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Linking soil moisture and carbon-cycle processes in two understudied terrestrial ecosystems: Ecuadorian páramo grasslands and constructed agricultural wetlands

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I am submitting herewith a dissertation written by Julie Yvette McKnight entitled "Linking soil moisture and carbon-cycle processes in two understudied terrestrial ecosystems: Ecuadorian páramo grasslands and constructed agricultural wetlands." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Geography.

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Linking soil moisture and carbon-cycle processes in two understudied terrestrial ecosystems:

Ecuadorian páramo grasslands and constructed agricultural wetlands

A Dissertation Presented for the
Doctor of Philosophy
Degree
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Julie Yvette McKnight
May 2015
Dedication

This dissertation is dedicated to my father, mother, and sister, who gave me the freedom to discover, the inspiration to follow, and the support to accomplish my dreams and goals.
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Abstract

A better understanding of soil-water interactions and associated feedbacks in carbon-cycle processes is necessary for addressing knowledge gaps in the global carbon budget. This doctoral dissertation research investigated soil carbon-cycle processes in two ecosystems, Ecuadorian páramos and constructed agricultural wetlands, which are understudied in terrestrial carbon research. These sites represent ecosystems where land-use induced changes in soil moisture were expected to play an important role in soil carbon processes.

Soil carbon dioxide (CO$_2$) flux and extracellular enzyme (EE) activities were measured to assess changes in soil carbon processes in soil from four types of land use in Ecuadorian páramos. Soil CO$_2$ flux was greater at sites with tree cover, which had lower soil moisture content, than at grass páramo sites, with higher soil moisture content. The results suggest that soil CO$_2$ flux responds to biological soil moisture thresholds, but the relationship between CO$_2$ flux and moisture in aerobic and anaerobic conditions is unclear. Carbon-acquisition EE activity indicated that soil carbon resources were in high demand for microbial utilization under non-native pine trees. Despite high soil carbon content at the grass páramo site, high carbon-acquisition EE activity there indicated high microbial demand for carbon resources, possibly due to the development of more stable forms of pyrogenic soil organic matter from a history of burning for grazing management.

Field-based data and a controlled laboratory experiment were used to investigate the relationship between soil moisture and soil CO$_2$ flux in two constructed agricultural wetlands with different hydrologic regimes, perennial and intermittent, in East Tennessee. The results suggest that constructed wetland hydrology plays an important role in soil moisture variability and mean CO$_2$ flux, and highlight the importance of the hydrologic design of constructed wetlands with respect to potential CO$_2$ emissions.

Results of this research suggest that soil moisture change due to land-use change can influence soil carbon decomposition and loss through CO$_2$ flux. The relationships between soil moisture, land-use change, and CO$_2$ flux have implications for land management and constructed wetland design.
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Chapter 1 Introduction

The significant increase in atmospheric carbon dioxide (CO$_2$) concentrations since the Industrial Revolution has created the scientific need to understand carbon-cycle processes and their role in global climate patterns (Revelle and Suess 1957; Baes et al. 1977; IPCC 2007). In spite of decades of research, balancing the global carbon budget remains a challenge (WBGU 1998). Globally, soil carbon pools are estimated to contain approximately three times more carbon than the atmosphere (Lal 2004) and to hold twice as much as the vegetative and atmospheric carbon pools combined (Davidson and Janssens 2006). Further, soil carbon emissions are estimated to annually contribute more than 10 times the amount of carbon to the atmosphere than anthropogenic burning of fossil fuels (Schlesinger 1977; Dornbush and Raich 2006). Consequently, changes in land use or land cover that alter soil carbon-cycle processes can significantly affect atmospheric carbon concentrations.

Houghton et al. (2012) estimated that land-use and land-cover change accounted for 12.5% of anthropogenic atmospheric carbon emissions between 1990 and 2010. Most of the estimates used by Houghton et al. (2012) included studies that reported soil CO$_2$ respiration. Numerous studies have shown that fluxes of soil CO$_2$ increase with changes in vegetation cover (Raich and Tufekcioglu 2000; Houghton et al. 2012). This is primarily a function of the effects that changes in land use and land cover can have on soil environmental conditions, such as soil temperature (Savva et al. 2010), soil moisture (Nosetto et al. 2005; Buytaert et al. 2006; Wang et al. 2012), and soil ecological properties, such as organic matter quality and quantity (Cambardella and Elliott 1992; Smith et al. 2014). Changes in soil CO$_2$ flux have been demonstrated for a variety of land-use changes, including the conversion of natural plant communities to agricultural land (Braimoh and Vlek 2004; Osman et al. 2013), the conversion of grasslands to forest or woody plant cover (Farley et al. 2004; Gibbon et al. 2010; Harden et al. 2013), and the drainage of wetlands for agricultural or urban development (Arai et al. 2015). The changes in soil properties that influence soil CO$_2$ vary with each type of land-use change. Further, the magnitude and significance of the effects of changes on soil properties can vary with the type of land-use change, geographic location, and ecosystem. Consequently, there is a need to better understand the mechanisms driving soil carbon-cycle processes within distinct ecosystems to better predict soil CO$_2$ flux responses to changes in land use and land cover.
1.1 Soil Carbon Processes

Numerous studies have shown that loss of soil C through CO$_2$ respiration is often a functional response to changes in soil temperature (Raich and Schlesinger 1992; Dornbush and Raich 2006; Wang et al. 2008) and soil moisture (Lloyd and Taylor 1994; Raich and Potter 1995; Bauer et al. 2008; Hernandez-Ramirez et al. 2009). Soil CO$_2$ flux response can reflect the influences of environmental conditions on soil microbial activity. Soil microbes play a critical role in the breakdown of soil organic matter and significantly influence soil carbon storage capacity and rates of soil carbon loss (Carriero et al. 2000; Simon et al. 2009). To acquire soil organic carbon resources, microbes produce extracellular enzymes that catalyze the breakdown of soil organic material (Schimel and Weintraub 2003; Huang et al. 2011). This process, known as depolymerization, yields simpler organic components, such as amino acids and sugars, which are the constituents of original soil organic material. Simpler organic components are then readily taken up and utilized by microbes in the form of dissolved organic carbon (Figure 1.1). Each extracellular enzyme is synthesized for the depolymerization of a specific substrate. The enzyme β-D-cellulobiohydrolase (CB), for example, facilitates carbon acquisition from cellulose-based organic matter, while β-glucosidase (BG) facilitates carbon acquisition from sugar degradation (Bell et al. 2013). The production of extracellular enzymes requires the investment of substantial energy and resources by the microbe. Consequently, microbes prioritize the production of specific extracellular enzymes according to their specific nutrient needs. As a result, microbes preferentially increase the synthesis of a specific type or group of extracellular enzyme specialized for the acquisition of the most limiting nutrient, or substrate (Tischer et al. 2014). Thus, greater activity of a specific enzyme, compared to activity levels of other enzymes, can indicate a limitation in the nutrient or substrate targeted by that enzyme.

The activity of specific extracellular enzymes can be used as a proxy for different aspects of ecosystem function. Soil extracellular enzyme activity has been shown to respond to changes in soil properties associated with land management practices, especially where changes in soil organic matter and nutrient composition occur (Acosta-Martínez et al. 2007; Kizilkaya and Dengiz 2010; Gonnety et al. 2012). Soil enzyme activity can also provide information about changes in carbon, nitrogen, and phosphorus processes in response to land-use change (Mganga et al. 2015). For instance, where labile forms of soil carbon—readily available for decomposition and uptake—have been depleted, microbial production and activity of labile carbon-acquisition
Figure 1-1 Conceptual model for microbial soil C processes. Labile soil carbon is defined here as water extractable organic carbon. Environmental controls on microbes affect all activity directly engaged by microbes, including death, uptake, enzyme production, and CO$_2$ respiration.
enzymes, such as BG and CB, is expected to be higher than production or activity of enzymes specialized for the degradation of more recalcitrant forms of carbon, such as phenol oxidase. Consequently, analysis of soil extracellular enzymes can help to determine whether changes in microbial activity result from changes in environmental (i.e. soil moisture and soil temperature) or ecological (i.e. stoichiometry and shifts in type of organic matter present) soil conditions. Where environmental drivers, such as soil temperature or moisture, are more influential, a decrease or increase in extracellular enzyme production would be expected without a significant shift in the abundance or distribution of different extracellular enzyme groups.

Microbial organisms involved in soil carbon depolymerization are dominantly aerobic and respire CO₂ when active. Consequently, changes in soil CO₂ flux can be used as a proxy for changes in soil microbial activity and related soil carbon processes. Many studies have shown that soil physical parameters, such as soil temperature and soil moisture, are significantly correlated with rates of microbial activity (Linn and Doran 1984; Allison et al. 2010; Carbone et al. 2011; Steinweg et al. 2013; A’Bear et al. 2014). Soil microbial activity is known to increase with warming soil temperatures (Raich and Schlesinger 1992; Dornbush and Raich 2006). A thermal biological threshold of 10 °C to 15 °C, below which soil microbial activity significantly decreases, was estimated by Richards et al. (1952). Trevors (1985) showed that microbial CO₂ production at 20 °C is three times greater than at 10 °C, and microbial soil respiration has been shown to increase exponentially at soil temperatures exceeding 20 °C (Yuste et al. 2007).

Microbial activity, and thus soil CO₂ flux responses to changes in soil moisture, is more complex than CO₂ flux responses to soil temperature. Water, required for microbial biological processes, can be limiting to soil microbial activity when water resources are scarce. Consequently, soil microbial activity and CO₂ respiration rates are positively correlated with soil water content when soil conditions are aerobic (i.e. when there is sufficient oxygen diffusion to support aerobic respiration). In anaerobic soil conditions, however, soil microbial activity associated with soil organic matter decomposition is limited and, as a result, soil CO₂ flux rates are significantly lower (Moore and Dalva 1993; Moyano et al. 2012; Suseela et al. 2012). The aerobic-anaerobic threshold is estimated to occur when water-filled pore space reaches 60–65%, with anaerobic conditions occurring at higher contents of water-filled pore space (Linn and Doran 1984). As a result, linear relationships between soil moisture and soil CO₂ flux may be
found when data are partitioned to represent aerobic and anaerobic conditions separately. However, when a wide range of soil moisture content (e.g. 25–75% water-filled pore space) is assessed with respect to soil CO₂ flux rates, a quadratic polynomial relationship is expected, showing a positive relationship below approximately 60% water-filled pore space and a negative relationship above 60% water-filled pore space (Figure 1.2; Linn and Doran 1984).

1.2 The Need for Coupled Water-Carbon Studies

Soil-water interactions clearly play an important role in terrestrial carbon-cycle processes and should be considered when evaluating terrestrial carbon budgets. However, knowledge gaps remain that constrain efforts to investigate water and carbon cycles as coupled systems (Lohse et al. 2009). Variations in water table level and soil moisture content have been shown to significantly influence soil carbon processes and atmospheric carbon fluxes (Moore and Dalva 1993; Blodau and Moore 2003; Chivers et al. 2009; Mander et al. 2011; Gazovic et al. 2013). In fact, some of the most significant global terrestrial carbon sinks attain their capacity for soil carbon storage through hydrologic controls on soil properties within the system. Wetlands, for instance, represent only about 5% of the global land area, but account for more than 30% (>500 Gt C) of global terrestrial carbon stores (WBGU 1998; Mitra et al. 2005; CBD 2007). High water table and very high soil moisture conditions required for the development of wetland hydric soils sustain anaerobic conditions that impede soil microbial activity. As a result, wetlands are often characterized by low rates of soil organic matter decomposition and soil CO₂ respiration. Although methanogenesis occurs in anaerobic conditions, wetland emissions of methane (CH₄) can be offset by continued plant uptake of atmospheric CO₂ and accumulation of soil organic matter within the wetland (Gazovic et al. 2013). Emissions of CH₄ can also be offset by the fixation of atmospheric CH₄ by methanotrophs—organisms capable of utilizing CH₄ for biological processes in upland ecosystems (Zhuang et al. 2013). Changes in land use, land cover, or climate conditions that induce long-term drought, or permanent drainage in wetland soils that induces aerobic conditions, can significantly alter these processes. The transition from anaerobic to aerobic soil conditions can stimulate soil organic matter decomposition by microbes and cause an associated loss of soil carbon via CO₂ respiration. In instances where soil moisture conditions are altered permanently, soil CO₂ emissions will equilibrate with CO₂ fluxes within the local
Figure 1-2 Relationship between percent water-filled pore space (WFPS) and relative microbial activity. The range of WFPS from 0% to 60% (water limiting) is representative of aerobic soil conditions. The range of WFPS from 60% to 100% (aeration limiting) is representative of anaerobic soil conditions. Relative microbial activity corresponds to expected trends in soil CO₂ flux rates. Source: Figure 1 in Linn and Doran (1984).
landscape surrounding the wetland (Blodau and Moore 2003). However, a significant loss of soil carbon from wetlands cannot be offset by carbon uptake within the landscape due to the disproportionately greater soil carbon storage capacity of wetlands, compared to the soil carbon storage capacity of upland ecosystems (Vicari et al. 2010). Soil-moisture induced biological thresholds are thus critical in the planning and development of constructed wetlands for maintaining the carbon sequestration potential of wetland ecosystems. This is only one example of the significant influence of soil moisture on terrestrial carbon processes.

1.3 The Case for Understudied Ecosystems

A better understanding of the linkages between hydrologic and biogeochemical cycles, especially in understudied ecosystems, is necessary to provide an account of the ‘missing’ carbon in terrestrial studies (Post et al. 1990). Gardner et al. (1980 p. 313) stated that “true values of transfer and variability of carbon cycle processes relative to time and space” are required to create informed carbon-cycling prediction models. While advancing knowledge of biogeochemical processes contributes to determining ‘true values,’ a better understanding of understudied ecosystems is needed to account for the transfer and variability of carbon-cycle processes. Much focus in terrestrial carbon research has been given to ecosystems that are recognized as globally significant carbon sinks and are most sensitive to global climate change, namely forests and wetlands in boreal and tropical climate zones (Gorham 1991; WBGU 1998). While these studies have contributed a wealth of knowledge to global carbon research, the complexity and connectivity of ecosystem processes across spatial scales require a more detailed account of hydrologic and biogeochemical processes in adjacent and understudied systems (Jones et al. 2013). Local and regional changes in surface water or soil hydrology, for instance, can have significant implications for carbon-cycle processes at landscape scales (Lohse et al. 2009). This is increasingly evident in ecosystems where land management practices were implemented prior to the existence of sufficient scientific information to accurately predict environmental responses. My doctoral dissertation research focuses on two ecosystems, constructed agricultural wetlands and Ecuadorian páramo grasslands, each of which represents a fraction of the larger landscape in which it occurs, yet has the potential to play a significant role in landscape-scale carbon-cycle processes.
1.4 Ecuadorian Páramo Grasslands

Páramo grasslands are high altitude ecosystems that extend from Costa Rica to the northern Andes in South America and occur between 3200 m and 4700 m in elevation (Horn and Kappelle 2009). Ecuadorian páramos are characterized by Andisol (volcanic ash) soils and perennially cool, moist climate conditions. Andisol soils in páramo grasslands have a high water-holding capacity. Previous studies have reported very high volumetric soil moisture content, ranging between 79 and 86% for páramo grasslands in Ecuador (Hartsig 2011; Harden et al. 2013). Soils in páramo grasslands in Ecuador are primarily non-allophanic Andisols dominated by Al-humus complexes. Due to the combination of climate and physical and chemical soil properties in Ecuadorian páramo grasslands, these ecosystems also have high soil carbon storage capacities. Previous studies have shown that páramo grasslands in Ecuador can store up to 143 tons C ha\(^{-1}\) (Farley et al. 2012) and yield 506–933 mm of water per year (Buytaert et al. 2007a). Water yield from páramos supports domestic water provision to many of the major cities in Ecuador. Additionally, due to the high retention of soil organic matter in páramo soils, these ecosystems act as significant regional carbon sinks. Consequently, as páramos are sensitive to disturbances, a better understanding of ecological responses in páramos to land-use change has significant implications for land management decisions in the Ecuadorian Andes.

The role of human activity in defining the boundaries of páramo ecosystems has been debated for some time (Ellenberg 1979; Keating 1998, 2007; Horn and Kappelle 2009). In recent decades, anthropogenic fire has been recognized to play an important role in defining boundaries between páramo grassland and montane forest (Keating 2007; Horn and Kappelle 2009). Previous studies suggested that some regional páramo ecosystem boundaries throughout Ecuador have been maintained by anthropogenically induced fire regimes for thousands of years (Keating 2007; White 2013). The use of fire as a land management tool has been suggested to be associated with grazer ecology (White 2013). Burning in páramo grasslands stimulates the growth of new plant shoots that can support more grazing animals (Lægaard 1992; Verweij and Budde 1992; Hofstede 2003), which have supported the livelihoods of local populations from the hunter-gatherer cultures of the late Holocene to modern agricultural societies (White 2013). Although fire has been associated with the expansion and maintenance of páramo grassland area, the effects of burning, at intervals from one to several years, on soil carbon processes in...
Ecuadorian páramo soils is poorly understood. Further, it has been suggested that the suppression of controlled anthropogenic burning may result in the encroachment of native forest and woody plant species into páramo grassland areas at higher altitudes (Farley 2010).

Afforestation in páramo grasslands, both as tree plantation and as native woody plant encroachment, has been promoted for a variety of purposes. Recently, the Ecuadorian Ministry of Environment has suppressed controlled burning to reduce direct anthropogenic impacts on páramo landscapes (Farley et al. 2011). In some areas in the Andes, this management decision may result in the encroachment of woody vegetation into grassland areas. In some instances, tree plantations, often pine or eucalyptus, have been established in páramo grasslands for a variety of reasons including, but not limited to, lumber production and the generation of international carbon credits. Previous studies have shown that both soil carbon content (Farley et al. 2012) and water yield (Buytaert et al. 2007) were significantly reduced where grassland was converted to forest cover. In spite of the increase in aboveground carbon stocks, the depletion of soil organic carbon stores was an unanticipated outcome that countered the original objectives of the carbon-focused afforestation programs. Further, because páramo ecosystems provide water resources to more than 10 million people in Ecuador (Buytaert et al. 2007), these findings have significant implications for existing or future plans for afforestation (Harden et al. 2013).

High soil organic matter in páramo grasslands in Ecuador has been significantly correlated with soil water retention (Buytaert et al. 2007b). However, to my knowledge, no known studies have investigated the role of land-use-induced changes in soil moisture on the loss of soil carbon stocks through increased soil carbon decomposition, or depolymerization, rates. Further, I am aware of no studies that report soil CO$_2$ flux rates for Ecuadorian páramo grasslands. This dissertation research provides a first insight into soil CO$_2$ flux rates and the relationship between soil moisture and soil carbon processes in Ecuadorian páramos.

1.5 Constructed Agricultural Wetlands

In spite of their significance to the global carbon budget (WBGU 1998; Mitra et al. 2005), wetland areas have been greatly diminished due to anthropogenic activity and land-use change. In the United States, agricultural development contributed to the loss of more than 50% of natural wetlands between the early 1600s and the 1900s (Dahl and Johnson 1991; Dahl and Allord 1999). In addition to the depletion of soil carbon stores where wetlands were drained, soil
carbon was further reduced in agricultural landscapes due to cultivation practices and animal
trampling (Caride et al. 2012; Hiltbrunner et al. 2012). Although recognition of agricultural
carbon loss instigated efforts to increase carbon sequestration in agricultural areas through
sustainable crop selection and cultivation, agricultural landscapes in the United States still
contribute approximately 460 Tg of CO₂ annually to atmospheric carbon concentrations (US
EPA 2004).

In recent decades, wetland construction has been increasingly used as a land management
practice for flood control and for the mitigation of nutrient and erosion runoff in crop and pasture
land (Hefting et al. 2013; Tanner et al. 2013). However, the role of constructed wetlands in
landscape carbon budgets is not well documented. Conversion of cropland to wetland has been
shown to increase soil carbon stocks (Euliss et al. 2006) and, in some cases, soil organic carbon
in constructed wetlands is up to 70% greater than in similar natural wetlands within the same
region (Anderson and Mitsch 2006; Bernal and Mitsch 2013). Bernal and Mitsch (2012) showed
that carbon sequestration capacities of freshwater wetlands are not always comparable. A better
understanding of the biogeochemical processes affecting soil carbon processes in constructed
wetlands in distinct geographic settings can provide insight into why these differences exist.
Consequently, to define the functional role of constructed wetlands in agricultural landscapes,
more information on biogeochemical processes in constructed agricultural wetlands, and on how
they compare to natural wetlands, is necessary. The number of previous studies that have
assessed soil carbon processes in constructed wetlands is limited, and even fewer studies have
assessed the relationship between wetland soil hydrology and soil carbon processes. Further,
those studies that have investigated the effects of soil moisture variation on soil carbon fluxes in
constructed wetlands have been primarily conducted using laboratory or mesocosm experiments,
while field studies in constructed wetlands are lacking. Finally, the majority of existing studies
that assess greenhouse gas emissions in constructed wetlands focus primarily on fluxes of
methane and nitrous oxide (Fey et al. 1999; Sovik et al. 2006; Maltais-Landry et al. 2009; Fuchs
et al. 2011). As a result, very few studies contribute to our understanding of soil moisture
controls on soil CO₂ fluxes in constructed wetlands, and no known field studies investigating
these processes have been conducted for agricultural landscapes in East Tennessee.

