Determining the Drivers of Spatial and Temporal Variation in Soil Greenhouse Gas Emissions from a Rehabilitated and Undisturbed Natural Riparian Forest in Southern Ontario

by

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A thesis

presented to University of Waterloo

in fulfillment of the

thesis requirement for the degree of

Master of Environmental Studies

in

Environment and Resource Studies

Waterloo, Ontario, Canada, 2017

Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

As the interface between terrestrial and aquatic ecosystems, riparian zones have been shown to provide extensive ecosystem services, particularly with respect to improved water quality, carbon sequestration, and wildlife habitat. Riparian rehabilitation is often implemented to restore or maintain these beneficial services following riparian removal or degradation. This rehabilitation usually occurs in agriculturally dominated landscapes to mitigate the negative impacts associated with agricultural land-use, such as leaching of nitrates from fertilizer. Although riparian zones are extremely effective at this mitigation, there is evidence that such high nitrate input and carbon availability may make these riparian zones hotspots for soil greenhouse gas emissions attributed to climate change, specifically nitrous oxide (N_2O) and carbon dioxide (CO_2). The objective of this study was to determine seasonal and temporal trends in greenhouse gas emissions, as well as how soil and vegetation drive these emissions, between an approximately 30 year old rehabilitated (RH) and an undisturbed natural (UNF) riparian forest in southern Ontario, Canada.

Mean soil CO_2-C emissions for the RH and UNF sites were 26.98 and 17.91 mg CO_2-C m^{-2} h^{-1}, respectively. Mean soil N_2O-N emissions for the RH and UNF sites were 7.62 and 5.93 µg N_2O-N m^{-2} h^{-1}, respectively. However, neither soil CO_2-C nor N_2O-N emissions varied significantly between the two land-uses ($p>0.05$). Both soil CO_2-C and N_2O-N emissions exhibited strong seasonal patterns for both land-uses, with elevated emissions during the summer. Soil CO_2-C emissions varied spatially within land-uses based on soil moisture, and the magnitude of this relationship was season-dependent. Spatial variability of soil N_2O-N emissions within land-uses appeared to follow anaerobic conditions ideal for denitrification, such as elevated soil moisture levels and nitrate/ammonium concentration. Soil greenhouse gas

emissions were significantly correlated to each other, and were most heavily influenced by soil temperature and soil moisture.

The results of this study provide evidence that although soil and vegetation can vary substantially between rehabilitated and natural riparian forests, differences in greenhouse gas emissions may be offset after 30 years of rehabilitation. Furthermore, soil greenhouse gas emissions in riparian systems are most directly affected by soil microclimate and seasonality, rather than spatial position or vegetation. Riparian greenhouse gas emissions rely on complex interactions between soil, vegetation, and atmospheric variables and further research on these interactions is needed to disentangle the drivers of emissions among different riparian ecosystems.

Acknowledgements

Firstly, I would like to express how grateful I am to my supervisor Dr. Maren Oelbermann, who has been an incredible mentor and from whom I have learned so much. The guidance, support, and confidence she has provided me throughout this Master's has been so important and I am so thankful for that. I would also like to thank my committee member Dr. Andrew M. Gordon, for his helpful insight and feedback throughout the process of this thesis. His unique point of view was extremely valuable in the process of conducting this research. I am also grateful to my external reader, Dr. Stephen Murphy, for his role in the thesis defense and providing feedback to this thesis. I would also like to thank Dr. Andrew Trant for his role as the chair of my thesis defense.

This thesis could not have been completed without infrastructure support provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canadian Foundation for Innovation (CFI), as well as financial support from the University of Waterloo. The generous support of these organizations is greatly appreciated.

I would like to thank my parents, Orlando and Anne Marie De Carlo, for constant love, support, and for believing in me throughout this Master's experience. Also, for trying their very best to not fall asleep as I explained my research to them for the thousandth time. I would also like to thank my three sisters, Jessica, Sara, and Nicole, for their confidence in my abilities and for telling me that I am smart (regardless of whether they believe it).

Andrea Bale, this research could not have been done without you. You have been an invaluable source of love, support, and companionship for the past 2 years. Thanks for keeping me from going insane and for doing everything you could to help me out in completing this

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Introduction

Terrestrial and aquatic ecosystems are among the world's most valuable resources; however, land-use change and exploitation of these resources has led to increased greenhouse gas emissions associated with climate change, loss of biodiversity, and reduced water quality (DeFries et al., 2004; Howells et al., 2013; Oliver & Morecroft, 2014). Riparian ecosystems, as the linkage between terrestrial and aquatic ecosystems, can provide a multitude of benefits to offset the negative impacts of land-use change such as improving water quality, sequestering carbon, and promoting increased species diversity (Capon et al., 2013; Catford et al., 2013). However, riparian ecosystems are some of the most highly degraded natural ecosystems because of increased urbanization and deforestation for agriculture (Perry et al., 2012). This degradation, along with increased awareness of the potential services provided by riparian ecosystems, has led to increased rehabilitation or restoration projects with approximately 17 % of projects conducted in riparian ecosystems (Capon et al., 2013; Palmer et al., 2014). Furthermore, there has also been increased evaluation of restoration success, with an increase in publications on restoration by approximately 30 times (Wortley et al., 2013).

Although riparian ecosystems can help to mitigate climate change, there has been recent interest in research on the potential environmental disservices that may arise (Mander et al., 2008). Restoration of riparian forests often occurs in agriculturally dominated areas to improve stream water quality from agricultural runoff, which causes nitrogen and phosphorus loading because of fertilizer application (Dosskey et al., 2010). This runoff, because of increased nitrate availability, is often associated with increased greenhouse gas emissions, specifically carbon dioxide and nitrous oxide, creating emission hotspots in riparian ecosystems (Mander et al., 2008; Shresthra et al., 2009). These emissions are often underestimated or overlooked in riparian

systems, as nitrous oxide is emitted in relatively low concentrations (Hefting et al., 2003; Mander, 2016). However, nitrous oxide is a potent greenhouse gas (~296 times the global warming potential of carbon dioxide) that can cause major atmospheric accumulation with small increases in emissions, leading to accelerated climate change (Forster et al., 2007; Mander, 2016).

Riparian greenhouse gas emissions have been shown to be spatially and temporally variable, as well as driven varying, and often contrasting, soil and vegetative characteristics (Raich & Tufekcioglu, 2000; Teiter & Mander, 2005). However little research has been conducted to disentangle the drivers of emissions in restored riparian forests (Groffman et al., 2000; Teiter & Mander, 2005; Bailey et al., 2009; Audet et al., 2013). Thus the objectives of this study are threefold: (1) to quantify and compare greenhouse gas emissions between a rehabilitated (RH) and undisturbed natural (UNF) riparian forest and identify spatial and temporal trends in emissions, (2) to determine the role of vegetation and soil characteristics in driving greenhouse gas emissions in the RH and UNF land-uses, and (3) to quantify and compare soil and vegetative characteristics related to greenhouse gas emissions in the RH and UNF land-uses and identify spatial and temporal trends in these characteristics.

1. Literature Review

1.1. Riparian Degradation and Rehabilitation

With increasing removal and degradation of natural ecosystems to accommodate the needs of a growing population (i.e., for food production, urban development), there has been a push towards restoration of ecosystems that provide ecosystem services (Verhoeven et al., 2006; Capon et al., 2013). Riparian zones (or buffers), because of their geographical location as the interface between aquatic and terrestrial ecosystems, provide many beneficial ecosystem services including, but not limited to, improving water quality, carbon sequestration (to mitigate climate change), providing wildlife habitat, and preventing soil erosion (Soosaar et al., 2011; Capon et al., 2013; Catford et al., 2013; Vasconcellos et al., 2013). As climate change continues to threaten natural ecosystems – specifically, riparian zones (Capon et al., 2013) – the rehabilitation (or restoration) of these ecosystems is paramount in mitigating potential loss of ecosystem services. Successful riparian rehabilitation will require enhanced land-use management and aquatic-based policies through an integrated effort from multiple stakeholders (i.e., government, non-governmental organizations, land owners) for each specific rehabilitation project (Seavy et al., 2009). The following will provide an overview of the historical land-use change, riparian degradation and rehabilitation/restoration of these ecosystems.

1.1.1. Historical Land-Use Change

Anthropogenic land-use change has been largely driven by the demand for food, timber, as well as urban development (DeFries et al., 2004). Much of this land-use change is for agriculture and industry, causing broad-scale deforestation. This deforestation has continued into the 21st century with an annual deforestation of ~14.6 million hectares of forest between 1990

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and 2000 (Food and Agriculture Organization of the United Nations (FAO), 2001). As of 2010, approximately 45% of the global population lives in areas with little to no vegetative cover (0-5%), 28% lives with some vegetative cover (5-10%), and only 27% live in an area with greater than 10% vegetative cover (FAO, 2014). In southern Ontario, Canada, approximately 80-90% of the land was covered in forests before the arrival of European settlers (Larson, 1999; Butt et al., 2005). By 1920, 90% of these forests had already been cleared for agricultural, residential, and industrial development (Larson, 1999). Today, the effects of deforestation in southern Ontario are clear, as there is less than 17% forest cover (Butt et al., 2005) remaining, with areas ranging from as little as 5% to as much as 20% forest cover (Riley & Mohr, 1994). Among the forests that were cleared, riparian forests may be some of the most important because of the ecosystem services they provide (Capon et al., 2013). The magnitude of riparian forest loss can be hard to estimate (Malanson, 1996); however, it was suggested that ~80-90% of riparian forests have been lost (Naiman et al., 1993; Blankenship, 1996), and this figure may be as high as 98% in agriculturally dominated landscapes (Oelbermann, 1999).

In the Grand River watershed, which is the focus of this study, the historical ecosystem was a forest-wetland complex with minimal human impact [Ontario Department of Planning and Development (ODPD), 1954]. However, much of these wetlands (up to 78%) have been drained and forests were cleared for agriculture and settlement (ODPD, 1954). This type of land-use transformation causes far-reaching implications for riparian and stream ecosystem functions such as increased runoff of nutrients and erosion, as well as changes in stream morphology (i.e., channel widening). Forested riparian zones – restored or natural – mitigate the impacts of historical land-use change on water quality, especially in agriculturally dominated landscapes. This mitigation has led to a push towards restoration in recent years. For example, the Grand

River Conservation Authourity has restored 920 hectares of degraded riparian zones to forests since 1997 (Grand River Conservation Authourity, 2016).

1.1.2. Degradation of Riparian Ecosystems

Riparian zones can be described as aquatic and terrestrial vegetation adjacent to watercourses, where soil dynamics and vegetative communities can be similar to wetland or upland forest communities (National Research Council, 1992; Knutson & Naef, 1997). Although riparian zones are relatively narrow strips of vegetation, they can provide substantial services based on their linkage between aquatic and terrestrial ecosystems (Gregory et al., 1991; Vasconcellos et al., 2013). The ecosystem services provided by riparian zones are highly valuable to humans, such as bank stabilization (maintaining stream morphology), clean drinking water, diverse faunal communities for recreation (i.e., hunting, bird-watching), and recharged water tables (Hooper et al., 2005). When there is a loss of these valued services, the ecosystem is usually defined as a degraded ecosystem (Richardson et al., 2007). Massive transformation and degradation has already occurred in riparian ecosystems, which are among the most highly affected (Perry et al., 2012), with the majority of natural riparian forests being cleared for agriculture (Naiman et al., 1993; Blankenship, 1996). This deforestation and degradation often leads to habitat fragmentation in riparian ecosystems, which limits connectivity (Denysius & Nilsson, 1994), increases biological invasions, and causes a shift towards lower diversity in vegetative communities (Wissmar & Beschta, 1998). England & Rosemond (2004) suggest that even small-scale deforestation can disrupt riparian function as the important link between aquatic and terrestrial ecosystems.

Riparian vegetation is still present in varying forms along many watercourses because of governmental guidelines (Lee et al., 2004), and the fact that riparian zones are difficult to

develop because of slopes and high soil moisture (Cey et al., 1999). These remaining riparian ecosystems act as 'critical transition zones', interacting heavily with surrounding land-uses (Ewel et al., 2001). These interactions can be seen in hydrological and chemical transfers (Burt & Pinay, 2005), and can extend into both the physical and biological aspects of the landscape (Gregory et al., 1991). One of the largest threats of riparian and stream quality degradation is adjacent agricultural land-use because of increased runoff, erosion, and flooding (Carpenter et al., 1998; Groffman et al., 2003).

There are many ways that riparian ecosystems act to maintain various aspects of terrestrial and aquatic environmental quality. Riparian forests provide microclimate controls for the terrestrial environment through regulating temperature, light, and humidity (Gregory et al., 1991). Along with regulating physical characteristics of the ecosystem, riparian vegetation also provides critical wildlife habitat and maintains a diverse ecosystem (Naiman et al., 1993). However, much of the focus of research on the benefits of riparian forests has been based around the mitigation of negative impacts on aquatic ecosystems from surrounding agriculture, specifically row-cropping (Gregory et al., 1991). The prevention of these negative impacts is usually positively correlated to the width of the riparian forest or buffer. Buffer width is especially important in small, headwater streams as the vegetation is used for shade, regulation of water flow rate, and to limit sedimentation and nutrient runoff (Broadmeadow & Nisbet, 2004; Dosskey et al., 2007). This has a large impact because of the narrow width and increased sensitivity of headwater (first-order) streams (Richardson & Danehy, 2007; Environment Canada, 2013).

With most (~80-90%) of the riparian vegetation in agricultural landscapes causes degradation of stream quality (i.e., wide, shallow streams) and fish habitat (Roth et al., 1996).

Shade and stream-bank stabilization provided by riparian vegetation can maintain low water temperatures (which is inversely correlated with dissolved oxygen), a strong indicator of water quality (Macdonald et al., 2003). Agricultural soils are susceptible to high levels of surface runoff from rain, which deposits sediment into adjacent aquatic ecosystems, causing reduced water quality (i.e., increased temperature and turbidity) (Schoonover et al., 2006). Riparian vegetation can act as a sediment trap through roots holding onto soil particles, slowing the surface runoff, and maintaining increased pore space that allows for infiltration of the runoff (Robinson et al., 1996). Sedimentation can also occur through stream-bank erosion caused by increased water levels and velocity from runoff, which is prevented through tree roots, stems, and branches being embedded in the bank (Harmon et al., 1986). Further, riparian vegetation has been found to provide a significant amount of the organic matter in aquatic ecosystems (Dosskey & Bertsch, 1994; Groffman et al., 2003; Dosskey et al., 2010). Finally, one of the largest benefits of riparian buffers in agricultural landscapes is the filtration of nutrients and pollutants that would otherwise run-off into streams or rivers, causing detrimental effects to aquatic life and drastically reducing water quality leading to eutrophication (Lowrance et al., 1997; Verhoeven et al., 2006).

Nitrogen is one of the major causes of concern regarding water quality impacts from surrounding agriculture (Dosskey et al., 2010). In areas with a high proportion of agricultural land-use, nitrate (NO_3^-) runoff often results in substantial degradation of aquatic ecosystems, caused by fertilizer use, animal waste, and tillage (Carpenter et al., 1998; Cey et al., 1999). Although nitrogen is essential for plant growth, it can cause eutrophication of watercourses in large quantities (Carpenter et al., 1998). Eutrophication can lead to reduced dissolved oxygen, fish population decline, and a loss of aquatic biodiversity and algae blooms (Vitousek et al.,

1997). Riparian vegetation, woody debris and wetland species, are especially effective in the uptake of NO_3^- (O'Neill & Gordon, 1994; Groffman et al., 2003). For example, riparian vegetation is effective at reducing the amount of NO_3^- runoff that reaches our watercourses (Osborne & Kovacic, 1993; Verhoeven et al., 2006) through being stored in the soil, microbial processes (denitrification), and plants utilizing the available nitrogen (Lowrance et al., 1997).

Another major contributor to riparian degradation is human-induced climate change. Riparian ecosystems are particularly susceptible to the impacts of climate change (Capon et al., 2013), which is likely intensified by the degradation that has already occurred (Perry et al., 2012), causing a positive feedback loop. It is well documented that riparian ecosystems impact adjacent aquatic ecosystems (Gregory et al., 1991; Soosaar et al., 2011; Catford et al., 2013; Vasconcellos et al. 2013); however, it is important to acknowledge that they are also impacted by these same aquatic ecosystems (Hupp & Osterkamp, 1996; Naiman & Decamps, 1997; Davies, 2010). This interaction may lead to issues with varying watercourse discharge (i.e., drought, flooding) associated with climate change (Palmer et al., 2008; Vorosmarty et al., 2010). These byproducts of climate change will likely cause altered soil moisture affecting microbial processes, increased carbon dioxide (CO_2) emissions, and species composition (Perry et al., 2012; Capon et al., 2013; Catford et al., 2013). Climate change is also expected to increase the range of invasive species, which is problematic as riparian ecosystems are among the most highly invaded (Richardson et al., 2007), and could cause further shifts in species composition and riparian function.

1.1.3. Riparian Rehabilitation/Restoration

As riparian zone alteration has continued to account for increased impervious surfaces, water diversion, and agriculture (Wissmar & Beschta, 1998), there has been a recent push

towards restoring or rehabilitating these degraded ecosystems to maintain or recover ecosystem services (Bernhardt et al., 2005; Verhoeven et al., 2006; Capon et al., 2013). Ecosystem restoration can be defined as the practice of reversing or mitigating damages to the ecosystem that have been caused by humans (Jackson et al., 1995). The focus of ecosystem restoration is often based around the notion of returning the dynamics of the ecosystem to the same state as before human disturbance had occurred (Osborne & Kovacic, 1993; Wissmar & Beschta, 1998; Seavy et al., 2009). However, this is nearly impossible to achieve because the underlying natural processes have been irreparably changed by disturbances and adjacent land-use (Osborne and Kovacic, 1993; Choi et al., 2008), especially in forest ecosystems (Larson, 1999; Chazdon, 2008). Furthermore, riparian forests show very different vegetative communities along the same watercourse because of varying land-use management and disturbances (i.e., residential development, agriculture, natural ecosystems) (Gregory et al., 1991; Naiman et al., 1993), making them difficult to replicate. Therefore, it is suggested that rather than trying to mimic the indigenous ecosystem, focus should be set on preventing further degradation and improving the services provided by the ecosystem (Osborne & Kovacic, 1993; Suding et al., 2004; Choi et al., 2008). Rehabilitation is a form of restoration, where efforts aim for an alternate state (other than the indigenous ecosystem) to provide ecosystem services quickly, while maintaining the original ecosystem's structures to allow the new system to be self-sustaining (Aronson et al., 1993).

There are usually two main approaches to restoration in a riparian setting: passive and active restoration. Passive restoration refers to reducing, protecting from, or stopping human practices affecting the ecosystem (i.e., altered grazing practices, increased buffer size, reduced fertilizer, etc.) (Watanabe et al., 2005). This is potentially the most important step, as it eliminates or reduces the forces driving degradation of the riparian ecosystem and allows for

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natural regeneration of species (Wissmar & Beschta, 1998; Watanabe et al., 2005). However,

sometimes the ecosystem will not regain ecosystem function (i.e., it has a crossed a threshold,

and will not naturally recover) (Suding et al., 2004), demonstrating the need for other measures.

In such cases, active restoration (such as removing invasive species or planting native species to

encourage or accelerate succession) is often necessary (Kauffman et al., 1997; Wissmar &

Beschta, 1998). The success of these restoration efforts can be difficult to evaluate (Wortley et

al., 2013). The amount of time it will take for riparian restoration to repair function is extremely

variable and usually relies on the intensity and length of degradation that has occurred (Dosskey

et al., 2010).

Another important aspect of riparian restoration success is the width of the riparian buffer

that has been restored. It is widely recognized that greater buffer widths contribute more

ecosystem services; however, the literature and governmental guidelines are highly variable in

outlining the necessary width (Castelle et al., 1994; Knutson & Naef, 1997; Broadmeadow &

Nisbet, 2004; Lee et al., 2004; Mayer et al., 2007). The wide range of buffer width guidelines

makes restoration of riparian ecosystems difficult and means that specific restoration will be

dependent on the goals of the project. For example, Broadmeadow and Nisbet (2004) provide

evidence that riparian zone width should be a minimum of 15 m for temperature and sediment

control and 27 m for litter contribution. Moreover, others argue that wildlife habitat, such as for

important reptile and amphibian species, will likely need to have more than 100 m buffers

(Semlitsch & Bodie, 2003). Along with width, the length of the watercourse that is vegetated is

also important. Holmes et al. (2004) found that the benefit-to-cost ratio was almost four times

higher in a 10,000 m restoration when compared to a 3,000 m restoration. It was suggested that

at least 75% of the length of the stream should be naturally vegetated to maintain stream quality

(Environment Canada, 2013). Choosing the correct vegetation for restoration will be highly dependent on the dynamics of each particular environment as well as the project goal (ACER, 2011), which is known to be highly variable. For example, Mayer et al. (2007) found that wetland vegetation was the most effective in nitrate removal, followed by a mix of woody and herbaceous vegetation. Therefore, if the goal of the restoration was to limit NO_3^- leaching into the watercourse, thus active planting of wetland species may be the best option.

With the clear importance of restoration efforts, it is likely that riparian management will continue to expand to larger scales to increase the yield of benefits (Holmes et al., 2004; Lee et al., 2004). However, it is important to note that headwater or first-order streams often make up the majority of the length of rivers in a given watershed (Naiman et al., 2005) and therefore need to be considered for their unique characteristics, functions, and sensitivities (Richardson & Danehy, 2007). Although riparian ecosystems have been shown to be sensitive to the effects of a changing climate because of their link to both the aquatic and terrestrial systems (Perry et al., 2012), it has also been argued that these ecosystems will be necessary for the mitigation of and adaptation to climate change (Seavy et al., 2009; Lal et al., 2011; Capon et al., 2013). With climate change threatening a massive loss of riparian ecosystem services and causing shifts in composition (Perry et al., 2012), riparian restoration will be critical to mitigate these impacts and maintain riparian function such as NO_3^- uptake, carbon sequestration, and protection from extreme events (Hill, 1996; Lal, 2005; Mayer et al., 2007; Lal et al., 2011; Soosaar et al., 2011; Catford et al., 2013).

