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Microbial and Organic Matter Characteristics of Restored Riparian Soils
by

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ABSTRACT

In the Prairie Pothole Region (PPR) of Canada wetlands once utilized for agricultural purposes are being restored to their pre-existing hydrological state. The overall objective of this research was to assess differences in microbial community structure and soil organic matter between native (reference) and restored riparian soils of varying times since restoration. Samples (0-6 cm) were taken from a total of 43 reference and restored wetlands. The soil microbial community was described using phospholipid fatty acid analysis and soil organic matter was characterized by isolating carbon pools using acid hydrolysis and physical separation techniques. Differences between younger restored (1-3 yrs, 4-6 yrs) and reference soils were observed in terms of microbial biomass and composition, and carbon concentration and distribution among pools. Although the carbon distribution in the older restored (7-11 yrs) and reference soils differed, similarities in other measured variables indicated a recovery within this time period.

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LIST OF SYMBOLS AND ABBREVIATIONS

^{13}C	Heavy carbon isotope with 7 neutrons
^{13}C NMR	^{13}C Nuclear Magnetic Resonance
ALK	Alkyl carbon
AROM	Aromatic carbon
AUR	Acid-unhydrolysable residue
C	Carbon
CARB	Carbonyl carbon
C/N	Carbon to nitrogen ratio
DNC	Dense nesting cover
DUC	Ducks Unlimited Canada
EGDD	Effective growing degree days
GC-FID	Gas chromatograph - Flame ionisation detector
ISA	Indicator species analysis
LF	Low-density fraction
MRPP	Multi-Response Permutation Procedure
MRT	Multivariate regression tree
NAWMP	North American Waterfowl Management Plan
NMS	Non-metric multidimensional scaling
O-ALK	O-alkyl carbon
PHEN	Phenolic carbon
PHJV	Prairie Habitat Joint Venture
PLFA	Phospholipid fatty-acid analysis
P-PE	Precipitation – potential evapotranspiration
PPR	Prairie pothole region
Sand	Sand associated organic matter
Silt + Clay	Silt + Clay associated organic matter
SOM	Soil organic matter

I. INTRODUCTION

1.1 Prairie Pothole Wetlands

The Prairie Pothole Region (PPR), formed by glacial action more than 10,000 years ago (Mitsch and Gosselink, 1993), extends throughout the Canadian provinces of Alberta, Saskatchewan and Manitoba southward to the United States, including North and South Dakota, Minnesota, and Iowa (Richardson et al., 1994). Wetlands in the PPR cover 11.4% of this landscape (Patterson, 1999). The potholes are kettle type depressions formed on a surface which has not yet developed an integrated surface drainage system. Due to the large quantity of shallow lakes and marshes, the fertile soils and warm summers, the wetlands within the PPR are considered one of the most important wetland areas in the world (Mitsch and Gosselink, 1993). The pre-agricultural prairie landscape benefited from these wetlands as they provided habitat for numerous plants and animals, local water storage capacity, and improved water quality through removal of sediments and nutrients (Galatowitsch and van der Valk, 1994).

In pothole wetlands, concentric zones of vegetation occur because plants with similar water depth or flooding tolerance grow together (Galatowitsch and van der Valk, 1994; Stewart and Kantrud, 1971). A classification of prairie potholes was developed by Stewart and Kantrud (1971) based on diagnostic vegetation found in the central or deepest zone of wetland basins, reflecting differences in water permanence. The zones, or wetlands classes, in order of increasing permanence are ephemeral pond (Class I, low prairie), temporary pond (Class II, wet meadow), seasonal pond (Class III, shallow emergent marsh), semipermanent pond (Class IV, deep emergent marsh), and permanent pond (Class V, open water). This study focused on those wetlands classified as either Class III or Class IV, where the ponding duration under normal conditions is 1-3 months to 5 months respectively.

1.2. Agriculture and wetland restoration

Eighty-percent of the agricultural land in Canada is contained within the PPR along with more than 4.5 million hectares (11 million acres) of wetlands (DUC, 2003). This land use pressure has led to substantial drainage of wetlands and their associated riparian areas for agricultural benefit, with estimates of 71-75% of wetlands being converted throughout the Canadian PPR (Anonymous, 1986; Patterson, 1999). Of those wetlands remaining, 70-90% are impacted by cultivation, brushing or burning (Neraasen and Nelson, 1999).

The loss of wetlands was first recognized on the basis of habitat loss for nesting waterfowl populations which motivated nations to cooperate and protect wetlands (Patterson, 1999). In 1986 the North American Waterfowl Management Plan (NAWMP) agreement was established in Canada and the United States, with Mexico joining in 1994 (Gray et al., 1999). The purpose of this plan was to achieve continental waterfowl population goals. The largest program under the NAWMP, the Prairie Habitat Joint Venture (PHJV) project, was then established to specifically address the habitat loss issues in the prairie region of Canada. This project placed an emphasis on habitat conservation efforts in degraded landscapes. In 1989 Ducks Unlimited Canada (DUC) became the principal habitat delivery organization for the PHJV and has been responsible for the majority of the restoration efforts to date. Between 1989 and 1997 DUC restored over 900 wetlands within the PPR of Alberta, Saskatchewan and Manitoba, which accounts for over 1700 ha of restored wetland area (Gray et al., 1999).

Restoration designs are dependent on the modifications necessary to create the desired hydrology in the wetland. These designs include excavating the basin, building dykes, removing tiles or plugging ditches (Galatowitsch and van der Valk, 1994). All of the wetlands included in this study were restored by installing a ditch plug. In this study restoration is defined as the reestablishment of a wetland through cessation of artificial drainage. An overview of the locations of sampling sites in the PPR and the sites sampling design can be found in Figures 1.1 and 1.2.

There has been much debate in regards to how to measure the success of wetland restoration (Ehrenfeld, 2000; Galatowitsch and van der Valk, 1996; Malakoff, 1998; Mitsch and Wilson, 1996). In PPR wetlands in Canada the primary goal of restoration is to provide habitat for breeding populations of waterfowl (Gray et al., 1999). Therefore, wetland restoration success is measured through such parameters as plant lists, percent vegetative cover and animal observations and this is done over relatively short time periods, generally from 3-5 years (Mitsch and Wilson, 1996). These measurements are used to judge whether or not the restored wetlands are functionally equivalent to the natural ecosystem in terms of providing wildlife habitat, but disregards the numerous other important functions of wetlands such as flood attenuation, groundwater recharge, and processing of nutrients.

Historically, the majority of studies conducted on restored and natural PPR wetlands has occurred in the United States and has focused on avian and plant communities (Knutsen and Euliss Jr., 2001). More recent studies have focused on the complexities of the water cycles within these systems, the array of microorganisms found in PPR wetlands (Keith-Roach et al., 2002) and the ability they have to both emit and sequester greenhouse gases (Gleason et al., 2009; Patterson, 1999; Wang et al., 1996). However, comparative studies conducted on soil biological and chemical characteristics of PPR wetlands are still scarce (Knutsen and Euliss Jr., 2001).

1.3. Riparian Soils

This study focused on soils occurring in what ecologists may refer to as the wet meadow zone (Stewart and Kantrud, 1971) and what soil scientists may refer to as the riparian area (Pennock et al., 2010) of the Prairie Pothole wetlands. The dominant soils in the PPR of Canada include Black, Dark Brown and Brown Chernozems (Udic, Typic and Aridic Borolls) which are differentiated from other soil great groups based on the primary pedogenic process of organic matter accumulation. In this study gleyed variants of the Chernozemic soils occurred in the riparian zone as evidenced by the appearance of drab gray colors and mottling

which indicate the influence of periodic or sustained reducing conditions during their genesis (Soil Classification Working Group, 1998).

Riparian zones are characterized by hydrophytic vegetation and the presence of hydric soils, which are formed when conditions of flooding, saturation or ponding occur long enough so that anaerobic conditions develop in the upper portions of the soil (Mausbach and Parker, 2001). Riparian soils occur in the transition zones between uplands and either standing water (lentic) systems, such as marshes and bogs, or running water (lotic) systems, such as rivers and streams (Lewis et al., 2003). Riparian areas and wetlands are considered more dynamic portions of the landscape as compared to uplands (Gregory et al., 1991; Lewis et al., 2003). These areas can change dramatically over short time periods due to flooding, deposition of sediment, particularly on streambanks and floodplains, and accumulation of organic materials in areas such as wet meadows, swamps and bogs. These areas also undergo large seasonal fluctuations and sharp gradients in environmental factors, such as temperature and soil moisture. The specific functions that riparian-wetland soils provide are to act as a catchment area to infiltrate water for gradual release, recharge aquifers, filter pollutants, and to initiate carbon sequestration, store nutrients, and act as a medium for nutrient cycling between plants and microorganisms (Lewis et al., 2003).

Soil moisture level is acknowledged as the primary factor controlling carbon and nitrogen fluxes in riparian soils (Freeman et al., 1997). When the soil undergoes periods of waterlogging the organic matter decomposition rate becomes less than the rate of production, resulting in the accumulation of carbon. Hence, the rate of carbon accumulation is controlled by low decomposition rates, rather than high rates of primary productivity (Aerts and Toet, 1997). With the reduction of waterlogging the soils in riparian areas may begin to exhibit characteristics similar to upland soils in that methane, nitrous oxide, and dissolved organic carbon release are suppressed while carbon dioxide fluxes increase (Freeman et al., 1997). Similarly, the hydrology of a wetland, and therefore the periodic waterlogging of the riparian soils is a dominant factor which also controls microbial processes (Balasooriya et al., 2008; Gutknecht et al., 2006;

Mentzer et al., 2006). Microbial community structure changes with resulting aerobic or anaerobic conditions. More specifically gram positive bacteria are associated with anaerobic conditions (Sundh et al., 1997) while gram negative tend to dominate in more aerated conditions (Ponder and Tadros, 2002).

The majority of studies on riparian zones has occurred in agriculture and forested lotic environments (Groffman et al., 2003; Lowrance et al., 1997). The focus of these studies has been on water quality and more specifically on the ability of riparian soils to function as sinks for groundwater pollutants, in particular (NO_3^-). Studies of PPR soils in Canada have focused on the impact of agriculture on greenhouse gas emissions along topographic gradients (Bedard-Haughn et al., 2006; Izaurralde et al., 2004; Pennock, 2003), but few have specifically addressed the riparian component. To date no study has examined the soil microbial community and processes of decomposition in riparian soils of Prairie Pothole wetlands in Canada.

1.4. Carbon and Microbial Communities

Historically, studies assessing wetland restoration success have been based on broad vegetation and wildlife parameters (Knutsen and Euliss Jr., 2001). Recognition that less apparent wetland functions such as flood attenuation and carbon storage need to be included in these assessments, combined with the development of more advanced measurement techniques that allow the study of *in situ* conditions, has resulted in a shift in focus to specific characteristics of the soil environment and to the changes which occur there due to restoration efforts (Bossio et al., 2006; D'Angelo et al., 2005; Euliss Jr. et al., 2006; Gleason et al., 2009) Determination of soil quality, measured through the utilization of techniques such as soil fractionation and examination of soil carbon composition through nuclear magnetic resonance (NMR), and measurements of the soil microbial community composition using techniques such as phospholipid fatty acid analysis (PLFA) have altered the way in which we now can determine the effects of restoration efforts.

Phospholipids, essential membrane components of living cells, are not found in storage products and degrade rapidly following cell death, thereby making their presence representative of the active microbial community (Bardgett et al., 1999; Zelles, 1999). Furthermore, the cell membranes of different organisms are made up of different types of fatty acids, the major constituents of the membranes of living cells, allowing for the identification of specific subsets of the microbial community, such as fungi, actinomycetes and gram (-) and gram (+) bacteria (Bardgett et al., 1999; Leckie, 2005). Therefore, the biochemical method of phospholipid fatty acid analysis allows us to examine the soil microbial community by measuring this active biomass and provides us with valuable information regarding the structure of the soil community (Bossio et al., 1998; Vestal and White, 1989).

The soil microbial community biomass and composition reflect the physical and chemical limitations of the soil ecosystem and respond to disturbance, thereby making the community a good indicator of soil quality (Peacock et al., 2001). PLFA analysis has been utilized to examine the soil microbial community characteristics in agricultural soils (Zelles et al., 1992) and mineral soils of forests (Frostegard et al., 1993; Hackl et al., 2005). It has also been used to determine the impacts of restoration on forested wetlands (D'Angelo et al., 2005), prairie soils impacted by agricultural activity (Bossio et al., 1998; McKinley et al., 2005), and permanently flooded wetlands (Bossio et al., 2006).

Soil organic matter imparts numerous beneficial biological, chemical and physical properties to the soil. It provides energy to support a diverse microbial community, increases the cation exchange capacity of the soil, and improves soil structure and aggregation (Wolf and Wagner, 2005). Numerous soil organic matter models and fractionation schemes have been developed to attempt to capture the heterogeneity of soil organic matter by distinguishing among several pools which vary in terms of decomposition rates or factors controlling decomposition (Christensen, 1992; Six et al., 2002). Decomposition is a sequential process in which complex organic substances are continuously degraded into simpler substances. Mechanisms responsible for the retention of

these substances in soils include chemical recalcitrance, stabilization by adsorption on mineral surfaces and physical barriers that protect substrates from decomposers (Christensen, 2001; Yadov and Malanson, 2007). Recently, Six et al. (2002) proposed a fractionation scheme which isolates three soil organic matter pools based on these retention mechanisms: the unprotected carbon pool, which contains root exudates and rapidly decomposed components of plant litter; the biochemically protected pool, which consists of non-hydrolyzable carbon and is considered the passive pool; and the physically protected pool, or slow pool, which is divided into microaggregate associated soil carbon and silt and clay associated soil carbon. For the purposes of this study a combination of density and particle-size separation techniques was used to isolate four fractions (Figure 1.3) to mimic the ones described by Six et al. (2002). The light fraction (2mm-53μm, <1 g cm^{-3}) is enriched in carbon and nitrogen and serves as a highly decomposable substrate for microorganisms (Gregorich and Ellert, 1993). It is highly sensitive to management practices and highly influenced by the cultivation history of the soil and may provide early indications of the consequences of different management practices (Six et al., 2002). The sand-sized fraction (2mm-53μm) contains unprotected carbon and is also readily affected by management practices. The clay and silt sized fraction (<53μm) is the physically protected carbon and is considered the slow pool. Finally, the biochemically protected carbon (<53μm), or unhydrolyzable fraction comprised of lignin and cellulose component, requires acid digestion or hydrolysis in order to extract components and is considered the passive pool (Six et al., 2002). Solid state ^{13}C nuclear magnetic resonance spectroscopy was used to characterize the chemical structure of the organic matter found in the light fraction (Figure 1.4). This process allows for quantification of the various types of carbon in the soil organic matter by identifying different chemical structures based on chemical shift values of the magnetic field of the spectrometer (Baldock et al., 1997) and has become a vital tool for examining decomposition processes (Baldock et al., 1997; Golchin et al., 1995; Preston, 1996). Variations in ^{13}C abundance were also examined which may offer insight into the underlying organic matter processes in terrestrial

ecosystems (Hannam et al., 2005; Quideau et al., 2003) by providing information about the extent of the microbial degradation in the soil organic matter.

A number of studies have been conducted focusing on the changes to soil organic matter after changes in management regimes, particularly in an agricultural setting (Golchin et al., 1994; Lopes de Gerenyu et al., 2008; McKinley, 2001; McKinley et al., 2005; Schnitzer et al., 2006). However, few have been conducted to study the effects of wetland restoration on soil organic matter (Aldous et al., 2005; D'Angelo et al., 2005; Galatowitsch and van der Valk, 1996).

1.5. Objectives of the Thesis

While this study is not intended to determine the "success" of restoration the main objective of this research is to provide information regarding the return of key soil characteristics, specifically linked to organic matter decomposition and microbial activity, in restored wetland riparian soils with varying years since restoration (3-11 years). We compared these soils to nearby undisturbed wetland riparian soils, which we refer to as reference wetlands, which have never been utilized for agricultural purposes and are intended to approximate a target ecosystem.

Following this first introductory chapter, this thesis is comprised of a total of three other chapters. The second chapter will address the changes in the soil carbon quantity and quality with time since restoration. Chapter three specifically looks at the changes in the soil microbial community, measured by phospholipid fatty-acid analysis (PLFA) with time since restoration. Finally, the fourth chapter will provide a synthesis of the findings presented here as well as recommendations for future research.

CHAPTER I: TABLES AND FIGURES

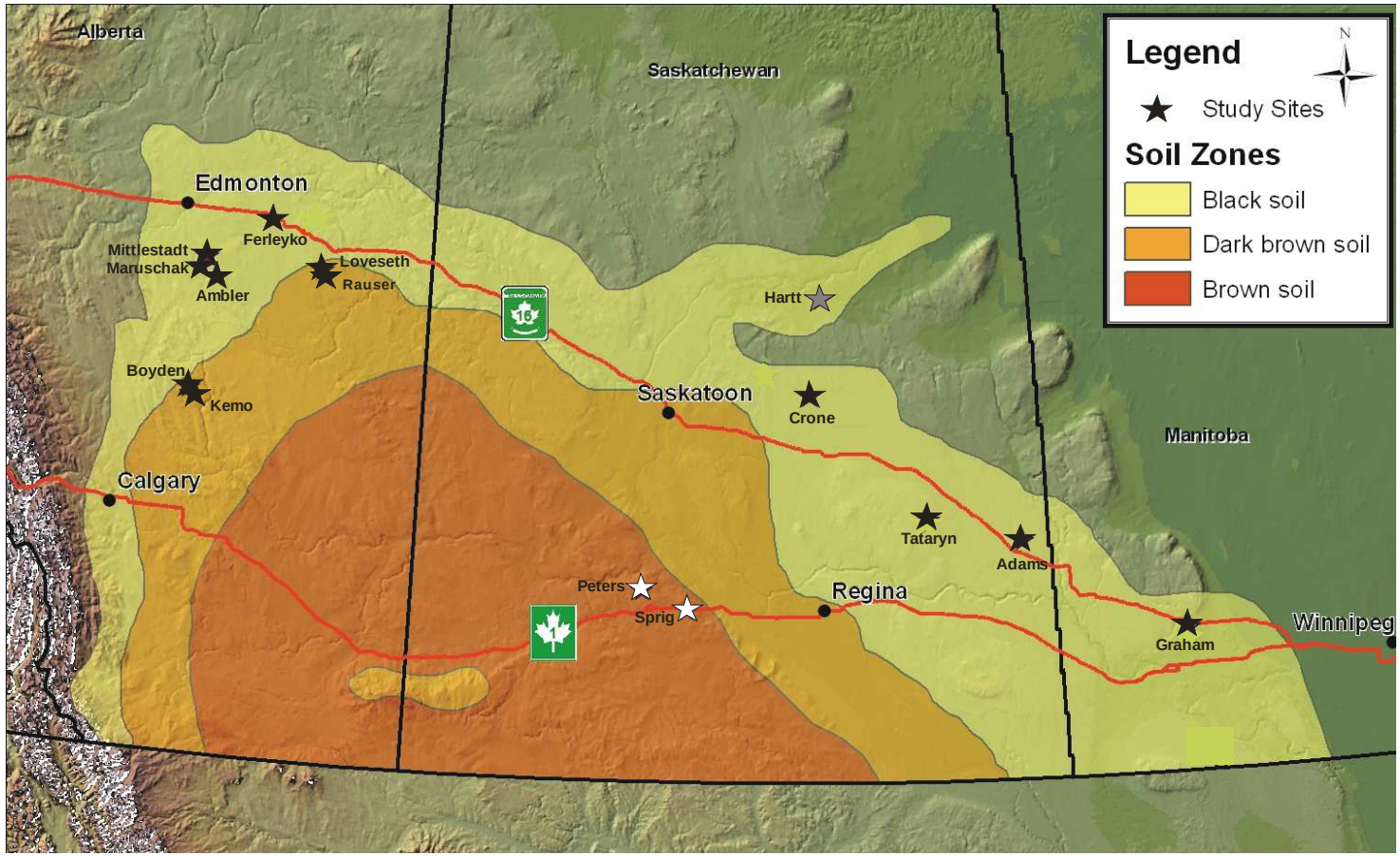


Figure 1. 1. Soil zone for each study site was determined by referencing the Canada Soil Inventory Maps (Agriculture Canada, 1988abc) and therefore differs slightly from the designations depicted in this Zonal soil map (Ecological Stratification Working Group, 1995). A black star represents the Black soil zone, a gray star the Dark Gray soil zone, and a white star the Brown soil zone.

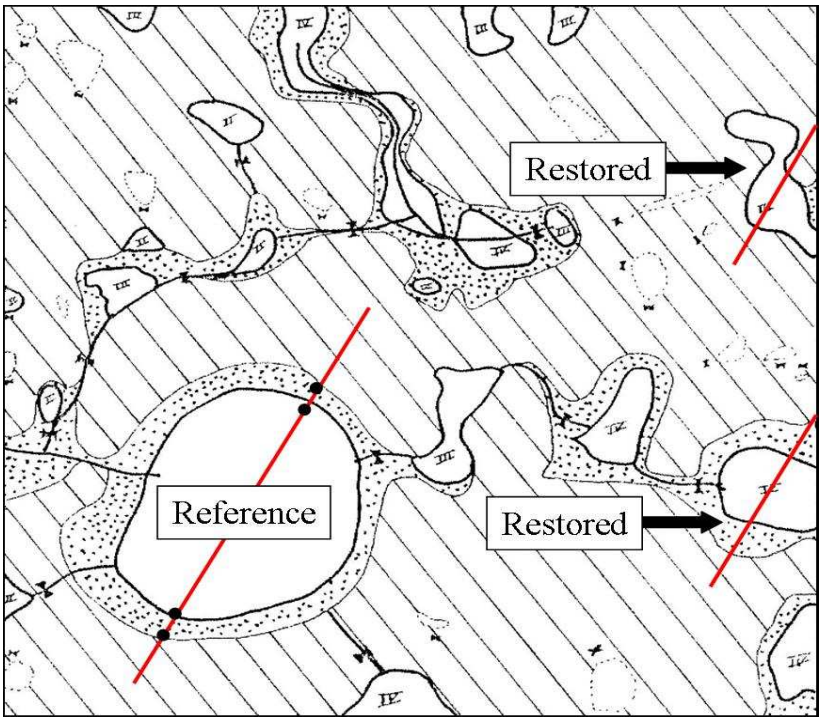


Figure 1. 2. Detailed map of a site, showing the reference wetland and two restored wetlands of the same age, the transects established across each wetland and the possible sampling points (as shown at the reference wetland). All wetlands at a site occur on one quarter section of land (160 acres or 2.59 km2), with the average size of the basins generally < 2 ha.

