ASSESSING MICROBIAL COMMUNITY DYNAMICS AND CARBON MINERALIZATION WITH DEPTH ACROSS AN ERODED AGRICULTURAL LANDSCAPE AT ST. DENIS NATIONAL WILDLIFE AREA

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ABSTRACT

Recent work has demonstrated that vast amounts of soil organic carbon (SOC) are redistributed and buried within Canadian croplands; however, the effects of redistribution on SOC dynamics and biological properties of the soil environment remain unknown. Because soil microorganisms are drivers of carbon (C) turnover in soil, the effects of such processes on microbial community dynamics are important in assessing the overall effects of redistribution and the stability of displaced C. This is particularly important in the face of future climate change scenarios and potential disturbances.

The objectives of this study were to examine microbial community dynamics with depth and among landscape positions in an eroded landscape, and to assess C mineralization response between surface and subsurface soil layers in a depositional position. Microbial abundance was highly influenced by SOC redistribution. This was most evident in the buried backslope position where substantial soil and SOC deposition had occurred, creating a very thick A horizon (ca. 80 cm). Phospholipid fatty acid (PLFA) analysis revealed substantial concentrations of microbial biomass located at depth (30-60 cm), which was greater than PLFA concentration at the soil surface and correlated with SOC concentration. Community structure analysis demonstrated the strong influence of landscape position and depth in structuring microbial communities near the soil surface (0-20 cm). Communities in positions that were predominantly erosional were the most different from those in the depositional position, accounting for the largest amount of variation (60%) in the overall analysis. The existence of distinct microbial communities found in depositional material (0-25 cm) and within the buried A horizon (30-80 cm) in the buried backslope position indicate a strong influence of depth and redistribution in structuring microbial communities.

The existence of significant viable biomass in the buried A horizon of the depositional position leads to question the persistence of highly concentrated, buried SOC over many decades. When soils from surface (0-5 and 20-25 cm) and subsurface (40-45 and 65-70 cm) depths were incubated in surface-like conditions, greater mineralization response in surface relative to subsurface soils, despite relatively similar SOC concentration, suggests that redistribution protects buried C from decomposition. Distinct microbial communities found at the onset and completion of the mineralization study between surface and subsurface soil layers may indicate the influence of microbial community structure on mineralization response. Depth

was the largest source of variation in microbial community structure, and although a shift occurred after exposure to incubation conditions, the effect of depth remained the strongest influence. This work indicates that SOC redistribution strongly influences microbial abundance and community structure development, primarily driven by altered substrate gradients occurring with depth, and suggests that C is less susceptible to decomposition once buried in depositional positions.

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LIST OF ABBREVIATIONS

AAFC	Agriculture and Agri-Food Canada
AMF	arbuscular mycorrhizal fungi
ANOVA	analysis of variance
AWCD	average well color development
B:F	bacterial:fungal
CLPP	community level physiological profiling
G-	Gram negative
G+	Gram positive
GC	gas chromatography
G+:G-	Gram positive:Gram negative
MRPP	multi-response permutation procedure
NMDS	nonmetric multidimensional scaling
PBS	phosphate-buffered saline
PLFA	phospholipd fatty acid
ref	reference
SDNWA	St. Denis National Wildlife Area
SOC	soil organic carbon
SOM	soil organic matter
T0	Day 0, destructive sampling occurrence
T1	Day 14, destructive sampling occurrence
T2	Day 62, destructive sampling occurrence
TCD	thermal conductivity detector
TOC	total organic carbon
WEOC	water extractable organic carbon
WEOM	water extractable organic matter

1.0 INTRODUCTION

The majority of agricultural lands within the interior plains of Canada are classified as hummocky, with complex patterns of knolls and depressions, estimated to cover 15.4 million hectares (Pennock, 2003). Because of this landform, these landscapes are naturally susceptible to soil redistribution, and are further perturbed by anthropogenic exacerbation of erosion and redistribution, such as cultivation. Soil organic carbon redistribution occurs in conjunction with soil redistribution, therefore the factors driving soil redistribution are important for influencing C concentration in the soil (Gregorich et al., 1998).

Agricultural landscapes undergo SOC redistribution via tillage, wind, and water erosion, where SOC is moved from erosional to depositional positions, becoming concentrated and buried (Pennock, 1997; Van Oost et al., 2007). Agricultural redistribution is thought to affect terrestrial carbon (C) flux to and from the atmosphere, but the direction and magnitude of flux is not agreed upon (Van Oost et al., 2007). The net effect of such processes is currently debated, with one argument supporting a net C loss due to increased mineralization during redistribution (Lal, 2003). In contrast, the opposing argument supports a net C gain due to sufficient C burial in depositional positions and dynamic replacement of C at eroding positions to constitute a net sink (Berhe et al., 2007; Van Oost et al., 2007).

Recent work has demonstrated that vast amounts of C are redistributed and buried within Canadian croplands. Using ^{137}Cs deposition data, SOC stocks, and horizonation data, VandenBygaart et al. (2012) observed extensive redistribution at agricultural sites across Canada. This process was evidenced by loss of soil material and SOC at eroding positions, and a substantial gain in soil material and SOC at depositional positions. Depositional positions were characterized by very deep profiles with multiple A horizons, displaying a higher concentration of SOC deep within the profile relative to the soil surface, suggesting that C decomposition was constrained since burial. Though C buried at depth is thought to be stabilized, uncertainty remains in the face of future climate change scenarios and potential disturbances (Van Oost et al., 2007). Further work to determine the dynamics of buried SOC and the net result of redistribution on terrestrial C flux in future climate change scenarios is needed.

Though the immense amount of SOC redistributed and contained within topographically variable agricultural landscapes across Canada has been quantified, less is known about the effects of redistribution on SOC dynamics and soil biological properties. The effect of

redistribution on microbial communities and the processes they mediate are important for understanding the turnover and stability of displaced C.

Previous work has been conducted on microbial community dynamics with depth in typical, non-eroding profiles. Briefly, microbial biomass, diversity, and activity generally decline with depth (Fierer et al., 2003b; Rumpel and Kögel-Knabner 2011), though biomass at depth (below 25 cm) has been found to significantly contribute to overall biomass (Fierer et al., 2003b). Additionally, significant structural differences in microbial communities with depth have been observed (Eilers et al., 2012; Fierer et al., 2003b) and are thought to affect microbially-mediated processes (Fierer et al., 2003a; Fierer et al., 2003b; Salome et al., 2010). It is unclear if the mechanisms governing such patterns and differences with depth in non-eroding profiles apply to those in eroding landscapes and in particular, depositional landscape positions where SOC and soil material become concentrated. Assessing microbial community dynamics in an eroded landscape will provide insight into the stability of displaced C and its susceptibility to decomposition in the face of potential land use and climate change.

The site selected for this study was an agricultural landscape displaying evidence of SOC redistribution within the St. Denis National Wildlife Area (SDNWA), approximately 40 km east of Saskatoon, SK. In fall 2010, three soil profiles at different positions in the landscape were excavated to examine the extent of SOC redistribution and to assess microbial community dynamics. The first pit was classified as an eroding shoulder, the second an eroded backslope, and the third a buried backslope, where substantial soil deposition occurred, resulting in a very thick A horizon (ca. 80 cm). The buried backslope position was sampled again in spring 2011 to obtain intact cores for a mineralization-incubation study. The main objectives were: 1) to examine microbial abundance and community composition with depth and between landscape positions and to relate microbial abundance and community composition to the associated physiochemical conditions and geomorphic controls, and 2) to determine the C mineralization and microbial community response of surface and subsurface soil layers in a depositional position to incubation in surface-like conditions and physical disturbance. The following thesis is compiled in a manuscript-style format, so that each objective is presented in a separate chapter (Chapters 3.0 and 4.0). Therefore, in order for each chapter to stand alone, some repetition of information may occur, particularly in the introduction and methodology sections.

2.0 LITERATURE REVIEW

2.1. Soil organic carbon in the global C cycle

Soil organic carbon is the largest active terrestrial reservoir of C and is thus an important part of the global C cycle. Current estimates of the SOC pool are estimated to be as much as 2300 Pg C (Eglin et al., 2010; Gruber et al., 2004), which amounts to ~ 75% of total C found in terrestrial ecosystems (Eglin et al., 2010; Houghton, 2007). The remaining C stored on land is found in vegetation and litter; approximately 550 and 300 Pg C resides in each of those pools, respectively (Houghton, 2007).

The terrestrial system has the potential to be either a sink or source of C in future climate change scenarios. Soil and living biomass are currently estimated to be a net C sink (3 Pg C from 2000-2008); however, the continuation of this scenario is uncertain with future climate instability, and the underlying mechanisms of this sink remain unclear (Eglin et al. 2010; Houghton, 2007). The balance of SOC is determined by C gains via photosynthesis and losses via respiration (plants, animals, and microbes) and disturbance. Changes in these two processes will affect the continuation of the terrestrial environment as a C sink (Houghton, 2007). There is much uncertainty in terms of SOC response to change in climate; therefore, understanding the dynamics of SOC will help to reduce the uncertainty of predicting potential climate change impacts.

2.1.1. Deep SOC in the global C cycle

Deep or 'subsurface' SOC generally refers to SOC located below the A horizon (i.e., the B and C horizons), but often refers to the depth below the rooting zone (below ~0-30 cm). As such, it can be considered to be operationally defined and somewhat dependent on ecosystem characteristics. Herein, subsurface will refer generally to both scenarios, and specific soil depths will be given where needed.

It is estimated that subsurface soil horizons account for as much as half of total SOC stocks (Batjes, 1996). Though significant amounts of C at depth have been identified, the dynamics and characteristics of deep soil C are not well understood, because most studies focus on C rich surface horizons that are typically thought of as most active biologically and in terms of C cycling (Rumpel and Kögel-Knabner, 2011; Fierer et al. 2003a). Insights regarding subsurface C

source, composition, and dynamics have been put forth, and it is becoming clear that subsurface and surface C have inherently different underlying stabilization/destabilization mechanisms. It has also been identified that soil redistribution in agricultural landscapes (from water, wind, and tillage erosion) can result in substantial C at depth. This occurs via detachment, movement, and burial of soil C in depositional positions, resulting in C-concentrated, buried horizons, found deep in the soil profile (VandenBygaart et al., 2012). In addition, little is known about the nature and dynamics of SOC once it is buried and located at depth. Due to the vast amount of C stored at depth and the large influence that this may have in the global C cycle, studies related to the dynamics of deep soil C are of increasing importance and vital to accurate modeling of response to potential climate change (Rumpel and Kögel-Knabner, 2011).

2.1.2. Carbon characteristics in surface vs. subsurface soil layers

2.1.2.1. Carbon source and composition

Differences have been observed in source and composition of soil C between surface and subsurface soil layers. In the subsurface, C input sources are plant roots, root exudates, dissolved organic matter from surface horizons, transported particulate organic matter, as well as bioturbation, the relative importance of which is not thoroughly understood, and largely dependent on climatic parameters (Rumpel and Kögel-Knabner, 2011). Subsurface sources originating from root material are generally less decomposable and more recalcitrant (higher in lignin content) than surface sources (Lorenz and Lal, 2005). In contrast, C sources in surface soils typically originate from more labile, fresh plant litter such as shoots and leaves; the decomposability characteristics for both surface and subsurface sources of C depend upon plant species (Lorenz and Lal, 2005; Rumpel et al., 2002).

Typically as depth increases, so does the radiocarbon age and turnover time of soil C, resulting in higher concentrations of stabilized soil organic matter (SOM) at depth, characterized by high mean residence times (comparatively "old" SOC). How higher proportions of stabilized C at depth occurs is not well understood, and may be the result of continual microbial recycling of 'old' soil C. Further, this contributes to the theory that subsurface C is higher in proportions of highly processed, microbially derived C (Rumpel and Kögel-Knabner, 2011).
Schimel et al. (2011) imposed a series of dry/wet cycles on surface and deep soils in a California grassland soil to study the age of C respired by modeling radiocarbon signatures (^{14}C and ^{13}C

CO_2 signatures). It was found that C respired from deep soils had average turnover times of 650-850 yr (dominated by older material), compared with the C respired from surface soils which showed an average turnover of 280-325 yr. Fontaine et al. (2007) found similar results, with subsoil C dominated by slow cycling carbon (2560 +/- 74 yr) and surface soils dominated by fast cycling carbon (320 +/- 27 yr). Moreover, in their review, Rumpel and Kögel-Knabner (2011) conducted a meta-analysis showing global radiocarbon ages of soil depths below 50 cm to be > 1000 yr.

2.1.2.2. Differences in C stabilization/destabilization mechanisms affecting mineralization

Differences in C dynamics between surface and subsurface soils have been observed. The nature of subsurface C has been cited for this discrepancy, including chemical recalcitrance, mineral bound C (in association with soil minerals), and physically protected C, while it has also been suggested that differences in microbial community structure (and inherent differences in microbial functioning) is an important control (Fierer et al., 2003b; Rumpel and Kögel-Knabner, 2011). In their review of current knowledge on SOM regulation and turnover, Dungait et al. (2012) highlighted the likelihood that mineral associations and SOM occlusion operate together in rendering soil C inaccessible to soil microorganisms. This, along with many other factors, including the ideal suite of abiotic factors conducive to decomposition, are proposed as likely controlling mechanisms on C turnover that can be applied in the context of C stabilization with depth. Microbial dynamics and how they relate to C dynamics with depth will be discussed further in Section 2.3.

A number of studies have tested the hypothesis that deep soil C is physically inaccessible to mineralization by soil microorganisms, and use various forms of disturbance to observe the response in surface vs. subsurface soil layers. Salome et al. (2010) found subsurface soils (80-100 cm) to be more affected by sieving treatment (<1 mm) than surface soils (5-10 cm). Sieving increased mineralization in subsurface soils by 75%, but had no effect on surface samples. The aforementioned study by Schimel et al. (2011) completed similar work using dry/wet cycles as the disturbance treatment on surface and deep soils. Due to the age of the C respired from subsurface soils, this work suggested that dry/wet cycles can mobilize deep C that typically does not contribute to soil C flux and supports the hypothesis that deep soil C is biologically, but not necessarily physically accessible to soil microorganisms. The finding of proportionally higher

concentrations of 'old C' (higher in radiocarbon age) at depth is postulated to be due to more mineral associations and subsequent protection with depth (Dungait et al., 2012). Mineralization in subsurface layers has similarly been shown to be more affected by nutrient inputs and temperature changes than surface layers. Fierer et al. (2003a) compared C mineralization rates in surface (0-25 cm) and subsurface soils (below 25 cm) with manipulations of soil moisture, nutrient, and temperature levels. The most prominent increases in respiration rates were observed with nutrient additions or temperature increases in subsurface soils. Average Q_{10} values (average increase in respiration rate for a 10°C increase in temperature) were 3.0 in surface soils and 3.9 in subsurface soils, and additions of nitrogen (N) and phosphorous (P) increased respiration rates as much as 4.5 fold in subsurface soils. Additionally, in a study by Fontaine et al. (2007), the 'priming effect' of soils at depth was demonstrated. The addition of cellulose to subsurface samples (60-80 cm) induced a significant increase in mineralization when compared to the control. Along with significantly more C respired in the cellulose-amended soil, a significant decrease in ^{14}C activity in the amended soil occurred, indicating that the amendment stimulated the mineralization of 'old C', thus 'priming' the mineralization process.

2.2. Landscape scale SOC redistribution and the global C cycle

On a landscape scale, redistribution of SOC via tillage and water erosion is questioned for its net contribution to terrestrial-atmospheric C flux. Soil organic carbon redistribution often occurs in agricultural landscapes due to management practices, as well as wind and water erosion. Soil redistribution typically occurs in three stages: detachment, transport, and deposition (Berhe et al. 2007), where erosive forces selectively expose fine soil particles and associated SOC that was previously protected in the soil matrix at eroding positions, transporting redistributed material to lower lying depositional areas (Berhe et al. 2007; Gregorich et al., 1998). Once redistributed, burial is thought to constrain decomposition due to reduced aeration and increased soil wetness (Berhe et al., 2007). There is currently a debate concerning the contribution of these processes to soil loss and atmospheric CO_2 levels, due in part to difficulty in quantifying all contributing processes and their interactions (Berhe et al., 2007; Van Oost, 2007). One side of the debate supports SOC redistribution resulting in a net C sink. This process is referred to as the erosion-induced sink, and involves the dynamic replacement of SOC at eroded positions and the stabilization and storage of SOC in depositional positions, though the

magnitude of the sink is disputable (Berhe et al. 2009; Van Oost et al. 2007). Carbon replaced at erosional positions is indeed a C sink, but the net effect of this process is determined by the fate of the eroded SOC (Van Oost et al. 2007). The opposing view is the more conventional theory, which supports a net C loss in the redistribution process due to stimulated microbial activity in the transported material leading to enhanced mineralization, with insufficient C replacement occurring in erosional positions to constitute a net sink (Amundson, 2001; Lal, 2003). A disequilibrium between replacement of C in eroding positions and stabilization in depositional positions will determine if the outcome of redistribution results in a net C sink or source (Berhe et al., 2007). There is great interest in C dynamics in agricultural landscapes due to potential for soils to offset excess atmospheric CO_2 through land use and management changes (Gruber et al., 2004). Therefore, a better understanding of these processes within croplands is needed for accurate estimations of management effects on global C dynamics (VandenBygaart et al., 2012).

2.2.1. Buried horizon C dynamics

Soil redistribution from tillage and water erosion results in the movement of soil from upper slope positions to depositional positions (Gregorich et al., 1998), often concentrating SOC and soil material where it is redeposited (VandenBygaart et al., 2012). The deposited material forms much deeper profiles, and results in buried horizons (multiple A horizons), with relatively high SOC concentrations (VandenBygaart et al., 2012). The fate of this buried C is in question, and particularly, its potential role in C cycling in a warmer climate.

In a recent study, hillslopes within six agricultural landscapes across Canada were examined for vertical and lateral distribution of SOC, using ^{137}Cs activity, SOC stocks, and horizonation data (VandenBygaart et al., 2012). Horizonation and ^{137}Cs data displayed extensive redistribution at all sites, with loss of soil material and SOC at shoulder positions, and in contrast, the occurrence of aggraded profiles with a gain in soil material and SOC at backslope and footslope positions. At depositional positions, deep profiles were found with multiple A horizons, displaying a sharp increase in SOC concentration at the lower limits of the existing plow layer (Ap horizon). The SOC deposition rate at aggraded positions in all of the sites was estimated to be from 2 to 4 Mg ha^{-1} yr^{-1}. The vast amount of buried SOC in agricultural landscapes across Canada supports the need for the next progression in this field of research: studying the fate and dynamics of buried SOC.

It is yet to be determined whether mechanisms proposed to influence subsurface C stabilization/destabilization mentioned previously (section 2.1.2) apply to SOC that originated at the surface and subsequently became situated in buried horizons; it is thought that once burial occurs, decomposition is constrained due to decreased oxygen, cooler and wetter conditions with less variability, and physical protection of soil C within newly formed aggregates (Dungait et al., 2012; Gregorich et al., 1998).

A recent study by Doetterl et al. (2012) examined C cycling in eroded landscapes, by quantifying SOC and C pool distribution (different functional SOC pools associated with varying levels of stability) at differing depths and positions along hillslopes within two agricultural landscapes in Belgium. Consistent with VandenBygaart et al. (2012), higher C concentrations were found in subsoils at depositional positions when compared to eroding positions. Relatively more C in passive pools in the subsoils, greater C associated with microaggregates and silt size particle fractions, and lower specific respiration rates indicated that buried C became physically protected in the burial process. As a result, the authors concluded that physical protection plays a dominant role in buried C dynamics. These shifts in C stability occurring due to redistribution demonstrate the need for more studies of this nature and inclusion of these processes in C models.

2.3. The microbial role in the SOC cycle

Soil microorganisms regulate C turnover in soil (Castro et al., 2010), equating to approximately 80-90% of all organic material decomposition (Ekschmitt et al., 2008), and are therefore essential to the consideration of terrestrial C impacts on the global C cycle (Bardgett et al., 2008). Climate change is predicted to elicit a change in soil microbial functioning, but this response and how it may affect the global C cycle is not fully understood (Castro et al., 2010; Trumbore, 2006). Changes in temperature and moisture content are likely to cause the strongest direct effects on microbial functioning. This would occur either by altering the physiology of current microbial populations (increasing or decreasing process rates) or by causing a shift in microbial community composition; the latter could lead to a change in process rates due to the presence or absence of different taxa with different physiologies (Singh et al., 2010). These potential changes in soil microbial functioning are important, considering CO_2 emissions from autotrophic and heterotrophic soil respiration are an order of magnitude greater than emissions

from anthropogenic sources (Dungait et al., 2012), and as such, even a small change in decomposition rates or C stocks could elicit increased CO_2 flux to the atmosphere (Nielsen et al., 2011).

2.3.1. Microbial dynamics in surface vs. subsurface soil layers

The structure and composition of microbial communities in subsurface soil layers is not well characterized, despite likely influencing a number of biogeochemical processes at depth. Most studies typically focus on surface layers where biomass is the greatest, and because of this, little is understood about microbial communities in subsurface soil layers (Eilers et al., 2012). However, differences in microbial community structure with depth have been identified (Eilers et al., 2012; Fierer et al., 2003b), and are hypothesized as one control on differences in subsurface microbial-driven C dynamics (Fierer et al., 2003a, 2003b; Salome et al., 2010).

2.3.1.1. Abundance and activity

Microbial abundance and activity has generally been found to decrease with depth; however, deep microbial biomass is still metabolically active (Rumpel and Kögel-Knabner, 2011), and has been found to significantly contribute to overall biomass (Fierer et al., 2003b). In a study conducted on soils near Santa Barbara, CA, Fierer et al. (2003b) found approximately 35% of total microbial biomass located below 25 cm in two profiles studied to a depth of 2 m. They observed that a decreasing gradient in overall biomass typically follows the same decreasing pattern as resource gradients; in particular, C concentration and quality decrease with depth (Fierer et al., 2003b). In contrast, limited results have shown varying and irregular relative distributions of fungi, Gram positive, and Gram negative bacteria with depth (Ekschmitt et al., 2008).

Though microbial biomass at depth is less concentrated than at the surface, microbial activity in subsurface soil layers has shown to be more affected by various treatments than surface samples. As discussed in Section 2.1.2., microbial activity (measured via respiration) at depth has been shown to be more affected by physical disturbance via sieving (Salome et al., 2010), dry/wet cycles (Schimel et al., 2011; Xiang et a., 2008), nutrient additions (Fontaine et al., 2007), and temperature changes (Fierer et al., 2003a). These results have repercussions for climate change scenarios in which moisture and temperature changes could occur, potentially mobilizing the vast C pool found at depth (Xiang et al., 2008).

2.3.1.2. Community structure and diversity

It has long been thought that high levels of biodiversity in soil result in functional redundancy in certain ecosystem functions, that is, more than one species can carry out the same ecosystem process (Nielsen et al., 2011). This is thought to occur for processes where many microbial groups participate, such as C mineralization (Strickland et al., 2009a). However, recent work has demonstrated that differences in microbial community structure can influence function and more specifically, C cycling (Strickland et al., 2009a, 2009b). In a mineralization study utilizing the common garden approach (factorial design, with three compositionally distinct microbial inocula and three corresponding litter types), Strickland et al. (2009b) found that microcosms with shared history between microbial inocula and litter type resulted in the highest amount of mineralized C when compared with inocula foreign to the litter type, consistent with the 'home field advantage' hypothesis. These results suggest that microbial communities are not functionally equivalent and that community structure can in fact influence function. Consequently, these findings become important and can be applied when considering microbial community dynamics and C cycling with depth.

Though few studies have been conducted focusing on microbial community structure with depth, those that have show notable results. Fierer et al. (2003b) characterized microbial communities using phospholipid fatty acid analysis (PLFA) to a depth of 2 m in two profiles near Santa Barbara, CA, and found significant structural differences in community composition with depth. Diversity (richness) was found to decrease by approximately one third from the soil surface to the depth of measurement, and multivariate analysis of community fingerprints indicated that community composition was significantly affected by depth. Moreover, Eilers et al. (2012) found similar results in a study characterizing bacterial and archaeal community composition (using 16S rRNA gene analysis) with depth (1.5 m) in a forested watershed in Colorado. Bacterial diversity was found to decrease by approximately 20-40% from the surface to the depth of sampling. Interestingly, when microbial community composition with depth was compared with communities found in surface soils from different biomes, it was found that the depth-related gradient was comparable in structural differences to that seen across different biomes, signifying soil depth as an important determinant in microbial community structure.

The relationship between diversity (richness) and resiliency is often discussed in ecology. From their review on soil biodiversity and C cycling, Nielsen et al. (2011) found that low-diversity experiments were the most susceptible to changes in richness, and that the relationship between diversity and C cycling was mostly of an idiosyncratic nature. That is, process rates were controlled by the presence or absence of a particular species or species-specific traits, rather than the overall richness of the system. This, coupled with the finding that subsurface microbial communities are less diverse and structurally distinct from surface communities, could have implications for climate change scenarios (such as loss of species), as processes at depth are likely more specialized and less subject to functional redundancy.

Substantial C concentrations have been identified in subsurface horizons, and in particular, in subsurface horizons in eroded landscapes; however, less is known regarding the dynamics of deep C, as well as its response to future climate change scenarios. Additionally, the dynamics of microbial communities associated with deep C are not understood, and are a key component in understanding overall terrestrial C dynamics because they mediate C turnover. This project was created in response to these uncertainties, focusing on the characterization of microbial communities with depth in an eroded landscape, and their associated C mineralization response in a depositional landscape position.

3.0 LANDSCAPE POSITION AND DEPTH AFFECT MICROBIAL ABUNDANCE AND COMMUNITY COMPOSITION IN AN AGRICULTURAL LANDSCAPE WITH EVIDENCE OF SOIL ORGANIC CARBON REDISTRIBUTION

3.1. Introduction

Soil organic carbon is the largest actively cycling pool of C on land and is vital to understanding the global C cycle (Janzen, 2004). In the context of the global C cycle, subsurface soil C is increasingly gaining attention due to the realization that substantial amounts of C are found at depth, equating to approximately half of global SOC stocks (Batjes, 1996). Use of the terms 'subsurface' or 'deep' generally refers to SOC found in B and C horizons, or below the rooting zone (below ~0-30 cm). It is not yet understood what role subsurface soil C could play in climate change scenarios, but it is increasingly recognized as an important component in global C models (Rumpel and Kögel-Knabner, 2011). Additionally, there is interest in the potential for deep soil C sequestration, a mechanism through which less C-concentrated subsoils may act as a C sink through various methods of deep C deposition, such as increased root biomass (Lorenz and Lal, 2005; Rumpel and Kögel-Knabner, 2011). Another important consideration for global C models is the irregular C distribution that exists within landscapes with varying topography. Increased understanding in the accounting and dynamics of C stored in topographically variable landscapes will contribute to increased accuracy in assessments of C storage in terrestrial environments (VandenBygaart et al. 2012), as well as an increased understanding of future global C dynamics.