1.2. Soil Greenhouse Gas Emissions and Potential Environmental Disservices

1.2.1. Soil Respiration & Nitrous Oxide Emissions

Carbon dioxide (CO_2) is accountable for more than half of the human-induced radiative forcing in the atmosphere (Houghton et al., 1990). Atmospheric CO_2 concentrations have risen an estimated 40% since pre-industrial times, and are at the highest concentration in 800,000 years (IPCC, 2013). The rate at which CO_2 is emitted is also at its highest in greater than 20,000 years (IPCC, 2013). Although much of the focus on reducing emissions is based around fossil fuel combustion, soil and vegetation respiration accounts for more total CO_2 emissions (Raich & Schlesinger, 1992; Smith et al., 2003). Soil respiration – the release of CO_2 from the soil – is considered the additive emissions from root respiration and microbial breakdown of organic matter in the soil (Raich & Potter, 1995). Emissions from soil respiration have continued to increase annually and are considered one of the largest terrestrial contributors of CO_2 to the atmosphere (Raich & Potter, 1995; Schlesinger & Andrews, 2000; Bond-Lamberty & Thomson, 2010). This continual increase of emissions is thought to be responding to increased temperatures caused by climate change, creating a positive feedback loop (Smith et al., 2003; Bond-Lamberty & Thomson, 2010).

Within an ecosystem there are different variables that can drive soil respiration (Raich & Tufekcioglu, 2000). Soils with higher organic matter content have increased decomposition that causes soil respiration (Bailey et al., 2009). Soil organic matter is the largest contributor to terrestrial carbon storage (Jobbagy & Jackson, 2000); however, soil microbes readily decompose increased soil carbon which leads to increased emissions (Schlesinger & Andrews, 2000). It has also been documented that soil texture can affect decomposition, where finer soil texture will decrease the rate of decomposition and therefore soil respiration (Bronick & Lal, 2005; Dilustro

et al., 2005; Bailey et al., 2009). Increasing temperature has been shown to increase respiration (Davidson et al., 1998), likely due to increased microbial activity leading to respiration (Smith et al., 2003). Raich & Potter (1995) suggest that the positive relationship between soil temperature and soil respiration is the greatest determinant of emissions, followed by soil moisture. However, some studies found that in areas with wet soils, soil moisture has a greater control over soil respiration (Pacific et al., 2008). Soil moisture is usually positively linked to soil respiration by facilitating plant growth and microbial activity; however, in areas of very high and very low water tables, soil moisture can limit soil respiration (Smith et al., 2003). High water tables increase the amount of water-filled pore space limiting emissions; whereas low water tables lack the moisture needed for microbial activity and plant growth (Smith et al., 2003). It has been well documented that soil respiration usually follows a seasonal pattern of temperature and moisture, where rates are usually highest in the spring and the summer, drop off in the fall, and are negligible in the winter (Picek et al., 2007; Shresthra et al., 2009; Soosaar et al., 2011). Determining which of these drivers of soil respiration is most important can be very difficult because of the clear interaction between soil moisture and temperature (Davidson et al., 1998). For example, Davidson et al. (1998) found that soil respiration declined in the summer months because of drought except in a hardwood swamp (high soil moisture). Therefore, although temperatures were high, soil moisture acted as a confounding factor and limited respiration.

Along with CO_2, nitrous oxide (N_2O) also substantially contributes to climate change, with atmospheric concentrations increasing by an estimated 20% since pre-industrial times (IPCC, 2013). Although N_2O is found in much smaller concentrations (Groffman et al., 1998), it has a global warming potential approximately 296 times higher than CO_2 (IPCC, 2007), contributes to radiative forcing, and has a long atmospheric lifespan (Mander et al., 2008). Based

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on current projections, anthropogenic N_2O emissions will be almost twice as high by 2050

(Davidson & Kanter, 2014). Soil emissions are the largest source of N_2O to the atmosphere,

which poses the largest risk for ozone depletion (Ravishankara et al., 2009; Butterbach-Bahl et

al., 2013; Oertel et al., 2016). There are two predominant processes that lead to these emissions

from the soil: denitrification and nitrification (Tiedje, 1982; Smith et al., 2003; Mander et al.,

2008; Audet et al., 2014), both of which are highly sensitive to land-use change and management

(Butterbach-Bahl et al., 2013).

It was suggested that denitrification is the predominant process that leads to soil N_2O

emissions in riparian zones (Mander et al., 2008). Denitrification is the microbial reduction of

nitrate (NO_3^-) and nitrite (NO_2^-) to dinitrogen gas (N_2) (Tiedje, 1982; Wrage et al., 2001; Smith

et al., 2003). Denitrification occurs when vegetation consumes the available oxygen (O_2) in a

given microsite, creating ideal conditions for anaerobes to convert NO_3^- to N_2 gas (Smith et al.,

2003). If the denitrification process is incomplete, N_2O is produced as an intermediate and is

emitted (Tiedje, 1982; Farquharson & Baldock, 2008). As denitrification is the reduction of

NO_3^-, a source of NO_3^- is necessary for the process and has been shown to increase soil N_2O

emissions in soils (Walker et al., 2002; Farquharson & Baldock, 2008; Mander et al., 2008). Soil

organic carbon (SOC) can provide necessary energy for denitrifying microorganisms to facilitate

denitrification (Farquharson & Baldock, 2008), and has been linked to increased soil N_2O

emissions (Tufekcioglu et al., 1999; Mander et al., 2008). SOC can also further create ideal

anaerobic microbial activity through a reduction in local O_2 stores by increasing aerobic

microbial activity (i.e., increased biochemical oxygen demand) (Farquharson & Baldock, 2008).

As anaerobic microorganisms drive denitrification, periods of increased soil moisture are often

associated with the process as increased water-filled pore space creates local anaerobic

conditions (Smith et al., 2003; Hopfensperger et al., 2009). However, if there is no free pore space, N_2O gas cannot diffuse into the atmosphere and will likely be reduced to N_2 (Farquharson & Baldock, 2008). The relationship between temperature and N_2O emissions has been extensively studied, but remains very complex. Much of the research shows an increase in microbial activity and N_2O emissions with increasing temperature (Smith et al., 2003; Shresthra et al., 2009; Soosaar et al., 2011). However, the ideal temperature for N_2O emissions will vary based on site because of the interaction with the driving forces of denitrification and nitrification (i.e., soil moisture, organic matter decomposition, N mineralization) (Farquharson & Baldock, 2008).

Nitrification, on the other hand, is the oxidation of ammonium (NH_4^+) to nitrate (NO_3^-). It has been shown to cause soil N_2O emissions as a byproduct (Smith et al., 2003; Audet et al., 2014) through decomposition or incomplete oxidation of intermediates such as NH_2OH and NO_2^- (Wrage et al., 2001). This process occurs under aerobic conditions and is limited by available oxygen (Smith et al., 2003; Barnard et al., 2005). Nitrification rates are positively correlated with soil moisture, until the moisture is so high that aerobic conditions are no longer available (Wrage et al., 2001; Smith et al., 2003). Increased soil NH_4^+ concentrations are also often associated with increased nitrification rates (Barnard et al., 2005). Soil NH_4^+ can enter a system by the mineralization of organic nitrogen or through NH_4^+ fertilization, leading to increased N_2O emissions (Farquharson & Baldock, 2008). The resulting NO_3^- can also cause further N_2O emissions through denitrification; this is called coupled nitrification-denitrification (Wrage et al., 2001; Farquharson & Baldock, 2008). However, this is not common, as it requires adjacent soils to have contrasting conditions (i.e., aerobic and anaerobic) that are suitable for both processes (Wrage et al., 2001).

Although CO_2 and N_2O are often the focus of riparian research, recent evidence has shown that soil methane (CH_4) emissions may also be a substantial contributor global climate change (IPCC, 2007). This contribution may be attributed to the high radiative forcing (second only to CO_2) (Ramaswamy et al., 2001), as well as a global warming potential that is 25 times higher than that of CO_2 (IPCC, 2007). Soil CH_4 emissions are caused by methanogenesis, that is the anaerobic decomposition of organic matter (Le Mer & Rogers, 2001; Smith et al., 2003). This process usually requires long periods of saturation or waterlogging in natural ecosystems (Smith et al., 2003), making wetlands the largest natural contributor of soil CH_4 emissions (IPCC, 2007).

1.2.2. Riparian Land-Use and Greenhouse Gas Emissions

Riparian land-use can play a large role in altering soil characteristics in a manner that drives CO_2 and N_2O emissions and contributes to climate change (Teiter & Mander, 2005; Capon et al., 2013; Audet et al., 2014). Restoration of these riparian systems towards a naturalized forest state can have major impacts on soil microclimate, nitrogen cycling (i.e., plant uptake of available nitrogen), and the input of organic matter, which lead to changes in soil respiration and denitrification/nitrification processes (Gregory et al., 1991; Osborne & Kovacic, 1993; Dosskey et al., 2010). Riparian zones are most commonly restored or maintained in areas adjacent to or in areas of high agricultural land-use to mitigate the leaching of NO_3^- into the watercourse (Verhoeven et al., 2006). Although riparian zones are extremely effective at this filtration, there is evidence that such high NO_3^- input and carbon availability may make riparian zones hotspots for soil greenhouse gas emissions (Mander et al., 2008; Kim et al., 2009; Shresthra et al., 2009).

Although CO_2 emissions in riparian soils have been relatively well studied, the results have been variable, showing a range of emissions from 20.6 kg ha^{-1} yr^{-1} to 11,400 kg ha^{-1} yr^{-1}

(Soosaar et al., 2011). Some have even suggested that riparian zones can act as both sources and sinks of CO_2 (Teiter & Mander, 2005; Hopfensperger et al., 2009; Soosaar et al., 2011; Audet et al., 2014). The influence of land-use on soil respiration has been observed when comparing grassland to forested riparian zones, where grasslands exhibited higher CO_2 production, likely due to increased temperature from lack of canopy (Raich & Tufekcioglu, 2000; Picek et al., 2007; Bailey et al., 2009; Shresthra et al., 2009). The relationship between age of a riparian forest and soil respiration is debated, as immature and mature riparian forests can create conditions ideal for soil respiration. Mature forests likely contribute more litter that increases soil organic matter and decomposition, leading to increased CO_2 emissions (Raich & Tufekcioglu, 2000). However, it has been documented that immature riparian zones have higher soil respiration than undisturbed natural forest riparian zones (Bailey et al., 2009). It has also been suggested that reduced canopies in immature forests affects soil temperature and moisture, which have stronger influences on CO_2 emissions (Pacific et al., 2008; Bailey et al., 2009).

Studies that measured N_2O emissions in riparian zones found they are extremely variable and can differ substantially temporally and spatially (Mander et al., 2008; Audet et al., 2014). Specifically, researchers found high (Walker et al., 2002; Kim et al., 2009), negligible (Picek et al., 2007), and negative (Audet et al., 2014) emissions of N_2O in riparian zones. With respect to N_2O emissions, it has been suggested that riparian vegetative buffers can act to slow microbial denitrification, through uptake of available nitrogen and reduced soil temperature (Fortier et al., 2010). However, Picek et al. (2007) suggested that greenhouse gas emissions may be higher with the presence of vegetation due to accelerated microbial decomposition of root exudates. As denitrification is driven by available carbon in the soil (McLain & Martens, 2006; Mander et al., 2008), permanent vegetation increases root biomass which provides carbon for microbial

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denitrification (Tufekcioglu et al., 1999). Furthermore, increased N_2O emissions have been observed in grassed or no riparian buffers compared to forested riparian zones (Picek et al., 2007; Kim et al., 2009; Shresthra et al., 2009). It has been hypothesized that high NO_3^- availability, high temperature, and optimal soil moisture could explain these findings (Shresthra et al., 2009). It has been suggested that restoration of degraded riparian ecosystems to forests could reduce the amount of soil N_2O emissions up to 75% (Shresthra et al., 2009).

Soil CH_4 emissions in natural systems have been shown to vary with vegetation type and water table depth (Smith et al., 2003). Little research has been conducted on soil CH_4 emissions from riparian ecosystems; however, it has been argued that these ecosystems can act as sources or sinks of CH_4 depending on the drainage of the soils (with sinks being well-drained) (Teiter & Mander, 2005). As riparian ecosystems are seasonally flooded, there may be periods of increased soil CH_4 emissions (Teiter & Mander, 2005). However, Altor & Mitsch (2006) found that restored wetlands with seasonal flooding decreased soil CH_4 emissions by 30% when compared permanently waterlogged wetland soils. Although soil CH_4 emissions are likely impacted by riparian land-use, they were not included in this study.

1.2.3. Soil and Greenhouse Gas Emissions: Environmental Disservices and Knowledge Gaps

While the benefits of riparian ecosystems have been well documented, very few studies have assessed the potential environmental disservices that may be present (Mander et al., 2008; Shresthra et al., 2009). Riparian buffers have the potential to act as greenhouse gas emission hotspots and are not completely accounted for in the calculations of global greenhouse gas emissions by the Intergovernmental Panel on Climate Change (IPCC) (Groffman et al., 2000). Although sources and sinks of greenhouse gases have become a focus of research in terrestrial

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ecosystems (Merbold et al., 2013), little is known about the emission of greenhouse gases in riparian ecosystems (Groffman et al., 1992; Groffman et al., 2000; Teiter & Mander, 2005). N_2O, specifically, needs further investigation to understand what drives emissions and how these emissions may offset carbon sinks (Merbold et al., 2013).

Of the studies that have considered greenhouse gas emissions in riparian zones, many have looked at grassland buffers (Kim et al., 2009; Shresthra et al., 2009), constructed wetlands (Teiter & Mander, 2005; Picek et al., 2007), or homogenous riparian stands (Mander et al., 2008; Soosaar et al., 2011). Very little evidence has been provided on restored/rehabilitated riparian forests in terms of environmental disservices in the form of greenhouse gas emissions, apart from reclaimed, mined soil (Shresthra et al., 2009), and wetlands constructed for wastewater treatment (Teiter & Mander, 2005; Picek et al., 2007; Jahangir et al., 2016). Furthermore, little research has been conducted on riparian greenhouse gas emissions in agriculturally dominated, north temperate landscapes such as southern Ontario (Petrone et al., 2008; Oelbermann et al., 2015), especially in restored riparian forests. Although the spatial variability of greenhouse gas emissions in riparian zones has been well documented (Tufekcioglu et al., 2001; Teiter & Mander, 2005; McLain & Martens, 2006; Petrone et al., 2008), there has been very little description of how this variability occurs in relation to the adjacent land-use, watercourse, or natural hydrology. Tufekcioglu et al. (2001) suggest that future studies consider the position effect (i.e., closer to the stream or to adjacent land-use) of riparian greenhouse gas emissions.

As soils are extremely important contributors to atmospheric CO_2 and N_2O (Raich & Potter, 1995), it is crucial to determine the drivers of spatial and temporal variation of potential emissions hotspots, such as riparian ecosystems. Furthermore, with an increasing push towards riparian rehabilitation/restoration, understanding the effectiveness of these efforts in improving

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or maintaining ecosystem function is also critical (i.e., nutrient uptake, soil microclimate). However, the majority of restoration efforts are not monitored to evaluate success (Bernhardt et al., 2005). Research is now needed to understand the influence of riparian restoration on greenhouse gas emissions and their correlative variables.

1.3.   Research Objectives and Hypotheses

The objective of this thesis are three-fold:

1. To quantify and compare greenhouse gas (CO_2-C and N_2O-N) emissions between a rehabilitated forest (RH) and an undisturbed natural forest (UNF) riparian land-use, including the identification of spatial and temporal trends.

2. To determine the role of vegetation and soil characteristics in driving greenhouse gas emissions in the RH and UNF land-uses

3. To quantify and compare soil and vegetative characteristics related to greenhouse gas emissions in the RH and UNF land-uses and identify spatial and temporal trends in these characteristics.

Hypotheses:

H1: There will be a significant difference in GHG emissions, soil, and vegetation characteristics between the rehabilitated (RH) and undisturbed natural (UNF) forest riparian land-uses.

H_0: There will be no significant difference in GHG emissions, soil, and vegetation characteristics between the RH and UNF riparian land-uses.

H2: Variation in riparian soil greenhouse gases is driven by soil and vegetation characteristics.

H_0: Soil and vegetation characteristics will not be significantly correlated to soil greenhouse gas emissions

H3: Greenhouse gas emissions and soil characteristics will significantly vary spatially and temporally for both the RH and UNF riparian land-uses.

H$_0$: Greenhouse gas emissions will not significantly vary spatially or temporally for both the RH and UNF riparian land-uses.

2. Materials and Methods

2.1. Study Site – Washington Creek, ON, Canada

The study sites used for this research are found along Washington Creek in the Township of Blandford-Blenheim, Oxford County, Ontario, Canada. Washington Creek is a spring-fed, first-order tributary of the Nith River that stretches 9 km, and is part of the Grand River Watershed. Washington Creek flows into the Nith River south of Plattsville, Ontario (43˚18'N 80˚33'W). Oxford County is located in the peninsular region of southwestern Ontario, and thus the climate is greatly affected by the Great Lakes (Wicklund & Richards, 1961). The climate can be described as temperate with cold winters, hot and humid summers, and have 208 frost free days annually (Environment Canada, 2016). The mean annual temperature for this area is 7.3˚C and the mean annual precipitation is 918.7 mm (Environment Canada, 2016).

The soil parent material found at the study sites is glacial till (Pleistocene) overlying limestone bedrock (Silurian) (Oelbermann et al., 2008). Soils adjacent to the study site are predominantly grey-brown Luvisol and can be classified as Guelph loam, Brisbane sandy loam, and Fox loamy sand (Wicklund & Richards, 1961; Mozuraitis & Hagarty, 1996); however, the soil found along Washington Creek is considered Bottomland (OMAFRA, 2008). Bottomland soils are comprised of alluvial sands, silts, and clays from the streambeds, as well as from the spring thaw (Wicklund & Richards, 1961). The surface soil at the study sites can be classified as silt loam (Oelbermann et al., 2008) and sandy loam (Wicklund & Richards, 1961).

The landscape surrounding Washington Creek is dominated by agriculture, specifically for corn (*Zea mays* L.), soybeans (*Glycine max* (L.) Merr.), or utilized as pasture for livestock. However, there is still a remnant of riparian land-use with undisturbed, natural forest (UNF), as

well as a rehabilitated forest (RH). The comparison of these two land-uses is the focus of this

research.

Figure 2.1. Aerial photograph of the overall study site location including both the UNF and RH sites (Google Earth, 2016)

2.1.1. Undisturbed, Natural Riparian Forest (UNF)

The UNF site is a riparian buffer of greater than 100 m that has not been disturbed since

the mid-1800's. The predominant tree species found in this forest are sugar maple (*Acer*

saccharum L.), American basswood (*Tilia americana* L.), eastern hemlock (*Tsuga canadensis*

L.), American beech (*Fagus grandifolia* E.), American hophornbeam (*Ostrya virginiana* P.), and

hickory (*Carya* spp.) (Oelbermann et al., 2015). The adjacent land-use to the UNF site is row

cropping (soybean-corn rotation), with one adjacent farm transitioning from unused pasture to

row cropping within one year of the start of sampling. The soil texture, to a 20 cm depth, can be

classified as a loam, being comprised of 53.7% sand, 39.0% silt, and 7.9% clay (Oelbermann et

al., 2015). The pH at the UNF site was 7.28 and the bulk density was 0.75 g cm^{-3} (Oelbermann et al., 2015).

2.1.2. Rehabilitated Riparian Forest (RH)

Approximately 600 m downstream from the UNF site, there is a 1.6 km long, rehabilitated riparian forest along Washington Creek. Until 1985, vegetation was cleared up to the creek for soybean-maize rotation row cropping. With very little vegetative buffer to prevent erosion and filter out pollutants, land and stream degradation were large concerns (Gordon et al., 1996). This degradation led to a 6-year long rehabilitation effort that aimed to restore a riparian buffer of 30-50m. In 1985 and 1986, tree and shrub planting began with multiple alder species (*Alnus incana* subsp. *Rugosa* (Du Roi) R.T. Clausen, *Alnus glutinosa* (L.) Gaertn., and *Alnus rubra* Bong.), hybrid poplars (*Populus* x *canadensis* Moench), and silver maples (*Acer sacharinum* L.). In 1990 and 1991, additional silver maples were planted as well as multiflora rosevine (*Rosa multiflora* Thunb.), Russian olive (*Elaegnus angustifolia* L.), and red-osier dogwood (*Cornus sericea* subsp. *Sericea* L.). Tree plantings were conducted in four rows of 3 x 3 m spacing or in blocks based on the size and shape of the riparian buffer. The plantings created a tree density of 3.14 trees m^{-2} and a productivity of 3.9 Mg ha^{-1} y^{-1} (oven-dry) in 1991 (Gordon et al., 1992). The adjacent land-use to the RH site is row cropping (soybean-corn rotation). The soil texture at the RH site, to a 20 cm depth, can be classified as a loam, being comprised of 43.6% sand, 48.8% silt, and 8.4% clay (Oelbermann et al., 2015). The pH at the RH site was 8.01 and the bulk density was 0.99 g cm^{-3} (Oelbermann et al., 2015). Much research has been conducted on the RH site since the rehabilitation in 1985 focusing on flora, fauna, soil dynamics, and stream quality (Mallory, 1993; Gordon et al., 1996; Oelbermann and Gordon, 2000;

Oelbermann & Gordon, 2001; Oelbermann et al., 2008; Plascencia-Escalante, 2008; Raimbault, 2011; Oelbermann et al., 2015).

2.2.   Experimental Design

This study utilizes a unique opportunity to compare an approximately 30-year old rehabilitated riparian forest (RH), with a relatively undisturbed, natural forest (UNF) along a first-order stream in an agriculturally dominated landscape. To capture the temporal variation of greenhouse gas emissions (CO_2 and N_2O) and soil characteristics, there were 20 sampling dates conducted throughout one year between May 28, 2015 and May 19, 2016. To incorporate the effect that seasonality has on greenhouse gas emissions and soil characteristics, sampling dates were broken up into seasons as the following:

1. Summer (n=6): May 28, 2015 – September 24, 2015
2. Fall (n=6): October 7, 2015 – December 27, 2015
3. Winter (n=4): January 15, 2016 – March 4, 2016
4. Spring (n=4): March 22, 2016 – May 19, 2016

Seasons were separated in this manner to isolate the winter season as the dates when the topsoil was frozen and soil samples were unable to be taken. Due to a mild winter, this freeze did not occur until the January 15, 2016 sampling date.