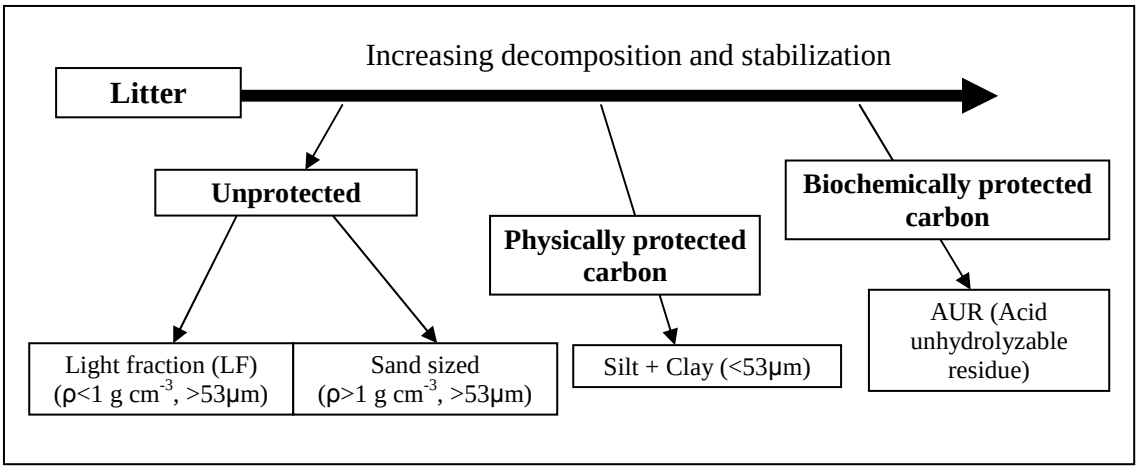
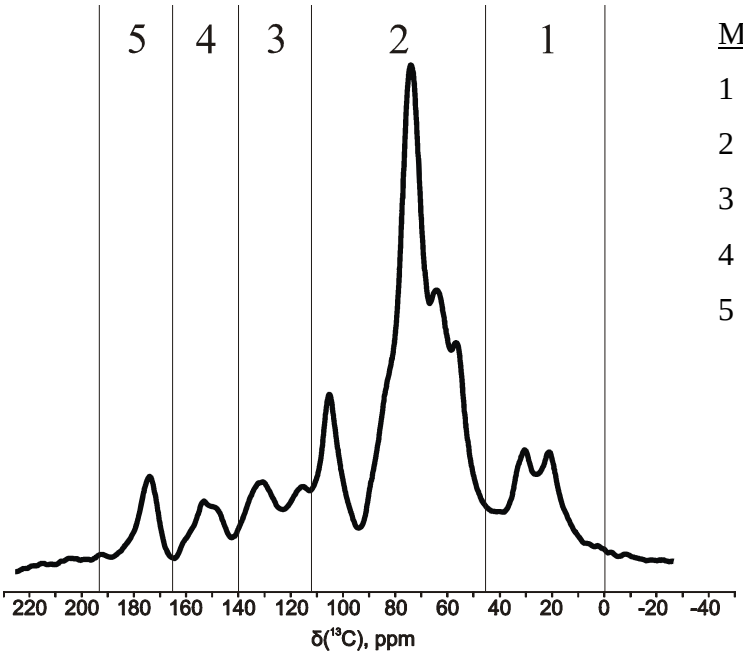


Figure 1. 3. Diagram showing the decomposition and stabilization of organic matter and its relationship to the fractionation protocol.



Macromolecular yields:

1 = Aliphatics (ALK)

2 = Carbohydrates (O-ALK)

3 = Aromatics (AROM)

4 = Phenolics (PHEN)

5 = Carbonyls (CARB)

Figure 1. 4. Example of RAMP-CP 13C NMR spectral divisions as applied to restored and reference wetland riparian soil material.

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II. CARBON CHARACTERISTICS IN RESTORED RIPARIAN SOILS OF THE CANADIAN PRAIRIE POTHOLE REGION

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1. Introduction

The North American prairie pothole region (PPR) is a major non-forested landform which covers approximately 900,000 km^2 (Euliss Jr. et al., 2006). More than 67 percent of the PPR and more than 42 percent of the wetland area in the PPR occurs in Canada. Historically, land use pressure has resulted in the drainage of these wetlands, with estimates of 71-75% of wetlands being converted to agriculture throughout the Canadian PPR (Anonymous, 1986; Patterson, 1999). The impact of this land conversion was first recognized due to the loss of habitat for nesting waterfowl populations and subsequent action was taken to achieve continental waterfowl population goals, primarily through the recovery of habitat (DUC, 2003). The impact of wetland drainage and cultivation on soil carbon (C) has also been acknowledged, and estimated to result in an average loss of 10^{-1} Mg of C ha^{-1} (Euliss Jr. et al., 2006). However, very few quantitative data are available with regards to the potential of restored prairie wetlands to sequester soil C.

Historically, assessing the implications of wetland restoration focused on visual cues of aboveground indicators such as soil erosion and plant diversity and coverage (Mitsch and Wilson, 1996; Mummy et al., 2002). More recently, the implications of restoration have been examined by focusing on the changes that occur within the soil environment and more specifically within the soil organic matter (Bruland and Richardson, 2006; Lopes de Gerenyu et al., 2008; McLauchlan et al., 2006). While a number of studies have focused on the changes to grassland and wetland soils following restoration (D'Angelo et al., 2005; Galatowitsch and van der Valk, 1996; McKinley, 2001; McKinley et al., 2005), none have looked at riparian soils of wetlands within the Prairie Pothole Region

(PPR) of Canada. We chose to focus on riparian soils as they are dynamic components of the landscape (Gregory et al., 1991; Lewis et al., 2003), are unique because they undergo large seasonal fluctuations in temperature and moisture and finally because information on specific SOM characteristics in these wetland areas in Canada is lacking.

Numerous SOM models and fractionation schemes have been developed in an attempt to capture the heterogeneity of SOM by distinguishing among several pools which vary in terms of decomposition rates or factors controlling decomposition (Christensen, 1992; Six et al., 2002). Traditionally extraction methods using chemical solvents were used to determine humic and fulvic acids, the functionality of which has since come into question (Oades, 1988; Tiessen and Stewart, 1983). More recently, the importance of SOM stabilization in the context of the physical and biological realm has been recognized and physical fractionation techniques have been utilized (Six et al., 2002). In particular, a combination of density and particle-size separation techniques can be used to isolate various fractions. The labile light and sand-sized fractions, which are highly sensitive to management practices may provide early indications of the consequences of disturbance (Six et al., 2002). The clay and silt sized pool is comprised of physically and chemically protected C and is considered the slow pool. Finally, the acid-unhydrolyzable fraction, which is often defined as the passive pool (Six et al., 2002) as it contains biochemically protected C comprised of lignin cutin and condensed tannins (Preston et al., 2006).

Solid state ^{13}C nuclear magnetic resonance (NMR) spectroscopy has become a vital tool for examining SOM macromolecular composition and decomposition processes (Baldock et al., 1997; Golchin et al., 1995; Preston, 1996). Studies utilizing ^{13}C NMR have focused on characterizing SOM in specific density and particle size fractions (Mahieu et al., 1999; Preston, 1996), examining the influence of environmental factors such as climate and vegetation (Baldock et al., 1992), and, in particular, the influence of forest vegetation types on the structure of organic matter (Hannam et al., 2004; Preston et al., 1999; Quideau et al., 2001; Zech et al., 1992). Specifically looking at the variation in ^{13}C abundance

(Preston et al., 2006) combined with NMR can further aide in determining the decomposition processes occurring in a soil.

The overall objective of this study was to examine SOM quantity and quality in riparian soils of restored wetlands. Furthermore, we determined the return of functionality in these restored soils of varying ages since restoration (3-11 yrs) by making comparisons with reference wetlands, which were representative of the pre-disturbance conditions and were intended to approximate a target ecosystem. Specific objectives involved the quantification of C distribution among physically and chemically separated SOM pools, and SOM characterization through isotopic and solid state NMR analyses. Since native prairie soil is renowned for its fertility and high SOM content (McKinley et al., 2005), we expected that reference soils would have higher C than the restored soils. We also hypothesized that with increasing time since restoration the C characteristics would become more similar to the reference soils.

2. Materials and Methods

2.1. Study sites and sampling protocol

The Canadian PPR includes approximately 480,000 km^2 and encompasses southeastern Alberta, southern Saskatchewan and southwestern Manitoba (Johnson et al., 1989). The region was once covered with glacial drift deposits during the Wisconsin glacial advance (Kantrud et al., 1989) and the parent material of the region consists of glacio-lacustrine sediments. The topography of this area is mostly flat to gently rolling. The PPR is characterized by warm summers and cold winters, with snow covering the ground 30 to 50 % of the time. Precipitation varies in the prairie region from 350 to 450 mm per year (Greenwood et al., 1995), and this region is characterized as having a negative water balance, with evaporation exceeding precipitation. Temperatures are generally cold, with mean daily temperatures at or below 0°C for 5 months of the year (Forcey et al., 2007). Summer air temperatures throughout the region are similar with a July mean of approximately 18°C (Greenwood et al., 1995). Air temperatures in the winter can drop below -60°C and can exceed 40°C in the

summer (Euliss Jr. et al., 1999). The PPR encompasses two physiographic zones, the aspen parkland and prairie or grassland (Greenwood et al., 1995). The aspen parkland is transitional between the boreal forest and prairie, where the natural vegetation is grassland interspersed with aspen and oak bluffs. The prairie or grassland region is characterized by dry mixed grassland and tallgrass prairie. The dominant soils are Udic, Typic, and Aridic Borolls (Soil Survey Staff, 2006), or Black, Dark Brown and Brown Chernozems according to the Canadian classification (Agriculture Canada, 1988abc; Soil Classification Working Group, 1998).

Ducks Unlimited Canada (DUC) has been the primary organization responsible for wetland restoration to date, with over 900 wetlands (or 1700 ha) in Alberta, Saskatchewan and Manitoba being restored between 1989 and 1997 (DUC, 2003). Restoration designs are dependent on the modifications necessary to create the desired hydrology in the wetland, including excavating the basin, building dykes, removing tiles or plugging ditches (Galatowitsch and van der Valk, 1994). All of the wetlands included in the present study were restored by installing a ditch plug. In this study restoration is further defined as the reestablishment of a wetland through cessation of artificial drainage. Restored wetlands were chosen from an inventory of over 898 wetlands based on a series of criteria, including site accessibility, age class, and presence of a reference wetland within a quarter section of the restored wetland. All transects were established in 2003 by Ducks Unlimited Canada to provide an extensive survey of soil organic C storage and greenhouse gas emissions, specifically emissions of methane (CH_4) and nitrous oxide (N_2O) from wetlands across the PPR. In total 15 reference wetlands and 28 restored wetlands were selected and sampled in August of 2005. Soil classification at each site was determined by referencing the Canada Soil Inventory Maps for each province (Agriculture Canada, 1988abc), with gleyed variants of these soils dominating the riparian zones. Twenty-four of these wetlands were located in the aspen parkland ecoregion and Black Chernozem (Udic Boroll) soil zone of Alberta (Table 2.1). Sixteen wetlands were located in Saskatchewan and included wetlands in the Black, Dark Gray and Brown

Chernozem (Udic, Boralfic and Aridic Boroll) soil zones as well as in the aspen parkland ecoregion and prairie ecoregion. Finally, 3 wetlands were located in the Aspen Parkland ecoregion and Black Chernozem (Udic Boroll) soil zone of Manitoba.

A classification of prairie potholes was developed by Stewart and Kantrud (1971) based on diagnostic vegetation found in the central or deepest zone of wetland basins, reflecting differences in water permanence. The zones, or wetlands classes, in order of increasing permanence are ephemeral pond (Class I, low prairie), temporary pond (Class II, wet meadow), seasonal pond (Class III, shallow emergent marsh), semipermanent pond (Class IV, deep emergent marsh), and permanent pond (Class V, open water). This study focused on wetlands classified as either Class III or Class IV, where the ponding duration under normal conditions is 1-3 months to 5 months respectively. Also included in this study, was one wetland in Southern Saskatchewan classified as a Class II wetland or temporary pond. The restored wetlands ranged in age from 3 to 11 years since restoration (Table 2.1) and all were restored with the placement of a ditch plug. After installation of the ditch plug, reseeding with a dense nesting cover mix (DNC), which is generally comprised of different species of wheatgrasses (*Agropyron* spp.), meadow brome (*Bromus biebersteinii*) and other species of native prairie grasses, occurred to provide erosion protection but no fertilization took place.

Each study site included one reference wetland, which based on air photo interpretation and discussion with land owners, had not been purposely drained for agricultural purposes, and one or more restored wetlands of the same restoration age. Within a site, wetlands were located on the same quarter section of land (160 acres or 2.59 km^2). They varied in size, but were all less than 10 ha in area (Figure 2.1). Sufficient distance between reference and restored wetlands insured their hydrological independence. A transect had been delineated at each wetland for a detailed greenhouse gas study that occurred at the sites from 2003 to 2005 (Dan Pennock, personal communication, 2007). Soil samples for C analysis were collected using a bulk density corer and were taken within 1 meter of the

original transect position to the left or right depending on trampling or disturbance. Fresh litter and live plant material was removed from the area where the core was taken. Four sampling points were selected within the riparian area through identification of hydrophytic vegetation (eg. *Carex* spp. and *Juncus* spp.) and position in relation to the basin (Figure 2.1). Four cores were taken at each of the four sampling points, composited, and placed in large Ziploc bags. The two bags corresponding to similar positions on the transect in relation to the basin were further composited to yield two samples per wetland. Cores were taken to a depth of 6 cm as this is where we expected changes to soil characteristics to have occurred over the short term interval of our study (Karlen et al., 1999; McKinley, 2001; McKinley et al., 2005). All samples were placed in coolers immediately after sampling occurred.

2.2. Laboratory analyses

Fractionation techniques included both physical and chemical separation and were applied to air-dried (<2mm) soil samples. Sonication, wet sieving based on particle size, density separation and proximate analysis procedures were modified from various methodologies (Baldock et al., 1992; Oades et al., 1987; Ryan et al., 1990). For each sample, a mixture of 10 grams of soil and 50 ml of distilled water was placed on a reciprocal shaker for 1 hr and then sonicated for 5 min at 60 J/cm^3. Samples were then wet sieved through a 53 μm mesh. Material >53 μm was further fractionated by density separation in water by floatation, which yielded the light fraction (LF) organic matter and the sand associated organic matter (Sand). Multiple fractionations of soil samples were completed in order to obtain enough light fraction organic matter material for elemental analysis and ^{13}C NMR spectroscopy. Material <53 μm comprised the silt and clay associated organic matter (Silt + Clay). In addition, one gram subsamples from the Silt + Clay fraction were chemically fractionated to isolate the acid-unhydrolyzable residue, AUR (Ryan et al., 1990). Briefly, 1 g of soil and 10 ml of 72% sulphuric acid were shaken in a flask for one hour and then diluted to 2.5% sulphuric acid using distilled water. The flask was autoclaved at 121 °C for 1 hr,

and the acid unhydrolyzable fraction was isolated by vacuum filtration using a 0.45 μm glass fiber filter. Material remaining on the filter was dried overnight at 65 °C and the final weight of the acid unhydrolyzable material recorded. The LF and Sand fractions were oven dried at 50°C while the Silt + Clay fraction was freeze-dried. All soil fractions were finely ground using a Brinkmann ball grinder (Retsch, MM200) and weighed. Average recovery of the original 10 g was 94.9%.

Total C and N analyses were completed on all soil fractions as well as on the whole soil by dry combustion using a Costech ECS 4010 CHNS-O Elemental Combustion System (Costech Analytical Technologies Inc., Valencia, CA). All fractions were also analyzed for C isotopic composition ($\delta^{13}C$) on a Costech ECS 4010 CHNS-O Elemental Combustion System coupled to a Finnigan Deltaplus Advantage^{TM} Isotope Ratio Mass Spectrometer (ThermoFinnigan, Bremen Germany). Results were expressed using the δ-notation, the ‰ variation from the standard Pee Dee Belemnite (PDB) reference material, and the isotopic composition calculated from:

$$\delta^{13}C = [(R_{sample} / R_{standard}) - 1] * 1000$$

where R_{sample} and $R_{standard}$ are the ratios of $^{13}C/^{12}C$ in the sample and standard, respectively.

The LF chemical composition was further characterized using ramped-cross-polarisation (RAMP-CP) ^{13}C NMR spectroscopy on a Bruker Avance 400 (B_0=9.4 T, $\nu_L(^{13}C)$=100.6 MHz) spectrometer as previously described by Thiffault et al. (2008). Spectra were acquired using a ^{1}H 90° pulse width of 4.0 μs, a 1 ms contact time and 5 s pulse delay with spinning at 13kHz. The Hartmann-Hahn matching condition was determined using the COO signal of glycine. For each sample 4,000-8,000 scans were collected and line broadening set at 200Hz. The ^{13}C chemical shifts were referenced relative to tetramethylsilane (δ_{iso} = 0.0 ppm) using adamantine as a secondary reference.

Bruker's WIN-NMR package was used to estimate the relative integrated areas of five regions between 0 and 194 ppm. The areas included were: 0 to 45 ppm, attributed to alkyl C (ALK); 45 to 112 ppm, attributed to O-Alkyl C (O-ALK); 112 to140 ppm, attributed to aromatic C (AROM); 140 to 165 ppm,

attributed to phenolic C (PHEN), and 165 to 192 ppm, attributed to carbonyl C (CARB). Corrections for spinning sidebands were applied under the assumption that both sidebands of each signal have the same intensity, and spectral divisions were assigned based on local minima.

2.3. Statistical analyses

Differences between transect positions as well as among age groups were analyzed for C concentration, distribution, C/N and ^{13}C results (all soil fractions) using a two-way ANOVA with PROC GLM (version 9.1, SAS Institute Inc.). Position will not be discussed in the remainder of this manuscript as there were no significant effects of position and no significant interactions between position and age groups. Post-hoc comparisons were performed using the SNK test and determined to be significant at $p \leq 0.05$. Carbon concentration data for the bulk soil, Sand, Silt + Clay, and AUR fractions as well as C distribution values for the Sand and Silt + Clay had to be log transformed to meet the assumptions of normality.

Soil C characteristics in reference and restored riparian soils were further analyzed through a non-metric multidimensional scaling (NMS) ordination technique, followed by Multi-Response Permutation Procedures (MRPP) using the PC-ORD software (version 4, MjM Software Design, Gleneden Beach, OR). Ordination allows items to be graphically organized as to summarize complex relationships and then to extract dominant patterns from many possible outcomes (McCune and Grace, 2002). In particular, NMS runs numerous iterations from which the best possible way to represent the data is reduced to either two or three dimensions, with the distance between the data points indicative of the similarity between those points. NMS does not necessitate normal distribution of data nor does it assume a linear relationship between variables. The Sorensen (Bray-Curtis) distance measure was used for the analysis.

For the NMS analysis of the NMR data, the first matrix contained the five integrated NMR spectral areas. Outlier analysis was carried out on the NMR data and 7 samples were removed as 6 samples were greater than 2 standard deviations

from the mean and one was removed as it no longer had a corresponding reference sample. The first matrix for analysis of the C fractionation data contained the C concentrations, C/N, and ^{13}C values for the LF, Sand, Silt + Clay, and AUR fractions. Data in the first matrices were relativized by column and standardized using the arcsine square-root function. The second matrix contained chosen site and soil parameters in order to map vectors ($r^2 \geq 0.40$) over the first matrix: age group, position on transect, soil zone, wetland class, pH, and climatic data consisting of moisture deficit (precipitation – potential evapotranspiration; P-PE) and effective growing degree days (EGDD) values (Baier and Robertson, 1965; Canadian Climate Impact Scenarios Project, 2005). The climate data used here are from 10 km gridded databases which are formed from climate data from actual stations and interpolated to the 10 km x 10 km grid. In addition, the second matrix for the C fractionation data contained parameters derived from the NMR analysis, including all spectral areas as well as the ratios of Alkyl/O-Alkyl C and Aromatic/O-Alkyl C.

All parameters were tested for significance in the NMS analysis using a multi-response permutation procedure (MRPP). MRPP is a non-parametric procedure used to determine if there are differences among 2 or more groups (McCune and Grace, 2002). Significance was determined from the output of three values: the p value, indicated overall significance of the comparisons, the test statistic (*T*) indicated the separation between groups, with a more negative T value indicative of stronger separation and the agreement statistic (*A*), which indicated the within group homogeneity versus the random expectation, with a higher A value indicative of higher homogeneity. The r^2 value considered significant for inclusion of vectors was set at 0.4.

3. Results

3.1. Composition of the light fractions

All light fractions (LF) isolated from soils collected both from the restored and reference sites exhibited remarkably similar NMR spectra. Figure 2.2

provides an illustration of spectra from a restored soil and two reference soils, one from a Black Chernozem (Udic Boroll) and one from a Brown Chernozem (Aridic Boroll). Specifically, for all samples a dominant peak was found at 74 ppm, indicative of the C-2, C-3, and C-5 carbons found in cellulose and hemicelluloses (Hannam et al., 2004), while the presence of a shoulder at 64 ppm is indicative of the C-6 carbons (Quideau et al., 2001). A peak at 105 ppm also occurred, as is characteristic of anomeric carbons in cellulose and hemicelluloses (Gregorich et al., 1996). In the ALK region of the spectra there were two small main peaks at 21 and 30 ppm. The peak at 21 ppm represents terminal methyl groups (Mikutta et al., 2006), while the one at 30 ppm represents polymethylene C in long chain aliphatic structures, like those found in lipids and cutin (Kogel-Knabner, 2002). A peak at 130 ppm along with a shoulder at 115 ppm on some of the spectra was found in the AROM region of the spectra, indicating C-substituted aromatic carbons such as C-1 carbon of lignin guaiacyl and syringyl units. The PHEN region contained a small peak at 153 ppm also likely arising from lignin (Preston et al., 1999). Finally, the CARB region of the spectra contained a peak at 174 ppm, which may include carboxyl groups from organic acids or amide groups (Quideau et al., 2001).

The NMS ordination of the NMR spectral data produced a two dimensional solution with a final stress of 6.7 after 50 iterations. Axis 1 and 2 explained 16 and 82% of the variation respectively. Results from the ordination of the NMR spectra indicated that differences found among samples were not related to time since restoration as no significant differences were found among age groups and reference sites ($T= 0.1$, $A= -2.2 \times 10^{-3}$, $p= 4.8 \times 10^{-1}$). However, significant differences were found when we grouped the NMR spectra by soil zone (Figure 2.3). Specifically, significant differences were found between the Black and Brown Chernozem (Udic and Aridic Borolls) soil zones ($T= -10.2$, $A= 0.1$, $p= 2.0 \times 10^{-5}$) and the Dark Gray and Brown Chernozem (Boralfic and Aridic Borolls) soil zones ($T= -3.6$, $A= 0.1$, $p= 6.2 \times 10^{-3}$). No significant difference was found between the Black and Dark Gray Chernozem (Udic and Boralfic Borolls) soil zones ($T= -1.8$, $A= 0.1$, $p= 0.1$). Results from the vector analysis revealed a

relationship of the NMR data to climate, and climatic variables of precipitation – potential evapotranspiration (P-PE) and effective growing degree days (EGDD) were included as vectors with r^2 values of 0.44 and 0.49, respectively (Figure 2.3). The direction of the P-PE vector indicated a positive relationship with the Black Chernozem (Udic Borolls) soil zone, while the EGDD vector was positively related to the Brown Chernozem soil zone (Aridic Borolls).

