AMINO ACID NUTRITION IN SHORT-ROTATION TREE PRODUCTION: THE EFFECTS ON SOIL NUTRIENT DYNAMICS, MICROBIAL INTERACTIONS, AND TREE PHYSIOLOGY

By

Alexa R. Wilson

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Forestry

2012

ABSTRACT

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Plants have the ability to assimilate and use amino acids as part of their nitrogen (N) nutrition. This has been observed in boreal, temperate, tundra, and alpine ecosystems, but further studies are needed to elucidate amino acid nutrition in forestry and agricultural production systems. This research evaluates the effects of amino acid nutrition on soil nutrient dynamics, microbial interactions, and tree physiology in short-rotation tree production of three economically important tree species. Two conifer species—Fraser fir (*Abies fraseri* [Pursh] Poir.) and Red pine (*Pinus resinosa* Aiton)—and one hardwood, hybrid poplar (*Populus nigra* L. x *Populus maximowiczii* A. Henry 'NM6') were fertilized with varying rates (0, 50, 100, 200, and 300 lbs N ac^{-1}) of an amino acid fertilizer containing arginine. Results indicate that competition may be occurring in the year of establishment, as arginine applications rates two to three times greater than the inorganic control were necessary to achieve similar growth and foliar N. In subsequent research, similar biomass and nutrient partitioning and no improvements in NUE were observed, indicating that nutrients are not severely limiting likely because arginine is functioning as a slow release fertilizer. CEC and microbial activity were not improved, likely due to the short duration of the study. Results also indicate that photosynthesis is likely more affected by biochemical processes than nutrient availability or microbial interactions. We suggest that amino acids have the potential to be a viable, alternative nutrient source, though further research should continue to elucidate the effects of amino acid nutrition in production systems.

ACKNOWLEDGEMENTS

There are so many people I could not have done this without. First and foremost, I would like to thank my advisor Dr. Pascal Nzokou. It has been such a pleasure working with him for these past few years. I am so thankful and appreciative for all he has taught me, all of the opportunities he has given me, funding me for two years, and for pushing me to be the best I can be. I would not be the researcher or person I am today without his guidance. I would also like to thank my committee members, Dr. Bert Cregg and Dr. Laurent Matuana for all of their encouragement, excellent advice, and for taking the time to read my thesis.

Of course I would also like to thank my parents, Brad and Fran Wilson, two of the most academically encouraging, supportive, and intelligent people I know. Without them putting up with my intellectual curiosity and infinite questions for the past 23 years, I most certainly would not be where I am today. I am so appreciative of their encouragement and always believing in me! I would like to thank my grandma Dar for reminding me that life is best approached one day at a time and my grandma Kathleen for keeping me in her prayers. I also would like to thank my amazing boyfriend, Zach, who has been so encouraging throughout this process and so patient with my crazy busy schedule these past few months!

I most definitely need to thank Coretta Kamdem, the best undergraduate assistant anyone could ask for. She has not only kept me sane in times of insanity, but has helped me immensely in doing fieldwork, lab work, entering data, and editing my writing. I am so appreciative of her willingness to work hard and I could not have done this without her. I would also like to thank my fellow graduate students, Ismail Koç and Yingqian Lin for collaborating with me and helping me with everything from fieldwork to statistics.

I would like to thank Paul Bloese and Randy Klevickas for all of their help at the TRC and Jerry and Josh Peterson for donating the Fraser firs. Lastly, I would like to thank Deniz Güney and Şemsettin Kulaç for kick-starting this research.

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LIST OF ABBREVIATIONS

AM……..Arbuscular Mycorrhizae
ANUE…….Assimilatory Nutrient Use Efficiency
A.. ……..……Photosynthetic Rate
B……...……Boron
C…….Carbon
C_i……..Intercellular CO_2 concentration
Ca……..……Calcium
Cl……..……Chlorine
CO_2……...Carbon Dioxide
Co……Cobalt
Cu……...Copper
EcM……Ectomycorrhizae
g_s……Stomatal Conductance
Fe……..……...Iron
K……Potassium
LWR…..……Leaf Weight Ratio
Mg……...…Magnesium
Mn……Manganese
Mo……Molybdenum
N……..Nitrogen
Na……..Sodium
NH_4^+-N……...……...Ammonium
Ni…….Nickel
NO_3^--N……..……..……..……...……..……...Nitrate
N/RW……Index of Nitrogen Availability
NUE…….Nutrient Use Efficiency
OM……..……..……..…..……..Organic Matter
P……...Phosphorus
PFNR……Plant-Fertilizer Nutrient Ratio
RWR…..……...Root Weight Ratio
TR……Transpiration Rate
S……...Sulfur
Si…..……Silicon
SRWC……..……..…….Short Rotation Woody Crops
Zn……....Zinc

INTRODUCTION

INTRODUCTION

It has traditionally been accepted that plants only take up inorganic nitrogen (N) sources at rates limited by microbial mineralization, since soil microbial communities out-compete plants for organic N sources (Schimel and Bennett 2004). This belief has resulted in the intensive use of inorganic fertilization in agriculture and tree plantations to provide plants with nutrients to grow and develop. In 2008, the United States used 54.9 million tons of fertilizer (TFI 2011). Inorganic fertilizers are largely produced by the Haber-Bosch process, which produces ammonia-based fertilizers by fixing atmospheric N (Epstein and Bloom 2005), and this process is known to cause significant changes to the N biogeochemical cycle, contributing to anthropogenic accelerated global climate change (Näsholm et al. 2009).

Inorganic N sources found in fertilizers include nitrate (NO_3^--N) and ammonium (NH_4^+-N)—both of which can have detrimental impacts on the environment and the growing system. The incentive to apply N fertilizers at high rates to improve tree growth is accompanied with nitrogen losses, which translates to money lost by growers. Nitrate ions are vulnerable to leaching through the root zone and contaminating groundwater and surrounding bodies of water (EPA 2009). Nitrate pollution of aquatic ecosystems can result in eutrophication (Jagus and Rzetala 2011), which results in overabundant nutrient availability and can lead to algal blooms and disrupt the functionality of these systems. Toxic levels of nitrate in drinking water can also have negative impacts on human health (Goodrich et al. 1991). Conversely, ammonium ions can induce stress in the soil profile due to the acidic exudates released when uptaken by roots. This can lead to ammonium toxicity (Griffin et al. 1995), reduced fine root growth, and reduced uptake of plant-essential cations (Rothstein and Cregg 2005).

Research in the past few decades has challenged the theory of inorganic N being the only N supply used by plants in demonstrating that plants can use organic N and compete well with microbes, depending on the N status of the microsite (Schimel and Bennett 2004). Among organic N sources that can be assimilated and utilized by plants are amino acids. Amino acid uptake by plants has been observed in natural settings where mineralization rates are low, such as in arctic tundra (Kielland 1995), boreal (Persson and Näsholm 2001), and alpine (Raab et al. 1996) ecosystems.

Organic fertilization can provide many benefits to plants, the growing system, and the environment. Some examples of organic fertilizers include amino acids, peptides, manure, bone meal, blood meal, fishmeal, compost, and green manures. Organic fertilizers have been shown to increase arbuscular mycorrhizae occurrences (Gryndler et al. 2006), enhance microbial activity due to the associated carbon input (Schobert et al. 1988), improve soil structure and moisture availability (Rosen and Allan 2007; Havlin and Tisdale 2005), increase nutrient availability (Havlin and Tisdale 2005), increase the number of cation and anion exchange sites (Havlin and Tisdale 2005), and function to release nutrients over time due to chemical and biological soil properties (Rosen and Allan 2007). Because of their organic nature, the availability of nutrients is regulated inherently by the biological and chemical properties of the system, thus leading to potential reductions in nutrient losses from the system via runoff or leaching.

These principles are becoming increasingly relevant applications in agriculture and forestry production as agronomists continue to seek environmentally friendly alternatives in selecting N sources. Amino acids used as a N source in controlled container studies have been shown to improve fine root growth of Scots pine (*Pinus sylvestris L.*) and Norway spruce (*Picea*

abies (L.) Karst.) seedlings (Öhlund and Näsholm 2001), which can lead to successful establishment and survival, and additionally enhance recovery of N in plant tissue and growth substrate (Öhlund and Näsholm 2002). Under controlled conditions, conifer tree seedlings can take up the amino acids glycine and arginine at rates similar to NO_3^--N and NH_4^+-N (Öhlund and Näsholm 2001). However, amino acid fertilization has seldom been tested in field production systems.

Amino acid transporters have been identified in plants, ecto- and arbuscular- mycorrhizal fungi (Näsholm et al. 2009). However, species differences in amino acid uptake rates exist and have been suggested to be due to different transport system affinities (Persson and Näsholm 2001). Mycorrhizal fungi have been proven to aid in the assimilation of amino acids in soils (Näsholm et al. 2009; Dannenmann et al. 2009), but have also been suggested to be of little importance to amino acid acquisition (Persson and Näsholm 2001).

When amino acids are applied to or present in soils, rapid mineralization may occur due to their short half lives (Jones 1999). This can result in reduced availability to plants if mineralization is not synchronized with plant demand. Amino acids also bind to anion and cation exchange sites (Rothstein 2010), soil aggregates, and are uptaken by microbes until saturation occurs (Jones 1999). This mediates the rate at which amino acids are available for mineralization (Reeve et al. 2008; Gonod et al. 2006), reducing losses to leaching, but also decreasing amino acids available to plants (Näsholm et al. 2009). Rapid turnover of microbial communities can result in N releases over time, thus increasing the window in which amino acids will become available and used by plants (Dannenmann et al. 2009).

While much research has been conducted on amino acid nutrition, these principles have seldom been tested in production systems. More research is needed to understand the effects of

amino acids on biological and chemical soil dynamics, tree growth response, nutrient physiology including nutrient use efficiency, and tree metabolism. Greater understanding of amino acids as a nutrient source for tree crops could help to improve the sustainability of the production of short rotation woody crops, ornamentals, landscape trees, Christmas trees, and even agricultural and horticultural species. Three economically important species were selected for this study, including Fraser fir (*Abies fraseri* [Pursh] Poir.), Red pine (*Pinus resinosa* Aiton), and hybrid poplar (*Populus nigra* L. x *Populus maximowiczii* A. Henry 'NM6'). Fraser fir is a species primarily grown for Christmas tree production and are intensively fertilized to improve productivity and shorten the rotation in plantations. Red pine is landscape tree widely grown for pulp, paper, and for conservation purposes. Hybrid poplar (NM6) is widely grown for sustainable woody biofeedstock production in which high productivity can be realized over very short rotations (Dickmann 2006). This study explores the use of the amino acid, arginine, in short-rotation tree production to evaluate its ability to fulfill tree nutritional needs and its behavior in production soils.

The specific objectives of this study are to:

1- Determine the contribution of arginine to soil inorganic N pools and N losses and evaluate the influence on tree growth response and N physiology.

2- Evaluate the effect of arginine nutrition on biomass and nutrient partitioning and the effects on nutrient use efficiency.

3- Determine the influence of arginine on cation exchange capacity, microbial respiration, and mycorrhizal infection and evaluate the interactions with tree nutrient status and photochemical processes.

We hypothesize that:

1- Arginine applied to soils will not be fully available to plants due to binding to cation exchange sites and immobilization in microbial biomass, which will also reduce mineral nutrient losses and contributions to mineral nutrient pools.

2- Microbial respiration and mycorrhizal infection will be enhanced by arginine fertilization.

3- Application of arginine will improve tree growth response, nutrient use physiology, and photosynthesis.

REFERENCES

REFERENCES

Dannenmann MJ Simon, R Gasche, J Holst, PS Naumann, I Kogel-Knabner, H Knicker, H Mayer, M Schloter, R Pena, A Polle, H Rennenberg, and H Papen (2009) Tree girdling provides insight on the role of labile carbon in nitrogen partitioning between soil microorganisms and adult European beech. Soil Biology and Biochemistry 41: 1622-1631.

Dickmann DI (2006) Silviculture and biology of short-rotation woody crops in temperate regions: then and now. Biomass & Bioenergy 30:696-705. USDA/Forest Service General Technical Report SO-26.

EPA (2009) Fertilizer Applied for Agricultural Purposes. Retrieved December 30, 2011, from http://cfpub.epa.gov/eroe/index.cfm?fuseaction=detail.viewInd&lv=list.listByAlpha&r=216629&subtop=312.

Epstein E and A Bloom (2005) Mineral Nutrition of Plants: Principles and Perspectives. Sunderland: Sinauer Associates, Inc. (2nd edition).

Gonod LV, DL Jones, and C Chenu (2006) Sorption regulates fate of the amino acids lysine and leucine in soil aggregates. Eur. J. Soil Sci. 57: 320-329.

Goodrich JA, BW Lykins Jr., and RM Clark (1991) Drinking water from agriculturally contaminated groundwater. Journal of Environmental Quality 20:707-717.

Griffin KL, WE Winner, and BR Strain (1995) Growth and dry matters partitioning in Loblolly and Ponderosa pine seedlings in response to carbon and nitrogen availability. New Phytol. 129: 547-556.

Gryndler M, J Larsen, H Hrselova, V Rezacova, H Gryndlerova, and J Kubat (2006) Organic and mineral fertilization, respectively, increase and decrease the development of external mycelium of arbuscular mycorrhizal fungi in a long-term field experiment. Mycorrhiza 16:159-166.

Havlin JL and SL Tisdale (2005) Soil Fertility and Fertilizers. Upper Saddle River: Pearson Education, Inc. (7th edition).

Jagus A and M Rzetala (2011) Influence of agricultural anthropopression in water quality of the dam reservoirs. Ecological Chemistry and Engineering 18: 359-367.

Jones DL (1999) Amino acid biodegradation and its potential effects of organic nitrogen capture by plants. Soil Biology and Biochemistry 31: 613-622.

Kielland K (1995) Landscape patterns of free amino acids in arctic tundra soils. Biogeochemistry 31: 85-98.

Näsholm T, K Kielland, and U Ganeteg. (2009) Uptake of organic nitrogen by plants. New Phytol. 182: 31-48.

Öhlund J and T Näsholm. (2001) Growth of conifer seedlings on organic and inorganic nitrogen sources. Tree Physiology 21: 1319-1326.

Öhlund J and T Näsholm (2002) Low nitrogen losses with a new source of nitrogen for cultivation of conifer seedlings. Environ. Sci. Technol. 36: 4854-4859.

Persson J and T Näsholm (2001) Amino acid uptake: a widespread ability among boreal forest plants. Ecology Letters 4: 434-438.

Raab TK, DA Lipson, and RK Monson (1996) Non-mycorrhizal uptake of amino acids by roots of the alpine sedge *Kobresia myouroides*: implications for the alpine nitrogen cycle. Oecologica 108: 488-494.

Reeve JR, JL Smith, L Carpenter-Boggs, and JP Reganold (2008) Soil-based cycling and differential uptake of amino acids by three species of strawberry (*Fragaria* spp.) plants. Soil Biology and Biochemistry 40: 2547-2552.

Rothstein DE (2010) Effects of amino-acid chemistry and soil properties on the behavior of free amino acids in acidic forest soils. Soil Biology and Biochemistry 42: 1743-1750.

Rothstein DE and BM Cregg (2005) Effects of nitrogen form on nutrient uptake and physiology of Fraser fir (*Abies fraseri*). For. Ecol. Manage. 219: 69-80.

Rosen CJ and DL Allan (2007) Exploring the benefits of organic nutrient sources for crop production and soil quality. HortTechnology 17: 422-430.

Schimel JP and J Bennett (2004) Nitrogen mineralization: Challenges of a changing paradigm. Ecology 85: 591-602.

Schobert C, W Kockenberger, and E Komor (1988) Uptake of amino acids by plants from soil: A comparative study with castor bean seedlings grown under natural and axenic soil conditions. Plant Soil 109: 181-188.

TFI, The Fertilizer Institute (2011) Statistics FAQs. Retrieved December 30, 2011, from http://www.tfi.org/statistics/statistics-faqs.

CHAPTER ONE: LITERATURE REVIEW

Proper plant nutrition in production systems is achieved by applications of fertilizers containing plant essential nutrients. Organic and inorganic fertilizers have different effects on the chemical and biological soil nutrient dynamics and plant-mycorrhizae symbioses. These soil properties, in turn, will affect plant nutrient physiology and photosynthesis. This literature review is a discussion of the existing published literature on plant nutrition, plant essential nutrients, fertilizer sources, amino acids as a nutrient source, plant-mycorrhizae symbioses, nutrient use physiology, and photosynthesis.

1. Plant Nutrition

Plants acquire nutrients from their growth media, and their ability to assimilate and incorporate nutrients into their tissues will impact their growth and performance (Epstein and Bloom 2005). In turn, a plant's nutrient status will dictate its growth and development because limitations in plant essential nutrients disrupt normal physiological activity (Epstein and Bloom 2005). In natural ecosystems, plants have developed means of coping with nutrient limitations. These adaptation strategies primarily function to increase the surface area of the root, where nutrient acquisition occurs. Alterations in root morphology (Vance et al. 2003; Hodge 2004; Gloser et al. 2008), allocations of resources to roots (Poorter et al. 2012), symbioses with mycorrhizal fungi (Larcher 2003), and associations with nitrogen fixing bacteria (Havlin and Tisdale 2005) are common examples of how nutrient limitations are overcome.

Trees are produced for a variety of uses including production of fruit and nut crops, use as ornamentals, landscape trees, Christmas trees, wood products, and biofuels. In 2007, Christmas trees and short rotation woody crops (SRWC) were grown on 343,374 and 228,335 acres, respectively, with a market value of cut Christmas trees and harvested SRWC totaling

$384.6 million in the United States and $29.2 million in Michigan alone (Vilsack and Clark 2009).

In the case of agricultural and tree production systems where plants are grown in rotation, soil nutrients are depleted over time. Because the goal of growers is to achieve maximum growth and yield of their crops to optimize their profit, growers must ensure that plant essential nutrients are present in soils at concentrations that are conducive to optimum plant growth. Plant nutrition principles are founded on Carl S. Sprengel (1787-1859) and Justus von Liebig's (1803-1873) "law of the minimum," which states that if a plant is lacking any single essential element, growth and development will be impeded (Epstein and Bloom 2005). This principle is the driving force for use of soil amendments in crop production systems. For perennial crops, like trees grown in short rotation in intensive systems, the nutritional requirements depend primarily on the species being grown and the stage of the rotation.

Short rotation woody crops, including hybrids and clones of *Populus spp.* and *Salix spp.*, will have different nutritional requirements based on the combination of the species/clone and the production site (Dickmann 2006). Site characteristics that will affect the growth of *Populus spp.* include soil depth, texture, and structure, water table depth, topographic position, field history, pH, and the geologic source of nutrients (Baker and Broadfoot 1979). In a previous study, SRWC clones and hybrids were shown to be unaffected by fertilization in the first rotation, however, they are reported to require nutrients once harvesting begins as nutrients in the soil are depleted over time (Dickmann 2006). Coleman et al. (2006) found nitrogen (N) fertilization of hybrid poplars increased biomass by 43 to 83%, and suggested that regular low-dose applications of fertilizers could effectively sustain high N concentrations in hybrid poplar biomass.

In Christmas tree production, the recommended N application rate for 6-year-old Fraser firs is almost 200 kg N ha^{-1} in one year (Table 1.1, Koelling 2002), contrasting to Fixen and West's (2002) recommendation of 145 kg N ha^{-1} per year in the production of corn (Rothstein 2005; Nikiema et al. 2011). Christmas tree species are intensely fertilized to shorten the rotation and achieve desired growth, morphological, and foliar characteristics (Koelling 2002). In a two-year study by Rothstein (2005), 4-year-old Fraser firs were fertilized with 0, 50, 100, and 150% of the recommended application rate (95 kg N ha^{-1}, Table 1.1) for 4-year-old Fraser fir and no reductions in the growth or quality of the firs was found. Rothstein (2005) also found an increase in nitrate leached with increasing N rate applied, with the highest N rate yielding N concentrations in leachate that were 20-30 times higher than levels considered to be safe in drinking water.

2. Plant Essential Nutrients

An element can be defined as essential if it is imperative to the normal growth and development of a plant and is involved in the plant's metabolism or structure (Epstein and Bloom 2005). Nutrients are characterized as macronutrients and micronutrients based on the relative amount needed to satisfy plant demand. Macronutrients include nitrogen (N), potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), and sulfur (S) (Epstein and Bloom 2005). Micronutrients include chlorine (Cl), iron (Fe), nickel (Ni), boron (B), manganese (Mn), sodium (Na), zinc (Zn), molybdenum (Mo), copper (Cu), and cobalt (Co) (Epstein and Bloom 2005). For some species, especially grasses, silicon (Si) is considered to be essential (Epstein and Bloom 2005). The availability of these nutrients in soils is highly dependent on soil physical and

chemical properties. Soil characteristics that influence nutrient availability include the soil type, pH, water, and the chemical properties of the nutrient (e.g., charge) (Havlin and Tisdale 2005). If any of these nutrients are unavailable in soils or cannot be assimilated by the plant, the plant's growth and development will be hampered (Epstein and Bloom 2005). Plants assimilate nutrients from the soil solution, which is very dynamic as nutrients are continuously being removed by plants or lost from the system, but replenished by natural soil processes including desorption from binding sites and mineralization by microbes (Havlin and Tisdale 2005). Because some nutrients are mobile within the plant, and others are not, deficiencies in young versus old tissues can help indicate the deficient nutrient; however, foliar tests are recommended because nutrients can have similar deficiency symptoms (Havlin and Tisdale 2005). The macro- and micronutrients important to this study are discussed in detail below.