To encapsulate the spatial variation in greenhouse gas emissions and soil characteristics, three transects were set up running parallel to Washington Creek (Picek et al., 2007). Each transect was made up of three sampling replicates, placed 15 m apart, resulting in a 3 x 3 grid (n=9) for each treatment (land-uses) (Figure 2.2). A 2 m buffer was set up between the grid and the stream, as well as the adjacent land-use (Oelbermann et al., 2015) (Figure 2.2). Sampling was

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performed between 10:00 h and 16:00 h to capture the peak and most consistent greenhouse gas

emissions (Petrone et al., 2008). To avoid bias, the sampling order of the treatments and

replicates was randomized on each of the sampling dates. With no other first-order stream with

comparable land-use management within the Grand River watershed (i.e., three streams with the

same soil and groundwater chemistry, vegetative communities, and similarly aged rehabilitated

and natural riparian forests), this study is pseudo-replicated. This pseudo-replication, and the

hydrological link between the two sites, makes the sample size effectively one, thus limiting the

universality of the results.
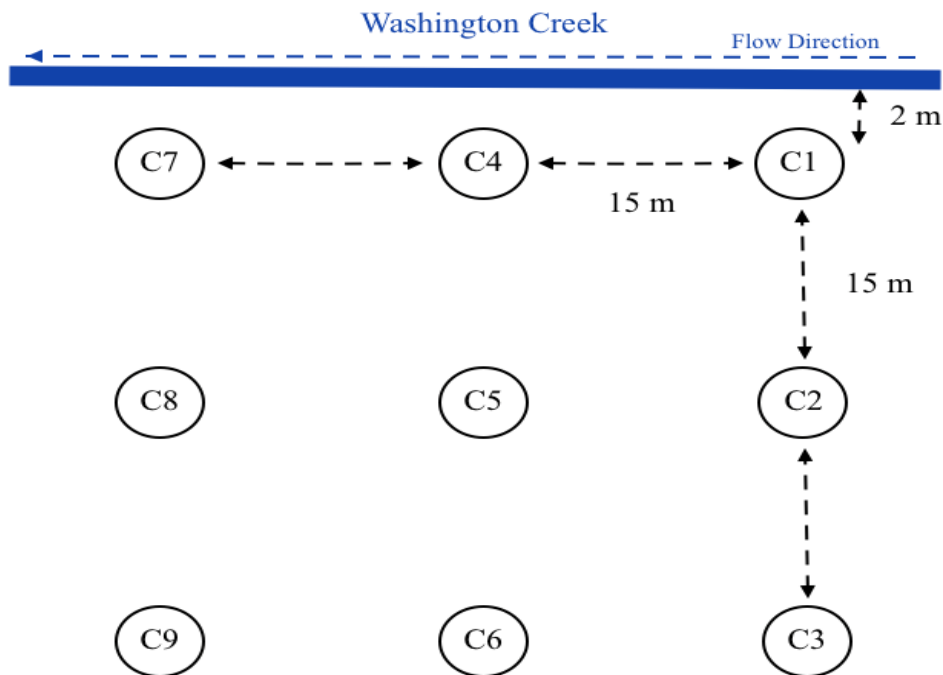
Figure 2.2. Greenhouse gas chamber (C1-C9) placement starting for both the RH and UNF land-uses along Washington Creek in southern Ontario, Canada in 2015. This 3x3 transect design was adapted from Picek et al. (2007).

2.3.   Soil Sampling Design & Analysis

Soil samples were collected from the RH and UNF sites during the summer, fall, and

spring season sampling dates. Soil was collected to a depth of 10 cm using a spade according to

Estefan et al. (2013). Soil sampling was conducted randomly within 1 m of the sampling

replicate point to avoid using continuously disturbed soils. Soils (0-10 cm) for each replicate

were then put into labeled bags, placed in a cooler, and transported back to the Soil Ecosystem

Dynamics Laboratory, University of Waterloo, Waterloo, Ontario. Soils were then frozen

immediately to maintain the concentrations of available nitrogen within the samples (Marx et al.,

1996; Horneck et al., 2011).

2.3.1. Soil Physical Characteristics

Soil temperature (˚C) and moisture (%) were measured at each soil sampling location

using an HH2-WET Sensor (Delta T Devices, Cambridge, UK). Thermocouples were

constructed and deployed at each of the replicates on December 3, 2015 to continue to collect

soil temperature throughout the winter season. Due to technical failures in the field, only 42 of

72 temperature measurements were obtained throughout the winter months. Temperature was not

used in statistical analysis because of small sample size and standard error in thermocouple

readings (+/- 1.5 ℃); however, they were included in graphical analysis to show the general

trend present. Ambient air temperature (˚C) at time of sampling was determined using the closest

weather station available, which was the Kitchener/Waterloo (43°27'39.000" N 80°22'43.000"

W) weather station.

2.3.2. Soil Chemical Characteristics

To determine the soil chemical characteristics, soils were thawed overnight in a fridge,

allowed to air-dry, ground using a mortar and pestle, and then sieved (2 mm). Soil nitrate (NO_3^-)

and ammonium (NH_4^+) concentrations were determined for summer, fall and spring, whereas soil

organic carbon (SOC) and total nitrogen (TN) was determined for the May 28, 2015 sampling

date. SOC was only measured once as it accumulates at less than 1 % intra-annually on temperate zone forest floors based on the predicted annual accumulation (Poeplau et al., 2011) and the availability of SOC values from the RH and UNF sites from 2010 (Oelbermann et al., 2015). TN was only sampled once because it is not common to conduct extensive sampling of the TN (Walworth, 2011), therefore the focus was shifted towards the forms of nitrogen that drive denitrification and nitrification (NO_3^- and NH_4^+, respectively) and therefore N_2O emissions.

NO_3^- and NH_4^+ was extracted from the soil by mixing 10 g of air-dried soil into snap cap containers with 50 mL of 2.0 M KCl. Using a reciprocating shaker, the solution was mixed for 15 minutes at 180 rpm. The extraction was filtered from the soil through Whatman 42 filter paper. Soil NO_3^- and NH_4^+ was analyzed using a Shimadzu 1800 UV-Vis Spectrophotometer (Shimadzu Corp., Kyoto, Japan) at 540 nm after 12 h of color development (Miranda et al., 2001; Doane & Horwath, 2003) and at 650 nm after 1 h of color development (Verdouw et al., 1978; Foster, 1995), respectively.

To remove carbonates from the soil for SOC analysis, 3 g of soil was combined with 30 mL of 0.5 M HCl and, using a reciprocating shaker, mixed for 1h at 300 rpm. The acid was then removed and the process was repeated three more times (Lutes et al., 2016). These soils were then rinsed with distilled water and baked in an oven at 40˚C for 24 h. Soils were then ground further with a ball-mill (Retsch, Haan, Germany). Of these soils, 15 mg was packed into tin capsules and analyzed for SOC and TN using a Costech CHNS-O 4010 Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, USA).

2.4.   Vegetative Characteristics

Vegetative characteristics were measured during the July 17, 2015 sampling date as it falls within the time-period when the leaf area index for the RH and UNF sites were at a

maximum (Comeau, 2000). Stand density was determined using three random quadrats of 5 m x 5 m to ensure that substantial number of trees would fall within the quadrats (Fidelibus & Mac Aller, 1993). The number of stems was counted for each quadrat. Stand density was calculated as the average number of stems per 10 m^2 area (Fidelibus & Mac Aller, 1993). To determine whether microsite features impact N_2O and CO_2 fluxes, the distance (m) from each greenhouse gas chamber to the nearest stem was determined (Butterbach-Bahl et al., 2002). Illuminance (Lux), an indicator of canopy cover (Wu et al., 2013), was measured at each of the sampling replicates for both the RH (n=9) and UNF (n=9) sites using a Digital Illuminance Meter (Nicety LX802, China). To ensure uniform results, open sky readings were taken within five minutes of below canopy readings (at each sampling replicate) around midday and the difference was calculated to determine the light intensity (or percentage of light penetration) (Comeau, 2000).

2.5.   Greenhouse Gas Sampling & Analysis

To measure greenhouse gases (CO_2 and N_2O) at each sampling replicate location, for both the RH and UNF sites (n=18), greenhouse gas chambers were deployed on May 21, 2015, one week before the first sampling date to prevent disruption of the soils that could affect greenhouse gas emissions. Greenhouse gas chambers were made up of a permanent anchor and a detachable chamber cap. The chambers were constructed using white, non-reactive PVC piping (with a diameter of 10 cm and a length of 25 cm). At the time of deployment, the chamber anchor was inserted 10 cm into the soil, allowing for 15 cm of chamber space above the soil surface (Smith et al., 2003; Dyer et al., 2012; Lutes et al., 2016). The permanent anchor was left embedded in the soil for the duration of the sampling and removed on May 19, 2016. Chamber caps were made from non-reactive PVC, covered in reflective insulation, and a sampling port was drilled in the cap and fitted with a rubber septum (1 cm diameter) to allow for gas sampling

(Dyer et al., 2012; Lutes et al., 2016). The chamber caps were also fitted with a 10 cm vent tube (9 mm diameter), which reduces the variation in pressure during gas sampling (Dyer et al., 2012; Lutes et al., 2016). Chamber caps were removed after each sampling and the anchors were left open to the atmosphere in between sampling dates. Vegetation and litterfall that might influence greenhouse gas emissions were cleared from the chamber 24 h in advance of the sampling date.

To capture observed temporal variability of greenhouse gas emissions (Mander et al., 2008; Parkin and Venterea, 2010; Lutes et al., 2016), sampling occurred throughout all seasons, in one full year (n=20). Sinusoidal diurnal temperature variation impacting soil respiration (Smith et al., 2003) was accounted for by sampling between 10:00 h and 16:00 h, reducing bias (Parkin and Venterea, 2010). To determine CO_2 and N_2O fluxes, samples were taken from the chamber at 0, 15, and 30 min (Dyer et al., 2012; Lutes et al., 2016) using a 10 ml air-tight syringe (Luer-Lock Tip. BD, Franklin Lakes, NJ, USA), and deposited into evacuated 3 mL vials (LabCo Ltd., High Wycombe, UK) until over-pressurized (Parkin and Venterea, 2010).

Greenhouse gas samples were brought back to the Soil Ecosystem Dynamics Lab, University of Waterloo, Waterloo, Ontario, and analyzed using an Agilent 6890 Gas Chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA). To measure N_2O emissions (ppm), a capillary column connected to a micro-electron capture detector (ECD) was utilized, whereas a thermo-conductivity detector (TCD) was used to measure CO_2 emissions (ppm). CO_2 and N_2O emissions (ppm) were converted to fluxes (μg N_2O-N $m^{-2}\,h^{-1}$ and mg CO_2-C $m^{-2}\,h^{-1}$, respectively) by determining whether the fluxes follow a linear or curvilinear response (Hutchinson and Mosier, 1981). If a linear response was determined, then a linear regression slope was used to determine the greenhouse gas emission; however, if a curvilinear response was found, the equation outlined by Hutchinson and Mosier (1981) was used. The result of this

calculation was the greenhouse gas flux in µL of gas (CO_2 and N_2O for this study) m^{-2} h^{-1} (Parkin and Venterea, 2010). Using the ideal gas law, the result was converted to μmol m^{-2} h^{-1}, and then the molecular mass was used to translate this value to μg m^{-2} h^{-1} (Lutes et al., 2016). All of these equations can be found described in depth by Lutes (2016).

2.6.   Statistical Analysis

To avoid violating the assumption that the data has no outliers, the data were tested for outliers according to Weinberg and Abramowitz (2008) and were removed. Levene's and Shapiro-Wilk tests were run on all datasets to test for homogeneity of variance and normality, respectively (Steel et al., 1997). When data violated the assumption of normality, they were log-transformed and tested again. If after log-transformation, the distribution was still not normal, parametric tests were still performed, since in sample sizes greater than 30 or 40 (such as the seasonal data), such a violation should not substantially impact the results (Elliot & Woodward, 2007; Pallant, 2007), and in sample sizes in the hundreds (such as the yearly data) the distribution can be disregarded altogether (Altman & Bland, 1995).

Linear mixed models were run for comparisons where values within and/or between samples were not independent (i.e., repeated measures) (Seltman, 2012). These linear mixed models were used to find temporal differences in greenhouse gases (CO_2 and N_2O) and soil characteristics (soil NO_3^- concentrations, NH_4^+ concentrations, moisture, and temperature). To determine the model with the best balance between complexity and fit, the Bayesian Information Criteria (BIC) were compared, and the model with the smallest BIC was chosen (Seltman, 2012). To compare temporally, the model was set up with chamber (n=18) as the subject and random effect (Seltman, 2012), and the sampling date (n=20 for greenhouse gases, n=16 for soil characteristics) as the repeated measure with the autoregressive (homogenous/heterogeneous)

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repeated covariance type based the homogeneity of variance of the response variable (Kincaid, 2005). The fixed effect for the temporal model was the season (n=8 for greenhouse gas emissions, n=6 for soil characteristics) and the Sidak post hoc test was used to determine significant differences (Kowalchuk, 2000) between the seasons.

To determine the spatial variation in greenhouse gases and soil characteristics, the chambers were grouped by parallel and perpendicular locations to show variation along the gradient of Washington Creek to determine the impact of the riparian buffer as well as the natural flow of runoff towards the creek. The parallel groups were broken into the following three groups (locations): upstream (chambers one, two, and three), middle (chambers four, five, and six), and downstream (chambers seven, eight, and nine) (Figure 2.3). The perpendicular groups were broken into the following three groups (locations): farthest (chambers three, six, and nine), middle (chambers two, five, and eight), and closest (chambers one, four, and seven) (Figure 2.3). A one-way analysis of variance (ANOVA) was run to see if there was a statistical difference between soil moisture, temperature, NO_3^- concentration, NH_4^+ concentration, and greenhouse gas emissions between the independent locations (parallel and perpendicular) for each land-use for the whole sampling period, as well as by season. If variances were not homogenous per Levene's test, the Welch test was used to determine significant differences between locations (Tomarken & Serlin, 1986). If significant, Tukey's (homogenous variance) and Games-Howell (heterogeneous variance) post hoc tests were used to determine where the significant differences were found (Hilton & Armstrong, 2006).

To determine the correlations among greenhouse gases, soil characteristics and vegetative characteristics, the Pearson Product-Moment Correlation was used, as all are random variables (Zou et al., 2003). Correlation analysis was run for all variables to greenhouse gas emissions for

each season and land-use to account for seasonal and geographical variation. All variables were run against each other to see if there was a correlation throughout the whole year of sampling and for both land uses to determine if there was an overall significant correlation (for the whole system). To compare data collected on only one sampling date (SOC, TN, illuminance, average distance to nearest tree, and density), the student *t* test was used. All statistical analysis was performed using SPSS Statistics for Mac Version 23.0 (IBM Corp., 2015). The Type I error rate for all statistical analysis was p<0.05.
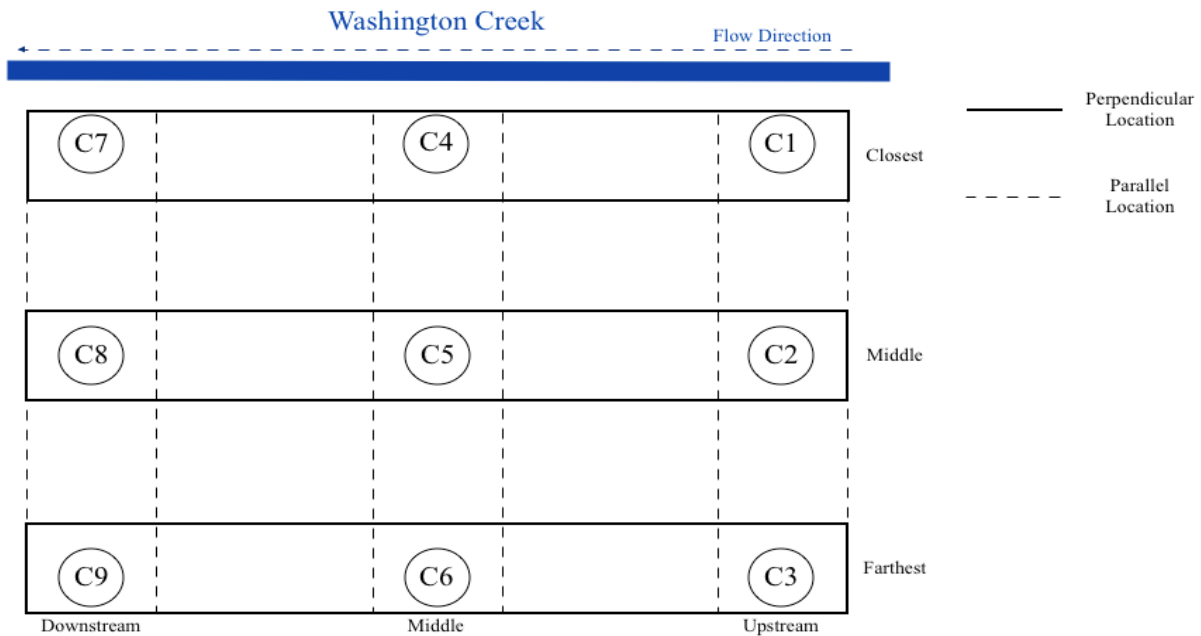
Figure 2.3. Spatial groupings (perpendicular and parallel) of greenhouse gas chambers and soil sampling sites for both the RH and UNF land-uses along Washington Creek in southern Ontario, Canada in 2015.

3. Results

3.1. Soil Physical and Chemical Characteristics

3.1.1. Soil Temperature and Moisture

The mean annual air temperature and soil temperatures for the RH and UNF sites during the sampling period were 10.9˚C +/- 2.3˚C, 11.7˚C +/- 0.5˚C, and 10.9 +/- 0.4, respectively (Figure 3.1). Based on the linear mixed model, soil temperature (˚C) was significantly different temporally among seasons [$F_{(5, 73.4)}$=19.7, $p<0.001$] (Figure 3.1; Table 3.1). Within the RH land-use, soil temperature was significantly higher in the summer, when compared to the fall and spring (Figure 3.1; Table 3.1). Within the UNF land-use, the summer also had significantly higher soil temperature than the fall and spring (Figure 3.1b; Table 3.1). Between land-uses, soil temperature was significantly higher during the summer for both land-uses than the other land-use's fall and spring (Table 3.1). The soil moisture (% vol) was also significantly different temporally among seasons [$F_{(5, 107.5)}$=58.5, $p<0.001$] (Table 3.1). Within the RH land-use, soil moisture was significantly lower in the summer than in the fall and spring (Table 3.1). Within the UNF land-use, soil moisture did not vary significantly (Table 3.1). Between land-uses, all seasons at the UNF site had significantly higher soil moisture than all seasons at the RH site (Table 3.1). The mean annual soil moisture for the RH and UNF sites during the sampling period were 37.1% +/- 1.0% and 60.8% +/- 0.8%, respectively (Figure 3.1).
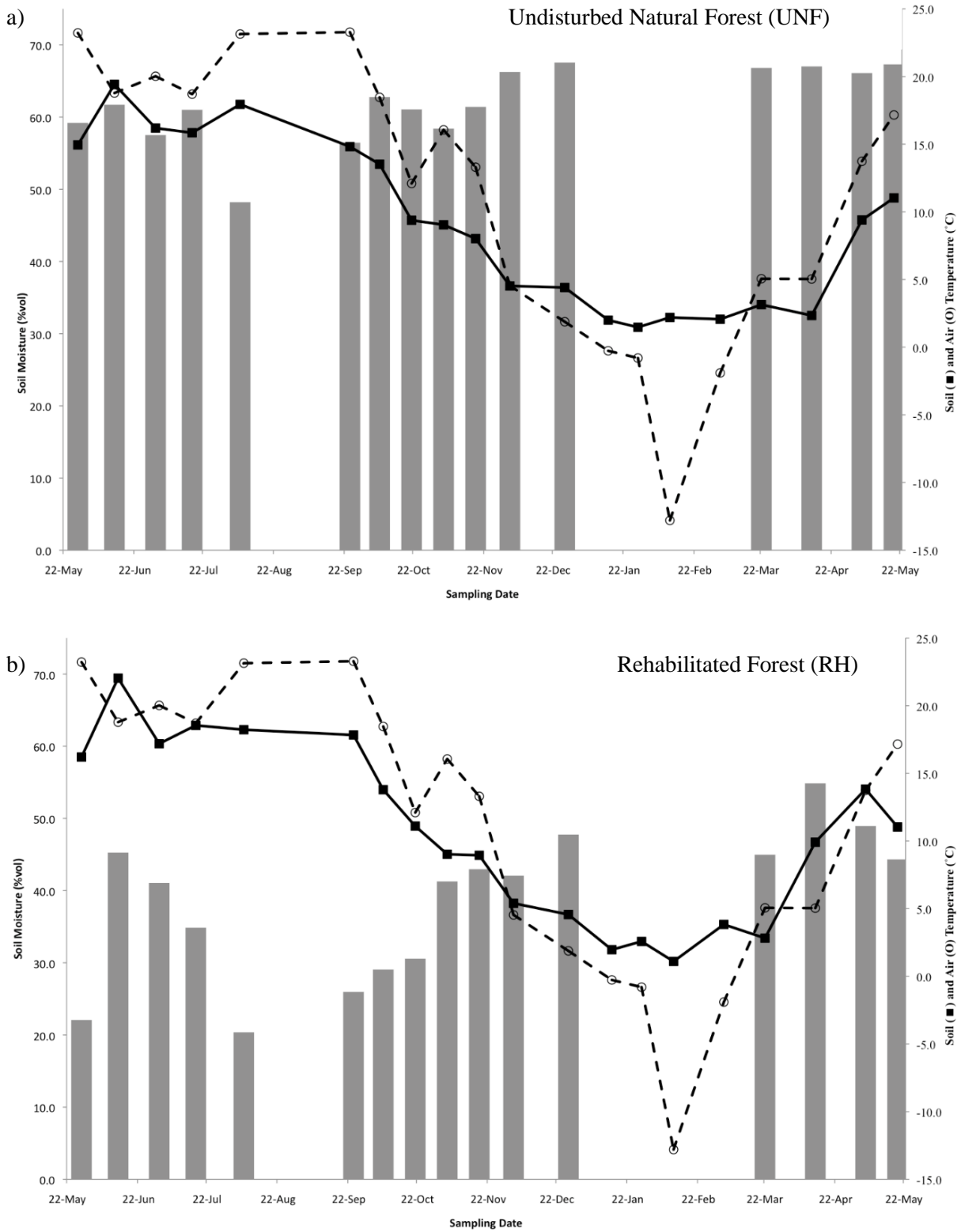
Figure 3.1. Mean ambient air temperature, soil temperature and soil moisture for the a) undisturbed natural forest (UNF) and b) rehabilitated forest (RH) sites along Washington Creek in southern Ontario, Canada for all sampling dates between May 28, 2015 and May 19, 2016.