Agricultural erosion is thought to affect terrestrial C flux, but the direction and magnitude of flux is not agreed upon (Van Oost et al., 2007). Agricultural landscapes are often subject to SOC redistribution (via tillage, wind, and water erosion) where SOC is moved from erosional to depositional positions, becoming concentrated and buried (Van Oost et al., 2007), often resulting in very deep profiles (VandenBygaart et al., 2012). Globally, it's been estimated that 16-21 Pg C have been buried in agricultural landscapes due to erosion in the last 50 years (Van Oost et al., 2007). The response buried SOC will have to climate warming is unknown, as is its response to other potential perturbations, such as land use change, or further erosion or excavation (Berhe et al., 2007; Ito, 2007). While some progress has been made in quantifying buried C (Doetterl et al., 2012; VandenBygaart et al., 2012), less is known about C turnover in C-rich subsurface soil environments.

As the drivers of C turnover, soil microorganisms and their associated dynamics are an important component of terrestrial C dynamics. Similar to subsurface SOC, subsurface microbial communities have gained recent attention due to the recognition that substantial amounts of biomass are found at depth (Fierer et al., 2003b). Furthermore, distinct microbial communities have been found between surface and subsurface soil layers (Eilers et al., 2012; Fierer et al., 2003b); these differences are proposed to affect microbially-driven processes, such as C mineralization and turnover (Fierer et al., 2003a, 2003b; Salome et al., 2010). As such, studies that characterize microbial community composition provide the basis for insight into links between structure and function in terms of C cycling. In particular, little work has been done studying microbial community dynamics associated with buried SOC material. Microbial abundance generally decreases with depth, typically displaying a 50-85% decrease in biomass from the soil surface to below the rooting zone (Allison et al., 2007a; Fierer et al., 2003b). This pattern was attributed to a similar decrease in resource gradients. In the agricultural landscape in this study, redistributional processes have affected typical resource gradients with depth, by SOC removal at eroding positions, and subsequent concentration and burial at depositional positions (VandenBygaart et al., 2012). As such, the goal of this study was to determine the effects of SOC redistribution on microbial community dynamics. The specific study objectives were to examine soil microbial abundance and community composition with depth and between landscape positions, and to relate abundance and community structure to the associated physiochemical conditions and geomorphic controls.

3.2. Materials and methods

3.2.1. Site description

The study site is located north of St. Denis, Saskatchewan, approximately 40 km east of Saskatoon, within the St. Denis National Wildlife Area (SDNWA). The SDNWA is in the aspen parkland ecoregion and is characterized by undulating to hummocky landscapes dominated by Dark Brown Chernozemic soils (Hogan and Conley, 2002). There are approximately 200 wetland areas scattered throughout restored grasslands, native grasslands, and cultivated lands. The study site is a southwest facing slope located on cultivated agricultural land, which was under annual crop production using direct seeding, rotating oats (*Avena sativa*), barley (*Hordeum vulgare*), and canola (*Brassica napus*) (Environment Canada, 2013b). Agricultural lands are

usually leased for 1-3 years to local farmers (Hogan and Conley, 2002). Though a complete historic record of management practices are not available for cultivated land within the SDNWA, records kept since the formation of the area indicate the study site has been under annual cultivation since 1967 and likely many years prior (Environment Canada, 2013b; Hogan and Conley, 2002).

3.2.2. Sampling methodology

Samples were collected in the fall after harvest, on Oct. 29 and 30, 2010. To characterize SOC redistribution and examine microbial community dynamics, three positions within the landscape were chosen, and numbered from upper to lower slope (Fig. 3.1). Pit 1 was classified as an eroded shoulder, pit 2 a depositional backslope, and pit 3 a buried backslope. Geomorphic shape classifications were: pit 1, divergent backslope; pit 2, convergent backslope; and pit 3, footslope (A. Bedard-Haughn, personal communication, 2013). The current geomorphic surface form is likely smoother than the pre-cultivation surface. At each position, a large pit was excavated and a pit face cleared. In each excavated pit, four bulk samples (~ 1 kg) were removed per 5-cm depth increment, from the soil surface to the depth of the A horizon (Fig. 3.2). Each bulk sample was homogenized and sub-sampled in the field for microbial analyses (100 g). Sub-samples were transported back to the laboratory on ice and frozen at -80°C.

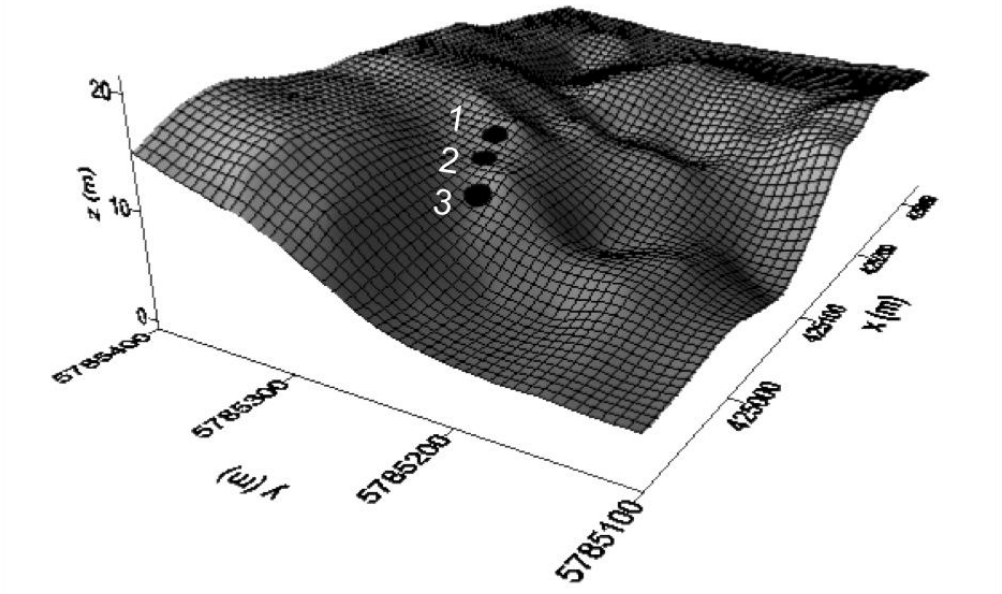

Fig. 3.1 Digital elevation model displaying the study site located within the St. Denis National Wildlife Area. Pits are numbered 1-3 from upper to lower slope.

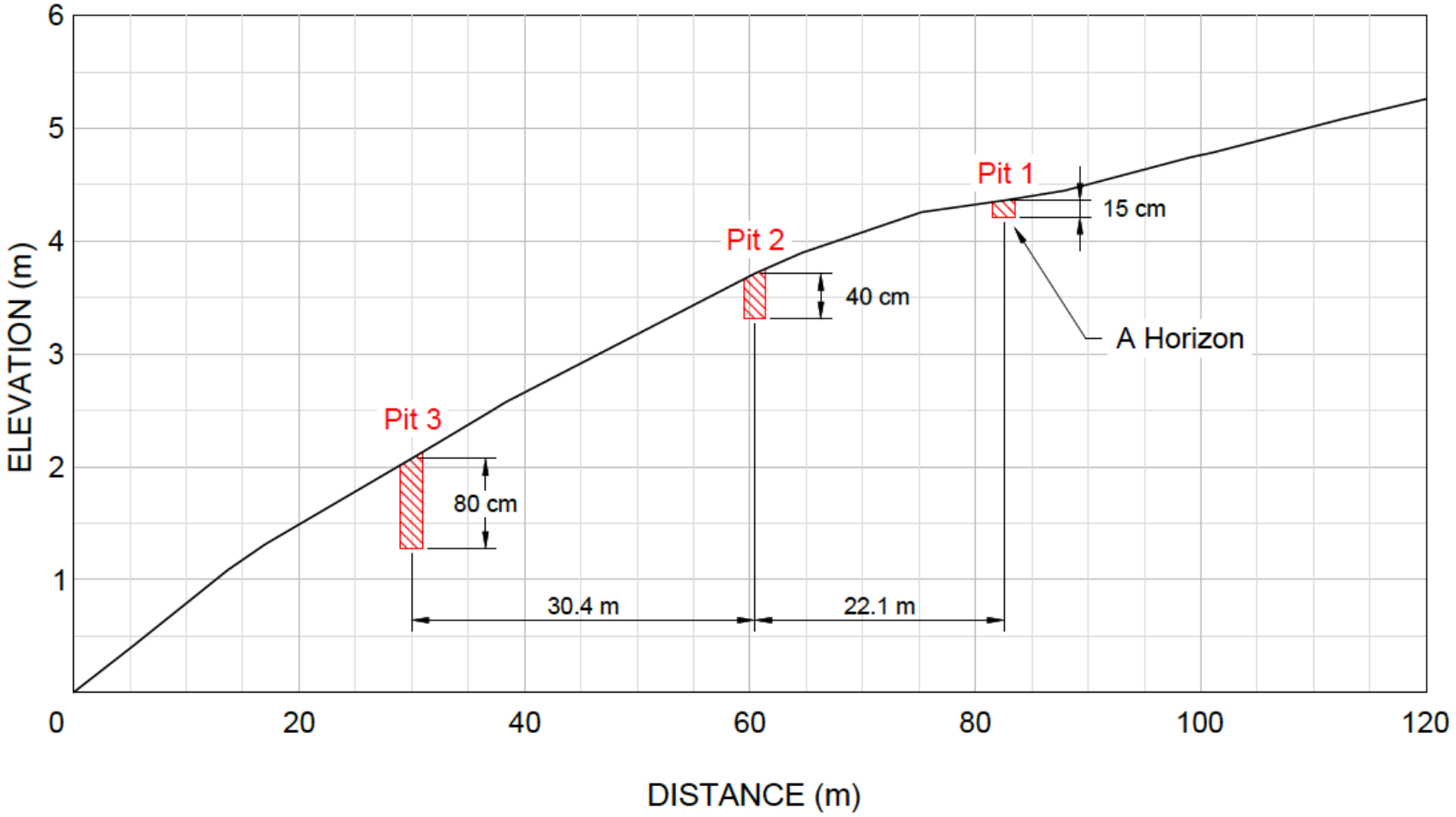

Fig. 3.2 Cross section scale drawing of the study landscape displaying pit locations and A horizon depths.

3.2.3. Site characterization

This work is related to an ongoing national Agriculture and Agri-Food Canada (AAFC) study focused on SOM dynamics in a warming climate. One facet of the study assessed soil C erosion and burial in agricultural landscapes across Canada; SDNWA served as one of six sites. Field sampling for both this study and the abovementioned study was done concurrently, and analyses were completed on corresponding 5-cm increments at the Eastern Cereals and Oilseeds Research Centre (ECORC) in Ottawa, ON. The following site characterization data are included with permission from the authors. This and additional information can be found in VandenBygaart et al. (2012).

3.2.3.1. Horizonation and SOC redistribution

A-horizon characteristics and ^{137}Cs deposition analysis were used to assess soil erosion and redistribution within the study landscape. Each pit was pedologically characterized and classified according to the Canadian System of Soil Classification (Soil Classification Working Group, 1998). Table 3.1 describes the A horizon characteristics of each pit. The depth and thickness of the A horizon increases from the upper slope position to lower slope, as does the darkness (value and chroma) of the A horizon.

The ^{137}Cs deposition data can be used as an indicator of soil erosion in agricultural landscapes, due to its strong absorption to clay minerals and SOM. Figure 3.3 displays the ^{137}Cs profile to the depth of A horizon in each pit. The ^{137}Cs profile from pit 1 shows reduced activity (in the depth profile) compared to pit 3, indicating soil accumulation in pit 3. Approximately 40 cm of soil deposition has occurred since 1955 in pit 3, shown by little to no ^{137}Cs activity near the 40 cm depth, which approximates the start of ^{137}Cs activity in soils in 1955 (Fig. 3.3). Pit 1 predominately displays erosional properties according to ^{137}Cs and horizonation data, whereas pit 2 displays the presence of a thin buried horizon and a slightly deeper ^{137}Cs profile, indicating previous deposition occurred at pit 2. Total SOC concentration was also measured to assess redistribution. Soil organic carbon concentration is incorporated with microbial community data in Results Section 3.3.1 and is displayed individually in Fig. A.1.

Table 3.1 Pit designation and A horizon characteristics for pits 1-3. Adapted from VandenBygaart et al. (2012).

Pit number	Position	Horizon Designation†	Depth	Colour	Texture‡
			cm		
1	Eroded shoulder	Ap	0-15	10YR3/2	CL
		AB	15-29	10YR4/2	L
2	Depositional backslope	Apk	0-17	10YR3.5/2	L
		Apk	17-27	10YR2/2	
		Ahbk	27-37	10YR2/2	
		ABk	37-59	10YR3/3	
3	Buried backslope	Apk	0-26	10YR3/1	L
		Apkb	26-36	10YR2/2	
		Ahkb	36-81	10YR2/1	

† Horizon designation classified according to the Canadian System of Soil Classification (Soil Classification Working Group, 1998).

‡ CL, Clay loam; L, Loam

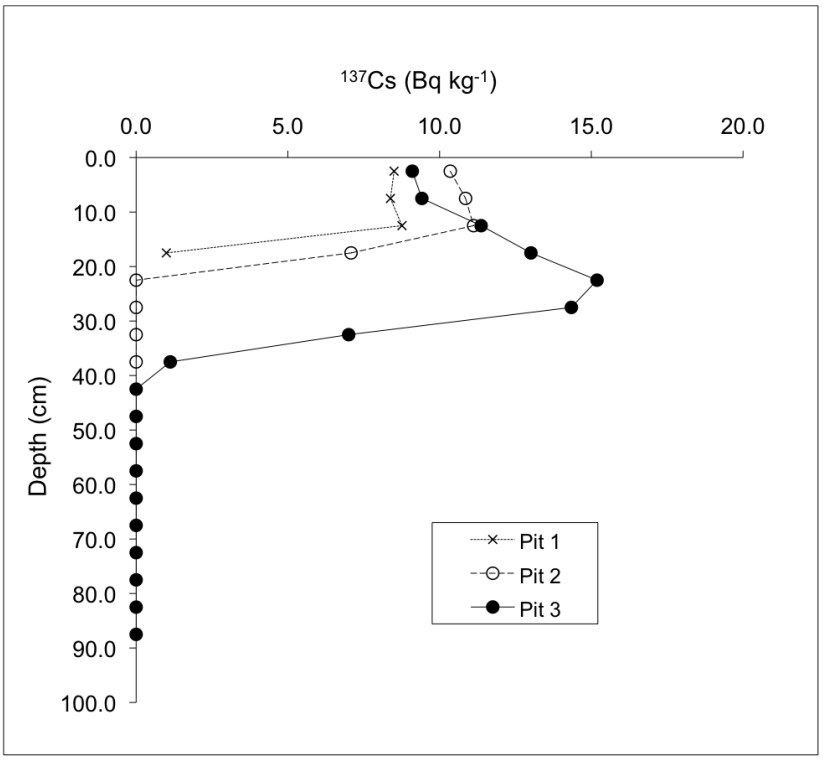

Fig. 3.3 The ^{137}Cs profile in A horizons of pits 1-3. Approximately 40 cm of soil deposition occurred at pit 3 within the last 60 years. Adapted from VandenBygaart et al. (2012).

3.2.4. Phospholipid fatty acid analysis

Phospholipid fatty acid analysis (PLFA) is a commonly used method for the study of microbial community structure and abundance and was used to characterize microbial communities in this study. This method is robust due to its relative ease of extraction, cost-effectiveness and sensitive and reproducible results (Frostegard et al., 2011). Additionally, PLFA is a valuable indicator of viable soil microorganisms, as phospholipids are only present in the cell membrane of living soil microorganisms. Briefly, all lipid fractions contained within cell membranes are extracted from the soil sample and separated from one another using solid phase extraction. The isolated phospholipid fraction is then methylated, quantified using an internal standard, and measured by gas chromatography (GC) (Drenovsky et al., 2008; Kaur et al., 2005). Functional groups including fungi, bacteria, and actinomycetes found in soil have specific PLFA biomarkers associated with them, allowing an estimate of relative abundance. Similarly, a community fingerprint can be derived from PLFA, based upon the presence of individual phospholipid molecules and their relative abundance (Drenovsky et al., 2008).

Within two weeks of sampling, frozen PLFA soils were freeze-dried, ground and stored at -80°C to prevent degradation of the lipids prior to extraction. The modified PLFA extraction method of White et al. (1979), adapted from the original method of Bligh and Dyer (1959) was used to perform the extraction, per Helgason et al. (2010). Briefly, fatty acids were extracted from 4 g of lyophilized, ground soil using a phosphate buffered extractant solution and separated on a solid phase extraction column (0.50 g Si; Varian Inc. Mississauga, ON). Following separation, fatty acids were methylated and identified using a GC-FID (Hewlett Packard 5890 Series II, Hewlett Packard Scientific Instruments, Palo Alto, CA) and MIDI software (MIDI Inc., Newark, DE). Total microbial biomass as well as biomass of various functional groups was calculated according to Hedrick et al. (2005), based on the addition of a known internal standard (methyl nonadecanoate; 19:0). Specific individual biomarkers and corresponding functional groups were assessed; biomarkers used are found in Table 3.2. Biomarker ratios cy17:0:16:1ω7c and cy19:0:18:1ω7c, herein referred to as stress 1 and stress 2, were used as measures of physiological stress (Grogan and Cronan, 1997).

Table 3.2 Biomarkers and the functional groups they represent as used to determine relative abundance.

Functional group	Biomarker
Gram positive bacteria†	i14:0, i15:0, i16:0, i16:1 i17:0, a17:0, 18:1ω7c
Gram negative bacteria‡	16:1 2OH, cy17:0, cy19:0
Arbuscular mycorrhizal fungi†	16:1ω5c
Stress 1 & 2§	cy17:0/16:1ω7c, cy19:0/18:1ω7c
General fungal biomarkers†	16:1 ω5c, 18:1ω9c, 18:2 ω6,9c
General bacterial biomarkers†	14:0, i14:0,15:0, i15:0, a15:0, 16:0, i16:0,16:0 10Me,16:1 2OH, 17:0, i17:0, a17:0, 17:1 ω8c, cy17:0,17:0 10Me, 18:1ω7c
Actinobacteria¶	16:0 10Me, 17:0 10Me, TBSA 10Me 18:0

†Adapted list based on Helgason et al. (2010)
‡Adapted list based on Moore-Kucera and Dick (2008)
§Grogan and Cronan (1997)
¶Adapted list based on Kaur et al. (2005)

3.2.5. Statistical analyses

Data exploration, significance testing, and correlations were completed using SPSS Statistics v. 19 (SPSS Inc., 2010). To accommodate for non-independence and an unbalanced experimental design (unequal samples per pit), and to designate depth as a repeated measure, a linear mixed model was used to perform an analysis of variance (ANOVA) on total PLFA (microbial abundance) and microbial functional group values. Pit (landscape position) and depth were designated as fixed effects. Prior to analysis, PLFA data were log(x+1) transformed to account for non-normality and to improve homogeneity of variance (Steel and Torrie, 1980). Data are presented as untransformed data. Pearson correlation coefficients were calculated for relationships between SOC and microbial functional groups.

Ordination analysis was used to examine and compare microbial community structure in the top 20 cm of pits 1-3, as well as communities found in pit 3 alone. Ordination is a non-parametric statistical method that iteratively searches and graphically arranges data points so that the strongest patterns or gradients within a data set are displayed along one or a few axes.

Nonmetric multidimensional scaling (NMDS) is a powerful ordination technique suitable for use with ecological data sets due to its inclusion of non-normal data and for its use on data that may exist on arbitrary scales (McCune and Grace, 2002). Phospholipid fatty acid data (mol %) was analyzed using NMDS in PC-ORD v.5 (MjM Software, 2006). Data were log(mol%+1) transformed prior to analysis in order to focus on relative distributions of microbial groups and to reduce the magnitude of high values. The Sorensen distance measure was used and the analysis was run on the medium autopilot setting with a random starting point (McCune and Grace, 2002). Final stress values calculated in the ordination analysis represent a departure from monotonicity (Helgason et al., 2010). Low stress values are sought in order to provide more confidence in the interpretability of ordination results. Stress values equal to or less than 10 are satisfactory, while values greater than 20 should be interpreted with caution (McCune and Grace, 2002). Multi-response permutation procedure (MRPP) was the non-parametric method used to test for pre-defined group differences. Reported *p*-values indicate the likeliness that the observed difference is due to chance, and the A-value describes within-group homogeneity, when compared with random expectation. A value of 1 is the highest possible value for A (indicating all subjects within a group are identical). An A value of <0.1 is common in ecological studies, whereas a value >0.3 is quite high (McCune and Grace, 2002). Group differences for the variables 'depth' and 'landscape position' were carried out using PC-ORD v.5.

3.3. Results

3.3.1. Microbial abundance and SOC

3.3.1.1. Total abundance

Total PLFAs and SOC were quantified per 5-cm increment to the depth of A horizon (Fig. 3.4). Pits 1 and 2 displayed typical PLFA profiles that have been documented previously, with the highest concentration observed at the soil surface and decreasing with depth (Blume et al., 2002; Fierer et al., 2003b). When compared to the 0- to 5-cm depth, total PLFA's decreased by approximately 78 and 90% at the lower limits of the A horizon for pit 1 and 2, respectively. Pit 2 displayed a slight increase in total PLFA at the 25- to 30-cm depth, corresponding with the thin buried horizon (Ahbk horizon). The PLFA profile in the surface Ahk horizon (~0-30cm) in pit 3 was similar to that found in pit 1 and 2, but as the A horizon in pit 3 was 81 cm deep, an unusual pattern in microbial abundance ensued in the lower, buried horizons. In fact, the highest

concentrations were contained within the buried horizon (30-60 cm) in this position, indicating the presence of viable biomass, despite conditions which were likely sub-optimal for microbial growth and metabolism. Total PLFAs were found to differ significantly by sampling depth (p<0.001) and by landscape position (p<0.01)(Table 3.3). Total PLFA and SOC displayed similar relative vertical patterns with depth in pits 1-3 (Fig. 3.4), and were significantly correlated (r = 0.40; p<0.001)(Table 3.4). Pairwise comparisons showed that total PLFAs found in the buried horizon in pit 3 were not significantly different than the top 10 cm (p>0.05), indicating that conditions in the buried horizon could promote microbial abundance greater than what is typically found at depth.

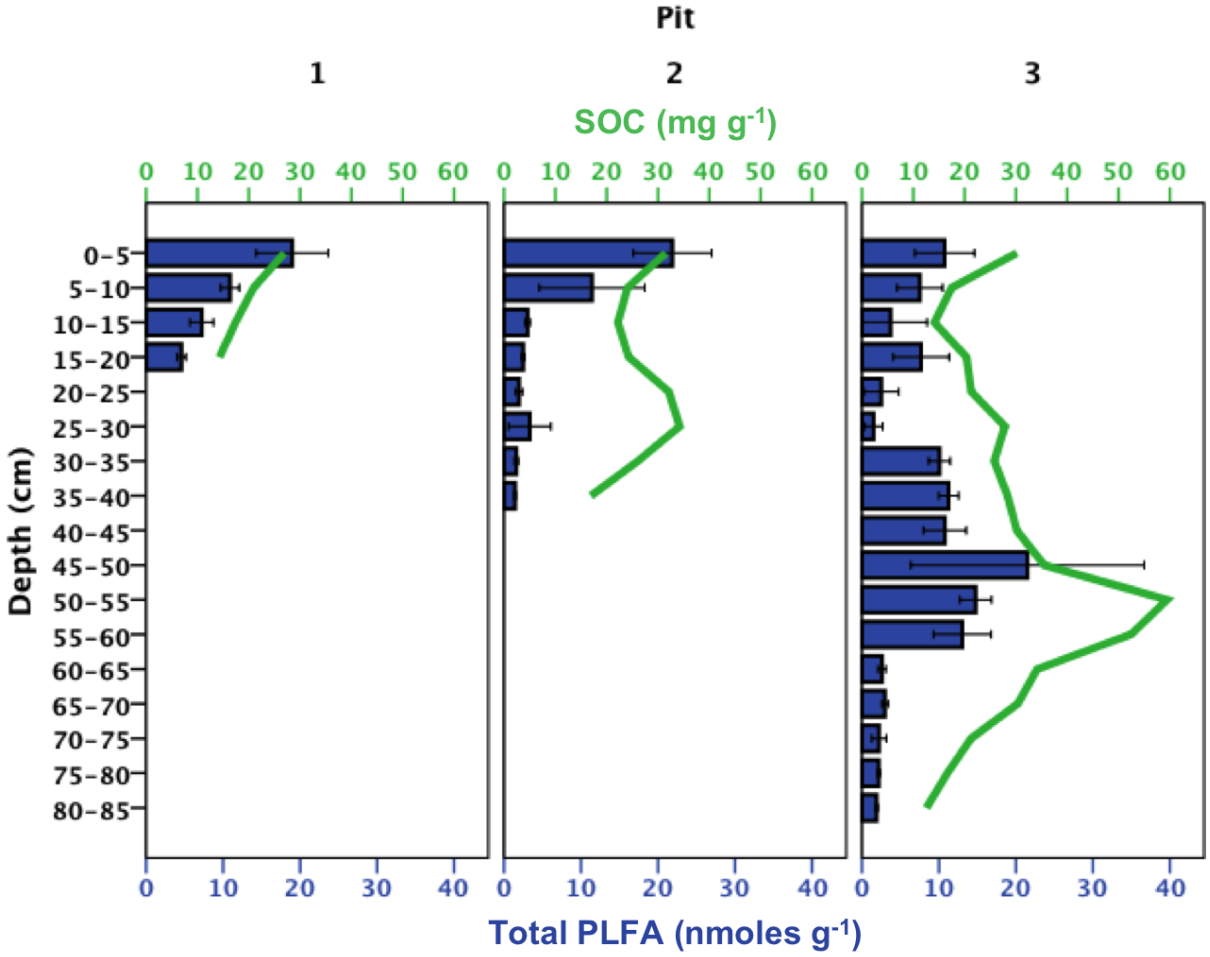

Fig. 3.4 Microbial abundance (total phospholipid fatty acids) and soil organic carbon (SOC) in the A horizon of pits 1-3. The top axis displays SOC (green lines; n=4; included with permission from VandenBygaart et al., 2012); the bottom axis displays total phospholipid fatty acids (blue bars; n=4). Errors bars represent +/- 2 SE.

