SOIL MICROBIAL CONTRIBUTION TO GREENHOUSE GAS EFFLUX FROM A SECONDARY FOREST IN CENTRAL MISSOURI

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by
NIGEL HOILETT

Dr. Robert Kremer, Dissertation Supervisor
Dr. Keith W. Goyne, Co-Advisor

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The undersigned, appointed by the dean of the Graduate School, have examined the dissertation entitled

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presented by Nigel Hoilett,
a candidate for the degree of doctor of philosophy,

and hereby certify that, in their opinion, it is worthy of acceptance.

Professor Robert J. Kremer, Dissertation Supervisor, United States Department of Agriculture, Agricultural Research Service, and University of Missouri

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Professor. Keith W. Goyne, co-advisor, University of Missouri Department of Soil, Environmental, and Atmospheric Sciences

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Professor Frieda Eivazi, Lincoln University and University of Missouri Department of Soil, Environmental, and Atmospheric Sciences

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Professor John M. Kabrick, U.S. Forest Service and University of Missouri

DEDICATION

This dissertation is dedicated to the people in my life who inspired me to do this Ph.D.: my wife Natalie, my children Aldane, Nahiemah, Kimoy, Nayeli, and Nesean. Thanks for all the love, support and sacrifices. To my mother, Clear Hoilett, I thank her for her unwavering belief in me all these years. Most importantly to GOD the Father and Creator: through him all things are possible.

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LIST OF NOMENCLATURE

Abbreviations	Full Name
CEC	Cation Exchange Capacity
CH_4	Methane
CO_2	Carbondioxide
CFE	Chloroform Fumigation Extraction
DGGE	Denaturing Gradient Gel Electrophoresis
EL-FAME	Ester-linked FAME
FAME	Fatty Acid Methyl Esters
GHG	Greenhouse Gases
DOC	Dissolved Organic Carbon
MBC	Microbial Biomass Carbon
MBN	Microbial Biomass Nitrogen
MPN	Most Probable Number
N_2O	Nitrous Oxide
NO_3	Nitrate
NO_2	Nitrite
PCR	Polymerase Chain Reaction
PLFA	Phospholipid Fatty Acid
SOM	Soil Organic Matter
SOC	Soil Organic Carbon
TN	Total Nitrogen
TOC	Total Organic Carbon
WHC	Water-holding capacity

ABSTRACT

Greenhouse gas emissions (GHG) vary with the interactions among physical, chemical, and biological characteristics of soil and microclimate. Soil microorganisms are involved in almost all soil processes, mediating soil organic matter decomposition and nutrient cycling; and are also involved GHG dynamics between the soil and atmosphere. The objective of this study was to examine the relationship between GHG efflux and soil microbial community and activity across a forested landscape. The experimental site was divided into five landscape sampling positions to also test the influence of topography on enzyme activity, phospholipid lipid fatty acid profiles (PLFA), and soil microbial diversity, based on polymerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGGE) and real-time PCR analysis. Soil samples were incubated for 30 days at a control temperature of 25^0C and a temperature reflecting the time of year when the samples were collected: 35^0C for samples collected in July 2008, 15^0C for samples collected in November 2008, and 20^0C for samples collected in May 2009. The moisture levels used in the incubation study were a control moisture level of 60% water-holding capacity (60%WHC) and the field moisture (FM) content at the time sampling was performed (based on gravimetric moisture determination). Our research revealed temporal differences in microbial population and GHG efflux, indicating that time of year when samples were collected is an important factor. Temperature also had a significant effect on soil microbial population and GHG efflux. Moisture also impacted some GHG measurements; however, the influence was not as great as the temperature effect. In addition, correlation between GHG and measured biological properties and GHG and soil temperature and moisture in the incubated soils implied that microbial properties as well

as soil temperature and moisture may affect GHG efflux from these forest soils. However, the low correlation coefficient (r values) and the lack of correlation within some sampling periods indicated that the relationship among soil microorganisms, soil conditions and GHG efflux is highly complex and cannot be fully explained by direct correlations among the measured properties and GHG efflux.

CHAPTER 1

GENERAL INTRODUCTION

Significance of Study

Earth's atmosphere is dominated by nitrogen (78%) and oxygen (21%) gases with the remaining 1% comprised of a number of 'trace gases.' Trace gases with greatest impact on the environment and human health include carbon dioxide (CO_2) (Zhou and Shangguan, 2006; Heinemann et al., 2006; Xuexia et al., 2006), nitrous oxide (N_2O) (Guo-yuan et al., 2006), and methane (CH_4) (Paul and Kimble, 1995; Jensen et al., 2000). These trace gases absorb and re-radiate infrared rays resulting in warming of the Earth's surface. This is known as 'greenhouse effect' and these trace gases are often referred to as 'greenhouse gases (GHG).' Nitrous oxide and CH_4, when transported to the stratosphere, can react with ozone destroying the atmospheric protective layer responsible for reducing ultraviolet radiation reaching Earth. The destruction of this protective layer poses increased levels of risk to human health.

The main drivers of GHG emissions are anthropogenic activities (IPCC, 1996) such as deforestation, increased fossil fuel consumption, and intensive agriculture production to meet the demands of the growing world population (Smith et al., 1998). The anthropocene era is expected to contribute significantly to global warming over the coming decades through increased rates of CO_2, N_2O and CH_4 emissions (Zhou and Shangguan, 2006; Mosier, 1998). For example, the United States comprises only 5% of the world population yet accounts for approximately 25% of all GHG emissions (Jackson and Schlesinger, 2004), mostly from increases in combustion of fossil fuels and

agricultural practices to meet lifestyle and production needs of the population (IPCC, 2007).

In 2005, the global concentrations of the three main GHGs were estimated at 379 ppm, 1774 ppb, and 319 ppb for CO_2, CH_4, and N_2O respectively (IPCC, 2007). Increases in radiative forcing due to increased GHG concentrations has led to an overall warming of the Earth's surface at an average annual rate of 0.13^0C per decade (IPCC, 2007). The main contributor of CO_2, CH_4, and N_2O to the atmosphere is believed to be the terrestrial carbon pool. The rate of exchange between the terrestrial pool and the atmosphere is generally affected by various soil biological, physical, and chemical properties (Guo-yuan et al., 2006; Conrad, 1996; Ihssen et al., 2003), including soil organic matter content, soil enzymes (Yuan et al., 2006), soil microorganisms (Xuexia et al., 2006) and management practices (Conrad, 1996; Adamsen and King, 1993; Nkongolo et al., 2006). Relationships among soil physical and chemical properties and greenhouse gas effluxes are documented by Agehara and Warncke (2005), Jackson and Schlesinger (2004), Fung et al. (2005), Paul and Kimble (1995), Ginting et al. (2003), and Avrahami et al. (2002). The effects of soil chemical and physical properties as well as some interactions of soil enzymatic activities, soil organic matter content, soil water content, and soil biological properties were also reviewed by Conrad (1996). Adamsen and King (1993) assessed methane consumption relative to soil temperature, vertical zonation, soil water content and nitrogen content. Others including Jensen et al. (2000), Benstead et al. (1998), Brusseua et al. (1994), and Dunfield et al. (1998) examined the role of methanotrophs in CH_4 oxidation. Graaff et al. (2006), Yuan et al. (2006) and Jason et al. (2006) examined the effects of CO_2 enrichment and irrigation on bacterial contribution to

nitrogen cycling. They all concluded that soil microbial population and activities were integral to GHG exchange between soil and atmosphere. However, these previous research reports focus primarily on either subsections of the soil population, or on a "snap-shot" period of time or space within their study site; with only a precursory attempt made to assess the role of season, topography, or spatial variation on the GHG dynamics.

Seasonal, topographic, and spatial patterns in soil physical and chemical properties have been reported to affect GHG efflux. Notable at our mid-Missouri study site, Paro et al. (2007) observed significant correlations between soil thermal properties and spatial and seasonal variation in greenhouse gas efflux. Johnson et al. (2007) also observed similar spatial and seasonal variation in greenhouse gas efflux in a managed pasture. Lu and his team of researchers (2000) attributed seasonal patterns of methane (CH_4) emissions to variations in dissolved organic carbon (DOC), which they linked to differences in DOC released from plant roots. Similar results were found by Jarecki and Lal (2003), Uselman et al. (2007), and Froberg et al. (2007). Ding et al. (2007) addressed the interaction of soil temperature and soil moisture, and their combined influence on CO_2 emissions from soils in Henan, China. They found significant correlations between seasonal CO_2 fluxes and soil temperature and moisture. Landform is also noted to impact soil properties (Rezaei and Gilkes, 2005) and GHG exchange with the atmosphere (Hanson et al., 1993). Rezaei and Gilkes (2005) observed differences in TOC mineralization due to landscape position and aspect of slope. Preliminary results from field studies in our research also inferred differences due to landscape position. Therefore, investigation of the effects of landscape position is critical to the full understanding of GHG dynamics from the soil. From the literature is it clear that

greenhouse gases produced and released by soil microorganisms are influenced by soil properties, land management and agricultural practices. However, the full extent of the role of microorganisms in GHG efflux has not been explored and interactions are not fully understood and there clearly exists a need for closer examination of the relationships among soil microbial properties, and greenhouse gas efflux mechanisms.

Rationale

Increasing atmospheric concentration of greenhouse gases poses a serious threat to human health and the environment. Preliminary work by Nkongolo and Schmidt (unpublished data), Paro et al. (2007, 2008), Johnson et al. (2007), and Hoilett et al. (2008) examined the role of soil as a source or sink for greenhouse gases at forest, pasture, and cropland sites in central Missouri. They found that GHG efflux was affected by soil physical, chemical, thermal, and biological properties. Ongoing research is assessing the contribution of soil physical and chemical properties on greenhouse gas cycling at these sites. Prior knowledge has established the significance of soil microorganisms to nutrient cycling and to the biological, physical, and chemical processes in the soil. Therefore, further assessment of the role of soil biological properties in GHG efflux will provide valuable information that can assist decision makers in regard to GHG efflux and global climate change.

Objectives

General Objective:

This research examined the interaction of soil microbial populations and activities with greenhouse gas emissions within a secondary forest in Central Missouri. Soils at this site were mapped as a Gatewood (Oxyaquic Hapludalfs)-Moko (Oxyaquic Hapludoll) complex. The Gatewood-Moko complex is gravelly alluvium over clayey

residuum derived from dolostone. Soil properties, inclusive of biological populations are known to vary within ecosystems (Arunachalam et al., 1999; Donald et al; 1999) and are influenced by differences in landscape positions (Fu and Chen, 2000). The effect of landscape position on soil properties and function also varies with time (Barreto et al. 2000). Additionally soil fungi and bacteria influence the amount and rate of greenhouse gases emitted from soils (Alvarez et. al., 1998). This work examines the distribution of soil microorganisms and attempts to establish possible links between variations of soil microbial populations and variations in greenhouse gas emissions within this secondary forest.

To successfully identify possible links between microbial population distribution and greenhouse gas emissions the following specific objectives and hypotheses were addressed:

Objective 1: Determine soil functional activity by measuring selected enzymatic activities: dehydrogenase, β-glucosidase, and amino acid arylamidase enzyme activities along the landscape based on position of each landscape unit.

Hypothesis 1: Soil properties vary within ecosystems, due partly to variations in vegetation, land management, topography, soil organic matter content, and other biotic and abiotic factors. Additionally, soil microbial populations are influenced by soil properties such as temperature, moisture, organic carbon, and nutrient status. Subsequently, microbial populations and their activity (e.g., enzyme activity) will reflect the influence of landscape position within an ecosystem.

Objective 2: Examine the potential of enzyme activity to establish a relationship between microbial activity and GHG efflux**.**

Hypothesis 2: Enzyme activity is often used as a measure of soil health and as an indication of the soils ability to carry out its ecological function. In addition, enzymes act as catalysts for the cycling of carbon and nitrogen by soil microorganisms. Therefore enzyme activity can indicate microbial activity and its contribution to carbon and nitrogen dynamics in the soil.

Objective 3: Determine the influence of landscape position on phospholipid fatty acid profiles, total phospholipid fatty acid concentration, and GHG efflux within a secondary forest.

Hypothesis 3: The composition of the microbial community is influenced by soil properties inclusive of soil texture, structure, organic carbon, and nutrient status. Therefore, phospholipid fatty acid (PLFA) profiles of the soil microbial communities in an ecosystem are possible indicators of differences in soil properties within the ecosystem.

Objective 4: Assess the potential of using PLFA profiles as indices of variation in GHG efflux from a secondary forest in mid-Missouri.

Hypothesis 4: Differences in soil properties and microbial communities at the different slope positions impact GHG efflux from this forest site.

Objective 5: Use PCR-DGGE to examine the effect of topographic position on soil microbial diversity by molecular analysis of fungal and bacterial populations.

Objective 6: Quantify fungal (*Fusarium*) DNA along the landscape using real-time PCR and DGGE.

Objective 7: Explore the potential of DGGE profiles and real time PCR to determine the role of bacteria and fungi in GHG efflux at different landscape positions in a secondary forest.

Hypothesis 5 -7: The composition of the microbial community is influenced by the heterogeneity in soil properties inclusive of temperature, moisture, organic carbon, and nutrient status as influenced by topography. Therefore species type and richness in an ecosystem are possible indicators of differences in soil properties within ecosystems

Dissertation Outline, Linkages to Objectives and Potential Publications

This dissertation consists of seven chapters in the following sequence.

Chapter 1: Introduction.

Chapter 2: Literature Review relevant to research project.

Chapter 3: Materials and Methods: Field and Laboratory Procedures

Chapters 4: Relationship of three enzymes and greenhouse gas efflux from a secondary forest in mid-Missouri (objectives 1 & 2).

Chapter 5: Soil microbial communities and greenhouse gas efflux in forest soils: an incubation study (objectives 3 & 4).

Chapter 6: Using PCR-DGGE to assess the association between soil microbial community and greenhouse gas emissions in a forest ecosystem (Objectives 5, 6 and 7).

Chapter 7: Summary of the results from the Chapters 4, 5, and 6 in addition to suggestions for future research areas that would assist in providing possible answers to questions generated from the results of this and other research work discussed in the dissertation.

Formatting: Chapters 4, 5, & 6 were formatted following the style of *Soil Science Society of America Journal*, in anticipation of publishing the chapters in refereed journals.

CHAPTER 2

LITERATURE REVIEW

Greenhouse Gas Emissions

Carbon, nitrogen, oxygen, and hydrogen are the key elements of life on Earth as they are major components of atmosphere, soil air and soil organic matter (Paul and Kimble, 1995). The processes that maintain the balance of carbon and nitrogen between the atmosphere and soil are the carbon and nitrogen cycles respectively (Keeling, 1997; Paul and Kimble, 1995). Carbon and nitrogen enter the atmosphere in the forms of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O), which are byproducts of coal, natural gases, and petroleum combustion. Additional contributions of CO_2 and N_2O to the atmosphere are made through industrial and agricultural processes (Keeling, 1997) with changes in land use affecting the nutrient cycles and the exchange rates of gases between the soil and the atmosphere (Glatzel et al., 2004; Subbarao et al., 2006).

Continued worldwide concern with increases in greenhouse gases and their effect on global climate change and the environment requires better understanding of the processes that govern greenhouse gas efflux (Fang and Moncrieff, 2000). Carbon dioxide (Zhou and Shangguan, 2006; Heinemann et al., 2006; Xuexia et al., 2006), nitrous oxide (McLain and Martens, 2005; Guo-yuan et al., 2006) and methane (Keeling, 1997) are the gases of major concern. The atmospheric concentrations of these gases are increasing at rates of 0.5%, 0.75% and 0.75% respectively on an annual basis (Paul and Kimble, 1995). The United States with annual emission rates of ≈ 1.58 petagrams (pg) accounts for approximately 25% of the global production of CO_2 (Jackson and Schlesinger, 2004).

Nitrous oxide, with a longevity 300 times that of CO_2 (Kusa et. al., 2005; Rodhe, 1990), is potentially more damaging to the atmosphere.

The implications of increased levels of greenhouse gases in the atmosphere are shifts in climate resulting in possible increased global temperature, rising sea levels and reductions in carbon sequestration and terrestrial and oceanic carbon pools (Parry et. al., 2007). The reduction in terrestrial and oceanic carbon sequestration could possibly have a positive feedback effect on greenhouse gas emissions (Fung et al., 2005), thereby, amplifying the rate of global climate change.

Soil organic matter

Terrestrial carbon sequestration reverses the loss of soil organic carbon resulting from agricultural practices, e.g., plowing (Jackson and Schlesinger, 2004; IPCC, 2007). Soil organic matter (SOM) acts as a reservoir for large amounts of carbon (C) (Purakayastha et. al., 2008; Alvarez and Alvarez, 2000), thus factors that affect the dynamics of SOM are of major concern (Kemmitt et. al., 2007). Soil organic matter is integral to the development and maintenance of soil properties (Yin and Cai, 2006); and is known to influence soil fertility (Ross et al., 1999), soil chemical properties, soil water content (Rawls et al., 2003), plant growth, soil biota, greenhouse gas emissions, and nutrient cycling (Bastviked et. al., 2007). Soil organic matter also influences soil temperature, microbial activity, and the ability of the soil to sequester carbon and act as a sink for environmental contaminants (Fung et al., 2005).

Although the CO_2 released during respiration is primarily utilized in the process of photosynthesis, shifts in land-use practices such as deforestation, wetland drainage, and cultivation tend to upset the delicate equilibrium (van Hees et al., 2005) resulting in

increases in atmospheric CO_2 mostly due to the increased decomposition of organic carbon. Research by Burford and Bremner (1975), Standford et al. (1975), Bastviked et al., (2007) and others have found significant correlation between soluble soil carbon and rates of denitrification. Similar relationships exist between soluble soil carbon and other microbial activities. For example Leita et al. (1999) found a linear relationship between soil microbial biomass and total organic carbon (TOC). Their results were similar to those of Brown et al. (1997), Bardegett and Shine (1999), and Nsambimana et al. (2004) who concluded that the size, activity, and diversity of the soil microbial community are altered by changes in land management. Brown et al. (1997), Bardegett and Shine (1999), and Nsambimana et al. (2004) also concluded that microbial biomass is influenced by the quality of the organic matter. Studies showed that metabolic quotient (Leita et al., 1999; Bardgett and Shine 1999; Nsambimana et al., 2004), respiration, decomposition (Bardgett and Shine, 1999; Nsabimana et al., 2004) and enzymatic activities (Nsabimana et al., 2004) were affected by quantity and quality of soil carbon. From their results it can be inferred that in most soils, soil organic carbon is an important substrate for soil microorganisms (Lorenzo et al., 2007).

Soil Microorganisms

Microbial processes mediate the efflux of CO_2 (MacDonald et al., 1995; Paul and Kimble, 1995), N_2O (Cao et al., 2006) and CH_4 (Paul and Kimble, 1995). With microbial processes contributing over 70% of N_2O emitted to the atmosphere (Conrad, 1996) it is, therefore, important to understand how anthropogenic activities impact soil microbial population and greenhouse gas efflux and subsequently global climate changes. Additionally, N_2O emission from soil is a major environmental concern owing to its

longevity. The rate of N_2O emission is a function of the nitrogen (fertilizer) content of the soil (Cao et al., 2006), temperature, soil moisture (Agehara and Warncke, 2005; Raich & Tufekcioglu, 2000), available carbon (Raich & Tufekcioglu, 2000), and available nitrogen (N). Nitrous oxide fluxes are influenced by nitrification and denitrification processes mediated by soil microorganisms (McLean and Martens, 2005; Hoyle et al., 2006); and is of concern as it contributes to global warming due to its ability to deplete the stratospheric ozone layer (Cao et al., 2006).

Nitrification only accounts for a minor portion ($\leq 2 - 4\%$) of N oxides lost to the atmosphere (Duxbury and McConnaughey, 1986); however, the process of nitrification is significant as it is a precursor to denitrification (Norton, 1999). Subbarao et al. (2006) describes the nitrification process as the transformation of relatively immobile ammonia to the highly mobile NO_3^- form by *Nitrosomonas and Nitrobactor species*. The NO_3^- is then easily converted to gaseous forms (N_2, N_2O, and NO) by denitrifiers such as the bacteria *Bacillus subtilis, E. coli, Achromobacter aerogenes, Pseudomonas spp., and Micrococcus sp,* and some fungi including *Fusarium oxysporium* (Fujii and Takaya 2008), *F. lini, F. decemcellulare, F. solani, Gibberella fujikuroi* (Shoun et al., 1992), *Aspergillus flavus and Penicillium atrovenetum* (Yoshida and Alexander, 1970).

The denitrification process is carried out by both fungal and bacterial species (Laughlin and Stevens, 2002). However, fungal populations are potentially able to conduct denitrification under both aerobic and anaerobic conditions versus bacteria that require anaerobic conditions for denitrification (Laughlin and Stevens, 2002). In addition, most fungal species lack the N_2O-reductase enzyme; hence N_2O is generally the final product of fungal denitrification (Laughlin and Stevens, 2002). The microbial

biomass of most soils is predominantly fungal biomass (Ruzicka et al., 2000; Norton, 1999); therefore, the ratio of fungal biomass to bacterial biomass will affect the dynamics of greenhouse gas efflux within the ecosystem.

Soil Enzymes

Soil enzymes have often been used as indicators of soil quality and health because of their key roles in regulating carbon, nitrogen, and other nutrient cycles through organic matter decomposition, and C and N immobilization and mineralization (Tate, 2002; Bandick and Dick, 1999). The sensitivity of enzymes to changes or differences in soil conditions, the ease of assay, and their role in soil biological functions (Ekenler and Tabatabai, 2004) contribute to their added usefulness as indicators of biological activity in soils (Bandick and Dick, 1999). Over the last 50 years enzyme activities have been used to monitor soil response to management practices (Eivazi and Tabatabai, 1990; Deng and Tabatabi, 1996), heavy metal pollution (Lorenz and Kandeler, 2006; Belen-Hinojosa et al., 2004; Tscherko and Kandeler, 1999), chemicals used in pest and disease control (Rahmansyah et al., 2009) and to assess soil functional diversity (Tscherko et al., 2003).

Despite considerable research on soil enzyme activity, few attempts (e.g. Pant, 2009; Qin et al., 2010; Wingate et al., 2009) have demonstrated the use of enzyme activity as a tool for assessing microbial contributions to GHG efflux. Skujins (1967) attempted to use soil enzymes as a measure of CO_2 evolution; however, as discussed by Dick (1994), lack of efficient methods at the time could have impacted the sensitivity of the results. Frankenberger and Dick (1983) had some success in establishing direct relationships between soil respiration and enzyme activity. However, the main focus of

soil enzymology over the years has been on using enzyme activity to quantify and qualify microbial activity.

Phospholipid Fatty Acid Profiles

Soil microorganisms utilize the products of photosynthesis, i.e., plant material and rhizodeposition substances, for growth. The products of microbial respiration include organic matter fractions of organic residues at different stages of decomposition and CO_2. The rate of residue accumulation and CO_2 production is dependent on the type of substrate, soil conditions, land management, and the composition of the microbial community. Macro-organisms often initiate the decomposition process by fragmenting and incorporating plant material into the soil. Microorganisms such as fungi, bacteria, and actinomycetes are then able to further decompose the shredded material (Wagner and Wolf, 1998). However, within a landscape the above mentioned differences in soil properties often foster heterogeneity in the distribution of soil organisms and their respiratory products. This complexity of the soil microbial community is continuously been assessed using various techniques.

A critical limitation in understanding the soil community is that some techniques used to characterize microbial communities such as plating and counting on artificial media, most probable numbers (MPN), and other culture-based techniques, although somewhat successful, often only captures a minor proportion (<10%) of the soil microbial community (Kennedy, 1994). Phospholipid fatty acid (PLFA) profiles represent the living microbial community and have been successfully used to identify differences in microbial communities in the environment (Boggs et al., 1998; Ibekwe & Kennedy, 1998). The PLFA technique is based on the principle that the structure and

form of phospholipids for each genera or group of organisms is unique to that group (Sinsbaugh et al., 1999). Therefore different groups of organisms have specific phospholipid fatty acid profiles which allow for the characterization and quantification of the soil microbial community (Peterson and Klug, 1994). Other information obtained from PLFA profiles such as fungal to bacterial ratio is often used to measure differences in microbial biomass (Baath, 2003). The ratio of saturated to monounsaturated fatty acids and the ratio of the cyclopropyl (cy 17:0 & cy 19:0) to their monoenoic precursors (16:1w7 & 18:1w7) are also often used as indicators of physiological and nutritional stress (Bassio and Scow, 1998; Kieft et al., 1997).

Soil DNA and Molecular Characterization

Soil communities include bacteria, fungi, actinomycetes, and other soil flora and soil fauna. Soil bacteria tend to be most dominant in terms of number and species diversity (Wollum, 1998); however, soil fungi often account for a greater biomass especially in forest soils (Busse et al., 2009; Kageyama et al., 2008). Although microscopic in size the influence of soil bacteria and fungi are often exerted at the macro-scale, and are known to influence their surrounding environment (Dighton et al., 2005). The distribution of these microorganisms in forest soils however is still in need of further investigation (Morris, 1999), especially in regard to their relationship with GHG efflux. Morris (1999) and Morris and Boerner (1999) have demonstrated spatial variability in soil microbial properties in Ohio and have concluded that vegetation, moisture, and landscape position were among the factors influencing biological distribution within these ecosystems. However, some concerns exist with the type of assessment used to

quantify and characterize the microbial population within soil systems (Morris and Robertson, 2005; Filion et al., 2003; Jensen et al., 2000).

Conventional techniques such as plating, serial dilution and direct counts have successfully been used to identify and quantify microorganisms from soils (Morris and Robertson, 2005). However, these techniques are often limited to subsections of the soil microbial population (Filion et al., 2003). Microorganisms in general (Jensen et al., 2000), and fungi in particular are often difficult to propagate from soil, with some species having very specific growth requirements (Filion et al., 2003). It would require a herculean effort to isolate the various groups of soil fungi using growth media (Dighton et al., 2005).

Fusarium species represent a common fungal genus that proliferate throughout soils in the USA (Leslie et al., 1990) and around the world (Wakelin et al., 2008; Saremi et al., 1999); and have been identified in agricultural (Latiffah et al., 2009; Rajput and Rao, 2007) and forest soils (Latiffah et al., 2007; Lim and Chew, 1972). *Fusarium* species are renowned for the economic loss associated with crop infestation and toxicity in food and feed (Walkelin et al., 2008). However, on the other hand, some *Fusarium* species are also beneficial to the ecosystem in which they persist. In symbiotic relationships fungi influence the productivity of plants by providing additional surface area for nutrient and water uptake (Morris and Robertson, 2005). Species such as *Fusarium* are precursors in the decay of woody material (Walkin et al., 2008) and thus are often important players in carbon cycling in forest ecosystems (Rajput and Rao, 2007). *Fusarium* species have also been associated with the denitrification process in soils by previous researchers (Laughlin and Stevens, 2002; Tayaka et al., 2002; Shoun et

al., 1992) and are therefore of interest in the study of GHG efflux. However, as with other fungal species, the difficulty and limited effectiveness of conventional culturing techniques restrict the rapid quantification of *Fusarium* species in soil. Latiffah et al. (2009) were only able to isolate two *Fusarium* species during their research of a Malaysian forest. In contrast Summerell et al. (1993) were able to isolate a total of 13 *Fusarium* species from an Australian forest using the same plating technique as Latiffah and his group. The differences in the two results may be related to differences in soil conditions e.g. vegetation, moisture, soil microbial community composition, or the inherent variability of the technique.

Denaturing Gradient Gel Electrophoresis is proving to be useful in microbial ecology studies when combined with other molecular techniques. The DGGE technique is based on the separation of PCR - amplified gene fragments, not according to size but owing to variation in targeted nucleotide sequences (Hasting, 1999). Various authors (see Anderson and Cairney, 2004) have shown that PCR can be used to quantify soil fungi. Wakelin et al. (2008), Filion et al. (2003), Smit et al (1999), Li and Hartman (2003), Fujii and Takaya (2008), Tellenbach et al. (2010) and Yergeau et al. (2005) were able to successfully quantify or discriminate *Fusarium* species from varied soil systems using PCR, qPCR and/or DGGE techniques. The results from the above listed research not only established the usefulness of PCR-based techniques in mycology, but also reinforced the importance of *Fusarium* taxa in different soil environments. However of the research mentioned above, only Fujii and Takaya (2008) tried to associate the *Fusarium* communities with GHG efflux.

Although not exhaustive, our search of the literature clearly highlighted a need for more work on the role of *Fusarium* species in soil systems and in particular their contribution to and influence on GHG efflux. We hypothesize that characterizing the microbial community using PCR-DGGE profiles will provide information critical to a better understanding of the role soil microorganisms in soil processes e.g. GHG efflux.

CHAPTER 3

MATERIAL AND METHODS: FIELD AND LABORATORY PROCEDURES

This research was conducted as part of an ongoing project at Lincoln University-Missouri to investigate the effect of soil biological, chemical, and physical properties under pasture (Washington Carver Farms), forest (Busby Farms), and cropping systems (Freeman Farms) on greenhouse gas emissions. This chapter outlines the field and laboratory procedures which were used to address objectives 1 through 7.

Materials and Methods

Description of Research Sites and field sampling

The study site, Busby Farm (BF), is a permanent secondary forest near Jefferson City, Missouri; geographic coordinates are 38 34' 53"N and 92 08' 07" W. The site is dominated by oak (*Quercus alba*) and hickory (*Carya ovate*) trees. Soils at this site were mapped as a Gatewood (Oxyaquic Hapludalfs)-Moko (Oxyaquic Hapludoll) complex. The Gatewood-Moko complex is gravelly alluvium over clayey residuum derived from dolostone. The BF plot is 0.49 ha with a total of 20 chambers distributed along an elevation range of approximately 29.2 meters. Topography of the landform allowed for division into five landscape units, summit (SS), shoulder (SH), backslope (BS), footslope (FS), and drainageway (DR) (Boerner and LeBlanc, 1995). The summit is defined by a gentle slope less than 15 percent slope, the shoulder also slopes gently 15 – 20 percent slope, the steepest section, the backslope, drops as much as 40 percent slope in some places; and the footslope tapers to an almost flat valley. The drainage area is separated from the other landscape units by a gully and is characterized by slopes of 20 – 25

percent slope. The field design also took into consideration soil characteristics, such as total organic carbon, total nitrogen, and CEC from preliminary results (Table 3.1).

Soil Sampling

Soils were sampled (0 - 20 cm depth) three times, July 2008 (JSP), November 2008 (NSP), and March 2009 (MSP), along the five landscape units (Figure 3.1). The landscape units SS, SH, BS, FS, and DR were further sub-divided into three pseudo-replicates yielding a total of 15 sub-units. Samples from these 15 sub-units were sieved (<2mm) and roots and other plant material removed by hand.

Incubation Procedure

The incubation study used a 2 x 2 factorial design (Table 3.2) to determine the impact of soil temperature 25^0C and 35^0C (JSP), 15^0C (NSP) and 20^0C (MSP); and water content (60%WHC and field moisture content (FM) at time of sampling; Table 3.3), on the soil microbial contribution to GHG efflux. Each landscape sampling position was replicated 24 times for a total of 120 experimental units.

Samples from each field replication (ca. 300 g oven dry [o.d] equivalent) were placed in 0.5- liter mason jars. Soils were adjusted to appropriate moisture, i.e., field moisture (FM) content at time of sampling (based on gravimetric water determination) or 60% water holding capacity. For example, samples for the field replication one from SS taken in July of 2008 were adjusted to 28.33% to reflect soil moisture at time of sampling (Table 3.3). Samples were preconditioned for seven days to account for disturbances due to sample preparation activities such as sieving and moisture adjustment. Samples were incubated for a total of 30 days at the appropriate temperature based on treatment combinations (Table 3.2). Simultaneously, 30 g (o.d. equivalent) of soil was incubated as described above in 60 ml vials to monitor respiration in the respective soils. During

incubation, jars and vials were covered with perforated Parafilm® to reduce moisture loss while allowing for O_2 exchange. Headspace gas from 60 ml vials were sampled and analyzed for CO_2, N_2O, and CH_4 on a Shimadzu GC14 Gas Chromatograph (Shimadzu, USA) after covering vials for one hour. The chromatograph was equipped with an electron capture detector (ECD) for CO_2 and N_2O; and a flame ionization detector for CH_4. The columns on the chromatograph were Propak Q and N 80/100 mesh. Simultaneously, soils were taken from the mason jars and processed as described below. Sampling was done on days 1 (24 hours after the start of the incubation), 5, 10, 20, 30.

Laboratory Procedures

Analytical Procedures

Composite soil samples taken from the site were analyzed for soil nutrient status to provide a baseline for the phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), pH, cation exchange capacity, base saturation and other such indices for the soil (Table 3.1).

Soil water content – gravimetric soil water content for each sampling date at each sampling location was determined in the laboratory following the method described by Zancan et al. (2006). Freshly sieved (<2mm) soils from each sample location were weighed in aluminum boats and heated at 105^0C until no further weight loss was observed. Results were used to convert relevant data from quantity/rate per gram of field moist soil to quantity/rate per gram of dry soil.

Soil pH – was measured in H_2O at a 1:2.5 ratio soil to H_2O (Raiesi, 2006).

Total organic carbon and total nitrogen – were determined through combustion to CO_2 and N_2 at 950^0C using a LECO carbon – nitrogen analyzer (McLauchan and Hobbie, 2004).

Soil Biological Properties

Enzyme Activity: Enzyme activities were measured using standardized protocols at incubation temperatures of 37°C, at fixed time periods, over specific buffer pH ranges, and included relevant cofactors for each enzyme assay. Activities are expressed as µg product g^{-1} soil h^{-1} using calibration curves for the appropriate product and absorbance values and extraction solution as blanks. Soil blanks and controls were analyzed concurrently. Absorbance for each enzyme assay was measured on a Thermo Genesys10 (Thermo Scientific, PA) spectrophotometer at the appropriate wavelength for each enzyme assay.

β-glucosidase activity was measured by placing 1.0 g of field moist soil (<2mm) in a 25 ml Erlenmeyer flask, adding 0.25 ml of toluene, 4 ml of modified universal buffer (MUB; pH 6.0), and 1.0 ml of 0.5 mol L^{-1} p-nitrophenyl–β-D-glucoside (PNG) solution. Flasks were then swirled to fully mix contents, stoppered, and incubated at 37^0C for 1 hour. After incubation 1.0 ml of 0.5 mol L^{-1} $CaCl_2$ and 4 ml of 0.1 mol L^{-1} tris (hydroxymethyl)aminomethane (THAM) buffer pH 12 was added to stop the reaction. Suspensions were filtered through Whatman # 2 filter paper using a vacuum source, and absorbance of the filtrates were measured at 410 nm wavelength. Results are reported on a dry weight basis in units of µg pNP g^{-1} soil h^{-1} (Tabatabai 1994)

Dehydrogenase activity was determined according to Von Mersi and Schinner (1991). Briefly, field moist soil equivalent to 1 g oven-dried soil was weighed into 25 ml Erlenmeyer flask, 1.5 ml TRIS buffer (1M; pH 7.0) and 2 ml of 0.5% (w:v) aqueous solution of indonitrotetazolium chloride (INT;10 mg ml^{-1}) were added. Samples were mixed thoroughly and incubated at 40^0C in the dark for 2 hours, after which 10 ml of an

extracting solution (N,N-dimethylformamide/ethanol in a 1:1 ratio) was added. To extract the developed iodonitrotetrazolium formazan (INTF), samples were kept in the dark for 1 hour, shaken vigorously at 20 min intervals, filtered through Whatman #2 filter paper, and absorbance measured at 464 nm using the extraction solution as a blank. Results for dehydrogenase activities are reported as μg $INTFg^{-1}$ soil $2hr^{-1}$.

Arylamidase activity was determined based on the method of Acosta-Martinez and Tabatabai (2000). To one gram (< 2mm) of soil in a 25-ml Erlenmeyer flask 3 ml of 0.1 M THAM buffer (pH 8.0) and 1 ml of 8.0 mM L-leucine β-naphthylamide hydrochloride were added. The flask was swirled gently to mix contents and incubated at 37^0C for 1 hour. After incubation, the reaction was stopped by adding 6 ml of ethanol (95%). The soil suspension was then mixed and transferred to a centrifuge tube and centrifuged for 1 minute at 17000 x g. The supernatant was transferred to a new test tube to prevent any further hydrolysis of the substrate. Then 1ml of the supernatant was further transferred to a clean test tube and 1 ml of ethanol, 2 ml of acidified ethanol, and 2 ml of p-dimethylaminocinnamaldehyde reagent added. Samples were vortexed after addition of each of the reagents. The intensity of the resulting red azo compound was measured at 540 nm. Results are reported on a dry weight basis in units of μg β-naphthyamine g^{-1} soil h^{-1}.