2.1 Macronutrients

Nitrogen exists in soils as nitrate (NO_3^--N), ammonium (NH_4^+-N), and in organic forms, all of which can be used by plants (Larcher 2003). Availability of N in the soil is regulated by microbial activity and the degree to which it is bound in the soil (Larcher 2003). Plants take up N by mass flow and diffusion (Havlin and Tisdale 2005). Once in the plant, N will accumulate in young tissues, but can easily be translocated within the plant, especially when it is organically bound (Larcher 2003). When roots take up NO_3^--N it is reduced by nitrate reductase into nitrite (NO_2^--N) and further reduced to NH_4^+-N by nitrite reductase in root cells (Epstein and Bloom 2005; Below 2002). These reactions are fueled by energy produced in photosynthesis (Epstein and Bloom 2005). Once N exists in the NH_4^+-N form, it is converted to glutamine by glutamine

synthetase and then to glutamate by glutamate synthase (Epstein and Bloom 2005; Below 2002). At this point, it can be converted into other organic compounds including proteins, nucleic acids, chlorophyll, and growth regulators (Below 2002). Nitrogen is especially important in plant metabolism because of its intimate relationship with photosynthesis and incorporation in enzymes. Some plant species have symbiotic relationships with *Rhizobia*, which fix atmospheric N (N_2-N), making it available to the plant (Havlin and Tisdale 2003).

Conifers' foliage typically contains 1.3-3.5% N (dry weight) when healthy (Table 1.2, Landis et al. 2010), which is lower than that of broadleaf foliage which usually contains an average of 2-4% N (dry weight) (Cregg 2005). Nitrogen deficiencies result in conifer foliage having a yellowish appearance (Cregg 2005). When growing in media with increasing relative concentrations of NO_3^-: NH_4^+, Fraser firs were found to have improved photosynthesis, uptake of N, P, and exchangeable cations, and foliar nutrition (Rothstein and Cregg 2005). A study found significantly less fine root growth when hybrid poplar species were fertilized with ammonium as opposed to nitrate fertilizer, because ammonium reduced the ability of poplars to take up water (Domenicano et al. 2011). This could be a result of the release of hydrogen ions by plant roots with the uptake of NH_4^+ creating an acidic environment not conducive to root growth. Liu and Dickmann (1996) found significant increases in photosynthesis and stomatal conductance of hybrid poplars under flooded conditions when N was applied.

Phosphorus is present in soils in organic matter or in Ca, Fe, and Al phosphates, but only labile forms of P are considered available to plants (Larcher 2003; Havlin and Tisdale 2005). Most P in soils is non-labile and is present in chelated complexes, parent material, or organic matter (Havlin and Tisdale 2005). Labile P is primarily adsorbed to the soils and becomes

available at rates largely dependent on adsorption and desorption because microbial mineralization of P is not significant (Havlin and Tisdale 2005). Plants take up P as orthophosphate (HPO_4^{-2}-P) or dihydrogen phosphate ($H_2PO_4^-$-P) via diffusion and mass transport (Havlin and Tisdale 2005). Once in the plant P tends to accumulate in reproductive organs, but can easily be translocated when organically bound (Larcher 2003). Phosphorus is essential to plant metabolism and is present in nucleic acids, phospholipids in membranes, adenosine phosphates including ATP and ADP, and phytin (Epstein and Bloom 2005; Larcher 2003). Phosphorus typically constitutes 0.20-0.60% of conifer dry weight (Table 1.2, Landis et al. 2010), and needles will have a purplish color when P deficient (Cregg 2005).

The majority of K in soil is in mineral form in feldspar micas, but with weathering, K^+-K can be found in the soil solution, and in clay minerals due to its positive charge, which allows it to bind negatively charged sites (Havlin and Tisdale 2005). Potassium in clay particles is considered nonexchangeable or exchangeable based on its ability to equilibrate with the soil solution, thus becoming available to plants (Havlin and Tisdale 2005). Plants take up K ions primarily by mass flow (Havlin and Tisdale 2005) and K will accumulate in the meristem, parenchyma of bark, and locations where there is young tissue or high metabolic activity (Larcher 2003). Potassium can readily be transported throughout the plant, and is important for balancing electrochemical potentials and activating enzymes, especially in photosynthesis and in the reduction of nitrate (Larcher 2003). For conifers, potassium is about 0.70-2.40% of their dry weight (Table 1.2, Landis et al. 2010). Potassium is important to wood formation and biomass production in trees, including poplar, playing a key role in controlling the expansion of xylem cells (Ache et al. 2010; Fromm 2010).

Calcium and magnesium in soils come primarily from organic matter and from weathering of parent material (Havlin and Tisdale 2005). When released from parent material and organic matter, Ca and Mg exist as divalent cations (Ca^{2+} and Mg^{2+}, respectively) in the soil solution, which remains in equilibrium with the exchangeable Ca^{2+} and Mg^{2+} that are adsorbed and desorbed from clay minerals (Havlin and Tisdale 2005). Ca^{2+} and Mg^{2+} are bound in carbonate gypsum and carbonate (dolomite), respectively (Larcher 2003), which are materials used in liming soils to raise the pH (Havlin and Tisdale 2005). Ca^{2+} and Mg^{2+} tend to be deficient in acidic soils (Larcher 2003; Havlin and Tisdale 2005) where conifers prefer to grow (pH 5.5) (Landis 1989).

Calcium is primarily transported in cationic form by mass flow and root interception (Havlin and Tisdale 2005) and accumulates in the foliage and bark of plants, but it is not readily transportable in the plant (Larcher 2003). Calcium is essential for maintaining cell wall structure and stability, enzyme activation, intercellular signaling especially in signaling stress, and in stomatal aperture (Epstein and Bloom 2005). Magnesium is transported in cationic form via mass flow and diffusion (Havlin and Tisdale 2005) and accumulates in the foliage, but can be transported once in the plant (Larcher 2003). Magnesium is essential to plants because it is an important component of chlorophyll and is important in the activation of enzymes involved in transferring phosphates (Epstein and Bloom 2005). Conifer dry weight tends to be 0.10-.30% Mg and 0.30-1.00% Ca (Table 1.2, Landis et al. 2010), with Mg deficiencies resulting in yellowed needle tips (Landis 1989). Calcium has been demonstrated to be essential in wood formation of trees by reactivating cambial activity following dormancy in the winter (Fromm 2010).

2.2 Micronutrients

Manganese (Mn^{2+}) is supplied to soils primarily from organic matter and becomes available via mineralization (Havlin and Tisdale 2005). Manganese is in equilibrium with the soil solution due to dissolution and precipitation of primary and secondary manganese minerals and adsorption and desorption of labile Mn^{2+} (Havlin and Tisdale 2005). It is taken up by the plant in cationic form and transported into the plasmalemma across an electrical gradient (Havlin and Tisdale 2005). Once in the plant, Mn^{2+} accumulates in the leaves and is not easily transported (Larcher 2003). Manganese is essential in activating enzymes, especially in the citric acid cycle, and is a component of the enzyme complex that splits water in Photosystem II (Epstein and Bloom 2005). In healthy conifers, Mn tends to constitute about 100-250 ppm of dry weight (Table 1.2, Landis et al. 2010).

3. Nutrient sources

In production systems, amendments are made to soils to ensure that nutrient concentrations in soils are conducive to optimum plant growth. Nutrient concentrations in the soil solution, where plants acquire their resources, are in equilibrium with the surrounding soil environment, thus soil nutrient dynamics are very complex (Havlin and Tisdale 2005). This equilibrium is complicated by soil chemical and biological properties, nutrient losses through the soil profile and via runoff, and by plant uptake of nutrients (Havlin and Tisdale 2005). Fertilizers are added to soils in an effort to increase the amount of nutrients available to plants, thus improving plant growth and physiological processes if nutrients can be assimilated. Soil

amendments have been used for thousands of years, but it was not until the inception of the Haber-Bosch process in 1913 that fertilizers became widely available for commercial use (BASF 2012).

A diversity of inorganic and organic fertilizer is available, with varying amounts of nutrients and nutrient combinations and physical states (gas, liquid, solid) (Havlin and Tisdale 2005). Fertilizers also include liming materials, such as dolomite or carbonite gypsum, which add Mg^{2+} and Ca^{2+}, respectively, which increase the pH of soils and can increase availability of certain nutrients (Havlin and Tisdale 2005). Rock powders are the sources for phosphorus and in some cases, potassium (e.g., biotite, feldspar, potassium sulfate), which are considered to be "organic," but do not necessarily meet organic certification standards (Card et al. 2011). Fertilizers are typically selected based on the results of soil tests, species being grown, anticipated plant demand, and associated nutrients in the fertilizer mix, which can also be important for plant demand or altering soil chemical properties (e.g., pH). Among fertilizers used in the United States in 1996, 91% were N-P-K fertilizers, 4% were liming materials, and only 1% were organic fertilizers (EPA 1999).

3.1 Inorganic N Fertilization

Inorganic fertilizers are synthetically created nutrient sources (Blessington et al. 2009), which contain mineral nutrients that can be readily used by plants. These fertilizers have gained popularity because they are easily accessible, less expensive than organic sources, contain nutrients that are readily available to plants, and are available in a variety of resources. Because inorganic fertilizers contain nutrients that are in a chemical form that can be readily taken up by plants, applying inorganic fertilizers in production systems when plants are not able to use them

immediately can result in serious nutrient losses via nitrification, leaching, and runoff (Havlin and Tisdale 2005). However, slow-release fertilizers, which are also commercially available, have a chemical coating that regulates the rate at which nutrients are released. Infusing wood chips with ammonium nitrate was recently demonstrated to be an effective slow-release fertilizer (Ahmed et al. 2011). Because they are inorganic, these nutrients are not inherently regulated by the growing system.

Among nitrogen solutions used in the United States in 2004, 25% were urea-ammonium-nitrate, 25% were ammonia, and 20% were urea (Kramer 2004). The inorganic N fertilizer containing the greatest amount of N is anhydrous ammonia, which is in the gas state and contains 82% N (Havlin and Tisdale 2005). Urea ($CO(NH_2)_2$) is a solid ammonium-based fertilizer that contains 45-46% nitrogen (Havlin and Tisdale 2005). Some other examples of ammonium-based fertilizers include ammonium nitrate (NH_4NO_3) containing 33-34% N, mono- and diammonium phosphate ($NH_4H_2PO_4$ and $(NH_4)_2HPO_4$, respectively) containing 11% and 18-21% N and 48-55% and 46-54% P, respectively, and ammonium sulfate ($(NH_4)_2SO_4$) which contains about 21% N and 24% S. There are also nitrate-based fertilizers including calcium nitrate ($Ca(NO_3)_2$), potassium nitrate (KNO_3), and sodium nitrate ($NaNO_3$). Ammonium sulfate is a fertilizer appropriate for growing conifers because this fertilizer can reduce the pH of soils, mimicking the growing conditions of their natural environment, thus optimizing growth (Cregg 2005).

3.2 Organic N Fertilization

While organic fertilization has been used for thousands of years, its recent popularity is driven by increasing environmental concerns and a greater body of knowledge on the negative impacts of intensive application of inorganic fertilizers on their surroundings. Organic fertilizers are produced from natural sources and do not include synthetically produced nutrient sources (Blessington et al. 2009; Card et al. 2011). Organic nutrient sources that are currently used in agriculture and tree plantation production systems include various animal manures, green manure leguminous cover crops, sewage sludge, bone meal, blood meal, fish meal, fish emulsion, kelp, and compost (Card et al. 2011). Like inorganic fertilizers, different organic nutrient sources contain varying levels of nutrients (Table 1.3, Dumroese et al. 2009).

There are many benefits of organic fertilization. Because the nutrients exist naturally, they do not have an associated greenhouse gas emission in their production (Blessington et al. 2009). Organic fertilizers provide nutrients that become available for plant use over time by microbial decomposition, therefore nutrient losses from organic systems can be reduced (Card et al. 2011; Blessington et al. 2009). However, because most of these organic fertilizers do not contain nutrients in forms that can readily be used by plants, growers must take into account this time lapse in their nutrient management regimens (Card et al. 2011). Organic fertilizers have been demonstrated to work as well as inorganic fertilizers (Card et al. 2011). For example, Baldi et al. (2010) found improved root growth and lifespan when using organic fertilizers for peach trees. Organic fertilizers can also increase soil quality and nutrient use efficiency over time (Blessington et al. 2009).

Problems with organic fertilizers include increased costs and the potential to contaminate the surrounding environment (Blessington et al. 2009). Organic nutrient sources have an associated carbon (C) input, which can stimulate soil microbial activity due to alterations of the

soil C/N ratio (Schobert et al. 1988), which could lead to non-targeted organisms intercepting the nutrient source, and reduced growth (Gronli et al. 2005).

4. Amino acids as a nutrient source

Amino acids have been identified as an important nutrient source for plants growing in a variety of environments including arctic tundra (Kielland 1995), boreal (Näsholm et al. 1998; Persson and Näsholm 2001), temperate (Gallet-Budynek et al. 2009; Metcalfe et al. 2011), and alpine (Raab et al. 1996) ecosystems (Table 1.4). In these systems, amino acids tend to be the dominant form of available nitrogen because of low N turnover rates (Kielland 1995). However, Lipson and Näsholm (2001) reported that organic nitrogen, mainly in the form of amino acids, is potentially important nutrient sources in a greater diversity of ecosystems including tropical savanna woodland, subtropical rainforest (Schmidt and Stewart 1999), desert ephemeral pools (Schiller et al. 1998), and agricultural systems (Jones and Darrah 1994; Yamagata and Ae 1996; Näsholm et al. 2000) (Table 1.4).

4.1 Amino acid availability in soils

Organic matter from plant material and microbial biomass turnover are the main sources of proteins and peptides in soil (Lipson and Näsholm 2001). Free amino acids are present in soils as a result of the depolymerization of organic matter, including proteins and peptides, which are broken down into monomers such as amino acids and nucleic acids (Schimel and Bennett 2004). Extracellular enzymes play the most significant role in this process of releasing "free amino acids" (Lipson and Näsholm 2001). It has also been demonstrated that plants excrete amino acids at the root tip and they can reabsorb them if they remain free amino acids (Jones and

Darrah 1994). Free amino acids are rapidly mineralized due to their short half-lives, which have been estimated to be between 1 and 12 hours (Jones 1999).

Free amino acids can bind to anion and cation exchange sites (Rothstein 2010) and soil aggregates, and are taken up by microbes until saturation occurs (Jones 1999) (Figure 1.1). These biological and chemical processes mediate the rate at which amino acids become available for mineralization (Reeve et al. 2008; Gonod et al. 2006), which has the potential to reduce losses to leaching, although amino acids can leach through the soil profile (Raab et al. 1996). In a container study fertilizing Scots pine (*Pinus sylvestris* (L).) with amino acids, there was improved nitrogen recovery in growth substrate and plant tissues (Öhlund and Näsholm, 2002). When amino acids are bound in the soil or immobilized in microbial biomass, their availability for plant use is limited (Näsholm et al. 2009). Amino acids also serve as a substrate for mineralizing bacteria (Kielland 1995). When amino acids are mineralized, they may be taken up by plants, adsorbed to soils, fixed in microbial biomass, or leached below the rootzone (Kielland et al. 2007) (Figure 1.1).

It has been reported that initial competition between plants and microbes exists for amino acids in soils (Andresen et al. 2009). This can likely be attributed to the C input associated with amino acids, which stimulates soil microbial activity (Schobert et al. 1988). The intensity of the competition is variable by microsite depending on the nitrogen form and availability at the root-microbe interface (Schimel and Bennett 2004). However, challenges faced by plants in accessing amino acids can be overcome. When amino acids are present in high concentrations, plant uptake is enhanced and plants are more successful competitors (Jones et al., 2005). Mycorrhizal fungi have also been proven to aid in the assimilation of amino acids in soils

(Näsholm et al. 2009; Dannenmann et al. 2009). Amino acid transporter genes have been identified in both ecto- and endomycorrhizal fungi (Näsholm et al. 2009).

4.2 Amino acid uptake by plants

There are 20 different amino acids with a variety of different characteristics including acidic, basic, neutral, positively charged, negatively charged, non-polar, and polar. As a result amino acid transporters are as diverse as the amino acids they are transporting (Table 1.5). High affinity and low affinity amino acid transporters have been identified in *Arabidopsis* (Tegeder and Rentsch 2010), and studies indicate that amino acid transporters in plants are ubiquitous (Lipson and Näsholm 2001). Amino acids have two stereoisomers with different chirality, an L-enantiomer and a D-enantiomer, but plants can only effectively use the L-enantiomer form of amino acids (Näsholm et al 2009).

Amino acid transporters in plant roots have primarily been identified in studies using complementation, knockout and overexpression, and isotope labeling experiments (Tegeder and Rentsch 2010). Based on knockout and overexpression, amino acid and peptide transporters have been classified into gene families based on their function in plants (Tegeder and Rentsch 2010) (Table 1.5). The two gene families involved in the uptake of cationic amino acids, like arginine, are the "amino acid permease" (AAP) and "lysine-histidine-like transporters" (LHT) families (Tegeder and Rentsch 2010) (Table 1.5). AAP genes are expressed in the epidermis of root hairs and tips, but a study by Birnbaum et al. (2003) indicates that the AtAAP5 gene was expressed in all root cells of *Arabidopsis* (Tegeder and Rentsch 2010).

Using T-DNA knockout mutants of *Arabidopsis*, it was discovered that the *AAP5* mutant had an effect on L-arginine transport when growing in media with high levels of arginine, which

indicated a low-affinity transporter (Svennerstam et al. 2008). The presence of a high affinity transporter in *Arabidopsis* was determined using ^{15}N labeling and it was discovered that the *AAP5* mutant affected L-arginine transport when growing in media with low levels of arginine (Svennerstam et al. 2008). When both the *AAP5* and *LHT1* genes were knocked out, the uptake of all amino acids was affected and 78% less amino acids were taken up than by the wild type, indicating that these genes are significant in the transport of amino acids by plants (Svennerstam et al. 2008).

Species differences have been observed in amino acid uptake and this has been attributed to differing transport system affinities for amino acid (Persson and Näsholm 2001). However, in a container study using Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* [L.] Karst.) seedlings, uptake of glycine and arginine was similar to that of ammonium and nitrate (Öhlund and Näsholm 2001).

5. Plant-Mycorrhizae Symbioses

Mycorrhizal fungi are an important component of plant nutrition because they are plant symbionts that increase the surface area of the root, thus increasing the area over which nutrients can be intercepted (Anderson and Cordell 1979). In exchange for providing plants with nutrients, plants provide mycorrhizal fungi with organic C (Smith and Read 2008). Mycorrhizae are of utmost importance when nutrient acquisition is hampered (Hobbie 2006); however, they provide other services to plants including reduced susceptibility to root diseases (Anderson and Cordell 1979) and improved performance when exposed to stress (Nguyen at al. 2006; Anderson and Cordell 1979). Mycorrhizae have been recognized by forest managers to be beneficial and economically significant (Anderson and Cordell 1979). The two classes of mycorrhizae are

ectomycorrhizae and arbuscular mycorrhizae. Both of these types of mycorrhizae transfer nutrients to new roots that are not equipped to effectively assimilate nutrients, and mycorrhizae rely on plants to function as their host, which allows them to grow and reproduce (Brundrett 2009).

Fossil evidence suggests that ectomycorrhizal associations date back 50 million years (LePage 1997). Ectomycorrhizae are especially important to forest species growing in environments characterized by low soil fertility, low species richness, or harsh environments (Malloch et al. 1980). There are an estimated 6,000 plant species, 285 of which are gymnosperm species, with known ectomycorrhizal associations (Brundrett 2009). Conifers in the Pinaceae family have an estimated 250 tree species, primarily growing in boreal ecosystems, with ectomycorrhizal relationships (Brundrett 2009). Ectomycorrhizae do not generally penetrate the root cortex, but form mantles or hyphal sheaths, which surround roots (Agerer 2006). From the mantle extends mycelium that is uniquely organized by different mycorrhizal fungi species (Agerer 2006). The root-ectomycorrhizae interface is the Hartig net, which in certain instances can consist of hyphae that are intracellular and cause root cells to enlarge (Brundrett 2009). Plants control this symbiotic relationship by altering the root architecture and growth (Brudrett 2009), which can cause roots to swell and fork (Anderson and Cordell). Nutrient transfer from plants to fungi is evidenced by considerable mantle development and fungal fruiting (Brundrett 2009). Ectomycorrhizal fungi have been demonstrated to transport P, NH_4^+, NO_3^-, and K to the plant (Marschner and Dell 1994). It has been suggested that 0-22% of the total C flux in plants is allocated to ectomycorrhizal fungi (Hobbie 2006). It has also been suggested that mycorrhizae function as C sinks with an estimated 10-20% of C from photosynthesis provided to ectomycorrhizae (Smith and Read 2008).