Table 3.3 Significance levels from ANOVA of the effect of depth (D) and landscape position (LP) on dependent variables: total PLFA, bacterial (B), fungal (F), Gram positive (G+), and Gram negative (G-) microbial abundance, as well as bacterial:fungal (B:F) and Gram positive:Gram negative (G+:G-) ratios.

Factor	Total PLFA	B	F	B:F	G+	G-	G+:G-
Depth	***	***	***	***	***	***	***
Landscape position	**	*	***	***	*	ns	***
D x LP	***	***	***	***	***	***	***

*,**,***, Significant at $p \leq 0.05, 0.01, 0.001$.

3.3.1.2. Functional group abundance

Bacterial and fungal abundance displayed in Fig. 3.5 were both significantly affected by depth ($p<0.001$), and landscape position ($p<0.05$ and $p<0.001$, respectively) (Table 3.3). The vertical distribution of bacterial and fungal functional groups (Fig. 3.5) generally followed a similar pattern to that of total microbial abundance (Fig. 3.4). Bacterial abundance and SOC displayed a stronger correlation than that of total PLFA and SOC ($r = 0.50$; $p<0.01$), whereas no significant relationship between SOC and fungal abundance with depth was observed (Table 3.4). Bacterial and fungal abundance decreased with depth in pit 1, whereas in pits 2 and 3 (depositional backslope and buried backslope, respectively) abundance decreased with depth until the buried horizons were reached (~25 and ~30 cm for pits 2 and 3, respectively), where an increase in both SOC and PLFA occurred. The increase in bacterial concentration in the buried horizons (panel A; Fig. 3.5) is far more prominent than the increase observed in fungal concentration in the buried horizons (panel B; Fig. 3.5). This is also indicated by a significant increase in the bacterial:fungal (B:F) ratio in the buried horizons (Fig. 3.6), suggesting that conditions were more favorable for bacteria relative to fungi. Depth and landscape position were both significant in affecting the B:F ratio ($p<0.001$)(Table 3.3). A significant relationship between SOC and the B:F was also observed ($r = 0.60$; $p<0.01$)(Table 3.4).

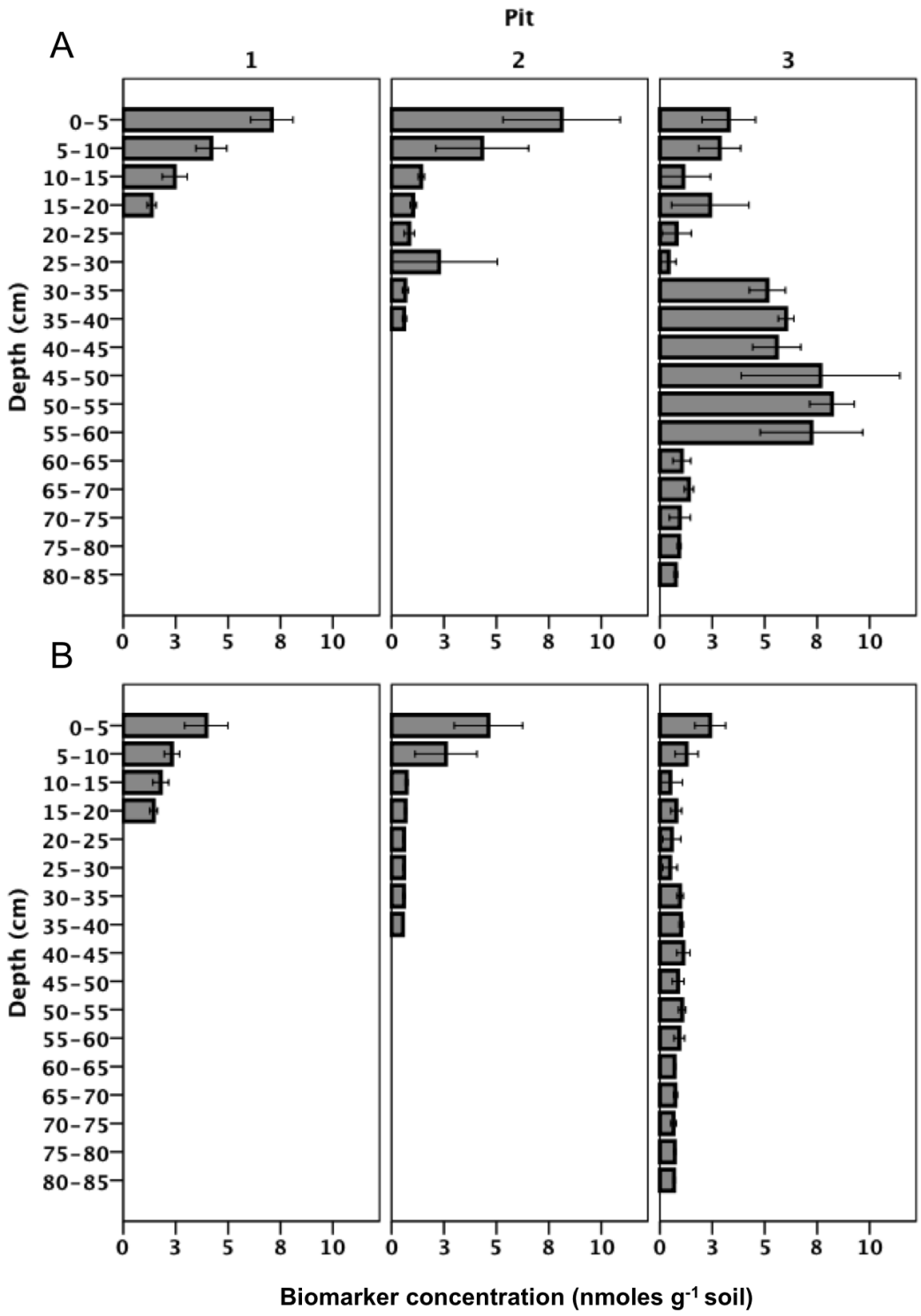

Fig. 3.5 Abundance of A) bacteria; and B) fungi with depth in pits 1-3. Bars represent the mean (n=4) and errors bars represent +/- 2 SE.

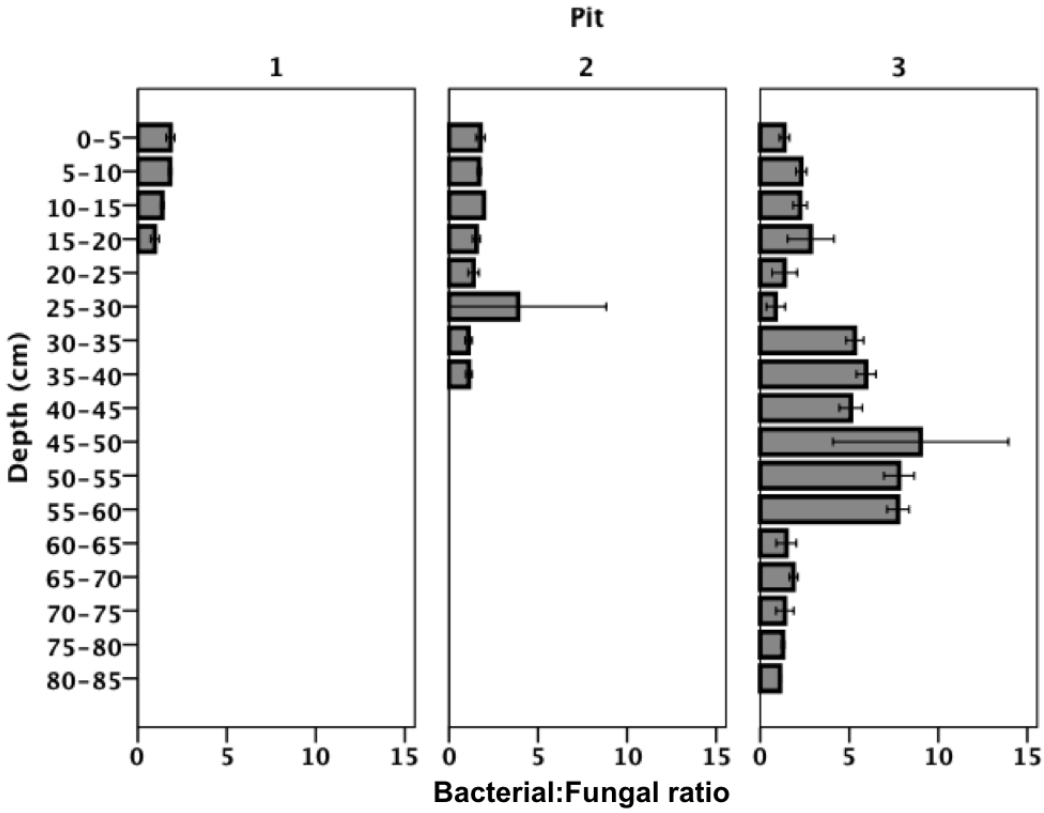

Fig. 3.6 Bacterial:Fungal (B:F) ratio in pits 1-3. Bars represent the mean (n=4) and errors bars represent +/- 2 SE.

Table 3.4 Pearson correlation coefficients between SOC and microbial abundance data: total PLFA, bacterial (B), fungal (F), Gram positive (G+), and Gram negative (G-) microbial abundance, as well as bacterial:fungal (B:F) and Gram positive:Gram negative (G+:G-) ratios.

	Total PLFA	B	F	B:F	G+	G-	G+:G-
SOC	0.393**	0.499**	0.064	0.575**	0.458**	0.518**	-0.49

*,**,***, Significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Gram positive (G+) and Gram negative (G-) bacterial abundance displayed in Fig. 3.7 were both significantly affected by depth ($p<0.001$) (Table 3.3). Gram positive bacterial abundance was significantly affected by landscape position ($p<0.05$), whereas G- bacterial abundance was not. Both G+ and G- bacterial abundance displayed similar relative vertical distributions with depth in pits 1-3 to that of total PLFA (Fig. 3.4), and bacterial and fungal biomarkers (Fig. 3.5). Both G+ and G- biomarkers displayed a significant relationship with SOC ($r = 0.50$, 0.52 respectively; $p<0.01$) (Table 3.4). Despite similar relative distributions with

depth, the Gram positive to Gram negative ratio (G+:G-) decreased with depth in all three landscape positions (Fig. 3.8), indicating environmental conditions were less favorable for G+ growth with increasing depth, and more favorable for G- growth. The G+:G- was significantly affected by depth and landscape position ($p<0.001$).

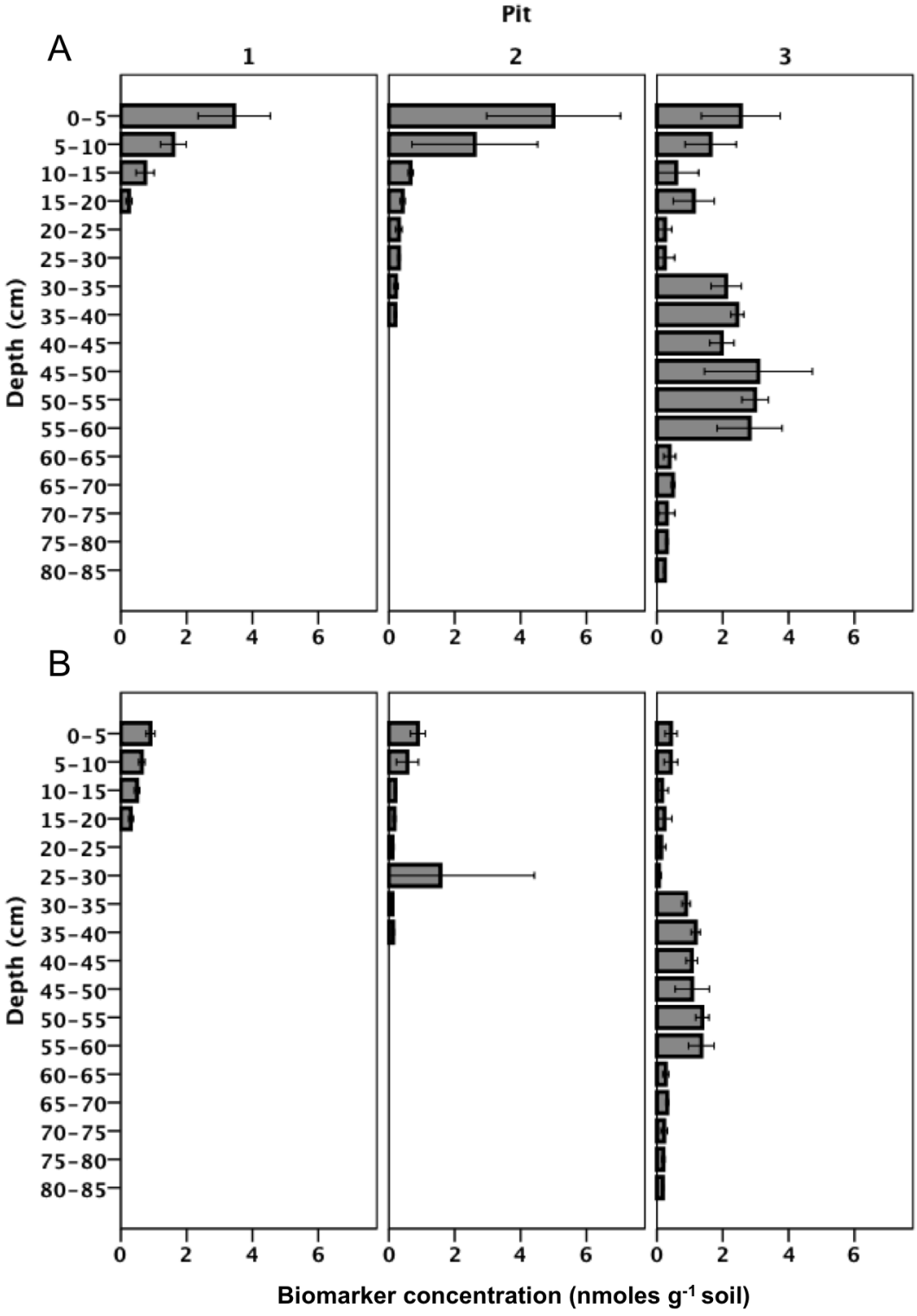

Fig. 3.7 Abundance of A) Gram positive; and B) Gram negative bacteria with depth in pits 1-3. Bars represent the mean (n=4) and errors bars represent +/- 2 SE.

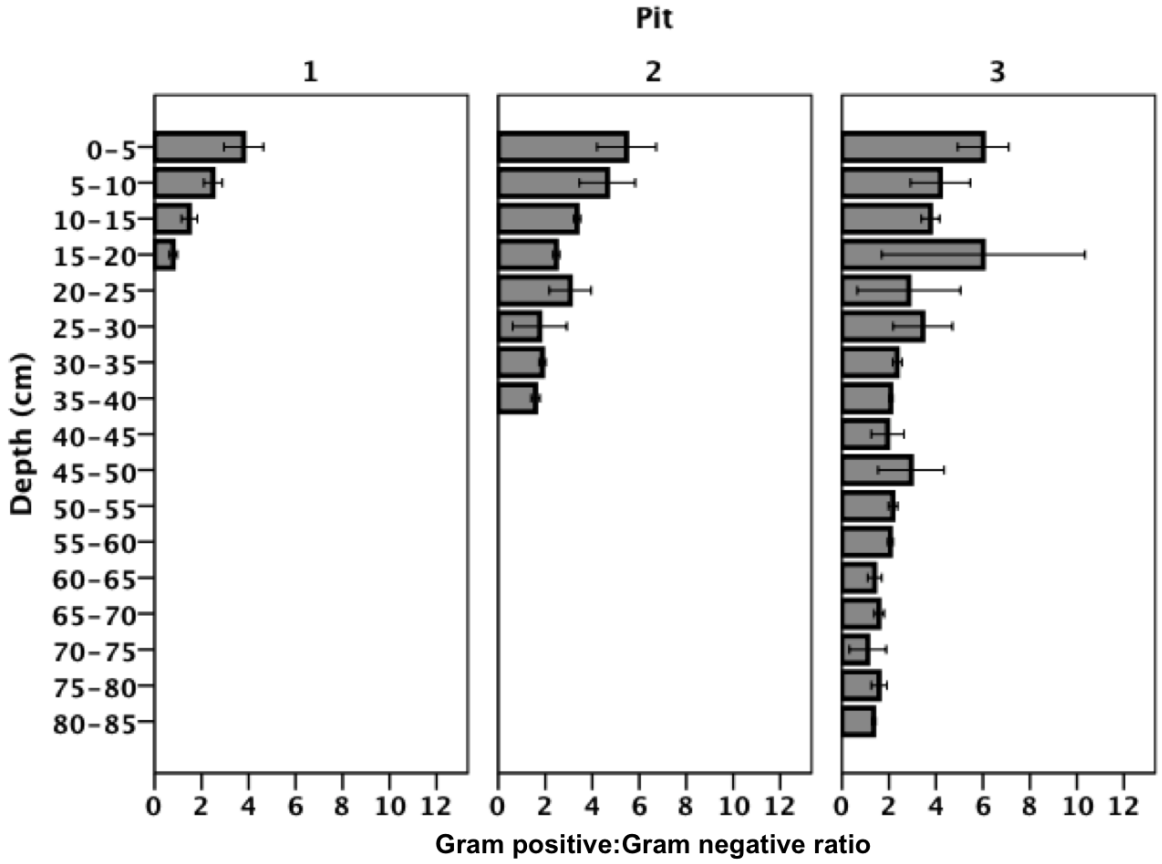

Fig. 3.8 Gram postive:Gram negative (G+:G-) ratio with depth in pits 1-3. Bars represent the mean (n=4) and errors bars represent +/- 2 SE.

3.3.1.3. Stress biomarkers

Stress 1 and stress 2 were both significantly affected by depth (p<0.001), but not by landscape position, or an interaction of depth and landscape position. Both stress biomarkers generally increased with increasing depth, showing mean values of 0.20 and 0.17 in the 0- to 5-cm depth for stress 1 and 2 respectively, increasing by approximate factors of 3 and 5 at the lowest depth of sampling (80-85 cm) (Fig. 3.9). Stress 1 showed a more variable pattern with depth.

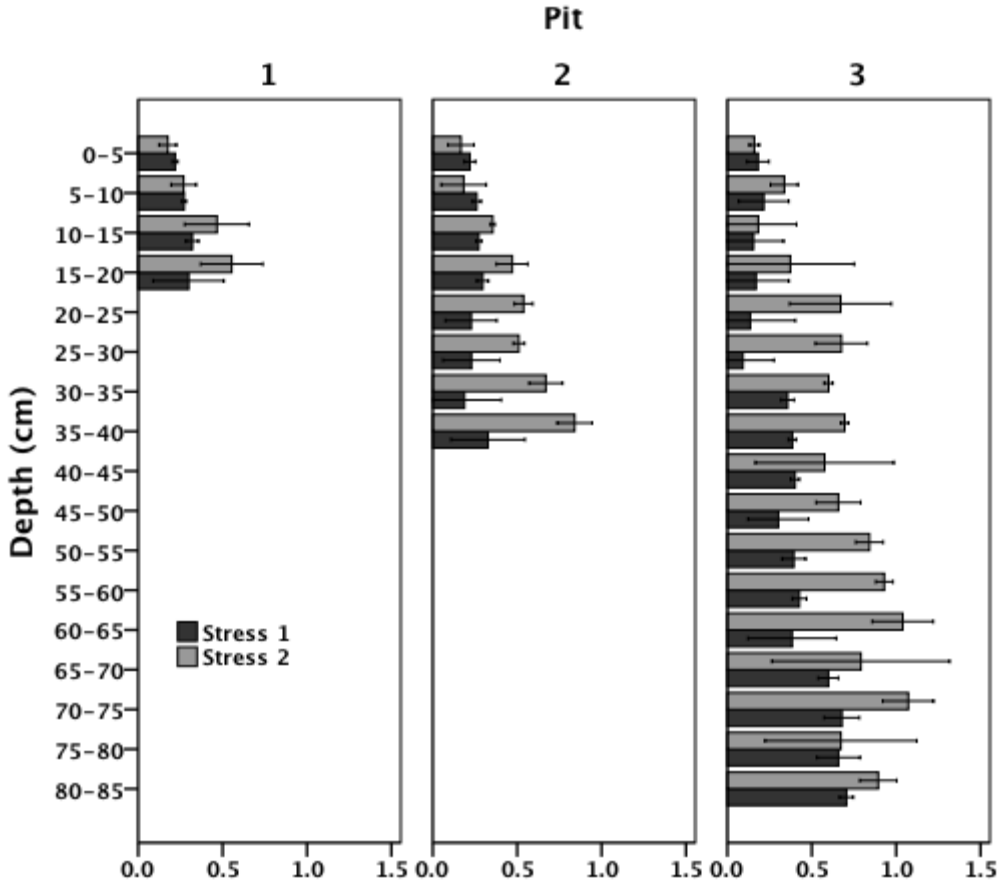

Fig. 3.9 Stress 1 and stress 2 biomarkers with depth in pits 1-3. Stress 1 = cy17:0:16:1 ω7c; stress 2 = cy19:0:18:1 ω7c. Bars represent the mean (n=4) and errors bars represent +/- 2 SE.

3.3.2. Microbial community structure

3.3.2.1. Microbial community structure in pits 1, 2, and 3(0-20 cm)

Nonmetric multidimensional scaling and MRPP analysis of microbial community structure in the top 20 cm of pits 1, 2, and 3 resulted in a 3-dimensional solution with a final stress of 8.7. Along axis 1, microbial communities separated according to landscape position, representing 61% of variation accounted for in the final solution (panel A; Fig. 3.10). Pit 1 and 2 points (open and grey symbols, respectively) overlapped and were positioned separately from pit 3 points (black symbols), indicating that the community structure in the buried backslope position was most different from the other landscape positions. This effect of landscape position was confirmed by MRPP analysis (A= 0.112; p<0.001).

Microbial communities differed significantly by depth, with a clear depth gradient seen along axis 2, representing 20% of variation in the final solution (panel B; Fig. 3.10). Slight overlapping of communities occurred, but the significant effect of depth was confirmed by MRPP (A= 0.135; $p<0.001$). Significant correlations between variables and depth occurred: SOC ($r = 0.65$), arbuscular mycorrhizal fungi (AMF; $r = 0.63$), and G+ bacteria ($r = 0.65$) were all positively correlated with 0-5 cm communities, and the stress 2 biomarker ($r = 0.65$) was positively correlated with the 15-20 cm communities.

Pit 3 communities provided a dominant source of variation in community structure in this solution. The dispersion of pit 3 communities (black symbols) along axis 3 in panel A (and to an extent in panel B; representing 15% of variation in the final solution) is indicative of highly varied community structure within the pit 3 communities (Fig. 3.10).

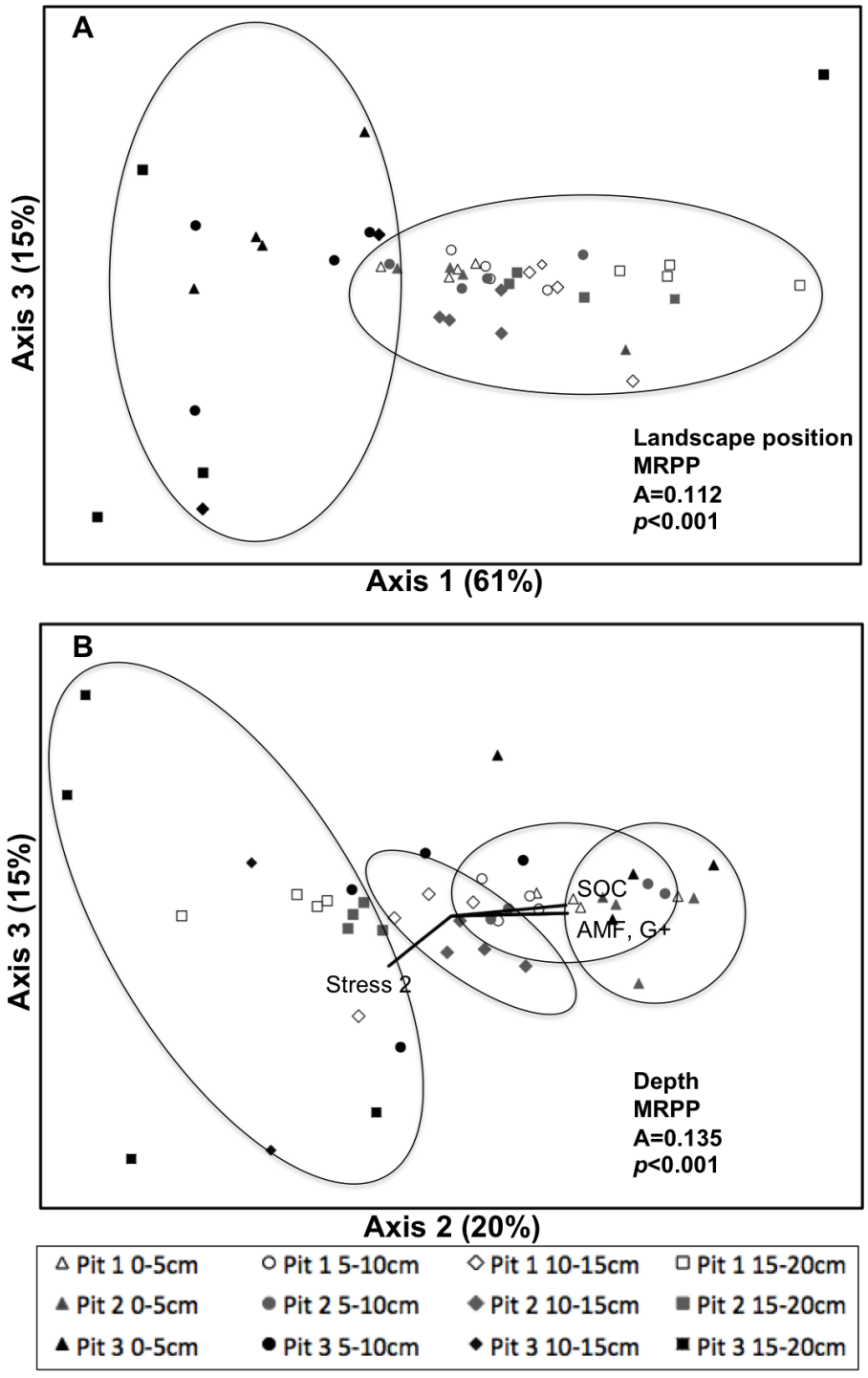

Fig. 3.10 Nonmetric multidimensional scaling (NMDS) analysis and multi-response permutation procedure (MRPP) analysis of microbial communities (mol% data) found in the top 20 cm of pits 1, 2, and 3. Landscape position (pits 1-3) is represented by gray scale and depth increments by different shapes. The 3-dimensional solution had a final stress of 8.7. Panel A (top) displays axis 1 vs. 3 and the effect of landscape position, and panel B (bottom) displays axis 2 vs. 3 and the effect of depth (clusters enclosed in ovals). Labeled vectors indicate correlation (Pearson's *r*; *r* >0.4) between variables and ordination scores.