PLFA

Soil samples were extracted and PLFA quantified following a modified version of the method described by Petersen and Klug (1994). See Chapter 5 for details.

Soil DNA

DGGE

Total DNA was extracted from 0.5 g air dry soil using a commercially available soil isolation DNA kit. DNA concentration in each purified extract was quantified by UV spectroscopy and stored at -80C until PCR analysis. Denaturing Gradient Gel Electrophoresis (DGGE) analysis was performed following the procedures described by Hastings (1999) for bacteria and Haugland et al. (2002) and Kabir et al. (2003) for fungi. DGGE technique is based on PCR amplification of gene fragments according to variation in targeted nucleotide sequences. (See Chapter Six for detailed description)

Quantitative PCR (qPCR)

Additionally, qPCR was used following the nested approach outlined in Yergeau et al. (2005) and Walkin et al. (2008) applying modifications as suggested by Walkin (personal communication). An additional adjustment to the number of cycles use in step 1 of the PCR amplification was made based on Tellenbach et al. (2010). Details of the PCR conditions are given in Chapter Six.

General Experimental Details

Table 3.1 Some soil characteristics (0 – 20 cm) for each landscape sampling position at Busby Farms. Textural Class (SiL silt loam, CL clay loam), total organic carbon (TOC), total nitrogen (TN), and cation exchange capacity (CEC). Clay, silt, and sand measured as percentage of total soil.

	Slope Position				
	Summit	Shoulder	Back slope	Footslope	Drainageway
Textural Class	SiL	SiL	SiL	CL	SiL
Clay (<.002 mm)	16.27	15.23	14.27	27.23	23.03
Silt (.002 - .05 mm)	74.23	74.17	68.60	43.50	60.53
Sand (.05 - 2.00 mm)	9.50	10.60	17.13	29.27	16.43
TOC (%)	1.43	1.97	2.47	3.20	3.20
TN (%)	0.12	0.17	0.22	0.29	0.27
pH (salt)	4.77	5.03	6.10	6.17	6.50
pH (water)	5.23	5.33	6.40	6.40	6.73
Extractable Bases	5.50	7.23	12.57	20.57	21.87
CEC ($Cmol_c/Kg$)	11.83	14.63	16.73	25.30	26.43

Table 3.2 Two X two factorial design for incubation study

	T1	T2
M1	T1M1	T2M1
M2	T1M2	T2M2

Where: T1 = Optimal temperature for microbial activity (~25^0C)
M1 = Field moisture at the time of sampling (Gravimetric water determination)
T2 = Temperature at time of field sampling
M2 = Optimal moisture for microbial activity (~ 60% WHC)

Table 3.3 Incubation moisture conditions for soils from each landscape sampling position during each sampling period. Soils were incubated at 60% water-holding capacity (60%WHC) and the field moisture content (FM) at the time of sampling. Sampling was performed three times over a one year period July 2008 (JSP), November 2008 (NSP) and May 2009 (MSP).

	60% WHC	Gravimetric water content		
		JSP	NSP	MSP
----------Percentage (%) ----------				
Summit	28.33	28.03	19.76	29.33
Shoulder	35.33	9.31	18.44	30.15
Backslope	35.00	30.83	23.95	30.82
Footslope	40.33	33.14	31.46	40.29
Drainageway	40.67	31.60	31.48	35.88

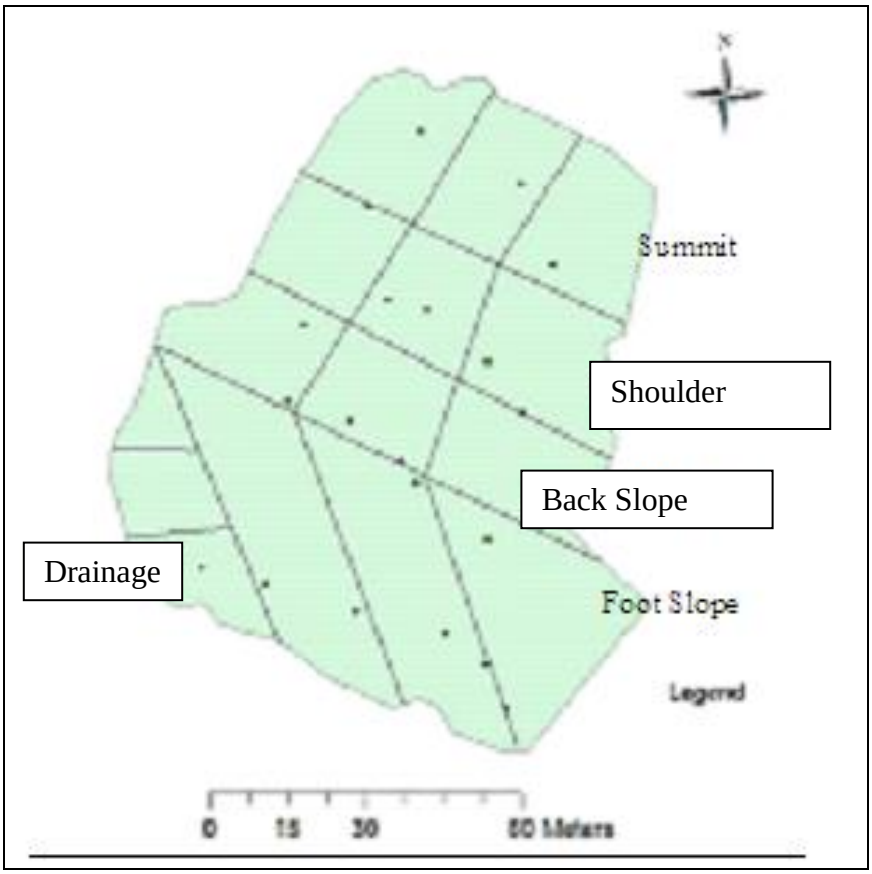

Figure 3.1: Map of Busby site showing landscape sampling positions and replications for sampling

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Chapter 4

Relationship between soil microbial enzyme activities and greenhouse gas efflux from forest soils

Abstract

Soil enzymes are often used as indicators of soil quality and microbial activity in soils. However, as far as we know, no previous attempt has been made to relate enzyme activity to greenhouse gas (GHG) efflux from forest soils. The objective of this study was to examine the relationship between enzyme activity and GHG efflux across a forested landscape. Activities of selected microbial enzymes, total organic carbon, total nitrogen, and gaseous efflux of carbon dioxide, nitrous oxide, and methane were measured in soils from a secondary forest in central Missouri, during an incubation study. The experimental site was divided into five landscape sampling positions to test the influence of topography on enzyme activity and GHG efflux. Soil samples were incubated for 30 days at a control temperature of 25^0C and temperature reflecting the time of year when the samples were collected, i.e. 35^0C for samples collected in July 2008, 15^0C for samples collected in November 2008, and 20^0C for samples collected in May 2009. The moisture levels used in the incubation study were a control moisture level of 60% water holding capacity (60%WHC) and the field moisture (FM) content at the time sampling was performed (based on gravimetric moisture determination). The results from the study identified spatial and temporal variations in enzyme activities and GHG efflux. However, significant, although weak, correlation between the activities of three enzymes and GHG efflux indicate that enzymes may provide useful information on the role of soil microorganisms in GHG efflux from forest soils. For July 2008 sampling

period all three enzymes, β-glucosidase (r -0.20; $p<0.01$), dehydrogenase (r -0.39; $p<0.001$), and arylamidase (r -0.22; $p<0.01$) activities correlated with CH_4 efflux. In addition, significant differences among soils from various landscape sampling positions, combined with significant interactions of landscape position with other main effects for the measured properties, highlight the potential of topographic position to influence both enzyme activity and GHG efflux.

Introduction

The role of GHG in affecting temperature changes and subsequently global warming is a highly discussed topic of current interest. Although considerable research has addressed these issues, we still do not fully understand the dynamics of GHG efflux to the atmosphere. The difficulty in understanding GHG efflux is most likely due to the high number and complexity of contributors of GHGs; the most complex of which is likely the soil-plant interface with the atmosphere. Within the soil matrix, soil organisms play an integral role due to their participation in organic matter decomposition (Xuexia et al., 2006). However, understanding GHG efflux dynamics between the soil and atmosphere is often challenging due to the natural spatial and temporal variability of soil properties within a landscape (Yanai et al., 2003; Conrad, 1996; Broos et al., 2007).

Variability in soil properties is often affected by abiotic soil properties that subsequently influence soil biota (Vanhala, 2002). The interrelationship among soil biotic and abiotic properties makes it difficult to resolve the contribution of each factor to soil processes (Jensen et. al., 1997; Speratti and Whalen, 2008; Bowden et al., 1998). Lerios et al. (1999), Carreiro and Koske (1992), Makiranta et al. (2008), and Sainz Rozas et al. (2001) have documented significant variations in soil biological properties and

functions resulting from variations in soil temperature and moisture. For example, nitrifying (Yanai et al., 2003), denitrifying (Merino et al., 2004), and methanotrophic (Bowden et al., 1998) activities are moisture and/or temperature dependent. Similarly, Turner et al. (2008) and Zhong and Makeschin (2006) associated spatial and temporal variations in GHG efflux with heterogeneity in soil moisture content. Merino et al. (2004) found strong correlation between soil microbial C and CO_2 emissions. They further concluded that microbial activity had a greater influence on GHG efflux than did soil temperature and moisture. In contrast, Jensen et al. (1997) found that temperature, rather than soil microbial biomass C, explained the majority of the variability in CO_2 emissions. Preliminary results from our study site found moisture, temperature, and soil thermal properties to be more pronounced influences on GHG efflux than biological properties (Hoilett et al., 2008). Therefore, to fully understand the relationship among the many variables influencing GHG efflux, it is necessary to elucidate the contributions of the different factors in an in-depth study (Conrad, 1996).

Franzluebbers et al. (2000), Maag and Vinther (1999), and Khorsandi and Nourbakhsh (2008) have suggested incubation studies at constant temperatures and moistures as probable methods of reducing the compounding influences of moisture and temperature. For example, Vanhala (2002) found that incubation at 60% water holding capacity reduced the variation in soil respiration rates. However, optimum conditions of moisture (50% - 60% WHC) and temperature (25 - 35^0C) used in incubations are not always present in the field (Knoepp and Swank, 2002). Additionally, optimum conditions often result in an under- or over-estimation of soil biological properties or responses. Carreiro and Koske (1992) observed that fungi species isolated during

incubation were dependent on incubation conditions including temperature. Sainz Rozas et al. (2001) found incubation at 87 – 94% water-filled pore space favored the denitrification process. Nevertheless, incubation studies continue to provide useful information that is not readily obtainable from field studies.

It is common knowledge that soil microorganisms regulate carbon, nitrogen, phosphorus and sulfur cycles through organic matter decomposition, and immobilization and mineralization of these nutrients. These cycles are almost completely regulated by enzymatic activity (Tate, 2002; Bandick and Dick, 1999), therefore an understanding of the different soil enzymes involved in nutrient cycling processes is critical to understanding the processes themselves (Acosta-Martinez and Tabatabai, 2000). For example, amino acid arylamidase [EC 3.4.11.2] catalyzes the hydrolysis of an N-terminal amino acid from arylamides (Acosta-Martinez and Tabatabi, 2001, 2002; Bandick and Dick, 1999); while β-glucosidase [EC 3.2.1.21] is involved in the hydrolysis of cellobiose (Tabatabai, 1994). Dehydrogenase enzymes, which are important in soil organic matter oxidation (Camina et al., 1998), are only found in living cells (Dick, 1994) and, therefore, provide a measure of the viable microbial population. The sensitivity of enzymes to changes or differences in soil conditions, the ease of assay, and their role in soil biological functions (Ekenler and Tabatabai, 2004) accede with their use as indicators of biological activity in soils (Bandick and Dick, 1999). Bandick and Dick (1999) observed that β-glucosidase activity consistently identified differences in carbon cycling on their plots. Dehydrogenase activity has provided useful information on microbial populations and their activity in soils (von Mersi and Schinner, 1991). Deng et al. (2000) found strong a correlation between amidase activity and N mineralization from active N pools.

Over the last 50 years activities of various enzymes have been used as indicators of soil quality (Eivazi and Tabatabai, 1990), soil health (Deng and Tabatabi, 1996), and soil response to anthropogenic changes (Lorenz and Kandeler, 2006)

Despite the considerable research on soil enzyme activity, few attempts (e.g. Pant, 2009; Qin et al., 2010; Wingate et al., 2009) have demonstrated the use enzyme activity as a tool for assessing microbial contributions to GHG efflux. Thus, our primary objective was to investigate the relationship between microbial activity and GHG efflux using soil enzyme indicators. As variation in soil properties also impact enzyme activity and GHG efflux our second and third objectives, respectively, were to determine the influences of temperature and moisture on spatial and temporal variation in enzyme activities and GHG effluxes. This is particularly important as biogeochemical cycling facilitated by enzymes often indicate the ability of the soil to carry out its ecological functions (Tscherko and Kandeler, 1999; Dick, 1994). Therefore, an understanding of the relationship between soil enzyme activity and GHG efflux could provide policy makers with useful information to assist in making meaningful recommendations to minimize GHG contributions from the soil ecosystem.

Material and Methods

***Study site and experimental design*.** Soil samples were collected from the Lincoln University Busby Research Farm near Jefferson City, Missouri (38° 34’ 53” N, 92° 08’ 07” W). The site (0.49 ha) is a secondary forest that is dominated by oak (*Quercus alba*) and hickory (*Carya ovata*) trees on soils mapped as Gatewood (Oxyaquic Hapludalfs)-Moko (Oxyaquic Hapludolls) silt loam complex. Other notable tree species include green-ash (*Fraxinus pennsylvanica*), white ash (*Fraxinus americana*), American elm

(*Ulmus americana*) and common hackberry (*Celtis occidentalis*). Topography at the site permits division into the following landscape positions: summit (SS); shoulder (SH); backslope (BS); and footslope (FS). Samples were also collected from a drainageway (DR) that was separated from the other four landscape positions by a gully. Each landscape position was divided into three pseudo-replicates following transects established across each landscape position. On average, three to six subsamples were collected to a depth of 0-20 cm from each landscape sampling position within a pseudo-replicate. Subsamples were then pooled to create 15 composite samples per sampling period (i.e., one composite sample per landscape position within each of the three pseudo-replicates). Samples were collected in July and November of 2008 and May of 2009, sieved (<2mm) and stored below 4^0C prior to analysis.

Incubation Procedure. The three field sampling periods (July 2008, November 2008, and May 2009) were treated as independent experiments and were incubated separately. Samples collected in the July 2008 sampling period (JSP) were incubated at 35°C; samples from the November 2008 sampling periods (NSP) were incubated 15°C; and samples from the May 2009 sampling period (MSP) were incubated at 20°C.

Samples from each field replication within each sampling period (*ca.* 300 g oven dry equivalent) was placed in 0.5 liter mason jars, and adjusted to appropriate moisture, i.e., field moisture content at time of sampling (FM) or 60% water holding capacity (60%WHC). Samples were preconditioned for 7 days at 4^0C to account for disturbances due to sample preparation activities such as sieving and moisture adjustment. Samples were then incubated for a total of 30 days. In addition to the above incubation temperatures for each sampling period (JSP 35^0C, NSP 15^0C, MSP 20^0C) a subsample

from each period was concurrently incubated at 25°C as a control. Simultaneously, 30 g oven dry (o.d.) equivalent of soil from each field replicate was incubated as described above in 60 ml vials to monitor GHG efflux. During incubation, jars and vials were covered with perforated Parafilm® to reduce moisture loss while allowing for gas exchange with the atmosphere. Moisture was adjusted weekly on a mass basis (McLauchlan and Hobbie, 2004). On days 1, 5, 10, 20, and 30 of incubation, headspace gas from 60-ml vials was analyzed for CO_2, N_2O, and CH_4 on a Shimadzu GC14 Gas Chromatograph (Shimadzu USA) after covering vials for 1 hour (modified from Nkongolo et al., 2008). Detectors on the GC14 were ECD for CO_2 and N_2O (column Porapak Q 80/100 mesh 1m x 3mm I.D.) and FID (column Porapak N 80/100 mesh 2m x 3mm I.D.). Injector temperature was 100^0C, and column temperature was 60^0C with a 4 minute hold time. Concurrently, sub-samples of soil were taken from the mason jars and processed as described below.

Enzyme Assays. Standardized protocols including incubation at 37°C, at fixed time period, specific buffer pH range, and inclusion of needed cofactors were followed for each of the following enzyme assays. Enzyme activities are expressed as µg product g^{-1} soil h^{-1} using calibration curves for the appropriate product and absorbance values and extraction solution as blanks. Soil blanks and controls were analyzed concurrently. β-glucosidase activity was measured by the product p-nitrophenol using a colorimetric method (Tabatabi and Fung, 1992). Dehydrogenase activity was determined following methods described by Von Mersi and Schinner (1991) based on the colorimetric measurement of iodonitrotetrazolium formazan (INTF). Arylamidase activity was determined by colorimetric measurement of β-naphthylamine product using the method

of Acosta-Martinez and Tabatabai (2000). Absorbance was measured using a Thermo Genesys (Thermo Scientific, PA) spectrophotometer.

Soil moisture and chemical properties. Soil gravimetric water content was determined for each landscape sampling position (Zancan et al., 2006). Total organic carbon (TOC) and total nitrogen (TN) were determined by dry combustion at 900 ^{o}C (Nelson and Summers, 1996) using a LECO Tru-Spec carbon – nitrogen analyzer (LECO Corporation, St. Joseph, MI, USA). Carbonates were not found in any of these samples; therefore, total carbon values were considered to be TOC. Soil pH was measured in water at a 1:2.5 ratio soil to water (Raiesi, 2006).

Statistical Analysis

The data was subjected to analysis of variance (ANOVA) of a split-split plot design using PROC MIXED in statistical software package SAS version 9.2 (SAS, 2008) to detect effects of landscape sampling position, incubation temperature, soil moisture, incubation time and their interactions on enzyme activity and GHG efflux. Analysis of variance was also conducted separately for each sampling period to test differences among landscape sampling position, temperature, moisture, incubation time and their interactions. Means were compared at $p \leq 0.05$ or the values given at means ± standard error of three replicates. Relationships among soil and environmental properties with soil enzyme activities were determined using a Pearson correlation analysis (PROC CORR).

Results

Carbon and nitrogen. Total organic carbon and TN content varied depended on the time of year samples were collected and generally followed the order: MSP> NSP > JSP. Additionally, TOC and TN content differed across landscape sampling positions, with soils collected from lower landscape positions (i.e. BS, FS and DR) generally having a greater TOC and TN content than those collected from higher in the landscape (i.e., SS and SH) (Figs. 4.1a and 4.1b). Between day 1 and day 30 of incubation for each sampling period there was generally a slight decrease in TOC and TN, however the differences were not significant. Carbon to nitrogen ratio (C/N) for the three sampling periods JSP, NSP, and MSP, averaged 12.9, 13.6, and 13.2 respectively, well within the typical range for forest soils (add ref). However, C/N varied across landscape sampling positions (Figure 4.1c), SH and DR having slightly higher C/N than SS, BS, and FS.

Enzyme Activity. Soil enzyme activities varied during each incubation period, similar to the trends observed for TOC and TN contents. Mean arylamidase activity was greatest for MSP and least for JSP (Table 4.1); in contrast, activities for dehydrogenase and β-glucosidase followed the order JSP>MSP>NSP (Table 4.1). Enzyme activities also differed significantly based on the effects of temperature, moisture, landscape sampling position and incubation time (Table 4.2). For MSP arylamidase activity was significantly affected by all main effects and their respective interactions except for moisture; also for JSP and NSP, moisture and some of its interactions with other main effects did not significantly impact arylamidase activity. Similar trends were also observed for β-glucosidase and dehydrogenase activities.

The interaction among landscape sampling position, incubation time, and soil moisture was significant over all three sampling periods for arylamidase activity. Generally, for all three sampling periods at both FM and 60%WHC, amino acid arylamidase activity tended to be greater in samples collected from lower landscape positions (BS, FS, DR) than at SH and SS landscape positions (Figure 4.2).

The interaction among landscape sampling position, incubation time, and temperature was significant for β-glucosidase and dehydrogenase. The activity of these enzymes tended to be greater at the lower landscape positions than at the summit and shoulder positions (Figures 4.3 and 4.4 respectively). Additionally, in all three sampling periods warmer incubation temperatures resulted in increased activity for β-glucosidase (Figure 4.3) and dehydrogenase (Figure 4.4).

Correlation analysis showed that activities of the three enzymes were correlated with one another ($p \leq 0.05$), as well as TOC and TN content in all three sampling periods (Table 4.3). The main observation from Table 4.3 was that TOC and TN influenced enzyme activity throughout the incubation. The correlation matrix also defined the relationship between enzyme activities and other soil properties as positive or negative (Table 4.3). β-glucosidase, for example, was positively correlated with arylamidase activity in all three sampling periods; however, the relationship was generally weaker between β-glucosidase and dehydrogenase. Possible explanations for the differences could be due to the relationship of these enzymes with TOC and TN content, or the differences may have resulted from the inherent variability within the soil. In addition, enzyme activity was greatly dependent on landscape sampling position as enzyme activity at lower positions tended to be significantly greater than at upper landscape

positions (see figures 4.2 - 4.4). These results suggest that enzyme activities, which can be used as a measure of soil biological activity, tend to vary in time and space (Dick and Burns, 2011).

Greenhouse Gases. Following previously observed trends in soil properties and enzyme activity; all three GHGs varied dependent on time of year soil was sampled. In general N_2O and CO_2 were emitted from soil to the atmosphere (Figure 4.5 and Figure 4.6 respectively), however in our research, as is typical for forest soils (Jensen et al., 2000; Merino et al; 2004), the soil tended to act as a sink for atmospheric CH_4 (Figure 4.7) most likely due to CH_4 oxidation by methanotrophic bacteria (Alluvione et al., 2009). Methane uptake by the soils studied was significantly affected by temperature and its interaction with other independent variables in all three sampling periods (Table 4.2). The number of days after incubation and the interactions among temperature, landscape sampling position, and moisture were also significant for CH_4 efflux. Similarly, CO_2 efflux was significantly affected by interactions among incubation time, landscape sampling position, temperature, and moisture (Table 4.2). Nitrous oxide efflux, however, was not significantly affected by any of the main effects or their interactions during NSP and was only significantly affected by moisture and landscape sampling position for MSP. There were however, no clearly defined patterns across the three sampling periods for GHG efflux (Figures 4.5 to 4.7). Nevertheless, it was evident that the efflux of CH_4 was affected by the number of days of incubation, temperature and landscape sampling position (Figure 4.7). Additionally, incubations of soil collected at all sampling periods showed CH_4 uptake was greater under ambient temperature (25^0C) than at other incubation temperatures. Incubation temperature was also significant for CO_2 efflux in

each sampling period, with gas efflux greater at warmer incubation temperatures; efflux of CO_2 was also significantly different based on landscape sampling position and number of days of incubation (Figure 4.6). However, as shown by the other GHG, there was no obvious pattern to the emission of CO_2 across the different landscape sampling positions. The variation of N_2O was even greater than for CH_4 and CO_2, however compared to the other sampling periods, MSP had the greatest N_2O efflux (Figure 4.5).

Correlation of greenhouse gases and enzyme activities

As previously noted, activities of the three enzymes correlated with one another and other soil properties within this study (see Tables 4.3 and 4.4). In addition, enzyme activity also correlated significantly with GHG efflux in all three sampling periods. β-glucosidase significantly correlated with CO_2 for NSP and MSP. All three enzymes also significantly correlated with CH_4 efflux. There were, however, no significant correlations between N_2O and any of the enzymes for any of the sampling periods; neither did dehydrogenase nor arylamidase activities correlated with any GHG for NSP. However, both dehydrogrenase and arylamidase activities correlated with CO_2 for JSP and MSP.

Discussion

Soil carbon contents, measured periodically, often reveal seasonal variations with spring and fall having higher TOC than summer (Leinweber et al., 1994; Leiros et al., 1999). In a long-term (850 d) incubation study Follett et al. (2007) observed that a 3 – 7^0C difference in temperature increased loss of carbon mainly due to higher microbial respiration. They further hypothesized that temperature was the determinant factor in TOC utilization in their study. In a 250 d incubation study, Townsend et al. (1997)

observed that greater amounts of carbon were respired at higher temperature. However, in both cases the researchers concluded that the initial carbon respired was from the small (1 – 5%) active carbon pool; since respiration rate tended to stabilized after approximately 40 d (Townsend et al., 1997) and 100 d (Follett et al., 2007). In our incubation study NSP and MSP which were incubated at lower temperatures, tended to have greater amounts of TOC and TN than JSP throughout the 30 d incubation indicating a possible temperature effect. However, the differences in TOC and TN due to incubation temperatures were not significant. Indicating there is most likely a large recalcitrant carbon pool (Townsend et al., 1997) at our research site.

On the other hand, significant differences were however observed for TOC and TN based on position along the landscape where samples were taken (Figure 4.1), indicating that topography affected carbon and nitrogen distribution at the sampling site. Topographic effect on C and N distribution has previously been reported (Tsui et al., 2004; Martin et al., 2010; Papiernik et al., 2009; Miralles et al., 2007); which were attributed to movement of material downslope due to runoff and/or below surface seepage.

Enzyme Activity

Miralles et al. (2007) observed high coefficients of variation in enzyme activities, which was postulated to be due to differences in altitude, vegetation, and the variability in TOC and TN. They observed that biochemical soil properties followed the same variation pattern as soil carbon and nitrogen. Niemi et al. (2005), Boerner et al. (2005), Mungai et al. (2005) also observed temporal and spatial variation in soil enzyme activity. Our study also revealed high spatial and temporal variation in enzyme activities. Miralles et al.

(2007) detected topographic influences on enzyme activity with lower landscape positions having greater enzyme activity than landscapes positions at or near the summit. Similar topographic influences were observed in our study (Figures 4.2 – 4.4). Miralles et al. (2007), Mungai et al. (2005), Boerner et al. (2005), Niemi et al. (2005) attributed enzyme activity variation to differences in vegetation, temperature, soil moisture, TOC and TN. Correlations observed in our study between soil enzyme and TOC and TN highlight the relationship between soil carbon and biochemical soil properties.

Variations in correlation between soil enzyme and temperature and/or moisture for each sampling period indicate that additional factors affected enzyme activities. Therefore, variation may be better explained in reference to soil carbon and nitrogen contents since all three enzymes were significantly correlated to TOC and TN in all three sampling periods (Table 4.3). Bandick and Dick (1999) and Eivazi and Tabatabai (1990) both concurred that differences in soil enzyme activity was related to carbon content and the substrate quality of the soil. Leiros et al. (1999) observed significant seasonal influences of substrate availability on SOM mineralization, implying that temperature and moisture were limiting factors in the mineralization process. This suggests that enzyme activity, which is crucial to SOM degradation and transformation (Karaca et al; 2011), would also vary due to differences in temperature, moisture and substrate availability.

Soil pH is also noted to influence soil enzyme activity (Gianfreda et al, 2005; Acosta-Martinez and Tabatabai, 2000b) with enzymes showing either positive, negative or no correlation with soil pH. In our study both enzyme activity (Figures 4.3 – 4.4) and

pH generally increased moving from higher to lower landscape sampling positions (for e.g. see Table 3.2 in Materials and Methods Chapter).

Greenhouse Gases

Carbon dioxide efflux correlated with temperature in all three incubation periods suggesting that CO_2 efflux was temperature dependent. In addition, CO_2 for NSP, N_2O for MSP, and both CO_2 and N_2O for JSP correlated with moisture (Table 4.5). Hanson et al. (1993) observed similar variability in GHG efflux from an upland oak forest. They also observed topographic differences in CO_2 efflux, however, similar to our findings, there was no discernable pattern to the GHG efflux based on topographic location. In general, soils from the summit landscape position tended to have less CO_2 efflux than soils sampled from other landscape positions. However, significant interactions between landscape sampling position, temperature, and moisture suggest that abiotic factors can affect GHG activity at the different topographic positions. Leiros et al. (1999) suggested that differences in substrate availability could result from changes in moisture and temperature. The lack of correlation between GHG efflux and TOC and TN implies that only selected pools of C and N may be impacting GHG efflux. McLain and Martens (2005) observed meaningful relationships between the C and N content of amino acids added to soils and GHG efflux in an incubation study. In their experiment higher C content tended to suppress N_2O emission and increase CO_2 emission. In experiments with agroforestry residues, Miller and Baggs (2004; 2005) noted that residue composition affected GHG efflux. Miller and Baggs (2004) observed significant positive correlation between C/N ratio and CO_2 efflux, however lignin content, polyphenol content, protein binding capacity and ligin:N ratio were negatively correlated to GHG efflux. Miller and

Boggs (2005) also observed that soluble C and N fractions enhanced CO_2 emissions, but was negatively correlated with N_2O emissions. In our experiment it is therefore possible that variation in lignin, polyphenol, and soluble C and N contents across the landscape may be impacting GHG efflux. However, further research is needed to verify the interactions of these factors on GHG efflux.

Nevertheless, the positive correlations among GHG and nutrient cycling enzymes also suggests that substrate availability affected GHG efflux since mineralizing enzymes are integral in the release of nutrients from organic matter. For example, arylamidase enzyme, which catalyzes the hydrolysis of an N-terminal amino acid from peptides, amides, or arylamides in soil, showed significant positive (CO_2), negative (CH_4), or no correlation (N_2O) with GHG efflux. This indicates that the products of protein mineralization were impacting GHG efflux. The greater efflux of N_2O and CO_2 for MSP also indicates that nutrient availability was impacting gas efflux, since OM stabilized in soil during the winter months could act as a substrate for microorganisms (Leinweber et al., 1994) during the spring months when MSP samples were collected.

However, the low r values for the relationships between enzyme activity and GHG efflux also indicates that other factors were influencing GHG efflux from the soils at the study site. This could be related to the high specificity of enzymes that may be restricted to only a small percentage of the active microbial pool at the time of sampling (Dick, 1994). This pool of microorganisms may not fully represent the overall microbial community responsible for CO_2 and the other GHG produced via respiration and microbial metabolic activity. In addition, as further explained by Dick (1994), high extracellular (abiontic) enzyme, content of the soil may be contributing to the low

correlation between enzyme activity and GHG efflux. Finally, differences in correlation between enzymes and GHG efflux for the three sampling periods imply that other factors such as temperature, moisture, and rhizodeposition can impact both enzyme activity and GHG efflux.

Conclusion

Soil enzymes have routinely been studied as good indicators of soil environmental quality and biological activity (Dick, 1994). The groups of enzymes assayed in this research are only a few of numerous enzymes active in the soil matrix. Other enzymes involved in nutrient cycling include: amylase (EC 3.2.1), cellulase (EC 3.2.14), lipase (EC 3.1.1.3) and invertase for the C cycle; and proteases, urease, and deaminases for the N cycle. Other enzymes reportedly associated with microbial respiration are alkaline phosphatase and catalase (Frankenberger and Dick, 1983). Thus, additional analysis of other enzymes that play active roles in the catalysis of soil organic matter would likely complement the information gathered in this research. However, the current research showed that the activities of the analyzed enzymes correlated with GHG efflux and could, with further investigation, provide meaningful information on the role of soil biology in GHG efflux.

Although landscape sampling position was not significant for GHG efflux, the significance detected for enzyme activity, pH, and carbon and nitrogen distribution, combined with significant interactions with soil moisture and temperature indicates the importance of landscape features in understanding GHG efflux. Differences in substrate quality and quantity, and movement of material downslope can influence microbial activity and nutrient distribution. This in-turn could affect GHG efflux within a

landscape, and contribute to the high variation in GHG efflux over the broader site (Conrad 1996).

In this study we determined that activities of enzymes could potentially be used as indicators of GHG efflux. We also identified the importance of landscape position, temperature and moisture on GHG efflux. The results from the research will increase our understanding of the role of soil biota in GHG efflux, and the possible effects of forest systems on climate change.

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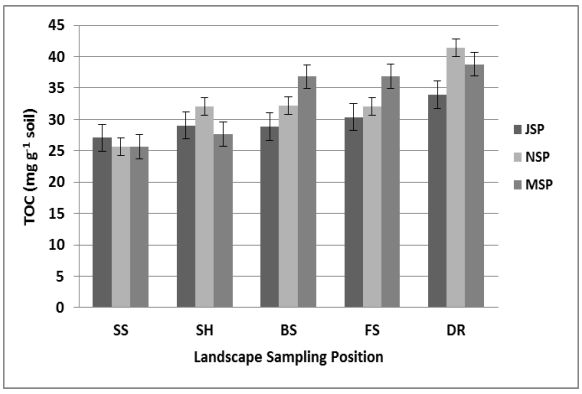

Figure 4.1A Total organic carbon at the different landscape sampling positions for soils incubated from July 2008 (JSP), November 2008 (NSP), and May 2009 (MSP) sampling periods. Landscape sampling positions summit (SS), shoulder (SH), backslope (BS), footslope (FS), and drainage (DR). Bars indicate standard error of means.

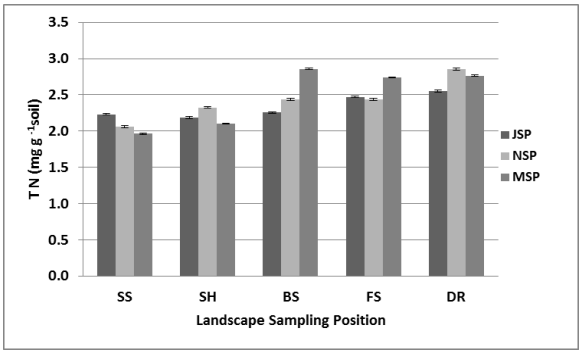

Figure 4.1B Total nitrogen at the different landscape sampling positions for soils incubated from July 2008 (JSP), November 2008 (NSP), and May 2009 (MSP) sampling periods. Landscape sampling positions summit (SS), shoulder (SH), backslope (BS), footslope (FS), and drainage (DR). Bars indicate standard error of means.

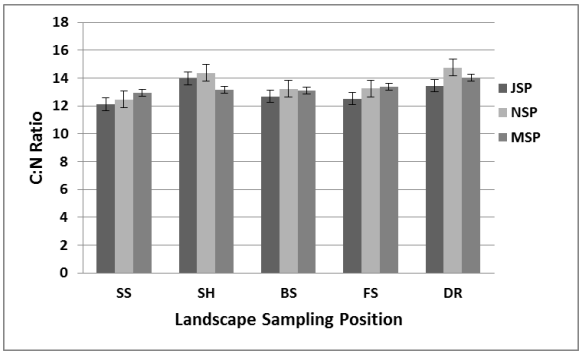

Figure 4.1C Carbon to nitrogen ratio at the different landscape sampling positions for soils incubated from July 2008 (JSP), November 2008 (NSP), and May 2009 (MSP) sampling periods. Landscape sampling positions summit (SS), shoulder (SH), backslope (BS), footslope (FS), and drainage (DR). Bars indicate standard error of means.