Table 3.1. Mean seasonal soil temperature (˚C) and moisture (% vol) for the rehabilitated forest (RH) and undisturbed natural forest (UNF) sites along Washington Creek in southern Ontario, Canada during 2015-2016. Standard errors are given in parentheses.

	Season	RH	UNF
Soil Temperature (˚C)	Summer	16.2 (0.8)[A]	14.3 (0.8)[A]
	Fall	10.3 (0.7)[B]	10.1 (0.7)[B]
	Spring	9.5 (0.8)[B]	8.3 (0.8)[B]
Soil Moisture (% vol)	Summer	30.2 (1.7)[A]	57.7 (1.9)[C]
	Fall	38.3 (1.6)[B]	63.9 (1.7)[C]
	Spring	43.8 (1.9)[B]	65.2 (1.9)[C]

[A] Means followed by different uppercase letters are significantly different ($p<0.05$) among seasons and land-uses.

The full-year spatial comparison showed that there was no significant effect of perpendicular location on soil temperature for the RH [$F_{(2,135)}=0.304$, $p=0.739$] and UNF sites [$F_{(2,141)}=0.108$, $p=0.898$] (Table 3.2). When separated into seasons, the RH site showed a significant effect of location on soil temperature for the summer [$F_{(2,46)}=4.4$ $p=0.018$], with the closest location having a significantly lower temperature than the farthest ($p=0.01$) (Table 3.2). However, there was no significant effect of location on soil temperature observed during the fall [$F_{(2,50)}=0.312$, $p=0.733$] or spring [$F_{(2,33)}=0.039$, $p=0.962$] seasons (Table 3.2). The seasonal effect of perpendicular location on soil temperature at the UNF site was not significant for the summer [$F_{(2,52)}=0.370$, $p=0.692$], fall [$F_{(2,52)}=0.001$, $p=0.999$], or spring [$F_{(2,33)}=0.186$, $p=0.831$] seasons (Table 3.2).

The full-year spatial comparison showed that there was a significant effect of perpendicular location on soil moisture for the RH site [$F_{(2,92.3)}=15.409$, $p<0.001$], with the closest location having significantly lower soil moisture than the middle ($p=0.007$) and farthest ($p<0.001$) locations (Table 3.2). However, soil moisture did not vary significantly among the

three locations at the UNF site [$F_{(2,78.2)}=0.155$, $p=0.857$] (Table 3.2). When separated into seasons, the RH site showed a significant effect of location on soil moisture for the summer [$F_{(2,51)}=6.0$ $p=0.005$], with the closest location having a significantly lower moisture than the farthest ($p=0.005$) (Table 3.2). During the fall, all locations were significantly different [$F_{(2,30.8)}=16.691$, $p<0.001$], showing a trend of increasing moisture with increasing distance from the watercourse (Table 3.2). There was also a significant effect of location on soil moisture observed during the spring [$F_{(2,33)}=7.087$ $p=0.003$] (Table 3.2), with the closest location having significantly lower moisture than the middle ($p=0.003$) and farthest ($p=0.029$) locations (Table 3.2). The seasonal effect of perpendicular location on soil moisture at the UNF site was not significant for the summer [$F_{(2,30.6)}=1.819$, $p=0.179$] or spring [$F_{(2,33)}=2.539$, $p=0.094$] seasons (Table 3.2). However, a significant effect was observed in the fall [$F_{(2,43)}=5.943$, $p=0.005$], with the closest location having significantly lower moisture than the middle ($p=0.004$) (Table 3.2).

The full-year spatial comparison showed that there was no significant effect of parallel location on soil temperature for the RH [$F_{(2,136)}=0.114$, $p=0.892$] and UNF sites [$F_{(2,141)}=0.168$, $p=0.994$] (Table 3.3). When separated into seasons, the RH site showed no significant effect of location on soil temperature for the summer [$F_{(2,29.8)}=0.601$ $p=0.555$], fall [$F_{(2,51)}=0.004$, $p=0.996$], or spring [$F_{(2,33)}=0.031$ $p=0.970$] seasons (Table 3.3). The seasonal effect of parallel location on soil moisture at the UNF site was not significant for the summer [$F_{(2,51)}=0.047$, $p=0.954$], fall [$F_{(2,51)}=0.082$, $p=0.921$], or spring [$F_{(2,33)}=0.003$, $p=0.997$].

The full-year spatial comparison showed that there was no significant effect of parallel location on soil moisture for the RH site [$F_{(2,141)}=1.909$, $p=0.152$]. However, soil moisture did vary significantly among the three locations at the UNF site [$F_{(2,88.0)}=17.567$ $p<0.001$] (Table

3.3), with the upstream location having significantly lower moisture than the middle ($p<0.001$)

and downstream ($p<0.001$). When separated into seasons, the RH site showed no significant

effect of location on soil moisture for the summer [$F_{(2,51)}=1.164$ $p=0.321$], fall [$F_{(2,51)}=0.269$,

$p=0.765$], or spring [$F_{(2,33)}=2.587$ $p=0.090$] (Table 3.3). The seasonal effect of parallel location

on soil moisture at the UNF site was significant for the summer [$F_{(2,32.7)}=7.960$, $p=0.002$], fall

[$F_{(2,26.9)}=11.943$, $p<0.001$], and spring [$F_{(2,33)}=11.500$, $p<0.001$] (Table 3.3). The upstream

location was significantly lower than the middle (summer: $p=0.015$; fall: $p=0.001$; spring:

$p<0.001$) and downstream (summer: $p=0.001$ fall: $p<0.001$; spring: $p<0.013$) locations (Table

3.3), showing an increasing trend in soil moisture moving downstream in the UNF site.

Table 3.2. Mean annual and seasonal soil temperature (˚C) and moisture (% vol) for three perpendicular locations (closest, middle, and farthest) at the rehabilitated forest (RH) and undisturbed natural forest (UNF) sites along Washington Creek in southern Ontario, Canada during 2015-2016. Standard errors are given in parentheses.

	Season	RH			UNF		
		Closest	Middle	Farthest	Closest	Middle	Farthest
Soil Temperature (˚C)	Summer	18.6 (1.5)[A]	17.7 (1.5)[A]	17.2 (0.9)[A]	16.2 (0.5)[A]	16.7 (0.4)[A]	16.5 (0.4)[A]
	Fall	8.9 (0.8)[A]	8.9 (0.8)[A]	8.2 (0.7)[A]	8.2 (0.8)[A]	8.3 (0.8)[A]	8.3 (0.8)[A]
	Spring	7.6 (1.4)[A]	7.3 (1.3)[A]	7.9 (1.4)[A]	5.9 (1.1)[A]	6.9 (1.2)[A]	6.7 (1.1)[A]
	Overall	12.1 (0.9)[A]	11.7 (0.8)[A]	11.2 (0.8)[A]	10.6 (0.8)[A]	11.1 (0.8)[A]	11.0 (0.8)[A]
Soil Moisture (% vol)	Summer	23.9 (2.5)[A]	29.6 (2.8)[AB]	37.1 (2.8)[B]	58.5 (1.3)[A]	54.8 (3.3)[A]	54.4 (2.1)[A]
	Fall	30.3 (2.3)[A]	38.5 (2.3)[B]	45.0 (5.1)[C]	61.5 (1.1)[A]	67.5 (1.4)[B]	64.2 (0.9)[AB]
	Spring	41.4 (1.7)[A]	50.9 (1.5)[B]	48.5 (2.3)[B]	63.0 (1.3)[A]	67.0 (1.5)[A]	66.3 (1.2)[A]
	Overall	30.6 (1.6)[A]	38.3 (1.8)[B]	42.8 (9.9)[C]	61.0 (0.8)[A]	62.0 (1.8)[A]	60.9 (1.2)[A]

[A] Means followed by a different uppercase letter are significantly different (p<0.05) within the same season and land-use comparing among closest, middle, and farthest locations.

Table 3.3. Mean annual and seasonal soil temperature (˚C) and moisture (% vol) for three parallel locations (upstream, middle, and downstream) at the rehabilitated forest (RH) and undisturbed natural forest (UNF) sites along Washington Creek in southern Ontario, Canada during 2015-2016. Standard errors are given in parentheses.

	Season	RH			UNF		
		Upstream	Middle	Downstream	Upstream	Middle	Downstream
Soil Temperature (˚C)	Summer	17.7 (0.4)^A	17.6 (0.3)^A	18.2 (0.5)^A	16.5 (0.4)^A	16.5 (0.4)^A	16.6 (0.4)^A
	Fall	8.8 (0.8)^A	8.9 (0.8)^A	8.8 (0.8)^A	8.2 (0.8)^A	8.4 (0.7)^A	7.9 (0.8)^A
	Spring	7.4 (1.3)^A	7.9 (1.5)^A	7.6 (1.3)^A	6.5 (1.2)^A	6.5 (1.1)^A	6.4 (1.2)^A
	Overall	11.5 (0.8)^A	11.5 (0.8)^A	12.0 (0.8)^A	10.9 (0.8)^A	10.9 (0.8)^A	10.8 (0.8)^A
Soil Moisture (% vol)	Summer	26.7 (3.4)^A	31.3 (2.4)^A	32.7 (2.9)^A	48.6 (2.7)^A	57.7 (1.4)^B	61.7 (1.8)^B
	Fall	36.3 (2.4)^A	37.7 (2.2)^A	38.8 (2.7)^A	56.0 (2.0)^A	64.8 (0.7)^B	67.1 (1.1)^B
	Spring	43.9 (2.1)^A	46.4 (2.3)^A	50.5 (1.7)^A	61.4 (1.2)^A	68.9 (0.6)^B	66.1 (1.4)^B
	Overall	34.6 (1.9)^A	37.4 (1.6)^A	39.4 (1.8)^A	54.5 (1.5)^A	63.1 (0.9)^B	64.8 (0.9)^B

[A] Means followed by a different uppercase letter are significantly different (p<0.05) within the same season and land-use comparing among upstream, middle, and downstream locations.

3.1.2. Nitrate and Ammonium

Soil NO_3^- concentration (mmg N kg_{soil}^{-1}) for the RH and UNF land-uses ranged from 3.02 to 87.10 mmg N kg_{soil}^{-1} and 2.33 to 106.22 mmg N kg_{soil}^{-1}, respectively. The mean annual soil NO_3^- concentration was 32.97 +/- 1.52 mg N kg_{soil}^{-1} for the RH site and 38.99 +/- 2.25 mg N kg_{soil}^{-1} for the UNF site. Based on the linear mixed model, soil NO_3^- concentration was significantly different temporally among seasons [$F(5, 93.1)=12.8$, $p<0.001$]. Within the RH land-use, soil NO_3^- concentrations did not vary significantly among seasons, but showed a decreasing trend from summer to spring (Table 3.4). Within the UNF, both the summer and fall had significantly higher soil NO_3^- concentrations than the spring (Table 3.4). When comparing between RH and UNF, soil NO_3^- concentrations were significantly higher during the summer and fall for both land-uses (Table 3.4). The summer at the UNF site also had significantly higher soil NO_3^- concentrations than the fall at the RH site (Table 3.4). There were no significant differences between land-uses for any of the seasons (Table 3.4).

Soil NH_4^+ concentration (mg N kg_{soil}^{-1}) for the RH and UNF land-uses ranged from 2.59 to 11.42 mg N kg_{soil}^{-1} and 3.29 to 62.12 mg N kg_{soil}^{-1}, respectively. The mean annual soil NH_4^+ concentration was 6.31 +/- 0.16 mg N kg_{soil}^{-1} for the RH site and 16.30 +/- 1.01 mg N kg_{soil}^{-1} for the UNF site. The soil NH_4^+ concentration (mg N kg_{soil}^{-1}) was also significantly different temporally among seasons [$F(5, 89.7)=36.2$, $p<0.001$] (Table 3.4). Within the RH land-use, soil NH_4^+ concentration did not vary significantly (Table 3.4). Within the UNF land-use, soil NH_4^+ concentration during the summer was significantly higher than the fall and spring (Table 3.4). Between land-uses, the summer at the UNF site was significantly higher than all seasons in the RH site and fall at the UNF site was significantly higher than the summer and fall in the RH site (Table 3.4).

Table 3.4. Mean seasonal soil NO$_3^-$ and NH$_4^+$ concentrations (mg N kg$_{soil}^{-1}$) for a rehabilitated forest and undisturbed natural forest site on Washington Creek, in southern Ontario, Canada during 2015-2016. Standard errors are given in parentheses.

	Season	RH	UNF
NO$_3^-$ (mg N kg$_{soil}^{-1}$)	Summer	37.0 (3.5)^{AB}	50.3 (3.5)^{B}
	Fall	31.8 (3.4)^{AC}	42.6 (3.4)^{BC}
	Spring	22.2 (4.0)^{AD}	11.9 (4.4)^{D}
NH$_4^+$ (mg N kg$_{soil}^{-1}$)	Summer	5.6 (1.1)^{A}	23.7 (1.0)^{B}
	Fall	6.3 (1.1)^{AD}	12.0 (1.2)^{C}
	Spring	7.5 (1.3)^{AC}	10.8 (1.3)^{CD}

[A] Means followed by different uppercase letters are significantly different (p<0.05) among seasons and land-uses.

The full-year spatial comparison showed that there was a significant effect of perpendicular location on soil NO$_3^-$ concentration for the RH [$F_{(2,85.0)}$=10.885, p<0.001] and UNF sites [$F_{(2,130)}$=9.439, p<0.001], with the closest location having a significantly lower concentration than the middle (RH: p=0.003; UNF: p<0.001) and farthest (RH: p<0.001; UNF: p=0.034) locations (Table 3.5). When separated into seasons, the RH site showed a significant effect of location on soil NO$_3^-$ concentration for the summer [$F_{(2,51)}$=7.636, p=0.001] and fall [$F_{(2, 28.0)}$=20.453, p<0.001], with the closest location having significantly lower concentrations than the middle (summer: p=0.001; fall: p=0.003) and farthest (summer: p=0.016; fall: p<0.001) locations (Table 3.5). However, there was no significant effect of location on soil NO$_3^-$ concentration observed during the spring [$F_{(2,32)}$=0.702, p=0.503] (Table 3.5). The seasonal effect of location on soil NO$_3^-$ concentration in the UNF site was significant for the summer [$F_{(2,51)}$=5.760, p=0.006], fall [$F_{(2,29.9)}$=10.187, p<0.001], and spring [$F_{(2,16.9)}$=5.632, p=0.012] (Table 3.5). The closest location had significantly lower soil NO$_3^-$ concentration than the middle location in all three seasons (summer: p=0.004; fall: p<0.001; winter: p=0.036) and the farthest location in the fall (p=0.002) (Table 3.5).

The full-year spatial comparison showed that there was a significant effect of perpendicular location on soil NH_4^+ concentration for the RH [$F_{(2,140)}=5.028$, $p=0.008$] and UNF sites [$F_{(2,83.7)}=7.361$, $p=0.001$], with the closest location having a significantly lower concentration than the middle ($p=0.007$) location for the RH site and farthest location (RH: $p=0.010$; UNF: $p=0.007$) for both sites (Table 3.5). When separated into seasons, the RH site showed no significant effect of location soil NH_4^+ concentration for the summer [$F_{(2,47)}=2.763$, $p=0.073$] and fall [$F_{(2, 46)}=0.708$, $p=0.422$] (Table 3.5). However, there was a significant effect of location on soil NH_4^+ concentration observed during the spring [$F_{(2,30)}=6.680$, $p=0.004$], with the farthest location having a significantly higher concentration than the middle ($p=0.006$) and closest ($p=0.018$) locations (Table 3.5). The seasonal effect of location on soil NH_4^+ concentration in the UNF site was significant for the summer [$F_{(2,29.1)}=10.024$, $p<0.001$] and spring [$F_{(2,19.1)}=6.597$, $p=0.007$]. The closest location had significantly lower soil NH_4^+ concentration than the middle location for both summer ($p=0.014$) and spring ($p=0.018$), and the farthest location in the summer ($p=0.007$) (Table 3.5).

Table 3.5. Mean annual and seasonal soil NO_3^- and NH_4^+ concentrations (mg N kg_{soil}^{-1}) for three perpendicular locations (closest, middle, and farthest) at the rehabilitated forest (RH) and undisturbed natural forest (UNF) sites along Washington Creek in southern Ontario, Canada during 2015-2016. Standard errors are given in parentheses.

	Season	RH			UNF		
		Closest	Middle	Farthest	Closest	Middle	Farthest
NO_3^- (mg N kg_{soil}^{-1})	Summer	31.2 (2.8)^A	50.2 (4.5)^B	45.8 (3.3)^B	35.8 (5.1)^A	64.3 (6.1)^B	54.0 (7.2)^{AB}
	Fall	26.5 (1.4)^A	39.5 (3.1)^B	41.2 (2.0)^B	29.3 (4.2)^A	54.0 (3.6)^B	48.0 (2.5)^B
	Spring	12.8 (3.2)^A	13.3 (4.0)^A	18.8 (4.5)^A	6.3 (1.1)^A	20.9 (5.0)^B	10.9 (2.2)^{AB}
	Overall	24.8 (1.8)^A	36.9 (3.1)^B	37.4 (2.5)^B	27.4 (3.1)^A	49.6 (3.8)^B	41.1 (4.3)^B
NH_4^+ (mg N kg_{soil}^{-1})	Summer	5.9 (1.3)^A	6.3 (1.7)^A	6.6 (1.9)^A	13.3 (1.8)^A	27.1 (3.4)^B	28.1 (3.9)^B
	Fall	4.8 (1.4)^A	5.6 (1.9)^A	6.1 (1.6)^A	14.6 (2.2)^A	13.2 (1.1)^A	15.0 (1.9)^A
	Spring	7.1 (0.3)^A	6.8 (0.3)^A	8.8 (0.6)^B	7.2 (0.7)^A	13.0 (1.7)^B	10.5 (1.2)^{AB}
	Overall	5.8 (0.2)^A	6.1 (0.3)^A	7.0 (0.3)^A	12.0 (1.1)^A	18.4 (1.7)^B	18.9 (2.0)^B

^{A} Means followed by a different uppercase letter are significantly different ($p<0.05$) within the same season and land-use comparing among closest, middle, and farthest locations.

The full-year spatial comparison showed that there was no significant effect of parallel location on soil NO_3^- concentration for the RH site [$F_{(2,138)}=0.806$, $p=0.449$]. However, there was a significant effect in the UNF site [$F_{(2,86.2)}=4.601$, $p=0.013$], with the middle location having a significantly higher concentration than the upstream ($p=0.022$) and downstream ($p=0.018$) locations (Table 3.6). When separated into seasons, the RH site showed no significant effect of location on soil NO_3^- concentration for the summer [$F_{(2,51)}=1.341$, $p=0.272$], fall [$F_{(2,47)}=0.551$, $p=0.580$], and spring [$F_{(2,31)}=0.128$, $p=0.880$) (Table 3.6). The seasonal effect of location on soil NO_3^- concentration in the UNF site was significant for the summer [$F_{(2,49)}=6.534$, $p=0.003$] and the fall [$F_{(2,51)}=3.431$, $p=0.040$], with the concentration being significantly higher at the middle than the upstream location for both seasons (summer: $p=0.009$; fall: $p=0.036$), and downstream location in the summer ($p=0.008$) (Table 3.6). Soil NO_3^- concentration also varied significantly with parallel location in the spring [$F_{(2, 14.9)}=4.362$, $p=0.032$], with the upstream location being significantly higher than the downstream location ($p=0.028$) (Table 3.6).

The full-year spatial comparison showed that there was no significant effect of parallel location on soil NH_4^+ concentration for the RH [$F_{(2,129)}=0.208$, $p=0.813$] and UNF [$F_{(2,133)}=0.368$, $p=0.693$] sites (Table 3.6). When separated into seasons, the RH site showed no significant effect of location on soil NO_3^- concentration for the summer [$F_{(2,30.7)}=0.744$, $p=0.484$], fall [$F_{(2,46)}=0.024$, $p=0.977$], or spring [$F_{(2,19.0)}=2.028$, $p=0.069$] (Table 3.6). The seasonal effect of location on soil NO_3^- concentration in the UNF site was not significant for the summer [$F_{(2,50)}=0.655$, $p=0.524$] or spring [$F_{(2,32)}=1.041$, $p=0.365$] (Table 3.6). However, the effect was significant in the fall [$F_{(2,29.4)}=6.997$, $p=0.003$] with the concentration being

significantly lower at the downstream site than the middle (p=0.040) and the upstream location (p<0.001), showing an decreasing trend running along the watercourse (Table 3.6).

3.1.3. Soil Organic Carbon and Total Nitrogen

Soil organic carbon content for the RH and UNF sites ranged from 27.97 g SOC kg_{soil}^{-1} to 68.71 g SOC kg_{soil}^{-1} and 134.29 g SOC kg_{soil}^{-1} to 240.35 g SOC kg_{soil}^{-1}, respectively. The average SOC content was 52.21 +/- 4.58 g SOC kg_{soil}^{-1} for the RH site and 184.10 +/- 16.24 g SOC kg_{soil}^{-1} for the UNF site. The UNF site had significantly higher SOC content than the RH site (t(15)=8.241, p<0.001). Total nitrogen content for the RH and UNF sites ranged from 2.63 g N kg_{soil}^{-1} to 6.52 g N kg_{soil}^{-1} and 8.96 g N kg_{soil}^{-1} to 18.07 g N kg_{soil}^{-1}, respectively. The average TN content was 4.96 +/- 0.44 g N kg_{soil}^{-1} for the RH site and 12.50 +/- 2.04 g N kg_{soil}^{-1} for the UNF site. The UNF site had significantly higher TN content than the RH site (t(11)=5.191, p<0.001).