3.3.2.2. Microbial community structure in pit 3

The A horizon in pit 3 (buried backslope) was very deep (81 cm), with multiple A horizons occurring due to redistribution processes in the landscape. Because of this, pit 3 PLFA profiles were analyzed separately via NMDS to study community structure. Nonmetric multidimensional scaling analysis of microbial communities found in pit 3 resulted in a 2-dimensional solution with a final stress of 13.8 (Fig. 3.11). Three main depth-dependent clusters occurred, spread along both axis 1 and axis 2 (clusters enclosed within ovals). Ordination points clustered according to soil origin: one cluster was communities found in depositional material (Apk horizon; circles) and two clusters were ordination points originating from original, buried material (Ahkb horizon; upper Ahkb, diamonds; lower buried horizon, x's). Total PLFA (r = 0.70) and bacterial biomarkers (r=0.78) were positively correlated with the communities found in the upper half of the Ahkb horizon, and proportional fungal biomarkers were negatively correlated (r = 0.77) along axis 1.

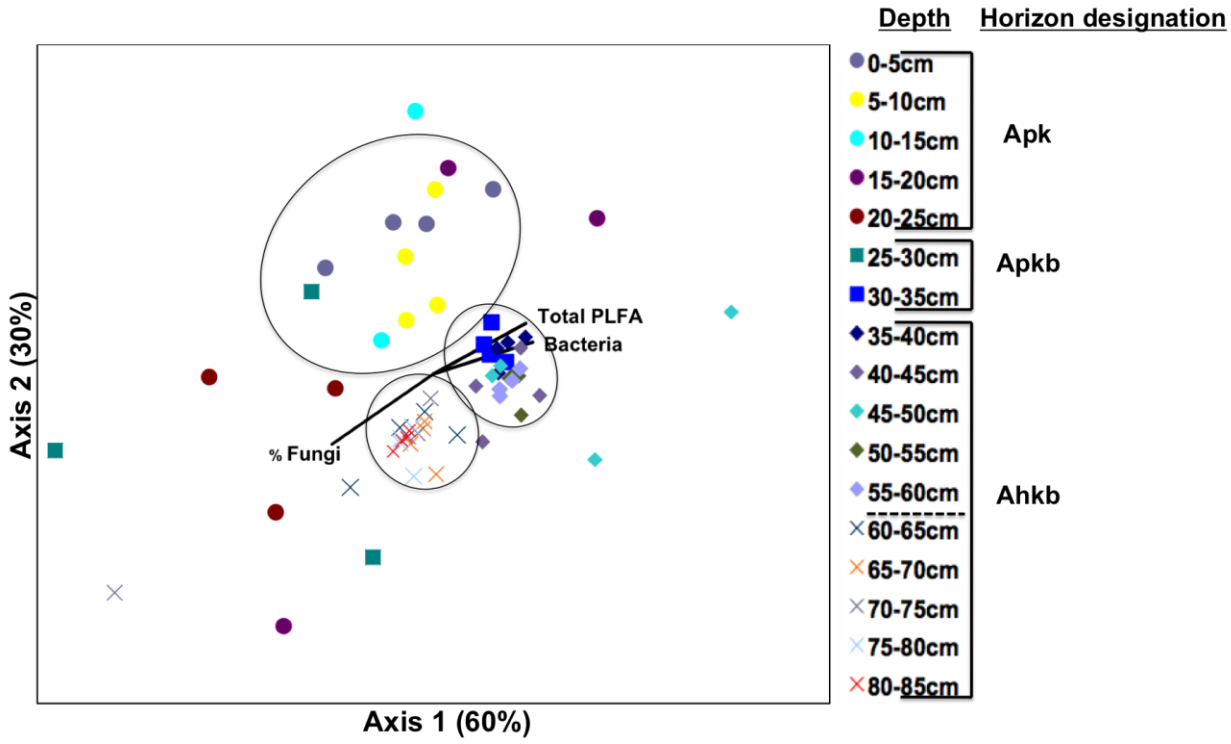

Fig. 3.11 Nonmetric multidimensional scaling (NMDS) analysis of microbial communities (mol% data) found in pit 3 (buried backslope; measured at 5 cm increments). The 2-dimensional solution had a final stress of 13.8. Labeled vectors indicate correlation (Pearson's *r*; *r* >0.40) between variables and ordination scores.

3.4. Discussion

3.4.1. Microbial abundance with depth

Soil organic carbon distribution is the main controlling factor thought to control microbial biomass distribution with depth (Allison et al., 2007a; Blume et al., 2002; Eilers et al., 2012; Fierer et al., 2003b; Fritze et al., 2000). A substantial decrease in microbial abundance was seen from the soil surface to the depth of A horizon in pit 1 and 2 (Fig. 3.4), very clearly following the same relative pattern as SOC (r = 0.40; p<0.001). Markedly, this also occurred for the present day A horizon layer (depositional SOC material, depth 0-30 cm) in pit 3 (Fig. 3.4). Carbon inputs are concentrated and relatively spatially consistent at the soil surface in agricultural and grassland ecosystems; the quantity and quality of SOC is largely dependent upon the amount of residue retained from the plant, fertilizer use (Lorenz and Lal, 2005), plant species, and climatic variables (Jobbagy and Jackson, 2000). Plant root distributions also affect vertical distribution and placement of SOC, with C deposition and placement in the form of root exudates and sloughed root material dependent upon rooting depth (Jobbagy and Jackson, 2000). Accordingly, the highest SOC concentrations were observed near the soil surface in this study (excluding the buried portions of the A horizons), due to the highest amount of plant litter deposition. This corresponded with the highest microbial biomass concentrations (Fig. 3.4). The results seen in this study are consistent with other studies, where similar decreases in abundance were seen with depth, attributed to corresponding gradients in SOC with depth in grassland (Allison et al., 2007a; Eilers et al., 2012; Fierer et al., 2003b) and agricultural ecosystems (Blume et al., 2002). Similar patterns were also observed in a study examining microbial abundance with depth in podzolic forest soils (Fritze et al., 2000).

Soil organic carbon quality also typically decreases with increasing depth, likely affecting microbial abundance due to less biodegradable, more stabilized and processed C at depth (Rumpel and Kögel-Knabner, 2011). This is in contrast to higher amounts of fresh, labile C sources occurring at the soil surface (Lorenz and Lal, 2005; Rumpel et al., 2002). Using a structural equations modeling (SEM) technique, Allison et al. (2007a) suggested that C quantity influenced total biomass distribution along a depth gradient more than C quality, though the relative importance of C vs. N as nutrient sources was not distinguishable in their model.

Environmental gradients with depth also influence microbial abundance (and potentially activity). Moisture and temperature are more temporally variable at the soil surface (Brady and Weil, 2002), though typically more optimal for microbial metabolism; oxygen and pH gradients, as well as textural changes likely occur with depth, but their effect on total biomass remains uncertain. The results of this study suggest SOC is a dominant controlling factor, due to the significant viable microbial biomass found at depth (~60 cm), which corresponded with an increase in SOC in the buried horizon layer in pit 3 (buried backslope).

3.4.2. Viable biomass at depth

Erosional/depositional processes occurring in the landscape largely shaped the most notable morphological characteristics in pit 3 (buried backslope), as well as the occurrence of significant C concentrations located at depth (Fig. 3.4). Such processes can affect the biochemical nature of SOC as it undergoes transport from erosional, exposed positions in the landscape, to depositional positions (Berhe et al., 2007). Erosive forces via wind and water erosion preferentially select for finer and less dense soil particles and associated SOC, which is likely light fraction C (more labile, easily degradable C); this fraction also serves as habitat and substrate for soil microorganisms (Gregorich et al., 1998). In this scenario, soil redistribution preferentially transfers light fraction C and associated microbial biomass, resulting in a concentration of substrate for microbial growth in the depositional positions. In contrast, depending on time in transport, and the degree of erosive forces in detaching, breaking up soil aggregates, and successive soil particle movement, labile fractions of C may be quickly mineralized, leaving a higher proportion of recalcitrant SOM (depleted in labile SOM) to be buried in depositional positions (Berhe et al., 2007). Erosion and redistribution that occur due to cultivation often result in bulk soil transport and fast burial of soil and SOC material (VandenBygaart et al., 2012) that would result in subsequent burial of varying qualities of SOC. In this study, substantial viable biomass was found at depth in pit 3, indicating that substrate of sufficient quality for microbial growth was present, though the lability of C at depth in this position is unclear. A growth advantage may be conferred to redistributed microbial biomass according to the ‘home field advantage’ hypothesis (Strickland et al., 2009a). Microbial communities may be best adapted to past resources; in this instance, allowing them to continue effective growth in a new, and

potentially drastically different environment with substrate they are adapted to utilize, helping to overcome other potential constraints on their growth.

3.4.3. Functional group abundance

Though total microbial abundance is generally well correlated with substrate concentration, individual functional group abundance does not always display the same relationship (Ekschmitt et al., 2008). Other considerations such as substrate quality, edaphic factors, and physiological growth requirements may influence functional group abundance more so than substrate concentration. Redistribution processes that have changed typical substrate quantity and likely quality seen with depth in this study also act to further confound functional group abundance.

Microbial habitat requirements can help to explain the distribution of microbial groups with depth in this study, and specifically the existence of substantial bacterial biomass with depth. Bacterial substrate utilization is thought to rely on microscale solute transport (Ekschmitt et al., 2008), meaning that if necessary conditions are present at depth (e.g. sufficient soil water content, vicinity of substrate to bacteria), bacterial growth should not be limited. In contrast, oxygen is required for the enzymatic reaction involved in lignin utilization, which fungi are thought to dominate, potentially restricting fungal activity to upper portions of the soil profile where oxygen is not as limiting (Ekschmitt et al., 2008). Accordingly, bacterial abundance was significantly correlated with SOC (r = 0. 50, p<0.01), a stronger correlation than that observed for total microbial abundance and SOC (r = 0. 40, p<0.01). The highest fungal abundance was found at the soil surface in all three landscape positions (Fig. 3.5), despite a drastic increase in SOC within the buried horizon in pit 3 (Fig. 3.4). The lack of a significant relationship between fungal abundance and SOC indicated that factors other than substrate concentration are influencing fungal distribution with depth, such as an oxygen gradient. The significant increase in bacterial abundance (Fig. 3.5) and the B:F ratio in the buried horizon of pit 3 (seen to some extent in the buried horizon of pit 2 as well; Fig. 3.6) indicated that bacteria were better able to thrive at depth than fungi and that C utilization in the buried A horizon is more strongly governed by microbial habitat conditions than substrate lability (Ekschmitt et al., 2008).

Previous studies have shown evidence of significant bacterial biomass existing at depth in non-eroding profiles, but very low to zero concentrations of fungal biomass have been detected at similar depths (Ekelund et al., 2001; Ekschmitt et al., 2008). Ekschmitt et al. (2008) reviewed two forest and two grassland ecosystem studies (typical SOC profiles, in landscapes not subject to redistribution): fungi was found mostly concentrated in the top 20 cm of the profile, whereas bacterial groups varied in concentration with depth. Similarly, fungal abundance was also observed to be concentrated in the top horizons (organic and eluvial horizons) in podzolic soil profiles (Fritze et al., 2000).

Trophic interactions between bacteria and micro fauna and between fungi and bacteria may affect abundance in the depth profile. The largest influence that protozoa have on C cycling is the predatory regulation of bacteria in surface depths, where protozoa are concentrated (Brady and Weil, 2002). Restricted growth of protozoa at depth (Ekelund et al., 2001) may better allow bacteria to thrive in the absence of predation. Similarly, bacterial suppression in a short-term decomposition study caused fungal dominance in decomposition with no change in respiration rates (Rousk et al., 2008). If the same is true for the opposite scenario (e.g. low fungal abundance), reduced competition could promote bacterial dominance during decomposition explaining the significant viable bacterial biomass at depth in the buried backslope position.

Phospholipid fatty acid analysis allows further identification of the bacterial domain to G+ and G- functional groups. Assessing the relative distribution of G+ and G- bacterial groups can provide information about environmental conditions in soil and the quality of substrate available. Physiological stress responses and 'life strategies' of these organisms can also provide information on small scale processes in soil that affect C and N flows, and can be extended to the ecosystem scale (Schimel et al., 2007). Microbial community structure in depth profiles have not been extensively studied, but a few common findings regarding G+ and G- distribution have been documented. Gram negative bacteria are generally higher in relative abundance than G+ at the soil surface. For example, in a study of grassland soils, G- bacteria were found in higher proportional abundance at the soil surface, whereas G+ bacteria increased in proportional abundance with depth (Fierer et al., 2003b). Similarly, in an agricultural soil under corn rotation, the ratio of G+ to G- bacteria approximately doubled from the soil surface to the depth of sampling (Blume et al., 2002). These distributions with depth could be partly explained by observed G+ and G- substrate preferences. For example, in a study of microbial C transformation

at three depth intervals (0-20, 20-40, and 40-60 cm), G- bacteria preferentially used plant-related C, which is typically the highest at the soil surface, whereas G+ bacteria preferentially used more recalcitrant, bulk SOM-C, which increases in proportion with depth (Kramer and Gleixner, 2008). In their study of a typical depth profile, these preferences were maintained independent of soil depth. Additionally, G- bacteria have been observed in relatively higher densities in the rhizosphere versus bulk SOM, where G+ bacteria were found in higher proportion (Soderberg et al., 2004).

In this study, the opposite distribution of G+ and G- bacteria was observed; the G+:G- ratio was the highest at the soil surface, and steadily decreased with depth (Fig. 3.8) . Soil redistribution has affected SOC distribution with depth in terms of quantity and quality, resulting in a departure from typically observed microbial patterns. Redistribution results in removal of surface soil at eroding positions, and subsequent burial of surface soil material with relatively more labile SOM components in depositional positions: after repeated redistribution events, displaced surface soil will become a subsoil (Gregorich et al., 1998). Additionally, in eroding positions, former subsoils become surface soils, whereas older more recalcitrant SOM is incorporated with new, plant-derived materials, drastically changing typical C gradients seen with depth (Berhe et al., 2007). Therefore, SOC located at the surface in all three landscape positions is not mainly composed of fresh, labile substrate, and SOC at depth is likely of varying recalcitrance. These altered substrate gradients, in combination with altered edaphic gradients such as increased water content, reduced oxygen availability, and temperature changes, essentially constrain decomposition (Berhe et al., 2007) and ultimately microbial transformation and growth are affected in depositional positions. Similarly, typical gradients are altered at erosional positions with the continual removal of surface soils and exposure of subsurface soil materials. Both G+ and G- bacteria were significantly correlated with SOC (r = 0.50, 0.52 respectively; p<0.01) indicating they responded to SOC concentration with depth. The decreasing G+:G- ratio observed indicates that G+ bacteria are relatively more abundant at the soil surface where SOC quality is perhaps atypical, and G- bacteria are capitalizing on substrate concentrated in the buried horizon that may not be as recalcitrant or poor in quality as predicted.

Stress response characteristics, or life strategies of G+ and G- bacteria may also provide insight into the conflicting finding observed in our study. Gram positive bacteria are thought to be inherently more resistant to both chronic (e.g. drought and freezing) and pulse stress events

(e.g. drying and rewetting), due to their thick cell wall structure, whereas G- bacteria must acclimate to stressful conditions (Schimel et al., 2007). Both strategies are energetically expensive, but if stresses are too acute, G- bacteria may not have the time or resources to initialize their defenses (Schimel et al., 2007). The response of G- bacteria in the buried horizon to SOC concentration, coupled with increasing stress biomarkers with depth (that are related to G- biomarkers, see next paragraph), would indicate that physiological stresses were present, but not so severe that growth was hindered.

The ratios of cyclopropyl fatty acids: cyclopropyl precursors that are associated with G- bacteria have been used as indicators of multiple stresses, such as temperature, pH, and starvation (Kaur et al., 2005). The shift from one structure to another (an increase in the ratio) occurs under stressful conditions to form a more stable, stress-resistant membrane (Kaur et al., 2005). Fierer et al. (2003b) used this ratio in their study of microbial community structure with depth in two California grassland profiles and found an increase in the ratio with increasing depth in both profiles. They attributed the increase in stress to a resource limitation with depth (decreasing C concentration and presumably quality). Similarly, in this study, an increase in stress occurred with depth in all three landscape positions (Fig. 3.9). In contrast to Fierer et al. (2003b), the present work indicates that C concentration is not limiting nor inducing the increase in stress biomarkers with depth, due to the corresponding increase in SOC and PLFA concentration in the buried horizon (Fig. 3.4). Due to redistributional processes in the landscape, C concentration at depth is equal or greater to that found at the surface (with the exception of pit 1). It is likely that substrate quality becomes limiting (and variable) with depth, and is inducing the observed stress response, depending on the level of recalcitrance of C substrate during and after the transport and burial process, and potential constraints on decomposition/utilization once buried. The linear increase in both stress ratios could also indicate a similar linear environmental gradient with increasing depth occurring that is inducing stressful physiological conditions.

3.4.4. Microbial community structure in an eroded landscape

Studying microbial community composition as a whole, rather than by abundance of individual functional groups or biomarkers allows additional insight into whole community compositional changes that occur across gradients. Ordination analyses of community data

allows the strongest gradients or patterns in community composition found in the data set to be visually described, providing a finer scale of resolution (McCune and Grace, 2002).

Environmental conditions or abiotic factors have been studied for their influence on shaping of microbial community composition and distribution, on both regional (de Vries et al., 2012) and biome scales (Decaëns, 2010), as well as on a landscape scale (Allison et al., 2007a, 2007b; de Vries et al., 2012). Specific dominant effects of pH (Rousk et al., 2010) and land use (Steenwerth et al., 2002) have been documented as well as the interacting factors of water content and microbial substrate availability (Drenovsky et al., 2004). Differences in community structure seen among landscape positions or along hillslopes are less studied, but were predicted to occur in the present study due to important soil characteristics known to vary on such scales, such as solum depth, SOM content, nutrient concentration, bulk density, and in particular, moisture regimes (Gregorich and Anderson, 1985). Due to these varying characteristics, topographically variable landscapes can have soils with markedly different properties occurring within short distances (Gregorich et al., 1998).

The sampled landscape positions within the study site occur along a hillslope and have been shaped by erosion and subsequent SOC redistribution, as well as the continuous influence of differing water regimes due to differences in position along the hillslope. This has resulted in different morphologies, soil properties, and varying substrate quality occurring among different positions. These differences are the most evident in the NMDS analysis of the microbial communities in the top 20 cm of pits 1-3 (Fig. 3.10), where pit 1 and 2 communities are the most different from pit 3 communities. Assessment of soil and SOC redistribution in the landscape indicated that pit 2 had both erosional and depositional properties, but when community structure was analyzed in the top 20 cm of all three positions, pit 2 communities were more similar to pit 1 communities than to those from pit 3. These results demonstrate the strong influence of landscape position on microbial community structure, accounting for the largest percentage of variation (60%; Fig. 3.10) in the overall solution.

Along a hillslope in an arid climate, knolls remain relatively dry due to water redistribution, whereas lower slope positions collect and receive higher amounts of water (Famiglietti et al., 1998), and can be more productive in terms of plant biomass, increasing C input to the soil (Gregorich et al., 1998). Soil moisture content has been found to influence soil microbial community composition and may have contributed to differences seen in microbial

community structure among landscape positions at the study site. de Vries et al. (2012) concluded that mean annual precipitation was highly significant in determining community composition in their study of grassland microbial communities across various climatic conditions. Furthermore, soil moisture content affects substrate availability for soil microorganisms, affecting respiration (Davidson et al., 2006) and microbial growth, and subsequently community composition.

The significant effect of depth in the NMDS analyses of microbial community composition in pits 1-3 (Fig. 3.10) supports its strong influence as a factor in structuring microbial communities. Significant changes in microbial community structure with depth have been documented, though which specific biotic or abiotic factors drive these differences remain unknown. Gradients in abiotic factors occur with depth, some of which are postulated to influence differences in microbial community structure (either directly or indirectly), such as soil water content (Allison et al., 2007a) and soil temperature, as well as C quantity and quality changes (Eilers et al., 2012; Fierer et al., 2003b). It is possible that these gradients are acting within our study landscape to influence microbial community structure with depth. Carbon quantity decreases with depth in pit 1 and 2 and at the surface in pit 3, with C quality likely displaying an atypical pattern due to redistribution processes that have occurred in the landscape. Due to the many environmental factors that change with depth, such as substrate and nutrient availability as well as soil moisture, it is difficult to determine which factors drive such structural changes (Eilers et al., 2012).

Changes in microbial community structure with depth have been found to be stronger than those changes seen between different land uses and restoration ages (Allison et al., 2007a), and have been found to be comparable to structural differences seen across different biomes (Eilers et al., 2012). In this study, soil depth was secondary to landscape position in terms of influence on soil microbial community structure. Our study design included A horizon samples only from each position, meaning that we may have missed capturing more influential differences in structure seen with depth in lower B and C horizons. Additionally, only the top 20 cm of each landscape position was included in the comparative analyses of landscape position (pit 2 and 3 had deeper A horizons and were sampled accordingly), as a means of comparing equal soil depths against each other among positions in the NMDS analyses. As such, the large variation in microclimate at each landscape position overrides the differences in community

structure seen in near surface soils. However, depth was still highly significant in influencing community structure, as seen in Fig. 3.10.

It is clear from our analyses that microbial communities throughout the stratified A horizon of the buried backlsope position (pit 3) responded to abiotic gradients occurring with depth, as well as C origin and quality. As observed in Eilers et al. (2012), near surface communities showed the most difference in community structure, attributed to variable environmental conditions occurring at the soil surface (soil moisture and temperature). Highly varied community structure was also seen in our study for communities found in the 0-30 cm increment in pit 3 (Fig. 3.11), attributed to more variable environmental conditions at the soil surface compared to the subsurface. Also to be considered, are the confounding effects of SOC movement in the landscape, which has eroded and deposited SOC in this position, suggesting that a range of substrate lability is occurring here. Within the buried horizon (Ahkb), communities are likely more insulated from surface extremes, which is evident from more closely related communities seen in the NMDS analysis (Fig. 3.11). The presence of distinct communities within buried (original) and depositional portions of the profile demonstrates the influence of C origin and depositional processes on microbial community composition. It is possible that further differentiation in communities within the buried horizon occurring near the 60 -65 cm depth increment is driven due to a decrease in SOC. Additionally, a sharp decrease in the B:F ratio occurs there, also supported by the correlation of % fungi along axis 1 in Fig. 3.11.

3.5. Conclusions

Erosion and redistribution greatly shaped microbial community dynamics within the study site, particularly in the buried backslope position (pit 3) where the magnitude of SOC redistribution events were the most apparent. Similar to non-eroding profiles, depth significantly affected both microbial biomass and functional group abundance. In contrast to non-eroding profiles, substantial viable microbial biomass was found in the buried horizon of pit 3 (30-60 cm), which was correlated with buried C, suggesting soil microorganisms are active at depth once buried. Landscape position and depth were significant in shaping microbial community structure, with the most variability in near surface communities seen between the buried backslope (pit 3) and the eroded shoulder and depositional backslope (pits 1 and 2, respectively), demonstrating the influence of differing moisture regimes and microclimates on microbial

community structure development. Depth and C origin influenced microbial community structure in the buried backslope position (pit 3), where a highly varied community was found in depositional soil material, and distinct communities were found within the buried horizon, driven by changes in SOC and functional group abundance. The results of this study show that erosion and redistribution not only affect SOC content within an eroded landscape, but also greatly affect microbial community dynamics; more research is needed to determine these effects on soil microbial community functioning.

4.0 C MINERALIZATION AND MICROBIAL COMMUNITY STRUCTURE IN SURFACE AND SUBSURFACE SOIL LAYERS IN A DEPOSITIONAL PROFILE: EFFECT OF DEPTH AND DISTURBANCE

4.1. Introduction

The SOC pool is large, acting as the second largest global pool of C (second only to the oceanic storage pool), and the largest actively cycling pool of C in the terrestrial environment (Janzen, 2004). As such, there is great potential for SOC to contribute to future climate change scenarios, either by exacerbating or mitigating CO_2 flux to and from the atmosphere. 'Deep' or subsurface C is increasingly recognized as vital in such scenarios, due in part to the vast amount of C located at depth, amounting to approximately half of total SOC stocks (Batjes, 1996).

Differences in the factors controlling C dynamics between surface and subsurface soils could necessitate the need for different strategies in C modeling and climate change mitigation strategies. Of the three main C stabilization mechanisms: physical protection within aggregates, organomineral associations, and biochemical stabilization (Six et al., 2002), physical protection is thought to play an increasingly important role in subsurface C dynamics (Gillabel et al., 2010). This is in contrast to previous theories where biochemical stabilization (recalcitrance) was thought to be largely responsible (Dungait et al., 2012; Gillabel et al., 2010). For example, recent studies have shown that relative to surface soils, C mineralization in subsurface soils is more affected by physical disturbance via sieving (Salome et al., 2010) and dry/wet cycles (Xiang et al., 2008). Physical disturbance acts to increase contact among microorganisms, enzymes, and C substrates (as well as increasing oxygen diffusion) all of which can be occluded or physically protected and thus inaccessible to one another within microaggregates in undisturbed soils (Dungait et al., 2012). Moreover, it is thought that organomineral associations and occlusion within aggregates occur together to physically protect and render C inaccessible to microbial degradation (Dungait et al., 2012).