Field-moist water content 60% Waterholding capacity

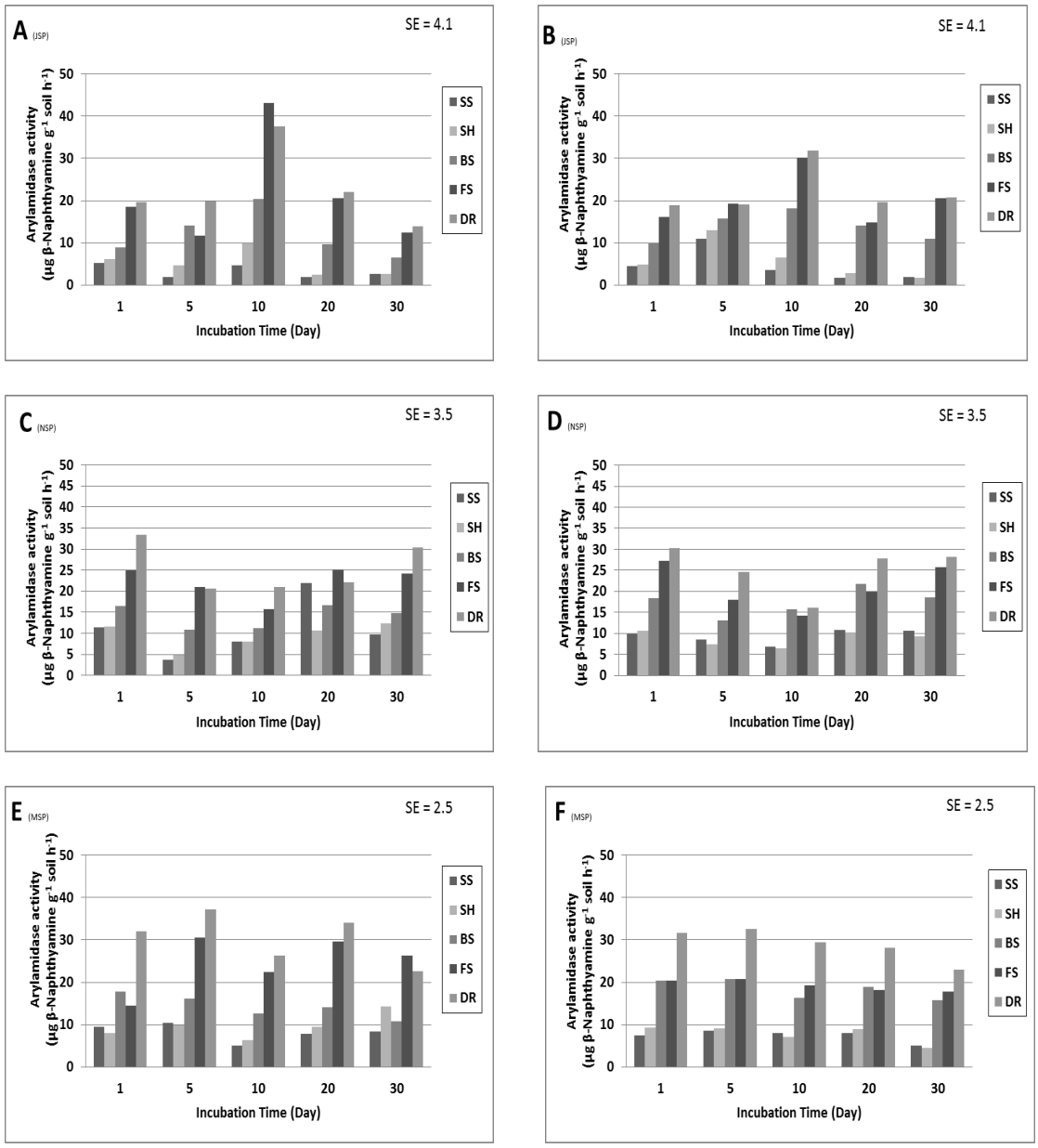

Figure 4.2 Effect of landscape sampling position and moisture on arylamidase activity for JSP (A & B), NSP (C & D), and MSP (E & F). Samples incubated at field moisture (A, C, D) or 60% waterholding capacity (B, D, F). Landscape sampling positions summit (SS), shoulder (SH), backslope (BS), footslope (FS), and drainage (DR).

Control Temperature

Incubation Temperatures

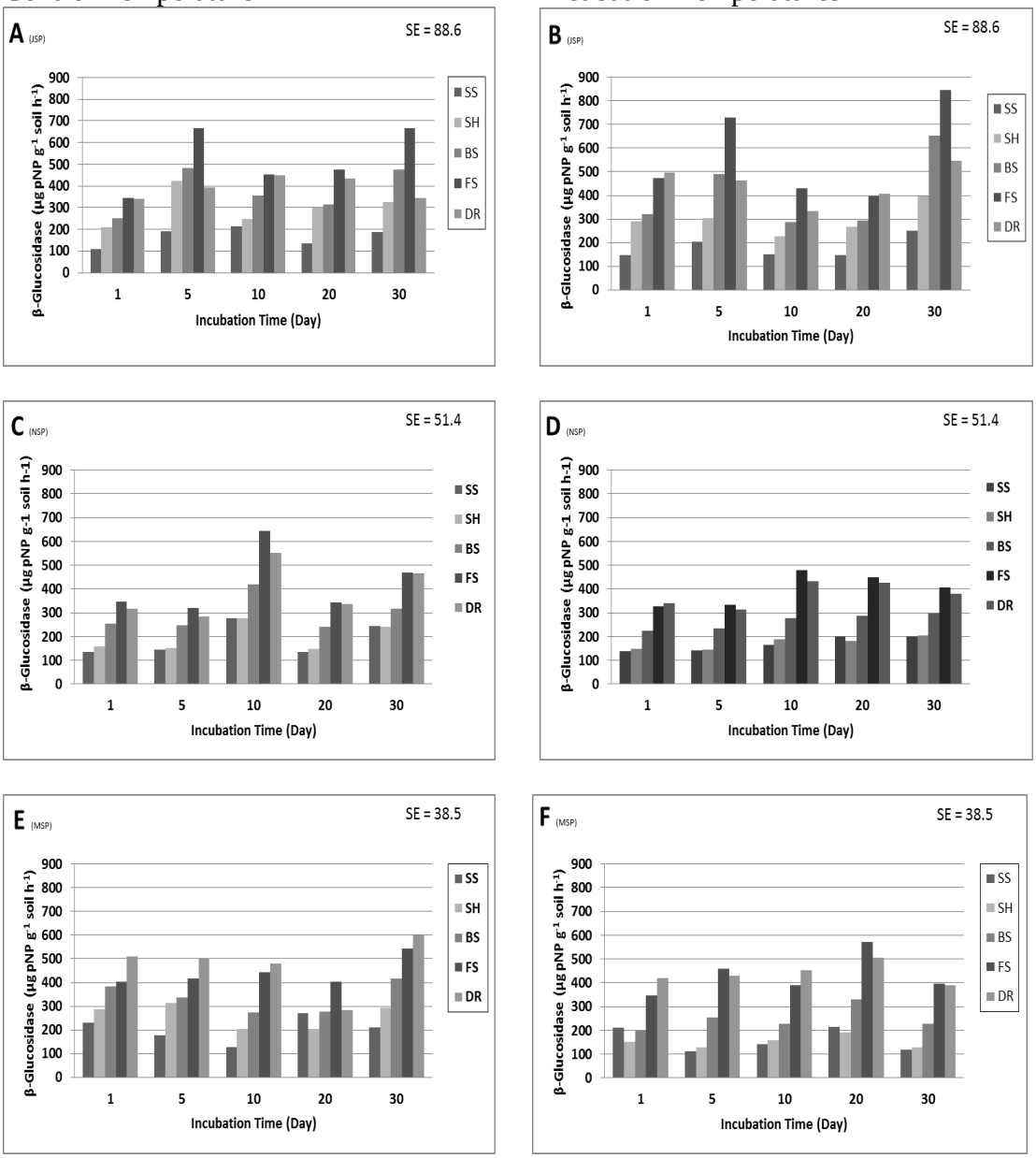

Figure 4.3 Effect of landscape sampling position and temperature on β-Glucosidase activity during 30 d of incubation for JSP incubated at either 25^0C (A) or 35^0C (B); NSP incubated at either 25^0C (C) or 15^0C (D); MSP incubated at 25^0C (E) or 20^0C (F). Landscape sampling positions summit (SS), shoulder (SH), backslope (BS), footslope (FS), and drainage (DR).

Control Temperature Incubation Temperatures

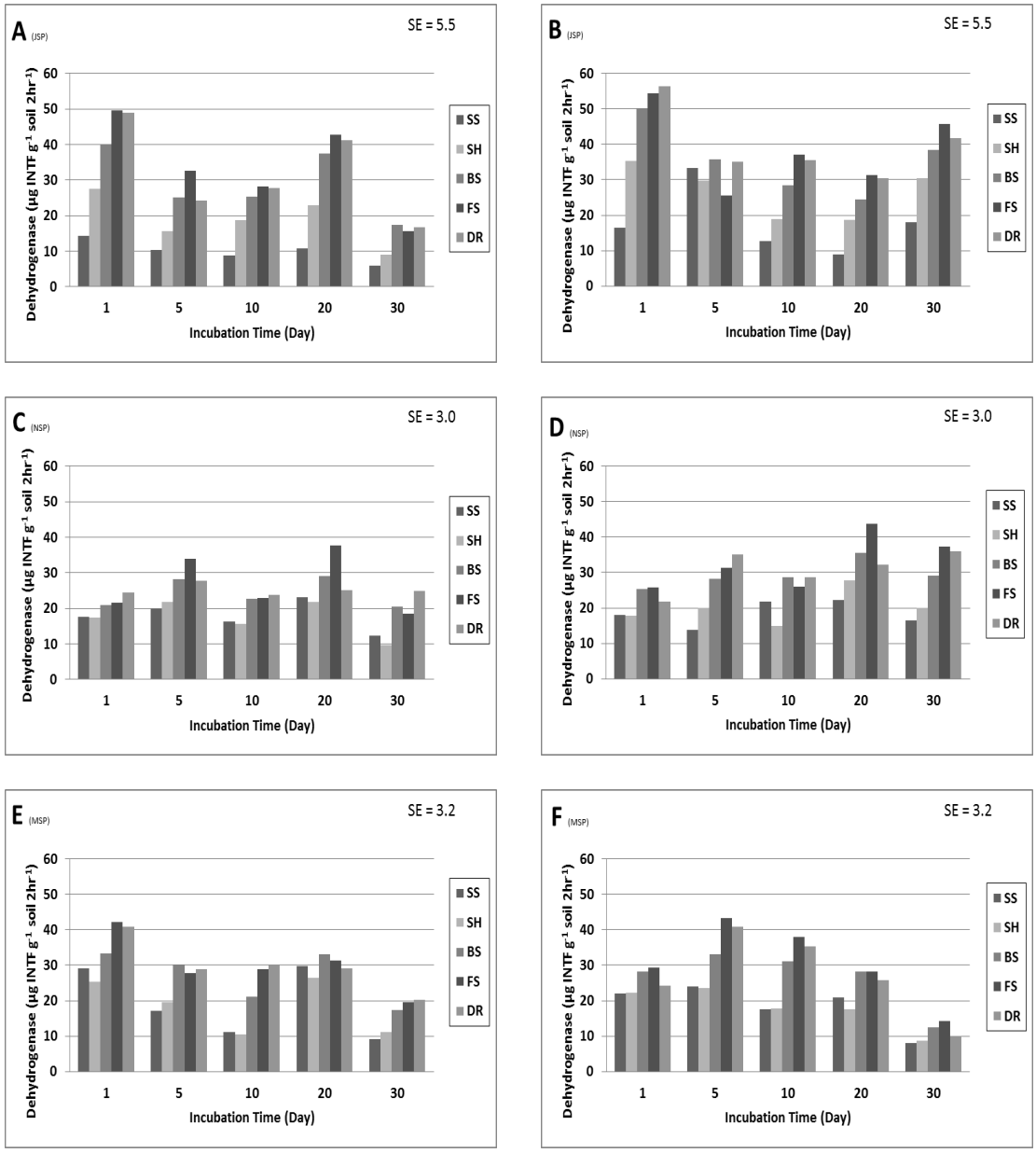

Figure 4.4 Effect of landscape position and temperature on dehydrogenase activity during 30 d of incubation for JSP incubated at 25^0C (A) or 35^0C (B); NSP incubated at either 25^0C (C) or 15^0C (D); MSP incubated at 25^0C (E) or 20^0C (F). Landscape sampling positions summit (SS), shoulder (SH), backslope (BS), footslope (FS), and drainage (DR).

Field-moist water content

60% Water holding capacity

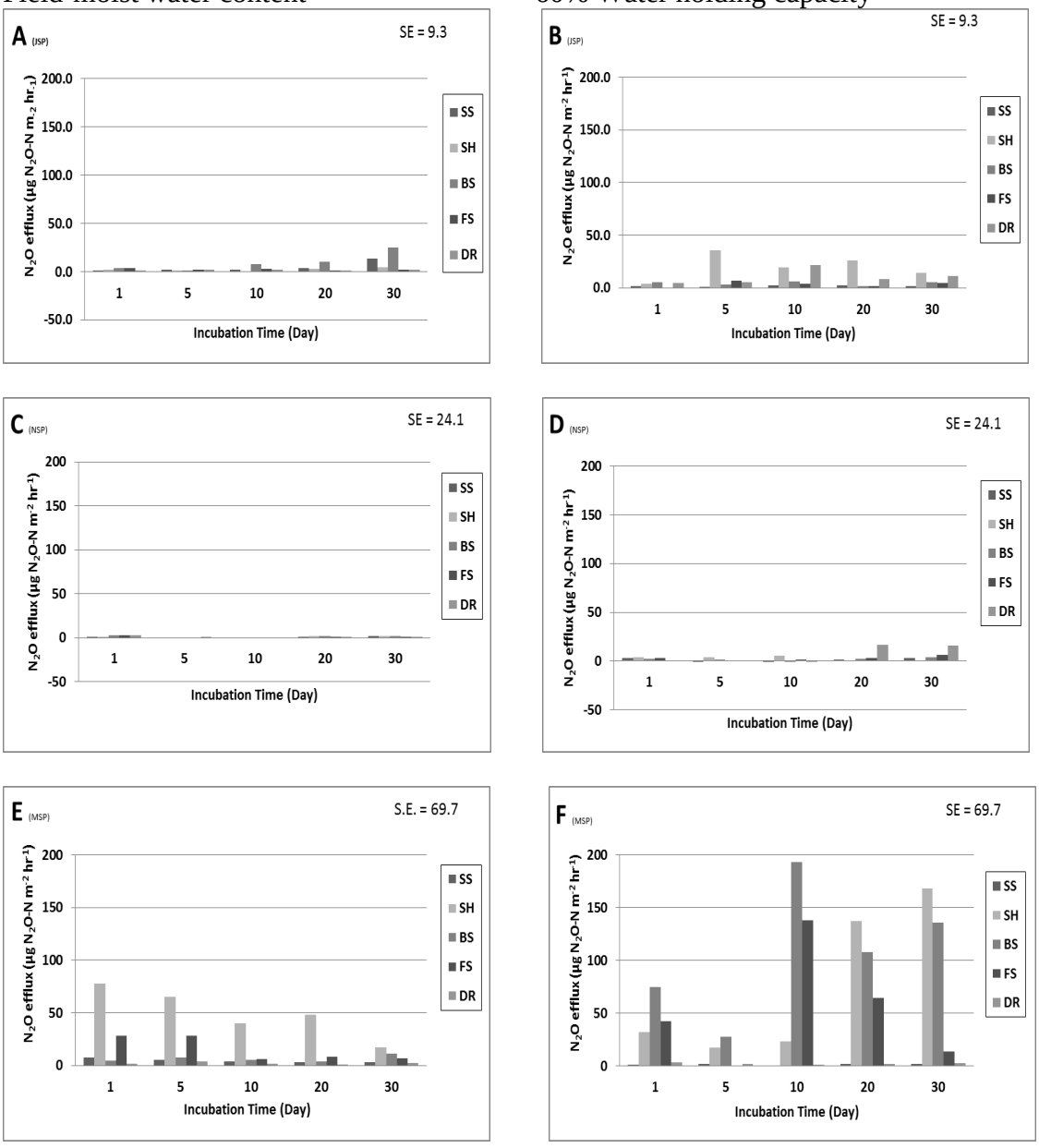

Figure 4.5 Effect of landscape sampling position and moisture on N_2O efflux during 30 d of incubation for soils collected in July 2008 (JSP) (A & B), November 2008 (NSP) (C & D), and May 2009 (MSP) (E & F) sampling periods. Samples incubated at field moisture (A, C, D) or 60% waterholding capacity (B, D, F). Landscape sampling positions summit (SS), shoulder (SH), backslope (BS), footslope (FS), and drainage (DR).

Control Temperature

Incubation Temperatures

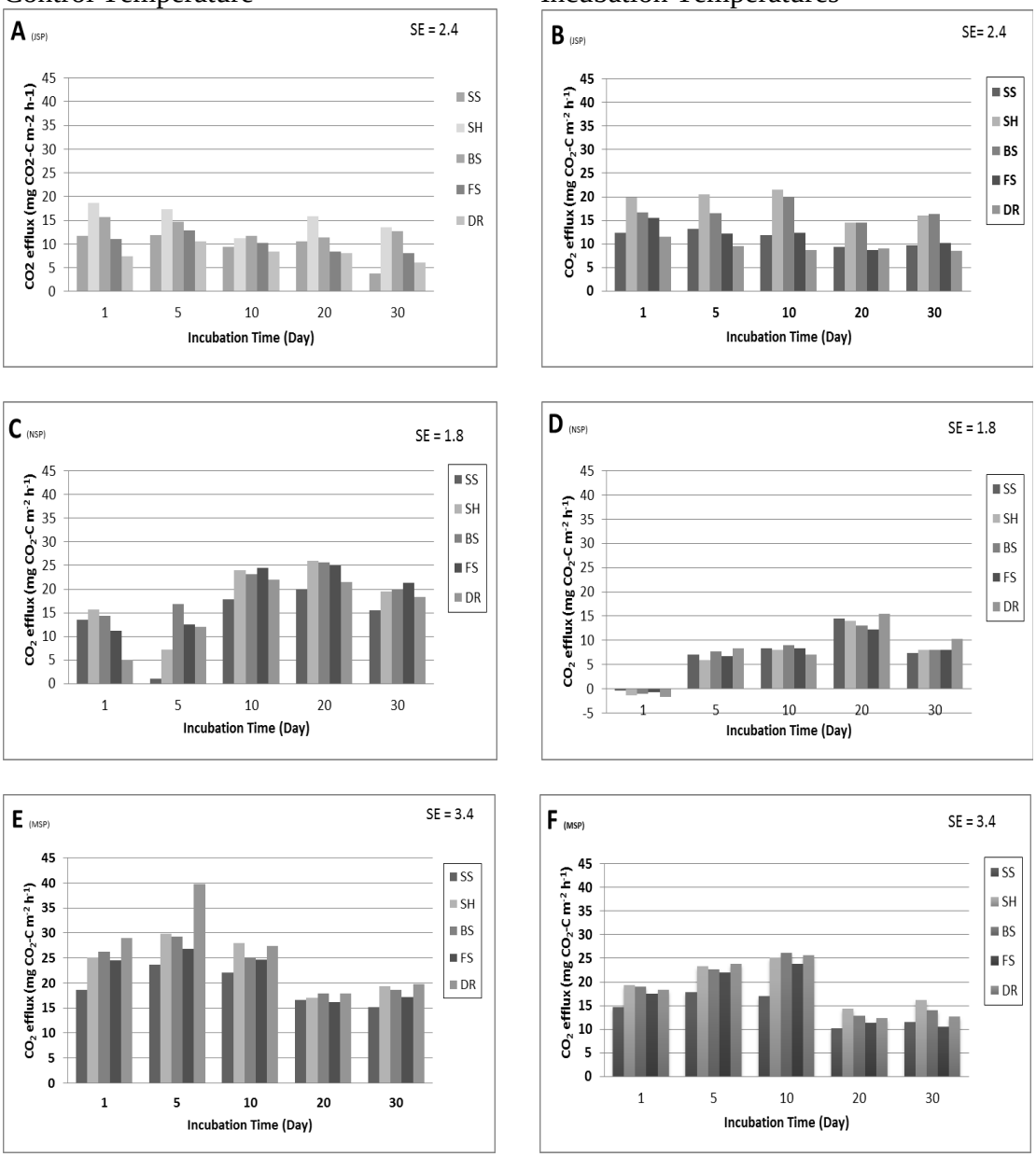

Figure 4.6 Effect of landscape position and temperature on CO_2 efflux during 30 d of incubation for JSP incubated at 25^0C (A) or 35^0C (B); NSP incubated at either 25^0C (C) or 15^0C (D); MSP incubated at 25^0C (E) or 20^0C (F). Landscape sampling positions summit (SS), shoulder (SH), backslope (BS), footslope (FS), and drainage (DR).

Control Temperature

Incubation Temperatures

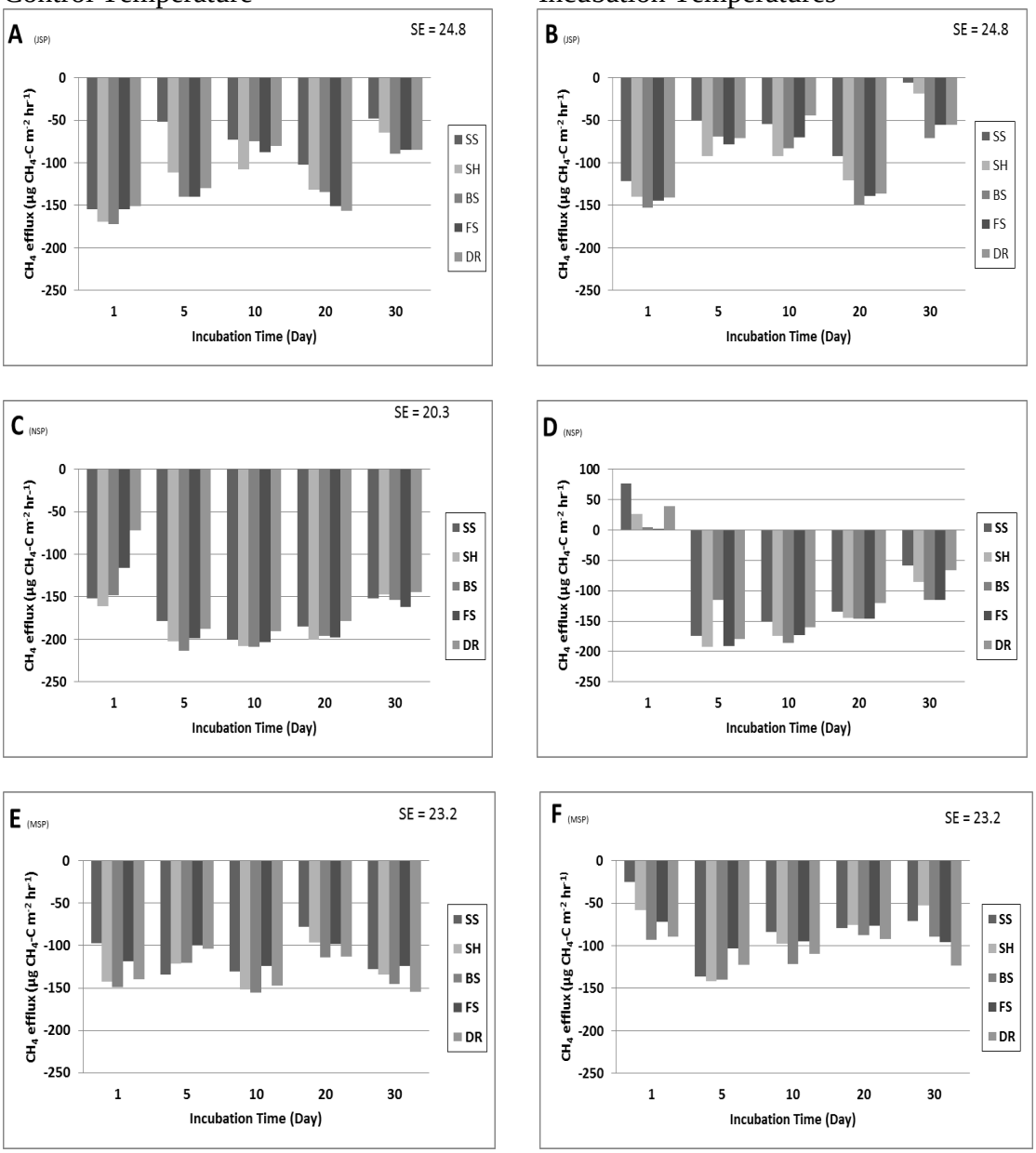

Figure 4.7 Effect of landscape sampling position and incubation temperature on CH_4 efflux during 30 d incubation for JSP incubated at either 25^0C (A) or 35^0C (B); NSP incubated at either 25^0C (C) or 15^0C (D); MSP incubated at either 25^0C (E) or 20^0C (F). Landscape sampling positions summit (SS), shoulder (SH), backslope (BS), footslope (FS), shoulder (SH), and drainage (DR).

Table 4.1 Descriptive statistics for total organic carbon (mg g^{-1} soil), total nitrogen (mg g^{-1} soil), β-glucosidase (µg pNP g^{-1} soil h^{-1}), arylamidase (µg β-Naphthyamine g^{-1} soil h^{-1}), dehydrogenase (µg INTF g^{-1} soil $2h^{-1}$), N_2O (µg N_2O-N m^{-2} h^{-1}), CO_2 (mg CO_2-C m^{-2} h^{-1}) and CH_4 (µg CH_4-C m^{-2} h^{-1}) during 30 d incubation. Samples were incubated at 25^0C and 35^0C, 15^0C, or 20^0C for July 2008 (JSP), November 2008 (NSP), and May 2009 (MSP) respectively.

July 2008

Soil Property	n	Mean	Minimum	Maximum	S.D.
TOC	299	29.9	11.9	52.1	11.9
Total N	299	2.3	0.6	4.9	0.9
β-Glucosidase	300	366.83	67.80	1429.67	218.38
Arylamidase	300	13.07	0.23	68.12	12.25
Dehydrogenase	300	28.19	3.08	76.21	16.47
N_2O	300	-30.73	-82.04	209.13	40.85
CO_2	300	13.20	-4.29	42.92	6.00
CH_4	300	-93.68	-208.77	104.10	61.15

November 2008

Soil Property	n	Mean	Minimum	Maximum	S.D.
TOC	300	32.7	3.5	86.0	13.0
Total N	300	2.4	0.8	6.5	0.9
β-Glucosidase	300	287.84	92.40	1032.75	150.53
Arylamidase	300	16.40	1.28	49.26	10.59
Dehydrogenase	300	24.30	2.17	65.57	11.60
N_2O	300	6.73	-13.40	978.08	59.85
CO_2	300	12.32	-6.75	42.07	9.26
CH_4	298	-140.49	-249.13	314.08	79.96

May 2009

Soil Property	n	Mean	Minimum	Maximum	S.D.
TOC	300	33.1	16.0	61.2	12.1
Total N	300	2.5	1.1	6.5	0.9
β-Glucosidase	300	314.99	54.74	808.70	167.94
Arylamidase	300	16.91	1.89	47.24	11.14
Dehydrogenase	300	24.58	3.35	64.14	12.59
N_2O	300	59.64	-0.96	2157.23	235.81
CO_2	300	20.36	4.72	84.92	8.44
CH_4	300	-109.03	-253.87	47.65	51.92

Table 4.2 ANOVA results for total organic carbon (TOC), total nitrogen (TN), β-glucosidase (BG), amino acid aryl-amidase (AA), dehydrogenase (DH), and greenhouse gases, carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4) based on landscape sampling position (S), temperature (T), moisture (M), and incubation time (D). Statistical significance: *P < 0.05; ** P < 0.01; ***P < 0.001; ns not significant.

July 2008

Source of variation	Num DF	Den DF	TOC	TN	BG	AA	DH	N_2O	CO_2	CH_4
T	1	150	ns	ns	**	ns	***	ns	***	***
M	1	150	ns	ns	ns	ns	ns	*	***	ns
T*M	1	150	ns	ns	***	*	ns	ns	ns	ns
S	4	10	ns	ns	ns	*	ns	ns	ns	ns
S*T	4	150	ns	ns	ns	ns	ns	*	ns	ns
S*M	4	150	*	ns	ns	ns	ns	*	ns	***
S*T*M	4	150	ns	ns	ns	ns	ns	*	***	***
D	4	40	ns	ns	***	***	***	ns	***	***
D*T	4	150	ns	ns	***	***	***	ns	***	***
D*M	4	150	ns	ns	*	***	***	ns	***	***
D*T*M	4	150	ns	ns	***	***	*	ns	*	ns
D*S	16	40	**	*	**	**	*	*	ns	*
D*S*T	16	150	ns	ns	ns	ns	***	ns	*	**
D*S*M	16	150	***	*	ns	*	*	*	***	***
D*S*T*M	16	150	***	***	*	ns	ns	ns	*	***

November 2008

source of variation	Num DF	Den DF	TOC	TN	BG	AA	DH	N_2O	CO_2	CH_4
T	1	150	ns	ns	***	*	***	ns	***	***
M	1	150	ns	ns	***	ns	***	ns	***	ns
T*M	1	150	ns	ns	*	ns	*	ns	***	*
S	4	10	*	*	*	*	*	ns	ns	ns
S*T	4	150	***	ns	ns	ns	*	ns	***	ns
S*M	4	150	***	*	*	*	ns	ns	*	*
S*T*M	4	150	ns	ns	ns	ns	ns	ns	*	ns
D	4	40	ns	ns	***	***	***	ns	***	***
D*T	4	150	ns	ns	***	***	***	ns	***	***
D*M	4	150	ns	ns	***	ns	***	ns	***	*
D*T*M	4	150	ns	ns	***	***	***	ns	***	***
D*S	16	40	***	ns	**	*	***	ns	*	ns
D*S*T	16	150	***	ns	ns	***	*	ns	*	*
D*S*M	16	150	***	*	*	**	***	ns	***	ns
D*S*T*M	16	150	**	ns	ns	***	***	ns	*	ns

Table 4.2 (continued) ANOVA results for total organic carbon (TOC), total nitrogen (TN), betaglucosidase (BG), amino acid aryl-amidase (AA), dehydrogenase (DH), and greenhouse gases, carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4) based on landscape sampling position (S), temperature (T), moisture (M), and incubation time (D). Statistical significance: *P < 0.05; ** P < 0.01; ***P < 0.001; ns not significant.

May 2009

Source of variation	Num DF	Den DF	TOC	TN	BG	AA	DH	N_2O	CO_2	CH_4
T	1	150	ns	ns	***	*	ns	ns	***	***
M	1	150	ns	ns	ns	ns	***	*	ns	**
T*M	1	150	ns	ns	**	*	*	ns	ns	ns
S	4	10	***	***	***	***	*	ns	ns	ns
S*T	4	150	***	***	**	***	ns	ns	ns	ns
S*M	4	150	***	***	**	***	ns	*	*	***
S*T*M	4	150	***	***	***	***	ns	ns	ns	***
D	4	40	ns	ns	*	***	***	ns	***	***
D*T	4	150	ns	ns	***	***	***	ns	*	***
D*M	4	150	ns	ns	***	*	***	ns	ns	***
D*T*M	4	150	ns	ns	***	**	***	ns	ns	***
D*S	16	40	***	**	*	*	*	ns	ns	ns
D*S*T	16	150	***	**	**	**	ns	ns	ns	ns
D*S*M	16	150	***	*	ns	*	ns	ns	ns	ns
D*S*T*M	16	150	***	***	***	***	***	ns	ns	***

Table 4.3 Correlation (r values) of enzyme activities, TOC and TN during 30 d incubation of soils collected in three sampling periods July 2008 (JSP), November 2008 (NSP), and May 2009 (MSP).

July 2008

	β-Glu	Dhy	Amid	TOC	TN
β-Glu	—				
Dhy	0.47***	—			
Amid	0.43***	0.40***	—		
TOC	0.09	0.18*	0.15*	—	
TN	0.07	0.17*	0.18*	0.90***	—

November 2008

	β-Glu	Dhy	Amid	TOC	TN
β-Glu	—				
Dhy	0.27***	—			
Amid	0.45***	0.32***	—		
TOC	0.21**	0.13*	0.23***	—	
TN	0.26***	0.14*	0.20**	0.87***	—

May 2009

	β-Glu	Dhy	Amid	TOC	TN
β-Glu	—				
Dhy	0.40***	—			
Amid	0.55***	0.41***	—		
TOC	0.44***	0.20**	0.41***	—	
TN	0.39***	0.21**	0.36***	0.92***	—

Statistical significant correlations: *P < 0.05; **P < 0.01; ***P < 0.001. B-Glu: B-glucosidase; Dhy: dehydrogenase; Amid: amino acid aryl-amidase; TOC: total organic carbon; TN total nitrogen.

Table 4.4 Correlation coefficience (r values) of enzyme activities and greenhouse gases during 30 d incubation of soils collected in three sampling periods July 2008 (JSP), November 2008 (NSP), and May 2009 (MSP).

July 2008

	Temp	Moist	N_2O	CO_2	CH_4
β-Glu	0.07	-0.03	-0.06	0.002	**-0.20****
Dhy	**0.21****	-0.03	-0.04	**0.14***	**-0.39*****
Amid	0.001	0.02	-0.002	**-0.15***	**-0.22****

November 2008

	Temp	Moist	N_2O	CO_2	CH_4
β-Glu	-0.07	**-0.21****	-0.02	**0.19***	-0.02
Dhy	**0.18***	0.10	-0.04	0.10	0.03
Amid	0.06	-0.01	-0.04	-0.05	-0.04

May 2009

	Temp	Moist	N_2O	CO_2	CH_4
β-Glu	**-0.17***	0.03	0.04	**0.14***	0.01
Dhy	-0.03	**0.17***	0.01	**0.28*****	-0.01
Amid	0.06	-0.05	0.03	**0.16***	-0.08

Statistical significant correlations: *P < 0.05; **P < 0.01; ***P < 0.001. β-Glu: β-glucosidase; Dhy: dehydrogenase; Amid: amino acid aryl-amidase; N_2O: nitrous oxide; CO_2: carbon dioxide; CH_4: methane.

Table 4.5 Simple correlations (r values) among some soil properties and greenhouse gases during 30 d incubation of soils collected in three sampling periods July 2008 (JSP), November 2008 (NSP), and May 2009 (MSP).

July 2008

	Temp	Moist	TOC	TN
N_2O	0.03	**0.15***	0.01	0.03
CO_2	**0.20****	**0.14***	-0.08	-0.09
CH_4	**0.20****	0.02	-0.07	-0.06

November 2008

	Temp	Moist	TOC	TN
N_2O	-0.10	0.10	0.08	0.01
CO_2	**-0.54*****	**0.20****	-0.007	0.03
CH_4	0.06	-0.06	-0.05	-0.06

May 2009

	Temp	Moist	TOC	TN
N_2O	-0.09	**0.14***	0.03	0.07
CO_2	**-0.32*****	0.01	0.01	0.02
CH_4	**-0.30*****	-0.10	-0.09	-0.07

Statistical significant correlations: *P < 0.05; **P < 0.01; ***P < 0.001. TOC: total organic carbon; TN: total nitrogen N_2O: nitrous oxide; CO_2: carbon dioxide; CH_4: methane.

CHAPTER 5
Soil microbial communities and greenhouse gas efflux in forest soils: an incubation study

Abstract

Greenhouse gas emissions (GHG) vary with the interactions among physical, chemical, and biological characteristics of soil and microclimate. Soils from a secondary forest in central Missouri were collected over an 10-month period (July and November of 2008 and May of 2009), representing three different seasons, from across the landscape and incubated at different temperatures and moisture content to evaluate GHG efflux and soil microbial communities. We conducted 30 d incubation studies at several air temperatures and soil moisture contents to determine the influence of landscape sampling position on soil GHG efflux. We evaluated the soil microbial community using phospholipid fatty acid (PLFA) analysis. Our hypothesis was that variations in soil properties across the landscape would be reflected in GHG efflux and soil community composition in this forest ecosystem. From this incubation study, we found that season of sampling and the topographic position influenced GHG and the microbial composition of the sampled soils. In addition, soil temperature influenced CO_2, N_2O, and CH_4 emission and microbial community composition. Temperature had a dramatic influence in altering the microbial community. Biomarkers for Gram-negative, Gram-positive, and anaerobic bacteria markers; and ratio of saturated to unsaturated fatty acids (Sat/Mono) and monounsaturated lipids were related to GHG efflux. Nitrous oxide efflux was negatively correlated with Gram-negative and anaerobic bacteria; whereas CO_2 efflux was positively correlated with Gram-positive markers and negatively correlated with Sat/Mono and monounsaturated lipids. Methane efflux was negatively correlated with

biomarkers for fungi and Gram-positive bacteria markers, and positively correlated with Gram-negative bacteria, mycorrhizae, Sat/Mono and monounsaturated lipids. Moisture influenced some measurements but in general did not impact as many factors as topographic position and temperature. Ordinate analysis showed biomarker group separations were based on season of sampling, landscape sampling position and temperature, but not moisture. This research indicates that CO_2 and CH_4 emissions from incubated soil are correlated to a limited number of microbial components while N_2O efflux correlated to a majority of components. These correlations vary with season of sampling, landscape position and incubation temperature and soil moisture to a lesser extent. It is evident that GHG emissions are very complex and cannot be fully explained by direct correlations with soil microbial community structure and soil environmental temperature and moisture regimes.