As agricultural lands comprise more than 50% of the conterminous United States (USDA
2011), the growing use of constructed wetlands for water quality best management practices
(BMPs) will likely have significant effects on regional and landscape carbon-cycle processes. Quantifying soil and aquatic carbon flows and their response to hydrologic feedbacks is therefore essential to the integration of constructed wetlands into agricultural and landscape carbon budget models.

1.6 Research Questions

My doctoral dissertation research investigated soil carbon-cycle processes in páramo grasslands in the Ecuadorian Andes and in two constructed agricultural wetlands in East Tennessee. These study sites represent two understudied ecosystems in which land-use induced changes in soil moisture were expected to play an important role in soil carbon processes. The primary research questions for this dissertation are:

1) What is the relationship between land-use induced changes in soil moisture and soil CO$_2$ flux rates among four different land-use types in the Ecuadorian páramos? (Chapter 2)
2) How does soil extracellular enzyme carbon-acquisition activity differ between four different types of land use in Ecuadorian páramos? (Chapter 3)
3) What is the importance of soil moisture variability on soil CO$_2$ flux rates in two constructed agricultural wetlands with different hydrologic regimes, perennial versus intermittent, in East Tennessee? (Chapter 4)

1.7 Organization of Dissertation

This dissertation consists of five chapters. Chapter 1 has provided an introduction to coupled water-soil interaction studies in terrestrial carbon research and put into context the value and need for the research being presented. Chapters 2 through 4 are included as stand-alone manuscripts that will be submitted for publication to peer-reviewed journals.

In Chapter 2, differences in soil moisture and soil CO$_2$ flux among four different types of land use are assessed. This research reports the first known data for soil CO$_2$ flux in the Ecuadorian Andes and provides a new view of soil carbon processes in high altitude páramo landscapes.
Chapter 3 reports an investigation into the effects of land-use and soil moisture changes on soil extracellular enzyme carbon-acquisition activity at four sites representing different land uses in Ecuadorian páramo landscapes. Microbial and nutrient acquisition activity, as indicated by measures of extracellular enzyme activity, provides valuable insight in soil carbon degradation and loss through biological pathways.

In Chapter 4, differences in soil moisture variability and soil CO₂ flux in two East Tennessee constructed agricultural wetlands, with different hydrologic regimes, are investigated. This research was intended to contribute to a better understanding of the relationship between wetland hydrology and soil carbon processes in constructed agricultural wetlands in East Tennessee. This knowledge is also intended to better inform the design and construction of man-made wetlands, especially for land managers engaged in reducing or offsetting greenhouse gas emissions in agricultural landscapes.

Chapter 5 summarizes the findings of Chapters 2 through 4, discusses the potential implications of this research with respect to land management and the broader scientific community, and provides suggestions for future research.
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Chapter 2 Differences in and relationship between soil moisture and soil carbon dioxide flux among four types of land use in Ecuadorian páramos
A version of this chapter will be submitted for publication to Biogeochemistry by Julie McKnight, Carol P. Harden, and Sean P. Schaeffer.

**Abstract**

Páramo grasslands are important carbon sinks in the Ecuadorian Andes. The organic matter content of the páramo Andisols is significantly correlated with their capacity for water retention, but no published studies have previously investigated the effects of land-use induced soil moisture change on soil carbon processes in these ecosystems. This study assesses the differences in and relationship between soil moisture and soil CO$_2$ flux among four different types of land use in Ecuadorian páramo landscapes: grass páramo, recently burned grass páramo grassland (< 6 months), native montane forest, and pine plantation (age 25 years). Soil CO$_2$ flux and soil moisture were measured over a 3-week period in December 2013. The highest (5.79 g CO$_2$-C m$^{-2}$ d$^{-1}$) and lowest (3.61 g CO$_2$-C m$^{-2}$ d$^{-1}$) mean soil CO$_2$ flux rates were measured at the pine and recently burned páramo sites, respectively. Soil CO$_2$ flux rates were higher (p<0.5) at the forest and pine sites than at the grassland sites, and CO$_2$ flux was higher (p<0.05) at the grass páramo than at the recently burned páramo site. Lower soil CO$_2$ flux rates at both grassland sites were associated with anaerobic conditions (water-filled pore space > 60%). Soil CO$_2$ flux appears to respond to biological soil moisture thresholds; however, there is no clear trend within aerobic and anaerobic soil moisture gradients. Our results suggest that decreases in soil moisture content with land-use change from páramo grassland to forest or pine plantation can lead to higher soil CO$_2$ flux rates in the páramo of the Mazar Wildlife Reserve, but further research is required to better define the relationship between soil moisture and CO$_2$ flux within specific types of land use. To our knowledge, these are the first soil CO$_2$ flux rates reported for Ecuadorian páramo grasslands.

1. **Introduction**

Globally, soil carbon accounts for more than three times the amount of carbon in the atmosphere and almost twice as much carbon as is present in the atmospheric and biotic pools combined (Lal 2004; Davidson and Janssens 2006). Soil CO$_2$ flux is a primary mechanism for soil carbon loss to the atmosphere, as it is a product of the breakdown and utilization of soil carbon resources by soil microorganisms. Changes affecting soil carbon-cycle processes thus have significant implications for terrestrial carbon fluxes. In fact, land-use change is second only
to fossil fuel burning in terms of anthropogenic carbon dioxide (CO₂) emissions, with an estimated loss of 25–50% of soil organic carbon (SOC) in the conversion of native vegetation to cropland alone (IPCC 2014; Scharlemann et al. 2014). Still, net flux of terrestrial carbon from land-use and land-cover change is among the most uncertain of factors in the global carbon budget, partly due to knowledge gaps regarding the amount and distribution of carbon in lands undergoing change (Houghton et al. 2012). Although estimates of carbon emissions from land-use change commonly include emissions resulting from deforestation and agricultural management, some ecosystems (e.g. peatlands), known to be significant carbon sinks and sensitive to land-use change, are not well represented in those estimates (Houghton et al. 2012). In addition, estimates of carbon emissions induced by land-use change poorly represent terrestrial carbon fluxes associated with unintended changes in land cover that result from land management, such as woody encroachment as a response to fire suppression (Hurtt et al. 2002). Consequently, to more accurately represent the role of land-use change in regional and global carbon budgets, studies that estimate carbon flux related to land use must incorporate direct and indirect terrestrial carbon responses and include a broader range of ecosystems.

Soil carbon dioxide (CO₂) flux, a primary mechanism for soil carbon loss, can be used as a proxy to reflect rates of soil microbial activity. Microbes require the transformation of soil organic matter into simpler organic components, such as amino acids and sugars, for uptake and utilization. This transformation process, known as depolymerization, is associated with aerobic microbial activity, from which CO₂ is an end product via respiration. As a result, changes in soil CO₂ flux rate can be used to indicate changes in soil microbial activity, which can be related to rates of soil carbon depolymerization. Soil CO₂ flux is a function of biological activity; thus, flux rates vary along gradients of environmental conditions that influence biological processes and that can create biological thresholds. Specifically, numerous studies have shown that soil CO₂ flux is sensitive to changes in soil temperature (Raich and Schlesinger 1992; Dornbush and Raich 2006; Wang et al. 2008) and soil moisture (Bauer et al. 2008; Hernandez-Ramirez et al. 2009; Sainju et al. 2010), both of which can be significantly altered by land-use change. Linn and Doran (1984) showed that soil CO₂ flux variation can be a function of soil moisture, as microbial processes are affected by aerobic or anaerobic conditions. This response is two-fold: too little water can be limiting, as it is vital to support biological activity; however, too much
Figure 2-1 Relationship between percent water-filled pore space (WFPS) and relative microbial activity. The range of WFPS from 0% to 60% (water limiting) is representative of aerobic soil conditions. The range of WFPS from 60% to 100% (aeration limiting) is representative of anaerobic soil conditions. Relative microbial activity corresponds to expected trends soil CO₂ flux rates. Source: Figure 1 in Linn and Doran (1984).
water can create anaerobic conditions, which hinder microbial processes (Figure 2-1). Linn and Doran (1984) showed that soil microbial CO₂ respiration responded positively to increasing soil moisture content until reaching the anaerobic threshold, estimated to be at approximately 60% water-filled pore space (WFPS). In soil moisture conditions that exceed 60% WFPS, microbial CO₂ respiration decreases because microbial activity responds negatively to anaerobic conditions. Accordingly, where land-use change results in significant changes in soil moisture, especially where conditions shift from anaerobic to aerobic or vice versa, the rate of soil CO₂ flux rate is expected to change.

In the Ecuadorian Andes, páramo grasslands are valuable ecosystems that represent less than 10% of the total land area but facilitate significant ecosystem services, including their functional role as regional carbon sinks (Hofstede 1995; Luteyn 1999). Páramo grasslands in Ecuador occur between elevations of 3200 m and 4700 m (Luteyn 1992), where a high-altitude equatorial climate facilitates consistently cool temperatures and high precipitation, including fog-moisture capture. Histic Andisols (organic-rich volcanic ash soils) are the dominant soils in Ecuadorian páramo grasslands (Buytaert et al. 2007a). Due to the high soil-water retention of histic Andisols and the perennially low temperatures, soil conditions in the Ecuadorian páramos are conducive to low decomposition rates of soil organic matter. The chemical characteristics of Andisols also contribute to soil organic matter retention, as properties such as low pH and the development of aluminum (Al)-complexes help to stabilize organic carbon in the soil (Poulenard et al. 2003). High soil organic matter retention, low decomposition rates, and thick A horizons (0.5–1 m) result in the storage of large amounts of soil carbon in the form of organic matter. This supports the role of Ecuadorian páramo grasslands as a carbon sink and, in fact, they have been shown to store up to 77 tons C ha⁻¹ (~7 kg m⁻²) within the first 10 cm of the soil profile (Farley et al. 2013). Páramo grasslands are sensitive to climate change and anthropogenic disturbances, which can result in soil carbon loss via leaching or soil organic matter decomposition and uptake. Consequently, changes in land use or land cover that affect physical or chemical soil properties can have significant implications for soil carbon storage and for the functional role of páramos as an atmospheric carbon sink or source.

In the Ecuadorian páramo, soil water retention is significantly correlated with organic carbon content (Buytaert et al. 2007a). Soil moisture in the Ecuadorian páramo grasslands is very high.
Previous studies have reported volumetric soil moisture content ranging between 79 and 86% for páramo grasslands in central-south Ecuador (Harden et al. 2013; Hartsig 2011). Working from the low bulk densities and the volumetric water contents reported in these earlier studies, WFPS can be estimated to be approximately 90 to 95%. According to the model proposed by Linn and Doran (1984), the high moisture contents of páramo soils (Harden et al. 2013; Hartsig 2011) support the expectation that, in the case of Ecuadorian páramos, a decrease in soil moisture content would likely correspond to an increase in soil CO$_2$ flux due to higher soil organic matter decomposition rates associated with more aerobic conditions. The increased rate of decomposition and uptake of soil carbon would result in a net decrease in soil carbon stocks. Previous studies in south Ecuadorian páramos have shown that land-use change, such as the transition from grassland to tree plantation, can significantly reduce both soil water content (Buytaert et al. 2006; Buyteart et al. 2007b; Harden et al. 2013) and soil carbon storage (Farley et al. 2013) by up to 50% and ~35%, respectively. This suggests that land-use-induced changes in soil moisture play an important role in soil carbon processes in Ecuadorian páramo landscapes; however, this hypothesis has not been tested explicitly.

Apart from agriculture, afforestation and controlled burning are prominent types of land management practices associated with land-use change in the Ecuadorian páramos. Afforestation, including both tree plantation and woody native forest encroachment, has been promoted for páramo grasslands for a variety of purposes in Ecuador, including compensation for ecosystem service programs and the generation of international carbon credits (Farley et al. 2011). Previous research has used tree plantations in space-for-time substitution studies to assess the effects of afforestation on páramo soil hydrology and carbon stocks to better understand the ecological benefits and consequences of afforestation of paramo grasslands. These studies found that, while aboveground carbon was greater in pine tree plantations, soil carbon content and water yield in the plantation, 42 tons C ha$^{-1}$ and 506–933 mm y$^{-1}$, respectively, were much lower than in páramo grassland, at 77 tons C ha$^{-1}$ (Buytaert et al. 2007b) and 175 mm y$^{-1}$ (Farley et al. 2013), respectively.

Anthropogenic fire has played a significant role in defining páramo grassland boundaries (Keating 2007; Horn and Kappelle 2009) and is thought to have been a prevalent land management practice throughout the Andean páramos for more than 10,000 years (White 2013).
Fire suppression in páramos thus has the potential to promote native woody forest encroachment into grassland areas (White 2013; Luteyn 1999). Consequently, recent efforts by the Ecuadorian Ministry of Environment to limit anthropogenic burning have the potential to result in larger areas of grassland becoming forest via succession of native woody species. Soil moisture and soil organic matter have been shown not to vary significantly between grassland and montane forest vegetative communities (Hofstede et al. 2002). As a result, according to the work of Linn and Doran (1984), then, soil CO₂ flux would not be expected to differ between grassland páramo and areas where grassland has been converted to native forest cover. As no studies have reported soil carbon content for montane forest in areas that were previously páramo grassland, and no soil CO₂ flux rates have been reported for either páramo grassland or montane forest, this hypothesis has also not been explicitly tested. A better understanding of soil carbon-cycle processes in páramo landscapes is required to anticipate the effects of fire suppression on soil carbon stocks in native montane forests in the Ecuadorian Andes.

Due to the significant correlation between soil moisture and organic carbon content of páramo soils (Buytaert et al. 2007a), there is a critical need to assess the effects of land-use-induced changes in soil moisture on soil carbon fluxes. The objectives of this research were to compare soil CO₂ flux and soil moisture among sites representing four types of land use in Ecuadorian páramo landscapes, and to examine the relationship between CO₂ flux and soil moisture across these four sites. The four study sites were chosen to represent four different types of land use common to the Ecuadorian Andes: páramo grassland (last burned 5–6 years prior to the study), recently burned páramo grassland (burned 6 months prior to the study), pine plantation (25 years old), and native woody forest. Soil CO₂ flux rates for the two grassland sites were not expected to differ (Hypothesis 1). Further, CO₂ flux at the native forest site was not expected to differ from flux at the grassland sites (Hypothesis 2), due to evidence that soil moisture and soil organic matter content are similar between these two land-cover types (Hofstede et al. 2002). Because grassland and native forest sites have previously been shown to have higher moisture contents than pine plantations (Hofstede et al. 2002; Buytaert et al. 2006; Buytaert et al. 2007b; Harden et al. 2013), soil CO₂ flux was expected to be highest at the pine site (Hypothesis 3), and soil CO₂ flux was expected to differ between the pine plantation and native forest sites (Hypothesis 4). Finally, soil CO₂ flux was expected to follow the trend outlined by Linn and Doran (1984) in response to soil moisture differences across the four sites:
a non-linear relationship was expected in which soil CO$_2$ flux would respond positively to higher levels of soil moisture until reaching a WFPS of 60%, at which point soil CO$_2$ flux would begin to decrease (Hypothesis 5).

2. Materials and Methods

2.1 Site Description

This study was conducted at the Mazar Wildlife Reserve (MWR) in the Sangay National Park (Figure 2–2). The MWR is a privately owned 1,800-ha reserve, located in the Nudo del Azuay, which spans an elevation gradient from 1,200 m to 4,500 m above sea level. The MWR supports the conservation of Andean cloud forest and páramo grassland ecosystems that occur within this elevation range and scientific research that contributes to a better understanding of these systems (FCT 2008). The MWR provides an ideal location for studying the effects of different types of land use on soil-water and soil-carbon processes. Using an aerial photo from 1977 and expert knowledge of the MWR owner and manager of 30 years (S. White, personal communication, December 2013), we identified and described four study sites with different histories of land use. Historically, the area of the MWR was predominantly characterized by páramo grassland maintained by regular cattle grazing and controlled burning approximately every 4 to 6 years (S. White, personal communication, December 2013). A variety of uses and land-management practices have been implemented in the MWR páramo, which is now a heterogeneous landscape containing native forest, pine plantations (25 years old), alpaca-grazed páramo (~350 ha), and páramo with varying burn histories (with most recent burn ranging from 6 months to >40 years earlier).

We chose four sites, representing different types of land use: a páramo grassland site with no burning during the previous 4 to 6 years, a páramo grassland site that had recently burned within 6 months of the study, a site with native woody species forest cover, and an afforested site with a stand of *Pinus patula* established 25 years prior to sampling (Figure 2–3; Table 2-1). At each study site, a location was selected arbitrarily to establish a plot center. Four data collection points were established, each 5 m from the plot center, at 0°, 90°, 180°, and 270°. Data collection points were used for ongoing field measurements during the study period. All field data
Figure 2-2 Map of study area. This study was conducted in the Mazar Wildlife Reserve located in the Nudo del Azuay east-northeast of the city of Cuenca. (Source: Harden et al. 2013)
Figure 2-3 Scanned topographic map (IGM 1969) with letters indicating the location of the (A) pine plantation, (B) native forest, (C) páramo grassland, and (D) recently burned páramo grassland sites. Arrows from each site location provided on the maps point to a photo of each of the four study sites. Grid cells on the maps are 1 km by 1 km. Green shading on the map forest areas do not accurately support forest cover area and should be disregarded.
collection and sample collections were taken over a three-week sampling period from December 12 to December 31, 2013.

Our research was based out of the MWR Jalagus field camp (UTM: S 02.57081, W 078.74585) located at 3,300 m elevation. Average daily temperatures at Jalagus range from 7°C to 21°C, and the mean annual precipitation exceeds 1,500 mm, according to a time series analysis (1998–2009) by Bookhagen (in review) and on-site measures of precipitation by the FCT (Bremer 2012). Native forest vegetation is tropical montane cloud forest. Páramo grassland is dominated by tussock grasses and *Puya clava-herculis*, with little to no bare-soil interspace. The MWR sites in this study are between 3,200 m and 3,450 m in elevation and are characterized by non-allophanic Andisol soils dominated by Al-humus complexes (Bremer 2012; Poulénard et al. 2003).

2.2 Field Methods

To measure soil CO$_2$ flux, one PVC soil collar (20 cm diameter) was installed at each of the four data collection points within each of the four study sites (4 x 4 = 16 collars). All soil collars were installed at least 24 hours prior to the first soil CO$_2$ flux measurement, to minimize flux variations from site disturbance, and were removed upon completion of the study. A total of nine field CO$_2$ measurements were taken at each soil collar over the course of three weeks (the number of measurements being constrained by electrical power limitations). On each of the nine data collection days, CO$_2$ flux was measured at all data collection points. All field CO$_2$ measurements were taken between 10:00 and 14:00 to minimize effects of diurnal variation (Mielnick and Dugas 2000; Laporte et al. 2001; Petrone et al. 2008). At the time of each measurement, a LI-8100 20-cm Survey Chamber (chamber) was placed atop an installed soil collar, and the soil CO$_2$ flux rate was measured using a LICOR 8100A Automated Soil CO$_2$ Flux Infrared Gas Analyzer (LI-8100). Settings for each site measurement included a total measurement length of 120 seconds, a deadband (time between measurements) of 30 seconds, and purge of time 60 seconds. At the time of soil flux measurement, soil temperature and volumetric soil moisture were also measured, adjacent to the soil collar, using an Omega Type E Soil Temperature Probe and EC H$_2$O Soil Moisture Probe, respectively. For data analysis, volumetric soil moisture values were converted to water-filled pore space (WFPS) to compare results with predicted trends in WFPS and soil CO$_2$ flux rates as reported in previous studies.
Table 2-1 MWR soil properties and site descriptions. Results of laboratory analyses (pH, total soil carbon, soil organic carbon, soil organic matter content) and calculated estimates (bulk density, porosity) reported as mean values. For each study site: N=4 for pH; N=6 for bulk density, porosity, soil organic carbon, and soil organic matter; N=8 for total soil carbon.