Table 3.6. Mean annual and seasonal soil NO_3^- and NH_4^+ concentrations (mg N kg_{soil}^{-1}) for three parallel locations (upstream, middle, and downstream) at the rehabilitated forest (RH) and undisturbed natural forest (UNF) sites along Washington Creek in southern Ontario, Canada during 2015-2016. Standard errors are given in parentheses.

	Season	RH			UNF		
		Upstream	Middle	Downstream	Upstream	Middle	Downstream
NO_3^- (mg N kg_{soil}^{-1})	Summer	35.6 (3.5)[A]	46.2 (4.5)[A]	45.5 (3.8)[A]	42.0 (6.7)[A]	68.1 (6.0)[B]	41.8 (5.1)[A]
	Fall	33.9 (3.0)[A]	32.2 (2.4)[A]	36.4 (2.8)[A]	36.7 (4.5)[A]	53.9 (5.5)[B]	41.8 (4.2)[AB]
	Spring	12.3 (3.4)[A]	14.5 (3.9)[A]	15.0 (4.1)[A]	12.8 (0.7)[A]	10.4 (2.3)[AB]	8.4 (1.3)[B]
	Overall	29.9 (2.3)[A]	33.1 (2.9)[A]	34.5 (2.6)[A]	34.4 (3.6)[A]	50.0 (3.7)[B]	34.4 (3.3)[A]
NH_4^+ (mg N kg_{soil}^{-1})	Summer	5.1 (0.3)[A]	5.7 (0.4)[A]	5.6 (0.5)[A]	23.9 (3.1)[A]	20.8 (3.0)[A]	26.6 (4.5)[A]
	Fall	6.3 (0.4)[A]	6.2 (0.4)[A]	6.3 (0.5)[A]	17.0 (2.1)[A]	13.1 (1.1)[B]	9.3 (0.9)[B]
	Spring	8.3 (0.6)[A]	7.4 (0.3)[A]	6.9 (0.3)[A]	8.8 (1.1)[A]	11.3 (1.5)[A]	11.0 (1.4)[A]
	Overall	6.4 (0.3)[A]	6.3 (0.3)[A]	6.2 (0.3)[A]	17.7 (1.7)[A]	15.6 (1.5)[A]	16.8 (2.2)[A]

[A] Means followed by a different uppercase letter are significantly different (p<0.05) within the same season and land-use comparing among upstream, middle, and downstream locations.

3.2. Vegetative Characteristics

3.2.1. Stand Density, Light Intensity, and Microhabitat Characteristics

Stand density for the RH and UNF land-uses was 3.6 +/- 0.8 trees/10 m^2 and 2.4 +/- 0.3 trees/10m^2, respectively. There was no significant difference in stand density between the RH and UNF sites ($t(4)=1.376$, $p=0.241$). The light intensity ranged from 1.00% to 43.27% and 1.48% to 6.96% for the RH and UNF land-uses, respectively. The average light penetration was calculated as 14.78 +/- 4.54% for the RH site and 3.16 +/- 0.59% for the UNF site. The RH site had significantly more light penetrating the canopy than the UNF site ($t(16)=2.537$, $p=0.034$). The distance from each chamber to the nearest tree (microhabitat features) ranged from 0.88 m to 4.81 m and 0.68 m to 2.15 m for the RH and UNF sites, respectively. The average distance to the nearest tree for the RH site was 2.92 +/- 0.47 m and 1.45 +/- 0.19 m for the UNF site. The UNF site had significantly shorter distances to the nearest tree than the RH site ($t(16)=2.883$, $p=0.016$).

3.3. Soil Greenhouse Gas Emissions

3.3.1. Soil Carbon Dioxide Emissions

Soil CO_2-C emissions (mg CO_2-C m^{-2} h^{-1}) for the RH and UNF land-uses ranged from -59.15 to 138.51 mg CO_2-C m^{-2} h^{-1} and -84.19 to 142.23 mg CO_2-C m^{-2} h^{-1}, respectively. The mean annual CO_2-C emissions were 26.98 +/- 3.06 mg CO_2-C m^{-2} h^{-1} for the RH site and 17.91 +/- 3.51 mg CO_2-C m^{-2} h^{-1} for the UNF site. Based on the linear mixed model, CO_2-C emissions were significantly different temporally among seasons [$F(7, 111.7)=7.0$, $p<0.001$] (Figure 3.2;Table 3.7). Within the RH land-use, CO_2-C emissions were significantly different among seasons, with emissions being significantly greater in the summer compared to the fall, winter,

and spring (Table 3.7). Within the UNF land-use, CO_2-C emissions were significantly different among seasons, with emissions being significantly higher in the summer compared to the fall and winter (Table 3.7). Between land-uses, CO_2-C emissions were not significantly different for the same season; however, the summer at the RH site was significantly greater than the fall, winter, and spring at the UNF site (Table 3.7).

Table 3.7. Mean seasonal soil CO_2-C emissions (mg CO_2-C m^{-2} h^{-1}) for the rehabilitated forest (RH) and undisturbed natural forest (UNF) sites on Washington Creek, in southern Ontario, Canada during 2015-2016. Standard errors are given in parentheses.

Season	RH	UNF
Summer	49.1 (5.8)[A]	36.6 (5.8)[AB]
Fall	21.4 (5.9)[BC]	10.1 (5.8)[C]
Winter	13.1 (7.1)[BC]	3.2 (7.2)[C]
Spring	15.2 (7.0)[BC]	14.6 (7.3)[BC]

[A] Means followed by different uppercase letters are significantly different (p<0.05) among seasons and land-uses.
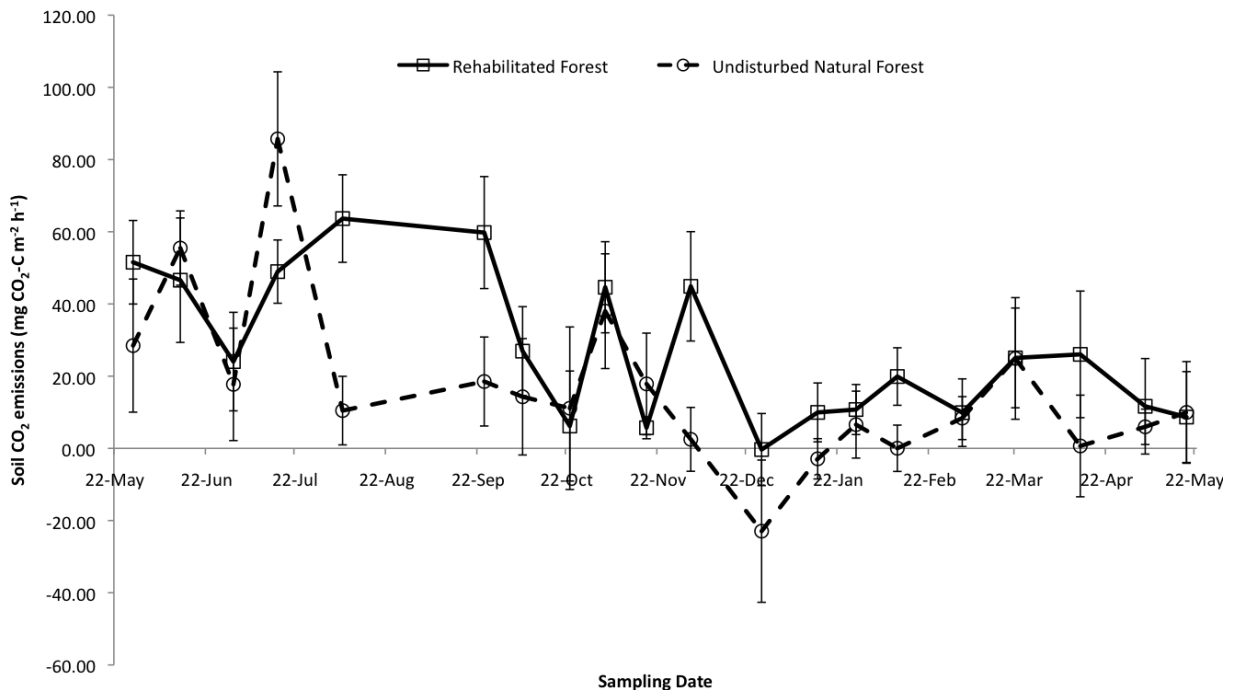
Figure 3.2. Mean soil CO_2-C emissions (mg CO_2-C m^{-2} h^{-1}) for each sampling date for the rehabilitated forest (RH) and undisturbed natural forest (UNF) sites along Washington Creek in southern Ontario, Canada for the full-year sampling period (May 28, 2015 to May 19, 2016).

The full-year spatial comparison showed that there was a significant effect of perpendicular location on CO_2-C emissions for the RH site [$F(2,171)=4.471$, $p=0.013$], although the effect was not significant for the UNF site [$F(2,177)=2.237$, $p=0.110$] (Table 3.8). In the RH site, there were significantly lower emissions at the farthest location when compared to the middle ($p=0.032$) and closest ($p=0.022$) locations, showing a decreasing trend (Table 3.8). When separated into seasons, the RH site showed no significant effect of location on CO_2-C emissions for the summer [$F(2,48)=1.721$, $p=0.190$], winter [$F(2,32)=1.250$, $p=0.300$], or spring [$F(2,19.0)=1.307$, $p=0.294$] (Table 3.8). However, there was a significant effect during the fall [$F(2,50)=4.703$, $p=0.013$], with the farthest location having significantly higher emissions than the middle ($p=0.039$) and closest ($p=0.020$) locations. The seasonal effect of location on CO_2-C emissions in the UNF site was not significant for the fall [$F(2,52)=0.727$, $p=0.488$] and spring [$F(2,33)=0.213$, $p=0.810$] (Table 3.8). The results showed a significant effect of perpendicular location on CO_2-C emissions for the UNF site in the summer [$F(2,52)=3.970$, $p=0.025$] and winter [$F(2,33)=3.593$, $p=0.039$], with the farthest location having significantly higher emissions than middle location in the summer ($p=0.022$) and the closest location in the winter ($p=0.036$) (Table 3.8).

Table 3.8. Mean annual and seasonal soil CO_2-C emissions (mg CO_2-C m^{-2} h^{-1}) for three perpendicular locations (closest, middle, and farthest) at the rehabilitated forest (RH) and undisturbed natural forest (UNF) sites along Washington Creek in southern Ontario, Canada during 2015-2016. Standard errors are given in parentheses.

Season	RH			UNF		
	Closest	Middle	Farthest	Closest	Middle	Farthest
Summer	41.5 (10.8)[A]	61.9 (8.1)[A]	43.7 (6.4)[A]	28.9 (10.6)[AB]	14.5 (12.5)[A]	60.1 (11.6)[B]
Fall	36.8 (10.8)[A]	32.8 (9.1)[A]	-4.7 (11.4)[B]	31.2 (13.9)[A]	8.1 (11.0)[A]	13.5 (17.3)[A]
Winter	26.5 (11.7)[A]	8.1 (9.2)[A]	7.9 (7.0)[A]	-11.7 (8.4)[A]	15.0 (9.9)[AB]	25.3 (11.6)[B]
Spring	31.2 (16.6)[A]	18.7 (13.0)[A]	4.7 (6.7)[A]	25.3 (13.7)[A]	14.7 (20.6)[A]	29.8 (15.6)[A]
Overall	34.9 (6.0)[A]	33.8 (5.4)[A]	13.8 (5.0)[B]	19.6 (6.5)[A]	12.7 (6.6)[A]	33.1 (7.6)[A]

[A] Means followed by a different uppercase letter are significantly different (p<0.05) within the same season and land-use comparing among closest, middle, and farthest locations.

Table 3.9. Mean annual and seasonal soil CO_2-C emissions (mg CO_2-C m^{-2} h^{-1}) for three parallel locations (upstream, middle, and downstream) at the rehabilitated forest (RH) and undisturbed natural forest (UNF) sites along Washington Creek in southern Ontario, Canada during 2015-2016. Standard errors are given in parentheses.

Season	RH			UNF		
	Upstream	Middle	Downstream	Upstream	Middle	Downstream
Summer	50.6 (7.3)[A]	43.4 (6.7)[A]	61.7 (9.6)[A]	59.7 (5.2)[A]	51.7 (14.4)[AB]	14.3 (7.8)[B]
Fall	4.9 (6.2)[A]	30.8 (9.6)[A]	26.5 (11.4)[A]	12.7 (13.4)[AB]	28.5 (10.9)[A]	-16.4 (9.6)[B]
Winter	8.5 (5.8)[A]	1.1 (2.8)[A]	11.2 (10.9)[A]	4.3 (6.1)[A]	-2.9 (8.4)[A]	8.4 (4.7)[A]
Spring	32.9 (15.0)[A]	27.9 (14.4)[A]	-3.8 (6.8)[A]	40.6 (16.2)[A]	8.0 (5.0)[A]	8.7 (6.0)[A]
Overall	25.1 (5.1)[A]	28.4 (5.0)[A]	28.2 (6.0)[A]	30.0 (6.5)[A]	27.0 (6.7)[A]	2.6 (4.3)[B]

[A] Means followed by a different uppercase letter are significantly different (p<0.05) within the same season and land-use comparing among upstream, middle, and downstream locations.

The full-year spatial comparison showed that there was no significant effect of parallel location on CO_2-C emissions for the RH site [$F_{(2,161)}=0.143$, $p=0.867$], although the effect was significant for the UNF site [$F_{(2,102.4)}=8.348$, $p<0.001$] (Table 3.9). In the UNF site, there were significantly lower emissions at the downstream location when compared to the middle ($p=0.008$) and upstream ($p=0.002$) locations, showing a decreasing trend. When separated into seasons, the RH site showed no significant effect of location on CO_2-C emissions for the summer [$F_{(2,46)}=1.341$, $p=0.272$], fall [$F_{(2,15.6)}=0.937$, $p=0.413$], winter [$F_{(2,32)}=1.250$, $p=0.300$], or spring [$F_{(2,32)}=2.554$, $p=0.094$] (Table 3.9). The seasonal effect of location on CO_2-C emissions in the UNF site was significant for the summer [$F_{(2,29.3)}=11.630$, $p<0.001$] and fall [$F_{(2,49)}=3.847$, $p=0.028$], with the downstream location having significantly lower emissions than the upstream location ($p<0.001$) in the summer, and the middle location ($p=0.023$) in the fall. The results showed no significant effect of parallel location on CO_2-C emissions for the UNF site in the winter [$F_{(2,30)}=0.807$, $p=0.456$] or spring [$F_{(2,17.5)}=1.812$, $p=0.193$] (Table 3.9).

3.3.2. Soil Nitrous Oxide Emissions

Soil N_2O-N emissions (μg N_2O-N m^{-2} h^{-1}) for the RH and UNF land-uses ranged from -20.59 to 49.48 μg N_2O-N m^{-2} h^{-1} and -23.92 to 38.14 mg μg N_2O-N m^{-2} h^{-1}, respectively. The mean annual N_2O-N emissions were 7.62 +/- 0.87 μg N_2O-N m^{-2} h^{-1} for the RH site and 5.93 +/- 0.78 μg N_2O-N m^{-2} h^{-1} for the UNF site. Based on the linear mixed model, N_2O-N emissions (μg N_2O-N m^{-2} h^{-1}) were significantly different temporally among seasons and land-uses [$F_{(7, 153.4)}=3.0$, $p=0.005$] (Figure 3.3; Table 3.10). Within the RH land-use, N_2O-N emissions did not vary significantly among seasons; however in the UNF site, emissions were significantly higher in the summer than in the winter and spring (Figure 3.3; Table 3.10). Between land-uses, N_2O-N

emissions were not significantly different for the same season; however, the summer at the RH

site was significantly greater than the winter and spring at the UNF site (Table 3.10).

Table 3.10. Mean seasonal soil N$_2$O-N emissions (µg N$_2$O-N m^{-2} h^{-1}) for the rehabilitated forest (RH) and undisturbed natural forest (UNF) sites along Washington Creek in southern Ontario, Canada during 2015-2016. Standard errors are given in parentheses.

Season	RH	UNF
Summer	11.3 (1.5)[A]	9.3 (1.6)[A]
Fall	5.2 (1.5)[AB]	6.2 (1.5)[AB]
Winter	7.3 (1.8)[AB]	3.6 (1.8)[B]
Spring	5.6 (2.0)[AB]	3.4 (1.8)[B]

[A] Means followed by different uppercase letters are significantly different ($p<0.05$) among seasons and land-uses.
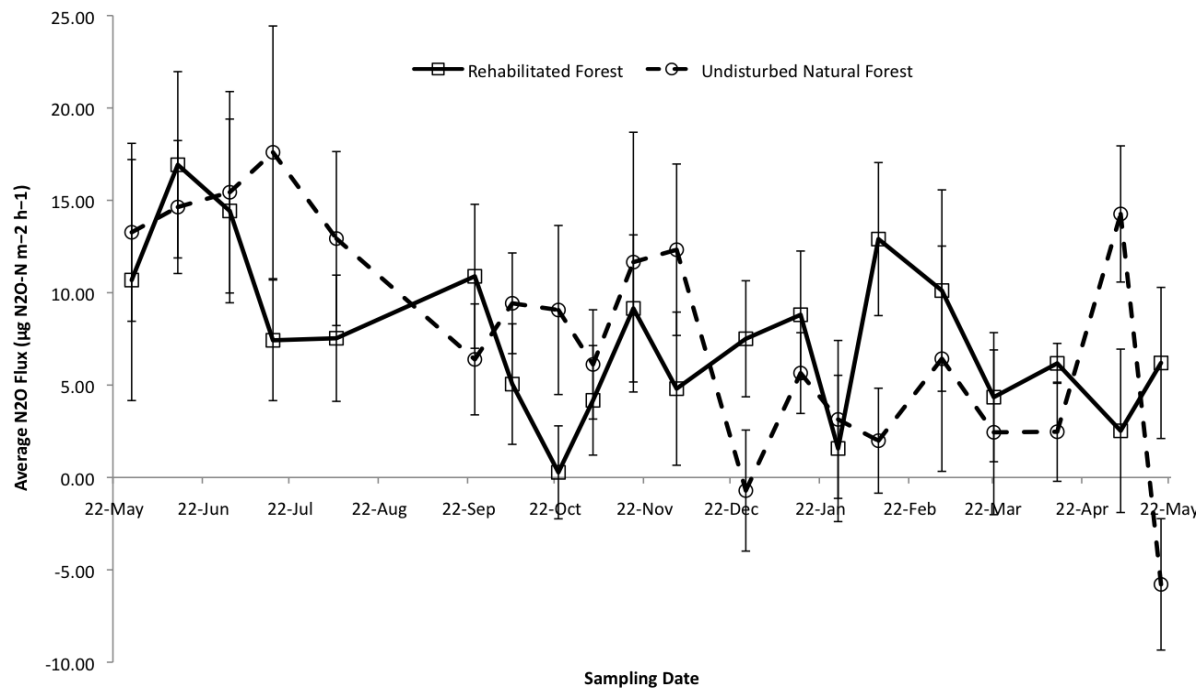
Figure 3.3. Mean soil N$_2$O-N emissions (µg N$_2$O-N m^{-2} h^{-1}) for each sampling date at the rehabilitated forest (RH) and undisturbed natural forest (UNF) site along Washington Creek in southern Ontario, Canada for the full-year sampling period (May 28, 2015 to May 19, 2016).

The full-year spatial comparison showed that there was no significant effect of perpendicular location on N_2O-N emissions for the RH [$F_{(2,98.5)}=1.330$, $p=0.269$] and UNF [$F_{(2,163)}=0.329$, $p=0.720$] sites (Table 3.11). When separated into seasons, the RH site showed no significant effect of location on N_2O-N emissions for the summer [$F_{(2,25.9)}=0.567$, $p=0.574$], fall [$F_{(2,27.6)}=1.082$, $p=0.353$], winter [$F_{(2,19.5)}=0.977$, $p=0.394$], or spring [$F_{(2,18.2)}=1.221$, $p=0.318$] (Table 3.11). The seasonal effect of location on N_2O-N emissions in the UNF site was significant in the summer [$F_{(2,26.1)}=11.300$, $p<0.001$], with the closest location having significantly lower emissions than the middle ($p=0.001$) and farthest locations ($p=0.014$) (Table 3.11). However, there was no significant effect in the fall [$F_{(2,47)}=2.646$, $p=0.081$], winter [$F_{(2,31)}=0.206$, $p=0.815$], or spring [$F_{(2,33)}=1.841$, $p=0.269$] (Table 3.11).

Table 3.11. Mean annual and seasonal soil N_2O-N emissions (μg N_2O-N m^{-2} h^{-1}) for three perpendicular locations (closest, middle, and farthest) at the rehabilitated forest (RH) and undisturbed natural forest (UNF) sites along Washington Creek in southern Ontario, Canada during 2015-2016. Standard errors are given in parentheses.

Season	RH			UNF		
	Closest	Middle	Farthest	Closest	Middle	Farthest
Summer	10.6 (3.6)[A]	11.6 (3.6)[A]	8.2 (1.0)[A]	6.8 (1.7)[A]	12.7 (0.9)[B]	16.4 (3.2)[B]
Fall	2.2 (1.0)[A]	6.1 (3.0)[A]	4.5 (2.2)[A]	11.0 (2.2)[A]	6.4 (2.4)[A]	3.8 (2.0)[A]
Winter	11.4 (5.2)[A]	7.3 (3.1)[A]	4.2 (2.1)[A]	3.2 (1.2)[A]	4.7 (2.6)[A]	2.3 (3.5)[A]
Spring	4.3 (1.3)[A]	9.4 (3.4)[A]	6.2 (1.1)[A]	6.3 (3.7)[A]	6.0 (3.2)[A]	-2.3 (2.6)[A]
Overall	7.6 (1.8)[A]	8.6 (1.7)[A]	5.7 (0.9)[A]	6.7 (1.3)[A]	7.8 (1.2)[A]	6.2 (1.7)[A]

[A] Means followed by a different uppercase letter are significantly different ($p<0.05$) within the same season and land-use comparing among closest, middle, and farthest locations.

