d sampling period were selected. Samples from 78 d were thought to be too confounded through internal recycling of the ^{13}C labelled by consumers of ^{13}C scavenged organisms of soil food webs. An incubation study conducted by Haichar et al. (2007) using ^{13}C labelled cellulose observed changes in DNA profiles between heavy and light DNA fractions from seven days onwards. The changes occurring in the heavy fraction DGGE profiles persisted until 30 d.

5.4.5.2.1 DNA extraction

Total DNA was extracted using PowerSoil DNA isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA) and DNA was quantified using a Qubit® 2.0 Fluorometer with the Qubit®

dsDNA HS assay kit (Thermo Fisher Scientific, Waltham, MA USA). Three technical replicates were used to extract the DNA from each microcosm. The final volume of each DNA technical replicate was 30 µL.

5.4.5.2.2 Isopycnic centrifugation and gradient fractionation

DNA fractionation was conducted following the method described by Dunford and Neufeld, (2010) to separate heavy and light DNA fractions. Briefly, 0.5 µg of DNA was mixed with gradient buffer (final solution contained 0.1 M Tris, 0.1 M KCl and 1 mM EDTA). DNA containing gradient buffer was mixed with 7.163 M CsCl in a sterilized tube to obtain a final density of 1.725 g mL^{-1} (the equation described by Dunford and Neufeld, (2010) was used to calculate the volume of gradient buffer needed and the volume of CsCl required is dependent on the density of the stock solution). The DNA containing solution was mixed by inverting 10 times. Using a sterilized pasture pipette, the DNA mixture was gently transferred into a sterilized polypropylene (5.1 mL) ultracentrifuge tube (Beckman Coulter Canada, LP, Mississauga, ON, Canada) to the tube shoulder. After filling all the tubes, the mass of each tube was measured precisely to two decimal points. Based on the mass, tubes were paired within 0 to 10 mg weights to balance during centrifugation. Following the manufacturer's instructions tubes were sealed using a tube topper. Using a Vti 65.2 (Beckman Coulter Canada, LP, Mississauga, ON, Canada), tubes were centrifuged at 44,100 rpm for 40 hr at 20ºC.

Immediately following centrifugation, tubes were gently removed from the rotor and subjected to gradient fractionation. A sterile 60 mL syringe with a 2.5 cm needle was filled with sterilized bromophenol blue (dd H_2O) solution and loaded into the pump and the flow rate was adjusted to 425 µL min^{-1}. A centrifuge tube was gently fixed to a clamp and the bottom edge was carefully pierced to allow collection of the DNA fractions by displacement. Each fraction was collected into a sterile tube and the tube was replaced once in every minute. At the end, there were 12 fractions with 425 µL from each sample. The density of each fraction was determined by measuring the weights of triplicate 100 µL aliquots and corrections were done by comparing with equal volumes of water weight (Neufeld et al., 2007; Dunford and Neufeld, 2010). Density values were used to separate light and heavy fractions. DNA precipitation was done by adding 1 µL (20 µg) of glycogen (Invitrogen™ UltraPure™) and 2 mL of filter sterilized polyethylene

glycol solution (30% polyethylene glycol 6000, 1.6 M NaCl) to each fraction and mixing by inversion. The mixture was incubated overnight at room temperature to precipitate the DNA.

Centrifugation was done at 13, 000 g rpm at 20ºC for 30 min to separate the DNA pellet from gradient buffer CsCl solution which was discarded. The pellet was washed with 500 mL 70% ethanol and centrifuged at 13, 000 g rpm at 20ºC for 10 min. After discarding the supernatant, the DNA pellets were dried at room temperature for 15 min. Then the pellet was suspended in 30 µL of filter sterilized TE buffer (10 mM Tris-HCl with pH 8, 1 mM EDTA with pH 8 and sterile dd H_2O). Final DNA concentrations were measured using a Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) with the Qubit® dsDNA HS assay kit (Thermo Fisher Scientific, Waltham, MA USA). These DNA samples were stored at -80ºC.

Heavy and light fraction identifications were performed using sample density measurement (Fig.5.2). The density range for the heavy fraction slightly varied with soil type where the heavy fraction had densities of 1.76 to 1.75 g mL^{-1} in the CC soil, 1.77 to 1.75 g mL^{-1}in the PL soil, 1.76 to 1.75 g mL^{-1} in the DTF soil and 1.78 to 1.74 g mL^{-1} in the RM soil (Fig. 5.2). Despite this density variation the fractions 5, 6 and 7 were identified as heavy while 9, 10, 11 and 12 as light fractions for all soils. Further, fraction selection was done for 16S *rRNA* genes using DGGE (denaturing gradient gel electrophoresis) fingerprinting. The amplification of 16S *rRNA* genes was performed using primers U341 (with GC-clamp) and U758 primers (Phillips et al., 2006). PCR was done in a reaction volume of 50 µL using of 3 µL of DNA from each fraction, 0.5 µL each primer (µM), 0.25 µL BSA (10 mg mL^{-1}) (Amersham Biosciences, Mississauga, ON, Canada) and 10 µL of Hot Star Master Mix (Qiagen, Mississauga, Ontario, Canada). Amplification was performed for 10 cycles of 1 min denaturing at 94ºC, 1 min annealing at 65-55ºC and 1 min extension at 72ºC, followed by 20 cycles using an annealing temperature of 55ºC. PCR products were purified and loaded onto an 8% acrylamide gel with a 40-60% denaturing gradient and electrophoresis was done at 80 V for 16 h and the resulting gels were stained with SYBR Safe and visualized under UV light. DGGE analysis indicated different band profiles in heavy and light DNA fractions (Fig. C.1). Particularly, heavy fractions (5 to 7) contained bands that did not appear in light fractions.

Based on densities and DGGE analysis, selected heavy fractions (5 to 7) were pooled together and V4 region of the 16S *rRNA* gene was sequenced (amplicon sequencing) at Génome Québec (Montréal (Québec) to profile bacterial and archaeal communities. In addition to heavy DNA fractions, genomic DNA extracted from all microcosms at 30 and 78 d was also subjected to amplicon sequencing (Génome Québec, Montréal, Québec). Sequencing analysis of heavy fractions and genomic DNA was performed using the Illumina Miseq platform as described in Chapter 4 (Section 4.4.3).

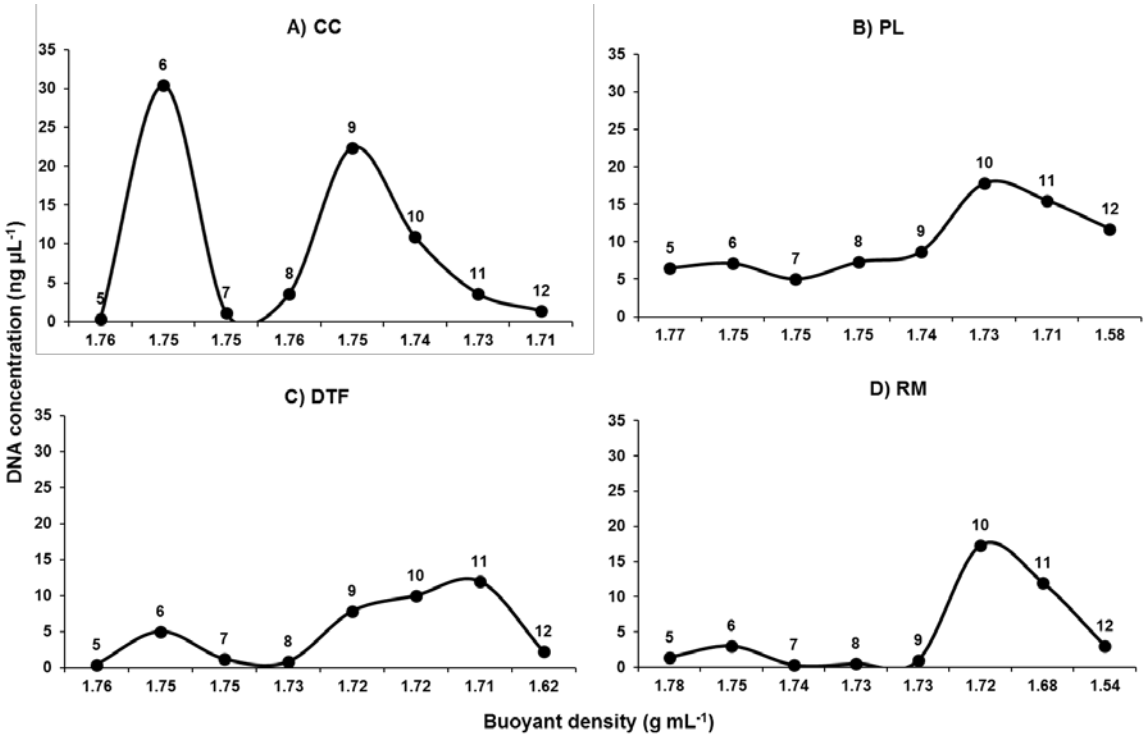


Fig. 5.2. Separation of light and heavy fractions after CsCl density gradient centrifugation and DNA distribution. A= CC (cereal cultivated), B= PL (pastureland), C= DTF (dryland tilled fallowed) and D= RM (30 t ha^{-1} manured).

5.4.5.3. Sequence data processing

Illumina sequencing data were processed using the QIIME (Quantitative Insights Into Microbial Ecology) pipeline (v1.9.1) (Caporaso et al., 2010). Forward and reverse sequences were first joined using paired_ends.py script in QIIME (reads were ranged between 873 and 107360) and the joined sequences were then subjected to quality filtering by removing short sequences (< 250 bp) and low-quality reads (Phred score < 20). This criterion resulted in

discarding three samples with low-quality data. Following the removal of singletons and doubletons, chimeric sequences were removed using the UCHIME algorithm (Edgar et al., 2011). Closed operational taxonomic units (OTUs) picking was performed using UCLUST (Edgar, 2010) and the taxonomic assignment was performed using the RDP classifier (v2.12) and Greengenes (v13.8) at a 97% similarity threshold. The rarefaction curves related to whole and decomposer community are displayed in Figs C.4 and C.5.

Alpha diversity of the bacterial communities was evaluated using Phylogenetic diversity, Chao 1 and observed species metrics within QIIME. Shannon diversity index, OTU richness, and inverse Simpson diversity indices were computed using the Vegan package in R statistical software (R i386 3.2.3) (https://www.r-project.org/). Beta diversity was conducted using weighted and unweighted UniFrac matrices (Lozupone and Knight, 2005) generated in QIIME followed by principle coordinate analysis (PCoA).

5.4.6. Statistical analysis

ANOVA and the MIXED procedure in SAS 9.3 (SAS Institute Inc, NC, USA) were used to identify the statistical differences between the different transplanted soils, the N fertilizer history, and ^{13}C cellulose application rates. The block and the interaction between block and transplanted soil type were considered as random effects and soil type and N fertilizer rate as fixed effects. Statistical significance was declared at Type III error rate of $P = 0.05$ with a 95% confidence interval. For analysis of interaction effects, the multiple mean comparison method LS means (Least Square Means) was used. If the main treatment effects were significant, LSD (Least Square Difference) was conducted.

Principal Coordinate Analysis (PCoA) for total PLFA and ^{13}C PLFA biomarkers was computed using PCOrd software version 6 (Gleneden Beach, OR 97388, USA). A multi-response permutation procedure (MRPP) test was conducted to identify between group differences among the transplanted soils, N fertilizer levels, and ^{13}C cellulose rate. Permutational Multivariate Analysis of Variance (PERMANOVA) analysis was conducted using the Origin statistical software package (OriginLab, Northampton, MA) to observe the significant interaction effects between the studied factors on whole and decomposer microbial communities of soil

measured through PLFA and OTU relative abundance profiles. Statistical differences of the clustering were tested using analysis of similarity (ANOSIM) within the QIIME platform.

5.5. RESULTS

5.5.1. Cumulative CO_2 evolved in the soil microcosms

Soil origin (P<0.0001) and history of N fertilizer application (P<0.003) were the main driving forces of OM mineralization in transplanted soils at the beginning of the incubation (3 d). The effect of ^{13}C cellulose addition became significant (P<0.001) from 6 d until the end of the incubation (78 d). Daily respiration rate peaked on 1 d after incubation and gradually declined overtime. The daily respiration emissions were significantly influenced by soil origin and N fertilizer history, where higher emissions were found in soils with a history of N fertilizer application. At the beginning of the incubation, the PL soil had the highest daily respiration rate (Fig 5.3). Later on, RM soil had a similar CO_2 flux indicating variable respiration responses with soil origin and past land use history.

The cumulative C-CO_2 emission at 78 d was significantly affected by soil origin and past land use history (P<0.001), N fertilization history (P<0.02) and ^{13}C cellulose application (P<0.0001) and was greatest at 78 d in PL and RM soils (Fig 5.3A). High fertility CC and PL soils were estimated to have mineralized greater amounts of added cellulose at higher rate compared to DTF and RM soils (Fig. 5.3B). For all of the soils, cellulose mineralization reached a plateau after 21 d. One day after ^{13}C cellulose application, DTF soils had the lowest emission rate; however, from the second day onwards it had cumulative CO_2 emissions that were comparable to CC and RM soils. Cumulative CO_2 emissions were greater in soils with a history of N fertilizer application and the pattern of emissions varied among the four transplanted soils (Fig. 5.4).

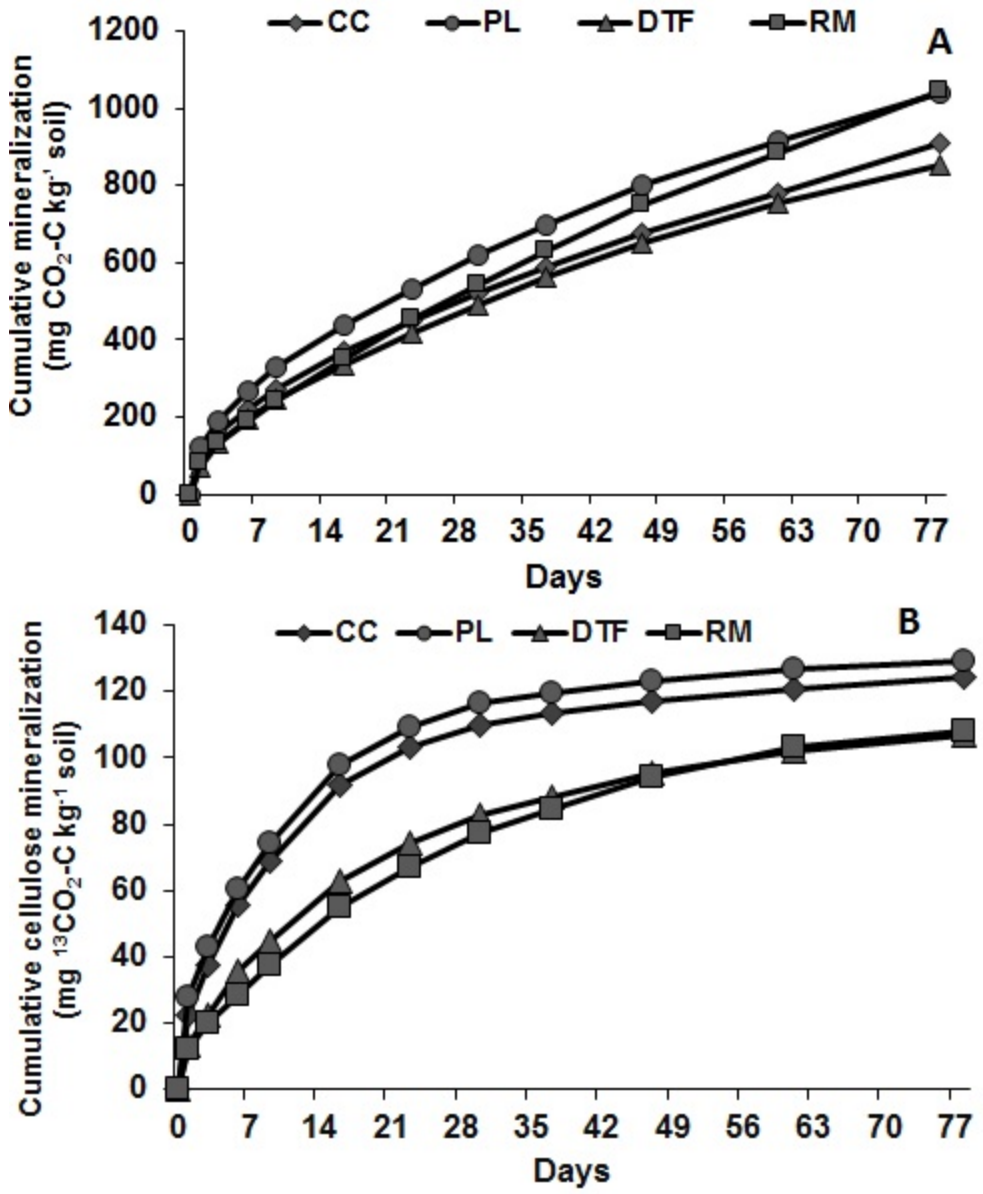


Fig. 5.3. Cumulative mineralization for A) soil OM and cellulose and B) cellulose in transplanted soils. CC (cereal cultivated) PL (pastureland), DTF (dryland tilled fallowed) and RM (30 t ha^{-1} manured).

Statistical analysis of individual soils revealed variable responses towards N and C (^{13}C cellulose) amendment at different stages of incubation without any N rate × ^{13}C interaction effect. In all soils, respiration of $^{13}CO_2$ was significantly influenced by soil origin at the beginning (6 d to 9 d period) (Fig. C.2). Nitrogen fertilizer application did not have any influence on CC soil ^{13}C cellulose mineralization (Fig. 5.4A). However, regardless of N history (N^- and N^+), microcosms that had C (^{13}C cellulose) addition showed greater respiration than the control

microcosms (^{13}C cellulose unamended), demonstrating N as the most limiting factor in the CC soil. In contrast, PL soil microcosms with a history of N fertilizer application had higher cumulative respiration following ^{13}C cellulose application (Fig. 5.4B). The lowest cumulative CO_2 emission was observed in low fertility DTF soil (Fig. 5.4C). Gains in soil OC content over the 21 yr post transplantation might have fulfilled the C need of the DTF soil microbial community; however, microcosms with a history of N fertilizer application had higher cumulative respiration after ^{13}C cellulose addition. A similar response to N fertilizer was observed for RM soil (Fig. 5.4D).

High fertility soils (CC and PL) with C (^{13}C cellulose) addition and N fertilizer application history had reduced respiration, indicating incorporation of C into microbial biomass (Table 5.2). ^{13}C cellulose decomposition in the CC soil was less impacted by N fertilization compared to the PL soil. Respiration of low fertility DTF soil relied on ^{13}C cellulose application and N history as the microbial community was apparently C and N limited for their metabolism. The RM soil was more dependent on N history (N fertilizer application) with ^{13}C cellulose addition.^{13}C cellulose use efficiency was pre-determined by soil fertility level, which was related to the origin and N fertilization history (Table 5.3). Soil respiration in the RM and DTF soils appeared to be influenced mainly by ^{13}C cellulose application; when supplied with N fertilizer and ^{13}C cellulose, respiration rates were increased in both soils. Specific respiration [i.e., soil respiration with respect to TC and ^{13}C amounts] was significantly influenced by soil origin/land use history ($P<0.0001$) and N fertilization history ($P<0.01$) (Table 5.4). A greater percentage of C was respired in low fertility DTF soil (6%) followed by RM soil (4%) compared to high fertility soils CC (2%) and PL (2%) when considered with TC amounts.

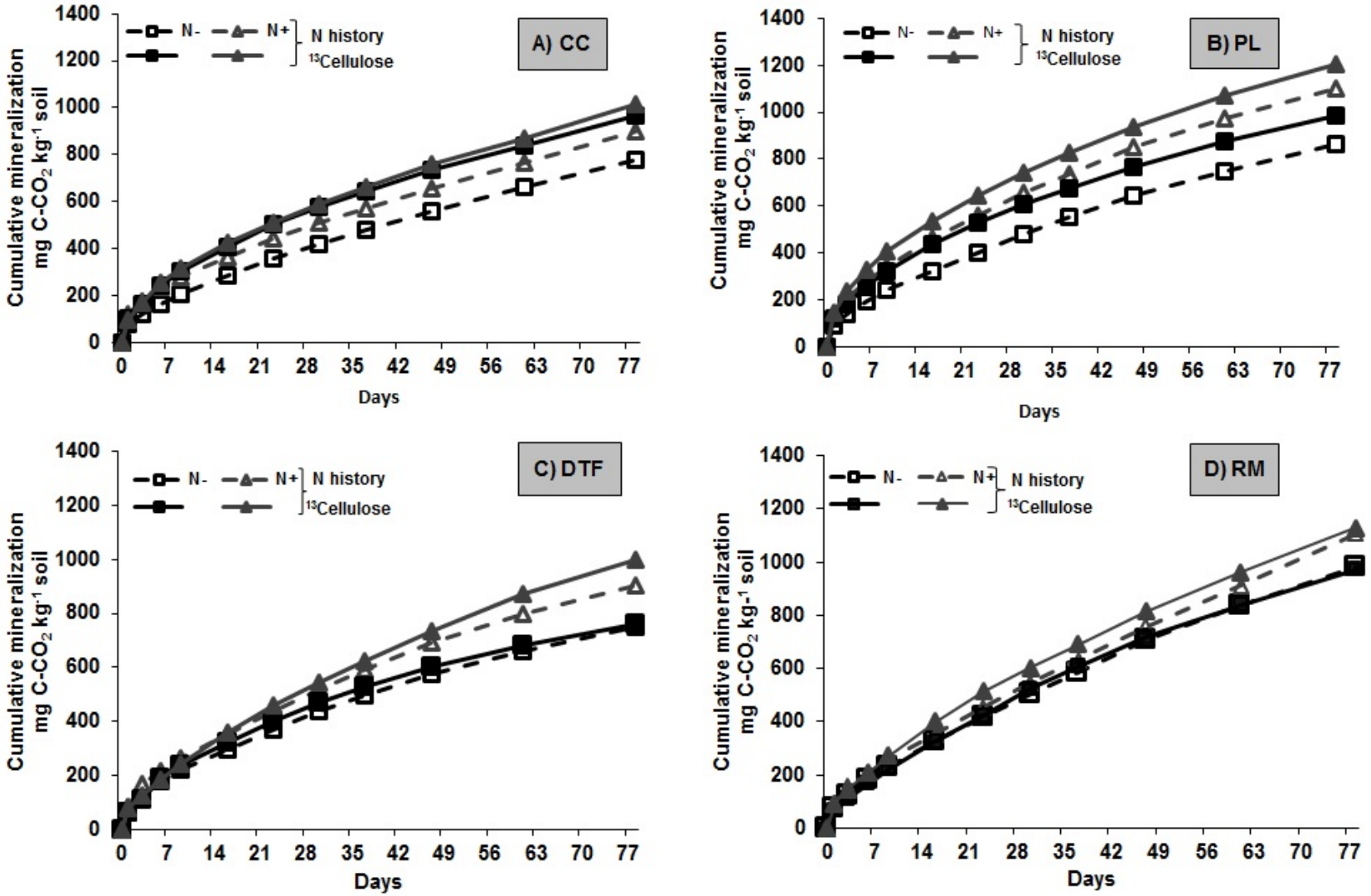


Fig. 5.4. Cumulative mineralization of soil OM in transplanted soils with N fertilizer history and ^{13}C cellulose application. A) CC (cereal cultivated) B) PL (pastureland), C) DTF (dryland tilled fallowed) and D) RM (30 t ha^{-1} manured).

Table 5.2. Effect of N fertilizer application history on cumulative mineralization.

Soil† origin	^{13}Cellulose unamended			^{13}Cellulose amended		
	N history		N effect %	N history		N effect %
	N^-	N^+		N^-	N^+	
CC	775	898	16	966	1014	5
PL	863	1102	28	984	1205	23
DTF	750	902	20	762	999	31
RM	979	1106	13	970	1129	16

†CC (cereal cultivated) PL (pastureland), DTF (dryland tilled fallowed) and RM (30 t ha^{-1} manured).

Table 5.3. Effect of ^{13}C cellulose application on cumulative mineralization.

Soil† origin	No N history			with N history		
	^{13}C cellulose		C effect	^{13}C cellulose		C effect
	0C	0.2C		0C	0.2C	
	C-CO_2 mg kg^{-1} soil		%	C-CO_2 mg kg^{-1} soil		%
CC	775	966	24	898	1014	13
PL	863	984	14	1102	1205	9
DTF	750	762	2	902	999	11
RM	979	970	-1	1106	1129	2

†CC (cereal cultivated) PL (pastureland), DTF (dryland tilled fallowed) and RM (30 t ha^{-1} manured).

Table 5.4. Specific soil mineralization in relation to soil TC.

Soil† origin	N^-		N^+	
	^{13}C cellulose		^{13}C cellulose	
	0C	0.2C	0C	0.2C
	$\left[\frac{C\ respiration\ mg/\ kg\ soil}{(TC + 13C\ added) mg/\ kg\ soil}\right] \times 100$			
CC	1.7	2.1	1.9	2.1
PL	1.7	1.9	2.1	2.4
DTF	5.8	5.8	6.3	6.9
RM	3.6	3.5	4.0	4.0

†CC (cereal cultivated) PL (pastureland), DTF (dryland tilled fallowed) and RM (30 t ha^{-1} manured).

Most of the time, soils experienced a negative priming response to cellulose addition indicating preferential substrate utilization after receiving cellulose, which is an easily degradable substrate. During days 6 to 9, negative priming was observed for all soils without any significant influence from the soil origin and N history (Fig. 5.5). A similar trend was found for 30 to 37 d period. However, PL soil without N history had a trend of positive priming (i.e., did not differ significantly from other treatments) which emphasized microbial reliance on soil OM. At the end of the incubation study (60 to 78 d), a significant interaction effect of soil origin and N history on priming effect (PE) of CC and RM soils was observed. A positive priming effect was observed in N unfertilized CC soil while N fertilizer application prevented the microbial use of soil OM. RM soil with a N history had higher negative PE than soil without a N history. Organic matter mineralization differences were mainly governed by microbial community differences. An examination of microbial community compositions and diversity provided better explanations for observed functional differences in the transplanted soils.

5.5.2. Microbial community dynamics after ^{13}C cellulose application

PLFA analysis revealed no significant effects of sampling date, N fertilization history, soil origin or ^{13}C cellulose application on total microbial biomass, G^- bacteria and fungi abundances of transplanted soils. The relative abundance of fungi was higher at 30 d (Fig.C.4) indicating a dominant role played by fungi in cellulose decomposition. The abundance of bacteria and *Actinobacteria* did not vary at sampling days 30 and 78 with N fertilization history or ^{13}C cellulose application. Bacteria (30 d $P<0.04$, 78 d $P<0.001$) and *Actinobacteria* (30 d $P<0.001$, 78 d $P<0.001$) abundances were significantly different between soils of different origin at each sampling day and followed similar patterns. Low fertility DTF soil contained the lowest bacterial and *Actinobacteria* abundances compared to other soils (Fig. 5.6) while *Actinobacteria* abundance was greatest in CC soil at both sampling days.

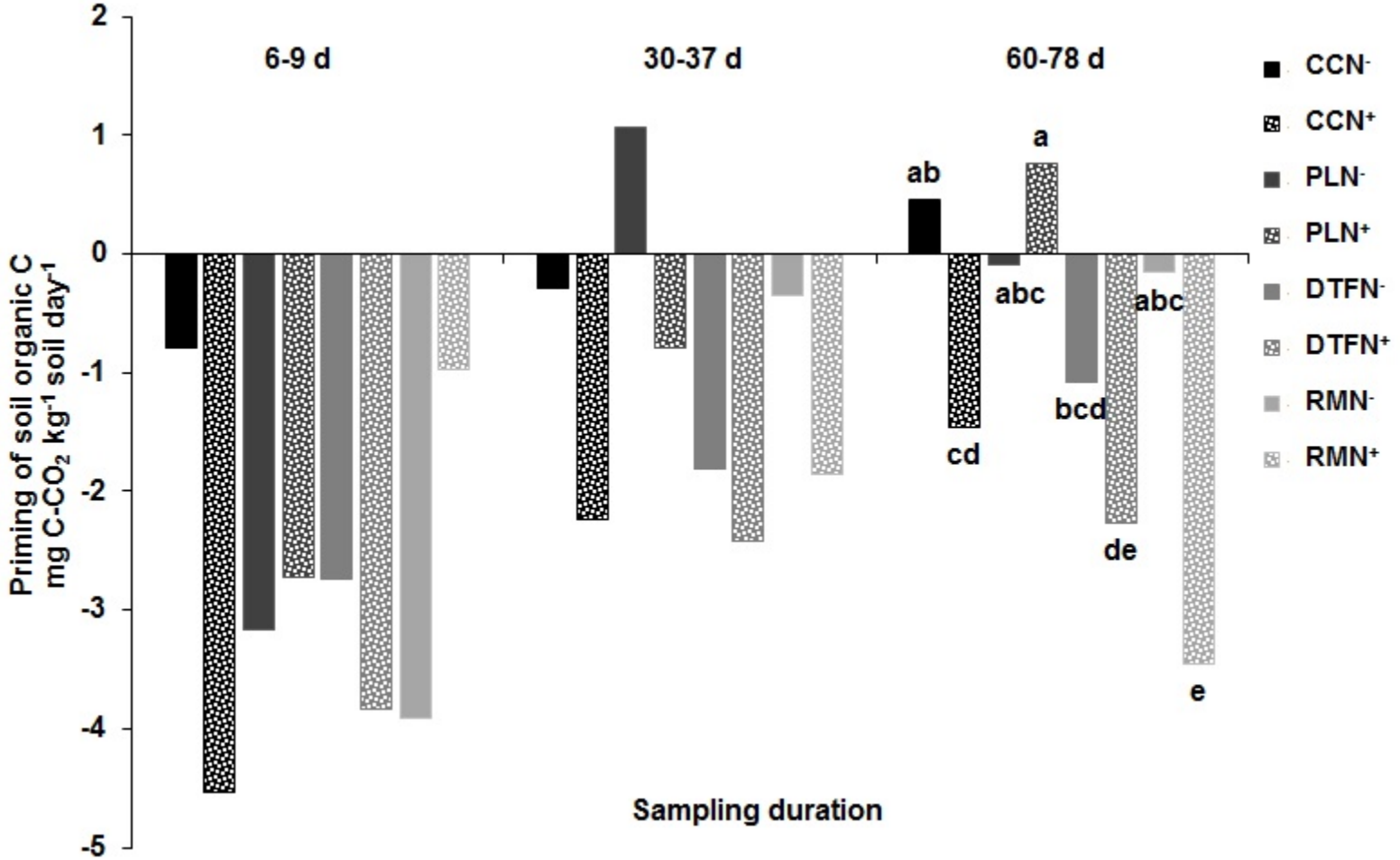


Fig. 5.5. Daily priming effect on soil OM decomposition at 6 to 9 d, 30 to 37 d and 60 to 78 d periods. Black to grey colors represent the fertility gradient from high to low. CC (cereal cultivated) PL (pastureland), DTF (dryland tilled fallowed) and RM (30 t ha^{-1} manured) soils. N history N^- -0 kg N ha^{-1} and N^+ -60 kg N ha^{-1}.