Manipulations of temperature and nutrient availability have also affected mineralization in subsurface soil layers more than surface soils. Fierer et al. (2003a) found subsurface soils to be more sensitive to temperature increases and nutrient additions (N and P), with both manipulations increasing rates of mineralization in subsurface soils to a greater extent than surface soils. Differences in microbial community structure with depth are also hypothesized as an explanation for changes in mineralization rate (Fierer et al., 2003a), suggesting that distinct

microbial communities found between surface and subsurface soils, and inherent differences in microbial functioning, will affect process rates (Allison et al., 2007a; Eilers et al., 2012).

Previous differences in C dynamics between surface and subsurface soils were measured in profiles of a 'typical', non-eroding nature (Fierer et al., 2003a; Salome et al., 2010). Far less is known about how erosion and redistribution affect stabilization mechanisms, where subsurface soil horizons become exposed in eroding positions, and soils that were previously at the surface become buried in depositional positions (Berhe et al., 2007; Gregorich et al., 1998), creating 'new' surface and subsurface soils. Additionally, eroding landscapes are thought to stabilize C through the abovementioned mechanisms, with C burial and constrained decomposition at depositional positions, and 'dynamic replacement' of C at eroding positions, resulting in a net storage of C (Berhe et al. 2007; Van Oost et al. 2007). An opposing theory is also supported by some, with these processes thought to result in a net loss of C to the atmosphere, as the result of redistribution causing increased mineralization during transport, and insufficient storage at depositional positions to constitute a C sink (Amundson, 2001; Lal, 2003).

The process of redistribution in agricultural landscapes often results in the development of deep profiles in depositional positions, forming multiple buried A horizons, with a net increase in C concentration (VandenBygaart et al., 2012). It is globally estimated that 16-21 Pg C have been buried in agricultural landscapes in the last 50 yr (Van Oost et al, 2007). Though thought to be stabilized at depth, the stability of buried C is unclear in the face of future climate change scenarios and potential disturbances (Van Oost et al., 2007). The work of the present study aims to contribute to the research question – what is the fate of redistributed and buried C?

To date, little work has been done assessing C dynamics in landscapes subject to redistribution. Additionally, no known studies have assessed microbial community dynamics associated with such landscapes. This incubation study examined C mineralization of surface and subsurface soils from a depositional position that contained a buried A horizon. The specific study objectives were to: determine C mineralization and microbial community response of surface and subsurface soil layers in a depositional position to 1) incubation in surface-like conditions, and 2) physical disturbance via sieving.

4.2. Materials and methods

4.2.1. Sample selection and collection

The primary site of interest for this study was a depositional profile located along a hill slope in an agricultural landscape within the SDNWA. This particular landscape position was chosen for the incubation study due to substantial SOC deposition and ensuing morphological characteristics that were observed in previous studies (Fig. 4.1) (see section 3.2.1 for site description, this document; VandenBygaart et al., 2012). Sample depths selected for incubation were based on ^{137}Cs deposition data (Fig. 3.3), horizon classification (Pit 3, buried backslope; Table 3.1), and texture analysis (Fig. B.1) completed by VandenBygaart et al. (2012). The selected depths aimed to include soil origin differences (original and recently deposited material) and to include a change in clay content found in the Ahkb horizon. The first two depths (0-5 and 20-25 cm) occurred in recently deposited material (Apk horizon) and aimed to capture the surface microbial community believed to be the most active and abundant (Fig. 4.2). The third and fourth depths (40-45 and 65-70 cm) occurred within the Ahkb horizon to include original A horizon soil material and a change in clay content seen at approximately 65 cm (Fig. B.1). A control location (or 'reference soil'; abbreviated as 'Ref' in figures) was also sampled from a level upland area in the same landscape where minimal soil redistribution had occurred. This soil was included in the incubation and used as a reference for a more 'typical' surface soil in terms of microbial community dynamics and SOC quantity and quality. The reference location and the primary site of interest have been managed under the same agricultural and cropping practices.

Fig. 4.1 Image of pit 3, buried backslope position. The dark, original A horizon can be seen approximately half way down the profile, overlain by depositional material.

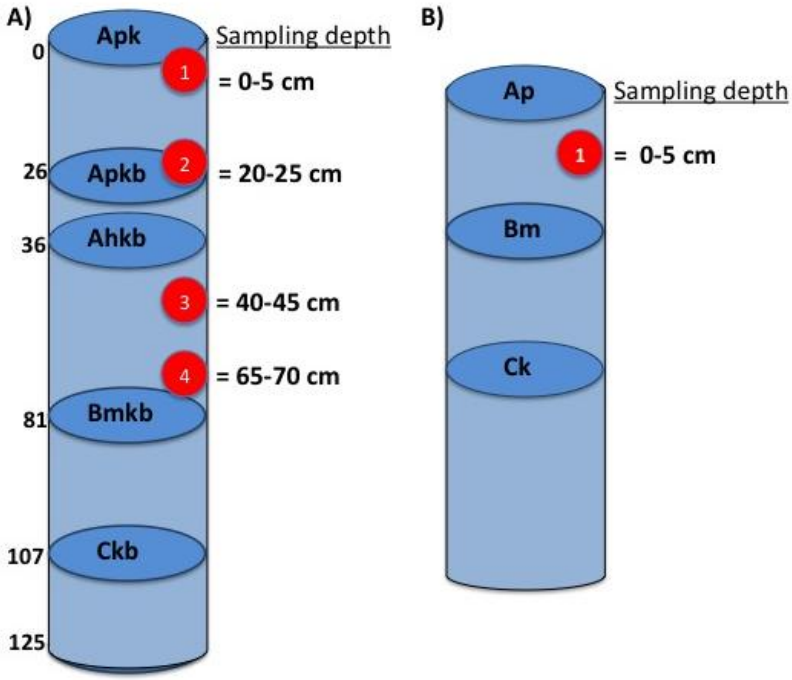

Fig. 4.2 Selected depths for the incubation experiment. Image depicts horizonation as it would be seen in a sampled soil core: A) the four selected depths in the buried backslope position; B) the reference soil from a level upland position.

Sampling occurred in spring 2011 (May 20) prior to seeding. Samples were collected in soil cores (5.08 cm diam.) with a plastic liner insert using a truck mounted hydraulic punch system (Giddings Machine Co, Inc., Fort Collins, CO). Once collected, samples were transported in a cooler and placed in a 4°C fridge until processing (10 d) and the commencement of the incubation.

4.2.2. Experimental design

4.2.2.1. Treatments

To determine effects of spatial substrate accessibility and to simulate disturbance, a disturbed treatment was imposed on incubated soils. The disturbed soil cores were sieved to 2 mm to study the effects of spatial separation between microorganisms and C substrates (Salome et al., 2010). In contrast, to simulate *in situ*, undisrupted conditions, undisturbed soil cores were also included in the study. To access selected depths, cores were cut at required increments. Sieving was completed 24 h prior to incubation start, and was performed in a 4°C chamber to minimize C mineralization.

4.2.2.2. Incubation conditions

Microcosms were kept in a shelved incubator (Adaptis a1000, Conviron, Winnipeg, MB) for the duration of the study. Microcosms were held at a constant gravimetric water content (70% of field capacity; by weight) and constant temperature (25°C). Temperature was monitored using two Tinytag ULTRA 2 data loggers (Gemini Data Loggers Ltd., Chichester, UK), evenly positioned throughout the incubator. Microcosms were monitored for gravimetric water content by weighing at time of sampling, and maintained accordingly, adjusted typically twice per 7 d.

4.2.2.3. Sampling schedule

The incubation lasted 62 d and included three destructive samplings: day 0, day 14, and day 62 (hereafter referred to as T0, T1, and T2 'time intervals', respectively). Soil removed at each destructive sampling underwent any required processing (freeze-drying and grinding for PLFA) and were stored at -80°C until further analysis. Carbon respiration (CO_2 evolution) was assessed on days 1, 3, 5, 7, and 9, and twice a week thereafter for the duration of the study. Analyses completed on soils from all 3 destructive samplings were: total SOC, PLFA, and community level physiological profiling (CLPP) using BIOLOG Ecoplate™ analysis. Water extractable organic matter (WEOC) was completed on T0 and T2 soils only.

4.2.3. Gas sampling methodology and analysis

To sample headspace, a 35 mL sample was extracted using a syringe, the plunger compressed to 25 mL and inserted into a pre-evacuated 12 mL Exetainer® (Labco Inc., Lampeter, UK) (evacuated to 0.007 kPa). Samples were stored at room temperature until analysis, which was typically completed in less than 2 weeks.

Two gas samples were collected per sampling period: an initial sample at the onset of the accumulation period and a final sample taken at the end of the accumulation period. After each reading, microcosms were allowed to equilibrate with ambient air by briefly removing jar lids and flushing the jar with ambient air. After flushing, the jar lids were reapplied, the headspace was flushed with CO_2 free air (at a rate of 1 L min^{-1} for 7 min), and the initial reading of the next sampling period taken. This was done to avoid potential CO_2 saturation effects on mineralization.

Samples were analyzed for CO_2 concentration using a gas chromatograph equipped with a thermal conductivity detector (TCD) (Varian Canada Inc. Mississauga, ON) and CombiPAL autosampler. The TCD temperature was 130°C with a filament temperature of 220°C. The injector was set at 100°C and the column oven at 80°C. The carrier gas for CO_2 was helium (He), with a flow rate of 30 mL min^{-1}. Carbon dioxide was quantified by comparing sample peak area with that of a 400 µL L^{-1} CO_2 custom standard (Praxair Specialty Gases, Edmonton, AB).

Respiration rates were calculated as the difference in CO_2 between the initial and final sample, divided by the accumulation period. To calculate CO_2 concentration, headspace volume was estimated as follows:

Headspace volume = Jar volume - (soil volume + moisture volume + vessel volume)

Carbon dioxide concentrations were then corrected using the ideal gas law (Pare et al., 2005). In doing so, pressure was assumed to be 101.3 kPa, and temperature held constant at 25°C (incubator temperature). Carbon mineralization was calculated on a per mass of soil basis (mg CO_2-C kg^{-1} soil), presented both cumulatively and by daily flux rate. Only those mineralization values from microcosms that were incubated for the entire duration of the study (T2 samples) were used for cumulative analysis.

4.2.4. Soil and microbial analyses

4.2.4.1. Total SOC and WEOC

Samples were freeze-dried and ground prior to total SOC analysis. Total SOC was measured by combustion at 840°C (Wang and Anderson, 1998) on a C 632 Carbon Determinator (LECO Corporation, St. Joseph, MI).

Water extractable organic carbon (WEOC) was used as a measure of C quality. Water extractable organic carbon is considered a labile and readily available fraction of organic matter that is extracted with a weak salt or water solution (Chantigny, 2003; Mycock, 2011). The extraction methods of Chantigny et al. (2008) and Mycock (2011) were followed. In brief, field moist samples were sieved to 4.75 mm and 10 g of soil (dry mass basis) was extracted with a 5 mM $CaCl_2$ solution at a 1:2 soil to solution (mL) ratio. Samples were lightly stirred for 1 min and extracted using 0.45 µm syringe filters. Samples were stored at -20 °C until time of analysis (3-4 wk). Water extractable organic carbon was measured by wet combustion-oxidation on a TOC-V CPH/CPN (Shimadzu Corporation, Kyoto, Japan).

4.2.4.2. PLFA

Phospholipid fatty acid analysis followed the methods outlined in Section 3.2.4 (p. 16, this document). Immediately after each destructive sampling, samples were frozen and stored at -80°C, where they were stored until further processing. Samples were removed from the freezer, freeze-dried and ground, and replaced at -80°C to prevent degradation of the lipids prior to extraction. To increase detection on the gas chromatograph, two 4 g samples per depth were extracted and combined (to account for low concentration in some samples). Final PLFA values were presented as concentration per g of dry soil.

4.2.4.3. Community level physiological profiling: Biolog EcoPlate™ analysis

Community level physiological profiling using the Biolog microtiter EcoPlate™ is a method used to study functional diversity of whole microbial communities (Garland, 1997). This involves direct inoculation of environmental samples in plates containing individual C substrates and studying substrate utilization to distinguish microbial communities based on metabolic profiles. Carbon substrate utilization is quantified by color production in each individual well; color develops from the reduction of a tetrazolium violet dye as C substrates are used and respiration occurs (Garland & Mills, 1991). This study used the Biolog EcoPlate™ (Biolog, Inc.,

Hayward, CA), which contains 31 different C substrates and one water blank (Table 4.1), replicated three times per plate (96 wells in total).

Biolog EcoPlate™ inoculation was performed as described by Dunfield and Germida (2003). Plates were inoculated at each destructive sampling (0, 14, and 62 d). Briefly, soil (5 g, wet wt.) was added to 95 mL of phosphate-buffered saline (PBS) solution and shaken on a rotary shaker for 20 min at 200 rpm (22°C). After shaking, 10^{-2}, 10^{-3}, and 10^{-4} dilutions were made using sterile PBS, and the 10^{-2} and 10^{-4} dilutions kept for Biolog EcoPlate™ inoculation. The 10^{-2} and 10^{-4} soil dilutions were selected for plate inoculation to encompass the range of cell densities predicted to be found in samples originating from different depths (two plates inoculated per sample, one of each dilution). For each plate, 100 µL of inoculum was added to each well. Plates were incubated at 28°C for 5 d. To study reaction patterns, color development (optical density measured at 590 nm) was recorded every 24 h over the 5 d incubation period, on a SpectraMax Plus 384 microplate reader (Molecular Devices, Sunnyvale, CA).

4.2.5. Statistical analyses

A linear mixed model was used to analyze cumulative mineralization, WEOC, and SOC values. For final cumulative mineralization values, only the four depths found in the backslope position were included in the model, with depth treated as a repeated factor. For WEOC and TOC values, 'time interval' was treated as a repeated factor, representing samples measured at different destructive sampling occurrences (T0, T1, T2).

Statistical analysis of PLFA was done as per Section 3.2.5 (p. 16; this document). A linear mixed model was used to analyze differences in total PLFA and specific functional groups with depth and over time. In this instance, 'time interval' was treated as a repeating factor. To achieve normality, values were log(x+1) transformed prior to analysis; actual values are presented. Nonmetric multidimensional scaling was used to study community structure differences with depth and over time. Phospholipid fatty acid data was log(mol%+1) transformed prior to NMDS analysis and only fatty acids that represented >1% of the total microbial community (mol%) were included.

Table 4.1 Biolog Ecoplate™ carbon substrates and guild groupings.

Well no.	Carbon source	Guild grouping†
1	Water (control)	
2	Pyruvic acid methyl ester	Carbohydrate
3	Tween 40	Polymer
4	Tween 80	Polymer
5	Alpha-cyclodextrin	Polymer
6	Glycogen	Polymer
7	D-cellobiose	Carbohydrate
8	Alpha-D-lactose	Carbohydrate
9	Beta-methyl-D-glucoside	Carbohydrate
10	D-xylose	Carbohydrate
11	i-erythritol	Carbohydrate
12	D-mannitol	Carbohydrate
13	N-acetyl-D-glucosamine	Carbohydrate
14	D-glucosaminic acid	Carboxylic and acetic acid
15	Glucose-1-phosphate	Carbohydrate
16	D,L-alpha-glycerol phosphate	Carbohydrate
17	D-galactonic acid-gamma-lactone	Carboxylic and acetic acid
18	D-galacturonic acid	Carboxylic and acetic acid
19	2-Hydroxy benzoic acid	Carboxylic and acetic acid
20	4-Hydroxy benzoic acid	Carboxylic and acetic acid
21	Gamma-hydroxybutyric acid	Carboxylic and acetic acid
22	Itaconic acid	Carboxylic and acetic acid
23	Alpha-ketobutyric acid	Carboxylic and acetic acid
24	D-malic acid	Carboxylic and acetic acid
25	L-arginine	Amino acid
26	L-asparagine	Amino acid
27	L-phenylalanine	Amino acid
28	L-serine	Amino acid
29	L-theonine	Amino acid
30	Glycyl-L-glutamic acid	Amino acid
31	Phenylethylamine	Amine/Amide
32	Putrescine	Amine/Amide

†Guild groupings adapted from Weber & Legge (2009).

4.2.5.1. Biolog EcoPlate™ statistical analysis: Normalization using AWCD

To determine the overall rate of color development (substrate utilization) and identify a standardized point for further functional diversity analysis, average well color development (AWCD) was calculated as described by Garland and Mills (1991): subtracting color development in the control well from each substrate well and calculating a mean. When negative absorbance values were recorded, a value of zero was assigned to that well (Harch et al., 1997). Negative values occurred at early reading times (24-48 h) and were the result of similar color development in substrate wells to that of the control well. The 10^{-4} dilution was not selected for any further analysis due to low color development, likely reflecting low inoculum density. Analysis was only completed on undisturbed samples to study the *in situ* substrate utilization with depth.

In order to compare community profiles among sample depths and among the three time intervals, absorbance values were normalized to eliminate the influence of different inoculum densities. To do this, plates were first chosen where AWCD fell within a similar range in each time interval (red circles, 0.4-0.8; Fig. B.2). From there, on each chosen plate, each substrate well was divided by its AWCD (Garland and Mills, 1991).

4.2.5.2. Community level physiological profiling and guild grouping

Each of the 31 C substrates in the Biolog Ecoplate™ can be sorted into groups or guilds (Weber and Legge, 2009). In doing this, the data set is reduced to fewer dimensions which is an effective way of analyzing substrate usage in larger functional groups over time. For the following analyses, guild groupings proposed by Weber and Legge (2009) were followed, as seen in Table 4.1. The guild groupings (proportional guild usage, % of total absorbance) and the normalized absorbance values for each of the 31 C substrates were included in the CLPP analysis, completed using NMDS in PC-ORD v.5.

4.3. Results

4.3.1. C characterization

4.3.1.1. Total SOC

Total SOC concentration for each incubated depth is displayed in Fig. 4.3. The effect of time interval and treatment were not significant, whereas the effect of depth was significant (p<0.001; Table 4.2). No major changes to total SOC concentration over the 62 d period were expected, because the size of the total SOC pool is only affected on much longer time scales than the duration of this study. The deepest depth sampled (65-70 cm) displayed the highest SOC concentration, higher than both the reference (ref) and 0-5 cm soil. The highest concentration found in the 65-70 cm depth corresponds with the SOC 'bulge' seen in the buried horizon in the previous fall sampling at the same site (Fig. 3.4).

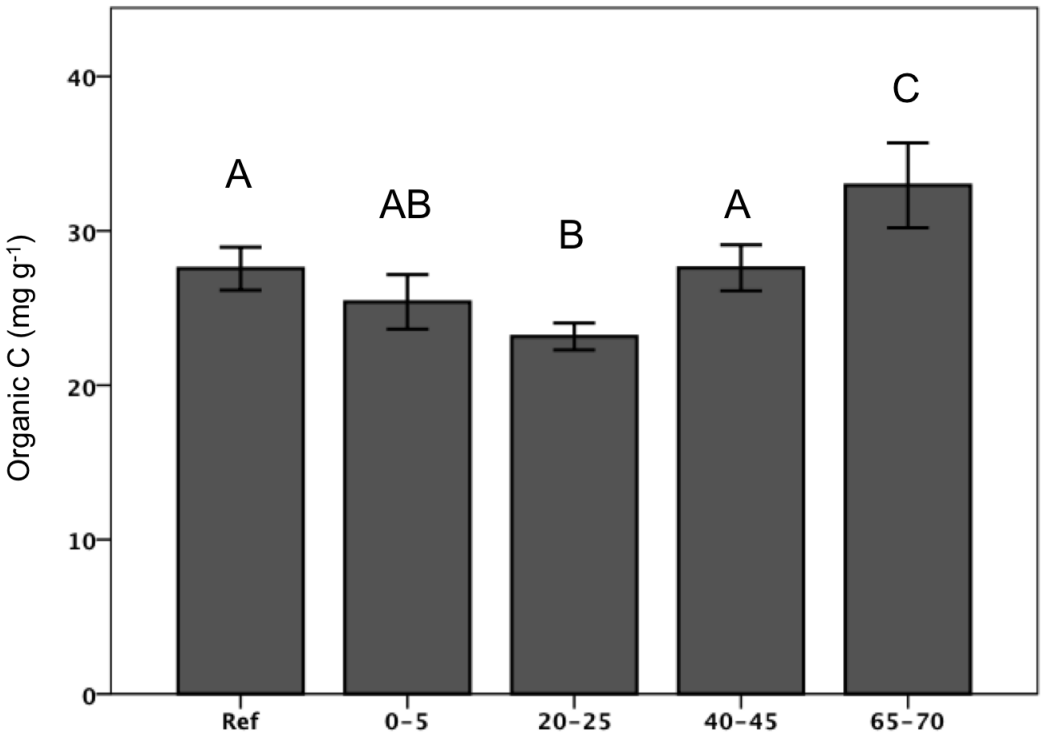

Fig. 4.3 Total SOC for the five incubated soil depths (reference soil, Ref; 0-5, 20-25, 40-45, and 65-70 cm). Bars represent the mean and errors bars represent +/- 2 SE of the mean. Uppercase letters denote statistical differences among incubated depths.

4.3.1.2. WEOC

Water extractable organic carbon in disturbed samples was lower at T2 compared to the onset of the incubation (Fig. 4.4). Time interval and the disturbance treatment both significantly affected WEOC (p<0.001; Table 4.2), whereas depth did not. Two-way interactions between depth and time interval (p<0.001) and depth and treatment occurred (p<0.05), as well as a 3-way interaction among all factors (p<0.001).

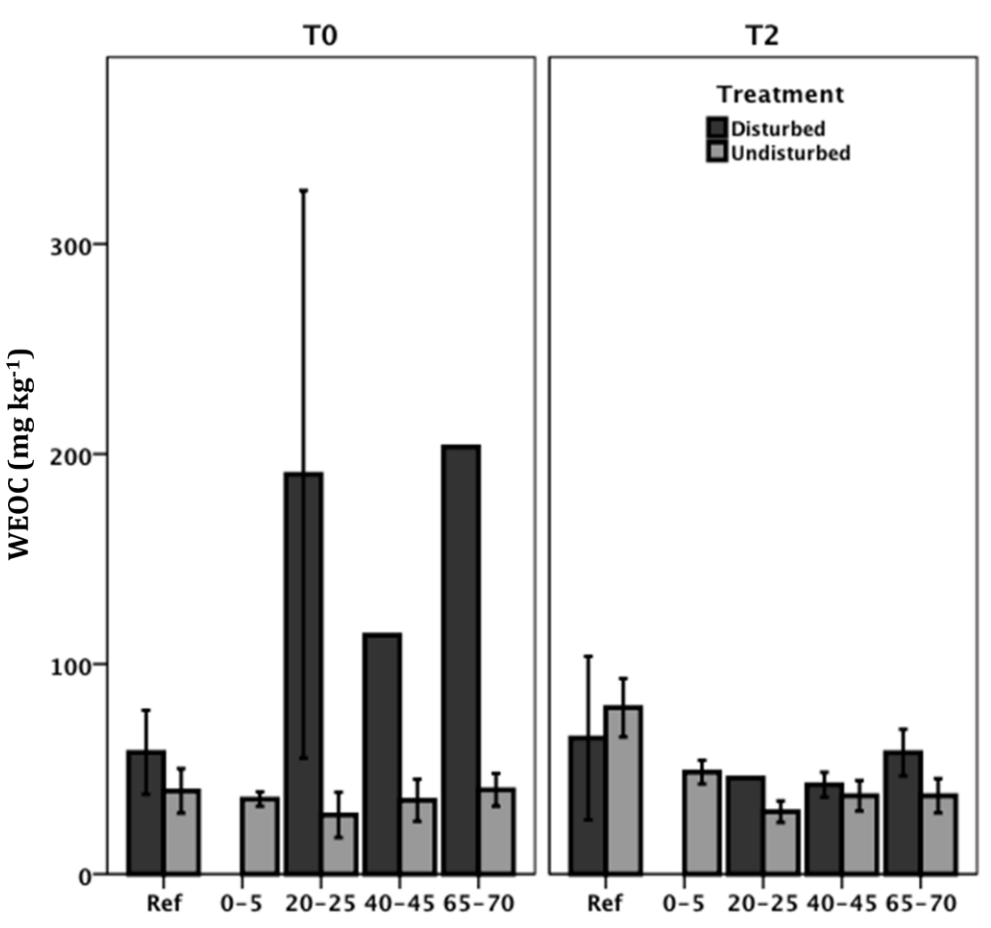

Fig. 4.4 Water extractable organic carbon concentration for the five incubated depths (reference soil, Ref; 0-5, 20-25, 40-45, and 65-70 cm) from T0 (left panel) and T2 (right panel) destructive samplings, with the effect of disturbance displayed. Bars represent the mean (n=3) and error bars represent +/- 2 SE of the mean. Disturbed 0-5 cm samples were not available for analysis. Error bars are not present where n=1.

4.3.2. C mineralization

Figure 4.5 displays cumulative mineralization over the 62-d incubation period for each depth increment. Final cumulative values differed with depth (p<0.001), but not disturbance treatment (Table 4.2). Cumulative mineralization in the 0-5 cm depth (Fig. 4.5; panel B) was as much as three times higher than the deeper depths (panel C; note the change in scale). Disturbed samples were slightly higher than undisturbed samples, but not significantly so. The reference soil (panel A) mineralized substantially more C than both samples found in the depositional and original soil material, suggesting more available carbon in the level upland position when compared with the depositional position. Daily respiration rates were highly variable for the reference and the 0-5 cm soil (Fig. B.3). All incubated samples displayed the highest rates at the onset of the incubation and gradually tapered off; the 20-25, 40-45, and 65-70 cm samples were at their lowest rate by day 7, and fluctuated above and below that level until the end of the incubation.

Table 4.2 Significance levels from ANOVA of the effect of depth (D), time interval (TI), and disturbance treatment (DT) on dependent variables: total soil organic carbon (SOC), water extractable organic carbon (WEOC), and cumulative respiration (Cumulative-C) on incubated samples.

Factor	SOC	WEOC	Cumulative-C
Depth	***	ns	***
Time Interval	ns	***	ns
Disturbance Treatment	ns	***	ns
D x TI	ns	***	na
D x DT	ns	*	ns
TI x DT	ns	***	na
D x TI x DT	ns	**	na

*,**,***, Significant at $p \leq 0.05, 0.01, 0.001$.