The full-year spatial comparison showed that there was no significant effect of parallel location on N_2O-N emissions for the RH [$F_{(2,161)}=0.923$, $p=0.399$] and UNF [$F_{(2,160)}=2.465$, $p=0.088$] sites (Table 3.12). The UNF site did show a trend of decreasing emissions when comparing upstream to downstream locations; however, this was not significant ($p=0.080$).

When separated into seasons, the RH site showed no significant effect of location on N_2O-N emissions for the summer [$F(2,50)=1.334$, $p=0.273$], winter [$F(2,30)=0.001$, $p=0.999$], or spring [$F(2,16.7)=0.923$, $p=0.296$] (Table 3.12). However, there was a significant effect of parallel location on N_2O-N emissions during the fall [$F(2,49)=6.437$, $p=0.003$). In the fall, emissions were significantly higher downstream than at the middle ($p=0.040$) and upstream ($p=0.003$) locations, showing a decreasing trend from upstream to downstream. The seasonal effect of location on N_2O-N emissions in the UNF site was not significant for the summer [$F(2,42)=0.612$, $p=151$], fall [$F(2,49)=2.646$, $p=0.081$], winter [$F(2,15.9)=0.730$, $p=0.498$], or spring [$F(2,32)=1.618$, $p=0.214$] (Table 3.12). All seasons, except for winter, showed a decreasing trend from upstream to downstream; however, this was not significant.

Table 3.12. Mean annual and seasonal soil N_2O-N emissions (µg N_2O-N m^{-2} h^{-1}) for three parallel locations (closest, middle, and furthest) at the rehabilitated forest (RH) and undisturbed natural forest (UNF) sites along Washington Creek in southern Ontario, Canada during 2015-2016. Standard errors are given in parentheses.

Season	RH			UNF		
	Upstream	Middle	Downstream	Upstream	Middle	Downstream
Summer	12.6 (2.7)[A]	13.0 (3.2)[A]	7.0 (2.5)[A]	10.5 (1.4)[A]	10.1 (2.3)[A]	7.8 (1.8)[A]
Fall	1.3 (2.1)[A]	4.7 (2.1)[A]	12.6 (2.6)[B]	8.3 (2.1)[A]	8.1 (2.7)[A]	2.5 (2.2)[A]
Winter	5.5 (2.2)[A]	5.5 (4.7)[A]	5.3 (2.5)[A]	4.2 (2.9)[A]	1.4 (0.7)[A]	3.5 (2.4)[A]
Spring	6.5 (2.1)[A]	4.4 (0.9)[A]	9.4 (3.3)[A]	6.7 (2.7)[A]	1.6 (3.9)[A]	-1.6 (3.1)[A]
Overall	6.8 (1.3)[A]	7.4 (1.5)[A]	8.9 (1.4)[A]	7.5 (1.2)[A]	6.2 (1.5)[A]	3.6 (1.2)[A]

[A] Means followed by a different uppercase letter are significantly different ($p<0.05$) within the same season and land-use comparing among upstream, middle, and downstream locations.

3.4. Greenhouse Gas, Soil, and Vegetative Characteristics Correlation Analysis

3.4.1. Seasonal and Land-Use Based Correlations to Greenhouse Gas Emissions

When considering seasonal and geographical variation, greenhouse gas emissions (N_2O-N, CO_2-C) were not significantly correlated to soil temperature, moisture, NO_3^- concentration, or NH_4^+ concentration for either land use (RH, UNF) during the summer (Table 3.13). During the fall, there were no significant relationships between the soil characteristics and CO_2-C emissions for either land-use or N_2O-N emissions for the UNF land-use. Within the RH site during the fall, N_2O-N emissions had a significant positive correlation to soil moisture (r=0.277, n=54, p=0.043) and significant negative relationships to soil temperature (r=-0.385, n=54, p=0.004) and NO_3^- concentration (r=-0.385, n=52, p=0.005) (Table 3.13). Although there were no significant correlations to greenhouse gas emissions during the spring, soil temperature and moisture were closely linked to the CO_2-C emissions (r=-0.327, n=34, p=0.059) in the RH site and N_2O-N emissions in the UNF site (r=-0.323, n=36, p=0.055), respectively (Table 3.13).

When determining correlation based solely on land-use, the RH site showed no significant correlations between N_2O-N emissions and soil temperature, moisture, NO_3^- concentration, NH_4^+, SOC content, TN content, light intensity, or distance to the nearest tree (Table 3.14). However, CO_2-C emissions were found to have a significant positive correlation to soil temperature (r=0.242, n=131, p=0.005) and soil NO_3^- concentration (r=0.178, n=134, p=0.040), and a negative significant relationship to soil moisture (r=-0.239, n=137, p=0.005) (Table 3.14). For the UNF land-use, soil temperature was found to have a significant correlation to both N_2O-N (r=0.218, n=135, p=0.011) and CO_2-C emissions (r=0.234, n=138, p=0.006) (Table 3.14). Soil CO_2-C emissions for the UNF site were also significantly correlated to SOC (r=0.739, n=8, p=0.036) and TN (r=0.972, n=4, p=0.028). No other variables were significantly

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correlated to greenhouse gas emissions in the UNF site. When broken down by season for the

whole system, greenhouse gas emissions were not significantly correlated to soil temperature,

moisture, NO_3^- concentration, or NH_4^+ concentration for the summer, fall, or spring seasons.

Table 3.13. Pearson product-moment correlation R-values for seasonal soil characteristics and greenhouse gas emissions for the rehabilitated forest (RH) and undisturbed natural forest (UNF) sites along Washington Creek in southern Ontario, Canada during 2015-2016.

Season	Summer				Fall				Spring			
Land-Use	RH		UNF		RH		UNF		RH		UNF	
	N_2O-N	CO_2-C	N_2O-N	CO_2-C	N_2O-N	CO_2-C	N_2O-N	CO_2-C	N_2O-N	CO_2-C	N_2O-N	CO_2-C
Soil Temperature	0.017	-0.051	0.166	0.073	-0.385**	0.094	0.253	0.183	0.074	-0.327	-0.090	-0.131
Soil Moisture	0.038	-0.232	0.261	0.200	0.277*	-0.006	-0.161	-0.169	-0.236	0.137	-0.323	-0.263
NO_3^-	0.037	-0.119	0.177	0.008	-0.385**	0.132	0.085	-0.255	0.139	0.075	-0.008	0.052
NH_4^+	0.111	0.040	-0.028	-0.096	0.068	-0.100	0.079	0.013	0.222	-0.032	-0.165	-0.258

*R-values followed by a * or ** are significant at $p<0.05$ and $p<0.01$, respectively.

Table 3.14. Pearson product-moment correlation R-values for annual soil and vegetative characteristics and greenhouse gas emissions for the rehabilitated forest (RH) and undisturbed natural forest (UNF) sites along Washington Creek in southern Ontario, Canada during 2015-2016.

Land-Use	RH		UNF	
	N_2O-N	CO_2-C	N_2O-N	CO_2-C
Soil Temperature	0.130	0.242**	0.218*	0.234**
Soil Moisture	-0.018	-0.239**	-0.141	-0.114
NO_3^-	0.031	0.178*	0.164	0.052
NH_4^+	0.008	-0.138	0.061	0.096
SOC	0.087	-0.289	0.142	0.739*
TN	0.060	-0.276	0.106	0.972*
Light Intensity	0.415	0.201	-0.105	0.198
Distance to Nearest Tree	-0.097	-0.483	0.012	0.228

*R-values followed by a * or ** are significant at $p<0.05$ and $p<0.01$, respectively.

Table 3.15. Pearson product-moment correlation R-values for seasonal soil characteristics and greenhouse gas emissions for the rehabilitated forest (RH) and undisturbed natural forest (UNF) sites along Washington Creek in southern Ontario, Canada during 2015-2016.

Season	Summer		Fall		Spring	
	N_2O-N	CO_2-C	N_2O-N	CO_2-C	N_2O-N	CO_2-C
Soil Temperature	0.099	0.082	-0.087	0.153	-0.105	-0.129
Soil Moisture	-0.007	-0.128	0.105	-0.142	-0.014	-0.046
NO_3^-	0.006	0.048	-0.063	-0.168	-0.062	0.122
NH_4^+	-0.076	-0.091	0.086	-0.108	-0.095	-0.186

3.4.2. Overall Annual Correlations

All seasons and land-uses were combined and a Pearson's product-moment correlation was conducted to show the overall annual correlations between greenhouse gas emissions, soil, and vegetative characteristics (Figure 3.16.). N_2O-N emissions showed a positive significant relationship to CO_2-C emissions (r=0.182, n=320, p=0.001) and soil temperature (r=0.175, n=264, p=0.004). CO_2-C emissions also showed a positive and negative significant relationship with soil temperature (r=0.241, n=269, p<0.001) and moisture (r=-0.188, n=264, p=0.002), respectively. Along with CO_2-C emissions, soil moisture was found to have a significant negative correlation to soil temperature (r=-0.374, n=271, p<0.001) and distance to the nearest tree (r=-0.470, n=18, p=0.049), as well as a significant positive correlation to soil NH_4^+ concentration (r=0.364, n=257, p<0.001), SOC content (r=0.842, n=17, p<0.002), and TN content (r=0.837, n=13, p<0.001). Soil temperature had a significant positive relationship to soil NO_3^- (r=0.346, n=271, p<0.001), NH_4^+ (r=0.207, n=261, p=0.001) concentrations, as well as the distance to the nearest tree (r=0.658, n=18, p=0.003). However, soil temperature was negatively correlated with SOC (r=-0.729, n=17, p=0.001) and TN (r=-0.708, n=13, p=0.007). Soil NO_3^- and NH_4^+ concentrations were significantly correlated to each other (r=0.163, n=257, p=0.009). Soil NO_3^- and NH_4^+ concentrations were also significantly correlated to SOC (NO_3^-: r=0.826, n=17, p<0.001, NH_4^+: r=0.627, n=16, p=0.009) and TN content (NO_3^-: r=0.817, n=13, p=0.001, NH_4^+: r=0.823, n=12, p=0.001).

Table 3.16. Pearson product-moment correlation matrix containing R-values for greenhouse gas emissions, soil characteristics, and vegetative characteristics for the rehabilitated forest (RH) and undisturbed natural forest (UNF) sites along Washington Creek in southern Ontario, Canada, for the full sampling period (May 28, 2015-May 19, 2016).

	N_2O-N	CO_2-C	Soil Moisture	Soil Temper-ature	NO_3^-	NH_4^+	SOC	TN	Light Intensity (%)	Distance to Nearest Tree
N_2O-N	1.000									
CO_2-C	0.182*	1.00								
Soil Moisture	-0.092	-0.188**	1.00							
Soil Temperature	0.206**	0.241**	-0.374**	1.00						
NO_3^-	0.100	0.083	0.018	0.346**	1.00					
NH_4^+	0.012	-0.003	0.364**	0.207**	0.163**	1.00				
SOC	-0.014	-0.082	0.842**	-0.729**	0.826**	0.627**	1.00			
TN	-0.083	-0.220	0.837**	-0.708**	0.817**	0.823**	0.975**	1.00		
Light Intensity	0.370	-0.135	-0.315	0.180	-0.028	-0.357	N/A	N/A	1.00	
Distance to Nearest Tree	0.047	-0.323	-0.470*	0.658**	-0.153	-0.431	N/A	N/A	0.180	1.00

*R-values followed by a * or ** are significant at p<0.05 and p<0.01, respectively.

4. Discussion

4.1. Soil Physical and Chemical Characteristics

4.1.1. Soil Temperature and Moisture

Over the entire sampling period, soil temperature was consistently higher in the RH site than the UNF site but not significantly, which is consistent with the results found by Oelbermann et al. (2015), who studied the same sites in 2010. This pattern was also observed on the forest floor of a newly established (25 year old) and mature (125 year old) forest in boreal riparian plant communities (Schwendenmann, 2000). Paul et al. (2004) found that canopy cover, as well as the understory, can often explain variation in soil temperature. Taller canopies are usually associated with cooler understory microclimates, providing supporting evidence for lower temperatures in the UNF site (Rambo & North, 2009). Furthermore, decreased light penetrating the canopy in the UNF site may have also maintained a cooler microclimate in the UNF site. However, the dense natural regeneration that occurs during succession in rehabilitated forests could provide sufficient ground cover to maintain similar soil temperatures to a natural forest (Ritter et al., 2005). Alternatively, fast-growing tree species are often used in restoration projects to rapidly create a relatively high canopy, although these species are usually short-lived and are unlikely to reach the heights of natural forest canopies (Chazdon, 2008). This may explain the lack of a significant difference between the two sites, as after over 30 years the trees in the RH site have created a relatively high canopy, but have not replicated the effectiveness of the UNF site's canopy. Soil organic matter can also drive soil temperature, where an increased organic layer maintains cool soil temperatures (Lal, 2004). Organic matter accumulates more quickly in waterlogged soils, which were visually observed in the field at the UNF site in this study, thus reducing soil temperature (Rayment & Jarvis, 2000). This supports a lower soil temperature in

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the UNF site as it has been shown to have higher soil organic matter and increased litter input than the RH site which is supported in the study conducted by Oelbermann et al. (2015).

Temporally, soil temperature was higher in the summer for both sites than in the fall and spring, although there were no differences between land-uses for any given season. These results showed that, regardless of season, temperature did not vary significantly between land-uses. Soil temperature is also highly affected by leaf area index (Kang et al., 2000), meaning that the difference in canopy was likely not substantial enough to cause a significant difference in soil temperatures between the land-uses. The summer had the largest difference between the land-uses in average soil temperature of 1.9˚C; this could be because leaf area index is highest in the summer, which may cause a larger disparity between the amounts of sunlight reaching the forest floor between land-uses (Comeau, 2000). The summer had the highest air temperatures, which is known to have a strong positive relationship with soil temperature (Kang et al., 2000). Rey et al. (2002) also found that soil temperature was the highest in the summer in an oak coppice forest in central Italy. Although soil temperature was not significantly different between the fall and spring seasons, temperatures were slightly higher in the fall. Soil temperatures were also found to be similar in the fall and spring by Davidson et al. (1998) in a temperate mixed hardwood forest. Lower temperatures in the spring could be seen because of low-temperature runoff from melted snow.

Over the entire sampling period, soil temperature was not affected by perpendicular or parallel location for either land-use. Seasonally, there were no differences between location and soil temperature for any season in either land-use, with the exception of perpendicular location for the summer in the RH site. During this season, there was a decrease in soil temperature with increasing distance from the stream. This was likely not caused by decreased canopy cover

closer to the watercourse as the average percentage of light reaching the forest floor were measured as 7.6%, 19.3%, and 16.4% from closest to furthest, respectively. It was noted, however, that there was substantially more vegetation in the understory and along the forest floor in the farthest and middle locations in the RH site. Plant cover is known to have a large effect on soil temperature (Raich & Tufekcioglu, 2000). Ritter et al. (2005) suggested that soil temperature varies based on its relationship to forest floor vegetation because of shade, evaporative cooling, and insulation. This trend might also be explained by the inverse relationship with soil moisture, which followed the opposite trend of temperature for the RH site, being the highest at the farthest location. Redding et al. (2003) found that this inverse relationship is the most influential in determining soil temperature on forest floors.

Throughout the sampling period, the UNF site showed consistently higher soil moisture than the RH site, averaging 61% and 37%, respectively. This trend is consistent with the findings of Oelbermann et al. (2015) in 2010 for the same sites. These results are comparable to a study in Louisiana that found higher soil moisture in natural wetlands than in restored wetlands that were previously cleared for intensive agriculture likely due to disturbance of the natural hydrology (Hunter & Faulkner, 2001). Shwendenmann (2000) also found higher soil moisture in a mature boreal riparian forest when compared to the newly established forest. Soil organic matter – specifically SOC – is strongly correlated to soil moisture because of its high water-holding capacity (Hudson, 1994; McGlynn et al., 1999; Schwendenmann, 2000; Hunter & Faulkner, 2001). Thus, increased soil moisture at the UNF site is expected as it has a higher SOC content (Oelbermann et al., 2015).

Within the RH site, soil moisture was significantly lower during the summer season when compared to the fall or spring. However, there were no differences in soil moisture among

seasons in the UNF site. Both land-uses did show an increasing trend of soil moisture from summer to spring. This trend was also observed in forest, meadow and wetland riparian systems (Hunter & Faulkner, 2001; Clement et al., 2002), and can likely be explained by the inverse relationship between soil temperature and moisture observed in temperate climates (Redding et al., 2003). Hunter & Faulkner (2001) suggest the decrease in soil moisture during the later months of the summer is caused by lack of precipitation in natural and reconstructed wetlands. McGlynn et al. (1999) found increased soil moisture from post-melt runoff in riparian ecosystems along a headwater catchment in Vermont. This could explain increased soil moisture in the spring after the snowmelt. Illston et al. (2004) measured soil moisture over six years at 115 different meteorological stations in Oklahoma, and found soil moisture follows a sinusoidal pattern. Their results were consistent with those found in the present study, where the major drop in soil moisture occurred during the summer months, and was consistently high in the spring and fall months. The moisture plateau can be attributed to reduced evaporation, sunlight, and functional vegetation; however, moisture begins to drop because of increased evapotranspiration and a lack of precipitation (Illston et al., 2004).

Soil moisture seemed to have a positive relationship with distance in the RH site. The RH site had significantly lower soil moisture at the closest location than the middle and farthest locations over the whole sampling period. This trend held true during the spring, although the middle was not significantly different than the closest location during the summer. In the fall, increasing soil moisture values from closest to farthest were observed; this season also showed the most variation between the two locations. These results show opposite results than that of a study on a 50 year old riparian forest along a small stream in Georgia, where soil moisture increased significantly in areas closer to the watercourse (Ettema et al., 1999). Clement et al.

(2002) also found that soil moisture was highest at the closest location to the stream when compared to a similar middle and farthest location in a riparian wetland. Ma et al. (2014) also suggest that spatial variation in soil moisture within temperate forests is driven by vegetation and canopy characteristics rather than soil temperature. This can explain the trend seen in the RH site; although soil temperature did not vary in the same way, canopy cover was higher at the closest location than the middle or farthest. Another explanation, suggested by Hefting et al. (2003), is that elevation causes a shift towards higher soil moisture closer to streams. In the UNF site, there was no clear relationship between perpendicular location and soil moisture, with the exception of the fall season, where the middle location had significantly higher soil moisture than the closest location. Hunter & Faulkner (2001) found similar results, concluding that proximity to a watercourse did not affect soil moisture in natural and 20-25 year old reconstructed wetlands. These results seem to indicate that in areas of high soil moisture (such as the UNF site, or wetlands more broadly), spatial variation of soil moisture may not be related to distance from a watercourse.

In terms of parallel locations, soil moisture tended to increase towards the downstream location for all seasons and year-round for both sites. However, this relationship was not significant in the RH site and only the upstream location was significantly different than the middle and downstream locations in the UNF site. Clement et al. (2002) also found that soil moisture increased downstream in a riparian wetland regardless of vegetative differences. This can be expected as soil moisture is often negatively correlated to elevation (Hefting et al., 2003; Harms & Grimm, 2008), and streams flow from higher elevations to lower elevations (i.e., upstream to downstream) (National Resource Council, 2002). However, hydrology does not

always follow a linear trajectory (Penna et al., 2011), and confounding factors such as vegetation or soil texture may play a role in the lack of significance in the RH site.

4.1.2. Nitrate and Ammonium

Soil NO_3^- concentration was significantly greater in the UNF land-use when compared to the RH land-use. Based on the literature, the opposite relationship between land-use and soil NO_3^- concentration would be expected. Gift et al. (2010) found that restored riparian zones had higher NO_3^- concentrations than naturally forested riparian forests. However, these restored riparian zones had only been established for ~10 years and took place in predominantly suburban watersheds (Gift et al., 2010). In the present study, the RH site was ~30 years old and in an agriculturally dominated watershed where adjacent land-use with chronic input of nitrogen-based fertilizers. Soosaar et al. (2011) found that the buffering capacity of restored riparian zones diminished over time, which could bridge the gap between the UNF and RH land-uses. It has been suggested that riparian land-uses with lower water tables, are less effective at NO_3^- filtration (Gold et al., 2001; Groffman et al., 2002; Gift et al., 2010). There is likely a lower water table at the RH site as observed via visual inspection in the field and through decreased soil moisture readings (McMillan & Srinivasan, 2015). However, this is often related to availability of NO_3^- and soil organic matter, leading to increased denitrification (Groffman et al., 1992; Groffman et al., 2002; Gift et al., 2010). Groffman et al. (1992) found higher soil NO_3^- in areas with a higher water table, such as the UNF site, likely because of the interaction of the groundwater (carrying NO_3^-) and the top layer of soil from which samples from this study were taken (Groffman et al., 2002). Furthermore, Groffman et al. (2002) found that a downstream riparian forest had lower soil NO_3^- than an upstream counterpart within the same suburban

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watershed in Baltimore, supporting the increased soil NO_3^- concentration in the UNF (upstream) site.

Riparian zones with greater width have often been associated with greater NO_3^- filtration, although Mayer et al. (2007) found no statistical difference between riparian zones that were wider than 50 m (i.e., UNF) and those that were 26-50 m (i.e., RH). Vegetation, as the primary nitrogen sinks in riparian systems (Groffman et al., 1992), can have a major influence on soil NO_3^- concentration (Compton et al., 2003). It has been suggested that the inclusion and greater proportions of N-fixing species (such as alder species found in the RH land-use) can reduce soil NO_3^- through increased uptake (Compton et al., 2003; Hefting et al., 2003). However, studies have also showed that vegetation type – even alder species – does not always increase nitrogen uptake from the soil in riparian zones because of confounding factors such as adjacent land-use (Hefting et al., 2005; Mayer et al., 2007). Alternatively, the RH site may be exhibiting increased uptake of nitrogen because of increased growth rates and nitrogen uptake when compared to the UNF site. At the same sites along Washington Creek, it has been found that the RH site showed increased nitrogen uptake (Oelbermann & Gordon, 2000; Oelbermann et al., 2015), which might explain reduced soil nitrogen concentrations. Another explanation for increased NO_3^- in the UNF site is the conversion of previously unmaintained "grassland" to new crop fields adjacent to the riparian zone, which likely included increased fertilizer application and substantial tillage. These practices would likely cause increased nitrogen runoff affecting the UNF site (Cey et al., 1999).