INTRODUCTION

Terrestrial systems (agricultural, forest and wetland soils) are sources of greenhouse gases (GHG) accounting for 7 percent of the total annual global GHG (EPA, 2011). Increasing atmospheric levels of the three major greenhouse gases (GHG): nitrous oxide (N_2O), carbon dioxide (CO_2), and methane (CH_4) and their potential impact on global temperatures are cause for concern (IPCC, 2001). Greenhouse gas emissions can be the result of physical, chemical, biological activities in the soil (Guo-yuan et al., 2006; Conrad, 1996; Ihssen et al., 2003). The efflux of GHGs is dependent upon the balance between the uptake and/or release of N_2O, CO_2, and CH_4 by vegetation, soil biota, and processes such as nitrification and denitrification (Blais et al., 2005). Research has been conducted to understand, model, and predict the influence of soil properties on GHG

efflux from terrestrial systems (Agehara and Warncke, 2005; Jackson and Schlesinger, 2004; Fung et al., 2005; Paul and Kimble, 1995; Ginting et al., 2003; and Avrahami et al., 2002). Ding et al. (2007) found correlations between seasonal CO_2 fluxes and soil temperature and moisture from soils in Henan, China. Paro et al. (2007) reported monthly variations in greenhouse gas effluxes, which were attributed to fluctuations in soil thermal properties. Lu et al. (2000) attributed seasonal patterns of methane (CH_4) emissions to variations in dissolved organic carbon (DOC), which were linked to differences in DOC released from plant roots (Jarecki and Lal, 2003; Uselman et al., 2007; Froberg et al., 2007). Because DOC serves as substrates for soil microorganisms, Lu et al. (2000) concluded that microbial activity influenced methane emissions. However, the microbial contribution to gas efflux could not be clearly ascertained due to the compounding effects of other soil factors including temperature and moisture.

Wander and Bollero (1999), Broos et al. (2007), and Bandick and Dick (1999) have discussed the difficulty of differentiating individual contributions of soil physical, chemical, and biological properties to greenhouse gas efflux. This is partially due to the high variability among soil properties both at field level (Yanai et al., 2003) and ecosystem level (Blais et al., 2005). Preliminary work at a mid-Missouri forest site revealed indirect associations between gas effluxes and soil biological properties (Hoilett et al., 2008). While many researchers also conclude that the complexity of the interactions among dominant soil properties (moisture and temperature) makes it difficult to understand the relationships between soil biota and GHG; it is agreed that soil biological properties influences GHG efflux (Wander and Bollero, 1999; Broos et al., 2007; Bandick and Dick, 1999).

Soil properties are also influenced by landscape and the distribution of nutrients and organic matter across the slope (Rezaei and Gilkes, 2005; Collins et al., 2011). Rezaei and Gilkes (2005) determined that properties of rangeland soils (e.g. pH, salinity, EC and TOC) differed across the landscape. Exposed slopes had higher temperatures, moisture loss and greater SOM mineralization, which resulted in lower TOC. The size and activity of the soil microbial biomass (MB) depends on soil conditions, thus differences in soil conditions will therefore impact microbial population and activity (Arunachalam et al., 1999).

Phospholipid fatty acid (PLFA) profiles are indicators of the living soil microbial community and have been used to describe soil microbial communities in different ecosystems (Frostegård and Bååth, 1996; Bååth, 2003; Carpenter-Boggs et al., 1998; Ibekwe & Kennedy, 1998, 1999; Butler et al., 2011; Snajdr et al., 2008; Liu et al., 2009; Feng and Simpson 2009). The various groups of microorganisms have specific phospholipid fatty acid profiles, which allow for the characterization and quantification of soil microbial community (Peterson and Klug, 1994).

The objectives of this research were i) to determine the influence of landscape position on soil biological activity and greenhouse gas efflux and ii) to assess the potential of using PLFA profiles as indices of variation in GHG efflux from forest soils. We hypothesize that differences in SOM, soil chemical properties and microbial communities of the different landscape sampling positions cause differences in GHG effluxes from these forest soils.

MATERIAL AND METHODS

Study site and experimental design. Soil samples were collected from the Lincoln University Busby Research Farm, South of Highway 54 in Jefferson City, Missouri (Latitude: 38.510157° Longitude: -92.242314°). The site is a 0.50 ha secondary forest dominated by oak (*Quercus alba*) and hickory (*Carya ovata*) trees on soils mapped as Gatewood (Oxyaquic Hapludalfs)-Moko (Oxyaquic Hapludolls) silt loam complex. Based on topography the site was divided into the following landscape sampling positions: summit (SS), shoulder (SH), backslope (BS), and footslope (FS). Samples were also collected from a drainage area (DR) that was separated from the other four landscape sampling positions by a gully. Each landscape position was divided into three pseudo-replicates following transects established across each landscape position (Figure 3.1). An average of three to six subsamples were collected to a depth of 0-20 cm from each landscape sampling position within a pseudo-replicate. Subsamples were then pooled to create 15 composite samples per sampling period (i.e., one composite sample per landscape position within each of the three pseudo-replicates). Samples were collected in July and November of 2008 and May of 2009, sieved (<2mm dia. mesh) and stored below 4^0C prior to analysis.

Soil moisture and chemical properties. After each field sampling, gravimetric soil water content was determined for each landscape at each location (Zancan et al., 2006). Soil pH was measured in distilled water using a 1:2.5 (soil:water) suspension (Smith and Doran, 1996). Total organic carbon (TOC) and total nitrogen (TN) were determined through combustion using a LECO TruSpec carbon/nitrogen analyzer (LECO

Corporation, St. Joseph, MI, USA). Carbonates were not found in any of these samples; therefore, total carbon values were considered to be TOC.

Incubation study. A sub-sample of soil (*ca.* 300g oven dry equivalent) from each landscape sampling position on each sample date was adjusted to either the field moisture (FM; gravimetric water content) at the time of sampling or to 60% water holding capacity (60%WHC) (Table 3.3). The soils were equilibrated for 7 d at 4^{o}C in 0.5 L mason jars. Jars were then incubated for 30 d at 25°C for the control temperature for each sampling period, as well as at 35°C for July 2008 samples (JSP), 15°C for November 2008 samples (NSP), and 20°C for May 2009 samples (MSP). Each sampling period (JSP, NSP, and MSP) was treated as a separate experiment and incubated independently. Simultaneously, 30 g oven dry (o.d.) equivalent of soil from each field replicate was incubated as described above in 60 ml vials to monitor GHG efflux. During incubation, jars and vials were covered with perforated Parafilm® to reduce moisture loss while allowing for gas exchange with the atmosphere. The soils in jars and vials were adjusted by adding water weekly on a soil mass basis to maintain the desired moisture content (McLauchlan and Hobbie, 2004).

GHG Analyses. At the end of 30 d, vials were capped for 1 h. Headspace gas was sampled through a septum in the lid of each vial. Gas samples were analyzed for CO_2, N_2O, and CH_4 on a Shimadzu GC14 Gas Chromatograph (Shimadzu USA) after covering vials for 1 hour (modified from Nkongolo et al., 2008). Detectors on the GC14 were ECD for CO_2 and N_2O (column Porapak Q 80/100 mesh 1m x 3mm I.D.) and FID for CH_4 (column Porapak N 80/100 mesh 2m x 3mm I.D.). Injector temperature was 100^{0}C,

and column temperature was 60^0C with a 4 min hold time. Concurrently, sub-samples of soil were taken from the mason jars and processed for PLFA analysis.

Phospholipid fatty acids analysis. For phospholipid fatty acids, soil samples were extracted following the method of Petersen and Klug (1994) with modifications described in Pritchett et al. (2011). All reagents were HPLC grade and purchased from Sigma (St. Louis, MO). Briefly, 2 g of soil was placed in Teflon-lined screw cap tubes (16 mm x 100 mm) with 2 mL phosphate buffer (50 mM; pH 7.4) and 7.5 mL methanol: dichloromethane (2:1) and shaken on a Whirly mixer. Fatty acid methyl ester analysis is based on saponification of soil at 100^oC, acid methylation at 80^oC, an alkaline wash, and an extraction of methyl esters of long-chained fatty acids and similar compounds in hexane. The extract was centrifuged to separate the organic phase. Phospholipids in the organic phase were isolated by solid-phase extraction using 100 mg silicic acid columns (Varian, Harbor City, CA). Columns were conditioned under slight vacuum (>0 in Hg) with 3 ml hexane, 1.5 ml hexane: chloroform (1:1) and 100 ml chloroform. Columns were sequentially rinsed with 1.5 ml chloroform: 2-propanol and 2% acetic acid. Phospholipids were then eluted from the columns with 2 ml methanol, and evaporated under N_2 (g) in preparation of PLFA extraction. The combined organic phase was evaporated to dryness under N_2 (g) and then redissolved in 75µl hexane: methyl tertiary butyl ether (1:1). PLFA samples were analyzed on a gas chromatograph (Agilent Technologies GC 6890, Palo Alto, CA) with a fused silica column and equipped with flame ionizer detector and integrator. ChemStation (Agilent Technologies GC 6890, Palo Alto, CA) operated the sampling analysis, and integration of samples. Extraction efficiencies were based on the nonadecanoic acid peak as an internal standard. Peak

identification and integration of areas was performed under the Eukary method parameters by software supplied my Microbial Identification Systems, Inc. (Newark, DE).

Peak chromatographic responses were translated into mole responses using the internal standard and responses were recalculated as needed. Peaks that corresponded to C chain lengths of 12-20 Cs are generally associated with microorganisms. Fatty acids are designated by the number of carbon atoms, followed by a colon, the number of double bonds, and then by the position of the first double bond from the methyl (ω) end of the molecules. Branched fatty acids are indicated by 'i' and 'a' for iso and anteiso branching, respectively. The prefix 'cyc' designates cyclopropane fatty acid. Peaks used as markers for bacteria were 12:0 3OH, i14:0, 15:0, a15:0, i15:0, i15:0 g, cyc15:1, i16:0, 16:1ω7, trans16:1ω7, a17:0, cyc17:0, i17:0, 17:1ω6, i17:1ω7, 18:1ω7, cis18:1ω7, cis18:1ω9, cyc19:0 C11-12, cyc19:0, cis19:1ω9 (Vestal and White, 1989; Bååth, 2003; Frostegård and Bååth, 1996; Zelles, 1999). Fungal markers were 16:1ω5, cis16:1ω5, 18:1ω9, 18:2ω6, cis18:2ω6, 18:2ω9, 18:3ω3, 18:3ω6, cis18:3ω6 (Frostegård et al., 1993, Zelles, 1995; Frostegård and Bååth, 1996; Sundh et al., 1997; Grigera et al., 2007). Microbial biomass was calculated from mole response calculations using relationships determined by Bailey et al. (2002) and expressed in nanomoles of PLFA g^{-1} of dry weight of soil (nmol g^{-1} soil). Mole percent values for biomarkers representing bacteria (Bac), aerobic (Aer), Gram-negative (Gneg) and Gram-positive (Gpos) bacteria and total fungi ((Fun) and mycorrhizal (Myc) components were summed individually, and bacteria to fungi ratios calculated for each sample. The ratio of bacterial to fungal fatty acids (B/F) often indicates changes in the soil microbial community structure (Fierer et al., 2003).

For Gram-positive bacteria, markers were i14:0, i15:0, a15:0, i15:0 g, i16:0, i17:0 and cis18:1ω9 (Zelles et al., 1995; Sundh et al., 1997). Markers for Gram-negative bacteria were 15ω6c, cis16:1ω7t, cyc17:0, cis18:1ω7, cyc19:0, cis19:0ω9 (Zelles et at., 1995; Sundh et al., 1997). Mycorrhizal markers were 16:1ω5, cis16:1ω5, 18:2ω6, cis18:2ω6, 18:2ω9 (Balser et al., 2005; Belen Hinojosa et al., 2005; Madan et al., 2002; Olsson, 1999). Ratios were calculated for cyclopropyl fatty acids to monoenoic precursors and total saturated to unsaturated fatty acids (Sat/Mono; Kieft et al., 1994; Bossio and Scow, 1998; Fierer et al., 2003). Specific peaks used to calculate cyclopropyl fatty acids to monoenoic precursors were cyc17:0 to cis16:1ω7 and cyc19:0 to cis18:1ω7. The ratio of total saturated to monounsaturated fatty acids used the ratio of the sum of 14:0, 15:0, 16:0, 18:0 and 20:0 to sum of cis16:1ω11, cis16:1ω9, cis16:1ω7, cis16:1ω5, cis17: ω9, scis17:1ω8, cis17:1ω7, and cis17:1ω5. The ratio of saturated to monounsaturated fatty acids gives an indication of changes in substrate availability and/or anaerobic conditions (Larkin, 2003). Monounsaturated fatty acids (Mono) from 14:0 to 19:0 were also evaluated (Bossio and Scow, 1998).

Statistical analysis. Data were log transformed when necessary to improve normality (Khattree and Naik, 1999). We conducted Pearson's correlation to determine if there were relationships among the data. The data was then analyzed using the PROC MIX procedure in SAS. Principal component analysis (PCA) was performed on the data to reduce dimensionality by using components associated with eigenvalues greater than one to reduce the number of variables retained for further analysis. Selected groups of response variables were used in a series of MANOVAs, followed by ANOVAs when suggested by the multivariate results. These analyses tested for differences (at $P<0.05$)

among treatments within PLFA profiles and GHG efflux (Jolliffe, 1986; SAS Institute, 1999). Means separation was done using LSMeans. The whole dataset (see Appendix Table 1) was used to identify those variables responsible for separations among treatments and correlation among variables (Khattree and Naik, 1999; SAS Institute, 1999).

RESULTS

GHG correlations. Pearson correlations were used to determine if relationships existed among the data for each sampling period. Total organic carbon significantly correlated with total nitrogen within each sampling period (Table 5.1), but TOC and TN did not correlate with GHG efflux from any sampling period. Of the GHGs studied, efflux of CO_2 correlated with N_2O for JSP and NSP; CO_2 correlated with CH_4 for MSP. Overall we saw that for MSP, all PLFA groups significantly correlated with TOC and TN, except for biomass, Sat/Mono and Mono. Bacteria, B/F, Gneg, Gpos, Aer, and Ana negatively correlated with TOC for MSP; and with TN for MSP and NSP. Fungi and Myc were positively correlated with TOC for MSP; TN for NSP and MSP; N_2O for JSP; and CH_4 for NSP. In addition, Myc was positively correlated to CO_2 for JSP (Table 5.1).

GHG vs. microbial groups. Total organic carbon and TN negatively correlated with B/F, Gneg and Ana in NSP (Table 5.1). Total nitrogen also positively correlated with Fungi and negatively correlated with Ana and Mono markers for NSP. No significant correlations were found for JSP between TOC or TN and any of the PLFA groups. However, N_2O positively correlated with Fun, Gpos, and Myc and negatively correlated with B/F, Gneg, Ana, Sat/Mono, and Mono for JSP. A positive correlation between MB and N_2O for MSP and a negative correlation between N_2O and CH_4

occurred for JSP, otherwise no other significant correlations were observed for MB in this study. Carbon dioxide only correlated with Myc for JSP and Mono for NSP. Correlations between CH_4 and MB (negative) and CH_4 and Sat/Mono and Mono were detected for JSP. Total fungi, Myc, Sat/Mono and Mono (neg) were associated with CH_4 for the NSP and MSP. Overall TN and TOC correlated to the greatest extent with N_2O for NSP. In general, correlations among GHG effluxes were weak. This suggests that the biological activity or microbial group composition were related to content and availability of TOC and TN. Greenhouse gas efflux did not appear to be a result of general metabolic activity of the various microbial groups detected by PLFA analysis but likely is influenced by many interacting environmental factors including biological activity.

GHG vs. PLFA chain length. Correlation of TOC, TN and GHG with relevant PLFA peaks was conducted to reveal potential relationships between gas efflux and individual taxonomic groups. Correlations among TOC, TN, CO_2 and CH_4 were weak and sporadic (Table 5.2). Few correlations were found in JSP and NSP for TOC and TN; however TOC and TN correlated with several PLFAs for MSP, which were associated with microbial growth. Nitrous oxide efflux correlated with several groups in JSP and MSP (Table 5.2); however, N_2O efflux did not correlate with any group for NSP. Carbon dioxide efflux correlated with chain length 17: anti iso for all three sampling periods (Table 5.2). Methane efflux was least related with PFLA chain lengths, although the detected associations occurred with higher C chain lengths, including chain length 19:00 that was significant for all three sampling periods. The chain length 16:00, which is found in many living organisms was negatively correlated with TOC and TN for MSP

and positively correlated with N_2O for JSP. Although biological groups followed similar patterns as TOC and TN, they did not correlate with GHG efflux in these systems.

ANOVA Results. Analysis of variance of GHG efflux and PLFA profiles indicated temporal effects and multiple interactions among sampling periods, landscape sampling position, soil temperature, and soil moisture (Table 5.3). Overall, sampling period influenced all variables tested except TOC and TN. In this study, MSP and JSP samples were incubated at temperatures $5 – 20^0$C higher than the NSP samples. Subsequently, each sampling period (JSP, NSP, and MSP) was analyzed independently to determine the main effects and interactions within each sampling period.

ANOVA- GHG. Greenhouse gas efflux was affected by main effects and many interactions across all three sampling periods (Table 5.4). Nitrous oxide efflux was affected by all main effects and their interactions for NSP; however, for JSP, only temperature, moisture, and the temperature moisture interaction affected N_2O efflux. For MSP, there was no effect of any of the factors on N_2O efflux. Carbon dioxide was affected by temperature, moisture, and the landscape sampling position by moisture interaction for NSP. For MSP, CO_2 efflux was affected by temperature, and interactions of moisture with landscape position and temperature. Similarly for JSP, CO_2 was affected by temperature, moisture, and interactions of moisture with landscape position and temperature. Temperature also affected CH_4 for NSP, MSP, and JSP. Methane efflux was also affected by the landscape sampling position by temperature and landscape by moisture interactions for NSP. Landscape sampling position by moisture interactions were also found for MSP and JSP for CH_4 efflux (Table 5.4).

ANOVA -Biomarker Groups. Similar to greenhouse gas efflux, soil biological properties varied with each sampling period (Table 5.4). In addition, landscape sampling position affected all the variables in this data set. Biomarkers for fungi, Gram-positive bacteria (Gpos), mycorrhizal fungi (Myc), Sat/Mono, monounsaturated lipids (Mono), N_2O and CO_2 varied with temperature. Biomarkers for bacteria to fungi ratio (B/F), Sat/Mono, Mono, and GHG, N_2O and CO_2 varied with moisture. Interactions were not consistent, although landscape sampling position interacted with temperature to affect markers of B/F, Gram-negative bacteria (Gneg), anaerobic bacteria (Ana) and Sat/Mono. Landscape sampling position by moisture interactions influenced fungi, B/F, Myc, and Mono lipids. Temperature by moisture interactions were found for fungi, B/F, Gneg, Gpos and Ana bacteria and Myc, Sat/Mono and Mono biomarkers. Total organic carbon and TN differed for only two variables. In addition, MB, and Bac and Aer biomarkers and B/F ratios changed only slightly with treatments and will not be included in these data.

GHG values. Gas production from NSP ranged from -1.4 to 28.9 µg N_2O-N $m^{-2}h^{-1}$; 2.6 to 30.8 mg CO_2-C m^{-2} h^{-1}; and -200 to -6.5 µg CH_4-C m^{-2} h^{-1}. For MSP N_2O ranged from 0.8 to 15.5 µg N_2O-N $m^{-2}h^{-1}$; CO_2 ranged from 6.2 to 30.3 mg CO_2-C m^{-2} h^{-1}; and CH_4 ranged from -253.9 to 47.6 µg CH_4-C m^{-2} h^{-1}. For JSP values ranged from 0.8 to 17.8 µg N_2O-N $m^{-2}h^{-1}$; 1.4 to 21.6 mg CO_2-C m^{-2} h^{-1}; and -158 to 83.9 µg CH_4-C m^{-2} h^{-1}.

GHG across landscape sampling positions. Over all the sampling periods, the amount of N_2O released generally followed similar trends across landscape sampling positions. For example the JSP samples incubated at 35^0C and 60% WHC resulted in

greater efflux then other temperature by moisture combinations at all landscape sampling positions (Figure 5.1a). Nitrous oxide efflux for JSP at all landscape sampling positions was less at the control temperature of 25^0C than at 35^0C (Figure 5.1a). For NSP the trend was for samples incubated at 25^0C and 60% WHC to have greater N_2O efflux than other temperature by moisture combinations (Figure 5.1 b). In addition, N_2O efflux was greatest for NSP from SH and DR incubated at 25^0C. For MSP, the greatest N_2O efflux was from BS samples incubated at 25^0C and 60% WHC. Samples from NSP had greater N_2O losses at BS, SH and DR when incubated at 25^0C and 60% WHC. For MSP, N_2O release did not change with temperature except for BS and SH landscape sample positions (Figure 5.1c). Nitrous oxide efflux for MSP was least at SS and FS for samples incubated at 20^0C and 60% WHC. The greatest N_2O efflux was observed at BS incubated at 25^0C and 60% WHC. For NSP, soils from SH and DR landscape sampling positions released more N_2O when incubated at 25^0C and 60% WHC (Figure 5.1b). Carbon dioxide efflux for JSP was greatest at incubation of 35^0C at 60% WHC for all landscape sampling positions (Figure 5.2a). Also, for JSP CO_2 efflux appeared spatially variable, where, in general, greatest and most reduced efflux rates occurrd at SH and DR positions, respectively. However, the effect of landscape sampling position on GHG efflux was less obvious for NSP and MSP (Figures 5.2b & c).

GHG with temperature. In general, warmer temperatures resulted in greater CO_2 efflux during all three sampling periods. Samples collected during NSP showed a temperature effect across all landscape sampling positions with the greatest CO_2 efflux occurring when soils were incubated at 25^0C. In addition soils incubated at 15^0C under FM conditions had the least CO_2 efflux, except BS (Figure 5.2b). Samples from MSP

incubated at 25^0C generally released more CO_2 than at 20^0C. The effect of moisture was less obvious; however the overall tendency was for greater CO_2 efflux from soils incubated at 60% WHC than soils incubated at FM (Figure 5.2c). Carbon dioxide efflux from SH was not affected by incubation temperature for MSP. For JSP soils incubated at 35^0C had greater CO_2 efflux than at the control temperature (25^0C) for all landscape sampling positions. For JSP CO_2 release from soils incubated at 35^0C and 60% WHC was greatest from BS and SH. Reduced CO_2 efflux from soils incubated at 25^0C and 60% WHC was noted for SS and was only greater than DR soils incubated at 25^0C under FM conditions

Methane emission was not detected at the landscape sampling positions for JSP except for SS where emission occurred under all treatments except for soils incubated at 25^0C and 60% WHC (Figure 5.3a). Emission of CH_4 during JSP also occurred for soils from the SH landscape sampling position when soils were incubated at 35^0C and 60% WHC. In addition, soils incubated at control temperature (25^0C) had generally greater CH_4 oxidative capacity than soils incubated at 35^0C (Figure 5.3b). Temperature effect was also evident for NSP; samples incubated at 25^0C generally had greater CH_4 oxidative capacity than soils incubated at 15^0C. However, for MSP samples incubated at 20^0C had greater oxidative capacity than samples incubated at 25^0C. Moisture differences also affect CH_4 efflux; however, the effect of moisture was not as pronounced as the effect of temperature. The greatest moisture effect on CH_4 efflux was observed at DR for JSP, BS for NSP, and BS for MSP. During MSP, CH_4 efflux from the DR sampling position was not affected by either temperature or moisture and was the only landscape sampling

position for MSP that emitted CH_4 (Figure 5.3c). For NSP landscape sampling positions were not a source of CH_4 to the atmosphere.

Biological groups. Soil biological properties varied with each sampling period and were affected differently by the main effects of landscape sampling position, temperature, moisture and their interactions (Figures 5.4 to 5.8). Gram positive and Aer biomarkers were greatest for MSP samples, while Fungi and Myc were greater for NSP and MSP samples than for JSP. Gram negative and Ana biomarkers were least for NSP samples. Bacteria to fungi, Sat/Mono and Mono lipids were greatest for JSP. In addition, Ana and Myc were similar to other biomarkers previously reported (See Appendix 1) and were therefore, not discussed in details.

Biomarkers and Landscape. Biomarkers also varied with landscape (Figures 5.4 – 5.8) during all three sampling periods. For JSP biomarkers for fungi were least in SS and SH, and greatest in BS and DR (Figure 5.4b). The general trend for fungi biomarkers for JSP was a slight increase from SS to DR. For NSP, fungi biomarkers were less spatially defined, however, there was also a tendency of fungi biomarkers for NSP to increase slightly from SS to DR. Biomarkers for B/F (Appendix 1) and Gneg were greatest in SH and SS and least in FS and DR for both JSP (Figure 5.6a) and MSP (Figure 5.6c). Aerobic biomarkers were highest in SH, SS and BS and lowest in DR (Appendix 1). Interestingly, Ana biomarkers were greartest in SH and SS and lowest in DR and FS (Appendix 1). For JSP, Gneg and Ana biomarkers, in addition to BtoF ratio were affected by landscape sampling position.

Biomarkers and Temperature. Temperature affected B/F, Fun, Gneg, Bac, Ana, Myc and Mono in NSP samples (Table 5.4). Temperature also affected B/F, Gneg, Ana, and Mono in MSP. For JSP, all soil biological properties, except B/F, were affected by temperature; moisture was also significant for most soil biological properties except MB, Gpos and Mono for JSP. Total fungi, Gpos, and Myc biomarkers were greatest at 35^0C while Sat/Mono and Mono were greatest at 25°C. Bacteria to fungi ratio, Sat/Mono and Mono lipids were greatest at FM; all other groups were not affected by moisture. Landscape sampling position also influenced PLFA biomarkers detected in the JSP. For example Sat/Mono was less in SS soils incubated at 35^0C compared to 25^0C. Fungi biomarkers were greater at all landscape sampling positions when samples were incubated at 35^0C and 60% WHC. At all other temperature-moisture combinations for JSP fungi biomarkers varied at each landscape sampling position (Figure 5.4a). Similarly, for NSP the temperature-moisture combination of 15^0C and 60% WHC had greater fungi biomarkers across all landscape sampling positions except for DR (Figure 5.4b). For JSP Sat/Mono and Mono were generally greater at SS and SH landscape sampling positions than at BS, FS, and DR (Figures 5.7a and 5.8a). Gram negative biomarkers (Figure 5.6a) was greatest in SH and SS for JSP, following a trend similar to sat/Mono and Mono biomarkers. However, for Gpos, the effect of landscape sampling position was not as apparent (Figure 5.5a), as occurrence of this group was not notably different among landscape sampling positions. Interestingly, the effect of temperature and moisture on Gpos biomarkers was still obvious within the different landscape sampling positions in JSP. For the most part incubation at 35^0C and 60% WHC revealed greater Gpos biomarkers than the other temperature-moisture combinations for JSP (Figure 5.5a) at all

landscape sampling positions except SH; in contrast, the 25^0C-60% WHC temperature-moisture combination for JSP showed the least Gpos biomarkers at all landscape sampling locations except SH (Figure 5.5a).

Interactions between temperature and moisture with landscape sampling position also influenced soil biological properties. For NSP, fungi were lowest at BS and DR landscape sampling positions at 15^oC and 60% WHC. In general for the FS, SH and SS landscape sampling positions, fungi were almost always greater at 15^oC incubation than at 25^0C. In contrast, response of fungi was variable at 20^oC, either increasing in BS or decreasing in FS soils for MSP (Figure 5.4c). For JSP, fungi were less at 35^oC than at 25^oC for both SS and SH landscape sampling positions; however, at other landscape sampling positions fungi were greater at 35^oC (Figure 5.4a). For NSP, landscape sampling position by temperature interactions influenced the biomarkers Gpos, Myc, Mono and Sat/Mono. Similarly, MB at MSP and B/F, Gneg, Ana, Myc and Sat/Mono for JSP were affected by the landscape sampling position and temperature interaction. Landscape sampling position by moisture interactions were found for JSP for Mono and Sat/Mono. Landscape position by moisture interactions were not found for fungi in any of the sampling periods.

Biomarkers and Moisture. Moisture was the one factor that elicited the least change in the variables assayed in this study. Moisture affected B/F, Sat/Mono, Mono, Bac for JSP; Gneg for NSP; and Ana for MSP (Table 5.4). Additionally, temperature by moisture interactions influenced B/F, Fun, Myc, Gneg, Mono and Sat/Mono for NSP, and Ana and Gneg for MSP. Temperature by moisture interactions was evident for all other soil biological properties except for MB and total bacterial PLFA for MSP.

For NSP at 25^{o}C, Mono was greater at 60% WHC than at FM; however, at 15^{o}C the values reversed with greater Mono at FM in all soils (Figure 5.8b). For MSP greater Mono concentrations were detected at BS and DR landscape sampling positions at 60% WHC than FM at 25^{o}C; however for the other landscape sampling positions for MSP incubated at the same 25^{0}C, 60% WHC tended to have less Mono concentrations than FM. In contrast, Mono was less at 60% WHC than FM at the 20^{o}C incubation temperature at the BS and DR landscape sampling positions. Also, monounsaturated PLFA at FS and SH landscape sampling positions were greater at 20^{o}C under 60% WHC than FM. The trend for monounsaturated PLFA in BS and DR soils to be at their greatest levels at 60% WHC at 25^{o}C continued for JSP although this biomarker decreased more in soils incubated at 35^{o}C at WHC than at FMfor all landscape sampling positions. For MSP and JSP, the ratio of saturated to monounsaturated PLFA, which is often used as a stress indicator, was affected by the three-way interaction of landscape sampling position, temperature, and moisture (Table 5.4). Gram-negative bacterial PLFAs, along with bacteria to fungi ratio also differed for JSP due to the landscape sampling position, temperature, and moisture three-way interaction. Although significant differences were not consistently detected for Gpos as a result of the landscape sampling position, temperature, moisture interaction in both NSP and MSP, groups were highly influenced by temperature and moisture, in particular at SH and SS (Figure 5.5a and b).

Samples from NSP showed Fun (Figure 5.4b), B/F (Appendix 1) and Myc (Appendix 1) markers were greater at 60% WHC in SS and SH soils. Bacterial markers showed mixed responses to moisture for landscape sampling position (e.g. Figures 5.5 and 5.6). Mono biomarkers also had mixed responses to moisture for all three sampling

periods (Figure 5.8). Anaerobic bacteria biomarkers were not affected by the interaction of temperature with moisture for NSP (Appendix 1). Mycorrhizae biomarkers were greater at SS and SH at 60% WHC for NSP and SH for MSP, but least at SH at 60% WHC for JSP (Appendix 1).

Fungi biomarkers were generally greater at the 60% WHC at FS and SH for JSP (Figure 5.4a) and NSP (Figure 5.4b). Bacteria to fungi ratio was greater at 60% WHC at SS and SH and Gneg was least at those two landscape sampling positions for JSP (Appendix 1). Anaerobic bacteria were greater at the lower moisture at SS for JSP and MSP. Mycorrhizae biomarkers increased with moisture at SH for MSP. Monounsaturated biomarkers were greater however at the FM for JSP. For MSP, fungi were greater when soils were incubated at FM from only the BS landscape. Bacteria to fungi and Myc were higher in FM soils from SH samples; Gneg was higher when soils from SH landscape sampling positions were incubated at WHC.

GHG and PLFA chain length Multivariate analysis. The first two ordinates explained over 95% of the variation from all three sampling periods. Methane exhibited strong positive loadings on PC1 for all three sampling periods, whereas nitrous oxide and carbon dioxide had strong positive loadings on PC2 for MSP and JSP. The influence of CO_2 and N_2O on PC2 for NSP although positive was low, and therefore had only minor influence on variation in PC2. There were no strong associations between PLFA and PC1 in any sampling period; there was a weak positive association between PC2 and PLFA 18ω9t. Principal component three was positively associated with PLFA 18:1ω9t and negatively associated with PLFA 19:0 for NSP; with PLFA 18:1ω19t for JSP; and PLFAs 18:0, 19:1ω11c, 18:1ω9cA, 18:1ω9tA, and 19:1ω11c for MSP. However, PC3

only accounted for approximately 1.25% of the variation in any sampling period. Principle component analysis of the data showed good separation of sampling periods based on temperature, moisture and the temperature by moisture interaction. In all three cases MSP and JSP with similar temperature and/or moisture tended to cluster together, whereas NSP tended to separate based on both temperature and/or moisture.

Ordinate analysis across landscape sampling positions showed definite separation for MSP and JSP, with the upper landscape positions (summit and shoulder) grouped together for JSP (Figure 5.9A); and the lower landscape position tended to group to the right (Figure 5.9A). We found separations due to sampling period, landscape sampling position and temperature, but not due to moisture. Microbial communities as determined by PLFA data did not show uniform patterns across each sampling period and landscape sampling position. No clear pattern was evident among the PLFAs and GHGs in each sampling period. This analysis indicated that abiotic properties played a more influential role in the variation of GHG efflux than the soil microbial groups studied.

DISCUSSION

GHG Efflux. Greenhouse gas effluxes tend to vary due to differences in moisture and temperature and their combined effects on substrate quality and quantity (Hanson et al., 2000; Merino et al., 2004). In our research, temperature or its interaction with other independent variables affected efflux of all three GHG in each sampling period. Hanson et al. (2000) observed that CO_2 efflux in a temperate forest was diminished at low temperature and greater moisture content. Kim et al. (2010), Alluvione et al. (2009) also observed that CH_4 oxidative capacity in forest soils was greatest in the summer months under high temperature and low moisture. We also observed temperature effects on GHG

efflux with warmer temperatures associated with higher CO_2 efflux in the three incubation periods. For example, NSP and MSP had greater CO_2 efflux at 25^oC than at the respective seasonal temperatures of 15^oC and 20^oC; likewise for JSP CO_2 efflux was greater at 35^oC than at 25^oC. However, moisture effects varied across the three sampling periods with higher moisture contributing to greater CO_2 efflux for NSP and JSP, but not for MSP. Similarly, N_2O efflux was greater at warmer temperature in all three sampling periods. In addition N_2O efflux tended to be greater at WHC for NSP regardless of incubation temperature. The rate of microbial respiration is dependent on the combined effect of temperature, moisture, and carbon availability (Alluvione et al., 2009). The effect of temperature and moisture on GHG efflux observed in our research suggests an increase in microbial activity and turnover rate at warmer temperatures (Steinweg et al., 2008).

Total organic carbon and TN were correlated with MB, bacteria, fungi, B/F, Gram-negative, aerobic, and anaerobic for NSP and MSP. There were significant correlations between N_2O and most biomarker groups for JSP. Also CO_2 correlated with Myc for JSP and Mono for NSP. Correlation with Gram-positive, Sat/Mono, Mono, was observed with N_2O and CH_4 for NSP and MSP. Methane was correlated with MB, fungi, B/F, Gram-negative, Gram-positive, Sat/Mono and Mono within the three sampling periods. The variability in GHG efflux in relation to temperature and moisture, combined with the low correlations between GHG and biomarker suggest that interacton between other interacting mechanisms between soil microbial community and the soil environment (Kumaresan et al., 2009) could be affecting GHG efflux. In their study, Kumarensan et al. (2009) related temporal effects on methane oxidation potential

seasonal changes in soil abiotic factors. Differences in temperature and moisture for the different sampling periods, and at the different sampling locations could inadvertently result in variability in soil microbial activity and GHG efflux.

Landscape, sampling period, and soil factors. We found that landscape sampling position and temperature had greater influence on soil microbial community composition compared to other factors. For example for JSP and NSP BS and DR had higher concentrations of fungi biomarkers than SS, SH, and FS (Figures 5.4 a and b). Soil moisture also influenced the soil community; however this was mostly for JSP. In previous research Guner et al. (2010), Kumaresan et al. (2009) and Shrestha et al. (2004) commented on the temporal influence of moisture on microbial communities and their activities. Temperature can also be a source of temporal differences (Guner et al., 2010; Martin et al., 2010; Shrestha et al., 2004) observed increased microbial activity at greater moisture and temperature. In this section and in parallel research in this study, we observed a tendency towards greater microbial population and activity (Chapters 4 and 6) at greater incubation temperatures and moisture. However, variations across the landscape and throughout the sampling periods indicated that there may be synergistic and/or antagonistic responses by groups within the microbial community to environmental conditions of the study (Kumaresan et al., 2009).