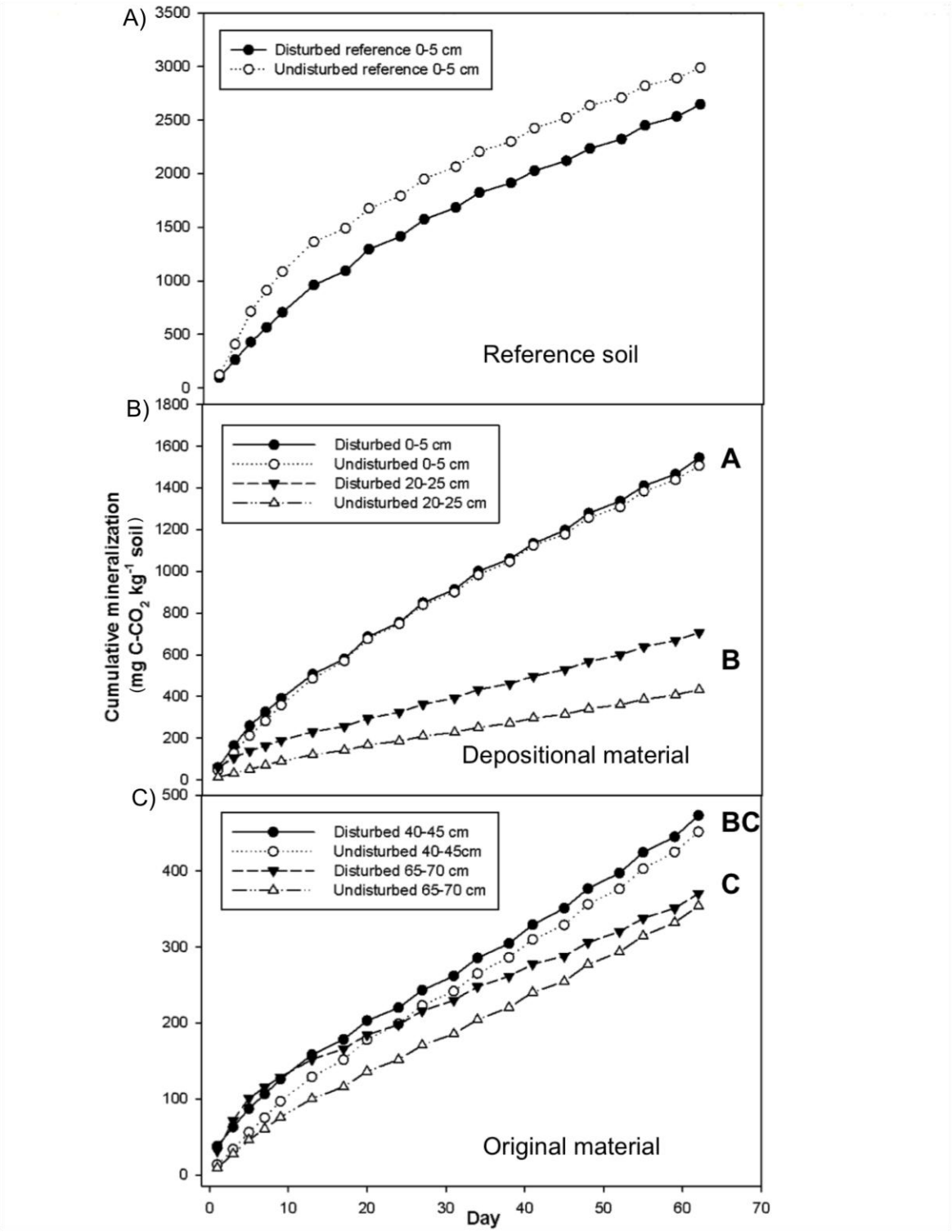

Fig. 4.5 Cumulative mineralization during a 62 d soil incubation experiment. Five soil depths with the disturbance treatment are shown. Samples are displayed by soil origin; panel A displays the reference soil, panel B the depositional material, and panel C the original material. Letters denote significant differences among depth increments for final cumulative mineralization values. The reference soil was not included in the statistical model. Note the change in scale between axes. Symbols represent the mean (n=3; T2 soils only).

4.3.3. Microbial community analyses

4.3.3.1. Total PLFA

Depth affected total PLFA (p<0.001); the reference and the 0-5 cm soils were consistently different from the 20-25, 40-45, and 65-70 cm soils across all three time intervals, as seen in Fig. 4.6 (Table 4.3). Differences in total PLFA among time intervals also occurred (p<0.001). Total PLFA concentration remained similar for all depths and for both treatments from the first time interval to the second time interval (14 d); however, an increase in total PLFA concentration occurred from the second time interval to the third (62 d) for all depths. Disturbance did not affect total PLFA.

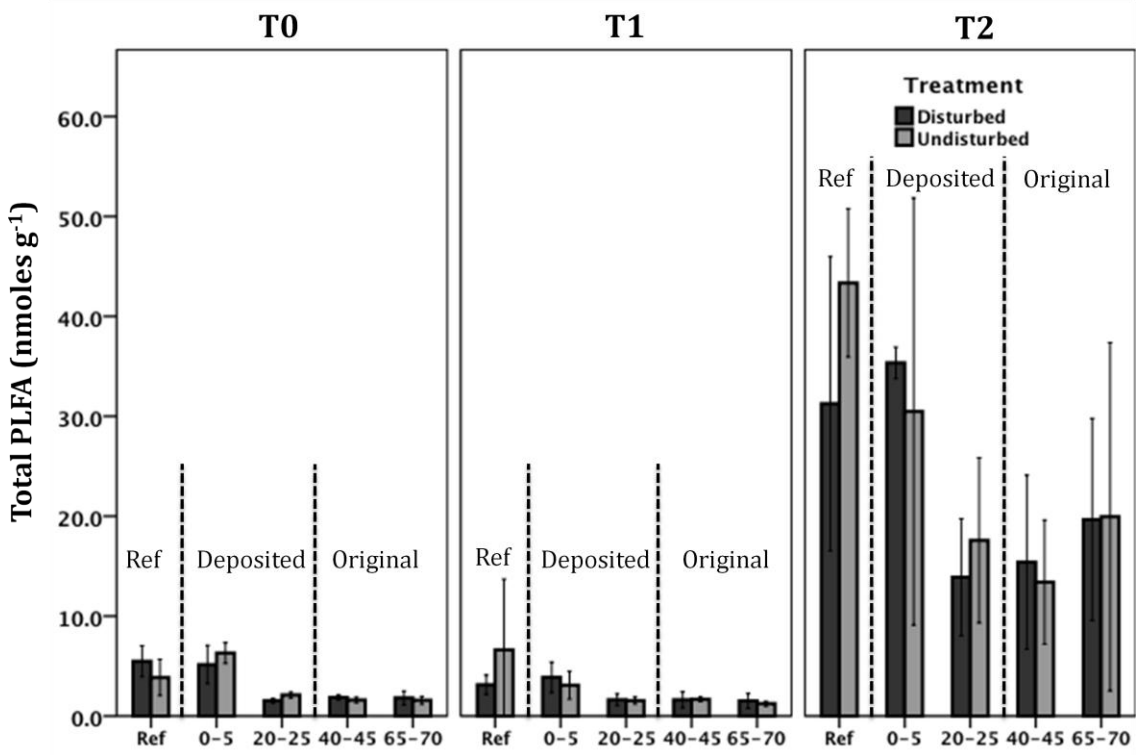

Fig. 4.6 Total PLFA measured at three destructive samplings (T0, T1, T2, left to right) for five incubated depths (reference soil, Ref; 0-5, 20-25, 40-45, and 65-70 cm) with the effect of disturbance treatment displayed. Bars display means (n=3) and error bars represent +/- 2 SE of the mean. Broken lines designate soil origin of extracted PLFAs (reference, deposited and original soil material).

Table 4.3 Significance levels from ANOVA of the effect of depth (D), time interval (TI), and disturbance treatment (DT) on total microbial abundance (Total PLFA), fungal abundance (F), bacterial abundance (B), bacterial:fungal ratio (B:F), Gram positive abundance (G+), Gram negative abundance (G-), Gram positve:Gram negative ratio (G+:G-), and stress biomarkers stress 1 (S1) and stress 2 (S2) for incubated samples.

Factor	Total PLFA	F	B	B:F	G+	G-	G+:G-	S1	S2
Depth	***	***	***	***	***	*	***	***	***
Time Interval	***	***	***	***	***	***	***	***	ns
Disturbance Treatment	ns	ns	ns	ns	ns	ns	ns	ns	ns
D x TI	ns	*	ns	*	ns	*	***	ns	ns
D x DT	ns	ns	ns	ns	ns	ns	ns	ns	ns
TI x DT	ns	ns	ns	ns	ns	ns	ns	ns	ns
D x TI x DT	ns	ns	ns	ns	ns	ns	ns	ns	ns

*,**,***, Significant at $p \leq 0.05, 0.01, 0.001$.

4.3.3.2. Functional group abundance

Bacterial and fungal abundance displayed the same relative pattern and statistical significance as total PLFA (Fig. 4.7). The effect of depth was significant ($p<0.001$; Table 4.3); the reference soil and the 0-5 cm soil were greater than the 20-25, 40-45, and 65-70 cm soils. Bacterial and fungal biomass was similar from T0 to T1 (14 d), and increased from T1 to T2 (62 d). There was no effect of the disturbance treatment. Interestingly, the B:F ratio remained similar between T0 and T1, at ca. 2 for all depths and showed a significant increase by the third destructive sampling (T2; between ca. 4-11) (Fig. 4.8). As well as an increase in the B:F ratio at T2, a different pattern among depths was seen. An increase in the ratio with increasing depth increment occurred, illustrating the increase in bacterial abundance relative to fungi in the deeper depth increments.

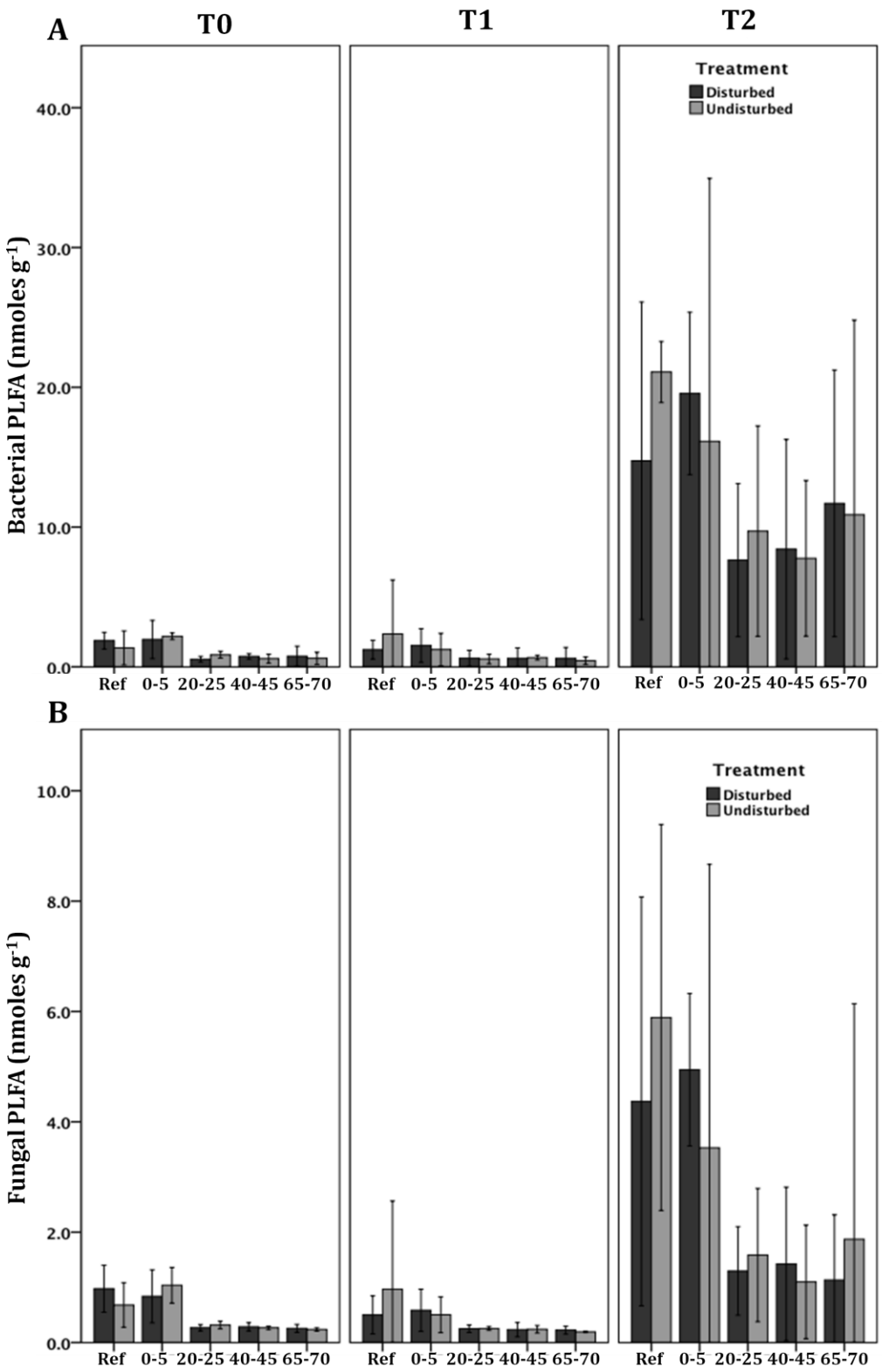

Fig. 4.7 Bacterial (A) and fungal (B) abundance measured at three destructive samplings (T0, T1, T2, left to right) for five incubated soil depths (reference soil, Ref; 0-5, 20-25, 40-45, and 65-70 cm) with the effect of disturbance displayed. Bars display means (n=3) and error bars represent +/- 2 SE of the mean.

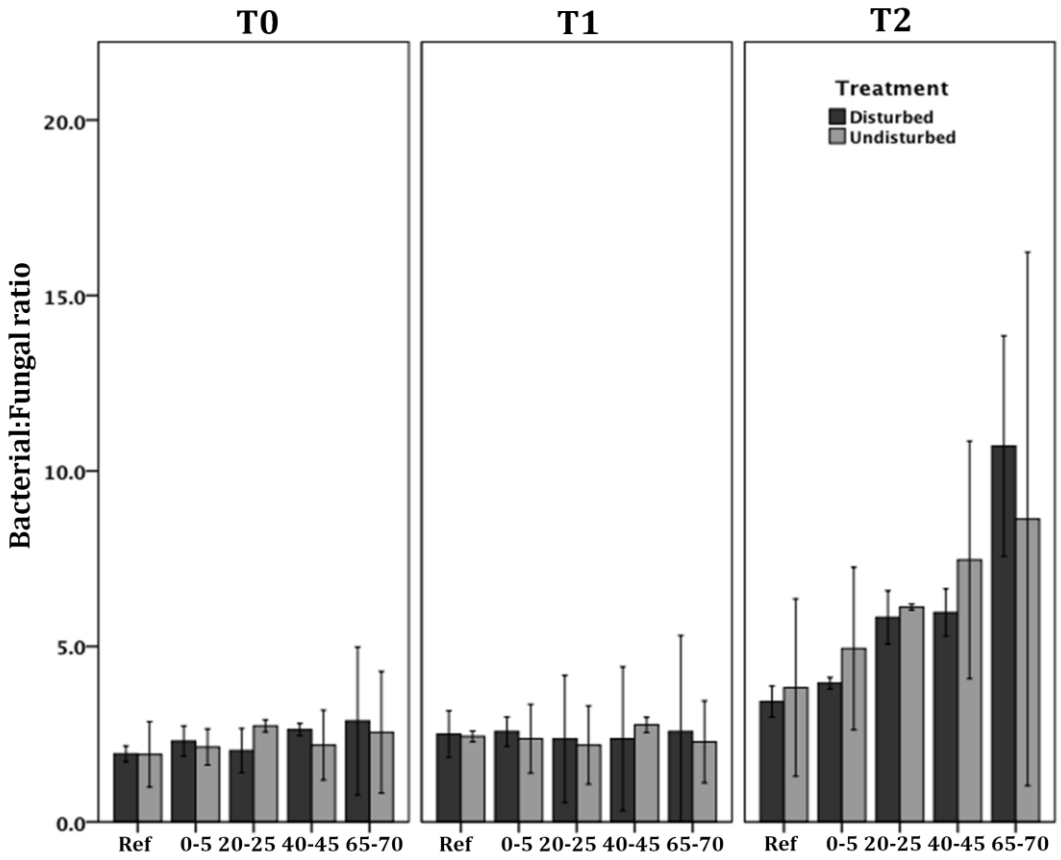

Fig. 4.8 The bacterial:fungal ratio measured at three destructive samplings (T0, T1, T2, left to right) for five incubated soil depths (reference soil, Ref; 0-5, 20-25, 40-45, and 65-70 cm) with the effect of disturbance displayed. Bars display means (n=3) and error bars represent +/- 2 SE of the mean.

Gram positive and G- bacterial functional groups were affected by depth ($p<0.001$, $p<0.05$, respectively) and time interval ($p<0.001$)(Fig. 4.9; Table 4.3). Similar to bacterial and fungal abundance, G+ and G- abundance were the highest in the reference and the 0-5 cm soil, and significantly different from the 3 deepest incubated depths (20-25, 40-45, 65-70 cm). Similar to total bacterial biomass, by the third time interval (62 d), both G+ and G- abundance had increased ($p<0.001$). The G+:G- ratio decreased with increasing depth across all three time intervals, displaying a consistently greater G+ dominance in the surface depth increments (reference and 0-5 cm depths; Fig. 4.10). The ratio was also variable over time, with no clear pattern apparent. Depth and time interval were both significant in affecting the ratio ($p<0.001$).

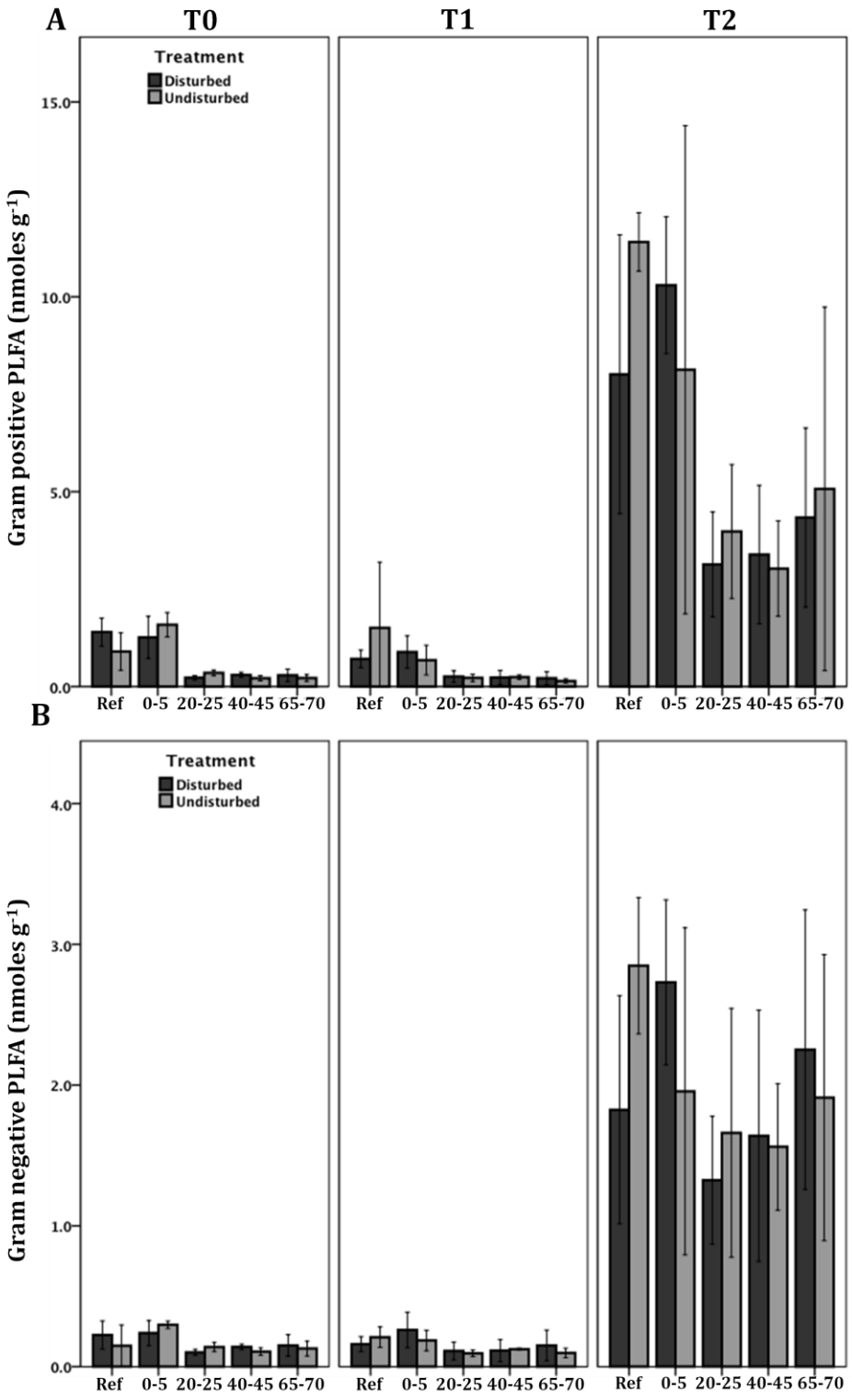

Fig. 4.9 Gram positive (A) and Gram negative (B) bacterial abundance measured at three destructive samplings (T0, T1, T2, left to right) for five incubated soil depths (reference soil, Ref; 0-5, 20-25, 40-45, and 65-70 cm) with the effect of disturbance displayed. Bars display means (n=3) and error bars represent +/- 2 SE of the mean.

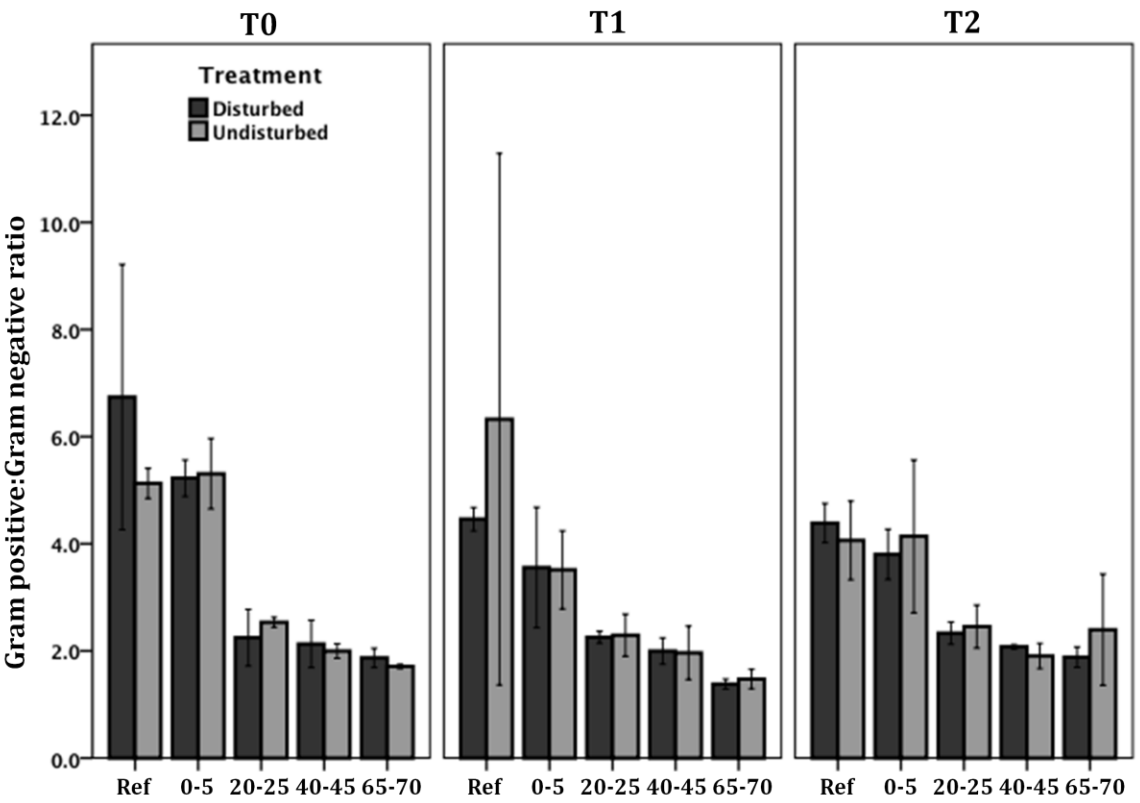

Fig. 4.10 The Gram positive:Gram negative ratio measured at three destructive samplings (T0, T1, T2, left to right) for five incubated soil depths (reference soil, Ref; 0-5, 20-25, 40-45, and 65-70 cm) with the effect of disturbance displayed. Bars display means (n=3) and error bars represent +/- 2 SE of the mean.

The ratio of fatty acids indicative of physiological stress were affected by depth ($p<0.001$), displaying an increase in stress with increasing depth increment, despite exposure to identical incubation conditions (Fig. 4.11). Stress 2 displayed a larger incremental difference between the surface samples (reference and 0-5 cm) and the deeper incubated depths. Stress 1 was additionally affected by time interval ($p<0.001$), showing a slight increase from the onset of the incubation to the last destructive sampling.