Variations in G^+ bacteria with soil origin at 30 d and the interaction effect of soil origin and ^{13}C cellulose rate at 78 d were evident (Fig. 5.6). In CC and PL soils, G^+ bacteria abundance increased at 78 d in microcosms that cellulose unamended. However, with ^{13}C cellulose application to the PL soil, G^+ bacterial abundance declined and became comparable to DTF and RM soils at 78 d. In the DTF and RM soils, G^+ bacteria were less responsive and remained steady with sampling dates and cellulose application. There was no response of G^- bacterial biomarkers to ^{13}C cellulose application, N fertilizer application history or sampling date. Physiological stress 1 biomarkers significantly varied with soil origin without any effects from cellulose and N fertilizer additions (Fig. C.3).

Microbial community compositions varied among microcosms of different soil origins and had varied responses to N fertilizer history and ^{13}C cellulose application (Fig. 5.7). Based on MRPP analysis, heterogeneity among microbial community composition with soil origin (A=0.001, P<0.00001), ^{13}C cellulose application (A=0.07, P<0.000001) and sampling period (A=0.04, P<0.0001) were apparent without any difference with N fertilizer history (A=0.001, P=0.34). High fertility, PL, and CC soil community compositions were closely arranged in the ordination demonstrating inherent soil fertility level as a key determinant of microbial community composition. In CC soil, microbial community composition remained steady with N history and ^{13}C cellulose application over time. Replicates of PL soil demonstrated differences which may be due to the patchy distribution of nutrients (e.g. from cow dung patches) in pastureland. Regardless of ^{13}C cellulose application, N history altered the microbial community structure at each sampling date for PL soil. The PL soil responded to ^{13}C cellulose application by a shift in the microbial community composition detected at 30 d. Microbial community differences due to ^{13}C cellulose application diminished at 78 d as decomposer communities triggered by the initial cellulose application did not persist with cellulose depletion. Microbial community compositions changed between 30 d and 78 d in low fertility DTF soil, even when cellulose was not applied. ^{13}C cellulose addition created differences in DTF soil microbial community structures in both N fertilizer levels (0 and 60 kg N ha^{-1}). Within 30 d, the microbial community was similar in cellulose amended and non-amended soils with a history of N fertilizer addition; however, communities diverged at 78 d which may be due to variations in C assimilation pathways following C amendment. Microbial community composition of N

unfertilized soil altered with cellulose application, likely because the microbial community was more responsive to added C in the N control soil. This divergence in N unfertilized soil did not persist at 78 d. Similar trends of DTF were observed for RM soils both sampling dates.

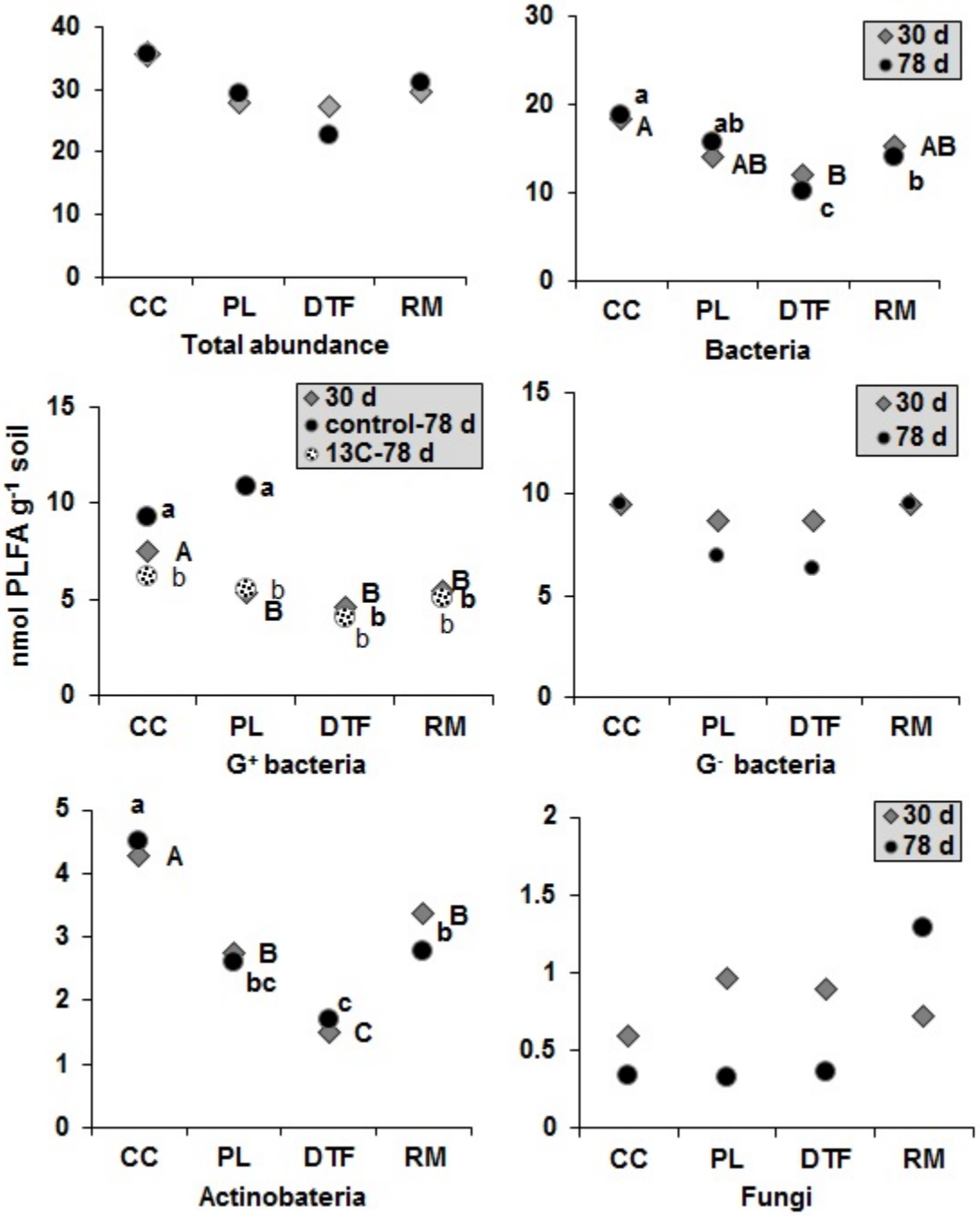


Fig. 5.6. Abundance of total PLFA and different microbial groups (bacteria, G^+, G^-, Actinobacteria and fungi) at 30 and 78 d. Means with different letters (A to C for 30 d and a to c for 78 d) are statistically different at $P<0.05$. CC-cereal cultivated, PL- pastureland soil, DTF- dryland tilled fallow and RM- manured 30 t ha^{-1} soils.

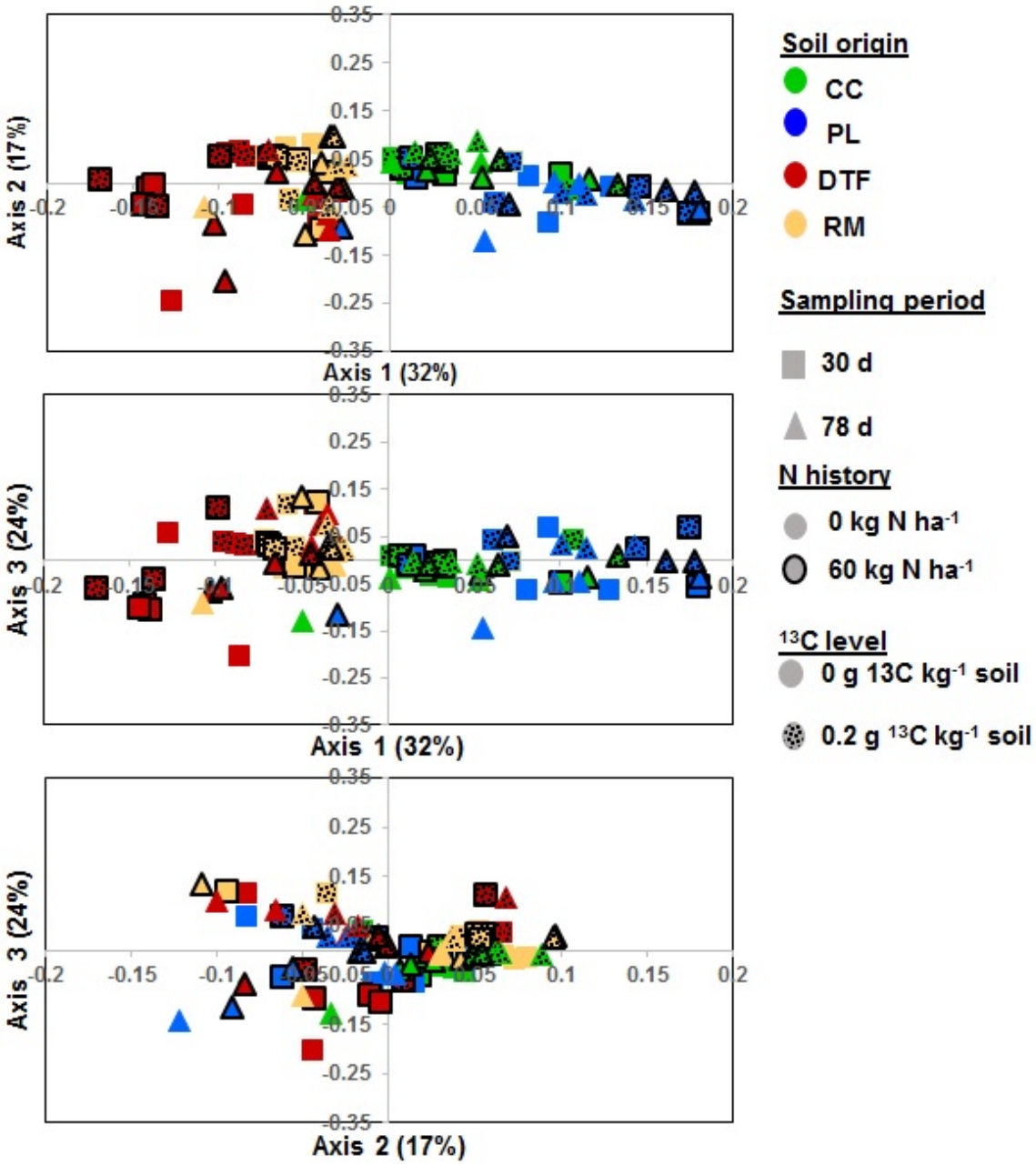


Fig. 5.7. Principle coordinate analysis of soil microbial PLFA (total community) profiles of ^{13}C amended and control soils at 30 d and 78 d. CC-cereal cultivated, PL- pastureland soil, DTF-dryland tilled fallow and RM- manured 30 t ha^{-1} soils.

5.5.3. Active decomposer community dynamics

Active (^{13}C labelled) decomposer communities identified at 30 d and 78 d had different microbial profiles. The ^{13}C cellulose addition did not alter the total microbial abundance of a particular soil between 30 d and 78 d (P=0.31). However, ^{13}C cellulose created differences in total microbial abundances between soils of different origin at 30 d (P<0.0058). Nitrogen fertilizer history further influenced total microbial abundance at 30 d (P<0.04) in the CC and DTF soils. The high fertility CC soil had the highest total active decomposer abundance (Fig. 5.8), while PL soil had lowest, indicating an effect of soil origin on the decomposition of ^{13}C cellulose. ^{13}C cellulose application appeared to create temporary variations among soils and differences in total microbial abundance that did not persist at 78 d. Similar trends and patterns were observed for total bacteria, and G^+ and G^- bacterial groups.

The fungal decomposer community was steady throughout incubation and did not vary with soil origin. A higher fungal population at 30 d than 78 d indicated an initial assimilation of ^{13}C cellulose by fungi. Other than the PL soil, trends of increased fungal abundance at the 30 d sampling was further supported by a relative abundance of different biomarkers (Fig. C.5).

The distribution of ^{13}C derived from ^{13}C labeled cellulose within microbial groups varied depending on soil origin. ^{13}C cellulose incorporation was significantly influenced by soil origin at 30 d (P<0.01) without any difference at 78 d (P=0.8). The high fertility CC (53%) and manured RM (49%) soil bacterial populations contained the highest concentrations of ^{13}C at 30 d (Fig. 5.9), whereas high fertility PL (41%) and low fertility DTF (42%) soil bacterial communities contained lower ^{13}C amounts and varied with soil origin (P<0.005).

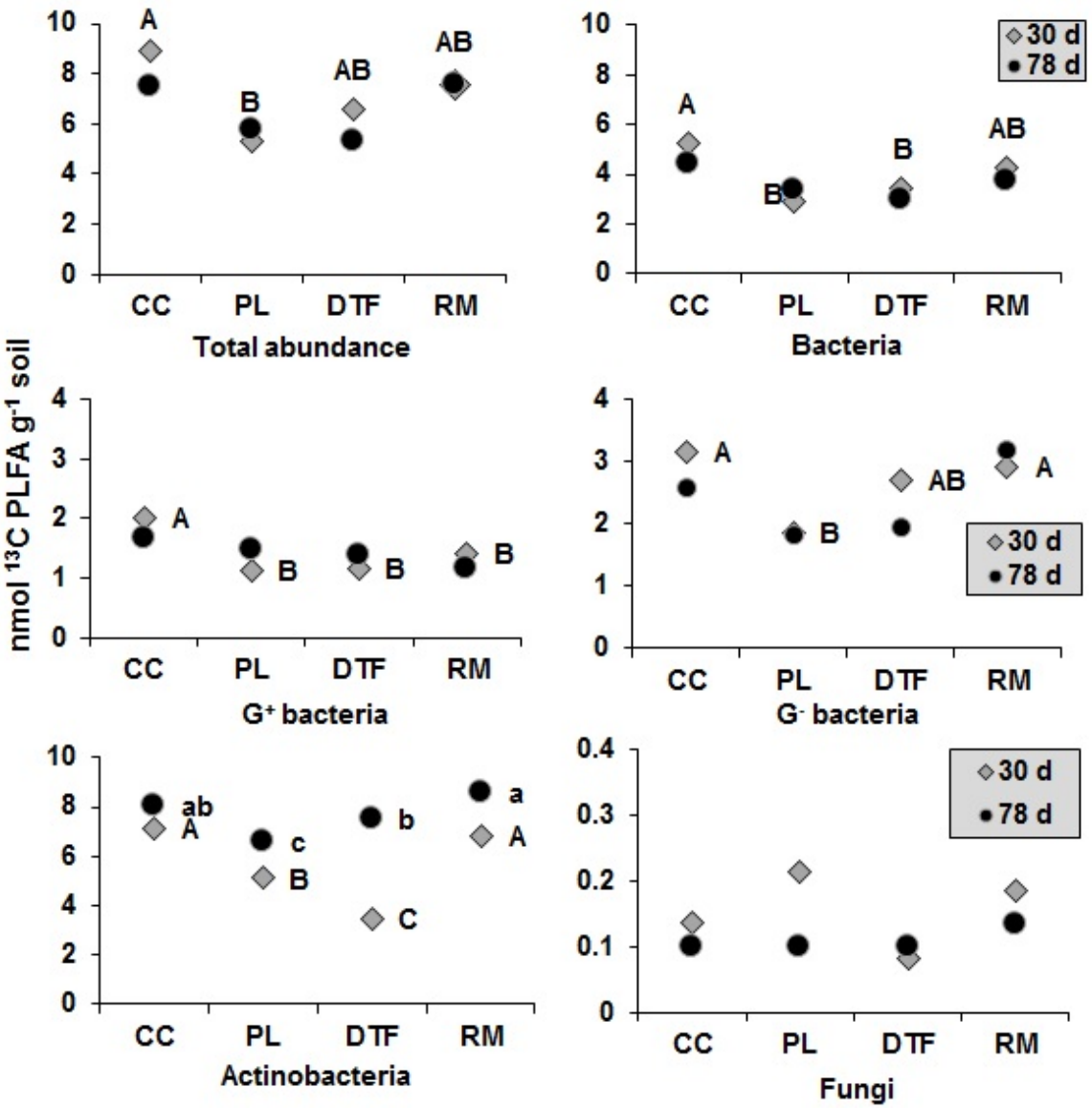


Fig. 5.8. Abundance of total ^{13}C PLFA and active decomposer microbial groups (bacteria, G^+, G^-, Actinobacteria and fungi) during 30 and 78 d. Means with different letters (A to C for 30 d and a to c for 78 d) are statistically different at $P<0.05$. CC-cereal cultivated, PL- pastureland soil, DTF- dryland tilled fallow, RM- manured 30 t ha^{-1} soils.

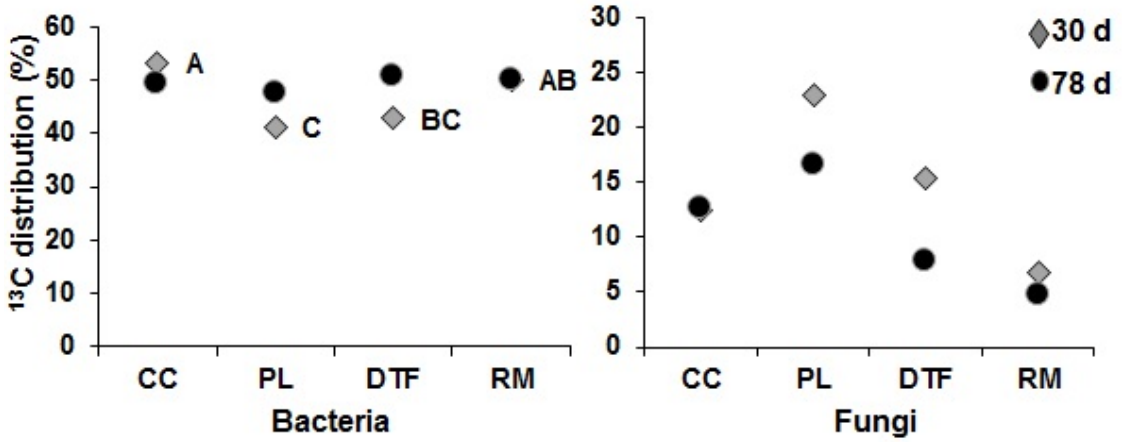


Fig. 5.9. Proportion of ^{13}C derived C within bacteria and fungi communities at 30 d and 78 d. Means with different letters (A to C for 30 d and a to c for 78 d) are statistically different at P<0.05. CC-cereal cultivated, PL- pastureland soil, DTF- dryland tilled fallow, RM- manured 30 t ha^{-1} soils.

Distinct ^{13}C labelled active decomposer community compositions were associated with soil origin and land use history (Fig. 5.10). Differences in decomposer community compositions with N fertilizer history and sampling day were apparent. The MRPP analysis further confirmed the variability between microbial communities with soil origin (A=0.3, P<0.0001), N history (A=0.009, P<0.18) and sampling day (A=0.05, P<0.002). However, these community differences were predetermined by soil origin, as variable community responses were associated with N fertilizer history and ^{13}C cellulose application for each soil. The active (^{13}C labelled) decomposer microbial community of CC remained stable with both N fertilizer histories (0 and 60 kg N ha^{-1}) at 30 d. When incubation continued (78 d), CC soil had diverse decomposer communities related to N history. A similar pattern was found in RM soil microcosms. The composition of the decomposer community of PL soil was relatively distinct between N history levels at both sampling days. Low fertility DTF soil decomposer communities varied with sampling day and did not differ between soils with different N fertilizer history.

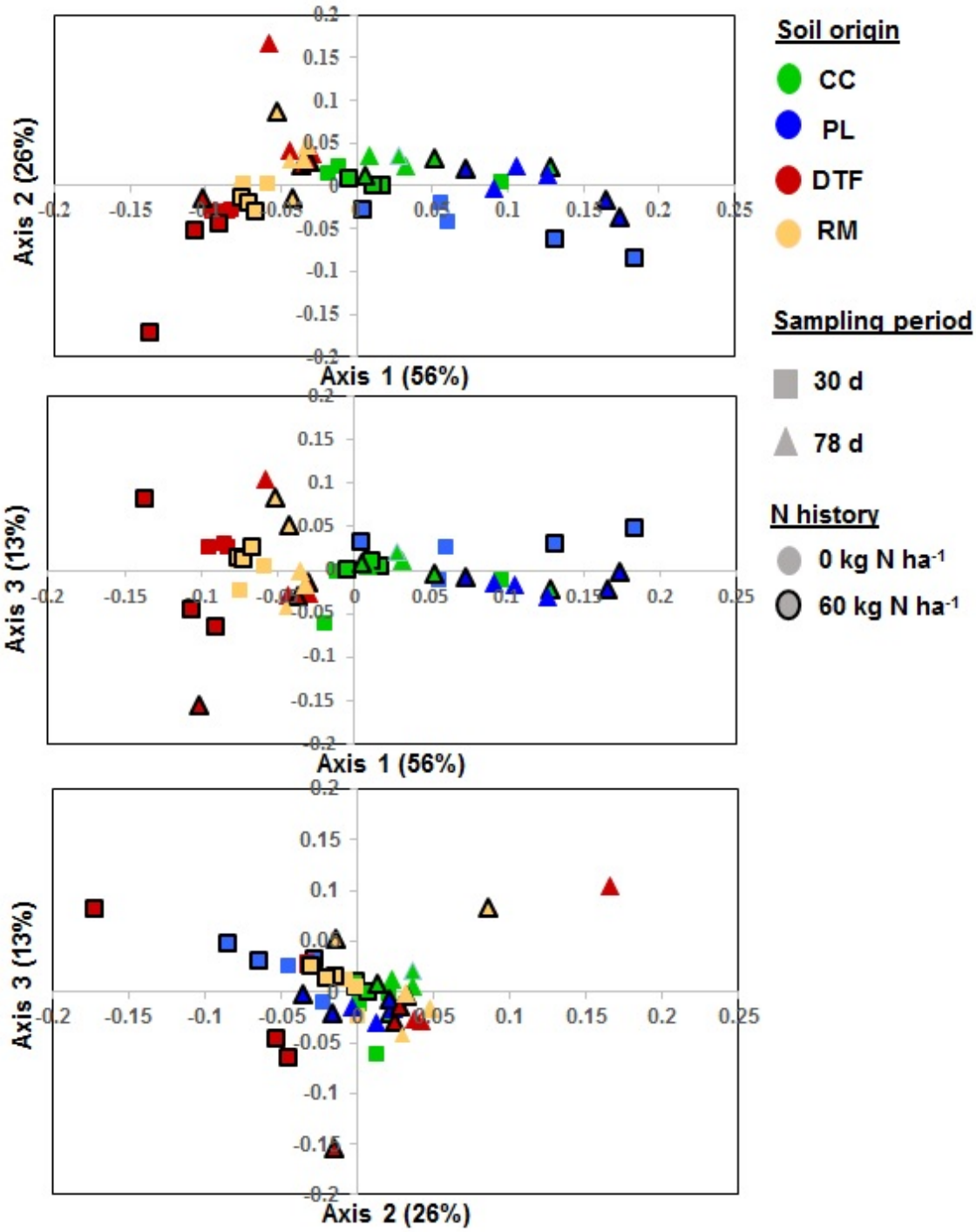


Fig. 5.10. Principle coordinate analysis of active decomposer microbial (^{13}C assimilated) PLFA profiles at 30 d and 78 d. CC-cereal cultivated, PL- pastureland soil, DTF- dryland tilled fallow and RM- manured 30 t ha^{-1} soils.

Illumina sequencing analysis of 16S *rRNA* gene showed similar trends as those found for PLFA. The community differences between high fertility soils (CC and PL) from DTF and RM soils were clearly visible in the sequencing analysis (Fig. 5.11) and diverged with ^{13}C cellulose application. However, the degree of community differences varied with soil origin. Also, the effect of N fertilization on soil communities varied with soil origin where N fertilizer application had noticeable alterations in high fertility soil (CC and PL) communities. The PL soil microbial community from microcosms without N fertilization background and cellulose application were closely related to DTF and RM soils. Analysis of similarities (ANOSIM) of microbial community structure indicated microbial community differences with respect to the soil origin (P<0.001) and ^{13}C cellulose rate (P<0.001). The PERMANOVA highlighted a significant interaction effect (P<0.023) of soil origin × N fertilizer application history × ^{13}C cellulose addition × sampling day on the transplanted soil microbial communities. Significant differences in the microbial composition of each transplanted soil with ^{13}C cellulose addition and sampling day were detected using a pairwise test conducted through PERMANOVA analysis.

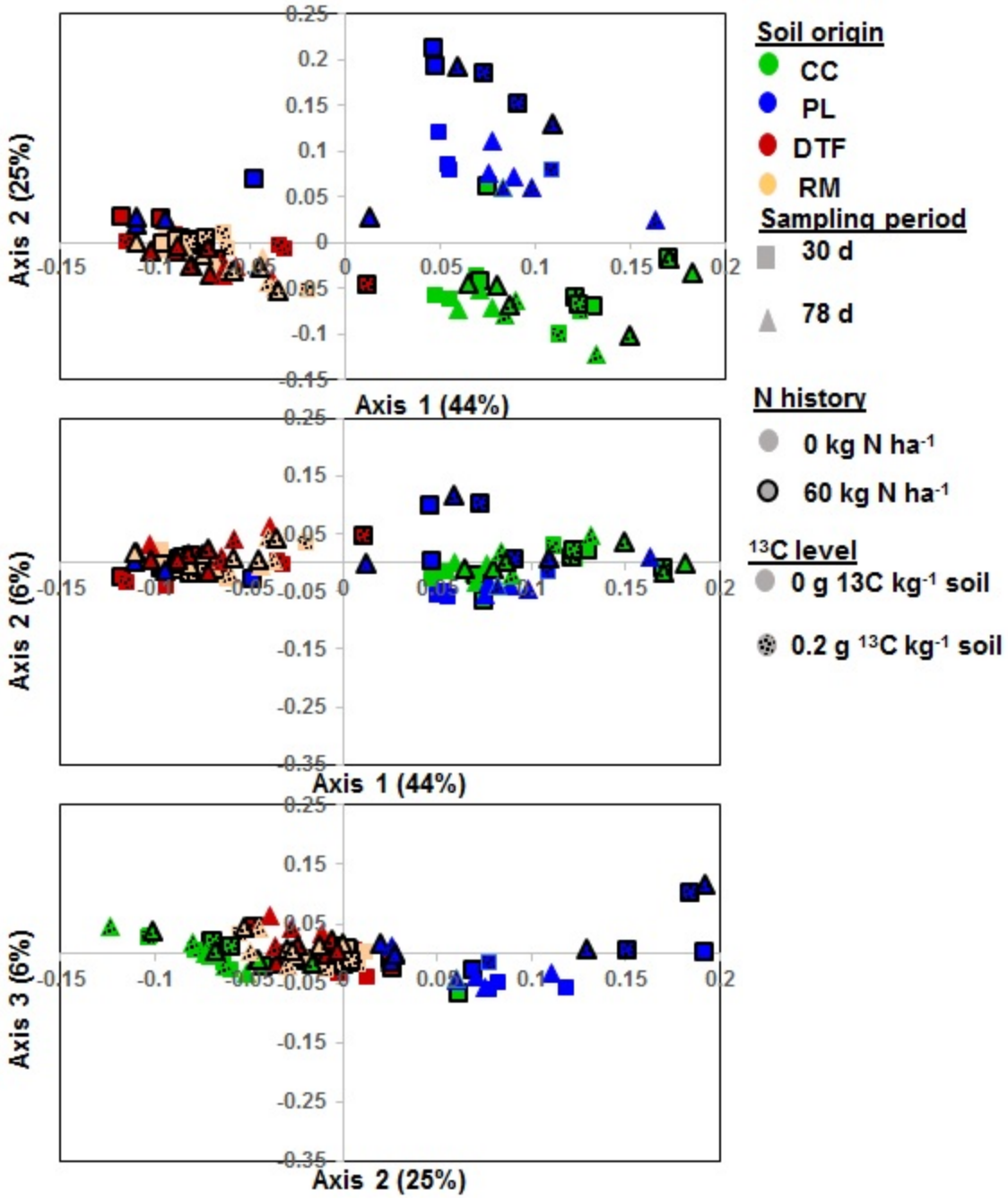


Fig. 5.11. Principle coordinate analysis of total microbial profiles at 30 d and 78 d and based on 16S *rRNA* genes sequencing. CC-cereal cultivated, PL- pastureland soil, DTF- dryland tilled fallow and RM- manured 30 t ha^{-1} soils.

As a part of the total soil microbial community captured by sequencing the 16S *rRNA* genes extracted from the soil, a subset of microbial groups actively participated in ^{13}C cellulose decomposition and was studied at 30 d. Decomposer community structures were different from

their total communities at 30 d and distinct to each transplanted soil (Fig. 5.12). The long-lasting effect of soil origin on ^{13}C cellulose active communities was confirmed by PERMANOVA analysis ($P<0.001$) with no detectable impact of N fertilizer application history. Pairwise analysis of N fertilizer application for each soil provided further evidence of no N fertilizer effect on the active decomposer communities. However, visual assessment of microbial profile ordinations indicated the active decomposer community of N fertilized soil was different from that of the control (0N). Higher variability was found within the replicates of soils with N fertilizer application. The active decomposer communities of CC, PL and RM soils without N application were distinctly different from each other at 30 d. Further, the active decomposer community of unfertilized PL soil was drastically different from the remaining soils.

Decomposer community diversity was investigated using a number of species observed (Sobs), Shannon and inverse Simpson indices (Table 5.5). The Sobs varied with soil origin and N fertilizer history. Species richness was high in high fertility PL soil without a N history. High fertility CC soil with no N fertilizer addition had the lowest species richness among transplanted soils. In CC and DTF soils, microbial species richness and evenness increased with N fertilizer application, whereas the decomposer community of RM soil was unaffected by N history. Nitrogen fertilizer application had a larger impact on decomposer diversity of high fertility PL soil where soil with N history contained less diverse decomposer community. The community differences were apparent at different taxonomic levels.

Table 5.5. Microbial diversity of the ^{13}C labelled active decomposer community at 30 d.

Diversity	CC		PL		DTF		RM	
	N^-	N^+	N^-	N^+	N^-	N^+	N^-	N^+
Sobs	429	681	712	498	552	654	565	500
Shannon	3.7	4.1	4.8	3.7	3.8	4.3	3.6	3.4
$1/D^{\dagger}$	21	30	59	24	25	37	18	16

†Inverse Simpson

CC-cereal cultivated, PL- pastureland soil, DTF- dryland tilled fallow, RM- manured 30 t ha^{-1}.