<table>
<thead>
<tr>
<th>Study Site</th>
<th>pH (SE)</th>
<th>Bulk Density g cm⁻³ (SE)</th>
<th>Porosity (SE)</th>
<th>Total Soil Carbon g kg⁻¹ (SE)</th>
<th>Soil Organic Carbon g kg⁻¹ (SE)</th>
<th>Soil Organic Matter (%)</th>
<th>Land-Use and Site Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native Forest</td>
<td>4.32 (0.07)</td>
<td>0.6017 (.0061)</td>
<td>0.7729 (.0023)</td>
<td>142.6 (5.4)</td>
<td>135.9 (2.1)</td>
<td>27.2 (0.4)</td>
<td>Native montane cloud forest at least 35 years old; dominant plant families: Araliaceae, Asteraceae, Clusiaceae, Cyatheaceae, Myrsinaceae</td>
</tr>
<tr>
<td>Páramo Grassland</td>
<td>4.39 (0.04)</td>
<td>0.5769 (.0075)</td>
<td>0.7823 (.0028)</td>
<td>176.5 (5.6)</td>
<td>145.2 (3.0)</td>
<td>29.1 (0.6)</td>
<td>Intermediate burn (6 years); Alpaca grazing; tussock grass &amp; <em>Puya clava-herculis</em></td>
</tr>
<tr>
<td>Recently Burned Páramo Grassland</td>
<td>4.62 (0.08)</td>
<td>0.6437 (.0052)</td>
<td>0.7571 (.0020)</td>
<td>137.0 (2.5)</td>
<td>121.9 (1.6)</td>
<td>24.4 (0.3)</td>
<td>Recent burn (&lt;6 months); Alpaca grazing; tussock grass &amp; <em>Puya clava-herculis</em></td>
</tr>
<tr>
<td>Pine Plantation</td>
<td>4.10 (0.06)</td>
<td>0.6219 (.006)</td>
<td>0.7653 (.0021)</td>
<td>138.0 (4.2)</td>
<td>129.0 (1.9)</td>
<td>25.8 (0.4)</td>
<td>Pine plantation (25 years old); previously páramo grassland used for grazing; <em>Pinus patula</em></td>
</tr>
</tbody>
</table>

*SE: Standard Error

bSoil porosity values reported here were determined using the standard mineral particle density (2.65 g cm⁻³). Additional data analyses, not reported here, were performed using a particle density of 1.5 g cm⁻³, reported for boreal wetland soils with very high organic matter (Redding and Devito 2005). These two particle densities are considered to bracket the actual particle density for the soils collected in the Mazar Wildlife Reserve, and calculations using both particle density values yielded the same trend between soil water-filled pore space and soil CO₂ flux.
Soil samples were collected from within each study site for soil carbon-content analysis. Samples consisted of soil cores taken adjacent to each of the four soil collars within each of the four study sites (16 soil cores in total). Analysis of the 16 individual cores was used to determine soil properties (pH, total soil carbon, soil organic carbon, bulk density, porosity, water-filled pore space) for each study site. Soil cores were taken with a 2.0-cm diameter Oakfield Model G soil sampler to a depth of 10 cm and placed on ice within 24 hours of collection for shipment to the University of Tennessee, Knoxville, Tennessee.

2.3 Soil Properties

Soil pH was determined in deionized water solution using a Denver Instrument UltraBASIC pH Meter. To determine soil carbon content with a Costech Combustion Module, subsamples of each soil were dried at 60°C for 48 hours (ASTM 2010) then homogenized into a fine powder. Approximately 7.0 mg of each soil sample was weighed into a 3 x 5 mm tin capsule and stored in a desiccator until analysis. For soil organic carbon content, soils were first acidified in 3.0 N phosphoric acid to remove the soil mineral component. Soil organic matter (%SOM by weight) content was estimated as:

\[
\%\text{SOM} = \%\text{soil organic carbon} \times 2
\]

which is a relationship used for soils with high organic matter content (Mitsch and Gosselink 2007). Soil organic matter content was then used to determine soil bulk density using the following method of Adams (1973):

\[
\text{BD} = 100 / [\left(\%\text{SOM} / \text{OM}_{\text{BD}}\right) + (100 - \%\text{SOM}) / \text{MS}_{\text{BD}}]
\]

where BD is bulk density, OM_{BD} is the average bulk density of organic matter (0.223 g cm\(^{-3}\)), and MS_{BD} is the average mineral bulk density (1.64 g cm\(^{-3}\)). Soil porosity was then determined for the calculation of the water-filled pore space (WFPS) using the following method:

\[
\text{Porosity} = (1 - \text{BD} / \text{Mineral particle density}) \times 100
\]

where mineral particle density was assumed to be 2.65 g cm\(^{-3}\). Water-filled pore space (WFPS) was then calculated as volumetric soil moisture (measured in situ) divided by Porosity.
2.4 Statistical Analyses

Data sets for all soil parameters at all sites were tested for normality using the Shapiro-Wilk test at each study site. The Shapiro-Wilk normality test was used as it is more powerful than the K-S (Kolmogorov-Smirnov) test and is recommended for small sample sizes (n<50) (Ghasemi and Zahediasl 2012). Due to inconsistencies in normality between the soil CO₂ flux, soil moisture, and soil temperature data sets, and to the inability to apply data transformations universally to achieve normality, non-parametric methods were selected for statistical analyses of these parameters. Differences in soil CO₂ flux, soil moisture, and soil temperature between the study sites were assessed using the Mann-Whitney Wilcoxon test (α = 0.05). To describe the relationship between soil water-filled pore space and soil CO₂ flux across all four sites, the Spearman’s Rho correlation test was first used to test for a linear relationship between the two variables. Where a significant linear relationship was not found, data were fitted using a polynomial regression. Statistical analyses were performed using the R statistical software (R 2013).

3. Results

Mean values of volumetric soil moisture, CO₂ flux, soil temperature, and percent water-filled pore space (WFPS) are provided in Table 2.2. The highest mean soil CO₂ flux rate, 5.79 g-C m⁻² d⁻¹ (standard error = 0.35 g-C m⁻² d⁻¹), was measured at the pine plantation site. The lowest mean soil CO₂ flux rate, 3.61 g-C m⁻² d⁻¹ (standard error = 0.23 g-C m⁻² d⁻¹) was measured at the recently burned páramo grassland site. WFPS is reported and discussed to allow for a more comprehensive examination of soil biological responses to soil moisture variation. Mean soil % WFPS was highest at the páramo, 69%, and recently burned páramo, 68%, sites and did not differ significantly between those grassland sites. Mean soil % WFPS at the grassland sites was significantly higher than the mean % WFPS at the forest and pine sites. The lowest % WFPS, ~30%, was measured at the pine plantation. The expected biological threshold for aerobic-anaerobic activity, 60% WFPS (Linn and Doran 1984), corresponded to a volumetric soil moisture of ~46% for MWR soils.

Contrary to Hypothesis 1, soil CO₂ flux in the páramo grassland was significantly (p<0.01) greater than flux in the recently burned páramo grassland site (Figure 2-3). CO₂ flux at
Table 2-2 MWR field data. Mean values are given for soil CO$_2$ flux, soil moisture (expressed in both volumetric water content and water-filled pore space), and soil temperature for páramo (PA), recently páramo burned (burned within 6 months, PAB), native forest (NF), and pine plantation (PI).

<table>
<thead>
<tr>
<th>Study Site (sample N)</th>
<th>Soil Flux g CO$_2$-C m$^{-2}$ d$^{-1}$ (SE$^a$)</th>
<th>% Volumetric Soil Moisture (SE)</th>
<th>% Water-Filled Pore Space (SE)</th>
<th>Soil Temperature °C (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF (36)</td>
<td>5.59 (0.19)</td>
<td>39.0 (2.2)</td>
<td>50.5 (2.8)</td>
<td>9.57 (0.04)</td>
</tr>
<tr>
<td>PA (35)</td>
<td>4.75 (0.23)</td>
<td>55.5 (0.8)</td>
<td>69.0 (2.2)</td>
<td>12.99 (0.08)</td>
</tr>
<tr>
<td>PAB (31)</td>
<td>3.61 (0.23)</td>
<td>51.7 (0.8)</td>
<td>68.3 (1.0)</td>
<td>12.82 (0.08)</td>
</tr>
<tr>
<td>PI (36)</td>
<td>5.79 (0.35)</td>
<td>22.8 (0.7)</td>
<td>29.8 (0.1)</td>
<td>9.87 (0.05)</td>
</tr>
</tbody>
</table>

$^a$ SE: Standard Error
Figure 2-4  (A) Soil CO$_2$ flux and (B) % water-filled pore space, measured at the Mazar Wildlife Reserve, Ecuador. Study areas represented are: native forest (NF), páramo grassland (PA), recently burned (<6 months) páramo grassland (PAB), and pine plantation (PI). Letters A-C indicate significantly different (p < 0.05) values of soil CO$_2$ flux and water-filled pore space. Lines within the boxes indicate median values.
the native forest was significantly (p<0.05) greater than flux at both grassland sites, causing Hypothesis 2 to be rejected. The highest mean CO$_2$ flux was measured under the pines, supporting Hypothesis 3. However, we rejected Hypothesis 4, as mean soil CO$_2$ flux did not differ significantly between the native forest and pine plantation sites. To further explore similarities and differences in soil CO$_2$ flux among the four study sites, we compared only the flux rates within a constrained range of soil moistures. Within the range of 20–40% WFPS, the Wilcoxon test showed no significant difference (p = 0.522) in soil CO$_2$ flux at the native forest and pine sites (Figure 2–7 A, in appendix). Within the range of 50–70% WFPS, soil CO$_2$ flux differed significantly (p < 0.01) among the native forest, páramo, and recently burned páramo sites. Soil CO$_2$ flux was highest at the native forest site and lowest at the recently burned páramo site (Figure 2–7 B, in appendix).

By sampling across four different types of land cover, we were able to capture a range of soil moisture content, from 13–74% WFPS, which corresponds to 23–56% volumetric water content. This range of WFPS was sufficient to test Hypothesis 5: that the response of soil CO$_2$ flux rate to land-use induced changes in soil moisture would follow the theoretical model proposed by Linn and Doran (1984). Although our results do not cause us to reject Hypothesis 5, the data do not provide robust support. As shown in Figure 2-4, the soil CO$_2$ flux rate across the four study sites appears to follow the expected trend. The polynomial regression line fit the data (p<0.01; $R^2 = 0.083$), indicating a significant but weak relationship between soil moisture change and soil CO$_2$ flux across the four different types of land use, and supporting Hypothesis 5. This trend suggests that flux rates increase with increasing soil moisture content within the aerobic WFPS range of 0–60% and decrease as WFPS contents increase beyond 60%. However, when the data were partitioned into two groups—aerobic and anaerobic—the Spearman’s Rho correlation test showed no significant linear relationship between soil CO$_2$ flux and soil moisture for either group (aerobic: p = 0.301, Rho = 0.134; anaerobic: p = 0.563, Rho = 0.069). Further, the shaded confidence interval ($\alpha = 0.05$) for the fitted polynomial line (Figure 2-5 A) shows that many measurements of CO$_2$ flux do not fall within the predicted values. The absence of significant correlations on the limbs of the curve suggests that our results differ from the theoretical trend proposed by Linn and Doran (1984) and do not support Hypothesis 5 in terms of the expected relationship within the partitioned aerobic and anaerobic gradients. However, the range of CO$_2$ flux is notably smaller at the inflection point of the fitted polynomial line, between
50–60% WFPS, with no flux rates measured below 4.5 g CO₂-C m⁻² d⁻¹ at that point. The absence of low flux rates at the inflection point supports the theoretical model (Linn and Doran 1984), in which CO₂ flux is higher where soil conditions are wet and aerobic. Correlation analysis of % WFPS, including both aerobic and anaerobic groups, and soil CO₂ flux rate across all four sites did reveal a weak, negative relationship between soil moisture and CO₂ flux (p<0.05, Rho = -0.17, n = 138).

Although this study did not include a treatment experiment to measure the response of soil CO₂ flux to the spatial variability of soil moisture within each land-use type, the range of volumetric soil moisture measured in the native forest, from approximately 13% to 54% (estimated WFPS of 17% to 70%) allowed us to conduct an exploratory analysis of soil CO₂ flux response to the gradient of % WFPS captured at that site. When a quadratic polynomial regression line is fitted to the soil CO₂ flux and soil moisture at the native forest site, the regression is not significant (p > 0.05), but the curve appears to show a weak parabolic trend: soil CO₂ flux rates are relatively higher as soil moisture increases from 17% to ~55%, at which point soil CO₂ rates appears to decrease above 55–60% (Figure 2-5 B).

4. Discussion

The significant difference in soil CO₂ flux between the páramo grassland and recently burned páramo grassland sites was unexpected due to similarities in plant community, soil organic matter, and soil moisture. Since the mean WFPS values for both grassland sites were greater than the 60% threshold defined by Linn and Doran (1984), soil CO₂ flux was expected to be similar between the sites due to the biological limitations on microbial activity associated with anaerobic conditions. Although soil temperature can also significantly affect soil CO₂ rates, soil temperature was not significantly different between the two grassland sites, suggesting this environmental parameter is likely not correlated with the difference in flux rate in the range of temperatures measured in this environment. Consequently, we hypothesize that the difference in soil CO₂ flux rate between the grassland sites is due to biochemical effects of fire at the recently burned páramo site.

Fire can result in the transformation of soil organic matter susceptible to microbial decomposition (labile carbon) into more recalcitrant forms of pyrogenic organic matter that are more resistant to microbial decomposition processes. Studies have shown that pyrogenic organic
Figure 2-5 Soil CO$_2$ flux response to variation in water-filled pore space at four sites representing different histories of land use in the Mazar Wildlife Reserve, Ecuador.
Figure 2-6 Relationships between soil moisture and soil CO\textsubscript{2} flux (A) across all sites and (B) at the native forest site, fitted with a quadratic polynomial regression equations.
material in recently burned grasslands can be more susceptible to transport and leaching than untransformed, labile forms of soil organic material (Knicker et al. 2012; Hilscher and Knicker 2011). Transformation and loss of pyrogenic organic material post-fire can result in the loss of labile carbon material, thus limiting soil carbon resources available for microbial decomposition and uptake by plants and microorganisms. In this case, lower soil CO\textsubscript{2} flux rates would reflect lower rates of soil microbial activity due to nutrient limitation. Plant activity and nutrient uptake are likely to be elevated at the recently burned páramo site because of post-burn regrowth and recovery of the plant community. Bell et al. (2014) showed that soil microbial activity—expressed as extracellular enzyme activity associated with nutrient acquisition, including microbial soil carbon uptake—does not always correspond positively to plant activity. In fact, soil microbial activity has been shown to decrease when plant nutrient utilization is higher (Bell et al. 2014), a response that could manifest as lower soil CO\textsubscript{2} flux rates. Although burning is considered a disturbance, and oxidation of organic material during the burn may temporarily contribute to atmospheric CO\textsubscript{2} release, lower soil CO\textsubscript{2} flux rates associated with lower microbial activity can indicate lower rates of soil organic matter decomposition after a fire. In addition, the development of pyrogenic organic matter, which is more resistant to microbial decomposition, can contribute to an increase in more stable forms of organic carbon in páramo grassland soils. These processes merit further examination in future research to explore the potential long-term benefits of different burning regimes on soil carbon storage in Ecuadorian páramos. Because conservation efforts and programs in Ecuador have begun to compensate highland communities for promoting ecosystem services (Farley et al. 2011; Bremer et al. 2014), the results of such research could be used to influence land management decisions.

It is important to note that, although all field study sites were located within 2 km of each other, keeping aspect and elevation consistent, potentially confounding factors exist between the páramo and recently burned páramo sites due to localized differences. Specifically, the recently burned páramo site is located in an area that has been more regularly grazed and may have thus been burned more frequently (every 2–3 years versus every 4–6 years) within the past 30 years. Due to its proximity to an alpaca-holding and grazing area, this site may also have been subjected to more direct anthropogenic interaction, resulting in a higher level of disturbance. As a result, the recently burned páramo site may have been more susceptible to erosion, or other physical processes, that could also have led to greater loss of soil carbon to leaching and runoff.
Future studies, including more extensive field data collection at multiple sites representative of each type of land use, are recommended.

The significant difference in CO$_2$ flux between the native forest sites and the grassland sites was also unexpected. The prediction that soil CO$_2$ flux would be similar between the forest and grassland sites (*Hypothesis 2*) was predicated on evidence from a previous study that showed soil moisture did not significantly differ between these land covers in páramo landscapes (Hofstede et al. 2002). Our results, expressed as % WFPS show that, in the MWR, soil water content was significantly lower at the forest site than at the grassland sites. Further, although the range of % WFPS at the native forest site included both aerobic and anaerobic conditions, more than half of the field measurements taken fell below the 60% WFPS anaerobic threshold (Linn and Doran 1984). Conversely, all but two soil moisture measurements taken at both grassland sites were greater than 60% WFPS. This likely contributes to the higher mean soil CO$_2$ flux rate at the native forest site, as CO$_2$ flux has been shown to be higher in aerobic conditions (Linn and Doran 1984; Moore and Dalva 1993).

As expected, the highest values of soil CO$_2$ flux appeared to be under the pine plantation; however, CO$_2$ flux did not differ significantly between the pine and native forest sites. Since mean %WFPS at the native forest site was significantly greater than at the pine plantation site, and mean %WFPS under the forest was ~50%—below the expected anaerobic threshold—soil CO$_2$ flux would be expected to be greater at the native forest site. However, the lack of significant difference in mean soil CO$_2$ flux rate between the forest and pine sites suggests that differences in vegetative community structure between tree-covered sites may have less bearing on drivers affecting soil CO$_2$ flux rate than do differences between grass cover and woody vegetation cover (Creamer et al. 2013). This is supported by the lack of difference in soil CO$_2$ flux between the native forest and pine site within a range 20–40% WFPS (Figure 2-7 A in appendix). Further, the differences in soil CO$_2$ flux between the native forest and páramo sites within a constrained range of %WFPS (50–70%) (Figure 2-7 B in appendix) suggests that soil CO$_2$ flux can differ under different land-cover types with similar soil moisture conditions. Changes in soil microbial community composition or structure with land-cover change from grassland to forest can have implications for organic matter decomposition and soil carbon utilization (Yannarell et al. 2014). To determine whether this is the case for the Ecuadorian
páramo, future work is needed to investigate differences in microbial community and soil carbon-related activity between grassland and forest sites.

The trend described between soil CO\textsubscript{2} flux and soil moisture across the four study sites is conceptually similar to the expected trend adapted from the work of Linn and Doran (1984). When we compared CO\textsubscript{2} flux for samples in the range of 55–60\% WFPS, we found that the range of flux was smaller compared to flux in the WFPS ranges below 55\% and above 60\%. Between 55–60\% WFPS, the lower boundary of soil CO\textsubscript{2} flux rate is 3.7 g CO\textsubscript{2}-C m\textsuperscript{-2} d\textsuperscript{-1}, compared to a lower flux boundary of 1.8 g CO\textsubscript{2}-C m\textsuperscript{-2} d\textsuperscript{-1} between 15–55\% and 60–78\% WFPS. Also, the range of CO\textsubscript{2} flux between 55–60\% WFPS was half that of the flux range measured below 55\% WFPS and above 60\% WFPS. The constrained lower boundary and smaller range of flux between 55–60\% WFPS coincides with the range of soil moisture conditions expected for microbial activity to peak within the transition from aerobic and anaerobic conditions (Linn and Doran 1984). However, the large range in soil CO\textsubscript{2} flux and lack of significant linear correlation with soil moisture within the range of aerobic and anaerobic moisture conditions at the MWR differed from the Linn and Doran (1984) theoretical model, indicating that soil moisture may not be a primary driver of soil CO\textsubscript{2} flux rates. The relationship expressed by Linn and Doran (1984) was derived in a controlled laboratory experiment and represents a theoretical trend for conditions in which all other environmental factors, apart from soil moisture content, were held constant. In our study, however, we present field measurements of soil CO\textsubscript{2} flux that represent the combined effects of potentially confounding factors, not identified or measured and different soil moisture contents. For instance, the difference in soil temperature between the two studies, ~28\°C in the study of Linn and Doran (1984) and 9–12\°C in our study, may contribute to the difference in magnitude of soil CO\textsubscript{2} flux response to soil moisture between the studies, as lower soil temperatures limit soil CO\textsubscript{2} flux (Dornbush and Raich 2006; Wang et al. 2008). This may explain why the magnitude of the CO\textsubscript{2} flux trend found across all study sites at MWR was more subtle than the relationship shown by Linn and Doran (1984). Our results suggest that biological activity resulting in CO\textsubscript{2} production responds to the biological soil moisture threshold expected with a transition from aerobic to anaerobic soil moisture conditions. However, no conclusive statements can be regarding the relationship between soil moisture and soil CO\textsubscript{2} flux in aerobic or anaerobic soil moisture gradients across the four study sites in the MWR.
In addition, the theoretical aerobic-anaerobic threshold of approximately 60% WFPS does not take into account the potential for microbial organisms that are either adapted (Pett-Ridge and Firestone 2005) or acclimated (Blodau and Moore 2002) to perform aerobic processes in anaerobic (>60% WFPS) soil moisture conditions. Future that investigates the community structure and biological thresholds of microorganisms in páramo soils at the Mazar Wildlife Reserve may help explain the range of soil CO₂ flux measured at the páramo grassland site. Further, the relationship between soil moisture and soil CO₂ flux may vary within each study site, though this was not tested in this study. The trend of CO₂ flux over a range of soil moistures at the native forest site was similar to that observed across all study sites, even though the flux-moisture relationship at the native forest was not significant. The significant relationship between soil moisture and flux across all sites may be due to the inclusion of a wider range of soil moisture as a result of different soil water contents under different types of land use. This is consistent with the weak, negative relationship found across all sites, which supports the concept of a decrease in soil CO₂ flux when shifting from more to less aerobic soil moisture conditions. From these results, we infer that, in páramo landscape of the MWR, decreases in soil moisture content with land-use change from grassland to forest or pine plantation can lead to higher soil CO₂ flux rates from soils in wooded areas. This inference is consistent with changes in soil CO₂ flux rates at other locations where decreases in soil moisture with land-use change created more aerobic conditions (Houghton et al. 2012).