Arbuscular mycorrhizal fungi have been dated back to 400 million years ago (Remy et al. 1994), and symbioses are common in forests with high species richness (Malloch et al. 1980). There are 150 known arbuscular mycorrhizal fungi species colonizing 300,000 plant species (Klironomos 2000), with angiosperms in the Salicaceae family (*Salix spp.* and *Poplar spp.*) having 385 tree and shrub species with mycorrhizal associations (Brundrett 2009). Arbuscular mycorrhizal fungi penetrate the root cell wall and form arbuscles, or bundle, coil-like structures. The root-fungi interface, or intercellular arbuscles, elicit an ephemeral response by root cells (Brundrett 2009). The plant mediates this relationship by altering growth of roots and plant digestion of arbuscles, which are primarily present in new roots (Brundrett 2009). Plant transfer of nutrients to mycorrhizae is evidenced by ample arbuscles and reproduction (Brundrett 2009). Gryndler et al. (2006) found a greater occurrence of arbuscular mycorrhizal fungi under organically fertilized conditions than under mineral fertilization. Arbuscular mycorrhizae have been shown to transport P, NH_4^+, K, Ca, SO_4^{2-}, Zn, and Cu (Marschner and Dell 1994). Under controlled conditions, arbuscular mycorrhizae could supply 80% of required P to plants (Marschner and Dell 1994). Snellgrove et al. (1982) found that mycorrhizal plants allocated approximately 7% more C to roots than non-mycorrhizal plants, while Pang and Paul (1980) estimated translocated C was 12% greater than in non-mycorrhizal plants. After review of multiple studies, it is estimated that up to 20% of the C assimilated by plants is allocated to mycorrhizae (Smith and Read 2008). The discrepancies in the carbon cost of this symbiosis are likely due to species and environmental differences.

6. Nutrient Physiology

How plants assimilate, allocate, and use resources is intimately linked to their physiological processes, including carbon assimilated in photosynthesis and carbon lost via respiration (Sheriff et al. 1995). The resources of utmost importance when evaluating the performance of plants include light, water, nutrients, and carbon (Sheriff et al. 1995). Many internal and external forces dictate how biomass is partitioned, energy is used, and where nutrients are accumulated. These forces may include resource availability and environmental conditions (e.g., nutrient, light, water, atmospheric ozone concentrations) and long-term or diurnal stresses (e.g., drought, salinity, heat) (Poorter et al. 2012). In a meta-analysis evaluating the environmental factors importance on biomass allocations, nutrient availability was found to be the most important factor (Poorter et al. 2012).

Nutrient use efficiency (NUE) is the plant biomass relative to the nutrient content and depends upon the ability of a plant to uptake a particular nutrient, transport and incorporate the nutrient into tissues, and remobilize nutrients within the plant (Baligar et al. 2001). There are many factors influencing NUE, but this parameter is particularly related to soil chemical and physical properties, which alter nutrient availability in soils (Baligar et al. 2001) (Table 1.6). Nutrient use by a particular plant is also believed to be genetically and physiologically controlled by the plant species (Baligar et al. 2001). Additionally, differences in the NUE between annual and perennial species and deciduous and evergreen species exist. Ripullone et al. (2003) found differences between the hardwood and conifer species observed, with greater growth and foliar N responses observed in hardwoods, due to greater allocation of N to photosynthetic structures. Bown et al. (2010) demonstrated that the form of N applied influenced the N use efficiency of conifers, altering photosynthetic rates, biomass production, and growth responses.

Because of the complexity of nutrient use physiology and its intimate relations with metabolic physiology and the surrounding environment, how plants utilize resources is often characterized by ratios to understand NUE (Sheriff et al. 1995). Ratios are used to describe the relationships between biomass production and resource use, and are not meant to be interpreted as absolute values (Sheriff et al. 1995). Greater nutrient use efficiencies will only result in improved productivity when the resource is limited; it is also important to understand tradeoffs in survival and reproduction versus productivity (Sheriff et al. 1995). There is a wide variety of ways in which NUE can be evaluated, depending on the objective being addressed. Nutrient use efficiency can be evaluated spatially at the leaf, plant, and ecosystem levels (Sheriff et al. 1995) and physiologically at the uptake, incorporation, and utilization stages (Baligar et al. 2001) using ratios.

Understanding NUE at the leaf level is directly related to C assimilation, and ratios used to evaluate this relationship include measures of C assimilation and foliar nutrient status (Sheriff et al. 1995). Individual nutrients will have different relationships with C assimilation because certain nutrients, like N, are more important in this physiological process (Sheriff et al. 1995). Assimilatory nitrogen use efficiency (ANUE) is the ratio that defines the relationship between C assimilated and the concentration of foliar N, and while it can be used to determine relationships with other nutrients, it is commonly expressed relative to N because of the strong positive correlation between foliar N concentration and C assimilation (Sheriff et al. 1995). ANUE is influenced by plant's ability to assimilate nutrients, nutrient status of the site, and internal regulation of plant demand for the specific nutrient as related to nutrient sinks (Sheriff et al. 1995).

Understanding how biomass and nutrients are partitioned within the plant can give insight on the forces that are most impeding to growth. The leaf weight ratio (LWR) compares the total foliar biomass to the biomass of the entire plant (Sheriff et al. 1995). Greater allocation of resources to foliage may indicate that lack of light is impeding growth of the plant or that C sources are limiting to metabolic activity (Poorter et al. 2012). Conversely, the root weight ratio (RWR) compares the total root biomass to the entire plant biomass (Sheriff et al. 1995). Allocation of biomass to the roots may indicate a lack of water or nutrient availability, which may be a result of competition or stress in the soil profile (Poorter et al. 2012). Lloyd et al. (2006) found that when root interception of essential nutrients becomes limiting to plant growth, allocation of resources to roots can occur, thus resulting in reduced shoot growth in crabapple (*Malus* 'Sutyzam').

Nutrient use physiology can be understood on a plant level with the use of ratios as well. NUE is a ratio defined by the total plant biomass relative to the total content of a particular nutrient and indicates the efficiency by which the nutrient is taken up by the plant (Sheriff et al. 1995). The index of nitrogen availability (N/RW) is a measure of foliar N biomass relative to the root biomass and indicates the N availability per unit root area (Sheriff et al 1995). If there is low relative allocation of biomass to roots, it can be compensated by a greater N/RW, which would indicate that a single unit of root biomass efficiently supplies greater N to the foliage.

Understanding how well plants can efficiently use nutrients in production systems can lend insight to growers when developing effective management strategies (Baligar et al. 2001). Ratios used to understand NUE in production systems typically relate factors including yield, nutrient status, biomass production, and nutrients applied to one another to determine, for example, the efficiency of fertilizer use and the effect on crop characteristics (Baligar et al.

2001). Currently, it has been estimated that a maximum of 50% N, 10% P, and 40% K of inorganic nutrient sources applied are actually used by the target crops, with the remaining fraction speculated to be lost from the growing system, thus contributing to production pollution (Baligar et al. 2001). Growers can improve the NUE of production systems by selecting species with different genotypes, making appropriate soil amendments, changing fertilization methods, and managing biological and environmental factors in the production system (Baligar et al. 2001). Adesemoye and Kloeper (2009) suggest that fertilizer use in production systems can be improved by the presence of microbes, thus reducing environmental damage; however, this would result in the trade-off of supplying nutrients to non-targeted species and would not improve the NUE of target crops.

7. Photosynthesis

Tree nutritional status and allocations of biomass and nutrients to photosynthetic tissues (primarily foliage) greatly impacts the photosynthetic capacity of the tree because nutrients, especially N, are required to create photosynthetic structures and are key components in photochemical enzymatic processes (Below 2002). This is important because photosynthesis is the process by which plants harness atmospheric C using light energy to synthesize carbohydrates used for anabolic production of biomass and catabolic reactions including metabolism and respiration (Larcher 2003). Photosynthesis occurs in the chloroplasts of mesophyll cells, which contain numerous thylakoids surrounded by the chloroplast stroma (Hudák 1997). The light reactions occur in the membranes of the chloroplasts (Hudák 1997). The ability of photosynthetic pigments, chlorophyll a and b, and the accessory pigments,

carotenoids and xanthophyll, to capture light energy is a critical requirement for photosynthesis (Larcher 2003).

In order for photosynthesis to occur, the plant's stomata, the organs where gas is exchanged with the atmosphere, must be open (Larcher 2003). Evergreen conifers have an average of 40-120 stomata per mm^2 leaf area and cover 0.3-1% of the leaf area (Larcher 2003). Potassium (K^+) transport into guard cells cause the stomata to open, while changes in concentrations of Ca^{2+} in the cytoplasm cause stomata to close (Larcher 2003). In conditions of adequate water potentials, optimum temperatures and partial pressure of CO_2, and low exposure to ozone and other pollutants, stomata will be open (Larcher 2003). Perhaps the most typical condition to elicit a change in stomatal aperture in Michigan is low water potentials from diurnal drought stress. Stomata are closed in this situation, despite other environmental or hormonal signals, to prevent further water loss from the plant (Larcher 2003).

When red light is detected on the chloroplast stroma side of the thylakoid membrane by Photosystem II, it triggers the water-splitting reaction in the thylakoid lumen, which liberates an electron (e^-) (Larcher 2003). The e^- travels in the membrane via the electron transport chain, passing through the plastiquinone and the cytochrome b_6f complex into Photosystem I (Larcher 2003). In Photosystem I, far red light excites the e^-, and ferredoxin reduces NADP to NADPH (Larcher 2003). The hydrogen ions liberated throughout this process into the thylakoid membrane create a proton gradient, which is the energy source for ATP synthase, thus adenine diphosphate (ADP) is converted to adenine triphosphate (ATP) (Larcher 2003).

This energy is then used in the Calvin-Benson cycle where carbon dioxide (CO_2) is intercepted and binds to pentose phosphate ribulose-1,5-bisphosphate (RuBP) (Larcher 2003). Rubisco then induces carboxylation of CO_2 and RuBP, producing a 6C molecule, which rapidly splits to form two 3C compounds called 3-phosphoglycerate (PGA) (Larcher 2003). NADPH and ATP are oxidized and PGA is reduced to glyceraldehyde 3-phosphate (GAP), which can be used to form other carbon-containing compounds, and Rubisco is regenerated (Larcher 2003).

The PGA produced in the reduction phase can be converted to glucose ($C_6H_{12}O_6$), the carbon source for the plant, and O_2 is released (Below 2002). Under conditions of too much light, too high of temperatures, too much O_2 or too little CO_2, Rubisco can intercept O_2 instead and photorespiration will occur, thus carbon will be released as CO_2 (Larcher 2003; Below 2002). This process only occurs in C3 plants. When Rubisco functions properly and CO_2 is intercepted and glucose is synthesized, it can be used for metabolism and respiration when glucose is split and CO_2 or it can be used to produce new plant tissues (Below 2002).

Because of the morphology of their foliage, conifers are considered to have only "moderate" photosynthetic rates compared to other tree and plant species (Larcher 2003). Additionally, shade-tolerant or shade-adapted species, like Fraser fir (*Abies fraseri* [Pursh] Poir.), tend to have relatively lower photosynthetic rates than shade intolerant species (Larcher 2003). Many other factors lead to the diversity of photochemical activity among plants. Räim et al. (2012) observed decreased photosynthesis in Norway spruce with increasing height and suggested it to be due to multiple mechanisms including limitations in sink strength, stomata, and N. Han (2011) similarly attributed reduced photosynthesis with height in *Pinus densiflora* Sieb.

& Zucc. to be related to the resistance of CO_2 diffusion. As previously mentioned, nutritional status also influences photochemical processes. It has been demonstrated that foliar N status of Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco) and poplar (*Populus* x *euroamericana* [Dole] Guinier)) has a positive correlation with chlorophyll content and photosynthetic parameters (Ripullone et al. 2003). Chandler and Dale (1995) found improved photosynthesis, stomatal conductance, and increased chlorophyll and carotenoid concentrations in Sitka spruce (*Picea sitchensis* [Bong.] Carrière) seedlings when supplied with N following deficiency.

Table 1.1. Suggested N application rates for Fraser fir Christmas Trees.

Years following planting	N Application ($kg\ ha^{-1}\ year^{-1}$)
2	47
3	70
4	95
5	140
6+	188
Harvest Year	470-570

From: Koelling (2002).

Table 1.2. Foliar nutrient ranges for conifers.

Nutrient	Symbol	Acceptable range
Macronutrients (%)		
Nitrogen	N	1.30 – 3.50
Phosphorus	P	0.20 – 0.60
Potassium	K	0.70 – 2.50
Calcium	Ca	0.30 – 1.00
Magnesium	Mg	0.10 – 0.30
Sulfur	S	0.10 – 0.20
Micronutrients (ppm)		
Iron	Fe	40 – 200
Manganese	Mn	100 – 250
Zinc	Zn	30 – 150
Copper	Cu	4 – 20
Boron	B	20 – 100
Molybdenum	Mo	0.25 – 5.00
Chloride	Cl	10 – 3,000

From: Landis et al. (2010).

Table 1.3. Nutrients supplied by various organic nutrient sources.

Source	**Nitrogen** (% N)	**Phosphorus** (% P_2O_5)	**Potassium** (%K_2O)
Manures			
Cow	0.35	0.2	0.1 – 0.5
Goat/Sheep	0.5 – 0.8	0.2 – 0.6	0.3 – 0.7
Pig	0.55	0.4 – 0.75	0.1 – 0.5
Chicken	1.7	1.6	0.6 – 1.0
Horse	0.3 – 0.6	0.3	0.5
Compost	0.2 – 3.5	0.2 – 1.0	0.2 – 2.0
Fish emulsion	5.0	2.0	2.0
Kelp	1.0	0.2	2.0

From: Dumroese et al. (2009).

Table 1.4. Ecosystems where organic N has been shown to be potentially significant to N nutrition of plants.

Community/Ecosystem	**Reference**
Agricultural	Jones and Darrah 1994; Yamagata and Ae 1996; Näsholm et al. 2000
Alaskan dry heath	Kielland 1994
Alaskan wet meadow	Kielland 1994
Alaskan tusock tundra	Kielland 1994
Alaskan shrub tundra	Kielland 1994
Boreal coniferous forest	Bajwa and Read 1985; Abuzinadah and Read 1989; Näsholm et al. 1998
Colorado alpine dry meadow	Raab et al. 1996, 1999
Colorado shortgrass steppe	Raab et al. 1999
Colorado subalpine fen	Raab et al. 1999
Desert ephemeral pools (Nambia)	Schiller et al. 1998
Heathland (UK)	Stribley and Read 1980; Abuarghub and Read 1988
Subantarctic herbfield	Schmidt and Stewart 1999
Subtropical herbfield	Schmidt and Stewart 1999
Subtropical coral cay	Schmidt and Stewart 1999
Subtropical rainforest	Schmidt and Stewart 1999
Subtropical wet heathland	Schmidt and Stewart 1999
Semiarid mulga woodland	Schmidt and Stewart 1999
Tropical savanna woodland	Schmidt and Stewart 1999

From: Lipson and Näsholm (2001).

Table 1.5. Amino acid transporters.

Function in plants	
Family	Gene, role, or effects in transgenic plants and publications
AAP	***AtAAP1***, root uptake, seed loading, Lee et al. 2007, Sanders et al. 2009; ***AtAAP5***, root uptake, Svennerstam et al 2008; ***AtAAP6***, phloem amino acid content, Hunt et al. 2010; ***AtAAP8***, seed development, Schmidt et al. 2007; ***StAAP1***, long-distance transport, Koch et al. 2003; ***VfAAP1***, seed size, seed protein, vegetative biomass, Rolletschek et al. 2005, Götz et al. 2007, Weigelt et al. 2008
LHT	***AtLHT1***, uptake in root and leaf Mesophyll cells, Himer et al. 2006, Svennerstam et al. 2007, 2008
ProT	***AtProT2***, uptake into roots, Lehmann and Rentsch unpublished; ***HvProT***, growth, tissue proline levels, Ueda et al. 2008
ANT	***AtANT1***, phloem amino acids content, Hunt et al. 2006
CAT	***AtCAT6***, sink supply, Hammes et al. 2006
OEP	***AtOEP16***, role in deetiolation and NADPH:protochlorophyllide oxioreductase A import (Pollmann et al. 2007), but not confirmed by other studies (Philippar et al. 2007; Pudelski et al. 2009)
DASS	***AtDiT2.1***, glutamate/malate exchange, Renné et al. 2003
PTR	***AtPTR1, 5***, root uptake, biomass, N content, uptake in pollen, Komarova et al. 2008; ***AtPTR2***, flowering, seed development, Song et al. 1997; ***AtPTR3***, seed germination on salt, pathogen defense, Karim et al. 2005, 2007
OPT	***AtOPT3***, seed development (Stacey et al. 2002), however, phenotype is due to a function of AtOPT3 in iron nutrition e.g. by transporting a peptide/modified peptide Fe chelator or Fe chelator complex (Stacey et al. 2003)
Arabidopsis, At *Arabidopsis thaliana*; barley, Hv *Hordeum vulgare*; potato, St *Solanum tuberosum*: Faba bean, Vf *Vicia faba*	

From: Tegeder and Rentsch (2010).

Table 1.6. Factors influencing nutrient use efficiency (NUE) in plants.

Plant Factors	**External Factors**
Genetic Control	*Fertilizers*
- Species/cultivar/genotypes	- Source
Physiological	- Ammonification, nitrification inhibitors
- Roots: length, and density of main, lateral, and root hair	- Time depth method of placement and application
- Higher shoot yield, harvest index internal demand	- Applying in combination
- Higher physiological efficiency	- Reduce losses (NH_3, NO_3)
- Higher nutrient uptake and utilization	- Use slow release form
Biochemical	*Climatic*
- Enzymes: nitrate reductase (N), phosphatase (P), pyruvate kinase (K), arginine residue (N), phytic phosphate (P), rhodotorubic acid (Fe)	- Adequate soil moisture
- Proline, aspharagine pinitol (salinity)	- Extreme temperature
- Abscisic acid, proline (drought)	*Elements*
- Matallothionein (trace element)	- Toxicities: acidic soil (Al, Mn, pH), saline (Na, Mg, Cl, SO_4) and alkaline (Na, Na_2, CO_3) soils
- Root exudate (citric, malic, transaccionitic acid)	- Deficiencies (N, P, K, micro)
	Others
	- Arbuscular mycorrhizae, beneficial microbes
	- Control of weeds, diseased, and insects
	- Incorporate crop residue, cover crops, crop rotation
Baligar and Bennett (1986a,b); Baligar and Fageria (1997); Duncan (1994), Fageria (1992)	

From: Baligar et al. (2001).