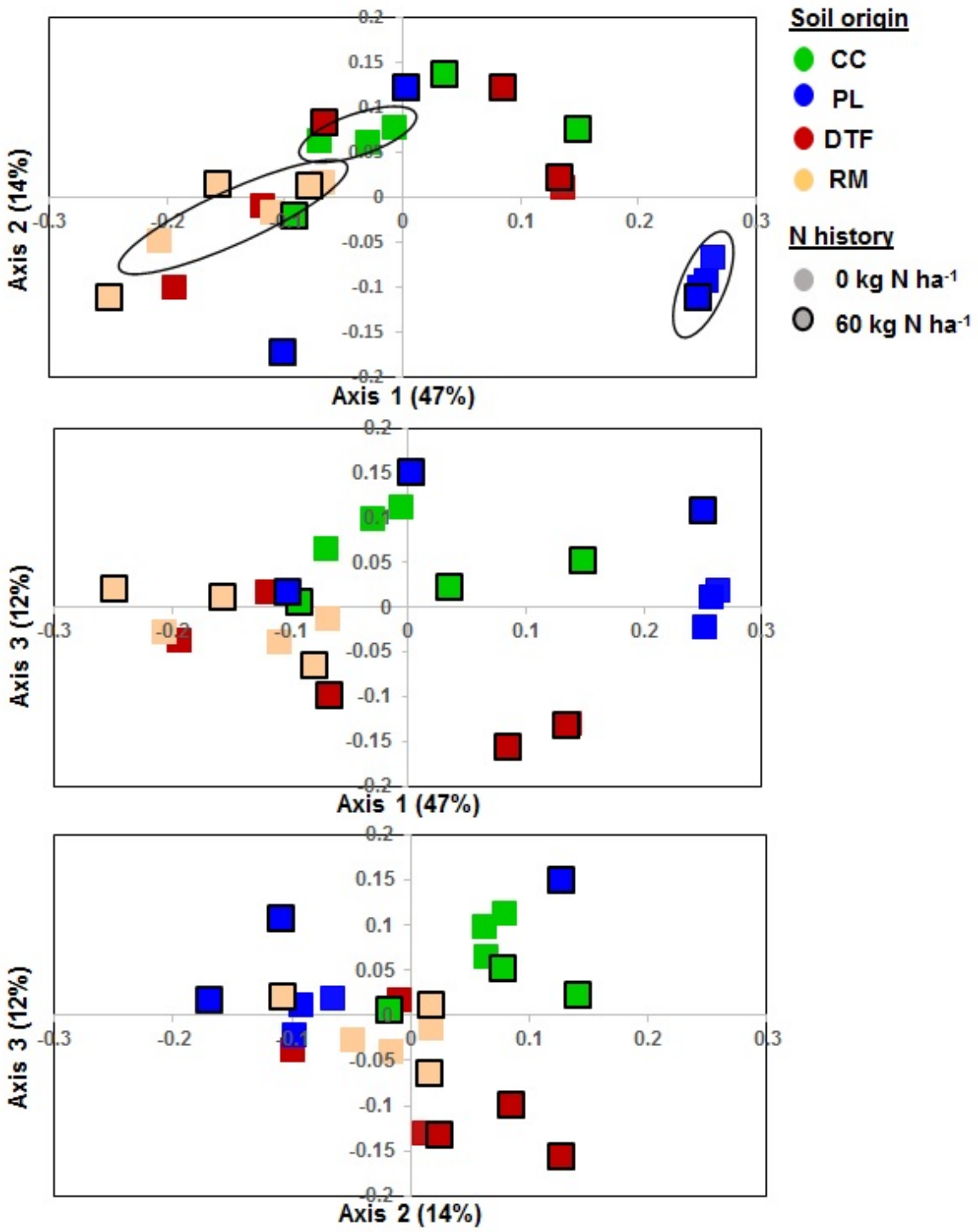


Fig. 5.12. Principle coordinate analysis of active decomposer community profiles at 30 d based on 16S *rRNA* genes sequencing. CC-cereal cultivated, PL- pastureland soil, DTF- dryland tilled fallow and RM- manured 30 t ha^{-1} soils.

The relative abundance of phyla shifted from 30 to 78 d (Fig.5.13). These community composition changes continued to the end of incubation. Major changes were confined to *Proteobacteria* and *Actinobacteria* at 30 d. This was further confirmed by the sequencing data for heavy (HF) DNA fractions (active cellulose decomposer C community) obtained from isopycnic centrifugation (HF in Fig. 5.13). Most of the ^{13}C cellulosed amended microcosms without N history had lower *Acidobacteria* populations. The cellulose decomposer community of high fertility CC and PL soils were mainly composed of the *Proteobacteria* and were not affected by a history of N fertilizer addition. Low fertility DTF soil *Proteobacteri*a abundance did not shift with N addition or C supplement, whereas *Proteobacteria* abundance remained stable with N history. The differences in *Actinobacteria* community abundance according to N history and ^{13}C cellulose addition were determined by soil origin.

Actinobacteria and *Proteobacteria* abundances and compositions in the active decomposer communities suggest that soil origin is the major controlling factor of the C-cycling and decomposition functions in soils (Fig. 5.14 and Fig. 5.15). Shifts in *Actinobacteria* and *Proteobacteria* composition emphasize the effect of a history of N fertilizer application on cellulose decomposition. *Streptomycetaceae* were the most abundant *Actinobacterial* family present in all the soils except for unfertilized PL soil which was dominated by *Gaiellaceae* and *Actinomycetales*. The *Actinobacteria* diversity of unfertilized PL soil was greater than N fertilized soil. Decomposer *Actinobacteria* and *Proteobacteria* diversity increased in unfertilized PL and DTF soils. The opposite trend to PL soil was found in CC ad RM soils where long-term N fertilization increased the active decomposer *Actinobacteria* diversity. Unlike in *Actinobacteria*, *Proteobacteria*, evenness was lesser influenced by N history. Regardless of N history, CC and RM decomposer *Proteobacteria* community was dominated by *Xanthomnadaceae*. Similarly, in DTF and RM soils, more than 20% of the active decomposer *Proteobacteria* community was compromised of *Myxococcales*.

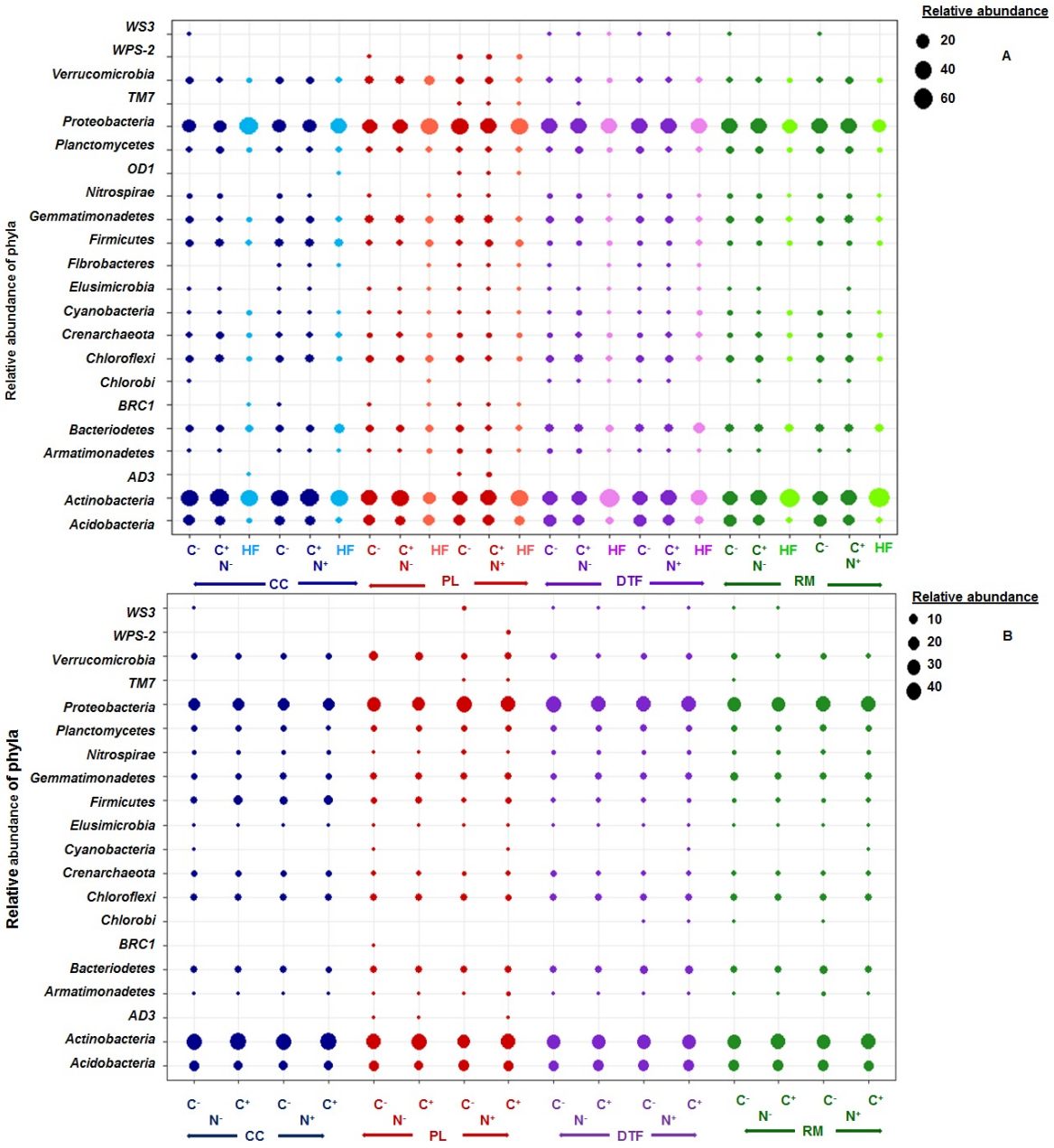


Fig. 5.13. Relative abundance of microbial phyla at A) 30 d and B) 78 d. C^- - 0 g ^{13}C kg^{-1}soil and C^+- 0.2 g ^{13}C kg^{-1}soil, N^- - 0 kg N ha^{-1} and N^+ - 60 kg N ha^{-1}, HF- ^{13}C assimilated active community.CC-cereal cultivated, PL- pastureland soil, DTF- dryland tilled fallow, RM-manured 30 t ha^{-1}.

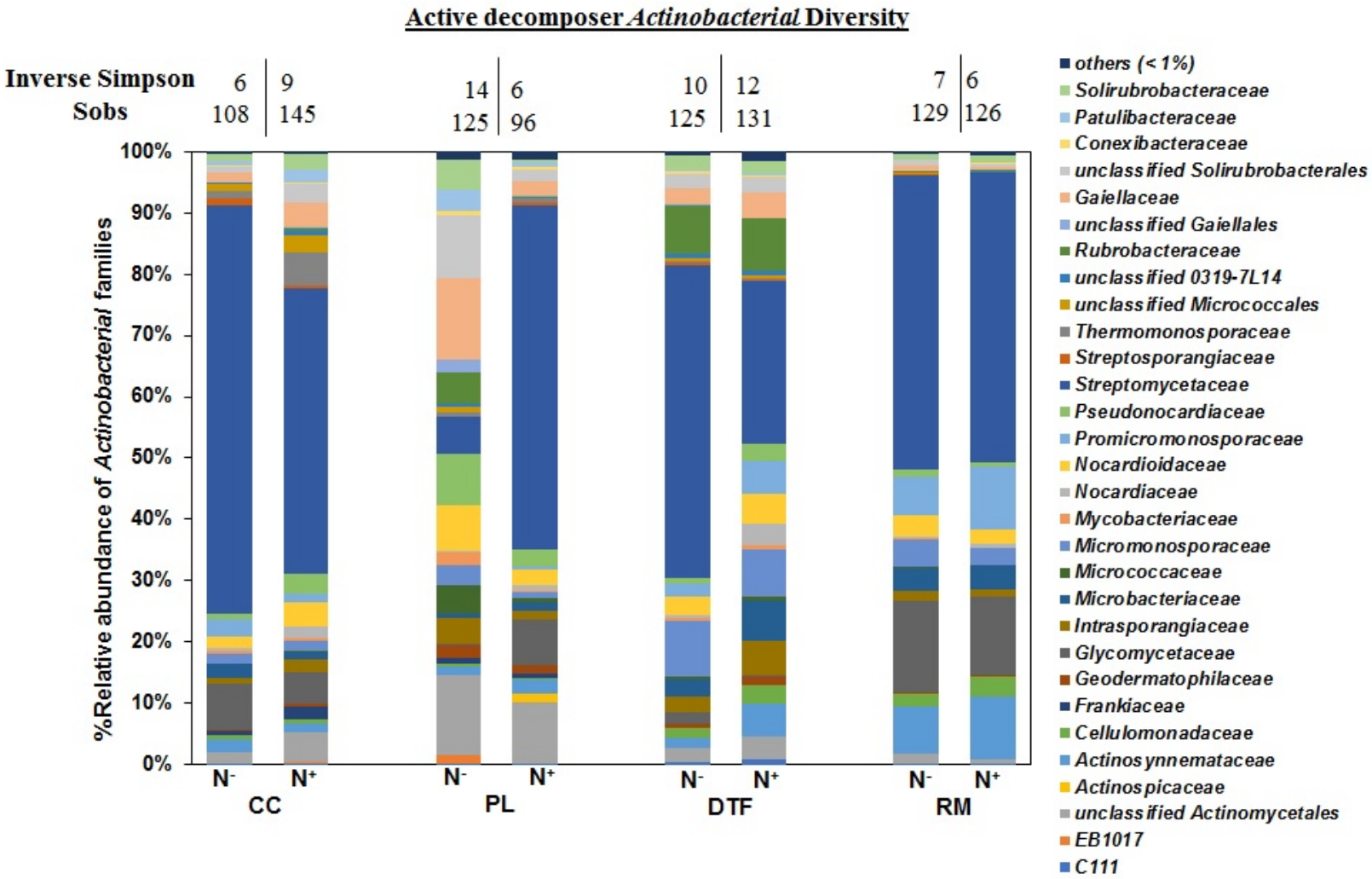


Fig. 5.14. Most abundant (> 1%) *Actinobacteria* families and diversity in active decomposer community at 30 d. CC-cereal cultivated, PL- pastureland soil, DTF- dryland tilled fallow, RM- manured 30 t ha^{-1}.

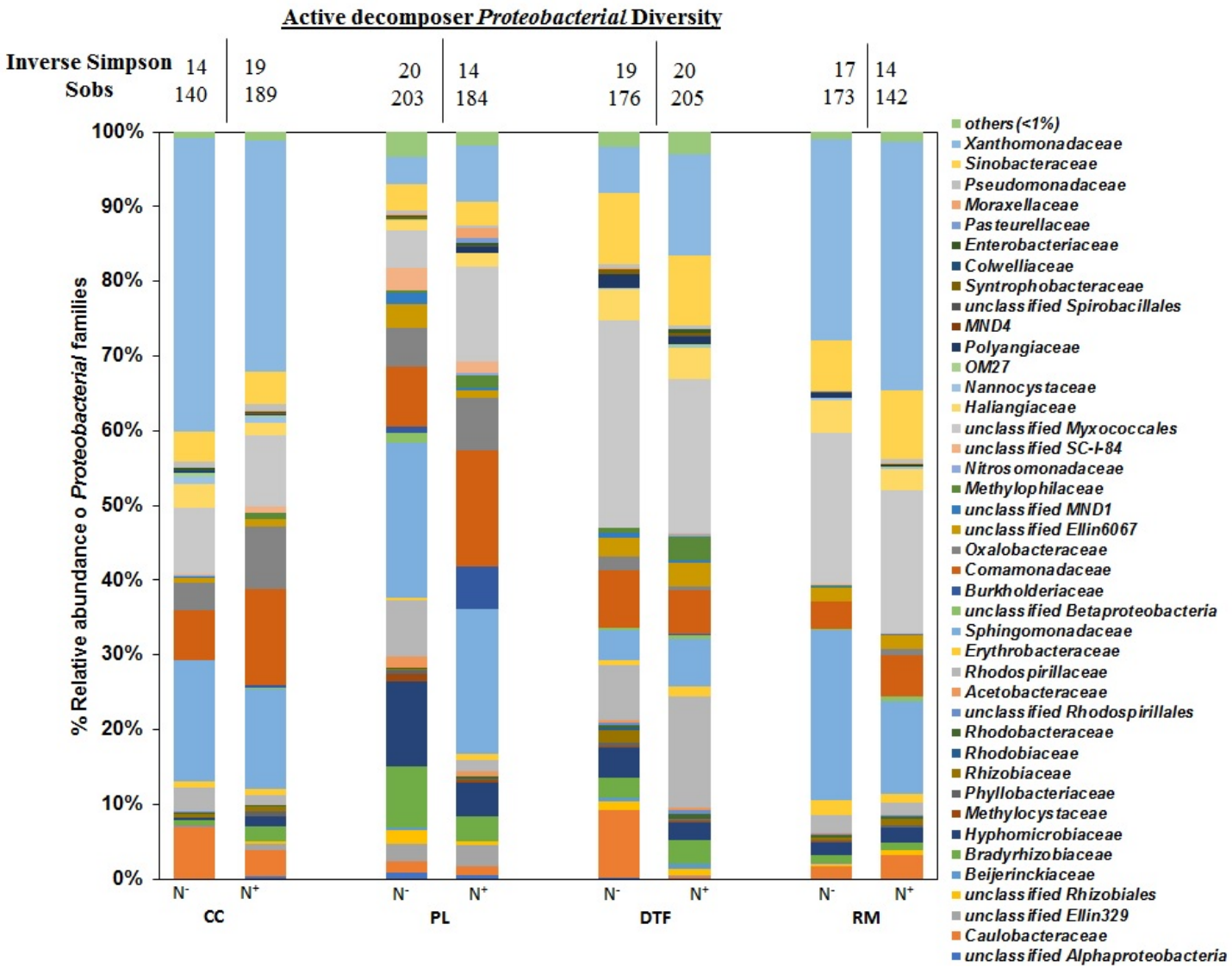


Fig. 5.15. Most abundant (> 1%) *Proteobacteria* families and diversity in active decomposer community at 30 d. CC-cereal cultivated, PL- pastureland soil, DTF- dryland tilled fallow, RM- manured 30 t ha^{-1}.

5.6. DISCUSSION

Results of the SIP cellulose decomposition study indicated a prolonged effect of soil origin and land use legacy on C dynamics of the transplanted soils. Land use legacy and soil origin effects were apparent both through the differences in the total community and in active decomposer communities (^{13}C decomposer community) of transplanted soils (Figs. 5.7, 5.10, 5.11, 5.12 and 5.13). In the total microbial community, total PLFA, bacterial and fungal abundances and bacteria: fungi ratio remained stable with cellulose addition and history of N fertilizer application at both sampling days (30 and 78 d) but varied with soil origin and land use legacy (Fig. 5.6). Similar microbial biomass between cellulose amended and unamended soils at 30 and 78 d did not emphasize the lack of microbial biomass response to added cellulose at a later stage of cellulose decomposition. However, it is expected that microbial biomass would respond to added cellulose at the very beginning and likely returned to their original state over time (30 d). In agreement with Zhao et al. (2014), the current research found no difference in abundance of total PLFA, bacterial and *Actinobacteria* with N fertilizer application at both sampling days (30 and 78 d). The total microbial community composition changes persisted prolonged and evident through dissimilarities between the microbial community structures associated with ^{13}C cellulose addition and N fertilizer application history (Figs. 5.7 and 5.11). Similar effects of soil origin and land use legacies have also been found in various ecosystems including forest, grassland, intensively managed wheat, and bioenergy crop production (corn and switch grass) systems (Foster et al., 2003; Jangid et al., 2011; Liiri et al., 2012; Kallenbach and Grandy, 2015) through soil microbial community composition (Jangid et al., 2011) and enzymatic activities (Kallenbach and Grandy, 2015) which influence soil nutrient cycling (Foster et al., 2003; McLauchlan, 2006; Liiri et al., 2012; Kallenbach and Grandy, 2015). Buckley and Schmidt (2001) found similar microbial community compositions between cultivated land and nearby abandonment land left for seven years without cultivating. Further, a study conducted at long-term ecological research site in Michigan identified more similarities between soil microbial compositions of early succession grassland (17 yr) to remaining original cropland (Jangid et al., 2011).

According to ^{13}C PLFA, the active decomposer community varied over time (30 d and 78 d) indicating temporal variations in the active community through the decomposition process (Fig. 5.10). Tardy et al. (2015) also found distinct decomposer communities at different sampling times and reported a long-term effect of land use history on the succession of the microbial population during wheat straw decomposition. In the current study, the microbial community structure differences were more profound in ^{13}C enriched (active decomposer) PLFA and DNA at 30 d than 78 d (Figs. 5.10 and 5.12). The variations in the microbial composition with cellulose addition and N fertilizer application confirmed the sensitivity of the microbial community structures to the current land use. However, distinct microbial communities in each soil suggested a strong influence of soil origin and land use history on the impact of current land use. Further, these findings confirmed a long-term effect of the inherent soil characteristics (i.e., physical, chemical and biological) and land use legacy on soil C dynamics that has persisted 21 yr after transplanting.

Differences in the decomposer communities were likely related to the initial fertility level and nutrient availability of the transplanted soils. The soil OC and TN are two main factors governing soil microbial community structure and functional diversity (Li et al., 2015). Physicochemical characteristics of the transplanted soils were highly variable at the time of transplanting (Olson et al., 1996; Zvomuya et al., 2008; Janzen, 2012; Yanni et al., 2016). Loss of TN, TC, and OC occurred under the new management practices in PL, CC and RM soils. Nitrogen fertilizer additions can minimize the reduction of TN, TC, and OC. Low fertility DTF soil indicated an increase in OC levels under the current management practices even with a limited supply of C (Chapter 3) (Yanni et al., 2016). The observed soil fertility changes suggested soil responses to land use changes occurred following the transplantation including a shift in the plant community, limited crop residue incorporation and N fertilizer application. Although soil chemical properties changed with current management practices, dissimilarities between soil physiochemical properties remained comparable to the original soil properties before transplantation. Similar to microbial communities, these alterations were predetermined by the land use history. Previous studies have also reported lasting effects of land use changes on soil C and nutrient contents (Fu et al., 2001; Monkiedje et al., 2006; Barančíková et al., 2016; Yanni et al., 2016). For example, Monkiedje et al. (2006) found higher OC and available

nutrients (N and P) in cultivated systems compared to native forest land signifying land use effects on nutrient availability. Further, conversion of croplands to forests and grasslands enhances the soil OM, TN and other nutrients (Fu et al., 2001; Barančíková et al., 2016). The aforementioned fertility changes may have affected the soil environment by altering physicochemical and microbial community (decomposer community) compositions.

The above-ground vegetation is strongly associated with belowground components and this connection is linked through the soil microbial community (Berg and Smalla, 2009; Kardol and Wardle, 2010; Bonito et al., 2014). Shifts in plant community composition have been shown to alter the litter quality (Kardol and Wardle, 2010) and host specificity and root exudates (Haichar et al., 2008; Huang et al., 2014) resulting in changes in the soil microbial community composition. The transplanted soils used in the present study originated from ecosystems and soils with diverse inherent characteristics and management histories (Zvomuya et al., 2008) and following transplantation, type and quantity of C inputs returning to soils were significantly changed as a consequence of new land use practices. Except for the CC soil transplanted from a continuous cereal system, other soils had different vegetation covers before the transplantation. For example, the PL soil was obtained from a native pastureland which had the highest plant diversity compared to CC, DTF, and RM soils. The DTF soil was obtained from a pathway of an experimental field, which only had minimum C inputs because the pathways were tilled to minimize plant growth, leaving only sparse and periodic weed growth. The RM soil was continuously cropped with barley and organic manure was applied through wet manure. After transplantation, continuous wheat cultivation changed C inputs to these soil systems. In addition to the changes that occurred in the vegetation cover, crop residue was continuously removed from the studied field for 23 yr. It has been reported that the removal of crop residues for a 4 yr period caused a substantial lost in the amounts of soil OC from the top soil (Chowdhury et al., 2015). Similarly, reductions in soil OC were observed for CC, PL and RM soils in the present study. In agroecosystems, crop residues are the key component of C for soil, and decomposer nutrient demands are one of the main factors governing the direction and intensity of new OM input decomposition (Rinkes et al., 2013).

The transplanted soils displayed negative priming at 6 d to 9 d, and 30 d to 37 d except for PL soil without N application, indicating a decrease in the decomposition of stabilised soil OC in

the transplanted soils (Fig. 5.5). Negative priming during the initial phase of decomposition has also been observed in previous studies (Kuzyakov and Bol, 2006; Wang et al., 2015). Negative priming is a sign of preferential substrate utilization, where soil microorganisms prefer to consume easily degradable compounds over existing soil OM (Fontaine et al., 2003). The presence of indigenous highly labile C reduces the PE in soils (van der Wal and de Boer, 2017). Similar to the current study, a negative priming (-4%) was observed by Qiao et al. (2016) when soils were amended with labile C that had a low C: N ratio. Continuous crop residue removal after harvesting reduced the soil C availability for microbial utilization and the remaining depleted soil OM. Remaining wheat stubble on the soil surface may slowly incorporate into the soil as experiment field is maintained with zero tillage. In addition, semi-arid climatic conditions at Lethbridge may have slowed down the incorporation of residues that remained on the soil surface (Helgason et al., 2014). Wheat roots decompose at a slower rate compared to aerial parts due to the difference in residue composition (Bertrand et al., 2006). In this scenario, the addition of ^{13}C cellulose may be preferred by soil organisms over wheat stubble and roots. Preferential substrate utilization in transplanted soils was supported by a microbial community shift after ^{13}C cellulose application and the resulting differences between total and active decomposer community structures observed in the present study (Figs. 5.7 and 5.11). The same phenomena have previously been reported in several other studies (Allison, 2012; Dijkstra et al., 2013; Qiao et al., 2016). The presence of a good quality substrate released soluble organic compounds, resulting in increased heterogeneity of the soil environment, and leading to changes in the microbial community (Nicolardot et al., 2007). Positive priming in the unfertilized high fertility soils (PL and CC) and N fertilized PL soil began at later decomposition stages. Positive priming may have occurred due to the mining of stable C by microorganisms to fulfill their C and N requirements after depletion of the added labile ^{13}C cellulose. Likewise, a dynamic shift in PE (negative to positive) has also been reported in an incubation study conducted to investigate the effect of fresh OM quality on microbial substrate utilization (Wang et al., 2015). Furthermore, the addition of fresh OM (cellulose) may have provided adequate energy for microorganisms to synthesize extra cellular enzymes which facilitate the decomposition of stable C (Wang et al., 2015).

The influence of long-term N fertilizer application was reflected through variations in the microbial composition of both total and active decomposer communities (Figs. 5.7, 5.11, 5.12 and 5.13). These changes were detected at lower taxonomic hierarchy (Figs. 5.14 and 5.15) but were less apparent at a higher taxonomic level (Fig. 5.13). For example, ^{13}C cellulose amendment and N fertilizer application influenced *Proteobacteria* and *Actinobacteria* communities. Except for unfertilized PL soil, family *Streptomycetaceae* was the dominant *Actinobacteria* family found in the active decomposer community of soils receiving cellulose (Fig. 5.14). *Streptomycetaceae* is known to involve in the later stage of cellulose degradation (Kämpfer et al., 2006). Regardless of N fertilizer application, the most dominant active *Proteobacterial* family varied with soil origin (Fig. 5.15). For example, *Xanthomonadaceae* was the most abundant *Proteobacterial* family in the CC and RM soils while *Sphingomonadaceae* and unclassified *Myxococcales* were commonly found in the PL and DTF soils, respectively. In the CC and DTF soils, active *Actinobacteria* and *Proteobacteria* diversity were greater in the N fertilized soils than those soils that did not receive N. Similarly, active decomposer community diversity responses to N fertilization history varied with soil type (soil origin and land use history) (Table 5.5). For example, N fertilizer application increased the active decomposer microbial diversity and their evenness in CC and DTF soils, while opposite trend was observed for the PL and RM soils. Diverse impacts of N fertilizer management on the microbial community composition and diversity also have been reported in previous studies (Nicol et al., 2004; Luo et al., 2015; Yu et al., 2016). Similar to CC and DTF soils, a 33-yr-old long-term N fertilized field had a higher microbial diversity than unfertilized soils (Luo et al., 2015). Campbell et al. (2010) also found a decrease in the bacterial diversity with long-term (24 yr) N fertilization in moist acidic tundra soils. A complex association between N fertilization and the active decomposer community composition has been shown, which can be influenced by soil factors such as increased available N (NO_3^- and NH_4^+) and lower soil pH (Zhang et al., 2008), length of N fertilization history (Yu et al., 2016) and fertilizer application rate (Fierer et al., 2012). These results suggest that the effect of long-term N fertilizer application on the microbial diversity is inconsistent across different soil origins and land use histories, due to the complex nature of soil fertility and microbial functional characteristics. Variations in the microbial composition and diversity might have altered microbial functions including OM mineralization.

All these findings demonstrate a lasting impact of soil origin and land use legacy in mediating the influence of the long-term N fertilizer application on soil C dynamics.

Differences in the active decomposer community composition and physicochemical properties of the transplanted soils may be the reason for cumulative C-CO_2 differences following cellulose addition in the transplanted soils (Figs. 5.3 and 5.4). Different microbial community structures and mineralization patterns in the transplanted soils were related to fertility differences, which resulted in C and/or N limited conditions for microorganisms. Therefore, diverse respiration patterns occurred as a result of complex differences in nutrient acquisition requirements of microorganisms. For example, soil transplanted from previously uncultivated PL (high-fertility) soil had the highest cumulative C-CO_2 emission, while low-fertility DTF had the lowest emission. A lower cumulative C-CO_2 emission in the high-fertility CC soil with greatest microbial biomass indicated higher C use efficiency. This may be a result of CC soil adaptations to cereal cultivation prior to transplanting. A litter decomposition study conducted by Strickland et al. (2009) identified a large impact of C resource history on the decomposition of current litter types.

In addition to differences in cumulative CO_2 emissions, OM mineralization had different patterns for each soil. Irrespective of the N fertilization history, ^{13}C cellulose addition always increased the soil respiration in high-fertility CC soil while in high-fertility PL soil, N fertilizer application alone enhanced respiration. Substrate availability is the key factor that governs C and N fluxes in soil (Lal, 2008; Zaehle, 2013). This suggest that N in CC soil and C in PL soil were the main limiting factors for the microbial communities in these high-fertility soils. Furthermore, high-fertility CC and PL soils had low C-CO_2 production when provided with both C and N compared to N alone (Table 5.2), implying higher C use efficiency in high-fertility soils with adequate C and N availability for microbial assimilation. Increased C released as CO_2 results in less C converted into microbial biomass and low stabilization of OM inputs in soil stable C pool (Manzoni et al., 2012). Based on 2012 field microbial analysis (Chapter 3), high-fertility CC and PL and RM soils contained a greater microbial biomass than DTF. Carbon use efficiency is greater when the microbial growth is limited with C availability (Sinsabaugh et al., 2013). Therefore, the addition of ^{13}C cellulose to the high-fertility soil (CC and PL) with N fertilization application may have incorporated most of the C into the microbial biomass.

In DTF and RM soils, the microbial communities were most limited by C availability as N fertilized soils always had higher CO_2 flux following cellulose addition, apparently due to scavenging of the stable OM pool to fulfill their C requirement (Figs. 5.4C and 5.4D). Availability of both N and C increased CO_2 emissions in DTF (low fertility) and RM soils. Low-fertility DTF soil OC, TC and TN contents were greater than in the original soils after >21 yr (Chapter 3). Among the studied soils, DTF contained the lowest microbial biomass. Therefore, the addition of ^{13}C cellulose to N fertilized soil may have contributed more C than the microbial energy requirement. As a result, greater CO_2 released might have occurred in cellulose and N added soils than the unfertilized soil to reach to native state (condition before cellulose addition) by neutralizing the effect of remaining C from cellulose. Also, ample amounts of C and N may have facilitated the microbial biomass, resulting in a greater respiration than in control soils (0 N kg ha^{-1} and 0 g ^{13}C kg^{-1} soil). Once the C availability increased to a particular level, excess is emitted as CO_2 (Liang et al., 2015).