Soil NO_3^- showed a decreasing trend from summer to spring, although within land-uses the only significant difference was in the UNF site, which showed increased concentration during the summer and fall compared to the spring. Similar to the results found at the RH site, Vidon & Hill (2004) found that there was no significant relationship between seasonality and

NO_3^- concentrations or filtration in seven riparian zones in southern Ontario. The lack of seasonality in soil NO_3^- and removal efficiency has also been seen in riparian zones throughout Europe (Pinay et al., 1993; Sabater et al., 2003), and specifically in alder-dominated riparian forests (Hefting et al., 2003). Although nitrogen removal occurs year-round, there is evidence for increased plant uptake and denitrification of soil NO_3^- during the summer and fall seasons (Pinay et al., 1993; Pinay et al., 1995). However, Pinay et al. (1993) suggest increases in nitrogen will occur during the summer months due to fertilizer application in the spring, elevating nitrogen levels. The seasonal trend of increased NO_3^- concentrations in the summer and fall when compared to the spring in the UNF site has been observed in multiple other studies conducted on riparian ecosystems (Pinay et al., 1995; Konohira et al., 2001; Bernal et al., 2003; Young & Briggs, 2005).

NO_3^- filtration by riparian buffer strips has been well documented in the literature (Osborne & Kovacic, 1993; Mayer et al., 2007). In the RH site, there was a clear trend of increased NO_3^- concentrations at the two locations farthest from the stream for all seasons and in the full-year comparison, with the exception of spring where the trend was present but not significant. This trend was consistent during the fall and when considering the full-year comparison in the UNF site as well. However, during the summer and spring, the middle location was the only location with significantly higher soil NO_3^- concentration. Consistent with the RH site, Hefting et al. (2003) found that a forested (alder) riparian zone in the Netherlands found a consistent decrease in NO_3^- concentration from farthest to closest locations for all seasons. Their study also found a greater drop in soil NO_3^- concentrations between their middle and closest locations than the middle and farthest (Hefting et al., 2003), which is consistent with both land-uses in the present study. The reduction of soil NO_3^- concentration closer to the stream as seen in

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this study has been common in riparian research (Ambus, 1998; Lowrance et al., 2000; Walker et al., 2002). The unexpected lack of significance between the farthest and closest locations in the UNF site might be based on microtopography (i.e., small-scale elevation changes affecting hydrology) within the land-use. It has been suggested that restoration practitioners do not usually incorporate terrestrial microtopographic features such as mounds and pools (Simmons et al., 2011). Simmons et al. (2011) found that soil NO_3^- concentrations were higher in pools when compared to mounds in the same riparian forests in Texas. This microtopography occurs naturally in ecosystems through tree fall, sediment build-up or erosion, and fauna activity (Ehrenfeld, 1995), and would likely be present in the UNF site but not necessarily be fully restored in the RH site. This potential difference in microtopography might explain the clear trend in the RH site and provide evidence for potential pools around the middle location in the UNF site, which led to increasing soil NO_3^- concentrations.

Based on the natural hydrology of riparian systems, it might be expected that the same filtration might occur as water flows parallel to the stream. Alternatively, the movement of groundwater carrying NO_3^- runoff might cause an accumulation of NO_3^- at downstream locations. Within the RH land-use, a slight increase in NO_3^- downstream was observed, although this relationship was not significant for any season or for the full-year comparison. Vellidis et al. (2003) found a decrease in NO_3^- in downstream locations in a restored riparian wetland in Georgia; however, they found that nitrogen retention and transformation was highest during the first eight years of the restoration project. The effectiveness of the NO_3^- removal in the RH site may have diminished (Parkyn, 2004) as it is much older than the restored wetland presented by Vellidis et al. (2003) and is likely closer to reaching a stable forest condition. The UNF land-use showed a clear trend of increased NO_3^- in the middle location when compared to the upstream

and downstream locations during summer, fall, and full-year comparison. However, the spring saw a significant decrease between the upstream to downstream locations, which is consistent with the findings of Vellidis et al. (2003). Mulholland (1992) suggests that immobilization of inorganic forms of nitrogen (such as NO_3^-) will cause a decrease in the NO_3^- concentration downstream. It has been suggested in that the immobilization rates are highest in the winter and can extend into the spring (Hefting et al., 2005), which could explain the decrease in soil NO_3^- concentration from upstream to downstream in the UNF site during the spring.

Soil NH_4^+ concentration was considerably lower than NO_3^- concentration along Washington Creek, which is common in riparian zones (Ambus, 1998; Hefting et al., 2003). However, similar to NO_3^- concentration, soil NH_4^+ showed a significant increase, as well as increased variability, in the UNF land-use compared to the RH land-use. Hefting et al. (2004) found that European riparian wetlands with higher water tables accumulated greater concentrations of NH_4^+ in the topsoil, probably due to decreased nitrification because of anaerobic conditions and the main product of nitrogen mineralization (conversion of organic nitrogen to a form available for plant uptake) was NH_4^+ in waterlogged soils. Therefore, those results support an increase in soil NH_4^+ concentration in the UNF site as it has a higher water table and is more often waterlogged than the RH site. Ambus (1998) observed a similar trend where soil NH_4^+ concentrations were higher and more variable in a temperate spruce forest compared to a beech forest. In that study, the spruce forest aligned closer to the UNF site with increased soil organic matter and moisture than the beech forest (Ambus, 1998).

Temporally, soil NH_4^+ concentrations showed no clear trend in the RH land-use; however, was highest during the summer in the UNF site when compared to fall and spring concentrations. Pinay et al. (1993) suggest that NH_4^+ concentrations will be elevated after spring

fertilization and found that months that fell within our summer season had consistently high

concentrations and dropped off starting at the beginning of fall until around mid-spring. This

trend was consistent with results from the UNF site and may be more evident because of

increased fertilizer application in the new adjacent crop fields. Soil NH_4^+ concentrations followed

a similar trend in other temperate forest and riparian research (Pinay et al., 1995; Ambus, 1998;

Bernal et al., 2003). Another possible explanation for elevated NH_4^+ concentrations is increased

nitrogen mineralization in the summer months increasing available NH_4^+ (Bernal et al., 2003).

However, this seasonal trend is not always clear. For example, Garten et al. (1994) found no

clear seasonal trend – with the exception of increased soil NH_4^+ in the winter – in forests within a

Tennessee watershed. Even though mineralization was found to be highest in the summer, plant

uptake was likely great enough to prevent a spike in soil NH_4^+ (Garten et al., 1994). It has been

shown that nutrient uptake diminishes with increasing age of forest stands and is highest during

rapid growth (Vellidis et al., 2003; Dosskey et al., 2007; Dosskey et al., 2010), which supports

increased plant uptake offsetting mineralization in the RH site but not the UNF.

Soil NH_4^+ concentration showed a decreasing trend towards the stream in the RH site,

although this relationship was only significant during the spring and when the full year was

considered. The seasonal significance in the spring may be attributed to greater runoff from the

snowmelt, as well as reduced plant uptake. The UNF site showed a similar trend, with soil NH_4^+

concentration being lowest closest to the stream; however, it was significant for all time-periods

with the exception of the fall. Hedin et al. (1998) noted a similar trend in NH_4^+ concentration in

riparian wetlands in southwestern Michigan; however, their study was on a much smaller scale

(3 m) in terms of distance from the stream. Although the spatial trends of removal and retention

are present in both the RH and UNF sites, the magnitude of this filtration is lower than that of the

soil NO_3^-. This decrease in spatial variability of NH_4^+ can be explained by lower mobility, retention at cation exchange sites, and lower plant removal efficiency than NO_3^- (Ettema et al., 1998; Gallardo, 2003; Vellidis et al., 2003). Furthermore, NH_4^+ concentrations in riparian forests have been shown to have a positive correlation to small changes in elevation by Harms & Grimm (2008), which supports increases in soil NH_4^+ concentration with increased distance from the stream in the UNF and RH sites. Consistent with the RH site's lack of significance, Knoepp & Clinton (2009) found no significant differences in soil NH_4^+ concentration at varying distances from the stream during the summer in a 30 m forested riparian buffer in North Carolina. This could be attributed to greater nitrogen mineralization closer to the stream (Knoepp & Clinton, 2009), leading to an offset of the plant uptake of available NH_4^+ from the soil.

Parallel location did not play a major role in delineating the spatial variation of soil NH_4^+ concentration in either land-use. However, one exception was noted in the UNF site, where the concentration was higher upstream than at the middle and downstream locations. Like perpendicular locations, the lack of significance may be related to a decrease in spatial variability as mentioned above (Ettema et al., 1998; Gallardo, 2003; Vellidis et al., 2003). The significance in only the fall was an unexpected result; however, soil NH_4^+ concentrations can be driven by elevation so a decreasing trend with distance downstream is not unprecedented (Harms & Grimm, 2008). Furthermore, the relatively late nature of the fall season in the present study is consistent with increased immobilization in the fall and winter seasons (Kaiser et al., 2011), which may have driven the decrease with distance downstream observed during the fall season at Washington Creek.

4.1.3. Soil Organic Carbon and Total Nitrogen

Soil organic carbon (SOC) and total nitrogen (TN) content showed a marked increase in the UNF site compared to the RH site along Washington Creek. SOC and TN content showed only a slight increase in the RH site from the values presented by Oelbermann et al. (2015) for the same land-use from 2010. However, SOC and TN content effectively doubled at the UNF site when compared to the same study (Oelbermann et al., 2015). However, the magnitude of this increase is unlikely in a 150+ year old riparian forest and is likely attributed to the spatial dependence of SOC and TN (Cambardella et al., 1994), and the soil heterogeneity between the sampling points in the present study and those sampled by Oelbermann et al. (2015). The difference in SOC and TN content between the RH and UNF sites is likely attributed to forest age (Corre et al., 1999; Hooker & Compton, 2006; Bush, 2008; Oelbermann et al., 2015). Corre et al. (1999) found that SOC increased at riparian forests greater than 60 years old compared to riparian forests that were around 30 years old in northeastern USA. Furthermore, Bush (2008) found similar results in naturally succeeding riparian forests; however, Bush (2008) also found that SOC and TN accumulation slowed later in succession. Hooker & Compton (2006) found that SOC and TN content continued to increase in temperate coniferous forests at a linear rate at ages over 100 years old. Knops & Tilman (2000) suggest that SOC and TN increase for ~200 years after in ecosystems after agricultural abandonment. Accumulation and storage of SOC and TN is often related to vegetation composition, with species such as forb and grasses decreasing the accumulation (Knops & Tilman, 2000), which is more indicative of the RH site. Furthermore, increased nitrogen in the litter has been shown to increase the accumulation of soil organic matter, and thus SOC and TN (Berg, 2000).

4.2. Vegetative Characteristics

4.2.1. Stand Density, Light Intensity, and Microhabitat Characteristics

Riparian forest density is often overlooked in restoration, although it plays a crucial role in determining the functionality of the ecosystem in terms of providing shade, nutrient uptake, and litterfall (National Research Council, 2002; DeWalle, 2010). Our results showed that the UNF land use had a lower stand density than the rehabilitated site, although this was not a significant difference. Spies & Franklin (1991) found that young (40 year old) Douglas fir (*Pseudotsuga menziesii*) forests had much higher tree density than mature and old growth forests, due to increased basal area of trees in the later stages of succession. It is common to see this inverse relationship between stand density and basal area in regenerating forests (Christensen & Peet, 1984; Tappeiner et al., 1997; Suzuki et al., 2002). However, the lack of significance can be attributed to high spatial variability in tree density within and between forest ecosystems (Tappeiner et al., 1997; Allen et al., 2002). Shear et al. (1996) also found that after 50 years, naturally and actively restored riparian forests maintained increased stem density in southwestern Kentucky. It is likely that with an increased sample size taken over a larger area, the difference in density between land-uses at Washington Creek would have been significant. However, this aspect was limited based on the size of the RH site and the size of the sampling area. Distance to the nearest tree stem is an important factor in nutrient, gas, and water dynamics in soil and soil solutions (Butterbach-Bahl et al., 2002). This is a facet of stand density but is much more spatially uneven. In the RH site (with increased density), there was an increase in average distance and variability in distance to the nearest stem from the greenhouse gas sampling points. Although counterintuitive, it provides evidence that even though overall density is greater at the RH site, it is likely more clumped together rather than evenly spread out.

Although stand density is positively correlated to canopy cover and shade (Lhotka & Loewenstein, 2006), our results showed that light penetration was significantly lower in the UNF site, which indicates increased canopy cover. Basal area is often used as a proxy for canopy cover and leaf area (Smith et al., 1992; Nagler et al., 2004) and can explain much of the variation in canopy cover in forest ecosystems (Mitchell et al., 1997). It is likely that increased basal area in the UNF site was higher due to an increase in size and age of tree individuals. Shear et al. (1996) found that basal area of trees in restored sites took around 40 years to reach the level of the mature forest. Parkyn et al. (2003) found that shade over watercourses provided by restored riparian forests was highest when remnant trees from the original forest ecosystems were present, which can take decades to be restored to a pre-disturbance state. Species frequently used in riparian restoration are often fast-growing, short-lived species that can colonize quickly and deal with soil conditions indicative of riparian ecosystems (Chazdon, 2008). Although these species grow fast, they rarely match the size of the local natural forest ecosystems such as the UNF site. The smaller tree individuals may allow for increased density, but will likely decrease canopy cover and allow light to penetrate more frequently (Parker & Russ, 2004). Tree height may also play a role in determining light intensity, as it is often negatively correlated with understory illumination (Martens et al., 2000), and tree height in restored forests usually takes longer than 30 years to equilibrate with the natural forest ecosystem (Davies-Colley et al., 2009). Anderson et al. (2009) found that canopy cover decreased with increasing distance from the stream, which was also true for the RH site. This may account for increased light penetration in the RH site as sampling reached the edge of the riparian forest, whereas the UNF site was wider decreasing the potential of light penetration from the forest's edge.

4.3. Soil Greenhouse Gas Emissions

4.3.1. Soil Carbon Dioxide Emissions

Soil CO_2-C emissions determined from the RH and UNF sites fell within the range of emissions documented in the literature for riparian zones (Soosaar et al., 2011; Mander, 2016). Emissions at Washington Creek were relatively low, falling in the lowest 15-20% of riparian soil CO_2-C emissions for riparian zones (Soosaar et al., 2011; Mander, 2016). The ranges in this study for both land-uses varied and even provided negative emissions. Negative CO_2-C emissions, although not commonly reported in the literature, have been seen in riparian ecosystems and have been attributed to woody and wetland plant composition leading to increased uptake of CO_2 (Scott et al., 2006; Picek et al., 2007). Other explanations for this phenomenon have been lichens, mosses, or cyanobacteria uptake and CO_2 dissolution in alkaline and carbonate-rich soils, like those at Washington Creek (Xie et al., 2009; Ma et al., 2013). Our results also show substantially lower emissions than those found at the same sites by Oelbermann et al. (2015), although this can be explained by the inclusion of seasons where lower soil CO_2-C emissions are expected (Shreshthra et al, 2009), as well as inter-annual variability of emissions (Raich et al., 2002; Epron et al., 2004).

On average, soil CO_2-C emissions were considerably higher in the RH site, when compared to the UNF site. The difference between the two land-uses was not significant, however it was extremely close (p=0.052). Although insignificant, the difference might still prove to be biologically meaningful. Oelbermann et al. (2015) found significantly higher soil CO_2-C production rates from the same two land-uses. However, the time gap between sampling periods provides evidence that it took ~30-years post-rehabilitation for soil CO_2-C emissions to even out for the two land-uses. These results are consistent with Shresthra et al. (2009), who

found that 28 year old reclaimed forest in Ohio had consistently higher soil CO_2-C emissions than the undisturbed reference forest, although the difference was also insignificant. Soil CO_2-C emission variation is often attributed to increased soil temperature, decreased moisture, organic carbon content, and vegetation type through root density (Raich & Potter, 1995; Raich & Tufekcioglu, 2000; McLain & Martens, 2006; Pacific et al., 2008; Bailey et al., 2009; Soosaar et al., 2011). Temperature is often the referred to as the primary control on soil CO_2-C emissions (Tufekcioglu et al., 2001; Pacific et al., 2008), therefore the slight increase in soil temperature at the RH site compared to the UNF site may account for the same increase in emissions found. Soil moisture is another key regulator of soil CO_2-C emissions, with a negative relationship in moderate-high moisture content soils (Davidson et al., 1998; Raich & Tufekcioglu, 2000; Tufekcioglu et al., 2001; Pacific et al., 2008). Therefore, the increased soil moisture at the UNF site may contribute to reduced soil CO_2-C emissions. Similarly, decreased canopy cover and increased density may have led to an increase in soil CO_2-C emissions at the RH site, as reduced canopy cover can lead to favorable soil conditions for respiration, and density can increase root respiration (Raich & Tufekcioglu, 2000; Shresthra et al., 2009). Increased litterfall and organic matter at the UNF site may have offset the other variables preventing a significant difference, as mature forests contribute more organic matter which leads to increased decomposition and therefore emissions (Raich & Tufekcioglu, 2000; Oelbermann et al., 2015).

The driving variables of soil CO_2-C emissions often lead to seasonal trends in soil CO_2-C emissions (Soosaar et al., 2011), which have been paralleled in the results at Washington Creek. Soil CO_2-C emissions were highest during the summer season for both land-uses, being significantly greater than all other seasons in the RH site and the fall and winter in the UNF site. Increased soil CO_2-C emissions in the summer are well documented in restored forest and

riparian research (Teiter & Mander, 2005; McLain & Martens, 2006; Picek et al., 2007; Shresthra et al., 2009; Soosaar et al., 2011). The winter season had the lowest soil CO_2-C emissions for both land-uses, being negligible in the UNF site. Shresthra et al. (2009) found that soil CO_2-C emissions were minimal in the winter months due to reduced soil temperature and thus microbial activity. Emissions were also relatively low in the fall and spring seasons at Washington Creek, which is consistent with dropping soil and air temperatures as well as increased soil moisture (Mander et al., 2008). Both land-uses followed similar trends in dropping soil CO_2-C emissions from summer to winter; however, the UNF site showed a marked jump from winter to spring not seen in the RH site. Later thawing of soils (releasing labile carbon stores) in the UNF site, because of a cooler microclimate and increased surface litter, may have caused a pulse of emissions (Tufekcioglu et al., 2001; Brooks et al., 2011).

Soil CO_2-C emissions have been shown to have substantial spatial variability within riparian ecosystems (Picek et al., 2007; Petrone et al., 2008); however, few studies have determined the effect of position (horizontally and vertically) along the stream (Tufekcioglu et al., 2001). In terms of perpendicular variation, the RH site showed no significant trends in terms of distance from the stream during the summer. However, the fall and full-year comparison showed that soil CO_2-C emissions were significantly lower at the farthest location from the stream. This trend was mirrored in the winter and spring, but was not significant. In the RH land-use, this trend might be explained by increased soil moisture seen at the farthest locations, as well as slightly lower temperatures creating less than ideal conditions for soil respiration. Pacific et al. (2008) found that soil CO_2 concentrations were higher in riparian zones than in the hillslope forests further from the stream in Montana. This aligns well with the findings of the present study, however the magnitude of differences is likely less in this study site, as their study

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outlines a transition from upland forest to grassed riparian zone which usually show the highest riparian emissions (Pacific et al., 2008; Shresthra et al., 2009; Oelbermann et al., 2015). The UNF site seemed to have almost an opposite spatial effect, where there was no significant difference in soil CO_2-C emissions during the fall, spring, and over the full sampling period. However, emissions at the farthest location were the highest during the summer and winter, being higher than the middle and closest locations, respectively. During the summer, the middle and farthest locations shared almost identical soil temperature and moisture, although the middle location had increased soil NO_3^- concentrations. Mo et al. (2008) found that addition of nitrogen fertilizer to a forest ecosystem caused a decline in soil respiration rates because of reduced autotrophic respiration and fine root biomass associated with increased nitrogen. Therefore, in the absence of temperature or moisture variation, it appears significantly increased nitrogen in the middle locations stunted emissions. As for the decrease in emissions at the closest location in the UNF site, it may be related to increased carbon availability at the farthest location. Brooks et al. (2005) found that winter soil CO_2-C emissions was positively related to available carbon, which can accumulate via freeze-thaw events before consistent snow cover.

In terms of parallel location, no significant trend was found in the RH site and values seemed to vary randomly. This lack of spatial variation in soil CO_2-C emissions may be related to the similar trend in soil temperature and moisture. However, the UNF site showed significantly decreased soil CO_2-C emissions at the downstream location during the summer, fall, and over the entire sampling period. As soil temperature did not vary in the UNF site, soil moisture seems to be the driving force of spatial variability in soil CO_2-C emissions in this land-use. Soil moisture was consistently the highest at the farthest location, reducing microbial decomposition of litter and thus soil CO_2-C emissions (Davidson et al., 1998). Although soil

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temperature is usually the primary control, soil moisture can overtake temperature in very wet soils (Pacific et al., 2008), such as the UNF site.

4.3.2. Soil Nitrous Oxide Emissions

Soil N_2O-N emissions measured along Washington Creek were relatively low, but fell within the range of emissions found in riparian ecosystems as outlined by Soosaar et al. (2011). Dhondt et al. (2004) found similar emission values in forested and mixed vegetation riparian zones in Belgium. The soil N_2O-N emission values were also similar to those found at reclaimed mine sites, riparian alder forests, and a boreal forest (Jacinthe & Lal, 2006; Shresthra et al., 2009; Soosaar et al., 2011; Tupek et al., 2015). Low soil N_2O-N emissions for the land-uses along Washington Creek may be attributed to relatively high pH found in the area (Oelbermann et al., 2015). N_2O reduction (to N_2) via microbial processes is lowest in areas with low pH values; however, the mechanism behind this relationship is not well understood (Hopfensperger et al., 2009; Van den Heuvel et al., 2011). Wide ranges of emissions were found in both land-uses along Washington Creek and indicate varying magnitudes of N_2O-N emissions and uptake (negative values), which has been observed in riparian ecosystems (Dhondt et al., 2004; Kim et al., 2009; Audet et al., 2013). Forest soils often act as sinks for N_2O-N and this uptake has been attributed to increases in soil moisture favoring denitrification, resulting in the conversion of N_2O to N_2 (Chapuis-Lardy et al., 2007). Another proposed explanation for negative fluxes in soil N_2O is consumption by nitrifiers in coupled nitrification-denitrification (Chapuis-Lardy et al., 2007).

