CARBON AND NITROGEN CYCLING IN INTENSIVE PRODUCTION SYSTEMS: TRADE-OFFS BETWEEN PRODUCTIVITY AND SUSTAINABILITY

By

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ABSTRACT

CARBON AND NITROGEN CYCLING IN INTENSIVE PRODUCTION SYSTEMS: TRADE-OFFS BETWEEN PRODUCTIVITY AND SUSTAINABILITY

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A solid understanding of the impacts of agricultural management practices on soil biology, nitrogen and carbon dynamics and net greenhouse gas (GHG) emissions is essential to evaluating agro-ecosystem sustainability. I established a suite of experiments at the Michigan State University's (MSU) Tree Research Center (TRC) in East Lansing and at two different sites in Northern Michigan to address the following three hypotheses. Hypothesis 1: Managing Fraser-fir plantations with cover crops in a low-input system will increase soil organic carbon, total soil nitrogen, microbial biomass and functional diversity as well as tree performance compared to a high-input conventional system. Hypothesis 2: Conversion of grassland to short-rotation woody crop (SRWC) bioenergy systems will increase nitrate leaching losses and soil emissions of nitrous oxide (N_2O), carbon dioxide (CO_2) and methane (CH_4), undermining the environmental benefits of substituting biomass energy for fossil fuels. Hypothesis 3: Adding N fertilizer to a switchgrass bioenergy system will improve the net GHG balance because enhanced CO_2 fixation will exceed direct and indirect emissions of GHGs associated with fertilizer use. To test the first hypothesis, a tree-cover crop intercropping trial involving Fraser fir, two leguminous (Dutch white clover and alfalfa) and a non-leguminous (perennial ryegrass) cover crops was conducted at the TRC. For each cover crop, two competition-management practices were evaluated.

The approach used to test the second hypothesis was to set-up experimental plots cleared of existing grassland vegetation, then cultivated and planted with either willow (*Salix dasyclados*) or poplar (*Populus nigra*). I measured soil greenhouse gas (GHG) fluxes and N leaching losses from these plots and compared these against undisturbed, reference grassland plots. I also established a switchgrass fertilization trial to test the third hypothesis. Nitrogen fertilization treatments included 0, 56 and 112 kg N ha^{-1} applied as urea, once early in the growing season. Direct and indirect GHG fluxes were performed and biomass yield of switchgrass was evaluated at the end of the growing season. Results indicate that cover crop treatments increased soil microbial N and available N by 35 to 80% and 1.5- and 2.2-fold, respectively, relative to the conventional treatment. Tree seedling survival and growth in cover crop treatments with strips and in the CONV plots were similar. These results demonstrate the potential for cover crops to quickly increase plantation soil nitrogen fertility and tree growth response. Converting grassland to SRWC resulted in 13.3 - 17.2-fold increases of N_2O, an additional loss of 3.3 - 9.0 Mg CO_2 ha^{-1} of CO_2 emissions and an extra N leaching loss of 36 - 51 kg N ha^{-1}, relative to the reference pasture control plots. Grassland conversion to SRWC systems incurred GHG debts of 9.4 and 14.2 Mg CO_2eq ha^{-1} for poplar and willow plots, respectively. Overall, N fertilizer application to a switchgrass contributed little to direct GHG emissions from soil but substantially increased aboveground biomass production which sequestered an additional 2.6 - 9.4 Mg ha^{-1} of atmospheric CO_2 relative to an unfertilized field. N fertilization of switchgrass in this region could reduce the land base needed for bioenergy production and reduce pressure on land required for food and forage crop production.

DEDICATION

Dedicated to my
Wife Pauline Kéré,
and sons
Ives Rodrigue Nikièma,
Jonanthan Rayende Nikièma,
Bonaventure Koka Nikièma,
Wend-Lassida Magloire Nikièma

In memory of my
Father, Raogo Denis Nikièma,
Mother, Guesyaoba Rose Kaboré,
Brother, Salif Gustave Nikièma,
Father-in-law, Gabriel Kéré,
Mother-in-law, Regina Sidyandé, and
Sister-in-law, Anne Marie Kéré

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LIST OF ABBREVIATIONS

ALFNS : Alfalfa with no strips

ALFS : Alfalfa with strips

ANOVA : Analysis of variance

AWCD : Average well color development

BN : Biomass nitrogen

BY : Biomass yield

CEC : Cation exchange capacity

CLPP : Community-level physiological profiling

CONV : Conventional

DM : Dry matter

DON : Dissolved organic nitrogen

DWCNS : Dutch white clover with no strips

DWCS : Dutch white clover with strips

FBIC : Forest Biomass Innovation Center

FS : First growing season

GC : Gas chromatography

GHG : Greenhouse gas

GREET : Greenhouse gases, Regulated Emissions, and Energy use in Transportation

GWP : Global warming potential

ICP-AES : Inductively coupled plasma-atomic emission spectrometry

IPCC : Intergovernmental panel on climate change

KBS : Kellogg Biological Station

LCA : Life Cycle Assessment

MSU : Michigan State University

NCR-SARE : North Central Region-Sustainable Research Education

NT : No strip

OD : Optical density

PC : Principal component

PRGNS : Perennial ryegrass with no strips

PRGS : Perennial ryegrass with strips

PVC : Polyvinyl chloride

RC : Root contribution to soil respiration

RGR : Relative growth rate

S : Strip

SMB : Soil microbial biomass

SOC : Soil organic carbon

SOM : Soil organic matter

SRWC : Short rotation woody crop

SS : Second growing season

TKN : Total Kjeldahl nitrogen

TRC : Tree Research Center

TS : Two growing seasons

WFPS : Water filled pore space

CHAPTER 1

GENERAL INTRODUCTION

1.1. INTRODUCTION

Achieving greater agricultural productivity without degrading land resources, environmental quality and ecosystem services has been a major challenge for the scientific community because of the interconnection and potential tradeoffs between productivity and resource conservation. Agricultural systems are essentially ecological systems which are profoundly modified and managed by mankind (e.g. cultivation, fertilizers, and herbicides) to produce food and fiber, generally with the aim of increasing their productivity. Whereas agricultural intensification has the potential to achieve maximum crop productivity, impacts of agricultural management can alter ecosystem processes and services that support sustainability.

Important areas of resource degradation arising from intensive management include: soil carbon (C) loss through accelerated soil organic matter (SOM) decomposition, groundwater contamination and surface water eutrophication through nitrate leaching and runoff, biodiversity decline, and enhanced greenhouse gas (GHG) emissions. Because the degradation of some of these environmental resources and processes may not be reversible and jeopardize functioning of the whole system, finding agricultural management options (e.g. low inputs) with the potential of keeping these trade-offs within reasonable limits appears necessary. To this end, studies on the impacts of agricultural management practices on ecosystem processes and services could be an important step towards finding strategies for sustainable land-use. Here, I focused my investigations on the response of soil microbial community metabolism, pools and fluxes of carbon and nitrogen, and GHG emissions to agricultural management practices such as tillage, N fertilization and cover cropping. I addressed the issues separately in three different intensive production systems: Christmas tree farming, short-rotation woody biomass plantations and perennial switchgrass bioenergy production systems.

Whereas use of anthropogenic inputs of reactive N and intensive tillage can potentially increase crop production, their inefficient use can lead to a number of problems. For instance, these agricultural management practices sometimes lead to accelerated soil erosion and depletion of soil organic matter, pollution as a result of runoff, lower efficiency of nutrient use and lower profitability. On the other hand, management practices such as cover cropping reduce soil erosion, add organic matter to the soil, conserve soil humus, improve soil aeration and structure, and improve soil nutrient status (Broughton 1977). Organic matter in turn acts as substrate for a variety of soil organisms that immobilize excess nutrients, mineralize them and make them available to plants as they are needed.

While the potential benefits of incorporating cover crops into cropping systems has been widely demonstrated for many agronomic, vegetable and orchard crops, to my knowledge, studies have not yet been conducted to evaluate the potential beneficial contribution of cover crops to Christmas tree production, which cover approximately 150,000 ha of land in production in the United States (Vilsack and Clark 2009) and represent one segment of the forestry sector that uses substantial fertilizer inputs comparable to agronomic systems (Rothstein, 2005). In chapter 2, *Soil microbial biomass and community catabolic diversity as affected by intercropping cover crops with Fraser fir (*Abies fraseri *[Pursh] Poir) – in Christmas tree plantation in Michigan, U.S.A*, I investigated the effects of cover crops on soil microbial properties in a Fraser fir production system in Michigan. The three cover crops used in this study were two legumes (alfalfa [*Medicago sativa*] and Dutch white clover [*Trifolium pratense*]) and a non-legume (perennial ryegrass [*Lolium perenne*]). I expected that incorporating cover crops into a Christmas tree plantation would increase the amount of organic matter returned to the soil, which is a key driver of microbiological processes. Because legumes have the ability to fix atmospheric N,

through symbiotic association with N-fixing bacteria, I also predicted that the leguminous cover crops would have more labile biomass residues and be more efficient in improving soil microbial biomass and activity. I used the chloroform fumigation-extraction method to determine soil microbial biomass C and N and the effectiveness of each cover crop to enhance soil microbial biomass and quotients, and thus soil fertility (Anderson and Domsch, 1986). I also sought to measure the functional diversity of the soil microbial community using the Biolog^{TM} Eco Plate system, which is based on the metabolic capabilities of bacteria during growth in the wells of the Biolog^{TM} micro-plates. Finally, I hypothesized that Managing Fraser-fir plantations with cover crops in a low-input system would increase soil organic carbon, total soil nitrogen, microbial biomass and microbial diversity compared to a high-input conventional system.

Whereas anthropogenic inputs of reactive N inputs are widely known to have a great potential for maximizing growth and producing more marketable trees in a shorter period of time in Christmas tree plantations, recent N studies have raised concerns about N leaching losses from excessive and exclusive use of inorganic fertilizers in these systems (Rothstein 2005; Pedersen et al. 2006). This shows the need to seek alternative N management practices with the potential to improve sustainability and enhance profitability for this system. Because of their several benefits, cover crops have been introduced successfully in many cereal and vegetable crop production systems to address similar issues. However, to my knowledge, studies have not been yet conducted to evaluate the potential beneficial contribution of cover crops to Christmas tree production.

In Chapter 3, *effects of groundcover management on soil properties, tree physiology, foliar chemistry and growth in a newly established Fraser fir (*Abies fraseri *[Pursh] Poir) plantation, in Michigan, U.S.A.* I assess whether soil fertility, Fraser fir survival and growth

could be improved by incorporating leguminous and non-leguminous cover crops into the production system. Because nitrogen input from the cover crop systems is from an organic source which would have to gradually be decomposed through microbial processes before it can be made available to the trees, I expected that the release of plant available nitrogen would be in synchrony with the rate of tree uptake, leading to an improved foliar N level and growth of the trees in the cover crop systems relative to their counterparts in the conventional system. On the other hand, I predicted that the cover crop would strongly compete with the trees for water and plant essential nutrients other than N that could lead to reduced growth performance unless proper competition-management practices are introduced. To test this prediction, I used two competition-management practices; no strips (NS) by growing each cover crop throughout the entire plot and strips (S) by creating a 0.61 m-wide bare zone centered on the tree rows. A conventionally-managed system was used as a control. Finally, I hypothesized that plots with strips would result in overall better tree foliar nutrition, water use efficiency and growth relative to the no-strip treatments. I also anticipated that trees in cover crop plots with strips would perform as well as or even better than their counterparts in the conventional system.

The second production system of interest in these investigations is bioenergy crop production. Environmental impacts associated with the use of fossil fuels, combined with increasing concerns about rising fossil fuel prices, depletion of the world fossil fuel supply and the need to enhance regional and national energy security have made the large-scale development and use of bioenergy and bio-based products a top priority worldwide. The potential for biofuel production and use to meet this growing renewable energy targets has been emphasized by the Intergovernmental Panel on Climate Change (IPCC 1997). Second-generation cellulosic feedstocks such as perennial grasses (e.g. switchgrass) and wood crops (e.g., poplar

and willow) have been suggested as a more sustainable alternative than first generation stand-based (e.g. Maize) feedstocks. However, the use of energy from biomass does not necessarily mean that their production is sustainable. For instance, there are still knowledge gaps, especially in terms of immediate direct impacts of agricultural management such as land clearing and cultivating as well as N fertilization for bioenergy crop production on the system net greenhouse gas (GHG) balance.

In Chapter 4, *greenhouse gas emissions and N leaching associated with conversion of grassland to short-rotation woody biomass crops in northern Michigan*; *U.S.A.* I tested the assumption that conversion of grassland land to short-rotation woody bioenergy systems would increase nitrate leaching to ground water and soil emissions of nitrous oxide (N_2O), carbon dioxide (CO_2) and methane (CH_4) due to increased SOM mineralization. I therefore expected that this land-use change would incur some levels of GHG "debt" that would undermine the potential environmental benefits of substituting biomass energy with fossil fuels and require years for the bioenergy system to payback this carbon debt. In this study, I sought to answer these two fundamental questions: (i) what is the magnitude of GHG emissions and "debt" associated with grassland conversion to SRWC systems? and (ii) what is the "payback period," the time required for the SRWC systems to overcome this GHG debt and begin providing net GHG benefits? Based on a set of assumptions regarding the contribution of root respiration to total CO_2 efflux and the yield potential of these bioenergy crops, I made an attempt to estimated different levels of GHG debt and the corresponding payback times.

In addition to tillage, the impacts of N fertilizer application on biomass yield and the magnitude of GHG released from agricultural soils have been the subject of a number of

investigations in recent years. However, most of these studies have focused on agronomic crops such as corn, wheat and potatoes which are not only managed differently but also may have different N requirements than bioenergy crops (Grant et al. 2006; Zebarth et al. 2008). In Chapter 5, *Nitrogen fertilization of switchgrass in northern Michigan, U.S.A increases biomass yield and improves net greenhouse gas balance*, I attempted to determine aboveground biomass production and GHG emission responses to inorganic N fertilizer application for switchgrass. I hypothesized that added fertilizer input would increase switchgrass production which would in turn allow offsetting any GHG emissions associated with switchgrass production. The overall goal of this study was to determine the correlation between N addition, and net GHG benefits in a perennial switchgrass system.

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CHAPTER 2

SOIL MICROBIAL BIOMASS AND COMMUNITY CATABOLIC DIVERSITY AS AFFECTED BY INTERCROPPING COVER CROPS WITH FRASER FIR (ABIES FRASERI [PURSH] POIR) – IN A CHRISTMAS TREE PLANTATION IN MICHIGAN, U.S.A.

2.1. ABSTRACT

Intercropping cover crops with tree crops may have important effects on soil productive capacity through their influence on soil organic matter and biology. I investigated soil microbial biomass carbon (SMB-C) and nitrogen (SMB-N) as well as microbial community-level physiological profiling (CLPP) in an intercropping system involving Fraser fir, two leguminous (Dutch white clover and alfalfa) and a non-leguminous (perennial ryegrass) cover crops. For each cover crop, two competition-management practices were evaluated: no strips (cover crop grown in continuous patch) and with strips (maintenance of bare ground strips centered on the tree rows). A conventionally-managed plot was used as a control. SMB-C, SMB-N and soil organic carbon (SOC) and total nitrogen (TKN) levels were assessed at the 0-15, 15-30 and 30-35 cm soil depths and CLPP at the 0-15 cm soil depth. At the 0-15cm depth, cover crop treatments increased SMB-C by 20-50% relative to the conventional plots; whereas changes in SMB-N ranged between 35 to 80% higher in the cover crop treatments than the CONV plots. Values for both SMB-C and SMB-N data followed this order: Legume cover crops > non-legume cover crop > conventional. Groundcover treatments significantly increased both soil SMB-C: SOC ratio ($P<0.001$) and SMB-N: TKN ratio ($P<0.01$) at the surface soil layer (0-15 cm), whereas no significant differences were recorded among treatments at the deeper soil layers. The legumes also increased microbial catabolic diversity compared to the conventional treatment. Cover crop treatment with strips had little effects on soil microbial biomass and diversity relative to the no strip treatments. These results suggest that cover cropping with proper management can provide a larger, more active, and more metabolically diverse soil microbial community and be an alternative approach to sustainable tree production.

2.2. INTRODUCTION

In recent years, there has been increasing interest in the use of management practices that maintain soil productivity and environmental quality while improving farm profitability (Baumann et al. 2001). Intercropping legume and/or non-legume cover crops is a practice that has been widely investigated and promoted as a practical way to achieve sustainable production (Walsh et al. 1996). Cover crops reduce soil erosion, add organic matter to the soil, conserve soil humus, improve soil aeration and structure, and improve soil nutrient status (Broughton 1977). Because of these benefits, cover cropping is increasingly becoming a common practice in cereal and vegetable crop production systems. However, to my knowledge, studies have not yet been conducted to evaluate the potential beneficial contribution of cover crops to Christmas tree production, which cover approximately 150,000 ha of land in production in the United States (Vilsack and Clark 2009) and represent one segment of the forestry sector that uses substantial fertilizer inputs comparable to agronomic systems (Rothstein 2005).

Soil management in cover cropping systems generally involves the use of mowed, tilled or killed cover crops, to increase soil organic matter levels and steadily release available nutrients to the crop plants as the organic matter breaks down (Sanchez et al. 2007). In this process, the action of soil organisms is a major determinant of nutrient cycling rates and plant growth. Planting either grass or legume cover crops in the interspaces of plantations increases plant residue inputs to soils (Dinesh et al. 2004) and therefore may stimulate soil microbial activity and increase mineralizable C and N (Mendes et al. 1999). Moreover, many studies have reported plant-induced quantitative and qualitative variations in nitrogen and carbon flow to the soil as different plant species may maintain a different microbial biomass and activity (Drury et al. 1991; Haynes and Francis 1993; Groffman et al. 1996; Chantigny et al. 1997; Angers and

Caron 1998; Mullen et al. 1998). It is also well documented that farming systems and management practices greatly influence microbial populations and activities in soil (Bossio et al. 1998; Mendes et al., 1999).

Soil microbial biomass and activity respond rapidly to changes in agronomic practices and other disturbances (Kennedy and Papendick 1995; Cong et al. 2006; Powlson et al. 1987; Lundquist et al. 1999), and have been used to ascertain early changes in soil fertility due to different soil management practices (Doran and Zeiss 2000; Wang and Wang 2008). In fact, changes in microbial activity and in the ratio of soil microbial biomass to soil organic matter (soil microbial biomass quotient) are often used as rapid indicators of changes in soil organic matter content and soil fertility (Ocio et al. 1991). To date, many studies have focused on the effects of cover crops on soil properties and plant productivity in several agronomic crop production systems, including vegetable, cereal crops and orchards (Sainju and Singh 1997; Macdonald et al. 2005; Lehmann et al. 2000; Hanninen 2002; Sanchez et al. 2007). However, very little information is available concerning the effects of cover crops and management practices on soil microbial biomass and activity in Christmas tree plantations.

This study was part of a research project designed to investigate the impact of cover crops on nutrient cycling, soil and water quality and Fraser fir (*Abies fraseri* [Pursh] Poir) performance. The objective of the present study was to examine the effects of various groundcover management practices on soil microbial properties in a Fraser fir production system in Michigan. Incorporating cover crops into the plantation is expected to increase the amount of organic matter returned and diversity of metabolic substrates to the soil that are key drivers of microbiological processes. Consequently, I hypothesized that the overall site chemical and biological conditions, particularly soil organic carbon and total nitrogen as well as soil microbial

biomass and catabolic diversity would be increased in cover crop-Fraser fir intercropping plots relative to a conventional production system. Additionally, I anticipated that, because plant residues from leguminous cover crops are more labile (having higher N content and smaller C:N ratios) than those of non-leguminous cover crops, plots with N-fixing cover crops would yield significantly higher soil microbial biomass and catabolic diversity than those with a non-N-fixing cover crop.

2.3. MATERIALS AND METHODS

2.3.1. Site description

A field experiment was established in the spring of 2007 at the Tree Research Center (TRC) (42.67^{o}N, 84.46^{o}W) on the campus of Michigan State University (MSU) in East Lansing, Michigan. The local climate is characterized by mean temperature of 15.5 °C and -6.6 °C during summer and winter periods, respectively. Annual mean precipitation is 853 mm with rainfall distributed fairly evenly throughout the year. Soil at this site is classified as a fine-loamy, mixed, active, mesic Aquic Glossudalf (USDA/NRCS-MAES, 1992). The general soil chemical characteristics of the site are: 35% silt + clay, pH 5.6 and 13 cmol kg^{-1} CEC (Rothstein 2005). Soil total C and N, inorganic N and Mehlich III extractable nutrients (P, K, Ca and Mg), measured at the initiation of the plantation establishment are provided in Table 2.1. The site had been used for crop cultivation, primarily maize with occasional rotations of wheat and soybeans, for at least the past 30-40 years. The experiment was located in a fenced area to prevent the impact of deer browsing.

Table 2.1 Physical and chemical characteristics of the topsoil (0–15 cm) of the study site

Soil Characteristics	Values
Sand (%)[a]	65
Silt + Clay (%)[a]	35
pH (soil/water ratio of 1:1)[a]	7.24
Soil organic C concentration (%)[b]	1.96 ±0.84
Soil total N concentration(%)[b]	0.16 ±0.08
Mehlich-3 (mg kg^{-1})[b]	35.46±4.38
Exch. K (mg kg^{-1})[a]	403.91±31.64

[a] Values are obtained from a previous study (Rothstein 2005)

[b] Samples were taken in May 2007, at tree planting and values are means (±se; n = 21)

2.3.2. Plant materials, experimental design and management

Fraser fir transplants (plug + 2) were obtained from a local commercial nursery (Peterson's Riverview Nursery), and machine-planted (Whitfield planter) at a spacing of 1.8 × 1.8 m. Fraser fir seedlings were planted into a chisel-plowed and dragged field soil on 8 May 2007. Seeds of common Dutch white clover, alfalfa (SS 100 brand) and perennial ryegrass (VNS) were purchased from Michigan State Seeds (Grand Ledge, Michigan, U.S.A.) and hand-seeded on 22 May 2007. The seeding rates used were 28 kg ha^{-1} for clover and alfalfa, and 16 kg ha^{-1} for perennial ryegrass.

The experiment was established in a randomized complete block design with three replications in a field measuring 32.4 m × 50.4 m. Blocks and experimental plots were 10.8 m × 50.4 m and 7.2 m × 10.8 m, respectively. Each rectangular plot contained a total of 35 trees (5 × 7 trees). Trees in border rows were used as buffers and not included in measurements, therefore restricting data collection to the remaining 15 interior trees in each plot. Each block had two plots per cover crop, managed either with no strips (NS) or with strips (S). The NS treatments consisted of growing each cover crop continuously over the entire plot. In contrast, in the S treatments, the assigned cover crop was intercropped between the tree rows while maintaining, through glyphosate application, a clear strip of 0.61 m wide, centered on the tree row. Control plots were managed conventionally (CONV); they contained no cover crop and weeds were completely controlled with glyphosate (active ingredient concentration =1.1kg ha^{-1}). Thus, the treatments were as follows: conventional system (CONV), Dutch white clover with strips (DWCS), Dutch white clover with no strips (DWCNS), alfalfa with strips (ALFS), alfalfa with no strips (ALFNS), perennial ryegrass with strips (PRGS) and perennial ryegrass with no strips (PRGNS).

No N fertilizer was applied to any plots in 2007 as it may injure first-year seedling roots (Koelling, 2002). However, during the second growing season, 16 g N per tree (50 kg N ha^{-1}) as ammonium sulfate [$(NH_4)_2SO_4$] was applied one time on 13 May 2008 to the control plots but not to the cover crop plots. The cover crop plots were not fertilized. Once the cover crops were fully established, mechanical mowing was performed at 3 cm above the ground every three to four weeks in 2007 (2 July, 26 July, 21 August and 18 September) and 2008 (27 May, 24 June, 17 July, 14 August, and 11 September) to control cover crop growth, minimize the competition with the trees, and add green manure to the soil surface. Glyphosate was sprayed twice during each growing season (2007 and 2008) to control weeds in the CONV plots and within the tree rows in the S plots. The whole field was protected with an electric fence to prevent deer browsing. A description of treatments and management practices applied in 2007 and 2008 can be found in Appendix –Table A.1.

2.3.3. Soil sampling and analysis

Fifteen randomly-selected soil sub-samples per plot were collected with a 2.5 cm diameter corer and composited into one sample per plot. Soil samples were collected in mid-October of 2007 and 2008, corresponding to the end of the first and second growing seasons, respectively. Of the 15 soil cores collected per plot, in the cover crop plots with strips, nine were taken within the cover crop zone and 6 within the bare ground zone, proportional to the area of strip zone. Soil was collected from 0-15, 15-30, and 30-45 cm depths. Samples were placed in double plastic zip-lock bags, securely tied, and kept on ice in a cooler before transporting them to the laboratory at Michigan State University for analysis. Soil moisture content was determined gravimetrically on oven-dried samples (105^{o}C for 48hrs). Soil pH (soil:water ratio of 1:1) values were measured

from the air-dried soil (passed through a 2 mm sieve) with a HORIBA pH/COND meter (model D-54; Spectrum Technologies, Inc. Japan). Soil organic C (SOC) and total N (TN) of soil samples were determined by combustion with an elemental analyzer (Model ECS 4010, COSTECH Analytical, Valencia, CA).

2.3.4. Microbial biomass analysis

Soil samples collected in 2007 were used for microbial biomass determination. Soil microbial biomass C (SMB-C) and N (SMB-N) were assessed from these samples by the chloroform fumigation-extraction method described by Brookes et al. (1985) and Beck et al. (1997). Soil solutions obtained from the fumigated and non-fumigated samples were analyzed for total dissolved C and N by oxidative combustion-infrared analysis and oxidative combustion-chemiluminescence, respectively (Shimadzu models TOC-V_{CPN} analyzer and TNM-1 unit, Kyoto, Japan). Microbial biomass C and N were calculated as the difference between C and N in the fumigated and non-fumigated samples using 0.45 as a correction factor for SMB-C and 0.54 as a correction factor for SMB-N (Brookes et al. 1985; Beck et al. 1997). Soil microbial quotients, the ratios of soil microbial biomass to soil organic C or N, were calculated as SMB-C: SOC and SMB-N: TN.

2.3.5. Microbial community-level physiological profile analysis

The functional diversity of the soil microbial community was measured on soil samples (0-15 cm) collected in 2007, using the Biolog^{TM} Eco Plate system (Biolog Inc., Hayward, CA, USA). The Biolog approach is based on relative changes in carbon source utilization. The Biolog micro-plates were chosen as they contain 31 of the most useful carbon sources for community analysis of mixed cultures. Substrate-utilization patterns of the soil microbial population were

determined by a procedure adapted from Garland and Mills (1991). Ten grams of field-moist soil were shaken with 90 ml of a sterilized saline solution (0.85% NaCl, w/v) for 60 min and then brought to 10^3 final dilutions. A 150 µL aliquot was inoculated into each micro-plate well. The plates were kept at a constant temperature (25 °C) in the dark. The absorbance of the content of each well at 595 nm was measured at 0, 24, 96, 120, 168, and 240 h using an automated plate reader (Dynatech, MR-7000, Dynatech Laboratories - U.S.A.). Readings of the plates at day 0 and readings generated from the control wells were subtracted from subsequent readings to eliminate background color generated by the substrates and the bacterial suspension.

Microbial activity in each micro-plate, expressed as average well color development (AWCD), was calculated for each sample at each time point by dividing the sum of the optical density data by 31 (number of substrates). I used an optical density (OD) of 0.25 as the threshold for a positive response (Garland 1997) to calculate richness (R), or the total number of oxidized C substrates, a Shannon-Weaver index (*H'*) of metabolic diversity and evenness of response (E). The Shannon-Weaver index was calculated as follows:

- $H' = -\sum p_i \ln(p_i)$ Eq.(2.1)

where p_i is the ratio of the activity on each substrate (OD_i) to the sum of activities on all substrates ($\sum OD_i$). Its value usually ranges from 0.4 to 4 and expresses a greater metabolic diversity when it is close to 4 and a lower metabolic diversity when close to 0.4 (Frontier and Pichod-Viale, 1988). Substrate evenness (E), which is a measure of the uniformity of activities across all substrates, was calculated as follows:

- $E = H'/\log(R)$ Eq.(2.2)

where *H'* is the Shannon Weaver diversity index and R is the number of different substrates used by the community (counting all positive OD readings). E values range between 0 and 1 with lower uniformity of activity when the values are close to 0 and a greater uniformity when the values get close to 1. Micro-plate readings measured at 0, 24, 96, 120, 168, and 240 h of incubation were used to calculate AWCD. However, plate readings at 120 h were used to calculate R, *H'* and E since it was the shortest incubation time that allowed the best resolution among treatments.

2.3.6. Data analysis

Data were analyzed as a randomized complete block design using Proc Mixed in Statistical Software Package SAS version 9.1 (SAS, 2002-2003). For soil pH, total C, total N, C:N ratio, SMB-C and SMB-N data were analyzed taking into account the three sampling depths. The statistical model thus included one random factor (block) and two (2) fixed factors: groundcover treatments and depths, with the latter variable considered as repeated measurements. The potential effect of depth on all parameters in the various ground cover treatments was initially assessed using an analysis of variance/ covariance (ANCOVA) structure. Because the interactions between groundcover treatments and depths on some of the parameters (e.g. total N in soil, SMB-C and SMB-N) were statistically significant, an evaluation of treatment effects was performed using a simple one-way analysis of variance (ANOVA) only for the 0-15 cm depth. I used Fisher's Least Significant Difference (LSD) test to make pair-wise comparisons of individual treatment means. Significance for the overall treatment effects and pair-wise comparisons was accepted at $\alpha = 0.05$.

Microbial community level-physiological profiling data were analyzed using the soil samples collected from 0-15 cm depth. The AWCD, R, *H'* and E data were subjected to a one-

way ANOVA in SAS. The standardized OD values obtained from each of the 31 substrates for each treatment were further analyzed using multivariate techniques (principal component analysis in Minitab 15.1.1.0) to differentiate among microbial communities based on substrate utilization profiles (Garland and Mills 1991; Winding 1994; Zak et al. 1994; Grayston and Campbell 1996).

2.4. RESULTS AND DISCUSSION

2.4.1. Soil properties

There were no measurable effect of treatments on the soil total C and N concentrations as well as soil pH (all $P>0.05$), and therefore, data were not presented here. The lack of treatment differences in the present study is a common finding in most short-term C change studies. Small gradual changes in SOM are generally difficult to detect because of high background carbon level and natural variability of soils (Mendes et al. 1999; Brye et al. 2002).