The current study identified the long-term influence of soil origin and land use legacy on C dynamics of soil relocate from diverse land use systems and managed under common conditions for 23 yr. A study conducted in High field site at Rothamsted research station, UK found that even after 50 yr of no input (i.e., tilled bare fallow) the microbial diversity was similar to that of the original grassland soil (Hirsch et al., 2009). Differences in active decomposer community composition and fertility levels between transplanted soils resulted in variable decomposition dynamics. Therefore, soil origin and land use history are important drivers of soil nutrient cycling and functioning and needed to be taken into account in future management decisions regarding agricultural lands.

5.7. CONCLUSIONS

This study showed that the microbial cellulose decomposer community structure, diversity and function are strongly linked to soil origin and land use legacy, even after more than two decades of common management, climatic and topographic conditions. There were influences from the current land management (continuous wheat mono-cropping, limited C input and N fertilizer application) as well as environmental conditions on soil OM mineralization and decomposer community dynamics.

Variable mineralization patterns among the transplanted soils confirmed that different decomposer communities processed the cellulose in functionally different ways. As a result, metabolic capabilities between soils may have altered the decomposition products of the common C input (cellulose). Further, microbial necromass is a significant component in soil stable C pool which can likewise be altered through changes in decomposer community structure and activity. For example, decomposer cell constituents built through anabolic activities affect the composition of necromass. Together these changes in the microbial community may lead to lasting differences in soil OM composition.

Despite 23 yr of common climatic and management conditions, decomposition of fresh and native soil OM did not appear to converge in the transplanted soils. This showed the critical role of the decomposer community, which together with the inherent properties of the soil matrix, had a stronger influence on C and N turnover than intermediate term agricultural management and environmental conditions. These results enhanced our understanding of the key role of soil microbial decomposers in terrestrial C dynamics by decoupling the effects of management, climate and topography from the dynamics of microbial C turnover in different soils. Sustainable soil management strategies need to include a comprehensive view of soil fertility that considers the role of land use legacy in shaping biological C and nutrient turnover.

6.0. SYNTHESIS AND CONCLUSIONS

Land use changes have large impacts on microbial communities and can alter ecosystem processes and functions (Lauber et al., 2013; Rampelotto et al., 2013; Paula et al., 2014; Zhang et al., 2016). Links between soil microbial communities and ecosystem processes are mainly governed by ecosystem characteristics (Van Der Heijden et al., 2008; Graham et al., 2016). Agriculture lands are considered dynamic ecosystems where diverse management practices can cause significant changes in the soil ecosystem. Agricultural management practices are responsible for shifts in microbial communities (Ye et al., 2009; Lauber et al., 2013; Carbonetto et al., 2014; Lammel et al., 2015; Ma et al., 2015). Microorganisms are the key regulators of ecosystem services. Therefore, understanding the fundamentals of microbial communities is important for a sustainable ecosystem management.

There are several studies, representing diverse climates that have investigated microbial community responses to agricultural land use; however, these findings were carried out for a particular soil environment (Fierer and Jackson, 2006). Heterogeneity in soil physical and chemical properties makes soil one of the most complicated environments to explore (Delmont et al., 2011). Further, land topography and hydrology are two main environmental variables that can change abruptly and influence several ecosystem components. A metadata analysis conducted in natural ecosystems confirmed that climate change is capable of affecting living systems (Parmesan et al., 2003). The effect of confounding factors (climate, topography, and hydrology) can influence research observations and hinder our ability to elucidate determinants on particular variables on microbial communities. A study conducted in forest ecosystems found nearly 17% of the variability in bacterial community structure was due to temporal variability (Freedman and Zak, 2015); whereas Hanson et al. (2012) described 26.9% of microbial composition difference to be accounted for by environmental variables. Therefore, the applicability of microbial community findings from one place to another can be complicated.

Environmental variability and other confounding factors may interfere with major findings which cause misinterpretation of the real story.

The long-term transplanted field experiment at AAFC, Lethbridge provided a unique opportunity for ecological research to be conducted by uncoupling the confound effects of climate, topography, and hydrology from soil microbial communities and functions. In establishing the experiment, top soils from diverse donor sites were relocated onto a flat area after removing surface soils. These transplanted Chernozemic soils from different locations had diverse physical, chemical and biological properties at the time of relocation (Olson et al., 1996; Zvomuya et al., 2008; Janzen et al., 2012). For > 21 yr, the soils were maintained under common climatic, topographic and management conditions. Based on the initial characteristics, 10 different soils with diverse properties were selected for investigation. Total microbial community abundance and N-cycling gene composition were analyzed in 2012 to identify the current status of the microbial communities. Further, archived soils from 1990 (original) and 2011 were used to understand the microbial community composition changes that occurred after transplantation. Finally, microbial functional attributes were assessed by conducting a SIP study using ^{13}C cellulose.

Soil microbial communities were assessed to compare microbial communities of different transplanted soils after 21 yr of identical conditions (Chapter 3). PLFA, qPCR, and DGGE techniques were used to characterize the microbial communities. The comparison of microbial communities between the original (1990) and 21 yr post-transplantation soils was conducted using archived soils to better understand the shifts in microbial communities with current land use practices (Chapter 4). DNA was extracted using a method modified by Clark and Hirsch (2008) and 16S *rRNA* genes target was sequenced using the Miseq Illumina platform. Based on the microbial surveys conducted in Chapters 3 & 4, four soils (CC, PL, DTF, RM) were selected for a SIP study using ^{13}C cellulose as the carrier molecule (Chapter 5). The objective of this study was to establish a link between microbial community compositions and C-cycling in transplanted soils to determine whether the broadly different total communities contained a subset which functioned similarly under the identical conditions following transplantation.

6.1. SUMMARY OF FINDINGS

Soil fertility legacy caused by soil origin, management and land use history prompted diverse microbial communities, N-cycling and C-cycling in transplanted soils after >21 yr of environmental and land use management shifts. Soil fertility legacy effect dominated the effect of environmental and land use shifts on microbial communities, OM decomposition and N cycling functions. More than two decades of identical climatic, topographic and management conditions were not strong enough to negate the land use legacy effects on microbial communities and soil fertility. The land use legacy effect on microbial communities was revealed through distinct community compositions related to each transplanted soil (Chapters 3, 4 and 5). Soil fertility differences were speculated as the major reason for different microbial profiles among transplanted soils. Although soil fertility (TC, TN, and OC) shifted with the post-transplant land use practices and envvironmental changes, fertility differences between transplanted soils remained distinct among the different soils. Diverse fertility even >21 yr after environmental and land use shifts suggests stronger influence of soil fertility legacy on microbial communities of transplanted soils. For example, total microbial biomass ($P<0.0002$) as well as, bacterial ($P<0.0001$) and fungal ($P<0.049$) abundances were significantly varied between transplanted soils (Chapter 3). Microbial community composition and microbial diversity changes related to the land use history have been reported previously (Jangid et al., 2011; Kallenbach and Grandy, 2015; Tardy et al., 2015).

The transplanted soil microbial composition shifted in response to environmental and land use changes after relocation; however, each soil had a unique microbial community depending on the soil origin and land use history. Diverse microbial communities after >21 yr of identical conditions may an indication of soil origin and land use legacy effect on microbial resilience. The effect of disturbances cause through continuous wheat monocropping, limited crop residue retention, long-term N fertilization and climatic shift were notable in both soil fertility and total microbial and active decomposer profiles (Chapter 3, 4 and 5). For example, microbial profiling using archived soils (1990 and 2011) revealed different communities between the original (1990) and soils sampled 21 yr (2011) after transplantation (Chapter 4). The disturbance caused by current environmental and land use conditions continuously occurred after transplantation.

However, diverse total and active decomposer communities emphasized a long-term effect of soil origin and land use legacy on resilience of microbial community that was greater than current environmental and management conditions.

Soils transplanted from native or uncultivated ecosystems (NG and PL) as well as sub-surface soils (BH and CH) had major shifts in microbial communities due to post transplant changes. This indicated prominent effects of current land use with the conversion from a natural ecosystem and uncultivated into the annually cropped agricultural land. In NG and PL soils, both soil diversity and evenness increased after transplantation. Among soils transplanted from uncultivated ecosystems, PL soil communities shifted but remained separated from other communities. It has been reported that the higher the diversity, the faster the ecosystem can to return to its original state (van Bruggen, 2000). The NG and PL soils microbial communities were dominated by a few species at the origin of the transplant experiment and lesser stability to current land use changes. However, these differences were observed through analyses of archived soils and can be related, in part, to prolonged storage. Further, analysis of active decomposer community indicated PL soil that did not receive N fertilizer had the highest microbial diversity compared to both N levels of CC, DTF, and RM soils. This also emphasized higher C functional diversity in PL soil. Exposure of sub-surface (BH and CH) soils to agricultural practices resulted in microbial community structures that were more similar to the surface agriculture soils. It was apparent that microbial community legacy influenced shifts in composition resulting from current land use and microbial community resilience to changing conditions. These findings showed the importance of soil microbial species richness and evenness on soil ecosystem stability.

Similar to microbial communities, soil fertility also shifted with land use and environmental changes. However, soil origin and land use history created long-term persistent effects on soil fertility from current environmental and management changes. Substantial reductions in nutrients and shifts in litter inputs after transplanting altered soil fertility and nutrient dynamics. Limited residue incorporation and continuous cultivation had negative effects on soil TC, TN, and OC of most transplanted soils except for the low fertility DTF and subsurface (BH and CH) soils. These fertility variations corresponded to land use legacy and current land use practices. For example, DTF soil originated from a pathway to an experimental field and had low levels of C inputs,

while subsurface soils (BH and CH) originated from an eroded land and contained relatively low levels of soil C. Increases in TC, TN, and OC in DTF soil and sub-surface soils were highest in N fertilized soils (Chapter 3). Increased soil fertility may be the reason for shifts in the microbial community of sub-surface soils towards those communities more typical of top soil. Fertility changes in TC, TN and OC were minimal in N fertilized soils than soils that did not receive N, suggesting that long-term N fertilizer application buffered the TC, TN and OC change with land use conversions. This suggested that N fertilizer application was able to reduce the soil fertility changes against agriculture exploitation. In agreement with the current study, Raun et al. (1998) reported increased TC and TN in long-term N fertilizer (>23 yr) treated soils. In response to N fertilization, wheat yield was increased in transplanted soils (Zvomuya et al., 2008), thus high amounts of C inputs returned to the soils diminished soil OM changes over time (Yanni et al., 2016). There is a strong link between soil fertility and microbial compositions. Soil microorganisms are the living component in soil OM and they regulate available nutrients in soil ecosystems, but they are often C and N limited (Griffiths et al., 2012).

A legacy of soil fertility regulated the N cycling functional gene responses for current land use including long-term N fertilizer application. Functional abundance and functional diversity are strong predictors of the direction of a change in an ecosystem process (Graham et al., 2016). Unlike soil fertility, whole microbial communities did not change with N fertilizer application (Chapter 3). Lack of microbial community response to N fertilizer application implied that soil N availability was not the most limiting factor for transplanted soil microbial communities. However, community structures of N-cycling genes shifted with N fertilizer application. Lack of N cycling gene abundance changes with N fertilizer application emphasize than gene abundance, N cycling gene structure is sensitive to long-term N fertilizer application. The phylogenetic difference of specific N-cycling genes observed through DGGE analysis can be related to N functional differences between transplanted soils. Between soils, bacteria *amoA*, *nirK* and *nirS* community structures of the high fertility soils shifted in response to the long-term N fertilizer application. Convergence in N fertilized high fertility (CC and PL) soil AOB communities suggested that soil fertility and long-term N fertilizer application were leading drivers of AOB community composition under similar agricultural management conditions.

Decomposition of fresh C inputs followed diverse mineralization patterns unique to each soil which were created by distinct active decomposer communities associated with trnsplanted soils. Under resource limited conditions, application of ^{13}C cellulose at a rate of 0.2 g C kg^{-1} soil shifted total microbial community structure indicating microbial responses to C amendments (Chapter 5). Although, long-term N fertilizer application alone did not significantly affect the total microbial community composition (Chapters 3 and 4), cellulose application to a soil with a history of N fertilizer application altered the total community structure (Chapter 5). Different active decomposer communities suggested that cellulose application stimulated a subset of microorganisms from the total community; however, greater differences between, rather than within transplanted soils showed that soil fertility legacy had a more dominant influence on decomposer community structure and their functions than current conditions.

Distinct decomposer community structures associated with each soil gave rise to variable soil mineralization patterns. Different mineralization pathways also emphasized that even after two decades of common conditions, transplanted soils followed different decomposition patterns when assimilating the cellulose. Negative priming at early decomposition indicated preferential substrate utilization by soil microorganisms (cellulose over wheat residue) (Qiao et al., 2016). Availability of cellulose prevented microbial dependency on stable soil OM suggesting a key role of fresh OM quality on soil C dynamics. Soil N and C were the most important nutrient and energy sources for soil microbes. Carbon use efficiency increased when C and N were added to a high fertility soil with high microbial biomass. A greater proportion of cellulose C incorporated into microbial biomass may end up in the stable OM pool upon microbial cell lysis. However, C composition of the necromass may vary with decomposer community structure and their metabolic capabilities did determine by the fertility legacy in transplanted soils. These results confirmed that soil origin and land use history have long-term persistent influence soil N and C dynamics and decomposer community structure of transplanted soils through fertility legacy.

Soil origin and land use legacy affect terrestrial ecosystem processes by influencing the impact of current land use practices. Soil microbial community composition, their C and N functions and fertility dynamics were significantly controlled by the soil origin land use history even two decades after environmental and management changes. Current land use responses were significantly modified by soil origin and land use legacy which had long-term persistent

influences on soil fertility and microbial composition. When interpreting responses to agricultural management practices soil history is an important factor as it interfluences the response to current land use practices. The development of effective soil fertility management strategies can be enhanced by considering the history of land use in agricultural systems.

6.2. FUTURE RESEARCH

This research enhances the fundamental understanding of microbial communities and soil fertility dynamics in agricultural soils by uncoupling the confounding effects of climate, topography, and hydrology. Future investigations of microbial metabolic pathways associated with C-cycling in transplanted soils will provide further insights that can build on the current findings. For this, an incubation study using a SIP technique can be conducted using ^{13}C labelled wheat residues. During the incubation, analysis of the decomposer community and ^{13}C labelled molecules present in soil OM at different sampling points will explain the relationship between microbial community structure and decomposition pathways determined through ^{13}C OM composition. Assessing N-cycling functional dynamics using ^{15}N labelled N sources and tracking ^{15}N within microbial communities and OM components will be beneficial to understand the fundamentals. Since the AOB community structure in the high fertility soils had interesting convergence with long-term N fertilizer applications, it may be beneficial to conduct an incubation study by amending NH_4^+ and NO_3^- sources and measuring the nitrification potential in transplanted soils. This would link the AOB and AOA community structures with soil nitrification process.

Soil organism responses to nutrient limitations can be captured through extracellular enzymatic activities because enzyme composition varies with microbial nutrient requirements (Schimel and Schaeffer, 2012; Allison, 2012). Determination of activities of β-glucosidase, N-acetyl glucosaminidase and phosphatase will give an idea about the factors influencing the availability of C, N, and P in transplanted soils.

Starting from establishment soil sampling was done in the transplanted field soils and stored at the dried condition for future use. Soil air drying can preserve microbial DNA for future use (Clark and Hirsch, 2008). Further, mapping of community changes chronologically using

archived soils will aid in exposing the microbial community successions relevant to land use changes. Understanding sequential changes in microbial communities with agriculture land use will allow better predictions in the future directions.

7.0. REFERENCES

Abeliovich, A. 2006. The nitrite oxidizing bacteria. p. 861–872. In Dworn, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., Stackebrandt, E. (eds.), The Prokaryotes. 3rd ed. Springer, New York.

Aislabie, J., and J.R. Deslippe. 2013. Soil microbes and their contribution to soil services. p. 143–161. In Dymond, J. (ed.), Ecosystem services in New Zealand - conditions and trends. Manaaki Whenua Press, Lincoln, New Zealand.

Alberta, and Soils and Animal Nutrition Laboratory. 1988. Recommended methods of soil analyses for Canadian prairie agricultural soils. Alberta Agriculture.

Allison, S.D. 2012. A trait-based approach for modelling microbial litter decomposition. Ecol. Lett. 15(9): 1058–1070.

Allison, S.D., Y. Lu, C. Weihe, M.L. Goulden, A.C. Martiny, K.K. Treseder, and J.B.H. Martiny. 2013. Microbial abundance and composition influence litter decomposition response to environmental change. Ecology 94(3): 714–725.

Allison, V.J., R.M. Miller, J.D. Jastrow, R. Matamala, and D.R. Zak. 2005. Changes in soil microbial community structure in a tallgrass prairie chronosequence. Soil Sci. Soc. Am. J. 69(5): 1412–1421.

Altschul, S.F., T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D.J. Lipman. 1997. Gapped BLAST and PS I-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25(17): 3389–3402.

Amundson, R. 2001. The carbon budget in soils. Annu. Rev. Earth Planet. Sci. 29: 535–562.

Anbessa, Y., and P. Juskiw. 2012. Nitrogen fertilizer rate and cultivar interaction effects on nitrogen recovery, utilization efficiency, and agronomic performance of spring barley. ISRN Agron. 2012: 1–8.

Arcand, M.M., B.L. Helgason, and R.L. Lemke. 2016. Microbial crop residue decomposition dynamics in organic and conventionally managed soils. Appl. Soil Ecol. 107: 347–359.

Auguet, J.-C., A. Barberan, and E.O. Casamayor. 2010. Global ecological patterns in uncultured Archaea. ISME J. 4(2): 182–90.

Bååth, E., and T.H. Anderson. 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. Soil Biol. Biochem. 35(7): 955–963.

Balk, M., A.M. Laverman, J.A. Keuskamp, and H.J. Laanbroek. 2015. Nitrate ammonification in mangrove soils: A hidden source of nitrite? Front. Microbiol.: 1–10.

Balser, T.C., and M.K. Firestone. 2005. Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. Biogeochemistry 73(2): 395–415.

Banning, N.C., L.D. Maccarone, L.M. Fisk, and D.V. Murphy. 2015. Ammonia-oxidising bacteria not archaea dominate nitrification activity in semi-arid agricultural soil. Sci. Rep. 5(May): 11146.

Barančíková, G., J. Makovníková, and J. Halas. 2016. Effect of land use change on soil organic carbon. Agric. 62(1): 10–18.

Barrios, E. 2007. Soil biota, ecosystem services and land productivity. Ecol. Econ. 64(2): 269–285.

Bartram, A.K., M.D.J. Lynch, J.C. Stearns, G. Moreno-Hagelsieb, and J.D. Neufeld. 2011. Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads. Appl. Environ. Microbiol. 77(11): 3846–3852.

Bastian, F., L. Bouziri, B. Nicolardot, and L. Ranjard. 2009. Impact of wheat straw decomposition on successional patterns of soil microbial community structure. Soil Biol. Biochem. 41(2): 262–275.

Baumann, K., M.F. Dignac, C. Rumpel, G. Bardoux, A. Sarr, M. Steffens, and P. Maron. 2012. Soil microbial diversity affects soil organic matter decomposition in a silty grassland soil. Biogeochemistry 114(1–3): 1–12.

Belay, A., A.S. Claassens, and F.C. Wehner. 2002. Effect of direct nitrogen and potassium and residual phosphorus fertilizers on soil chemical properties, microbial components and maize yield under long-term crop rotation. Biol. Fertil. Soils 35(6): 420–427.

Bending, G.D., M.K. Turner, F. Rayns, M.C. Marx, and M. Wood. 2004. Microbial and biochemical soil quality indicators and their potential for differentiating areas under contrasting agricultural management regimes. Soil Biol. Biochem. 36(11): 1785–1792.

Bennett, E.M., S.R. Carpenter, and N.F. Caraco. 2001. Human impact on erodable phosphorus and eutrophication: a global perspective. Bioscience 51(3): 227-234.

Berg, B. 2000. Litter decomposition and organic matter turnover in northern forest soils. For. Ecol. Manage. 133(1–2): 13–22.

Berg, G., and K. Smalla. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol. Ecol. 68(1): 1–13.

Bernard, L., C. Mougel, P.A. Maron, V. Nowak, J. Lévêque, C. Henault, F.E.Z. Haichar, O. Berge, C. Marol, J. Balesdent, F. Gibiat, P. Lemanceau, and L. Ranjard. 2007. Dynamics and identification of soil microbial populations actively assimilating carbon from 13C-labelled wheat residue as estimated by DNA- and RNA-SIP techniques. Environ. Microbiol. 9(3): 752–764.

Bertrand, I., B. Chabbert, B. Kurek, and S. Recous. 2006. Can the biochemical features and histology of wheat residues explain their decomposition in soil? Plant Soil 281(1–2): 291–307.

Blagodatskaya, E.V., S.A. Blagodatsky, T.H. Anderson, and Y. Kuzyakov. 2007. Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies. Appl. Soil Ecol. 37(1–2): 95–105.

Blair, N., R.D. Faulkner, A.R. Till, M. Korschens, and E. Schulz. 2006. Long-term management impacts on soil C, N and physical fertility. Part II: Bad Lauchstadt static and extreme FYM experiments. Soil Tillage Res. 91(1–2): 39–47.

Blake, L., K.W.T. Goulding, C.J.B. Mott, and P.R. Poulton. 2000. Temporal changes in chemical properties of air-dried stored soils and their interpretation for long-term experiments. Eur. J. Soil Sci. 51(2): 345–353.

Blanco-Canqui, H., and R. Lal. 2009. Crop residue removal impacts on soil productivity and environmental quality. Crit. Rev. Plant Sci. 28(3): 139–163.

Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37(8): 911-917.

Blum, W.E.H. 2013. Soil and land resources for agricultural production: general trends and future scenarios-a worldwide perspective. int. Soil Water Conserv. Res. 1(3): 1–14.

Bollag, J.M. and G. Tung. 1972. Nitrous oxide release by soil fungi. Soil Biol. Biochem. 4: 271-276.

Bonito, G., H. Reynolds, M.S. Robeson, J. Nelson, B.P. Hodkinson, G. Tuskan, C.W. Schadt, and R. Vilgalys. 2014. Plant host and soil origin influence fungal and bacterial assemblages in the roots of woody plants. Mol. Ecol. 23(13): 3356–3370.

Boschker, H.T.S., and J.J. Middelburg. 2002. Stable isotopes and biomarker in microbial ecology. FEMS Microbiol. Ecol. 40: 85–95.

Boyden, A., L. Hill, P. Leduc, and J. Wassermann. 2001. Field tests to correlate biomass, combine yield and recoverable straw. Prairie Agricultural Machinery Institute. Project No. 5000H.

Bremer, E. and C. Van Kessel. 1992. Seasonal microbial biomass dynamics after addition of lentil and wheat residues. Soil Sci. Soc. Am. J. 56: 1141-1146.

Brentrup, F., J. Kiisters, J. Lammel, and H. Kuhlmann. 2000. Methods to estimate on-field nitrogen emissions from crop production as an input to LCA studies in the agricultural sector. Int. J. Lca 5(6): 349–357.

Brock, C., A. Fließbach, H.-R. Oberholzer, F. Schulz, K. Wiesinger, F. Reinicke, W. Koch, B. Pallutt, B. Dittman, J. Zimmer, K.-J. Hülsbergen, and G. Leithold. 2011. Relation between soil organic matter and yield levels of nonlegume crops in organic and conventional farming systems. J. Plant Nutr. Soil Sci. 174(4): 568–575

Brockett, B.F.T., C.E. Prescott, and S.J. Grayston. 2012. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven bio geoclimatic zones in western Canada. Soil Biol. Biochem. 44(1): 9–20.

Bru, D., A. Ramette, N.P. Saby, S. Dequiedt, L. Ranjard, C. Jolivet, D. Arrouays, and L. Philippot. 2011. Determinants of the distribution of nitrogen-cycling microbial communities at the landscape scale. ISME J. 5(3): 532–542.

Buckley, D.H., and T.M. Schmidt. 2001. The structure of microbial communities in soil and the lasting impact of cultivation. Microb. Ecol. 42(1): 11–21.

Buckley, D.H., and T.M. Schmidt. 2003. Diversity and dynamics of microbial communities in soils from agro-ecosystems. Environ. Microbiol. 5(6): 441–452.

Burger, M., and L.E. Jackson. 2003. Microbial immobilization of ammonium and nitrate in relation to ammonification and nitrification rates in organic and conventional cropping systems. Soil Biol. Biochem. 35(1): 29–36.

Butt, K., M.J. Shipitalo, P.J. Bohlen, W.M. Edwards, and R.W. Parmelee. 1999. Long-term trends in earthworm populations of cropped experimental watersheds in Ohio, USA. Pedobiologia 43: 713–719

Butterbach-Bahl, K., P. Gundersen, P. Ambus, J. Augustin, C. Beier, P. Boeckx, M. Dannenmann, B. Sanchez Gimeno, A. Ibrom, R. Kiese, B. Kitzler, R.M. Rees, K.A. Smith, C. Stevens, T. Vesala, and S. Zechmeister-Boltenstern. 2011. Nitrogen processes in terrestrial ecosystems. p. 99–125. In Ambus, P., Aug, N., Beier, C., Boeckx, P., Dannenmann, M., Sanchez Gimeno, B., Ibrom, A., Kiese, R., Kitzler, B., Rees, R.M., Smith, K.A., Stvens, C., Vesala, T., Zechmeister-Boltenstern, S. (eds.), The European Nitrogen Assessment. Cambridge University Press, UK.

Butterbach-Bahl, K., E.M. Baggs, M. Dannenmann, R. Kiese, and S. Zechmeister-Boltenstern. 2013. Nitrous oxide emissions from soils: how well do we understand the processes and their controls? Philos. Trans. R. Soc. Lond. B. Biol. Sci. 368:16-21

Campbell, C.A., G.P. Lafond, R.P. Zentner, and V.O. Biederbeck. 1991. Influence of fertilizer and straw baling on soil organic matter in a thin black chernozem in Western Canada. Soil Biol. Biochem. 23(5): 443–446.

Campbell, B.J., S.W. Polson, T.E. Hanson, M.C. Mack, and E.A.G. Schuur. 2010. The effect of nutrient deposition on bacterial communities in Arctic tundra soil. Environ. Microbiol. 12(7): 1842–1854.

Canfield, D.E., A.N. Glazer, and P.G. Falkowski. 2010. REVIEW: The evolution and future of earth's Nitrogen cycle. Science (80-). 330: 192–196.

Caporaso, J.G., J. Kuczynski, J. Stombaugh, K. Bittinger, F.D. Bushman, E.K. Costello, N. Fierer, A.G. Peña, K. Goodrich, J.I. Gordon, G.A. Huttley, S.T. Kelley, D. Knights, E. Jeremy, R.E. Ley, C.A. Lozupone, D. Mcdonald, B.D. Muegge, J. Reeder, J.R. Sevinsky, P.J. Turnbaugh, and W. A. Walters. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7(5):335–336.

Caporaso, J.G., C.L. Lauber, W.A. Walters, D. Berg-Lyons, C.A. Lozupone, P.J. Turnbaugh, N. Fierer, and R. Knight. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. U. S. A. 108 Suppl (Supplement_1): 4516–4522.

Caporaso, J.G., C.L. Lauber, W. a Walters, D. Berg-Lyons, J. Huntley, N. Fierer, S.M. Owens, J. Betley, L. Fraser, M. Bauer, N. Gormley, J. a Gilbert, G. Smith, and R. Knight. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 6(8): 1621–1624.

Carbonetto, B., N. Rascovan, R. Álvarez, A. Mentaberry, and M.P. Vázquez. 2014. Structure, composition and metagenomic profile of soil microbiomes associated to agricultural land use and tillage systems in Argentine Pampas. PLoS One 9(6).

Cary, S.C., and N. Fierer. 2014. The importance of sample archiving in microbial ecology. Nat. Rev. Microbiol. 12(12): 789–790.

Cassman, K.G., and R.R. Harwood. 1995. The nature of agricultural systems: food security and environmental balance. Food Policy 20(5): 439–454.

Castro, H.F., A.T. Classen, E.E. Austin, R.J. Norby, and C.W. Schadt. 2010. Soil microbial community responses to multiple experimental climate change drivers. Appl. Environ. Microbiol. 76(4): 999–1007.

Cavigelli, M.A., and G.P. Robertson. 2001. Role of denitrifier diversity in rates of nitrous oxide consumption in a terrestrial ecosystem. Soil Biol. Biochem. 33(3): 297–310.

Cederlund, H., E. Wessèn, K. Enwall, C.M. Jones, J. Juhanson, M. Pell, L. Philippot, and S. Hallin. 2014. Soil carbon quality and nitrogen fertilization structure bacterial communities with predictable responses of major bacterial phyla. Appl. Soil Ecol. 84: 62–68.

Ceja-Navarro, J.A., F.N. Rivera-Orduña, L. Patiño-Zúñiga, A. Vila-Sanjurjo, J. Crossa, B. Govaerts, and L. Dendooven. 2010. Phylogenetic and multivariate analyses to determine the effects of different tillage and residue management practices on soil bacterial communities. Appl. Environ. Microbiol. 76(11): 3685–3691.

Chapin, F. S., P. A. Matson, and H. A. Mooney. 2002. Principles of terrestrial ecosystem ecology. Springer-Verlag, New York, New York, USA.

Chau, J.F., A.C. Bagtzoglou, and M.R. Willig. 2011. The Effect of soil texture on richness and diversity of bacterial communities. Environ. Forensics 12: 333–341.

Chen, F., M. Tan, Y. Yang, J. Ma, S. Zhang, and G. Li. 2015. The diversity changes of soil microbial communities stimulated by climate, soil type and vegetation type analyzed via a functional gene array. World J. Microbiol. Biotechnol. 31(11): 1755–1763.

Cheng, W. 1999. Rhizosphere feedbacks in elevated CO_2. Tree Physiol. 19: 313–320.

Chowdhury, S., M. Farrell, and N. Bolan. 2014. Priming of soil organic carbon by malic acid addition is differentially affected by nutrient availability. Soil Biol. Biochem. 77: 158–169.

Chowdhury, S., M. Farrell, G. Butler, and N. Bolan. 2015. Assessing the effect of crop residue removal on soil organic carbon storage and microbial activity in a no-till cropping system. Soil Use Manag. 31(4): 450–460.

Christopher, S.F., and R. Lal. 2007. Nitrogen management affects carbon sequestration in North American cropland soils. Crit. Rev. Plant Sci. 26(1): 45–64.

Chu, H., T. Fujii, S. Morimoto, X. Lin, K. Yagi, J. Hu, and J. Zhang. 2007. Community structure of ammonia-oxidizing bacteria under long-term application of mineral fertilizer and organic manure in a sandy loam soil. Appl. Environ. Microbiol. 73(2): 485–491.

Clark, I.M., and P.R. Hirsch. 2008. Survival of bacterial DNA and culturable bacteria in archived soils from the Rothamsted Broadbalk experiment. Soil Biol. Biochem. 40(5): 1090–1102.

Clark, I.M., N. Buchkina, D. Jhurreea, K.W.T. Goulding, and P.R. Hirsch. 2012. Impacts of nitrogen application rates on the activity and diversity of denitrifying bacteria in the Broadbalk wheat experiment. Philos. Trans. R. Soc. B Biol. Sci. 367: 1235–1244.