In view of the differences pointed out through the NMS analysis, further investigation of specific differences in the spectral regions between the Black and Brown Chernozems (Udic and Aridic Borolls) reference sites was conducted using a t-test (Table 2.2). A significant difference was found between soil zones for % Aromatic C ($p= 2.6 \times 10^{-2}$), where Black Chernozems (Udic Borolls) had higher amounts of aromatic C than the Brown Chernozems (Aridic Borolls). A significant difference was also found in the % Phenolic C ($p= 1.8 \times 10^{-3}$) between the soil zones. The Black Chernozems exhibited higher amounts of phenolic C than the Brown Chernozems. Finally, a significant difference was found in % Alkyl C ($p= 4.6 \times 10^{-2}$), with the Brown Chernozems having higher amounts than the Black Chernozems. No significant differences were found between the % O-Alkyl C and % Carbonyl C or the ratios of Alkyl/O-Alkyl C and Aromatic/O-Alkyl C among soil zones.

3.2. Carbon characteristics of soil fractions

The NMS ordination of the C data, including the C concentrations, C/N ratios and $\delta^{13}C$ for all fractions, produced a two-dimensional solution with a final stress of 10.07 after 92 iterations (Figure 2.4). Axis 1 and 2 explained 58% and 37% of the data, respectively. Results from the C ordination further indicated that these differences were indicative of time since restoration as significant differences were found among age groups and reference sites ($T= -8.0$, $A= 9.3 \times 10^{-2}$, $p= 5.7 \times 10^{-6}$). More specifically, the strongest separation in C characteristics was found between the reference sites and the 1-3 yr since restored sites ($T= -5.9$, $A= 0.11$, $p= 1.2 \times 10^{-3}$). A significant difference was also found between the reference sites and the 4-6 yr since restored sites ($T= -5.4$, $A= 0.09$, $p= 1.9 \times 10^{-3}$),

and the 7-11 yr since restored sites (T= -5.5, A= 0.05, p= 1.5 x 10^{-2}). Finally, a significant difference was found between the 7-11 yr since restored sites and both the 1-3 yrs since restored sites (T= -4.0, A= 0.05, p= 4.2 x 10^{-3}) and 4-6 yr since restored sites (T= -2.9, A= 0.03, p= 1.5 x 10^{-2}). On the other hand, no significant difference in C characteristics was found between the 1-3 yr and 4-6 yr since restored sites. Results from the vector analysis exercise did not reveal any significant correlation when r^2 was set at 0.4.

Particular differences in soil C characteristics included significant differences in the C/N ratios among age groups specifically in the Sand (p= 9.3 x 10^{-3}) fraction (Table 2.3). The C/N ratio in the Sand fraction was significantly higher in the 4-6 yr age group when compared with the 1-3 yr, 7-11 yr and reference groups, but no significant differences were found for either the bulk soils or the other (LF, Silt + Clay, and AUR) fractions. Significant differences were found in the $\delta^{13}C$ values among age groups for the AUR fraction (p= 3.7 x 10^{-3}), and although differences were not significant for the other fractions, there was a general trend of decreasing (more negative) $\delta^{13}C$ values with increasing age since restoration. The LF (p= 5.7 x 10^{-2}) in particular exhibited this trend. Specifically, $\delta^{13}C$ were found to be more depleted in the heavier isotope (more negative) in the 7-11 yr age group compared to the 1-3 yr age group in the LF fraction, while in the AUR fraction, the $\delta^{13}C$ were significantly more negative in the reference soils than in the 1-3 yr age group.

In terms of C concentrations (g kg^{-1}), significant differences were found among age groups for the bulk (p= 6.0 x 10^{-4}), Sand (p<1.0 x 10^{-4}), and Silt + Clay (p= 3.8 x 10^{-3}) fractions (Table 2.3). No significant differences were found in the LF and AUR fractions. The bulk fraction contained a significantly higher amount of C in the reference soils compared to the 1-3 and 4-6 yr age groups. Similarly, the C concentration was significantly higher in the 7-11 yr and reference age groups when compared to the younger 1-3 and 4-6 yr age groups in the Sand fraction. Finally, there was a significant difference in C concentration between the reference group compared to the youngest 1-3 yr age group in the Silt + Clay fraction.

When C concentrations were expressed as percentage of the total amount of C in soils, differences in the distribution of C became apparent among age groups in the Sand and Silt + Clay fractions (Figure 2.5). In the Sand fraction there was a significant increase in C with age, with the reference group (47%) having significantly higher amounts of C than the 7-11 yr (32%), the 4-6 yr (23%), and the 1-3 yr (17%) age groups. Alternatively, the Silt + Clay fraction showed a significant decreasing trend in C distribution with age, from 81% in the 1-3 yr age group to 75% (4-6 yr), 66% (7-11 yr), and finally 51% in the reference group. In all cases, only a small amount of total C (< 3%) was contained in the light fraction (LF), and there was no difference among sites for that fraction.

4. Discussion

The chemical structure of soil organic material is controlled primarily by two factors: the chemical composition of the litter inputs, and the soil environmental conditions (Golchin et al., 1994). In our study, the general structure of the LF fractions as seen by the NMR analysis is consistent with composition of wheat straw and other grasses in that the signal of aromatic carbons representative of lignin is less prominent than in other plant samples such as those derived from trees (Kogel-Knabner, 2002; Turcotte et al., 2009). As no difference was found in the chemical structure of this light organic matter fraction among age groups, we can conclude that restoration did not result in significant differences in the composition of litter inputs at the sites. On the other hand, differences in organic material composition were partially explained by differences in the soil environment as revealed by the multivariate analysis (Figure 2.3). Closer examination of the specific regions of the spectra in reference sites between soil zones supported this conclusion, and showed that Black Chernozems (Udic Borolls) contained significantly higher amounts of aromatic and phenolic C (Table 2.2), but lower amounts of alkyl C than the Brown Chernozems (Aridic Borolls). Furthermore, the vector analysis revealed the significance of the climatic variables when samples were grouped by soil zone, with the Brown Chernozems included in this study having more negative P-PE

values and a higher number of EGDD than the Black Chernozems. In addition, the C/N ratios of the LF fractions were lower in the Brown Chernozems (20.31 ± 1.54) when compared to the Black Chernozems (24.04 ± 1.03s.e), indicating that the Brown Chernozems were at a more advanced stage of decomposition (Otto et al., 2005). Aromatic compounds, and specifically lignin, are not easily decomposed under anaerobic conditions (Kogel-Knabner, 2002). The degradation of aromatics has been found to increase with increasing soil temperature (Alarcon-Gutierrez et al., 2008). In our study, it is likely that the riparian zones in the Black Chernozems, which are characterized by a subhumid climate (Soil Classification Working Group, 1998), on average experience longer periods of saturation (i.e. anaerobic conditions) and/or cooler soil temperatures than the Brown Chernozems, which are characterized by a subarid to semiarid climate, thereby explaining why they contain higher amounts of aromatic and phenolic carbons. On the other hand, the preferential accumulation of lipids (i.e. alkyl C), has been observed under both dry and anaerobic environments due to inhibited microbial activity (Otto et al., 2005). The higher percentage of Alkyl C found in the Brown Chernozems in our study would indicate that the drier environment at these sites was either more inhibiting to the degradation of lipids than periods of anaerobiosis in the Black Chernozems, or that the soil environment in these Brown Chernozems favored the decomposition of aromatic compounds when compared to lipidic moieties. In all cases, our results showed that organic matter composition in the light fractions was more sensitive to long-term variations in soil forming environmental conditions (as related to different soil groups) than to more localized variations associated with restoration practices.

Numerous studies have examined how the quantity and quality of soil C are affected by land use changes, in particular following the conversion of prairie soils to agriculture as well as following restoration of agricultural lands once these are no longer cultivated (Aldous et al., 2005; Golchin et al., 1994; Lemke et al., 1998; Lopes de Gerenyu et al., 2008; Schnitzer et al., 2006). When cultivated lands are allowed to revert back to natural prairie vegetation, soil C typically accumulates (Lopes de Gerenyu et al., 2008). Cultivation and tillage practices

degrade soil aggregation thereby accelerating microbial activity and increasing the turnover of soil C (Golchin et al., 1995). They also increase compaction and subsidence, and may disrupt the entire soil profile (Aldous et al., 2005). As a result a loss of 20 to >50% of C is typically observed following the conversion to cultivation (Anderson, 1995; Cihacek and Ulmer, 1995; Mann, 1986; McGill et al., 1988). For instance, in their study of Black Chernozems (Udic Borolls) in southern Saskatchewan, Schnitzer et al. (2006) found that virgin sites had higher amounts of C than cultivated sites, 23 g kg^{-1} in the cultivated soils compared to 47 g kg^{-1} in the virgin soils. In our study, it is thus not surprising that the younger restored sites (1-3 and 4-6 yr) that have been most recently affected by the impacts of agricultural activities were similar yet both significantly different from the older restored sites (7-11 yr) and reference sites (Table 2.3).

When looking at the impacts of restoration on soil C content, Lopes du Gerenyu et al. (2008) found that arable soil allowed to revert back to natural vegetation for 5 years still had lower soil C than soil not cultivated for 11, 21 and 77 years. Similarly, agricultural land and prairie restorations have been shown to differ from virgin prairie in terms of having less SOM even after as much as 30 years following restoration (McKinley, 2001; McKinley et al., 2005). Results of studies that more specifically target wetland soils that have been restored by destroying or filling portions of tile or ditch systems (Bruland and Richardson, 2006; D'Angelo et al., 2005; Galatowitsch and van der Valk, 1996) mirror these findings, with soil C being higher in natural wetlands when compared to restored wetlands. D'Angelo et al. (2005) found that soil C was twice as high in late-successional wetlands (>30 years) than early successional wetlands (<10 years after mitigation). In their study of hydric soils in the United States portion of the PPR, Galatowitsch and van der Valk (1996) reported that soils 3 years after reflooding had 3.8% soil C, compared to 10.1% in natural wetlands. In comparison to these studies, our results showed that for all soil fractions, excluding the LF and AUR fractions, the reference sites had significantly higher soil C than the youngest (1-3 yr) restored sites, but that the C contents in the bulk soil and sand-sized fractions 7-11 years after restoration were not significantly

different from the reference sites (Table 2.3). Thus C quantity in these soils appears to return to pre-disturbance conditions after 7-11 years, at least for C associated with the more labile, sand-sized fraction. In the AUR fraction, although not significantly different, there was a trend of increasing C concentration with time since restoration. This differs from the findings of Euliss Jr. et al. (2006), who found that restored wetlands would be able to sequester amounts of C comparable to reference wetlands in less than 4 years. Their conclusions were based on semi-permanent wetlands throughout the United States, whereas our findings included both seasonal and semipermanent wetlands in the Canadian PPR only. Climatic differences between Canada and the United States as well as shorter ponding durations characteristic of seasonal wetlands may be the cause of the extended time period of C storage recovery that we see in our study.

The distribution of C among particle size fractions has been examined to determine the effects of soil disturbance such as cultivation on SOM dynamics (Cambardella and Elliott, 1992; Tiessen and Stewart, 1983). Findings from these studies show that cultivation results in a higher amount of mineral associated C in tilled soils when compared to native soils (Cambardella and Elliott, 1992) and a decrease in the sand associated C due to cultivation (Tiessen and Stewart, 1983). In our study, the youngest restored sites (1-3 yrs) had significantly higher amounts of C associated with the Silt + Clay fraction than the 7-11 yr and reference sites while the reference sites had significantly higher amounts of C associated with the Sand fraction than the younger (1-3 and 4-6 yr) restored sites (Figure 2.5). This indicates that the impacts of cultivation have included preferential decomposition of the sand-sized organic matter to the point where approximately 80% of organic matter is now associated with the finer mineral fractions at the 1-3 yr sites. This impact is decreasing with time since restoration, but return to reference site levels has still not occurred after 7-11 years.

We observed a decrease in C/N ratios from the LF to Sand fraction, with the lowest ratios occurring in the Silt + Clay fraction for all age groups (Table 2.3). This decrease in C/N with decreasing particle-size has been well documented and has often been used as an index of decomposition (Schulten et

al., 1993; Stemmer et al., 1998). In general for all age groups in this study there, we also saw an enrichment in $\delta^{13}C$ with decreasing particle-size (Table 2.3). There was an enrichment of $\delta^{13}C$ in the 1-3 yr age group in both the LF and the AUR fraction when compared with the 7-11 yr age group and the reference age group. The LF is the labile fraction which consists of particulate and partly decomposed plant residues whereas the AUR fraction contains compounds specifically derived from lignin, cutin and condensed tannins (Preston et al., 2006). This enrichment in the heavier isotope may indicate that the C pool in the LF and AUR fractions of the younger restored soils was more affected by microbial degradation than in the reference soils (Hannam et al., 2005; Quideau et al., 2003). Specifically, microbial discrimination against $\delta^{13}C$ during catabolic processes; i.e. the emission of $\delta^{13}C$-depleted CO_2 by microbial respiration would result in an increase in $\delta^{13}C$ concentration in the residual soil C. It is interesting to note that although differences in $\delta^{13}C$ were observed among age groups, which may be related to variation in the degree of SOM decomposition, we were unable to detect any differences through NMR analysis.

5. Conclusions

In this study we found that with the use of ^{13}C NMR spectroscopy we were unable to find differences between restored and reference soils as the soil environment, impacted by climatic variables, was the primary influence on the chemical composition of the light fractions. However, with the use of ordination and examination of specific C characteristics we detected significant differences between recently restored riparian soils (1-3 and 4-6 yr), older restored soils (7-11 yrs), and reference riparian soils. Results from our study contribute to the paucity of information on Canadian PPR wetlands and the ability of elements of functionality, specifically C storage capacity, to return to pre-disturbance conditions following restoration. Riparian soils specifically, with their extreme fluctuations in temperature and hydrology, have the ability to reach soil C reference levels 7-11 years after restoration. However, additional studies of C

storage potential in the Canadian PPR are necessary to fully quantify this C sink and to develop optimal land-use strategies.

CHAPTER II. TABLES AND FIGURES

Table 2. 1. Soil classification, climatic variables and time since restoration for the study sites.

Province	Site Name	Latitude, Longitude (°N, °W)	Canadian soil Classification†	USDA Classification‡	Time Since Restoration (yr)	Age Class (yrs)	P-PE § (mm)	EGDD¶
Alberta	Ambler	53.09, -113.20	Black Chernozem	Udic Borolls	9	7-11	-205	1294
	Boyden	52.07, -113.18	Black Chernozem	Udic Borolls	11	7-11	-265	1242
	Ferleyko	53.54, -112.27	Black Chernozem	Udic Borolls	3	1-3	-233	1264
	Kemo	52.07, -113.18	Black Chernozem	Udic Borolls	5	4-6	-265	1242
	Loveseth	53.16, -111.50	Black Chernozem	Udic Borolls	4	4-6	-243	1270
	Maruschak	53.18, -113.12	Black Chernozem	Udic Borolls	11	7-11	-205	1294
	Mittlestadt	53.18, -113.13	Black Chernozem	Udic Borolls	11	7-11	-205	1294
	Rauser	53.11, -111.46	Black Chernozem	Udic Borolls	10	7-11	-243	1270
Saskatchewan	Adams	51.02, -101.87	Black Chernozem	Udic Borolls	7	7-11	-290	1337
	Crone	52.26, -104.71	Black Chernozem	Udic Borolls	5	4-6	-290	1352
	Hartt	53.08, -104.54	Dark Gray Chernozem	Boralfic Borolls	8	7-11	-306	1318
	Peters	50.62, -106.93	Brown Chernozem	Aridic Borolls	8	7-11	-401	1483
	Sprig	50.46, -106.33	Brown Chernozem	Aridic Borolls	8	7-11	-400	1481
	Tataryn	51.21, -103.12	Black Chernozem	Udic Borolls	3	1-3	-265	1307
Manitoba	Graham	50.21, -99.74	Black Chernozem	Udic Borolls	3	1-3	-252	1341

†Agriculture Canada (1988abc)
‡ Soil Survey Staff (2006)
§ Precipitation – potential evapotranspiration
¶ Effective growing degree days

Table 2. 2. Distribution (% total spectral area) of carbon species in light fractions from the Black Chernozems (Udic Borolls) and Brown Chernozems (Aridic Borolls) as determined from the integration of the solid-state 13C NMR spectra. Regions integrated are Alkyl (0-45 ppm), O-Alkyl (45-112), Aromatic (112-140 ppm), Phenolic (140-165 ppm), and Carbonyl (165-192 ppm).

Spectral Region (% total spectral area)	Black Chernozems	Brown Chernozems
Alkyl	13.3 (0.3)*	14.8 (0.9)
O-Alkyl	63.4 (0.7)	61.9 (1.1)
Aromatic	12.3 (0.3)*	10.9 (0.2)
Phenolic	5.9 (0.2)*	5.2 (0.1)
Carbonyl	5.2 (0.2)	6.0 (0.3)

* Means (standard error) within the same row followed by the asterisk are significantly different ($P < 0.05$) based on SNK test.

Table 2. 3. Carbon characteristics in riparian soil fractions from restored and reference sites**.**

		Age Groups			
Carbon Characteristic	**Soil Fraction**	**1-3 yrs**	**4-6 yrs**	**7-11 yrs**	**Reference**
C/N	**Bulk**	10.4 (0.3)#	11.4 (0.6)	10.2 (0.2)	10.9 (0.3)
	LF†	24.5 (1.9)	23.2 (0.5)	23.4 (0.7)	23.5 (0.9)
	Sand‡	15.4 (0.6)^{b††}	17.2 (1.0)^{a}	14.4 (0.4)^{b}	14.7 (0.4)^{b}
	Silt + Clay§	9.7 (0.2)	10.7 (0.5)	9.7 (0.2)	10.2 (0.3)
	AUR¶	10.1 (0.6)	11.4 (0.4)	10.8 (0.2)	11.1 (0.3)
$\delta^{13}C$ ($^{0}/_{00}$)	**Bulk**	-25.2 (0.3)	-24.7 (0.6)	-25.6 (0.3)	-25.6 (0.3)
	LF	-26.3 (0.2)	-26.6 (0.1)	-26.7 (0.04)	-26.5 (0.1)
	Sand	-25.4 (0.5)	-25.2 (0.6)	-26.3 (0.3)	-26.6 (0.3)
	Silt + Clay	-24.7 (0.2)	-24.6 (0.4)	-25.0 (0.2)	-25.1 (0.3)
	AUR	-25.6 (0.1)^{a}	-25.9 (0.1)^{ab}	-25.9 (0.1)^{ab}	-26.3 (0.2)^{b}
C concentration (g kg^{-1} fraction)	**Bulk**	47.6 (0.3)^{b}	56.2 (0.6)^{b}	73.1 (0.6)^{ab}	107.6 (1.1)^{a}
	LF	414.1 (1.7)	418.6 (2.0)	413.5 (1.1)	418.8 (1.5)
	Sand	27.1 (0.5)^{b}	43.9 (0.9)^{b}	97.3 (1.2)^{a}	159.9 (2.3)^{a}
	Silt + Clay	55.9 (0.7)^{b}	65.3 (0.4)^{ab}	64.2 (0.4)^{ab}	89.7 (0.6)^{a}
	AUR	37.9 (0.5)	40.0 (0.5)	43.4 (0.3)	63.1 (0.6)

† Light Fraction organic matter (ρ<1; >53μm).
‡ Sand associated organic matter (ρ>1; >53μm).
§ Silt and Clay associated organic matter (<53μ).
¶ Acid unhydrolyzable residue.
# Mean and standard error in parentheses (n=8 for 1-3 yr, n=11 for 4-6 yr, n=31 for 7-11 yr and n=25 for reference)
†† Means within the same row followed by the same letter are not significantly different ($P < 0.05$) based on SNK test.

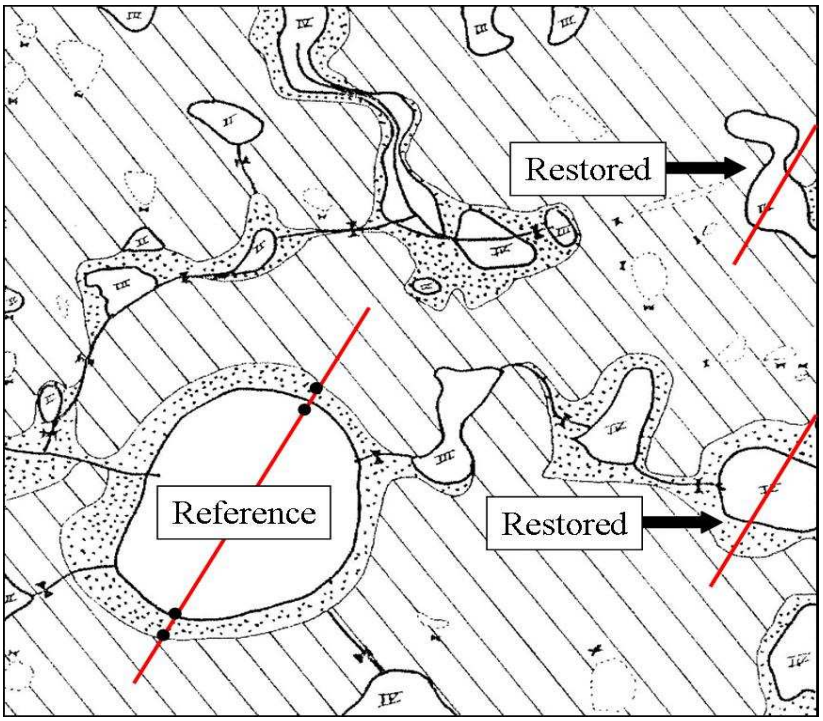

Figure 2. 1. Detailed map of a site, showing the reference wetland and two restored wetlands of the same age, the transects established across each wetland and the possible sampling points (as shown at the reference wetland). All wetlands at a site occur on one quarter section of land (160 acres or 2.59 km^2), with the average size of the basins generally < 2 ha.