2.4.2. Soil microbial biomass and quotients

Relative to the CONV treatment, all cover crop treatments significantly ($P<0.001$) enhanced soil microbial biomass (SMB-C and SMB-N) at the 0-15 cm depth (Figs. 2.1-a and 2.1-b). However, no statistical difference was observed among groundcover treatments for both SMB-C and SMB-N at the deeper soil layers (15-30 and 30-45 cm). At the 0-15cm depth, cover crop treatments increased SMB-C by 20-50% relative to the CONV plots; whereas changes in SMB-N ranged between 35 to 80% higher in the cover crop treatments than the CONV plots. Alfalfa and clover were more effective in improving SMB-C and SMB-N than perennial ryegrass. Increased organic C input from cover crop biomass is probably the dominant factor explaining the greater amount of SMB-C and SMB-N in all cover crop plots at the soil surface layer (0-15 cm) than CONV plots. Franzluebbers et al. (1999) showed that as the total organic C pool expands or contracts due to changes in C inputs to soil, the microbial pool also expands or contracts. A continuous uniform supply of C from crop residues serves as an energy source for microorganisms (Govaerts et al. 2007; Campbell et al. 1997). By mowing cover crops and leaving residues on the soil, organic carbon accumulates in the topsoil, and microbial substrates of different quality and quantity are made available (Govaerts et al. 2007).

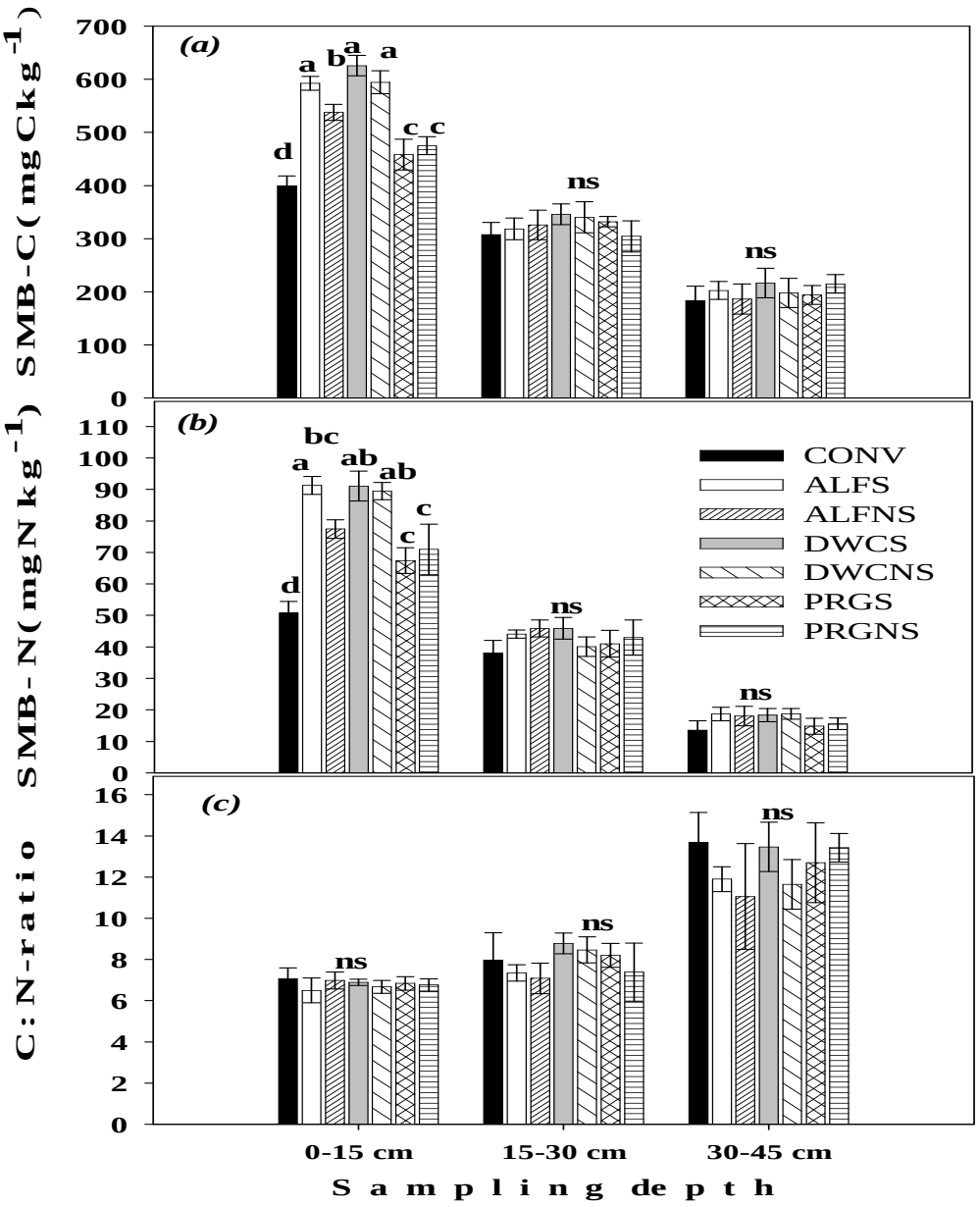

Figure 2.1 (a) Soil microbial biomass carbon microbial, (b) biomass nitrogen and (c) microbial biomass C:N ratio (C) as influenced by groundcover management. Treatments are: Conventionally managed (CONV), Dutch white clover with strips (DWCS), Dutch white clover with no strips (DWCS), alfalfa with strips (ALFS), alfalfa with no strips (ALFNS), perennial ryegrass with strips (PRGS), and perennial ryegrass with no strips (PRGNS.(ns =not significant)

Leaving cover crops residues on the soil surface as green manure also increases microbial abundance, because of improved conditions for growth and reproduction in the top soil layer (Govaerts et al. 2007). The fact that the residue is not incorporated into deeper soil layers most likely explains the similarity in SMB-C and SMB-N among the various treatments at 15-30 cm and 30-45 cm layers.

Whereas I expected greater cover crop residue inputs from plots with no strips compared to plots with strips, creating strips did not significantly affect SMB-C or SMB-N in the cover crop treatments (Fig. 2.2-c). SMB-C and SMB-N in plots managed with strips averaged 558.8 mg C kg^{-1} dry soil and 83.2 mg N kg^{-1} dry soil while plots with no strips averaged 535.8 mg C kg^{-1} dry soil and 79.3 mg N kg^{-1} dry soil, respectively. Legume cover crop treatments showed significantly ($P<0.001$) higher SMB-C (average of 587.7 mg C kg^{-1}) than the grass cover crop (466.6 mg C kg^{-1}) and CONV treatments (399.0 mg C kg^{-1}). Similarly, SMB-N was significantly ($P<0.001$) higher in legume cover crops treatments (87.3 mg N kg^{-1}) than in the non-legume cover crop (69.2 mg N kg^{-1}) and the CONV (50.7 mg N kg^{-1}) treatments.

Soil microbial biomass C: N ratio did not differ among groundcover treatments at any of the three soil depths. However, Soil microbial biomass C: N ratio significantly ($P<0.05$) increased with soil depth (Fig. 2.1-c), ranging from 6.5-7.1, 7.1-8.8, and 11.1-13.7 at the 0-15, 15-30 and 30-45 cm soil depths, respectively. This trend is the opposite of that observed for soil total C: N and could indicate a shift in community composition, perhaps from a bacterial-dominated community on the top soil layer to a fungal-dominated community in deeper soil layers. In fact, previous research have reported that a high soil microbial biomass C: N ratio

generally indicates higher fungal versus bacterial populations in a soil (Jenkinson 1976; Moore et al. 2000).

Groundcover treatments significantly increased both soil SMB-C: SOC ($P<0.001$) and SMB-N: TKN ($P<0.01$) at the surface soil layer (0-15 cm), whereas no significant differences were recorded among treatments at the deeper soil layers (Table 2.2). At the surface soil layer where differences among treatments occurred, soil microbial quotients were significantly higher from soil collected under legume cover crop plots than the non-legume cover crop and the CONV treatments. The contrasting effect observed between legume and non-legume cover crops on soil microbial quotients might be due to differences in the amount and quality of organic matter produced by these two categories of cover crops. The relatively lower quotients in the CONV and perennial ryegrass plots may suggest lower availability of organic substrates in these environments, whereas a great supply of more labile organic substrate may explain the relatively higher microbial quotients in the leguminous cover crop plots.

Table 2.2 Microbial Biomass quotients (SMB-C:Corg and SMB-N:Norg) measured at 0-15, 15-30 and 30-45 cm soil depth for each groundcover management treatment. †Treatments are: Conventionally managed (CONV), Dutch white clover with strips (DWCS), Dutch white clover with no strips (DWCNS), alfalfa with strips (ALFS), alfalfa with no strips (ALFNS), perennial ryegrass with strips (PRGS), and perennial ryegrass with no strips (PRGNS); (n=3).

Groundcover treatments	SMB-C :Corg			SMB-N :Norg		
	0-15 cm	15-30cm	30-45cm	0-15 cm	15-30cm	30-45cm
CONV†	0.020 a	0.018 a	0.033 a	0.032 a	0.027 a	0.028 a
ALFS	0.029 c	0.022 a	0.049 a	0.051 c	0.031 a	0.039 a
ALFNS	0.025 b	0.019 a	0.024 a	0.049 bc	0.031 a	0.026 a
DWCS	0.031 c	0.026 a	0.042 a	0.049 bc	0.032 a	0.032 a
DWCNS	0.025 b	0.020 a	0.028 a	0.046 bc	0.027 a	0.028 a
PRGS	0.022 a	0.020 a	0.033 a	0.037 ab	0.027 a	0.028 a
PRGNS	0.021 a	0.021 a	0.045 a	0.039 abc	0.037 a	0.032 a
P-value	**P<0.001**	P>0.05	P>0.05	P=0.05	P>0.05	P>0.05

2.4.3. Community-level physiological profile of soil microbial communities

The color response in a given well is related to the number of microorganisms (functional diversity) which are able to use the substrate within the well as a sole carbon source, and is therefore used to assess microbial community structure in a given ecosystem (Garland and Mills, 1991). Average well color development (AWCD) recorded as optical density (OD) and the number of well responses expressed as the catabolic diversity from all treatments followed the same pattern (sigmoidal curve) throughout the incubation period (Figs. 2.2-a and 2.2-b), although the rate of increase varied with different treatments. However, both the AWCD and the catabolic diversity of communities from CONV plots were lower than the cover crop managed plots. The high AWCD values recorded from soil collected under the cover crop treatments relative to the CONV plots may suggest that cover crop treatments enhanced the diversity of microbial communities, resulting in a higher substrate utilization rate.

The number of well responses (catabolic richness) followed the same pattern as AWCD throughout the incubation (Fig. 2.2-b). For all treatments, only a few wells showed no color response after 96 h of incubation. Significant differences among treatments ($P<0.01$) were found in catabolic richness, Shannon diversity and evenness (Figs. 2.3-a, 2.3-b and 2.3-c). All cover crop treatments, both with S and NS, had significantly higher ($P<0.001$) microbial catabolic richness than the conventional treatments (Fig. 2.3-a). Similarly, plots managed with both legume cover crops, had significantly higher *H'* values than CONV plots. These results are in agreement with results of studies conducted elsewhere which found organic farming systems to significantly enhance soil microbial indexes compared with conventional systems (Mäder et al. 2002; Wu et al., 2008; Drinkwater et al,. 1995; Saison et al., 2006).

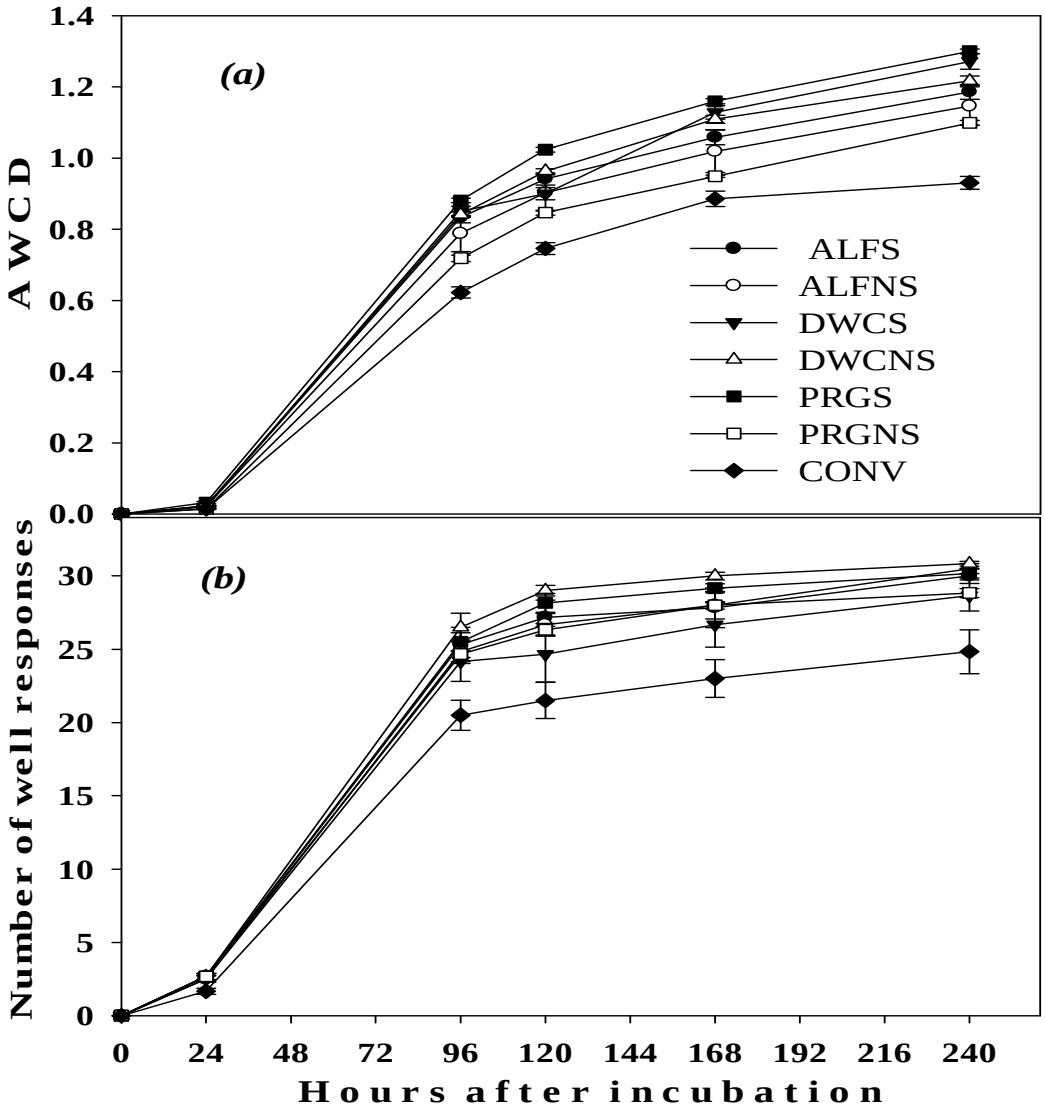

Figure 2.2 (a) Average well color development (AWCD) and (b) average catabolic diversity obtained from Biolog EcoPlate ^{TM} incubation of different groundcover treatments in a Fraser fir plantation. Treatments are: Conventionally managed (CONV), Dutch white clover with strips (DWCS), Dutch white clover with no strips (DWCNS), alfalfa with strips (ALFS), alfalfa with no strips (ALFNS), perennial ryegrass with strips (PRGS), and perennial ryegrass with no strips (PRGNS).

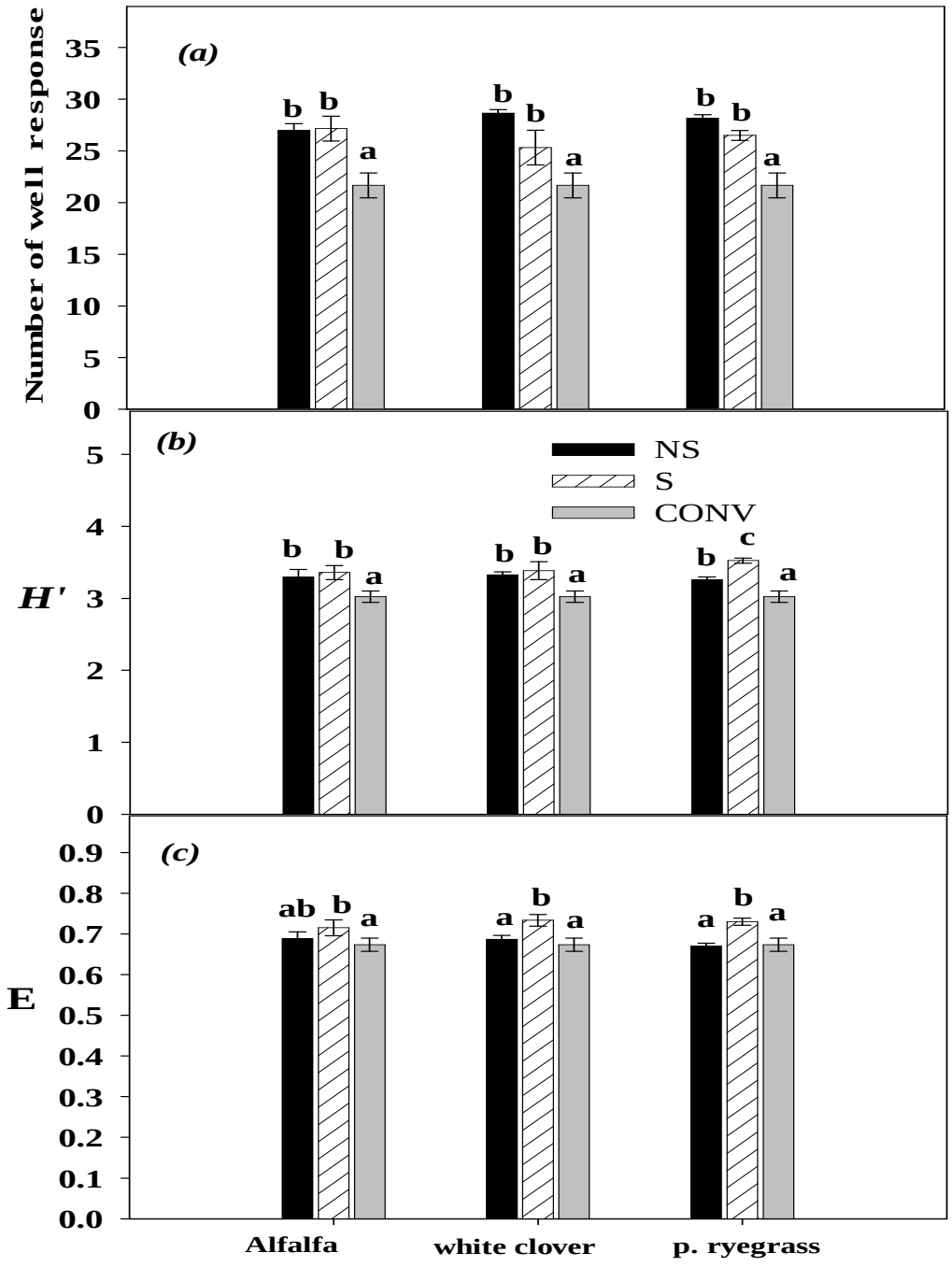

Figure 2.3 (a) Species richness, (b) Shannon index of diversity and (c) Evenness index as influence by different groundcover management (October/November 2007 data) †NS is no strips, S is strips and CONV is conventional treatments

In order to determine the extent of differentiation between the conventional and the cover crop treatments with regard to carbon source utilization, the OD data were subjected to multivariate analyses (principal component [PC] analysis). The trends observed on soil microbial biomass and diversity indexes were supported by results from the multivariate analysis. Contrasting patterns were apparent between the cover crop treatments and the CONV treatment (Fig. 2.4).

The results showed that PC1 had a high coordinate value (Eigen value of 17.04) and explained 56% of the total variance in the data; while the second principal component had a variance of 4.90 and accounted for 16% of the data variability. While PC1 is the axis that differentiates the striped legumes from the other treatments, PC2 differentiates the control plots from all of the cover crop treatments. In PC1 space, soils from the two leguminous cover crop plots, managed with strips, had distinct catabolic responses from the other treatments (i.e. CONV, PRGS, PRGNS, ALFNS and DWCNS). Carbohydrates and carboxylic acids were the potential carbon sources used by microorganisms in the leguminous cover crop plots with strips, while all phosphorylated chemicals and amino acids were the most utilized carbon sources in soil collected from the CONV plots and to some extent the non-leguminous cover crop plots (Table 2.3). The combination of the “strip effect” and the availability of high labile C substrates from the leguminous cover crops might have provided an ideal condition in the soil surface for specific groups of microbial populations with affinity to utilize these substrates.

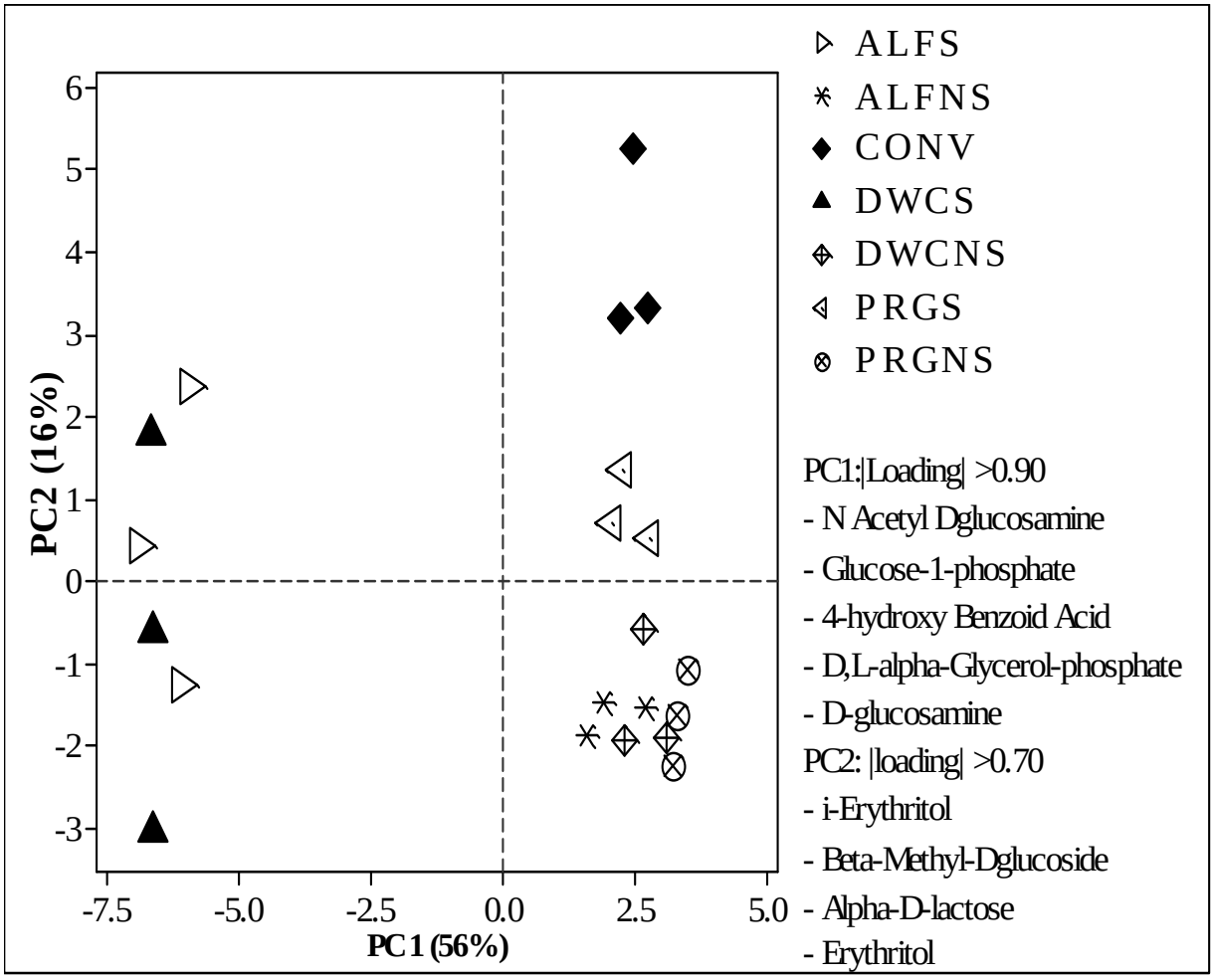

Figure 2.4 Principal component analysis (PCA) performed on BIOLOG™ Eco Plates from data microbial community substrate utilization patterns of soil extracts from different groundcover management at 120 h incubation period. †Treatments are: Conventionally managed (CONV), Dutch white clover with strips (DWCS), Dutch white clover with no strips (DWCNS), alfalfa with strips (ALFS), alfalfa with no strips (ALFNS), perennial ryegrass with strips (PRGS), and perennial ryegrass with no strips (PRGNS).

I observed higher rates of utilization of N-acetyl-D-glucosamine in soils collected from the leguminous cover crops, as compared to the control and the non-legume cover crop plots (Table 2.3). This could be an indication that the soil microbial decomposer community in these soils is dominated by fungal mycelia as this particular substrate is an important component of fungal tissues (Mäder et al. 2002). Fungal biomass generally contains significant amounts of chitin, which releases N-acetyl-D-glucosamine when enzymatically degraded. Suitable enzymes for degrading the chitin include chitinases and β-N-acetyl-glucosaminidases, which are generally secreted from eukaryotic or prokaryotic microorganisms (Bohlmann et al. 2004). The presence of ample amount of fungal biomass might have stimulated high numbers of microorganisms that possess these particular enzymes involved in chitin decomposition.

Table 2.3 List of all 31 C-substrates in Biolog EcoPlate™, classified by category of carbon and their relative substrate utilization in each groundcover management treatment. †Treatments are: Conventionally managed (CONV), Dutch white clover with strips (DWCS), Dutch white clover with no strips (DWCNS), alfalfa with strips (ALFS), alfalfa with no strips (ALFNS), perennial ryegrass with strips (PRGS), and perennial ryegrass with no strips (PRGNS). (n=3)

C-Categories	C-Substrates	Groundcover management treatments							P-value
		CONV†	ALFS	ALFNS	DWCS	DWCNS	PRGS	PRGNS	
		----------%----------							
amines	*Phenylethyl-amine*	4.3	1.6	5.4	2.1	6.0	5.0	4.2	0.01
	Putrescine	2.5	2.2	2.7	2.1	2.2	2.2	2.5	ns
Amino acids	*Glycyl-L-glutamic-acid*	1.1	1.4	2.3	1.2	0.8	1.8	2.7	ns
	L-arginine	7.0	3.5	6.5	4.1	6.0	6.1	6.2	0.001
	L-asparagine B1	7.9	4.9	6.8	5.0	6.6	6.6	7.5	0.001
	L-phenylalanine	5.8	2.9	3.8	1.4	5.0	5.0	4.6	<0.001
	L-serine	1.0	3.6	1.2	3.5	1.4	1.3	1.7	<0.001
	L-threonine	1.0	0.6	1.2	0.5	1.7	1.1	1.5	0.001
Carbohydrates	*D-cellobiose*	3.8	6.8	2.7	6.7	2.6	3.0	2.7	<0.001
	D-mannitol	5.0	6.1	3.4	5.5	4.0	3.6	4.4	0.001
	D-xylose	2.8	6.0	3.9	6.1	4.2	3.3	4.0	<0.001
	i-erythritol	1.3	2.2	3.1	1.6	2.9	3.2	2.8	0.01
	N-acetyl-D-glucosamine	0.4	6.9	7.4	7.4	5.5	4.3	5.3	<0.001
	α-D-lactose	2.4	2.5	3.2	2.8	2.6	3.5	3.1	ns
	β-methyl-D-glucoside	0.6	4.5	3.3	4.9	4.5	4.0	5.8	0.002
	D-galactonic acid γ-lactone	2.3	4.9	4.1	4.7	3.1	3.4	3.5	<0.001

Table 2.3 (Cont'd)

C-Categories	C-Substrates	Groundcover management treatments							P-value
		CONV†	ALFS	ALFNS	DWCS	DWCNS	PRGS	PRGNS	
		----------%----------							
Carboxylic acids	*4-hydroxy benzoic acid*	1.1	2.9	0.8	2.7	1.0	1.1	0.9	<0.001
	2-hydroxy benzoic acid	0.6	0.6	1.0	0.1	1.1	0.8	0.4	ns
	D-galacturonic-acid	0.5	5.5	2.1	5.4	1.3	1.9	1.5	<0.001
	D-glucosaminic-acid	0.1	2.5	1.2	2.0	0.6	0.2	0.3	<0.001
	D-Malic acid	2.2	2.3	2.9	3.6	2.7	2.7	2.3	0.041
	iItaconic acid	2.8	1.7	2.7	2.5	2.1	3.4	2.2	0.006
	α-Ketobutyric acid	1.0	0.5	1.5	0.5	1.5	1.7	1.8	<0.001
	γ-Hydroxybutyric acid	0.1	0.2	0.4	0.3	0.8	0.4	0.4	ns
Esters	*Pyruvic acid-methyl ester*	5.3	3.1	3.2	2.8	3.1	3.6	4.1	0.027
Phosphorylated chemicals	*D,L-α-glycerol phosphate*	4.0	3.8	0.9	3.7	1.1	3.8	1.9	<0.001
	glucose-1-phosphate	9.3	7.1	3.2	7.3	3.8	7.1	2.7	<0.001
Polymers	*Glycogen*	8.3	2.4	5.3	2.4	5.2	4.5	5.0	<0.001
	Tween 40	6.4	2.6	5.6	3.4	7.0	4.6	6.2	<0.001
	Tween 80	6.3	3.5	3.7	3.3	4.6	4.2	4.9	0.021
	α-Cyclodextrin	2.8	0.7	4.5	0.4	5.0	2.6	2.9	<0.001
	Total	100	100	100	100	100	100	100	

Also, Table 2.3 indicates that the rates of utilization of the two (2) phosphorylated substrates were the highest in soil from the control and the cover crop plots with strips. Glyphosate (N-phosphonomethyl glycine), a non-selective herbicide used for control of unwanted vegetation in these targeted areas is reported in previous studies to be rapidly and completely degradable by specific soil micro-organisms (Jacob et al. 1985; Krzysko-Lupicka and Sudol 2008; Kremer and Means 2009). The glyphosate applications may have selected specific microorganisms, most likely fungal species, capable of metabolizing phophorylated chemicals. For instance, in a recent culture-based study, different strains of a fungal species (*Fusarium* spp.) isolated from soil were able to successfully metabolized glyphosate and used it as a phosphorus source (Castro et al. 2007). In this experiment, although the glyphosate was not intentionally soil applied, a significant concentration of material might have reached the soil surface during broadcast; thus making it available to soil micro-organisms with the ability to utilize it as phosphorus source.