Comeau, L.P., R.L. Lemke, J.D. Knight, and A. Bedard-Haughn. 2013. Carbon input from 13C-labeled crops in four soil organic matter fractions. Biol. Fertil. Soils 49(8): 1179–1188.

Cong, J., Y. Yang, X. Liu, H. Lu, X. Liu, J. Zhou, D. Li, H. Yin, J. Ding, and Y. Zhang. 2015. Analyses of soil microbial community compositions and functional genes reveal potential consequences of natural forest succession. Sci. Rep. 5: 10007.

Conrad, R. 1996. Soil microorganisms as controllers of atmospheric trace gases (H2, CO, CH4, OCS, N2O, and NO). Microbiol. Rev. 60(4): 609–640.

Cotrufo, M.F., J.L. Soong, A.J. Horton, E.E. Campbell, M.L. Haddix, D.H. Wall, and W.J. Parton. 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nat. Geosci. 8(10): 776–779.

Costa, R., M. Götz, N. Mrotzek, J. Lottmann, G. Berg, and K. Smalla. 2006. Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. FEMS Microbiol. Ecol. 56(2): 236–249.

Cui, Z., X. Chen, and F. Zhang. 2010. Current Nitrogen management status and measures to improve the intensive wheat-maize system in China. Ambio 39(6): 376–384.

Dandie, C.E., S. Wertz, C.L. Leclair, C. Goyer, D.L. Burton, C.L. Patten, B.J. Zebarth, and J.T. Trevors. 2011. Abundance, diversity and functional gene expression of denitrifier communities in adjacent riparian and agricultural zones. FEMS Microbiol. Ecol. 77(1): 69–82.

Davidson, E.A., and I.L. Ackerman. 1993. Changes in soil carbon inventories following cultivation of previously untilled soils. Biogeochemistry 20(3): 161–193.

Davidson, E.A., and L.V. Verchot. 2000. Testing the hole in the pipe model of nitric and nitrous oxide emission from soils using the TRAGNET database. Global Biogeochem. Cycles 14(4): 1035–1042.

Davidson, E.A., and I.A. Janssens. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440(March): 165–173.

de Menezes, A.B., M.T. Prendergast-Miller, P. Poonpatana, M. Farrell, A. Bissett, L.M. Macdonald, P. Toscas, A.E. Richardson, and P.H. Thrall. 2015. C/N ratio drives soil actinobacterial cellobiohydrolase gene diversity. Appl. Environ. Microbiol. 81(9): 3016–3028.

De Nobili, M., M. Contin, and P.C. Brookes. 2006. Microbial biomass dynamics in recently air-dried and rewetted soils compared to others stored air-dry for up to 103 years. Soil Biol. Biochem. 38(9): 2871–2881.

de Vries, F.T., P. Manning, J.R.B. Tallowin, S.R. Mortimer, E.S. Pilgrim, K.A. Harrison, P.J. Hobbs, H. Quirk, B. Shipley, J.H.C. Cornelissen, J. Kattge, and R.D. Bardgett. 2012. Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. Ecol. Lett. 15(11): 1230–1239.

Delmont, T.O., P. Robe, S. Cecillon, I.M. Clark, F. Constancias, P. Simonet, P.R. Hirsch, and T.M. Vogel. 2011. Accessing the soil metagenome for studies of microbial diversity. Appl. Environ. Microbiol. 77(4): 1315–1324.

Denef, K., H. Bubenheim, K. Lenhart, J. Vermeulen, O. Van Cleemput, P. Boeckx, and C. Müller. 2007. Community shifts and carbon translocation within metabolically-active rhizosphere microorganisms in grasslands under elevated CO2. Biogeosciences 4(5): 769–779.

Dhillon, G.S., B.Y. Amichev, R. De Freitas and K. Van Rees. 2015. Accurate and precise measurement of organic carbon content in carbonate-rich soils. Comm. in Soil Science and Plant. (46):2707–2720.

Di, H. J., Cameron, K. C., Shen, J. P., Winefield, C., O'Callaghan, M., Bowatte, S., et al. (2009). Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. Nat. Geosci. 2: 621–624.

Di, H.J., K.C. Cameron, J.P. Shen, C.S. Winefield, M. O'Callaghan, S. Bowatte, and J.Z. He. 2010. Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. FEMS Microbiol. Ecol. 72(3): 386–394.

Dijkstra, F.A., Y. Carrillo, E. Pendall, and J.A. Morgan. 2013. Rhizosphere priming: A nutrient perspective. Front. Microbiol. 4(JUL): 1–8.

Ding, G.-C., Y.M. Piceno, H. Heuer, N. Weinert, A.B. Dohrmann, A. Carrillo, G.L. Andersen, T. Castellanos, C.C. Tebbe, and K. Smalla. 2013. Changes of soil bacterial diversity as a consequence of agricultural land use in a semi-arid ecosystem. PLoS One 8(3): e59497.

Djukic, I., F. Zehetner, A. Mentler, and M.H. Gerzabek. 2010. Microbial community composition and activity in different Alpine vegetation zones. Soil Biol. Biochem. 42(2): 155-161.

Dolfing, J., A. Vos, J. Bloem, P.A.I. Ehlert, N.B. Naumova, and P.J. Kuikman. 2004. Microbial diversity in archived soils. Science (80). 306(October): 811–813.

Dolfing, J., and Y. Feng. 2015. The importance of soil archives for microbial ecology. Nat. Rev. Microbiol. 13(3): 1.

Doran, J.W. 1987. Microbial biomass and mineralizable nitrogen distribution into-tillage and plowed soils. Biol. Fertil. Soils 5(1): 68–75.

Doran, J.W., and M.R. Zeiss. 2000. Soil health and sustainability: managing the biotic component of soil quality. Appl. Soil Ecol. 15: 3–11.

Drenovsky, R.E., D. Vo, K.J. Graham, and K.M. Scow. 2004. Soil water content and organic carbon availability are major determinants of soil microbial community composition. Microb. Ecol. 48(3): 424–430.

Drenovsky, R., K. Steenwerth, L. Jackson, and K.M. Scow. 2010. Land use and climatic factors structure regional patterns in soil microbial communities. Natl. Inst. Heal. 19(1): 27–39.

Dumont, M.G., and J.C. Murrell. 2005. Innovation: Stable isotope probing - linking microbial identity to function. Nat. Rev. Microbiol. 3(6): 499–504.

Dunford, E.A., and J.D. Neufeld. 2010. DNA stable-isotope probing (DNA-SIP). J. Vis. Exp. (42): 1–6.

Edgar, R.C. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26(19): 2460–2461.

Edgar, R.C., B.J. Haas, J.C. Clemente, C. Quince, and R. Knight. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27(16): 2194–2200.

Edgerton, M.D. 2009. Increasing crop productivity to meet global needs for feed, food, and fuel. Plant Physiol. 149(1): 7–13.

Elfstrand, S., B. Båth, and A. Mårtensson. 2007. Influence of various forms of green manure amendment on soil microbial community composition, enzyme activity and nutrient levels in leek. Appl. Soil Ecol. 36(1): 70–82.

Enriquez, S., C. Duarte, and K. Sand-Jensen. 1993. Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C: N: P content. Oecologia 94(4): 457–471.

Enwall, K., L. Philippot, and S. Hallin. 2005. activity and composition of the denitrifying bacterial community respond differently to long-term fertilization. Appl. Environ. Microbiol. 71(12): 8335–8343.

Enwall, K., K. Nyberg, S. Bertilsson, H. Cederlund, J. Stenström, and S. Hallin. 2007. Long-term impact of fertilization on activity and composition of bacterial communities and metabolic guilds in agricultural soil. Soil Biol. Biochem. 39(1): 106–115.

Erguder, T.H., N. Boon, L. Wittebolle, M. Marzorati, and W. Verstraete. 2009. Environmental factors shaping the ecological niches of ammonia-oxidizing archaea. FEMS Microbiol. Rev. 33(5): 855–869.

Fan, M., J. Shen, L. Yuan, R. Jiang, X. Chen, W.J. Davies, and F. Zhang. 2012. Improving crop productivity and resource use efficiency to ensure food security and environmental quality in China. J. Exp. Bot. 63(1): 13–24.

Ferguson S. J., D. J. Richardson, R. J. M. van Spanning 2007. Biochemistry and molecular biology of nitrification. p. 209–222. In Biology of the nitrogen cycle. Bothe H., Ferguson S. J., Newton W. E., (ed.), Elsevier, Amsterdam.

Fierer, N., J. Jackson, R. Vilgalys, and R.B. Jackson. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl. Environ. Microbiol. 71(7): 4117-4120.

Fierer, N., and R.B. Jackson. 2006. The diversity and biogeography of soil bacterial communities. Proc. Natl. Acad. Sci. U. S. A. 103(3): 626–631.

Fierer, N., M.A. Bradford, and R.B. Jackson. 2007. Toward an ecological classification of soil bacteria. Ecology 88(6): 1354–1364.

Fierer, N., J.W. Leff, B.J. Adams, U.N. Nielsen, S.T. Bates, C.L. Lauber, S. Owens, J.A. Gilbert, D.H. Wall, and J.G. Caporaso. 2012a. Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. Proc. Natl. Acad. Sci. 109(52): 21390–21395.

Fierer, N., C.L. Lauber, K.S. Ramirez, J. Zaneveld, M.A. Bradford, and R. Knight. 2012b. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. ISME J. 6(5): 1007–1017.

Fontaine, S., A. Mariotti, and L. Abbadie. 2003. The priming effect of organic matter: A question of microbial competition? Soil Biol. Biochem. 35(6): 837–843.

Fontaine, S., S. Barot, P. Barré, N. Bdioui, B. Mary, and C. Rumpel. 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450(8): 277–80.

Fontaine, S., C. Henault, A. Aamor, N. Bdioui, J.M.G. Bloor, V. Maire, B. Mary, S. Revaillot, and P.A. Maron. 2011. Fungi mediate long-term sequestration of carbon and nitrogen in soil through their priming effect. Soil Biol. Biochem. 43(1): 86–96.

Foster, D., F. Swanson, J. Aber, I. Burke, N. Brokaw, D. Tilman, and A. Knapp. 2003. The importance of land-use legacies to ecology and conservation. Bioscience 53(1): 77–88.

Franklin, R.B., and A.L. Mills. 2009. Importance of spatially structured environmental heterogeneity in controlling microbial community composition at small spatial scales in an agricultural field. Soil Biol. Biochem. 41(9): 1833–1840.

Freedman, Z., and D.R. Zak. 2015. Soil bacterial communities are shaped by temporal and environmental filtering: Evidence from a long-term chronosequence. Environ. Microbiol. 17(9): 3208–3218.

Frostegård, Å., A. Tunlid, and E. Bååth. 2011. Use and misuse of PLFA measurements in soils. Soil Biol. Biochem. 43(8): 1621–1625.

Fu, B.J., X.D. Guo, L.D. Chen, K.M. Ma, and J.R. Li. 2001. Soil nutrient changes due to land use changes in Northern China: a case study in Zunhua County, Hebei Province. Soil Use Manag. 17(November 1999): 294–296.

Fukami, T., H.J.E. Beaumont, X.-X. Zhang, and P.B. Rainey. 2007. Immigration history controls diversification in experimental adaptive radiation. Nature 446: 436–439.

Galloway, J.N., J.D. Aber, J.W. Erisman, S.P. Seitzinger, R.W. Howarth, E.B. Cowling, and B.J. Cosby. 2003. The Nitrogen Cascade. Bioscience 53(4): 341–356

Gan, Y., C. Liang, C.A. Campbell, R.P. Zentner, R.L. Lemke, H. Wang, and C. Yang. 2012. Carbon footprint of spring wheat in response to fallow frequency and soil carbon changes over 25 years on the semiarid Canadian prairie. Eur. J. Agron. 43: 175–184.

Garbeva, P., J.D. van Elsas, and J.A. van Veen. 2008. Rhizosphere microbial community and its response to plant species and soil history. Plant Soil 302(1–2): 19–32.

Gao, J., Y. Xie, H. Jin, Y. Liu, X. Bai, D. Ma, Y. Zhu, C. Wang, and T. Guo. 2016. Nitrous oxide emission and denitrifier abundance in two agricultural soils amended with crop residues and urea in the North China Plain. PLoS One 11(5): 1–15.

Gao, H., X. Chen, J. Wei, Y. Zhang, L. Zhang, J. Chang, and M.L. Thompson. 2016. Decomposition dynamics and changes in chemical composition of wheat straw residue under anaerobic and aerobic conditions. PLoS One 11(7): 1–17.

García-Orenes, F., A. Morugán-Coronado, R. Zornoza, and K. Scow. 2013. Changes in soil microbial community structure influenced by agricultural management practices in a mediterranean agro-ecosystem. PLoS One 8(11): e80522.

Giardina, C.P., M.G. Ryan, R.M. Hubbard, and D. Binkley. 2001. Tree species and soil textural controls on carbon and nitrogen mineralization rates. Soil Sci. Soc. Am. J. 65(4): 1272–1279.

Giles, M., N. Morley, E.M. Baggs, and T.J. Daniell. 2012. Soil nitrate reducing processes - Drivers, mechanisms for spatial variation, and significance for nitrous oxide production. Front. Microbiol. 3 (407): 1–16.

Girvan, M.S., J. Bullimore, J.N. Pretty, A.M. Osborn, and A.S. Ball. 2003. Soil Type Is the Primary Determinant of the Composition of the Total and Active Bacterial Communities in Arable Soils. Appl. Environ. Microbiol. 69(3): 1800–1809.

Gleixner, G. 2013. Soil organic matter dynamics: A biological perspective derived from the use of compound-specific isotopes studies. Ecol. Res. 28(5): 683–695.

Goldfarb, K.C., U. Karaoz, C.A. Hanson, C.A. Santee, M.A. Bradford, K.K. Treseder, M.D. Wallenstein, and E.L. Brodie. 2011. Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance. Front. Microbiol. 2(94): 1–10.

Gonzalez-Quiñones, V., E. a. Stockdale, N.C. Banning, F.C. Hoyle, Y. Sawada, A. D. Wherrett, D.L. Jones, and D. V. Murphy. 2011. Soil microbial biomass - Interpretation and consideration for soil monitoring. Soil Res. 49(4): 287–304.

Gougoulias, C., J.M. Clark, and L.J. Shaw. 2014. The role of soil microbes in the global carbon cycle: Tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems. J. Sci. Food Agric. 94(12): 2362–2371.

Govind, A., and J. Kumari. 2014. Understanding the terrestrial carbon cycle: An ecohydrological perspective. Int. J. Ecol. 2014.

Graham, E.B., J.E. Knelman, A. Schindlbacher, S. Siciliano, et al. 2016. Microbes as engines of ecosystem function: When does community structure enhance predictions of ecosystem processes? Front. Microbiol. 7: 1–10.

Griffiths, B.S., A. Spilles, and M. Bonkowski. 2012. C: N: P stoichiometry and nutrient limitation of the soil microbial biomass in a grazed grassland site under experimental P limitation or excess. Ecol. Process. 1(6): 1-11.

Griffiths, B.S., and L. Philippot. 2013. Insights into the resistance and resilience of the soil microbial community. FEMS Microbiol. Rev. 37(2): 112–129.

Grogan, D.W., and J.E. Cronan. 1997. Cyclopropane ring formation in membrane lipids of bacteria. Microbiol. Mol. Biol. Rev. 61(4): 429–441.

Groppo, J.D., S.R.M. Lins, P.B. Camargo, E.D. Assad, H.S. Pinto, S.C. Martins, P.R. Salgado, B. Evangelista, E. Vasconcellos, E.E. Sano, E. Pavão, R. Luna, and L.A. Martinelli. 2015. Changes in soil carbon, nitrogen, and phosphorus due to land-use changes in Brazil. Biogeosciences 12(15): 4765–4780.

Guenet, B., S. Juarez, G. Bardoux, V. Pouteau, N. Cheviron, C. Marrauld, L. Abbadie, and C. Chenu. 2011. Metabolic capacities of microorganisms from a long-term bare fallow. Appl. Soil Ecol. 51(1): 87–93.

Hai, B., N.H. Diallo, S. Sall, F. Haesler, K. Schauss, M. Bonzi, K. Assigbetse, J.L. Chotte, J.C. Munch, and M. Schloter. 2009. Quantification of key genes steering the microbial nitrogen cycle in the rhizosphere of sorghum cultivars in tropical agroecosystems. Appl. Environ. Microbiol. 75(15): 4993–5000.

Haichar, F.E.Z., W. Achouak, R. Christen, T. Heulin, C. Marol, M.F. Marais, C. Mougel, L. Ranjard, J. Balesdent, and O. Berge. 2007. Identification of cellulolytic bacteria in soil by stable isotope probing. Environ. Microbiol. 9(3): 625–634.

Haichar, F.E.Z., C. Marol, O. Berge, J.I. Rangel-Castro, J.I. Prosser, J. Balesdent, T. Heulin, and W. Achouak. 2008. Plant host habitat and root exudates shape soil bacterial community structure. ISME J. 2(12): 1221–1230.

Hallin, S., C.M. Jones, M. Schloter, and L. Philippot. 2009. Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. ISME J. 3(5): 597–605.

Hannam, K.D., S.A. Quideau, and B.E. Kishchuk. 2007. The microbial communities of aspen and spruce forest floors are resistant to changes in litter inputs and microclimate. Appl. Soil Ecol. 35(3): 635–647.

Hanson, C.A., J.A. Fuhrman, M.C. Horner-Devine, and J.B.H. Martiny. 2012. Beyond biogeographic patterns: processes shaping the microbial landscape. Nat. Rev. Microbiol. 10: 497–506.

Harris, D., W.R. Horwa, and C.Van Kessel. 2001. Acid fumigation of soils to remove carbonates prior to total organic carbon. 65: 1853–1856.

Harter, J., H.-M. Krause, S. Schuettler, R. Ruser, M. Fromme, T. Scholten, A. Kappler, and S. Behrens. 2013. Linking N 2 O emissions from biochar-amended soil to the structure and function of the N-cycling microbial community. ISME J. 8(10): 660–674.

Hartmann, M., B. Frey, J. Mayer, P. Mäder, and F. Widmer. 2015. Distinct soil microbial diversity under long-term organic and conventional farming. ISME J. 9(5): 1177–1194.

Hayatsu, M., K. Tago, and M. Saito. 2008. Various players in the nitrogen cycle: Diversity and functions of the microorganisms involved in nitrification and denitrification. Soil Sci. Plant Nutr. 54(1): 33–45.

He, J.Z., J.P. Shen, L.M. Zhang, Y.G. Zhu, Y.M. Zheng, M.G. Xu, and H. Di. 2007. Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria

and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. Environ. Microbiol. 9(9): 2364–2374.

Heil, J., H. Vereecken, and N. Brüggemann. 2016. A review of chemical reactions of nitrification intermediates and their role in nitrogen cycling and nitrogen trace gas formation in soil. Eur. J. Soil Sci. 67(1): 23–39.

Hedrick, D.B., A. Peacock, and D.C. White. 2005. Interpretation of Fatty Acid Profiles of Soil Microorganisms. p. 252–259. In Manual of Soil Analysis, Soil Biology. Springer Verlag, Berlin.

Helgason, B.L., F.L. Walley, and J.J. Germida. 2009. Fungal and bacterial abundance in long-term no-till and intensive-till soils of the Northern Great Plains. Soil Sci. Soc. Am. J. 73(1): 120–127.

Helgason, B.L., F.L. Walley, and J.J. Germida. 2010a. No-till soil management increases microbial biomass and alters community profiles in soil aggregates. Appl. Soil Ecol. 46(3): 390–397.

Helgason, B.L., F.L. Walley, and J.J. Germida. 2010b. Long-term no-till management affects microbial biomass but not community composition in Canadian prairie agroecosytems. Soil Biol. Biochem. 42(12): 2192–2202.

Helgason, B.L., E.G. Gregorich, H.H. Janzen, B.H. Ellert, N. Lorenz, and R.P. Dick. 2014. Long-term microbial retention of residue C is site-specific and depends on residue placement. Soil Biol. Biochem. 68: 231–240.

Henry, S., D. Bru, B. Stres, S. Hallet, and L. Philippot. 2006. Quantitative detection of the nosZ gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, narG, nirK, and nosZ genes in soils. Appl. Environ. Microbiol. 72(8): 5181–5189.

Hermansson, A., and P. Lindgren. 2001. quantification of ammonia-oxidizing bacteria in arable soil by real-time PCR. Appl. Environ. Microbiol. 67(2): 972–976.

Herridge, D.F., M.B. Peoples, and R.M. Boddey. 2008. Global inputs of biological nitrogen fixation in agricultural systems. Plant Soil 311(1–2): 1–18.

Hirsch, P.R., L.M. Gilliam, S.P. Sohi, J.K. Williams, I.M. Clark, and P.J. Murray. 2009. Starving the soil of plant inputs for 50 years reduces abundance but not diversity of soil bacterial communities. Soil Biol. Biochem. 41(9): 2021–2024.

Hobbie, J.E. 2003. Scientific Accomplishments of the long-term ecological research program: an introduction. Bioscience 53(1): 17.

Hou, A., H. Tsuruta, M.A. Mccreary, and Y. Hosen. 2010. Effect of urea placement on the time-depth profiles of NO, N2O and mineral nitrogen concentrations in an Andisol during a Chinese cabbage growing season. Soil Sci. Plant Nutr. 56(6): 861–869.

Hou, R., Z. Ouyang, Y. Li, D.D. Tyler, F. Li, and G. V. Wilson. 2012. Effects of tillage and residue management on soil organic carbon and total nitrogen in the North China Plain. Soil Sci. Soc. Am. J. 76(1): 230.

Hsu, S.-F., and D.H. Buckley. 2009. Evidence for the functional significance of diazotroph community structure in soil. Int. Soc. Microb. Ecol. J. 3(1): 124–136.

Hu, Y., D. Xiang, S.D. Veresoglou, F. Chen, Y. Chen, Z. Hao, X. Zhang, and B. Chen. 2014. Soil organic carbon and soil structure are driving microbial abundance and community composition across the arid and semi-arid grasslands in northern China. Soil Biol. Biochem. 77: 51–57.

Huang, X.-F., J.M. Chaparro, K.F. Reardon, R. Zhang, Q. Shen, and J.M. Vivanco. 2014. Rhizosphere interactions: root exudates, microbes, and microbial communities. Botany 92(4): 267–275.

Imhoff, M., L. Bounoua, T. Ricketts, C. Loucks, R. Harriss, and W.T. Lawrence. 2004. Global patterns in human consumption of net primary production. Nature 429(June): 870–873.

Inselsbacher, E., K. Ripka, S. Klaubauf, D. Fedosoyenko, E. Hackl, M. Gorfer, R. Hood-Novotny, N. Von Wirén, A. Sessitsch, S. Zechmeister-Boltenstern, W. Wanek, and J. Strauss. 2009. A cost-effective high-throughput microcosm system for studying nitrogen dynamics at the plant-microbe-soil interface. Plant Soil 317(1–2): 293–307.

Inselsbacher, E., N. Hinko-Najera Umana, F.C. Stange, M. Gorfer, E. Schüller, K. Ripka, S. Zechmeister-Boltenstern, R. Hood-Novotny, J. Strauss, and W. Wanek. 2010. Short-term competition between crop plants and soil microbes for inorganic N fertilizer. Soil Biol. Biochem. 42(2): 360–372.

Isobe, K., and N. Ohte. 2014. Ecological perspectives on microbes involved in N-cycling. Microbes Environ. 29(1): 4–16.

Jangid, K., M.A. Williams, A.J. Franzluebbers, J.S. Sanderlin, J.H. Reeves, M.B. Jenkins, D.M. Endale, D.C. Coleman, and W.B. Whitman. 2008. Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems. Soil Biol. Biochem. 40(11): 2843–2853.

Jangid, K., M.A. Williams, A.J. Franzluebbers, T.M. Schmidt, D.C. Coleman, and W.B. Whitman. 2011. Land-use history has a stronger impact on soil microbial community composition than aboveground vegetation and soil properties. Soil Biol. Biochem. 43(10): 2184–2193.

Janssen, P.H. 2006. MINIREVIEWS: identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA Genes. Appl. Environ. Microbiol. 72(3): 1719–1728.

Janzen, H.H., C.A. Campbell, R.C. Izaurralde, B.H. Ellert, N. Juma, W.B. McGill, and R.P. Zentner. 1998. Management effects on soil C storage on the Canadian prairies. Soil Tillage Res. 47(3–4): 181–195.

Janzen, H.H. 2004. Carbon cycling in earth systems - A soil science perspective. Agric. Ecosyst. Environ. 104(3): 399–417.

Janzen, H.H. 2009. Long-term ecological sites: Musings on the future, as seen (dimly) from the past. Glob. Chang. Biol. 15(11): 2770–2778.

Janzen, H.H., B.M. Olson, F. Zvomuya, F.J. Larney, and B.H. Ellert. 2012. Long-term field bioassay of soil quality summary major findings from the study. Prairie Soils Crop. 5: 165–168.

Jesus, E.C., T.L. Marsh, J.M. Tiedje, and F.M. Moreira. 2009. Changes in land use alter the structure of bacterial communities in Western Amazon soils. ISME J. 3(9): 1004–1011.

Jia, Z., and R. Conrad. 2009. Bacteria rather than Archaea dominate microbial ammonia oxidation in an agricultural soil. Environ. Microbiol. 11(7): 1658–1671.

Jiang, X., A.L. Wright, X. Wang, and F. Liang. 2011. Tillage-induced changes in fungal and bacterial biomass associated with soil aggregates: A long-term field study in a subtropical rice soil in China. Appl. Soil Ecol. 48(2): 168–173.

Jiménez-Bueno, N.G., C. Valenzuela-Encinas, R. Marsch, D. Ortiz-Gutiérrez, N. Verhulst, B. Govaerts, L. Dendooven, and Y.E. Navarro-Noya. 2016. Bacterial indicator taxa in soils under different long-term agricultural management. J. Appl. Microbiol. 120(4): 921–933.

Jin, T., T. Zhang and Q. Yan. 2010. Characterization and quantification of ammonia-oxidizing archaea (AOA) and bacteria (AOB) in a nitrogen-removing reactor using T-RFLP and qPCR. Appl. Microbiol. Biotechnol. 87(3): 1167–1176.

Johnston, A.E., P.R. Poulton, K. Coleman, 2009. Soil organic matter: its importance in sustainable agriculture and carbon dioxide fluxes. Adva. in Agro. 101: 1-57.

Kallenbach, C.M., and A.S. Grandy. 2015. Land-use legacies regulate decomposition dynamics following bioenergy crop conversion. GCB Bioenergy 7(6): 1232–1244.

Kardol, P., and D.A. Wardle. 2010. How understanding aboveground-belowground linkages can assist restoration ecology. Trends Ecol. Evol. 25(11): 670–679

Kaur, A., A. Chaudhary, A. Kaur, R. Choudhary, and R. Kaushik. 2005. Phospholipid fatty acid - A bioindicator of environment monitoring and assessment in soil ecosystem. Curr. Sci. 89(7): 1103–1112.

Kelly, J.J., K. Policht, T. Grancharova, and L.S. Hundal. 2011. Distinct responses in ammonia-oxidizing archaea and bacteria after addition of biosolids to an agricultural soil. Appl. Environ. Microbiol. 77(18): 6551–6558.

Kirkby, C.A., A.E. Richardson, L.J. Wade, G.D. Batten, C. Blanchard, and J.A. Kirkegaard. 2013. Carbon-nutrient stoichiometry to increase soil carbon sequestration. Soil Biol. Biochem. 60: 77–86.

Kleber, M. 2010. What is recalcitrant soil organic matter? Environ. Chem. 7(4): 320–332.

Konopka, A. 2009. What is microbial community ecology? ISME J. (3): 1223–1230.

Koorem, K., A. Gazol, M. Öpik, M. Moora, Ülle Saks, A. Uibopuu, V. Sõber, and M. Zobel. 2014. Soil nutrient content influences the abundance of soil microbes but not plant biomass at the small-scale. PLoS One 9(3): 1–9.

Kowalchuk, G. a, J.R. Stephen, W. De Boer, J.I. Prosser, T.M. Embley, and J.W. Woldendorp. 1997. Analysis of ammonia-oxidizing bacteria of the beta subdivision of the class Proteobacteria in coastal sand dunes by denaturing gradient gel electrophoresis and

sequencing of PCR-amplified 16S ribosomal DNA fragments. Appl. Environ. Microbiol. 63(4): 1489–1497.

Kowalchuk, G.A., and J.R. Stephen. 2001. Ammonia-oxidizing bacteria: a model for molecular microbial ecology. Annu. Rev. Microbiol. 55(1): 485–529.

Kozich, J.J., S.L. Westcott, N.T. Baxter, S.K. Highlander, and P.D. Schloss. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the Miseq Illumina sequencing platform. Appl. Environ. Microbiol. 79(17): 5112–5120.

Kersters K., P. De Vos, M. Gillis, J. Swings, P. Vandamme, E. Stackebrandt. (2006). Introduction to the Proteobacteria. pp 3–37. In Dwarkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds). The Prokaryotes. 3rd ed. vol. 5. Springer, New York.

Krull, E., J. Baldock, and J. Skjemstad. 2001. Soil texture effects on decomposition and soil carbon storage. NEE Work. Proc.: 103–110.

Kuzyakov, Y., and G. Domanski. 2000. Carbon input by plants into the soil. Review. J. Plant Nutr. Soil Sci. 163(4): 421–431.

Kuzyakov, Y., and R. Bol. 2006. Sources and mechanisms of priming effect induced in two grassland soils amended with slurry and sugar. Soil Biol. Biochem. 38(4): 747–758.

Lafond, G.P., M. Stumborg, R. Lemke, W.E. May, C.B. Holzapfel, and C.A. Campbell. 2009. Quantifying straw removal through baling and measuring the long-term impact on soil quality and wheat production. Agron. J. 101(3): 529–537.

Lal, R. 1974. Soil temperature, soil moisture and maize yield from mulched and unmulched tropical soils. Plant Soil 40(1): 129–143.

Lal, R. 2004. Is crop residue a waste? J. Soil Water Conserv. 59: 136–139.

Lal, R. 2008. Carbon sequestration. Philos Trans R Soc L. B Biol Sci 363(1492): 815–830.

Lalande, R., B. Gagnon, R.A. Chapman, and G.M. Barnett. 2005. Soil microbial populations, activity, and community structure in continuous corn or forage systems under organic or inorganic fertilization in eastern Canada. Can. J. Soil Sci. 85(1): 27–38.

Lambers, H., F. S. Chapin III, and T. L. Pons. 1998. Plant physiological ecology. 540 pp., Springer, New York.

Lammel, D.R., B.J. Feigl, C.C. Cerri, and K. Nüsslein. 2015. Specific microbial gene abundances and soil parameters contribute to C, N, and greenhouse gas process rates after land use change in Southern Amazonian Soils. Front. Microbiol. 6(Oct): 1–14.

Lauber, C.L., M.S. Strickland, M.A. Bradford, and N. Fierer. 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biol. Biochem. 40(9): 2407–2415.

Lauber, C.L., M. Hamady, R. Knight, and N. Fierer. 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl. Environ. Microbiol. 75(15): 5111–5120.

Lauber, C.L., K.S. Ramirez, Z. Aanderud, J. Lennon, and N. Fierer. 2013. Temporal variability in soil microbial communities across land-use types. ISME J. 7(8): 1641–1650.