In terms of environmental drivers that influence soil CO₂ flux rate, soil temperature is known to significantly affect flux rates where soil moisture is less influential. Numerous studies have shown that soil temperature is positively correlated with soil CO₂ flux, as soil biological agents, especially microbes, increase in activity and respiration in warmer temperatures (Bowden et al. 1998; Qi and Xu 2001; Laporte et al. 2002; Litton et al. 2011). In other environments, shrub encroachment into grassland has been shown to have a negative effect on soil CO₂ flux rates due to lower soil temperatures under shrub cover (Yannarell et al. 2014). These trends were not observed at the MWR sites in this study. Soil temperatures significantly higher at the two grassland sites than at the forest and plantation sites, but the lower mean soil temperatures at the forest and pine sites were associated with the highest mean soil CO₂ flux rates. The inverse interaction between soil temperature and soil CO₂ flux at the MWR sites may indicate that differences in soil temperature, ranging from 9°C to 13°C, do not significantly affect soil CO₂
flux rates in areas with the four types of land use included in this study. Consistently cool soil temperatures in this region likely play a significant role in limiting soil biological activity and soil organic matter decomposition. Due to the short time scale and equatorial location of this study, within-site variation in soil temperature was not significant. Therefore, it was not possible to assess the effects of soil temperature changes on soil CO₂ flux rates within each study site. Future research, using a controlled treatment experiment with constant conditions of soil moisture and land use to investigate the effects of soil temperature variation on soil CO₂ flux, could provide valuable insight into the potential soil-warming effects of climate change in páramo landscapes.

Changes in soil physical properties that result from land-use change can also affect soil CO₂ flux rates if they change rates of soil organic material decomposition and microbial soil carbon uptake. A recent study conducted at the MWR showed that soil properties are significantly altered in south Ecuadorian páramos where grassland is converted to pine plantation (Harden et al. 2013). Notable changes in soil properties included a shift from friable soil with moderate-to-strong sub-angular structures under grassland to very friable soil with granular structures under pine forest (Harden et al. 2013). Although aggregate size and stability were not analyzed by Harden et al. (2013), the shift to very friable, granular structures under the pine forest may have made soils in MWR páramos more susceptible to microbial decomposition. Changes in physical soil properties such as this can increase accessibility of soil organic matter to microbial decomposers, thus potentially increasing the rate of soil organic matter decomposition. Ecological succession from grassland to forest can also significantly alter microbial community structure, with implications for the amount and fractions of soil carbon utilized by decomposers. Yannarell et al. (2014) showed that the successional encroachment of woody shrub communities into prairie grasslands in the Midwestern United States significantly altered the composition of the soil fungi community, which contributes to utilization of different fractions of soil carbon pools. Further, compared to semi-wooded grasslands, woodlands have been shown to have higher soil carbon-acquisition microbial enzyme activity (Garcia-Morote et al. 2012), which corresponds to both increased soil CO₂ flux rates and decreases in soil organic carbon contents (Creamer et al. 2013). Consequently, the significant difference in soil CO₂ flux between the grassland and tree-covered sites may be a function of differences in microbial community and structure, in conjunction with the response of microbial activity to more aerobic
soil moisture conditions at afforested sites. To date, no studies have examined changes in microbial activity or microbial community with land-use change in Ecuadorian páramos.

5. Conclusion

Soil CO$_2$ flux rates were found to differ among types of land use in páramo landscapes of the Mazar Wildlife Reserve (MWR). Soil CO$_2$ flux was higher at a páramo grassland than at a recently burned páramo grassland. Although close in proximity, the recently burned páramo and páramo sites appeared to have some differences in history of land use. However, the difference in soil CO$_2$ flux between the sites merits further investigation that directly tests the effects of burning on pathways of soil carbon loss, including the development of more recalcitrant pyrogenic organic material. Mean soil CO$_2$ flux was similar between native forest and pine plantation communities, and flux measured at sites with tree cover was significantly greater than at páramo grassland sites. Our results suggest that soil CO$_2$ flux in the páramo of the MWR is susceptible to change where land-use change involves a transition from páramo grassland to tree cover. A negative, weak relationship between soil moisture and CO$_2$ flux, found across all four sites, suggests that the differences in CO$_2$ flux with land-use type may be a function of different soil moisture contents. CO$_2$ flux appeared to respond to biological soil moisture thresholds; however, no clear trend was detected between CO$_2$ flux and soil moisture within aerobic and anaerobic soil moisture gradients. Our results suggest that decreases in soil moisture content with land-use change from grassland to forest or pine plantation can lead to higher soil CO$_2$ flux rates, though we recognize that other mechanisms not tested in this study may also affect soil CO$_2$ flux rates under different land uses. We recommend that future research investigate potential shifts in soil microbial community and activity among different types of land use. Such shifts may correspond to different soil organic matter decomposition rates and, thus, different soil CO$_2$ flux rates.

To our knowledge, these are the first soil CO$_2$ flux rates reported for Ecuadorian páramos. Further, this study provides a first look at the role of soil CO$_2$ flux as a mechanism for soil carbon loss and the interaction between soil moisture and CO$_2$ flux rates in páramo landscapes of the MWR. In spite of being a substantial regional carbon sink in Ecuador, the páramo landscape is changing at a rapid rate due to human-environment interactions that change land-use and land-cover. A better understanding of the mechanisms driving fluxes of soil carbon
in Ecuadorian páramos is crucial to predicting shifts in terrestrial carbon pools and integrating this information into land management decisions in the Andes.
References


Dornbush, M.E., Raich, J.W., 2006. Soil temperature, not aboveground plant productivity, best predicts intra-annual variations of soil respiration in central Iowa grasslands. Ecosystems 9, 909-920


Qi, Y., Xu, M., 2001. Separating the effects of moisture and temperature on soil CO$_2$ efflux in a coniferous forest in the Sierra Nevada mountains.


Appendices
Figure 2-7 Photos of Mazar Wildlife Reserve field study sites. Photos of the four soil collar data collection points within each study site are provided in images A (native forest), B (páramo grassland), C (recently burned páramo grassland), and D (pine forest).
Figure 2–7 Continued
Figure 2–7 (continued)
Figure 2–7 (continued)
Figure 2–7 (continued)
Figure 2–7 Continued
Figure 2–7 Continued
Figure 2–7 Continued
Table 2-3 Measured total and organic soil carbon content and calculated values for soil organic matter content, bulk density, and porosity.

<table>
<thead>
<tr>
<th>Total Carbon (g kg⁻¹)</th>
<th>Organic Carbon (g kg⁻¹)</th>
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</thead>
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<td>137.93</td>
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<td>153.34</td>
<td>129.28</td>
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</table>

Mean | 176.51 | 136.98 | 142.61 | 138.02 | 135.88 | 135.25 | 121.86 | 128.93 | 128.93 |

SE | 5.58 | 2.54 | 5.44 | 4.25 | 2.12 | 2.96 | 1.63 | 1.89 |

<table>
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<th>Organic Carbon (%)</th>
<th>Organic Matter (%)</th>
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<td>15.46</td>
</tr>
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<td>12.55</td>
<td>14.29</td>
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</table>

Mean | 13.59 | 14.53 | 12.19 | 12.89 | 27.18 | 29.05 | 24.37 | 25.79 |

SE | 0.21 | 0.30 | 0.16 | 0.19 | 0.42 | 0.59 | 0.33 | 0.38 |

<table>
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SE | 0.0061 | 0.0075 | 0.0052 | 0.0056 | 0.0023 | 0.0028 | 0.0020 | 0.0021 |
Table 2-4 Field data collected at the Mazar Wildlife Reserve during December 2013. Four study sites are representative of four different land-use histories, with four data collection points established within each site. Soil carbon dioxide (CO$_2$) flux was measured with a LICOR 8100 Automated Soil Respiration Analyzer using a 20-cm chamber. Soil temperature and soil moisture were measured with an Omega Type E Soil Temperature Probe and EC H$_2$O Soil Moisture Probe, respectively. The date of data collection is year-month-day (yyyymmdd).

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Figure 2-8 Soil CO₂ flux comparisons among study sites (A) within the ranges of 20–40% and (B) 50–70% water-filled pore space. Lines within the boxes indicate the median value of soil CO₂ flux for each study site. Letters A-C indicate significant (p < 0.05) differences between study sites.
Chapter 3 Carbon-cycle effects of differences in soil moisture and soil extracellular enzyme activity at sites representing different land-use histories in high-elevation Ecuadorian páramo landscapes
A version of this chapter will be submitted to Soil Biology and Biogeochemistry for publication by Julie McKnight, Carol P. Harden, and Sean M. Schaeffer.

Abstract

Ecuadorian páramo grasslands are important regional soil carbon sinks. In the páramo of the Mazar Wildlife Reserve, changes in soil moisture and soil carbon stocks have been shown to be associated with land-use change. Differences in soil carbon content among different types of land use may reflect changes in soil carbon-acquisition related microbial enzyme activity after land cover and soil moisture are altered; however, this hypothesis has not been tested explicitly for Ecuadorian páramos. This study used a fluorescence enzyme assay to assess the activities of four different extracellular enzymes representing carbon acquisition: α-glucosidase (AG), β-glucosidase (BG), β-D-cellulohydrolase (CB), and β-xylosidase (XYL) in Andean páramo soils. Acquisition activities were also measured for nitrogen (N-acetyl-β-glucosidase (NAG) and leucine aminopeptidase (LAP)) and phosphorus (phosphatase (PHOS)) to assess stoichiometric differences between land-uses, which can limit or support soil microbial activity related to carbon acquisition. Soils were collected and analyzed from four land uses: native forest, grass páramo, recently burned grass páramo, and pine plantation. Carbon acquisition activity was highest at the pine site (678 nmol h$^{-1}$ g$^{-1}$) and lowest at the recently burned páramo site (252 nmol h$^{-1}$ g$^{-1}$), indicating the lowest and highest available soil carbon, respectively. Carbon-acquisition EE activity had not been expected to differ between the two grassland sites, but was significantly higher at the grass páramo site (595 nmol h$^{-1}$ g$^{-1}$) than at the recently burned páramo and native forest sites. Although carbon-acquisition activity was high at both the pine and grass páramo sites, soil carbon stocks were much higher in the grass páramo site. At the grass páramo site, with a history of burning as a management strategy, high carbon-acquisition EE activity could indicate the presence of pyrogenic soil organic matter, which is more resistant to microbial decomposition. Soils at the native forest and both grassland sites were phosphorus limited, and soil at the (non-native) pine site had higher nitrogen-acquisition activity, indicative of a shift to nitrogen-limited soil stoichiometric conditions. To our knowledge these are the first data reported for soil extracellular enzyme activities for Ecuadorian páramos.
1. Introduction

The high altitude páramo grasslands of northern South America are important regional carbon sinks despite only accounting for less than 5% of the total land area in the Andes mountain region. The high organic carbon content of páramos has been significantly correlated with water retention capacities of the histic Andisol soils that characterize these ecosystems (Buytaert et al. 2007a); however, the effects of land-use change on the interaction between soil moisture and soil carbon-cycle processes in páramos remain poorly understood. Soil microbial activity plays a significant role in soil organic matter decomposition, in which complex organic material is transformed into simpler organic components available for plant and microbial uptake. Soil organic matter decomposition is facilitated by the microbial production of extracellular enzymes that are synthesized to decompose, or depolymerize, specific substrates, or forms of organic matter. Environmental conditions, including soil moisture and soil temperature, are known to influence the rate of microbial activity due to thermal and aerobic biological thresholds (Richards et al. 1952; Wildung et al. 1975; Linn and Doran 1984; Dornbush and Raich 2006; Steinweg et al. 2012). The cool, moist conditions of the Ecuadorian páramos are conducive to low rates of microbial decomposition of soil organic matter. Previous studies have shown, however, that the transition from grassland to forest or tree plantation has a significant effect on soil moisture and soil carbon content in páramo landscapes (Hofstede et al. 2002; Buytaert et al. 2006; Buytaert et al. 2007b; Farley et al. 2012; Harden et al. 2013). Although soil organic matter content is significantly correlated with soil water-retention capacity (Buytaert et al. 2007b), the effect of different soil moistures, under different types of land use, on carbon-related soil microbial activity is not known for Ecuadorian páramo landscapes.

Soil microbes play a significant role in nutrient cycling through the release of extracellular enzymes (EE). Microbes synthesize specific EE to catalyze the depolymerization of specific substrates, or complex organic materials such as cellulose and chitin, into simpler organic components, such as sugars (Huang et al. 2011). The simpler organic components can then be readily taken up by microbes and plants, to be used as sources of energy and nutrition. Specific EE are specialized for the depolymerization of different types of organic compounds to yield specific nutrients. For instance, the extracellular enzyme β-D-cellulobiohydrolase breaks down hemicellulose for carbon acquisition, whereas N-acetyl-β-glucosidase breaks down chitin.
for nitrogen acquisition (Bell et al. 2013). The production of EE is expensive in terms of energy and resource allocation. Consequently, microbes preferentially produce EE that acquire the nutrient that is most needed, or is least abundant in the immediate soil environment (Tischer et al. 2014). Extracellular enzyme activity can be used as a proxy for microbial activity. Consequently, changes in extracellular enzyme activity can reflect changes in microbial activity associated with nutrient availability and deficiencies. Since microbes synthesize EE according to their specific nutrient, or substrate, needs, higher activity for a specific EE compared to others indicates a deficiency in the nutrient for which that EE is synthesized. This relationship can be used to indicate shifts in the stoichiometric balance of nutrients in a soil environment. As biological processes require carbon, nitrogen, and phosphorus, the least abundant of the three is considered the nutrient most limiting to growth and biological function, and is indicated by high levels of EE activity specific to the acquisition of that nutrient. For example, in a relatively phosphorus-limited system, the addition of other nutrients, such as nitrogen, will increase phosphorus-acquisition EE activity, whereas the application of phosphate corresponds to a decrease in phosphorus-acquisition EE activity (Treseder and Visoutek 2001). The measurement of EE activity is thus a proxy expression of nutrient resource deficiencies and needs of the microbial community.

The relationship between nutrient availability and EE activity can also be used to detect shifts in the abundance or availability of specific fractions of a single nutrient within the soil environment. For instance, the addition of nitrogen in a nitrogen-limited environment can increase microbial activity and induce higher rates of soil organic matter decomposition. In some cases, an increased rate of soil organic matter decomposition can deplete labile, i.e. easily degradable, soil organic carbon pools (Du et al. 2014). This process would be reflected in smaller pools of labile soil organic carbon and higher levels of EE activity, corresponding to increased acquisition of labile forms of carbon (e.g. cellulose and sugar). Where this process is taken to an extreme, for instance, if nitrogen is continuously added to the soil environment over long periods of time, the transformation of more recalcitrant forms of carbon into labile carbon pools (Du et al. 2014; Jiang et al. 2014) supports biological processes until soil carbon, or other nutrient pools, become limiting. Since EE activity reflects substrate-specific microbial activity, changes in EE activity can reflect changes in soil stoichiometry and limitations in nutrient abundance.
The transition of regional carbon sinks to atmospheric carbon sources has significant implications for global greenhouse warming effects. As a result, understanding the mechanisms for soil carbon retention in and loss from regional terrestrial carbon sinks is critical for global concerns regarding climate change. Páramo ecosystems are important regional carbon sinks due to their high capacity for soil organic carbon retention: soil carbon content can be up to 15% in South American páramo soils (Hofstede 1995). Although the physical and chemical properties of the andic soils that characterize Ecuadorian páramo ecosystems facilitate the stabilization of soil organic carbon (Matus et al. 2014), land management that affects soil properties can alter soil carbon-cycle processes. In the Ecuadorian páramos, conversion of grassland to tree plantation has been shown to correspond with a decrease in soil organic carbon content (Farley et al. 2004; Farley et al. 2012). This indicates a change in soil carbon-cycle processes, resulting in net soil carbon loss. Previous studies show decreases in soil organic carbon associated with decreased soil moisture content under pine plantations in Ecuadorian páramo landscapes (Poulenard et al. 2003; Farley et al. 2004; Hartsig 2011; Harden et al. 2013). Soil moisture changes from less to more aerobic conditions can stimulate greater carbon-utilization soil microbial activity resulting in a decrease in soil carbon content. Further, soil organic matter content has been shown to be correlated with soil water-holding capacity in Ecuadorian páramo soils (Buytaert et al. 2007a). A decrease in soil carbon content can thus contribute to a positive feedback in which soil-water holding capacity is also reduced. The relationship between changes in soil moisture and soil carbon-cycle processes has not been directly tested for Ecuadorian páramos. A better understanding of the relationship between these processes is crucial as land-use change that affects soil carbon processes and storage may also have important implications for water-provision ecosystem services provided by Ecuadorian páramos.

Studies conducted in montane forests and numerous laboratory experiments show that soil microbial activity, measured as soil CO₂ respiration and EE activity, is positively correlated with soil moisture content in aerobic soil conditions (A´Bear et al. 2014; Zhou et al. 2014; Krashevska et al. 2012). Linn and Doran (1984) showed that maximum soil microbial activity is supported at ~60% water-filled pore space (WFPS), with microbial activity decreasing at higher soil moisture contents due to a transition to anaerobic soil conditions (Figure 3-1). In the case of the Ecuadorian páramos, soil moisture content has been shown to exceed the 60% WFPS threshold (McKnight 2015; Hartsig 2011). Consequently, decreases in soil moisture that result
Figure 3-1 Relationship between percent water-filled pore space (WFPS) and relative microbial activity. The range of WFPS from 0% to 60% (water limiting) is representative of aerobic soil conditions. The range of WFPS from 60% to 100% (aeration limiting) is representative of anaerobic soil conditions. Relative microbial activity corresponds to expected trends soil CO$_2$ flux rates. Source: Figure 1 in Linn and Doran (1984).
from land-use change may create more aerobic soil conditions. Changes in soil moisture that result in more aerobic conditions have been associated with higher soil CO$_2$ flux rates in controlled water-table studies (Linn and Doran 1984; Moore and Dalva 1993), indicating a corresponding increase in soil microbial activity. Results from a field experiment conducted in Ecuadorian páramos support this trend—a forest and a pine plantation had higher soil CO$_2$ flux and more aerobic soil moisture conditions than nearby páramo grassland sites (McKnight 2015: Chapter 2). Consequently, land-use change that results in a decrease in soil moisture under páramo grassland cover can be expected to increase rates of microbial activity and soil organic matter decomposition.

This study investigates differences in soil microbial activity under four types of land use with different average soil moisture conditions. Four study sites were selected to represent common types of land use in Ecuadorian páramo landscape: a páramo grassland that has been grazed but not burned for 6 years; a recently burned páramo grassland (burned within 6 months of the study); a native forest (at least 40 years since conversion from grass páramo), and a pine tree plantation (at least 25 years since conversion from grass páramo). A native forest site was included in this study because White (2013) suggested that páramo grasslands are a hunter-gatherer landscape maintained by anthropogenic fire regimes and that, in the absence of controlled burning, some grassland areas may be susceptible to native forest encroachment. Because regular burning (typically at least every 4–6 years) is typical páramo grassland management, a recently burned grassland site was included to capture the short-term effects of fire on soil moisture and soil microbial activity (Lægaard 1992; Verweij and Budde 1992). Seven extracellular enzymes, representing labile carbon, nitrogen, and phosphorus acquisition activities, were analyzed in soil samples from each of the four sites.

In this paper, we report the results of two lines of investigation. First, we test whether carbon-acquisition activity differs among the four study sites. Carbon-acquisition EE activity was expected to be higher at the pine plantation and native forest sites compared to the two grassland sites (Hypothesis 1). This hypothesis was predicated upon results from McKnight (2015 Chapter 2) showing lower soil moisture contents, assumed to be associated with more aerobic soil conditions, under the plantation and forest sites. As no difference in soil moisture was expected between the grass páramo and recently burned páramo sites, we expected to find
no difference in carbon-acquisition activity (*Hypothesis 2*) between the two grassland sites. In the second line of investigation, we test whether the ratio and distribution of carbon-nitrogen-phosphorus acquisition activities differs among the four study sites. This measure provides insight into changes in microbial activity with respect to the stoichiometry of the soil. We expected no difference among the sites (*Hypothesis 3*).

2. Methods

2.1 Study Sites

This study was conducted at the Mazar Wildlife Reserve (MWR) in the Sangay National Park in Ecuador (Figure 3–2). The MWR is a privately owned 1,800 ha reserve, which is located in the Nudo del Azuay and spans elevations of 1,200 m to 4,500 m above sea level. The MWR supports the conservation of Andean cloud forest and páramo grassland ecosystems that occur within this elevational range and scientific research that contributes to a better understanding of these systems (Schloegel 2010; FCT 2015). The MWR provides an ideal location for studying the effects of land-use change and differences in land-use history on soil-water and soil-carbon processes. Historically, the area now managed as MWR was predominantly a páramo grassland maintained by regular cattle grazing and controlled by burning every 3 to 6 years (White and Maldonado 1991; Lægaard 1992; Verweij and Budde 1992). A variety of uses and land-management practices have been implemented in the MWR páramo, which is now a heterogeneous landscape containing native forest, pine plantations (>25 years old), alpaca-grazed grass páramo (~350 ha), and grass páramo with varying burn histories (most recent burn ranging from 6 months to >40 years prior to this study).