2.5. CONCLUSIONS

This study indicates that the leguminous cover crop systems are better in increasing soil microbial biomass and catabolic diversity than the non-leguminous cover crop and the CONV systems, probably due to the quantity and quality of the green manure input into the system. These preliminary results indicate that inclusion of leguminous or non-leguminous cover crops into Fraser fir production systems can lead to healthier soil and could be an alternative to the use of inorganic N fertilizers in Fraser fir Christmas tree plantations. Nitrogen mineralization, nutrient fluxes and N losses through leaching and their effects on tree nutrition and growth are being investigated to confirm these trends and determine the overall impact of these management practices on Fraser fir production.

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CHAPTER 3

EFFECTS OF GROUNDCOVER MANAGEMENT ON SOIL PROPERTIES, TREE PHYSIOLOGY, FOLIAR CHEMISTRY AND GROWTH IN A NEWLY ESTABLISHED FRASER FIR (*ABIES FRASERI* [PURSH] POIR*)* PLANTATION IN MICHIGAN, U.S.A

3.1. ABSTRACT

Incorporating cover crops into Fraser fir (*Abies fraseri*) Christmas tree plantations may improve soil fertility and tree performance. Dutch white clover (*Trifolium pratense*), alfalfa (*Medicago sativa*) and perennial ryegrass (*Lolium perenne*) were grown in a newly established Fraser fir plantation using two competition-management practices; no strips (NS) by growing each cover crop throughout the entire plot and strips (S) by creating a 61 cm-wide bare zone centered on the tree rows. A conventionally-managed system (CONV) was used as a control. The cover crop aboveground biomass and N content were assessed. Soil available N (NO_3^--N and NH_4^+-N) and N mineralization were measured at 0-15, 15-30 and 30-45 cm soil depths. Tree survival, height, diameter, chlorophyll fluorescence (F_v/F_m), stem water potential (Ψ_w) and foliar nutrients were also evaluated. Biomass production was as high as 13.9, 10.2 and 5.9 Mg DM ha^{-1} yr^{-1} for clover, alfalfa and ryegrass, respectively. Cover cropping increased soil available N by 1.5- and 2.2-fold relative to the CONV treatment in the top soil layer in 2007 and 2008. Tree seedling survival and growth in the S and CONV systems were similar. In contrast, NS treatments resulted in poor seedling survival and growth relative to the S and CONV plots. Plant stem Ψ_w decreased significantly for seedlings on the NS treatments relative to their counterparts on the S and CONV plots. Conversely, cover cropping had marginal effects on foliar nutrients and F_v/F_m. Cover cropping with strips can be an efficient strategy for maintaining productivity in Fraser fir Christmas plantations.

3.2. INTRODUCTION

In the past two to three decades, increasing crop productivity and conserving the environment have been major challenges facing the agricultural and forestry sectors. Whereas N fertilization can maximize Christmas tree growth and produce more marketable trees in a shorter period of time, excessive use of anthropogenic inputs of reactive nitrogen (N) and subsequent N losses through runoff and leaching, pose potential pollution risks to many ecosystems (Rothstein 2005). The issue is generally not important in forestry production systems, which receive far less fertilizer inputs than agronomic and horticultural production systems (Di and Cameron 2002; Rothstein 2005). It is, however, well documented that Christmas tree production represents one segment of the forestry sector that uses substantial fertilizer inputs comparable to agronomic systems, and these inputs can contribute to N pollution (Rothstein 2005; Pedersen et al. 2006). Given the large scope of the production of this specialty crop and its potential environmental risks, alternative management systems need to be sought.

In North America, approximately 33 to 36 million Christmas trees are produced each year. By comparison, the estimated total annual production from Christmas tree farms in Europe ranges between 50 and 60 million trees (Frampton and McKinley 1999). According to the United States 2007 census of agriculture, there were an estimated 17,367 Christmas tree farms in the U.S.A. and Michigan ranks third among all Christmas tree producing states and harvested about 1.6 million trees annually (Vilsack and Clark 2009).

A number of species are grown as Christmas trees in Michigan such as Scotch pine (*Pinus sylvestris*), Fraser fir (*Abies fraseri* [Pursh] Poir), Douglas-fir (*Pseudotsuga menziesii*), Colorado blue spruce (*Picea pungens*) and Balsam fir (*Abies balsamea*). However, demand for Fraser fir has increased, primarily due to its overall appearance, needle length, crown

architecture and fragrance. In general, Firs have gained market share in the last 10-15 yrs, and Fraser fir is the most popular Eastern/mid-western fir. By 2000, Fraser fir alone occupied about one fifth of the total Christmas tree acreage in Michigan and is still expanding (Chastagner and Benson 2000). On the other hand, Fraser fir is known to be more demanding with respect to soil fertility, and performs poorly when grown on marginal land that is suitable for other conifers such as spruces and pines (Leuty 2005). While weed control alone may be enough to adequately release enough nutrients for other conifer species, Fraser fir requires close attention to nitrogen availability (Koelling et al. 1998). Because of continued increases in the cost of fertilizers and the declining price of trees due to a nationwide oversupply (Sidebottom 2009), conventional fertilization in Fraser fir plantations may not be cost-effective and may reduce overall farm profitability.

Because of these economic and environmental considerations, it is important to seek alternative cropping systems for Fraser fir production that can improve sustainability and enhance profitability. One potentially useful strategy is the incorporation of cover crops (Ruffo and Bollero 2003). Cover crops offer a valuable source of nitrogen, especially if they are nitrogen fixers such as Dutch white clover (*Trifolium repens*) and alfalfa (*Medicago sativa*) (Smith et al. 1996). Such cover crops are regularly planted to improve soil fertility, increase crop nitrogen economy and obtain better growth in cereal, vegetable, and fruit production systems (Hanninen et al. 1999; Sanchez et al. 2007). However, very little published information is available concerning the use and potential benefits of cover crops in Christmas tree production systems.

To fill this gap, this research was established in the spring of 2007 to assess whether soil fertility, Fraser fir survival and growth could be improved by incorporating leguminous and non-

leguminous cover crops into the production system. This paper presents results on the effects of Dutch white clover, alfalfa and perennial ryegrass on soil fertility, tree physiology, foliar chemistry, survival and growth for the first two growing seasons following establishment of a Fraser fir Christmas tree plantation.

3.3. MATERIALS AND METHODS

3.3.1. Location

The study was conducted from May 2007 to October 2008 in a newly established Fraser fir plantation located at the Tree Research Center (TRC) (42.67ºN, 84.46ºW) on the campus of Michigan State University (MSU) in East Lansing, Michigan, U.S.A. Soil at this site is classified as a fine-loamy, mixed, active, mesic Aquic Glossudalf (USDA/NRCS-MAES 1992). The general soil chemical characteristics of the site are: 35% silt + clay, pH 5.6 and 13 cmol kg^{-1} CEC (Rothstein 2005). Soil total C and N, inorganic N and Mehlich III extractable nutrients (P, K, Ca and Mg), measured at the initiation of the plantation establishment are provided in Table 3.1. The site had been used for crop cultivation, primarily corn with occasional rotations of wheat and soybeans, for at least the past 30-40 years. Mean monthly temperature and total monthly rainfall from May to September of 2007, 2008 and the past 12-yr average (1996-2008) are presented in Figure 3.1.

Table 3.1 Initial soil chemical (mg kg^{-1}) characteristics (means ± se; n=3) at three soil depths (cm) of the experimental site, Michigan State University Tree Research Center, East Lansing, Michigan, US.A.

Soil depth	Total C	Total N	NO_3^- +NH_4^+	Mehlich III extractable nutrients			
				P	K	Ca	Mg
0-15	21.4 (0.8)	1.8 (0.1)	8.86 (0.56)	49.1 (4.8)	201(13)	1260 (53)	183 (7)
15-30	16.0 (0.7)	1.4 (0.1)	4.44 (0.13)	31.7 (4.2)	111 (9)	1186 (43)	155 (6)
30-45	06.5 (0.8)	0.6 (0.1)	2.73 (0.15)	14.1 (3.1)	78 (4)	1226 (59)	175 (10)

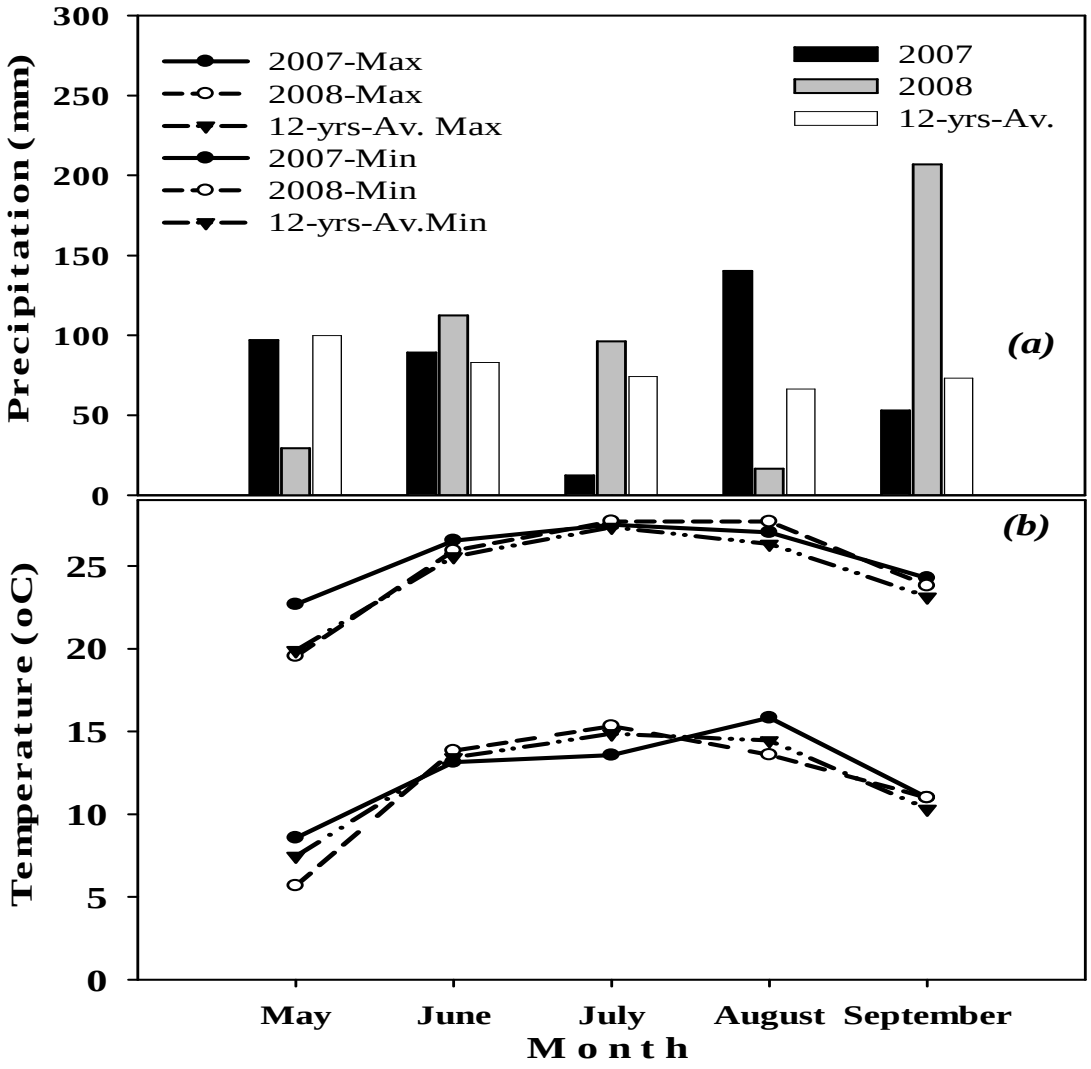

Figure 3.1 (a) Total monthly precipitation and (b) monthly minimum and maximum temperatures during the 2007 and 2008 growing seasons compared with the 12-year monthly average at MSU's Horticultural Farm, East Lansing, Michigan, U.S.A.

3.3.2. Experimental design and plot layout

The experiment was established in a randomized complete block design with three replicates in a field measuring 32.4 m × 50.4 m. Blocks and experimental plots were 10.8 m × 50.4 m and 7.2 m × 10.8 m, respectively. Each rectangular plot contained a total of 35 trees (5 × 7 trees). Trees in border rows were used as buffers and not included in measurements, therefore restricting data collection to the remaining 15 interior trees in each plot. Each block had two plots per cover crop, managed either with no strips (NS) or with strips (S). The NS treatment consisted of growing each cover crop continuously over the entire plot. In contrast, in the S treatment, the assigned cover crop was intercropped between the tree rows while maintaining, through glyphosate application, a clear strip of 0.61 m wide, centered on the tree row. Control plots were managed conventionally (CONV); they contained no cover crop and weeds were completely controlled with glyphosate (active ingredient concentration =1.1 kg ha^{-1}). Thus, the treatments were as follows: conventional system (CONV), Dutch white clover with strips (DWCS), Dutch white clover with no strips (DWCNS), alfalfa with strips (ALFS), alfalfa with no strips (ALFNS), perennial ryegrass with strips (PRGS) and perennial ryegrass with no strips (PRGNS). No N fertilizer was applied to any plot in 2007 as it may injure first-year seedling roots (Koelling 2002). However, during the second growing season, 16 g N per tree (50 kg N ha^{-1}) as ammonium sulfate [$(NH_4)_2SO_4$] was applied one time on 13 May, 2008 to the control plots. A description of treatments and management practices applied in 2007 and 2008 can be found in Appendix –TableA.1.

3.3.3. Plant materials and management

Fraser fir transplants (plug + 2) were obtained from a local commercial nursery (Peterson's Riverview Nursery), and machine-planted (Whitfield planter) at a spacing of 1.8 m × 1.8 m. Fraser fir seedlings were planted into a chisel-plowed and dragged field soil on 8 May 2007. Seeds of common Dutch white clover, alfalfa (SS 100 brand) and perennial rye (VNS) were purchased from Michigan State Seeds (Grand Ledge, Michigan, U.S.A.) and hand-seeded on 22 May, 2007. The seeding rates used were 28 kg ha^{-1} for clover and alfalfa, and 16 kg ha^{-1} for ryegrass. Once the cover crops were fully established, mechanical mowing was performed at 3 cm above the ground every three to four weeks in 2007 (2 July, 26 July, 21 August and 18 September) and 2008 (27 May, 24 June, 17 July, 14 August, and 11 September) to control cover crop growth, minimize the competition with the trees, and add green manure to the surface soil. Glyphosate was sprayed twice during each growing season (2007 and 2008) to control weeds in the CONV plots and within the tree rows in the S plots. The whole field was protected with an electric fence to prevent deer browsing.

3.3.4. Seedling growth and physiology

Seedling initial height and collar diameter, recorded at 1 cm from the ground, were measured two weeks after planting. In addition, in mid-October of 2007 and 2008, I measured tree survival as well as each seedling's height and collar diameter. Relative growth rate (RGR) was calculated from total height and collar diameter data using the formula by Hunt (1982):

$$RGR = (\ln W_1 - \ln W_2)/(t_1 - t_2) \quad \text{(eq.3.1)}$$

where W_1 and W_2 are tree sizes (height or diameter) measured at times t_1 and t_2.

The maximum quantum yield of photo-system II, also referred to as chlorophyll fluorescence (F_v/F_m), of current year needles was measured using a Handy PEA Chlorophyll Fluorometer (Hansatech Instruments, England). The stem water potential (Ψ_w) of new branches obtained from the upper third of the crown was also measured with a Pressure Bomb (PMS Instruments Company, Oregon, USA). Both F_v/F_m and Ψ_w were measured three times a year in 2007 (29 May, 18 July and 27 September) and 2008 (14 May, July 17 and 23 September).

3.3.5. Soil and plant sampling and nutrient analysis

In mid-October of 2007 and 2008, 15 randomly selected soil samples per plot were collected with 5.2-cm diameter PVC tubes and composited into one sample. In the striped cover crop plots, nine cores of soil were collected within the cover crop zone and six cores within the strips (bare ground zones), proportional to the area of the striped and cover crop zones. Soil samples were collected from 0-15, 15-30, and 30-45 cm depths.

From each field-moist soil sample, about 10 g of soil was used for determination of gravimetric moisture content by weighing the soil before and after oven drying at 105°C for 24 h. A second subsample was used for inorganic N determination while a third subsample was used for potential N mineralization determination after 4 weeks of incubation at 25^{o}C and maintaining the samples at 50% of field capacity by periodically adding distilled water. Nitrate (NO_3^--N) and ammonium (NH_4^+-N) concentrations of the non-incubated and incubated soil samples were extracted with 2 M KCL (5:1 extractant to soil ratio) and extracts were analyzed spectrophotometrically (Spectrophotometric plate reader, Model ELx Bio Tek Instruments, Inc. Winooski, Vermont, U.S.A.) for NO_3^--N and NH_4^+-N following the procedures described by

Doane and Horwath (2003) and Sinsabaugh et al. (2000), respectively. Soil macronutrients (e.g. P, K, Ca and Mg) were extracted with Mehlich III extractant (Mehlich 1984) and analyzed using an inductively coupled plasma atomic emission spectrometry (ICP-AES; PerkinElmer, Model Optima 2100DV, Shelton, CT, U.S.A.).

Before each mowing, the aboveground biomass of the cover crops was measured by using four quadrats of 0.3 m x 0.3 m, randomly placed between the tree rows in each plot. Vegetation was cut at 3 cm above the ground to mimic a mechanical mowing. Then, clippings were taken to the laboratory where cover crop fresh material was separated from weeds. Needle samples were also taken at the end of the growing season, in mid-October of 2007 and 2008 for determination of nutrient (e.g. N, P) concentrations. During this time, roots are dormant, trees have produced most of their annual woody growth, and nutrient levels in the trees are stable (Hart et al. 2004). Samples were collected by pinching five to eight needles of new or current season growth from different locations on the upper one third of the tree crown. This procedure was repeated for three to six trees within each plot. All plant tissue samples were gently washed with a non-ionic detergent and rinsed five times in distilled water before they were oven-dried at 65ºC for 48 hours. Dried weight of cover crop clippings was used for the determination of herbage yield at each mowing event. Then, each plant tissue sample was ground in a ball-mill and a sub-sample of 500 mg was acid-digested (4.5 mL of concentrated 70% nitric acid) for elemental analysis (ICP-AES). Additional subsamples of about 2.5 mg were used for total C and N determination by combustion using an elemental analyzer (Model ECS 4010, COSTECH Analytical, Valencia, California, U.S.A.).

3.3.6. Statistical analysis

All variables were analyzed with the statistical package Minitab 15 (Minitab Inc. 2006) using a randomized complete block design with three replications. For the soil data, treatment effects on measured variables were analyzed taking into account the three sampling depths. The statistical model thus included one random factor (block) and two fixed factors: groundcover treatments and depths, with the later variable considered as repeated measurements. Both cover crop and tree measurement data were subjected to a one way ANOVA. Fisher $LSD_{0.05}$ range test was used to separate treatment means found to be significantly different in the ANOVA (Little and Hill 1978).

3.4. RESULTS

3.4.1. Cover crop establishment, biomass yield and foliar nutrient concentration

Overall, all three cover crops germinated and grew well due to adequate rainfall during the first two months following seeding. As shown in Figure 3.1, precipitation in May and June of 2007 approximated the 12-year average for the corresponding months which promoted good establishment of the cover crop stands. Although July and September of 2007 were drier than the 12-year average, this drought had little effect on the successful establishment of cover crop stands. The three cover crops differed significantly (P=0.013) in their annual aboveground dry matter production as well as biomass N content (P=0.034) and tissue N concentration (P=0.021) (Table 3.2). Dutch white clover was the most productive (13.9 Mg DM ha^{-1} yr^{-1}), whereas perennial ryegrass had the lowest dry matter yield (5.9 Mg DM ha^{-1} yr^{-1}). Biomass N content followed the same trend as aboveground biomass yield.

Table 3.2 Cumulative cover crop biomass obtained after five mowing events and biomass N, P, K, Ca and Mg concentration during the 2008 growing season with different cover crops for a Fraser fir Christmas tree plantation in Michigan, U.S.A. BY= cover crop biomass dry matter yield, BN= cover crop biomass dry matter nitrogen yield

Cover crop	BY ($t\ ha^{-1}$)	BN ($kgha^{-1}$)	N (%)	C:N ratio	P (%)	K (%)	Ca (%)	Mg (%)
Alfalfa	10.2 ^{b}	494 ^{b}	4.1 ^{b}	10.9 ^{a}	0.36 ^{b}	3.22 ^{b}	1.19 ^{b}	0.31 ^{b}
Clover	13.9 ^{c}	563 ^{b}	4.0 ^{b}	11.3 ^{a}	0.30 ^{a}	3.32 ^{b}	1.15 ^{b}	0.36 ^{c}
Ryegrass	5.9 ^{a}	210 ^{a}	3.4 ^{a}	13.4 ^{b}	0.30 ^{a}	2.78 ^{a}	0.30 ^{a}	0.23 ^{a}
P-value	0.013	0.034	0.021	0.040	0.021	0.044	<0.001	<0.001

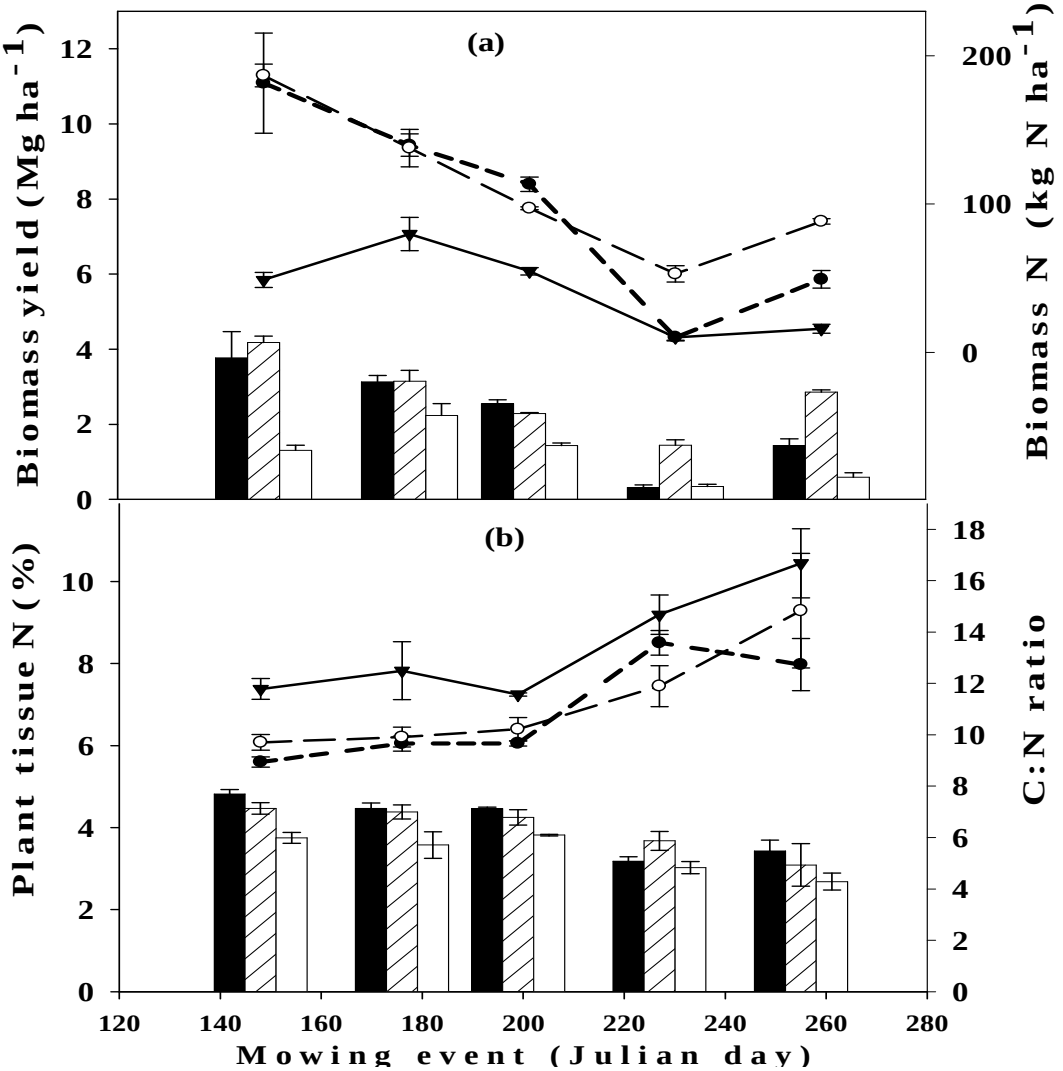

Figure 3.2 (a) Total aboveground biomass yield (*bars*) and biomass N content (*lines*), (b) tissue N concentration (*bars*) and C:N ratio (*lines*) of alfalfa (*black bars, black circles and short lines*), Dutch white clover (*gray bars, white circles and long dashed lines*) and perennial ryegrass (*white bars, down triangles and solid lines)* mowed five times in 2008 at 3 cm above the ground in a Fraser fir Christmas tree plantation in Michigan, U.S.A. Values are means (± se; n = 3)

The two leguminous cover crops accumulated the highest biomass N. With the exception of P, foliar nutrient concentrations were significantly ($P<0.05$ for N and K; $P<0.001$ for Ca and Mg) higher for the two legumes than the non-legume cover crop. The distribution of the 2008 biomass yield among mowing events varied in magnitude and rank for the three cover crops. In general, biomass yields per mowing event ranged from 0.3-3.8, 1.4-4.2 and 0.3-2.2 Mg DM ha^{-1} for alfalfa, Dutch white clover and perennial ryegrass, respectively (Fig. 3.2-a).There was a general trend toward a decrease of biomass yield for each consecutive mowing event. Likewise, biomass N content followed almost the same pattern as biomass yield, with a general trend of decline with each subsequent mowing event. Tissue N concentrations per mowing event were in the range of 3.2-4.8, 3.1-4.5, and 2.7-3.8%, whereas biomass N content ranged between 10-182, 53-187, and 10-80 kg N for alfalfa, Dutch white clover and perennial ryegrass, respectively (Fig. 3.2-a and 3.2-b). C: N ratios ranged from 8.9-13.6, 9.7-14.8 and 11.6-16.7 for alfalfa, Dutch white clover and perennial ryegrass, respectively (Fig. 3.2-b). Tissue N concentration also tended to decline with each subsequent mowing event, resulting in an increase of the C: N ratios.

3.4.2. Soil extractable nutrients

Across all three soil depths and groundcover treatments, P was generally significantly lower in NS than the other treatments (Table 3.3). However, extractable P concentration did not differ between CONV and S treatments at any of the three soil layers. Soil extractable K concentration decreased significantly ($P<0.001$) with increasing soil depth whereas treatment effect was not significant. Treatment and sampling depth had no significant effects on extractable Ca concentration (Table 3.3).

Table 3.3 Average concentration of Mehlich III extractable P, K, Ca and Mg (mg/kg^{-1}) measured in 2008 at three soil depths (cm) with different cover crops in a Fraser fir Christmas tree plantation in Michigan, U.S.A. Values are means (±se; n=3,). Treatments are: Conventionally managed (CONV), Dutch white clover with strips (DWCS), Dutch white clover with no strips (DWCNS), alfalfa with strips (ALFS), alfalfa with no strips (ALFNS), perennial ryegrass with strips (PRGS), and perennial ryegrass with no strips (PRGNS).

Soil depth	Management	Mehlich III							
		P		K		C		Mg	
0-15	CONV	43.4	(2.4) b	196	(61)	1480	(266)	207	(35)
	ALFS	40.2	(0.6) b	217	(8)	1079	(169)	161	(19)
	ALFNS	27.4	(3.6) a	144	(51)	1231	(242)	173	(29)
	DWCS	38.3	(3.0) b	204	(13)	1224	(160)	169	(19)
	DWCNS	29.7	(2.4) a	249	(34)	1380	(215)	191	(31)
	PRGS	41.3	(2.0) b	228	(24)	1160	(142)	174	(27)
	PRGNS	28.0	(3.6) a	165	(54)	1207	(178)	180	(22)
	P_value	0.002		ns		ns		ns	
15-30	CONV	14.4	(2.2) b	81	(36)	973	(234)	131	(50)
	ALFS	17.9	(1.6) b	139	(21)	1061	(165)	140	(9)
	ALFNS	8.0	(0.7) a	105	(10)	1232	(223)	153	(22)
	DWCS	16.5	(0.8) b	100	(3)	1352	(79)	168	(10)
	DWCNS	9.8	(0.2) a	122	(29)	1169	(112)	145	(11)
	PRGS	16.0	(1.6) b	127	(3)	1048	(174)	135	(21)
	PRGNS	7.3	(0.5) a	73	(22)	1128	(311)	156	(19)
	P_value	<0.001		ns		ns		ns	
30-45	CONV	4.9	(0.7) c	80	(12)	1481	(395)	191	(49)
	ALFS	3.1	(0.4) ab	77	(5)	1079	(148)	148	(6)
	ALFNS	2.2	(0.6) ab	68	(8)	1459	(168)	212	(4)
	DWCS	2.2	(0.4) ab	67	(2)	1204	(168)	190	(31)
	DWCNS	1.1	(0.4) a	70	(20)	921	(219)	130	(36)
	PRGS	3.3	(0.2) b	68	(16)	1025	(119)	113	(11)
	PRGNS	2.0	(0.1) ab	98	(43)	1251	(160)	207	(12)
	P_value	0.001		ns		ns		ns	

Table 3.4 Average soil available nitrate, available N, nitrification and net N mineralization (kg ha^{-1})at three depths, from samples collected at the end of the growing season of 2007 and 2008 with different cover crops in a Fraser fir Christmas tree plantation in Michigan, U.S.A. Treatments are: Conventional (CONV), Dutch white clover with strips (DWCS), Dutch white clover with no strips (DWCNS), alfalfa with strips (ALFS), alfalfa with no strips (ALFNS), perennial ryegrass with strips (PRGS), and perennial ryegrass with no strips (PRGNS).