Lazcano, C., M. Gómez-Brandón, P. Revilla, and J. Domínguez. 2013. Short-term effects of organic and inorganic fertilizers on soil microbial community structure and function: A field study with sweet corn. Biol. Fertil. Soils 49(6): 723–733.

Lazzaro, A., A. Gauer, and J. Zeyer. 2011. Field-scale transplantation experiment to investigate structures of soil bacterial communities at pioneering sites. Appl. Environ. Microbiol. 77(23): 8241–8248.

Le Guillou, C., D.A. Angers, P.A. Maron, P. Leterme, and S. Menasseri-Aubry. 2012. Linking microbial community to soil water-stable aggregation during crop residue decomposition. Soil Biol. Biochem. 50: 126–133.

Leininger, S., T. Urich, M. Schloter, L. Schwark, J. Qi, G.W. Nicol, J.I. Prosser, S.C. Schuster, and C. Schleper. 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442: 806–809.

Lemke, R.L., A.J. VandenBygaart, C.A. Campbell, G.P. Lafond, and B. Grant. 2010. Crop residue removal and fertilizer N: Effects on soil organic carbon in a long-term crop rotation experiment on a Udic Boroll. Agric. Ecosyst. Environ. 135(1–2): 42–51.

Levičnik-Höfferle, Š., G.W. Nicol, L. Ausec, I. Mandić-Mulec, and J.I. Prosser. 2012. Stimulation of thaumarchaeal ammonia oxidation by ammonia derived from organic nitrogen but not added inorganic nitrogen. FEMS Microbiol. Ecol. 80(1): 114–123.

Levin S. A. 1992. The problem of pattern and scale in ecology. Ecology. 73(6): 1943-1967.

Levy-Booth, D.J., C.E. Prescott, and S.J. Grayston. 2014. Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems. Soil Biol. Biochem. 75: 11–25.

Li, J., Y. Li, X. Yang, J. Zhang, Z. Lin, and B. Zhao. 2015. Microbial community structure and functional metabolic diversity are associated with organic carbon availability in an agricultural soil. J. Integr. Agric. 14(12): 2500–2511.

Liang, L.L., J.R. Eberwein, L.A. Allsman, D.A. Grantz, and G.D. Jenerette. 2015. Regulation of CO2 and N2O fluxes by coupled carbon and nitrogen availability. Environ. Res. Lett. 10(3): 34008.

Liiri, M., M. Häsä, J. Haimi, and H. Setälä. 2012. History of land-use intensity can modify the relationship between functional complexity of the soil fauna and soil ecosystem services - A microcosm study. Appl. Soil Ecol. 55: 53–61.

Liu, E., C. Yan, X. Mei, Y. Zhang, and T. Fan. 2013. Long-term effect of manure and fertilizer on soil organic carbon pools in dryland farming in northwest china. PLoS One 8(2).

Lozupone, C., and R. knight. 2005. unifrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71(12): 8228–8235.

Luo, P., X. Han, Y. Wang, M. Han, H. Shi, N. Liu, and H. Bai. 2015. Influence of long-term fertilization on soil microbial biomass, dehydrogenase activity, and bacterial and fungal community structure in a brown soil of northeast China. Ann. Microbiol. 65(1): 533–542.

Ma, W.K., A. Bedard-Haughn, S.D. Siciliano, and R.E. Farrell. 2008. Relationship between nitrifier and denitrifier community composition and abundance in predicting nitrous oxide emissions from ephemeral wetland soils. Soil Biol. Biochem. 40(5): 1114–1123.

Ma, B.L., T.Y. Wu, N. Tremblay, W. Deen, N.B. McLaughlin, M.J. Morrison, and G. Stewart. 2010. On-farm assessment of the amount and timing of nitrogen fertilizer on ammonia volatilization. Agron. J. 102(1): 134–144.

Ma, L., C. Guo, X. Lü, S. Yuan, and R. Wang. 2015. Soil moisture and land use are major determinants of soil microbial community composition and biomass at a regional scale in northeastern China. Biogeosciences 12(8): 2585–2596.

Macdonald, L.M., E. Paterson, L.A. Dawson, and A.J.S. McDonald. 2004. Short-term effects of defoliation on the soil microbial community associated with two contrasting *Lolium perenne* cultivars. Soil Biol. Biochem. 36(3): 489–498.

Malhi, S.S., R. Lemke, Z.H. Wang, and B.S. Chhabra. 2006. Tillage, nitrogen and crop residue effects on crop yield, nutrient uptake, soil quality, and greenhouse gas emissions. Soil Tillage Res. 90(1–2): 171–183.

Manzoni, S., P. Taylor, A. Richter, A. Porporato, and G.I. Ågren. 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytol. 196(1): 79–91.

Marschner, P., S. Umar, and K. Baumann. 2011. The microbial community composition changes rapidly in the early stages of decomposition of wheat residue. Soil Biol. Biochem. 43(2): 445–451.

Matsuzaka, E., N. Nomura, H. Maseda, H. Otagaki, T. Nakajima-Kambe, T. Nakahara, and H. Uchiyama. 2003. Participation of nitrite reductase in conversion of NO_2^- to NO_3^- in a heterotrophic nitrifier, *Burkholderia cepacia* NH-17, with denitrification activity. Microbes Environ. 18(4): 203–209.

Matus, F.J., C.H. Lusk, and C.R. Maire. 2007. Effects of soil texture, carbon input rates, and litter quality on free organic matter and nitrogen mineralization in Chilean rain forest and agricultural soils. Commun. Soil Sci. Plant Anal. 39(1–2): 187–201.

McLauchlan, K. 2006. The nature and longevity of agricultural impacts on soil carbon and nutrients: A review. Ecosystems 9(8): 1364–1382.

Meliani, A., A. Bensoltane, and K. Mederbel. 2012. Microbial diversity and abundance in soil: related to plant and soil type. Am. J. Plant Nutr. Fertil. Technol. 2(1): 10–18.

Miki, T., M. Ushio, S. Fukui, and M. Kondoh. 2010. Functional diversity of microbial decomposers facilitates plant coexistence in a plant-microbe-soil feedback model. Proc. Natl. Acad. Sci. U. S. A. 107(32): 14251–14256.

Miller, M.N., B.J. Zebarth, C.E. Dandie, D.L. Burton, C. Goyer, and J.T. Trevors. 2008. Crop residue influence on denitrification, N2O emissions and denitrifier community abundance in soil. Soil Biol. Biochem. 40(10): 2553–2562.

Miyadoh, S. 1997. Atlas of Actinomycetes. The Society for *Actinomycetes* Japan. Asakura Publishing Co., Ltd., Japan. Moncheva,

Monkiedje, A., M. Spiteller, D. Fotio, and P. Sukul. 2006. The effect of land use on soil health indicators in peri-urban agriculture in the humid forest zone of southern Cameroon. J. Environ. Qual. 35(6): 2402–2409.

Moore-Kucera, J., and R.P. Dick. 2008. Application of 13C-labeled litter and root materials for in situ decomposition studies using phospholipid fatty acids. Soil Biol. Biochem. 40(10): 2485–2493.

Moran, K.K., J. Six, W.R. Horwath, and C. van Kessel. 2005. Role of mineral-nitrogen in residue decomposition and stable soil organic matter formation. Soil Sci. Soc. Am. J. 69: 1730.

Moreno-vivián, C., P. Cabello, R. Blasco, M. Martinez-luque, and C. Castillo. 1999. MINIREVIEW Prokaryotic nitrate reduction: molecular properties and functional distinction among bacterial nitrate reductases. Jour. Of Bacte. 181(21): 6573–6584.

Muyzer, G., E.C. Dewaal, and A.G. Uitterlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S *rRNA*. Appl. Environ. Microbiol. 59(3): 695–700.

Muyzer, G., C. Wawer, G. Wanner, S. Ro, and W. Lubitz. 1996. Identification of bacteria in a biodegraded wall painting by denaturing gradient gel electrophoresis of PCR-amplied gene fragments coding for 16S *rRNA*. Microbiology 62(6): 2059–2065.

Navarro-Noya, Y.E., S. Gómez-Acata, N. Montoya-Ciriaco, A. Rojas-Valdez, M.C. Suárez-Arriaga, C. Valenzuela-Encinas, N. Jiménez-Bueno, N. Verhulst, B. Govaerts, and L. Dendooven. 2013. Relative impacts of tillage, residue management and crop-rotation on soil bacterial communities in a semi-arid agroecosystem. Soil Biol. Biochem. 65: 86–95.

Nemergut, D.R., S.K. Schmidt, T. Fukami, S.P. O'Neill, T.M. Bilinski, L.F. Stanish, J.E. Knelman, J.L. Darcy, R.C. Lynch, P. Wickey, and S. Ferrenberg. 2013. Patterns and processes of microbial community assembly. Microbiol. Mol. Biol. Rev. 77(3): 342–356.

Neufeld, J.D., J. Vohra, M.G. Dumont, T. Lueders, M. Manfield, M.W. Friedrich, and J.C. Murrell. 2007. RNA stable-isotope probing. Nat. Protoc. 2(4): 838–844.

Ng, E.L., A.F. Patti, M.T. Rose, C.R. Schefe, K. Wilkinson, R.J. Smernik, and T.R. Cavagnaro. 2014. Does the chemical nature of soil carbon drive the structure and functioning of soil microbial communities? Soil Biol. Biochem. 70: 54–61.

Nicol, G.W., G. Webster, L.A. Glover, and J.I. Prosser. 2004. Differential response of archaeal and bacterial communities to nitrogen inputs and pH changes in upland pasture rhizosphere soil. Environ. Microbiol. 6(8): 861–867.

Nicolardot, B., L. Bouziri, F. Bastian, and L. Ranjard. 2007. A microcosm experiment to evaluate the influence of location and quality of plant residues on residue decomposition and genetic structure of soil microbial communities. Soil Biol. Biochem. 39(7): 1631–1644.

Oskay, M. 2009. Comparison of Streptomyces diversity between agricultural and non-agricultural soils by using various culture media. Sci. Res. Essay Vol.4 4(10): 997–1005.

O Sullivan, C.A., A.W. Steven, I.R.P. Fillery, and M.M. Roper. 2013. Factors affecting ammonia-oxidising microorganisms and potential nitrification rates in southern Australian agricultural soils. Soil Res (51): 240–252.

Offre, P., J.I. Prosser, and G.W. Nicol. 2009. Growth of ammonia-oxidizing archaea in soil microcosms is inhibited by acetylene. FEMS Microbiol. Ecol. 70: 99–108.

Okano, Y., K.R. Hristova, C.M. Leutenegger, L.E. Jackson, R.F. Denison, B. Gebreyesus, D. Lebauer, and K.M. Scow. 2004. Application of real-time PCR to study effects of ammonium on population size of ammonia-oxidizing bacteria in soil. Appl. Environ. Microbiol. 70(2): 1008–1016

Olson, B. M., H. H. Janzen, F. J. Larney and L. J. Selinger. 1996. A proposed method for measuring the effect of soil quality on productivity. Agron. J. 88(3): 497–500.

Olsson, P.A., 1999. Signature fatty acids provide tools for determination of the distribution and interaction of mycorrhizal fungi in soil. FEMS Microbiol. Ecol. 29, 303–310.

Parmesan, C., G. Yohe, and Y. G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421(6918): 37.

Paterson, E., A. Sim, S.M. Osborne, and P.J. Murray. 2011. Long-term exclusion of plant-inputs to soil reduces the functional capacity of microbial communities to mineralise recalcitrant root-derived carbon sources. Soil Biol. Biochem. 43(9): 1873–1880.

Paula, F.S., J.L.M. Rodrigues, J. Zhou, L. Wu, R.C. Mueller, B.S. Mirza, B.J.M. Bohannan, K. Nüsslein, Y. Deng, J.M. Tiedje, and V.H. Pellizari. 2014. Land use change alters functional gene diversity, composition and abundance in Amazon forest soil microbial communities. Mol. Ecol. 23(12): 2988–2999.

Peacock, A.D., M.D. Mullen, D.B. Ringelberg, D.D. Tyler, D.B. Hedrick, P.M. Gale, and D.C. White. 2001. Soil microbial community responses to dairy manure or ammonium nitrate applications. Soil Biol. Biochem. 33(7–8): 1011–1019.

Pelster, D.E., F. Larouche, P. Rochette, M.H. Chantigny, S. Allaire, and D.A. Angers. 2011. Nitrogen fertilization but not soil tillage affects nitrous oxide emissions from a clay loam soil under a maize-soybean rotation. Soil Tillage Res. 115–116: 16–26.

Peralta, A.L., J.W. Matthews, and A.D. Kent. 2010. Microbial community structure and denitrification in a wetland mitigation bank. Appl. Environ. Microbiol. 76(13): 4207–4215.

Philippot, L., S. Hallin, and M. Schloter. 2007. Ecology of denitrifying prokaryotes in agricultural soil. Adv. Agron. 96(7): 249–305.

Philippot, L., J. Čuhel, N.P.A. Saby, D. Chèneby, A. Chroňáková, D. Bru, D. Arrouays, F. Martin-Laurent, and M. Šimek. 2009. Mapping field-scale spatial patterns of size and activity of the denitrifier community. Environ. Microbiol. 11(6): 1518–1526.

Philippot, L., S.G.E. Andersson, T.J. Battin, J.I. Prosser, J.P. Schimel, W.B. Whitman, and S. Hallin. 2010. The ecological coherence of high bacterial taxonomic ranks. Nat. Rev. Microbiol. 8(7): 523–529.

Philippot, L., A. Spor, C. Hénault, D. Bru, F. Bizouard, C.M. Jones, A. Sarr, and P. A. Maron. 2013. Loss in microbial diversity affects nitrogen cycling in soil. ISME J. 7(8): 1609–1619.

Phillips, L.A., C.W. Greer, and J.J. Germida. 2006. Culture-based and culture-independent assessment of the impact of mixed and single plant treatments on rhizosphere microbial communities in hydrocarbon contaminated flare-pit soil. Soil Biol. Biochem. 38: 2823–2833.

Pianka, R. 1970. On r and K-selection. Ame. Natu. 104(940): 592–597.

Powlson, D. 2001. The role of soil microorganisms in soil organic matter conservation in the tropics. Nutr. Cycl. Agroecosystems 61: 41–51.

Prosser, J.I. 2005. Nitrogen in Soils: Nitrification. Encycl. Soils Environ.: 32–39.

Prosser, J.I., and G.W. Nicol. 2012. Archaeal and bacterial ammonia-oxidisers in soil: The quest for niche specialisation and differentiation. Trends Microbiol. 20(11): 523–531.

Purahong, W., M. Schloter, M.J. Pecyna, D. Kapturska, V. Däumlich, S. Mital, F. Buscot, M. Hofrichter, J.L.M. Gutknecht, and D. Krüger. 2014. Uncoupling of microbial community structure and function in decomposing litter across beech forest ecosystems in Central Europe. Sci. Rep. 4: 7014.

Qiao, N., X. Xu, Y. Hu, E. Blagodatskaya, Y. Liu, D. Schaefer, and Y. Kuzyakov. 2016. Carbon and nitrogen additions induce distinct priming effects along an organic-matter decay continuum. Sci. Rep. 6: 19865.

Qian, P., J. Schoenau, and R.E. Karamanos. 1994. Simultaneous extraction of available phosphorus and potassium with a new soil test: A modification of kelowna extraction. Commun. Soil Sci. Plant Anal. 25(5-6): 627–635.

Rampelotto, P.H., A. de Siqueira Ferreira, A.D.M. Barboza, and L.F.W. Roesch. 2013. Changes in diversity, abundance, and structure of soil bacterial communities in Brazilian Savanna under different land use systems. Microb. Ecol. 66(3): 593–607.

Ramsey, P.W., M.C. Rillig, K.P. Feris, W.E. Holben, and J.E. Gannon. 2006. Choice of methods for soil microbial community analysis: PLFA maximizes power compared to CLPP and PCR-based approaches. Pedobiologia (Jena). 50(3): 275–280.

Ramirez, K.S., C.A. Lauber, R. Knight, M.A. Bradford, and N. Fierer. 2010. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. Ecology 91(12): 3463–3470.

Rastogi, G., and K.A. Sani. 2011. Molecular techniques to assess microbial community structure, function, and dynamics in the environment. p. 29–57. In Ahmad, I., Ahmad, F., Pichtel, J. (eds.), Microbes and microbial technology: Agricultural and environmental applications. Springer, New York.

Raun, W.R., G. V. Johnson, S.B. Phillips, and R.L. Westerman. 1998. Effect of long-term N fertilization on soil organic C and total N in continuous wheat under conventional tillage in Oklahoma. Soil Tillage Res. 47(3–4): 323–330.

Raun, W.R., and G.V. Johnson. 1999. Improving nitrogen use efficiency for cereal production. Agron. J. 91(June): 357–363.

Reeve, J.R., C.W. Schadt, L. Carpenter-Boggs, S. Kang, J. Zhou, and J.P. Reganold. 2010. Effects of soil type and farm management on soil ecological functional genes and microbial activities. ISME J. 4(9): 1099–1107.

Rincon-Florez, V.A., L.C. Carvalhais, and P.M. Schenk. 2013. Culture-independent molecular tools for soil and rhizosphere microbiology. Diversity 5(3): 581–612.

Rinkes, Z.L., R.L. Sinsabaugh, D.L. Moorhead, A. Stuart Grandy, and M.N. Weintraub. 2013. Field and lab conditions alter microbial enzyme and biomass dynamics driving decomposition of the same leaf litter. Front. Microbiol. 4(Sep): 1–14.

Rotthauwe, J.H., K.P. Witzel, and W. Liesack. 1997. The ammonia monooxygenase structural gene amoA as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing populations. Appl. Environ. Microbiol. 63(12): 4704–4712.

Rousk, J., E. Baath, P.C. Brookes, C.L. Lauber, C.A. Lozupone, J.G. Caporaso, R. Knight, and N. Fierer. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J. 4(10): 1340–51.

Santoro, A.E., C.A. Francis, N.R. De Sieyes, and A.B. Boehm. 2008. Shifts in the relative abundance of ammonia-oxidizing bacteria and archaea across physicochemical gradients in a subterranean estuary. Environ. Microbiol. 10(4): 1068–1079.

Sarathchandra, S.U., A. Ghani, G.W. Yeates, G. Burch, and N.R. Cox. 2001. Effect of nitrogen and phosphate fertilisers on microbial and nematode diversity in pasture soils. Soil Biol. Biochem. 33(7–8): 953–964.

Schimel, D. 1995. Terrestrial ecosystems and the carbon cycle. Glob. Chang. Biol. (October 1994): 77–91.

Schimel, J.P., and S.M. Schaeffer. 2012. Microbial control over carbon cycling in soil. Front. Microbiol. 3(Sep): 1–11.

Schlesinger, W.H., and E.S. Bernhardt (Eds). 2013. Biogeochemistry an analysis of global change. 3rd ed. Elesvier Inc, Waltham, MA.

Schloss, P.D., S.L. Westcott, T. Ryabin, J.R. Hall, M. Hartmann, E.B. Hollister, R.A. Lesniewski, B.B. Oakley, D.H. Parks, C.J. Robinson, J.W. Sahl, B. Stres, G.G. Thallinger, D.J. Van Horn, and C.F. Weber. 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75(23): 7537–7541.

Schnürer, J., M. Clarholm, and T. Rosswall. 1985. Microbial biomass and activity in an agricultural soil with different organic matter contents. Soil Biol. Biochem. 17(5): 611–618.

Schulten, R., and M. Schnitzer. 1998. Review article: The chemistry of soil organic nitrogen: a review. Agriculture 26(1): 1–15.

Schutter, M.E., J.M. Sandeno, and R.P. Dick. 2001. Seasonal, soil type, and alternative management influences on microbial communities of vegetable cropping systems. Biol. Fertil. Soils 34(6): 397–410.

Sekercioglu, C.H. 2010. Ecosystem functions and services. p. 45–72. In Sodhi, S., N., Ehrlich, P.R. (eds.), Conservation biology for all. 1st ed. Oxford University Press Inc., United Kingdom, Oxford.

Serna-Chavez, H.M., N. Fierer, and P.M. Van Bodegom. 2013. Global drivers and patterns of microbial abundance in soil. Glob. Ecol. Biogeogr. 22(10): 1162–1172.

Shaw, L.J., G.W. Nicol, Z. Smith, J. Fear, J.I. Prosser, and E.M. Baggs. 2006. *Nitrosospira* spp. can produce nitrous oxide via a nitrifier denitrification pathway. Environ. Microbiol. 8(2): 214–222.

Shen, J.P., L.M. Zhang, Y.G. Zhu, J.B. Zhang, and J.Z. He. 2008. Abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea communities of an alkaline sandy loam. Environ. Microbiol. 10(6): 1601–1611.

Shen, J.P., P. Cao, H.W. Hu, and J.Z. He. 2013. Differential response of archaeal groups to land use change in an acidic red soil. Sci. Total Environ. 461–462: 742–749.

Shi, W., and J.M. Norton. 2000. Microbial control of nitrate concentrations in an agricultural soil treated with dairy waste compost or ammonium fertilizer. Soil Biol. Biochem. 32(10): 1453–1457.

Shoun, H., S. Fushinobu, L. Jiang, S.-W. Kim, and T. Wakagi. 2012. Fungal denitrification and nitric oxide reductase cytochrome P450nor. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 367(1593): 1186–1194.

Signor, D., C. Eduardo, and P. Cerri. 2013. Nitrous oxide emissions in agricultural soils: a review 1. Pesqui. Agropecu. Trop. Goiania 2013: 322–338.

Sinsabaugh, R.L., S. Manzoni, D.L. Moorhead, and A. Richter. 2013. Carbon use efficiency of microbial communities: Stoichiometry, methodology and modelling. Ecol. Lett. 16(7): 930–939.

Smalla, K. 1999. Methods of microbial community analysis: Introduction to the symposium. p. 1–2. In Bell, C.R., Brylinsky, M., Johnson-Green, P. (eds.), 8th International Symposium on Microbial Ecology Bell. Atlantic Canada Society for Microbial Ecology, Halifax.

Smit, E., P. Leeflang, S. Gommans, J. Van Den Broek, S. Van Mil, and K. Wernars. 2001. Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. Appl. Environ. Microbiol. 67(5): 2284–2291.

Smith, C.J., and A.M. Osborn. 2009. Advantages and limitations of quantitative PCR (Q-PCR)-based approaches in microbial ecology. FEMS Microbiol. Ecol. 67(1): 6–20.

Smith, J., C. Wagner-Riddle, and K. Dunfield. 2010. Season and management related changes in the diversity of nitrifying and denitrifying bacteria over winter and spring. Appl. Soil Ecol. 44(2): 138–146.

Steenwerth, K.L., L.E. Jackson, F.J. Calderón, M.R. Stromberg, and K.M. Scow. 2002. Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. Soil Biol. Biochem. 34(11): 1599–1611.

Stephen, J.R., Y. Chang, S.J. Macnaughton, G.A. Kowalchuk, K.T. Leung, C.A. Flemming and D.C. White. 1999. Effect of toxic metals on indigenous soil β -subgroup proteobacterium ammonia oxidizer community structure and protection against toxicity by inoculated metal-resistant bacteria. Appl. Environ. Microbiol. 65(1): 95–101.

Sterngren, A.E., S. Hallin, and P. Bengtson. 2015. Archaeal ammonia oxidizers dominate in numbers, but bacteria drive gross nitrification in n-amended grassland soil. Front. Microbiol. 6(Nov): 1–8.

Sun, P.P., Y.P. Zhuge, J.B. Zhang, and Z.C. Cai. 2012. Soil pH was the main controlling factor of the denitrification rates and N-2/N2O emission ratios in forest and grassland soils along the Northeast China Transect (NECT). Soil Sci. Plant Nutr. 58(4): 517–525.

Sun, B., X. Wang, F. Wang, Y. Jiang, and X.X. Zhang. 2013. Assessing the relative effects of geographic location and soil type on microbial communities associated with straw decomposition. Appl. Environ. Microbiol. 79(11): 3327–3335.

Sun, B., F. Wang, Y. Jiang, Y. Li, Z. Dong, Z. Li, and X.X. Zhang. 2014. A long-term field experiment of soil transplantation demonstrating the role of contemporary geographic separation in shaping soil microbial community structure. Ecol. Evol. 4(7): 1073–1087.

Sun, R., X. Guo, D. Wang, and H. Chu. 2015. Effects of long-term application of chemical and organic fertilizers on the abundance of microbial communities involved in the nitrogen cycle. Appl. Soil Ecol. 95: 171–178.

Swallow, M., S.A. Quideau, M.D. MacKenzie, and B.E. Kishchuk. 2009. Microbial community structure and function: The effect of silvicultural burning and topographic variability in northern Alberta. Soil Biol. Biochem. 41(4): 770–777.

Sylvia, D.M., J.J. Fuhrmann, P.G. Hartel, D.A. Zuberer, 1999. Principles and applications of soil microbiology. In Prentice Hall, Upper Saddle River, NJ.

Strickland, M.S., E. Osburn, C. Lauber, N. Fierer, and M.A. Bradford. 2009. Litter quality is in the eye of the beholder: Initial decomposition rates as a function of inoculum characteristics. Funct. Ecol. 23(3): 627–636.

Szukics, U., E. Hackl, S. Zechmeister-Boltenstern, and A. Sessitsch. 2012. Rapid and dissimilar response of ammonia oxidizing archaea and bacteria to nitrogen and water amendment in two temperate forest soils. Microbiol. Res. 167(2): 103–109.

Tardy, V., A. Chabbi, X. Charrier, C. de Berranger, T. Reignier, S. Dequiedt, C. Faivre-Primot, S. Terrat, L. Ranjard, and P.-A. Maron. 2015. Land use history shifts in situ fungal and

bacterial successions following wheat straw input into the soil. PLoS One 10(6): e0130672.

Throbäck, I.N., K. Enwall, Å. Jarvis, and S. Hallin. 2004. Reassessing PCR primers targeting nirS, nirK and nosZ genes for community surveys of denitrifying bacteria with DGGE. FEMS Microbiol. Ecol. 49: 401–417.

Tilman, D., K.G. Cassman, P.A. Matson, R. Naylor, and S. Polasky. 2002. Agricultural sustainability and intensive production practices. Nature 418(6898): 671–677.

Tilman, D., C. Balzer, J. Hill, and B. Befort. 2011. Global food demand and the sustainable intensification of agriculture. Proc. Natl. Acad. Sci. 108(50): 20260–20264.

Torsvik, V., L. Øvreås, and T.F. Thingstad. 2002. Prokaryotic diversity-magnitude dynamics, and controlling factors. Science. 296(5570): 1064–1066.

Tourna, M., T.E. Freitag, G.W. Nicol, and J.I. Prosser. 2008. Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms. Environ. Microbiol. 10(5): 1357–1364.

Toyota, K., and S. Kuninaga. 2006. Comparison of soil microbial community between soils amended with or without farmyard manure. Appl. Soil Ecol. 33(1): 39–48.

Turner, B., and P.M. Haygarth. 2001. Phosphorus solubilization in rewetted soils. Nature 411: 258.

Tzeneva, V.A., J.F. Salles, N. Naumova, W.M. de Vos, P.J. Kuikman, J. Dolfing, and H. Smidt. 2009. Effect of soil sample preservation, compared to the effect of other environmental variables, on bacterial and eukaryotic diversity. Res. Microbiol. 160(2): 89–98.

Uitierlinden, A.G. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 53(3): 695–700.

Valentine, D.L. 2007. Adaptations to energy stress dictate the ecology and evolution of the Archaea. Nat. Rev. Microbiol. 5(4): 316–323.

van Bruggen, A. 2000. In search of biological indicators for soil health and disease suppression. Appl. Soil Ecol. 15(1): 13–24.

Van Der Heijden, M.G.A., R.D. Bardgett, and N.M. Van Straalen. 2008. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11(3): 296–310.

van der Wal, A., and W. de Boer. 2017. Dinner in the dark: Illuminating drivers of soil organic matter decomposition. Soil Biol. Biochem. 105: 45–48.

VandenBygaart, A. J., and D. A. Angers. 2006. Towards accurate measurements of soil organic carbon stock change in agroecosystems. Can. J. Soil Sci. 86: 465–471.

Vanhala, P., K. Karhu, M. Tuomi, K. Björklöf, H. Fritze, H. Hyvärinen, and J. Liski. 2011. Transplantation of organic surface horizons of boreal soils into warmer regions alters

microbiology but not the temperature sensitivity of decomposition. Glob. Chang. Biol. 17(1): 538–550.

Verhagen, F.J.M., H.J. Laanbroek, and J.W. Woldendrop. 1995. Competition for ammonium between plant roots and nitrifying and heterotrophic bacteria and the effects of protozoan grazing. Plant Soil 170(2): 241–250.

Vitousek, P.M., and R.W. Howarth. 2007. Nitrogen limitation on land and in the sea: how can it occur? Biobeoche. 13(2): 87–115.

Wagg, C., S.F. Bender, F. Widmer, and M.G.A. van der Heijden. 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proc. Natl. Acad. Sci. U. S. A. 111(14): 5266–5270.

Wang, Y., C. Tu, L. Cheng, C. Li, L.F. Gentry, G.D. Hoyt, X. Zhang, and S. Hu. 2011. Long-term impact of farming practices on soil organic carbon and nitrogen pools and microbial biomass and activity. Soil Tillage Res. 117: 8–16.

Wang, Z., T.-Z. Lei, X.-Y. Yan, Y. Li, X.-F. He, and J. Zhu. 2012. Assessment and Utilisation of agricultural residue resources in Henan Province, China. BioResources 7(3): 3847–3861.

Wang, H., T.W. Boutton, W. Xu, G. Hu, P. Jiang, and E. Bai. 2015. Quality of fresh organic matter affects priming of soil organic matter and substrate utilization patterns of microbes. Sci. Rep. 5: 10102.

Ward, B.B. 2013. Nitrification. Ref. Modul. Earth Syst. Environ. Sci. (March): 1–8.

Warrington R. 1878. On nitrification. J. Chem. Soc. 33:44–51.

Webster, G., T.M. Embley, T.E. Freitag, Z. Smith, and J.I. Prosser. 2005. Links between ammonia oxidizer species composition, functional diversity and nitrification kinetics in grassland soils. Environ. Microbiol. 7(5): 676–684.

Wessen, E., M. Soderstrom, M. Stenberg, D. Bru, M. Hellman, A. Welsh, F. Thomsen, L. Klemedtson, L. Philippot, and S. Hallin. 2011. Spatial distribution of ammonia-oxidizing bacteria and archaea across a 44-hectare farm related to ecosystem functioning. ISME J. 5(7): 1213–1225.

White, D.C., W.M. Davis, J.S. Nickels, J.D. King and R.J. Bobbie. 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40: 51-62.

Wickings, K., A. S. Grandy, S.C. Reed, and C.C. Cleveland. 2012. The origin of litter chemical complexity during decomposition. Ecol. Lett. 15(10): 1180–1188.

Wieland, G., R. Neumann, and H. Backhaus. 2001. Variation of microbial communities in soil, rhizosphere, and rhizoplane in response to crop species, soil type, and crop development. Appl. Environ. Microbiol. 67(12): 5849-5854.