Four study sites were chosen to represent the following types of land use: a páramo site with no burning in the past 6 years, a páramo site burned 6 months prior to the study, a site with native montane forest cover at least 35 years old, and an afforested site with a *Pinus patula* stand more than 25 years old (Table 3-1). All soil collection sites were selected arbitrarily. Our research was based out of the MWR Jalogus field camp (UTM: S 02.57081, W 078.74585) located at 3,300 m elevation. Average daily temperatures at Jalogus range from 7°C to 21°C, and the mean annual precipitation exceeds 1,500 mm, according to a time-series analysis (1998–2009) by Bookhagen (in review) and on-site measures of precipitation by the Fundación
Table 3-1 Site descriptions and soil properties for the four study sites: native forest (NF), páramo grassland (PA), recently burned páramo grassland (PAB), and pine plantation (PI). Mean values (and standard error) are provided for soil organic carbon (SOC), water-extractable soil organic carbon (WEC), volumetric soil moisture, and soil pH. Sample size, indicated by ‘n’, is reported for each individual site. (Source: McKnight 2015 Chapter 2)

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<th>SOC g kg(^{-1}) (SE) n=6</th>
<th>WEC(^a) n=2</th>
<th>% Volumetric Soil Moisture (SE) n(^b)</th>
<th>Soil Temperature °C (SE) n(^b)</th>
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<td>PA</td>
<td>176.5 (5.6)</td>
<td>83.1</td>
<td>55.5 (0.8)</td>
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<td>4.32</td>
<td>Intermediate burn (6 years); Alpaca grazing; Tussock grass &amp; Puya clava-herculis; Elevation = 3,428 m</td>
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<td>PAB</td>
<td>137.0 (2.5)</td>
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<td>51.7 (0.8)</td>
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<td>Recent burn (&lt;6 months); Alpaca grazing; Tussock grass &amp; Puya clava-herculis; Elevation = 3,521 m</td>
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<td>PI</td>
<td>138.0 (4.2)</td>
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<td>22.8 (0.7)</td>
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<td>4.08</td>
<td>Pine plantation (25 years old); Previously páramo grassland used for grazing; Pinus patula; Elevation = 3,435 m</td>
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</table>

\(^a\) Water-extractable organic carbon reported here is representative of a preliminary data set. Additional samples are prepared and in storage until additional analyses can be completed. With analysis of additional samples, the sample size to determine mean WEC for each site will increase to 16. The larger data set will be used for publication.

\(^b\) Sample sizes for mean values of % volumetric soil moisture and soil temperature for each study site are: NF=36, PA=35, PAB=31 and PI=36.
Cordillera Tropical (FCT) (Bremer 2012). The native montane forest is characterized by the following dominant plant families: Araliaceae, Asteraceae, Clusiaceae, Cyatheaceae, and Myrsinaceae (Martinez 2001; Salgado et al. 2007). The grass páramo site is dominated by tussock grasses (e.g. Calamagrostis intermedia) and by Puya clava-herculis. Although the same dominant plant species appeared to be present in both the grass páramo and recently burned páramo sites, the grass páramo site had a higher density of Puya than the recently burned páramo site. Further, the grass páramo site had little to no bare interspace, whereas the recently burned páramo site was characterized by more bare soil space between the bunch grasses. The MWR sites in this study are between 3,200 m and 3,450 m in elevation, with non-allophanic Andisols dominated by Al-humus complexes (Bremer 2012; Poulenard et al. 2003). Site descriptions, including soil properties, are provided in Table 3-1, and photos of each of the four sites are provided in Figure 3–3.

2.2 Soil Sampling

We obtained a composite soil sample from each study site to represent soil conditions under each of the four types of land use. Samples were collected to a depth of 10.0 cm at 0.5-m intervals along a horizontal, 12-m transect, with the transect mid-point at the site plot center. We used a 2.0-cm diameter Oakfield Model G soil auger, collected 25 soil cores along each transect, and combined cores to form a composite sample. All soil samples were collected on December 31, 2013 and placed on ice with 24 hours of collection for shipment to the University of Tennessee, Knoxville, Tennessee. Soils were stored at 4°C until analyzed.

2.3 Soil Properties

Volumetric soil moisture was measured in situ at the four data collection points established within each study site. To determine whether soil moisture differed among the four study sites, nine sets of soil moisture measurements were taken over a 3-week sampling period, from December 12, 2013 to December 31, 2013. Volumetric soil moisture was measured using an EC H₂O Soil Moisture Probe; all measurements were taken between 10:00 and 14:00 on the same days.

Water-extractable organic carbon (WEC) was determined using the extraction method followed in the Schaeffer soil laboratory at the University of Tennessee Institute of Agriculture.
Figure 3-2 Map of study area. This study was conducted in the Mazar Wildlife Reserve located in the Nudo del Azuay east-northeast of the city of Cuenca. (Source: Harden et al. 2013)
Figure 3-3 Scanned topographic map (IGM 1969) with letters indicating the location of the (A) pine plantation, (B) native forest, (C) páramo grassland, and (D) recently burned páramo grassland sites. Arrows from each site location provided on the maps point to a photo of each of the four study sites. Grid cells on the maps are 1 km by 1 km. Green shading on the map forest areas do not accurately support forest cover area and should be disregarded.
In a 50 mL conical centrifuge vial, 20 mL of deionized water was added to 5 g of field-moist soil, shaken for 1 hour on a mechanical shaker (Innova 2100 Platform Shaker), centrifuged at 4,500 rpm for 10 minutes on a Thermo-Scientific ST 16R centrifuge, and filtered through a No. 1 Whatman filter. The extraction solution was then mixed with 4 mL of phosphoric acid to remove inorganic content, allowed to sit for at least 8 hours, and vented to allow headspace CO$_2$ concentration to return to ambient concentrations. Then, 10 mL of extract was oxidized with 10 mL of sodium perchlorate at 90°C overnight in airtight sealed vials. The concentration of CO$_2$ in the headspace of the container for each oxidized sample was then measured on a LICOR 820 infrared gas analyzer.

To determine soil carbon content with a Costech Combustion Module, subsamples of each soil were dried at 60 °C for 48 hours then homogenized into a fine powder. Approximately 7.0 mg of each soil sample were weighed into a 3 x 5 mm tin capsule and stored in a dry place until analysis. For soil organic carbon content, subsamples were first acidified in 3.0 N phosphoric acid to remove mineral components. Soil pH was measured in deionized water solution using a Denver Instrument UltraBASIC pH Meter.

2.4 Extracellular Enzyme Activity

The activity of seven different EE, representing three microbial nutrient-acquisition groups, was measured in this study. A list of all EE measured, including the abbreviation and nutrient acquisition group assignment for each EE, is provided in Table 3-2. Extracellular enzyme activity was determined using a fluorescence microplate assay procedure. The fluorescent activity of each enzyme was measured as the amount of fluorescent dye released during an enzyme-catalyzed reaction that occurs when each soil sample reacts with a synthetic substrate, specific to the enzyme being assessed, bound with fluorescent dye. Two standard solutions, 4-methylumbelliferone (MUB) and 7-amino-4-methylcoumarin (MUC), were used in this study to create standard curves for each soil sample from which the fluorescence activity for each sample was calculated. MUB was used to create the standard curve for substrates used to measure the fluorescent activity of six of the enzymes measured: α-glucosidase (AG), β-glucosidase (BG), β-D-cellulobiohydrolase (CB), β-xylosidase (XYL), N-acetyl-β-glucosidase (NAG), and phosphatase (PHOS); and MUB was used to create the standard curve for the substrate used to measure the fluorescent activity of Leucine aminopeptidase (LAP). Soil slurries
### Table 3-2 General functions of extracellular enzymes by stoichiometric group (Source: Bell et al. 2013)

<table>
<thead>
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<th>Group</th>
<th>Enzyme ID</th>
<th>Enzyme</th>
<th>Function</th>
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<tr>
<td>Carbon Acquisition</td>
<td>AG</td>
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<td>Sugar degradation</td>
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<tr>
<td></td>
<td>BG</td>
<td>β-glucosidase</td>
<td>Sugar degradation</td>
</tr>
<tr>
<td></td>
<td>CB</td>
<td>β-D-cellulbiohydrolase</td>
<td>Cellulose degradation</td>
</tr>
<tr>
<td></td>
<td>XYL</td>
<td>β-xylosidase</td>
<td>Hemicellulose degradation</td>
</tr>
<tr>
<td>Nitrogen Acquisition</td>
<td>NAG</td>
<td>N-acetyl-β-glucosidase</td>
<td>Chitin degradation</td>
</tr>
<tr>
<td></td>
<td>LAP</td>
<td>Leucine aminopeptidase</td>
<td>Protein degradation</td>
</tr>
<tr>
<td>Phosphorous Acquisition</td>
<td>PHOS</td>
<td>phosphatase</td>
<td>Phosphorous degradation</td>
</tr>
</tbody>
</table>
were prepared by blending 2.75 g of each soil sample with 91 mL of sodium acetate buffer, adjusted to a pH of 4.5 to match the acidic conditions of the field moist soils. Then, 800 µL of the soil slurry was pipetted into three deep-well plates, two of which were used to create a standard curve for each soil sample using concentration gradients (0 µM, 2.5 µM, 5 µM, 10 µM, 25 µM, 50 µM, and 100 µM) of MUB or MUC. Substrates for the seven EE measured in this study were added to the third plate. Upon adding 200 µL of MUB standard, MUC standard, or EE substrate to the appropriate microplate well, the soils were mixed with the respective standard or substrate by inversion and incubated at 25°C for 3 hours. After 3 hours, the assays were removed from incubation, and centrifuged at 1,500 rpm for 3 minutes to separate soil particles from aqueous solution. Finally, 250 µL of aqueous solution was pipetted into corresponding, flat-bottomed, black, 96-well plates, and 5.0 µL of 0.5 N NaOH was added to each soil sample to cease the enzyme activity. Extracellular enzyme activity was measured on a BioTek Synergy H1 Hybrid microplate reader with the excitation and emission wavelengths set to 365 nm and 450 nm, respectively. Enzyme activity is reported in nmol g⁻¹-dry soil h⁻¹ for specific enzymes, total carbon (C)-acquisition activity (AG+BG+CB+XYL), total nitrogen (N)-acquisition activity (NAG+LAP), total phosphorus (P)-acquisition activity (PHOS), and total overall EE nutrient acquisition activity (sum of all seven enzymes measured).

2.5 Statistical Analysis

Statistical comparisons in this study were made to evaluate differences between sites with different histories of land use. Variables were checked for normality using the Shapiro-Wilk test. The Mann-Whitney Wilcoxon test was used to assess differences in EE activity between sites due to the presence of non-normal sets that could not be transformed using consistent methods. Statistical analyses were performed using R Statistical Software (R 2013).

3. Results

3.1 Soil Properties

Mean volumetric soil moisture differed significantly among the four study sites. The grass páramo and recently burned páramo sites had the greatest mean soil moisture contents, of 56% and 52%, respectively (Table 3-1). The pine plantation site had the lowest mean soil moisture content, of 23%. Although the mean volumetric soil moisture of the native forest site,
41%, was lower than that of the páramo grassland sites, our results show higher soil moisture contents under native forest species than under the non-native pines.

Soil organic carbon content at the grass páramo site, 177 g kg\(^{-1}\), was greater than soil organic carbon measured at all other sites. Although there was no significant difference in soil organic carbon among the native forest, pine, and recently burned páramo sites, soil organic carbon measured at the recently burned páramo site was greater than at either the native forest or pine sites (Table 3-1). Relative concentrations of water-extractable organic carbon (WEC) among the study sites showed a different pattern than did soil organic carbon content. The greatest WEC (83.1 mg L\(^{-1}\)) was measured at the grass páramo site; however, the lowest WEC (5.3 mg L\(^{-1}\)) was measured at the recently burned páramo site, despite this site having the second greatest soil organic carbon content. Further, the WEC at the native forest site (14.8 mg L\(^{-1}\)) was greater than at the pine site (9.3 g L\(^{-1}\)), despite the pine site having a slightly, though not significantly, higher soil organic carbon content.

3.2 Soil Extracellular Enzyme Activity

Carbon-acquisition EE activity at the native forest site, 383 nmol h\(^{-1}\) g\(^{-1}\)-dry soil, was greater than at the grassland sites, as predicted; however, carbon-acquisition EE activity at the pine site, 678 nmol h\(^{-1}\) g\(^{-1}\)-dry soil, was not significantly (p<0.05) greater than at the páramo grassland site, 595 nmol h\(^{-1}\) g\(^{-1}\)-dry soil (Hypothesis I). Contrary to what we hypothesized for carbon-acquisition EE activity at the two grassland sites (Hypothesis 2), carbon EE activity was significantly higher (p<0.05) at the páramo grass site than at the recently burned páramo site, 252 nmol h\(^{-1}\) g\(^{-1}\)-dry soil (Figure 3-3). Mean, maximum, and minimum values for activities measured for individual EE and EE nutrient groups are given in Table 3-3.

With respect to the labile carbon-acquisition EE group (AG, BG, CB, and XYL), BG accounted for most of the EE activity, representing 59% (pine) to 76% (native forest) of the labile-carbon-acquisition activity across all four sites. Activity for BG was greatest at the pine site (521 nmol h\(^{-1}\) g\(^{-1}\)-dry soil) and lowest at the recently burned páramo site (150 nmol h\(^{-1}\) g\(^{-1}\)-dry soil). BG activity varied significantly (p<0.5) among all sites, except between native forest and grass páramo, and between grass páramo and pine (Figure 3-4). XYL was the second most produced labile carbon-acquisition enzyme at native forest, páramo and recently burned páramo
Figure 3-4 Comparison of extracellular enzyme activity by nutrient acquisition group. Horizontal lines across each box indicate the median for each group. Study sites are: NF – native forest, PA – grass páramo, PAB – recently burned páramo (<6 months), PI – pine plantation. The carbon acquisition group is α-glucosidase (AG), β-glucosidase (BG), β-D-cellulbiohydrolase (CB), and β-xylosidase (XYL). The nitrogen acquisition group is N-acetyl-β-glucosidase (NAG) and leucine aminopeptidase (LAP). The phosphorus acquisition group is phosphatase (PHOS). Group activity is expressed as the sum of activity (nmol h\(^{-1}\) g\(^{-1}\)-dry soil) measured for all extracellular enzymes included that group. Letters a-c indicate differences between groups determined using the Mann-Whitney Wilcoxon test (p < 0.05). (This figure was created in JMP Pro 11.)
Table 3-3 Maximum, minimum, and mean values of extracellular enzyme activities across all sites (NF-native forest, PA – páramo, PAB – recently burned páramo, PI – pine) sampled in the Mazar Wildlife Reserve. Enzyme activity is expressed in nmol h$^{-1}$ g$^{-1}$-dry soil. Mean, maximum, and minimum values are given for each extracellular enzyme measured (see Table 3.2 for abbreviation descriptions) and for the carbon (C-Acq), nitrogen (N-Acq), and phosphorus (P-Acq) nutrient acquisition groups. Acquisition activities, shown in bold, represent the sum of activities for all extracellular enzymes in each nutrient acquisition.

<table>
<thead>
<tr>
<th></th>
<th>NF (n = 12)</th>
<th>PA (n = 12)</th>
<th>PAB (n = 12)</th>
<th>PI (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SE)</td>
<td>Max</td>
<td>Min</td>
<td>Mean (SE)</td>
</tr>
<tr>
<td>AG</td>
<td>1.9 (3.0)</td>
<td>12</td>
<td>-21</td>
<td>11 (3)</td>
</tr>
<tr>
<td>BG</td>
<td>293 (23)</td>
<td>439</td>
<td>166</td>
<td>355 (21)</td>
</tr>
<tr>
<td>CB</td>
<td>36 (3)</td>
<td>52</td>
<td>11</td>
<td>88 (4)</td>
</tr>
<tr>
<td>XYL</td>
<td>56 (3)</td>
<td>67</td>
<td>38</td>
<td>140 (11)</td>
</tr>
<tr>
<td>C-Acq</td>
<td>383 (27)</td>
<td>518</td>
<td>256</td>
<td>595 (35)</td>
</tr>
<tr>
<td>N-Acq</td>
<td>170 (18)</td>
<td>257</td>
<td>97</td>
<td>136 (10)</td>
</tr>
<tr>
<td>PHOS</td>
<td>905 (77)</td>
<td>1291</td>
<td>434</td>
<td>971 (90)</td>
</tr>
<tr>
<td>P-Acq</td>
<td>905 (81)</td>
<td>1291</td>
<td>434</td>
<td>971 (94)</td>
</tr>
</tbody>
</table>
Figure 3-5 Comparison of extracellular enzyme activity for the carbon-acquisition enzymes α-glucosidase (AG), β-glucosidase (BG), β-D-cellulobiohydrolase (CB), and β-xylosidase (XYL). Enzyme activity is reported as nmol h⁻¹ g⁻¹ dry soil. Study sites are: NF – native forest, PA – grass páramo, PAB – recently burned páramo (<6 months), PI – pine plantation. Letters a-c indicate results of Mann-Whitney Wilcoxon test for significant difference (p < 0.05) in mean extracellular enzyme activity between study sites. Horizontal lines within the boxes indicate median values. (This figure was created in JMP Pro 11.)
sites; however, at the pine site, CB activity was greater than XYL activity. XYL activity at the páramo site was greater than at any other site. Although AG activity was negligible compared to the activity levels of the other three labile carbon-acquisition enzymes, AG activity was lower in the native forest than at any other site (Figure 3-4).

In the N-acquisition group (NAG and LAP), NAG accounted for 66% (páramo) to 92% (pine) of the overall nitrogen-acquisition. For all sites, NAG activity was significantly greater than LAP activity. Mean NAG activity ranged from 34 nmol h⁻¹ g⁻¹-dry soil (recently burned páramo) to 347 nmol h⁻¹ g⁻¹-dry soil (pine) and differed significantly among all sites (Figure 3-5). Mean LAP activity ranged from 14 to 46 nmol h⁻¹ g⁻¹-dry soil at the recently burned páramo and páramo sites, respectively. Mean P-acquisition, represented by PHOS activity, ranged from 303 to 971 nmol h⁻¹ g⁻¹-dry soil at the pine and páramo sites, respectively (Table 3-3). Significant differences in PHOS activity were found between two pairs of sites—(1) recently burned páramo and pine and (2) native forest and páramo grassland—with no significant variation between sites within each pair. At the recently burned páramo and pine sites, phosphorus-acquisition EE activity was significantly lower (p<0.05) than at the native forest and páramo sites (Figure 3-6).

With respect to overall enzyme acquisition activity, PHOS accounted for approximately 60% of all nutrient-acquisition EE activity at the native forest, páramo, and recently burned páramo sites, with nitrogen-acquisition activity ranging only from 6% to 12% (Figure 3-7). However, at the PI site, phosphorus-acquisition activity only represented 20% of total EE nutrient activity, which was lower than the total nitrogen-acquisition activity, at 26%. This shift in phosphorus- and nitrogen-acquisition activities indicates a shift to a more nitrogen-limited stoichiometry under the pine plantation.

4. Discussion

The higher carbon-acquisition activities at the pine site suggest a relatively lower soil carbon availability in the pines, compared to that at the native forest and recently burned páramo grassland sites. This is consistent with the slower decomposition and nutrient-poor qualities of pine needles (Edmonds 1990), compared to organic matter inputs from the native forest and and recently burned páramo sites. Further, the lower mean soil moisture content at the pine site was
Figure 3-6 Mean extracellular enzyme activity for the nitrogen-acquisition enzymes N-acetyl-β-glucosidase (NAG) and leucine aminopeptidase (LAP). Enzyme activity is reported as nmol h⁻¹ g⁻¹-dry soil. Study sites are: NF – native forest, PA – grass páramo, PAB – recently burned páramo (<6 months), PI – pine plantation. Letters a-d indicate results of Mann-Whitney Wilcoxon test for significant difference (p < 0.05) in mean extracellular enzyme activity between study sites. The line within each box represents the median. (This figure was created in JMP Pro 11.)
Figure 3-7 Mean extracellular enzyme activity for the phosphorus-acquisition enzyme phosphatase (PHOS). Enzyme activity is reported as nmol h$^{-1}$ g$^{-1}$-dry soil. Study sites are: NF – native forest, PA – grass páramo, PAB – recently burned páramo (<6 months), PI – pine plantation. The letters a indicate results of Mann-Whitney Wilcoxon test for significant difference (p < 0.05) in mean extracellular enzyme activity between study sites. The line within each box represents the median. (This figure was created in JMP Pro 11.)
Figure 3-8 Relative distribution of carbon (C), nitrogen (N), and phosphorus (P) acquisition activities by percent of total enzyme activity measured for each nutrient group. Percent carbon acquisition is the sum of AG, BG, CB, and XYL activities. Percent nitrogen acquisition is the sum of LAP and NAG activities. Percent of phosphorus activity is the activity measured for PHOS. See Table 3.2 for a description of each enzyme abbreviation. Note ‘EE Acq Type’ in the legend is the nutrient type. (This figure was created in JMP Pro 11.)
shown by McKnight (2015 Chapter II) to correspond to higher soil CO$_2$ flux rates compared to those of the other sites. This suggests higher rates of soil carbon depolymerization at the pine site which, over time, can result in a depletion of soil labile-carbon pools. The deficiency in labile-carbon resources is thus expressed as elevated labile carbon-acquisition EE activity. The lack of difference in total carbon-acquisition EE activity between the pine and páramo grasslands was unexpected. Although carbon-acquisition EE activity was high at the páramo site, soil organic carbon and water-extractable organic carbon contents were both highest at this site. This suggests that more soil carbon resources are available for microbial decomposition and uptake at the páramo site, which should coincide with lower carbon-acquisition EE activity. One plausible hypothesis for the high carbon-acquisition activity at the páramo grassland is that the theoretical aerobic-anaerobic biological threshold of 60% water-filled pore space (Linn and Doran 1984) may not be applicable to microorganisms in the páramo soils of the Mazar Wildlife Reserve (MWR). Dynamic soil moisture conditions have been shown to cultivate soil microbial communities specifically adapted to fluctuating conditions and tolerant of anaerobic periods (Pett-Ridge and Firestone 2005). However, long intervals of high soil moisture content have been shown to reduce soil carbon use efficiency in prairie grasslands in North America (Tiemann and Billings 2011). These studies support the hypothesis that soil microbial communities in the páramos of the MWR may tolerate high soil moisture conditions, but utilize carbon less efficiently. A second hypothesis for the relatively high carbon-acquisition activity at the páramo grassland is the development of pyrogenic organic matter. However, frequent burning in highland grasslands has been shown to transform organic material into pyrogenic organic matter, which is more difficult for microorganisms to breakdown and thus more resistant to microbial decomposition (Knicker et al. 2012). The presence of pyrogenic organic matter may result in a relative deficiency of labile carbon, as the depolymerization of pyrogenic organic material is more difficult, and, therefore, a slower process, than the depolymerization of labile carbon pools. Future work investigating the microbial community structure and biological thresholds in the páramo soils at the MWR will be required to explain the lower levels of EE activity at the páramo grassland site.