Temporal differences in temperature and moisture in forest soils affect microbial activity and communities (Leckie, 2005). Differences in GHG efflux also vary due to soil temperature and moisture differences (Martin et al., 1993; Hoilett et al., 2007). Moore-Kucera and Dick (2008) concluded that PLFA profiling of temperate forest soils varied due to wide temporal shifts; therefore, samples should be analyzed over different

seasons to fully understand the dynamics of microbial populations. In our research GHG and PLFA biomarkers varied significantly for each sampling period.

Soil properties such as texture, moisture, nutrient content, SOM, and soil biological characteristics vary within topographic features (Ozatas et al., 2003; Martin et al., 2010; Kennedy and Schillinger, 2006). Microbial biomass is often correlated with clay content. High clay content may provide a protective environment for microorganisms against predation leading to reduced moisture related stress on Sat/Mono (substrate availability), while maintaining nutrient availability for microbial metabolism (Chodax and Niklinska 2010; Muller and Hoper, 2004). The variability of these soil properties affects the relationships among the soil biological characteristics, which may influence GHG efflux. We observed textural differences along the topographic sampling gradient with lower clay content found at shoulder and backslope positions. Other soil properties including TOC, TN, pH, extractable bases, CEC, and soil water (WHC and FM) increased as the landscape transitioned from summit to footslope (Table 3.1). Such variability may be due in-part to differences in soil texture and/or to the downslope movement of material (Martin et al., 2010) as a result of erosion (Papiernik et al., 2009), surface run-off and/or percolating water (Tsui et al., 2004). Soil texture differences across the landscape affected soil properties and soil biology of an organic farm in Washington State (Collins et al., 2011). The movement of subsurface water can redistribute soluble nutrients (Papiernik et al., 2009; Tsui et al., 2004). Differences in pH along a gradient can also influence soil properties (Rousk et al., 2009), with lower topographic positions (e.g. footslope) often more basic than summit (Tsui et al., 2004).

We used PLFA profile analysis to characterize soil communities at the different landscape sampling positions as PLFAs are often used as indicators to differentiate microbial groups (Frostegard et al., 1996; Ibekwe and Kennedy, 1999). In addition, "signature" PLFAs assists in identifying diverse groups of microorganisms associated with the different landscape sampling positions (Frostegard et al., 1996; Ibekwe and Kennedy, 1999). Differences in the composition of the PLFA profiles were expected to give insight on influences of various soil properties on the microbial community structure in different landscape positions. Landscape sampling position influenced several PLFA components, with total fungi ($p=0.0476$) and mycorrhizae ($p=0.0414$) significantly differing for MSP, and bacteria to fungi ratio ($p=0.0039$), gram negative ($p=0.0071$), and anaerobic ($p=0.0053$) bacterial PLFAs significantly differing for JSP. Leckie (2005) discussed the importance of moisture and temperature on the rate of organic matter decomposition and the subsequent effects on abiotic and biotic soil properties. Although lower landscape positions were greater in TOC, TN, and pH, the effect of temperature and moisture affected PLFA profiles more than any of these properties or landscape sampling position. Variations in microbial biomarkers in our research were therefore more likely related to differences in moisture and temperature and their interactive effects on SOM.

Temperature and moisture impact soil microbial community profiles (Leckie, 2005), soil properties (Rey Benayas et al., 2004), and GHG efflux (Hoilett et al., 2007; Shrestha et al., 2004). Soil microbial community structures (Rousk et al., 2009; Xing et al., 2010), soil properties (Oztas et al., 2003; Xing et al., 2010), and GHG efflux (Hanson et al., 1993) are also influenced by topography. However, the combined effects of

landscape sampling position, temperature, and moisture on soil microbial properties and GHG dynamics are yet to be fully explored. Hanson et al. (1993) observed temporal effects of temperature and moisture on CO_2 efflux; however, although topographic differences were observed during isolated sampling periods, no consistent patterns based on topography were detected. Additionally, reviews on the variability of heterotrophic contributions to GHG efflux have concluded that soil organisms are responsible for anywhere from 10 to 95% of soil respiration (Ryan and Law 2005, Tang et al. 2006, Hanson et al. 2000). The reviews also concluded that root respiration and plant metabolism are decisive players in soil respiration process. The complex interactions among soil processes make it difficult to determine the impact of individual factors on variable response. The issue is further complicated by seasonal effects on photosynthesis, soil temperature and soil moisture.

We hypothesized that laboratory incubation of soil after removal of roots would identify heterotrophic contributions to GHG efflux. However, as demonstrated by other researchers, we were unable to clearly identify defined patterns of response due to the effects of temperature, moisture, and their interactions and other factors on the microbial community using PLFA profiles. For example, the effect of the three-way interaction of temperature, moisture, and landscape sampling position on bacteria to fungi ratio varied across the three sampling periods but was only significant for JSP. At the 25^{o}C incubation temperature for NSP, bacteria to fungi ratio were generally greater for all landscape sampling positions at 60% WHC than under FM conditions. However, at 15^{o}C incubation temperature under FM conditions, bacteria to fungi ratios were generally greater at all landscape sampling positions, except DR. At warmer soil temperatures, B/F

ratios were not consistently related to soil moisture or landscape; these variations were likely due to shifts in bacterial populations resulting from differences in soil moisture during each sampling period, since fungal populations are less sensitive than bacteria to fluctuations in moisture. Similar variations in PLFA were observed for the Sat/Mono indicators, total bacterial, Gram-positive Gram-negative, and monounsaturated PLFAs. The soil water contents determined for FM and 60% WHC were very similar for both MSP and JSP; however, for NSP FM soil water content was much lower than 60% WHC. Therefore, soil water tended to show a greater effect on microbial PLFA composition for NSP, which exhibited drier soil moisture conditions at FM. Other possible explanations for the variations in PLFA concentrations could be differences in substrate quantity and/or quality (Ryan and Law, 2005) due to temporal changes in photosynthetic efficiency (Ryan and Law, 2005; Tang et al., 2006) with consequential effects on quantity and quality of root exudates (Lu et al., 2000; Jarecki and Lal, 2003). Temporal differences in the Sat/Mono indicators have previously been attributed to differences in substrate quality and quantity (Steinweg et al., 2008; Petersen and Klug, 1994), and aeration or moisture content (Moore-Kucera and Dick, 2008). Similarly, increases in Sat/Mono indicators during summer in soils under a Douglas-fir chronosequence were due to moisture stress (Moore-Kucera and Dick 2008). Diediou et al. (2009) noted that limited nutrient and organic C availability led to an increased ratio of saturated to unsaturated fatty acids.

In our research, soils generally had greatest saturated/unsaturated fatty acid ratios and least TOC contents during JSP. For JSP the increase in the Sat/Mono indicator could be a result of reduction in substrate availability due to high microbial activity at warmer

incubation temperatures (Steinweg et al., 2008). Similarly, Feng and Simpson (2009) observed shifts in microbial community composition due to temperature effects on substrate availability. We also found an effect due to a three-way interaction of landscape sampling position, temperature, and moisture in which soils collected in November and July and incubated at 60% WHC had increased cyc/pre ratios compared to FM at 25^{o}C; however, at 15^{o}C (NSP) and 35^{o}C (JSP) the cyc/pre ratio was reduced at 60% WHC than at FM. The increase in Sat/Mono indicators at 15^{o}C during NSP could be due to reduced microbial activity under reduced temperature and reduced moisture content at FM. This would indicate that moisture related stress, as indicated by the higher cyc/pre ratios, was impacting the activity and composition of the microbial community. Also O_2 supply relative to moisture content (Ryan and Law, 2005) may be important because increased moisture alters pore space O_2/H_2O ratio often resulting in a shift to more anaerobic microbial components (Moore-Kucera and Dick, 2008). Therefore, variations in PLFA profiles along landscape sampling positions under variable soil moistures and temperatures over time may be attributed to differences in aeration as well as available substrates and subsequent utilization by soil organisms. This suggests that factors other than the measured soil properties were influencing microbial properties.

CONCLUSION

Differences in GHG efflux from incubations of soils sampled across the three sampling periods and soil biological profiles as determined by PLFA analysis was most influenced by landscape position and soil temperature during a 10-month sampling period. Additionally, temporal differences in soil properties influenced GHG efflux and soil community composition, but these two variables generally weakly correlated at

occasional time periods. Several soil biological marker groups correlated with GHG efflux. These studies illustrate the contribution of temperature more than moisture in determining soil microbial activity and community. We found greater correlation of TOC and TN with microbial community groups indicating that GHG efflux may be partly a result of overall soil microbial activity rather than an outcome related to the individual groups measured in this study. Also specific microbial components potentially responsible for GHG emission (i.e., nitrifying bacteria, methanogenic bacteria) were not measured. These results show the complexity of interactions among time, space, temperature and moisture when investigating GHG and soil microbial community changes. In addition analysis of the factors influenceing microbial community structure and/or the influence of the microbial community on their environment are determined by the resolution used in the study (Kumaresan et al., 2009). In this section of the study we used PLFA profiles to characterize the community, it is therefore possible that a more taxonomically sensitive technique may contribute additional information to understanding the complex relationship between soil physical, chemical, and biological properties and GHG efflux in forest soils.

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Table 5.1 Pearson correlations among GHG (CO_2, N_2O, CH_4) and PLFA soil microbial group biomarkers after 30 day incubations of forest soils over three sampling periods (July and November 2008; May 2009), across five landscape sampling positions (summit, shoulder, backslope, footslope, and drainageway) and incubated at two temperatures (July (JSP) 25 and 35°C; November (NSP) 25 and 15°C; May (MSP) 25 and 20°C) and two moistures (field moisture and 60% water holding capacity). For each data set correlation coefficients are indicated with significance identified by asterisks. PLFA soil microbial group biomarkers included total (fun), mycorrhizae (Myc) fungi, bacteria to fungi ratio (B/F), Gram positive (Gpos), Gram negative (Gneg), aerobic (Aer), anaerobic (Ana) bacteria, stress indicators (Sat/Mono), monounsaturated (Mono) lipids.

	Carbon			**Nitrogen**			**N_2O**			**CO_2**			**CH_4**		
	JSP	**NSP**	**MSP**	**JSP**	**NSP**	**MSP**	**JSP**	**NSP**	**MSP**	**JSP**	**NSP**	**MSP**	**JSP**	**NSP**	**MSP**
Carbon	---	---	---	0.925**	0.907**	0.975**	---	---	---	---	---	---	---	---	---
Nitrogen	0.925**	0.907**	0.975**	---	---	---	---	---	---	---	---	---	---	---	---
N2O	---	---	---	---	---	---	---	---	---	0.616**	0.468**	---	---	---	---
CO2	---	---	---	---	---	---	0.616**	0.468**	---	---	---	---	---	-0.671**	---
CH4	---	---	---	---	---	---	---	---	---	---	-0.671**	---	---	---	---
Biomass	----	----	----	----	----	----	----	----	0.323*	----	----	----	-0.43**	----	----
Bacteria	----	----	-0.463**	----	----	-0.456**	----	----	----	----	----	----	----	----	----
Fungi	----	----	0.431**	----	0.265*	0.392*	0.545*	----	----	----	----	----	----	0.287*	----
BtoF	----	-0.344*	-0.514*	----	-0.366**	-0.479**	-0.361**	----	----	----	----	----	----	----	----
Gneg	----	-0.3*	-0.425**	----	-0.323**	-0.411**	-0.421**	----	----	----	----	----	----	----	----
Gpos	----	----	-0.41**	----	----	-0.43**	0.441**	----	----	----	----	----	----	----	----
Aer	----	----	-0.459**	----	-0.254*	-0.454*	----	----	----	----	----	----	----	----	----
Ana	----	-0.301*	-0.427**	----	-0.325*	-0.414**	-0.376**	----	----	----	----	----	----	----	0.293*
Myc	----	----	0.431**	----	0.265**	0.392**	0.582*	----	----	0.262*	----	----	----	0.287*	----
CMtoSM	----	----	----	----	----	----	-0.35**	----	----	----	----	----	0.263*	0.001**	----
Mono	----	----	----	----	-0.256*	----	-0.344**	----	----	----	0.31*	----	0.341**	-0.306*	----

---- Not significant.; * Significant at $p < 0.05$** Significant at $p < 0.01$.

Table 5.2 Pearson correlations among GHG (CO_2, N_2O, CH_4) and PLFA chain length after 30 day incubations of forest soils sampled over three sampling periods (July[JSP] and November [NSP] 2008; May [MSP] 2009), across five landscape sampling positions (summit, shoulder, back slope, foot slope, and drainageway) and incubated at two temperatures (July 25 and 35°C; November 25 and 15°C; May 25 and 20°C) and two moistures (gravimetric field moisture and 60% water holding capacity). For each data set correlation coefficients are indicated with significance identified by asterisks.

	Carbon			**Nitrogen**			**N_2O**			**CO_2**			**CH_4**		
	JSP	**NSP**	**MSP**	**JSP**	**NSP**	**MSP**	**JSP**	**NSP**	**MSP**	**JSP**	**NSP**	**MSP**	**JSP**	**NSP**	**JSP**
14:00	----	----	----	----	----	----	0.351**	----	----	0.267*	----	----	----	----	----
15:0ai	----	----	----	----	----	----	0.395*	----	----	----	----	0.358*	----	----	----
15:0i	----	----	----	----	----	----	0.461*	----	----	----	----	----	----	----	----
15:1iG	----	0.253*	----	----	0.263*	----	0.486**	----	----	0.327*	----	0.419**	----	----	----
16:00	----	----	-0.383**	----	----	-0.36**	0.364**	----	----	----	----	----	----	----	----
16:1w11c	----	----	----	----	----	0.257*	----	----	-0.259*	----	----	----	----	----	----
16:1w5c	----	----	0.311*	----	----	----	0.436**	----	----	----	----	----	----	----	----
16:1c7	----	----	----	----	----	----	----	----	0.358**	----	----	----	----	----	----
17:00	----	----	----	----	----	----	0.399**	----	----	0.416**	----	----	----	----	----
17:0ai	----	----	----	----	----	----	----	----	----	-0.282*	-0.374**	-0.277*	----	0.358**	----
17:0cy	----	----	-0.272*	----	----	-0.342**	----	----	-0.340**	----	----	----	----	----	----
17:0i	0.263*	----		----	----	----	----	----	----	----	----	-0.361**	----	----	----
17:17c	----	----	----	----	----	----	----	----	0.358**	----	----	----	----	----	----
17:18c	----	----	----	0.259*	----	----	0.417**	----	-0.295*	----	----	----	----	----	----
18:19c	----	----	-0.392**		----	-0.413**	----	----	----	----	----	----	----	----	0.328*
18:19t	----	----	----	----	----	----	----	----	----	----	----	0.289*	----	----	----
18:19tA	----	----	-0.303*	----	----	-0.305*	0.257*	----	----	----	----	----	----	----	----
18:26c	----	----	----	----	----	----	0.359**	----	0.2978*	0.392**	-0.274*	----	0.276*	----	----
19:00	----	----	----	----	----	----	-0.390**	----	----	----	0.310*	----	0.313*	-0.307*	-0.286*
19:0cyz2	----	----	-0.271*	----	----	----	----	----	----	----	----	----	0.305*	----	----
20:00	----	----	----	----	----	----	0.416**	----	----	----	0.318*	----	----	-0.276*	-0.279*

Table 5.3 ANOVA results showing p values for GHG (CO_2, N_2O, CH_4) and PLFA soil microbial group biomarkers after 30 day incubations of forest soils over three sampling periods (July and November 2008; May 2009), across five landscape sampling positions (summit, shoulder, backslope, footslope, and drainageway) and incubated at two temperatures (July 25 and 35°C; November 25 and 15°C; May 25 and 20°C) and two moistures (field moisture and 60% water holding capacity). PLFA soil microbial group biomarkers included total (fun), mycorrhizae (Myc) fungi, bacteria to fungi ratio (BtoF), Gram positive (Gpos), Gram negative (Gneg), aerobic (Aer), anaerobic (Ana) bacteria, stress indicators (CMtoSM), monounsaturated (Mono) lipids. Numbers in bold indicate significant differences.

	df	TOC	TN	N_20	CO_2	CH_4	MB	bac	fun	b/f	Gneg	Gpos	Aer	Ana	myc	Sat/Mono	Mono
		mol percent															
Sample period (Smpl prd)	2	0.3173	0.4217	**0.028**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
Landscape (lndscp)	4	**<.0001**	**0.0015**	**<.0001**	**<.0001**	**0.0017**	**<.0001**	**0.0124**	**<.0001**	**<.0001**	**<.0001**	**0.002**	**0.004**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
smpl prd*landscape	8	0.0716	0.7015	**0.0001**	**0.0061**	0.1998	0.2236	**0.0114**	0.083	0.083	**0.011**	**0.001**	**0.015**	**0.015**	**0.038**	**<.0001**	**<.0001**
Temperature (temp)	1	0.8777	0.5993	**0.2**	**<.0001**	0.0644	0.6505	0.281	**<.0001**	0.224	0.754	**0.002**	0.387	0.931	**<.0001**	**0.0002**	**<.0001**
Smpl prd *temp	2	0.9805	0.9911	**<.0001**	**<.0001**	**<.0001**	0.1777	0.2602	**0.008**	0.191	**0.002**	**<.0001**	0.234	**0.026**	**0.001**	**0.002**	**<.0001**
landscape*temp	4	**0.0029**	0.0686	0.09	0.6945	0.8456	0.3722	0.2216	0.761	**0.034**	**0.028**	0.22	0.167	**0.019**	0.983	**<.0001**	0.069
Smpl prd *lndscp*temp	8	0.1743	0.7654	**0.0001**	0.8383	0.7732	0.6159	0.9491	0.296	0.208	0.933	0.807	0.967	0.9	0.556	**0.0002**	0.335
Moisture (moist)	1	0.9488	0.9877	**<.0001**	**0.007**	0.6895	0.5536	0.3175	0.061	**0.041**	0.624	0.096	0.35	0.707	0.148	**0.0013**	**0.002**
Smpl prd *moist	2	0.753	0.7368	**<.0001**	**0.0088**	0.5094	0.3196	0.061	**0.019**	**0.003**	0.064	**0.009**	0.101	0.077	0.065	**0.0422**	**0.015**
lndscp*moist	4	0.9486	0.5032	**<.0001**	**0.0471**	**0.0115**	0.97	0.5027	**0.031**	**0.012**	0.307	**0.005**	0.492	0.327	0.091	**<.0001**	**0.0001**
Smpl prd *lndscp*moist	8	0.2945	0.5355	**0.002**	0.1844	0.8987	0.9946	0.1954	**0.031**	**0.012**	0.114	0.132	0.201	0.093	**0.034**	**0.0021**	**0.008**
temp*moist	1	0.944	0.9396	0.758	0.8864	0.6522	0.8541	0.6102	**0.0001**	**<.0001**	**<.0001**	**<.0001**	0.377	**<.0001**	**<.0001**	**<.0001**	**0.004**
Smpl prd *temp*moist	2	0.7902	0.7387	**<.0001**	**0.0194**	0.9692	0.3054	**0.0277**	**<.0001**	**<.0001**	**<.0001**	**0.007**	0.355	**0.0001**	**<.0001**	**<.0001**	**0.006**
lndscp*temp*moist	4	0.0671	0.2115	0.364	0.8429	0.8686	0.4969	0.9808	0.84	0.751	0.866	**0.041**	0.992	0.924	0.807	**<.0001**	0.089
Smpl prd *lndscp*temp*moist	8	0.3795	0.5194	**<.0001**	0.7787	0.9455	0.7802	0.7907	0.173	0.129	0.406	0.448	0.714	0.468	0.056	**<.0001**	0.647

Table 5.4a ANOVA results for July 2008 sampling period to determine the effect of landscape sampling position (slope), temperature (temp), moisture (moist), and their interactions on microbial properties and greenhouse gas efflux from forest soils during an incubation study. Numbers (p-values) in bold indicate significant differences.

July 2008							Pr > F			
Source of variance	Num DF	Den DF	MB	Bac	Fun	B/F	Gneg	Gpos	Aner	
Slope (Slp)	4	10	0.7089	0.1213	0.1981	**0.0039**	**0.0071**	0.6474	**0.0053**	
Temp	1	30	**0.0007**	**0.0017**	**0.0199**	0.0548	**0.0432**	**0.0011**	**0.0338**	
Slp*Temp	4	30	0.1481	0.9996	**<.0001**	**<.0001**	**0.0069**	**0.0013**	**0.0114**	
Moist	1	30	0.0576	**0.0009**	**0.0003**	**<.00001**	**<.0001**	0.7936	**0.0002**	
Slp*Moist	4	30	0.7428	0.7494	0.069	**0.0005**	0.0688	0.0601	0.0627	
Temp*Moist	4	30	0.9244	0.2247	**<.00001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	
Slp*Temp*Moist	4	30	0.5429	**0.0174**	0.0956	**0.0004**	**0.038**	0.0527	**0.0366**	
							Pr > F			
			MYC	Sat/Mono	Mono	TOC	TN	N_2O	CO_2	CH_4
Slp	4	10	0.2968	0.0833	0.8249	0.3356	0.4418	0.3556	**0.0162**	0.4584
Temp	1	30	**0.0475**	**0.0156**	**<.0001**	0.7284	0.6375	**<.0001**	**<.0001**	**0.0005**
Slp*Temp	4	30	**<.00001**	**0.0001**	**<.0001**	0.4863	0.6349	0.724	0.8492	0.5282
Moist	1	30	**0.0014**	**0.0108**	0.4239	0.4411	0.4103	**0.0002**	**0.0001**	0.1929
Slp*Moist	4	30	0.0698	**0.0012**	**0.0247**	0.8157	0.6158	0.3795	**0.0003**	0.0519
Temp*Moist	4	30	**<.0001**	**0.0002**	**0.0024**	0.8816	0.7094	**<.0001**	**0.0001**	0.5031
Slp*Temp*Moist	4	30	0.2072	**0.018**	0.2751	0.4536	0.3565	0.2121	0.3512	0.5886

Microbial biomass (MB), bacteria (Bac), fungi (Fun), bacterial to fungal ratio (B/F), gram negative bacteria (Gneg), gram positive bacteria (Gpos), anaerobic bacteria (Aner), mycorrhizae fungi (Myc), ratio saturated to monounsaturated (Sat/Mono), monosaturated (Mono) phospholip fatty acid profiles, and total organic carbon (TOC), total nitrogen (TN), nitrous oxide (N_2O), carbon dioxide (CO_2), and methane (CH_4) based on, slope position (Slp), temperature (Temp), and moisture (Moist). Samples were taken in July and November of 2008, and May of 2009 along five landscape sampling positions. Significant effects are in bold.

Table 5.4b ANOVA results for November 2008 sampling period to determine the effect of landscape sampling position (slope), temperature (temp), moisture (moist), and their interactions on microbial properties and greenhouse gas efflux from forest soils during an incubation study. Numbers (p-values) in bold indicate significant differences.

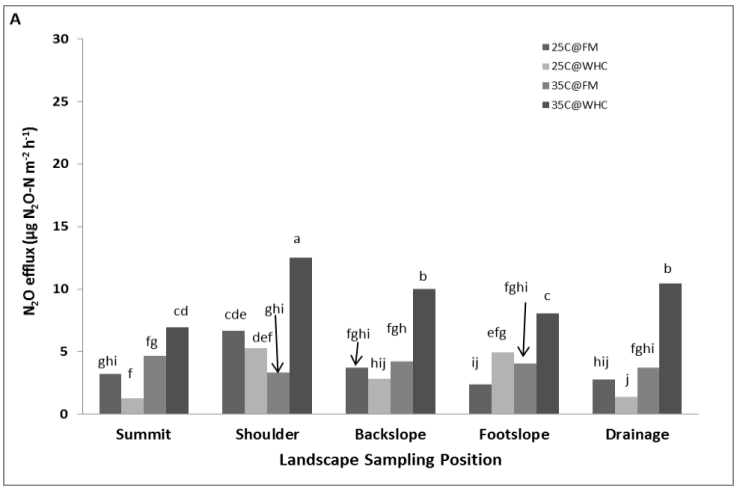
November 2008							Pr > F			
Source of variance	Num DF	Den DF	MB	Bac	Fun	B/F	Gneg	Gpos	Aner	
Slp	4	10	0.6039	0.9014	0.6544	0.6475	0.8163	0.5439	0.814	
Temp	1	30	0.9792	0.0569	0.0576	**0.0292**	**0.0056**	0.7455	**0.0125**	
Slp*Temp	4	30	0.2613	0.7807	**0.039**	0.2702	0.6308	**0.0253**	0.8143	
Moist	1	30	0.4984	0.0949	0.6362	0.5459	0.4418	**0.0329**	0.6024	
Slp*Moist	4	30	0.9069	0.8718	0.1351	0.3974	0.8128	0.1465	0.8239	
Temp*Moist	4	30	0.1309	0.3669	**0.0078**	**0.0373**	0.3285	**0.002**	0.3665	
Slp*Temp*Moist	4	30	0.5059	0.1711	0.5586	0.4845	0.4524	0.069	0.4439	
							Pr > F			
			MYC	Sat/Mono	Mono	TOC	TN	N_2O	CO_2	CH_4
Slp	4	10	0.671	0.161	0.0552	0.4674	0.7207	**0.0004**	0.6336	0.4226
Temp	1	30	**0.0263**	0.12	**0.0473**	0.9506	0.7332	**<.0001**	**<.0001**	**<.0001**
Slp*Temp	4	30	**0.0083**	**0.0013**	**0.0004**	**0.0115**	0.3187	**<.0001**	0.1042	**0.0112**
Moist	1	30	0.7124	0.3527	0.8246	0.8079	0.8929	**<.0001**	**<.0001**	0.0926
Slp*Moist	4	30	0.1614	0.2949	0.0776	0.865	0.9547	**<.0001**	**0.0148**	**0.0002**
Temp*Moist	4	30	**0.0098**	**0.0048**	**0.0003**	0.9746	0.9833	**<.0001**	0.613	0.8923
Slp*Temp*Moist	4	30	0.4759	0.5465	0.1937	0.2157	0.5518	**<.0001**	0.162	0.1824

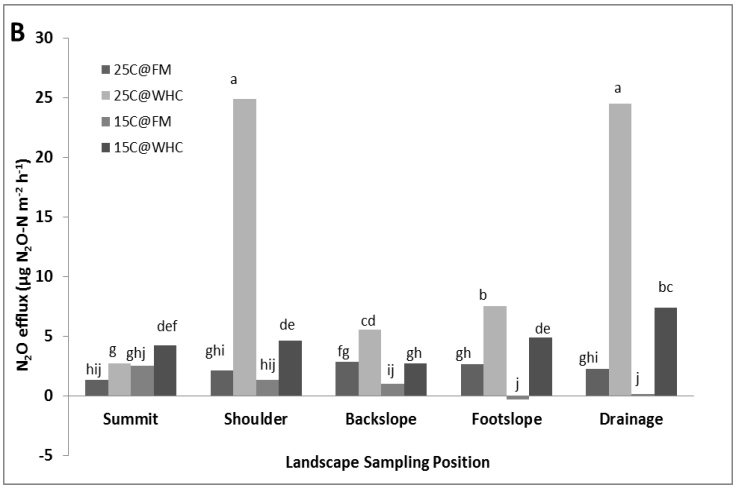
Microbial biomass (MB), bacteria (Bac), fungi (Fun), bacteria to fungi ratio (B/F), gram negative bacteria (Gneg), gram positive bacteria (Gpos), anaerobic bacteria (Aner), mycorrhizae fungi (Myc), ratio saturated to monosaturated (Sat/Mono), monosaturated (Mono) phospholipid fatty acid profiles, and total organic carbon (TOC), total nitrogen (TN), nitrous oxide (N_2O), carbon dioxide (CO_2), and methane (CH_4) based on, slope position (Slp), temperature (Temp), and moisture (Moist). Samples were taken in July and November of 2008, and May of 2009 along five landscape positions. Significant effects are in bold.

Table 5.4c ANOVA results for May 2009 sampling period to determine the effect of landscape sampling position (slope), temperature (temp), moisture (moist), and their interactions on microbial properties and greenhouse gas efflux from forest soils during an incubation study. Numbers (p-values) in bold indicate significant differences.

May 2009							Pr > F			
Source of variance	Num DF	Den DF	MB	Bac	Fun	B/F	Gneg	Gpos	Aner	
Slp	4	10	0.1094	0.6329	**0.0476**	0.3389	0.7583	0.1738	0.775	
Temp	1	30	0.6043	**0.0007**	0.5159	**0.0133**	**<.0001**	0.2098	**0.0002**	
Slp*Temp	4	30	**0.0262**	0.9723	0.189	0.1451	0.8651	0.8547	0.9372	
Moist	1	30	0.6027	0.1394	0.7059	0.1852	0.0713	0.6437	**0.0491**	
Slp*Moist	4	30	0.3237	0.5746	0.9413	0.8508	0.8893	0.4286	0.8326	
Temp*Moist	4	30	0.4597	**0.0134**	0.9401	0.1517	**0.0181**	0.1416	**0.0108**	
Slp*Temp*Moist	4	30	0.086	0.8366	0.2547	0.4025	0.5047	0.3782	0.5086	
							Pr > F			
			MYC	Sat/Mono	Mono	TOC	TN	N_2O	CO_2	CH_4
Slp	4	10	**0.0414**	0.1691	0.1338	**0.002**	**0.0261**	0.1805	0.6947	0.728
Temp	1	30	0.2809	0.1978	**0.041**	0.742	0.6881	0.7455	**<.0001**	**<.0001**
Slp*Temp	4	30	0.1894	0.1363	0.1147	**0.0143**	**0.0322**	0.3256	0.5381	0.3203
Moist	1	30	0.7997	0.9231	0.6487	0.4817	0.4239	0.8526	0.3382	0.3495
Slp*Moist	4	30	0.9839	0.6263	0.8705	**0.0028**	**0.005**	0.186	**0.0258**	**0.0097**
Temp*Moist	4	30	0.9708	0.4332	0.3424	0.3306	0.4239	0.7833	**0.0501**	0.6255
Slp*Temp*Moist	4	30	0.1307	**0.0238**	**0.0167**	**0.0035**	**0.0164**	0.742	0.5901	0.4362

Microbial biomass (MB), bacteria (Bac), fungi (Fun), bacterial to fungal ratio (B/F), gram negative bacteria (Gneg), gram positive bacteria (Gpos), anaerobic bacteria (Aner), mycorrhizae fungi (Myc), ratio saturated to monounsaturated (Sat/Mono), monounsaturated phospholip fatty acid profiles, and total organic carbon (TOC), total nitrogen (TN), nitrous oxide (N_2O), carbon dioxide (CO_2), and methane (CH_4) based on, slope position (Slp), temperature (Temp), and moisture (Moist). Samples were taken in July and November 2008, and May 2009 along five landscape positions. Significant effects are in bold.





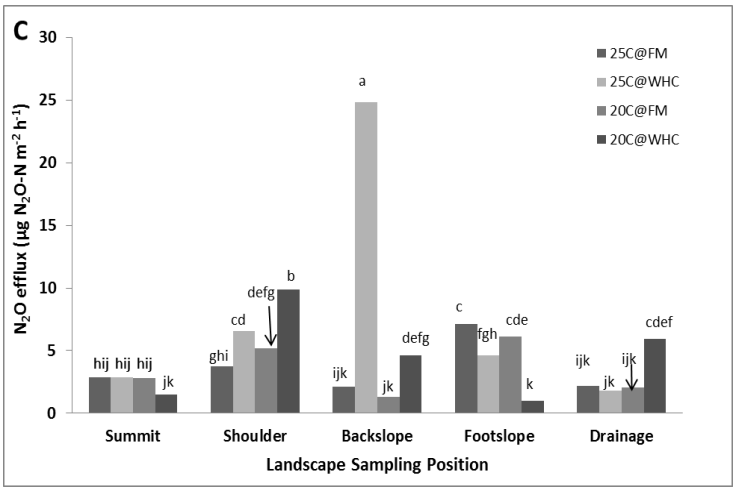
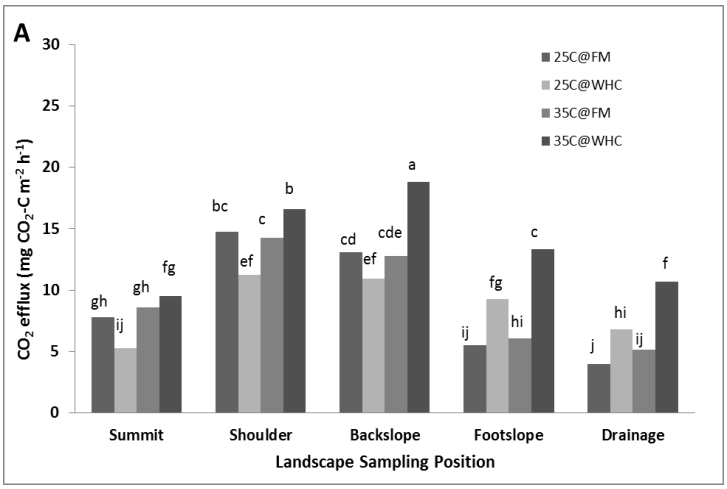
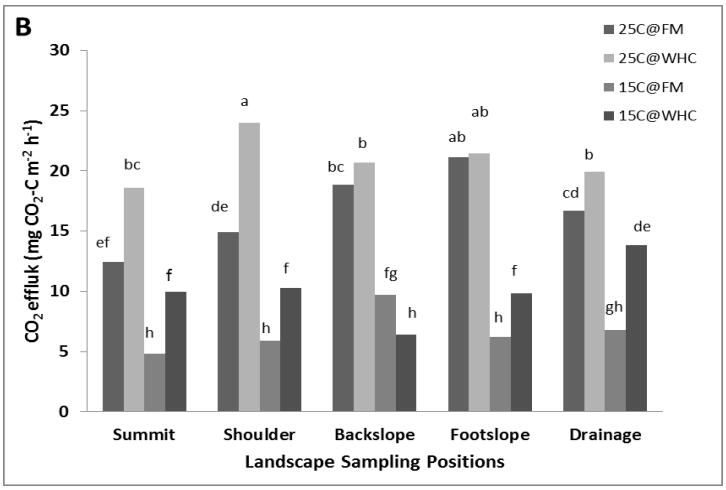


Figure 5.1 Effect of landscape sampling position, temperature, and moisture on N_2O efflux from forest soils sampled in July and November 2008 and May 2009. Soils collected in July 2008 (A) were incubated at either 25^0C or 35^0C; November 2008 (B) 25^0C or 15^0C; and May 2009 (C) 25^0C or 20^0C. In addition samples were either incubated at field moisture (FM) at time of sampling based on gravimetric water determination or 60% water holding capacity (60% WHC). Bar graphs followed by the same letters are not significantly different ($p < 0.05$).