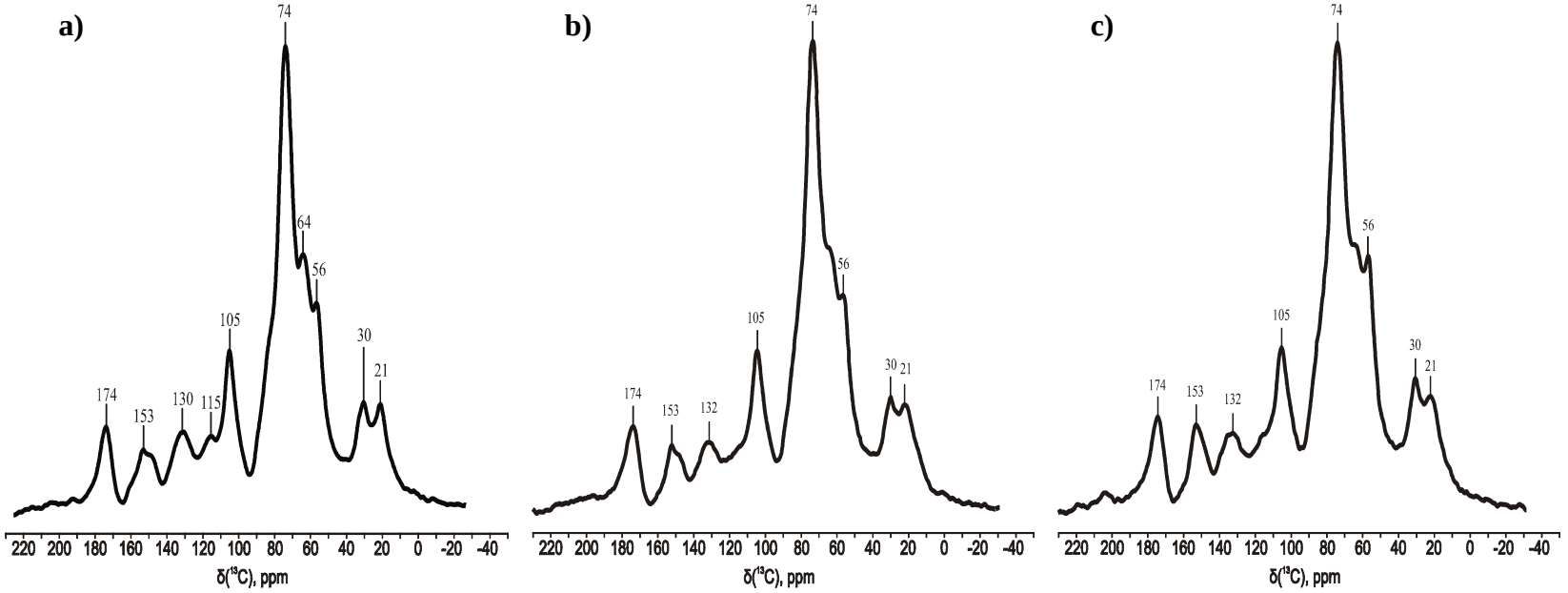

Figure 2. 2. Example of RAMP-CP 13 C NMR spectrum of light fraction material extracted from a) a reference Black Chernozem (Udic Boroll) riparian soil b) a reference Brown Chernozem (Aridic Boroll) riparian soil and c) a restored riparian soil.

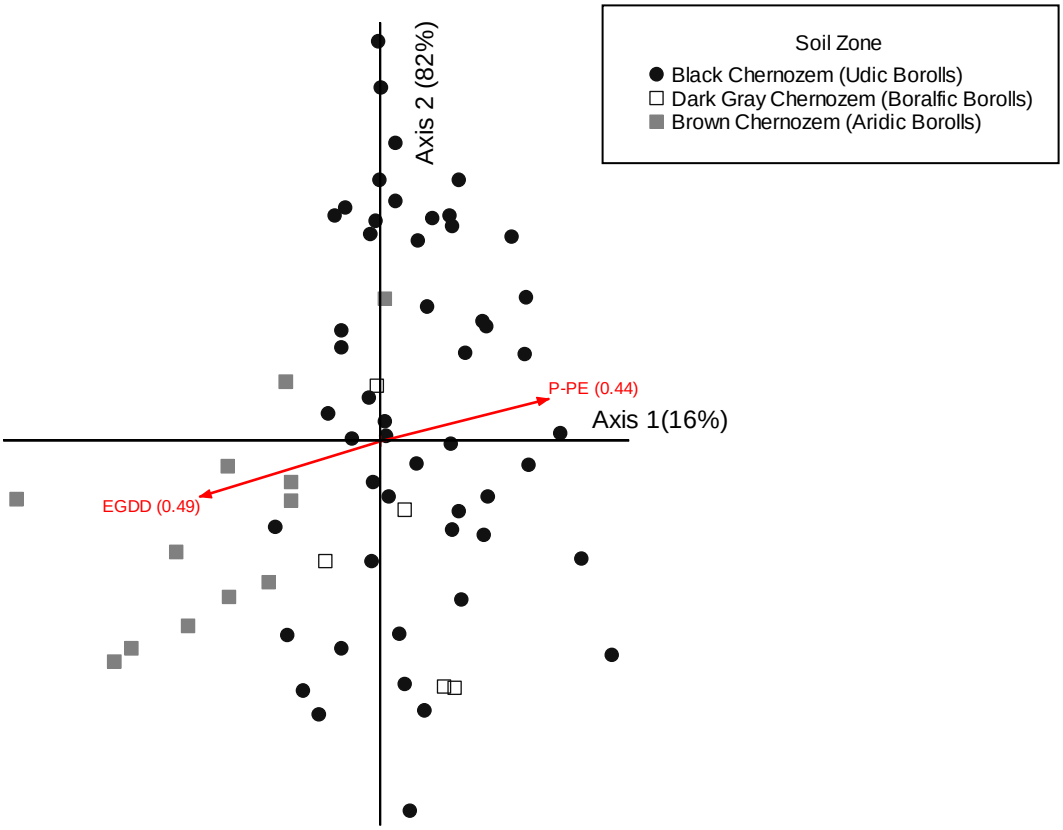

Figure 2. 3. Non-metric multidimensional scaling (NMS) ordination of the RAMP-CP 13C NMR spectra as coded by soil zone. Vectors correspond to precipitation – potential evapotranspiration (P-PE) and effective growing degree days (EGDD).

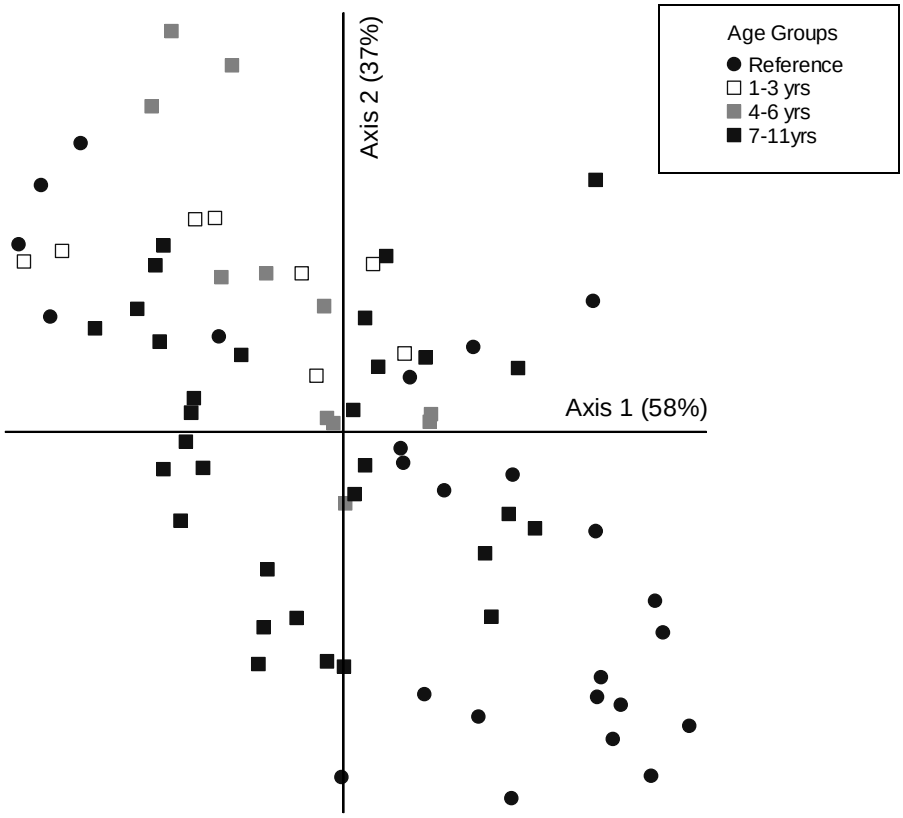

Figure 2. 4. Non-metric multidimensional scaling (NMS) ordination of soil carbon characteristics as coded by age groups.

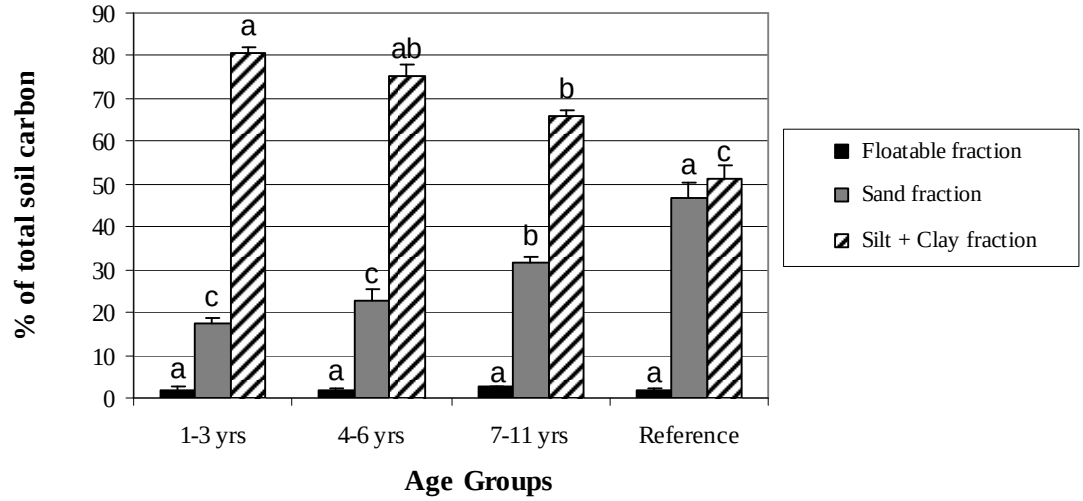

Figure 2. 5. Distribution of carbon in soil fractions from restored and reference soils. Bars indicate one standard error. For a given fraction, different letters indicate significant differences among age groups based on SNK test ($P < 0.05$).

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III. MICROBIAL COMMUNITY STRUCTURE IN RESTORED RIPARIAN SOILS OF THE CANADIAN PRAIRIE POTHOLE REGION

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1. Introduction

Judgment of "success" in restoration projects is often limited to visual cues of aboveground indicators such as wildlife use and plant diversity and coverage (Mitsch and Wilson 1996; Mummy et al., 2002). Since soils respond dynamically to stresses and disturbances, a closer look at belowground characteristics not only offers a more complete understanding of ecosystem dynamics, but also better determines the return of elements of functionality in order to gauge successful restoration efforts than more general plant and animal surveys (Harris, 2003). The soil microbial community is inherent in determining the biogeochemical cycles and organic matter turnover in soils (Zelles, 1999). It integrates both the physical and chemical aspects of the soil environment and is sensitive to anthropogenic activity, making it a suitable indicator of overall soil quality (Peacock et al., 2001a). The soil microbial community has been used in relation to various restoration projects to determine the state of the restored ecosystems when compared to "target" ecosystems (Harris, 2009). Nearby undisturbed sites are often chosen as "target" ecosystems as they may be considered biologically stable and representative of the pre-disturbance conditions (Mummy et al., 2002).

One method to examine the structural composition of the soil microbial community is phospholipid fatty acid (PLFA) analysis (Vestal and White, 1989). Because phospholipids are rapidly degraded following cell death, PLFA analysis is a good index of the living community (Bardgett et al., 1999). The measurement of PLFAs has been utilized to examine the microbial community in agricultural soils (Bossio et al., 1998; Zelles et al., 1992) and in restored prairie soils (McKinley et al., 2005). McKinley et al. (2005) found that with time the soil

characteristics and the PLFA profiles of restored prairie soils became increasingly similar to that of the native soils. Hence, PLFA profiling may be considered a sensitive method for determining changes in the overall microbial community during land restoration. However, to date, very few studies have examined the changes in microbial community structure of restored wetlands (D'Angelo et al., 2005) and specifically the changes that occur within riparian, or hydric, soils of these restored wetlands.

The Prairie Pothole Region (PPR) extends across north-central North America and covers approximately 715,000 km^2 (Euliss Jr. et al., 1999) with over 67% of the PPR occurring in Canada. The Canadian portion of the PPR contains 80% of the agricultural land in Canada along with more than 4.5 million hectares (11 million acres) of wetlands (DUC, 2003). Land use pressure has resulted in the drainage of these wetlands, with estimates of 71-75% of wetlands being converted to agriculture throughout the Canadian PPR (Anonymous, 1986; Patterson, 1999). The loss of wetlands was first recognized due to the loss of habitat for nesting waterfowl populations and subsequent action was taken to achieve continental waterfowl population goals, primarily through the recovery of habitat. Ducks Unlimited Canada (DUC) has been the primary organization responsible for the majority of wetland restorations to date, with over 900 wetlands (or 1700 ha) in Alberta, Saskatchewan and Manitoba being restored between 1989 and 1997. Restoration designs are dependent on the modifications necessary to create the desired hydrology including excavating the basin, building dykes, removing tiles or plugging ditches (Galatowitsch and van der Valk, 1994). All of the wetlands included in the present study were restored by installing a ditch plug. In this study restoration is further defined as the reestablishment of a wetland through cessation of artificial drainage.

The overall objective of this study was to examine the microbial community structure in riparian soils of restored wetlands. Furthermore, we determined the return of key characteristics linked to microbial activity in these restored soils of varying ages since restoration (3-11 yrs) by making comparisons with undisturbed, or reference, wetlands. Since native prairie soil is renowned for

its fertility, high soil organic matter content and high microbial biomass (McKinley et al., 2005), we expected that reference soils would have higher microbial biomass and diversity than restored soils. We also hypothesized that with increasing time since restoration the microbial community would become more similar to the reference soil microbial communities. Another objective of this study was to investigate the relationship of the microbial community structure to carbon characteristics (total carbon content, C/N and $\delta^{13}C$) of the soils. Specifically, we were interested in separating distinct soil carbon pools to explore their respective relations to microbial communities.

2. Materials and Methods

2.1. Study sites and sampling protocol

The Canadian PPR includes approximately 480,000 km^2 and runs through southeastern Alberta, southern Saskatchewan and southwestern Manitoba (Johnson et al., 1989). The parent material of the region consists of glacio-lacustrine sediments (Kantrud et al., 1989) and the topography of this area is mostly flat to gently rolling. Warm summers, with air temperatures throughout the region being similar with a July mean of approximately 18°C (Greenwood et al., 1995), and cold winters, where air temperatures can drop below -60°C (Euliss Jr. et al., 1999) characterize the PPR. Snow covers the ground 30 to 50% of the time and precipitation varies from 400 to 600 mm per year. The region typically experiences a negative water balance, with evaporation exceeding precipitation (Richardson et al., 2001). The PPR encompasses two physiographic zones: the aspen parkland, and prairie or grassland region (Greenwood et al., 1995). The aspen parkland is transitional between the boreal forest and prairie, where the natural vegetation is grassland interspersed with aspen and oak bluffs. The prairie or grassland region is characterized by dry mixed grassland and tallgrass prairie. The dominant soils are Udic, Typic, and Aridic Borolls (Soil Survey Staff, 2006), or Black, Dark Brown and Brown Chernozems according to the Canadian classification (Agriculture Canada, 1988abc; Soil Classification Working Group, 1998).

A total of 43 wetlands were sampled throughout the PPR of Alberta, Saskatchewan and Manitoba in August of 2005. These wetlands were chosen from an inventory of over 898 restored wetlands based on a series of criteria, including site accessibility, age class, and presence of a reference wetland within a quarter section of the restored wetlands. The wetlands in this study were classified as either Class III or Class IV, where the ponding duration under normal conditions is 1-3 to 5 months respectively (Stewart and Kantrud, 1971). Also included in this study was one wetland in Southern Saskatchewan classified as a Class II or temporary pond. In total 15 reference wetlands and 28 restored wetlands were sampled. The classification of soil zone for each site was determined by referencing the Canada Soil Inventory Maps for each province (Agriculture Canada, 1988abc), with gleyed variants of these soils dominating the riparian zones. Twenty-four of these wetlands were located in the aspen parkland ecoregion and Black Chernozem soil zone of Alberta (Table 3.1). Sixteen wetlands were located in Saskatchewan and included wetlands in the Black, Dark Gray and Brown Chernozem soil zones as well as in the aspen parkland ecoregion and prairie ecoregion. Finally, 3 wetlands were located in the Aspen Parkland ecoregion and Black Chernozem soil zone of Manitoba. The restored wetlands ranged in age from 3 to 11 years since restoration (Table 3.1) and all were restored with the placement of a ditch plug. After installation of the ditch plug, reseeding with a dense nesting cover mix (DNC), which is generally comprised of different species of wheatgrasses (*Agropyron* spp.), meadow brome (*Bromus biebersteinii*) and other species of native prairie grasses, occurred to provide erosion protection but no fertilization took place.

Each study site included one reference wetland, which based on air photo interpretation and discussion with land owners, had not been purposely drained for agricultural purposes, and one or more restored wetlands of the same restoration age. Within a site, wetlands varied in size but were all less than 10 ha in area (Figure 3.1) and all wetlands were located on the same quarter section of land (160 acres or 2.59 km^2). A transect had been delineated at each wetland for the detailed greenhouse gas study that occurred at the sites from 2003 to 2005

(Pennock, personal communication, 2007). Soil samples for microbial community (PLFA) determination were collected using an Oakfield corer (diameter = 3.18 cm) and were taken within 1 m of the original transect position to the left or right depending on trampling or disturbance. Fresh litter and live plant material was removed from the area where the core would be taken. Four sampling points were selected within the riparian area through identification of hydrophytic vegetation and position in relation to the basin (Figure 3.1). Four cores, approximately 30 cm apart, were taken at each sampling point and composited in sterile bags. Nitrile gloves were worn by samplers and equipment was wiped with 95% ethanol solution between positions to prevent contamination of the sample and cross-contamination between samples. Cores were taken to a depth of 6 cm as this is where we would expect to see most changes to soil characteristics over the short term (Karlen et al., 1999; McKinley, 2001; McKinley et al., 2005). Soil samples for pH and texture determination were collected using a bulk density corer (diameter = 7.5 cm) and followed the same procedure as outlined above. All samples were placed in a cooler and kept on ice until they were transported to the laboratory within a 12 hr period. Samples for PLFA analysis were stored at -86 °C until analyzed.

2.2. Laboratory analyses

Air-dried, sieved (<2mm) soil samples from the four positions along each transect were composited prior to analysis and pH was determined on composited samples using a 1:2 soil: 0.01M $CaCl_2$ ratio and a settling time of 30 minutes (Kalra and Maynard, 1991; Peech, 1965) on a Fisher Scientific Accumet pH meter. Loss on ignition was used to estimate soil organic matter content following the procedure outlined by Ball (1964) and Kalra and Maynard (1991) using samples from reference wetlands. Based on the results from the loss on ignition method, pre-treatment of all samples to remove organic matter was carried out on soils when organic matter was determined to be greater than 5% wt (Gee and Or, 2002). Texture of the samples was then determined using the hydrometer method as outlined in Gee and Or (2002) and textural class was determined using the

Canadian System of Soil Classification (Soil Classification Working Group, 1998).

Air-dried (<2mm) soil samples were fractionated using a combination of density (ρ), particle-size separation (μm), and acid hydrolysis (Baldock et al., 1992; Oades, 1987; Ryan, 1990) to yield the following fractions: the light fraction ($\rho<1$ g cm^{-3} and >53μm), the Sand fraction ($\rho>1$ g cm^{-3} and >53μm), the Silt + Clay fraction (<53μm), and the acid-unhydrolyzable residue (AUR) fraction, which is the residue following digestion with H_2SO_4. Total C and N analyses were completed on all soil fractions by dry combustion using a Costech ECS 4010 CHNS-O Elemental Combustion System (Costech Analytical Technologies Inc., Valencia, CA). Values for C isotopic composition ($\delta^{13}C$), again on all soil fractions, were obtained on a Costech ECS 4010 CHNS-O Elemental Combustion System coupled to a Finnigan Deltaplus Advantage^{TM} Isotope Ratio Mass Spectrometer (ThermoFinnigan, Bremen Germany). Results were expressed using the δ-notation, the ‰ variation from the standard Pee Dee Belemnite (PDB) reference material, and the isotopic composition calculated from:

$$\delta^{13}C = [(R_{sample} / R_{standard}) - 1] * 1000$$

where R_{sample} and $R_{standard}$ are the ratios of $^{13}C/^{12}C$ in the sample and standard, respectively.

Samples for PLFA analysis were freeze-dried and then analyzed according to the procedure outlined in Hannam et al. (2006). Using a modified Bligh and Dyer (1959) extraction, lipids were extracted from 300 mg aliquots of freeze-dried mineral soil. A pre-packed silicic acid column (Agilent Technologies, Wilmington, NE), conditioned with 5 ml of chloroform, was used to extract polar lipids from neutral and glycolipids. Neutral fatty acids were eluted from the column by addition of chloroform and glycolipids were eluted with the addition of acetone. The polar lipids, which include the phospholipid fraction, were eluted from columns using methanol, which was then evaporated under nitrogen. Phospholipids were then subjected to mild alkaline methanolysis to form fatty acid methyl esters (FAMEs). Using an Agilent 6890 Series capillary gas chromatogaph (Agilent Technologies, Wilmington, NE) equipped with a 25m

Ultra 2 (5%-phenyl)-methylpolysiloxne column the FAMEs were separated and quantified. Hydrogen was used as the carrier gas. Peaks were identified based on bacterial fatty acid standards and MIDI peak identification software (MIDI, Inc., Newark, DE).

Fatty acids were designated as X:YωZ, with X indicating the number of carbon atoms, Y indicating the number of double bonds, and Z indicating the position of the first double bond from the aliphatic (ω) end of the molecule. The suffixes 'c' and 't' indicate cis and trans geometry. The prefixes 'i', 'a', and 'me' refer to iso, anteiso and mid-chain methyl branching, and 'cy' refers to cyclopropyl rings.

2.3. Statistical analyses

Based on a preliminary analysis of the data performed to test for differences among all restoration ages, three age groupings were selected to reflect restoration stages: 1-3, 4-6, and 7-11 years. Data corresponding to similar positions on the transect in relation to the basin were combined to yield two separate transect positions per wetland. Differences between transect positions as well as among age groups in the microbial community, as defined by the microbial indices (Table 3.2) were analyzed using a two-way ANOVA with PROC GLM (version 9.1, SAS Institute Inc.). Position will not be discussed in the remainder of this manuscript as there were no significant effects of position and no significant interactions between position and age groups. All indices were found to meet the assumptions of normality. Post-hoc comparisons were performed using the SNK test and determined to be significant at $p \leq 0.05$. Microbial community structure (PLFA) patterns in reference and restored wetland soils were further analyzed through a non-metric multidimensional scaling (NMS) ordination technique, followed by multi-response permutation procedures (MRPP), using the PC-ORD software (version 4, MjM Software Design, Gleneden Beach, OR). Ordination allows items to be graphically organized as to summarize complex relationships and then to extract dominant patterns from many possible outcomes (McCune and Grace, 2002). In particular, NMS runs

numerous iterations from which the best possible way to represent the data is reduced to either two or three dimensions, with the distance between the data points indicative of the similarity between those points. NMS does not require normal distribution of data nor does it assume a linear relationship between variables. The Sorensen (Bray-Curtis) distance was used for these analyses. All parameters were tested for significance in the NMS analysis using a multi-response permutation procedure (MRPP). MRPP is a non-parametric procedure used to determine if there are differences between 2 or more groups (McCune and Grace, 2002). Significance was determined from the output of three values: the p value, which indicates overall significance of the comparisons; the test statistic (*T*) which specifies the separation between groups, with a more negative T value indicative of stronger separation; and the agreement statistic (*A*), which indicates the within group homogeneity versus the random expectation, with a higher A value signifying higher homogeneity.