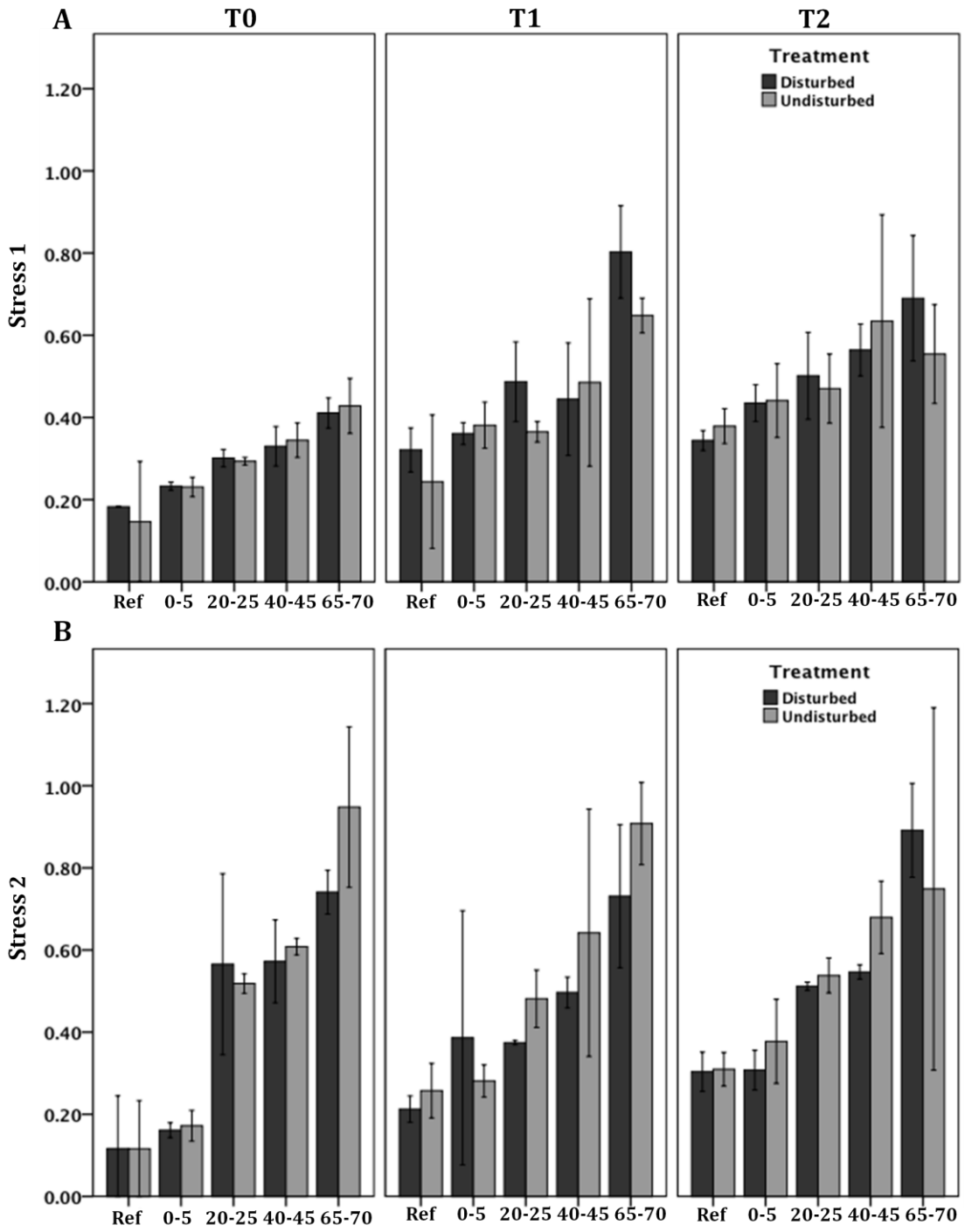

Fig. 4.11 Stress 1 (A) and stress 2 (B) measured at three destructive samplings (T0, T1, T2, left to right) for five incubated soil depths (reference soil, Ref; 0-5, 20-25, 40-45, and 65-70 cm) with the effect of disturbance displayed. Bars display means (n=3) and error bars represent +/- 2 SE of the mean. Stress 1 = cy17:0:16:1 ω7c; stress 2 = cy19:0:18:1 ω7.

4.3.3.3. Microbial community structure

Nonmetric multidimensional scaling analysis was completed on PLFA profiles from the incubation experiment to observe any structural gradients due to depth, time, or the disturbance treatment (Fig. 4.12). There was no effect of disturbance treatment observed across all depths and all time intervals (data not shown). Differences in community structure could be attributed to changes with time and differences among depths. The largest amount of variation accounted for in the solution (58%) is seen along axis 1 and represents differences in community structure among depths (represented by different colors). This is confirmed by a strong A-value for depth (A=0.23, $p<0.001$) resulting from MRPP analysis, indicating within-group similarity. Within the depth gradient, from the bottom left of the ordination space to the top right, three main clusters occur: reference and 0-5 cm (red and green points), 20-25 and 40-45 cm (light blue and purple points), and 65-70 cm (blue points), indicated by solid line ovals.

Variation in community structure also occurred among different time intervals (represented by different shapes), which can be seen along axis 2, accounting for 37% of variation in community structure in the final solution. There is clear separation between T2 (squares; enclosed within the broken line oval) and the earlier time intervals (T0 and T1, triangles and circles, respectively), with an overlapping of T0 and T1 points. Within each time interval grouping, the depth gradient is also seen. A slightly lower, but significant A-value was observed for the effect of time interval (A=0.14; $p<0.001$), confirming the visual representation of the observed gradient. Stress 1 ($r = 0.78$) and stress 2 ($r = 0.84$) were positively correlated with 65-70 cm soils, and % bacteria ($r = 0.93$) were positively correlated with 65-70 cm soils from T2. Lastly, % fungi ($r = 0.75$) were correlated with T0 and T1 soils.

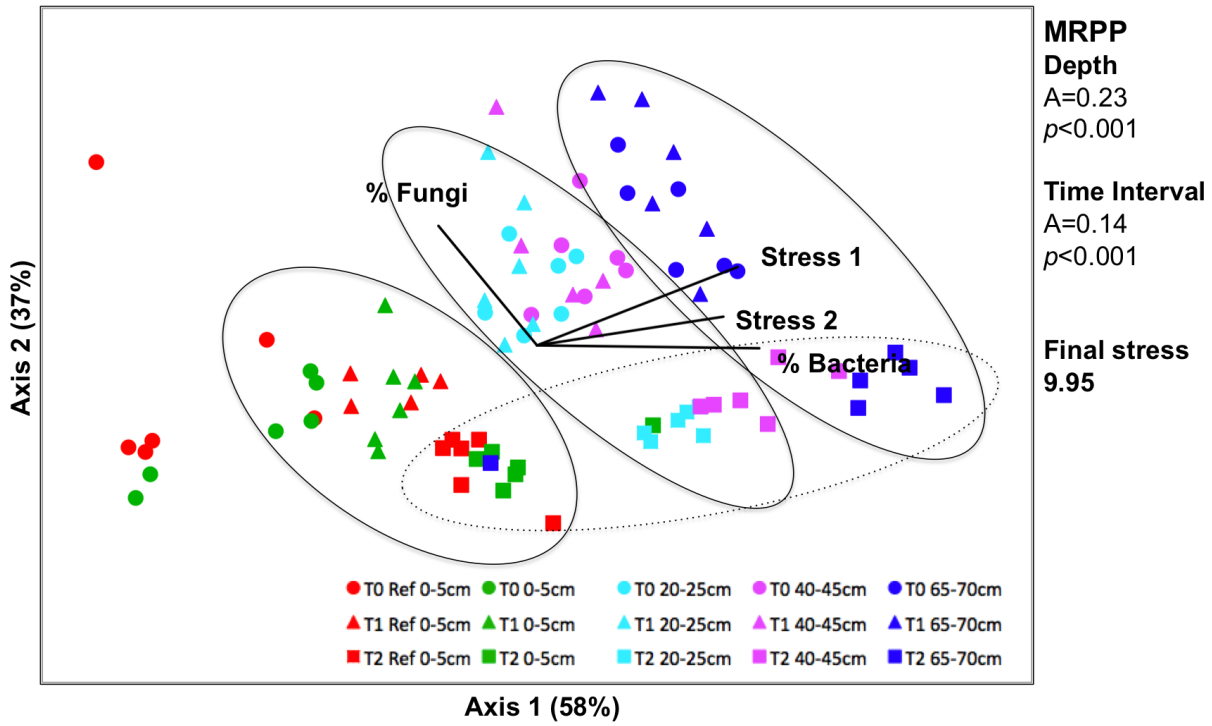

Fig. 4.12 Nonmetric multidimensional scaling (NMDS) analysis and multi-response permutation procedure (MRPP) analysis of depth and time interval on PLFA profiles from five depths (colors), incubated for 62 d and destructively sampled at three time intervals (shapes; T0, T1, and T2). The final solution was 2-dimensional and had a stress of 9.95. Labeled vectors indicate correlation (Pearson's *r*; *r* >0.4) between variables (functional group abundance; nmol g^{-1}) and ordination scores.

4.3.3.4. Community level physiological profiling using the BIOLOG EcoPlate™

Microbial community substrate usage could be attributed to changes with time (measured at three destructive samplings) and differences among depths (Fig. 4.13). Changes in substrate usage over time is represented along axis 1, accounting for 40% of variability in the solution (time interval clusters indicated by ovals in panel B). Samples from T2 are more varied in their substrate usage (squares), indicated by a greater magnitude of spread along axis 1, when compared to T1 (triangles) and T0 (circles). As a percentage of total substrate usage, polymer (r = 0.55) and amino acid guilds (r = 0.46) were positively correlated with T2 soils, whereas the amine/amide (r = 0.40) and carboxylic acid guilds (r = 0.46) were positively correlated with T1 soils. Differences in substrate usage among depths is represented along all axes and is visible in both panels (represented by different colored points). Though changes in substrate usage over time is evident, such changes occur at a different magnitude for each depth. The 65-70 cm soils

(blue points) are clustered relatively close together, indicating minor changes in usage over the incubation period. The remaining depths spread both vertically and horizontally across the ordination space, indicating more variability or adaptability in usage over the incubation period. As indicated by MRPP analysis, both the effect of depth (A=0.08, $p<0.001$) and time interval (A=0.09, $p<0.001$) were significant, indicating the differences between groups are greater than those expected to occur by chance (McCune and Grace, 2002).

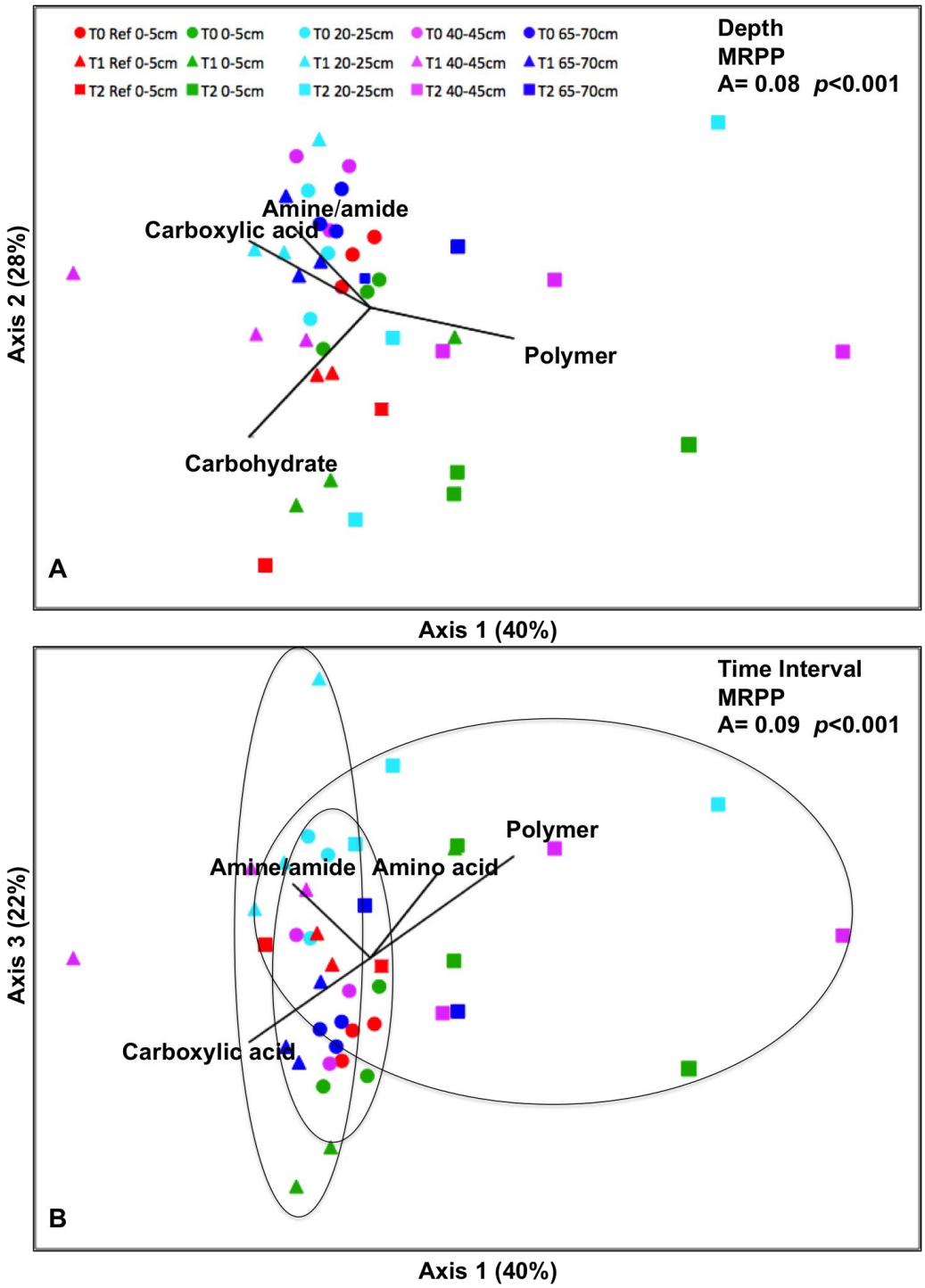

Fig. 4.13 Nonmetric multidimensional scaling (NMDS) analysis and multi-response permutation procedure (MRPP) analysis of Biolog Ecoplate™ substrate usage. The effect of depth (colors) and destructive sampling interval (shapes) and the use of 31 C substrates from a 62 d incubation experiment were included in the analysis. The 3-dimensional solution had a final stress of 11.0. The top panel (A) displays axis 1 vs. 2, and the bottom panel (B) displays axis 1 vs. 3. Labeled vectors indicate correlation (Pearson's *r*; *r* >0.40) between variables (% guild usage) and ordination scores.

4.4. Discussion

4.4.1. Surface and subsurface C mineralization response

Greater mineralization (Fig. 4.5) in surface soils (0-5 and 20-25 cm) relative to subsurface soils (40-45 and 65-70 cm) indicated that buried surface soil C has decreased respiration potential, suggesting that physical protection mechanisms may be occurring during/after the burial process. Water extractable organic carbon data (Fig. 4.4) indicated that similar labile C pools existed among all depths. However, this was not reflected in the mineralization response, indicating that WEOC concentration was not a good measure of C quality or availability in these soils. It is also possible that other nutrients could be limiting in subsurface soils and affecting mineralization. In a study of C dynamics in an eroding landscape affected by long term tillage disturbance, Doetterl et al. (2012) found a similar result in mineralization response in depositional positions, with surface soils (0-25 cm) respiring at a higher rate than buried surface soils located at 25-100 cm. By incorporating C pool distribution data (SOC fractionated into functional pools of varying stability), they found subsurface C to be associated with microaggregates and silt-sized particles or 'intermediate' cycling SOC fractions. In contrast, surface soils were higher in proportion of macroaggregate C, or the 'fast' cycling fraction. This suggests that physical protection is stabilizing buried, former surface soil C against decomposition. In our study, more fresh C from recently deposited plant litter likely contributed to a higher proportion of weakly protected, highly available C in surface soils, contributing to higher cumulative respiration in surface soils at both the depositional and non-eroding position (reference position). Soil organic matter formation is relatively uninterrupted in non-eroding positions, with a continuous fresh SOM supply promoting the formation of macroaggregates, associated with available C with faster turnover times (Doetterl et al., 2012). The breakdown of macroaggregates during redistribution in eroding landscapes, and the lack of fresh SOM supply once buried, results in smaller, highly stabilized microaggregates remaining (Doetterl et al., 2012; Six et al., 2004).

The physical protection mechanisms stabilizing C in the subsoil also act to restrict accessibility and interactions among soil microorganisms, enzymes, and C substrate, likely decreasing decomposition rates. Microorganisms sorb and form close bonds with mineral surfaces in the same way C substrate does, essentially reducing connectivity between soil

microorganisms and C substrate (Dungait et al., 2012). Additionally, C occluded within microaggregates is likely protected from microbial and enzymatic attack, coupled with low oxygen availability within microaggregates (Dungait et al., 2012). Further, these mechanisms create micro-habitats that are non-preferred by soil microorganisms (Ekschmitt et al. 2008). This spatial relationship and interaction among soil microorganisms, enzymes, and C substrate in the subsoil are thought to be one potential controlling mechanism affecting mineralization response in subsurface soils. Of the few studies conducted, most have assessed this in non-eroding profiles. Salome et al. (2010) observed a 75% increase in mineralization after sieving (<1 mm) in subsurface soils (80-100 cm) whereas no significant difference was seen in surface soils (0-5 cm). In our study, a slight non-significant increase in cumulative C was observed in disturbed samples from both surface and subsurface soils. It is possible that the imposed disturbance treatment elicited only a slight increase in accessibility between microorganisms and substrate through minimal soil structure breakdown and subsequent release of labile C. This was observed for all depths with the exception of the reference topsoil, where the undisturbed soil mineralized more C than its disturbed counterpart. At this time there is no logical explanation for this, as disturbing soil structure typically releases previously inaccessible C within macroaggregates, making it available for decomposition and increasing mineralization (Franzluebbers, 1999; Six et al., 2004). Thus, it is likely that our <2 mm sieving disturbance treatment did not substantially affect microaggregate stability or enhance connectivity between enzymes, microbes, and substrate, leading to the lack of mineralization response from the imposed disturbance treatment.

4.4.2. Surface and subsurface microbial community response: abundance

Differences in C availability rather than concentration influenced microbial abundance with depth in spring-sampled soils (2011) at the onset of the incubation study (Fig. 4.6), in contrast to patterns observed in soils sampled in fall 2010 (Fig. 3.4). The spring-sampled soils displayed a typical profile of microbial abundance with depth. As previously discussed (Section 4.4.1), a higher proportion of more available, recent plant derived C in surface soils (reference and 0-5 cm) supports greater microbial abundance. In contrast, older C that may be more stabilized and less available, and that may be associated with microaggregate-C (Doetterl et al., 2012) resulted in lower abundance in subsurface soils (20-25, 40-46, and 65-70 cm). The effect of disturbance was not significant in affecting total PLFA across all depths and time intervals,

presumably due to the ineffectiveness of the sieving treatment in releasing large amounts of protected C.

The comparable increase in microbial abundance observed by T2 (62 d) for both surface and subsurface soils indicated a similar response to warming of the different soil layers. In warming experiments assessing microbial and C dynamics on non-eroding surface soils, biomass has shown to be unaffected by warming (Schindlbacher et al., 2011; Zhang et al., 2005), or to display an overall decrease in biomass (Frey et al., 2008). Decreases in biomass have been accompanied by a similar decrease in respiration rate, attributed to labile C depletion (Feng and Simpson, 2009). In contrast, we observed an increase in biomass at T2, with a corresponding decrease and stabilization of respiration rates (Fig. B.3). Our study was 62 d in length, a relatively short study compared to previously completed warming studies (Feng and Simspon, 2009; Frey et al., 2008; Schindlbacher et al., 2011). In a long-term warming study (1 yr), an overall decreasing pattern in biomass displayed variability, with periods of slight increase in biomass observed, before continuing to decrease and stabilize for the duration of the study (Feng and Simpson, 2009). It is possible that we captured a relatively short-term increase in microbial abundance which may have receded over time as available C declined.

Increased C use efficiency by decomposer organisms due to warming could explain the increase in biomass observed at T2 in this study (Fig. 4.6), despite stabilized low rates of respiration (Fig. B.3). In an *in situ* soil warming study on non-eroding surface soils, Schindlbacher et al. (2011) suggested that warming had decreased the C use efficiency of the decomposer community. They attributed this to an increase in metabolic activity (soil respiration rate per unit of microbial biomass C) despite relatively steady concentrations of biomass throughout the study (i.e., warming increased metabolic activity, but not biomass). Bardgett et al. (1999) observed results in contrast to this in a manipulated terrestrial model system exposed to a warming treatment. Over three simulated plant growth generations, total PLFA concentration and microbial respiration reached a maximum at the end of the second plant generation, followed by stabilization in PLFA and decrease in respiration by the third generation. The authors concluded that while increased temperature and generation time increased active biomass, there was no effect on microbial metabolic activity and subsequent C turnover, due to nutrient limitations in the terrestrial model system. It is possible that decreased metabolic activity and increased C use efficiency as a response to increased temperature explains the increase in

biomass observed at T2 in this study. Similarly to Bardgett et al. (1999), nutrient limitations in the buried horizon may also be influencing respiration and subsequent turnover of C.

Finally, it is possible that warming increased C availability/accessibility and subsequently increased biomass in these soils. Abundance at T2 under controlled conditions was similar to microbial abundance observed in soils sampled in fall 2010 directly from the field (Fig. 3.4) suggesting a seasonal effect on PLFA concentration. Samples for this study were sampled in the spring (May 2011) and subjected to a warming treatment of 25°C, which is comparable to the high end of daily maximum temperatures occurring in the summer and early fall months in this region (18-25°C from May-September; Environment Canada, 2013a). Spedding et al. (2004) observed seasonal variations in microbial community dynamics, with an overall significant increase in total PLFA occurring from spring to fall sampling. Thus, incubation conditions reflected to some extent the seasonal warming that occurs.

4.4.3. Surface and subsurface microbial community response: functional group abundance

Soil warming has been shown to have variable effects on specific microbial functional group abundance in non-eroding surface soils. Study designs range from *in situ* studies with slight warming increases above ambient temperature (Castro et al., 2010; Frey et al., 2008; Schindlbacher et al., 2011; Zhang et al., 2005) to laboratory incubations with more extreme warming scenarios (Feng and Simpson 2009; Wu et al., 2012), all of which vary considerably in length. While an increase or decrease in relative abundance of specific microbial groups have been observed, temperature mediated substrate effects rather than the effect of temperature independently are attributed to causing both positive and negative responses (Schindlbacher et al., 2011).

Relative and absolute fungal abundance has decreased with warming during incubation at 2-20°C above MAT (Feng and Simpson, 2009) and after 12 yr of *in situ* warming 5°C above ambient temperature (Frey et al., 2008). These decreases were attributed to exhaustion of available labile C compounds, exacerbated due to warming. In contrast, relative fungal abundance has been shown to increase when exposed to warming. Zhang et al. (2005) observed an increase in fungal abundance and an increase in the fungal:bacterial ratio with *in situ* warming, attributed to warming induced plant-soil interactions causing an increase in plant productivity and subsequent C deposition. In contrast, Castro et al. (2010) observed no detectable

change to the fungal:bacterial ratio among different treatments in a multi-factor climate change experiment (interactive and direct effects of different ambient CO_2, temperature, and precipitation). Fungal and bacterial response to warming is varied and influenced by site and study specific factors; therefore, a more consistent approach to measuring functional group response to warming is needed.

In the present study, it is possible that the increase in bacteria relative to fungi (B:F ratio; note the change in ratio syntax to the abovementioned studies) with depth at T2 (Fig. 4.8) is due to exhaustion of fungal-preferred substrate, but it is more likely that substrate quality or complexity is influencing relative microbial abundance. Fresh SOM additions to subsurface soils are limited; therefore, it is possible that continual microbial cycling of substrate (accelerated from incubation warming) has created highly processed, complex substrate (Rumpel and Kögel-Knabner, 2011) that bacteria are better able to exploit than fungi. Temperature mediated changes in environmental factors can affect microbial metabolism, such as a decrease in soil moisture and subsequent effects on oxygen levels. However, these parameters were kept optimal during the incubation, therefore temperature mediated substrate changes are thought to have exerted the greatest effect on microbial abundance and composition.

Substrate quality/complexity also influenced G+ and G- relative distributions (Fig. 4.10) and stress biomarkers (Fig. 4.11) in incubated soils. Similar G+:G- ratios have been observed for corresponding depths in the previous chapter where microbial communities were assessed in samples taken directly from the field (Fig. 3.8), which reflected atypical relative G+ and G- bacterial proportions. Previous studies have observed G- bacteria higher in proportion at the soil surface in association with more labile C fractions, and G+ bacteria becoming more dominant with depth (Blume et al., 2002; Fierer et al., 2003b). In contrast, we observed G+ bacteria to be the most dominant in surface depths, thought to occur due to altered substrate gradients. The G+:G- ratio displayed significant differences with incubation time, but no consistent pattern was observed, suggesting that incubation conditions had no major effect on G+ and G- relative proportions. Similar effects of warming have been observed in other studies, where no significant shift in G+ and G- relative proportions was observed from *in situ* studies (Schindlbacher et al., 2011). In contrast, Feng and Simpson (2009) observed a decrease in G- biomarkers relative to G+ and fungi, attributed to a decrease in labile C at higher incubation temperatures. They also observed a decrease in G- bacteria relative to G+ bacteria with

increasing incubation temperature. Our results and those of the abovementioned studies would suggest that moderate warming does not cause substantial effects on G+:G- ratios, but may be affected by more intense warming and major changes in substrate quality and quantity.

Increasing stress ratios with depth were observed (Fig. 4.11), which may be a reflection of nutrient stress with increasing depth. Our measurements show similar C concentrations exist between depths, but a lack of fresh substrate supply at deeper depths could cause the deficiency of other essential nutrients, such as N. Increases in stress biomarkers with warming have been reported from both incubation and *in situ* experiments (Feng and Simspon, 2009; Schindlbacher et al., 2011). In our study, only the stress 1 biomarker displayed a significant increase over time, with both ratios remaining similar to stress values observed in freshly sampled field communities from fall 2010 (Fig. 3.8). Because conditions were kept optimal in terms of soil moisture and temperature for microbial metabolism, it is probable that this reflects an increase in physiological stress due to exhaustion of available substrate or increased nutrient stress over time.

4.4.4. Surface and subsurface microbial community response: microbial community structure

Microbial community structure development is strongly influenced by surrounding environmental factors that impact microbial functioning. Distinct microbial communities between surface and subsurface depths may have also influenced differences observed in cumulative C respiration. The NMDS analysis of community structure in incubated soils confirmed that distinct communities existed among depths in the depositional position (Fig. 4.12), observed previously in Chapter 3.0 (Fig. 3.11). It is thought that such differences may affect microbially-mediated processes such as C mineralization (Fierer et al., 2003a, 2003b), but an underlying link between distinct microbial communities in subsurface soils and differences in function is yet to be confirmed. A shift in community structure at T2 vs. T0 and T1 was observed, but PLFA profiles remained reflective of the original differences seen among depths at the onset of the incubation. That is, after 62 d of exposure to incubation conditions, a shift in community structure occurred, but the effect of depth remained the strongest in influencing community structure. The shift seen in PLFA community structure was also confirmed by a shift in substrate use at T2 observed from CLPP analysis (Fig. 4.13), where all but the 65-70 cm depth became more varied (more dissimilar) in substrate usage. Therefore, given that microbial

community composition remained distinct among depths from T0 to T2, differences in cumulative C respired among depths may be attributed to differences in community structure (also, relative differences in C respired among depths remained consistent from T0 to T2).