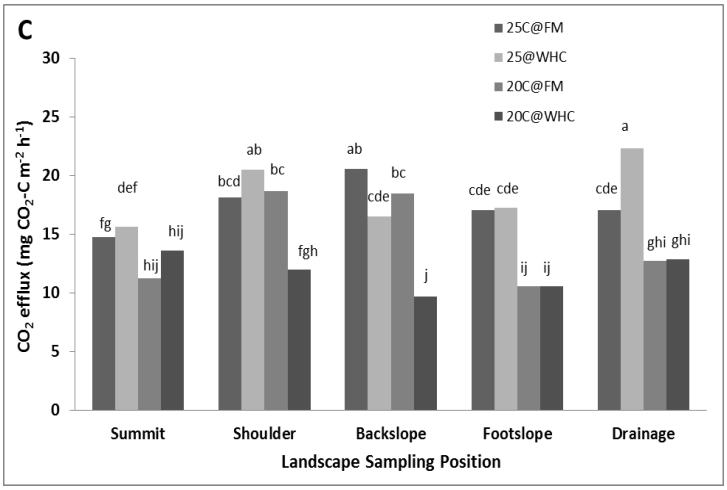
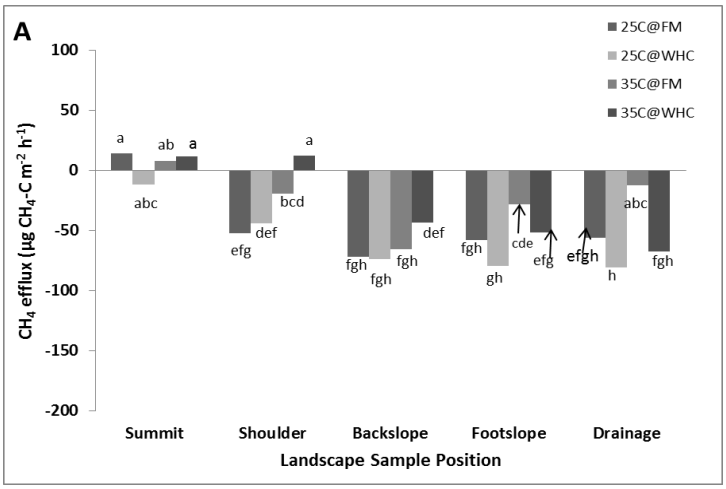
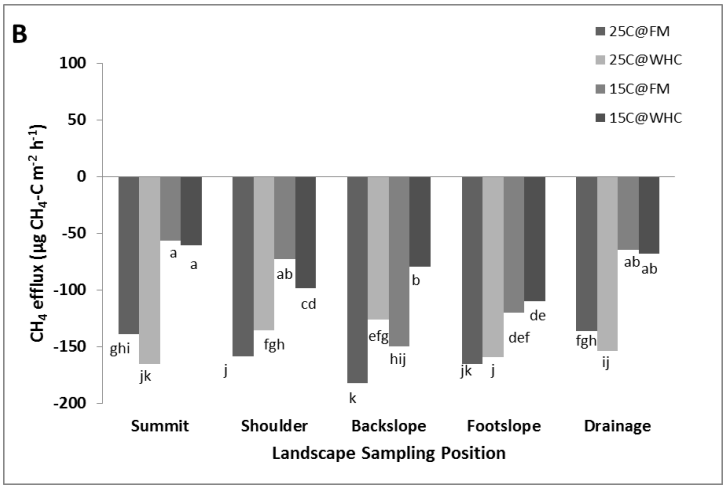


Figure 5.2 Effect of landscape sampling position, temperature, and moisture on CO_2 efflux from forest soils sampled in July and November 2008 and May 2009. Soils collected in July (A) were incubated at either 25^0C or 35^0C; November (B) 25^0C or 15^0C; and May (C) 25^0C or 20^0C. In addition samples were either incubated at field moisture (FM) at time of sampling based on gravimetric water determination or 60% water holding capacity (60% WHC). Bar graphs followed by the same letters are not significantly different ($p < 0.05$).





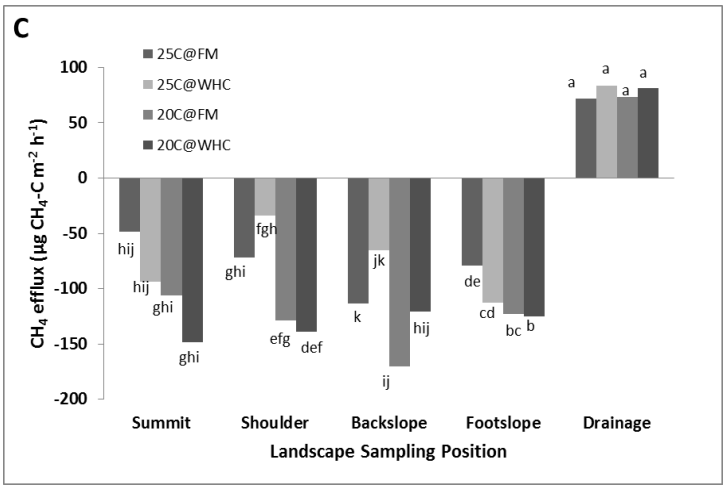
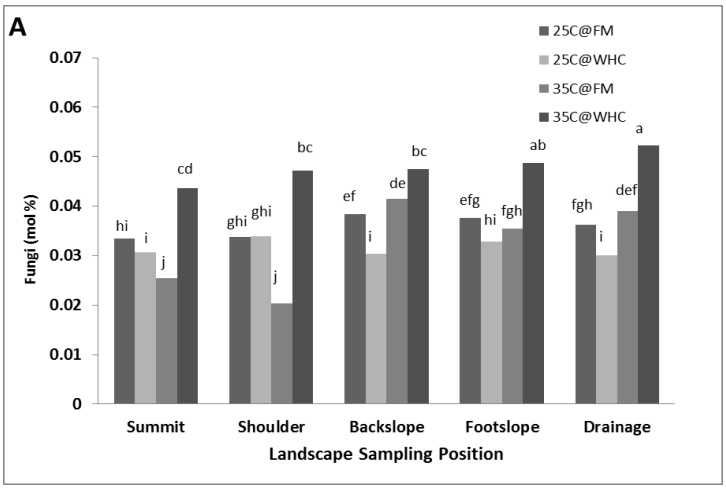
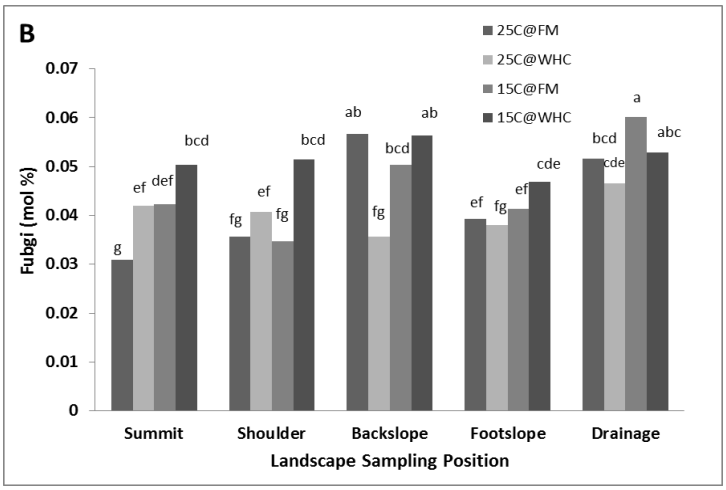


Figure 5.3 Effect of landscape sampling position, temperature, and moisture on CH_4 efflux from forest soils sampled inJuly and November 2008 and May 2009. Soils collected in July (A) were incubated at either 25^0C or 35^0C; November (B) 25^0C or 15^0C; and May (C) 25^0C or 20^0C. In addition samples were either incubated at field moisture (FM) at time of sampling based on gravimetric water determination (FM) or 60% water holding capacity (60% WHC). Bar graphs followed by the same letters are not significantly different ($p < 0.05$).





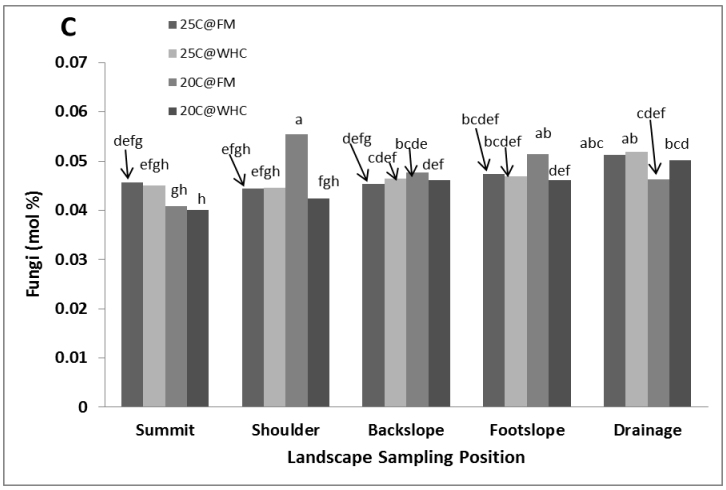
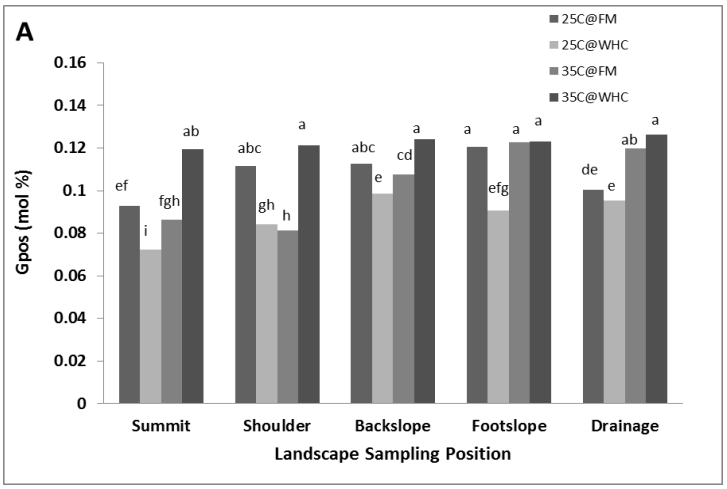
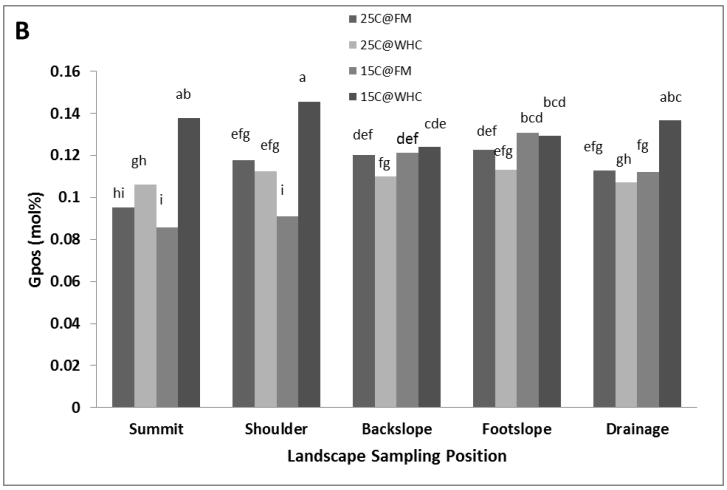


Figure 5.4 Effect of landscape sampling position, temperature, and moisture on fungi biomarkers from forest soils. Sampling was performed three times over a one year period (July and November 2008 and May 2009). Soils collected in July (A) were incubated at either 25^0C or 35^0C; November (B) 25^0C or 15^0C; and May (C) 25^0C or 20^0C. In addition samples were incubated at either field moisture (FM) at time of sampling based on gravimetric water determination or 60% water holding capacity (60% WHC). Bar graphs followed by the same letters are not significantly different ($p < 0.05$).





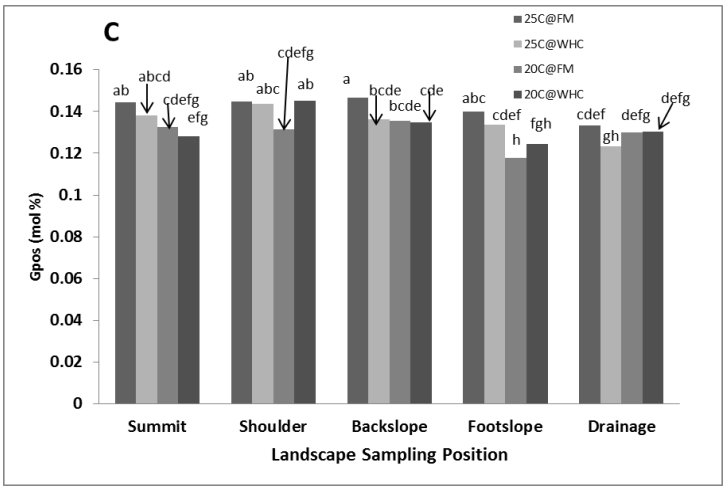


Figure 5.5 Effect of landscape sampling position, temperature, and moisture on Gram positive bacteria biomarkers from forest soils. Sampling was done three times over a one year period (July and November 2008 and May 2009). Soils collected in July (A) were incubated at either 25^0C or 35^0C; November (B) 25^0C or 15^0C; and May (C) 25^0C or 20^0C. In addition samples were incubated at either field moisture (FM) at time of sampling based on gravimetric water determination or 60% water holding capacity (60%WHC). Bar graphs followed by the same letters are not significantly different ($p < 0.05$).

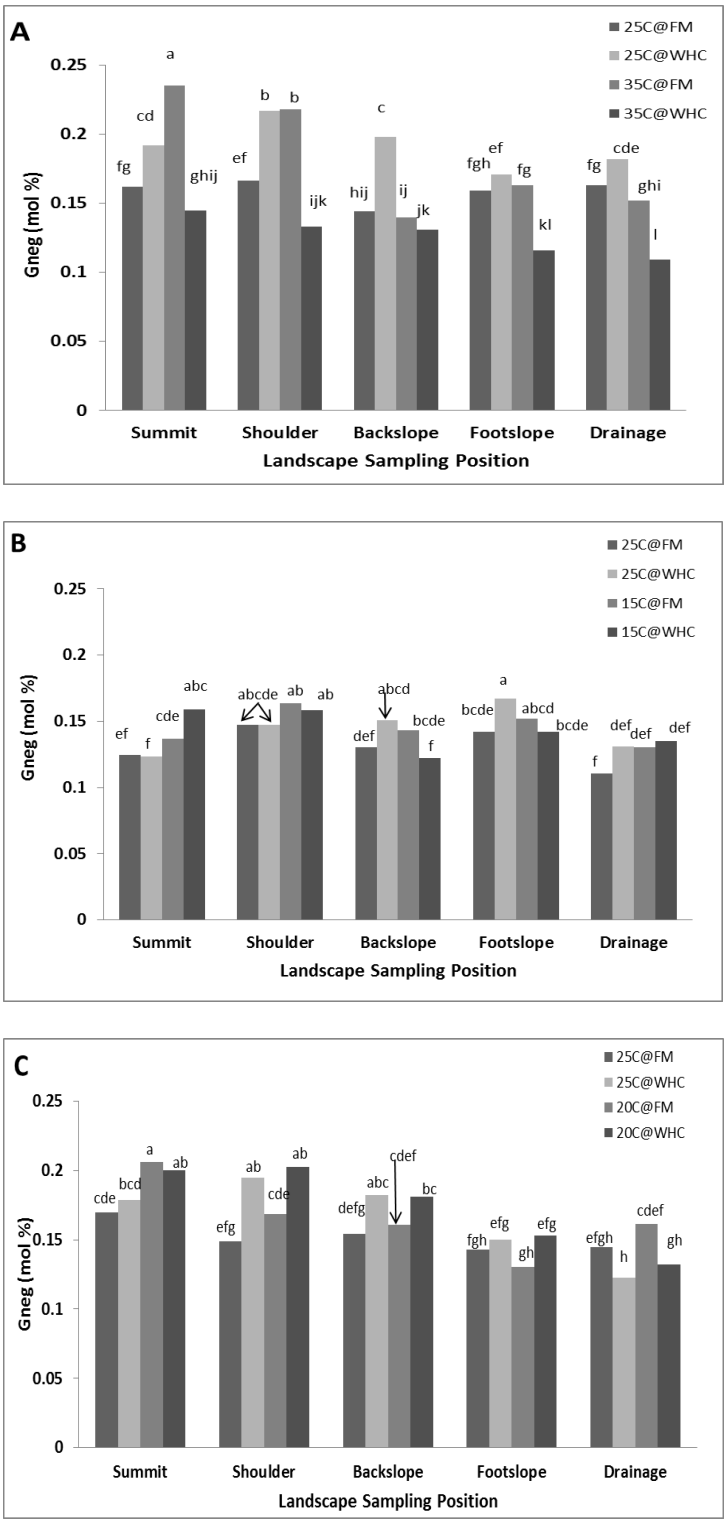


Figure 5.6 Effect of landscape sampling position, temperature, and moisture on Gram negative bacteria biomarkers from forest soils. Sampling was performed three times over a one year period (July and November 2008 and May 2009). Soils collected in July (A) were incubated at either 25^0C or 35^0C; November (B) 25^0C or 15^0C; and May 25^0C (C) or 20^0C. In addition samples were either incubated at field moisture (FM) at time of sampling based on gravimetric water determination or 60% water holding capacity (60% WHC). Bar graphs followed by the same letters are not significantly different ($p < 0.05$).

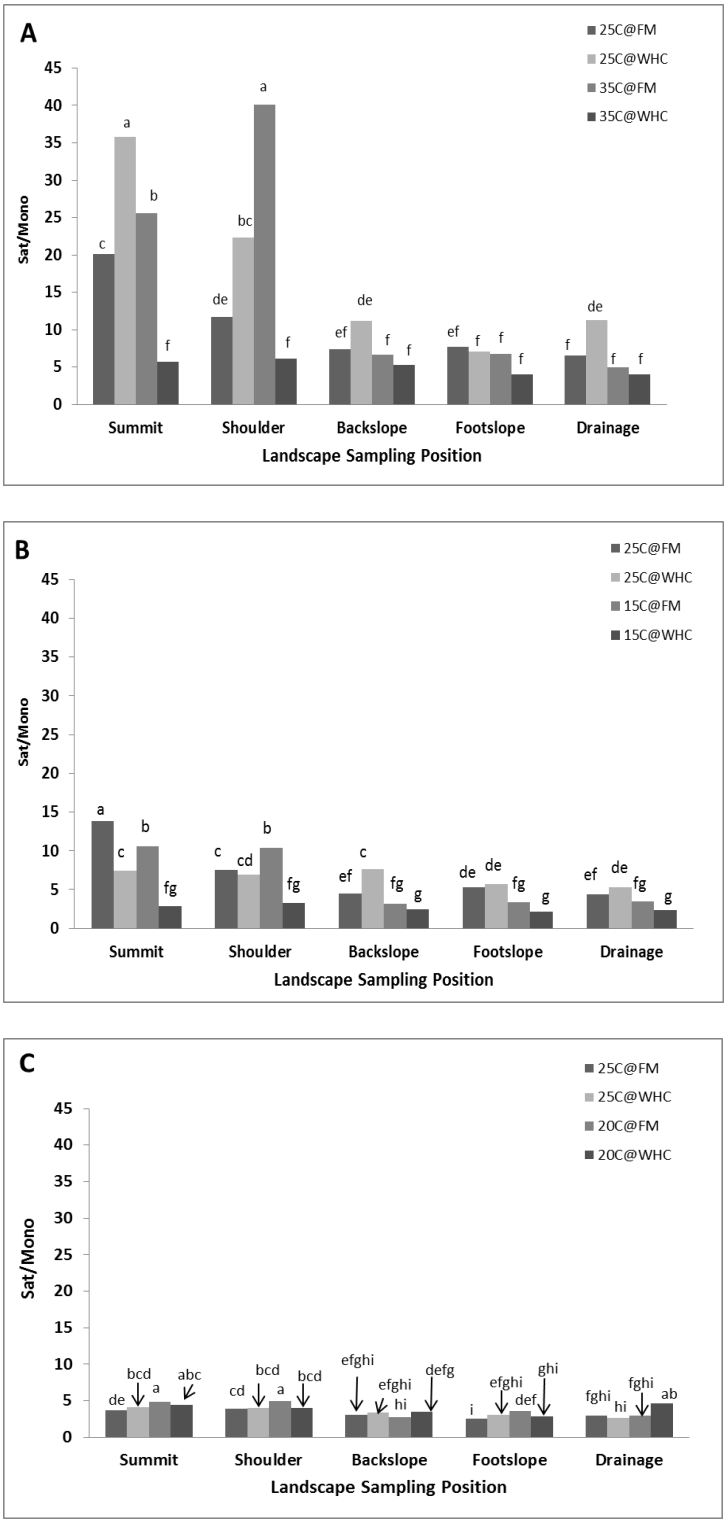
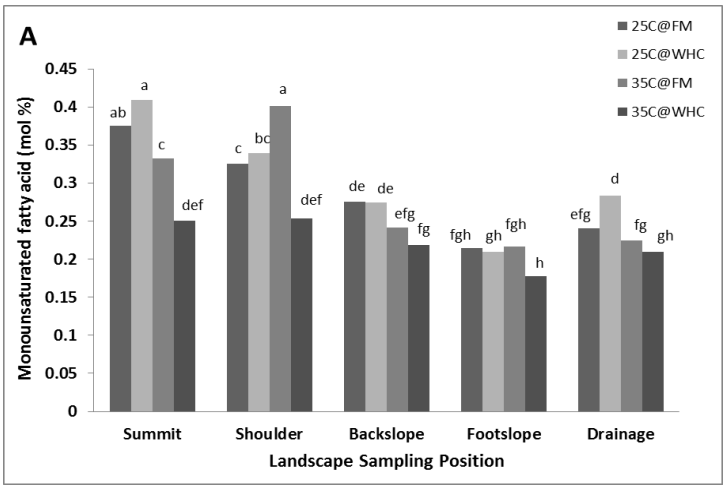
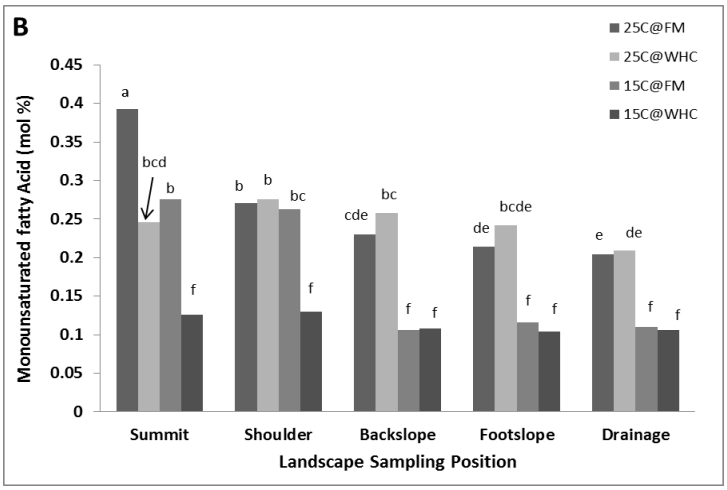


Figure 5.7 Effect of landscape sampling position, temperature, and moisture on stress indicators (Sat/Mono) from forest soils. Sampling was performed three times over a one year period (July and November 2008 and May 2009). Soils collected in July (A) were incubated at either 25^0C or 35^0C; November (B) 25^0C or 15^0C; and May (C) 25^0C or 20^0C. In addition samples were either incubated at field moisture (FM) at time of sampling based on gravimetric water determination or 60% water holding capacity (60% WHC). Bar graphs followed by the same letters are not significantly different ($p < 0.05$).





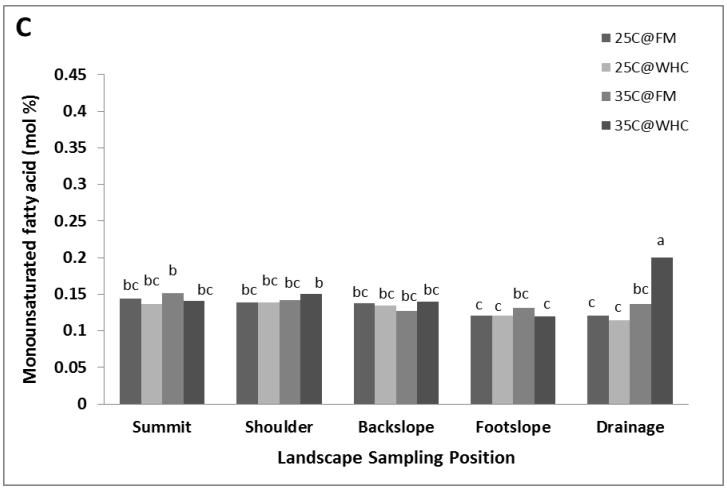


Figure 5.8 Effect of landscape sampling position, temperature, and moisture on monounsaturated fatty acid biomarkers from forest soils. Sampling was done three times over a one year period (July and November 2008 and May 2009). Soils collected in July (A) were incubated at either 25^0C or 35^0C; November (B) 25^0C or 15^0C; and May (C) 25^0C or 20^0C. In addition samples were either incubated at field moisture (FM) at time of sampling based on gravimetric water determination or 60% water holding capacity (60% WHC). Bar graphs followed by the same letters are not significantly different ($p < 0.05$).

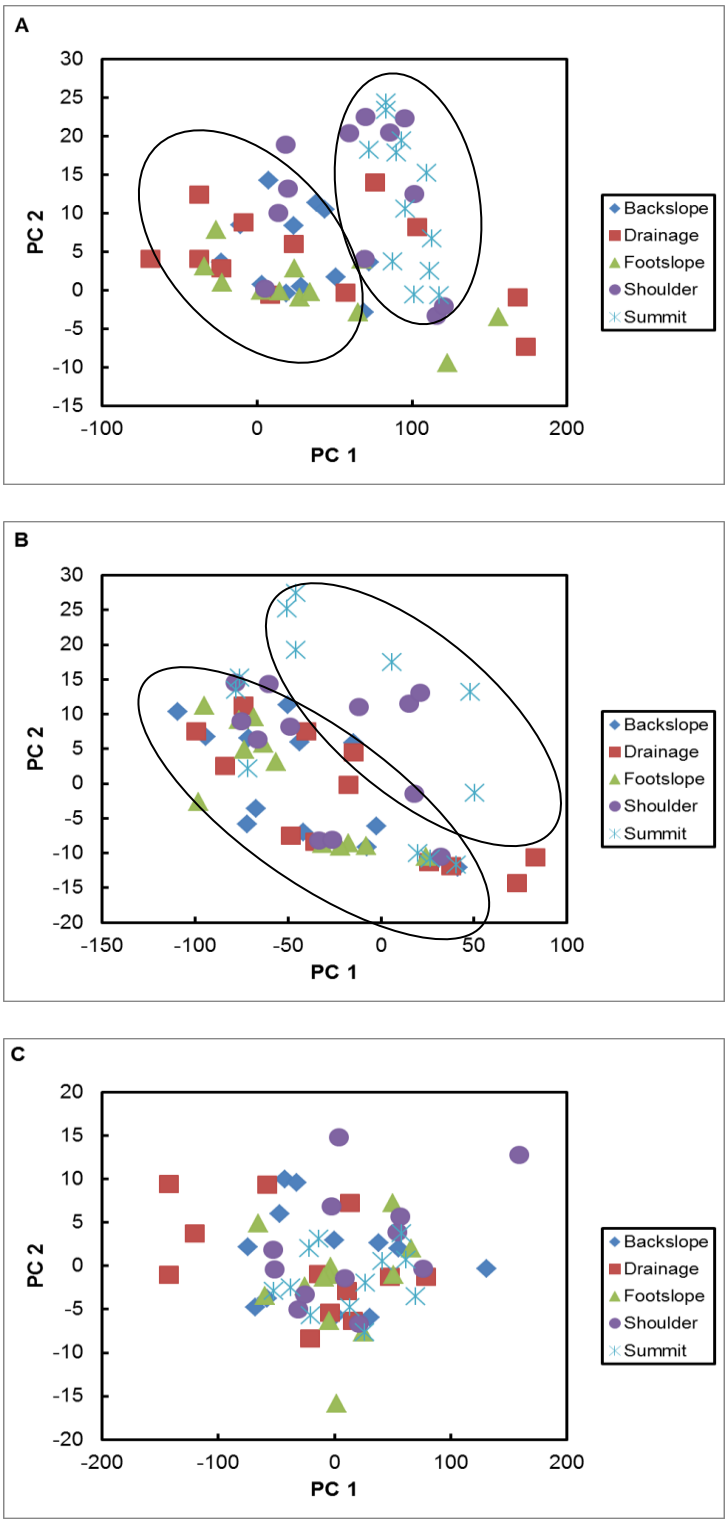


Figure 5.9 Ordination results from canonical analysis of GHG (N_2O, CO_2, CH_4) and PLFA chains from forest soils after 30 d incubations. Sampling was performed three times over a one year period (July and November 2008 and May 2009). Soils collected in July (A) were incubated at either 25^{0}C or 35^{0}C; November (B) 25^{0}C or 15^{0}C; and May (C) 25^{0}C or 20^{0}C. In addition samples were either incubated at field moisture (FM) at time of sampling based on gravimetric water determination or 60% water holding capacity (60% WHC).

Chapter 6

Using PCR-DGGE to assess soil microbial communities associated with greenhouse gas efflux from a secondary forest in central Missouri

Abstract

Soil microorganisms are involved in almost all soil processes, mediating soil organic matter decomposition and nutrient cycling; and are also involved in greenhouse gas (GHG) dynamics between the soil and atmosphere. Soil properties and field conditions such as topographic position also influences GHG activity. Therefore, understanding the spatial and temporal distribution of soil bacteria and fungi will advance our understanding of the role these organisms play in GHG efflux from forest soils. In our research we collected samples three times over a one year period (July and November 2008 and May 2009) from across the landscape. Samples were incubated at different temperature and moisture over a 30 day period to determine how topographic position influenced soil microbial community and GHG efflux. Soil microbial diversity was evaluated using polymerase chain reaction (PCR) based denaturing gradient gel electrophoresis (DGGE) and real-time PCR analysis. Our research revealed temporal differences in microbial population and GHG efflux; indicating that time of year when samples were collected is important. Temperature also had a significant effect on soil microbial population and GHG efflux. Warmer incubation temperatures generally had greater *Fusarium* population and greater GHG efflux for all three sampling periods. Moisture also impacted some GHG measurements; however the influence was not as great as the temperature effect. In addition, correlation between GHG and threshold cycle and GHG and soil temperature and moisture in the incubated soils implied that microbial properties as well as soil temperature and moisture affected GHG efflux from

these forest soils. However, the low R values and the lack of correlation within some sampling periods indicated that the relationship among soil microorganism, soil conditions and GHG efflux is highly complex; and cannot be fully explained by direct correlations among the measured properties and GHG efflux.

Introduction

Continued increases in GHG efflux due to anthropogenic activities are speculated to contribute significantly to global warming over the next few decades (Smith et al., 1998). The rates of GHG increase are estimated at 0.5%, 0.75% and 0.75% $year^{-1}$ for CO_2, N_2O and CH_4 respectively (Paul and Kimble, 1995). Soil and the microbial processes within soils are major drivers of GHG dynamics (MacDonald et al., 1995; Paul and Kimble, 1995; Cao et al., 2006; Conrad, 1996). The type of production systems established on a landscape also dictates the efflux of GHG within an area (Haider and Schaffer 2009). For example agricultural soils under conventional management regimes may contribute more GHG to the atmosphere than land in natural vegetation (van Hees et al., 2005). Natural habitats are most often sinks for GHG; however, they can also act as sources for GHG entering the atmosphere (Haider and Schaffer, 2009).

Soil microbial communities reflect the conditions such as nutrient availability, temperature, and moisture (Petersen and Klug, 1994; Petersen et al., 2002) of the soil environment in which they live. Additionally, microorganisms influence their environment (Ajwa et al., 1999) by mediating nutrient cycling and other soil processes (Keith-Roach et al., 2002). Soil biological properties and their susceptibility to changes in the environment are reported to be good indicators of soil quality (Bending et. al., 2000). Therefore understanding the biological and biochemical processes of the soil are paramount for reliably assessing the intricate relationships between soil communities and greenhouse gas effluxes.

Soil communities include bacteria, archeabacteria, actinomycetes, fungi, and other soil flora and fauna. Soil bacteria tend to be most dominant in terms of numbers (Haider

and Schaffer, 2009; Sylvia et al., 2005) and species (Haider and Schaffer, 2009); however, soil fungi often account for a greater biomass (Sylvia et al., 2009) especially in forest soils (Giller et al., 1997). Although microscopic in size, the influence of soil bacteria and fungi are often exerted at the macro-scale, and are known to influence their surrounding environment (Dighton et al., 2005).

Although the importance of bacteria in soil environments is well established (Sylvia et al., 1999; Tate, 1995) we are still lacking information on their overall role in GHG efflux. Rosch et al., (2002), McLain and Martens (2005) discussed the role of bacteria in nitrogen cycling and N_2, N_2O and NO efflux between soils and the atmosphere through the nitrification and denitrification processes. Carbon dioxide emissions due to soil respiration and substrate mineralization (Thompson et al., 2010) are often used to estimate microbial activity. Similarly, methane oxidations by soil organisms have been used to estimate the potential of the soil to act as a sink for methane (Kumaresan et al., 2009; Dunfield et al., 2007). However, the specific contribution of the different groups of bacteria to GHG efflux has still not been fully investigated or understood.

Additionally, topography influences GHG efflux (Nishina et al., 2009) and microbial distribution (Florinsky et al., 2004) within a landscape. However, there is still uncertainty as to how spatial distribution of bacteria due to topographic influences affects GHG efflux from forest soils. Characterizing bacterial communities in forest soils will undoubtedly add unique information on the influence of topography and the role of bacteria in GHG efflux from forest soils.

Although effectively used in the past to study bacterial communities, drawbacks with cultivation-based techniques have been noted (Smalla et al., 2001; Morris and

Robertson, 2005; Filion et al., 2003). Firstly, only a small sub-section (≤ 10%) of bacterial population can be recovered from soils using culture-based techniques (Filion et al., 2003). In addition, the time and cost associated in isolating and identifying different species using culturing procedures is prohibitive (Dighton et al., 2005). The used of molecular based techniques have gained prominence in microbial work (Miller et al., 2009; Frostegard et al., 1999; Thompson et al., 2010), because a fairly rapid analysis of large number of samples in comparatively short time can be accommodated (Smalla et al., 2007; Smalla et al., 2001; Rosch et al., 2002). The use of PCR and DGGE to analyze 16S rDNA extracted directly from soil and environmental samples has enhanced the ability of researchers to study spatial and temporal variations in bacterial populations (Dunbar et al., 2001).

Fungi are noted for forming symbiotic relationships with plants, which enhance plant growth and survival by providing additional surface area for nutrient and water uptake (Morris and Robertson, 2005). Fungi are also major players in carbon cycling in forest ecosystems (Rajput and Rao, 2007); as precursors in the decay of woody material (Walkin et al., 2008) and, as primary decomposers of litter (Hättenschwiler et al., 2005), they are important in organic matter turn-over in temperate forests. Species such as *Fusarium graminerium*, *F. proliferatum* from the Ascomycota phylum are noted for their ability to degrade woody materials (Regalado et al., 1997) inclusive of lignin.

Traditionally, *Fusarium* species are world renown for economic losses associated with crop disease infestations and mycotoxins in food and feed (Walkin et al., 2008). More recently, species from the genus *Fusarium* have received renewed attention for their role in denitrification (Shoun et al., 1991; Takaya and Shaun, 2000), an indication

that *Fusarium* spp. could potentially impact GHG emissions to the environment. Subsequently, Laughlin and Stevens (2002), Tayaka et al. (2002), and Shoun et al. (1992) have associated *Fusarium* spp. with GHG efflux. The full role of *Fusarium* in GHG efflux is however under-explored. Although *Fusarium* spp. are generally abundant in soils, the taxonomy of the *Fusarium* genus is currently in a state of flux; however, with advancement in molecular techniques, differentiation into new species is continuously occurring (Summerell et al., 2003).

The distribution of *Fusarium* spp. in forest soils is therefore still in need of further investigation both at the spatial (Morris, 1999) and temporal (Gömöryová, 2004) scales, especially in regards to their relationship with GHG efflux. Further investigation is necessary if we are to provide answers regarding the relationship between *Fusarium* populations and GHG efflux. Some of the unanswered questions include: How does *Fusarium* species diversity influence GHG efflux? How does topography affect the distribution and function of *Fusarium* spp. within a landscape and, by extension, GHG efflux? To answer these questions we propose to use molecular based techniques to quantify and characterize *Fusarium* spp. in a forest site under evaluation for GHG efflux.

Morris (1999) and Morris and Boerner (1999) have demonstrated spatial variability in soil microbial properties in Ohio and concluded that vegetation, moisture, and landscape position were among the factors influencing biological distribution within various ecosystems. However, they were concerned about the influence of spatial scale and sampling design on distribution and function of soil microbiota. Other researchers (e.g., Morris and Robertson, 2005; Filion et al., 2003; Jensen et al., 2000) have expressed

their concern with the type of assessment used to quantify and characterize microbial population within soil systems.

Conventional cultural techniques and direct microscopic counts have successfully been used to identify and quantify microorganisms from soils (Morris and Robertson, 2005), however, only a small portion of the soil microbial population is assessed (Filion et al., 2003). Microorganisms in general (Jensen et al., 2000), and fungi in particular, are often difficult to propagate from soil, with some species having very specific growth requirements (Filion et al., 2003). The large number of different growth media that would be required to isolate the various groups of soil microorganisms (Dighton et al., 2005) renders the task virtually impossible.