The first matrix for analysis of the microbial community data contained all PLFA measured and was expressed on a mol% basis, relativized by row, and transformed using the arcsine square-root function. The second matrix contained chosen site and soil descriptive variables in order to map these vectors over the first matrix: time since restored (yr), age group, position on transect, soil zone, textural class, wetland class, pH, and climatic data. Climatic data consisted of precipitation – potential evapotranspiration (P-PE) and effective growing degree days (EGDD) values derived from 10 km x 10 km data grids (Baier and Robertson, 1965; Canadian Climate Impact Scenarios Project, 2005). The second matrix for the microbial community data also contained parameters derived from PLFA analysis, including all calculated microbial indices. In addition, carbon characteristics such as organic carbon (OC) concentrations, C/N ratios, and $\delta^{13}C$ values for the bulk soil, light fraction (LF), sand-sized, silt-clay sized and acid-unhydrolyzable (AUR) fractions were included.

Further analysis of the data included the use of multivariate regression trees (MRT) using the R package (version 2.8.0, The R Foundation for Statistical Computing) and the mvpart library (Therneau and Atkinson, 2005). Trees were

constructed to compare the microbial community structure (PLFA) to categorical environmental variables (De'ath, 2002). In brief, MRT is a non-parametric method which can handle non-linear relationships and complex ecological datasets; it divides data into groups using least square splitting criteria based on the environmental variables so that the dissimilarity between groups is maximized and dissimilarity within groups is minimized. Each split in the MRT is represented graphically as a branch in the tree, and the length of each branch represents the variation in the data explained by that split.

The categorical environmental variables included in the MRT analysis were age groups (1-3 yrs, 4-6 yrs, 7-11 yrs and reference wetlands), position on transect, P-PE, textural class and wetland class. The species data used were the same mol% PLFA data used in the ordination as described above. The groupings of PLFAs resulting from the MRT analysis were then tested in ordination space using MRPP, as described above. Indicator species analysis was utilized to determine which species characterized the soil microbial community of grouping variables based on MRT clusters (McCune and Grace, 2002). Finally, these indicator values were tested for significance using a randomized Monte Carlo technique.

3. Results

Analysis of the individual microbial indices (Table 3.2) by age groups showed significant differences between the reference sites, and the 1-3 yr and 4-6 yr restored sites. Specifically, the reference sites had higher values for PLFA biomass (calculated based on the sum of 96 PLFAs), evenness and diversity (Figure 3.2). No significant differences were found between the older restored sites (7-11 yrs) and the reference sites for any of the indices calculated. Although no significant difference was found in PLFA richness between age groups the graph shows an increasing trend with age. A significant difference in the percent of actinomycetes was found between the reference sites and the 4-6 yr age grouping, with the 4-6 yr age grouping having a significantly higher percentage of actinomycetes than the reference sites ($p=0.009$). No other significant differences

among age groups were found for any of the other microbial indices calculated, including those indicative of stress and sulfate reducing or anaerobic bacteria.

MRTs with endings of five groups were consistently produced with multiple cross validations and explained 54% of the variation in the PLFA data (Figure 3.3). After 1000 cross validations, trees with five branches occurred 294 times. The first branching of the MRT was caused by the climatic factor precipitation – potential evapotranspiration (P-PE), which explained 23% of the variation. Groups 1 and 2 consisted of the "wet" sites, with the P-PE ranging from -205 mm to -252 mm. These groups in the "wet" sites were split further by age groups, which explained 29% of the variation and divided between sites 1-3 yrs and 4-6 yrs restored, and 7-11 yrs restored and reference sites. The "dry" sites consisted of groups 3, 4 and 5 with the P-PE ranging from -265 mm to -401 mm. The groupings in the "dry" sites were split further by textural class, which explained 26% of the variation and separated the clay loam (average of 33% sand and 31% clay) and silt loam (average of 32% sand and 12% clay) soils from the loamy textured soils (average of 42% sand and 21% clay). Finally, the last split of the loamy soils was due to age groups, which explained 58% of the variation and divided the 1-3 and 4-6 yr restored sites from the 7-11 yr and reference sites.

The NMS ordination of microbial community structure produced a three-dimensional solution with a final stress of 10.3 after 135 iterations (Figure 3.4). Axis 2 (18%) and 3 (35%) represented the strongest correlation structure in the data and provided the most interpretable ordination. The MRPP analysis for time since restoration (Table 3.3) showed both strong separation between groups and strong within group homogeneity (T=-9.67, A=0.11, $p = <10^{-8}$). Significant differences in microbial community structure between sites of varying time since restoration were found (Table 3.3), with the reference and older sites (9, 10, and 11 yrs) grouping closer in ordination space, and farther away from the younger sites (3 yrs). This particular ordination graph was not included here as a clearer interpretation of the data set occurred after inclusion of groups derived from the MRT (Figure 3.4). When the MRPP was re-run on the groupings from the MRT, larger significant differences were found (Table 3.3). In particular, the largest

difference based on the MRPP was associated with the climatic variable corresponding to precipitation – potential evapotranspiration (P-PE), which had the lowest T value (-17.98), highest A value (0.14) and most highly significant p-value ($1.0*10^{-6}$). A significant difference was also found within the soil microbial community of the "wet" sites (P-PE ranging from -205 mm to -252 mm), as shown on Figure 3.4, between Group 1, the younger restored sites (1-3 and 4-6 yrs) and Group 2, the older restored (7-11 yrs) and reference sites. In addition, a significant difference was found based on textural class with Group 3 (clay loam and silt loam textures), separating from the loamy textured Groups 4 and 5.

The relationships of the microbial community structure to environmental conditions including pH and soil carbon characteristics was interpreted through a correlation analysis, where vectors were considered significant at r^2=0.4 (Figure 3.4). These vectors included the organic carbon (OC) concentrations of the sand fraction, which was associated with the "wet" sites (P-PE > -252 mm), while the $\delta^{13}C$ (13C) of the sand and silt+ clay fractions, and pH were associated with the "dry" sites (P-PE < -265 mm).

Strong indicator species association values were found for all of the major branches indicated in the MRT (Table 3.4). The primary separation in the MRT, P-PE, was found to have two biomarkers with strong indicator species association values for the "dry" sites, one of actinomycetal origin (10ME18:0), and one of fungal origin (18:2ω6:9c). Within the "wet" sites, an indicator value associated with Gram (-) bacteria (14:1ω5c) and an indicator value for fungi (18:2ω6:9c) was strongly associated with the older restored (7-11 yrs) and reference sites. The same two biomarkers that were strongly associated with the "dry" sites in the primary P-PE branching, one of actinomycetal origin (10ME18:0), and one of fungal origin (18:2ω6:9c), were also found to be strongly associated with the clay loam and silt loam branch of the MRT.

4. Discussion

Distinct differences in microbial community composition between reference and restored wetlands of varying ages were found in this study. Results

from the PLFA analysis show that the microbial community in restored wetland riparian soils approaches that of reference wetland riparian soils after seven years in terms of PLFA biomass, diversity, evenness, and richness (Figure 3.2). Total PLFA biomass has previously been found to correlate positively with time since restoration in a study comparing a virgin prairie grassland with a recently (7 yr) and older (24 yr) restored agricultural site (McKinley et al., 2005). Total PLFA biomass also was reported to be higher in reference soils and those that experienced lower levels of anthropogenic disturbance (Peacock et al., 2001a). Similarly, microbial diversity and evenness were also found to be higher in an older created wetland (8 yr) when compared with 1 to 5 year old created wetlands (Ahn and Peralta, 2009).

Results from the MRT analysis indicated that the primary influence over the soil microbial community composition was the climatic variable, P-PE (Figure 3.3). Specifically, sites that had more negative P-PE values were significantly different from the sites with less negative P-PE values, indicating that significant differences existed in the hydrologic conditions at the sites. Moisture is a well known determinant of soil microbial community composition as it regulates the rates of aerobic or anaerobic processes in the soil and the resulting community structure (Gutknecht et al., 2006). Two specific biomarkers, one of actinomycetal origin (10ME18:0) and one of fungal origin (18:2ω6:9c) were found to be indicator species for the "dry" sites, or those sites with more negative P-PE values. Flooding has been shown to decrease the abundance of fungi in an agricultural field (Bossio and Scow, 1998) and in a freshwater wet prairie ecosystem (Mentzer et al., 2006). The finding in the present study, that fungi are more abundant in drier ecosystems, mirrors findings in the studies mentioned above (Bossio and Scow, 1998; Mentzer et al., 2006). Fungi are also known to withstand higher levels of osmotic stress than bacteria in tallgrass prairie soils (McKinley et al., 2005), thus making them more likely to prevail in dry environments. Furthermore, a significantly higher abundance of actinomycetes was found in the 4-6 year restored riparian soils, compared with the reference riparian soils (Figure 3.2). Peacock et al. (2001a) similarly found that heavily

trafficked areas contained more PLFAs associated with actinomycetes and attributed this to their ability to grow conidia and thus survive harsh soil conditions. The lower P-PE values found at the sites with actinomycete biomarkers as indicator species (Figure 3.3) are indeed consistent with a harsher soil environment. Further, we would expect that the harshest soil environmental conditions, increased heat and desiccation, would be demonstrated at the younger (1-3 yrs and 4-6 yrs) restored sites as hydrologic conditions, plant community and soil chemical properties may not have yet recovered from the agricultural disturbance.

The branch in the MRT that included all the "dry" sites was further divided into two leaves separated based on texture. It was found that clay loam and silt loam soils had significantly different microbial communities than the loamy soils (Figure 3.3). Further investigation using indicator species analysis (ISA) found that an actinomycetal biomarker (10ME18:0) and a fungal biomarker (18:2ω6:9c) were strong indicators for the clay loam and silt loam textured soils. Although the groups 3, 4 and 5 in the MRT are all considered "dry", the increased desiccation due to the higher percentage of sand in the loam soil has seemingly resulted in the microbial community reaching an upper limit of tolerance of desiccation and heat, as both fungi and actinomycete are able to withstand these harsh conditions better than other microbes.

Within the "wet" sites, the microbial community was split further to show significant differences between the younger restored sites (1-3 and 4-6 yrs), and the older restored (7-11 yrs) and reference sites (Figure 3.3). This supports our hypothesis that with increasing time since restoration the microbial community became more similar to the reference microbial community. Microbial communities have been reported to be significantly different between early and late successional wetlands (D'Angelo et al., 2005), between virgin prairie, restored prairie and agricultural sites (McKinley, 2001), and between agricultural sites, recent and long-term restorations and virgin prairie sites (McKinley et al., 2005). Two biomarkers, a gram negative bacterial biomarker (14:1ω5c) and a fungal biomarker (18:2ω6:9c), were found to be strong indicator species for the

older restored (7-11 yrs) and reference sites (Table 3.4). In contrast to our findings, D'Angelo et al. (2005) found that the same indicators of gram negative bacteria and fungi, both representative of aerobic microbial communities, were both more abundant in early successional wetlands (<10 years since mitigation) and attributed this to oxygen availability being higher at the early sites compared to the late successional wetlands, which experienced higher instances of anaerobiosis. This suggests that differences observed in our study between the younger restored sites, and the older restored and reference sites (Figure 3.3) were not primarily influenced by the hydrologic conditions at the sites. Instead, the longer period without disturbance, found in the 7-11 yr sites, and the absence of disturbance found in the reference sites may be the dominating influence. Lower levels of Gram negative bacterial populations have been associated with soils more recently subjected to disturbance (Peacock et al., 2001a) and disturbance is known to be extremely detrimental to fungal populations (Mummy et al., 2002).

The NMS ordination of the soil microbial community (Figure 3.4) showed that the vectors for pH and $\delta^{13}C$ were strongly correlated with the "dry" and younger restored sites (1-3 and 4-6 yrs). In our study, differences in $\delta^{13}C$ may be attributable to differences in decomposition processes since litter inputs did not differ among sites. The ^{13}C enrichment of soil organic matter during decomposition has been linked to discrimination against ^{13}C during the catabolic breakdown of organic substrates by soil microbes and/or accumulation of ^{13}C in microbial biomass and in humic substances of microbial origin (Quideau et al., 2003; Hannam et al., 2005). Hence, the higher $\delta^{13}C$ values (i.e.; more enriched in the heavier isotope) in the "dry" and younger restored sites may indicate organic material at a more advanced stage of decomposition. The more highly decomposed material and lower carbon availability at these sites hence could have impacted the structure of the microbial community, which was also lower in biomass and lower in diversity (Figure 3.2). Microbial biomass has been found to decrease with increasing acidity in an agricultural setting because most bacteria have pH optima between 6.0 and 7.5 (McKinley, 2001). However, in our study, the higher microbial biomass occurs in the "wet" sites which are lower in pH (5.8)

than the “dry” sites (pH=6.6). It is likely that a combination of soil moisture, pH and carbon availability impacted the microbial community, with not just one factor being predominant. Also, it is interesting to note that actinomycetes, an indicator species for the “dry” sites, have been found to tolerate soils with higher pH values and be sensitive to soil acidity (Alexander, 2005; Frostegard et al., 1993; Pennanen, 2001).

Composition of the soil microbial community at the older restored (7-11 yrs) and reference sites was correlated with higher OC concentrations for the sand fraction (Figure 3.4). It has been well documented that cultivation practices degrade soil aggregates and accelerate microbial activity therefore increasing the turnover of soil carbon (Golchin et al., 1995). Numerous studies have shown that with longer periods without disturbance, agricultural land allowed to revert back to natural vegetation as well as restored wetlands that were previously drained exhibit an increase in soil organic carbon (Bruland and Richardson, 2006; D'Angelo et al., 2005; Galatowitsch and van der Valk, 1996; Lopes de Gerenyu et al., 2008; Schnitzer et al., 2006). In agreement with the carbon vectors discussed above (Figure 3.4), gram-negative bacteria have been found to increase with an accumulation of soil carbon (Peacock et al., 2001b). In our study, the gram-negative bacteria (14:1ω5c) was found to be an indicator species for the older restored and reference sites (Table 3.4).

5. Conclusions

In this study, the examination of the soil microbial community indicated that there were distinct differences between the younger (1-3 yrs, 4-6 yrs) restored wetland riparian soils and the older restored (7-11 yrs) and reference soils. These differences were apparent even with significant influences of climate (P-PE) and texture. Specifically, the microbial communities of the older restored soils and the reference soils were found to have similar PLFA biomass, evenness, and diversity. Similar PLFA indicator species were found in the older restored and reference sites, demonstrating that the wetland ecosystem begins to recover within this time period and overcome the effects of agricultural disturbance. On the other

hand, the distinct dissimilarity between the younger restored soils and the reference soils indicates the need for further investigation into the ecological differences between the soils to determine what management changes could potentially be made to accelerate the restoration process.

CHAPTER III: TABLES AND FIGURES

Table 3. 1. Soil classification, climatic variables and time since restoration for the study sites.

Province	Site Name	Latitude, Longitude (°N, °W)	Canadian soil Classification†	USDA Classification‡	Time Since Restoration (yr)	Age Class (yrs)	P-PE § (mm)	EGDD¶
Alberta	Ambler	53.09, -113.20	Black Chernozem	Udic Borolls	9	7-11	-205	1294
	Boyden	52.07, -113.18	Black Chernozem	Udic Borolls	11	7-11	-265	1242
	Ferleyko	53.54, -112.27	Black Chernozem	Udic Borolls	3	1-3	-233	1264
	Kemo	52.07, -113.18	Black Chernozem	Udic Borolls	5	4-6	-265	1242
	Loveseth	53.16, -111.50	Black Chernozem	Udic Borolls	4	4-6	-243	1270
	Maruschak	53.18, -113.12	Black Chernozem	Udic Borolls	11	7-11	-205	1294
	Mittlestadt	53.18, -113.13	Black Chernozem	Udic Borolls	11	7-11	-205	1294
	Rauser	53.11, -111.46	Black Chernozem	Udic Borolls	10	7-11	-243	1270
Saskatchewan	Adams	51.02, -101.87	Black Chernozem	Udic Borolls	7	7-11	-290	1337
	Crone	52.26, -104.71	Black Chernozem	Udic Borolls	5	4-6	-290	1352
	Hartt	53.08, -104.54	Dark Gray Chernozem	Boralfic Borolls	8	7-11	-306	1318
	Peters	50.62, -106.93	Brown Chernozem	Aridic Borolls	8	7-11	-401	1483
	Sprig	50.46, -106.33	Brown Chernozem	Aridic Borolls	8	7-11	-400	1481
	Tataryn	51.21, -103.12	Black Chernozem	Udic Borolls	3	1-3	-265	1307
Manitoba	Graham	50.21, -99.74	Black Chernozem	Udic Borolls	3	1-3	-252	1341

†Agriculture Canada (1988abc)
‡ Soil Survey Staff (2006)
§ Precipitation – potential evapotranspiration
¶ Effective growing degree days

Table 3. 2. Microbial indices.

Indices	PLFA marker
Total Biomass	Sum of all fatty acids, in nmol/g soil (DW)
Diversity	Shannon Index (H´), $\Sigma(p_i(\ln p_i))$
Richness	Total number of non-zero PLFAs with total carbon length <20
Evenness	Measure of the variability in abundance of different PLFAs within a sample, Diversity/ln(Richness)
% Gram (+)[a,b,c]	a11:0, i14:0, i15:0, a15:0, i16:0, a17:0, i17:0/ Total Biomass
% Gram (-)[a,b,d,e]	14:1ω5c, 16:1ω5c, 16:1ω9c, 18:1ω5c, 18:1ω7c, 18:1 9c/ Total Biomass
% Fungi[f,g,h]	18:2ω6,9c/ Total Biomass
% Actinomycetes[i]	10Me18:0/ Total Biomass
Sulfate reducing bacteria[j,k]	10 Me16:0/ Total Biomass
Sulfate reducing bacteria[l]	17:1ω8/ Total Biomass
Anaerobic bacteria[m]	cy19/ Total Biomass
Fungal/Bacterial biomass[i,n,o]	18:2ω6,9c / i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω5, i17:0, a17:0, cy17:0, 17:0, 18:1ω7, and cy19:0
Gram (+)/Gram (-)[p]	a11:0, i14:0, i15:0, a15:0, i16:0, a17:0, i17:0 / 14:1ω5c, 16:1ω5c, 16:1ω9c, 18:1ω5c, 18:1ω7c, 18:1ω9c
iso to anteiso[p]	i15:0, i17:0/a15:0, a17:0

[a] Mentzer et al. (2006)
[b] O'Leary and Wilkinson (1988)
[c] D'Angelo et al. (2005)
[d] Sundh et al. (1997)
[e] Wilkinson (1988)
[f] Lechevalier and Lechevalier (1988)
[g] Frostegard and Baath (1996)
[h] Zelles (1999)
[i] Frostegard et al. (1993)
[j] Dowling et al. (1986)
[k] Parkes and Taylor (1983)
[l] Bossio and Scow (1998)
[m] Guckert et al. (1985)
[n] Tunlid et al. (1989)
[o] Degrood et al. (2005)
[p] McKinley et al. (2005)

Table 3. 3. Multi-response permutation procedure (MRPP) results for microbial community structure based on PLFA analysis, including groupings based on MRT results reported in Figure 3.3.

Comparison		T†	A‡	P§
Time Since Restored		-9.67	0.11	$<10^{-8}$
MRT group				
P-PE¶	1,2 vs. 3,4,5	-17.98	0.14	$<10^{-6}$
Age Group	1 vs. 2	-8.61	0.13	$7.5 * 10^{-5}$
Texture	3 vs. 4,5	-5.73	0.086	$1.4 * 10^{-3}$

† Test statistic (*T*) which specifies the separation between groups
‡ Agreement statistic (*A*), which indicates the within group homogeneity versus the random expectation
§ Indicates overall significance of the comparisons
¶ Precipitation – potential evapotranspiration

Table 3. 4. PLFA indicator species associated with the multivariate regression tree (MRT) groups as reported in Figure 3.3.

P-PE‡			Indicator value		Monte Carlo ($p < 0.05$)
PLFA	Origin	Mean†	MRT Group		
			1,2	3,4,5	
10ME18:0	actinomycete	52.2(1.77)	38	62	0.0002
18:2ω6:9c	fungi	52.8(2.18)	38	62	0.001
Age groups			Indicator value		Monte Carlo ($p < 0.05$)
PLFA	Origin	Mean	MRT Group		
			1	2	
14:1 ω5c	Gram (-)	49.3(4.97)	13	73	0.0002
18:2ω6:9c	fungi	54.3(3.15)	29	71	0.0002
Texture			Indicator value		Monte Carlo ($p < 0.05$)
PLFA	Origin	Mean	MRT Group		
			3	4,5	
10ME18:0	actinomycete	52.9(2.31)	63	37	0.0006
18:2ω6:9c	fungi	53.8(2.84)	65	35	0.0006

† Means and standard deviations in parentheses are based on Monte Carlo test of observed indicator values for each species based on 1000 randomizations (McCune and Grace 2002).
‡ Precipitation – potential evapotranspiration

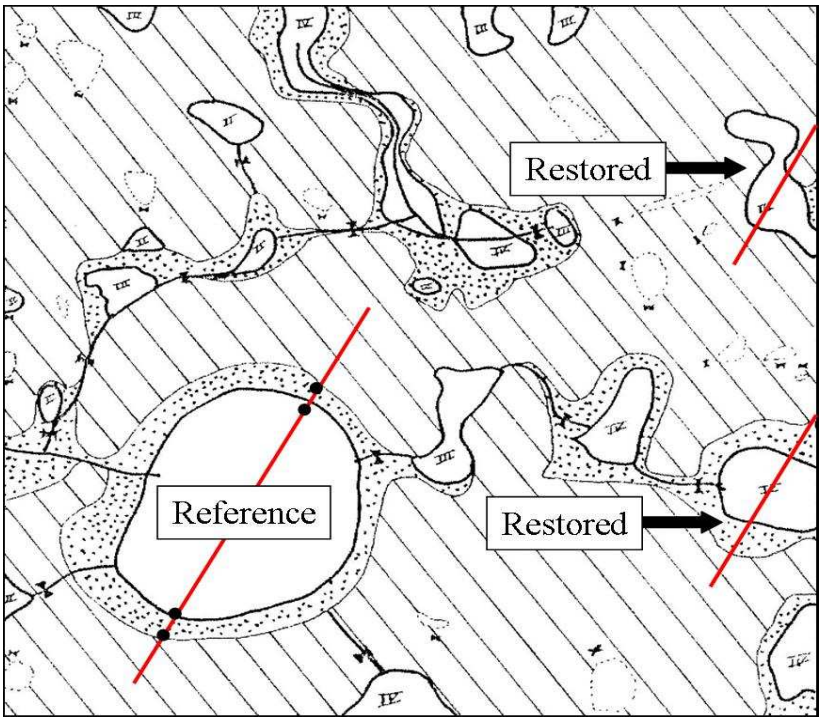

Figure 3. 1. Detailed map of a site, showing the reference wetland and two restored wetlands of the same age, the transects established across each wetland and the possible sampling points (as shown at the reference wetland). All wetlands at a site occur on one quarter section of land (160 acres or 2.59 km^2), with the average size of the basins generally < 2 ha.