In terms of individual carbon-acquisition EE activity, CB-acquisition was greater at the pine site than at the other three sites. The decomposition of pine needles is notably slow and dominated by the depolymerization of cellulose-based cell wall structural material (Edmonds
The slower decomposition rate and greater abundance of the more recalcitrant cellulose-based organic material of the pine needles likely induces a deficiency of labile carbon resources under the pines, which is consistent with higher CB-acquisition activity. Lignocellulose and lignin, which decompose more slowly than cellulose (Melillo et al. 1989), are also abundant in pine tree organic material and contribute to lower labile soil organic carbon inputs. Carbon-acquisition EE measured in this study are limited to labile carbon substrates that do not include lignin depolymerization enzymes, such as phenol oxidase.

We originally hypothesized that carbon-acquisition EE activity would be very similar between the two páramo grassland sites. However, our results showed that EE activity at the recently burned páramo site was lower than at the páramo sites for all enzymes except AG. Based on preliminary WEC results, low WEC content at the recently burned páramo site supports the hypothesis that soil organic carbon within the first 10 cm of soil is lost to combustion or leached as pyrogenic organic matter (McKnight 2015 Chapter 3; Hilscher and Knicker 2011). The rapid and significant loss of labile carbon resources may have resulted in a temporary shift in microbial allocation of resources from the acquisition of labile to recalcitrant soil carbon, including pyrogenic organic matter carbon resources. This would be expressed as low EE activity for labile carbon-acquiring enzymes, such as AG, BG, CB, and XYL, and high EE activity for recalcitrant carbon-acquiring enzymes, which were not measured in this study. Another plausible hypothesis for the low carbon-acquisition activity at the recently burned páramo site is that EE activity has been shown to be low when plant nutrient uptake is elevated (Bell et al. 2014). As the plant community recovers from the recent burn, plant nutrient uptake would be elevated to support biomass growth. Lower EE activity at the recently burned páramo site could thus be a function of soil microbial response to plant activity.

The lack of variation in BG activity between the native forest and páramo sites is consistent with the findings of Nierop et al. (2007), who showed that differences in vegetation between native cloud forest and grassland páramo do not significantly affect soil organic matter composition in Andean ecosystems in Ecuador. The four labile C-acquisition EE measured in this study are representative of microbial production of enzymes allocated for the decomposition of sugar- (BG and AG) and cellulose- (CB and XYL) based soil organic materials. Although sugars, or polysaccharides, are simple organic materials readily available for microbial uptake,
the predominantly high activity of BG measured at all four sites suggests that polysaccharides are low in abundance. Allophane is known to stabilize polysaccharides in soil organic matter pools (Parfitt et al. 1999), but non-allophanic Andisols, like those in the MWR, are associated with lower abundances of polysaccharides, including the nitrogen-containing polysaccharide chitin (Nierop et al. 2009). Still, with respect to native forest, páramo, and recently burned páramo sites, overall carbon-acquisition activity was highest at the páramo site and lowest at the recently burned páramo site. Lower carbon-acquisition EE activity at the native forest site, compared to that at the páramo site, indicates that labile carbon is more readily accessible for microbial use in the native forest. This corresponds to a significantly higher mean soil CO₂ flux at the native forest compared to the páramo grassland site reported by McKnight (2015 Chapter 2) in a complementary study, in which field measurements of soil CO₂ flux were compared among the same four MWR sites used in this study. The higher CO₂ flux reported for the native forest site by McKnight (2015 Chapter 2) indicates elevated microbial activity, most likely in response to more aerobic soil conditions (i.e. lower soil moisture compared to the páramo grassland sites). This is consistent with the lower concentration of WEC measured at the native forest, because higher rates of soil microbial carbon uptake and CO₂ flux can deplete labile carbon resources.

Tischer et al. (2014) reported the effects of land-use change on the activity of five of the same extracellular enzymes measured in this study (AG, BG, CB, XYL, and NAG) and a phosphorus-acquisition enzyme (phosphomonoesterase). The study sites used by Tischer et al. (2014), although located in the Ecuadorian Andes, represented lower montane forest (Melastomataceae, Lauraceae, and Rubiaceae), shrubland (Asteraceae, Melastomataceae, and Lycopodium), and pasture lands (Setaria sp.), 1900–2150 m in elevation. Although study sites used by Tischer et al. (2014) are not located in páramo landscapes, we compare their results for EE activity to the results in our study due to geographic proximity and the scarcity of soil EE data for the Ecuadorian Andes. The distributions of EE activity for BG (0.23-0.61 µM g⁻¹ h⁻¹), AG (21-61 nM g⁻¹ h⁻¹), and CB (22-116 nM g⁻¹ h⁻¹) reported by Tischer et al. (2014) are comparable to our results for the native forest, páramo, and recently burned páramo sites in the MWR. The similarity in the order of EE—highest to lowest—activity between the native forest, páramo, and recently burned páramo sites at MWR and the Tischer et al. (2014) sites suggests that EE activity trends are comparable between forested sites and sites with no tree cover in the
central Ecuadorian Andes. In the case of the pine site at the MWR, however, the maximum and mean EE activity rates are much higher than those reported for native vegetation sites at MWR and in the Tischer et al. (2014) study. This suggests a potential shift in soil microbial activity and soil organic matter decomposition rates under the (non-native) pines. In general, EE activity rates measured at MWR were lower than those reported for the lower elevation sites by Tischer et al. (2014). This is likely due to combined negative effects of cooler temperatures and higher precipitation (Allison et al. 2010; A’Bear et al. 2014) associated with the higher elevation páramo landscapes within the MWR.

The low LAP activity at the MWR indicates presence of protein-based N across all study sites. On the other hand, the higher NAG activity suggests a deficiency in chitin-based nitrogen resources. Chitin is very common in nature, as it is an important structural component of organisms such as insects and fungi (Gooday 1990). However, non-allophanic Andisols have been shown to be depleted in polysaccharides (Nierop et al. 2009), including chitin, a nitrogen-containing polysaccharide. Assessment of the significantly greater NAG activity at the pine site compared to the other three study sites can be complicated by the ability of NAG to assist in the decomposition of non-chitin biopolymers and, in some instances, act as a carbon-acquisition enzyme (Koyama et al. 2013). But because previous studies have shown that significantly less nitrogen is available under mature pine plantations, compared to grasslands (Farley and Kelly 2004) and native forest (Hofstede et al. 2002), greater NAG activity at the pine site likely compensates for nitrogen, not carbon, limitations under the pine plantation. This is further supported by the stoichiometric shift in overall enzyme allocation at the pine site, where nitrogen- and phosphorus-acquisition activities are approximately equal. The dominance of phosphorus-acquisition activity at the native forest, páramo, and recently burned páramo sites is consistent with the previously known phosphorus-limited conditions of South American páramo Andisols (e.g. Hofstede 1995).

5. Conclusions

Our results suggest that soil extracellular enzyme activity differs among types of land use in the páramo landscapes of the Mazar Wildlife Reserve (MWR) in Ecuador. Although carbon-acquisition activity was high at both the pine and grass páramo sites, soil carbon stocks were much lower under the non-native pines. High carbon-acquisition EE activity in the pine
plantation suggests that less soil carbon is available for use at this site compared to the native forest and páramo grassland sites. Soil CO₂ flux reported by McKnight (2015 Chapter 2) for the MWR supports the hypothesis that the depletion of soil carbon resources under the pines is a function of higher rates of soil organic matter decomposition and microbial carbon uptake under the drier, more aerobic, soil moisture conditions in the pine site. However, high carbon-acquisition activity at the grass páramo suggests that extracellular enzymes are still produced and active at higher soil moisture contents. Further, because soil organic carbon and soil water-extractable carbon were both highest at the grass páramo site, the more abundant total carbon resources there should correspond with lower EE activity. We hypothesize that, due to the history of burning at intervals of 4 to 6 years—confirmed for the last 25 years—at the MWR páramo grassland site, pyrogenic organic material has developed in the soil profile and is more resistant to microbial decomposition than other forms of soil organic matter. If that is the case, in spite of higher total carbon resources, the amount of carbon available for microbial decomposition and uptake under páramo grassland is more comparable to that of the pine site, due to higher recalcitrance of organic matter present. The ratio of carbon, nitrogen, and phosphorus EE activities suggested that the native forest and both grassland sites were more phosphorus limited than the pine site. However, higher nitrogen-acquisition activity at the pine site indicates a shift to nitrogen-limited soil stoichiometric conditions under (non-native) pine cover.

This study provides a first look at soil extracellular enzyme activity in páramo soils in the MWR. Our results suggest that differences in aerobic conditions related to differences in soil moisture under different types of land use affect nutrient-acquisition extracellular enzyme activity. Changes in extracellular enzyme activity following changes in land use have implications for rates of carbon uptake by soil microbial activity and for soil carbon storage in the MWR and similar páramo landscapes.
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Chapter 4 Soil CO$_2$ flux and soil moisture variability in two constructed agricultural wetlands with different hydrological patterns, perennial versus intermittent, in East Tennessee
A version of this chapter will be submitted to Wetlands Ecology and Management for publication by Julie McKnight, Carol P. Harden, and Sean M. Schaeffer.

Abstract

Constructed wetlands are integrated into land management strategies to mitigate nutrient and erosion runoff in agricultural landscapes. Wetlands with an intermittent hydrologic design are commonly constructed in agricultural landscapes due to their efficiency for runoff filtration; however, little is known about the effects of intermittent inundation on emissions of soil CO$_2$ in constructed wetlands. This study investigated the relationship between soil moisture variability and soil CO$_2$ flux rates in two constructed agricultural wetlands—one perennially inundated and one intermittently inundated—in East Tennessee. Although mean soil moisture for the field sampling season was not significantly different between the two sites, soil moisture content was more variable at the intermittently inundated wetland. Patterns in soil CO$_2$ flux were similar throughout the field season; however, the magnitude of change in CO$_2$ flux rates was greater at the intermittent wetland. Greater variability in both soil moisture and soil CO$_2$ flux rates through the field season corresponded to a higher mean soil CO$_2$ flux at the intermittent wetland (306 mg CO$_2$-C m$^{-2}$ h$^{-1}$) compared to the mean soil CO$_2$ flux at the perennial wetland (212 mg CO$_2$-C m$^{-2}$ h$^{-1}$). As soil temperature is a known driver of soil CO$_2$ flux rates in East Tennessee uplands, a soil incubation experiment was conducted to assess the effects of soil moisture on soil CO$_2$ flux at a constant temperature. Carbon dioxide production rates were low in soil treatments in which no water was added, whereas soil CO$_2$ flux rates increased with the addition of water. For both wetlands, the highest soil CO$_2$ flux rates were measured in saturated samples, even up to five days following saturation. Our results highlight the importance of soil moisture in soil carbon-cycle processes in constructed wetlands.

1. Introduction

Significant effects of recent changes in climate on human and natural systems underscore the importance of generating a better understanding of terrestrial carbon fluxes (IPCC 2014). Wetlands play an important role in the global carbon budget. Although only representative of approximately 6–7% of the global land surface area (Lehner and Döll 2004), wetlands account for approximately 25% of global soil organic carbon stocks (Gorham 1991; Lal 2008). Anthropogenic activity has resulted in significant losses in wetland areas. In the United States alone, development and land-use change have reduced wetland area by more than 50% since the
1600s (Dahl and Allord 1997). This loss of wetland area has contributed significantly to anthropogenic carbon dioxide (CO$_2$) emissions (Houghton et al. 2012; Arai et al. 2015). Specifically, the oxidation of wetland soils following draining results in an increase of soil organic matter decomposition, or depolymerization, in which complex forms of soil organic material are transformed into simpler organic soil carbon components available for plant and microbial uptake. When wetland soils are oxidized, soil carbon is utilized more rapidly than it is deposited, resulting in a net loss of soil carbon content. The end biological product of microbial utilization activity is CO$_2$, which is emitted to the atmosphere via respiration. Changes in soil CO$_2$ flux can reflect changes in rates of microbial activity associated with soil organic carbon decomposition and uptake (Hillman 1997; Wosten et al. 1997). Endeavors to create and restore wetlands where loss of wetland area has occurred have significant implications for carbon sequestration efforts.

In the United States, agricultural development has played a significant role in increasing carbon-based greenhouse gas emissions through land-use change. Soil CO$_2$ respiration and export of soil through erosion are the primary mechanisms for soil carbon loss resulting from land-use change. In spite of efforts to reduce soil carbon losses and increase carbon sequestration through sustainable crop selection and cultivation practices, many crop and pasture lands often still function as atmospheric carbon sources. Even with the inclusion of sustainable practices, agricultural landscapes in the United States contribute approximately 460 Tg of carbon dioxide (CO$_2$) to the atmosphere annually (US EPA 2004). In recent decades, the construction of agricultural wetlands has been included in land management strategies for flood control and mitigation of nutrient and sediment runoff from cropland and pastures. Constructed wetlands have been shown to accumulate up to 240 g C m$^{-2}$ yr$^{-1}$ (Bernal and Mitsch 2013), demonstrating their potential to function as reestablished carbon sinks. To better assess the role of constructed wetlands as carbon sinks or sources, however, atmospheric fluxes of carbon in constructed wetlands need to be better quantified with respect to wetland type and climate.

In spite of their carbon sequestration potential, wetlands in some environmental conditions are recognized as potential sources of atmospheric carbon. Wetlands are generally characterized by high water tables and saturated soils, which produce anaerobic conditions. Because methanogenesis is an anaerobic process, wetlands have high potential for methane emissions (Kang et al. 1998). Consequently, wetland carbon flux research has had a strong
emphasis on assessing methane emissions (Sovik et al. 2006; Melton et al. 2013; Mander et al. 2014), with fewer studies reporting CO$_2$ flux. The paucity of information regarding soil CO$_2$ flux in constructed wetlands can be problematic, as hydrologic designs that reduce methane emissions can increase CO$_2$ emissions. Wetlands characterized by relatively lower water tables—such that surface soil horizons support greater oxygen exchange and are more aerobic—are typically associated with lower methane emissions (Moore and Dalva 1993). Consequently, wetlands with a flowing or pulsed hydrology may be created for their potential to reduce methane emissions without significantly increasing CO$_2$ flux (Altor and Mitsch 2008). However, more aerobic soil moisture conditions are conducive to higher emissions of CO$_2$ (Moore and Dalva 1993; Boon et al. 1997) and, in some instances, flowing and pulsing hydrology has been shown to increase CO$_2$ flux (Moore and Dalva 1993; Altor and Mitsch 2008; Mander et al. 2011). In these instances, constructed wetlands can become a source of atmospheric carbon via CO$_2$ emissions.

Soil CO$_2$ flux rates in wetlands have been linked to hydrologic variability, with emission rates increasing significantly with declining water depth (Moore and Knowles 1989; Mander et al. 2011). Controlled laboratory studies have shown that CO$_2$ emissions can increase when soil is exposed to wetting-drying cycles (Fierer and Schimel 2002; Borken et al. 2003). In a mesocosm study with two constructed wetlands, Mander et al. (2011) found that intermittent inundation of a constructed wetland can result in significantly greater CO$_2$ emissions than measured in a permanently inundated constructed wetland. These studies show that soil moisture variation plays an important role in soil CO$_2$ flux. Consequently, the hydrologic design of created wetlands should be considered, especially where carbon sequestration is a desired ecosystem service.

Many of the studies that assess soil moisture variation and soil CO$_2$ flux are laboratory or mesocosm studies, in which many other environmental factors can be held constant. These studies are useful, but do not replace the need for field-based research that applies laboratory-based knowledge to constructed wetland systems that have been created with a hydrologic design, but left to integrate into the landscape. Although numerous studies report field-based information regarding interactions of soil moisture and soil respiration, many of these studies have been conducted on natural wetland systems, leaving constructed wetlands underrepresented in the literature. Carbon-cycle processes have been shown to be significantly different between natural and constructed wetlands in some cases (Bernal and Mitsch 2013). As differences occur between natural wetland systems, relationships found in natural wetlands should not be assumed.
for constructed wetlands. Further, regional variability in wetland carbon-cycle processes should be considered, as soil moisture-soil CO$_2$ flux interactions may vary with climatic conditions (e.g. boreal versus tropical wetlands). Due to their significance in the global carbon budget, boreal wetlands dominate carbon-based wetland research, while temperate wetlands remain underrepresented, despite their potential to significantly influence regional carbon budgets (Clair et al. 2002; Bernal and Mitsch 2013). Further, to our knowledge, no studies have assessed the relationship between wetland hydrology, soil moisture, and soil CO$_2$ flux in constructed wetlands in the southeastern United States (U.S.). Wetland creation and restoration is a growing management strategy in agricultural landscapes in the southeastern U.S. This presents an opportunity to restore carbon-based ecosystem services by constructing wetlands; however, a better understanding of atmospheric carbon flux is required to determine net carbon exchange in constructed wetlands. This is especially true for flux rates of CO$_2$, as little is known about CO$_2$ flux in constructed wetlands in the southeastern U.S.

This study investigates the effects of soil moisture on soil CO$_2$ flux in two constructed agricultural wetlands in East Tennessee. Both wetlands hold water in storage year round and can be classified as permanent to semi-permanent. However, one wetland has a perched water table, which maintains a higher water surface level (perennial wetland), whereas the second wetland does not have a perched water table, resulting in a more variable water surface level and more variable wetting-drying cycles (intermittent wetland). To assess the relationship between soil moisture variation and soil CO$_2$ flux rates in the two wetlands, we measured soil CO$_2$ flux and soil moisture weekly throughout the 2014 growing season.

Using field data and a laboratory experiment, we tested five hypotheses to gain knowledge of soil CO$_2$ flux and soil moisture in a perennial and an intermittent wetland. Mean soil CO$_2$ flux was expected to be higher at the intermittent wetland (Hypothesis 1). Mean soil moisture content was expected to be higher at the perennial wetland (Hypothesis 2). Mean soil moisture trends were expected to follow similar temporal patterns in both wetlands due to their close proximity and exposure to similar precipitation patterns. However, the magnitude of soil moisture variation was expected to be greater at the intermittent wetland (Hypothesis 3) due to differences in hydrologic responses to wetting and drying events. For this reason, soil CO$_2$ flux was expected to be higher at the wetland site with more variability in soil moisture (Hypothesis 4). This hypothesis is based on the assumption that greater variability in soil moisture at the
intermittent wetland—i.e. more intense wetting and drying cycles—throughout the field season would stimulate soil microbial activity, resulting in higher rates of soil CO$_2$ flux related to respiration. Because soil temperature was expected to be an influential factor on soil CO$_2$ rates, and therefore a potentially confounding environmental factor, a constant temperature laboratory incubation experiment was conducted in which soil samples from both wetlands were air dried and rewetted to measure the soil CO$_2$ flux rate response to water addition, with soil temperature held constant. At a constant temperature, soil CO$_2$ flux rate was expected to increase with the addition of water (*Hypothesis 5*). This study was undertaken to contribute to a better understanding of soil CO$_2$ flux responses to constructed wetland hydrology, which has important implications for constructed wetland design in East Tennessee and other similar locations.

2. Materials and Methods

2.1 Study Site

This study was conducted in two constructed agricultural wetlands located at the East Tennessee Research & Education Center Little River Animal and Environmental Unit (LRU). The LRU is an offsite experimental farm managed by the University of Tennessee, Institute of Agriculture. The 214-ha tract is a functioning dairy farm, with 81 ha used for crop research (corn, wheat, and soy rotations) and 50 ha used for pasture. While the primary emphasis of the LRU is Holstein dairy production, this area is also used for a variety of scientific studies that evaluate the interaction between agriculture and the environment.

The LRU is located approximately 40 km south of Knoxville, Tennessee. The temperate-subtropical climate of East Tennessee is characterized by annual mean temperature of 14°C, with an average maximum temperature of 26°C between July and August and an average minimum temperature of 3°C in January. The average annual precipitation is approximately 120 cm, with little variation between months. (NOAA 2015) Soils at the LRU are predominantly silt loam, with young hydric soils developing in both wetland areas (NRCS 2015).