Depth (cm)	Management practice	NO_3^--N		(NO_3^-+NH_4^+)-N		Nitrification		Net N-mineralization	
		2007	2008	2007	2008	2007	2008	2007	2008
0-15	CONV	8.3 a	7.9 a	12.4 a	13.6 a	25.8 a	28.4 a	22.9 a	20.0 a
	ALFS	13.0 b	24.5 c	17.8 bc	35.5 c	44.3 c	98.1 d	40.9 c	87.4 de
	ALFNS	13.1 b	21.6 bc	22.1 c	32.3 bc	37.8 bc	98.1 d	30.6 ab	88.5 de
	DWCS	11.0 b	24.0 c	16.9 b	32.0 bc	41.0 bc	101.4 d	36.5 bc	94.1 e
	DWCNS	11.3 b	20.5 bc	17.3 b	26.1 b	32.7 ab	77.3 c	27.9 ab	70.2 cd
	PRGS	11.1 b	16.4 b	16.3 ab	26.2 b	31.9 ab	57.5 b	28.6 ab	49.5 b
	PRGNS	11.8 b	16.5 b	15.6 ab	24.8 b	33.3 ab	67.3 bc	31.4 ab	61.4 bc
	P_value	0.050	0.004	0.010	0.001	0.029	<0.001	0.021	<0.001
15-30	CONV	3.7 a	5.3 a	10.6	8.1 a	11.4 a	14.7 a	5.7 a	12.3 a
	ALFS	7.8 b	18.5 bc	11.6	24.9 c	25.0 c	39.5 b	22.1 bc	33.6 b
	ALFNS	6.5 b	20.5 c	10.4	27.3 c	25.8 c	46.0 b	22.8 c	39.9 b
	DWCS	5.7 ab	19.4 bc	9.0	27.5 c	24.4 bc	68.3 cd	22.2 c	60.6 cd
	DWCNS	8.0 b	15.1 bc	10.4	24.5 bc	25.4 c	55.6 bc	24.1 c	46.7 bc
	PRGS	6.0 ab	12.9 abc	10.1	20.2 bc	17.8 abc	57.2 bc	14.7 abc	51.0 bc
	PRGNS	6.0 ab	10.2 ab	10.1	15.3 ab	15.2 ab	79.6 d	12.1 ab	75.2 d
	P_value	0.021	0.038	ns	0.005	0.027	<0.001	0.010	<0.001
30-45	CONV	5.4	4.9 ab	6.6	7.0 a	5.0	16.9 a	4.4	8.9 a
	ALFS	2.9	7.9 cd	6.5	15.6 c	8.6	41.1 bc	5.6	32.5 c
	ALFNS	4.9	8.5 d	6.4	12.7 bc	7.1	24.3 ab	6.2	17.4 ab
	DWCS	4.9	7.2 abcd	6.6	13.6 bc	10.0	43.5 c	9.2	27.8 bc
	DWCNS	3.0	7.7 bcd	4.8	10.1 ab	13.0	34.7 abc	11.5	31.4 c
	PRGS	6.5	4.5 a	7.9	10.9 abc	9.5	38.1 bc	8.5	33.5 c
	PRGNS	4.6	5.1 abc	6.2	11.5 abc	10.5	29.6 abc	9.5	23.3 bc
	P_value	ns	0.032	ns	0.022	ns	0.021	ns	0.012

By the end of the first growing season (2007), cover crop treatments significantly increased soil NO_3^--N concentration relative to CONV in the top two soil layers (P=0.05 and 0.021, respectively). There was more than 2% increase in soil NO_3^--N levels at both 0-15 and 15-30 cm soil depths (Table 3.4). Similarly nitrification rate and net N mineralization increased substantially in all cover crop treatments relative to CONV at the two top soil depths. In 2008, however, treatment differences were apparent at all three soil depths for soil NO_3^--N, nitrification rate and net N mineralization. In 2007, cover cropping also significantly (P=0.01) increased soil available N levels relative to the CONV treatment at the top soil layer, whereas treatments differences were not significant at the two deeper soil layers. In 2008, cover crop treatments significantly enhanced soil available N by more than 2% at all three soil layers.

3.4.3. Seedling survival

By the end of 2008 growing season, tree survival ranged from 13 to 68% (Fig. 3.3). PRGNS and DWCNS had the lowest tree survival of all groundcover treatments (13 and 20%, respectively). In both 2007 and 2008, no significant differences were found between the CONV and the S plots. After two growing seasons, survival rates within the S plots decreased in the following order: alfalfa > perennial ryegrass > Dutch white clover. In general, most seedling mortality occurred during the first growing season. It is noteworthy that on average, 91% of the seedlings that survived the first year also survived the second year in the CONV and S treatments. In the NS plots, however, second-year survival rate was 70%, with the lowest survival (47%) recorded in Dutch white clover plots. In 2007, some dead seedlings were sent for analysis at MSU's Center for Integrated Plant Systems and the results indicated that the mortality was not related to any disease problems.

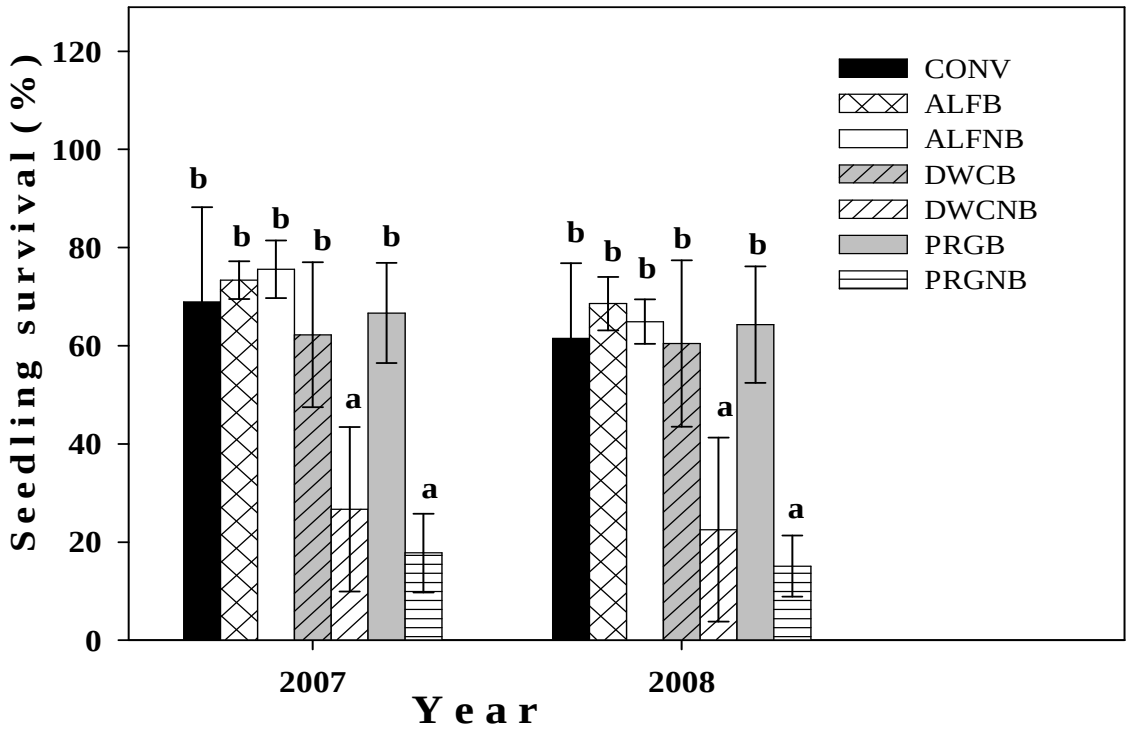

Figure 3.3 Tree survival in 2007 and 2008 with different cover crops in a Fraser fir Christmas tree plantation in Michigan, U.S.A. Treatments are: Conventionally managed (CONV), Dutch white clover with strips (DWCS), Dutch white clover with no strips (DWCNS), alfalfa with strips (ALFS), alfalfa with no strips (ALFNS), perennial ryegrass with strips (PRGS), and perennial ryegrass with no strips (PRGNS). Values are treatment means (± se; n = 3).

3.4.4. Relative diameter and height growth of seedlings

Relative diameter growth for the first growing season (FS) was not significantly affected by groundcover treatments. However, relative diameter growth for the second growing season (SS) and the combined two growing seasons (TS) differed significantly (P=0.003 and 0.009, respectively) among groundcover treatments. With the exception of ALFNS, seedlings grown in NS treatments had lower relative diameter growth than their counterparts in the CONV and S treatments (Table 3.5). In contrast, FS-, SS- and TS-relative height growth did not significantly differ among groundcover treatments. By the end of the second growing season, relative diameter growth of seedlings in the ALFNS, DWCNS and PRGNS treatments was reduced by 30, 52 and 70%, respectively, relative to their counterparts in the CONV plots.

Table 3.5 Relative growth of collar diameter and height of Fraser fir seedlings for the first growing season (FS), second growing season (SS) and the two growing seasons (TS) in a low input soil management with different cover crops for Fraser fir Christmas tree plantation in Michigan, U.S.A. Values are means (± se; n =3). Treatments are: Conventionally managed (CONV), Dutch white clover with strips (DWCS), Dutch white clover with no strips (DWCNS), alfalfa with strips (ALFS), alfalfa with no strips (ALFNS), perennial ryegrass with strips (PRGS), and perennial ryegrass with no strips (PRGNS).

Treatment	Relative diameter growth			Relative height growth		
	FS	SS	TS	FS	SS	TS
CONV	0.07 (0.04)	0.38 (0.03) b	0.23 (0.02) c	0.15 (0.03)	0.06 (0.03)	0.09 (0.02)
ALFS	0.10 (0.03)	0.34 (0.02) b	0.22 (0.02) c	0.09 (0.03)	0.01 (0.02)	0.08 (0.02)
ALFNS	0.10 (0.04)	0.20 (0.02) a	0.16 (0.02) bc	0.08 (0.03)	0.01 (0.02)	0.05 (0.02)
DWCS	0.07 (0.04)	0.38 (0.02) b	0.22 (0.02) c	0.12 (0.04)	0.00 (0.02)	0.07 (0.02)
DWCNS	-0.03 (0.04)	0.19 (0.02) a	0.11 (0.02) ab	0.06 (0.04)	0.00 (0.03)	0.02 (0.02)
PRGS	0.09 (0.03)	0.33 (0.02) b	0.21 (0.02) c	0.19 (0.03)	-0.02 (0.02)	0.08 (0.02)
PRGNS	-0.10 (0.03)	0.22 (0.01) a	0.07 (0.02) a	0.07 (0.04)	-0.05 (0.02)	0.01 (0.02)
P-value	ns	0.003	0.009	ns	ns	ns

3.4.5. Seedling chlorophyll fluorescence and stem water potential

Cover crop treatments had marginally significant effects on seedling F_v/F_m during the course of the experiment. In 2007, F_v/F_m differed significantly (P=0.048) among groundcover treatments, with the NS treatments generally having lower F_v/F_m values than the other treatments (Fig. 3.4). However, in 2008, F_v/F_m did not differ among groundcover treatments. Similar to seedling survival and F_v/F_m, in both 2007 and 2008, plant stem Ψ_w were significantly lower for seedlings on the NS treatments relative to their counterparts in the S and CONV plots (Fig.3.5). However, mean Ψ_w of seedlings in the S plots did not differ from that of their counterparts on the CONV plots. Average Ψ_w ranged from -2.6 to -1.8 MPa in 2007 and -1.6 to -1.1 MPa in 2008. Mean Ψ_w values ranged from -2.1 to -1.7 MPa in all S and CONV plots and from -2.4 to -2.9 MPa for seedlings in all NS treatments in 2007. Although lower mean Ψ_w values were recorded in 2008 than in 2007, similar patterns were observed among treatments in both years.

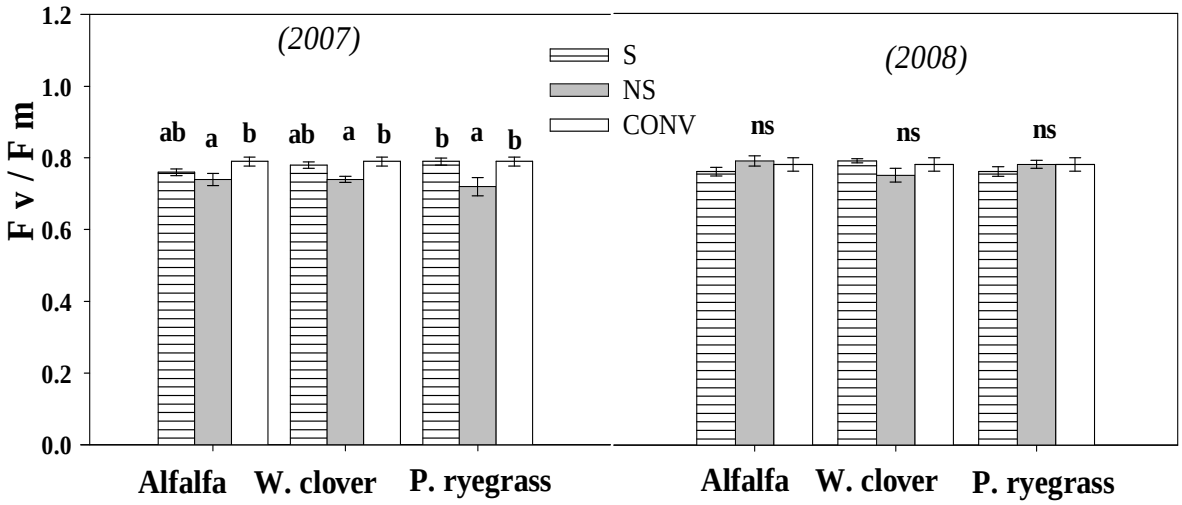

Figure 3.4 Average maximum quantum yield (F_v/F_m) measured in 2007 and 2008 with different cover crops in a Fraser fir Christmas tree plantation in Michigan, U.S.A. Mean values within each year and the same cover crop species, followed with the same letters are not statistically different. The long dashed line represents the threshold of F_v/F_m for healthy foliage. S, NS and CONV are strips, no strips and conventional treatments, respectively; ns = not significant.

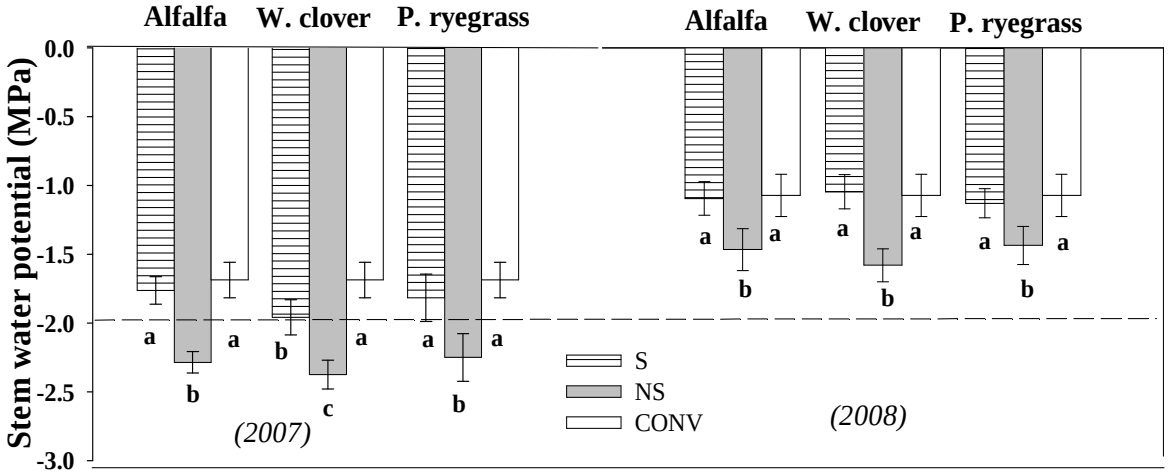

Figure 3.5 Average stem water potential (Ψ_w) measured in 2007 and 2008 with different cover crops in a Fraser fir Christmas tree plantation in Michigan, U.S.A. Mean values within each year and the same cover crop specie, followed with the same letters are not statistically different. The dashed line represents the threshold of Ψ_w at which stomatal opening and photosynthesis in young conifers are inhibited. S, ST and CONV are strips, no strips and conventional treatments, respectively

3.4.6. Seedling foliar chemistry

Needle N concentration ranged from 1.60 to 2.07% and 1.12 to 1.50% in 2007 and 2008, respectively (Fig 3.6-a). In both years, PRGNS treatments had the greatest adverse effects on needle N concentration, although treatment differences were not significant during the first growing season. In 2007, needle N concentration averaged 1.74 % for seedlings in the NS treatments, whereas a mean value of 2.00 % was recorded for seedlings in the S and CONV plots. In 2008, N concentrations averaged 1.30, 1.44 and 1.50 % for seedlings in the NS, S and CONV plots, respectively. In general, Needle P concentration tended to decrease for seedlings in the PRGS and PRGNS plots relative to all other treatments but differences were not significant (Fig 3.6-b).

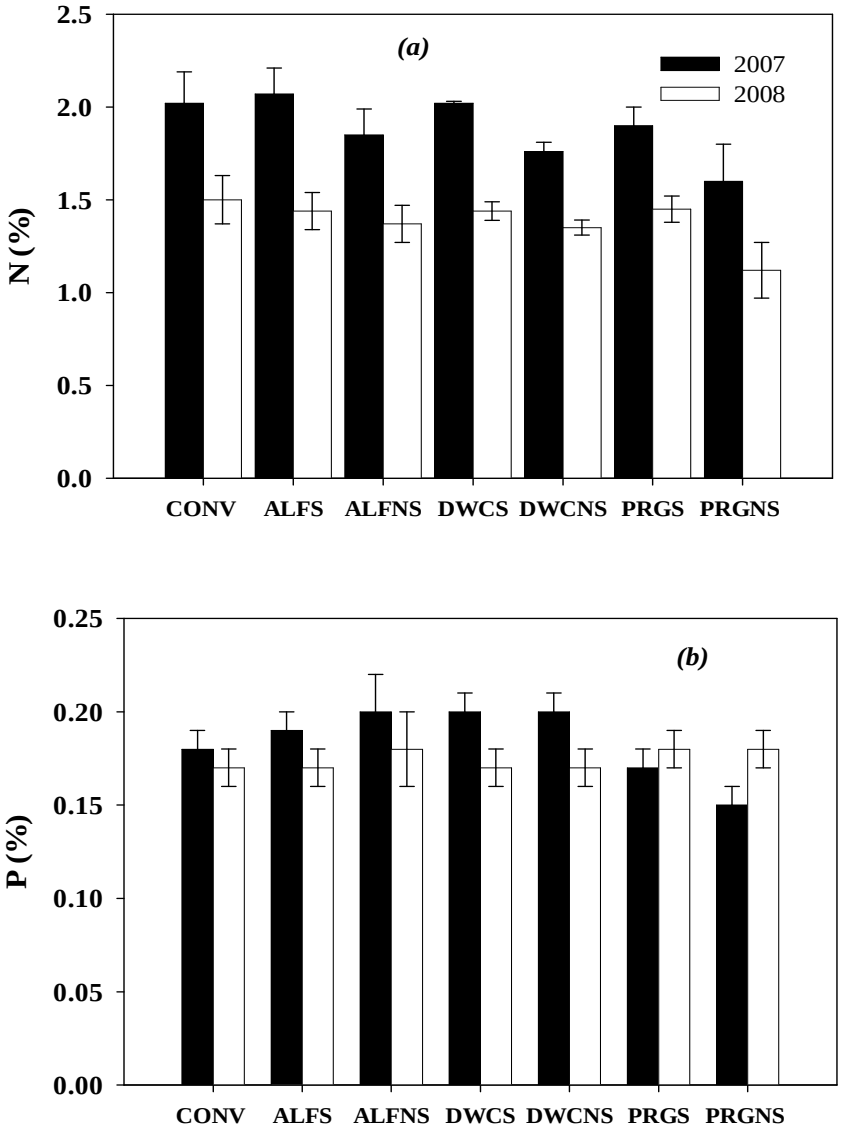

Figure 3.6 Needles (a) nitrogen, and (b) phosphorus concentrations of Fraser fir seedling in 2007 (*black bars*) and 2008 (*white bars*) with different cover crops for a Fraser fir Christmas tree plantation in Michigan, U.S.A. Treatments are: Conventionally managed (CONV), Dutch white clover with strips (DWCS), Dutch white clover with no strips (DWCNS), alfalfa with strips (ALFS), alfalfa with no strips (ALFNS), perennial ryegrass with strips (PRGS), and perennial ryegrass with no strips (PRGNS).Values are means (±se; n=3).

3.5. DISCUSSION

In this study, I compared the effects of different cover crops and competition-management on soil chemical properties, tree physiology, foliar chemistry, survival and growth in a newly established Fraser fir plantation. Over the first two growing seasons, I found that despite differences in external N fertilizer inputs and competition control, Fraser fir seedling survival and growth in the S and CONV plots tended to be similar. In contrast, however, allowing the cover crops to grow continuously over the entire plot led to poor performance of seedlings. This suggests that incorporating cover crops into Fraser fir Christmas tree production with partial competition control could be an alternative to the conventional system. On the other hand, cover cropping with no competition control should not be recommended for Fraser fir production system, especially at the early stage of the plantation establishment.

The primary benefits of cover crops include their ability to add organic matter to soil and provide better recycling of plant nutrients. After five consecutive mowing events in 2008, the two leguminous cover crops outproduced the non-legume cover crop probably because I did not fertilize the plots, which is in agreement with findings of other studies (Newman et al. 2007; Torbert et al. 1996). Aboveground biomass yield of alfalfa in this study is comparable to that obtained by Rock et al. (2009) in Minnesota, U.S. In contrast, biomass yields for white clover and perennial ryegrass, however, were higher than those reported by Elgersma and Schlepers (1997) probably due to differences in management, climatic, or edaphic conditions as these factors highly influence cover crop herbage yield (Odhiambo and Bomke 2001; Cherr et al. 2006).

Tissue N concentration of all three cover crops decreased with each subsequent mowing event, whereas the C: N ratio increased. These results are congruent with the results of studies

conducted elsewhere (Odhiambo and Bomke 2001; Dahlin et al 2005). It is generally agreed that cover crop amendments with C: N ratios lower than 25:1 lead to rapid N mineralization (Ranells and Wagger 1996). The C: N ratios for all three cover crops were below this critical value, suggesting that biomass N would be quickly decomposed and released within weeks for plant (trees and cover crops) uptake. The high tissue N concentration and low C: N ratios obtained in this study could be attributed to the short mowing frequency that allowed for the re-growth of fresh, immature plant material throughout the growing season.

These measurements of soil mineral N and N mineralization in 2007 and 2008 provided an indication of the fate of N from the cover crop green manures. Although more extensive research would be needed to follow the dynamic of the cover crop biomass N in the plantation soil, a single sampling of soil during a growing season may provide an indication of treatment differences. Although high available N and N mineralization rates were generally obtained in the S and NS plots, these did not necessarily lead to enhanced tree foliar N nutrition in these plots relative to the control. On the other hand, I did observe marginal effects of treatments on soil available nutrients (except for N and P) and seedling foliar nutrition (except for N). The reason for the lack of response of seedling foliar chemistry to groundcover treatments could be that water stress might have reduced the ability of the trees to effectively absorb plant available nutrients from the soil solution.

As indicated by the weather data, July and September of 2007 were drier than the 12-year average. Similarly, May and August of 2008 received 70 and 75%, respectively, less rainfall than the 12-yr average. The expected low soil moisture contents during these periods may have reduced the effective diffusion of elements in the soil solution. In addition, plant water stress may in turn, have reduced the mass flow and plant uptake of elements. Similar to moisture

availability, root volume and nutrient uptake and utilization are linked. Prior to planting, seedlings were root pruned to 20 cm following standard planting practices. This resulted in substantial root loss, especially the absorbing roots, and may also limit seedling nutrient uptake. As reported by Knapp and Smith (1982), root penetration to greater depths is an important factor determining the survival of conifer seedlings. Although I did not investigate seedling root development in this study, it is also likely that the root system was still shallow during the first year of the plantation establishment. The newly transplanted seedlings might have suffered from a combination of high temperature, drought, root damage and shallow rooting that contributed to poor nutrition and high mortality of seedlings (Dalton and Messina 1994), especially in the NS plots. However, most of the seedlings that survived during the first growing season also survived during the second year. Second year seedlings likely had ample time to restore their root system to the pre-transplant size, making them less susceptible to environmental stresses.

The presumed reduction of belowground competition between Fraser fir seedlings and cover crops by creating strips was expected to influence stem Ψ_w. The strip treatment substantially raised the mean Ψ_w of Fraser fir seedlings relative to the NS treatments, suggesting that seedlings in S plots had less water stress than their counterparts in the NS plots. Despite differences in the degree of competition control, seedlings in the S plots displayed similar Ψ_w to that of their counterparts on the CONV systems. This may be partially due to likelihood that root systems in the S plots have not grown much beyond the strip cleared of competing vegetation. It is noteworthy that seedlings in NS plots had their Ψ_w consistently below the threshold of -2.0 MPa, which is considered severe water stress and critical for stomatal opening and photosynthesis in seedlings and saplings of a variety of conifers (Tseng et al. 1988). In 2008,

mean Ψ_w was -1.1 MPa for seedlings in S and CONV treatments and -1.5 MPa for their counterparts in the NS plots. This suggests that seedlings in NS plots suffered more severe water stress than their counterparts in the S and CONV plots. The lower mean Ψ_w values of seedlings recorded during the first growing season versus the second growing season is in agreement with Muyi and Smith (1991) who also reported increasing Ψ_w value with increasing age in subalpine fir (*A. lasiocarpa*) seedlings.

The capacity to perform photosynthesis, given appropriate environmental conditions, is reflected by measurements of the maximum quantum yield of photosystem II (F_v/F_m). Environmental conditions such as extreme temperature, drought or nutrient deficiency can cause photo-oxidation or photo-inhibition, changing the efficiency of non-photochemical quenching and decreasing F_v/F_m (Westin et al. 1995; Maxwell and Johnson 2000). Similar to seedling survival and Ψ_w, I observed a significant response of F_v/F_m to groundcover treatment in 2007. Seedlings from NS treatments had their needles F_v/F_m below the 0.8 threshold considered characteristic of healthy foliage (Lambers et al. 1998) whereas mean F_v/F_m values for seedlings in the S and CONV treatments were greater or equal to this threshold. Competition management through creating strips, however, led to enhanced F_v/F_m level comparable to the CONV plots. I found a positive relationship between Ψ_w and F_v/F_m in both 2007 and 2008, suggesting that water stress was responsible for the decline in seedling F_v/F_m on the NS plots. Water stress, in turn, can reduce the ability of tree foliage to produce energy (carbohydrates), diminish growth, and leave the tree susceptible to many other environmental stresses. Together, these problems,

might have contributed to transplant stress that led to reduced seedling survival and growth in the NS plots.

Continued root and shoot growth during the establishment period depends on a number of factors such as the availability of nutrients and moisture, a well-established root system taking-up water and nutrients as well as healthy leaves producing high levels of carbohydrates during the growing season (Lambers et al. 1998). Relative diameter and height growth were not affected by groundcover treatment during the first growing season probably because root systems were still in the recovery phase, during which more carbohydrates are allocated for root growth than shoot growth. During the second growing season, however, unlike height, relative diameter growth of seedlings in all NS treatments was significantly reduced relative to their counterparts in the S and CONV plots. As reported in previous studies, height is generally less sensitive than diameter, and height growth differences may not become evident until the second or third growing season (Dunlap and Helms 1983; Dalton and Messina 1994).

3.6. CONCLUSIONS

Results of this study not only underline the major role of cover cropping in improving soil N fertility level but also underscore the overriding importance of competition management to ensuring survival and improving early growth of newly planted Fraser fir Christmas trees. Cover crop treatments increased soil NO_3^--N levels by 1.5- and 2.2-fold relative to CONV in the top soil layer in 2007 and 2008, respectively. Nitrification rate also increased substantially in all cover crop treatments relative to the CONV treatment at all three soil depths. Seedlings in the NS treatment performed poorly compared to their counterparts in the S and CONV plots, and water stress appeared to be the key factor on the poor performance of seedlings. However, seedlings on the S treatments performed as well as those on the conventional system, suggesting that incorporation of cover crops into Christmas tree plantation with partial weed competition control could be a viable alternative to the conventional system.

Although incorporating cover crops into Christmas tree plantations appeared to be a promising approach, an economic evaluation of the costs and benefits should be conducted to determine its profitability before recommending such a practice to growers. The study should focus on determining the costs and efforts required to purchase and sow the cover crop seed, periodically mow the cover crop, and maintain the strips. These costs should be compared to the costs and labor involved in the conventional system such as complete weed control and inorganic N fertilization. The potential contribution of the cover crops to soil carbon sequestration over the entire plantation rotation period should also be investigated, so that growers who adopt this approach could be adequately rewarded for the carbon sequestration services they provide.

Another possible option would be to simply create strips and allow local vegetation to fill in between the rows and serve as a “wild cover crop.” This would remove the cost of purchasing

and establishing a cover crop. While it may not provide as much nitrogen as a leguminous cover crop, it should approximate the advantages of perennial ryegrass. Additionally, a lysimeter study of nitrate leaching should be conducted to determine whether the cover crop system reduces NO_3^--N leaching below the tree rooting zone relative to the conventional system.

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3.7. BIBLIOGRAPHY

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CHAPTER 4

GREENHOUSE GAS EMISSIONS AND N LEACHING ASSOCIATED WITH CONVERSION OF GRASSLAND TO SHORT-ROTATION WOODY BIOMASS CROPS IN NORTHERN MICHIGAN, U.S.A.