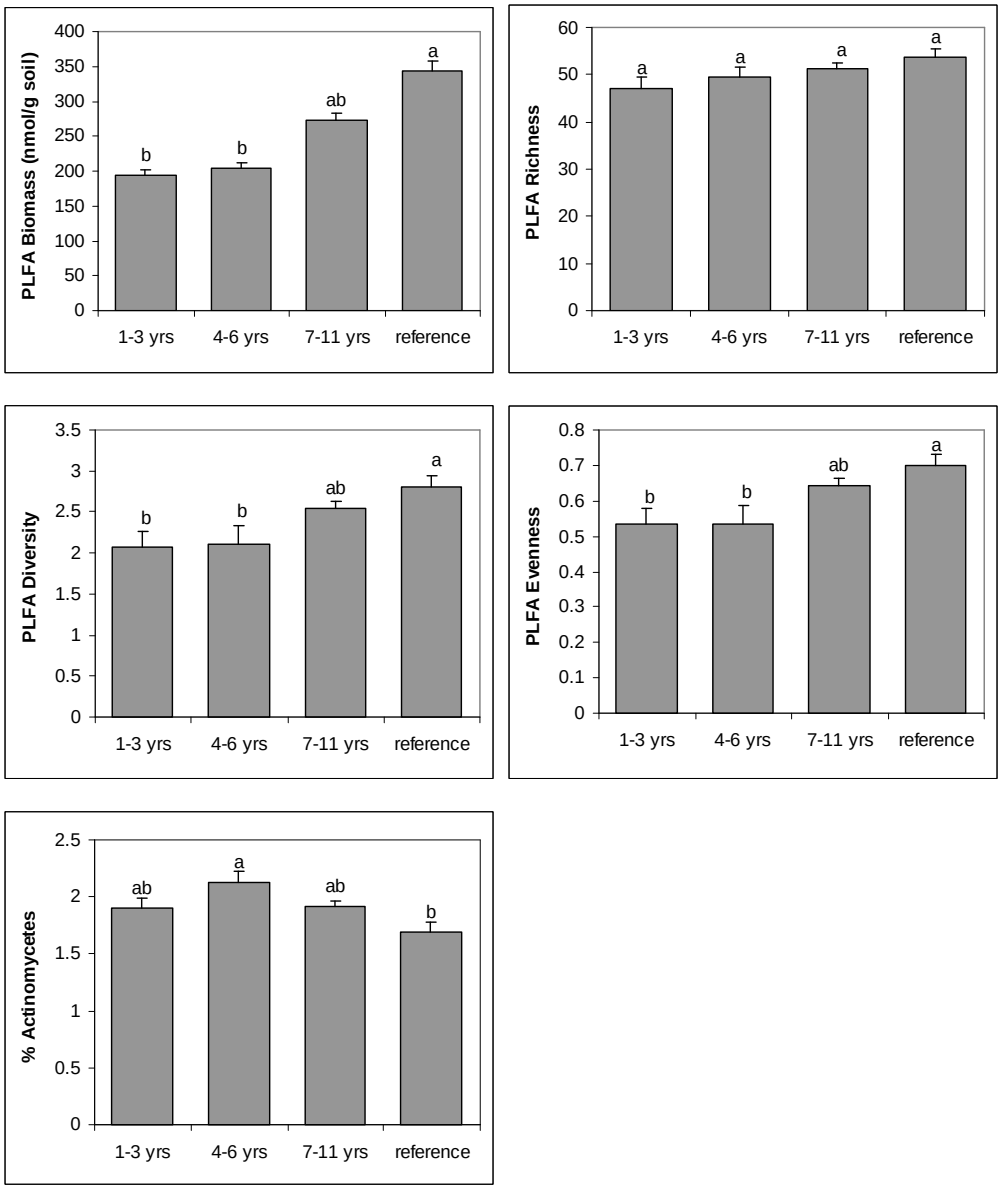

Figure 3. 2. Graphs of PLFA indices (mean and standard error bars with n=8 for 1-3 yrs, n= 11 for 4-6 yrs, n= 31 for 7-11 yrs, and n=25 for Reference sites) comparing the restored wetland soils of varying times since restoration and reference wetland soils. Bars with the same letter are not significantly different based on SNK test ($P<0.05$).

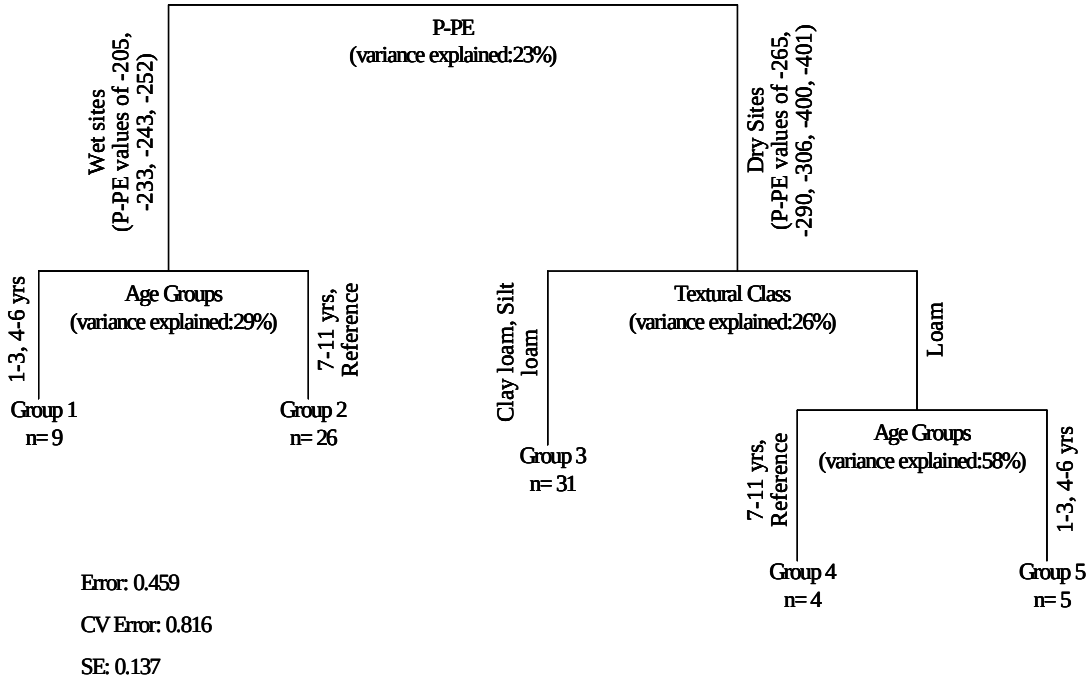

Figure 3. 3. Multivariate regression tree of mol% PLFA data from riparian soils of restored and reference wetlands. Groups are combinations of age groups (1-3 yrs, 4-6 yrs, 7-11 yrs, reference), climate (precipitation- potential evapotranspiration) and textural class.

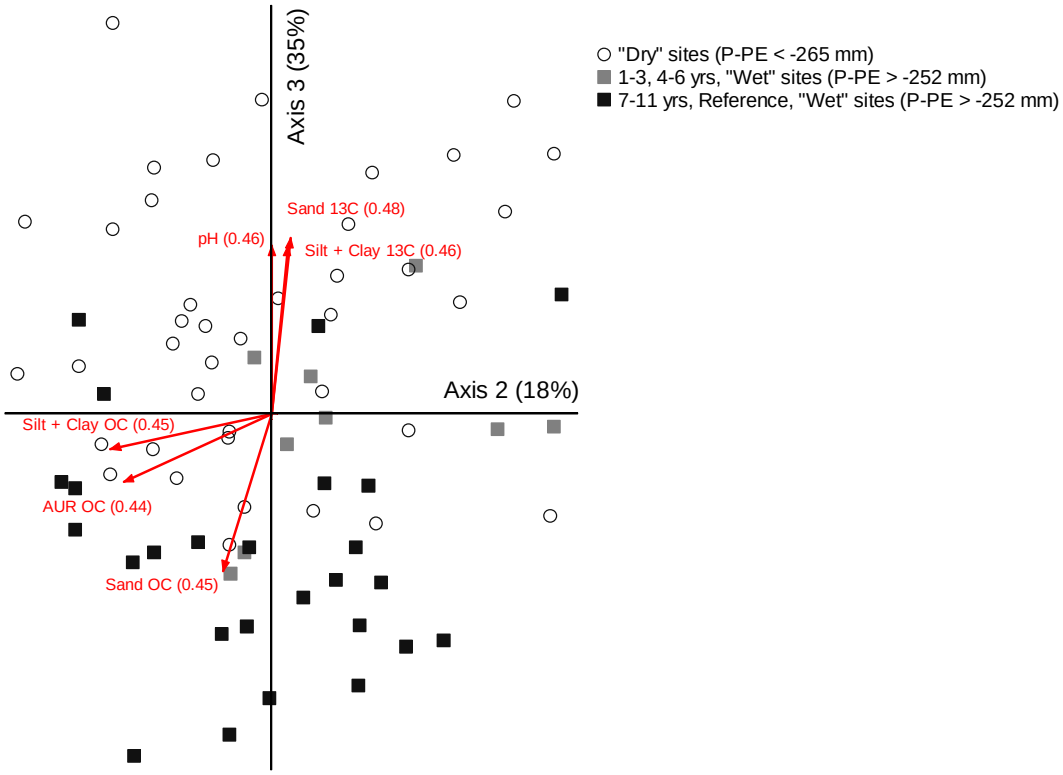

Figure 3. 4. NMS ordination of wetland riparian soil PLFAs delineated by clusters based on results from the multivariate regression tree (Figure 3.3). Ordination points labeled as ○ and Δ represent the "dry" sites (Groups 3,4,5) and points labeled as ■ and ■ represent the "wet" sites (Groups 1 and 2). Dry sites are those with P-PE (Precipitation – potential evapotranspiration) < -265 mm. Carbon vectors include $\delta^{13}C$ values and OC (organic carbon) values of various fractions.

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IV. SYNTHESIS

1.1. Objectives and experimental approach

This study investigated the return of elements of functionality, decomposition processes and microbial community composition by comparing restored riparian soils of varying ages since restoration (3-11 yrs) to reference riparian soils which were intended to approximate a target ecosystem. Decomposition processes, as indicated by soil organic matter quality and quantity, were characterized by measuring C concentration and distribution, ^{13}C, and C/N in the light (LF), Sand, Silt + Clay and AUR fractions, which were isolated using a combination of density and particle-size separation techniques. The composition of material in the light fraction was further quantified using solid state ^{13}C nuclear magnetic resonance (NMR) spectroscopy Measurements of soil microbial biomass, evenness, diversity, and richness as well as measurements of specific microbial indices were obtained using phospholipid fatty acid (PLFA). Differences between restored and reference soils were statistically analyzed using non-metric multidimensional scaling (NMS), multivariate regression trees (MRT) and indicator species analysis (ISA).

1.2. Soil organic matter quantity and quality

Using NMR spectroscopy, we were unable to detect any differences between reference and restored riparian soils. Climatic variables were the primary influence over the composition of organic materials, with the hotter and drier soils of the Brown Chernozems containing higher amounts of Alkyl C than the cooler and more moist soils in the Black Chernozems, which contained higher amounts of Aromatic and Phenolic C. In this study we were able to differentiate between reference and restored soils based on levels of the organic C concentration, where in the bulk, Sand, Silt + Clay, and AUR fractions reference sites contained higher amounts of organic C than the younger (1-3 yr since) restored soils. The younger restored soils (1-3 yrs) showed significantly lower C concentrations than the reference soils in the bulk samples and both size separates (Sand and Silt + Clay).

There also was an enrichment in ^{13}C in the LF and acid-unhydrolyzable residue (AUR) fractions of these younger restored soils compared to the older restored (7-11 yrs) and reference soils, suggesting that this material was more decomposed. Total C concentrations in the restored soils increased with time. Older restored soil (7-11 yrs after restoration) had amounts of organic C in the bulk and sand fractions comparable to reference soils, but significantly less in the AUR fractions, indicating that a longer time period is needed for these C levels to reach that of reference soils.

1.3. Soil microbial community structure

The microbial community of the younger restored soils (1-3 and 4-6 yrs) was found to differ significantly from the reference soils, with the reference soils having higher microbial biomass, evenness, and diversity. Richness was found to have an increasing trend with age. Composition of the older restored soils (7-11 yrs) was comparable to that of the reference soils. Results indicated the importance of climatic factors (P-PE and EGDD) in explaining the variation found in the soil microbial communities. Specifically, drier sites had strong indicator species values associated with PLFAs of actinomycetal origin and fungal origin. Within the wetter sites, the older restored sites (7-11 yrs) and reference sites had strong indicator species values associated with PLFAs of Gram negative bacteria and fungal origin. Investigation of the relationship of the microbial community structure to carbon characteristics (total carbon content, C/N and $\delta^{13}C$) of the distinct carbon pools in the soils revealed that the vectors for pH and $\delta^{13}C$ were strongly correlated with the “dry” and younger restored sites (1-3 and 4-6 yrs). Composition of the soil microbial community at the older restored (7-11 yrs) and reference sites was correlated with higher OC concentrations for the sand fraction.

1.4. Project limitations and future research

The primary objective of this study was not to deem the restorations as successful or not, but to examine the return of elements of functionality at the soil

C and microbial levels. This study supports a shift in wetland ecology and management to a more ecosystem based perspective which focuses on restoring ecosystem processes (Euliss Jr. et al., 2008). The goal of each restoration and the interpretation of "success" are defined by the stakeholders. In this case, the primary stakeholder, DUC may have determined that the restored wetlands had reached targets levels of waterfowl use. However, there needs to be a shift in the assessment of success, which should be primarily influenced by lessons from practice and by general theory from relevant disciplines (Heneghan et al., 2008), This is especially true as we now have started to acknowledge the important role PPR wetlands play in the global C cycle and C storage in terrestrial ecosystems.

In this study, we sampled once over the course of the study and this sampling occurred in 2005, which was part of a drought cycle in the prairie provinces. Therefore, the findings of this study, which were derived from a dry phase, may not be applicable to the same wetlands during a wet phase (Euliss Jr. et al., 2004). Future research should include sampling throughout the year to capture variability due to seasonal changes. There is a paucity of information on the impacts of restoration in Canadian prairie pothole wetlands and in particular the ability of these ecosystems to contribute to C storage capacity. Therefore, a more extensive study of complete transects in restored wetlands would aid in contributing to the general understanding of these ecosystems compared to reference wetlands and would contribute to the lack of data available to determine their C storage capacities. Our study was limited to a maximum of 11 years therefore a longer term study would determine if C characteristics in restored soils, specifically the distribution of C in the sand and silt + clay fractions, ever reaches that of the levels found in the reference soils. It would also contribute to a better understanding of long-term development of restored wetland ecosystems (Zedler, 2000).

With the use of NMR we were unable to detect differences linked to restoration, as climate was the primary influence, therefore, measurement of this variable is not necessary to determine the return of elements of functionality. However, PLFA indices and C concentration and distribution were useful

indicators of changes within the wetland ecosystem between age groups making the monitoring of these elements important to determine how restoration is proceeding.

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APPENDICES A. LEGAL LAND DESCRIPTION OF SITES

SITE	PROVINCE	X (LONGITUDE)	Y (LATITUDE)	LEGAL LAND DESCRIPTION
Ambler	AB	-113.1986	53.0939	SE 31-47-22 W4M
Boyden	AB	-113.1764	52.0736	SE 11-36-23 W4M
Ferleyko	AB	-112.2744	53.5392	NE 34-52-16 W4M
Kemo	AB	-113.1764	52.0664	NE 2-36-23 W4M
Loveseth	AB	-111.5047	53.1595	NW 23-48-11 W4M
Maruschak	AB	-113.1207	53.1763	NW 26-48-22 W4M
Mittlestadt	AB	-113.1263	53.1765	NE 27-48-22 W4M
Rauser	AB	-111.4607	53.1059	1= SE 06-48-10 W4M 2= SW 06-48-10 W4M
Adams	SK	-101.8675	51.0164	E 27-023-32-W1
Crone	SK	-104.7099	52.2630	SW 18-038-19-W2
Hartt	SK	-104.5428	53.0772	S 27-47-18-W2
Peters	SK	-106.9336	50.6215	N 20-19-07-W3
Sprig	SK	-106.3314	50.4597	SE 27-017-03-W3
Tataryn	SK	-103.1175	51.2111	SE 06-026-08-W2
Graham	MB	-99.7403	50.2128	SW 28-14-17-W1

APPENDICES B. RESULTS OF TWO-WAY ANOVAs

Table B. 1. Results of Two-Way ANOVAs for soil carbon characteristics

Carbon Characteristic	Soil Fraction	Age groups	Position	Age groups x position
C/N	Bulk	p=0.092	p=0.72	p=0.53
	LF	p=0.91	p=0.58	p=0.96
	Sand	p=0.0093	p=0.38	p=0.82
	Silt + Clay	p=0.20	p=0.35	p=0.92
	AUR	p=0.57	p=0.24	p=0.98
$\delta^{13}C$ ($^{0}/_{00}$)	Bulk	p=0.29	p=0.36	p=0.41
	LF	p=0.057	p=0.25	p=0.71
	Sand	p=0.055	p=0.32	p=0.49
	Silt + Clay	p=0.53	p=0.27	p=0.52
	AUR	p=0.0037	p=0.28	p=0.72
C concentration (g kg^{-1} fraction)	Bulk	p=0.0006	p=0.79	p=0.70
	LF	p=0.99	p=0.42	p=0.79
	Sand	p<0.0001	p=0.39	p=0.49
	Silt + Clay	p=0.0038	p=0.94	p=0.87
	AUR	p=0.0006	p=0.63	p=0.81

Table B. 2. Results of Two-Way ANOVAs for soil microbial indices

Soil Microbial Characteristic	Age groups	Position	Age groups x position
PLFA Biomass	p=0.0012	p=0.87	p=0.73
Diversity	p=0.0047	p=0.99	p=0.58
Richness	p=0.094	p=0.86	p=0.56
Evenness	p=0.0032	p=0.94	p=0.58
% Gram (+)	p=0.56	p=0.26	p=0.93
% Gram (-)	p=0.50	p=0.14	p=0.93
% Fungi	p=0.79	p=0.57	p=0.99
% Actinomycetes	p=0.012	p=0.24	p=0.49
Sulfate reducing bacteria (10Me16:0)	p=0.52	p=0.49	p=0.65
Sulfate reducing bacteria (17:1ω8)	p=0.59	p=0.26	p=0.69
Anaerobic bacteria (cy19)	p=0.77	p=0.55	p=0.85
Fungal/Bacterial biomass	p=0.81	p=0.62	p=0.98
Gram (+)/Gram (-)	p=0.40	p=0.20	p=0.95
iso to anteiso	p=0.0052*	p=0.39	p=0.97

*Note: Results of SNK test showed no significant differences among age groups

APPENDICES C. POTENTIAL CARBON STORAGE CAPACITY CALCULATIONS

Table C. 1. Potential carbon storage capacity for the bulk soil fraction based on a range of bulk density values as reported in Pennock et al. (2010).

	Age Groups							
	1-3 yrs		**4-6 yrs**		**7-11 yrs**		**Reference**	
Bulk density*	0.78	0.93	0.78	0.93	0.78	0.93	0.78	0.93
g C/m^2	2227.68	2656.08	2630.16	3135.96	3421.08	4078.98	5035.68	6004.08
Mg C/ ha	22.28	26.56	26.30	31.36	34.21	40.79	50.36	60.04

*Pennock, D., T. Yates, A. Bedard-Haughn, K. Phipps, R. Farrell, and R. McDougal. 2010. Landscape controls on N_2O and CH_4 emissions from freshwater mineral soil wetlands of the Canadian Prairie Pothole region. Geoderma 155:308-319.

APPENDICES D. GENERAL PROPERTIES OF SAMPLES

Table D. 1. Regional and site specific properties of samples.

					Regional Setting			Site Specific	
Sample	Site name	Years Since Restoration	Wetland	Position on Transect	Soil Zone	P-PE	EGDD	Textural Class	pH
Adams C-4	Adams	Reference	Reference	4	Black	-290	1337	CL	7.78
Amb C-4/9	Ambler	Reference	Reference	4/9	Black	-205	1294	SiL	6.4
Amb C-5/8	Ambler	Reference	Reference	5/8	Black	-205	1294	SiL	6.7
Boy C-4/9	Boyden	Reference	Reference	4/9	Black	-265	1242	CL	5.92
Boy C-5/8	Boyden	Reference	Reference	5/8	Black	-265	1242	SiL	5.4
Crone C-2/11	Crone	Reference	Reference	4/9	Black	-290	1352	L	7.13
Crone C-3/10	Crone	Reference	Reference	5/8	Black	-290	1352	L	6.59
Fer C-4/9	Ferleyko	Reference	Reference	4/9	Black	-233	1264	SiL	5.21
Fer C-5	Ferleyko	Reference	Reference	5	Black	-233	1264	SiL	4.63
Graham C-4/9	Graham	Reference	Reference	4/9	Black	-252	1341	SiCL	7.58
Graham C-5	Graham	Reference	Reference	5	Black	-252	1341	SiL	6.82
Hartt C-4/9	Hartt	Reference	Reference	4/9	Dark Gray	-306	1318	SiL	6.97
Kem C-4/9	Kemo	Reference	Reference	4/9	Black	-265	1242	CL	6.52
Kem C-5/8	Kemo	Reference	Reference	5/8	Black	-265	1242	CL	6.76
Lov C-4/9	Loveseth	Reference	Reference	4/9	Black	-243	1270	L	7.08
Mar C-4/9	Maruschak	Reference	Reference	4/9	Black	-205	1294	SiC	5.89
Mar C-5/8	Maruschak	Reference	Reference	5/8	Black	-205	1294	SiL	5.64
Mitt C-4/9	Mittlestadt	Reference	Reference	4/9	Black	-205	1294	SiC	5.22
Mitt C-5/8	Mittlestadt	Reference	Reference	5/8	Black	-205	1294	Si	5.11
Pet C-4/9	Peters	Reference	Reference	4/9	Brown	-401	1483	CL	7.18
Pet C-5/8	Peters	Reference	Reference	5/8	Brown	-401	1483	CL	7.12
Rau C-3	Rauser	Reference	Reference	4	Black	-243	1270	L	6.84
Sprig C-4/9	Sprig	Reference	Reference	4/9	Brown	-400	1481	CL	7.19
Sprig C-5/8	Sprig	Reference	Reference	5/8	Brown	-400	1481	CL	7.2

Table D.1. (con't) Regional and site specific properties of samples.