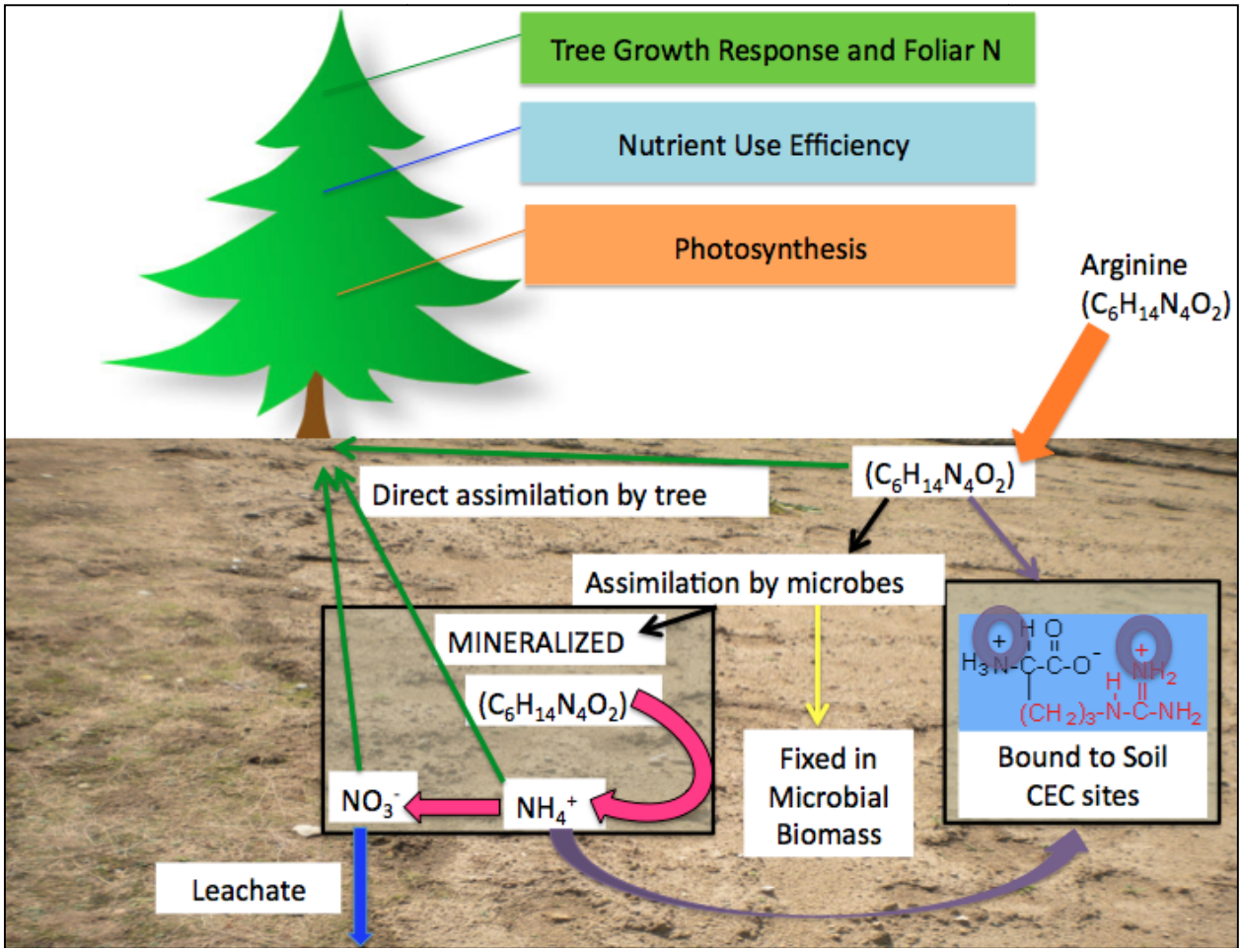

Figure 1.1. Amino acids in production soils. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

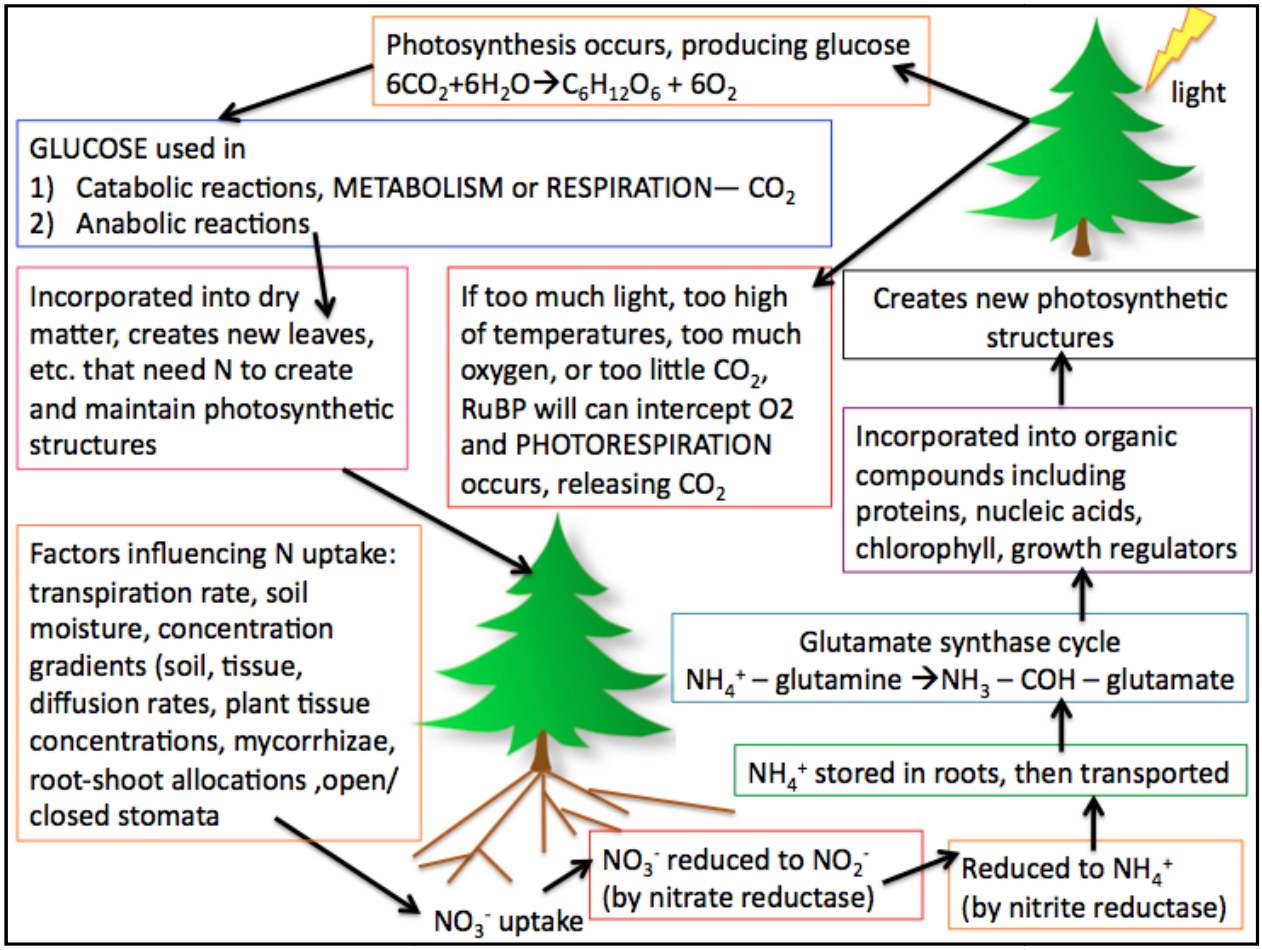

Figure 1.2. Nitrogen uptake and photosynthesis of C3 plants.

REFERENCES

REFERENCES

Ache P, J Fromm, and R Hendrich (2010) Potassium-dependent wood formation in poplar: seasonal aspects and environmental limitations. Plant Biology 12: 259-267.

Adesemoye AO and JW Kloepper (2009) Plant-microbes interactions in enhanced fertilizer-use efficiency. Appl. Microbiol. Biotechnol. 85:1-12.

Agerer R (2006) Fungal relationships and structural identity of their ectomycorrhizae. Mycol. Progress 5:67-107.

Ahmed SA, JI Kim, KM Park, and SK Chun (2011) Ammonium nitrate-impregnated woodchips: a slow-release nitrogen fertilizer for plants. J Wood Sci 57:295-301.

Anderson RL and CE Cordell (1979) How to recognize and quantify ectomycorrhizae on conifers. USDA Forest Service, Forestry Bulletin SA-FB/P8.

Andresen LC, Michelsen A, Jonasson S, Beier C, Ambus P (2009) Glycine uptake in heath plants and soil microbes responds to elevated temperature, CO_2 and drought. Acta Oecol 35: 786-796.

Baker JB and WM Broadfoot (1979) A practical field method of site evaluation for commercially important southern hardwoods.

Baldi E, M Toselli, DM Eissenstat, and B. Marangoni (2010) Organic fertilization leads to increased peach root production and lifespan. Tree Physiology 30: 1373-1382.

Baligar VC, NK Fageria, and ZL He (2001) Nutrient use efficiency in plants. Communications in Soil Science and Plant Analysis 32:921-950.

BASF (2012) BASF Historical Milestones. Retrieved January 10, 2012, from http://www.basf.com/group/corporate/en/about-basf/history/index.

Below FE (2002) Nitrogen metabolism and crop productivity. In Pessaraki, M (Ed.) *Handbook of Plant and Crop Physiology* (2nd edition) (pp 385-406). New York: Marcel Dekker, Inc.

Birnbaum K, DE Shasha, JY Wang, JW Jung, GM Lambert, DW Galbraith, and PN Benfey (2003) A gene expression map of the Arabidopsis root. Science. 302, 1956–1960.

Blessington TM, DL Clement, and KG Williams (2009) Organic and inorganic fertilizers. University of Maryland Cooperative Extension, Fact Sheet 837.

Bown HE, MS Watt, PW Clinton, EG Mason (2010) Influence of ammonium and nitrate supply on growth, dry matter partitioning, N uptake and photosynthetic capacity of *Pinus radiata* seedlings. Trees 24: 1097-1107.

Brundrett MC (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320:37-77.

Card A, D Whiting, C Wilson, and J Reeder (2011) Organic Fertilizers. Colorado State University Extension, CMG GardenNotes #234.

Chandler JW and JE Dale (1995) Nitrogen deficiency and fertilization effects on needle growth and photosynthesis in Sitka spruce (*Picea sitchensis*). Tree Physiology 15:813-817.

Coleman M, D Tolsted, T Nichols, WD Johnson, EG Wene, and T Houghtaling (2006). Post-establishment fertilization of Minnesota hybrid poplar plantations. Biomass and Bioenergy 30:740-749.

Cregg B (2005) Conifer Nutrition. Conifer Corner, September/October 2005.

Dannenmann M, Simon J, Gasche R, Holst J, Naumann PS, Kogel-Knabner I, Knicker H, Mayer H, Schloter M, Pena R, Polle A, Rennenberg H, Papen H (2009) Tree girdling provides insight on the role of labile carbon in nitrogen partitioning between soil microorganisms and adult European beech. Soil Biol Biochem 41: 1622-1631.

Dickmann DI (2006) Silviculture and biology of short-rotation woody crops in temperate regions: then and now. Biomass & Bioenergy 30:696-705. USDA/Forest Service General Technical Report SO-26.

Domenicano S, L Coll, C Messier, and F Berninger (2011) Nitrogen forms affect root structure and water uptake in the hybrid poplar. New Forests 42:347-362.

Dumroese RK, T Luna, TD Landis, editors (2009) Nursery manual for native plants: A guide for tribal nurseries — Volume 1: Nursery management. Agriculture Handbook 730. Washington, D.C.: U.S. Department of Agriculture, Forest Service. 302 p.

Epstein, E and A Bloom (2005). Mineral Nutrition of Plants: Principles and Perspectives. Sunderland: Sinauer Associates, Inc. (2nd edition).

EPA (1999) Background report in fertilizer use, contaminants, and regulations. EPA 747-R-98-003.

Fixen PE and FB West (2002) Nitrogen fertilizers: meeting contemporary challenges. Ambio 31:169-176.

Fromm J (2010) Wood formation of trees in relation to potassium and calcium nutrition. Tree Physiology 30: 1140-1147.

Gallet-Budynek A, E Brozostek, VL Rodgers, JM Talbot, S Hyzy, and AC Finzi (2009) Intact uptake by northern hardwood conifer trees. Oecologica 160:129-138.

Gloser V, K Libera, and CM Orians (2008) Contrasting below- and aboveground responses of two deciduous trees to patchy nitrate availability. Tree Physiology 28:37-44.

Gonod LV, DL Jones, and C Chenu (2006) Sorption regulates fate of the amino acids lysine and leucine in soil aggregates. Eur. J. Soil Sci. 57: 320-329.

Gronli KE, A Frostegard, LR Bakken, and M Ohlson (2005) Nutrient and carbon additions to the microbial soil community and its impact on tree seedlings in a boreal spruce forest. Plant and Soil 278:275-291.

Gryndler M, J Larsen, H Hrselova, V Rezavoa, H Grynderlerova, and J Kubat (2006) Organic and mineral fertilization, respectively, increase and decrease the development of external mycelium of arbuscular mycorrhizal fungi in a long-term field experiment. Mycorrhiza 16:159-166.

Han Q (2011) Height-related decreases in mesophyll conductance, leaf photosynthesis and compensating adjustments associated with leaf nitrogen concentrations in *Pinus densiflora*. Tree Physiology 31:976-984.

Havlin JL and SL Tisdale (2005) *Soil Fertility and Fertilizers*. Upper Saddle River: Pearson Education, Inc. (7^{th} edition).

Hobbie EA (2006) Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. Ecology 87:563-569.

Hodge A (2004) The plastic plant: root responses to heterogeneous supplies of nutrients. New Phytologist 162:9-24.

Hudák J (1997) Photosynthetic apparatus. In Pessarakli M, *Handbook of Photosynthesis* (pp 27-48). New York: Marcel Dekker, Inc.

Jones DL (1999) Amino acid biodegradation and its potential effects on organic nitrogen capture by plants. Soil Biol Biochem 31: 613-622.

Jones DL and PR Darrah (1994) Amino-acid influx at the soil-root interface of *Zea mays* L. and its implications in the rhizospehere. Plant and Soil 163:1-12.

Jones DL, Shannon D, Junvee-Fortune T, Farrar JF (2005) Plant capture of free amino acids is maximized under high soil amino acid concentrations. Soil Biol & Biochem 37: 179-181.

Kielland K (1995) Patterns of free amino acids in arctic tundra soils. Biogeochemistry 31: 85-98.

Kielland K, McFarland JW, Ruess RW, Olson K (2007) Rapid cycling of organic nitrogen in taiga forest ecosystems. Ecosystems 10: 360-368.

Klironomos JN (2000) Host-specificity and functional diversity among arbuscular mycorrhizal fungi. In: Bell, CR, Brylinsky, M, Johnson-Green, P (eds). Microbial Biosystems: New Frontiers. Proceedings of the 8th International Symposium on Microbial Ecology. Atlantic Canada Society for Microbial Ecology, Halifax, Canada. Pp 845-851.

Koelling MR (2002) Fertilization recommendations for Fraser fir: part II-Established plantings. Michigan Christmas Tree Journal 49:5-8.

Kramer DA (2004) Nitrogen. U.S. Geological Survey Minerals Yearbook pp.53.1-53.18.

Landis TD (1989). Mineral nutrients and fertilization. In: Landis TD, RW Tinus, SE McDonald, and JP Barnett. The Container Tree Nursery Manual, Volume 4. Agric. Handbk. 674. Washington, DC: US Department of Agriculture, Forest Service: 1-67.

Landis TD, RK Dumroese, and DL Haase (2010) The container tree nursery manual, Volume 7, seedling processing, storage, and outplanting. Agric. Handbk. Washington, DC: U.S. Department of Agriculture Forest Service. 200p.

Larcher, W (2003) *Physiological Plant Ecology* (4th edition). Berlin: Springer-Verlag Berlin Heidelberg.

LePage BA, RS Currah, RA Stockey, and GW Rothwell (1997) Fossil ectomycorrhizae from the Middle Eocene. American Journal of Botany 84:410-412.

Liu Z and DI Dickmann (1996) Effects of water and nitrogen interaction on net photosynthesis, stomatal conductance, and water-use efficiency in two hybrid poplar clones. Physiologia Plantarum 97:507-512.

Lipson D and T Näsholm (2001) The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. Oecologica 128:305-316.

Lloyd JE, Herms DA, Rose MA, Van Wagoner J (2006) Fertilization rate and irrigation scheduling in the nursery influence growth, insect performance, and stress tolerance of 'Sutyzan' Crabapple in the landscape. HortScience 41: 442-445.

Malloch DW, KA Pirozynski, and PH Raven (1980) Ecological and evolutionary significance of mycorrhizal symbioses in vascular plants (A review). Proc. Natl. Acad. Sci. USA 77:2113-2118.

Marschner H and B Dell (1994) Nutrient uptake in mycorrhizal symbiosis. Plant and Soil 159:89-102.

Metcalfe RJ, J. Nault, and BJ Hawkins (2011) Adaptations to nitrogen form: comparing inorganic nitrogen and amino acid availability and uptake by four temperate forest plants. Can J For Res 41:1626-1637.

Näsholm T, A Ekblad, A Nordin, R Giesler, M Högber, and P Högber (1998) Boreal forest plants take up organic nitrogen. Nature 392:914-916.

Näsholm T, Huss-Danell K, Hogberg P (2000) Uptake of organic nitrogen in the field by four agriculturally important plant species. Ecology 81: 1155-1161.

Näsholm T, K Kielland, and U Ganeteg (2009) Uptake of organic nitrogen by plants. New Phytologist 182: 31-48.

Nguyen M, MC Polanco, and JJ Zwiazek (2006) Gas exchange and growth responses of ectomycorrhizal *Picea mariana, Picea glauca,* and *Pinus banksiana* seedlings to NaCl and Na_2SO_4. Plant Biol. 8:646-652.

Nikiema P, P Nzokou, and D Rothstein (2011) 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, United States of America. New Forests. DOI 10.1007/s11056-011-9274-8.

Öhlund J, Näsholm T (2001) Growth of conifer seedlings on organic and inorganic nitrogen sources. Tree Physiology 21: 1319-1326.

Öhlund J, Näsholm T (2002) Low nitrogen losses with a new source of nitrogen for cultivation of conifer seedlings. Environ Sci Technol 36: 4854-4859.

Pang PC and EA Paul (1980) Effects of vesicular-arbuscular mycorrhizae on ^{14}C and ^{15}N distribution in nodulated fababeans. Canadian Journal of Soil Science 60:241-250.

Persson J, Näsholm T (2001) Amino acid uptake: a widespread ability among boreal forest plants. Ecology Letters 4: 434-438.

Poorter H, KJ Niklas, PB Reich, J Oleksyn, P Poot, and L Mommer (2012) Biomass allocation to leaves, stems, and roots: meta-analyses of interspecific variation and environmental control. New Phytologist 193: 30-50.

Raab TK, Lipson DA, Monson RK (1996) Non-mycorrhizal uptake of amino acids by roots of the alpine sedge *Kobresia myosuroides*: implications for the alpine nitrogen cycle. Oecologia 108: 488-494.

Räim O, E Kaurilind, L Hallik, and E. Merilo (2012) Why does needle photosynthesis decline with tree height in Norway spruce? Plant Biology 14:306-314.

Reeve JR, JL Smith, L Carpenter-Boggs, and JP Reganold (2008) Soil-based cycling and differential uptake of amino acids by three species of strawberry (*Fragaria* spp.) plants. Soil Biology and Biochemistry 40: 2547-2552.

Remy W, TN Taylor, H Hass, and H Kerp (1994) Four hundred-million-year-old vesicular arbuscular mycorrhizae. Proc. Natl. Acad. Sci. USA 91:11841-11843.

Ripullone F, G. Grassi, M. Lauteri, and M. Borghetti (2003) Photosynthesis-nitrogen relationships: interpretation of different patterns between *Pseudotsuga menziesii* and *Populus* x *euroamericana* in a mini-stand experiment. Tree Physiology 23: 137-144.

Rothstein DE (2005) Nitrogen management in a Fraser fir (*Abies fraseri* [Pursh] Poir.) Christmas tree plantation: effects of fertilization on tree performance and nitrogen leaching. Forest Science 51:175-184.

Rothstein DE (2010) Effects of amino-acid chemistry and soil properties on the behavior of free amino acids in acidic forest soils. Soil Biology and Biochemistry 42: 1743-1750.

Rothstein DE, Cregg BM (2005) Effects of nitrogen form on nutrient uptake and physiology of Fraser fir (*Abies fraseri*). For Ecol Manage 219: 69-80.

Schiller P, H Heilmeier, and W Hartung (1998) Uptake of amino acids by the aquatic resurrection plant *Chamaegigas intrepidus* and its implication for N nutrition. Oecologica 117:63-69.

Schimel JP and J Bennett (2004) Nitrogen mineralization: Challenges of a changing paradigm. Ecology 85: 591-602.

Schmidt S and GR Stewart (1999) Glycine metabolism by plant roots and its occurrence in Australian plant communities. Aust. J. Plant Physiol. 26:253-264.

Schobert C, Kockenberger W, Komor E (1988) Uptake of amino acids by plants from soil: A comparative study with castor bean seedlings grown under natural and axenic soil conditions. Plant Soil 109: 181-188.

Sheriff DW, HA Margolis, MR Kaufmann, and PB Reich (1995) Resource use efficiency. In WK Smith and TM Hinckley (Eds.), *Resource physiology f conifers: Acquisition, allocation, and utilization* (pp. 143-178). San Diego: Academic Press.

Smith SE and DJ Read (2008) *Mycorrhizal Symbioses* (3rd edition). London: Elsevier.

Snellgrove RC, WE Splittstoesser, DP Stribley, and PB Tinker (1982) The distribution of carbon and the demand of the fungal symbiont in leek plants with vesicular-arbuscular mycorrhizas. New Phytol. 92:75-87.

Svennerstram H, U Ganeteg, and T Näsholm (2008) Root uptake of cationic amino acids by Arabidopsis depends on functional expression of amino acid permease 5. New Phytologist 180: 620-630.

Tegeder M and D Rentsch (2010) Uptake and partitioning of amino acids and peptides. Molecular Plant 3:997-1011.

Vilsack T and CZF Clark (2009) 2007 Census of agriculture: United States summary and state data. Volume1, Part 51. AC007-A-51.

Vance CP, C Uhde-Stone, and D.L. Allan (2003) Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytologist 157: 423-447.

Yamagata M and N. Ae (1996) Nitrogen uptake response of corns to organic nutrition. Soil Sciences and Plant Nutrition 42:389-394.

CHAPTER TWO: GROWTH RESPONSE AND NITROGEN USE PHYSIOLOGY OF FRASER FIR (*Abies fraseri*), RED PINE (*Pinus resinosa*), AND HYBRID POPLAR UNDER AMINO ACID NUTRITION

Published: Wilson AR, P Nzokou, D Güney and S Kulaç (2012) Growth response and nitrogen use physiology of Fraser fir (*Abies fraseri*), red pine (*Pinus resinosa*), and hybrid poplar under amino acid nutrition. New Forests, doi: 10.1007/s11056-012-9317-9.

Abstract

Plants can assimilate amino acids from soils. This has been demonstrated in controlled environments and soils of various forest ecosystems. However, the role of root-absorbed amino acids in plant nitrogen nutrition is still poorly understood. We investigated the agroecological performance and nutrient use physiology of two conifers (*Abies fraseri* and *Pinus resinosa*) and one hardwood species (hybrid poplar) under amino acid fertilization. Arginine fertilizer (arGrow® Complete) was applied at varying rates (0, 56, 112, 224, and 336 kg N/ha) and compared to an inorganic control treatment (ammonium sulfate 112 kg N/ha). Parameters monitored included tree growth response, foliar nitrogen concentration, and inorganic nitrogen leaching below the rootzone. Results obtained indicate a significant growth and foliar nitrogen response to amino acid treatments, with increasing amino acid application leading to greater growth and foliar nitrogen. However, rates two to three times higher than that of the inorganic control were necessary to provide similar growth and foliar nitrogen responses. These observations were suggested to be due to competition with soil microbes for organic nitrogen, growth inhibition due to the presence of large concentrations of amino acids, or adsorption to cation exchange sites. Amino acid applications did not increase the leaching of inorganic nitrogen due either to the binding of positively charged arginine cations to exchange sites or rapid mineralization followed by plant assimilation. Mineral nitrogen collected in leachate samples increased with the application rate suggesting at least some mineralization in high amino acid application rates. We conclude that growth response and nitrogen use physiology of these species when treated with arginine are largely controlled by soil processes including microbial competition and adsorption. Further studies are being conducted to confirm these hypotheses.