Will, C., A. Thürmer, A. Wollherr, H. Nacke, N. Herold, M. Schrumpf, J. Gutknecht, T. Wubet, F. Buscot, and R. Daniell. 2010. Horizon-specific bacterial community composition of German grassland soils, as revealed by pyrosequencing-based analysis of 16S rRNA genes. Appl. Environ. Microbiol. 76(20): 6751–6759.

Wilhelm, W.W. 2004. Crop and soil productivity response to corn residue removal: a literature review. Agron. J. 96.

Wolsing, M., and A. Priemé. 2004. Observation of high seasonal variation in community structure of denitrifying bacteria in arable soil receiving artificial fertilizer and cattle manure by determining T-RFLP of nir gene fragments. FEMS Microbiol. Ecol. 48(2): 261–271.

Wu, J., and P.C. Brookes. 2005. The proportional mineralisation of microbial biomass and organic matter caused by air-drying and rewetting of a grassland soil. Soil Biol. Biochem. 37(3): 507–515.

Wu, Z., S.E. Haack, W. Lin, B. Li, L. Wu, C. Fang, and Z. Zhang. 2015. Soil microbial community structure and metabolic activity of *Pinus elliottii* plantations across different stand ages in a subtropical area. PLoS One 10(8): 1–11.

Wu, L., C. Wen, Y. Qin, H. Yin, Q. Tu, J.D. Van Nostrand, T. Yuan, M. Yuan, Y. Deng, and J. Zhou. 2015. Phasing amplicon sequencing on Illumina Miseq for robust environmental microbial community analysis. BMC Microbiol. 15(1): 125.

Xu, X., Z. Shi, D. Li, A. Rey, H. Ruan, J.M. Craine, J. Liang, J. Zhou, and Y. Luo. 2016. Soil properties control decomposition of soil organic carbon: Results from data-assimilation analysis. Geoderma 262: 235–242.

Xu, Z., G. Yu, X. Zhang, J. Ge, N. He, Q. Wang, and D. Wang. 2014. The variations in soil microbial communities, enzyme activities and their relationships with soil organic matter decomposition along the northern slope of Changbai mountain. Appl. Soil Ecol. 86: 19–29.

Xue, K., L. Wu, Y. Deng, Z. He, J. Van Nostrand, P.G. Robertson, T.M. Schmidt, and J. Zhou. 2013. Functional gene differences in soil microbial communities from conventional, low-input, and organic farmlands. Appl. Environ. Microbiol. 79(4): 1284–1292.

Yang, C., C. Hamel, and Y. Gan. 2015. Incongruous variation of denitrifying bacterial communities as soil N level rises in Canadian canola fields. Appl. Soil Ecol. 89(3): 93–101.

Yanni, S.F., H.H. Janzen, E.G. Gregorich, B.H. Ellert, F.J. Larney, B.M. Olson, and F. Zvomuya. 2016. Organic carbon convergence in diverse soils toward steady state: A 21-year field bioassay. Soil Sci. Soc. Am. J. 80(0): 1653–1662.

Ye, R., A.L. Wright, K. Inglett, Y. Wang, A. V Ogram, and K.R. Reddy. 2009. Land-use effects on soil nutrient cycling and microbial community dynamics in the Everglades agricultural area, Florida. Commun. Soil Sci. Plant Anal. 40(July 2008): 2725–2742.

Yin, C., F. Fan, A. Song, Z. Li, W. Yu, and Y. Liang. 2014. Different denitrification potential of aquic brown soil in Northeast China under inorganic and organic fertilization accompanied by distinct changes of nirS- and nirK-denitrifying bacterial community. Eur. J. Soil Biol. 65: 47–56.

Yu, H., Q. Gao, Z. Shao, A. Ying, Y. Sun, J. Liu, W. Mao, and B. Zhang. 2016. Decreasing nitrogen fertilizer input had little effect on microbial communities in three types of soils. PLoS One 11(3): 1–12.

Zaehle, S. 2013. Terrestrial nitrogen-carbon cycle interactions at the global scale. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 368(1621): 1-9.

Zak, D.R., W.E. Holmes, D.C. White, A.D. Peacock, S. Ecology, and N. Aug. 2014. Plant diversity, soil microbial communities, and ecosystem function: are there any links? Ecology. 84(8): 2042–2050.

Zhang, P., J. Zheng, G. Pan, X. Zhang, L. Li, and T. Rolf. 2007. Changes in microbial community structure and function within particle size fractions of a paddy soil under different long-term fertilization treatments from the Tai Lake region, China. Colloids Surfaces B Bio-interfaces 58(2): 264–270.

Zhang, N., S. Wan, L. Li, J. Bi, M. Zhao, and K. Ma. 2008. Impacts of urea N addition on soil microbial community in a semi-arid temperate steppe in northern China. Plant Soil 311(1–2): 19–28.

Zhang, Y., X. Han, N. He, M. Long, J. Huang, G. Zhang, Q. Wang, and X. Han. 2014. Increase in ammonia volatilization from soil in response to N deposition in inner Mongolia grasslands. Atmos. Environ. 84: 156–162.

Zhang, Q., J. Wu, F. Yang, Y. Lei, Q. Zhang, X. Cheng, A. Don, et al. 2016. Alterations in soil microbial community composition and biomass following agricultural land use change. Sci. Rep. 6(Oct): 36587.

Zhao, M., B. Sun, L. Wu, Q. Gao, F. Wang, C. Wen, M. Wang, Y. Liang, L. Hale, J. Zhou, and Y. Yang. 2016. Zonal soil type determines soil microbial responses to maize cropping and fertilization. mSystems 1: e00075-16.

Zhao, M., K. Xue, F. Wang, S. Liu, S. Bai, B. Sun, J. Zhou, and Y. Yang. 2014. Microbial mediation of biogeochemical cycles revealed by simulation of global changes with soil transplant and cropping. ISME J. 8(10): 2045–2055.

Zhao, S., S. Qiu, C. Cao, C. Zheng, W. Zhou, and P. He. 2014. Responses of soil properties, microbial community and crop yields to various rates of nitrogen fertilization in a wheat-maize cropping system in north-central China. Agric. Ecosyst. Environ. 194: 29–37.

Zhou, G., J. Zhang, C. Zhang, Y. Feng, L. Chen, Z. Yu, X. Xin, and B. Zhao. 2016. Effects of changes in straw chemical properties and alkaline soils on bacterial communities engaged in straw decomposition at different temperatures. Sci Rep 6(Feb): 22186.

Zumft, W.G. 1997. Cell biology and molecular basis of denitrification. Microbiol. Mol. Biol. Rev. 61(4): 533–616.

Zvomuya, F., H.H. Janzen, F.J. Larney, and B.M. Olson. 2008. A long-term field bioassay of soil quality indicators in a semiarid environment. Soil Sci. Soc. Am. J. 72(3): 683.

APPENDIX A MICROBIAL COMMUNITY ANALYSIS AFTER 21 YEARS RELATED METHODS AND DATA

A.1. DETAIL DESCRIPTION OF QPCR METHODS

Abundance of 16S *rRNA* gene was quantified using the EUB 338 and EUB 518 primers. Each reaction (25 µL) contained 10 µL of Platinum SYBR Green qPCR Supermix (Life Technologies, Burlington, ON), 0.5 µL of each primer (10 µM) (Sigma Aldrich, Oakville, Ontario, Canada), 0.62 µL of 10 mg μL^{-1} diluted bovine serum albumin (BSA) (Life Technologies), 0.40 µL of ROX dye (10 fold dilution in Rnase free water), 6.8 µL of H_2O and 2 µL of 0.1 ng μL^{-1} template (due to inhibitors, 10 ng μL^{-1} DNA concentrations were diluted 100 fold). Conditions were 95°C for 5 min followed by 40 cycles of 95°C for 1 min, 53°C for 30 sec, 72°C for 1 min, 80°C 1 min (data collection) and 1 cycle 95°C for 15sec, 60°C for 1 min, ramping at 0.3°C per min (data collection) to 95°C for 15 sec. Annealing temperature duration was modified based on the preliminary analysis.

Quantification of bacterial *amoA* gene copy number was performed using the amoA1F and amoA2R primers. Each reaction (25 µL) contained 12.50 µL of Platinum SYBR Green qPCR Supermix (Life Technologies, Burlington, ON), 1.50 µL of each primer (10 µM) (Sigma Aldrich, Oakville, Ontario, Canada), 0.78 µL of 10 mg μL^{-1} diluted bovine serum albumin (BSA) (Life Technologies), 0.50 µL of ROX dye (10 fold dilution in Rnase free water), 4 µL of H_2O and 4 µL of 0.1 ng μL^{-1} template (due to inhibitors 10 ng μL^{-1} DNA concentrations were diluted 100 fold). PCR thermo cycling conditions from were modified according to the preliminary analysis (annealing temperature and number of thermos cycling number). Conditions were 95°C for 5 min followed by 46 cycles of 95°C for 45 sec, 55°C for 45 sec, 72°C for 45 sec, 80°C 1 min (data collection) and 1 cycle 95°C for 15sec, 60°C for 30 sec, ramping at 0.3°C per min (data collection) to 95°C for 15 sec.

Archaeal *amoA* gene copy number was quantified by using a modified method of Tourna et al., 2008. Each reaction (20 µL) contained 10 µL of Platinum SYBR Green qPCR Supermix (Life Technologies, Burlington, ON), 0.25 µL of each primer (10 µM) (Sigma Aldrich, Oakville, Ontario, Canada), 0.625 µL of 10 mg μL^{-1} diluted bovine serum albumin (BSA) (Life

Technologies), 0.40 µL of ROX dye (10 fold dilution in Rnase free water), 4 µL of H_2O, 0.4 µL $MgCl_2$ (50 mM) and 4 µL of template (10 ng μL^{-1}). Conditions were 95°C for 5 min followed by 45 cycles of 95°C for 1 min, 55°C for 1 min, 72°C for 1 min, 80°C 1 min (fluorescent data collection) and 1 cycle of 95°C for 15sec, 60°C for 1 min, ramping at 0.5°C per min (fluorescent data collection), to 95°C for 15 sec.

Abundance of the *nirS* gene was quantified by using Cd3aF and R3cdR as the primer pair. Each reaction (25 µL) contained 12.50 µL of Platinum SYBR Green qPCR Supermix (Life Technologies, Burlington, ON), 2.50 µL of each primer (10 µM) (Sigma Aldrich, Oakville, Ontario, Canada), 0.78 µL of 10 mg μL^{-1} diluted bovine serum albumin (BSA) (Life Technologies), 0.50 µL of ROX dye (10 fold dilution in Rnase free water), 4.22 µL of H_2O and 2 µL of template (10 ng μL^{-1}). Conditions were 95°C for 5 min followed by 6 cycles of 95°C for 15 sec, 65°C for 30 sec (-1°C per cycle), 72°C for 30 sec, and 85°C for 15 sec (data collection), followed by 40 cycles of 95°C for 15 sec, 60°C for 30 sec, 72°C for 30 sec, and 85°C for 15 sec (data collection) and 1 cycle of 95°C for 15sec, 60°C for 1 min, ramping at 0.5°C per min (fluorescent data collection), to 95°C for 15 sec.

Abundance of *nirK* gene was quantified by using F1aCu and R3Cu as the primer pair. Each reaction (25 µL) contained 12.50 µL of Platinum SYBR Green qPCR Supermix (Life Technologies, Burlington, ON), 1.25 µL of each primer (10 µM) (Sigma Aldrich, Oakville, Ontario, Canada), 0.78 µL of 10 mg μL^{-1} diluted bovine serum albumin (BSA) (Life Technologies), 0.50 µl of ROX dye (10 fold dilution in Rnase free water), 7.50 µL of H_2O and 1.25 µL of template (10 ng μL^{-1}). Conditions were 95°C for 5 min followed by 6 cycles of 95°C for 15 sec, 63°C for 30 sec (-1°C per cycle), 72°C for 30 sec, and 80°C for 30 sec (data collection), followed by 40 cycles of 95°C for 15 sec, 58°C for 30 sec, 72°C for 30 sec, and 80°C for 15 sec (data collection) and 1 cycle of 95°C for 15sec, 60°C for 1 min, ramping at 0.3°C per min (fluorescent data collection), to 95°C for 15 sec.

Abundance of *nosZ* gene was assessed using nosZ2F and nosZ2R as the primer pair. Each reaction (25 µl) contained 12.5 µL of Platinum SYBR Green qPCR Supermix (Life Technologies, Burlington, ON), 2.5 µL of each primer (10 µM) (Sigma Aldrich, Oakville, Ontario, Canada), 0.78 µL of 10 mg μL^{-1} diluted bovine serum albumin (BSA) (Life

Technologies), 0.50 µL of ROX dye (10 fold dilution in Rnase free water), 4.72 µL of H_2O and 1.5 µL of template (10 ng μL^{-1}). Conditions were 95°C for 5 min followed by 6 cycles of 95°C for 15 sec, 65°C for 30 sec (-1°C per cycle), 72°C for 30 sec, and 80°C for 15 sec (data collection), followed by 40 cycles of 95°C for 15 sec, 60°C for 30 sec, 72°C for 30 sec, and 80°C for 15 sec (data collection) and 1 cycle of 95°C for 15 sec, 60°C for 1 min, ramping at 1 °C per min (fluorescent data collection), to 95°C for 15 sec.

Table A.1. Ammonia oxidizers (AOB, AOA), *nirS*, *nirK* and *nosZ* gene abundance analysis for individual soils

Soil origin[†]	*P* value				
	AOB	AOA	*nirS*	*nirK*	*nosZ*
NG	0.04	0.35	0.11	0.32	0.04*
DW	0.52	0.40	0.55	0.59	0.59
CC	0.16	0.90	0.19	0.09	0.30
PL	1.00	0.50	0.04*	0.05	0.02*
DTF	0.001**	0.0001***	0.55	0.05	0.34
ITF	0.03*	0.93	0.43	0.21	0.42
RM	0.01*	0.73	0.49	0.62	0.28
HM	0.18	0.99	0.59	0.73	0.42
BH	0.07	0.17	0.0001***	0.03*	0.06
CH	0.02*	0.78	0.29	0.11	0.40

[†]NG- native grassland, DW- dryland wheat, CC-cereal, DTF- dryland tilled fallowed, RM- manured 30 t h-1, HM- manured 90 t ha^{-1}, ITF- irrigated tilled fallow, PL- pastureland, BH- B horizon, CH-C horizon)

Significant values represent *P*-value *, $P<0.05$; **, $P< 0.001$; ***, $P<0.0001$

.

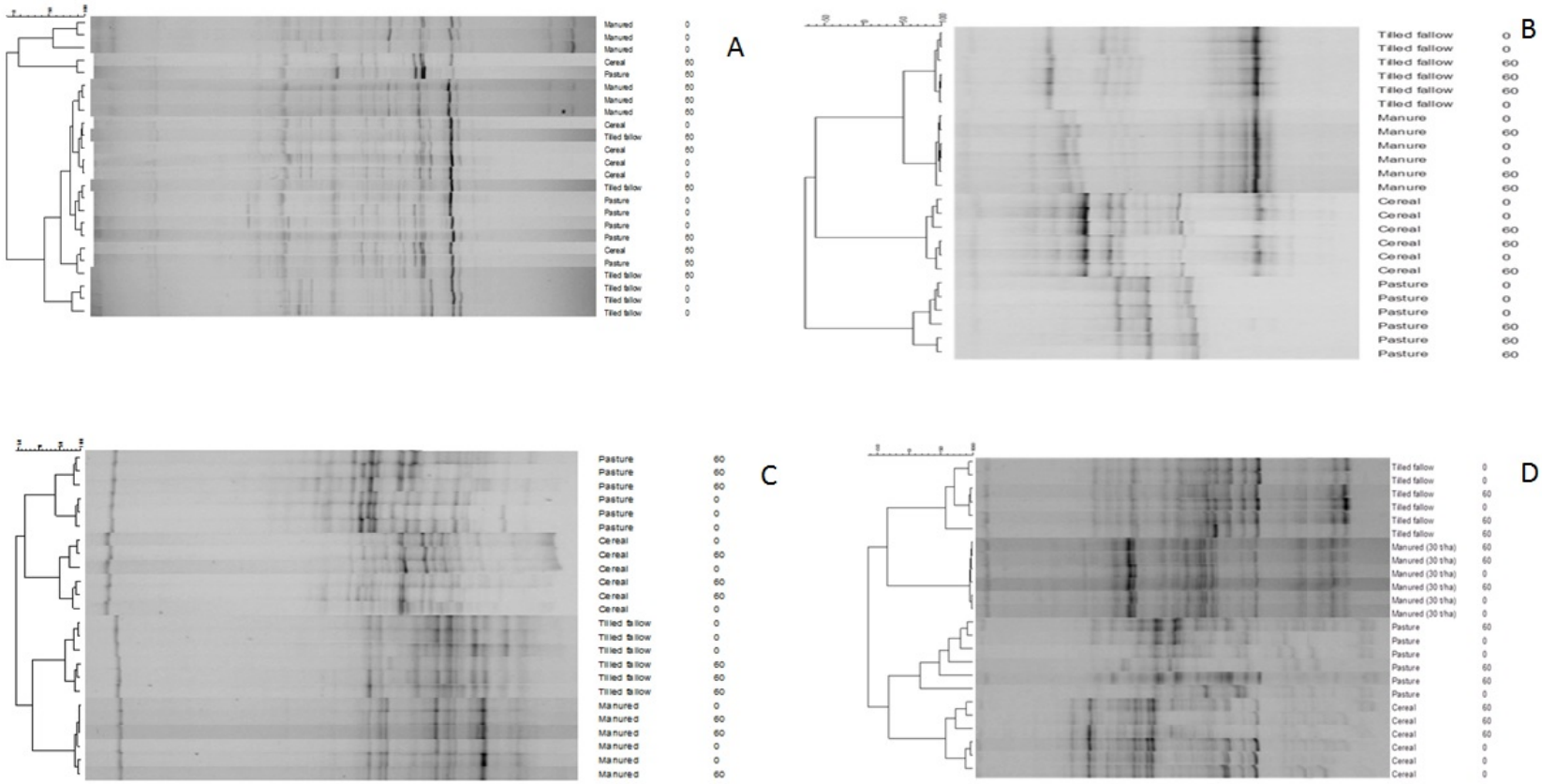


Fig. A.1. Dendrogram analysis using Pearson's correlation coefficient analysis for DGGE banding patterns of A) bacterial *amoA*, B) archaeal *amoA*, C) *nirK* and D) *nirS* gene containing microbial communities.

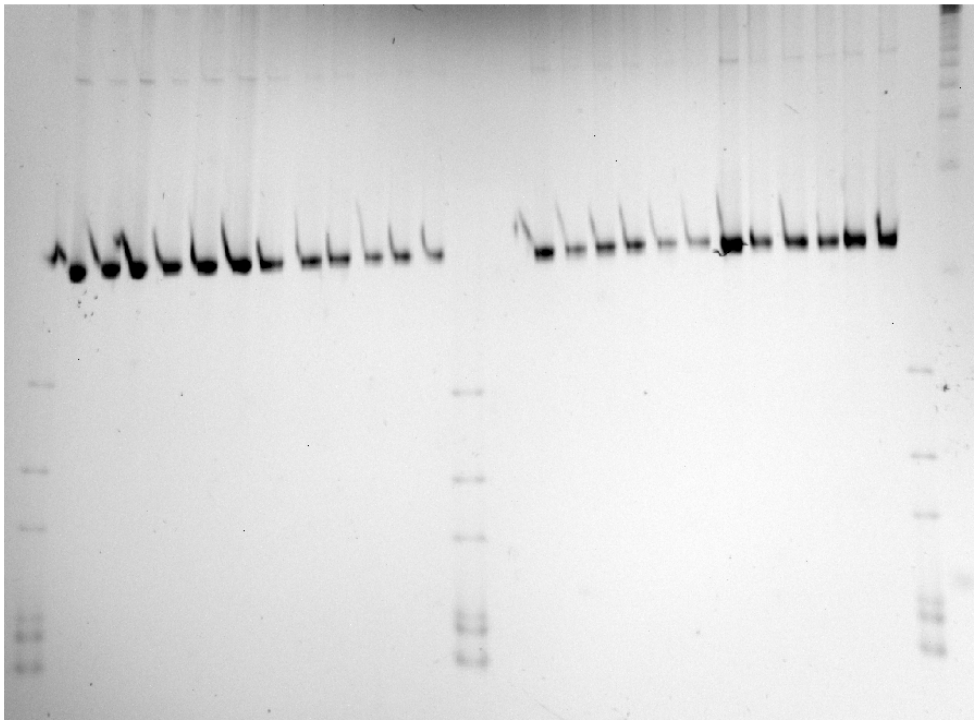
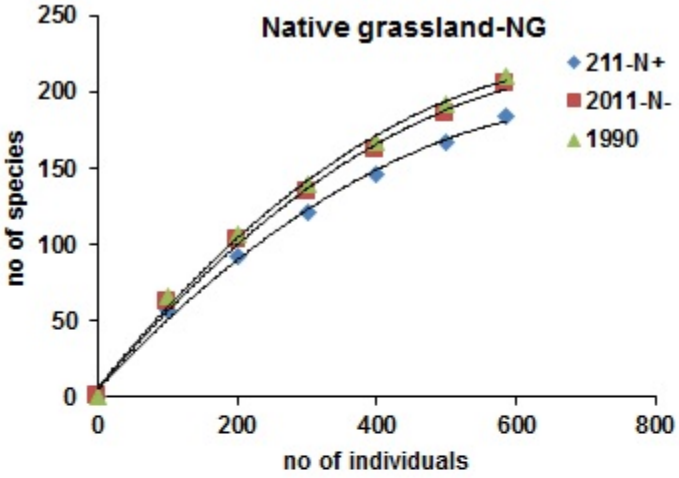
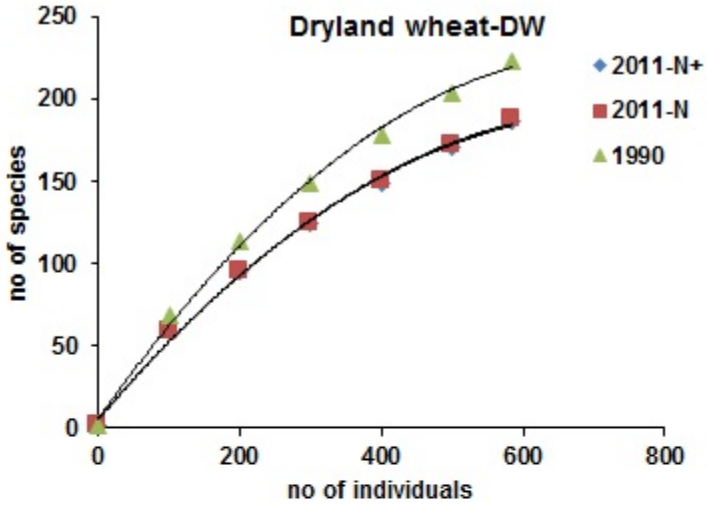
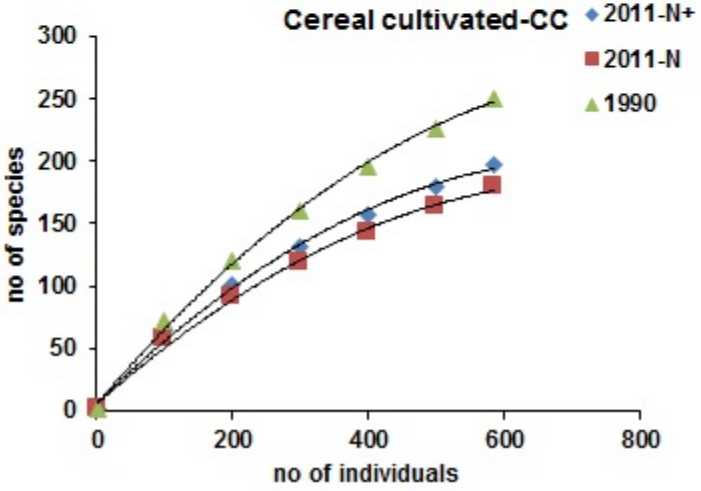
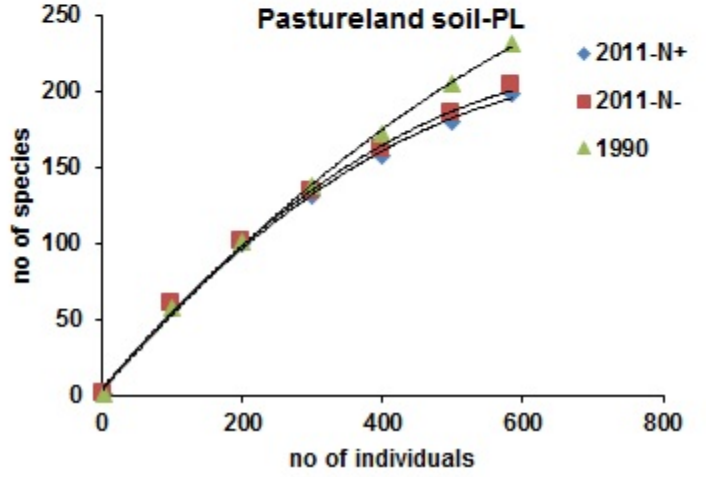


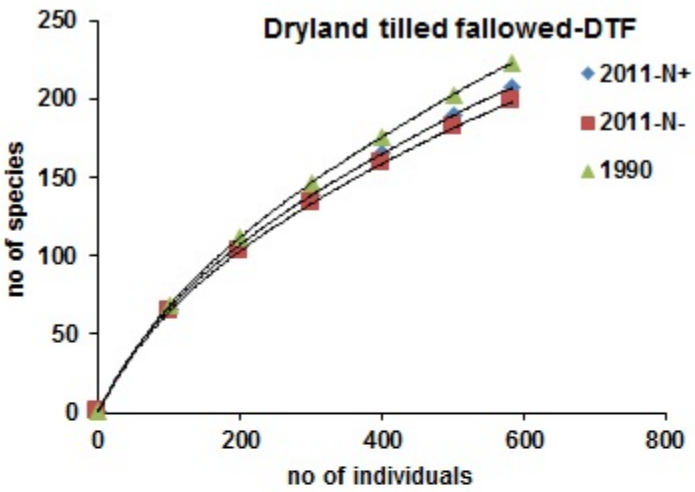
Fig. A.2. *NosZ* community profile measured through DGGE

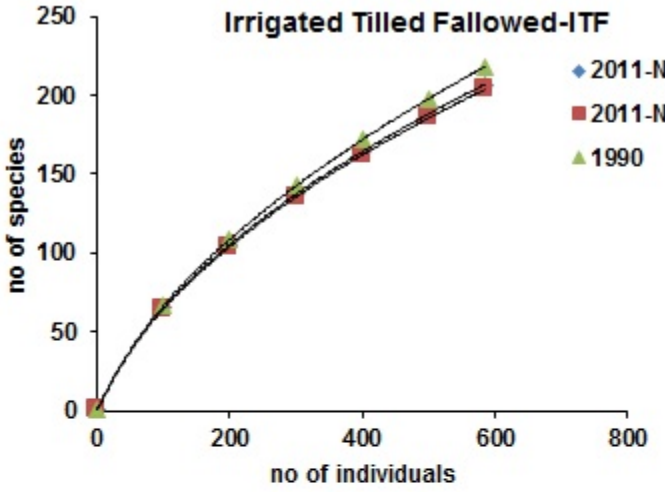

Native grassland-NG
211-N+
2011-N-
1990
no of species
no of individuals
0
50
100
150
200
250
0
200
400
600
800

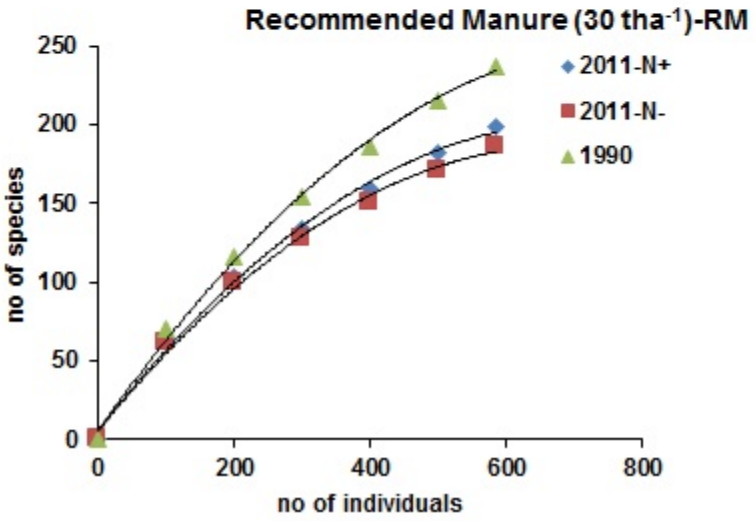

Dryland wheat-DW
2011-N+
2011-N
1990
no of species
no of individuals
0
50
100
150
200
250
0
200
400
600
800

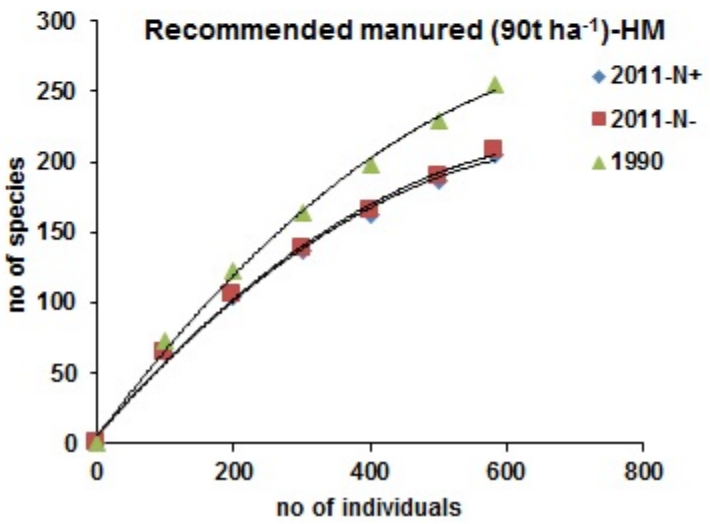

Cereal cultivated-CC
2011-N+
2011-N
1990
no of species
no of individuals
0
50
100
150
200
250
300
0
200
400
600
800


Pastureland soil-PL
2011-N+
2011-N-
1990
no of species
no of individuals
0
50
100
150
200
250
0
200
400
600
800


Dryland tilled fallowed-DTF
2011-N+
2011-N-
1990
no of species
no of individuals
0
50
100
150
200
250
0
200
400
600
800


Irrigated Tilled Fallowed-ITF
2011-N+
2011-N-
1990
no of species
no of individuals
0
50
100
150
200
250
0
200
400
600
800


Recommended Manure (30 tha-1)-RM
2011-N+
2011-N-
1990
no of species
no of individuals
0
50
100
150
200
250
0
200
400
600
800


Recommended manured (90t ha-1)-HM
2011-N+
2011-N-
1990
no of species
no of individuals
0
50
100
150
200
250
300
0
200
400
600
800

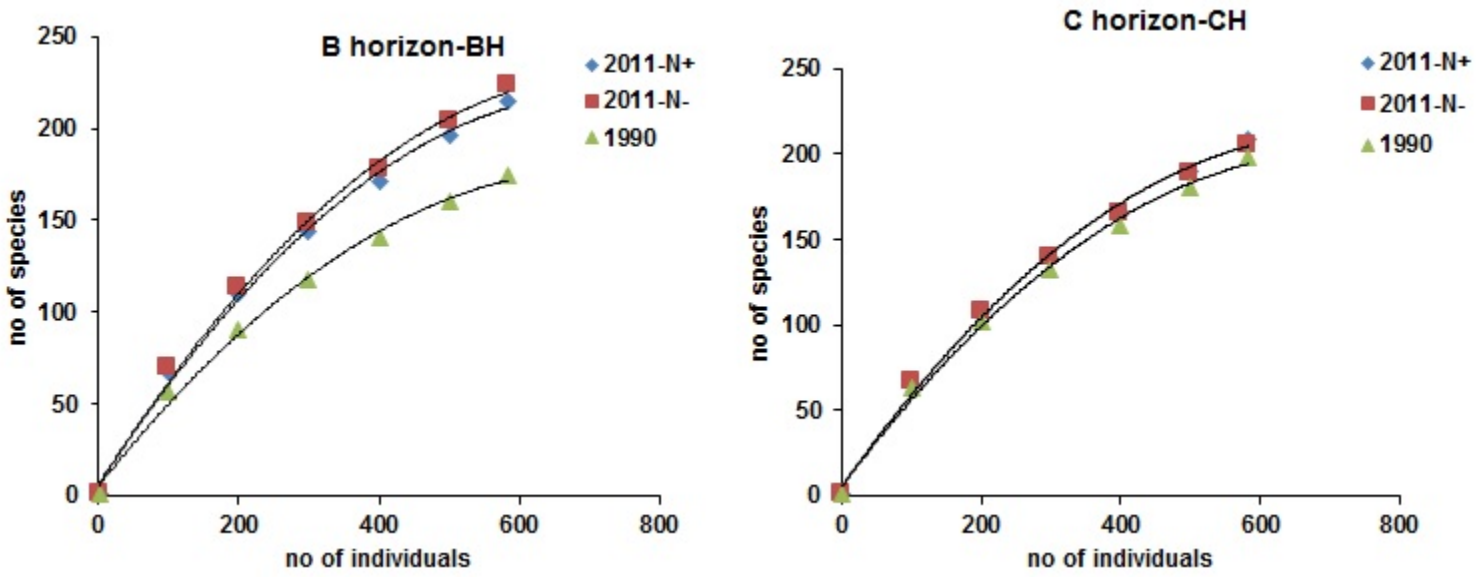


Fig. B.1. Rarefaction curves for 16S *rRNA* gene Illumina Sequencing data of 1990 and 2011 archived soil.