4.1 ABSTRACT

Pastures or grasslands are widely believed to be suitable ecosystems for growing bioenergy feedstocks as this may avoid conflict with food crop production or impacts to existing forests. However, there are still considerable knowledge gaps regarding the impacts of converting such lands into short-rotation woody crop (SRWC) bioenergy production. In the present study, I measured the short-term effects of converting pastureland to poplar (*Populus*) and willow (*Salix*) bioenergy plantations on soil greenhouse gas (GHG) fluxes and N leaching losses, on a moderately well-drained fine sandy loam soil, in northern Michigan, U.S.A. The study site had been cleared of forest ca. 100 y ago, and transitioned into pastureland in 2005. In spring of 2009, experimental plots were cleared of existing pastureland vegetation, cultivated and planted with either willow or poplar. Soil GHG fluxes and N leaching losses from these plots were compared against undisturbed, reference pasture plots. Based on a set of assumptions regarding the contribution of root respiration to total CO_2 efflux, I estimated that the cultivated poplar and cultivated willow plots lost an extra 3.3 and 9.0 Mg CO_2 ha^{-1}, respectively, compared to the reference pastureland during their establishment year. Emissions of N_2O also increased markedly following cultivation with rates as high as 250 ng N_2O-N cm^{-2} hr^{-1}. Consequently, cumulative direct N_2O emissions were 17.2- fold and 13.3-fold higher in the poplar and willow plots, respectively relative to the undisturbed pasture reference sites. Similarly, grassland conversion resulted in 20.3-fold and 14.6-fold increases in cumulative indirect N_2O emissions (from NO_3^--N leaching loads) in poplar and willow plots, respectively as compared with the reference plots. Although fluxes of methane were a small component of the net GHG balance,

data suggest that the undisturbed plots were a sink for CH_4 whereas the cultivated plots were a source for CH_4. Moreover, land conversion resulted in marked increases in NO_3^--N concentrations of soil leachate which were negligible under the controls (0.1 ppm), but regularly exceeded 5 ppm in the plantations. Dissolved organic nitrogen (DON) represented an important portion (69%) of N leaching losses of both bioenergy plantations. Stimulation of nitrification following cultivation appeared to be the driving factor behind increased N_2O and NO_3^--N fluxes. Overall, pastureland conversion incurred GHG debts of 9.4 and 14.2 Mg CO_2eq ha^{-1} for poplar and willow plots, respectively during the establishment year alone of the SRWC bioenergy program. These figures do not include any GHG debts associated with fossil fuel inputs for land preparation. Often neglected in most LCA studies, these GHG debts compares with estimated 11.3 Mg CO_2eq ha^{-1} of N_2O released from N fertilizer application and leaf litter decomposition over an entire live cycle of 23 years of SRWC bioenergy systems. Before embarking in a large scale deployment of SRWC plantations on similar pastureland, alternative cropping practices such as no-tillage, minimum tillage or high density tree planting that have potential to minimize GHG emissions and nutrients losses should be implemented for more sustainable feedstock production.

4.2. INTRODUCTION

There is an increasing interest worldwide in displacing fossil fuels with alternative renewable energies as the latter can potentially contribute to reduce atmospheric CO_2 emissions while enhancing national energy security (Cook and Beyea 2000; Ravindranath et al. 2008). The potential for biofuel production and use to meet growing renewable energy targets has been emphasized by the Intergovernmental Panel on Climate Change (IPCC 2007) methodology and supported by several life cycle assessment (LCA) studies (Graham et al. 1992; Heller et al. 2003; Heller et al. 2004; Volk et al. 2004; Keoleian and Volk 2005; Cherubini et al. 2009; Goglioa et al. 2009). To meet the world bioenergy needs, the promotion of large-scale cultivation of bioenergy crops on a wide range of grassland ecosystems including permanent pastures, "marginal or degraded land", "set-aside or reserved agricultural land", "idle or abandoned land", has been suggested as a sustainable strategy for biomass feedstock (De La Torre Ugarte et al. 2003; Perlack et al. 2005). However, there are still knowledge gaps, especially in terms of immediate direct impacts of clearing and cultivating such grassland ecosystems for bioenergy crop production on the net greenhouse gas (GHG) balance. The short-term direct effects of land use conversion, often neglected by many LCA studies, could result in significant GHG emissions and, thus, substantially reduce the climate benefits of bioenergy production.

Some researchers have argued that land-use conversion from native ecosystems to biofuel crop production could result in significant GHG emissions and a negative carbon balance, or carbon-debt, for many years (Cowie 2006; Pineiro et al 2009). These studies focused primarily on the issue of CO_2 release from ecosystem biomass and soils, but relatively little attention has been given to the GHG emissions associated with disruptions to the nitrogen (N) cycle associated

with land conversion. Based on the conceptual model of molecular soil C dynamics, Grandy and Neff (2008) ecosystem disturbances (e.g. N fertilization and tillage) which enhances soil organic matter (SOM) decomposition rates may have a series of direct and indirect effects on C stabilization in soil, leading to the release of large amounts of CO_2 and nitrous oxide (N_2O), a greenhouse gas 298 times more powerful than CO_2. Furthermore, nitrate (NO_3^--N) produced following cultivation can be lost through leaching which will have implications for the quality of drinking water and surface water eutrophication.

In the United States, permanent grasslands and pastures represent 58.7% of the total (1,174 million ha^{-1}) agricultural land (Lubowsky et al. 2005). To meet the range of renewable energy targets (7-20% over various time periods) announced by the different states of the U.S.A., the expansion of bioenergy crops is likely to come from these available permanent grasslands (Ravindranath et al. 2008). In the state of Michigan alone, it is estimated that at least 3 million hectares of idle agricultural and pasture lands are suitable for growing perennial bioenergy crops (Kelly 2002). Pastures and hayfields that represent a significant portion of the landscape of northern Michigan are particularly targeted for bioenergy plantations such as short-rotation woody crops (SRWC) of willow and poplar. However, grassland soils are generally believed to have large stores of SOM that may become susceptibility to accelerated mineralization and nitrification/ denitrification once they are disturbed (Six et al. 1998; Soussana et al. 2004) through conversion to SRWC production, which involves the use of conventional agricultural methods, such as soil plowing, disking and herbicide application (Tubby and Armstrong 2002).

Cultivation has widely been recognized to create a soil environment that increases SOM availability, stimulates aerobic microbial activity, and accelerates organic C oxidation to CO_2

(Blanco-Canqui and Lal 2007; Yadav and Malanson 2007). In addition, mineralization of SOM enhance soil N turnover which favors N_2O production and emissions in soils directly as a consequence of both nitrification and denitrification (Smith et al. 2003; Grandy and Robertson 2006). Indirect emission of N_2O can also occur when N is lost from soil in forms other than N_2O (e.g. NO_x, NH_3, NO_3^--N) and later converted to N_2O (Wrage et al 2005). The third most important GHG associated with land conversion is methane (CH_4). Soils can be an important sink or source for methane. Under normal conditions, native grasslands are reported to be a small sink for CH_4 whereas cultivation of such sites tends to inhibit the activity of CH_4 oxidizing bacteria and reduce natural rates of CH_4 consumption (Hutsch 2001; Robertson and Grace 2004). Finally, independent of GHG accounting, NO_3^--N leaching is important from a water-quality perspective because it contributes to aquatic eutrophication and can pose a health risk to humans.

This study was initiated to investigate the potential effects of grassland conversion to SRWC plantation on the net emissions of three primary GHGs associated with agriculture (CO_2, N_2O, and CH_4) at a site in the central Upper Peninsula (UP) of Michigan. It was hypothesized that the initial conversion from a grassland to a SRWC system would incur some level of GHG "debt" due to increased SOM mineralization, resulting in direct and indirect (through N leaching and subsequent conversion to N_2O) emissions of GHG. In this study, I posed two fundamental questions: (i) what is the magnitude of GHG emissions and "debt" associated with grassland conversion to SRWC systems? and (ii) what is the "payback period", the time required for the SRWC systems to overcome this GHG debt and begin providing net GHG benefits?

4.3. MATERIALS AND METHODS

4.3.1. Study site characteristics

This experiment was conducted in a former grassland located in the central Upper Peninsula of Michigan, U.S.A. (latitude 46^{o}36´N, longitude -87^{o}27´E, elevation 290 m above sea level) (Fig 4.1). It is noteworthy that this is the site of the first commercial SWRC plantation in Michigan's Upper Peninsula. In the spring of 2009 all but 2.6 ha of the entire field was converted from grassland to a willow plantation designed to provide feedstock for a new wood-fired power plant in the nearby city of Marquette, MI. At the same time a clonal-yield trial was established in a 2.6 ha portion of the field by the Michigan Agricultural Experiment Station in order to evaluate the performance of 11 clones of hybrid poplar and 20 clones of willow for use in bioenergy production. I used this clonal yield trial to measure soil GHG fluxes and N leaching associated with converting grassland to either hybrid poplar or willow.

The site was cleared of the original hardwood forest approximately 100 years ago and was used for a variety of purposes including potatoes, small grains, hay, and pasturing cattle. When the farm was transitioned into intensive grazing in 2005 it was seeded with birdsfoot trefoil (*Lotus corniculatus)* along with a small amount of timothy (*Phleum alpinum*). The current landowner has owned the site for the past 25 years during which time the land was used primarily for hay alternating with small grain production and intensive grazing. During that time period, the site regularly received cow manure applications of about 23 500 L ha^{-1} annually. Prior to establishing the willow and poplar yield trials in spring of 2009, the site had not been tilled since the spring of 2005. The most abundant plant species in the grassland prior to conversion to willow and poplar yield trials were birdsfoot trefoil, canary reed grass (*Phalaris arundinacea*) and quack grass (*Elytrigia repens).*

Figure 4.1 Experimental site location in Michigan, U.S.A. “For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.”

The area has lake-enhanced normal seasonal precipitation of 762 mm, and daily minimum winter temperatures fall to −18°C or lower an average of 26 times in a season. The growing season averages 91 frost free days and means annual maximum and minimum temperatures are 11 and -3°C. The soil type is a moderately well-drained Munising fine sandy loam (Eichenlaub et al. 1990).

4.3.2. Experimental set-up

The clonal yield trial was established in the spring of 2009 in a randomized block design with 5 replicate, 64-tree plots of poplar (19 x 22 m) and 4 replicate, 78-tree plots of willow (6 x 8 m). It was not feasible to investigated differences among all 31 clones so I selected a priori, promising clones of willow (*Salix dasyclados* SV1]) and of poplar (*Populus nigra x P. maximowiczii* [NM6]). The poplar plots were planted at a spacing of 2.4 m between rows and 2.1 m between trees within rows, while the willow plots were planted in double spacing with 0.8 m between narrow rows, 1.5 m between wide rows and 0.6 m between trees within rows (Bergkvist and Ledin 1998). Additionally, four plots situated adjacently (at least 10 m away from the cultivated and plantation zones) to the poplar and willow trials, in the undisturbed pastureland were used as controls.

Field management practices for converting the grassland to SRWC started with weed control on 23 May 2009 which consisted of applying *glyphosate* (2.11L active ingredient ha^{-1}) to all poplar and willow plots. Next, plots were plowed and disked on 31 May 2009 to about 20-25 cm depth, followed by a second cultivation with a tiller on 12 June 2009 to 15-20 cm depth. On 17 and 18 June, 2009, all willow and poplar plots were again sprayed with the herbicides *simazine* (1.13 kg active ingredient ha^{-1}) and *oxyflurofen* (1.13 L active ingredient ha^{-1}).

Herbicide application rates were determined from local experience at MSU's Forest Biomass Innovation Center (FBIC). Descriptions of cultural management practices applied to poplar and willow yield trials are provided in Appendix –Tables A.-2 &-3. No fertilizers were applied during this first year of plantation establishment. Planting of willow and poplar cuttings (about 0.6 to 1.3 cm in diameter and 25 cm in length) occurred on 16 and 18 June 2009, respectively and cuttings were obtained from Hramor Nursery, Michigan. The whole experiment was protected with an electric fence on 13 July 2009 to prevent the impact of deer browsing. On 2 September 2009, the poplar plantation soil was again shallow-cultivated between tree rows. Willow shoots were coppiced on 20 October 2009 using a Troy-bilt sickle bar mower powered by a walk-behind system. The stems after cutting were typically 2.5 to 5.0 cm in height depending on the micro-relief of the area.

4.3.3. Measurement of GHG fluxes

Emissions of CO_2, N_2O and CH_4 were measured 11 times in 2009 (May –November) and two times in spring of 2010 using one (26.2 cm – diameter and 26.5 cm tall) static chamber (high-density polyethylene plastic) per plot. Chambers consisted of bases driven 10 cm into the soil and gas tight lids fitted with rubber septa to sample the approximately 10 L of headspace volume. Chambers were left uncovered except during the periods when gas samples were being collected. Aboveground vegetation in each chamber was clipped immediately prior to sampling in order to avoid respiratory fluxes of CO_2 from aboveground plant parts. Removing plants from the chamber was performed to solve the problems associated with subtracting the fraction of the CO_2 release originating from the aboveground vegetation which is not taking into account when calculating global warming potential. Gas samples were collected from the headspace between

10.00 and 15.00 h to minimize changes of soil GHG fluxes associated with diurnal cycles. Headspace gas samples (10 mL) were drawn using a 10 mL syringe at 0, 20, 40 and 60 minutes following chamber closure, and then immediately over-pressurized to pre-flushed 5.9 mL flat-bottom exetainer vials (Labco, Unlimited, Buckinghamshire,U.K.).

The concentration of CO_2, N_2O and CH_4 in the headspace gas samples were determined using a Gas Chromatograph (GC-2014, Shimadzu Corporation) equipped with electron-capture and flame-ionization detectors. Gas flux measurements were initiated on 22 May 2009 before field management and planting took place and continued until 4 May 2010, except during the winter period when the ground was continually covered with snow. A total of 13 sampling events were captured over the course of this one year investigation, of which 11 were conducted in 2009 (22 May, 5 and 25 June, 7 and 28 July, 11 and 24 August, 14 September, 6 and 17 October and 12 November) and two in 2010 (30 March and 4 May).

4.3.4. Measurements of Nitrogen leaching potential

Two pairs of tension lysimeters (Soil Moisture Equipment, Santa Barbara, CA) were installed within each plot in the poplar and willow plantations, and a single lysimeter in each undisturbed grassland control plot. Lysimeter cups were installed at 50 cm below the soil surface; one within a tree row and the second between two consecutive rows of trees. A tension of -50 kPa was applied to each lysimeter two to three weeks prior to sampling. Water samples were taken height times the day of gas sampling, starting from 11 August 2009 throughout 4 May 2010 (except during the winter period when the soil was permanently covered with snow). Solution samples were analyzed for NO_3^-–N and ammonium (NH_4^+–N) spectrophotometrically (Spectrophotometric plate reader, Model ELx Bio Tek Instruments, Inc. Winooski, Vermont,

U.S.A.) following the procedures described by Doane and Horwath (2003) and Sinsabaugh et al. (2000), respectively. Total dissolved N was determined by oxidative combustion-chemiluminescence (Shimadzu model TOC-V_{CPN} analyzer and, Kyoto, Japan). Dissolved organic nitrogen (DON) was calculated as the difference between total N and mineral N (NO_3^- +NH_4^+–N).

4.3.5. Nitrification Potential

Soil nitrification potentials were assayed according to the "shaken slurry method" developed by Hart et al. (1994) in order to evaluate the activity of the NH_4^+-N oxidizer community in soil as affected by grassland conversion to SRWC production. Soil samples collected one week prior (22 May 2009) and eight weeks after (22 July 2009) land conversion, were use to measure nitrification potential. The10-cm deep soil cores collected during GHG flux measurements were kept in plastic zip bags and stored in a cooler or fridge (5^oC) and transported to the Forest Biogeochemistry laboratory at MSU. Field-moist soils were sieved (<2mm) after removing all vegetation and coarse root parts, before placing subsamples of 10 g of soil into an acid-washed Erlenmeyer flasks with 100 mL of buffer solution (0.384 ppm of 1M KH_2PO_4, 0.614 ppm of 1MK_2HPO_4 and 15 ppm of $(NH_4)_2SO_4$ solutions) with the pH adjusted to 7.2. Then aliquots of 1 mL were taken from the shaken slurry incubation at 2-h, 4-h, 20-h and 24-h period, and 0.1mL of flocculant solution added to it. Samples were analyzed for NO_3^--N using the microplate assay as described in the previous section after centrifuging at 3000 rpm for 10 min.

4.3.6. Ancillary data

Climatic data (precipitation and air temperature) were obtained from the weather station at MSU's Agricultural Experimental Station at Chatham (Enviro-Weather) located 27 km east of the field site. Soil temperature measurements were taken at 7 cm soil depth during each sampling event in close proximity (about 10 cm) to each of the chambers, using a temperature probe (HI-145, Hanna Instruments) I used 3.8-cm diameter PVC soil corers to take a 10-cm deep soil core in close proximity to the gas flux chamber at each sampling period. Gravimetric water content was determined for each chamber per sampling date, and then multiplied by the bulk density (B_D in Mg m^{-3}) to determine soil volumetric water. Total porosity was determined as ($1– B_D X P_D^{-1}$) while P_D is soil particle density assumed to be 2.65 Mg m^{-3}. The ratio of volumetric soil water to total porosity was used to calculate soil water-filled pore space (WFPS).

Soil samples collected at each gas sampling point were also used for determination of NH_4^+-N and NO_3^--N concentrations after extracting the sieved soil (<2mm) with 2 M KCL (5:1 extractant to soil ratio) and analyzing extracts spectrophotometrically. All soils were processed within 24 h after collection and stored in a fridge (5 deg). Soil pH was measured (in a mixture of soil and 0.01 M $CaCl_2$) from air-dried soil with a Corning pH Meter 430 (Corning Incorporated, Science Products Division, New York, 14831). Total C and N contents were determined on the same soil samples by dry combustion with an elemental analyzer (Model ECS 4010, Costech Analytical, Valencia, CA).

4.3.7. Nitrogen leaching and GHG flux calculations

To estimate nitrogen leaching, I used a modified bucket model (Fetter 1988) to estimate water yield at 50-cm depth from potential evapotranspiration (modified Penman–Monteith

method), rainfall, and soil water holding capacity. On days with a net water yield, I estimated leaching losses as the product of water yield (L m^{-2}) and soil solution NH_4^+-N, NO_3^--N and DON concentrations (mg L^{-1}). Based on the Inter-governmental Panel on Climate Change (IPCC 1997) methodology, I assumed that 2.5% of the N leached would eventually be denitrified to N_2O in water ways.

GHG fluxes were estimated from the concentration change in the chamber headspace over the 60 min sampling period. Chamber methods for measuring trace gas fluxes are widely reported to alter the diffusivity of trace gases at the soil surface following chamber deployment, leading to an underestimation of actual gas fluxes (Livingston et al. 2006; Rochette and Bertrand 2007). To account for this bias due to the chamber deployment, I used the simplified linear method for quantifying theoretical underestimation of chamber-based gas fluxes developed by Venterea et al. (2009) to calculate the GHG emission rates from my experimental plots. Estimation of cumulative CO_2, N_2O and CH_4 emissions during the study period was done by successive linear interpolation between adjacent sampling dates assuming that emission followed a linear trend during the periods when no sample was taken.

To compare the net effect of land use conversion from long-term grassland to SRWC systems on the atmospheric GHG budget, I used global warming potential factors of 25 and 298 based on a 100-year horizon for CH_4 and N_2O, respectively, to convert the soil emissions of these two GHGs to CO_2eq (IPCC 2007). The global warming potential (GWP), a measure of how much a given mass of GHG is estimated to contribute to global warming, of the different land use types was calculated using the following equation (Watson et al. 1996).

$$\text{Net } GHG_{flux} = CO_2 + 25*(CH_{4flux}) + 298*(N_2O_{flux}) \quad \text{Eq. (4.1)}$$

Soil CO_2 flux in grasslands is composed of root respiration (or the respiration of underground portion of green plants) and respiration due to microbial decomposition of SOM (refers to as heterotrophic respiration). With regard to the CO_2-driven greenhouse effect, however, only SOM decomposition contributes to changes in atmospheric CO_2 concentration (Wang and Fang 2009). Hanson et al. (2000) reviewed published articles on root contribution (RC) to soil respiration and found that grassland RC to total soil CO_2 effluxes ranged between 10 and 90% with an average near 50%. Therefore, I used three scenarios for calculating the net GWP of the bioenergy systems: the first scenario (best case) assumed grassland root contribution (RC) total soil CO_2 efflux to be 10%; a second scenario (worst case) assumed that grassland RC is 90% of the total soil CO_2 efflux and the third scenario used an average value of 50% for RC to total soil CO_2 efflux. Because trees in the plantations were still young and weeds were aggressively controlled during the study period, root respiration (from poplar and willow seedlings and from weeds) was assumed to be negligible in both bioenergy systems. I evaluate the validity of these assumptions and scenarios in the Discussion section below. To calculate cultivation induced GHG debt and the payback time, I also made the assumption that yields as harvestable wood biomass in the region would be in the range of 5-20 Mg DM ha^{-1} yr^{-1} with a current average of 11 Mg DM ha^{-1} yr^{-1} based on results of previous studies (Kelly 2002, Ronald et al. 2009). Based on Gasol et al. (2009) poplar biomass is 50.3% carbon and I assumed the same C content in the biomass of willow.

4.3.8. Data analysis

I tested for the effect of treatment and sampling date on fluxes of CO_2, CH_4, N_2O and the net GHG flux from soil. I used a mixed model, repeated measures analyses of variance (Little et al. 1996) in SAS (SAS Institute, Cary, NC), unequal variance model to test for the effect of land use type, sampling date and the interaction between land use type and sampling date for each GHG, soil temperature and WFPS. Mean values of flux rates for N leaching and for each trace gas and net GHG flux as CO_2eq were calculated for each sampling date. Each GHG flux rate was also converted in terms of CO_2eq using the GWP coefficients referenced above. Soil inorganic N concentration and N species concentrations in leachate water were also analyzed for differences among treatments with a mixed model, repeated measures analyses of variance, which included the random effects of treatment. Differences were accepted as significant at the 0.05 level.

4.4. RESULTS

4.4.1. Soil chemical properties

Before tillage on 22 May of 2009, soil total C concentrations in the top 10 cm of soil were 3.3%, 3.7% and 3.1% in the undisturbed pasture, poplar and willow plots, respectively, and did not differ between treatments (Table 4.1). Soil total N averaged 0.24%, 0.27% and 0.22% in the undisturbed pasture, poplar and willow plots, respectively, with no significant difference between treatments. Similarly, there were no significant differences in soil C: N ratio and pH before tillage. There were no detectable changes in total soil C and N levels a year following land cultivation (data not shown here). Soil bulk density, measured on 7 July of 2009 after initial tillage, was significantly lower in the poplar plantation than in the willow and untilled grassland.

4.4.2. Environmental variables

The annual (from May of 2009 to April of 2010) rainfall was 575 mm which was much less than the long-term average of 762 mm. Of the amount of rainfall received during the study period, it was estimated that 225 mm (39%) percolated below the rooting zone (50 cm). During the first growing season, the driest period was from 30 August to 20 September of 2009 when there was no rainfall (Fig 4.2-a).

WFPS showed highly significant ($F_{2,\ 17.6}$=22.0, P<0.001) main effects of treatment and was highest in the undisturbed grassland, intermediate in the willow, and lowest in the poplar plots. Before cultivation on 31 May 2009, WFPS was indistinguishable ($F_{2,\ 64}$=1.7, P=0.189) among treatments. WFPS also varied temporally ($F_{12,\ 99.3}$=7.5, P<0.001), the temporal patterns being influenced by rainfall variability (Figs 4.2-a, and 4.2-b). Most notable was the drop in WFPS coincident with the dry period in early September, 2009.

Table 4.1 Selected soil characteristics measured from 0-10 cm of soil depth in a long-term grassland as well as in poplar and willow biomass bioenergy plantations in the central Upper Peninsula of Michigan, U.S.A.

Treatment	Total C[1]	Total N[1]	C:N[1]	NH_4^+-N [3]	NO_3^--N[3]	Bulk Density[2]	Porosity[2]	pH[1]
	(mg kg^{-1})	(mg kg^{-1})	(Ratio)	(mg kg^{-1})	(mg kg^{-1})	(g m^{-3})	(%)	
Grassland	32.8 (3.5)a	2.4 (0.4)a	13.9 (0.2)a	2.9 (2.5)a	5.3 (0.9)a	1.13 (0.05)b	57 (3) a*	5.24 (0.17)a
Poplar	37.2 (3.0)a	2.7 (0.3)a	14.2 (0.1)a	3.0 (3.7)a	96.1(13.7)b	0.89 (0.06)a	66 (2) a	5.33 (0.04)a
Willow	31.0 (5.4)a	2.2 (0.3)a	13.8 (0.1)a	2.6 (3.2)a	67.0 (14.7)b	1.10 (0.04)b	59 (1) a	5.29 (0.12)a

1 *Pre-cultivation;* ***2****Post-cultivation and* [3]*one year average; *Means in columns followed by the same letter are not significantly different at the 5% level using the means separation test*

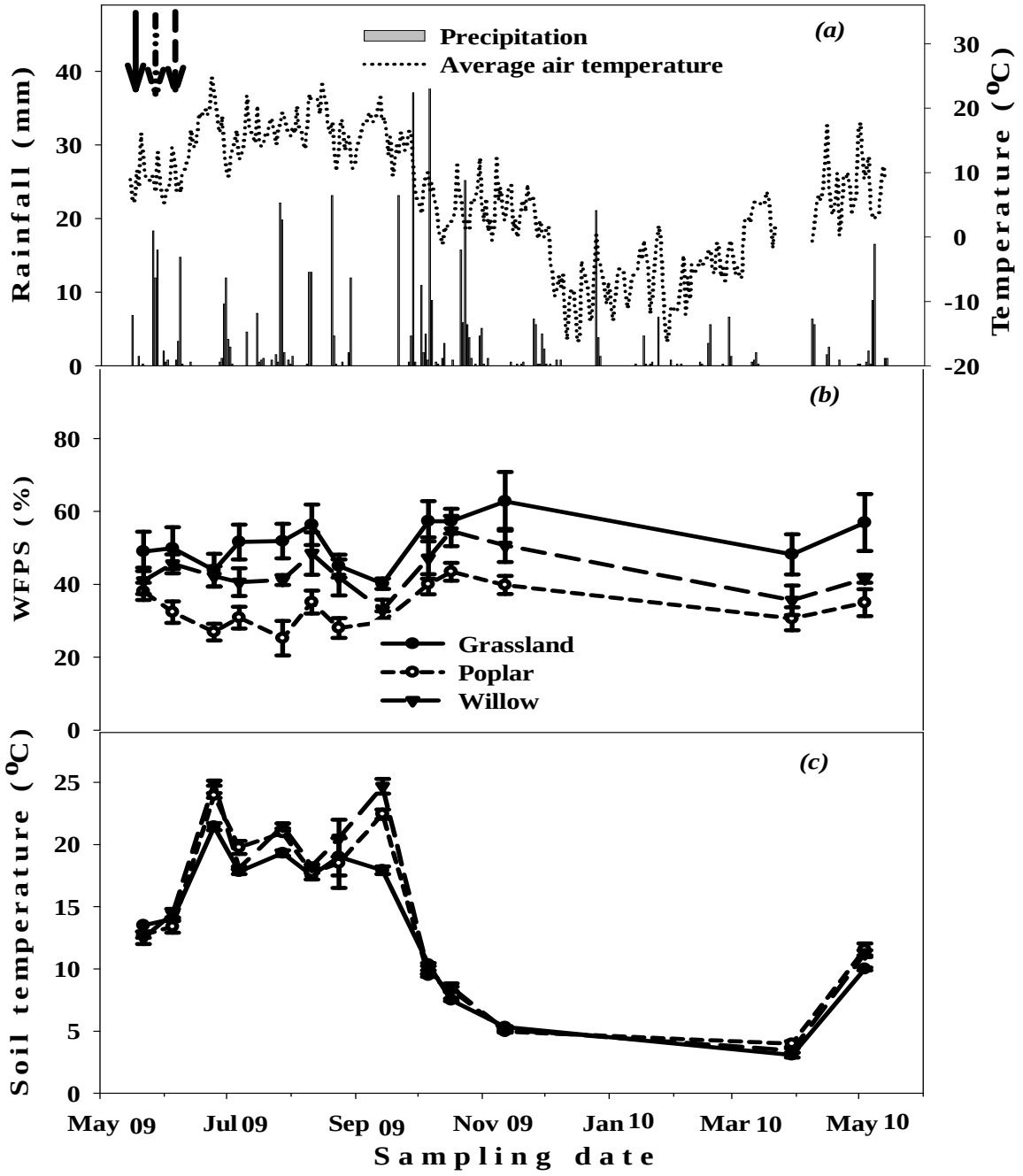

Figure 4.2 (a) Precipitation and average air temperature, (b) soil water filled pore space and (c) soil temperature measured from a poplar and willow biomass bioenergy plantations and adjacent grassland during the first year of a long term grassland conversion to bioenergy systems at Skandia in the Upper Peninsula of Michigan, USA. Black solid, dashed dot-dotted and long dashed arrows indicate dates for field application of herbicide, plowing and planting respectively

However, there was no significant ($F_{24,\ 97.6}$=0.9, P=0.655) treatment*date interaction effect for WFPS. Soil temperatures were significantly ($F_{2,\ 33.1}$=67.5, P<0.001) cooler in the undisturbed grassland compared to the tilled poplar and willow plantation soils. However, soil temperature was statistically indistinguishable (P=0.112) between the poplar and willow biomass plantations at any time during the study period. Soil temperature also varied across all sampling dates; being the greatest in the summer, then decreased over time from autumn to winter (Fig 4.2-c).

4.4.3. Soil nitrogen availability

Soil extractable NH_4^+-N concentration did not differ significantly ($F_{2,\ 37.5}$=1.9, P=0.170) between the undisturbed grassland and the tilled SRWC plantations and was unaffected ($F_{24,\ 96}$=1.4, P=0.133) by the interactions between treatment and sampling date. In contrast, soil NH_4^+-N levels varied significantly ($F_{12,\ 97}$=11.7, P<0.001) across the sampling dates, being greatest early on in the experiment (June-September) (Fig 4.3-a).

Initial nitrification potentials, measured one week prior to the grassland conversion (22 May, 2009), were statistically indistinguishable between the three sites (Fig 4.3-b). On average, nitrification rates were 0.1, 0.3 and 0.5 mg N kg^{-1} soil day^{-1} in the willow, grassland and poplar plots, respectively prior to conversion. However, eight weeks after cultivation, nitrification potential was significantly enhanced and was seven-fold higher in both SRWC plantations compared to the control grassland (Fig 4.3.-b).