Introduction

Inorganic fertilizers are traditionally used in intensive tree production and agroecosystems for providing nutrients to support plant growth. Among inorganic nutrients, nitrogen (N) has long been demonstrated as the most critical element for enhanced productivity. Nitrate (NO_3^-) and ammonium (NH_4^+) are the nitrogen forms usually taken up by plants (Hawkins and Robbins 2010). However, negatively charged nitrate ions in soils are vulnerable to leaching and can contaminate groundwater while ammonium can lead to ammonium toxicity in soils (Griffin et al. 1995), inhibition of fine root growth, and reduction of nutrients and cations taken up by plants (Rothstein and Cregg 2005).

During the past few decades, agronomists have looked for environmentally friendly alternatives and recognized organic N sources as significant for plants, especially when growing in conditions where mineralization rates are low. Recent studies have confirmed amino acids as a primary N source for vegetation in the arctic tundra (Kielland 1995), boreal (Persson and Näsholm 2001), and alpine (Raab et al. 1996) landscapes. Direct amino acid assimilation by plants saves the valuable energy costs of mineralization (Öhlund and Näsholm 2002; Liu et al. 2008) and has been demonstrated to improve fine root growth under laboratory conditions (Öhlund and Näsholm 2001), which can lead to successful establishment and survival.

Despite the known ability of forest plants to take up amino acids as a N source, the underlying physiological mechanisms, soil biological and chemical processes, and plant morphological and chemical responses related to this assimilation under field conditions are not well understood. For example, Persson and Näsholm (2001) observed large differences in assimilation rates between species and suggested those to be the result of differing affinities in amino acid transport systems in various plants. Furthermore, most of the published literature on

amino acids taken up by plants has been conducted in growing media or containers; only limited reports are available on field experimental applications. Amino acid application in field settings could alter the plant-microorganism balance due to an enhanced carbon environment (Schobert et al., 1988), leading to unexpected variations in N taken up by plants. Mycorrhizal fungi have a proven ability to degrade polymeric N compounds such as amino acids (Näsholm et al. 2009), but a large proportion of amino acids in soils can become unavailable to plants due to adsorption processes and immobilization in soil microbial biomass (Lipson and Näsholm 2001). In addition, intense microbial activity combined with increased soil proteolytic activity could lead to rapid mineralization of amino acids into inorganic forms that are either taken up by the plant, fixed to ion exchange sites, or leached below the rootzone (Kielland et al. 2007). Applied amino acids serve both as a direct source of N for plants and as a substrate for mineralization (Kielland 1995; Näsholm et al. 2009). Practical questions related to plant chemical responses to amino acid fertilization under field conditions, interactions with microbial populations, and the environmental fate of amino acids when applied to field soils need to be further investigated.

In this study we assess the plant response to amino acid nutrition under field conditions and evaluate the nutrient dynamic of amino acids applied to an intensive horticultural production system. The species investigated include Fraser fir (*Abies fraseri* [Pursh] Poir.), red pine (*Pinus resinosa* Aiton), and a fast growing hybrid poplar clone called 'NM6' which is a cross between black poplar (*Populus nigra* L.) and Japanese poplar (*Populus maximowiczii* A. Henry). Fraser fir is a major species mainly grown for Christmas tree production in intensively managed plantations where regular fertilization programs are used to enhance productivity and shorten the rotation. Red pine is a major landscape tree in the Midwestern United States and its transplants are raised and sold through commercial nurseries. Hybrid poplar (NM6) is the premiere species

used in sustainable woody biofeedstock production systems in which high productivity can be realized over very short rotations (Dickmann 2006). Successful establishment of amino acids as a nutrient source for intensive tree production systems will undoubtedly have a significant impact on the production of these tree species.

The goal of the study was to investigate the growth and physiological response of short rotation conifers and hardwoods to amino acid fertilization and determine the dynamics of amino acids in intensive production agroecosystems. The specific objectives were to evaluate the effects of amino acid fertilization on 1) tree growth response and foliar nitrogen physiology, 2) soil inorganic nitrogen, and 3) mineral nitrogen losses below the rootzone.

Materials and Methods

Site Description

This two-year study was conducted during the growing seasons of 2009 and 2010 at the Tree Research Center (TRC) on the campus of Michigan State University in East Lansing, Michigan, United States of America (42.65°N latitude and 84.42°W longitude). The climate in East Lansing is typically warm and rainy in the spring and mildly hot with sporadic rainfall late in the summer. Soils are classified as sandy with a pH of approximately 5.6 and a CEC of 3.2 meq/100g soil.

Species Selection and Management

The three species selected for this study were Fraser fir, red pine, and a hybrid poplar. The two conifers' seeds were sown and grown in 412B Styroblock® containers (112 cells/block, 95 mL/cell) in the greenhouse in 2008 and were transplanted into the nursery bed on May 22, 2009.

The potting mix was the Fafard 52 mix (Conrad Fafard, Inc.), which contains approximately 60% pine bark along with Canadian sphagnum peat, perlite, vermiculite, dolomitic limestone and gypsum. Fafard 52 is slightly acidic with a pH of 5.5–6.5. Hybrid poplar was grown from cuttings obtained in 2008 and also planted into the nursery on May 22, 2009. At the end of the 2009 growing season, poplar plants were harvested a few centimeters above the ground, allowing stumps and roots to remain in the soil. Second year results from poplar plots were collected from new growth on stumps left from the 2009 season.

Weeds were controlled either manually or by applying glyphosate at a rate of 35.84 kg/ha using a CO_2-powered backpack sprayer. Seedlings were irrigated with approximately 1.8 cm of well water per week (as needed) in the absence of adequate rainfall.

Nutrient Treatments

The amino acid source used was arGrow® Complete, an amino acid based fertilizer, which contains arginine (SweTree Technologies, Umea, Sweden). Elemental composition of arGrow® Complete as provided by the product label includes 70 g/L N, 12 g/L P, 49 g/L K, 4 g/L Mg, 10 g/L S, and smaller quantities of B, Cu, Fe, Mn, Mo, and Zn. A granular formulation of ammonium sulfate containing 21% N was used as a grower standard treatment for comparison. Fertilizer treatments included arGrow Complete® applied at 0 (Control), 56 (AA50), 112 (AA100), 224 (AA200), 336 (AA300) kg N/ha, and ammonium sulfate applied at 112 kg N/ha (AS100). The recommended dilution rate for arGrow is 1/200. To achieve the seasonal application rates for each treatment, the total solution volume to be applied in each plot was divided into weekly applications. In 2009, arginine fertilizer was applied twice per week over 10 weeks (June 1, 2009 to August 7, 2009). In 2010, arginine fertilizer was applied twice per week

over 14 weeks (May 24, 2010 to August 27, 2010). In accordance with normal farming practices, ammonium sulfate was broadcasted in single applications at the beginning of the season on June 1, 2009 and May 25, 2010. Thus, all treatments were applied for two consecutive years.

Experimental Design

The experiment was a randomized complete block design with blocking done for each species, and 6 fertilizer treatments and four replications per treatment. Each experimental plot contained 16 trees for conifers and 24 trees for the hybrid poplar; trees were planted approximately 30.5 cm from neighboring trees.

Tree Growth Response

Height and root collar diameter (RCD) were measured twice (beginning and end) during each of the two growing seasons. Growth for each year was calculated as the difference between the final and initial measurement for each parameter in each growing season.

Foliar Biomass and Nitrogen Concentration

Foliar samples were collected on July 29, 2009 and August 26, 2010 for analysis of foliar nutrient concentrations. Tissues were randomly obtained from all plants in each plot and combined to produce a composite sample for that plot. Samples were oven dried at 65°C for at least 48 h. Biomass was quantified by the dry weight of 100 needles for conifer species or 10 leaves of the hybrid poplar. Following biomass determination, tissue samples were ground into a fine powder and approximately 0.3 g from each sample was transferred to a 75 mL digestion tube and pre-digested overnight in a mixture of H_2SO_4 (4.5 mL) and H_2O_2 (1.5 mL). Digestion

tubes were then placed in a digestion block (AIM600) and heated to 340°C +/- 10°C for 60 min. After complete digestion, samples were diluted with distilled water on a vortex. An aliquot of digest solution was then analyzed on a SAN++ segmented flow analyzer (Skalar, Inc., Buford, GA, USA) for determination of the total N concentration.

Soil Nitrate and Ammonia

Soil samples were collected twice (beginning and end of season) in 2010 using a soil auger. One composite sample from each plot was collected from two random locations within 30 cm of a tree at 0-15 cm depth. Samples were placed in double-lock Ziploc bags and transported to the laboratory where they were stored in a cooler at 4°C until further analysis. Soil samples were extracted with potassium chloride (KCl) and directly analyzed for NO_3^- and NH_4^+ on the SAN++ segmented flow analyzer. Gravimetric moisture content of a soil sub-sample was determined and the NO_3^- and NH_4^+ concentrations obtained were corrected to account for water content.

NO_3^- Leachate

Suction lysimeters with their ceramic tips reaching 90 cm into the soil were installed in each plot and used to collect water leached below the rootzone. The leachate was collected weekly and the total volume in each plot determined. An aliquot from each plot was collected and refrigerated at 4°C until further analysis. The NO_3^- concentrations were determined by analysis of leachate aliquots on the SAN++ segmented flow analyzer. Total amount of NO_3^-

leached was determined by multiplying the total volume leached by the NO_3^- concentration for each plot.

Data analysis

Treatment effects on foliar N concentration, foliar N content, and biomass production in 2010 were analyzed using vector diagrams as described by Timmer (1991). Relative unit biomass is depicted on the z-axis. Each data point was calculated and plotted relative to the unfertilized control and again to the grower standard (ammonium sulfate) as an indication of the relative magnitude and type of treatment response.

A general factorial analysis of variance combining main effects and interaction was used to analyze species choice and arginine treatment levels as follows:

$$Y = b_0 + b1A + b2B + b12AB + \varepsilon$$

Where b_0 is the model intercept, b_n is the coefficient associated with factor n, and the letters A and B represent the factors in the model. Significant differences among means for response variables were determined using the Fisher's Least-Significant-Difference test at α=0.05. This allowed for comparison of response to amino acid treatments and the ammonium sulfate growers control (not included in the factorial analysis). Treatment effect on NO_3^- leaching was analyzed by repeated measures analysis of variance. The factorial design and corresponding statistical analysis were done using Design-Expert 7.1.3 from Stat-Ease, Inc. (Minneapolis, MN). Simple and repeated measures ANOVA and pairwise comparison was performed using SYSTAT 13 (Systat Software Inc., Chicago, Illinois, USA).

Results

Growth

A. fraseri responded significantly to treatments in both years of the study (Table 2.1). In 2009, plants fertilized with arginine at a rate of 336 kg N/ha (AA300) had significantly more height growth than the unfertilized control and did not differ from the grower standard (AS100). In 2010, all seedlings in arginine treatments had a height growth response similar to the grower standard (AS100), with the exception of the AA50 treatment.

For *P. resinosa* in 2009, all seedlings in arginine treatments had a height growth response similar to the grower standard (AS100) except the AA50 treatment; in 2010 there was no difference in seedling height growth among treatments (Table 2.1).

Hybrid poplar height growth was significantly greatest in the AA300 and grower standard (AS100) treatments in 2009, but in 2010 height growth was similar among treatments (Table 2.1).

The root collar diameter (RCD) growth response to fertilization treatment was statistically significant for all three species in both 2009 and 2010 (Table 2.1). Similar to the trend observed in height growth response, plants in AS100 and higher arginine rate treatments had the greatest RCD growth for all species. However, except in the case of hybrid poplar in 2009, the treatment means did not show a clear trend of increasing RCD with increased amounts of amino acid applications.

Foliar nitrogen status

Foliar N concentrations were significantly affected by amino acids treatments for all three species in 2009 (Table 2.1). Foliar N concentrations were greatest in AS100 plants in 2009, though they did not differ from the AA300 treatment for *P. resinosa* and the hybrid poplar. In

2010, only *P. resinosa* seedlings had a statistically significant foliar N treatment response with AA100 and AA200 seedlings having the greatest N concentrations.

Vector analysis for all species in 2010 indicated plants treated with amino acids or ammonium sulfate had foliar N concentrations and N contents higher than plants in unfertilized control treatments, with the exception of hybrid poplar plants in AA50 and AA100 treatments which had lower foliar N contents than the control (Figure 2.1a, Figure 2.2a, Figure 2.3a). However, when using the grower standard (AS100) as reference for the vector analysis, the directions of the vectors in *Abies fraseri* treatments showed lower relative N concentration, N content, and foliar biomass for all seedlings fertilized with amino acids (Figure 2.1b). Observations of the direction of the change in relative foliar biomass did not reveal any specific trend when using either control (Figure 2.1a) or AS100 (Figure 2.1b) as reference. Vector analysis for *P. resinosa* relative to the control treatment (Figure 2.2a) indicated relatively higher biomass in seedlings fertilized with ammonium sulfate, while seedlings in all amino acid treatments had lower foliar biomass. When using AS100 as reference (Figure 2.2b), *P. resinosa* seedlings grown in AA100 and AA200 treatment plots had relatively higher N concentrations while seedlings in AA300, AA50, and the control treatment plots were lower. However, relative foliar biomass and foliar N content was lower in all plants from amino acid treatments and the control when compared to AS100. Vector analysis for hybrid poplar using AS100 as reference indicated that only plants in AA300 and AA200 treatments had foliar N concentrations higher than the reference; foliar N concentrations of plants in AA100, AA50, and control treatments were lower (Figure 2.3a). The relative foliar biomass was variable when using control as reference (Figure 2.3a), but plants in all treatments had lower foliar biomass when using AS100 as reference (Figure 2.3b).

Mineral nitrogen leaching

Nitrate-N (NO_3^--N) losses in *A. fraseri*, *P. resinosa*, and hybrid poplar plots in 2009 were statistically similar among treatments (Figure 2.4a, 2.5a, 2.6a). The grower standard (AS100) had the greatest NO_3^--N losses and arginine treatments tended to have NO_3^--N losses proportional to the N applied. In 2010, similar trends were observed in NO_3^--N losses from *P. resinosa* plots, with no statistical differences among treatments (Figure 2.5b). For *A. fraseri* plots in 2010, AS100 treatments leached significantly greater amounts of NO_3^--N than all other treatments (Figure 2.4b). NO_3^--N leached in hybrid poplar plots in 2010 was similar among treatments, however NO_3^- -N losses were significantly less substantial than in 2009 (Figure 2.6b).

Discussion

Effect on tree growth response

Amino acid nutrition had a significant effect on height and RCD growth of all three species in the first year; however during the second growing season, only *A. fraseri* had a statistically significant height growth response to treatments (Table 2.1). Our results indicate that arginine applications two to three times greater than the grower standard (AS100) are needed to achieve similar growth responses of plants. This observation suggests that there are limitations in availability or plant assimilation of amino acids in soils due to other biological or chemical factors.

Studies of plant and microbial assimilation of inorganic and organic N sources have demonstrated that plants are inferior to microbes in their ability to take up N, irrespective of the N form (Näsholm et al. 2009; Harrison et al. 2008). Therefore, competition for organic N between microbes and plants can limit growth in arginine treatments. Furthermore, it has been suggested that the low capacity of plants to metabolize amino acids for growth is an effect of the restricted capacity of root absorption (Näsholm 2009; Bonner and Jensen 1997). In the first year of our study, this was observed in the unfertilized control and lower arginine application treatments (AA50 and AA100) as height growth of plants was significantly lower than plants fertilized with ammonium sulfate (AS100) (Table 2.1). When root interception of essential nutrients becomes limiting to plant growth, allocation of resources to roots can occur, thus resulting in reduced shoot growth (Lloyd et al. 2006). Öhlund and Näsholm (2001) found greater shoot/root ratios and shorter, stubbier roots in NH_4^+ treated seedlings than in amino acid treatments, but attributed this observation to ammonium assimilation being an acidifying process, which stunts root growth. While we did not monitor root growth in this study, it is likely that limitations in the soil resulted in allocation of resources to root growth.

Another important factor is the possible adsorption of arginine to soil colloids. It has been reported that when applied to soils, large proportions of amino acids become unavailable to plants as a result of adsorption processes and assimilation by soil microbial biomass (Näsholm et al. 2009; Lipson and Näsholm 2001). These processes limit the amount of amino acids available for root interception, which can also explain the reduced growth observed in lower rate arginine (AA50 and AA100) treatments. Because we observed similar height growth responses of plants in high-rate arginine treatments (AA200 and AA300) to plants in the grower standard (AS100) treatments, challenges in root interception of amino acids were clearly overcome when arginine

was present in higher concentrations. Andresen et al. (2009) reported initial competition between microbes and plants for assimilation of organic nitrogen; however, when amino acids are present at high concentrations in soils, plants are the favored competitors in capturing the free amino acids (Jones et al. 2005). The greater amounts of arginine required to achieve similar growth responses to the grower standard further support that a significant proportion of the arginine applied to soils in the field was used by non-targeted biological and chemical processes, thus limiting availability to plants.

Also of concern is the fact that results were statistically significant only for one of three species in the second year of the study (Table 2.1). This can be explained for hybrid poplar by the wide spread of roots across the field, caused by the fact that second year plants were grown from cut shoots, however reasons for lack of response in *P. resinosa* seedlings are unknown. In 2010, treatment had a significant effect on RCD response for all species. However, lack of a clear trend may indicate a confounding factor, such as water, that influenced diameter expansion (Nikiema et al. 2011).

Effects on tree nitrogen physiology

Foliar N increased with increasing amino acid application rate for all three species in 2009 (Table 2.1). However, in 2010, a significant response was observed only in *P. resinosa* seedlings. Previous studies have shown that increasing the amino acid concentration in soils has a positive effect on amino acids taken up by plants (Jones et al. 2005; Reeve et al. 2008; Persson and Näsholm 2002). Although we did not experiment with labeled amino acids to positively characterize assimilation by plants in this study, we can conclude that increasing arginine applications generally improved foliar N concentrations. Öhlund and Näsholm (2001) observed

a similar trend in a container study, however the percentage of N in seedlings fertilized with arginine and NH_4^+ were similar. This also lends support to our hypothesis that in our study, biological and chemical soil dynamics are influencing plant N interception, thus plant growth and foliar N concentrations.

In production soils, organic N sources positively influence microbial activity due to the carbon input associated with organic fertilization. However, increases in microbial activity can either lead to inhibition or enhancement of plant physiological performance, depending on the nature of the resulting plant-microbe interactions (Öhlund and Näsholm 2002; Andresen et al. 2009). While the capacity of mycorrhizal fungi to degrade polymeric N compounds is well established (Smith and Read 2007), it has been observed that plants and microbes will compete intensely for amino acids (Dannenmann et al. 2009), which can limit N availability to plants. Competition for the organic N was likely one of the underlying processes that explain the reduction in nutrients taken up when comparing amino acid treatments with inorganic ammonium sulfate control in the first year of the study.

The reasons for the non-significant foliar N response for *A. fraseri* and hybrid poplar in 2010 are not known (Table 2.1). However, we suspect in the case of hybrid poplar that growing the second year plants from stumps created conditions where the belowground root distribution effectively covered areas encompassing more than the initial plots, allowing root nutrition beyond the treatment area. Foliar N levels in plants treated with the highest amino acid rates (AA200 and AA300) were generally similar to those grown with the inorganic positive control (AS100), suggesting levels of amino acid N two to three times higher than the inorganic fertilizer are necessary to provide similar foliar N concentrations.

The vector analysis using ammonium sulfate as reference provides further evidence of reduced foliar N and biomass production of plants in amino acid fertilized treatments when compared to the inorganic standard (Figure 2.1b, Figure 2.2b, Figure 2.3b). Findings of previous studies conducted under more controlled conditions indicate the ability of plants to assimilate amino acids such as arginine and glycine at rates similar to inorganic sources such as ammonium (Öhlund and Näsholm 2001). However, differences in rates of amino acid uptake by different plants species has been observed and attributed to different affinities of amino acid transport systems (Persson and Näsholm 2001). Additionally, in the present study, high accumulation of applied amino acids within plants might have caused the inhibition of the synthesis of other amino acids (Näsholm et al, 2009). It has also been reported that as the amino acid concentration of the growth substrate increases, the rate of intact amino acids taken up by plants decreases (Sauheitl et al. 2009). This could have affected nutrients taken up by plants in high amino acid treatments. Similar to Näsholm (2009), we suggest that the effective limitation of plant assimilation in amino acid treatment is more controlled by soil processes in the rhizosphere rather than by specific plant physiological characteristics.