The change in community structure displayed at T2 may be reflective of a shift towards a more bacterial dominated community, supported by the increase in the B:F ratio and the correlation between community structure and bacterial PLFA at T2 in the 65-70 cm depth. Additionally, bacterial growth is known to be higher than fungal growth on Biolog plates (Zhang et al., 2005); therefore, the increased variability in usage at T2 is likely a bacterial response.

Recent studies have shown that microbial community structure influences decomposition (Strickland et al., 2009a) and thereby affects the chemical structure of SOM derived from plant litter inputs (Wickings et al., 2012). Strickland et al. (2009a) found evidence that distinct microbial communities are not functionally equivalent, but functionally dissimilar. After incubating different litter types with distinct soil microbial inocula, it was found that shared history between inoculum and litter habitat demonstrated the highest rates of decomposition, compared to communities that were not native to the particular habitat. Their results suggest that microbial communities display pre-adaptation to past exposure of litter habitats, meaning that the ability to decompose particular C compounds may be dependent upon past exposure to them (Strickland et al., 2009a). In this study, subsurface microbial communities that were once at the soil surface and are now buried were able to survive and remain viable because they were buried along with substrate they were adapted to utilize. After continual microbial cycling with no recent inputs of labile C, it is likely that a complex substrate now exists at depth, that may be less desirable or available to soil microorganisms, resulting in lower respiration rates in subsurface samples. This occurred despite similar C concentration among depths. It is also possible that distinct subsurface communities at depth have different functional capacities (thermal optima), affecting the rate at which they decompose C (Fierer et al., 2003a).

Recent work by Wickings et al. (2012) proved both the importance of litter quality and decomposer community characteristics on litter complexity. It was determined that litter chemistry was influenced by its initial quality, as well as by distinct microbial communities carrying out the decomposition process, highlighting the interaction between litter quality and decomposers. It is likely that such interactions affected C composition at depth, particularly considering the distinct microbial communities observed following incubation in the present

study. However, at this point it is difficult to determine which factors (e.g. substrate quality, physical protection mechanisms, or microbial community structure) or combination of factors is most influential in controlling mineralization response between surface and subsurface soil layers. However, if microbial communities can regulate the chemical structure of SOM inputs to soil and have the potential to affect SOM stability and formation (Wickings et al., 2012), this in turn can affect vulnerability to decomposition.

4.5. Conclusions

Greater cumulative mineralization in surface soils, relative to subsurface soils demonstrates that C dynamics between soil layers in a depositional position are controlled by complex underlying mechanims. Carbon substrate composition (e.g. availability and complexity) rather than quantity plays a dominant role in potential mineralizability. The existence of distinct microbial communities in surface and subsurface soil layers prior to and after 62 d of exposure to surface-like conditions may indicate a microbial community structure influence on C mineralization potential. The lack of a mineralization response to the imposed disturbance treatment suggests that increased contact between substrate and soil microbes did not occur, implying that C may be occluded in microaggregate fractions that are unaffected by sieving to <2 mm. The results of this study suggest that redistributed buried C is less susceptible to decomposition than depositional surface soils, supporting the theory that burial constrains decomposition. Further work is needed to clarify the mechanisms through which buried C is stabilized and to determine its chemical composition and potential longevity in the deep soil environment.

5.0 SUMMARY AND CONCLUSIONS

Agricultural erosion and redistribution have the potential to drastically reshape agricultural landscapes. Due to the vast amount of SOC buried globally in such landscapes, a better understanding of the effect of redistribution on C dynamics and turnover will provide more accurate estimates of terrestrial C flux in the context of the global C cycle. Microorganisms are the drivers of C turnover in soil; therefore, assessing microbial community dynamics in relation to redistributed C will contribute to a better overall understanding of the realized and potential effects of redistribution.

The first objective of this study was to examine microbial abundance and community composition with depth and between landscape positions and to relate microbial abundance and community composition to the associated physiochemical conditions and geomorphic controls. A departure from typical microbial abundance patterns with depth was found, particularly in the depositional position (pit 3), where considerable SOC deposition and burial had occurred. Substantial concentrations of viable microbial biomass were found at depth (30-60 cm) in the buried backslope position. Microbial biomass displayed a significant relationship with SOC, signifying that microbial abundance patterns were highly affected by SOC redistribution. Near-surface microbial community structure was significantly affected by landscape position, displaying the most difference between the buried backslope (pit 3) and the eroded shoulder and depositional backslope (pits 1 and 2, respectively). This suggested the influence of differing abiotic factors and subsequent microclimates among landscape positions. The strong effect of depth was seen in microbial community structural analysis, particularly in the buried backslope position, where distinct microbial communities were found within the buried horizon. Distinct communities clustered according to C origin, displaying the strong effect of redistribution on microbial community structure development.

The second objective of this study was to determine the C mineralization and microbial community response of surface and subsurface soil layers in a depositional position to incubation in surface-like conditions and physical disturbance. Greater mineralization response in surface relative to subsurface soils despite similar C concentrations among depths confirmed that complex underlying mechanisms control mineralization between soil layers in depositional positions, and suggests that redistribution renders buried C protected from decomposition. Differences in microbial community structure may have influenced C mineralization response, as

evidenced by distinct microbial communities found between soil layers at the onset and the completion of the study.

In conclusion, we found that redistribution significantly influenced microbial abundance and community structure, and altered SOC dynamics within our study site. Concentrated and buried C at depth supported viable microbial biomass; greater than that observed at the soil surface, despite suboptimal environmental conditions for microbial growth and metabolism. Though the mechanisms are yet to be clarified, our results suggest that C turnover in redistributed and buried soils is constrained and less susceptible to decomposition than surface soils.

5.1. Future Research Directions

Further analysis of microbial community distribution using more discrete molecular methods of identification, such as DNA fingerprinting, would assist in determining which specific microbial taxa are the most influenced by the effects of redistribution, depth, and landscape position. This would provide higher resolution in terms of the above effects on specific organisms compared with the assessment of broad microbial groups (e.g. fungi and bacteria) that PLFA analysis provides. Similarly, incorporation of spectroscopy or other molecular methods that elucidate the chemical structure of C compounds would be important in determining the role that redistribution and burial play in C composition and subsequent susceptibility to decomposition. In contrast to methods that broadly measure C concentration and availability, this would allow more discrete characterization of C on a molecular level, in terms of age and stage of decomposition i.e., determining if C is composed mostly of recent biomass or older, highly processed compounds. Testing other potential constraints (e.g., N) on mineralization in buried soils would also be valuable. Lastly, conducting additional studies in eroding landscapes across Canada and globally would allow a broader confidence in the role that redistribution plays in microbial community and SOC dynamics.

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APPENDIX A

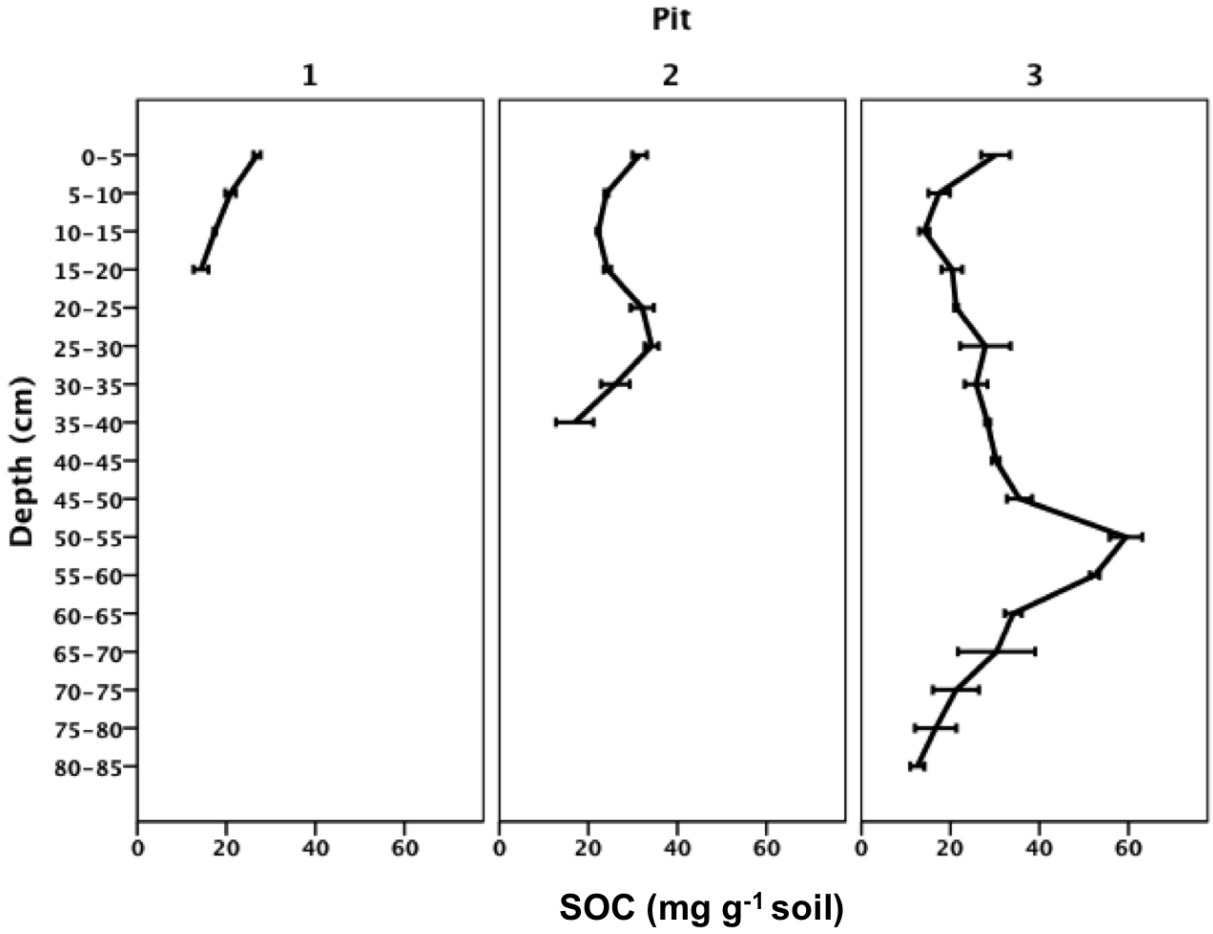

Fig. A.1 Mean soil organic carbon (SOC) concentration in pits 1-3, measured per 5 cm increment (n=4). Adapted from VandenBygaart et al. (2012). Error bars display +/- 2 SE.

APPENDIX B

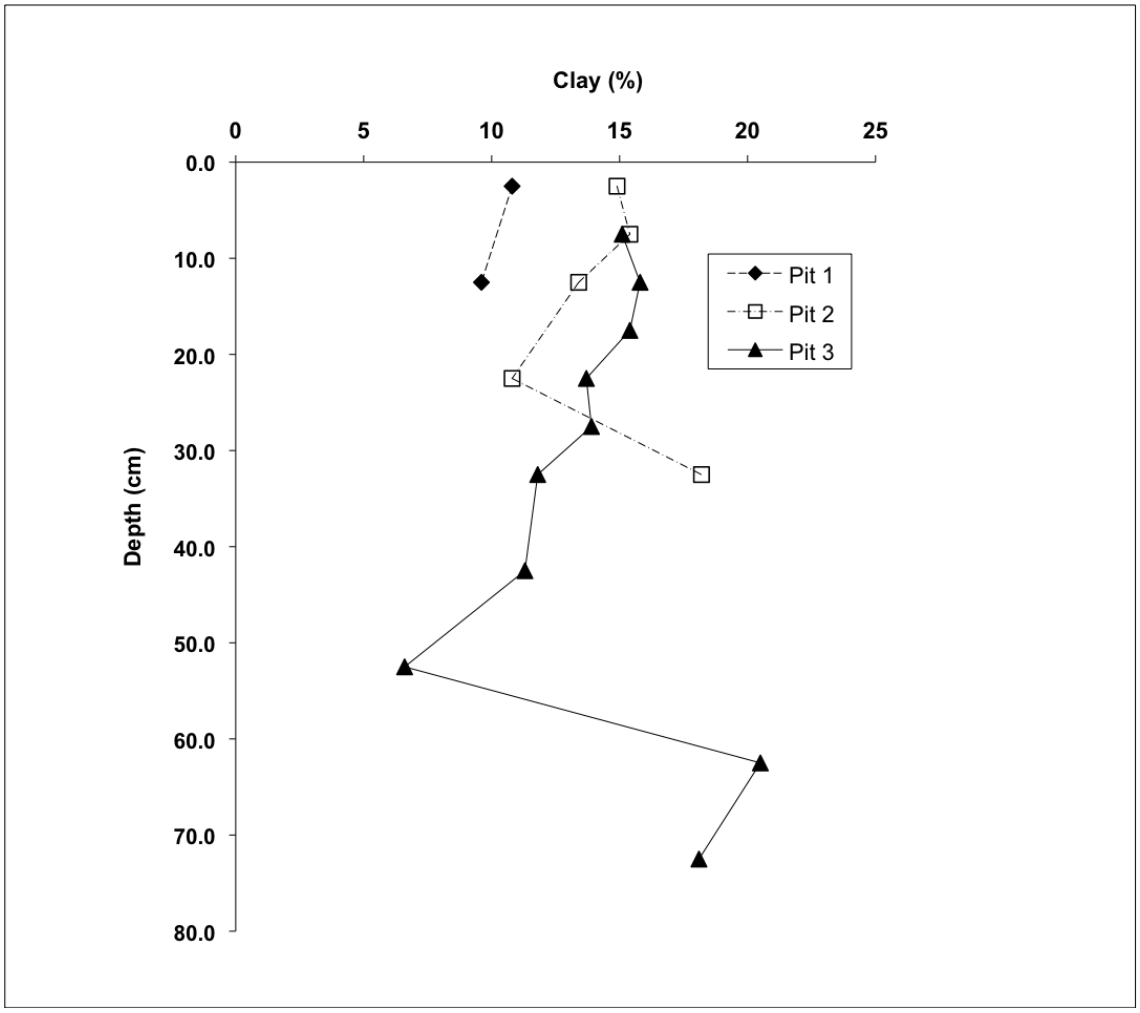

Fig. B.1 Percent clay content for pits 1-3. For the incubation experiment conducted using four depths increments from pit 3, the deepest depth sampled (65-70 cm) was chosen to capture an increase in clay content observed at ca. 65 cm. Adapted from VandenBygaart et al. (2012).

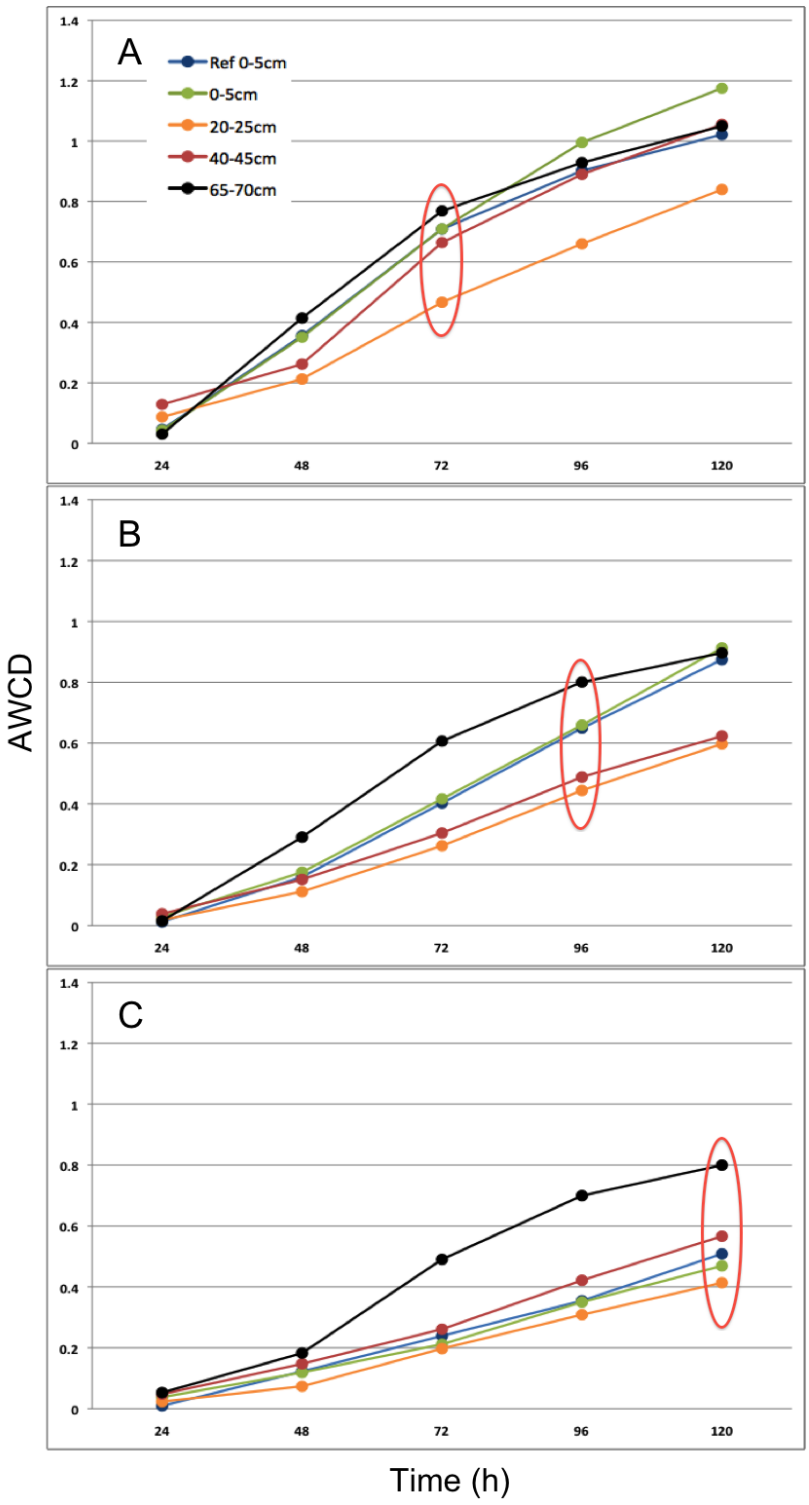

Fig. B.2 Biolog EcoPlate™ average well color development (AWCD) over the 5 d incubation period displayed for three destructive samplings: A) T0, B) T1, and C) T2. Red circles show similar ranges in AWCD where readings were chosen for further normalization and subsequent metabolic profiling.

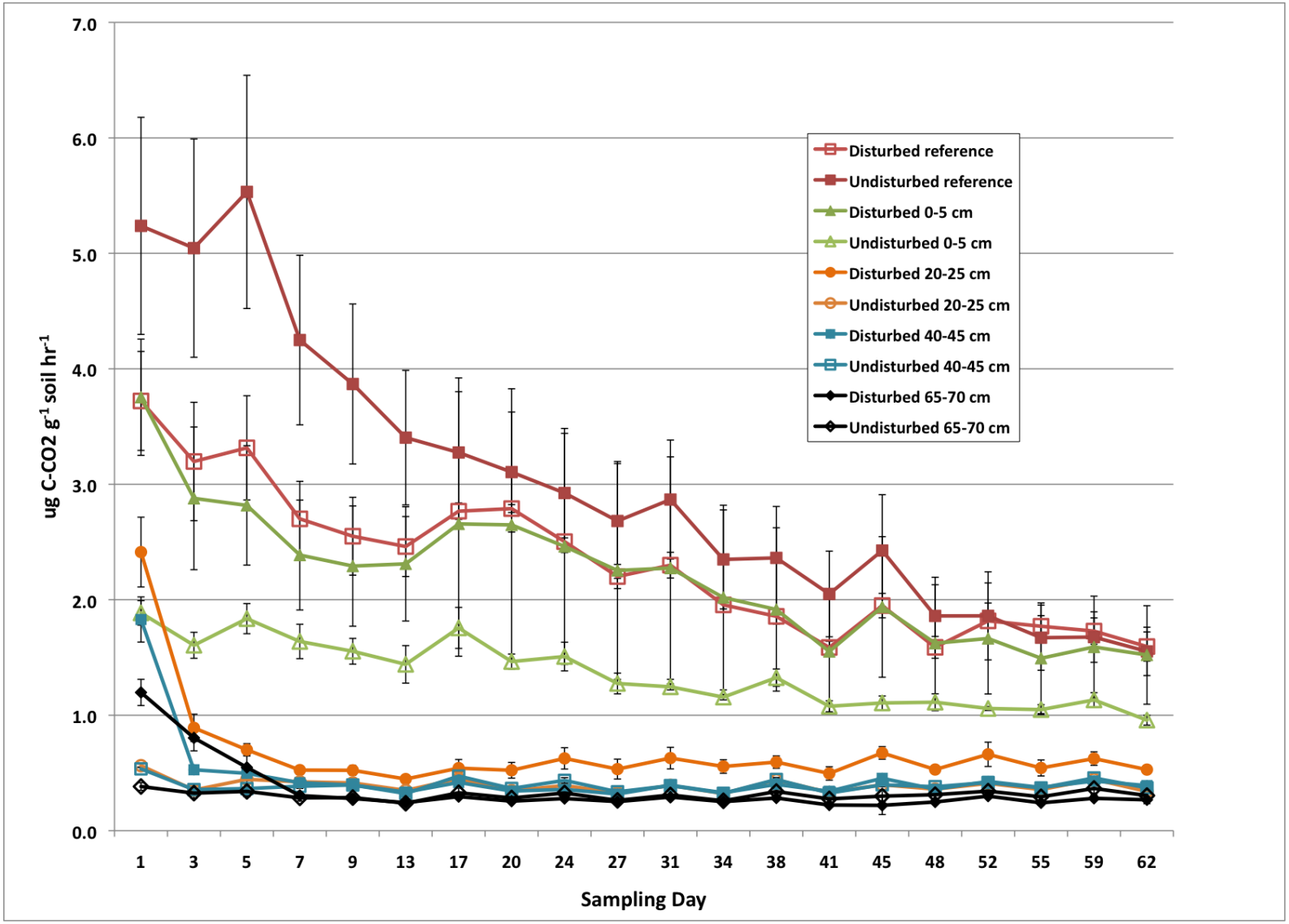

Fig. B.3 Daily respiration rates for five incubated samples over the 62 d incubation period. Symbols represent the mean (n=6) and error bars display +/- 1 SE.

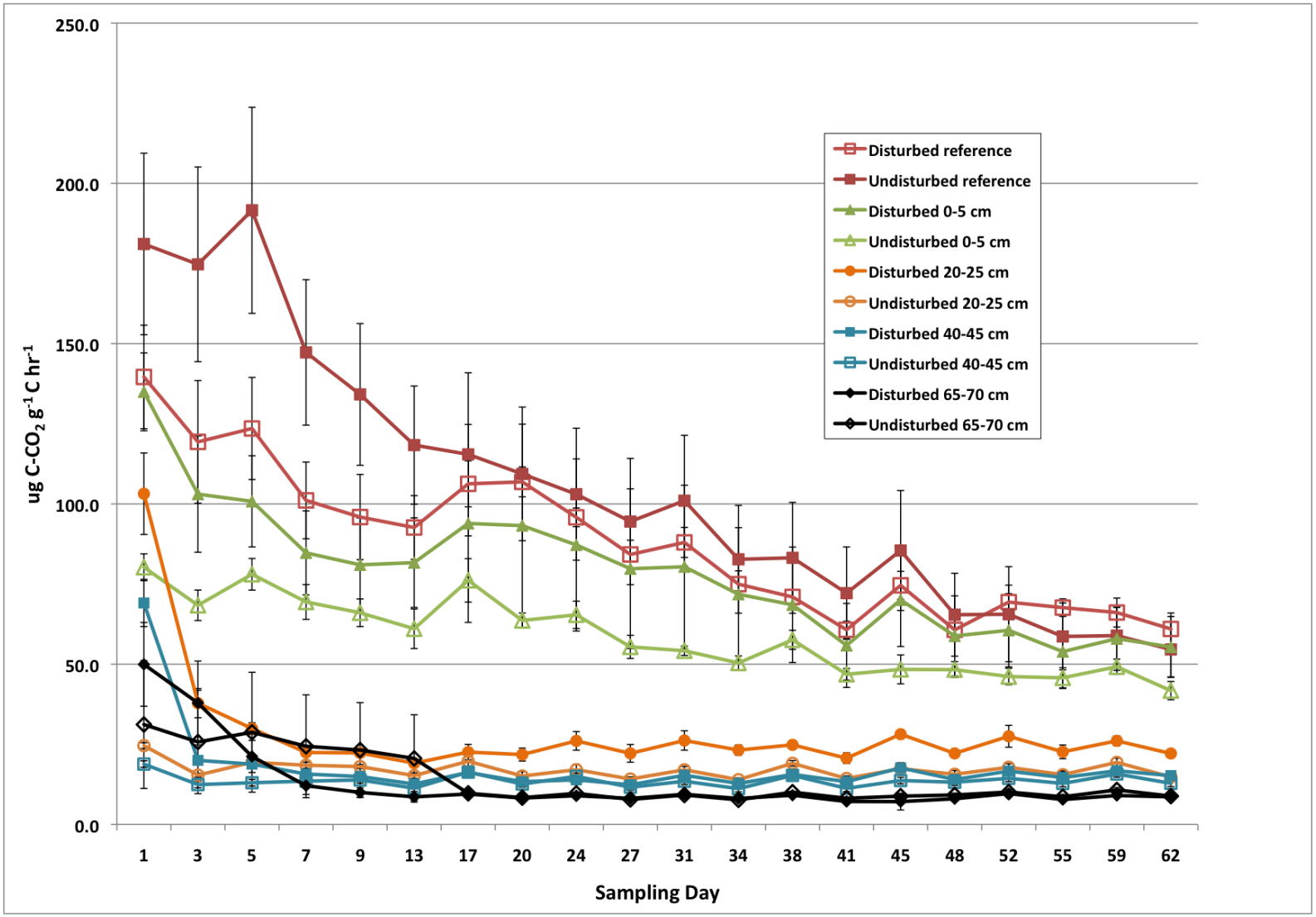

Fig. B.4 Daily respiration rates for five incubated samples over the 62 d incubation period displayed per g of soil C per hr. Symbols represent the mean (n=6) and error bars display +/- 1 SE.