					Regional Setting			Site Specific	
Sample	Site name	Years Since Restoration	Wetland	Position on Transect	Soil Zone	P-PE	EGDD	Textural Class	pH
Tat C-4/9	Tataryn	Reference	Reference	4/9	Black	-265	1307	CL	7.39
Fer R1-4	Ferleyko	3	Restored 1	4	Black	-233	1264	SiL	4.52
Fer R1-5	Ferleyko	3	Restored 1	5	Black	-233	1264	SiL	5.28
Fer R2-5/8	Ferleyko	3	Restored 2	5/8	Black	-233	1264	SiL	6.2
Graham R1-4/9	Graham	3	Restored 1	4/9	Black	-252	1341	SiCL	7.22
Graham R1-5/8	Graham	3	Restored 1	5/8	Black	-252	1341	SiL	7.12
Graham R2-4/9	Graham	3	Restored 2	4/9	Black	-252	1341	SiCL	7.12
Tat R1-4/9	Tataryn	3	Restored 1	4/9	Black	-265	1307	CL	7.13
Tat R1-5/8	Tataryn	3	Restored 1	5/8	Black	-265	1307	L	7
Lov R1-4/9	Loveseth	4	Restored 1	4/9	Black	-243	1270	L	5.58
Lov R1-5/8	Loveseth	4	Restored 1	5/8	Black	-243	1270	CL	6.19
Lov R2-3/10	Loveseth	4	Restored 2	4/9	Black	-243	1270	L	6.68
Crone R1-4/9	Crone	5	Restored 1	4/9	Black	-290	1352	L	7.08
Crone R1-5/8	Crone	5	Restored 1	5/8	Black	-290	1352	L	7.13
Crone R2-4/9	Crone	5	Restored 2	4/9	Black	-290	1352	L	6.61
Crone R2-5/8	Crone	5	Restored 2	5/8	Black	-290	1352	L	7.32
Kem R1-4/9	Kemo	5	Restored 1	4/9	Black	-265	1242	CL	5.6
Kem R1-5/8	Kemo	5	Restored 1	5/8	Black	-265	1242	CL	5.28
Kem R2-4/9	Kemo	5	Restored 2	4/9	Black	-265	1242	CL	5.38
Kem R2-8	Kemo	5	Restored 2	8	Black	-265	1242	CL	5.23
Adams R2-4	Adams	7	Restored 2	4	Black	-290	1337	CL	7.25
Hartt R1-4/9	Hartt	8	Restored 1	4/9	Dark Gray	-306	1318	SiL	7.04
Hartt R1-5/8	Hartt	8	Restored 1	5/8	Dark Gray	-306	1318	CL	7.45
Hartt R2-4/9	Hartt	8	Restored 2	4/9	Dark Gray	-306	1318	SiL	7.2

Table D.1. (con't) Regional and site specific properties of samples.

					Regional Setting			Site Specific	
Sample	Site name	Years Since Restoration	Wetland	Position on Transect	Soil Zone	P-PE	EGDD	Textural Class	pH
Hartt R2-5/8	Hartt	8	Restored 2	5/8	Dark Gray	-306	1318	CL	7.25
Pet R1-4/9	Peters	8	Restored 1	4/9	Brown	-401	1483	CL	6.46
Pet R1-5/8	Peters	8	Restored 1	5/8	Brown	-401	1483	CL	6.69
Pet R2-4/9	Peters	8	Restored 2	4/9	Brown	-401	1483	CL	6.84
Pet R2-5/8	Peters	8	Restored 2	5/8	Brown	-401	1483	CL	6.98
Sprig R1-4/9	Sprig	8	Restored 1	4/9	Brown	-400	1481	CL	6.68
Sprig R1-5/8	Sprig	8	Restored 1	5/8	Brown	-400	1481	CL	6.67
Sprig R2-4/9	Sprig	8	Restored 2	4/9	Brown	-400	1481	CL	6.75
Sprig R2-5/8	Sprig	8	Restored 2	5/8	Brown	-400	1481	CL	5.88
Amb R1-4/9	Ambler	9	Restored 1	4/9	Black	-205	1294	SiL	5.41
Amb R1-5/8	Ambler	9	Restored 1	5/8	Black	-205	1294	SiL	5.44
Amb R2-4/9	Ambler	9	Restored 2	4/9	Black	-205	1294	SiL	5.77
Amb R2-5/8	Ambler	9	Restored 2	5/8	Black	-205	1294	SiL	5.19
Rau R1-10	Rauser	10	Restored 1	9	Black	-243	1270	L	7.22
Rau R2-3/10	Rauser	10	Restored 2	4/9	Black	-243	1270	L	5.92
Rau R2-4/9	Rauser	10	Restored 2	5/8	Black	-243	1270	CL	5.6
Boy R1-4/9	Boyden	11	Restored 1	4/9	Black	-265	1242	L	5.65
Boy R1-5/8	Boyden	11	Restored 1	5/8	Black	-265	1242	SiL	5.17
Boy R2-4/9	Boyden	11	Restored 2	4/9	Black	-265	1242	L	6.06
Boy R2-5/8	Boyden	11	Restored 2	5/8	Black	-265	1242	SiL	5.28
Mar R1-4/9	Maruschak	11	Restored 1	4/9	Black	-205	1294	SiC	4.83
Mar R1-5/8	Maruschak	11	Restored 1	5/8	Black	-205	1294	SiL	4.94
Mar R2-5/8	Maruschak	11	Restored 2	5/8	Black	-205	1294	SiL	4.85
Mitt R1-4/9	Mittlestadt	11	Restored 1	4/9	Black	-205	1294	SiC	4.93

Table D.1. (con't) Regional and site specific properties of samples.

					Regional Setting			Site Specific	
Sample	Site name	Years Since Restoration	Wetland	Position on Transect	Soil Zone	P-PE	EGDD	Textural Class	pH
Mitt R1-5/8	Mittlestadt	11	Restored 1	5/8	Black	-205	1294	Si	5.16
Mitt R2-4/9	Mittlestadt	11	Restored 2	4/9	Black	-205	1294	SiC	5.2
Mitt R2-5/8	Mittlestadt	11	Restored 2	5/8	Black	-205	1294	Si	4.94

APPENDICES E. SOIL CARBON PROPERTIES OF SAMPLES

Table E. 1. Soil carbon properties of the bulk soil and the floatable and sand-sized fraction.

			Soil Carbon Properties								
			Bulk Soil			Floatable Fraction			Sand-sized Fraction		
Sample	Years Since Restoration	Position on Transect	Total % C	δC^{13}	Total C/N	Total % C	δC^{13}	Total C/N	Total % C	δC^{13}	Total C/N
Adams C-4	Reference	4	8.18	-22.37	12.73	40.45	-25.68	21.49	4.24	-23.85	13.24
Amb C-4/9	Reference	4/9	18.77	-27.00	11.82	45.60	-26.48	24.92	24.31	-27.97	12.83
Amb C-5/8	Reference	5/8	17.35	-27.04	8.40	42.56	-26.54	24.81	26.63	-28.22	11.69
Boy C-4/9	Reference	4/9	11.85	-26.81	12.28	48.23	-27.02	34.86	13.73	-27.38	15.17
Boy C-5/8	Reference	5/8	18.00	-27.13	14.00	38.64	-26.41	23.73	18.41	-27.45	16.39
Crone C-2/11	Reference	4/9	7.83	-26.27	10.37	42.25	-26.90	27.81	8.73	-26.88	13.88
Crone C-3/10	Reference	5/8	9.84	-26.59	10.23	58.43	-26.52	24.10	5.55	-26.06	13.85
Fer C-4/9	Reference	4/9	16.59	-26.38	11.52	49.51	-26.63	26.86	30.86	-27.68	18.94
Fer C-5	Reference	5	11.71	-26.35	11.29	30.21	-26.63	26.37	34.73	-27.94	20.45
Graham C-4/9	Reference	4/9	10.23	-24.66	11.30	31.22	-26.19	20.65	10.44	-25.83	15.25
Graham C-5	Reference	5	15.72	-26.10	10.00	34.58	-25.88	23.00	25.01	-27.55	13.64
Hartt C-4/9	Reference	4/9	13.08	-26.09	9.00	32.61	-27.07	19.21	18.53	-26.97	11.25
Kem C-4/9	Reference	4/9	8.60	-26.18	10.59	41.82	-26.34	20.21	8.49	-26.63	14.21
Kem C-5/8	Reference	5/8	9.13	-26.62	10.45	41.63	-26.61	20.50	10.40	-26.86	13.63
Lov C-4/9	Reference	4/9	7.18	-24.20	11.42	38.31	-26.66	29.44	5.47	-24.39	11.51
Mar C-4/9	Reference	4/9	14.32	-26.26	10.03	36.93	-24.95	13.05	27.46	-27.61	16.24
Mar C-5/8	Reference	5/8	6.62	-25.59	10.29	45.99	-26.43	22.42	33.84	-27.91	16.25
Mitt C-4/9	Reference	4/9	11.12	-26.34	9.51	33.75	-26.12	26.10	25.79	-27.61	15.52
Mitt C-5/8	Reference	5/8	20.37	-26.53	10.11	57.15	-26.78	25.28	34.17	-27.99	14.95
Pet C-4/9	Reference	4/9	2.51	-24.75	10.24	52.18	-26.79	23.73	1.59	-25.99	13.36
Pet C-5/8	Reference	5/8	3.45	-24.93	10.14	31.58	-26.55	22.04	3.10	-25.36	13.10
Rau C-3	Reference	4	2.88	-22.43	15.88	37.67	-27.26	24.92	3.36	-25.62	16.89
Sprig C-4/9	Reference	4/9	3.02	-22.97	10.40	42.96	-26.73	17.18	1.56	-22.52	16.17

Table E.1. Soil carbon properties of the bulk soil and the floatable and sand-sized fractions.

			Soil Carbon Properties								
			Bulk Soil			Floatable Fraction			Sand-sized Fraction		
Sample	Years Since Restoration	Position on Transect	Total % C	δC^{13}	Total C/N	Total % C	δC^{13}	Total C/N	Total % C	δC^{13}	Total C/N
Sprig C-5/8	Reference	5/8	5.74	-25.53	9.35	44.47	-26.40	18.30	4.63	-25.10	13.48
Tat C-4/9	Reference	4/9	14.95	-25.24	11.54	48.31	-26.58	26.48	18.77	-26.37	15.40
Fer R1-4	3	4	4.05	-24.79	11.35	42.17	-27.09	32.90	1.93	-25.90	14.97
Fer R1-5	3	5	4.00	-25.21	10.07	49.57	-26.84	31.89	1.95	-26.13	15.06
Fer R2-5/8	3	5/8	4.25	-25.11	11.51	36.47	-25.87	19.40	2.50	-26.10	16.68
Graham R1-4/9	3	4/9	6.26	-24.05	10.70	46.00	-26.20	24.64	2.51	-22.17	17.78
Graham R1-5/8	3	5/8	7.27	-24.63	10.32	38.40	-26.63	21.28	4.72	-24.37	15.85
Graham R2-4/9	3	4/9	7.10	-25.21	9.33	42.47	-25.62	25.65	5.24	-26.13	12.24
Tat R1-4/9	3	4/9	2.31	-25.99	10.13	41.62	-25.71	18.23	1.06	-26.08	14.48
Tat R1-5/8	3	5/8	2.85	-26.31	9.91	34.60	-26.24	22.29	1.77	-26.40	16.31
Lov R1-4/9	4	4/9	7.73	-26.19	10.37	33.94	-26.88	22.84	6.18	-26.42	14.29
Lov R1-5/8	4	5/8	7.84	-26.26	10.09	43.45	-26.85	21.09	6.27	-26.54	14.13
Lov R2-3/10	4	4/9	7.96	-25.69	10.10	40.44	-25.62	22.35	6.48	-26.35	14.73
Crone R1-4/9	5	4/9	3.17	-22.16	14.03	38.21	-26.37	23.65	1.90	-21.81	24.46
Crone R1-5/8	5	5/8	2.99	-22.72	13.13	35.03	-26.67	24.87	1.55	-22.97	21.15
Crone R2-4/9	5	4/9	5.16	-24.93	10.65	43.26	-26.71	25.99	2.84	-25.39	15.83
Crone R2-5/8	5	5/8	2.76	-20.99	15.39	43.83	-26.85	23.86	1.25	-21.32	20.94
Kem R1-4/9	5	4/9	5.56	-25.27	10.23	51.87	-26.44	20.72	2.74	-26.44	15.55
Kem R1-5/8	5	5/8	5.16	-25.72	10.23	46.16	-26.68	21.28	1.78	-26.29	16.49
Kem R2-4/9	5	4/9	6.15	-25.94	11.07	51.83	-26.80	24.32	6.90	-26.56	15.11
Kem R2-8	5	8	7.30	-26.17	10.25	32.51	-26.59	24.18	10.42	-26.79	16.37
Adams R2-4	7	4	7.73	-18.10	16.60	40.61	-26.13	17.56	4.01	-18.82	18.78
Hartt R1-4/9	8	4/9	4.73	-24.80	10.24	33.16	-26.80	30.36	2.55	-25.31	12.45

Table E.1. Soil carbon properties of the bulk soil and the floatable and sand-sized fractions.

			Soil Carbon Properties								
			Bulk Soil			Floatable Fraction			Sand-sized Fraction		
Sample	Years Since Restoration	Position on Transect	Total % C	δC^{13}	Total C/N	Total % C	δC^{13}	Total C/N	Total % C	δC^{13}	Total C/N
Hartt R1-5/8	8	5/8	7.34	-25.46	11.30	50.07	-27.16	36.88	7.23	-25.91	12.20
Hartt R2-4/9	8	4/9	3.29	-25.49	11.15	39.00	-26.51	19.72	2.11	-25.78	13.64
Hartt R2-5/8	8	5/8	7.56	-25.65	10.40	39.37	-26.75	20.69	4.94	-26.15	12.89
Pet R1-4/9	8	4/9	4.73	-24.96	9.88	40.93	-26.69	20.80	5.58	-26.62	12.00
Pet R1-5/8	8	5/8	5.69	-26.06	9.38	31.67	-26.45	23.32	8.03	-27.29	11.89
Pet R2-4/9	8	4/9	5.46	-25.77	10.50	38.55	-26.61	24.12	6.60	-26.68	12.22
Pet R2-5/8	8	5/8	6.32	-25.91	8.85	43.45	-26.52	24.11	12.37	-27.17	12.75
Sprig R1-4/9	8	4/9	3.80	-24.99	9.03	43.15	-26.56	24.36	3.00	-25.55	13.53
Sprig R1-5/8	8	5/8	3.64	-25.29	9.10	33.59	-26.88	21.45	3.41	-25.70	13.37
Sprig R2-4/9	8	4/9	3.48	-24.94	10.20	46.69	-26.76	28.10	4.18	-26.20	14.48
Sprig R2-5/8	8	5/8	5.50	-25.54	10.24	48.08	-26.91	24.14	8.07	-27.11	14.28
Amb R1-4/9	9	4/9	6.98	-26.08	10.05	43.85	-26.15	29.04	9.91	-26.15	15.24
Amb R1-5/8	9	5/8	7.32	-26.10	10.30	42.90	-26.07	28.83	11.15	-26.57	13.91
Amb R2-4/9	9	4/9	13.03	-26.47	9.14	40.92	-26.87	20.15	11.51	-27.15	11.89
Amb R2-5/8	9	5/8	11.14	-26.82	9.64	41.34	-26.73	20.82	12.10	-27.00	12.10
Rau R1-10	10	9	13.02	-26.40	9.80	34.29	-26.92	17.78	17.46	-26.46	12.32
Rau R2-3/10	10	4/9	6.58	-26.41	10.26	48.57	-26.84	18.69	6.52	-26.83	17.16
Rau R2-4/9	10	5/8	10.59	-26.85	10.94	40.49	-26.90	17.73	13.96	-26.98	14.91
Boy R1-4/9	11	4/9	3.63	-25.63	9.03	55.10	-26.94	27.64	1.87	-26.31	14.02
Boy R1-5/8	11	5/8	5.89	-25.70	10.30	37.00	-26.74	23.04	2.50	-26.41	14.60
Boy R2-4/9	11	4/9	6.70	-25.75	11.02	38.04	-26.85	26.45	4.47	-26.33	15.63
Boy R2-5/8	11	5/8	7.18	-25.99	10.53	43.00	-26.71	26.58	5.00	-26.60	17.94
Mar R1-4/9	11	4/9	19.48	-26.75	11.26	51.71	-26.42	22.37	9.77	-25.75	13.27

Table E.1. Soil carbon properties of the bulk soil and the floatable and sand-sized fractions.

			Soil Carbon Properties								
			Bulk Soil			Floatable Fraction			Sand-sized Fraction		
Sample	Years Since Restoration	Position on Transect	Total % C	δC^{13}	Total C/N	Total % C	δC^{13}	Total C/N	Total % C	δC^{13}	Total C/N
Mar R1-5/8	11	5/8	6.63	-25.74	10.66	48.08	-26.41	23.08	19.97	-26.77	17.66
Mar R2-5/8	11	5/8	7.83	-25.91	9.91	47.99	-26.54	22.64	16.46	-27.05	15.00
Mitt R1-4/9	11	4/9	7.25	-25.88	9.09	30.63	-26.85	19.46	20.23	-26.44	16.25
Mitt R1-5/8	11	5/8	7.56	-25.92	8.84	36.99	-26.56	19.23	22.35	-27.27	16.79
Mitt R2-4/9	11	4/9	7.06	-25.09	9.74	34.43	-27.04	21.41	19.30	-26.76	16.78
Mitt R2-5/8	11	5/8	9.28	-26.33	9.57	38.33	-26.76	23.54	25.11	-27.25	17.07

Table E. 2. Soil carbon properties of the Silt + Clay and acid unhydrolyzable fractions.

			Soil Carbon Properties					
			Silt + Clay Fraction			Acid Unhydrolyzable Fraction		
Sample	Years Since Restoration	Position on Transect	Total % C	δC^{13}	Total C/N	Total % C	δC^{13}	Total C/N
Adams C-4	Reference	4	11.61	-22.31	11.81	8.13	-24.41	13.27
Amb C-4/9	Reference	4/9	11.17	-27.02	9.74	8.76	-27.42	11.01
Amb C-5/8	Reference	5/8	11.11	-26.79	9.56	9.29	-27.51	11.30
Boy C-4/9	Reference	4/9	10.25	-26.28	10.20	7.98	-26.96	12.05
Boy C-5/8	Reference	5/8	13.51	-26.57	10.30	10.41	-27.35	13.44
Crone C-2/11	Reference	4/9	7.63	-25.22	9.79	5.26	-26.76	12.35
Crone C-3/10	Reference	5/8	8.34	-25.25	10.01	5.09	-26.58	11.44
Fer C-4/9	Reference	4/9	12.70	-25.81	10.45	8.91	-26.80	13.30
Fer C-5	Reference	5	8.38	-25.52	10.48	6.26	-26.36	12.71
Graham C-4/9	Reference	4/9	8.79	-23.95	11.36	7.01	-26.11	12.48
Graham C-5	Reference	5	10.95	-26.18	10.23	9.09	-26.63	12.86
Hartt C-4/9	Reference	4/9	12.45	-25.33	9.93	4.67	-25.89	9.32
Kem C-4/9	Reference	4/9	8.77	-25.56	9.12	5.23	-26.53	10.61
Kem C-5/8	Reference	5/8	8.66	-25.59	9.78	6.38	-26.71	10.63
Lov C-4/9	Reference	4/9	9.39	-24.14	10.76	5.53	-26.30	11.95
Mar C-4/9	Reference	4/9	6.83	-25.93	8.66	4.89	-26.58	9.44
Mar C-5/8	Reference	5/8	10.91	-26.03	9.44	8.50	-26.79	12.04
Mitt C-4/9	Reference	4/9	8.77	-25.57	8.66	6.64	-26.46	8.62
Mitt C-5/8	Reference	5/8	11.90	-25.91	9.04	9.22	-26.78	10.41
Pet C-4/9	Reference	4/9	2.83	-24.33	9.19	1.85	-25.36	9.50
Pet C-5/8	Reference	5/8	3.88	-24.54	8.42	1.89	-25.38	9.54
Rau C-3	Reference	4	3.57	-22.16	15.23	1.42	-26.46	9.90
Sprig C-4/9	Reference	4/9	3.88	-22.79	10.21	1.97	-24.57	8.83

Table E.2. (con't) Soil carbon properties of the Silt + Clay and acid unhydrolyzable fractions.

			Soil Carbon Properties					
			Silt Clay Fraction			Acid Unhydrolyzable Fraction		
Sample	Years Since Restoration	Position on Transect	Total % C	δC^{13}	Total C/N	Total % C	δC^{13}	Total C/N
Sprig C-5/8	Reference	5/8	5.94	-23.91	11.33	2.96	-25.84	9.95
Tat C-4/9	Reference	4/9	11.98	-24.27	11.68	10.42	-26.10	11.40
Fer R1-4	3	4	4.83	-24.46	9.99	3.15	-25.45	13.05
Fer R1-5	3	5	4.82	-24.55	9.77	2.73	-25.48	12.23
Fer R2-5/8	3	5/8	6.52	-24.57	10.12	4.04	-25.40	12.90
Graham R1-4/9	3	4/9	7.20	-24.27	9.48	5.45	-25.51	10.59
Graham R1-5/8	3	5/8	7.95	-24.57	8.74	6.19	-25.51	9.85
Graham R2-4/9	3	4/9	7.01	-24.63	9.85	4.39	-25.71	9.23
Tat R1-4/9	3	4/9	3.29	-25.33	9.56	2.25	-25.83	9.08
Tat R1-5/8	3	5/8	3.08	-25.47	9.95	2.09	-25.94	9.57
Lov R1-4/9	4	4/9	7.70	-25.47	9.49	4.12	-26.48	10.98
Lov R1-5/8	4	5/8	8.57	-25.97	9.32	6.30	-26.58	11.50
Lov R2-3/10	4	4/9	8.97	-25.25	10.21	6.19	-25.63	10.96
Crone R1-4/9	5	4/9	5.97	-21.93	14.46	2.48	-25.45	10.72
Crone R1-5/8	5	5/8	4.61	-23.39	11.82	2.03	-25.97	12.51
Crone R2-4/9	5	4/9	5.34	-25.22	10.79	2.74	-25.97	12.13
Crone R2-5/8	5	5/8	5.08	-22.60	13.39	1.52	-25.91	13.02
Kem R1-4/9	5	4/9	6.83	-24.96	9.94	4.84	-25.59	9.69
Kem R1-5/8	5	5/8	5.85	-24.97	9.43	4.32	-25.05	9.18
Kem R2-4/9	5	4/9	6.30	-25.47	9.50	4.64	-26.18	13.48
Kem R2-8	5	8	6.67	-25.43	9.17	4.84	-26.10	11.02
Adams R2-4	7	4	11.37	-20.39	15.20	5.71	-23.82	11.48
Hartt R1-4/9	8	4/9	4.75	-24.09	11.26	3.33	-25.67	9.08

Table E.2. (con't) Soil carbon properties of the Silt + Clay and acid unhydrolyzable fractions.