Effect on NO_3^-*-N leaching*

Leachate NO_3^--N losses in high amino acid treatment rates were similar to the inorganic control treatment for all species in 2009 (Figure 2.4a, 2.5a, 2.6a) and *P. resinosa* and the hybrid poplar in 2010 (Figure 2.5b and 2.6b). Amino acids in soils can bind to CEC sites, be immobilized in microbial biomass, or leach through soils following rapid mineralization (Kielland 1995; Jones 1999). Binding of amino acids to soil exchange sites and sorption to soil aggregates mediates the rate at which microbes can access and mineralize amino acids (Reeve et

al. 2008; Gonod et al. 2006). Mineralization will also occur over time as a result of microbial cycling which releases previously immobilized organic N. However, ammonium sulfate does not have an associated carbon input to regulate the biogeochemistry of the system, thus imbalances in plant-available N release and plant N requirements can result in large nitrogen losses from the system. This was observed in *A. fraseri* in 2010 as the inorganic control (AS100) treatment lost significantly more NO_3^--N in leachate compared to all other treatments (Figure 2.4b). Overall, amino acid treatments did not significantly contribute to NO_3^--N leaching in this study, likely due to the strong affinity for arginine to bind to cation exchange sites. When amino acids are abundant in soils, they will adsorb to binding sites on soil aggregates and be taken up by microbes until saturation occurs (Jones et al. 2005; Gonod et al. 2006), although mineralization occurs quickly due to the short half lives of amino acids (Jones 1999). Once mineralized, the N becomes vulnerable to leaching. Because we observed insignificant mineral N leaching and no contributions to soil inorganic N pools (data not shown), our results suggest either low mineralization of applied amino acids or rapid assimilation of available mineralized inorganic N. The fact that leached NO_3^--N content increased proportionally to the rate applied, suggests that the saturation principle in high-rate arginine fertilizer treatments was a significant factor.

Conclusions

Trees under amino acid fertilization showed a significant growth and foliar N response. However, when comparing individual organic treatments with an inorganic control, results indicated a requirement of amino acid application rates two to three times greater to achieve a similar response. This trend is attributed to competition with soil microbial populations or soil

adsorption of significant portions of applied amino acids. Further research is underway to determine the fate of amino acids once applied to soil in the field and to quantify the relative importance of microbial communities and adsorption to cation exchange sites.

Acknowledgements

Special thank to SweTree Technologies for providing the Amino Acid Fertilizer used in this study and for technical support. This study was financially supported by the MSU AgBioResearch Station and the Michigan Seedlings Growers Association.

Table 2.1. Height growth (cm), root collar diameter (RCD) growth (mm), and foliar N concentrations (mg/g) of *Abies fraseri*, *Pinus resinosa*, and hybrid poplar as affected by amino acid treatments.

		Height growth (cm)		**RCD growth (mm)**		**Foliar N concentration (mg/g)**	
	Treatment	2009	2010	2009	2010	2009	2010
Abies fraseri	Ctrl	8.7±0.4 b	6.2±0.5 c	1.5±0.1 d	2.6±0.2 bc	9.2±0.3 c	13.8±2 a
	AA50	9.0±0.4 b	6.6±0.6 bc	1.5±0.1 d	2.9±0.2 ab	11.0±0.3 c	14.2±0.9 a
	AA100	9.4±0.4 b	8.5±0.5 a	1.8±0.1 c	2.9±0.2 ab	11.5±0.1 bc	15.6±0.9 a
	AA200	9.7±0.5 bc	7.9±0.7 ab	2.1±0.1 b	3.2±0.3 a	13.6±1.6 b	15.4±1.6 a
	AA300	10.7±0.4 ac	7.3±0.5 ac	1.8±0.1 c	2.3±0.2 c	14.0±0.9 b	15.8±0.7 a
	AS100	11.0±0.4 a	8.9±0.7 a	2.6±0.1 a	3.0±0.2 ab	17.7±1.3 a	16.6±0.9 a
	p-value	**p=0.000**	**p=0.004**	**p=0.000**	**p=0.042**	**p=0.000**	**p=0.662**
Pinus resinosa	Ctrl	5.0±0.3 b	6.5±0.4 a	2.4±0.1 b	3.7±0.2 b	11.7±0.6 c	12.3±0.3 c
	AA50	5.2±0.2 b	5.7±0.4 a	2.5±0.1 b	3.8±0.2 ab	13.6±0.9 b	12.9±0.4 bc
	AA100	5.6±0.2 ab	5.9±0.4 a	2.5±0.1 b	4.0±0.2 ab	13.8±0.5 b	14.1±0.5 ac
	AA200	5.7±0.3 ab	6.3±0.4 a	2.6±0.1 b	3.6±0.2 b	14.3±0.5 ab	14.3±0.7 a
	AA300	6.1±0.3 a	7.3±0.4 a	3.0±0.1 a	4.4±0.2 a	14.9±0.1 ab	13.7±0.6 ab
	AS100	6.2±0.3 a	6.6±0.4 a	2.5±0.1 b	4.3±0.2 a	15.6±0.6 a	13.9±0.2 ab
	p-value	**p=0.009**	**p=0.099**	**p=0.001**	**p=0.026**	**p=0.003**	**p=0.049**
Populus hybrid	Ctrl	127±3.0 c	273±6.4 a	8.8±0.2 e	8.9±0.6 b	14.5±0.9 d	22.9±2.9 a
	AA50	140±3.7 b	283±6.2 a	9.4±0.2 de	10.8±0.7 a	15.9±0.8 cd	22.9±1.5 a
	AA100	147±4.2 b	287±6.2 a	10.1±0.3 dc	9.2±0.6 ab	18.4±1.4 bc	23.0±0.3 a
	AA200	150±4.0 b	268±7.1 a	10.6±0.2 bc	8.6±0.5 b	18.4±0.9 bc	23.5±1.5 a
	AA300	169±4.4 a	268±6.8 a	11.9±0.3 a	9.6±0.6 ab	21.9±0.7 ab	24.2±1.2 a
	AS100	163±5.3 a	285±5.9 a	11.0±0.3 b	10.9±0.7 a	25.4±1.9 a	23.1±1.0 a
	p-value	**p=0.000**	**p=0.093**	**p=0.000**	**p=0.038**	**p=0.000**	**p=0.991**

Values followed by the same letter are not statistically different (α=0.05) according to Fisher's Least-Significant-Difference Test.

Treatments: Ctrl= Control, AA50 = 56kg N/ha, AA100= 112kg N/ha, AA200= 224kg N/ha, AA300= 336kg N/ha, and AS100= Ammonium Sulfate 112kg N/ha.

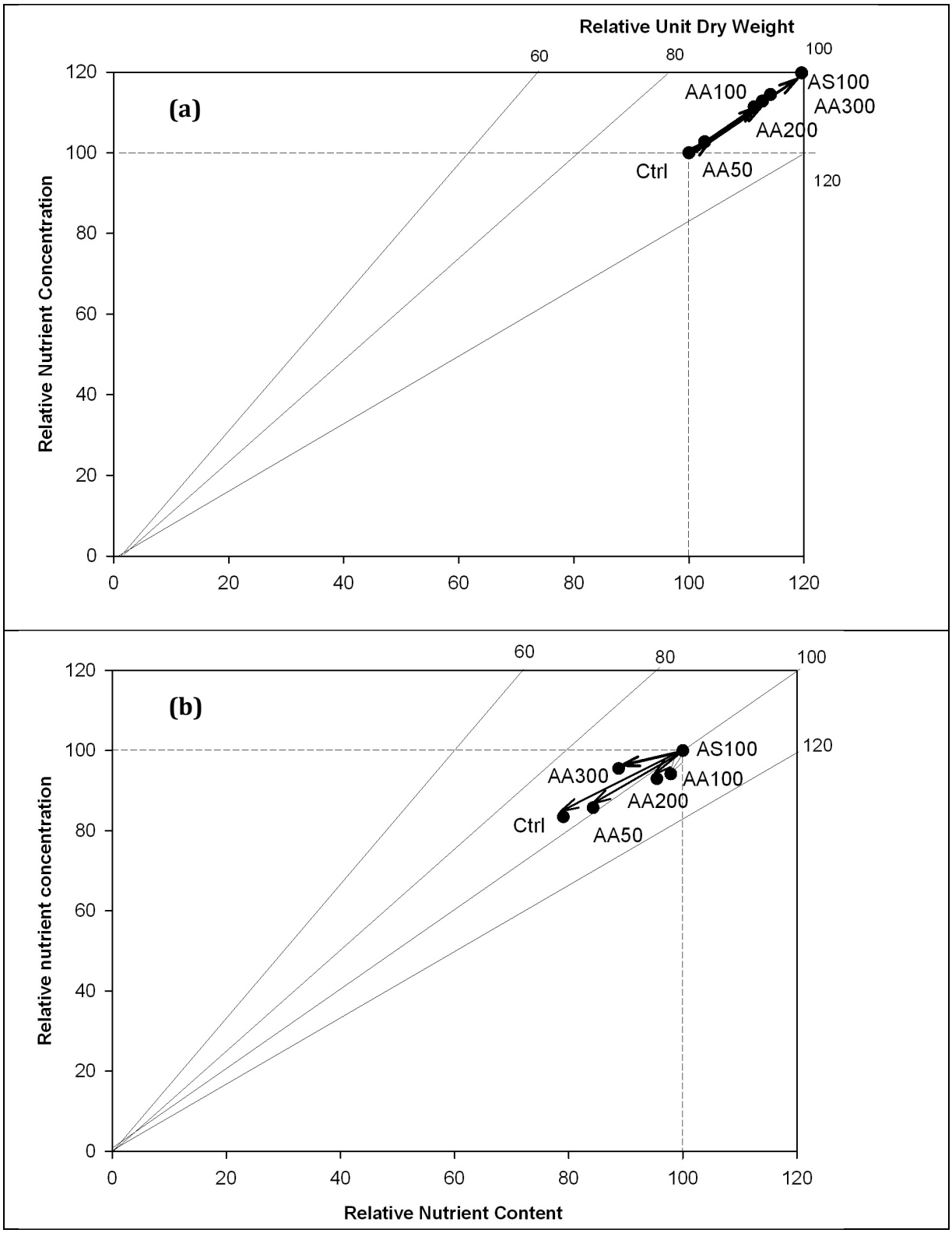

Figure 2.1. Vector analysis of foliar N of *Abies fraseri* in response to amino acid treatments in 2010. References used are untreated control (a) or ammonium sulfate grower standard (b) Treatments: Ctrl= Control, AA50 = 56kg N/ha, AA100= 112kg N/ha, AA200= 224kg N/ha, AA300= 336kg N/ha, and AS100= Ammonium Sulfate 112kg N/ha.

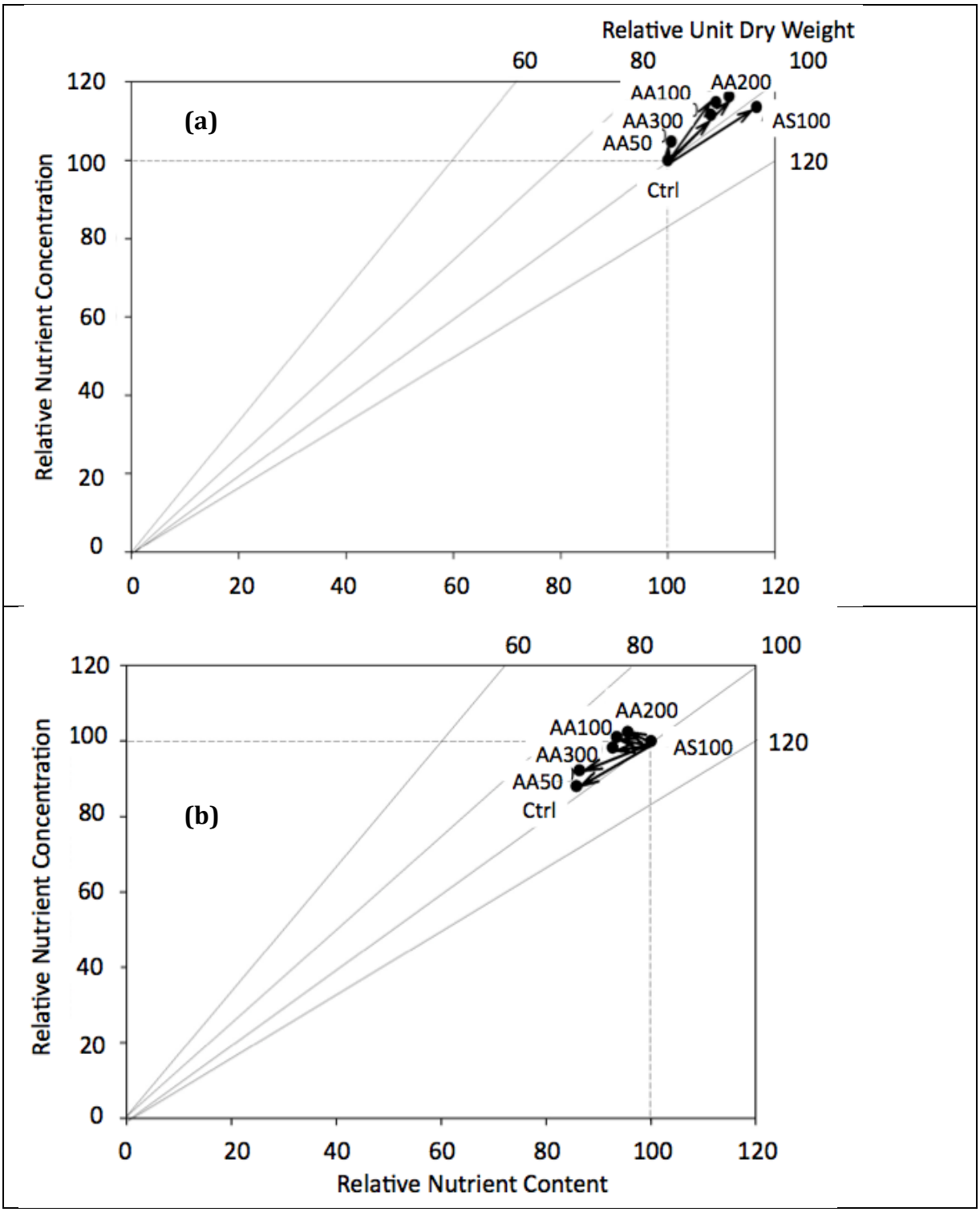

Figure 2.2. Vector analysis of foliar N of *Pinus resinosa* in response to amino acid treatments in 2010. References used are untreated control (a) or ammonium sulfate grower standard (b). Treatments: Ctrl= Control, AA50 = 56kg N/ha, AA100= 112kg N/ha, AA200= 224kg N/ha, AA300= 336kg N/ha, and AS100= Ammonium Sulfate 112kg N/ha.

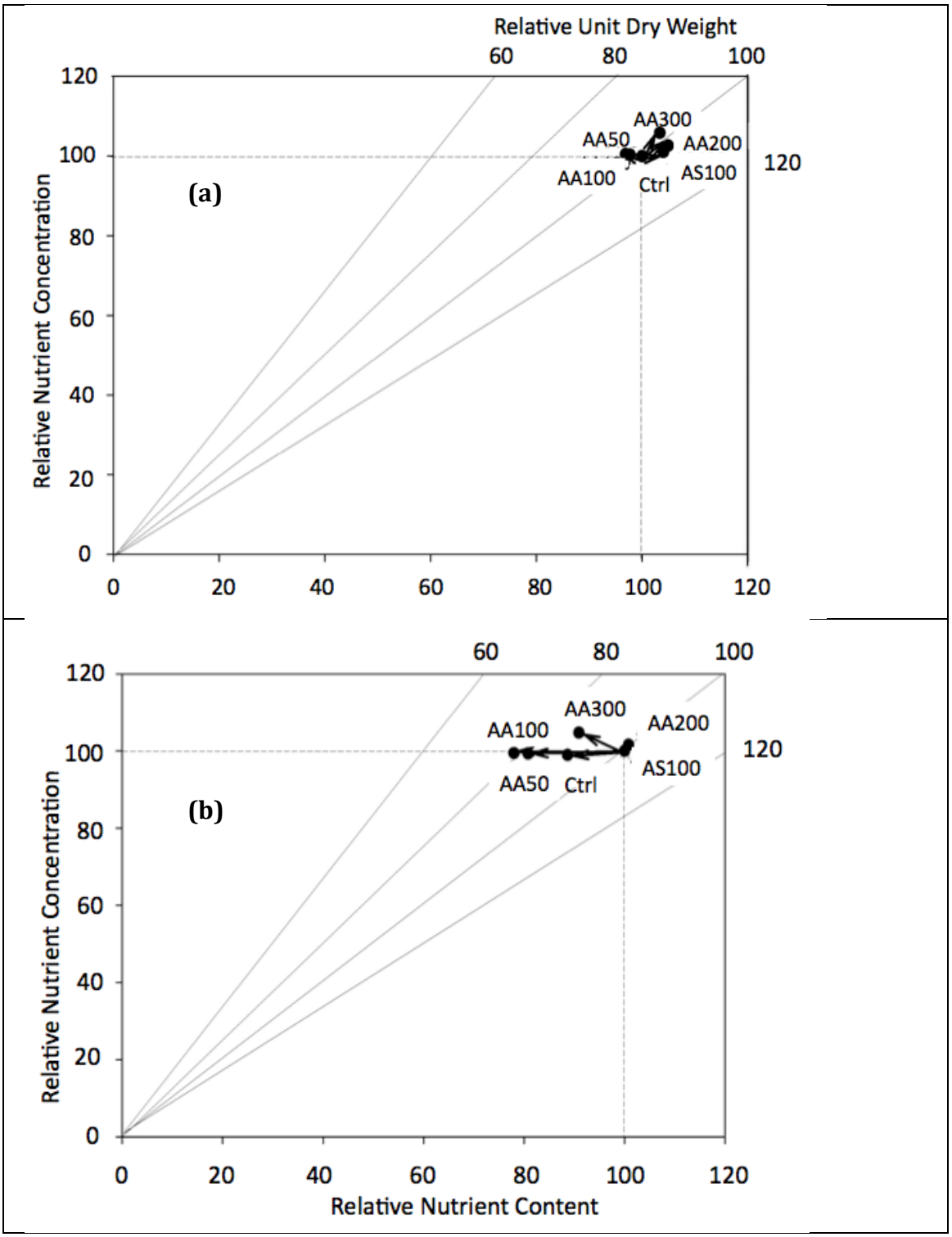

Figure 2.3. Vector analysis of foliar N of hybrid poplar in response to amino acid treatments in 2010. References used are untreated control (a) or ammonium sulfate grower standard (b). Treatments: Ctrl= Control, AA50 = 56kg N/ha, AA100= 112kg N/ha, AA200= 224kg N/ha, AA300= 336kg N/ha, and AS100= Ammonium Sulfate 112kg N/ha

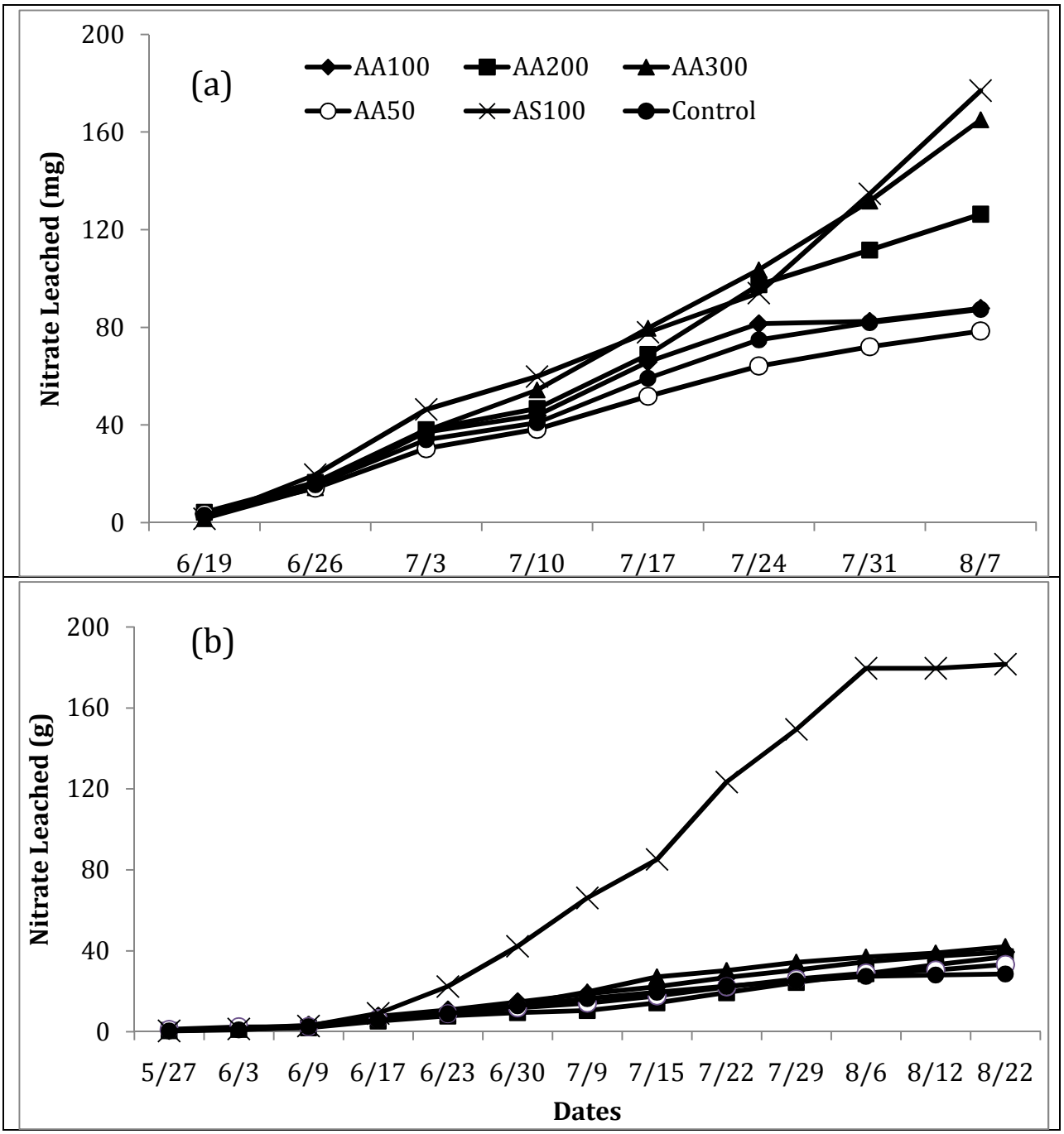

Figure 2.4 Cumulative NO_3^- leached in *Abies fraseri* in 2009 and 2010. *Abies fraseri* cumulative nitrate content was not significantly different in 2009 (**a)** (p=0.084), but was significantly different between treatments in 2010 (**b**) (p=0.002).