When comparing land-uses along Washington Creek, the RH and UNF sites did not differ significantly, although the RH site did exhibit slightly higher N_2O-N emissions. Shresthra et al. (2009) also found that a 28 year old reclaimed forest did not have significantly different soil

N$_2$O-N emissions when compared to an undisturbed reference forest. Furthermore, Kim et al. (2009) provide evidence that although riparian forest vegetation reduces soil N$_2$O-N emissions when compared to agricultural soils, three different riparian forests showed no major variations in soil N$_2$O-N emissions. Similar soil N$_2$O-N emissions between the RH and UNF land-uses is not surprising, as they seem to trade off increases in variables that can lead to N$_2$O-N emissions. Increased soil temperature, light penetration, and decreased soil moisture attributed to increased soil N$_2$O-N emissions (Hopfensperger et al., 2009; Shrestha et al., 2009; Soosaar et al., 2011; Mander, 2016) can be seen in the RH site, whereas, increased soil NO$_3^-$, NH$_4^+$, and soil organic carbon which drive the microbial processes leading to soil N$_2$O-N emissions (Walker et al., 2002; Teiter & Mander, 2005; Hopfensperger et al., 2009) were seen in the UNF site.

Throughout the literature there is significant seasonality in soil N$_2$O-N emissions in riparian ecosystems, with peaks in emissions in the summer and considerable drop-offs in the fall through spring (Dhondt et al., 2004; Teiter & Mander, 2005; McLain & Martens, 2006; Shresthra et al., 2009). The results for the RH and UNF site seem to follow a similar trend, with increased soil N$_2$O-N emissions in the summer. However, the only significant increases were found in the UNF site, which showed higher emissions during the summer when compared to the winter and spring. In the UNF site, increased soil N$_2$O-N emissions during the summer and decreasing until the spring seems to be driven by changes in soil temperature and available nitrogen (NO$_3^-$, NH$_4^+$). However, soil moisture did not seem to play a role in determining soil N$_2$O-N emissions in this land-use. This is likely due to the static and saturated nature of the soils at the UNF, in that these types of systems provide consistent anaerobic conditions for denitrification (Hopfensperger et al., 2009). Thus, soil N$_2$O-N emissions will be facilitated by soil temperature

and available NO_3^- as seen in post-mining reclaimed forests and recovering riparian ecosystems (Walker et al., 2002; Shresthra et al., 2009).

The lack of a significant trend in the RH site is not unprecedented in riparian zones (Burgin & Groffman, 2012), and might be attributed to inter-annual variability of seasonal impacts on soil N_2O-N emissions as observed by Soosaar et al. (2011). Increased soil N_2O-N emissions during the winter in the RH site may be caused by freeze-thaw cycles that can account for upwards of 70% of soil N_2O-N emissions in riparian systems (Kim et al., 2009). Furthermore, the magnitude of winter emissions from frozen soils is likely ecosystem dependent (Merbold et al., 2015), but has been shown to be a major contributor (Wolf et al., 2010). With a warmer microclimate, it is possible that a greater number of freeze-thaw events occurred at the RH site, leading to increased winter emissions. Based on soil characteristics, the trend seen in the RH site is not surprising. There was increased soil temperature and decreased soil moisture during the summer season, whereas the fall and spring shared similar values, which are mirrored in the soil N_2O-N emissions. The lack of a significant trend may be attributed to constantly available nitrogen, as there was no significant seasonal trend in soil NO_3^- or NH_4^+ concentrations for the RH site. Evidence has shown that variation in soil N_2O-N emissions in riparian ecosystems are strongly impacted by available nitrogen sources for nitrification and denitrification (Walker et al., 2002; Teiter & Mander, 2005; Verhoeven et al., 2006).

With respect to perpendicular distance, spatial variability of soil N_2O-N emissions did not follow any particular trend and varied by season. This held true for all seasons at both land-uses, with the exception of the summer in the UNF site where a significant increase in soil N_2O-N emissions was observed at the middle and farthest locations. This significant trend provides further evidence for the importance of available nitrogen for soil N_2O-N emissions, as soil

moisture and temperature were consistent throughout the summer in the UNF site, although NO_3^- and NH_4^+ decreased at the closest location. Dhondt et al. (2004) observed a similar trend, where uphill portions of riparian zones increased soil N_2O-N emissions compared to downhill portions. Conversely, Hefting et al. (2003) found the opposite effect, where emissions increased closer to the stream. However, this trend was driven by denitrification (Hefting et al., 2003), which supports the results during summer at the UNF site where increased emissions followed available nitrogen under ideal anaerobic denitrification conditions. Furthermore, Hefting et al. (2006) found increased soil N_2O-N emissions with distance from the stream in the summer in a natural alder riparian forest with high- NO_3^- removal efficiency, although most spatial trends found were insignificant and variable based on the driving factors of denitrification (i.e., soil moisture, NO_3^- concentration). As soil N_2O-N emissions can come from multiple processes and are driven by a complex suite of variables, the lack of a tangible trend in emissions is common within the literature and is usually attributed to a high degree of microsite variation and large standard error values within samples, preventing significance (Hefting et al., 2003; McLain & Martens, 2006; Mander et al., 2008; Butterbach-Bahl et al., 2013).

Soil N_2O-N emissions only varied in a parallel direction during the fall in RH site, with increased emissions downstream when compared to the upstream and middle locations. The significant trend found in the fall may have been related to slightly elevated soil NO_3^- concentrations downstream and increased soil moisture during the fall season, which created ideal anaerobic conditions for denitrification. Although soil moisture was also increased during the spring season in the RH site, the same trend may not be seen in soil N_2O-N emissions due to a drop-off in soil NO_3^- concentrations during the spring. Ma et al. (2008) found that elevation did not play a role in determining soil N_2O-N emissions in cultivated wetlands at lower soil moisture

contents, but found that at higher soil moisture (~70%) the relationship was more substantial. In general, the opposite effect (decreased emissions downstream) was observed in the UNF site; however, the relationship was not significant. Similar trends of slightly decreased soil N_2O-N emissions at lower elevations at the UNF site have been seen within temperate riparian wetlands and forests (Dhondt et al., 2004; Hernandez & Mitsch, 2006). This phenomenon, in the absence of significant hydrological differences (such as water table dropdown), has been attributed to increased soil NO_3^- concentrations (Hernandez & Mitsch, 2006).

4.4. Greenhouse Gas, Soil, and Vegetative Characteristics Correlations

4.4.1. Seasonal and Land-Use Based Correlations to Greenhouse Gas Emissions

The relationship between soil greenhouse gas emissions and soil and vegetation characteristics has been observed extensively in the literature (Raich & Tufekcioglu, 2000; Teiter & Mander, 2005; Hopfensperger et al., 2009). However, the magnitude of these relationships is highly dependent on the specific ecosystem and the spatial and temporal trends within the ecosystem. The results from this study show that correlations between soil characteristics and greenhouse gas emissions within the same season and land-use are few and far between. Correlations within the literature show extremely variable relationships and call for more information on correlations in different riparian systems (Teiter & Mander, 2005; Picek et al., 2007). The only significant seasonal correlations were found during the fall in the RH site, where soil N_2O-N emissions were positively correlated to soil moisture and negatively correlated to soil temperature and NO_3^- concentration. It is common to see soil N_2O-N emissions positively correlated to soil temperature and soil NO_3^- concentrations within the literature (Walker et al., 2002; Kitzler et al., 2006); however, these variables do not always correlate to emissions (Teiter & Mander, 2005; Audet et al., 2013). The negative relationship with temperature can be

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explained by a greater impact of soil moisture on soil N_2O-N emissions in the RH site, where negative temperatures are associated with increased soil moisture. Although decreased soil moisture has been seen to increase emissions (McLain & Martens, 2006), Dhondt et al. (2004) observed a similar positive relationship between soil moisture and soil N_2O-N emissions. In the RH site, the increased soil moisture may have stimulated increased denitrification (Hopfensperger et al., 2009; Butterbach-Bahl et al., 2013). Hefting et al. (2003) provide evidence that increasing soil moisture in riparian ecosystems can lead to higher denitrification rates, and subsequently soil N_2O-N emissions. The negative relationship to soil NO_3^- concentration may also provide evidence for denitrification as the driving force of emissions during the fall in the RH site. It has been shown that increased denitrification will lead to reduced soil NO_3^- concentrations and may lead to increased soil N_2O-N emissions (Schipper et al., 2010). Alternatively, soil N_2O-N emissions at the UNF site showed a strong negative relationship (although not significant, $r=-0.323$, $p=0.055$) to soil moisture, which is consistent with patterns observed in riparian systems (Teiter & Mander, 2005; McLain & Martens, 2006; Mander et al., 2008). Furthermore, this relationship could be related to the height of the water table at the UNF site during the spring. Hefting et al. (2004) found that denitrification increased when the water table decreased slightly in soils with high water tables in riparian wetlands. This phenomenon may have occurred during spring in the UNF due to increased runoff and precipitation early in the spring.

For the full sampling period, soil N_2O-N emissions in the RH site showed no significant correlations to any of the soil or vegetation variables. The lack of clear correlative variables indicates the complexity in soil N_2O-N emissions, specifically in soils with intermediate soil moisture like the RH site where N_2O-N emissions are likely driven by competing factors at

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different points in time (i.e., pulses of denitrification and nitrification) (Hefting et al., 2004; Butterbach-Bahl et al., 2013). In the UNF site, soil N_2O-N emissions had a significant positive relationship with soil temperature but no other soil or vegetative characteristics. This relationship is often observed within the literature (Shresthra et al., 2009; Butterbach-Bahl et al., 2013), specifically linked to increased emissions in the summer (McLain & Martens, 2006; Mander et al., 2008). Denitrification has been seen to increase with elevated temperatures because of oxygen depletion (creating anaerobic conditions) through high soil respiration rates (Schaufler et al., 2010; Butterbach-Bahl et al., 2013). The already near-optimal denitrification conditions in the UNF site (Butterbach-Bahl et al., 2013) may explain why we see this relationship between soil temperature and soil N_2O-N emissions in the UNF site (i.e., more sensitive to changes in temperature) and not the RH site.

Soil CO_2-C emissions for both the RH and UNF sites had a positive correlation to soil temperature. These correlations between soil CO_2-C emissions and soil temperature are consistent with the seasonal trends and correlations in soil respiration that are commonly observed in riparian and forest ecosystems (Teiter & Mander, 2005; McLain & Martens, 2006; Bailey et al., 2008; Petrone et al., 2008; Shresthra et al., 2009; Soosaar et al., 2011). This can be explained by higher microbial activity during periods of increased temperatures (Smith et al., 2003). In the RH site, soil CO_2-C emissions were also significantly correlated to soil moisture (negatively) and NO_3^- concentrations (positively). These correlations are likely related to the inverse relationship between soil temperature and moisture found at the RH site, as well as decreased decomposition during times of high soil moisture preventing respiration (Davidson et al., 1998). The relationship between NO_3^- from fertilizer on soil respiration has been argued as both positive and negative (Kim et al., 2012); however, very little information is available on the

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impact of NO_3^- from agricultural runoff on soil CO_2-C emissions. It has been suggested that fertilizer application can increase soil CO_2-C emissions through increased heterotrophic respiration (Kim et al., 2012), microbial activity, and root growth (Gauder et al., 2012). In the UNF site, soil CO_2-C emissions were positively correlated to SOC and TN as well. The relationship between soil CO_2-C emissions and SOC, because of increased decomposition, is well documented in riparian and forest ecosystems in the literature (Raich & Tufekcioglu, 2000; Tufekcioglu et al., 2001; Bailey et al., 2008; Shresthra et al., 2009). The significant relationship between soil CO_2-C emissions and TN content has been observed in stands of *Acacia spp.* in Burkina Faso (Traore et al., 2007) and is likely related to increased microbial activity, as it is limited first by SOC, then by available nitrogen (Teklay et al., 2005).

When isolating the impact of soil characteristics on soil greenhouse gas emissions within each season for the whole system (both land-uses) along Washington Creek, no significant relationship was found. These results provide further evidence for the overwhelming impacts of seasonality on greenhouse gas emissions from riparian soils. Variation in the factors that drive emissions, such as soil moisture and temperature, seem to require larger disparity than that which has been seen within a given season on a landscape scale (Pacific et al., 2008). This phenomenon may also be explained by the variation in soil conditions between the RH and UNF sites. For example, the effect of soil temperature and moisture within the fall season for the RH site was significantly correlated to soil N_2O-N emissions. However, integration of the UNF site creates a bimodal distribution of soil characteristics. This distribution, because of similar soil N_2O-N emissions between the land-uses, negates the strong relationship observed. Soil N_2O-N emissions may also be a product of two different microbial processes under opposite soil conditions (Smith

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et al., 2003; Butterbach-Bahl et al., 2013), which may also explain the lack of relationship between greenhouse gas emissions and soil characteristics.

4.4.2. Overall Annual Correlations

To consider correlations between greenhouse gas emissions, soil, and vegetative characteristics on a larger spatial and temporal scale, both land-uses were combined for the full sampling period. Soil N_2O-N emissions were found to increase with soil CO_2-C emissions significantly. This positive relationship may be due to similar conditions driving both greenhouse gas emissions, such as increased soil temperature and varying soil moisture content (Smith et al., 2003; Bailey et al., 2009; Shresthra et al., 2009; Soosaar et al., 2011). Both greenhouse gas emissions were also positively correlated to soil temperature, providing evidence for this explanation. Furthermore, it has been observed that increased soil respiration creates a positive feedback with soil CO_2-C and N_2O-N emissions by depleting oxygen making anaerobic conditions for denitrification (Butterbach-Bahl et al., 2013). Soil CO_2-C emissions were also correlated negatively with soil moisture, providing evidence for the importance of soil moisture in determining emissions, which is common in the literature (Hopfensperger et al., 2009; Samaritani et al., 2011). Furthermore, soil CO_2-C emissions increased at a shorter distance from the nearest tree. This may be related to increased root respiration, leading to higher soil CO_2-C emissions (Tufekcioglu et al., 1999). It may also be related to increased water uptake from adjacent trees, which has been seen in poplar species (Snyder & Williams, 2007), reducing soil moisture on a local scale and promoting soil respiration. However, this relationship likely occurs at depths below the 0-10 cm that soil samples were collected at.

Soil temperature, as expected, had a significant inverse relationship with soil moisture. Soil temperature and moisture were also positively and negatively correlated with distance to the

nearest tree. This may be related to gaps in the canopy with increased distance from the nearest tree, which affects the microclimate of the soil (Paul et al., 2004). Furthermore, closer proximity to trees increases litter fall, which may promote organic matter build-up that affects the microclimate of the soil (Butterbach-Bahl et al., 2002). SOC and TN had an opposite relationship with soil temperature and moisture, where increases in SOC and TN lowered temperature and elevated moisture. This is consistent with the relationship between organic matter and soil microclimate (Hunter & Faulkner, 2001; Lal, 2004). Soil temperature was positively correlated to both soil NO_3^- and NH_4^+ concentrations, which is likely a function of seasonal fertilization (Pinay et al., 1993) and increased nitrogen mineralization rates at higher temperatures (Peterjohn et al., 1994). Soil moisture also had a positive relationship with soil NH_4^+ concentrations, which could be explained by similar seasonal trends as described above for soil temperature, as well as increased soil NH_4^+ concentrations in the saturated soils at the UNF site. Soil NO_3^- and NH_4^+ concentrations were positively correlated to each other. This is likely attributed to topographical pooling (Simmons et al., 2011), nitrogen mineralization (Hefting et al., 2004), and nitrification (i.e., areas of high NH_4^+ concentrations increasing NO_3^- concentration) (Barnard et al., 2005; Farquarson & Baldock, 2008). Finally, soil NO_3^- and NH_4^+ concentrations were positively correlated to SOC and TN, which can be attributed to increased soil moisture and nitrogen mineralization in the presence of elevated organic matter (Hunter & Faulkner, 2001; Bernal et al., 2003).

5. Conclusions and Recommendations

Riparian ecosystems are able to effectively mitigate adverse effects of adjacent land-uses on stream quality (Verhoeven et al., 2006; Mayer et al., 2007). However, extensive degradation of these riparian ecosystems from agriculture and urbanization has substantially reduced the services they provide (Perry et al., 2012). Riparian restoration or rehabilitation has the potential to restore ecosystem services; however, they also have the potential to act as hotspots for greenhouse gas emissions (Bailey et al., 2008; Pacific et al., 2008; Capon et al., 2013). Greenhouse gas emissions – specifically N_2O – are usually exacerbated by proximity to adjacent agriculture through fertilizer runoff (Mander et al., 2008; Shresthra et al., 2009). Riparian systems are often underestimated or unaccounted for in global calculations of greenhouse gas emissions and may substantially impact climate change and ozone depletion (Mander, 2016).

This study suggests that 30 years post-rehabilitation, the RH and UNF sites did not produce substantially different levels of greenhouse gas emissions. Although greenhouse gas emissions did not vary, soil and vegetation characteristics were quite distinctive between the two land-uses. The RH site had a warmer and drier soil microclimate than the UNF site, although it had decreased soil nitrogen and organic carbon. The RH site also had greater stand density, although density was more heterogeneous and thus exhibited reduced canopy cover compared to the UNF site. With regards to the first hypothesis, the null hypothesis can be accepted for greenhouse gas emissions and rejected for soil and vegetation characteristics. Soil CO_2 emissions seemed to be most affected by soil temperature, moisture, and NO_3^- concentration at the RH site. Whereas, soil CO_2 emissions were driven by soil temperature, organic carbon, and total nitrogen in the UNF site. Soil N_2O emissions did not show any clear trends with soil or vegetation in the RH site; however, they were driven by soil temperature in the UNF site. Based

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on the correlation analyses, the second null hypothesis can be accepted for vegetation characteristics. However, soil characteristics were found to drive soil greenhouse gas emissions, therefore the second null hypothesis is rejected.

Soil greenhouse gas emissions followed a strong seasonal trend with elevated emissions during the summer and decreased emissions in all other seasons. This variation followed seasonal trends in soil characteristics, such as increased temperatures, available NO_3^- and NH_4^+, and decreased soil moisture. Correlations within seasons for both land-uses and the whole system did not yield any substantial trends, except during the fall in the RH site where production of soil N_2O emissions was associated with increased soil moisture and declining soil temperatures and NO_3^- concentrations. Spatial variation of soil CO_2 emissions within land-uses was highly sensitive to changes in soil moisture. Areas within land-uses with increased soil moisture showed reduced soil CO_2 emissions for both land-uses. Soil N_2O emissions did not follow any discernible spatial trends in emissions within land-uses, except for during the fall and summer for the RH and UNF site, respectively. The observed trends in soil N_2O emissions within land-uses followed conditions ideal for denitrification, specifically increased soil NO_3^- concentration and moisture. For the whole system, greenhouse gas emissions were positively correlated to each other, as well as temperature. Soil CO_2 emissions also had a negative relationship with soil moisture. Temporal trends in greenhouse gas emissions and soil characteristics were clear, therefore the temporal aspect of the third null hypothesis is rejected. However, only soil characteristics, specifically in the UNF site, showed consistent variation spatially leading to the rejection of the third null hypothesis.

The results from this study provide evidence that preserving natural riparian ecosystems may be important in reducing greenhouse gas emissions and maintaining soil quality. However,

riparian forest rehabilitation may be just as effective in reducing greenhouse gas emissions in an agricultural landscape. Based on this study, it may take 30 years or more for a rehabilitated riparian forest to match natural riparian forests in agriculturally dominated landscapes. Research as recent as five years, has shown that the RH site has not restored soil characteristics or CO_2 emissions to the levels before degradation (Placencia-Escalante, 2008; Oelbermann et al., 2015). The results from this study support that soil conditions still need more time to recover, although soil CO_2 emissions are starting to equilibrate. These results present an interesting conclusion, that although rehabilitation alters soil characteristics, differences in greenhouse gas emissions may be negligible after as little as 30 years. Furthermore, this research supports the literature in terms of the importance of seasonality in greenhouse gas emissions (Soosaar et al., 2011). The results of this study provide a greater insight into how greenhouse gas emissions vary spatially along a stream and the importance of soil characteristics in driving these variations. Although soil and vegetation characteristics are often related to soil greenhouse gas emissions emissions, the results of this study outlines the complexity of these interactions and how emissions are highly dependent on climatic variables and are ecosystem dependent. Although the literature often refers to riparian ecosystems as potential hotspots of greenhouse gas emissions, the results of this study provides evidence for hot 'moments' of emissions.

This research has provided some indication of the drivers of spatial and temporal trends soil greenhouse gas emissions between a rehabilitated and undisturbed natural riparian forest; however, it also illustrates the extensive variation in emissions that can occur within the same land-use and along the same watercourse. Thus, future research is still needed on greenhouse gas emissions in restored or rehabilitated riparian ecosystems to better predict future trajectories of hotspots and/or moments, as well as to implement effective management of these systems.

Specifically, it would be useful to conduct research on a landscape scale, and thus avoiding the pseudo-replicated nature of this study. It is likely that the results of this study would hold true in other riparian systems; however, the ability to generalize these results is limited. Although the results of this study show that greenhouse gas emissions along Washington Creek were relatively low, the inter-annual variability and ecosystem dependence of these emissions requires increased monitoring to truly understand the contribution of riparian ecosystems to climate change.

As riparian restoration and rehabilitation continues to gain ground, future research should continually monitor and inventory these systems to understand the effectiveness of these projects in reducing emissions, as well as how long it takes to gain beneficial services from these systems. Future research in riparian ecosystems should also consider other variables that have been connected to greenhouse gas emissions such as direct measures of elevation (Hernandez & Mitsch, 2006), local topography (Simmons et al., 2011), microbial community composition and structure (Samaritani et al., 2011), and understory vegetation composition (Hopfensperger et al., 2009). Furthermore, increased replication, riparian ecosystem types (i.e., grasslands, pasture), and incorporation of stream quality would increase the scope of this research. However, this study provides a good foundation on how riparian rehabilitation can effectively mitigate the adverse effects of a changing climate without mimicking the historical ecosystem.

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