In 2011, two wetlands were constructed at the LRU to implement water quality best management practices, specifically, filtration of stormwater pollutants and runoff from the LRU prior to flowing into Ellejoy Creek. Both LRU wetlands are located in excavated depressions adjacent to pasture and both receive runoff directed from pasture and cropland areas (Figure 4-1). The two wetlands are similar in size, with surface areas of 0.28 ha and 0.34 ha for the
perennial and intermittent wetlands, respectively (Figure 4-1). Macrotopographic features are similar at both wetland sites, and wetland surface area can be classified as 15% deep pool, 55% fringe high marsh, and 35% low marsh channels (Ludwig and Wright 2014). Marsh is defined by Mitsch and Gosselink (2007) as a permanently or periodically inundated site that has a mineral soil substrate and lacks peat accumulation. In the LRU wetlands, low marsh channels are seasonally or periodically flooded and are characterized by obligate and facultative wetland grasses and rushes. Fringe high marsh areas are considered in this study to be areas that occur along the fringe, or boundary, of the wetland. The vegetation zone of the fringe high marsh in this study is characterized by leafy emergent species and extends to the first appearance of blade grass, marking the uppermost boundaries of high water-table inundation zones. Both wetlands were planted with the same plant species, which had either been transplanted from nearby natural wetlands or seeded using a native seed blend (Ludwig and Wright 2014).

In spite of their similarities, hydrologic responses of the two wetlands to precipitation and drainage differ due to water-table controls. The ‘perennial wetland’ is located atop a dense clay lens, which restricts downward infiltration of water in the soil profile. As a result, the water table at this wetland is considered perched (Ludwig and Wright 2014), and higher water surface levels are maintained during dry periods. The ‘intermittent wetland’ does not have a perched water table, and vertical soil water movement is not restricted. Consequently, the water table and water surface levels are subject to greater variation during wetting and drying cycles.

2.2 Field Data Collection

Field data collection sites were established using soil collars, which were placed in corresponding wetland zones in each wetland. At the onset of the project, six soil collars were placed in each wetland: two along the upper boundary of the fringe high marsh, two in low depressions in the low marsh channels, and two at the approximate boundary between the fringe high marsh and low marsh channel (identified by the transition from obligate to facultative emergent plant species). These zones were of particular interest because they represent the intermittently inundated hydrologic zone for the LRU sites. Soil measurements could not be taken in the deep pool areas due to permanent inundation. The exact locations for soil collar installation were chosen arbitrarily once the appropriate zone was identified. One of the two soil collars installed in low marsh channel depressions in the perennial wetland was completely inundated for most of the field season. As a result, what few data were collected from this site
Figure 4-1 Map of Study Area. Aerial photos showing the perennial wetland (A) and intermittent wetland (B) study sites (Google Earth 2014). As a reference for scale, the yellow line in the aerial photos is 15 meters. A detailed topographic map is also provided for the perennial wetland (C); however, a topographic map was not drafted for the intermittent wetland. Both sites are located at the University of Tennessee Little River Animal Unit (LRU) located in Walland, TN.
were not included in this study.

Field measurements of soil CO$_2$ flux were taken between March 17th and October 8th of 2014. To measure soil CO$_2$ flux, 20-cm diameter PVC soil collars were inserted into the soil to a depth of approximately 3 cm. Five soil collars were installed at the perennial wetland and six soil collars were installed at the intermittent wetland. All soil collars were installed at least 24 hours prior to the first soil CO$_2$ flux measurement, to minimize flux variations from site disturbance, and remained in place until the completion of the study. Soil CO$_2$ flux was measured using a LICOR 8100A Automated Soil CO$_2$ Flux Infrared Gas Analyzer (LI-8100) outfitted with a LI-8100 20-cm Survey Chamber (chamber) fitted to each installed soil collar. Each flux measurement was taken over 120 seconds, with a deadband (time between measurements) of 30 seconds and purge of time 60 seconds. Weekly field measurements were taken between 09:00 and 12:00 to minimize effects of diurnal variation (Mielnick and Dugas 2000). Volumetric soil moisture and soil temperature (°C) were also recorded at the time of each soil CO$_2$ flux measurement, using an EC H$_2$O Soil Moisture Probe and an Omega Type E Soil Temperature Probe, respectively.

2.3 Soil Samples and Properties

Soil samples were collected from the fringe high marsh and low marsh channels to support a controlled laboratory incubation experiment and to determine soil properties. For the incubation experiment, nine replicate soil samples were collected for each of five soil moisture treatments for both wetlands for a total of 90 soil samples (9 replicates x 5 treatments x 2 wetlands). Soil samples were collected using an Oakfield Model G soil sampler (2.0 cm in diameter) to a depth of 5.0 cm. Each of the 90 soil samples was a composite sample that consisted of two cores combined to obtain approximately 50 g of field moist soil for each incubation sample. In addition to the 90 incubation soil samples, a third core was taken at each sampling location to determine gravimetric soil moisture and soil bulk density for the soil samples in each incubation jar. All soil samples were collected on September 10, 2014 and immediately transported to the University of Tennessee for the laboratory experiment.

To determine bulk density, the dry weight of each subsample was divided by the sample volume. Bulk density was determined for a total of 45 soil subsamples from each wetland. From these samples, an average bulk density was calculated for each wetland, and water-filled pore space was estimated from volumetric soil water measurements using the following equation:
WFPS (%) = \( \left( \frac{V_w}{1 - (BD/\text{PD})} \right) \times 100 \)

where WFPS (%) is percent water-filled pore space; \( V_w \) is volumetric water content; BD is bulk density (g cm\(^{-3}\)); and PD is the mineral particle density expressed as the standard value of 2.65 g cm\(^{-3}\).

2.4 Soil Incubation Experiment

The laboratory incubation experiment was conducted to assess the response of the soil CO\(_2\) flux rate to the addition of water, with soil temperature held constant at 22°C. Five soil moisture treatments were administered, including one (ambient) in which soil moisture was held constant at the field-moisture level observed at the time of soil sampling to serve as a control. Four of the five soil moisture treatments represent a gradient of soil moisture conditions, ranging from dry to saturated: air-dried soils (Dry), and 33%, 66%, and 100% (saturation) soil water content (SWC). As water-filled pore space (WFPS) has been shown to be significantly correlated with microbial activity, resulting in heterotrophic soil CO\(_2\) respiration (Linn and Doran 1984), the 33%, 66%, and 100% soil moisture treatments are defined as percent WFPS. In all four moisture treatments, soil samples were air dried until no change in soil weight was observed, then brought to the desired treatment soil moisture by the addition of deionized water. Soil CO\(_2\) pulses following the wetting of dry soil have been shown to indicate biological soil quality (van Gestel et al. 1991; Franzluebbers et al. 2000). The control treatment, a soil sample held at constant field soil moisture from the time of collection, provided a baseline CO\(_2\) respiration rate without a pulse from rewetting.

Each incubation sample was transferred to a 0.47-L container, weighed to determine field moist weight, and allowed to air dry until no change in weight was observed for at least 24 hours. Upon collection, subset soil samples were weighed to determine wet soil weight (g), dried in an oven at 105°C for 48 hours, and reweighed to determine dry soil weight (g). These data were used to determine gravimetric and volumetric soil moisture content, bulk density, porosity (assuming a particle density of 2.65 g cm\(^{-3}\)), and water-filled pore space (WFPS). Using this information, the wet soil weight to be maintained was determined for each individual sample. The wet soil weight determined for each soil sample treatment was attained by adding deionized water to each incubation jar. Each jar was capped with a sealed lid to prevent water loss due to evaporation. To ensure the desired WFPS was maintained, the weight of each incubation jar was
monitored at the time of each flux measurement. The total length of the incubation experiment was 5 days. Heterotrophic soil CO₂ respiration was measured using a LICOR 820 Infrared Gas Analyzer on days 0, 1, 2, 3, and 5. All treatments were kept at a constant temperature of 22°C for the entire length of the experiment.

2.5 Analytical Methods

A graphical, visual inspection of all data sets was completed using a scatter plot and histogram to identify any visually distinguishable trends, and the Shapiro-Wilk test was used to test the normality of each data set distribution. Although some data sets were found to be normally distributed, the inability to apply a consistent data transformation approach to attain a normal distribution in all data sets resulted in the use of non-parametric statistical methods. The Mann-Whitney Wilcoxon test was used to assess differences between groups. A Spearman correlation test was used to determine the strength of a linear association between soil moisture and soil CO₂ flux. Where a weak linear relationship was found, polynomial regression was used to fit non-linear data. A time-series graph for each wetland was visually inspected to compare frequency and intensity of changes in soil moisture content and soil CO₂ flux. For the incubation experiment, the Mann-Whitney Wilcoxon test was used to assess significant differences in soil CO₂ flux rates between each of the five soil moisture treatments. All statistical analyses were performed in JMP Pro 11.1.1 from SAS Software (JMP Pro 2013).

3. Results

Results of the Mann-Whitney Wilcoxon test supported Hypothesis 1, that mean soil CO₂ flux for the 2014 field season (March to October) was greater at the intermittent wetland (7.32 µmol-CO₂-C m⁻² d⁻¹) than at the perennial wetland (5.12 µmol-CO₂-C m⁻² d⁻¹) (p < 0.01) (Table 4-1; Figure 4-2). The mean volumetric soil moisture, however, did not differ significantly between the two wetland sites, as expected (Hypothesis 2) and was approximately 35% in both wetlands. Soil temperature ranged from 5°C to 27°C, with temperatures remaining above 15°C from early May in both wetlands. The highest soil temperature measured for each wetland was ~25°C, measured on July 3rd (Julian Day 184) at both sites. Soil temperature did not vary significantly between the two wetlands (α = 0.05; N = 114 for perennial wetland; N = 132 for
Table 4-1 Soil CO\textsubscript{2} flux rate and \% volumetric soil moisture. Mean and range values are representative of the 2014 field data collection season (March to October). Wetland 1 is the perennial wetland (perched water table) and Wetland 2 is the intermittent wetland.

<table>
<thead>
<tr>
<th>Site</th>
<th>Soil CO\textsubscript{2} Flux (µmol CO\textsubscript{2} m\textsuperscript{-2} s\textsuperscript{-1})</th>
<th>Soil CO\textsubscript{2} Flux (mg CO\textsubscript{2}-C m\textsuperscript{-2} h\textsuperscript{-1})</th>
<th>% Volumetric Soil Moisture</th>
<th>Vegetation/Hydrozone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SE) Range Sample Size (n)</td>
<td>Mean (SE) Range Sample Size (n)</td>
<td>Mean (SE) Range Sample Size (n)</td>
<td></td>
</tr>
<tr>
<td>Wetland 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collar 1</td>
<td>4.9 (0.2) 12.6 130</td>
<td>212 (9.2) 543 130</td>
<td>34.7 (0.9) 45.1 122</td>
<td>All soil collars</td>
</tr>
<tr>
<td>Collar 2</td>
<td>4.9 (0.5) 9.6 28</td>
<td>213 (21) 415 28</td>
<td>40.7 (1.3) 28.4 26</td>
<td>Fringe high marsh</td>
</tr>
<tr>
<td>Collar 3</td>
<td>5.7 (0.5) 12 29</td>
<td>245 (21) 520 29</td>
<td>36.2 (1.6) 27.6 27</td>
<td>Fringe high marsh</td>
</tr>
<tr>
<td>Collar 4</td>
<td>4.7 (0.5) 8.9 26</td>
<td>203 (21) 384 26</td>
<td>32.6 (1.8) 31.3 26</td>
<td>Fringe high marsh</td>
</tr>
<tr>
<td>Collar 5</td>
<td>4.3 (0.4) 9.9 24</td>
<td>184 (19) 429 24</td>
<td>33.0 (2.1) 42.2 22</td>
<td>Low channel marsh</td>
</tr>
<tr>
<td>Collar 6</td>
<td>4.8 (0.4) 7 23</td>
<td>208 (19) 300 23</td>
<td>30.1 (2.2) 40.1 21</td>
<td>Low channel marsh</td>
</tr>
<tr>
<td>Wetland 2</td>
<td>7.1 (0.3) 18.3 153</td>
<td>306 (13) 791 153</td>
<td>34.2 (0.8) 41.6 142</td>
<td>All soil collars</td>
</tr>
<tr>
<td>Collar 1</td>
<td>5.2 (0.6) 14 29</td>
<td>225 (24) 604 29</td>
<td>35.4 (1.5) 36.7 27</td>
<td>Fringe high marsh</td>
</tr>
<tr>
<td>Collar 2</td>
<td>7.5 (0.7) 17.1 29</td>
<td>324 (30) 739 29</td>
<td>35.8 (1.6) 30.0 27</td>
<td>Fringe high marsh</td>
</tr>
<tr>
<td>Collar 3</td>
<td>6.9 (0.6) 12.7 27</td>
<td>300 (27) 549 27</td>
<td>35.5 (1.9) 31.1 26</td>
<td>Fringe high marsh</td>
</tr>
<tr>
<td>Collar 4</td>
<td>8.4 (0.9) 17.8 29</td>
<td>362 (40) 771 29</td>
<td>34.9 (2.0) 40.6 27</td>
<td>Low channel marsh</td>
</tr>
<tr>
<td>Collar 5</td>
<td>6.8 (0.8) 13.2 19</td>
<td>292 (34) 570 19</td>
<td>32.4 (2.0) 29.0 17</td>
<td>Low channel marsh</td>
</tr>
<tr>
<td>Collar 6</td>
<td>7.8 (0.8) 15.2 20</td>
<td>337 (34) 658 20</td>
<td>30.2 (2.5) 39.0 18</td>
<td>Low channel marsh</td>
</tr>
</tbody>
</table>
Figure 4-2 Distribution of mean values of soil CO$_2$ flux and % volumetric soil moisture between the perennial wetland (W1) and intermittent wetland (W2). The letters a-b represents a significant difference in soil CO$_2$ flux between the two wetlands as determined by the Wilcoxon Test (p<0.05). The line within each box represents the median.
intermittent wetland), and the mean soil temperature for the full field season was 22°C at both sites.

Soil moisture trends followed the same general temporal pattern of alternating wet and dry periods (Figure 4-3). The mean soil moisture content did not differ significantly between the two wetlands (α = 0.05, N = 114 for perennial wetland; N = 132 for intermittent wetland). However, the magnitude of change in soil moisture content was greater in the intermittent wetland, supporting Hypothesis 3 (Table 4.2 in appendix). The time-series graph (Figure 4-3) was created using the mean value of weekly field measurements of soil moisture (perennial wetland, N = 5; intermittent wetland, N = 6) for each wetland over the 2014 field season (March to October). On many occasions, the magnitude of change in soil moisture content during wet and dry periods was greater at the intermittent wetland (Figure 4-3; Table 4.2 in appendix). The time series graph also shows that, on at least two occasions, soil moisture at the intermittent wetland increased (Julian Day 134) or decreased (Julian Day 239) by more than 5% from the previous week, whereas soil moisture at the perennial wetland remained approximately the same (soil moisture change of less than 2%) over the same time period (Table 4.3 in appendix). This further supports the expectation of greater soil moisture variability at the intermittent wetland.

Increases and decreases in soil CO₂ flux followed the same temporal patterns in both wetlands. As with soil moisture, the magnitude of change in soil CO₂ flux in response to alternating wetter and dryer periods was greater at the intermittent wetland. Total change in soil CO₂ flux for the entire season was 35.6 µmol CO₂-C m⁻² s⁻¹ and 63.2 µmol CO₂-C m⁻² s⁻¹ for the perennial and intermittent wetlands, respectively (Table 4.2 in appendix). The greater magnitude of soil CO₂ flux changes in the intermittent wetland contributed to a significantly greater (p < 0.01) mean soil CO₂ flux rate (7.3 µmol CO₂-C m⁻² s⁻¹) compared to that of the perennial wetland (5.1 µmol CO₂-C m⁻² s⁻¹) for the 2014 field season, supporting Hypothesis 4.

The quadratic polynomial regression, used to describe the interaction between soil moisture and soil CO₂ flux, showed that volumetric soil moisture accounted for 10% and 16% of soil CO₂ flux variation at the perennial wetland and intermittent wetland, respectively (p<0.01) (Figure 4-4).

Results of the incubation study showed that the highest CO₂ flux rate response was measured in the 100% soil moisture treatment for both the perennial and the intermittent
Figure 4-3 Time series graph of soil CO\textsubscript{2} flux (solid line) and volumetric soil moisture content (dashed line) for the perennial (A) and intermittent (B) wetlands. The dotted line represents the estimated aerobic-anaerobic threshold for soil moisture conditions. This threshold is defined as 60% water-filled pore space. Day of Year is the Julian Day. For the LRU wetland, pore space was determined by dividing the volumetric water content by soil porosity. One mean value of soil porosity was used to represent each wetland site. It was calculated using bulk density (measured for 45 soil samples for each wetland) and a standard mineral particle density of 2.65 g cm\textsuperscript{-3}. (Created in JMP Pro 11)
Figure 4-4 Fitted quadratic polynomial curves to describe the relationship between % volumetric soil moisture and soil CO$_2$ flux for the (A) perennial and (B) intermittent LRU wetland sites. (Figure created in JMP Pro 11)
wetlands. For both wetlands, the trend in flux rate was such that the order of treatment responses from greatest to least was 100% > 66% > 33% > Ambient > Dry. This relationship supported Hypothesis 5, that soil CO₂ flux would increase with the addition of water (Figure 4-5).

4. Discussion

The carbon dioxide flux rates reported in this study are consistent with CO₂ flux values reported in previous studies for temperate constructed wetlands, which range from approximately 50–325 mg CO₂-C m⁻² h⁻¹ (Picek et al. 2007; Altor and Mitsch 2008; VanderZaag et al. 2010; Mander et al. 2011). Mean values presented here for the LRU wetland sites, 212 mg CO₂-C m⁻² h⁻¹ for the perennial wetland and 306 mg CO₂-C m⁻² h⁻¹ for the intermittent wetland, more closely resemble those reported for constructed temperate wetlands in Ohio (Altor and Mitsch 2008). Other researchers have also reported higher CO₂ flux rates in wetlands with intermittent, as opposed to perennial, hydrology. Mander et al. (2011) showed that, between two constructed wetland mesocosms in Estonia, the wetland with a pulsed hydrology had a significantly higher median soil CO₂ flux compared to the wetland with a stable water table. Mander et al. (2011) also showed that the wetland mesocosm with the fluctuating water table had greater variability in soil CO₂ flux rates, with a range of 30–175 mg CO₂-C m⁻² h⁻¹, compared to 30–60 mg CO₂-C m⁻² h⁻¹ in the wetland with the stable water table. Further, in a controlled experiment with soils from boreal peatlands in Canada, Moore and Dalva (1993) showed a greater variation in soil CO₂ flux with rising and falling water tables. These studies support the notion that a greater range in soil CO₂ flux rates can contribute to a significantly higher mean soil CO₂ flux than that measured for a wetland with a stable water table.

The finding that there was no significant difference in mean soil moisture between sites in the two wetlands was unexpected. The original hypothesis (Hypothesis 2) that mean soil moisture would be greater in the perennial wetland was based on the characteristically higher water surface levels maintained by the perched water table at the perennial wetland. In contrast, trends in the time series plot (Figure 4-3) for the field season show greater magnitude in soil moisture variability at the intermittent wetland, suggesting that the non-perched water table at the intermittent wetland was more susceptible to variation in water surface levels during periods of wet and dry conditions. Further, between 2012 and 2013, prior to the present study, the perennial wetland received up to three times more inflow than the intermittent wetland during episodic storm events (Ludwig and Wright 2014). Consequently, it was anticipated that the perennial
Figure 4-5 Comparison soil CO₂ flux rates between five soil moisture treatments administered in a laboratory incubation study. Soil CO₂ flux rate response was measured for five soil moisture treatments: air-dried (Dry) soil, soil with moisture maintained to match ambient level at time of collection (Amb), and soils to which water was added to achieve three different levels of percent water-filled pore space (33%, 66%, and 100%). Soils were maintained at treatment moisture levels, and soil CO₂ flux rate response was measured over 5 days. Letters a-d represent significant differences between treatment means determined using the Wilcoxon test (p<0.05; n=9 for each treatment in each wetland). The horizontal lines across each box represent the median. (Figure created in JMP Pro 11)
wetland would have significantly higher mean soil moisture content throughout the 2014 field season.

Although mean soil moisture content values were similar between the two wetlands, trends in soil moisture variability throughout the field season, as shown in the time series plot in Figure 4-3, indicate differences in the frequency and magnitude of change in soil moisture content with periods of wet and dry conditions. Less variability in water-table levels is consistent with perched water-table hydrology (Rains et al. 2005; Deane et al. 2012) and contributes to less variability in soil moisture conditions. This supports the idea that greater frequency and magnitude of changes in soil moisture content at the intermittent wetland, where both wetland experienced the same periods of wet and dry conditions, is likely the expression of the different soil hydrologic properties and responses between the two wetland sites.