Treatments: Ctrl= Control, AA50 = 56kg N/ha, AA100= 112kg N/ha, AA200= 224kg N/ha, AA300= 336kg N/ha, and AS100= Ammonium Sulfate 112kg N/ha.

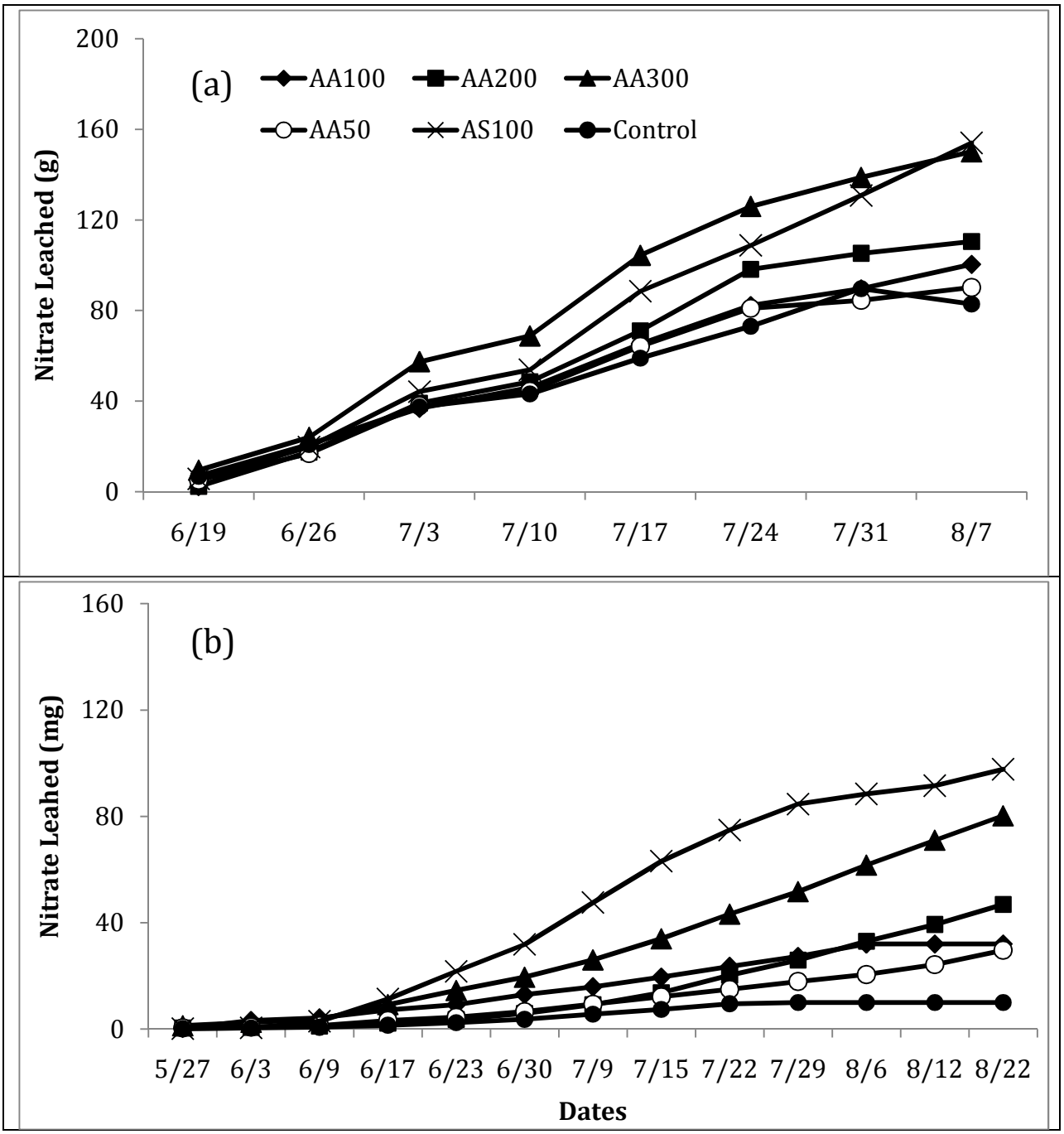

Figure 2.5 Cumulative NO_3^- leached in *Pinus resinosa* in 2009 and 2010. *Pinus resinosa* cumulative nitrate content in leachate was not significantly different across treatments in 2009 (**a**) (p=0.485) or in 2010 (**b**) (p=0.248).

Treatments: Ctrl= Control, AA50 = 56kg N/ha, AA100= 112kg N/ha, AA200= 224kg N/ha, AA300= 336kg N/ha, and AS100= Ammonium Sulfate 112kg N/ha.

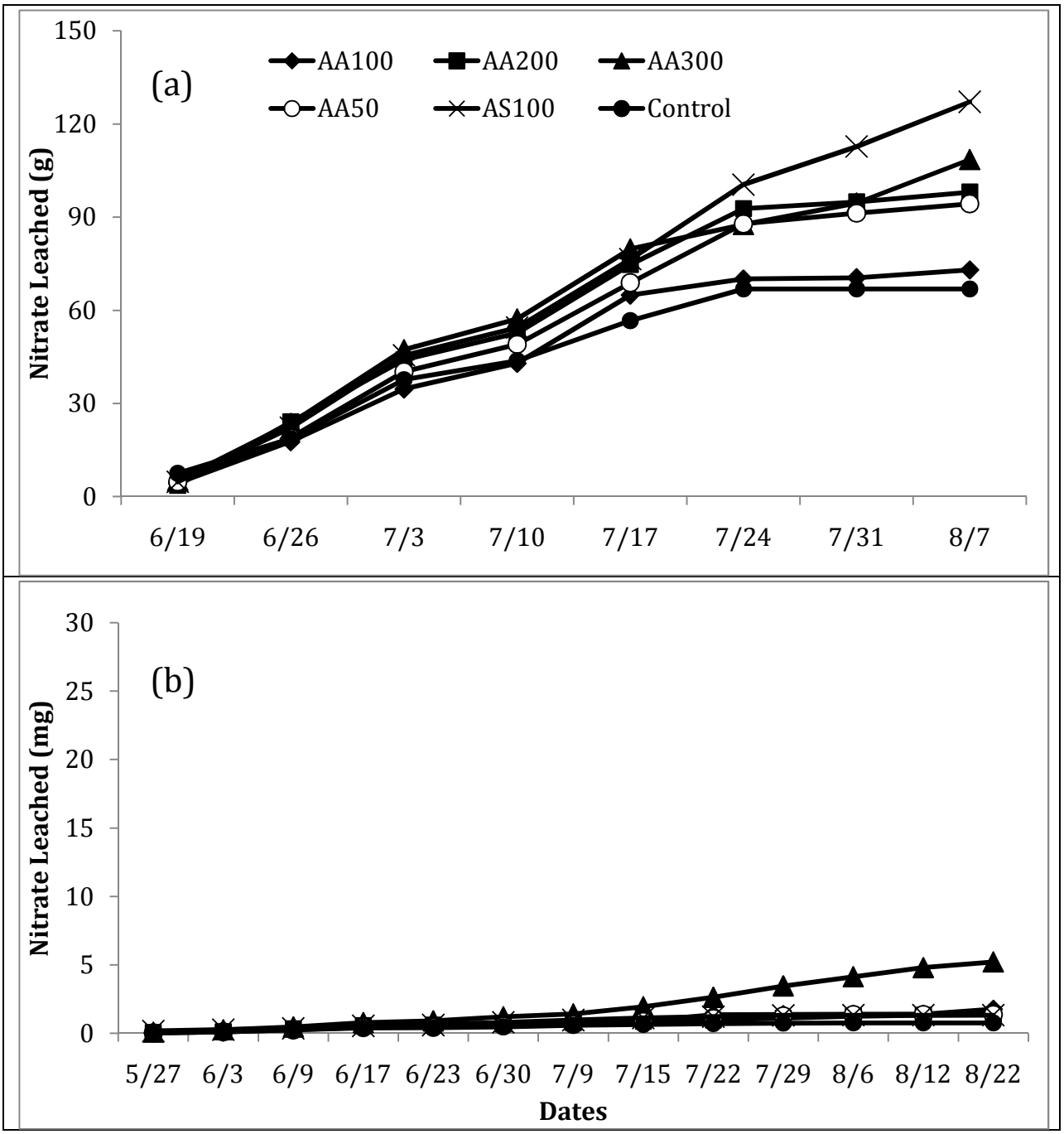

Figure 2.6 Cumulative NO_3^- hybrid poplar in 2009 and 2010. Hybrid poplar cumulative nitrate content in leachate was not significantly different across treatments in 2009 (**a**) (p=0.629) or in 2010 (**b**) (p=0.330). Treatments: Ctrl= Control, AA50 = 56kg N/ha, AA100= 112kg N/ha, AA200= 224kg N/ha, AA300= 336kg N/ha, and AS100= Ammonium Sulfate 112kg N/ha.

REFERENCES

REFERENCES

Andresen LC, Michelsen A, Jonasson S, Beier C, Ambus P (2009) Glycine uptake in heath plants and soil microbes responds to elevated temperature, CO_2 and drought. Acta Oecol 35: 786-796.

Bonner CA, Jensen RA (1997) Recognition of specific patterns of amino acid inhibition of growth in higher plants, uncomplicated by glutamine-reversible 'general amino acid inhibition.' Plant Science 130: 133-143.

Dannenmann M, Simon J, Gasche R, Holst J, Naumann PS, Kogel-Knabner I, Knicker H, Mayer H, Schloter M, Pena R, Polle A, Rennenberg H, Papen H (2009) Tree girdling provides insight on the role of labile carbon in nitrogen partitioning between soil microorganisms and adult European beech. Soil Biol Biochem 41: 1622-1631.

Dickmann DI (2006) Silviculture and biology of short-rotation woody crops in temperate regions: then and now. Biomass and Bioenergy 30: 696-705.

Ge T, Song S, Roberts P, Jones DL, Huang C, Iwasaki K (2009) Amino acids as a nitrogen source for tomato seedlings: The use of dual-labeled (^{13}C, ^{15}N) glycine to test for direct uptake by tomato seedlings. Environ Exp Bot 66: 357-361.

Gonod LV, Jones DL, Chenu C (2006) Sorption regulates the fate of amino acids lysine and leucine in soil aggregates. Eur J Soil Sci 57: 320-329.

Griffin KL, Winner WE, Strain BR (1995) Growth and dry matter portioning in Loblolly and Ponderosa pine seedlings in response to carbon and nitrogen availability. New Phytol 129: 547-556.

Harrison KA, Bol R, Bardgett RD (2008) Do plant species with different growth strategies vary in their ability to compete with soil microbes for chemical forms of nitrogen? Soil Biol & Chem 40: 228-237.

Hawkins BJ, Robbins S (2010) pH affects ammonium, nitrate and proton fluxes in the apical region of conifer and soybean roots. Physiol Plant 138: 238-247.

Jones DL (1999) Amino acid biodegradation and its potential effects on organic nitrogen capture by plants. Soil Biol Biochem 31: 613-622.

Jones DL, Shannon D, Junvee-Fortune T, Farrar JF (2005) Plant capture of free amino acids is maximized under high soil amino acid concentrations. Soil Biol & Biochem 37: 179-181.

Kielland K (1994) Amino acid absorption by arctic plants: implications for plant nutrition and nitrogen cycling. Ecology 75: 2373-2383.

Kielland K (1995) Patterns of free amino acids in arctic tundra soils. Biogeochemistry 31: 85-98.

Kielland K, McFarland JW, Ruess RW, Olson K (2007) Rapid cycling of organic nitrogen in taiga forest ecosystems. Ecosystems 10: 360-368.

Lipson D, Näsholm T (2001) The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. Oecologica 128: 305-316.

Liu X, Ko K, Kim S, Lee K (2008) Effect of amino acid fertilization on nitrate assimilation of leafy radish and soil chemical properties in high nitrate soil. Commun Soil Sci Plant Anal 39: 269-281.

Lloyd JE, Herms DA, Rose MA, Van Wagoner J (2006) Fertilization rate and irrigation scheduling in the nursery influence growth, insect performance, and stress tolerance of 'Sutyzan' Crabapple in the landscape. HortScience 41: 442-445.

Näsholm T, Ekblad A, Nordin A, Giesler R, Hogberg M, Hogberg P (1998) Boreal forest plants take up organic nitrogen. Nature 392: 914-916.

Näsholm T, Huss-Danell K, Hogberg P (2000) Uptake of organic nitrogen in the field by four agriculturally important plant species. Ecology 81: 1155-1161.

Näsholm T, Kielland K, Ganeteg U (2009) Uptake of organic nitrogen by plants. New Phytol 182: 31-48.

Nikiema P, P Nzokou, and D Rothstein (2011) 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, United States of America. New Forests. DOI 10.1007/s11056-011-9274-8.

Öhlund J, Näsholm T (2001) Growth of conifer seedlings on organic and inorganic nitrogen sources. Tree Physiology 21: 1319-1326.

Öhlund J, Näsholm T (2002) Low nitrogen losses with a new source of nitrogen for cultivation of conifer seedlings. Environ Sci Technol 36: 4854-4859.

Persson J, Näsholm T (2001) Amino acid uptake: a widespread ability among boreal forest plants. Ecology Letters 4: 434-438.

Persson J, Näsholm T (2002) Regulation of amino acid uptake in conifers by exogenous and endogenous nitrogen. Planta 215 :639-644.

Raab TK, Lipson DA, Monson RK (1996) Non-mycorrhizal uptake of amino acids by roots of the alpine sedge *Kobresia myosuroides*: implications for the alpine nitrogen cycle. Oecologia 108: 488-494.

Reeve JR, Smith JL, Carpenter-Boggs L, Reganold JP (2008) Soil-based cycling and differential uptake of amino acids by three species of strawberry (*Frageria* spp.) plants. Soil Biol Biochem 40: 2457-2552.

Rothstein DE, Cregg BM (2005) Effects of nitrogen form on nutrient uptake and physiology of Fraser fir (*Abies fraseri*). For Ecol Manage 219: 69-80.

Sauheitl L, Glaser B, Weigelt A (2009) Uptake of intact amino acids by plants depends on soil amino acid concentrations. Environ Exp Bot 66: 145-152.

Schimel JP, Chapin FS (1996) Tundra plant uptake of amino acid and NH_4^+ nitrogen in situ: Plants compete well for amino acid N. Ecology 77: 2142-2147.

Schobert C, Kockenberger W, Komor E (1988) Uptake of amino acids by plants from soil: A comparative study with castor bean seedlings grown under natural and axenic soil conditions. Plant Soil 109: 181-188.

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edition. New York, USA. Academic Press.

Timmer VR (1991) Interpretation of seedling analysis and visual symptoms. In: van den Driessche R., Mineral Nutrition of Conifer Seedlings. CRC Press, Boca Raton, pp. 113-134.

CHAPTER THREE: BIOMASS ALLOCATION AND NUTRIENT USE EFFICIENCY OF FRASER FIR AND RED PINE SEEDLINGS IN RESPONSE TO AMINO ACID FERTILIZATION

Submitted to Tree Physiology.

Abstract

Plants have the ability to assimilate and use amino acids as a primary nitrogen (N) source in forest and agricultural ecosystems. This process has been reported in the arctic, boreal and temperate forests, and in controlled environments where plants are raised in containers. We report on a two-year study aimed at understanding nutrient use physiology and biomass allocation of short rotation trees to amino acid fertilization. Conifer transplants (Fraser fir [*Abies fraseri* (Pursh.) Poir] and red pine [*Pinus resinosa* Aiton]) were installed in a nursery bed and treated with varying rates (0, 56, 112, 222, and 336 kg N ha^{-1}) of an amino acid fertilizer containing arginine as a N source and other plant-essential nutrients. Granular ammonium sulfate applied at 100 kg N ha^{-1} was used as a positive control. Parameters monitored include biomass and nutrient partitioning (N, P, K, Ca, Mg) in addition to nutrient use efficiency (NUE) and other biomass and nutrient allocation ratios. It was hypothesized that allocation of biomass to roots would occur and nutrient use efficiency would increase under amino acid nutrition due to nutrient limitations in the rhizosphere. We did not observe changes in allocations of biomass or nutrients to roots, indicating that nutrient limitations in the rhizosphere were not severe. This suggests the organic N source functioned as a slow-release fertilizer and released nutrients over time. Improved NUE in ammonium sulfate treatments was likely due to pH changes with nutrient uptake, nutrient losses to leaching, and different N forms being used by seedlings. Our results suggest that 1) competition in the rhizosphere is alleviated once seedlings establish in the field and 2) large proportions of applied arginine is being immobilized in microbial biomass or bound to soils.

Introduction

Nutrient use efficiency (NUE) is the plant biomass relative to the nutrient content and depends on a plant's ability to assimilate nutrients from the soil, transport and incorporate them into plant tissues, and also remobilize, translocate and use them once they are present in the plant (Baligar et al. 2001). It is estimated that plants only capture a maximum of 50% nitrogen (N), 10% phosphorus (P), and 40% potassium (K) applied as inorganic fertilizers, with the remainder being lost via runoff, leaching through the rootzone, immobilization or binding to soils, and volatilization (Baligar et al. 2001). The relative proportion of nutrients lost through various mechanisms varies, but leaching can easily be quantified. For example, in a container study of three-year-old Fraser fir (*Abies fraseri*) seedlings fertilized with a slow release N fertilizer, only 1.6-6.8% of the N applied was lost in leachate, with increased N losses at higher fertilization and irrigation levels (Nzokou and Cregg 2010). Nutrient losses significantly reduce NUE (Baligar et al. 2001), contaminate drinking water (Goodrich et al. 1991), and have adverse effects on aquatic ecosystems (Jagus and Rzetala 2011; Antikainen et al. 2008).

NUE is influenced by nutrient source and supply, environmental conditions, physical, biological, and chemical soil characteristics, and the interaction of all these factors with plant physiological and biochemical processes (Baligar et al. 2001). For example, hydroponically-grown *Pinus radiata* seedlings fertilized with different mixtures of ammonium (NH_4^+-N) and nitrate (NO_3^--N) had significantly greater N use efficiency when the amount of NH_4^+-N in the mixture decreased, because of luxury consumption of NH_4^+-N induced by greater relative NH_4^+-N concentrations (Bown et al. 2010). In a study of Red pine (*Pinus resinosa*) seedlings grown in a greenhouse under varying light, N (NO_3^--N and NH_4^+-N sources), and P conditions,

it was suggested that *P. resinosa* alters NUE as a result of changes in light and nutrient availability, with the greatest NUE found under the combination of high light, low N, and high P (Elliot and White 1994). Reduced nutrient availability to conifers has been demonstrated to result in allocations of biomass to the roots (Poorter et al. 2011; Bown et al. 2010; Kaakinen et al. 2004; Proe and Millard 1994) and increases in NUE (Bown et al. 2010; Elliot and White 1994).

The picture is completely different when organic nutrients are used. Organic nutrition has the potential to improve NUE because it is characterized as a slow-release nutrient source where nutrients become plant-available over time as a result of complex biological and chemical interactions in the soil (Blessington et al. 2009; Rosen and Allan 2007)). Amino acids are used as an organic nutrient source in boreal (Näsholm et al. 1998; Persson and Näsholm, 2001), alpine (Raab et al., 1996), arctic tundra (Kielland, 1995), temperate (Gallet-Budynek et al. 2009; Metcalfe et al. 2011), and agricultural (Näsholm et al. 2000, Wilson et al. 2012) ecosystems. They are particularly attractive because of their strong polarity and ability to bind to cation and anion exchange sites and soil aggregates (Rothstein 2010). Amino acids in soil are taken up by soil microbial communities until saturation occurs (Jones 1999), thus the rate at which amino acids are mineralized to inorganic forms is inherently regulated (Reeve et al. 2008; Gonod et al. 2006).

Studies have shown that plants can assimilate amino acids intact (Öhlund and Näsholm 2001; Näsholm et al. 2000; Ge et al. 2009); however, the amino acid use efficiency will likely be limited by the quantity binding to soil colloids and the level of immobilization in microbial biomass (Näsholm et al. 2009). A previous study found that amino acid application rates two to three times greater than the inorganic control were necessary to achieve similar growth responses

and foliar N concentrations in trees, and this result was attributed to nutrient limitations in the soil (Wilson et al. 2012). Additionally, the carbon input associated with organic nutrient sources can increase microbial community activity (Schobert et al. 1988), which could improve the NUE of the tree production system, similar to the increased fertilizer use efficiency observed in systems with microbial inoculants applications (Adesemoye and Kloepper 2009).