One molecular technique, DGGE is useful in microbial ecology studies when combined with other techniques. The DGGE technique is based on the separation of polymerase chain reaction (PCR) - amplified gene fragments, not according to size, but owing to variation in targeted nucleotide sequences (Hasting, 1999), which influences migration characteristics of the molecule when subjected to chemical gradients and an electrical field. Another useful technique recently adapted to quantify targeted microbial populations including *Fusarium* spp. is real-time PCR (see Schena et al., 2004; Filion et al., 2003; Wakelin et al., 2008; Yergeau et al., 2005). Amplicons from conventional PCR can be separated on agarose gels to verify the PCR protocol successfully amplified the target genes (Thompson et al., 2010). However, the information obtained is not quantitative. Real time PCR (RT-PCR or qPCR) detects and measures the accumulation of amplified products as the reaction progresses, which allows for quantification of the targeted genes. Measurement is based on the inclusion of a florescence molecule that

increases in signal strength with proportional increases in DNA copy number. Specialized thermal cyclers able to detect the fluorescence monitor the amplification as it progresses over a known number of amplification cycles. Real time PCR expands the information obtained from conventional PCR as it is able to determine the starting template copy number of genomic DNA. This allows for both qualitative (absence/presence) and quantitative (number of DNA templates) evaluation of samples (Li et al., 2010).

We hypothesize that quantifying the *Fusarium* community using real-time PCR, and characterizing the bacterial community using PCR-DGGE profiles will provide information that will better allow us to understand relationships between soil microorganisms and GHG efflux from forest soils. This section of research involved investigation of relationships of differences in bacterial and fungal diversity along a landscape with GHG efflux.

Materials and Methods

Selected sub-samples of soils from the incubation study described in previous chapters were used to characterize the soil microbial community. In brief, the experiment used a two by two factorial design to determine how GHG efflux and soil microbial communities are affected by two factors, temperature and moisture. Soil samples were collected in July 2008, November 2008, and May 2009 to represent summer, fall, and spring seasons respectively. Samples from each collection date were incubated at the temperatures and moisture reflecting conditions at the time of sampling (seasonal conditions). Thus, samples collected in July 2008 where incubated at 35 ^{0}C (JSP); November 2008 samples at 15^{0}C (NSP); and May 2009 samples at 20^{0}C (MSP). In

addition during each sampling period (SP), subsets of samples were simultaneously incubated at 25^0C (control temperature). During each SP, samples were either kept at the moisture content at which they were collected in the field (based on gravimetric moisture determination) or at 60% WHC (control conditions). Assumed optimum conditions for soil microbial activity were maintained at 25^0C and 60% WHC (Horwath and Paul, 1994; Tate, 1995).

Bacterial extraction, PCR amplification and DGGE analysis

Total DNA was extracted from 0.5 g air-dried soil using the MoBio Soil Isolation Kit (MoBio Laboratories, CA) following the manufacture's protocol. Amplification of soil bacterial DNA was done following the protocol outlined by Hastings (1999). Universal bacterial primers (F984GC-R1378) which target 16S rDNA at positions 968 – 1401 bp (*E.coli* rDNA sequence) were used for PCR amplification (Heuer and Smalla, 1997). The final volume of the PCR mixture was 50 µl (i.e. 20 pmol of each primer, 10 – 25 ng DNA template, and 2x Red TaqReadyMix (Sigma-Aldrich Co., St. Louis, MO)). The PCR was performed in an Eppendorf Mastercycler Thermal Cycler (Perkin-Elmer, Norwalk, CT); using the following program 94^0C for 4 min, followed by 35 cycles of 94^0C for 1 min, 55^0C for 1 min, 72^0C for 2 min, and a final extension at 72^0C for 10 min, then held at 4^0 C. Product size and yield was confirmed on 1% agarose gel, before performing DGGE.

Denaturing gradient gel electrophoresis was performed using the DCode Universal Mutation Detection system (Bio-Rad) on polyacrylamide gel (8%) with a 35 – 55% urea formamide gradient at 160V for 6 h. After electrophoresis gels were stained

with SYBER Green I nucleic acid gel stain (Molecular Probes, OR) and photographed and digitized using the GeneGenius Gel Documentation System (Syngene, MD).

Fungal DNA extraction, *Fusarium* PCR amplification, and Real-Time PCR quantification

Total DNA was extracted from 0.25 g moist soil using the PowerSoil™ DNA isolation DNA kit following manufactures protocol (MoBio Laboratories Inc.). A slight modification was made to step three for fungal DNA extraction after the addition of the anionic solution (C1), samples were placed in a heating block at 65^0C for 15 min to enhance the lysing process. In addition, mechanical lysing of the samples was done on a FastPrep cell disruptor (Bio 101) instead of on a vortex.

Polymerized chain reaction (PCR) was done using a PTC-200 Thermal Cycler (MJ Research) specifically for *Fusarium* spp. following the nested approach of Yergeau et al. (2005) with modifications suggested by Walkin (personal communication). PCR was carried out in 25 µl volumes which included 5 µl of DNA template, 1 µl of each primer (EF1 & EF2 for 1st PCR; Alfie 1 and alfie 2 for 2nd PCR); 0.5 µl of 10 mM dNTP mixture; 0.2 µl of Taq DNA polymerase; 2.5 µl 10x reaction buffer; and the remaining 14.5 µl Millipore water. The sequence for the primers were EF1: 5' ATG GGT AAG GAR GAC AAG AC 3'; EF2 5'GGA RGT ACC AGT SAT CAT GTT 3'; Alfie1: 5' CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GTC GTC ATC GGC CAC GTC GAC TC 3'; Alfie2: CCT TAC CGA GCT CRG CGG CTT C 3'. The underlined section in Alfie1 primer indicates the GC clamp. For fungal DNA, the annealing temperature and amplification cycles were based on Wakelin et al. (2008) using the nested PCR approach of Yergeau et al. (2005). One cycle of 94^0C for 5 min,

one cycle at 95^0C for 45 sec, one cycle at 50^0C for 45 sec, and one cycle at 72^0C for 45 sec were use for initial denaturing, followed by 35 cycles of denaturing at 95^0C for 45 sec and a final cycle at 72^0C for 5 min was used for the first PCR. The second PCR cycles were 94^0C for 5 min, 95^0C for 1 min, 67^0C for 1 min, and 72^0C for 1 min; followed by 35 cycles at 95^0C for 1 min and a final extension cycle of 72^0C for 20 min.

Amplified PCR products were verified on 2% (w/w) agarose gel by electrophoresis. Agarose gel was prepared and ran in 1x TAE buffer. A 100 bp DNA molecular size ladder was used to estimate size of amplified product. Imaging of gel was performed using a Digital Gel Logic 4 camera (Kodak®).

Real-Time PCR amplification (qPCR)

The 1^{st} round PCR in the nested procedure used in the real-time PCR was similar to that describe above, except that the number of amplification cycles were reduced to 15. The product from this 1^{st} run (EF1/EF2 primers) PCR was used in the 2^{nd} round using the Alfie1 and Alfie2 primers. However, the GC clamp used previously on the Alfie1 primer was not used in the qPCR. The cycles used consisted of a denaturation step at 95^0C for 3 min and 40 cycles at 95^0C for 15s and a final cycle of 60^0C for 1 min. The annealing temperature for the qPCR was 65^0C. The reaction conditions such as volume of reaction, annealing temperature, primer concentration and amplicon temperature for measuring the fluorescence signal were maximized experimentally to optimize the qPCR protocol. Samples were run in triplicate on a iCycler IQ ThermalCycler (Bio-Rad) using iQ SYBR Green Supermix (BioRad).

Standard curves based on threshold cycles (Ct) for 10-fold dilution series of fungal genomic DNA ($1x10^0$, $1x10^{-1}$, $1x10^{-2}$, $1x10^{-3}$, $1x10^{-4}$ and $1x10^{-5}$ ng μl^{-1}) were

constructed for *Fusarium* cultures. The standard curve was generated by plotting the log DNA concentration (ng) against the Ct values obtained from amplification of each dilution. Threshold cycle of a reaction is based on the starting quantity of the DNA template during amplification. The higher the starting quantity the lower the threshold cycle required to obtain a signal for the reaction. This allows for quantification of DNA amplification in qPCR compared to qualitative data collected via conventional PCR.

Statistical Analysis

The data was analyzed using PROC MIXED in SAS version 9.2 (SAS 2008). ANOVA was performed to test the null hypothesis that landscape position, temperature and moisture do not have significant seasonal influence on *fusarium* population in forest soils. LS MEANS was used to compare means; and Pearson correlation (PROC CORR) was used to determine relationships among threshold cycles and GHG efflux.

Results

Bacteria DNA content and DGGE profiles

Figure 6.1 shows 16S rRNA gene fragments from three incubation periods that were resolved as DGGE patterns. Position and intensity of bands on DGGE gel as measured by GeneTool software were interpreted as distinct bacterial genotypes. The intensity of each band was further assumed to be the numerical abundance of each operational taxonomic unit for that genotype (Wakelin et al., 2008). Genotypic composition of the soil bacterial communities were compared within each sampling period among the different positions along the landscape where samples were collected (Figure 6.1). The DGGE banding patterns from our incubated samples showed similar strong bands for almost all landscape sampling locations within each sampling period.

Although, visual inspection of the DGGE gels showed apparent differences in the number of bands and band intensity collected at different position along the landscape, statistical analysis of number of bands revealed no significant differences among these locations (see Appendix 2). Additionally, the most dominant bands based on intensity were common in all sampling periods and at all sampling locations within each sampling period (Figure 6.1). When subjected to ANOVA, the numbers of bands were revealed to be significantly affected by temperature, moisture and the interactions (Appendix 3). Overall samples incubated at "seasonal" temperatures 35^0C (JSP), 15^0C (NSP), and 20^0C (MSP) generally had greater number of bands than the control temperature (25^0C) within each sampling period. Also, there was a tendency for samples incubated at 60% WHC to have greater number of bands than samples incubated at FM conditions.

Fusarium PCR banding patterns and qPCR quantification

Fusarium DNA isolated from the different landscape sampling positions in each sampling period was successfully amplified by the nested PCR described above and verified on 2% agarose gel (Figure 6.2). Gel analysis from the different sampling periods produced a single band (~600bp) indicating that *Fusarium* DNA was successfully amplified in all the samples studied.

Real time PCR was then conducted using the same primers EF1/EF2 and Alfie1/Alfie2 to complement results obtained by conventional PCR. This enabled us to quantify *Fusarium* DNA within our samples based on threshold cycles (Ct). Threshold cycles from *Fusarium* DNA amplification varied, although not significantly, during each sampling period. Soils for JSP had the greatest Ct value; whereas the least Ct value was observed in NSP. To further investigate the main effects and there interactions within

each sampling period, independent analyses were subsequently performed for each sampling period (JSP, NSP, and MSP).

Threshold cycle (Ct) values were significantly affected by the main effect of temperature and by the interaction of temperature, moisture, and landscape position of soils sampled during NSP (Figure 6.3 c and d). Although only significant for NSP (Figure 6.3 c & d), the tendency in all three sampling periods was for Ct values to be inversely related to temperature (Figure 6.3). This corresponds to other findings in this study where phospholipid fatty acid profiles were significantly affected by temperature (see Chapter 5). For NSP and MSP the samples incubated at the control temperature (25^0C) had greater Ct values than samples incubated at 15^0C (NSP) and 20^0C MSP; whereas in JSP the 35^0C incubation temperature had greater Ct values than the control temperature. There was no discernable pattern to the differences in Ct values in regard to the position along the landscape where samples were taken (Figure 6.3). For example during JSP, samples taken from the footslope and incubated at the 25^0C control temperature and at 60% WHC had significantly greater Ct values than all other landscape sampling positions incubated at 25^0C. On the other hand, samples collected from the shoulder and incubated at the same temperature (25^0C) and moisture (60% WHC) exibited smallest Ct values of all samples for JSP (Figures 6.3 a & b). Significant variations in Ct values were also observed for NSP, both between samples from the same landscape sampling positions as well as across landscape sampling position. For example, soils collected from SS and incubated at FM significantly differed from SS samples adjusted to 60% WHC and incubated at 25^0C; however, when SS samples were

incubated at 15^0C, no significant difference between the Ct values of FM and 60% WHC were detected.

GHG Efflux

Greenhouse gas efflux varied significantly depending on the conditions under which samples were incubated. For example, N_2O efflux was greatest for MSP and was significantly greater than N_2O flux from JSP and NSP (Table 6.1). Carbon dioxide effluxes also differed significantly for all three sampling periods; the greatest CO_2 efflux occurred for NSP and was significantly greater than JSP and MSP (Table 6.1). In contrast, methane was least for NSP and was significantly less than both JSP and MSP (Table 6.1).

Each sampling period was analyzed separately to determine how incubation temperature and moisture influenced *Fusarium* populations in soils collected from different landscape position within each sampling period. For NSP the main effects of temperature and moisture and the three-way interaction of landscape sampling position, temperature, and moisture were significant for N_2O efflux (Figure 6.3). Samples incubated at 25^0C generally had greater N_2O efflux than samples incubated at 15^0C, 20^0C, or 35^0C. Similarly, samples incubated at 25^0C had generally greater CO_2 efflux than samples incubated at the "seasonal" temperatures of 15^0C, 20^0C, or 35^0C (Figure 6.4). For both gases incubation at 60% WHC also trended greater GHG efflux than FM. Unlike the other two GHG, CH_4 efflux showed no clear pattern in regard to temperature or moisture (Figure 6.5).

GHG Correlations

Pearson's correlation analysis showed significant correlations between Ct values and CO_2 efflux in JSP ($r = -0.34$; $p<0.05$) and NSP ($r = 0.34$; $p<0.05$). Significant correlation was also observed between Ct values and CH_4 in MSP ($r = -0.45$; $p <0.05$). The GHG also significantly correlated with each other for JSP and MSP; CO_2 correlated with both CH_4 and N_2O for NSP; there was also significant correlation between CH_4 and N_2O for NSP (Table 6.2). Correlation also occurred between CO_2 and N_2O and CO_2 and CH_4 for JSP and NSP; however, there was no significant correlation between N_2O and CH_4 for JSP. In addition, N_2O and CH_4 were significantly correlated in NSP (Table 6.2). During MSP no significant correlations were observed between GHG and Ct values (Table 6.2).

Discussion

Bacteria

Molecular techniques have proven to be effective in analyzing microbial communities in soil and environmental samples (Neufeld et al., 2006). Culture methods reflect the section of the population that readily grows on artificial media, which may not be the dominant population in numbers or functionality (Neufeld et al., 2006). Molecular techniques such as DGGE are able to characterize communities that would otherwise have limited or no growth on defined media (Bridge and Spooner, 2001). In our research PCR-based DGGE profiling was able to identify multiple bacterial bands from the different sampling locations (Figure 6.1); and also define the distribution of the bacterial species based on differences and similarity in band intensity reflected by the respective sampling locations (Figure 6.1). Bacterial populations in this incubation study did not

show significant differences due to the position along the landscape where the samples were collected. Likely, this is due to high soil microbial diversity at any given site which makes statistical differentiation between communities at the same site challenging. In addition, vegetation often greatly influences soil microbial community composition (Nusslein and Tiedje, 1999). Similarity in vegetation throughout our sampling area may have selected for groups of organisms adapted to the environment in which the samples were collected (Nusslein and Tiedje, 1999).

Fusarium

Standard curves were generated from 10-fold serial dilution of *Fusarium* isolated from pure cultures of *Fusarium graminearum* (USDA-ARS Culture Collection, NCAUR, Peoria. IL) by plotting threshold cycle number (Ct) against log of starting quantity for each dilution (Figure 6.7). The Ct of a reaction is dependent on the amount of template present at the start of the amplification reaction. Large amounts of template at the start of the reaction requires relatively few amplification cycles to detect the threshold fluorescent signal above the background signal. In other words, the lower the starting quantity of DNA template the greater the Ct value required to detect the fluorescent signal. In our research, the standard curves had average R^2 values of 0.998, confirming that there was a linear relationship between the *Fusarium* DNA concentrations of the dilution series and the Ct of the qPCR. This allowed us to use the standard curves in the quantification of DNA from our soil samples. Also the amplification efficiency of the qPCR was within the acceptable range 90 – 105%. This combined with the single amplicons of the melting curve (Figure 6.8) for pure culture extracts and DNA extracted

from our soil samples confirmed the potential of the qPCR method to differentiate *Fusarium* DNA concentration among our samples.

Temporal differences were more pronounced in our research than spatial differences, probably reflecting differences in soil conditions such as suitable carbon substrate, nutrient status and soil moisture between sampling periods. The main effects of temperature and their interactions were significant for almost all the dependent variables in all three sampling periods. In addition comparisons among the sampling periods also identified significant differences in the Ct values of each sampling period due to temperature and moisture effects. This would be expected as seasonal fluctuations in soil properties often result in seasonal differences in soil microbial population. Saremi et al. (1999) conducted a greenhouse study and observed that differences in *Fusarium* population density were related to temperature; some species appeared more prolific at higher temperatures, while others were more abundant at lower temperatures.

In addition, to their observed differences in *Fusarium* populations due to temperature, Saremi et al. (1999) also postulated, based on the results from other research, that differences in the *Fusarium* population was also related to other factors including the influence of carbonaceous inputs such as root exudates, litter, and crop residue. In incubation studies, Wakelin et al. (2008) demonstrated that differences in the type of crop residue significantly altered soil community structure. In our research, we did not observe any significant differences in the distribution of *Fusarium* DNA concentration (Figure 6.3) due to topography. Similarity in the distribution of tree species across the landscape from which our samples were taken most likely resulted in

highly similar carbon inputs that may have reduced the variability in soil *Fusarium* population along the landscapes where samples were collected.

Landscape sampling position did not significantly affect Ct, CO_2 or CH_4 during any of the sampling periods. The lack of spatial variability of Ct values ($p = 0.171$) among landscape sampling positions possibly indicates the low variation in total *Fusarium* DNA concentration among the different landscape sampling positions. In a field study, Wakelin et al. (2008) found that although residue addition increased soil *Fusarium* concentration, the ability of qPCR to identify significant differences among the different residue amendments was limited by large variability between replicates. Additionally, if spatial heterogeneity within each landscape sampling position is greater than or equal to the spatial variability across the entire sampling area, it becomes difficult to distinguish statistically between microbial communities amongst different landscape positions (Nusslein and Tiedje, 1999). This would be a more logical explanation for soils sampled in our research, since we observed similarly high variability in other parameters measured (see previous chapters).

Temperature and Moisture Effects

Soil moisture and temperature are known to induce changes in microbial populations (Schnürer et al., 1986). In a field experiment Schnürer et al. (1986) observed an increase in bacterial and fungal populations due to increased moisture. Carbon input from plants, i.e., litter throughfall and root exudates, also directly impacts microbial populations. In their research Schnürer et al. (1986) deduced that increases in bacterial populations were directly proportional to the amount of surface litter.

Preliminary results from our field studies showed that soil moisture and temperature influenced soil biological properties and GHG efflux (Hoilett et al., 2008). It was inferred that an indirect association of GHG with soil biology existed considering the similarity of responses to soil physical conditions. Pearson's correlation applied to results of the incubation study further explored the possible relationships among GHG efflux, soil biology, and soil physical properties. Significant correlations were observed among the GHG and also between the GHG and Ct (Table 6.2) in our incubation study. Within each sampling period significant correlations were also identified between soil moisture and temperature and Ct values (Table 6.2). Observed correlations between GHG efflux and Ct values, although low, combined with observed association among GHG efflux and other soil properties indicates possible contribution of soil biology and, probably in this research, *Fusarium* species contribution to the overall dynamics of GHG efflux from forest soils.

Low correlation between GHG and soil biology may partially be explained by high variability in GHG efflux. Greenhouse gas effluxes from soils tend to vary immensely due to spatial heterogeneity in micro-topography, moisture, organic matter etc. (Rayment and Jarvis, 1999; Yanai et al., 2003). In the incubation and field studies, significant correlations were observed between GHG and soil moisture; indicating that soil moisture played an influential role in GHG efflux

Low correlations between GHG efflux and microbial populations found in this study may be related to the diversity of species within functional groups involved in organic matter decomposition and nutrient cycling e.g. denitrifying and nitrifying bacteria (Hättenschwiler et al., 2005; Jones et al., 1994). Functional redundancy is often observed

within and across microbial groups; therefore, the role of both *Fusarium* and bacterial species within our study site may be imbedded in the activity of the much larger group of microorganisms within the soils.

Conclusion

Soil microbial activity is impacted by the soil conditions and environment in which they operate. Soil physical properties including temperature and moisture are known to influence the dynamics of soil microbial populations. The genera or consortia of organisms within a soil ecosystem, e.g. forest systems, are also influenced by the vegetative contribution to soil carbon either through plant residues and or root exudates. In the incubation study, both PCR and DGGE analyses were able to successfully identify differences in microbial populations due to soil physical properties such as temperature and moisture. Results from our study were also in agreement with complementary research which demonstrated links between soil biology and GHG efflux (see Chapters 5 and 6), ascertaining that studying the soil community composition and activity are key factors in understanding gaseous exchange between the soil and atmosphere.

Identification of significant differences between sampling locations along the topography where samples were collected for our study were inconclusive due to variation within the scale of sampling. Differences in band numbers and intensity observed in DGGE profiles suggest possible differences in the microbial populations due to topography. To further explore this possibility we suggest additional assessment of the soil microbial community through cloning and sequencing to identify bacterial species involved in GHG generation and *Fusarium* spp. involved in primary decomposition that are dominant at these locations. In addition, analysis should also be done to better

understand the influence of scale on soil microbial associations with GHG efflux. This information would prove invaluable as we try to gain insight on the factors influencing GHG efflux and climate change phenomena.

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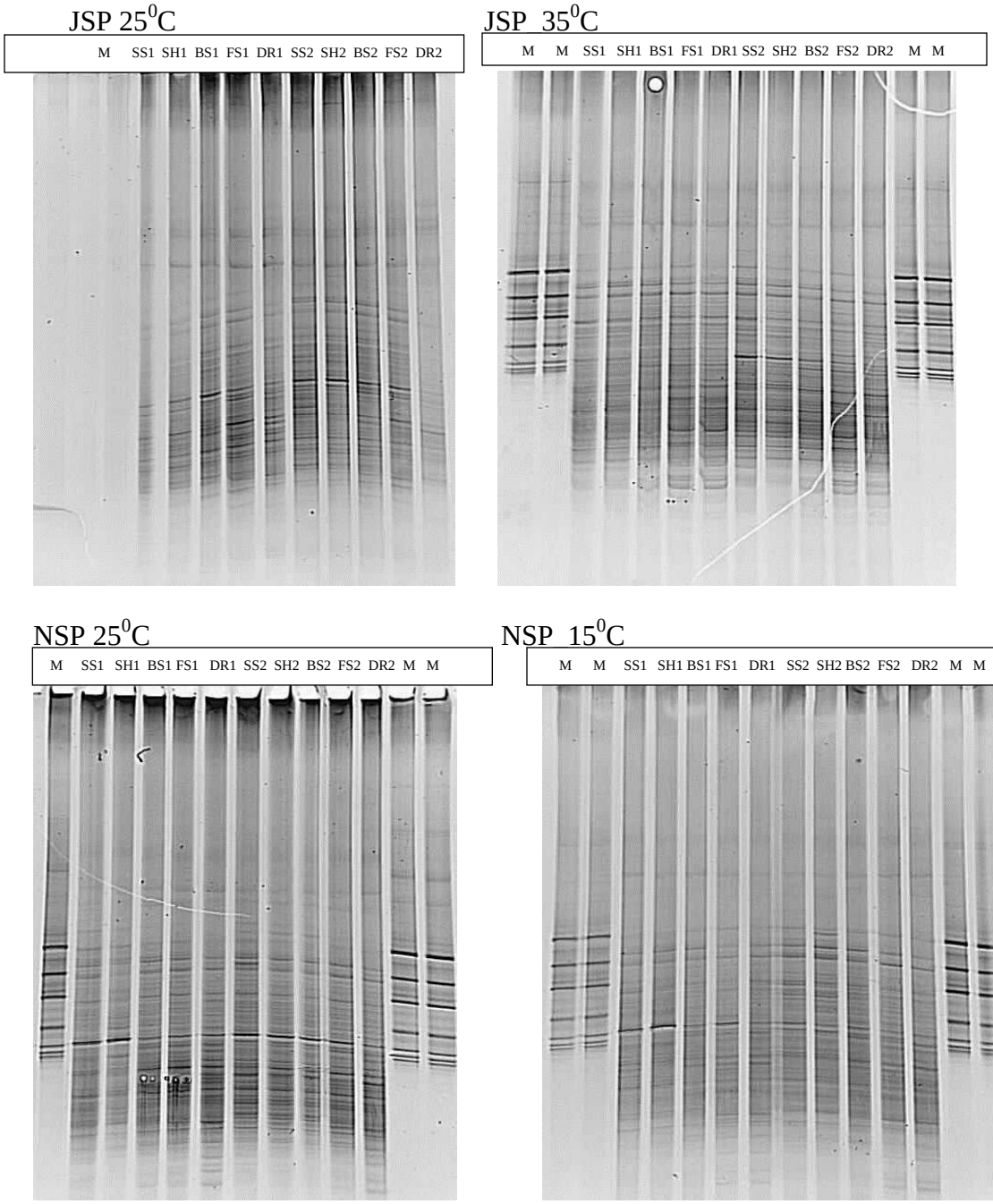


Figure 6.1 DGGE profiles for forest soils collected three times over a one year period and incubated at two temperatures during each sampling period. July 2008 (JSP) samples incubated at 25^{0}C or 35^{0}C; November 2008 (NSP) incubated at 25^{0}C or 15^{0}C; May 2009 (MSP) at 25^{0}C or 20^{0}C. Letters in lane headings indicate DNA markers (M) and landscape positions at which samples were collected: summit (SS), shoulder (SH), backslope (BS), footslope (FS) and drainage (DR). Numbers in lane headings indicate moisture level at which samples were incubated field moisture (FM) at time of sampling based on gravimetric water determination (1) and 60% waterholding capacity (2)

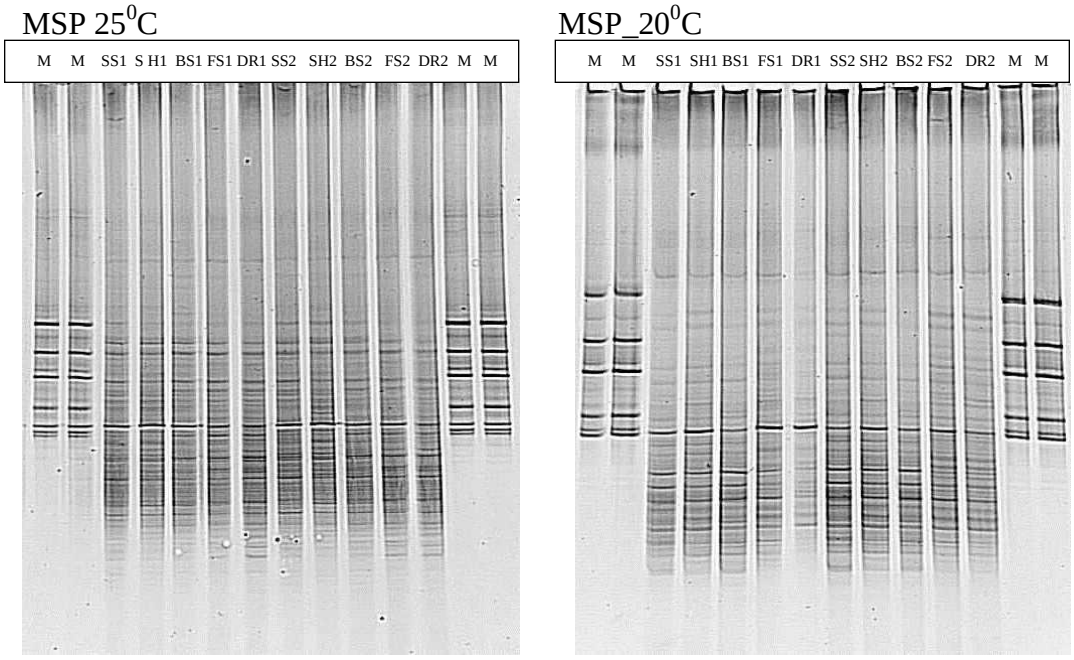


Figure 6.1(continued) DGGE profiles for forest soils collected three times over a one year period and incubated at two temperatures during each sampling period. July 2008 (JSP) samples incubated at 25^{0}C or 35^{0}C; November 2008 (NSP) incubated at 25^{0}C or 15^{0}C; May 2009 (MSP) at 25^{0}C or 20^{0}C. Letters in lane headings indicate DNA markers (M) and landscape positions at which samples were collected: summit (SS), shoulder (SH), backslope (BS), footslope (FS) and drainage (DR). Numbers in lane headings indicate moisture level at which samples were incubated, field moisture (FM) at time of sampling based on gravimetric water determination (1) and 60% waterholding capacity (2)

Table 6.1 Mean values (±SD) for GHG efflux and threshold cycles for each sampling period of a 30 d incubation study on soils collected from a secondary forest in Missouri. Headings in columns indicate the time of year samples were collected and also represent temperature at which samples were incubated during each sampling period. Samples collected in July 2008 (JSP) were incubated at 25^0C or 35^0C; November 2008 (NSP) at 25^0C or 15^0C; and May 2008 (MSP) at 25^0C and 20^0C. Different letters across rows immediately after the value indicate significant differences between sampling periods (LSMEANS, $P<0.05$)

	July 2008	November 2008	May 2009
N_2O (μg N_2O-N m^{-2} h^{-1})	2.9±4.8a	4.7±10.8a	7.3±12.8b
CO_2 (mg CO_2-C m^{-2} h^{-1})	11.3±4.4a	18.8±6.6b	14.1±4.3c
CH_4 (mg CH_4-C m^{-2} h^{-1})	-133.8±39.6a	-163±38.2b	-93.9±31.8c
Ct	32.3±1.2a	31.8±2.6a	31.7±1.4a

Table 6.2 Pearson correlation coefficients (r) for GHG efflux (CO_2, CH_4, and N_2O) and Ct values during a 30 d incubation of forest soils collected in mid-Missouri. Sampling periods in sub-headings of tables indicate time of year samples were collected and conditions under which samples were incubated. July 2008 samples were incubated at either 25^0C or 35^0C; November 2008 samples were incubated at 25^0C or 15^0C; May 2009 samples at 25^0C or 20^0C. * and *** $p < 0.05$ and $p < 0.001$.

July 2008

	Temperature	Moisture	Ct	CO_2	CH_4	N_2O
Temperature	-					
Moisture	-	-				
Ct	NS	NS	-			
CO_2	NS	NS	**-0.336***	-		
CH_4	NS	**-0.467***	NS	**-0.428***	-	
N_2O	NS	NS	NS	**0.499****	NS	-

November 2008

	Temperature	Moisture	Ct	CO_2	CH_4	N_2O
Temperature	-					
Moisture	-	-				
Ct	**-0.346***	NS	-			
CO_2	**-0.698*****	**0.364***	**0.341***	-		
CH_4	**0.747*****	NS	**-0.447***	**-0.705*****	-	
N_2O	**-0.444***	**0.313***	NS	**0.573*****	**-0.320***	-

May 2009

	Temperature	Moisture	Ct	CO_2	CH_4	N_2O
Temperature	-					
Moisture	-	-				
Ct	NS	NS	-			
CO_2	**-0.443***	NS	NS	-		
CH_4	**-0.327***	NS	NS	NS	-	
N_2O	NS	NS	NS	NS	NS	-

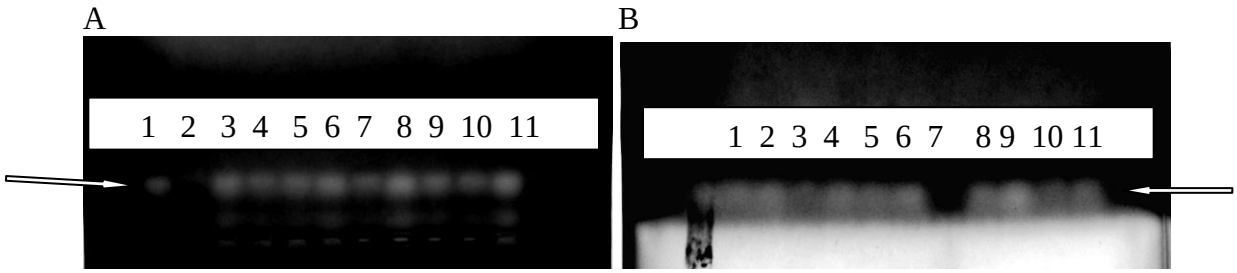
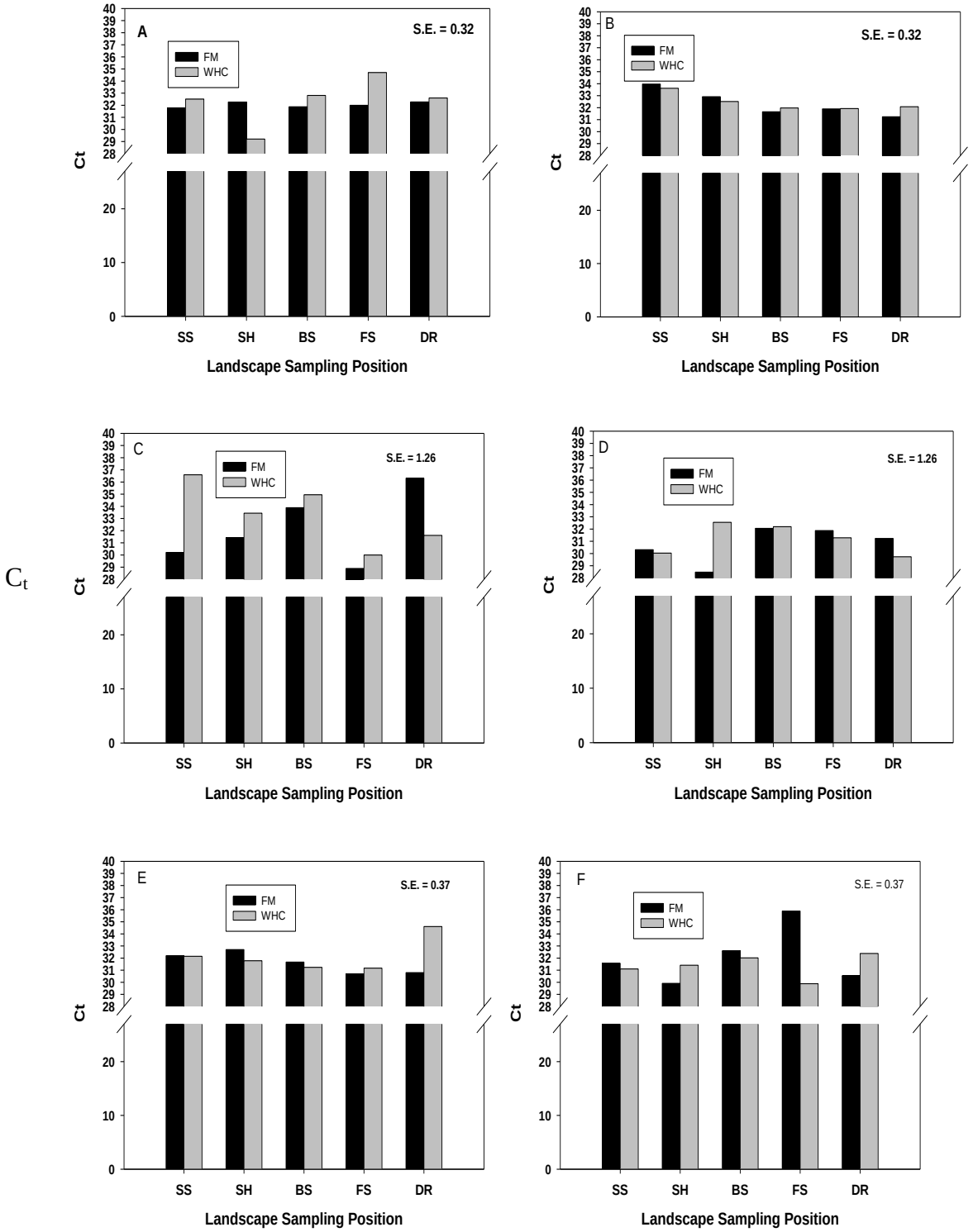


Figure 6.2 *Fusarium* DNA isolated from forest soils and amplified using nested PCR approach consisting of EF1/EF2 primer pair (1st round PCR) followed by Alfie 1/Alfie 2 primer pair (2nd round PCR) of the 1st round PCR product. Bands verified by agarose gel electrophoresis. (A) Lanes 1 – 5 samples incubated at 25^0C and FM; lanes 6 – 10 25^0C and 60% WHC. (B) Lanes 1 – 5 15^0C and FM; lanes 6 – 10 15^0C at 60% WHC. Arrows indicate position of fusarium spp. Bands (~650bp).