			Soil Carbon Properties					
			Silt Clay Fraction			Acid Unhydrolyzable Fraction		
Sample	Years Since Restoration	Position on Transect	Total % C	δC^{13}	Total C/N	Total % C	δC^{13}	Total C/N
Hartt R1-5/8	8	5/8	9.50	-24.97	10.65	5.77	-26.07	9.76
Hartt R2-4/9	8	4/9	4.80	-24.55	10.50	2.40	-25.88	10.98
Hartt R2-5/8	8	5/8	5.39	-24.36	10.66	3.25	-26.15	13.36
Pet R1-4/9	8	4/9	5.55	-24.55	8.13	3.08	-25.87	9.19
Pet R1-5/8	8	5/8	4.47	-25.21	8.83	2.94	-26.17	10.42
Pet R2-4/9	8	4/9	4.64	-25.35	9.16	2.90	-25.89	9.72
Pet R2-5/8	8	5/8	4.09	-25.48	8.91	2.89	-25.96	9.60
Sprig R1-4/9	8	4/9	3.86	-24.85	8.86	2.65	-25.41	10.08
Sprig R1-5/8	8	5/8	3.55	-24.96	8.81	2.46	-25.48	9.73
Sprig R2-4/9	8	4/9	3.94	-24.95	9.19	2.87	-25.50	9.91
Sprig R2-5/8	8	5/8	4.30	-25.38	9.02	2.81	-25.87	9.93
Amb R1-4/9	9	4/9	6.99	-25.29	9.88	4.61	-26.08	11.63
Amb R1-5/8	9	5/8	6.28	-25.29	10.11	4.62	-26.24	13.38
Amb R2-4/9	9	4/9	9.70	-26.00	9.89	7.00	-26.89	12.17
Amb R2-5/8	9	5/8	10.22	-26.29	9.66	7.75	-27.00	12.22
Rau R1-10	10	9	9.76	-25.58	9.48	6.70	-26.29	10.71
Rau R2-3/10	10	4/9	7.28	-25.62	10.05	4.77	-26.43	9.61
Rau R2-4/9	10	5/8	9.72	-26.01	9.44	6.66	-26.22	10.81
Boy R1-4/9	11	4/9	7.74	-25.22	10.32	5.14	-25.77	10.85
Boy R1-5/8	11	5/8	7.50	-25.23	9.77	5.49	-25.84	11.48
Boy R2-4/9	11	4/9	8.40	-25.26	10.64	6.07	-25.83	11.58
Boy R2-5/8	11	5/8	7.07	-25.13	9.51	5.39	-25.95	12.07
Mar R1-4/9	11	4/9	4.51	-24.78	9.48	2.97	-25.70	10.95

Table E.2. (con't) Soil carbon properties of the Silt + Clay and acid unhydrolyzable fractions.

			Soil Carbon Properties					
			Silt Clay Fraction			Acid Unhydrolyzable Fraction		
Sample	Years Since Restoration	Position on Transect	Total % C	δC^{13}	Total C/N	Total % C	δC^{13}	Total C/N
Mar R1-5/8	11	5/8	5.65	-25.05	9.32	4.02	-25.67	10.52
Mar R2-5/8	11	5/8	5.29	-25.00	9.25	3.65	-25.76	10.26
Mitt R1-4/9	11	4/9	5.40	-24.96	8.94	4.11	-25.79	9.03
Mitt R1-5/8	11	5/8	6.06	-25.09	8.67	4.57	-25.92	10.75
Mitt R2-4/9	11	4/9	5.13	-25.11	8.95	3.61	-25.92	12.20
Mitt R2-5/8	11	5/8	6.18	-25.42	9.04	4.35	-26.19	11.78

Table E. 3. NMR properties of the floatable fraction of each sample.

Sample	Years Since Restoration	Position on Transect	NMR Properties on Floatable Fraction					
			Alkyl	O-Alkyl	Aromatic	Phenolic	Carbonyl	Alkyl/ O-Alkyl
Adams C-4	Reference	4	12.81	60.51	12.12	6.17	6.59	0.21
Amb C-4/9	Reference	4/9	12.31	59.69	14.34	6.98	5.58	0.21
Amb C-5/8	Reference	5/8	12.47	62.01	14.02	6.24	4.72	0.20
Boy C-4/9	Reference	4/9	12.17	64.09	11.96	6.14	4.75	0.19
Boy C-5/8	Reference	5/8	14.14	63.78	11.72	5.18	4.69	0.22
Crone C-2/11	Reference	4/9	12.89	60.98	13.07	6.38	5.58	0.21
Crone C-3/10	Reference	5/8	14.21	62.36	12.16	5.66	5.04	0.23
Fer C-4/9	Reference	4/9	13.22	65.97	11.03	5.06	4.22	0.20
Fer C-5	Reference	5	12.84	66.12	10.75	5.36	4.6	0.19
Graham C-4/9	Reference	4/9	11.96	63.71	11.85	5.88	5.58	0.19
Graham C-5	Reference	5	13.66	59.23	13.29	6.57	6.09	0.23
Hartt C-4/9	Reference	4/9	14.38	60.89	12.71	6.03	5.32	0.24
Kem C-4/9	Reference	4/9	14.81	61.49	12.57	6.07	5.1	0.24
Kem C-5/8	Reference	5/8	12.08	58.13	14.26	7.77	6.32	0.21
Lov C-4/9	Reference	4/9	20.12	52.44	12.15	4.79	9.46	0.38
Mar C-4/9	Reference	4/9	12.2	66.22	10.75	5.08	4.88	0.18
Mar C-5/8	Reference	5/8	12.09	68.19	10.91	4.8	3.86	0.18
Mitt C-4/9	Reference	4/9	14.48	61.34	12.17	5.74	5.58	0.24
Mitt C-5/8	Reference	5/8	14.72	59.69	12.35	5.98	6.1	0.25
Pet C-4/9	Reference	4/9	14.64	62.9	10.69	5.16	5.59	0.23
Pet C-5/8	Reference	5/8	16.62	59.72	10.5	5.19	6.79	0.28
Rau C-3	Reference	4	13.42	65.85	11.18	4.84	4.35	0.20
Sprig C-4/9	Reference	4/9	15.54	60.65	11.4	5.45	5.97	0.26
Sprig C-5/8	Reference	5/8	12.57	64.39	11.11	5.15	5.57	0.20

Table E.3. (con't) NMR properties of the floatable fraction of each sample.

Sample	Years Since Restoration	Position on Transect	NMR Properties on Floatable Fraction					
			Alkyl	O-Alkyl	Aromatic	Phenolic	Carbonyl	Alkyl/ O-Alkyl
Tat C-4/9	Reference	4/9	16.27	60.14	12.64	5.41	4.87	0.27
Fer R1-4	3	4	17.16	57.66	12.6	6.14	6.28	0.30
Fer R1-5	3	5	12.36	61.34	12.27	7.22	5.56	0.20
Fer R2-5/8	3	5/8	11.64	65.84	11.5	5.54	4.64	0.18
Graham R1-4/9	3	4/9	11.68	65.98	11.39	5	5.14	0.18
Graham R1-5/8	3	5/8	12.52	61.73	12.01	5.87	6.25	0.20
Graham R2-4/9	3	4/9	13.36	60.29	13.12	6.07	5.79	0.22
Tat R1-4/9	3	4/9	15.2	57.78	12.65	5.83	6.78	0.26
Tat R1-5/8	3	5/8	14	63.58	10.89	5.52	5.1	0.22
Lov R1-4/9	4	4/9	16.91	51.48	15.21	8.05	6.53	0.33
Lov R1-5/8	4	5/8	16.41	51.22	16.7	7.33	6.67	0.32
Lov R2-3/10	4	4/9	16.85	57.34	12.7	6.34	5.99	0.29
Crone R1-4/9	5	4/9	17.26	56.77	12.79	6.26	5.72	0.30
Crone R1-5/8	5	5/8	17.26	58.17	12.19	5.93	5.45	0.30
Crone R2-4/9	5	4/9	16.55	55.4	13.74	7.31	5.7	0.30
Crone R2-5/8	5	5/8	14.8	62.41	11.1	5.95	5.08	0.24
Kem R1-4/9	5	4/9	13.03	65.82	11.32	5.19	4.44	0.20
Kem R1-5/8	5	5/8	11.91	68.84	10.58	4.65	3.76	0.17
Kem R2-4/9	5	4/9	12.02	63.81	11.76	6.28	4.93	0.19
Kem R2-8	5	8	10.94	64.62	12.08	6.02	5.09	0.17
Adams R2-4	7	4	14.13	58.47	12.54	6.1	7.28	0.24
Hartt R1-4/9	8	4/9	13.95	57.3	13.91	6.17	6.97	0.24
Hartt R1-5/8	8	5/8	13.58	63.11	11.98	5.14	5.65	0.22
Hartt R2-4/9	8	4/9	14.37	57.41	13.89	6.37	6.57	0.25

Table E.3. (con't) NMR properties of the floatable fraction of each sample.

Sample	Years Since Restoration	Position on Transect	NMR Properties on Floatable Fraction					
			Alkyl	O-Alkyl	Aromatic	Phenolic	Carbonyl	Alkyl/ O-Alkyl
Hartt R2-5/8	8	5/8	15.41	59.73	12.49	5.43	6.03	0.26
Pet R1-4/9	8	4/9	16.16	58.47	11.15	5.53	6.91	0.28
Pet R1-5/8	8	5/8	18.52	57.72	10.85	5.25	6.86	0.32
Pet R2-4/9	8	4/9	18.22	57.5	10.99	4.92	7.26	0.32
Pet R2-5/8	8	5/8	15.42	59.1	11.34	5.7	7	0.26
Sprig R1-4/9	8	4/9	15	60.95	11.13	5.42	6.19	0.25
Sprig R1-5/8	8	5/8	17.35	58.63	10.61	5.7	6.8	0.30
Sprig R2-4/9	8	4/9	15.83	61.6	10.56	5.21	6.11	0.26
Sprig R2-5/8	8	5/8	18.29	61.16	9.3	4.01	6.3	0.30
Amb R1-4/9	9	4/9	14.92	52.08	18.32	8.02	5.95	0.29
Amb R1-5/8	9	5/8	13.9	51.99	18.68	8.12	5.94	0.27
Amb R2-4/9	9	4/9	14.43	56.95	13.23	6.52	7.15	0.25
Amb R2-5/8	9	5/8	12.79	60.26	12.68	6.57	6.14	0.21
Rau R1-10	10	9	13.85	62.74	11.61	5.5	5.46	0.22
Rau R2-3/10	10	4/9	17.24	52.86	16.03	6.98	5.7	0.33
Rau R2-4/9	10	5/8	15.29	57.57	13.43	6.59	5.99	0.27
Boy R1-4/9	11	4/9	11.24	63.52	12.06	6.26	5.43	0.18
Boy R1-5/8	11	5/8	10.69	65.73	11.62	5.82	5.02	0.16
Boy R2-4/9	11	4/9	12.35	65.73	11.24	5.58	4.57	0.19
Boy R2-5/8	11	5/8	12.66	66.48	11.35	5.23	3.94	0.19
Mar R1-4/9	11	4/9	11.19	66.59	10.86	5.58	4.77	0.17
Mar R1-5/8	11	5/8	11.94	67.22	10.77	5.16	4.29	0.18
Mar R2-5/8	11	5/8	13.93	61.86	11.15	5.77	5.92	0.23
Mitt R1-4/9	11	4/9	14.23	62	12.32	5.64	5.07	0.23

Table E.3. (con't) NMR properties of the floatable fraction of each sample.

Sample	Years Since Restoration	Position on Transect	NMR Properties on Floatable Fraction					
			Alkyl	O-Alkyl	Aromatic	Phenolic	Carbonyl	Alkyl/ O-Alkyl
Mitt R1-5/8	11	5/8	11.79	66.01	12.02	5.21	4.51	0.18
Mitt R2-4/9	11	4/9	13.26	61.5	11.68	5.97	6.19	0.22
Mitt R2-5/8	11	5/8	12.28	64.21	11.54	5.46	5.24	0.19

APPENDICES F. SOIL MICROBIAL COMMUNITY PROPERTIES OF SAMPLES

Table F. 1. Soil microbial community properties of samples as determined by phospholipid fatty acid (PLFA) analysis.

			Soil Microbial Community Properties									
Sample	Years Since Restoration	Position on Transect	PLFA Biomass	PLFA Richness	PLFA Diversity	PLFA Evenness	% Actinomycetes	% Fungi	Fungi/bacterial biomass	% Gram (-) PLFA	% Gram (+) PLFA	% Gram (+) / % Gram (-)
Adams C-4	Reference	4	309.26	55.00	2.69	0.67	1.65	1.75	0.04	31.01	26.11	0.84
Amb C-4/9	Reference	4/9	471.41	67.00	3.63	0.86	1.62	2.85	0.08	23.86	24.82	1.04
Amb C-5/8	Reference	5/8	424.92	61.00	3.31	0.81	1.01	2.71	0.07	28.12	23.88	0.85
Boy C-4/9	Reference	4/9	292.59	53.50	2.77	0.70	2.12	3.13	0.08	26.71	25.07	0.94
Boy C-5/8	Reference	5/8	272.66	57.00	2.64	0.65	2.10	3.59	0.10	27.56	24.37	0.89
Crone C-2/11	Reference	4/9	196.84	53.00	2.18	0.55	1.64	2.16	0.06	29.26	25.45	0.87
Crone C-3/10	Reference	5/8	130.05	43.00	1.60	0.42	1.57	1.93	0.05	28.41	26.52	0.94
Fer C-4/9	Reference	4/9	523.79	60.00	3.56	0.87	2.06	2.19	0.06	24.73	29.81	1.21
Fer C-5	Reference	5	594.22	65.00	4.03	0.97	2.12	1.92	0.05	20.61	31.22	1.51
Graham C-4/9	Reference	4/9	332.31	54.00	2.73	0.69	1.57	1.55	0.04	28.85	26.84	0.93
Graham C-5	Reference	5	527.20	64.00	3.37	0.81	0.90	1.37	0.03	27.23	26.07	0.96
Hartt C-4/9	Reference	4/9	341.96	61.00	2.94	0.71	1.44	2.04	0.05	30.25	24.03	0.80
Kem C-4/9	Reference	4/9	317.55	56.50	2.79	0.69	1.19	4.31	0.11	30.56	22.59	0.74
Kem C-5/8	Reference	5/8	223.42	52.50	2.28	0.57	1.46	3.23	0.09	27.21	24.67	0.91
Lov C-4/9	Reference	4/9	394.25	46.50	2.95	0.77	1.29	1.75	0.04	29.93	27.95	0.93
Mar C-4/9	Reference	4/9	502.91	58.00	3.48	0.86	2.02	2.20	0.06	26.38	28.18	1.10
Mar C-5/8	Reference	5/8	457.77	59.00	3.50	0.86	2.06	2.18	0.06	25.58	28.04	1.11
Mitt C-4/9	Reference	4/9	452.32	57.00	3.40	0.84	1.78	3.18	0.08	25.03	27.71	1.11
Mitt C-5/8	Reference	5/8	531.65	56.00	3.46	0.86	2.39	2.99	0.08	25.83	29.20	1.13
Pet C-4/9	Reference	4/9	159.73	41.00	1.84	0.50	1.99	4.85	0.13	33.68	22.25	0.66
Pet C-5/8	Reference	5/8	160.23	42.50	1.88	0.50	2.01	2.96	0.08	33.24	23.94	0.72
Rau C-3	Reference	4	127.98	35.00	1.55	0.44	1.39	3.49	0.09	31.71	20.95	0.66
Sprig C-4/9	Reference	4/9	114.03	38.00	1.48	0.41	1.98	2.54	0.06	33.56	24.71	0.74
Sprig C-5/8	Reference	5/8	255.60	52.00	2.54	0.64	2.00	2.33	0.06	32.34	25.41	0.80

Table F.1. (con't) Soil microbial community properties of samples as determined by phospholipid fatty acid (PLFA) analysis.

			Soil Microbial Community Properties									
Sample	Years Since Restoration	Position on Transect	PLFA Biomass	PLFA Richness	PLFA Diversity	PLFA Evenness	% Actinomycetes	% Fungi	Fungi/bacterial biomass	% Gram (-) PLFA	% Gram (+) PLFA	% Gram (+) / % Gram (-)
Tat C-4/9	Reference	4/9	461.49	58.50	3.37	0.83	1.06	3.27	0.11	26.77	25.56	1.00
Fer R1-4	3	4	177.74	43.00	2.02	0.54	2.25	2.62	0.07	19.38	30.95	1.60
Fer R1-5	3	5	158.46	44.00	1.89	0.50	4.02	2.86	0.09	19.20	29.93	1.56
Fer R2-5/8	3	5/8	294.93	52.50	2.76	0.70	1.77	1.70	0.04	24.19	30.10	1.24
Graham R1-4/9	3	4/9	164.34	47.00	1.88	0.49	2.01	1.31	0.03	28.28	26.91	0.95
Graham R1-5/8	3	5/8	240.70	50.50	2.40	0.61	2.07	1.66	0.04	28.07	28.26	1.01
Graham R2-4/9	3	4/9	298.41	59.50	2.78	0.68	1.88	1.02	0.03	28.81	27.67	0.96
Tat R1-4/9	3	4/9	124.36	41.50	1.54	0.41	1.58	5.28	0.14	34.56	19.40	0.56
Tat R1-5/8	3	5/8	97.87	38.00	1.32	0.36	1.75	3.97	0.11	30.60	22.64	0.75
Lov R1-4/9	4	4/9	272.43	54.50	2.66	0.66	2.39	2.22	0.06	23.35	27.83	1.19
Lov R1-5/8	4	5/8	262.30	53.00	2.54	0.64	2.57	1.94	0.05	23.70	27.93	1.19
Lov R2-3/10	4	4/9	327.50	59.00	2.91	0.71	1.80	2.45	0.07	28.74	24.61	0.86
Crone R1-4/9	5	4/9	87.09	39.00	1.25	0.34	1.78	4.48	0.13	29.47	22.98	0.78
Crone R1-5/8	5	5/8	83.32	39.50	1.19	0.32	1.81	3.83	0.11	27.93	23.79	0.86
Crone R2-4/9	5	4/9	161.58	46.50	1.89	0.49	1.99	2.44	0.07	26.54	26.11	0.99
Crone R2-5/8	5	5/8	65.30	37.50	1.00	0.27	1.89	2.42	0.07	26.62	25.93	0.99
Kem R1-4/9	5	4/9	170.13	48.00	2.00	0.52	2.73	2.37	0.07	31.23	22.86	0.73
Kem R1-5/8	5	5/8	184.19	52.50	2.08	0.52	2.13	2.25	0.06	28.50	22.89	0.80
Kem R2-4/9	5	4/9	346.25	57.50	2.98	0.74	2.24	2.24	0.06	25.01	27.77	1.15
Kem R2-8	5	8	289.35	56.00	2.70	0.67	2.12	2.34	0.06	26.30	26.23	1.00
Adams R2-4	7	4	255.95	39.00	2.32	0.63	1.74	2.37	0.06	35.73	21.95	0.61
Hartt R1-4/9	8	4/9	141.54	44.00	1.71	0.45	1.90	3.06	0.08	34.70	22.87	0.66
Hartt R1-5/8	8	5/8	207.10	51.50	2.19	0.56	2.04	1.63	0.04	31.66	23.71	0.75
Hartt R2-4/9	8	4/9	127.10	43.00	1.60	0.42	2.10	2.38	0.06	36.07	23.11	0.64

Table F.1. (con't) Soil microbial community properties of samples as determined by phospholipid fatty acid (PLFA) analysis.

			Soil Microbial Community Properties									
Sample	Years Since Restoration	Position on Transect	PLFA Biomass	PLFA Richness	PLFA Diversity	PLFA Evenness	% Actinomycetes	% Fungi	Fungi/bacterial biomass	% Gram (-) PLFA	% Gram (+) PLFA	% Gram (+) / % Gram (-)
Hartt R2-5/8	8	5/8	176.36	49.50	1.99	0.51	1.95	2.24	0.06	34.39	23.58	0.69
Pet R1-4/9	8	4/9	246.81	49.50	2.47	0.63	1.90	3.08	0.08	28.91	26.59	0.92
Pet R1-5/8	8	5/8	209.49	44.50	2.25	0.59	2.24	1.56	0.04	23.67	29.20	1.23
Pet R2-4/9	8	4/9	258.29	52.00	2.57	0.65	1.85	2.67	0.08	26.20	27.58	1.06
Pet R2-5/8	8	5/8	241.57	52.50	2.46	0.62	1.69	2.57	0.07	24.43	25.85	1.06
Sprig R1-4/9	8	4/9	151.06	42.50	1.79	0.48	2.08	2.79	0.08	28.27	25.84	0.92
Sprig R1-5/8	8	5/8	133.93	31.00	1.56	0.45	2.21	3.34	0.09	26.68	28.66	1.08
Sprig R2-4/9	8	4/9	230.34	49.00	2.35	0.60	2.15	3.23	0.09	28.27	26.77	0.95
Sprig R2-5/8	8	5/8	296.39	57.50	2.86	0.71	2.31	3.26	0.09	25.70	27.23	1.06
Amb R1-4/9	9	4/9	368.76	55.00	2.98	0.74	1.68	2.79	0.07	25.65	26.46	1.04
Amb R1-5/8	9	5/8	386.99	54.00	2.98	0.75	1.58	2.57	0.07	24.89	25.29	1.02
Amb R2-4/9	9	4/9	347.50	50.50	2.97	0.76	1.66	3.62	0.10	26.05	25.62	1.03
Amb R2-5/8	9	5/8	432.93	62.50	3.48	0.84	1.58	2.93	0.08	20.70	27.49	1.34
Rau R1-10	10	9	353.03	55.00	2.96	0.74	1.05	3.76	0.10	27.50	23.80	0.87
Rau R2-3/10	10	4/9	318.40	56.00	2.75	0.68	1.88	9.06	0.30	22.92	23.67	1.04
Rau R2-4/9	10	5/8	419.15	61.50	3.22	0.78	2.16	2.24	0.06	26.70	26.93	1.01
Boy R1-4/9	11	4/9	198.63	49.50	2.19	0.56	2.37	2.22	0.06	29.09	25.78	0.89
Boy R1-5/8	11	5/8	198.17	49.50	2.18	0.56	2.17	2.99	0.08	29.30	24.76	0.85
Boy R2-4/9	11	4/9	222.47	50.50	2.32	0.59	1.90	2.56	0.07	27.13	26.00	0.98
Boy R2-5/8	11	5/8	283.65	57.50	2.77	0.68	1.92	3.19	0.09	28.34	24.40	0.86
Mar R1-4/9	11	4/9	283.88	54.00	2.67	0.67	1.62	3.99	0.11	20.91	29.27	1.40
Mar R1-5/8	11	5/8	261.37	54.00	2.56	0.64	1.49	2.93	0.08	18.99	29.84	1.57
Mar R2-5/8	11	5/8	384.28	59.00	3.25	0.80	1.92	3.32	0.09	20.30	25.87	1.27
Mitt R1-4/9	11	4/9	282.53	53.50	2.66	0.67	1.83	4.33	0.12	23.71	26.33	1.12

Table F.1. (con't) Soil microbial community properties of samples as determined by phospholipid fatty acid (PLFA) analysis.

Sample	Years Since Restoration	Position on Transect	Soil Microbial Community Properties									
			PLFA Biomass	PLFA Richness	PLFA Diversity	PLFA Evenness	% Actinomycetes	% Fungi	Fungi/bacterial biomass	% Gram (-) PLFA	% Gram (+) PLFA	% Gram (+) / % Gram (-)
Mitt R1-5/8	11	5/8	267.87	55.00	2.66	0.66	1.72	2.92	0.08	21.09	28.58	1.36
Mitt R2-4/9	11	4/9	346.99	53.00	2.93	0.74	2.05	1.59	0.04	22.66	29.38	1.30
Mitt R2-5/8	11	5/8	403.26	56.00	3.18	0.79	2.52	2.47	0.07	23.01	28.80	1.25