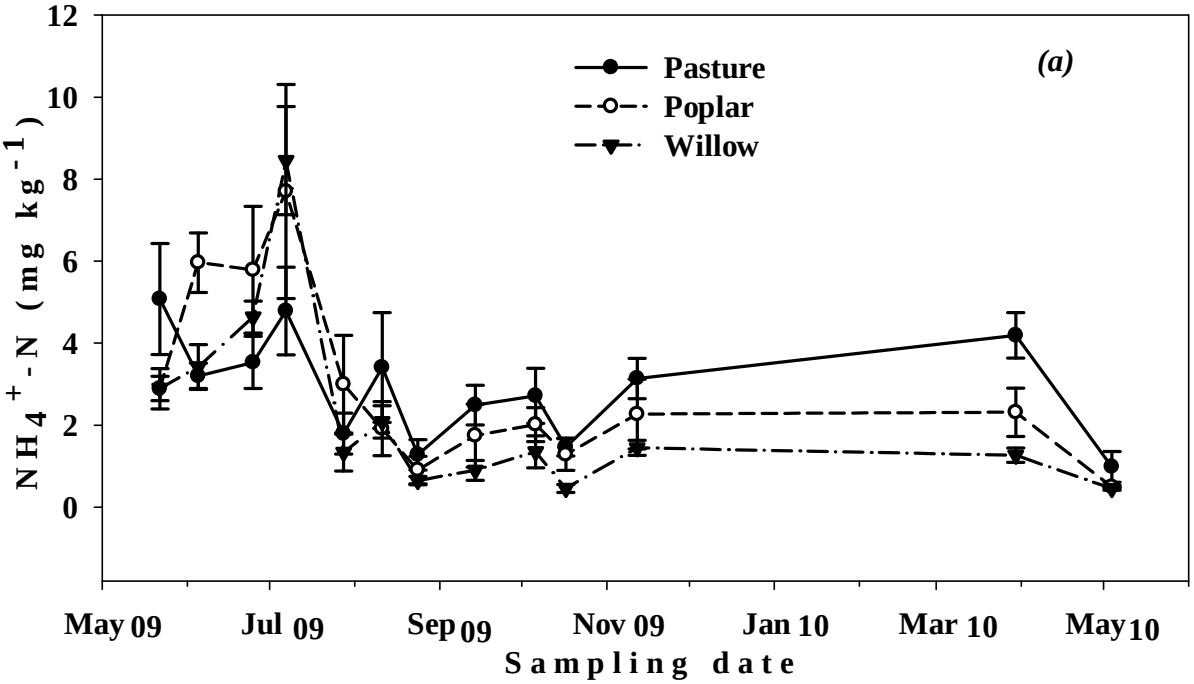

Figure 4.3 (a) Soil ammonium concentration, (b) nitrification rates and (c) nitrate concentration (KCL extraction) measured from 0-10 cm soil depth in the undisturbed grassland and in the poplar and willow biomass plantations prior and after cultivation at Skandia in the Upper Peninsula of Michigan, USA. Black solid, dashed dot-dotted and long dashed arrows indicate dates for field application of herbicide, plowing and planting respectively

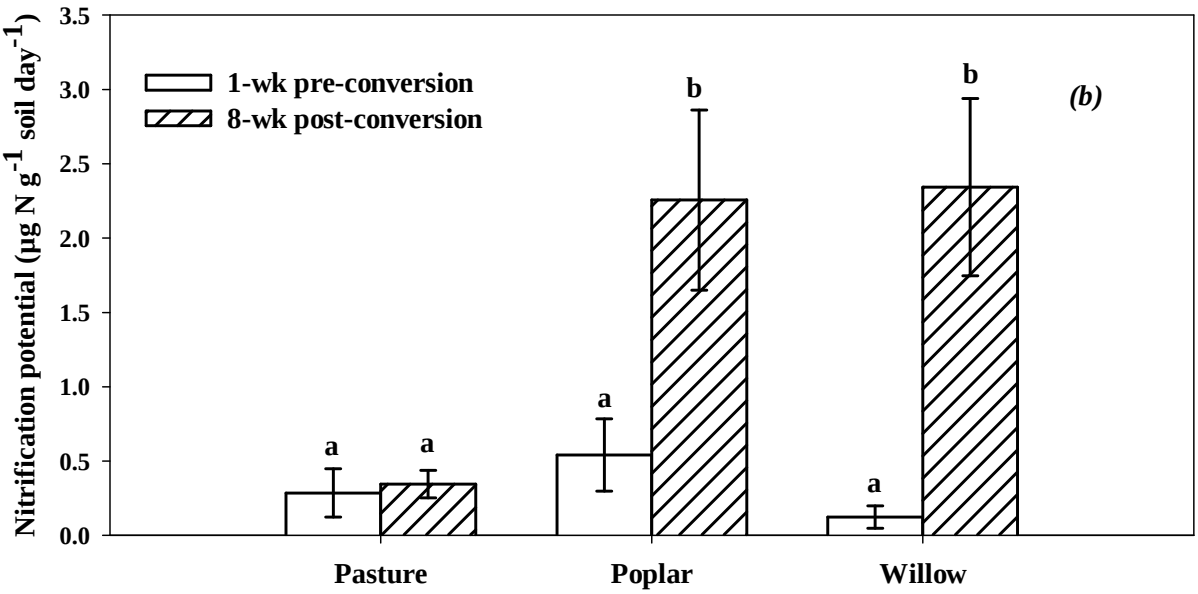

Figure 4.3 (cont'd)

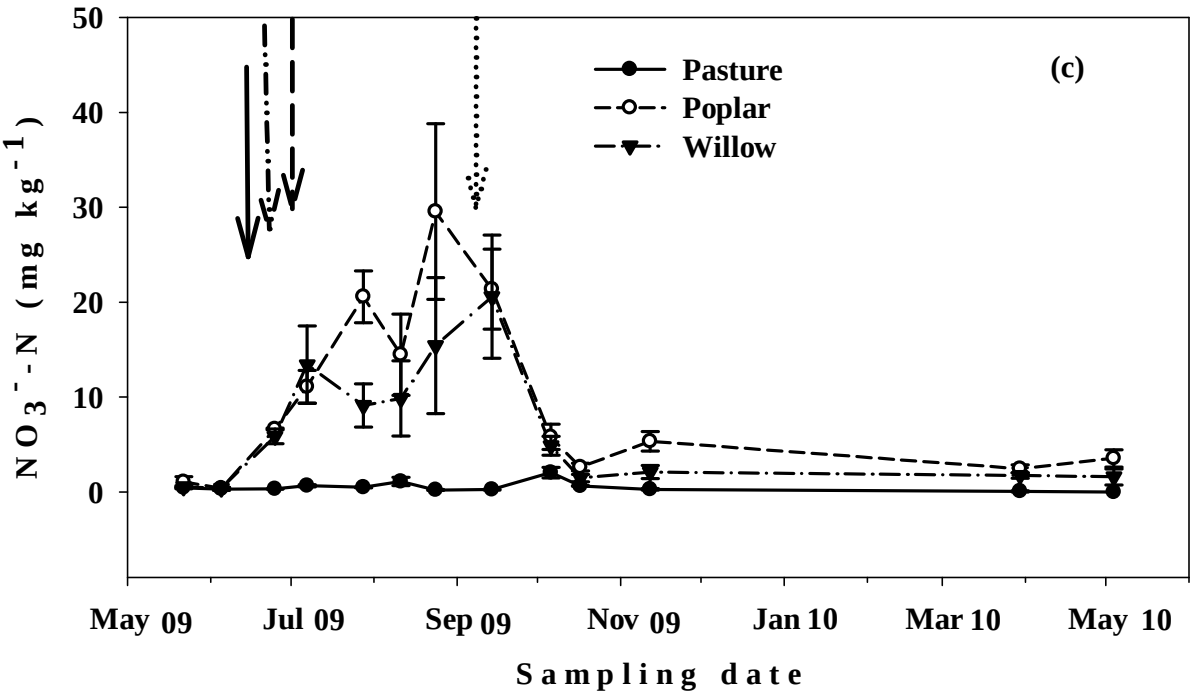

Figure 4.3 (cont'd)

The marked increase in nitrification potential following cultivation was reflected in substantial increases in soil extractable NO_3^--N pools. Soil NO_3^--N was significantly ($F_{2, 43.7}$=35.4, P<0.001) greater in both SRWC plantations relative to the adjacent grassland control. Soil nitrate also varied significantly ($F_{12, 99.3}$=9.9, P<0.001) across sampling dates. For instance, nitrate levels, measured on 22 May 2009 and on 5 June 200, were consistently low (< 1 mg N kg^{-1}) in all plots, with no significant differences among the three sites (P=0.963 and 0.116). Nitrate levels remained unchanged (P=0.520) in the undisturbed grassland throughout the study period (Fig 4.3-b). In contrast, soil NO_3^--N levels steadily increased after 5 July 2009 through mid-September 2009 with peaks as high as 21 and 30 mg kg^{-1} of soil in the tilled poplar and willow plantations, respectively, then declined rapidly through the autumn and the winter, and became indistinguishable to the grassland controls by spring 2010.

4.4.4. Nitrogen leaching

With the exception of the poplar plots at the first sampling date (11 August 2009), concentrations of NH_4^+-N in leachate were consistently low (<0.1 mg/L) at all sampling dates, regardless of treatments (Fig 4.4-a). In contrast, nitrate concentration in soil leachate was significantly (P<0.001) higher from both cultivated SRWC plantations than the undisturbed grassland control at all sampling dates (Fig 4.4-b). In the grassland, NO_3^--N levels were consistently negligible, ranging from 0.1 to 0.9 mg/L throughout the study period. There were marked increases of NO_3^--N concentration in soil leachate from the poplar and willow plantations due to cultivation, with values as high as 35 and 39 mg/L, respectively (Fig 4.4-b).

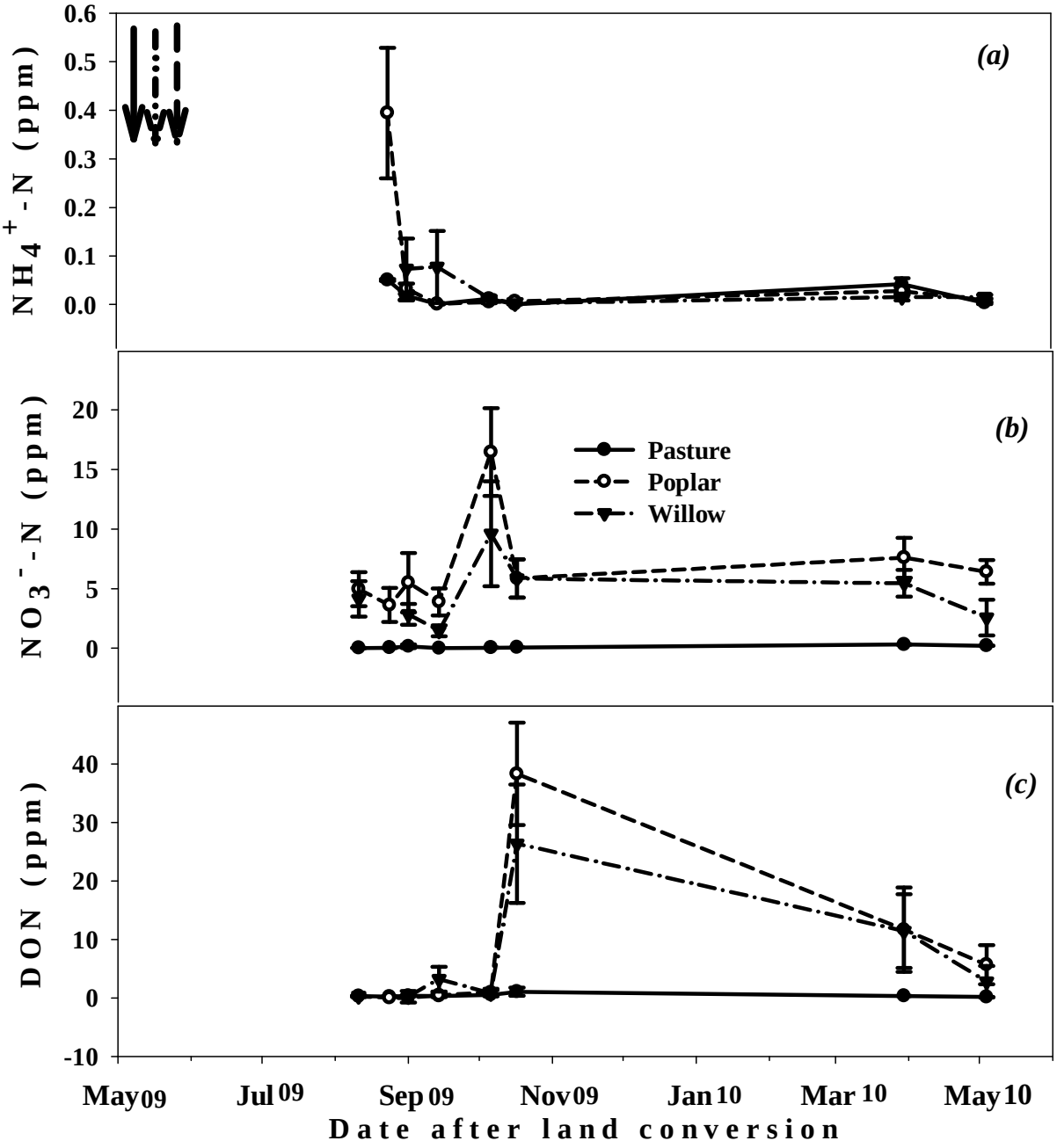

Figure 4.4 Concentrations of (a) NH_4^+-N, (b) NO_3^--N and (c) dissolved organic N in leachate water collected at 50 cm below the soil surface measured in the undisturbed grassland and in the poplar and willow biomass plantations after cultivation at Skandia in the Upper Peninsula of Michigan, USA. Black solid, dashed dot-dotted and long dashed arrows indicate dates for field application of herbicide, plowing and planting respectively.

Similar to nitrate, DON levels were low in the undisturbed grassland throughout the sampling period (Fig 4.4-c). In both cultivated willow and poplar plantations, however, DON levels increased 14.1- and 19.6- fold, respectively relative to the adjacent grassland controls following soil cultivation. It is noteworthy that, later in the season, DON increased more markedly than nitrate. The marked increase in NO_3^--N and DON concentrations following cultivation was reflected in substantial leaching loads of these two N pools. Annual nitrate leaching losses were 16 -and 22 -fold higher in the tilled willow and poplar plantations, respectively, relative to the adjacent undisturbed grassland (Table 4.2). Similarly, annual leaching loads of DON were 14 –and 20 –fold greater in the tilled willow and poplar plantations, respectively, relative to the grassland controls. Annual leaching of NH_4^+-N was negligible from both disturbed and undisturbed plots. For all nitrogen species, leaching losses from the cultivated poplar and willow plots amounted to 39 and 54 kg N ha^{-1}, whereas N loss from the undisturbed grassland was less than 3 kg N ha^{-1} for the study period.

Table 4.2 Hydrologic fluxes of N species at50 cm soil depth in a long-term grassland as well as in poplar and willow biomass bioenergy plantations in the central Upper Peninsula of Michigan, U.S.A., from August 2009 to May 2010.

System	NO_3^--N		NH_4^+-N		Dissolved N		Total N	
	----------(kg ha^{-1} yr^{-1})----------							
Grassland	0.8	(0.2)	0.02	(0.00)	1.8	(0.4)	2.6	(0.5)
Poplar	17.8	(3.0)	0.01	(0.01)	36.0	(5.1)	53.9	(6.0)
Willow	12.8	(2.5)	0.03	(0.01)	25.9	(4.4)	38.8	(5.2)

4.4.5. Carbon dioxide fluxes

Soil CO_2 fluxes were significantly ($F_{2,\ 28}$=5.0, P<0.014) affected by land conversion; soils from both tilled willow and poplar plantations emitted 29 and 42% less CO_2 relative to the adjacent undisturbed grassland controls. Soil CO_2 effluxes also varied significantly ($F_{12,\ 101}$=28.2, P<0.001) across sampling dates, with rates being higher in the summer and lower in autumn and winter (Fig 4.5-a). Examining the temporal patterns of CO_2 fluxes, before cultivation on May 22, 2009, CO_2 flux rates were low with no significant (P=0.645) differences between treatments. Then flux rates increased and stayed high during the growing season (June-September 2009) in both uncultivated and cultivated plots with peaks as high as 480 mg C m^{-2} h^{-1} in the uncultivated grassland plots (14 August 2009). Flux rates decreased sharply in autumn and remained low the following spring. In early spring of 2010, flux rates started rising again with more pronounced increases in the undisturbed grassland. The interacting effects between treatment and sampling date were also significant ($F_{24,\ 91.0}$=4.5, P<0.001) for CO_2 effluxes. Overall, annual cumulative CO_2 emissions from soil were 41.9 and 24.3 and 30.0 Mg CO_2 ha^{-1} yr^{-1} from the undisturbed grassland, the tilled poplar and tilled willow plantations, respectively (Table 4.3).

4.4.6. Nitrous oxide fluxes

Flux rates of N_2O were significantly ($F_{2,\ 39.6}$=15.9, P<0.001) higher in the disturbed soil (poplar and willow plantation) than in the undisturbed grassland control plots. Likewise, flux

rates in the disturbed plots were significantly affected by sampling date ($F_{12, 99.6}$=6.8, P<0.001). For instance, in both cultivated SRWC plantations, there was an initial pulse of N_2O flux rates a week following the cultivation event which increased until 11 August 2009, followed by a sharp decrease in mid-September of 2009. Thereafter, a second pulse occurred in both poplar and willow plantations through October 2009.

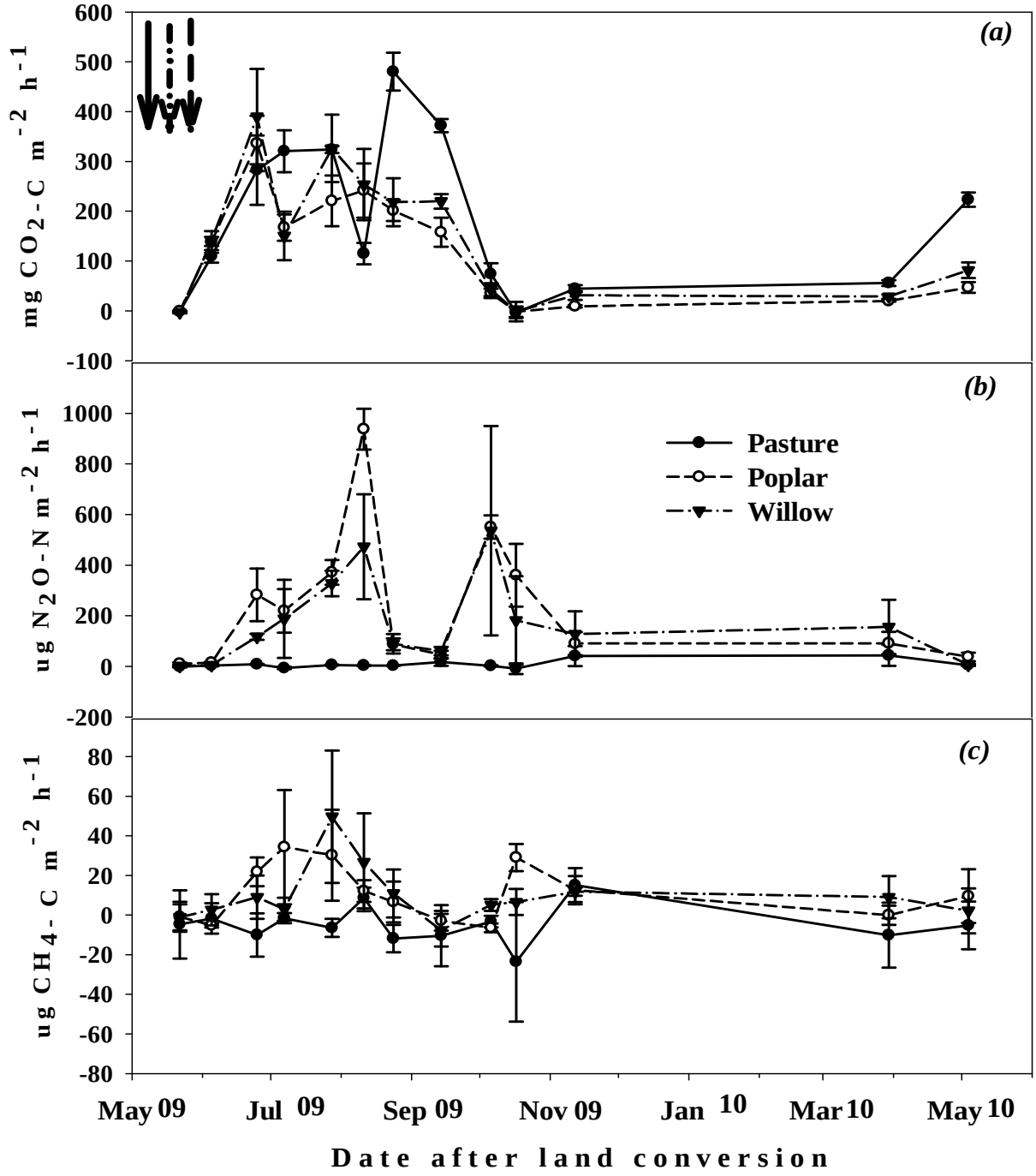

Figure 4.5 Fluxes of (a) CO_2, (b) N_2O and (c) CH_4 from soil measured in the undisturbed grassland and in the poplar and willow biomass plantations after cultivation at Skandia in the Upper Peninsula of Michigan, USA. Black solid, dashed dot-dotted and long dashed arrows indicate dates for field application of herbicide, plowing and planting, respectively.

Similarly, the interaction effects between the two factors (treatment and sampling date) were significant ($F_{24, 98.4}$=2.3, P=0.002). Annual cumulative N_2O emissions for the undisturbed grassland and the tilled poplar and willow plantations were 0.3, 5.8 and 4.5 Mg CO_2eq ha^{-1} yr^{-1}, respectively (Table 4.3).

4.4.7. Methane fluxes

The undisturbed grassland was a small sink for CH_4 (annual average consumption rate of -5.00 µg C m^{-2} h^{-1}) whereas soil disturbance resulted in CH_4 emissions of about 10.8 and 9.8 µg C m^{-2} h^{-1} from the poplar and willow plots, respectively; however, these differences, were not statistically significant ($F_{2, 26.2}$=2.9, P=0.071) (Fig 4.5-c and Table 4.3). In contrast to CO_2 and N_2O fluxes, methane flux rates did not differ significantly ($F_{12, 98.8}$=1.29, P<0.238) over the experimental period, nor was there a significant ($F_{24, 97.6}$=0.0.96, P=0.525) interaction between treatment and sampling date. Over the entire experimental period, undisturbed grassland cumulatively consumed as much as 10.6 kg CO_2eq ha^{-1} yr^{-1}, whereas the tilled poplar and willow plots emitted 21.1 and 18.3 kg CO_2eq ha^{-1} yr^{-1} (Table 4.3).

4.4.8. Global warming potential and payback period

In general, the poplar treatment resulted in a lower cultivation induced GWP and shorter payback period relative to willow. The best case scenario, based on the assumption that the contribution of roots to soil CO_2 effluxes in the grassland plots is 10% results in the reference grassland soil producing 19 and 7% greater CO_2 flux than the poplar and willow bioenergy systems,

respectively, for this initial phase of plantation establishment (Table 4.4). Under this scenario, initial cultivation would have resulted in a net soil C sequestration of 7.0 and 2.8 Mg CO_2eq ha^{-1} in the poplar and willow bioenergy plantation.

Table 4.3 Direct and indirect GHG emissions and total global warming potential estimated for a long-term grassland as well as in poplar and willow biomass bioenergy plantations in the central Upper Peninsula of Michigan, U.S.A. Data were collected during one year period following land conversion

System	Direct emission (CO_2eq kg ha^{-1})						Indirect emission (CO_2eq kg ha^{-1})		Cumulative GHG emission (CO_2eq kg ha^{-1})
	CO_2		N_2O		CH_4		NO_3^--N +DON		
Grassland	42185	(175)	342	(16)	-11	(7)	31	(2)	42547
Poplar	24391	(117)	5886	(236)	21	(14)	629	(22)	30927
Willow	30105	(135)	4558	(163)	18	(13)	453	(18)	35134

Table 4.4 Cultivation induced-cumulative greenhouse gas flux, -global warming potential and payback time for willow and poplar plantation establishment. Values are estimated based on raw data of direct GHG fluxes, indirect emissions of N_2O calculated from N leaching data and assumptions of grassland root contribution to soil CO_2 fluxes (see text for full description). Payback periods are calculated based on scenarios of minimum, average and maximum yields for SRWC in the region

Scenarios	Cultivation induced GWP (Kg CO_2eq ha^{-1} yr^{-1})		Payback time (year)					
			Poplar			Willow		
	Poplar	Willow	Min.	Avg.	Max.	Min.	Avg.	Max.
Scenario1 (RC=10%)	-7040	-2833	-0.8	-0.3	-0.2	-0.3	-0.1	-0.1
Scenario2 (RC=50%)	9835	14042	1.1	0.5	0.3	1.5	0.7	0.4
Scenario3 (RC=90%)	26709	30916	2.9	1.3	0.7	3.4	1.5	0.8

Min = expected minimum yield (5 Mg DM ha^{-1} yr^{-1}), Avg. = average yield (11 Mg DM ha^{-1} yr^{-1}), and Max= maximum yield (20 Mg DM ha^{-1} yr^{-1}).

When RC to soil CO_2 efflux in the grassland plots is assumed to be 50%, cultivation induced GWP as CO_2eq is in the magnitude of 9.8 and 14.0 Mg CO_2eq ha^{-1} for poplar and willow bioenergy systems, respectively. A willow plantation with yields ranging from 5 to 20 Mg DM $ha^{-1}yr^{-1}$ would require 0.5 – 1.5 years to payback this GHG debt. Finally, the worst case scenario, using a RC of 90% for grassland plots results in a GHG debt as high as 26.7 and 30.9 Mg CO_2eq ha^{-1} with payback times ranging from 0.7 to 2.9 years and 0.8 to 3.4 years for poplar and willow bioenergy plantations, respectively.

4.5. DISCUSSION

In the present study, I documented substantial emissions of CO_2 and N_2O as well as soil and soil leachate NO_3^--N following conversion of grassland to poplar and willow bioenergy plantations. Based on the most likely scenarios for root contribution to soil CO_2 flux, I estimate that the newly-established poplar and willow plantations lost an additional 3.3 and 9.0 Mg CO_2 ha^{-1}, respectively, during the establishment year. Emissions of N_2O also drastically increased following the grassland clearing and cultivation for the SRWC plantation establishment. Cumulative direct N_2O emissions measured in the cultivated poplar and willow plantations were 17.2- fold and 13.3-fold, respectively higher relative to the undisturbed grassland site. Similarly, land-use conversion resulted in 20.3-fold and 14.6-fold increases of cumulative indirect N_2O emissions (from N leaching losses) in poplar and willow plots, respectively, relative to the grassland site. Although fluxes of methane were a small component of the GWP of the both bioenergy plantations, data suggest that the undisturbed grassland was a sink for CH_4 while the cultivated poplar and cultivated willow plantations were sources for methane. Overall, this estimate of cumulative emissions of all three GHGs suggests that grassland conversion resulted in a GHG debt of 9.4 and 14.2 Mg CO_2eq ha^{-1} for poplar and willow plots, respectively in the establishment year alone. This GHG debt reflects solely the effects of plantation establishment, which are typically neglected in most LCA studies. In comparison, Keoleian and Volk (2005) estimated that N_2O released from N fertilizer application and leaf litter decomposition over an entire rotation cycle of 23 years of willow biomass production would total 11.3 Mg CO_2eq ha^{-1}.

Moreover, nitrogen leaching increased markedly following grassland conversion, resulting in additional losses of 51 and 36 kg N ha^{-1} in the poplar and willow plots, respectively. These N leaching loads can potentially contribute to eutrophication of surrounding ground and surface waters.

Physical disturbance of soil through clearing and cultivation of native ecosystems is widely recognized to result in the loss of soil organic C (Lal et al. 1998; Reicosky 2003). Tillage increases top soil aeration, mixes plant residues with the soil, increases substrate availability to microbial decomposition, thus releasing CO_2 into the atmosphere (Reicosky et al 1999; Lal 2004). Therefore, I was expecting that cultivation of the grassland for establishing poplar and willow bioenergy plantations would result in larger CO_2 emissions in the disturbed plots relative to the undisturbed plots. Instead, based on raw CO_2 flux data, I found lower CO_2 efflux in the poplar and willow treatments relative to the undisturbed grassland, suggesting that cultivation of the grassland for establishing bioenergy crops resulted in a net sink of C. This result is consistent with two other studies (Linn and Doran 1984; Yamulki and Jarvis 2002) that also found greater CO_2 production from non-tillage grassland soil than from cultivated plots.

However, based on the characteristics of this system, it seems highly implausible that grassland clearing and cultivation would lead to C sequestration rather than net release from the soil. An alternative explanation is that greater autotrophic respiration in the reference plots masked the mineralization of SOC following clearing and cultivation. In both poplar and willow plots, low occupancy by crop plants, coupled with aggressive weed control, suggests that heterotrophic rather than autotrophic respiration accounted for virtually all of the CO_2 emissions

in these sites. In contrast, the high CO_2 emissions measured in the undisturbed grassland could be an indication that root respiration, from the dense and actively growing grasses and forbs, might have contributed to a substantial share of CO_2 released from the control plots.

To address this issue, I used existing data from the literature (Hanson et al. 2000 summary of previous studies) to evaluate the potential influence of root contribution to total soil CO_2 efflux, as direct measurements are exceptionally difficult (Killham and Yeomans 2001).In the best case (scenario 1) using the minimum RC of 10% from the range provided by Hanson et al. (2000), I estimated that cultivating the grassland for poplar and willow bioenergy plantation establishment resulted in a net C sequestration in the soil of about 7.9 - 13.6 Mg CO_2 ha^{-1}. Assuming that RC is 50% (scenario 2), or near the worldwide average for grassland based on Hanson et al. (2000), land conversion would release as much as 3.3 and 9.0 Mg CO_2 ha^{-1} in the poplar and willow plots, respectively. In the worst case (scenario 3), using the upper end value of RC (90%) suggests that grassland conversion would lead to net emissions of 20.2 and 25.9 Mg CO_2 ha^{-1} from poplar and willow plots, respectively.

Based on the range of possible yields of the bioenergy crops, estimates from scenarios 2 and 3 suggest the payback time could range from one to three years. A net C sink arising from grassland cultivation as suggested by scenario 1 (RC is 10%) appears implausible considering that tillage has extensively been cited to accelerate SOM decomposition and release CO_2 to the atmosphere (Lal et al. 1998; Lal 2004; Reicosky et al. 1999; Reicosky 2003). Furthermore, Hanson et al. (2000) found that RC values close to the lower end of the range occurred in grassland ecosystems in which root density was generally low (e.g. old fields and crop studies),

whereas high values (at least 50%) were generally recorded from untilled or permanent grasslands.