Control Temperature

Incubation "Seasonal" Temperature



C_t

Figure 6.3 Effect of landscape position and moisture on threshold cycle (Ct) in forest soils collected over a one year period July 2008 (A and B), November 2008 (B and C), and May 2009 (D and E). Samples were incubated at either control temperature of 25^0C (A, C, E) or temperatures reflecting time of year samples were collected 35^0C, 15^0C or 20^0C (B, D, F respectively). Landscape sampling positions summit (SS), shoulder (SH), backslope (BS), footslope (FS), and drainage (DR).

Control Temperature

Incubation "Seasonal" Temperature

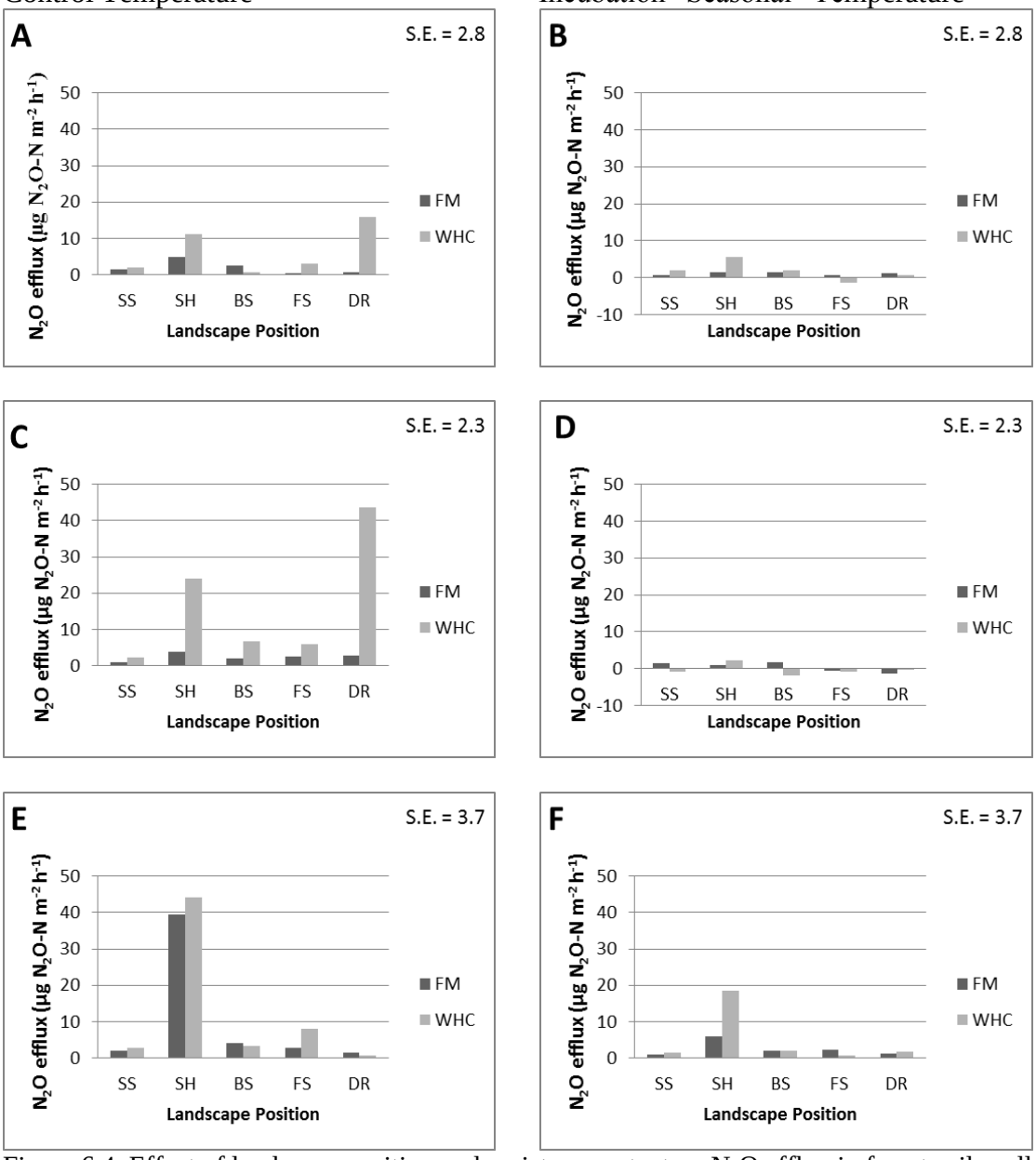


Figure 6.4 Effect of landscape position and moisture content on N_2O efflux in forest soils collected over a one year period July 2008 (A and B), November 2008 (B and C), and May 2009 (D and E). Samples were incubated at either control temperature of 25^0C (A, C, E) or temperatures reflecting time of year samples were collected 35^0C, 15^0C or 20^0C (B, D, F respectively). Landscape sampling positions summit (SS), shoulder (SH), backslope (BS), footslope (FS), and drainage (DR).

Control Temperature

Incubation "Seasonal" Temperature

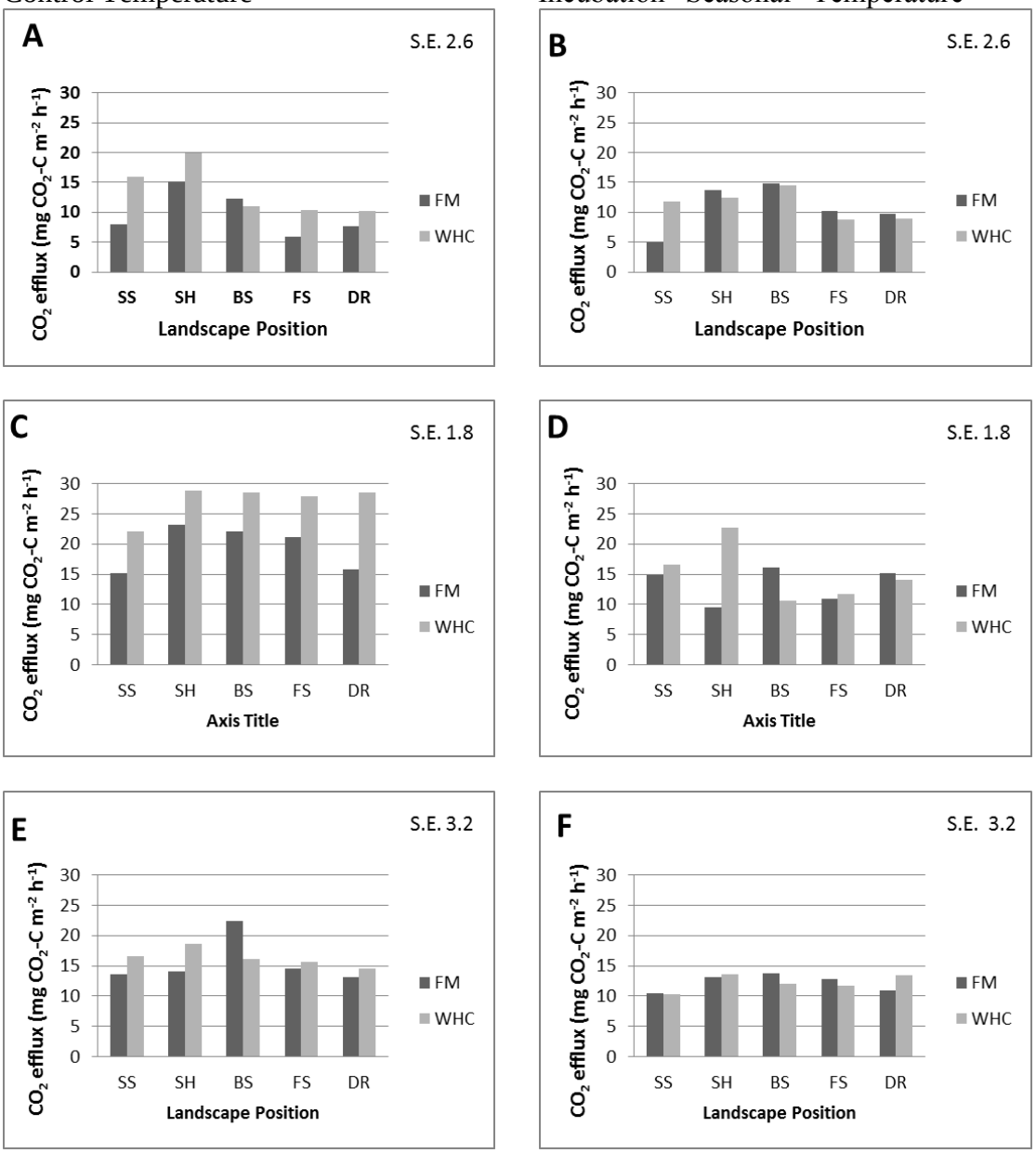


Figure 6.5 Effect of landscape position and moisture content on CO_2 efflux in forest soils collected over a one year period July 2008 (A and B), November 2008 (B and C), and May 2009 (D and E). Samples were incubated at either control temperature of 25^0C (A, C, E) or temperatures reflecting time of year samples were collected 35^0C, 15^0C or 20^0C (B, D, F respectively). Landscape sampling positions summit (SS), shoulder (SH), backslope (BS), footslope (FS), and drainage (DR).

Control Temperature

Incubation "Seasonal" Temperature

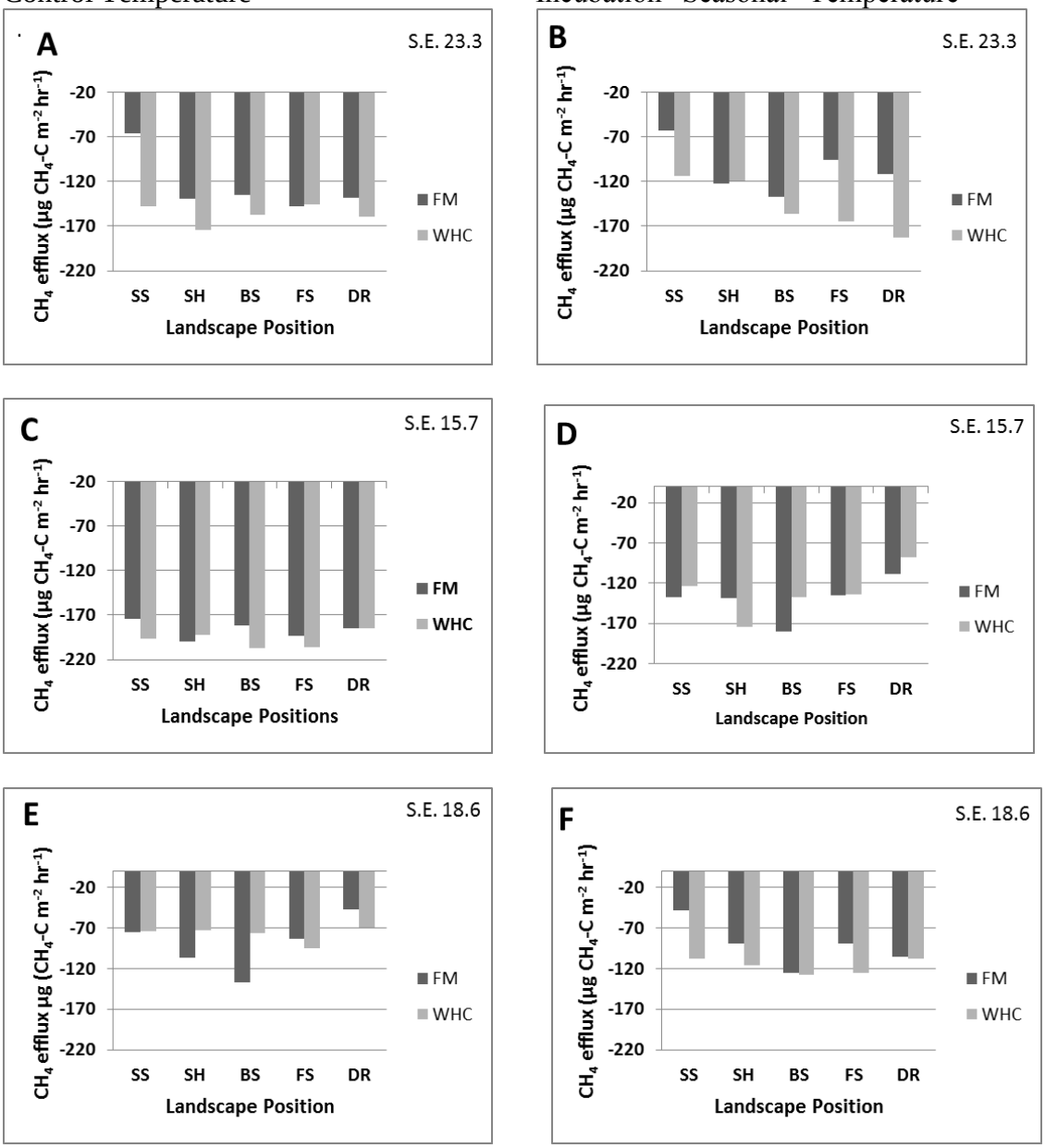


Figure 6.6 Effect of landscape position and moisture on CH_4 efflux in forest soils collected over a one year period July 2008 (A and B), November 2008 (B and C), and May 2009 (D and E). Samples were incubated at either control temperature of 25^0C (A, C, E) or temperatures reflecting time of year samples were collected 35^0C, 15^0C or 20^0C (B, D, F respectively). Landscape sampling positions summit (SS), shoulder (SH), backslope (BS), footslope (FS), and drainage (DR).

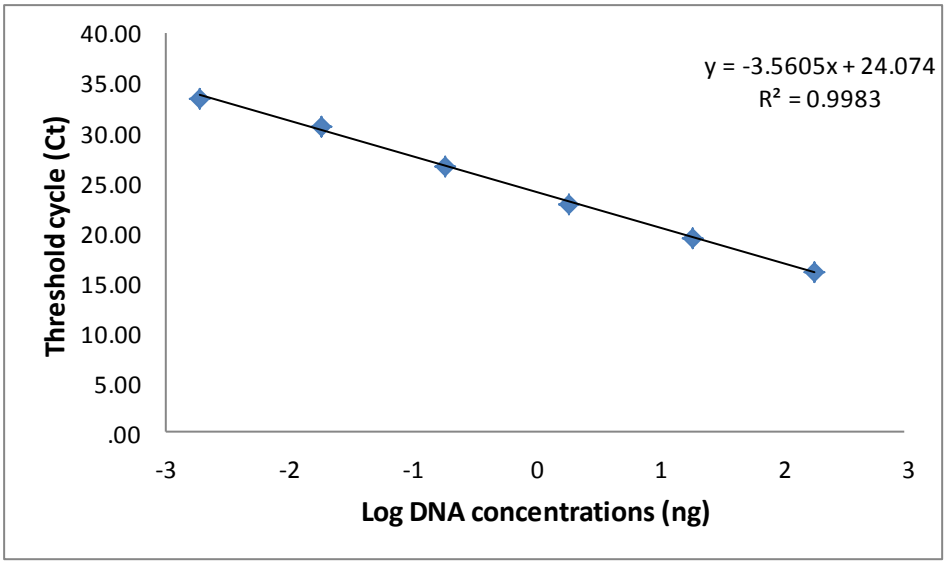


Figure 6.7 Standard curve of log DNA concentration versus the threshold cycle (Ct) number required to raise the fluorescence signal above the background level.

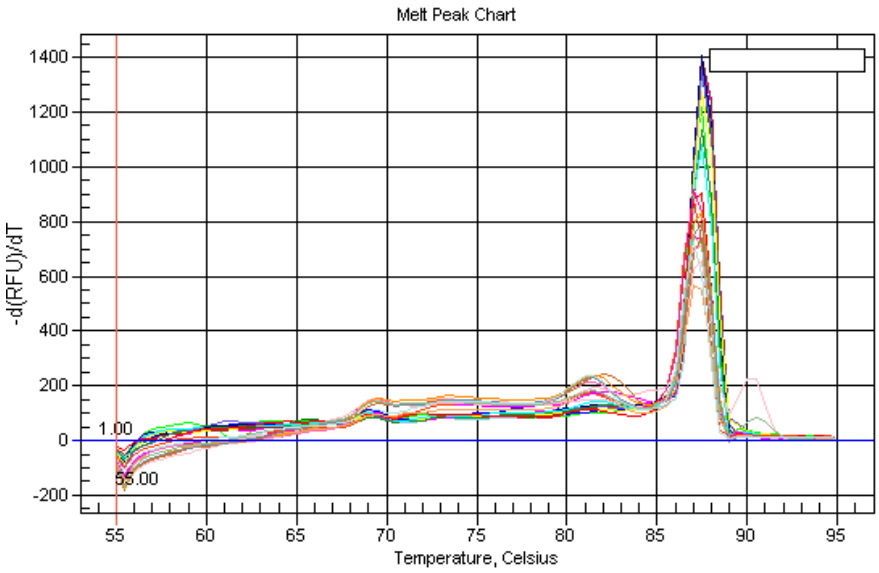


Figure 6.8 Melting curve profile for qPCR amplification of fusarium pure genomic DNA and DNA extracted from soil and amplified using a nested PCR approach.

Chapter 7

Summary

The results from Chapters Four, Five, and Six discussed the use of biological based analysis to evaluate the relationship between soil microbial communities and greenhouse gas efflux. A secondary component of this research was to explore the influence of topographic position on the microbial community structure and GHG efflux.

The main emphasis of Chapter Four was testing the influence of topography on enzyme activity and GHG efflux; and examining the relationship between enzyme activity and GHG efflux. Although we observed significant spatial and temporal variation in enzyme activity and GHG efflux, we were nevertheless able to recognize the potential of topographic position to impact enzyme activity and GHG efflux. Additionally, we were also able to infer that enzyme activity, if combined with other analysis, can be used as indicators of GHG efflux.

The results from Chapter Five, similar to Chapter Four, provided us with key information on the possibility of using PLFA analysis to gain insight on GHG dynamics in forest system. Our hypothesis for this chapter was that soil community composition and GHG efflux would reflect variations in soil properties across the landscape. From the results, we saw relationships among soil microbial community, topography, and GHG efflux. For example biomarkers for Gram negative, Gram positive and aerobic bacteria were positively correlated with GHG efflux. Ratios of cyclopropyl fatty acids to monoenoic precursers and total saturated to monounsaturated fatty acids, which are often indicators of stress related to substrated availability and moisture conditions, also correlated with GHG efflux. These correlations, in addition to significant effects of

temperature and moisture on GHG efflux and microbial community composition reiterated the importance of abiotic factors on microbial activity in these soils.

The use of molecular based analysis such as PCR, DGGE, and qPCR used in Chapter Six, showcases the ability of these techniques to identify differences in microbial communities due to abiotic factors. The results from these molecular analyses also agreed with observations in Chapters 4 and 5; and with previous findings of other researchers who associated GHG efflux with soil biological properties. Analysis of the data from the molecular analysis found correlations between *Fusarium* DNA concentrations and GHG efflux. Also, as observed in Chapters 4 and 5, temperature and temperature interactions with other main effects significantly affected microbial community compositions within each sampling period.

The general consensus from this research, therefore, is that biological tools such as enzyme assays, PLFA profiles, and molecular techniques are suitable for assessing soil microbial community and activities. However, as was noted by others, variability and complexity of the soil environment makes it difficult to conclusively determine how the different microbial groups contribute to GHG efflux. Additionally, the effect of soil abiotic factors impacts sections of the microbial community in different ways, sometimes contradictory to soil environmental changes. Nevertheless, biological asssesments can still be useful tools in exploring the impact of topography, temperature, and moisture on microbial community structure and their relationship with GHG efflux. However, the relationship between microbial communities, microbial activities, and GHG efflux is very complex; hence interpretation of this association must be approached with caution.

Future studies must therefore incorporate additional techniques such as cloning and sequencing; combined with measurements of wider ranges of biotic and abiotic factors.

Appendix 1. Means for PLFA soil microbial group biomarkers (mol %) after 30 day incubations of forest soils collected over three sampling periods (July and November 2008; May 2009), across five landscape sampling positions (summit, shoulder, backslope, footslope, and drainageway) and incubated at two temperatures (July (JSP) 25 and 35°C; November (NSP) 25 and 15°C; May (MSP) 25 and 20°C) and two moistures (gravimetric moisture and 60% water holding capacity). PLFA soil microbial group biomarkers included total fungi (fungi), bacteria to fungi ratio (B/F), Gram negative (Gneg), Gram positive (Gpos), anaerobic (Ana) bacteria, stress indicators (Sat/Mono), monounsaturated (Mono) lipids. Significantly different means in each column are indicated by different letters ($p < 0.05$).

July 2008

	Temp	Moist	Fungi	btof	gneg	gpos	ana	stress	mono
Summit	25	FM	0.033hi	7.881efgh	0.161fg	0.092ef	0.153fghi	20.097c	0.376ab
Summit	25	WHC	0.031i	8.5961de	0.192cd	0.072i	0.177cd	35.819a	0.409a
Summit	35	FM	0.0256j	12.717b	0.235a	0.086fgh	0.234a	25.579b	0.333c
Summit	35	WHC	0.044cd	6.229jk	0.144ghij	0.119ab	0.145ghij	5.692f	0.251def
Shoulder	25	FM	0.034ghi	8.376defg	0.166ef	0.112abc	0.163ef	11.739de	0.326c
Shoulder	25	WHC	0.034ghi	9.435cd	0.216b	0.084gh	0.206b	22.288bc	0.339bc
Shoulder	35	FM	0.020j	14.777a	0.217b	0.081h	0.211b	40.098a	0.401a
Shoulder	35	WHC	0.047bc	5.482kl	0.133ijk	0.121a	0.133j	6.163f	0.254def
Backslope	25	FM	0.039ef	6.927hij	0.144hij	0.112abc	0.140hij	7.380ef	0.276de
Backslope	25	WHC	0.030i	9.888c	0.197c	0.099e	0.186c	11.121de	0.275de
Backslope	35	FM	0.042de	6.371ijk	0.139ij	0.108cd	0.138ij	6.619f	0.242efg
Backslope	35	WHC	0.048bc	5.539kl	0.131jk	0.124a	0.131jk	5.323f	0.219fg
Footslope	25	FM	0.038efg	7.659efghi	0.159fgh	0.121a	0.154fgh	7.641ef	0.215fgh
Footslope	25	WHC	0.033hi	8.435def	0.170ef	0.091efg	0.169def	7.080f	0.209gh
Footslope	35	FM	0.035fgh	8.483def	0.163fg	0.123a	0.159efg	6.699f	0.217fgh
Footslope	35	WHC	0.049ab	5.137kl	0.116kl	0.123a	0.116kl	4.053f	0.178h
Drainage	25	FM	0.036fgh	7.272fghij	0.162fg	0.100de	0.155fgh	6.490f	0.240efg
Drainage	25	WHC	0.030i	9.272cd	0.181cde	0.095e	0.172cde	11.241de	0.283d
Drainage	35	FM	0.039def	7.190ghij	0.151ghi	0.119ab	0.146ghij	4.970f	0.225fg
Drainage	35	WHC	0.052a	4.741l	0.109l	0.126a	0.109l	4.034f	0.210gh

Appendix 1 continued

November 2008

	Temp	Moist	Fungi	btof	gneg	gpos	ana	stress	mono
Summit	25	FM	0.031g	7.227abc	0.125ef	0.095hi	0.125ef	13.873a	0.393a
Summit	25	WHC	0.042ef	6.001cde	0.123f	0.106gh	0.123ef	7.418c	0.246bcd
Summit	15	FM	0.042def	6.808abcd	0.137cde	0.086i	0.137bcde	10.590b	0.276b
Summit	15	WHC	0.050bcd	6.036cde	0.159abc	0.138ab	0.157ab	2.897fg	0.126f
Shoulder	25	FM	0.0357fg	7.681a	0.147abcde	0.118efg	0.147abcd	7.561c	0.270b
Shoulder	25	WHC	0.041ef	6.814abcd	0.147abcde	0.112efg	0.147abcd	6.903cd	0.276b
Shoulder	15	FM	0.035fg	8.019a	0.164ab	0.091i	0.164ab	10.389b	0.262bc
Shoulder	15	WHC	0.051bcd	6.172bcde	0.158abc	0.146a	0.155abc	3.256fg	0.130f
Backslope	25	FM	0.057ab	4.706f	0.130def	0.120def	0.130def	4.488ef	0.230cde
Backslope	25	WHC	0.036fg	7.568ab	0.151abcd	0.110fg	0.151abcd	7.629c	0.258bc
Backslope	15	FM	0.050bcd	5.539def	0.143bcde	0.121def	0.143bcde	3.195fg	0.106f
Backslope	15	WHC	0.056ab	4.810ef	0.123f	0.124cde	0.120ef	2.445g	0.108f
Footslope	25	FM	0.039ef	7.069abc	0.142bcde	0.123def	0.142bcde	5.328de	0.214de
Footslope	25	WHC	0.038fg	7.545ab	0.167a	0.113efg	0.167a	5.745de	0.242bcde
Footslope	15	FM	0.041ef	7.309abc	0.152abcd	0.131bcd	0.151abcd	3.387fg	0.116f
Footslope	15	WHC	0.047cde	6.194bcde	0.142bcde	0.129bcd	0.141bcde	2.129g	0.104f
Drainage	25	FM	0.052bcd	4.513f	0.111f	0.113efg	0.111f	4.405ef	0.205e
Drainage	25	WHC	0.047cde	5.466def	0.131def	0.107gh	0.131def	5.323de	0.209de
Drainage	15	FM	0.060a	4.716f	0.130def	0.112fg	0.129def	3.466fg	0.110f
Drainage	15	WHC	0.053abc	5.426def	0.135def	0.137abc	0.133cdef	2.337g	0.106f

Appendix 1 continued

May 2009

	Temp	Moist	fungi	btof	gneg	gpos	ana	stress	mono
Summit	25	FM	0.046defg	7.025bc	0.170cde	0.145ab	0.170cde	3.697de	0.144bc
Summit	25	WHC	0.045efgh	7.157bc	0.179bcd	0.138abcd	0.179bcd	4.086bcd	0.137bc
Summit	20	FM	0.041gh	8.331a	0.206a	0.135cdefg	0.204a	4.897a	0.152b
Summit	20	WHC	0.040h	8.272a	0.201ab	0.128efg	0.197ab	4.444abc	0.141bc
Shoulder	25	FM	0.044efgh	6.732bc	0.149efg	0.145ab	0.149efghi	3.868cd	0.139bc
Shoulder	25	WHC	0.045efgh	7.658ab	0.195ab	0.144abc	0.195ab	4.050bcd	0.139bc
Shoulder	20	FM	0.055a	6.148cde	0.168cde	0.131cdefg	0.165cdef	5.008a	0.142bc
Shoulder	20	WHC	0.042fgh	8.265a	0.203ab	0.145ab	0.199ab	4.054bcd	0.151b
Backslop	25	FM	0.045defg	6.870bc	0.155defg	0.147a	0.155defg	3.121efghi	0.137bc
Backslop	25	WHC	0.047cdef	6.956bc	0.182abc	0.136bcde	0.182abc	3.363efgh	0.134bc
Backslop	20	FM	0.048bcde	6.441cd	0.161cdef	0.136bcde	0.161cdef	2.780hi	0.127bc
Backslop	20	WHC	0.046def	7.018bc	0.181bc	0.135cde	0.180abc	3.510defg	0.140bc
Footslop	25	FM	0.047bcdef	6.219cde	0.143fgh	0.140abc	0.145fghi	2.535i	0.121c
Footslop	25	WHC	0.047bcdef	6.351cde	0.150efg	0.134cdef	0.150efghi	3.076efghi	0.121c
Footslop	20	FM	0.051ab	5.652def	0.130gh	0.118h	0.130ghi	3.626def	0.132bc
Footslop	20	WHC	0.046def	6.270cde	0.153efg	0.125fgh	0.152efghi	2.832ghi	0.119c
Drainage	25	FM	0.051abc	5.569def	0.145efgh	0.133cdef	0.145efghi	2.931fghi	0.121c
Drainage	25	WHC	0.052ab	4.963f	0.123h	0.123gh	0.123i	2.664hi	0.114c
Drainage	20	FM	0.046cdef	6.377cd	0.161cdef	0.130defg	0.161cdef	3.008fghi	0.137bc
Drainage	20	WHC	0.050bcd	5.3436ef	0.132gh	0.131defg	0.128hi	4.633ab	0.200 a

Appendix 2 Average number of bands identified by DGGE analysis of soil bacterial community for each sampling period during an incubation study. Samples were collected three times over a 10 month period July 2008 (JSP), November 2008 (NSP) and May 2009 (MSP). Within each sampling period soils were incubated for 30 days at 25^0C and 35C (JSP), 15C (NSP) or 20C (MSP). Two different moisture levels gravimetric water content at the time of sampling (FM) or 60% water holding capacity (WHC) was also used in the incubation study.

Sampling Period	Topographic sampling Position	Incubation Temp C	Incubation Moist	Number of bands
JSP	Summit	25	FM	17
JSP	Shoulder	25	FM	18
JSP	Backslope	25	FM	17
JSP	Footslope	25	FM	19
JSP	Drainage	25	FM	21
JSP	Summit	25	WHC	17
JSP	Shoulder	25	WHC	22
JSP	Backslope	25	WHC	16
JSP	Footslope	25	WHC	18
JSP	Drainage	25	WHC	16
JSP	Summit	35	FM	20
JSP	Shoulder	35	FM	22
JSP	Backslope	35	FM	24
JSP	Footslope	35	FM	18
JSP	Drainage	35	FM	17
JSP	Summit	35	WHC	28
JSP	Shoulder	35	WHC	27
JSP	Backslope	35	WHC	27
JSP	Footslope	35	WHC	29
JSP	Drainage	35	WHC	27
NSP	Summit	25	FM	14
NSP	Shoulder	25	FM	17
NSP	Backslope	25	FM	22
NSP	Footslope	25	FM	22
NSP	Drainage	25	FM	23
NSP	Summit	25	WHC	24
NSP	Shoulder	25	WHC	21
NSP	Backslope	25	WHC	17
NSP	Footslope	25	WHC	23
NSP	Drainage	25	WHC	21
NSP	Summit	15	FM	19
NSP	Shoulder	15	FM	16

Sampling Period	Topographic sampling Position	Incubation Temp C	Incubation Moist	Number of bands
NSP	Summit	15	WHC	25
NSP	Shoulder	15	WHC	22
NSP	Backslope	15	WHC	21
NSP	Footslope	15	WHC	19
NSP	Drainage	15	WHC	20
MSP	Shoulder	25	FM	16
MSP	Backslope	25	FM	20
MSP	Footslope	25	FM	20
MSP	Drainage	25	FM	18
MSP	Summit	25	WHC	26
MSP	Shoulder	25	WHC	26
MSP	Backslope	25	WHC	21
MSP	Footslope	25	WHC	23
MSP	Drainage	25	WHC	11
MSP	Summit	20	FM	19
MSP	Shoulder	20	FM	22
MSP	Backslope	20	FM	22
MSP	Footslope	20	FM	23
MSP	Drainage	20	FM	25
MSP	Summit	20	WHC	25
MSP	Shoulder	20	WHC	29
MSP	Backslope	20	WHC	24
MSP	Footslope	20	WHC	23
MSP	Drainage	20	WHC	23

Appendix 3. ANOVA results for July 2008, November 2008, and May 2009 sampling periods to determine the effect of temperature (temp), moisture (moist), and their interactions on bacterial population diversity (# of bands) from forest soils during an incubation study. Numbers (p-values) in bold indicate significant differences.

Source	July 2008	November 2008	May 2009
Temp	0.6961	**<.0001**	**0.0285**
Moist	0.0860	**0.0025**	0.0705
Temp x Moist	0.5853	**0.0007**	0.6865

Appendix 4a. ANOVA results for July 2008 sampling period to determine the effect of landscape sampling position (slope), temperature (temp), moisture (moist), and their interactions on greenhouse gases (N_2O, CO_2, CH_4) and threshold cycle (Ct) from forest soils during an incubation study. Numbers (p-values) in bold indicate significant differences.

Source of Variance	Num DF	Den DF	N_2O	CO_2	CH_4	Ct
Temp	1	15	**0.0352**	0.4783	0.0559	0.1913
Moist	1	15	0.0542	**0.0323**	**<.0001**	0.1327
Temp*Moist	1	15	0.1381	0.1032	0.4772	0.3658
Slope (slp)	4	5	0.2075	0.2063	0.4228	0.0789
Slp*Temp	4	15	0.3431	0.0798	0.4906	**<.0001**
Slp*Moist	4	15	0.2244	0.0914	0.1954	**<.0001**
Slp*Temp*Moist	4	15	0.2129	0.6855	0.0862	**0.0002**

Appendix 4b. ANOVA results for November 2008 sampling period to determine the effect of landscape sampling position (slope), temperature (temp), moisture (moist), and their interactions on greenhouse gases (N_2O, CO_2, CH_4) and threshold cycle (Ct) from forest soils during an incubation study. Numbers (p-values) in bold indicate significant differences.

Source of Variance	Num DF	Den DF	N_2O	CO_2	CH_4	Ct
Temp	1	15	**<.0001**	**<.0001**	**<.0001**	**0.0070**
Moist	1	15	**<.0001**	**<.0001**	0.8331	0.1906
Temp*Moist	1	15	**<.0001**	**0.0022**	0.1258	0.4847
Slope (slp)	4	5	**0.0055**	0.1549	0.2559	0.1621
Slp*Temp	4	15	**<.0001**	**0.0078**	0.0985	**0.0417**
Slp*Moist	4	15	**<.0001**	**0.0361**	0.6664	**0.0201**
Slp*Temp*Moist	4	15	**0.0002**	**0.0058**	0.0957	0.1022

Appendix 4c. ANOVA results for May2009 sampling period to determine the effect of landscape sampling position (slope), temperature (temp), moisture (moist), and their interactions on greenhouse gases (N_2O, CO_2, CH_4) and threshold cycle (Ct) from forest soils during an incubation study. Numbers (p-values) in bold indicate significant differences.

Source of Variance	Num DF	Den DF	N_2O	CO_2	CH_4	Ct
Temp	1	15	**0.0002**	**0.0004**	**0.0139**	0.2675
Moist	1	15	0.1825	0.6411	0.3785	0.5767
Temp*Moist	1	15	0.8194	0.6228	**0.0219**	**0.0005**
Slope (slp)	4	5	**0.0013**	0.8926	0.2887	0.5397
Slp*Temp	4	15	**<.0001**	0.4508	0.4001	**<.0001**
Slp*Moist	4	15	0.3404	0.1392	0.1375	**<.0001**
Slp*Temp*Moist	4	15	0.6855	0.4646	0.3433	**<.0001**

VITA

Nigel Hoilett was born in St. Mary, Jamaica. He graduated in 1990 with an Associate Degree in General Agriculture from the College of Agriculture, Portland, Jamaica. He came to Lincoln University, Missouri in 2001. He completed his B.S. in Agriculture in 2003, with an emphasis on Plant and Soil Science. He completed his M.S. degree in Soil Science through a joint agreement with Lincoln and MU under co-advisors Drs. J. Yang and R. Kremer, graduating in May of 2006. He decided to continue working under the supervision of Dr. Kremer and Dr. F. Eivazi for a Ph.D. degree. His doctoral research focuses on the relationship of soil microbial communities and activities with greenhouse gas emissions. Nigel and his wife Natalie and their children Nahiemah, Nayeli, and Nesean currently reside in Powell, Ohio. He is working in the capacity of laboratory manager and project supervisor under the supervision of Dr. Richard Dick at The Ohio State University.