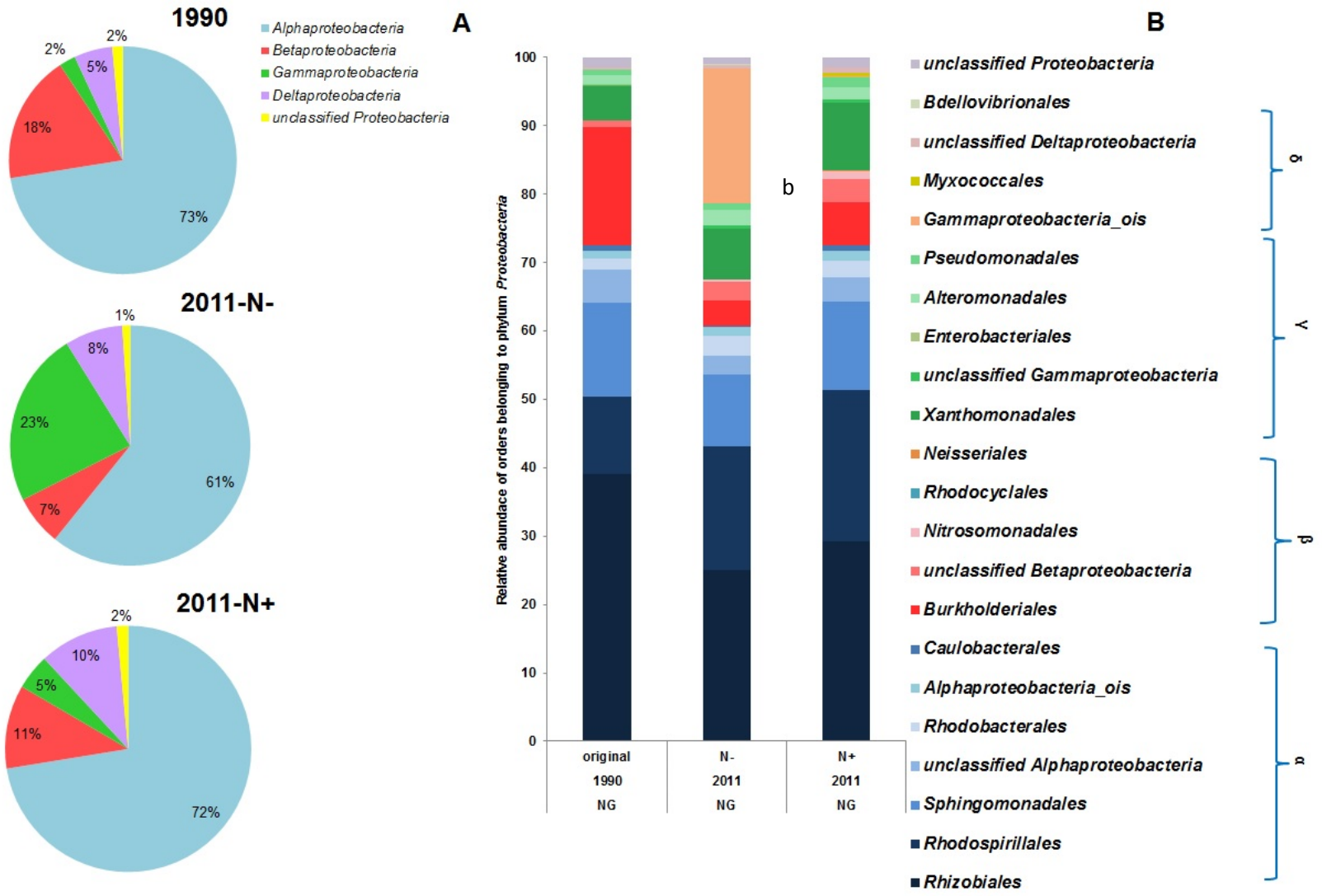


Fig. B.2. Phylogenetic comparison of phylum *Proteobacteria* at class (A) and order (B) levels for archived native grassland (NG) soils. N^- and N^+ are unfertilized and N fertilized (0 and 60 kg N ha^{-1}) for 2011 soils, respectively.

APPENDIX C SOIL ORIGIN REGULATES THE DECOMPOSITION DYNAMICS OF TRANSPLANTED CHERNOZEMS

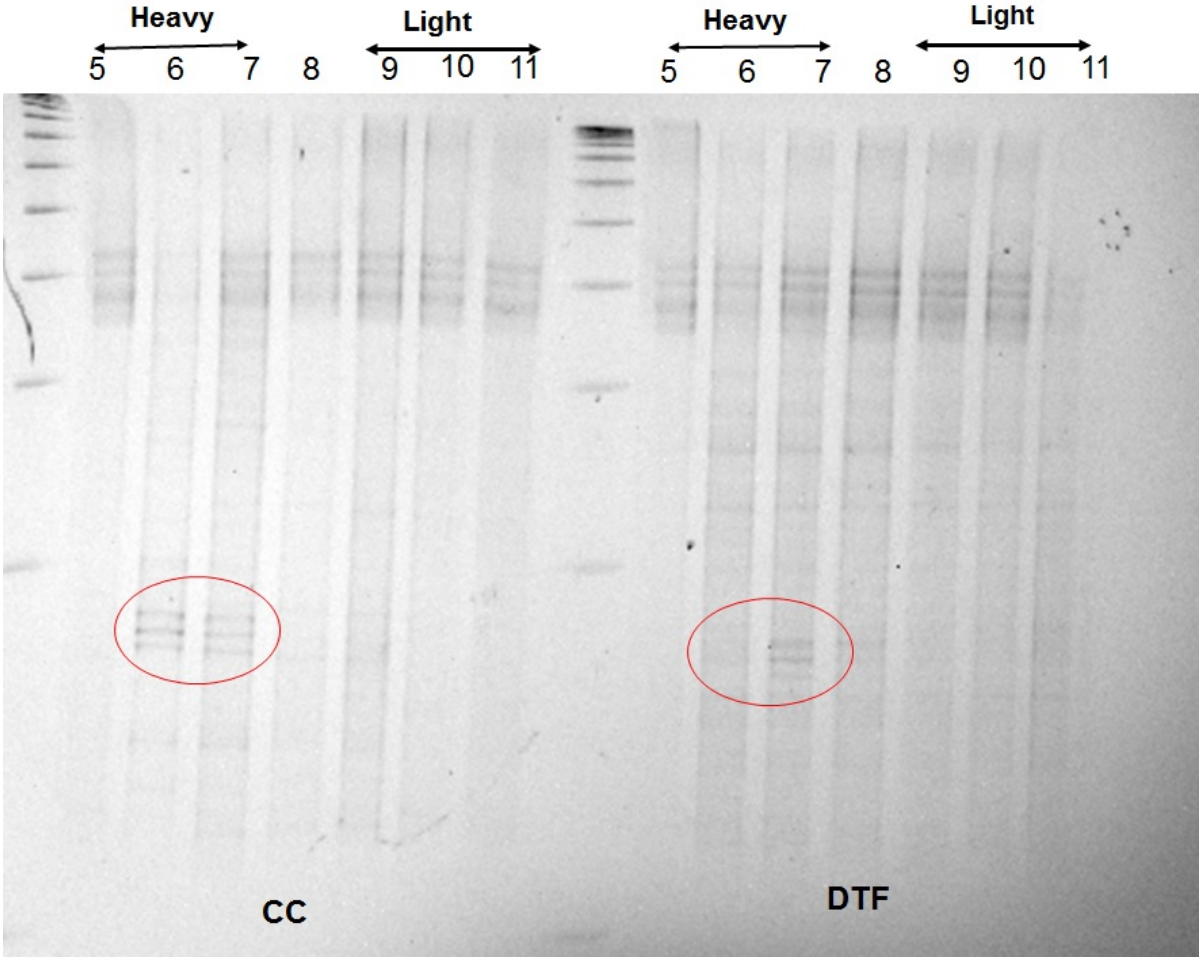


Fig. C.1. 16S *rRNA* gene DGGE banding profiles for heavy and light DNA fractions of ^{13}C cellulose labelled CC (cereal cultivated) and DTF (dryland tilled fallowed) soils.

Table C.1. ANOVA for daily C-CO_2 emission.

Sampling day	Soil	N level	Soil *N	C *P* values	Soil*C	N*C	Soil*N*C
1	0.0002	0.0033	0.82	0.70	0.63	0.24	0.58
3	0.0013	0.001	0.79	0.65	0.21	0.26	0.83
6	0.0001	0.0007	0.26	0.04	0.06	0.25	0.67
9	0.0001	0.0002	0.22	0.01	0.05	0.37	0.65
16	0.0003	0.0002	0.33	0.00	0.18	0.55	0.59
23	0.0016	0.0003	0.36	0.00	0.28	0.63	0.68
30	0.0033	0.0007	0.36	0.00	0.40	0.67	0.84
37	0.0041	0.0008	0.40	0.01	0.45	0.79	0.90
47	0.004	0.0005	0.43	0.01	0.49	0.88	0.91
61	0.0024	0.0002	0.43	0.02	0.56	0.97	0.88
78	0.0009	0.0001	0.51	0.03	0.50	0.93	0.88

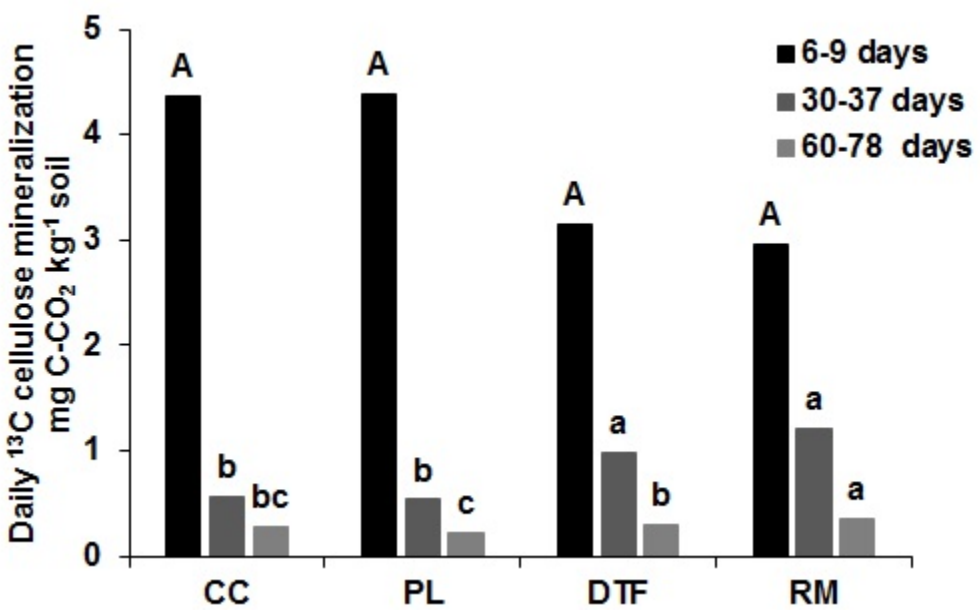


Fig. C.2. Daily ^{13}C cellulose mineralization. CC-cereal cultivated, PL- pastureland soil, DTF-dryland tilled fallow and RM- manured 30 t ha^{-1} soils.

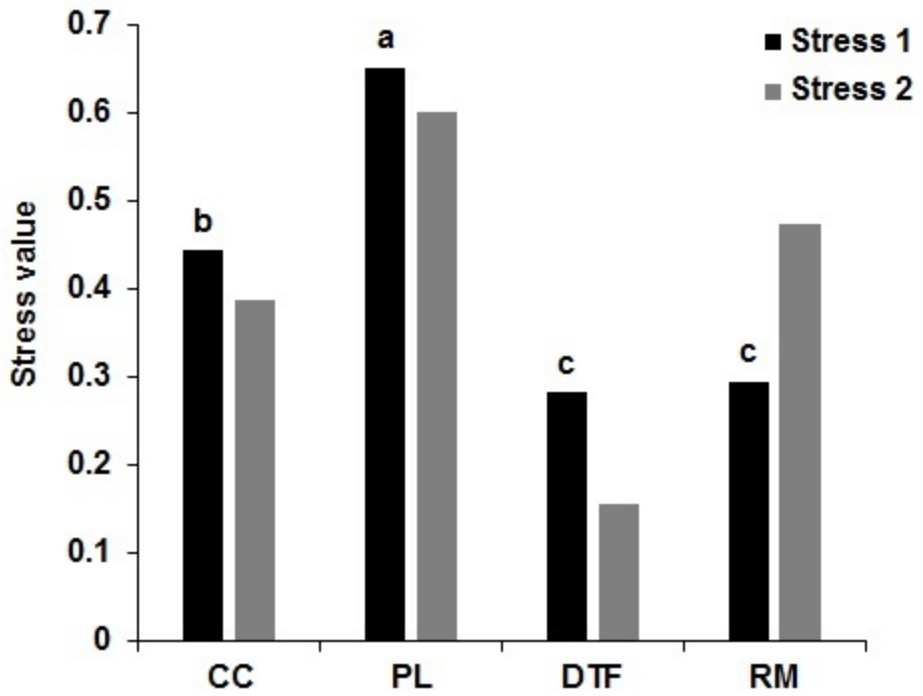


Fig. C.3. Physiological biomarker stress 1 and stress 2 for the total community. CC-cereal cultivated, PL- pastureland soil, DTF- dryland tilled fallow and RM- manured 30 t ha^{-1} soils.

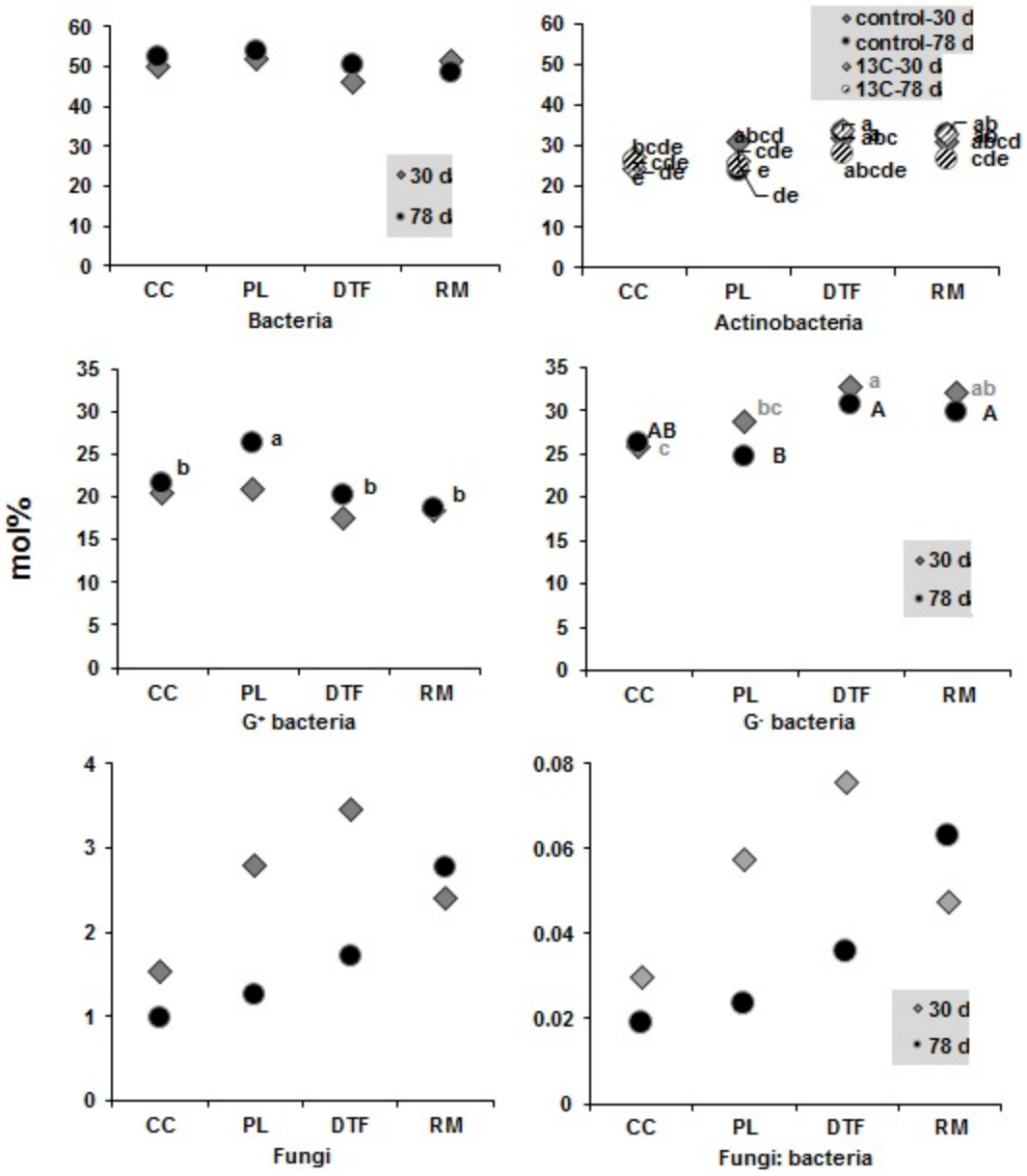


Fig. C.4. Relative abundance of different microbial groups (bacteria, G^+, G^-, *Actinobacteria* and fungi) during 30 and 78 d. CC-cereal cultivated, PL- pastureland soil, DTF- dryland tilled fallow and RM- manured 30 t ha^{-1} soils.

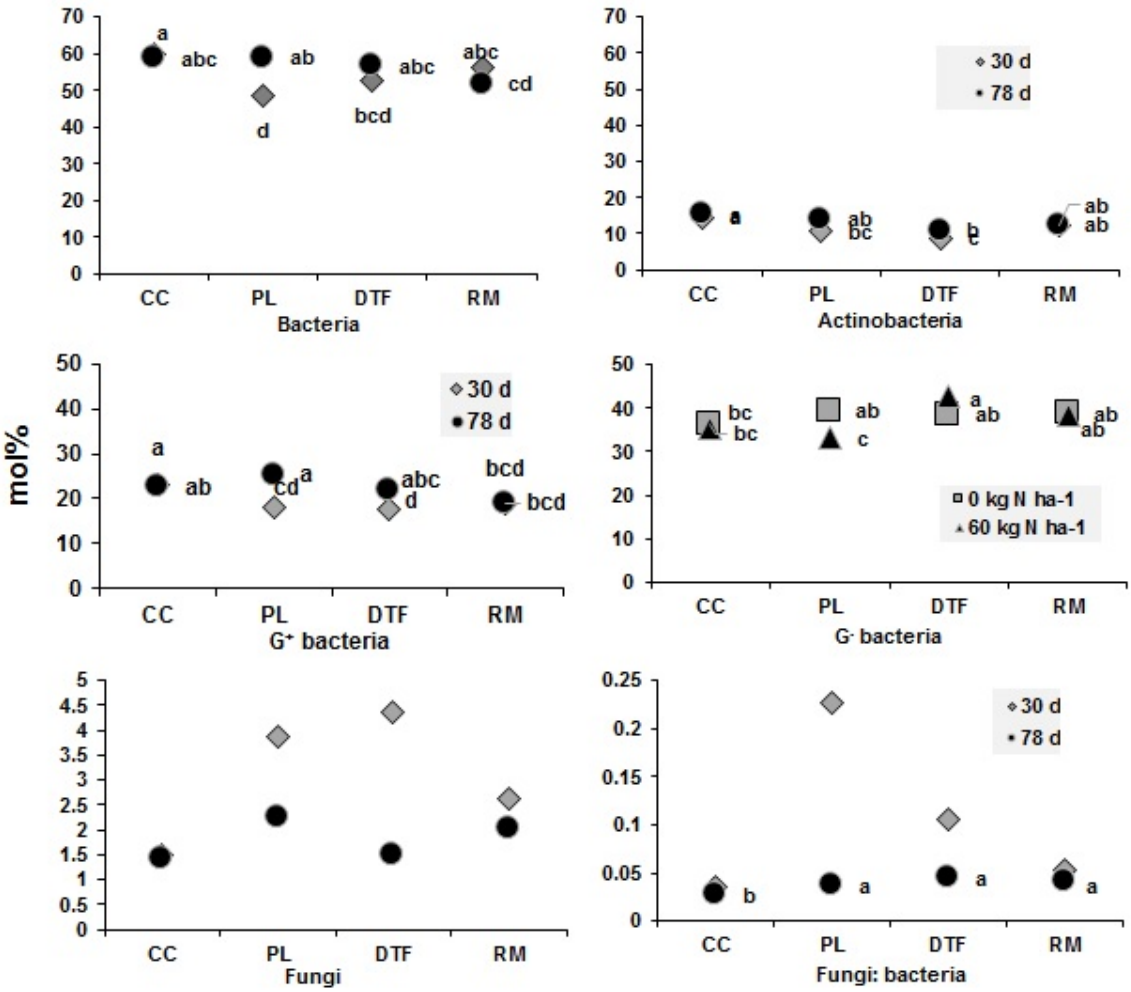


Fig. C.5. Relative abundance of active decomposer microbial groups (bacteria, G^+, G^- *Actinobacteria* and fungi) during 30 and 78 d. CC-cereal cultivated, PL- pastureland soil, DTF-dryland tilled fallow and RM- manured 30 t ha^{-1} soils.

Table C.2. Individual ANOVA for Total biomass, bacteria and fungal abundance measured through PLFA (nmol g^{-1} soil)

Total PLFA (*P* value)				
Effect	CC	PL	DTF	RM
N	0.56	0.26	0.21	0.77
C	0.12	0.01**	0.74	0.60
N*C	0.05	0.65	0.24	0.12
Day	0.99	0.76	0.44	0.80
N*Day	0.48	0.80	0.26	0.37
C*Day	0.04*	0.91	0.36	0.93
N*C*Day	0.32	0.39	0.65	0.23
Bacteria (*P* value)				
	CC	PL	DTF	RM
N	0.38	0.47	0.24	0.65
C	0.15	0.01**	0.78	0.46
N*C	0.11	0.78	0.38	0.51
Day	0.83	0.50	0.25	0.50
N*Day	0.57	0.52	0.35	0.11
C*Day	0.11	0.94	0.84	0.76
N*C*Day	0.33	0.46	0.17	0.44
Fungi (*P* value)				
	CC	PL	DTF	RM
N	0.09	0.34	0.17	0.42
C	0.32	0.47	0.22	0.36
N*C	0.07	0.35	0.38	0.31
Day	0.01*	0.20	0.05	0.51
N*Day	0.43	0.45	0.48	0.33
C*Day	0.65	0.41	0.37	0.36
N*C*Day	0.12	0.34	0.46	0.37

Significant values represent *P*-value *, $P<0.05$; **, $P< 0.001$; ***, $P<0.0001$
N- N fertilizer rate, C- cellulose application rate, CC (cereal cultivated) PL (pastureland), DTF (dryland tilled fallowed) and RM (30 t ha^{-1} manured)

Table C.3. Individual ANOVA for bacteria and fungal abundance measured through PLFA (mol%)

Bacteria (*P* value)				
Effect	CC	PL	DTF	RM
N	0.72	0.65	0.13	0.90
C	0.96	0.68	1.00	0.96
N*C	0.31	0.39	0.66	0.01*
Day	0.22	0.16	0.27	0.23
N*Day	0.78	0.85	0.59	0.41
C*Day	0.95	0.19	0.38	0.49
N*C*Day	0.27	0.28	0.56	0.12
Fungi (*P* value)				
	CC	PL	DTF	RM
N	0.36	0.56	0.53	0.56
C	0.09	0.37	0.28	0.37
N*C	0.15	0.40	0.56	0.40
Day	0.04*	0.73	0.04*	0.73
N*Day	0.98	0.43	0.52	0.43
C*Day	0.68	0.33	0.34	0.33
N*C*Day	0.16	0.51	0.62	0.51

Significant values represent *P*-value *, $P<0.05$; **, $P< 0.001$; ***, $P<0.0001$
N- N fertilizer rate, C- cellulose application rate, CC (cereal cultivated) PL (pastureland), DTF (dryland tilled fallowed) and RM (30 t ha^{-1} manured)

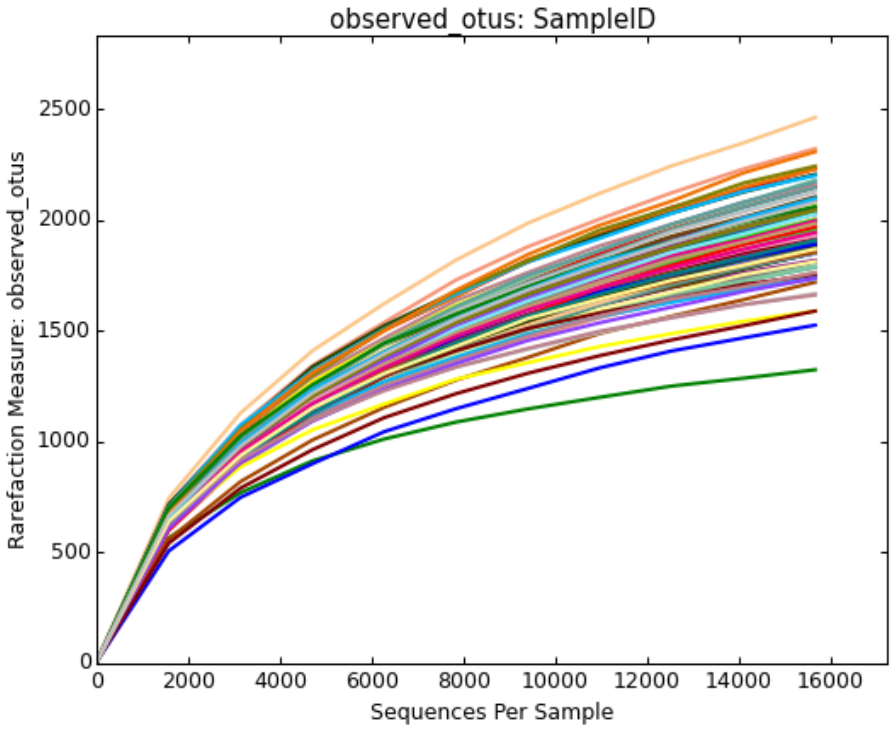


Fig. C.6. Rarefaction curves for total community of 16S *rRNA* gene Illumina sequencing data

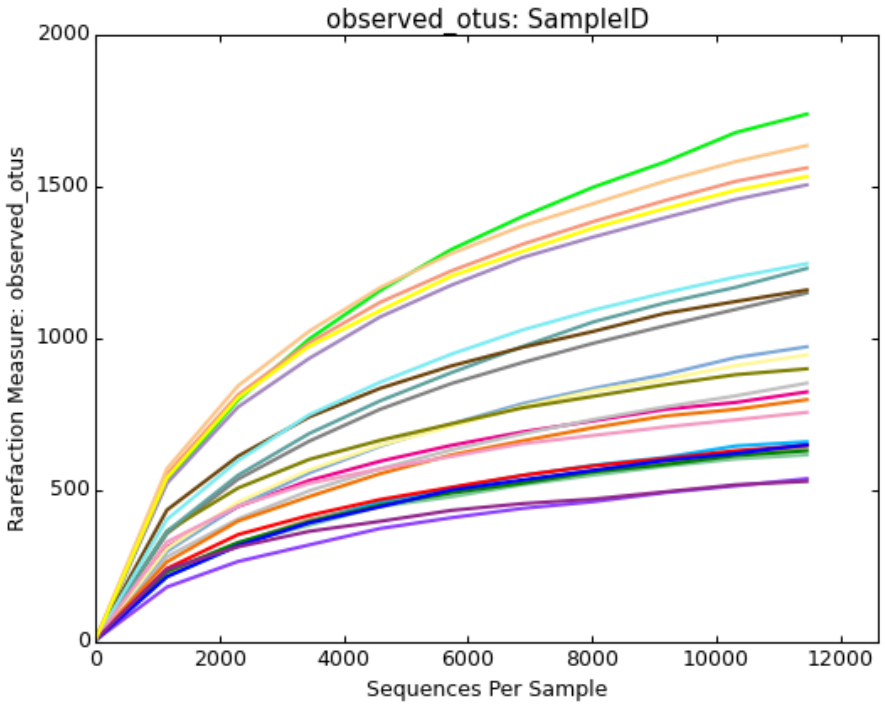


Fig. C.7. Rarefaction curves for decomposer community of 16S *rRNA* gene Illumina sequencing data.

APPENDIX D PERMISSION TO REPRODUCE

The photo illustrated in Figures 3.1 and 4.1as well as the data presented in Figures 3.2 and 3.3 and Tables 3.10 and 3.11 which was reproduced from the 1999 Alberta Agricultural Research Institute Final Technical Report “the resilience of soil quality under wheat production” Project No 970801 by Janzen and Ellert, were used with the permission of Dr. H.H. Janzen.

Dr. H.H. Janzen
Research Scientist
5403 - 1 Avenue South
PO Box 3000
Lethbridge, Alberta T1J 4B1
Telephone: 403-317-2223
Fax: 403-382-3156
Email: henry.janzen@agr.gc.ca