In order to get a better approximation of the RC value of the grassland of the present study site, I compared the root biomass of the studied grassland (measure from the top 10 cm in mid-summer) to data by Jackson et al. (1996) who conducted an extensive review of the literature on root distribution and biomass and found that temperate grassland root biomass averaged 600 g m^{-2} in the top 10 cm of the soil. This value is quite similar to root biomass of undisturbed grassland plots in this study, which averaged to 500 g m^{-2}. The fact that root biomass in the control plots approximated that of average values for temperate grassland suggests that scenario 2 (RC = 50%) is the more plausible for this site. Another assumption imbedded in my approach is that RC to soil CO_2 efflux in the poplar and willow plots is negligible. In a more recent study on similar soils in the UP we have found that root biomass at the end of the first growing season averages 0.4 g m^{-2} for poplar and 1.3 g m^{-2} for willow (Rothstein *unpublished data*) – nearly two orders of magnitude lower than that of our undisturbed grassland plots. Very low root biomass by SWRC crop species, coupled with aggressive weed control in conventional production practices, suggests that our assumption of negligible root respiration in willow and poplar plots is reasonable.

The higher CO_2 flux from willow compared to poplar plots (scenario 2 and 3) could possibly be due to differences in site preparation, tree planting density and growth habit. Unlike willow plots, poplar plots were shallow-cultivated in early September. Additionally, the higher planting density of willow relative to poplar might have resulted in a denser root development, thus leading to larger RC to soil CO_2 flux from the young willow trees. This may in part explain

the lower N_2O flux and NO_3^--N leaching loss found in the willow plots relative to the poplar treatments, as demand for NO_3^--N may be greater in willow than in poplar plots.

In contrast to CO_2 flux, conversion of grassland to willow and poplar plantings resulted in clear and dramatic increases in N_2O flux. On average, N_2O emissions was 2.6 µg N_2O-N m^{-2} h^{-1} in the reference control plots, while in the poplar and willow plots flux rates averaged 238.3 and 174.3 µg N_2O-N m^{-2} h^{-1}, respectively. Emission rates of N_2O measured from the reference grassland control plots in this study are comparable to those found (average of 2.9 µg N_2O-N m^{-2} h^{-1}) in grassland ecosystem at MSU's Kellogg Biological Station (KBS) (Grandy and Robertson 2006). However, N_2O flux rates in the cultivated plots in the present study were 8-11-fold higher than results obtained by Grandy and Robertson (2006) following initial cultivation of grassland at KBS. It is noteworthy that the tow grassland sites (at KBS and Skandia) have similar total soil C and N and differences in N_2O flux cannot be explained by SOM content of the sites. The grassland and cultivated plots in present study, however, are located in lowland area and there is the likelihood that higher moisture condition of the site has resulted in this elevated N_2O flux. Based on this field measurement data, land use conversion has produced an estimated GHG debt as N_2O of 4.6 and 6.1 Mg CO_2eq ha^{-1} for willow and poplar treatments, respectively. Of this, approximately 90% was attributed to direct N_2O emission from soil whereas the remaining 10% was attributed to indirect emissions through N leaching losses and its subsequent denitrification. The potential for significant GHG emissions due to establishment of SRWC is

typically ignored in LCA studies; however, N_2O emissions documented in this study over a single year exceeds the estimate of 4 Mg CO_2eq ha^{-1} of N_2O released from N fertilizer application over an entire rotation cycle of 23 years of willow bioenergy production (Heller et al. 2003; Koeleian and Volk 2005).

Elevated N_2O emissions in the poplar and willow plots relative to that of the undisturbed grassland appeared to be driven primarily by the high availability of soil N. Soil NO_3^--N concentrations averaged 9.6 and 6.7 mg kg^{-1} of soil in poplar and willow plots, respectively, whereas in the undisturbed reference control plots, NO_3^--N level averaged 0.5 mg kg^{-1} of soil. Seasonal N_2O emission rates and NO_3^--N concentrations followed a similar pattern (Figs 4.3-c and 4.5-b) with the exception of the low N_2O fluxes for sampling dates that occurred in September 2009. These findings are consistent with results from other studies which also observed that as soil NO_3^--N concentration increases following perennial grassland cultivation, N_2O flux also increases; with the initial increase generally following a short lag period (Pinto et al. 2004; Grandy and Robertson 2006). The low flux rates recorded in September 2009 were most likely due to low moisture availability at that particular month, demonstrating that elevated soil NO_3^--N levels are necessary, but not sufficient for stimulating N_2O emissions.

Elevated NO_3^--N levels in willow and poplar plots likely resulted from a combination of accelerated mineralization of N from SOM and grass residues, coupled with low vegetation occupancy of the sites during the planting year. These conditions likely supplied large quantities

of available NH_4^+ in soil, which, in turn, resulted in dramatic increase in the size or activity of the nitrifying bacteria community. Nitrifier response to grassland disturbance is clearly demonstrated in Figure 4.3-b, where soil nitrification potential measured one week before cultivation was low across the sites, but eight weeks following cultivation increased 7-fold in both cultivated sites relative to the uncultivated grassland treatments.

I have found that land-use conversion also resulted in large leaching fluxes of N, as both NO_3^--N and DON. I estimate that during this one year of poplar and willow establishment phase, total N leaching losses from land-use conversion were 36 kg N ha^{-1} and 51 kg N ha^{-1} willow and poplar plantations, respectively. These N leaching loads have the potential to contribute to eutrophication of river systems or negatively impact on the quality of water of the lakes in the study area. Impacts of N leaching associated with widespread grassland conversion for biofuels production is of particular concern for this region where nitrate accumulation in the Great Lakes is of pressing concern (Finlay et al. 2007). Moreover, using the IPCC (2007) approach, I estimated that indirect N_2O emissions associated with N leaching from willow and poplar plots contributed to 0.4 and 0.6 Mg CO_2eq ha^{-1}, respectively to the overall GHG debt. Nitrate leaching loads following land-use conversion, measured in this study, are within the range of 11 to 24 kg N ha^{-1} reported by Eriksen at al. (2008). Interestingly, DON dominated N leaching in both undisturbed and disturbed plots (69.2% and 66.8% of total N leaching as DON in uncultivated and cultivated plots, respectively), although absolute losses in the uncultivated soil were much lower. The magnitude of DON leaching loads in the cultivated poplar and willow plots is in agreement with results from Bhogal et al. (2000), who showed that significant DON

leaching may occur after grassland cultivation and can represent a significant pathway of N loss in these ecosystems once they are disturbed (Van Kessel et al. 2009). The late season DON spike in the cultivated plots was most likely due to a combination of factors such as colder soil, slowing of SOM mineralization and nitrification and high soil moisture at that sampling period.

Fluxes of methane were a small component of the GWP arising from SRWC plantation establishment. The undisturbed grassland was a small sink for CH_4 while the cultivated poplar and willow plantations were sources for CH_4; however, treatment differences were not statistically significant. The low P-value for this statistical comparison ($P = 0.071$), combined with the fact that similar results have been reported in other studies (Van den Pol-Van Dasselaar et al 1999; Regina et al. 2010; Steudler et al 1989, Moiser et al 1991), suggests that the transition from CH_4-sink to CH_4-source was biologically significant.

The ANOVA performed on total soil C and N data, collected at the top 0-10 cm soil layer over the sampling period, did not detect any C and N changes (likely due to spatial heterogeneity in soil), which is a common finding of short-term soil C studies (Cambardella and Elliott 1994, Grandy and Robertson 2006). However, the increase in nitrifier population size and NO_3^--N concentration in soil following cultivation, are evidence of accelerated SOM mineralization in the cultivated plots, which most likely resulted in a net loss of SOC in these plots. The decomposition rate of the labile pools of SOM, mainly composed of plant residues in different stages of decomposition, is reported to increase by 50 – 100% immediately following tillage (Six et al 1998; Lupwayi et al. 2004) and the preferential decomposition and depletion of these soil C pools represent a major portion of C loss in cultivated soils (Cambardella and Elliott 1994).

Given that fast shoot growth (Cambardella and Elliott 1994), rapid development of dense root structures, and high nutrient and water uptake (Rytter and Hansson 1996) are some of the growth traits of willow and hybrid poplar clones, I expect that a new equilibrium between nutrient release (through SOM mineralization) and plant demand will be reached rapidly as the trees grow and occupy the plots. The sizeable decrease of C and N fluxes to almost the levels of the undisturbed grassland controls by the end of this study, suggests that losses will continue to decrease gradually and perhaps become negligible during the next few years of the tree rotation cycles until a new disturbance event occurs. Results of other tillage studies also found that SOM mineralization and subsequent C and N losses following grassland ecosystem disturbance is a two-stage process with rapid decomposition over the first few months followed by a second phase of low decomposition with rates several times lower than in the first phase (Aronsson and Bergstrom 2001; Vertes et al. 2007).

It is widely recognized that accurate estimation of cumulative GHG flux remains a difficult task as flux rates may be highly variable, both temporally and spatially (Parkin 2008). The low frequency of measurement points taking in time may have potentially induced biases in our estimation of cumulative GHG flux for this system. First, by sampling GHG flux every two to three week time period, we may have not captured all episodic events (e.g. rainfalls, temperature, etc) which could have considerably contributed to increase the annual GHG emissions. For instance, Parkin and Kaspar (2006) who measured GHG flux in a corn field observed that almost 50% of the annual N_2O flux was due to two episodic pulses that occurred during the year. Another temporal uncertainty could come from the lack of GHG samplings during the winter period (December 2009 to February 2010) when the ground was continually covered with snow and GHG flux assumed to be negligible. Furthermore, all GHG sampling

were performed between 10.00 and 15.00 h when flux rates are supposed to represent the average daily fluxes. However, this midday time frame is generally the time period when air and soil temperature as well as biological activity are at their peaks, leading to higher GHG flux rates than diurnal flux rates which our approach did not capture.

Removing the aboveground parts of the vegetation prior to sampling is another factor that may have affected our estimation of cumulative GHG flux as maintaining plants in the chamber could have allowed the inclusion of the effects of growing plants on GHG emissions or uptake (Mosier et al 2006) in our measurements. However, because our chamber size could not accommodate the rapid shoot growth of switchgrass throughout the growing season, aboveground vegetation had to be removed to facilitate chamber installation and lid closure.

4.6. CONCLUSION

The present study has shown that clearing and cultivation of grassland for a bioenergy plantation led to significant GHG fluxes and nitrogen leaching losses in northern Michigan. The projected widespread conversion of idle agricultural lands to SRWC systems in the region, coupled with the magnitude of cultivation-induced GHG emissions emphasizes the need to consider these impacts explicitly when calculating life-cycle GHG balances for bioenergy systems. Opportunities clearly exist to mitigate the GHG and eutrophication impacts associated with SWRC establishment. In particular, I suggest that alternative cropping practices such as no-tillage, minimum tillage, cover cropping or high-density tree planting may have the potential to minimize GHG emissions and nutrients losses associated with SWRC establishment.

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4.7. BIBLIOGRAPHY

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CHAPTER 5

NITROGEN FERTILIZATION OF SWITCHGRASS IN NORTHERN MICHIGAN, U.S.A INCREASES BIOMASS YIELD AND IMPROVES NET GREENHOUSE GAS BALANCE.

5.1. ABSTRACT

Nitrogen (N) management can affect agro-ecosystem sustainability through impacts on biomass production and soil greenhouse gas (GHG) fluxes. In the present study, I investigated the effects of N fertilization on GHG emissions and biomass production of switchgrass grown for bioenergy production, in the central Upper Peninsula of Michigan, U.S.A. Nitrogen fertilization treatments included 0, 56 and 112 kg N ha^{-1} applied as urea, once early in the growing season. Field-based measurements of direct GHG fluxes (CO_2, N_2O and CH_4) were performed every two to three weeks using static chambers. Indirect GHG emissions associated with machinery operations in field activities, manufacturing of fertilizer and pesticides and transport of chemicals to the farm were derived from the literature. Biomass yield of switchgrass was evaluated at the end of the growing season. Overall, N fertilizer application contributed little to direct GHG emissions from soil. Relative to the unfertilized plots, there were an additional 0.74 and 1.5 Mg CO_2eq ha^{-1} yr^{-1} of net GHG emissions from soil in the 56 and 112 kg N ha^{-1} treatments, respectively. On the other hand, N fertilization greatly stimulated CO_2 uptake by the switchgrass bioenergy crop, resulting in 1.5- and 2.5-fold increases in aboveground biomass yield in 56 and 112 kg N ha^{-1} treatments, respectively. Biomass N contents were 26.6, 49.9 and 89.5 kg ha^{-1} for the 0, 56 and 112 kg N ha^{-1} treatments, respectively, with a relative recovery of applied N ranging from 43 - 56% in the fertilized plots. Nitrogen amendments improved the net GHG benefits by 2.6 and 9.4 Mg CO_2eq ha^{-1} relative to an unfertilized field. These results suggest that N fertilization of switchgrass in this region could reduce (15-50%) the land base needed for bioenergy production and decrease pressure on land needed for food and forage crop production.

5.2. INTRODUCTION

Of the many perennial grass species and fast-growing trees that have been intensively investigated for use as bioenergy crops, switchgrass (*Panicum virgatum* L.), a warm-season perennial grass native to North America, is believed to be one of the most promising and valuable crop for a wide range of sites in the U.S.A. (Alder et al. 2007; Froese et al. 2010; Wang et al. 2010). Relative to other bioenergy crops, desirable characteristics of switchgrass include its low establishment costs, high yielding potential, suitability for marginal lands, low nutrient and water requirements and positive environmental benefits (Sanderson and Alder 2008; McLaughlin et al. 2005; Wang 2010). However, nitrogen (N) availability has been reported as the most frequently limiting factor for growth of native grasses suggesting that N fertilization can be an important management practice for switchgrass biomass production and feedstock quality (Sanderson and Alder 2008; Wang et al. 2010). At the same time, production and use of exogenous N fertilizers in agricultural systems can contribute directly and indirectly to greenhouse gas (GHG) emissions, potentially reducing the net GHG benefits of bioenergy production (Hill et al. 2006; Lewandowski and Schmidt 2006). Yet, there is still a critical lack of information regarding biomass yield and net GHG benefit of adding N fertilizer for switchgrass bioenergy crop production, especially in northern Michigan's conditions.

The use of renewable energy from biomass has the potential to mitigate atmospheric CO_2 increase but achieving this potential will necessitate the development of high yielding feedstock production systems (McLaughlin et al. 1999). While a number of options are being investigated to achieve bioenergy crop yield potential (e.g. genetic improvement, irrigation, soil fertility management), it is most likely that N fertilization will play a central role in contributing to achieving high biomass production. This is because harvesting switchgrass biomass is likely to

remove high levels of N and adequate N inputs may be needed to replace the lost N from the soil. To date, N fertilization requirements for switchgrass are still not well established. While recent N fertilization studies suggest that growing switchgrass for use as feedstock without N fertilizer would be impracticable (Vogel et al. 2002; Sanderson and Alder 2008; Wang et al. 2010), some other studies have found no yield response to added N suggesting that N fertilization may not be require for this bioenergy crop (Christian et al. 2002). Mulkey et al. (2006), who conducted a study on N fertilization for a switchgrass bioenergy crop in South Dakota, recommended applying no more than 112 kg N ha^{-1} once annually to optimize biomass production.

Nitrogen fertilizer use in agricultural settings is generally cited as one of the largest potential sources of GHG emissions, especially if excessive soil N levels stimulate N_2O fluxes (Bouwman 1996; Bouwman et al. 2002; IPCC 2007; Robertson and Vitousek 2009). Although there is a large body of work that has investigated soil GHG emission response to N fertilizer addition, most of these studies have focused on agronomic crops such as corn, wheat and potatoes which are not only differently managed but also may have different N requirement than switchgrass (Grant et al. 2006; Zebarth et al. 2008). To account for N fertilizer input effects on GHG fluxes from soil, the IPCC (1996) methodology provides a N_2O emission factor as a percentage (1.325%) of the amount of N fertilizer applied that has been extensively used in most life cycle assessment (LCA) studies. This emission factor suggests that there is a linear relationship between N input and N_2O release (Bouwman 1996). Because perennial grasses generally have extensive root systems making them more efficient at taking up large amounts of soil inorganic N, N_2O fluxes may respond to N addition differently in perennial grassland ecosystems than in agronomic systems where this emission factor was derived. For instance, in a

nitrogen management for switchgrass biomass production study, Vogel et al. (2002) reported no change in soil N level when the N fertilizer rate was less than 120 kg N ha^{-1}, suggesting that below this threshold N_2O flux is unlikely to be persistent as the crop is taking up all of the N added to the soil.

In the present study, I investigated switchgrass yield and soil GHG (CO_2, N_2O, and CH_4) flux responses to different rates of N fertilizer application, at a site in the central Upper Peninsula of Michigan. I hypothesized that increases of CO_2 fixation into switchgrass biomass resulting from N additions would be more than compensate the direct and indirect GHG emissions associated with fertilizer production, transport and application. A better understanding of aboveground biomass production and GHG emission responses to N additions will help improve further LCA studies for this bioenergy crop.

5.3. MATERIALS AND METHODS

5.3.1. Site description and experimental set-up

This experiment was conducted at Michigan State University's Chatham Experiment Station located in central Upper Peninsula of Michigan, USA (46°33′ N, 86°55′ W), on a sandy loam, mixed, frigid Alfic Haplorthod soil. The study area has lake-enhanced precipitation with average annual precipitation of 880 mm (last 10 years). The rainfall for the study period (15 May 2009 to 14 May 2010) was 575 mm. Daily minimum winter temperatures generally fall down to −18°C or lower and there is a mean of 91 d frost free and average annual maximum and minimum temperatures of 11 and -3°C, respectively (Eichenlaub et al 1990)

The plant material that I used in the present study was switchgrass variety "Cave In Rock"; selected because of its ability to grow on marginal lands, high yield potential ease of processing after harvest (Varvel et al. 2008), high quality of pellets for heating or electricity generation and good conversion efficiency (McLaughlin et al. 1996). Moreover, because switchgrass is a perennial crop, it does not require annual seeding or soil tillage (Sampson et al. 2000).

The experimental field measured approximately 1.5 ha (200 m long in EW direction and 150 m wide in NS direction). Prior to beginning of the experiment, the field had been used for silage corn production for the past 30-40 years. Preparation for switchgrass establishment began on 10 June 2008 when the area was sprayed with 2.5 L ha^{-1} of glyphosate and 2.5 L of 2-D ester herbicides. The field was no-till planted on 23 June 2008, and the planting was performed in 18.8 cm row spacing 1.3 cm deep, at a seeding rate of 22.4 kg ha^{-1} (40% of pure live seeds). The area was sprayed again on 27 June 2008 with 2.5 L ha^{-1} of Atrazine 4L (pre-emerge herbicide).

Biomass was not harvested in 2008. On 1 June 2009, the field was sprayed with a combination of dicamba and atrazine at a rate of 2.5 L ha^{-1} for each herbicide. The switchgrass fertilization treatment was started in the spring of 2009. The experiment was a randomized block design with treatments consisting of 3 levels of N fertilizer as urea (46-0-0), replicated six times. The N fertilizer treatments used were N0 = 0 kg N ha^{-1}, N50 = 56 kg N ha^{-1} and N100 = 112 kg N ha^{-1}. Treatment plots were fertilized on 4 June 2009. Biomass was first mechanically harvested and baled on 11 November 2009.

5.3.2. Field measurements of direct GHG emissions

Field data collection started on 5 June 2009, a day following fertilizer application and continued until 4 May 2010. To measure emissions of CO_2, N_2O and CH_4, I used one static non-vented bucket chamber (Nº2 high-density polyethylene plastic - Letica, U.S. Plastic Corp.) per plot. The buckets used to design the chamber measured 26.2 cm in diameter, and chambers consisted of bases driven 10 cm into the soil and gas tight lids fitted with rubber septa to sample the approximately 10 L of headspace volume. To keep the chamber from heating up during the sampling period and altering GHG fluxes, foil covered bubble wrap (water heater blanket) was glued to the outside of the chamber. Aboveground vegetation in each chamber was clipped immediately prior to sampling in order to avoid respiratory fluxes of CO_2 from aboveground plant parts. Removing plants from the chamber was performed to solve the problems associated with subtracting the fraction of the CO_2 release originating from the aboveground vegetation which is not taking into account when calculating global warming potential. Gas samples were collected from the headspace between 10.00 and 15.00 h to minimize changes of soil GHG

fluxes associated with diurnal cycles. Headspace gas samples (10 mL) were drawn using a 10 mL syringe at 0, 20, 40 and 60 minutes following chamber closure, and then immediately over-pressurized to pre-flushed 5.9 mL flat-bottom exetainer vials (Labco, Unlimited, Buckinghamshire,U.K.). After each sampling event, chambers were moved to a new location, about 5 m away from the previous location, and left uncovered until the periods when gas samples were being collected on the next sampling date.

The concentration of CO_2, N_2O and CH_4 in the headspace gas samples were determined using a Gas Chromatograph (GC-2014, Shimadzu Corporation) equipped with electron-capture and flame-ionization detectors. Gas flux measurements were performed every 2-3 weeks, except during the winter period when the ground was continually covered with snow. A total of 12 sampling events were captured over the course of this one year investigation, of which 10 samplings were made in 2009 (5 and 25 June, 9 and 29 July, 10 and 25 August, 15 September, 5 and 27 October and 13 November) and two samplings in 2010 (3 March and 5 May).

5.3.3. Soil sampling and analyses

I used 3.8-cm diameter PVC soil corers to take a 10-cm deep soil core in close proximity to the gas flux chamber at each sampling period. Gravimetric moisture content was determined from field-moist soil after oven drying at 105 $^{\circ}$C for 48 hrs. Soil volume was calculated based on the core size. Soil bulk density (B_D in mg m^{-3}) was determined by dividing the oven-dried weight by soil volume after correcting the volume and weight for gravel contents in the sample. Total porosity was determined as ($1- B_D \times P_D^{-1}$) where P_D is soil particle density assumed to be 2.65 mg m^{-3}. The ratio of volumetric soil water to total porosity was used to calculate soil water-filled pore space (WFPS).

Soil samples collected at each gas sampling point were also used for determination of NH_4^+-N and NO_3^--N concentrations after extracting the soil with 2 M KCL (5:1 extractant to soil ratio) and analyzing extracts spectrophotometrically. Soil pH was measured (in a mixture of soil and 0.01 M $CaCl_2$) from air-dried soil with a Corning pH Meter 430 (Corning Incorporated, Science Products Division, New York, 14831). Total C and N contents were determined on the same soil samples by dry combustion with an elemental analyzer (Model ECS 4010, Costech Analytical, Valencia, CA).

5.3.4. Plant sampling and analyses

Switchgrass aboveground biomass was sampled on 27 October 2009. Standing aboveground biomass was sampled by randomly placing two 50×50 cm quadrats in each plot. Aboveground biomass samples were manually cut to a stubble height of 12.5 cm and all leaves and stems produced were collected, and a subsample was taken from each sample for moisture content determination after oven-drying at 65 ◦C for 48 hrs and weighing. All yields are expressed on a dry weight basis. The aboveground subsamples were chopped, finely ground with a mini-ball mill for C and N analysis. From the ground samples, other sub-samples of 500 mg were acid-digested (4.5 mL of concentrated 70% nitric acid) for elemental (P, K, Mg and Mn) analysis (ICP-AES). Additional subsamples of about 2.5 mg were used for total C and N determination by combustion using an elemental analyzer (Model ECS 4010, COSTECH Analytical, Valencia, California, U.S.A.).

5.3.5. Indirect GHG emissions and C sequestration

In this study, an attempt has been made to estimate indirect GHG emission associated with switchgrass bioenergy production. By indirect GHG emissions, I refer to non-soil related

GHG release prior to, and during farming processes, including energy use during farming, biomass harvesting and transportation, as well as fertilizer and pesticide manufacturing and use. Greenhouse gas emissions during feedstock transformation and power generation are not considered here. Farming energy and chemical use for growing switchgrass was based on the Greenhouse gases, Regulated Emissions, and Energy use in Transportation (GREET) default values (Wang 1999; Wu et al. 2006). The energy and emissions values for fertilizer manufacturing and transportation used in this study were obtained from Wang et al. (2003) and Wells (2001). Carbon dioxide emissions associated with urea, herbicide and lime production are 3.0, 0.4 and 16.2 kg CO_2 per element or active ingredient. I assumed that about the same amount of CO_2 will be release during distribution/ transportation of these chemicals to the farm. Fuel consumption data for farm activities were derived from Bone et al. 1999 (e.g. fertilizer spreading, herbicide application and liming = 3 L ha^{-1}; harvesting = 6 L ha^{-1}) and the IPCC (1996) carbon emission factor of 74.07 Mg of CO_2/TJ for the CO_2 emissions associated with farm activities. Because nutrient run-off or leaching data from switchgrass bioenergy crops are unavailable, I did not include such data in this assessment. Most perennial grasses have a strong ability to scavenge for soil available N and I expected N leaching losses to be negligible in the system. Cultivation of switchgrass on crop land leads to a significant soil C sequestration (Liebig et al. 2008). To account for the improvement in soil C content, I used the default value (48.5 kg CO_2 per Mg of switchgrass aboveground biomass) provided by Wu et al. (2006).

5.3.6. Greenhouse gas flux calculations

Fluxes of GHGs were estimated from the concentration change in the chamber headspace over the 60 min sampling period. I used the simplified linear method for quantifying theoretical underestimation of chamber-based gas fluxes developed by Venterea et al. (2009). The method takes into account the effects of chamber geometry and deployment time, the flux-calculation, and properties of the soil and the gas under consideration. I used linear interpolation between adjacent sampling dates (assuming that emission followed a linear trend during the periods when no sample was taken) to calculate cumulative CO_2, N_2O and CH_4 fluxes during the study period.

To compare the net effect of N fertilization on GHG gas budgets from the different treatments, I used global warming potential factors of 25 and 298 based on 100-y time horizons for CH_4 and N_2O, respectively, to convert the soil fluxes of these two GHGs to CO_2eq (IPCC 2007).

5.3.7. Data analysis

Chamber-based data were analyzed using ‘‘general linear model-repeated measures of ANOVA (Minitab 15, Minitab, U.S.A.) to assess the significance of the impacts of N fertilization, sampling date, and their interactions on the fluxes of CO_2, N_2O, and CH_4, as well as soil available N (NO_3^- and NH_4^+), temperature and WFPS in which sampling date was treated as a within-subject variable and N fertilizer rate was treated as a between-subject variable. For measurement of biomass yield and biomass nutrient concentration, the significant difference between the different N fertilization treatment was assessed by one-way ANOVA and least significance difference (LSD). All significances mentioned in the text are at the 0.05 level.

5.4. RESULTS

5.4.1. Soil C, N, pH, WFPS and temperature

Soil C content did not change with nitrogen application (P=0.417), whereas soil N levels increased significantly (P=0.033) in both N50 and N100 treatments compared with the N0 plots (Table 5.1). Soil porosity, bulk density and pH were not affected by N fertilization regimes. The total annual (from May of 2009 to April of 2010) rainfall was 575 mm and was much less than the long-term average of 880 mm. There was very little rainfall in June 2009. Also, the first three weeks of September 2009 were dry (no rainfall) while the last week of September and the entire month of October 2009 regularly received rainstorms (Fig 5.1-a). Soil temperature and soil WFPS were unaffected by the N-fertilizer treatments (Fig. 5.1-b & 5.1-c). However, both soil temperature and soil water content (expressed as WFPS) varied significantly (both P<0.001) across sampling dates. Soil WFPS levels responded to rainfall patterns over the study period. Soil WFPS measured at the depth of 0-10 cm varied between 16 to 45% with the lowest values recorded during the sampling dates that occurred on 25 June and 15 September of 2009.

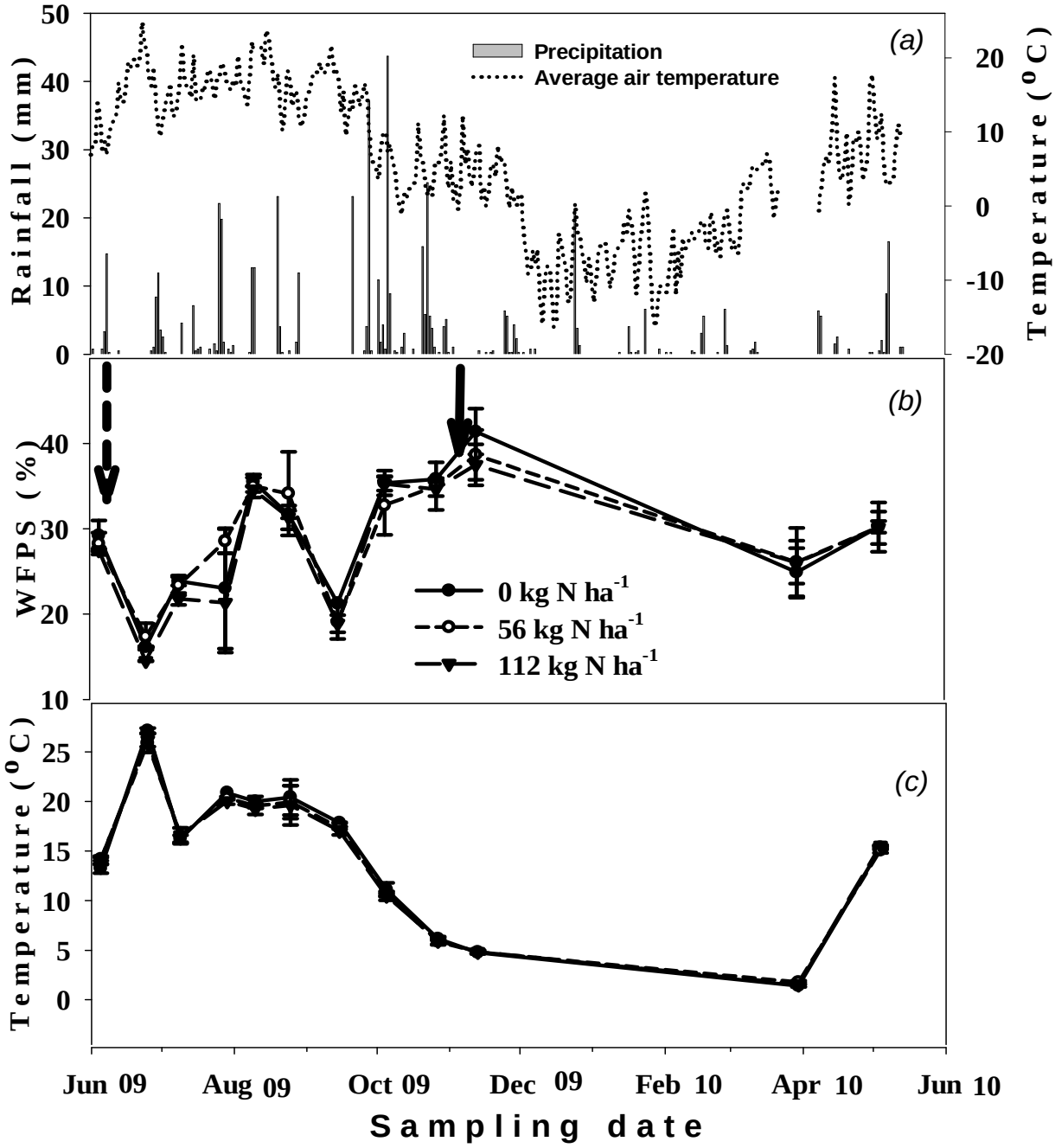

Figure 5.1 Precipitation and average air temperature (a), soil water filled pore space (b) and soil temperature (c) measured from switchgrass bioenergy yield plots, grown under three different N fertilization regimes, at Chatham, Upper Peninsula in Michigan, U.S.A. Black solid indicates the date for field application of N-fertilizer. The black, long dashed arrow indicates date of N fertilizer application and the solid arrow indicates date of biomass harvest.