In general, the temporal trends in soil moisture and soil CO$_2$ flux for both wetlands shown in Figure 4-3 corresponds to interactions reported in previous studies (Moore and Dalva 1993; Komulainen et al. 1999; Blodau et al. 2004). The rise and fall of soil CO$_2$ flux has been shown to be the result of biological respiration responses to soil moisture shifts between aerobic and anaerobic conditions, where other environmental conditions are held constant (Linn and Doran 1984; Moore and Dalva 1993). Our results show similar patterns in soil CO$_2$ flux rates in response to variation in soil moisture content when soil temperature is greater than 15°C, which has been shown to be a thermal biological threshold for soil microbial activity (Richards et al. 1952). The rapid increase in soil CO$_2$ flux rates at the beginning of the season corresponds to the increase in soil temperature as the seasons shifted from winter to spring. This observation indicates a clear response of soil CO$_2$ flux rates to thermal thresholds. The trends shown in Figure 4-3, at soil temperatures above 15°C (or later than Julian Day 127), however, are similar to those described with respect to microbial activity response to soil moisture content. The sensitivity of biological activity to change in soil moisture could explain the greater intensity of soil CO$_2$ flux response to soil moisture changes that shift around 35% volumetric soil moisture content. This is because, for both LRU wetland sites, 35% volumetric soil moisture is estimated to correspond to the 60% water-filled pore space aerobic-anaerobic threshold defined by Linn and Doran (1984). It is important to note that observed wetting and drying cycles in the LRU wetlands in this study were determined on the basis of weekly sampling. As soil CO$_2$ respiration can respond to changes in soil moisture within hours to days, a weekly sampling period is a
coarse representation of these events. Consequently, the time scale used in this study may underestimate the frequency of wetting-drying events in soils as well as soil CO\textsubscript{2} flux rate responses, especially at the intermittent wetland. These data are sufficient, however, as a proof of concept that soil hydrological responses at the perennial and intermittent wetland at the LRU site are different and are associated with fluctuations of soil CO\textsubscript{2} flux rates (Hypotheses 1 and 4).

The non-linear relationship between soil moisture and soil CO\textsubscript{2} content can be attributed to the wide range of soil moisture captured in this study. Although significant linear relationships have been described for the interaction between soil moisture and soil CO\textsubscript{2} flux (Lee et al. 2007; Sainju et al. 2010), these linear responses are generally constrained to aerobic or anaerobic ranges of soil moisture content. In 1984, Linn and Doran showed that soil microbial respiration of CO\textsubscript{2} in aerobic conditions is positively correlated with changes in soil moisture. Conversely, they also showed that in anaerobic conditions, soil microbial CO\textsubscript{2} respiration decreases as soil moisture increases and approaches saturation. CO\textsubscript{2} flux response to changes in soil moisture is a function of oxygen diffusion and availability at different soil moisture contents. As the majority of soil microbes that facilitate the breakdown of soil organic matter are aerobic organisms, soil CO\textsubscript{2} respiration, a measure of soil microbial activity, decreases once soil moisture surpasses a certain level. This threshold has been estimated to be at approximately 60\% water-filled pore space for soil microbial activity (Linn and Doran 1984). The equivalent of 60\% soil WFPS in terms of volumetric soil moisture content is estimated to be approximately 30–35\% for the LRU wetland soils in this study. The polynomial relationship described here shows soil CO\textsubscript{2} flux response to aerobic and anaerobic conditions, which further supports the fluctuation of soil CO\textsubscript{2} flux with variable soil moisture content at the LRU sites. Still, although the polynomial relationship between soil moisture and soil CO\textsubscript{2} flux was significant (p <0.01), soil moisture only explained 10\% and 16\% of CO\textsubscript{2} flux variation in the perennial and intermittent wetlands, respectively. This suggests that other factors, environmental or biological, also play important roles in soil CO\textsubscript{2} fluxes in the LRU wetlands.

Extensive research has demonstrated that soil respiration rates are positively correlated with temperature (e.g. Raich and Schlesinger 1992; Dornbush and Raich 2006) and soil moisture (e.g. Raich and Potter 1995; Bauer et al. 2008; Hernandez-Ramirez et al. 2009; Sainju et al., 2010). Soil temperature and the interaction between soil temperature and soil moisture content have been shown to explain approximately 80\% of soil CO\textsubscript{2} flux variability in agricultural
croplands in East Tennessee during the growing season (Soro 2011). Due to the dynamic role of soil moisture content in wetland carbon processes, however, variation in soil moisture with fluctuating water tables was expected to significantly influence soil CO$_2$ flux rates in soils of constructed wetland in East Tennessee. Results from the incubation study supported this hypothesis, as the addition of water to dried soil samples corresponded to significant increases in soil CO$_2$ emissions with temperature held constant.

These findings are consistent with those of other studies that show pulses of soil CO$_2$ emissions from agricultural landscapes with water addition through irrigation (Sainju et al. 2010). The high CO$_2$ flux response measured for the 66% and 100% soil moisture treatments do not agree with the Linn and Doran (1984) model, in which CO$_2$ flux is expected to decrease in high moisture, anaerobic conditions. However, large increases of CO$_2$ flux have been observed in soils that have been dried and rewetted, as soil organisms respond disproportionately to the sudden presence of a limiting resource, which, in this case of dried soils, is water. High CO$_2$ respiration rates have been measured for weeks after a flooding event in dried soils (Blodau and Moore 2003). Blodau and Moore (2003) suggested that soil CO$_2$ response to flooded conditions is a function of the magnitude and frequency of drying and rewetting events, and reported a 5-fold increase in anaerobic CO$_2$ production when soils were saturated following a dry period. In the present study, the only two treatments with no significant increase of soil CO$_2$ respiration in the incubation study were the only two treatments that did not receive a water addition (i.e. the dry and ambient-control treatments). Further, the concentration of CO$_2$ in the incubation chamber headspace for the dry treatment remained close to zero for most of the experiment. This suggests that drying events that result in very low soil moisture contents (<20% volumetric soil moisture) can cause soil CO$_2$ flux to significantly decrease. The significant increase in soil CO$_2$ flux within five days of water addition treatments highlights the sensitivity of CO$_2$-producing soil microbes in the LRU wetland soils to changes in soil moisture.

5. Conclusion

The results of this study suggest that hydrologic characteristics of constructed agricultural wetlands play important roles in soil CO$_2$ flux rate. More frequent and intense wetting and drying events can elicit microbial biological responses that result in pulses of elevated soil CO$_2$ flux rates. In contrast, a perennially (permanently) inundated wetland with more stable water levels appears to be more resistant to the effects of wetting and drying events on rates of soil CO$_2$ flux.
Mean soil CO\textsubscript{2} flux rates are thus likely to be higher in intermittent versus perennial constructed wetland conditions. Soil CO\textsubscript{2} flux rates reported here are similar to those reported in laboratory and mesocosm studies simulating constructed temperate wetlands. However, this is the first study investigating soil CO\textsubscript{2} flux in constructed wetlands with different hydrologic regimes in east Tennessee agricultural landscapes. Further, this is the first study to report soil CO\textsubscript{2} flux rates for constructed wetlands integrated into agricultural landscapes in East Tennessee.

This study supports the application of laboratory-based observations of soil moisture and soil CO\textsubscript{2} flux interactions to inform the design of constructed wetlands. However, this study also demonstrates the complexities of soil carbon processes and their interaction with soil moisture fluctuations. The results of the incubation experiment suggest that soils from the LRU wetland are sensitive to drying and rewetting cycles and that CO\textsubscript{2} production is possible for at least 5 days following a flooding event, even where anaerobic conditions are present. Wetland hydrology thus has bearing on the role of a constructed wetland as a carbon sink or source. This has implications for constructed wetland design where the reduction of greenhouse gas emissions is a desired ecosystem service. A wetland with a water table that is stable at a greater depth, to minimize anaerobic conditions, and constructed with a gradient to allow for continuous, slow flowing water export could facilitate the capture of runoff for nutrient and erosion filtration but also mitigate emissions of both methane and carbon dioxide. Future studies assessing wetland carbon budgets that provide insight into net emissions and sequestration of atmospheric carbon are recommended.
References


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Appendices
Table 4-2 Absolute values of change (Δ) in soil CO₂ flux and % volumetric soil moisture between weekly field measurements. The sum of all of changes is provided for comparison of total changes in CO₂ flux and soil moisture for the whole study period.

<table>
<thead>
<tr>
<th>Julian Day</th>
<th>Perennial Wetland</th>
<th>Intermittent Wetland</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Δ Soil CO₂ Flux</td>
<td>Δ Volumetric Soil</td>
</tr>
<tr>
<td></td>
<td>(µmol m⁻² s⁻¹)</td>
<td>Moisture (%)</td>
</tr>
<tr>
<td>76</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>91</td>
<td>0.25</td>
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<td><strong>Sum of</strong></td>
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Table 4-3 Mean daily values used for time series plot.

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<th>Intermittent Wetland (N=6 per day)</th>
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<td>CO₂ Flux (µmol m⁻² s⁻¹)</td>
<td>Volumetric Soil Moisture (%)</td>
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Chapter 5 Conclusion

The impetus for this research was the global concern for shifts in hydrologic cycles and distributions of soil carbon storage pools caused by human-landscape interactions and a changing climate. A better understanding of soil-water interactions and associated feedbacks in carbon-cycle processes is crucial to the development of comprehensive carbon budgets and predictive models. Additionally, a better understanding of understudied ecosystems is needed to account for the transfer and variability of carbon-cycle processes. Much focus in terrestrial carbon research has been given to ecosystems that are recognized as globally significant carbon sinks and are most sensitive to global climate change. However, the exclusion of carbon-cycle processes in ecosystems that account for less of the global terrestrial carbon, but are sensitive to changes in land use and land cover, can result in inaccurate estimations of terrestrial carbon flux at regional and global scales. To account for the complexity and connectivity of ecosystem processes across spatial scales, a more detailed account of adjacent and understudied ecosystems is necessary. This doctoral dissertation research focused on two ecosystems, Ecuadorian páramo grasslands and constructed agricultural wetlands, each of which represents a fraction of the larger landscape in which it occurs, yet has the potential to play a significant role in landscape-scale carbon-cycle processes.

5.1 Soil Carbon Dioxide Flux and Soil Moisture in Ecuadorian Páramo Landscapes

In the páramos of the Mazar Wildlife Reserve in the Ecuadorian Andes, soil CO$_2$ flux rates are susceptible to increase where land-use change involves a transition from páramo grassland to tree cover. In this study, soil CO$_2$ flux rates were higher at sites with tree cover (native forest or pine plantation) than in páramo grassland. The significant difference in soil CO$_2$ flux between the two grassland sites was unexpected. Soil CO$_2$ flux at the grass páramo site was higher than at a recently burned grass páramo site. Though we hypothesized that soil CO$_2$ flux would decrease as soils became anaerobic (water-filled pore space greater than 60%), our data did not unequivocally support this hypothesis. A polynomial regression showed a significant, but weak, relationship between soil moisture and soil CO$_2$ flux. The regression line shows that the range of CO$_2$ flux is notably smaller between 55–60% WFPS, with no flux rates measured below 3.7 g CO$_2$-C m$^{-2}$ d$^{-1}$ at that point. Thus, with respect to the response of soil CO$_2$ flux to soil moisture differences across types of land use, flux appeared to respond to the biological soil
moisture threshold (~60% WFPS) expected with a transition between aerobic and anaerobic soil moisture conditions, but there was no clear trend between CO$_2$ flux and soil moisture within aerobic and anaerobic soil moisture gradients.

A better understanding of the mechanisms driving fluxes of soil carbon in Ecuadorian páramos is crucial to predicting shifts in terrestrial carbon pools and integrating carbon budgets into land-management decisions. Future research is recommended to investigate potential changes in soil microbial communities and microbial activity among sites of different land uses because such changes may correspond to changes in rates of microbial soil carbon uptake and of soil CO$_2$ flux. A controlled laboratory study is recommended to test the effects of different ranges of soil moisture on soil CO$_2$ flux both within and between the four types of land use. This would provide new information about soil moisture and soil CO$_2$ flux interactions under ideal conditions in páramo soils and help determine whether (or how) the long-term changes in soil moisture content associated with land-use change alter that interaction. A long-term, field-based study is also recommended to assess annual and seasonal variations of soil CO$_2$ flux within and among types of land use, and to better understand the response of flux rates to in situ variation of soil moisture. Lastly, we recommend a study that monitors and assesses soil microbial activity, microbial community composition, soil CO$_2$ flux, and alterations to soil carbon type and distribution prior to and following a controlled burn. Such a study would provide new information about the effect of burning on soil microbial activity under grass páramo.

This study provides a first view of the role of soil CO$_2$ flux as a mechanism for soil carbon loss in Ecuadorian páramos and of the interaction between soil moisture and CO$_2$ flux rates in anthropogenically altered páramo landscapes. In terms of soil moisture variation with land use change, this research supports the finding of previous studies that show lower soil moisture content under pine plantation; however, this study also shows that soil moisture can be significantly lower under native forest cover than under páramo grassland in some páramo landscapes. Since two primary ecosystem services provided by Ecuadorian páramo grasslands are water provision and soil carbon sequestration, these findings have significant implications for land management decisions. Based on our results, which show that, under the non-native pines, soil moisture is lower and soil CO$_2$ flux is higher than at sites with other land uses, we do not
recommend pine plantations as a land management strategy in central Ecuador, especially when water provision or carbon sequestration are land management priorities.

Whether the practice of burning the páramo should be recommended depends on land management goals. Burning is a common strategy that benefits grazing animals and prevents loss of grass páramo area to native forest encroachment. Although soil within the first 10 cm at the recently burned site in this study contained less carbon than soil at the three other sites, this may be due to differences in the individual site histories of disturbance rather than to the time since burning. It is likely that soil carbon pools recuperate as the vegetation recovers after a burn and plant-microbial interactions equilibrate. Suppression of anthropogenic burning, and the subsequent encroachment of native forest, may result in soil carbon loss; however, increased aboveground carbon stores associated with woody vegetation may balance this loss and maintain the potential value of the land as a carbon sink. Still, the lower moisture content of soil under native forest than under grass páramo indicates a potential decrease in soil water yield with conversion of grassland to native forest cover, which may occur in the absence of burning, especially at lower elevations of páramo. This information can be used by land managers to inform decisions regarding land management objectives, whether the management objectives are water provision, carbon sequestration, or balancing these ecosystem services with consideration for human livelihoods.

5.2 Extracellular Enzyme Activity in Ecuadorian Páramo Landscapes

We investigated differences in soil carbon-acquisition activity, as measured by extracellular enzyme (EE) activity, and the distribution of acquisition activity for carbon, nitrogen, and phosphorus for four different types of land use in the páramos of the Mazar Wildlife Reserve in Ecuador: native forest, grass páramo, recently burned grass páramo (< 6 months), and pine plantation. Our results showed differences in soil extracellular enzyme activity among land uses in the Mazar Wildlife Reserve páramo landscapes. Although carbon-acquisition EE activity was high at the pine and grass páramo sites, soil carbon stocks were much lower at the pine site. High carbon-acquisition EE activity at the pine plantation site suggests that less soil carbon is available at this site than at the native forest and grass páramo sites. In contrast, high carbon-acquisition activity at the grass páramo site may be due to the development of pyrogenic organic material, which is more resistant to microbial decomposition, as result of a site history of
burning at intervals of approximately 4–6 years for at least the last 25 years. The ratio of carbon, nitrogen, and phosphorus EE activities showed that soils in the native forest and both grassland sites were phosphorus-limited. However, higher nitrogen-acquisition activity at the pine site indicates a shift to nitrogen-limited soil stoichiometric conditions after páramo grassland is converted to non-native pine cover.

As this study provides a first exploration of soil carbon-acquisition EE activity in Ecuadorian páramo landscapes, it contributes new information to help fill significant gaps in knowledge about soil carbon-cycle processes in Ecuadorian grass páramo ecosystems. Differences in soil carbon-EE acquisition activity under different types of land use can be related to changes in soil environmental conditions that affect soil microbial activity. The results of this study highlight the potential for changes in soil microbial activity as a mechanism for soil carbon loss, via soil CO₂ flux, associated with land-use and land-cover type. Stoichiometry of EE activity can show shifts in nutrient availability and deficiency. For example, because soil in much of the Ecuadorian páramo landscape is phosphorus-limited, pasture management includes the application of phosphorus fertilizer. Our results suggest, however, that soil stoichiometry can shift from a phosphorus- to a nitrogen-limited soil conditions under non-native pine plantations. With this information, land managers who wish to cultivate or rehabilitate areas planted with pine trees for more than 25 years can adjust their soil management strategy.

To test the response of EE activity to different soil moisture contents along moisture gradients within the aerobic and anaerobic ranges of soil water-filled pore space in páramo soils, we recommend a controlled, laboratory study. Such a study would provide information to determine whether the sensitivity of soil EE activity to soil moisture gradients is different among land uses. This study would also contribute to a better understanding of EE activity in response to changes in soil moisture within each type of land use. This information has implications for understanding the response of soil EE activity to changes in precipitation due to seasonal variations or longer term changes in the climate of the region. A second laboratory study is also recommended to test the response of carbon-acquisition EE activity to different types of soil organic matter, soil organic carbon contents, and water-extractable organic matter contents. These two recommended laboratory studies will increase knowledge of the effects of land-use decisions on carbon-acquisition EE activity by simulating different types of organic matter input.
from different plant communities associated with the native forest, grass páramo, and pine plantation. Results of these laboratory studies should increase understanding of the relationship between soil EE activity and differences in soil carbon stocks under different types of land use.

5.3 Effects of Constructed Wetland Hydrology on Soil Moisture and Soil CO₂ Flux

The objective of the final portion of this study was to assess differences in soil moisture and soil CO₂ flux in two constructed agricultural wetlands in East Tennessee, one with a perennial hydrology and one with an intermittent hydrology. Although mean soil moisture was similar between the two nearby wetlands during the field season, more variability in soil moisture content and soil CO₂ flux was measured in the intermittent wetland. The greater magnitude of CO₂ flux response to pulsing events contributed to a significantly (p<0.05) higher CO₂ flux rate for the intermittent wetland during the 2014 field season. An incubation study, which tested the response of soil CO₂ flux to different soil moisture treatments while holding soil temperature constant, supported the hypothesis that an increase in soil moisture following a dry period (air dried) stimulates CO₂ respiration and CO₂ flux rate.

We recommend long-term monitoring studies that assess both CO₂ and CH₄ emissions to create a more complete carbon flux budget for different hydrologic conditions in constructed wetlands. We also recommend future studies that assess gas fluxes at the water-surface/atmosphere interface to create a flux budget that is more representative of the total area of a wetland. Our data provide baseline measurements for CO₂ flux in the early stages of development of the Little River Unit constructed wetlands. This creates an opportunity for continued measurements of soil carbon content and flux to contribute to a better understanding of how these soil properties and processes change from the early to late stages of constructed wetland development.

This research has implications for the design and integration of constructed wetlands into agricultural landscapes in East Tennessee. If soil carbon sequestration is a desired outcome for the construction of a wetland, we recommend implementing a slowly flowing wetland with a stable, but not perched, water table. This approach will reduce soil CO₂ emissions associated with periods of wetting and drying, mitigate methane emissions by creating more aerobic
wetland conditions, and maintain nutrient removal and other water-quality oriented ecosystem services provided by constructed wetlands.

5.4 Concluding Remarks

The results of the three studies presented in this dissertation demonstrate the importance of taking into account the diversity and complexity of the natural world when assessing terrestrial carbon fluxes. Controlled laboratory studies provide valuable insight into various aspects of soil carbon processes and provide a framework for the development of carbon flux prediction models. However, there is a crucial need to link empirical information with field-based studies that incorporate the complexities of the natural world to reconcile differences in biogeochemical processes observed in controlled and natural system studies. In this dissertation research, the selection of field sites at the Mazar Wildlife Reserve provided a space-for-time representation of the potential effects of different types of land-use change on the interaction between soil moisture and soil carbon-cycle processes. Modern technology and the rapidly growing human population continue to drive accelerated rates of land-use change. Through a space-for-time approach, the effects of rapidly changing land uses on ecosystem processes can be assessed to predict the effects of plans for future land-use change. In this capacity, the space-for-time approach can be useful for land management decision-making without long-term, field-based studies, which require substantial resources, both in terms of equipment and time. The space-for-time substitution strategy used here has allowed for the identification and prioritization of meaningful lines of long-term research to better understand the interaction between soil moisture and soil carbon processes in Ecuadorian páramo grasslands. In a very different environment, the collection of soil moisture and soil-carbon-related data during the first few years of the Little River Unit constructed agricultural wetlands development laid a foundation for a long-term study that could provide a better understanding of soil carbon processes over time in constructed agricultural wetlands.
Vita

Julie McKnight was born in Somerset, Kentucky with a childhood split between the Appalachian Mountains and the Gulf Coast of Florida, which inspired her love and passion for the outdoors. Julie earned a Bachelor of Arts in Philosophy at the University of South Florida in 2007. During her time as an undergraduate, she completed an undergraduate thesis that introduced her to isotope ecology and the potential of a career in environmental science. Julie went on to complete a Master of Science in Geology where her thesis research assessed the use of $^{13}$C isotopes of amino acids in mollusk shells for paleoecological reconstructions of nearshore marine communities. During her Master’s program, Julie also engaged in ecohydrology and water resource research and management efforts in a small, coastal town in central Mexico. Through this experience, she built a rapport with non-profit environmental work and was offered a position working in the Great Basin of Nevada. As a result, Julie worked in the environmental non-profit sector for more than 2 years. As an Americorps Program Director, she managed collaborative efforts between the Bureau of Land Management, Natural Resources Conservation Service, and the Agricultural Resource Service to conduct land health assessments in Nevada rangelands.

Julie came to the University of Tennessee, Knoxville to pursue a Ph.D. through which she could synthesize her training and experience in biogeochemical and physical processes and further develop an expertise in soil-water interactions. She was awarded a Doctor of Philosophy degree in Geography with a Watershed minor in 2015. During her time at the University of Tennessee, she served as a Graduate Teaching Associate, Graduate Teaching Assistant, and a Research Assistant. She received several departmental awards, including the Outstanding Teaching Associate Award, and has served in numerous organizations and departmental groups. Julie will relocate to Thule, Greenland for a short-term postdoctoral research project and then to California to pursue further postdoctoral opportunities.