5.4.2. Soil nitrogen availability

Soil NH_4^+-N did not differ between fertilized and unfertilized plots at any sampling date. In contrast, N fertilizer application caused a sharp increase in soil NO_3^--N levels following N fertilization (Fig 5.2-a). For instance, during the sampling date that occurred on 25 June 2009, NO_3^--N levels were 2.3- and 2.7-fold higher ($P<0.001$) in plots fertilized with 56 and 112 kg N ha^{-1}, respectively, relative to the unfertilized plots. Similarly, on 9 July 2009, NO_3^--N concentrations were 1.6- and 2.0-fold higher ($P=0.01$) in plots fertilized with 56 and 112 kg N ha^{-1}. The spike of NO_3^--N following after fertilization application was followed by a steady decrease to the unfertilized soil level by 29 July 2009 (55 days following plots fertilization) (Fig 5.2-b).

Finally, on 5 December 2009, a 1.8-fold increase was noted in both fertilized plots relative to the unfertilized control. With the exception of these above mentioned dates, NO_3^--N concentration did not differ between fertilized and unfertilized plots at any sampling dates. On the other hand, nitrate level did not change and remained relatively steady in the control. N mineralization and nitrification, measured twice (5 June 2009 and 28 July 2009) during the growing season were not significant between treatments and data are not reported here.

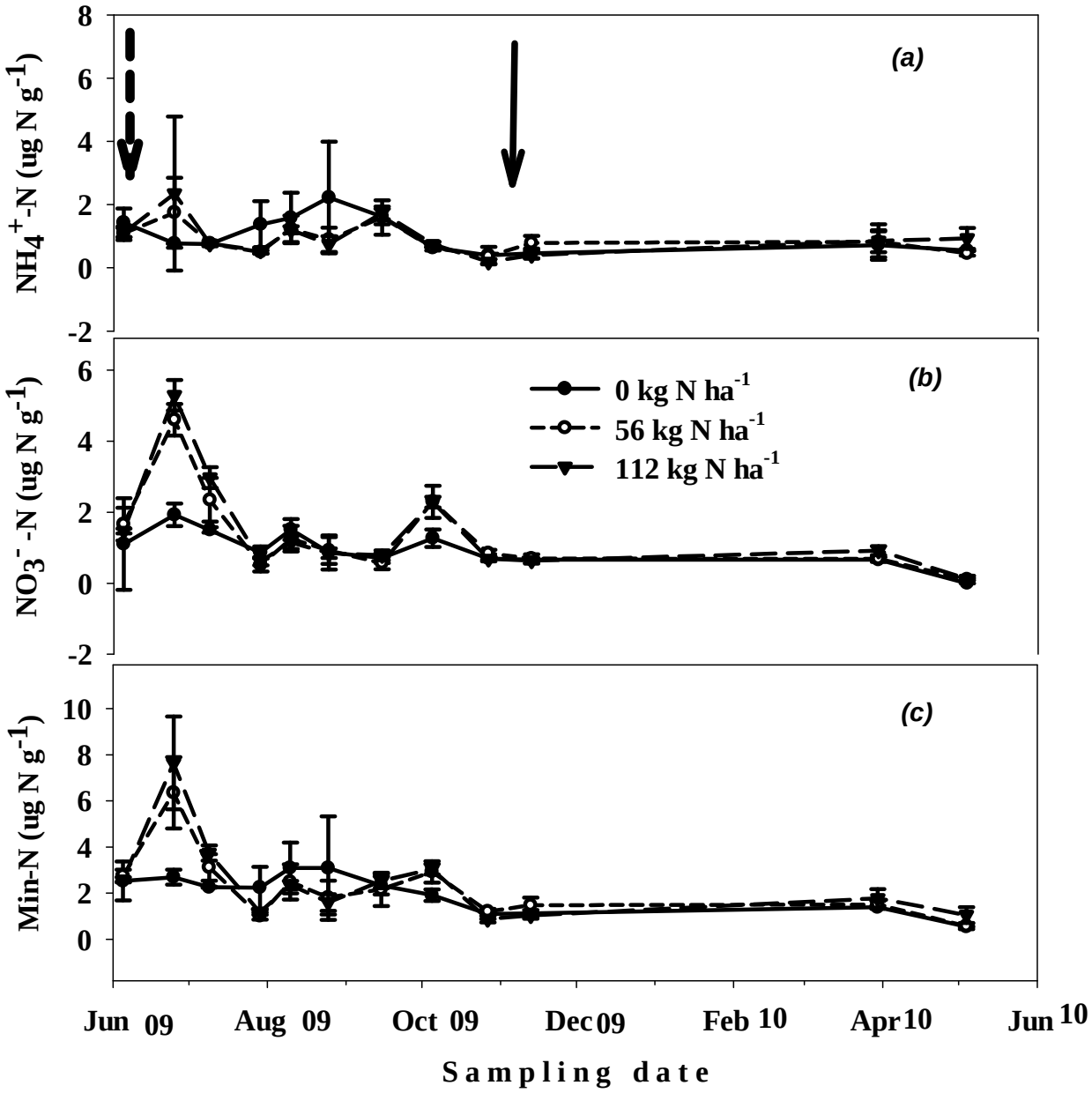

Figure 5.2 Soil ammonium (a), nitrate (b) and total mineral N (c) concentrations measured from 0-10 cm soil depth in switchgrass bioenergy yield plots, grown under three different N fertilization regimes, at Chatham, Upper Peninsula in Michigan, U.S.A. Black solid indicates date for field application of N-fertilizer. Black, long dashed arrows indicate date of N fertilizer application and the solid arrow indicates date of biomass harvest.

5.4.3. Biomass yield and nutrient concentrations

Switchgrass aboveground biomass yield increased significantly (P=0.005) with increased level of N fertilizer (Table 5.2). Average biomass production increased by 1.5- and 2.5-fold in the N50 and N100 treatments, respectively. The N concentration of harvested biomass significantly (P=0.05) increased with increasing N fertilization (Table 5.2). Consequently, biomass C:N ratio also significantly decreased with increasing fertilization level. Plant tissue P, Ca and Mg were unaffected by N addition. However, plants grown in plots that received the highest N fertilization level had significantly (P<0.01) greater K concentration than their counterparts in the N0 and N50 treatments.

5.4.4. Greenhouse gas flux and global warming potential

Nitrogen fertilization did not significantly (P=0.519) affect soil CO_2 flux as flux rates were indistinguishable between fertilized and unfertilized treatments. However, CO_2 flux significantly (P<0.001) varied with sampling date, with flux rates being high in the summer and lower in autumn and winter. The seasonal pattern of CO_2 fluxes was similar to that of soil temperature (Figs 5.1-c and 5.3-a). Soil CO_2 fluxes rapidly increased in both fertilized and unfertilized plots as soil temperature increased, peaked during the month of August, and then gradually decreased to almost zero by autumn 2009 throughout spring 2010.

Table 5.1 Selected soil characteristics measured beneath switchgrass grown under three different N fertilization regimes in the central Upper Peninsula, Michigan, U.S.A. Data were collected on July 29, 2009. Treatments are: N0= 0 kg N ha^{-1}; N50 =56 kg N ha^{-1} and N100 = 112 kg N ha^{-1}

Treatment	Total C (%)	Total N (%)	C:N ratio	Bulk density (g cm^{-3})	Porosity (%)	pH
N0	1.68 (0.03)	0.136 (0.002)	12.37 (0.01)	1.17 (0.01)	56 (0)	6.49 (0.06)
N50	1.71 (0.03)	0.141(0.002)	12.10 (0.05)	1.16 (0.05)	56 (2)	6.59 (0.02)
N100	1.72 (0.02)	0.142(0.002)	12.14 (0.02)	1.16 (0.02)	56 (1)	6.60 (0.10)
P-Value	0.417	**0.033**	0.939	0.939	0.939	0.540

Table 5.2 Yield and nutrient concentrations of aboveground biomass of switchgrass grown under three N fertilization regimes in the central Upper Peninsula, Michigan, U.S.A. Treatments are: N0= 0 kg N ha^{-1}; N50 =56 kg N ha^{-1} and N100 = 112 kg N ha^{-1}

Treatment	Biomass yield-DM	C	N	C:N	P	K	Ca	Mg
	(Mg ha^{-1})	----------%----------		(Ratio)	----------%----------			
N0	4.83 (0.21)	46.80 (0.06)	0.55 (0.03)	86 (4)	0.08 (0.01)	0.25 (0.02)	0.29 (0.01)	0.13 (0.01)
N50	7.13 (0.42)	46.57 (0.10)	0.70 (0.09)	69 (8)	0.08 (0.00)	0.25 (0.01)	0.28 (0.01)	0.13 (0.00)
N100	11.93 (0.53)	46.98 (0.11)	0.75 (0.03)	63 (2)	0.08 (0.01)	0.35 (0.02)	0.27 (0.01)	0.14 (0.01)
P-Value	**0.005**	0.057	0.055	0.026	0.757	**0.009**	0.740	0.720

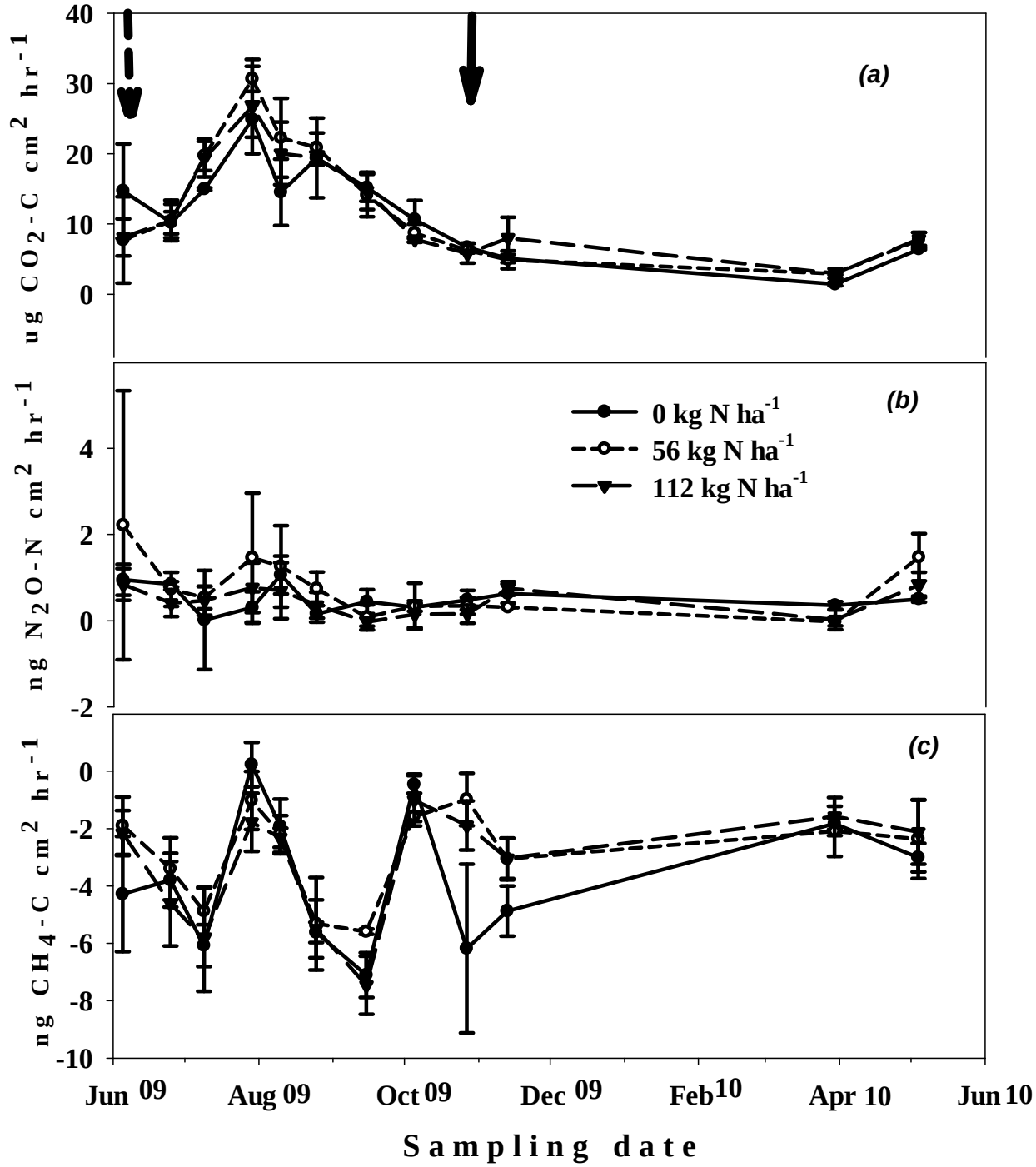

Figure 5.3 Fluxes of CO_2 (a), N_2O (b) and CH_4 (c) from soil, measured in switchgrass bioenergy yield plots, grown under three different N fertilization regimes, at Chatham, Upper Peninsula in Michigan, U.S.A. Black solid indicates date for field application of N-fertilizer. Black, long dashed arrows indicate date of N fertilizer application and the solid arrow indicates date of biomass harvest.

Carbon dioxide fluxes were statistically indistinguishable (all P>0.05) between fertilized and unfertilized plots at all sampling dates. The annual cumulative soil CO_2 flux for N0, N50 and N100 treatments were 24.6, 25.3 and 26.0 Mg CO_2 ha^{-1}, respectively. Nitrous oxide flux rates were not significantly affected by N-fertilization. However, the main effect of sampling date was significant (P=0.04) whereas the interaction between treatment and sampling date had no significant effect (P=0.850) on N_2O flux. Overall, average N_2O fluxes from all three treatments were very low. The highest flux rate, which occurred in one of the 112 kg N ha^{-1} treatments was 15 µg N_2O-N $m^{-2}hr^{-1}$ (Fig 5.3-b). There was a trend of decreasing CH_4 consumption with increasing N addition; however, this was not statistically significant (P=0.093). Cumulative N_2O fluxes were in the range of 0.2 -0.3 Mg CO_2eq ha^{-1} in the fertilized and unfertilized plots, but overall there was no significant difference among treatments. Although CH_4 consumption tended to decrease with increasing N fertilization, differences were not statistically significant between treatments. However, CH_4 fluxes were significantly affected by sampling date and the interaction between treatment and sampling date (P<0.001 and 0.004, respectively) (Fig 5.3-c). The seasonal flux of CH_4 followed almost the same pattern as WFPS, decreasing with decreasing soil WFPS and increasing with increasing WFPS. Overall, cumulative CH_4 fluxes ranged from -0.06 to -0.07 Mg CO_2eq ha^{-1} in the following order 0 kg N ha^{-1}>56 kg N ha^{-1}>112 kg N ha^{-1}.

Overall, indirect GHG emissions associated with agricultural input production and transport and field preparation coupled with direct GHG emissions from soil totaled 1.0, 2.4 and

4.1 Mg CO_2eq ha^{-1} for plots that received 0, 56, 112 kg N ha^{-1}, respectively (Table 5.4). Conversely, based on my calculation, CO_2 fixed in switchgrass biomass (above and belowground) and in soil totaled 8.5, 12.5 and 21.1 Mg CO_2eq ha^{-1} for 0, 56, 112 kg N ha^{-1}, respectively.

Table 5.3 Total annual CO_2, N_2O and CH_4 fluxes, cumulative GHG emissions and GWP all expressed in kg CO_2eq ha^{-1} (Total ± se) from switchgrass bioenergy plots grown with three different levels of N fertilizer in the central Upper Peninsula, Michigan, U.S.A. Treatments are: N0= 0 kg N ha^{-1}; N50 =56 kg N ha^{-1} and N100 = 112 kg N ha^{-1}

Fertilization level	CO_2		N_2O		CH_4		Cumulative GHG		GWP
N0	24427	(4019)	191	(100)	-68.3	(18.5)	24550	(4137)	
N50	25182	(5564)	167	(125)	-62.9	(19.7)	25287	(5709)	737
N100	25801	(4724)	302	(252)	-56.8	(18.2)	26047	(4995)	1497

Table 5.4 Greenhouse gas (GHG) flows per hectare of switchgrass bioenergy field in Northern Michigan, U.S.A. Positive figures represent CO_2 emissions and negative numbers are CO_2 sequestration. Treatments are: N0= 0 kg N ha^{-1}; N50 =56 kg N ha^{-1} and N100 = 112 kg N ha^{-1}

Source/ factor	N0	N50	N100
Indirect emissions			
Agricultural input production			
- Herbicide manufacture/ transport	+0.320	+0.320	+0.320
- N manufacture/ transport	+0.000	+0.300	+0.600
Diesel fuel for preparation of switchgrass			
- Field management	+0.023	+0.034	+0.057
- Harvest	+0.121	+0.178	+0.238
- transport	+0.580	+0.852	+1.432
Direct emissions from soil			
GHG emission from soil (measured)			
- CO_2		+0.755	+1.374
- N_2O		-0.024	+0.111
- CH_4		+0.005	+0.012
C sequestration			
- Aboveground biomass	-8.300	-12.200	-20.500
- Soil	-0.234	-0.346	-0.579
Net GHG balance	-7.490	-10.126	-16.935

5.5. DISCUSSION

In this study, I used actual field measurements to determine direct GHG emissions, GWP and biomass yield associated with N fertilization for a switchgrass bioenergy crop in northern Michigan. Indirect GHG emissions due to production, transportation, use of N fertilizer and machinery were derived from the literature. Overall, N fertilization had minimal impact on soil GHG emissions. Raw data obtained from field measurements indicate that the N50 and N100 treatments had GWP of 0.74 and 1.5 Mg CO_2eq ha^{-1} yr^{-1}, relative to the unfertilized baseline. On the other hand, N fertilization greatly stimulated CO_2 assimilation into aboveground biomass of the switchgrass crop, resulting in 1.5- and 2.5-fold increases in yield in the N50 and N100 treatments, respectively. Taking into account the total mix of activities required for growing switchgrass and transporting biomass to a processing plant, these estimates indicate that growing switchgrass on an unfertilized pastureland results in a net GHG sequestration of 7.5 Mg CO_2eq ha^{-1}. However, fertilizing switchgrass with 56 kg N ha^{-1} and 116 kg N ha^{-1} increased net GHG sequestration to 10.1 and 16.9 Mg CO_2eq ha^{-1}, respectively. These results suggest that application of N fertilizer to switchgrass bioenergy plantings can notably increase feedstock production and GHG sequestration. This, in turn, would reduce the land area required for a given level of feedstock production, thus reducing potential conflicts with land required for food and crop production, as well as avoiding additional GHG emission from land conversion.

Nitrous oxide emissions were not distinguishable between fertilized and unfertilized plots, suggesting that added N was either quickly taken up by the actively growing switchgrass plants or the site conditions did not favor N_2O production or the combination of both. Although

soil mineral N, especially NO_3^--N, spiked in the spring immediately after N fertilization, this increase was not accompanied by an enhanced N_2O emission in the fertilized plots. A day following N fertilization, both NH_4^+-N and NO_3^--N levels in the fertilized plots were indistinguishable from the unfertilized plots. The flush of soil NO_3^--N was followed by a sharp decrease of soil available NO_3^--N to the unfertilized plot level by 29 July 2009, demonstrating the ability of switchgrass to scavenge available soil N (Fike et al. 2006; McLaughlin and Kszos 2005). The elevated soil NO_3^--N which lasted about two months following N addition in the fertilized treatments implies that applied N must have exceeded the ability of the plant to take it up, at least, during that period of time, which may increase the risk of increased N_2O emissions. Snyder et al. (2007) reported that N_2O emissions usually occur when available soil N, especially NO_3^--N, exceeds crop demand. In the present study, however, the elevated NO_3^--N following N addition was not accompanied by an increase in N_2O emissions, suggesting that soil NO_3^--N level is not the single factor require to stimulate soil N_2O flux. Soil WFPS at the site was below 30% at this period and might explain why NO_2 flux did not increase as NO_3^--N increased. Results from Schmidt et al. (2000) study on the relationship between soil variables (including WFPS) and N_2O flux, suggests that minimum WFPS values at which soil N_2O flux start to increase is near 30% with highest fluxes occurring around 70% of WFPS.

In the present study, direct measurements of N_2O flux suggest that the N fertilizer rate of 56 kg ha^{-1} did not result in any noticeable N_2O flux from soil, supporting that there is no linear

relationship between N fertilizer addition and N_2O flux for this particular system, and that there will be a critical minimum amount of N rates above which N_2O flux occurs. The highest N fertilizer rate (112 kg ha^{-1}) resulted in an additional 0.1Mg CO_2eq ha^{-1} as N_2O, corresponding to an emission factor of 0.24% of the amount of applied N, a value 5.6 times lower than the IPCC emission factor.

Although the cumulative CO_2 flux tended to increase with increasing added N, this increase was not statistically significant. This result is consistent with Lee et al. (2007) who studied N fertilization and harvest frequency effects on CO_2 fluxes in an established switchgrass biomass crop field and found no effects of N fertilizer on soil CO_2 emission rates. Previous N fertilization studies have found that added N increased aboveground biomass yield but did not affect switchgrass root biomass (Ma et al. 2000; Jung 2010), and the lack of significant effect of N fertilization on soil CO_2 flux may suggest that there was no significant increase in root biomass due to adding N fertilizer.

Because perennial grass ecosystems are generally reported to be a small sink for atmospheric CH_4 (Schmer et al 2006), I expected CH_4 flux to make little contribution to net GHG emissions from switchgrass. Cumulative CH_4 measured in the present study indicate that as N fertilizer amount increases, CH_4 consumption decreases; however, overall fluxes were negligible. Methane oxidation decreasing with added N is in agreement with Moiser et al (1991) who reported an inhibition of CH_4-oxidization with agricultural management practices such as fertilizer application and tillage. The addition of N to perennial prairie grassland ecosystems has

been shown to increase above-ground biomass production (Collins et al. 1998) therefore suggesting that perennial biomass energy crops including switchgrass may have a similar response. In this study, increasing nitrogen fertilization linearly increased switchgrass aboveground biomass yield. In fact, results of previous N fertilization studies for switchgrass suggested that the highest N fertilization rate (112 kg N ha^{-1}) used in the present study is within the linear response range for this crop. For instance, using almost similar amount of N fertilizer (126 kg ha^{-1}) Bransby et al (1998) obtained 10.4 Mg ha^{-1} which is comparable to the biomass yield achieved with the highest N fertilizer rate in the present study. Muir et al. (2001) and Mooney et al. (2009) also found switchgrass yields to increase linearly up to 150-168 kg N ha^{-1}. Above this threshold, switchgrass yield responses to increasing N fertilizer level start to saturate (Muir et al. 2001; Lemus et al 2008).

In this study, application of 112 kg N ha^{-1} increased tissue K concentration by 40% relative to the unfertilized crops, suggesting that this level of N addition must have promoted the uptake of K by the plant (Table 5.3). Guretzky et al. (2010) also reported tissue K increase of a similar magnitude with comparable amount (134 kg N ha^{-1}) of N fertilization, thus suggesting that N fertilization of switchgrass crops may require more K addition to sustain productivity. Tissue N concentrations of switchgrass increased in response to increasing N addition thus agreeing with results obtained by Elbersen et al (2001). Switchgrass accumulated about 26.6 kg N ha^{-1} in the aboveground biomass when grown with no exogenous N added to the field. Nitrogen content, however, increased to as much as 49.9 and 89.5 kg ha^{-1} when 56 and 112 kg of N fertilizer were applied to the plots. This suggests a relative recovery of applied N of 43% and

56% in plots that received 56 and 112 kg N ha^{-1}during the year of application. Although, I did not find any specific information on N recovery of switchgrass bioenergy crop in the literature, available data for common agronomic crops (e.g. Maize and wheat) indicate that under conventional production practices, recovery of applied N is often no greater than 50-60% (Bransby et al. 1998). In the unfertilized plots, assuming that biomass yield remains constant, harvesting the biomass for energy production will remove about 26.6 kg N ha^{-1} from the soil annually which, over multiple harvest cycles, could deplete soil N pools if this N removal is not replaced by application of exogenous N. Therefore, producing switchgrass for bioenergy in the same field and without N fertilization may lead to yield decline over years (Muir et al. 2001).

5.6. CONCLUSIONS

The results of this study underscore the potential of switchgrass to sequester significant amounts of C and contribute to reducing atmospheric CO_2 levels. Results from this study clearly support my original hypothesis that increased CO_2 fixation into switchgrass biomass resulting from N fertilizer greatly exceeds the direct and indirect GHG emissions associated with fertilizer production, transport and application. Thus N fertilization of switchgrass (of at least 112 kg N ha^{-1}), increases net GHG sequestration and reduces the land base needed to meet feedstock production targets.

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5.7. BIBLIOGRAPHY

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CHAPTER 6

SUMMARY AND CONCLUSIONS

This study assessed the impacts of agricultural management practices such as cover cropping, nitrogen fertilizer inputs and cultivation on soil biology, nitrogen and carbon flows and soil greenhouse gas emissions. In particular, the study first assessed the possibility of incorporating cover crops into Fraser fir Christmas tree plantations as an alternative to the conventional use of N fertilizer to improve and maintain soil fertility and sustain tree growth. The study also evaluated the impacts of converting pastureland to short-rotation woody (poplar and willow) bioenergy systems on greenhouse gas emission from soil and nitrogen leaching losses during the establishment year. Finally, this study evaluated the effects of N fertilization to switchgrass bioenergy crop on yield and net greenhouse gas benefits. The results of the study are useful in designing effective strategies and policies to enhance management for these production systems of interest. My specific conclusions and recommendations are as follows:

1. Inclusion of leguminous or non-leguminous cover crops into Fraser fir production systems improved soil microbial properties and soil health, and can be used as an alternative to inorganic N fertilizers in Fraser fir Christmas tree plantations. However, I recommend that an economic evaluation of the costs and benefits should be conducted to determine its profitability before suggesting this practice to growers. I also propose that a study addressing the potential contribution of the cover crops to soil carbon sequestration over the entire plantation rotation period be conducted.
2. The results also indicate that pasture land-use conversion to SRWC bioenergy systems contributes to a significant part of the GHG budget of these systems. This implies that GHG emissions during the establishment year of any SRWC bioenergy systems should in further be included in any LCA studies for a complete estimate of net GHG budget of these bioenergy systems. It is also advisable that alternative cropping practices such as

no-tillage, minimum tillage or high density tree planting that have potential to minimize GHG emissions and nutrients losses should be implemented for more sustainable feedstock production.

3. Nitrogen fertilizer addition up to the levels used in this study markedly increased switchgrass yield with little effects on GHG emission, which allowed offsetting greater atmospheric CO_2 from fossil fuels. Nitrogen fertilization of switchgrass in this region could reduce the land base needed for bioenergy production and reduce pressure on land required for food and forage crop production.

APPENDICES

APPENDICES

Table A. 1 Description of groundcover management, competition management and cultural management practices at the cover crop-Fraser fir intercropping system at the MSU's Tree Research Center

Groundcover	Competition-Management	Treatments	Management practice	
			Year 2007	Year 2008
Dutch White Clover (DWC)	Strips (S)	DWCS	Cover crop seeded on 22 May 2007 at a rate of 28 kg ha^{-1}; N input from the cover crop mowed every three to four wks; glyphosate applied twice along the tree rows (strips)	N input from the cover crop mowed every three to four wks; glyphosate applied twice along the tree rows to maintain strips
	No strips (NS)	DWCNS	Cover crop seeded on 22 May 2007 at a rate of 28 kg ha^{-1}; N input from the cover crop mowed every three to four wks	N input from the cover crop mowed every three to four wks
Alfalfa (ALF)	Strips (S)	ALFS	Cover crop seeded on 22 May 2007 at a rate of 28 kg ha^{-1}; N input from the cover crop mowed every three to four wks; glyphosate applied twice along the tree rows (strips)	N inputs from the cover crop mowed every three to four wks; glyphosate applied twice along the tree rows to maintain strips
	No strips (NS)	ALFNS	Cover crop seeded on 22 May 2007 at a rate of 28 kg ha^{-1}; no N input; Cover crop mowed every three to four wks	N input from the cover crop mowed every three to four wks
Perennial Rye Grass (PRG)	Strips (S)	PRGS	Cover crop seeded on 22 May 2007 at a rate of 16 kg ha^{-1}; no N input; Cover crop mowed every three to four wks; glyphosate applied twice along the tree rows (strips)	No N input; Cover crop mowed every three to four wks; Glyphosate applied twice along the tree rows to maintain strips
	No strips (NS)	PRGNS	Cover crop seeded on 22 May 2007 at a rate of 16 kg ha^{-1}; no N input; Cover crop mowed every three to four wks	No N input; Cover crop mowed every three to four wks
Conventional	Bare ground	CONV	No nitrogen fertilization	Nitrogen fertilizer applied once at a rate of 50 kg N ha^{-1} as ammonium sulfate

Table A. 2 Description of cultural management practices applied at Skandia willow yield trial during the first year of the plantation establishment at Skandia, in Upper Peninsula of Michigan, USA

Date	Management	Pesticide
5/23/2009	Weed Control	glyphosate
5/31/2009	Weed Control	
6/12/2009	Weed Control	
6/18/2009	Weed Control	Simazine
6/18/2009	Planting	
6/18/2009	Weed Control	Goal
7/13/2009	Fencing	
10/20/2009	Coppice Cutting	

Table A. 3 Description of cultural management practices applied at Skandia hybrid poplar yield trial during the first year of the plantation establishment at Skandia, in Upper Peninsula of Michigan, USA

Date	Management	Pesticide
5/23/2009	Weed Control	glyphosate
5/31/2009	Weed Control	
6/12/2009	Weed Control	
6/16/2009	Planting	
6/17/2009	Weed Control	Pendulum Aqua Cap
6/17/2009	Weed Control	Scepter
7/13/2009	Fencing	
9/2/2009	Weed control	