A great deal of variability among species in amino acid uptake have been observed and suggested to be a result of differing transport system affinities among species (Persson and Näsholm 2001), which could alter nutrient use efficiency at the genetic level (Baligar et al. 2001).

While previous research suggest a good potential for amino acids in improving NUE of conifers, the underlying physiological processes associated with this principle need to be further tested and elucidated. We are hypothesizing that 1) arginine fertilization would result in increased biomass allocation to roots as compared to the inorganic control and 2) nutrient use efficiency of seedlings in arginine treatments would be increased due to nutrient limitations in the rhizosphere. The objectives of this study were to evaluate the effects of arginine nutrition on 1) biomass and nutrient partitioning and 2) nutrient use efficiency.

Methods

Site description

The study site was located in a nursery bed at the Tree Research Center (TRC) on the campus of Michigan State University in East Lansing, MI, USA (42.65°N and 84.42°W). Average daily maximum and minimum annual temperatures were 15.0 and 4.7 °C, respectively in 2010 with annual precipitation totaling 527.30 mm (Table 3.1). Soils in the nursery are

classified as sandy with a pH of approximately 5.6 and a CEC of 3.7meq/100g soil with exchangeable bases comprised of 7.1% potassium (K), 23.0% magnesium (Mg), and 69.9% calcium (Ca).

Species selection and management

Two conifer species, Fraser fir (*Abies fraseri* [Pursh] Poir) and Red pine (*Pinus resinosa* Aiton), were selected for this study. Seeds were sown and grown in a greenhouse in 2008, and the plug seedlings were transplanted in the nursery bed on May 22, 2009. Sixteen seedlings (4 x 4) were planted in each experimental plot and spaced 30.5 cm from neighboring trees.

Weeds were managed by application of glyphosate (35.84 kg/ha) using a CO_2 powered backpack sprayer at the beginning of the growing season while trees were still dormant and by hand removal throughout the growing season. Seedlings were irrigated with well water in the absence of rainfall.

Nutrient source

An amino acid fertilizer, arGrow complete® that contains the amino acid arginine was selected for this study (SweTree Technologies, Umea, Sweden). arGrow complete® contains 70 g/L nitrogen (N), 12 g/L phosphorus (P), 49 g/L potassium (K), 4 g/L magnesium (Mg), 10 g/L sulfur (S), 0.24 g/L boron (B), 0.03 g/L copper (Cu), 1.2 g/L iron (Fe), 0.6 g/L manganese (Mn), 0.05 g/L molybdenum (Mo), and 0.18 g/L zinc (Zn). arGrow complete® is a liquid fertilizer applied to soils in diluted form (1/200 dilution) twice per week in arginine treatment plots. Arginine was applied over 10 weeks from June 1 to August 7 in 2009 and over 14 weeks from May 24 to August 27 in 2010. Granular ammonium sulfate (21-0-0-14) was hand broadcasted to

positive control treatments at the beginning of each growing season on June 1, 2009 and May 25, 2010, in accordance with conventional practices.

Experimental design and treatments

Both species were treated with six different fertilizer treatments, replicated four times in a randomized complete block design. The six fertilizer treatments included arGrow Complete® applied at 0 (Control), 50 (AA50), 100 (AA100), 200 (AA200), and 300 (AA300) lb N/acre (0, 56, 112, 224, and 336 kg N/ha, respectively) and ammonium sulfate applied at 100 lbs N/ac (AS100) (112 kg N/ha).

Biomass determination and tissue analyses

One seedling from each treatment plot was harvested December 10, 2010 for both species. Care was taken to ensure that entire root systems were harvested. Roots were immediately separated from the shoot at the root collar. Samples were transported to the laboratory in double-lock Ziploc bags in a cooler and stored in a cooler at 4°C until analysis. Prior to freezing, roots were gently washed with deionized water to remove remaining soil.

Harvested biomass was oven-dried at 65° for at least 72 h. Following drying, needles were separated from the stem. Dry weight of roots, stem, and needles was then determined. Dried tissues were individually ground into a fine powder and approximately 0.3 g of dried tissue sample were digested with sulfuric acid (4.5 mL) and hydrogen peroxide (1.5 mL) in a 75 mL digestion tube. Tissue samples were pre-digested over night, then heated on a digestion block (AIM600 Block Digestion System) to 340°C +/- 10°C. After digestion of tissues, samples were diluted to 75 mL on a vortex and mixed thoroughly.

Nitrogen and phosphorus were determined by analysis of an aliquot of digested tissue on a SAN++ segmented flow analyzer (Skalar, Inc., Buford, GA). Potassium, calcium, and magnesium analysis was performed on an aliquot of digested tissue on an atomic absorption spectrometer (Aanalyst 400, Perkin Elmer, Waltham, MA).

Statistical Analysis

Biomass allocation and nutrient use efficiency parameters were calculated according to Sheriff et al. (1995) as follows:

RWR: Root weight ratio = (root biomass (g)/whole plant biomass (g))

LWR: Leaf weight ratio = (leaf biomass (g)/whole plant biomass (g))

NUE: Nutrient use efficiency of the whole plant = (whole plant biomass (g)/ g nutrient in biomass)

N/RW: Index of nitrogen availability = (foliar nitrogen (mg)/root biomass (g))

Additional parameters calculated include:

Shoot:Root: (aboveground biomass (g)/belowground biomass (g))

PFNR: Plant-fertilizer nutrient ratio = (plant nutrient content (g)/ nutrient supplied in two years (g))*100%

All statistical analyses were performed using Systat 13 (Systat Software Inc., Chicago, Illinois). Two-way analysis of variance (ANOVA) for single measures was used to test the effects of different fertilizer treatments on partitioning of biomass and nutrients in addition to

nutrient ratios. Tukey's Honestly-Significant-Difference Test was used in the pair-wise analysis of biomass and nutrient partitioning parameters and nutrient ratios. The significance threshold was assessed at α=0.05.

Results

Biomass allocation

A. fraseri seedlings fertilized with arginine at a rate of 112 kg N ha^{-1} (AA100) had greater root, stem, and needle biomass than all other treatments with one exception (Figure 3.1a). Only *A. fraseri* seedlings fertilized with arginine at a rate of 224 kg N ha^{-1} (AA200) produced similar needle biomass to the AA100 seedlings (Figure 3.1a).

P. resinosa seedlings in treatments fertilized with ammonium sulfate and arginine at a rate of 112 kg N ha^{-1} (AS100 and AA100, respectively) had significantly greater root growth than seedlings in control and AA50 plots (Figure 3.1b). A similar trend was observed for biomass allocated to stems, although seedlings fertilized with arginine at a rate of 112 kg N ha^{-1} (AA100) had similar stem biomass as seedlings in control and AA50 treatment plots (Figure 3.1b). Needle biomass for *P. resinosa* was significantly greater in ammonium sulfate treated seedlings than seedlings fertilized with arginine at a rate of 56 kg N ha^{-1} (AA50), but similar to the control and remaining amino acid fertilized seedlings (Figure 3.1b).

Nutrient partitioning

N content of *A. fraseri* seedlings was only different among treatments in stem and needle biomass (Table 3.2). *A. fraseri* seedlings fertilized with amino acids at a rate of 112 kg N ha^{-1} (AA100) had greater stem N content than all other treatments. The trend was similar with needle N content, but only seedlings in control and AA50 treatments had significantly less needle N content than AA100 seedlings. *A. fraseri* seedlings in AA100 treatments had greater root and needle P content than seedlings in AA50 and control treatments, but had greater stem P contents than seedlings in all other treatments. *A. fraseri* seedlings in AA100 treatment plots has greater root and stem K contents than those in AA50 treatments. K needle content of *A. fraseri* seedlings was greater in seedlings fertilized with amino acids at a rate of 112 kg N ha^{-1} (AA100) than those in AA50 and control treatments. Root Ca content was similar among treatments for *A. fraseri* seedlings, but stem Ca content was greater in seedlings of AA100 treatments than in AA50 treatment seedlings. Needle Ca content was greater in AA100 seedlings than seedlings in all other treatments. Root Mg content was similar among seedlings in all *A. fraseri* treatments. *A. fraseri* seedlings fertilized with arginine at a rate of 112 kg N ha^{-1} (AA100) had greater stem and needle calcium contents than seedlings in the unfertilized control, AA50, and ammonium sulfate (AS100) treatments.

P. resinosa seedlings fertilized with both ammonium sulfate and arginine at a rate of 112 kg N ha^{-1} had greater root N contents than seedlings in control and AA50 treatments (Table 3.3). Stem N content was lower in *P. resinosa* seedlings in control and AA50 treatments than seedlings in all other treatments with the exception of seedlings treated with arginine at a rate of 224 kg N ha^{-1} (AA200). Seedlings fertilized with ammonium sulfate (AS100) had similar

needle N contents to seedlings in all other treatments, except for the unfertilized control. Root P content of *P. resinosa* seedlings treated with arginine at a rate of 112 kg N ha^{-1} (AA100) and 336 kg N ha^{-1} (AA300) was greater than seedlings in the AA50 treatment. *P. resinosa* seedlings in all treatments had similar stem P contents. Ammonium sulfate (AS100) treated seedlings had similar needle P contents to seedlings in all other treatments except seedlings in the AA50 treatment. *P. resinosa* seedlings' root K contents showed a similar trend to that of root P content. Seedlings treated with ammonium sulfate (AS100) had similar stem K to seedlings in all treatments, except those in the AA50 treatment. Needle K content was similar among seedlings in all treatments for *P. resinosa*. Ca and Mg contents were only different among treatments in stem biomass. Ammonium sulfate (AS100) treated *P. resinosa* seedlings had greater stem Ca contents than seedlings in control and AA50 treatments. Seedlings in AA50 treatments had less stem Mg contents than seedlings in AS100 and AA100 treatments.

Root weight ratio, leaf weight ratio, and index of nitrogen availability

Fertilization did not affect RWR of *A. fraseri* seedlings, with an average of 23.5% of biomass allocated to roots (Table 3.4). LWR was also similar among *A. fraseri* seedlings, with an average of 35.3% of biomass allocated to roots. For *A. fraseri* seedlings, N/RW was also similar, ranging from 16.7 for seedlings in AA100 treatments to 23.0 for seedlings in control treatments. The average index of nitrogen availability among treatments was approximately 20.

P. resinosa seedlings had similar RWR, with an average of 14.1% allocated to roots—almost 10% less than that of *A. fraseri* seedlings (Table 3.4). LWR was also similar among treatments of *P. resinosa* seedlings, with an average of 60.9% of biomass allocated to foliage—almost double the biomass allocated to foliage in *A. fraseri* seedlings. N/RW was not

statistically different among treatments of *P. resinosa* seedlings, ranging from 46.1 in AA200 seedlings to 62.9 in AA300 seedlings, with an average of 53.5 for all treatments.

Nutrient use efficiency

Nutrient use efficiency was similar among treatments for *A. fraseri* seedlings for all nutrients except for Mg (Table 3.5). Seedlings in both the ammonium sulfate and arginine treatments applied at a rate of 112 kg N ha^{-1} (AS100 and AA100, respectively) had significantly greater Mg-NUE than all other treatments with the exception of seedlings in the AA300 treatment. Seedlings in the AA50 treatment had a lower Mg-NUE than all other treatments.

P. resinosa seedlings in the AA300 treatment had lower N-NUE than all other treatments (Table 3.5). Seedlings in the AA200 treatment had greater N-NUE than seedlings in unfertilized control treatments. P-NUE was greater in ammonium sulfate (AS100) treated seedlings than seedlings in the AA300 treatment, but similar to seedlings in all other treatments. For *P. resinosa*, K-NUE was similar among seedlings in all treatments. Ca-NUE was differed among seedlings in different treatments ($p=0.044$), however, pair-wise analysis was not sensitive enough to detect differences between treatments. Seedlings in ammonium sulfate treatments (AS100) had greater Mg-NUE than all other treatments. *P. resinosa* seedlings in AA50 treatments had greater Mg-NUE than seedlings in AA100 treatments, but seedlings in both treatments had Mg-NUE similar to seedlings in all other treatments with the exception of ammonium sulfate (AS100).

Plant-fertilizer nutrient ratio

For *A. fraseri*, N-PFNR was greater in ammonium sulfate and arginine seedlings fertilized at a rate of 112 kg n ha^{-1} (AS100 and AA100, respectively) than seedlings in AA200 and AA300 treatments (Figure 3.2a). P-PFNR was greater in seedlings of AA50 and AA100 treatments than in seedlings of AA200 and AA300 treatments (Figure 3.2b). This same trend was observed for *A. fraseri* Mg-PFNR (Figure 3.2d). *A. fraseri* seedlings in AA100 treatments had greater K-PFNR than seedlings in AA200 and AA300 treatments (Figure 3.2c).

P. resinosa seedlings in AA200 and AA300 treatments had lower N-PFNR than all other treatments (Figure 3.2e). P-PFNR was significantly greatest in seedlings of AA50 treatments and similar only to seedlings in AA100 treatments (Figure 3.2f). *P. resinosa* seedlings in the AA300 treatment had significantly less P-PFNR than seedlings in all treatments except AA200. K-PFNR was greater in seedlings in AA50 treatments than seedlings in AA300 treatments (Figure 3.2g). *P. resinosa* seedlings in AA50 and AA100 treatments had greater Mg-PFNR than seedlings in AA300 treatment plots (Figure 3.2h).

Discussion

Biomass and nutrient partitioning

Amino acid fertilization had a significant effect on biomass production for both *A. fraseri* and *P. resinosa* seedlings (Figure 3.1a,b). It was hypothesized that seedlings under arginine nutrition would have increased allocation of biomass to roots because of nutrient limitations due to chemical and biological soil processes. While root biomass differed among treatments for both species (Figure 3.1a,b), the expected trend was not observed. *A. fraseri* seedlings fertilized with arginine at a rate of 112 kg N ha^{-1} (AA100) had greater root biomass than all other

treatments (Figure 3.1a), and for *P. resinosa* seedlings, only AA100 and AS100 treatments had significantly greater root biomass than the unfertilized control and AA50 treatments (Figure 3.1b).

Nutrient limitations in the soil have been identified as the strongest force inducing allocation of biomass to the roots (Poorter et al. 2011) and can be exacerbated under amino acid nutrition by immobilization of nutrients in microbial biomass and binding to soil exchange sites (Näsholm et al. 2009). However, because RWR and shoot:root were similar among treatments for both species (Table 3.4), it can be concluded that allocation of biomass and nutrients did not occur in seedlings of any treatment. Additionally, nutrient contents in seedlings of control and AA50 treatments tended to be lower than seedlings of other arginine treatments and the inorganic control treatments (AS100) (Table 3.2 and 3.3). If nutrient limitations were occurring, we would expect to see allocations of biomass (Poorter et al. 2011) and N and P (Ericsson 1995) to the roots of seedlings in AA50 and unfertilized control treatments, presuming translocation of nutrients did not occur.

Therefore, we conclude that while nutrient limitations likely occurred, they were not severe enough to cause the hypothesized allocation patterns. We suggest that biomass and nutrient partitioning observed in this study is confounded by other physiological factors interacting with non-severe soil nutrient limitations. In carbon dioxide limiting conditions, carbon is maintained in the shoots and plant carbohydrate supply will diminish overtime if root growth is hampered (Ericsson 1995). Carbon limitations at the shoot level may have been the reason that we did not observe greater root biomass in AA50 and unfertilized control treatments, and could have been inhibited by nutrient supply. Loblolly pine (*Pinus taeda* (L)) seedlings grown in containers under N and P limitations had a significantly reduced ability to

photosynthesize when compared to seedlings grown in non-limiting N and P conditions (Thomas et al. 1994).

Greater root biomass in *P. resinosa* seedlings fertilized with ammonium sulfate could be due in part to nutrient limitations induced by greater NO_3^--N leaching (Wilson et al. 2012), no macro- or micronutrients applied in fertilizers, adequate carbon dioxide supply (Ericsson 1995), or nutrient limitations induced by the acidic soil environment associated with NH_4^+-N assimilation (Havlin and Tisdale 2005). A degree of nutrient limitation was also observed in ammonium sulfate treated *P. resinosa* seedlings as indicated by improved N-NUE, P-NUE, and Mg-NUE (Table 3.5). Greater root biomass observed in *P. resinosa* seedlings could also be due to the N species used by the plant (Bown et al. 2010). For example, NH_4^+-N nutrition can inhibit fine root growth (Rothstein and Cregg 2005), thus using NH_4^+-N once it is mineralized to NO_3^--N could have altered root biomass production.

As for arginine treatments, because amino acids have an associated carbon input, it can enhance microbial activity (Schobert et al. 1988), which can create a competitive environment in the rhizosphere (Dannenmann et al. 2009). However, plants are the favored competitors when amino acids are present in high concentrations (Jones et al. 2005), which could explain the reduced root biomass of seedlings in higher rate (AA200 and AA300) arginine treatments relative to AA100 treatments for both species (Fig 3.1a,b). The increased biomass (for all tissues) observed in seedlings of AA100 treatments for both species could be a result of more efficient amino acid uptake. It has been demonstrated that when concentrations of amino acids in soil increase, assimilation of intact amino acids decreases (Sauheitl et al. 2009). While we did not experiment with labeling, we hypothesize that arginine applications at a rate of 112 kg N ha^{-1}

could represent this threshold for intact assimilation. This is further supported by the significantly improved PFNR of seedlings in AA100 treatments as compared to high rate amino acid treatments (AA200 and AA300) (Figure 3.2) and superior nutrient content observed in root, stem, and needle biomass (Table 3.2 and 3.3).

The cause of the greater biomass production for all tissues of *A. fraseri* seedlings in AA100 treatments, but not for *P. resinosa* is not known, but could be a result of interactions between species (Persson and Näsholm 2001) and available amino acid concentration (Sauheitl et al. 2009). Because values of N/RW and RWR were similar among treatments for *A. fraseri* and foliar N of AA100 seedlings was similar to seedlings in high rate amino acid (AA200 and AA300) and AS100 treatments, we cannot conclude that root biomass or improved index of nitrogen availability led to improved N uptake. Low RWR can be compensated by N/RW, indicating a root unit can increase the N supply to foliage (Sheriff et al. 1995), but this did not occur in this study.

The greater root, stem, and needles biomass production and nutrient content of higher rate amino acid treatments (AA200 and AA300) for *P. resinosa* shows similar trends to height and foliar chemistry results in our previous studies (Wilson et al. 2012). The LWR ratio was similar among treatments for both species (Table 3.4), however significant differences in stem and needle biomass production and nutrient content of these tissues were observed (Figure 3.1, Table 3.3). Treatments with significantly greater root biomass tended to have significantly greater stem and needle biomass production (Figure 3.1) and nutrient contents (Table 3.2 and 3.3). It is unclear if this trend is a top-down or bottom-up mechanism, or interactions between carbon assimilation and soil nutrient supply (Ericsson 1995). Fertilizing conifers impacts foliar biomass production greater than carbon assimilation (Linder and Rook, 1984), thus greater root

biomass may have led to improved aboveground biomass production. However, knowledge of photochemical response to amino acid nutrition would help to understand the forces driving biomass partitioning in *A. fraseri* and *P. resinosa* seedlings and the carbon-nitrogen interactions occurring.

Nutrient use efficiency

We hypothesized that NUE would be improved in amino acid treatments due to nutrient limitations resulting from adsorption to soil and immobilization in microbial biomass, however this was not the case. For *A. fraseri*, NUE was similar for all nutrients except for Mg, where ammonium sulfate and AA100 treatments had significantly greater Mg-NUE (Table 3.5). For *P. resinosa*, AS100 treatments had significantly greater N-NUE and P-NUE than AA300, though similar to other treatments (Table 3.5). *P. resinosa* seedlings in AS100 treatments had significantly greater Mg-NUE than seedlings in all arginine treatments.

NUE of conifers is improved under nutrient limiting conditions (Elliot and White 1994; Bown et al. 2010). There are many factors that could have limited nutrient supply, thus improving NUE of ammonium sulfate-treated seedlings (AS100). N form has been found to have a significant effect on the N-NUE of *P. radiata* seedlings (Bown et al. 2010), thus whether *P. resinosa* seedlings in AS100 treatments utilized NH_4^+-N directly or once it was mineralized to NO_3^--N would alter NUE. In 2010, *A. fraseri* AS100 treatments had significantly greater Mg content in leachate (Nzokou et al. 2012), which may have limited Mg available to the plant, thus increasing nutrient use efficiency. Cation availability could have been limited under ammonium sulfate nutrition because assimilation of NH_4^+-N is an acidifying process or because the fertilizer

contains sulfate, which decreases soil pH rendering cations and P less available (Havlin and Tisdale 2005). Additionally, cation leaching in response to anion (including NO_3^--N) leaching occurs to balance charges in the soil, which could reduce cation availability (Havlin and Tisdale 2005). Congruencies have found between NO_3^--N leaching and cation leaching in similar studies of amino acid nutrition (Wilson et al. 2012; Nzokou et al. 2012). While care was taken to ensure ammonium sulfate application did not coincide with rainfall events, the monthly precipitation in May and June of 2010 was higher than all other months (Table 3.1), which could have induced N limitations if N was lost in leachate or via runoff.

The arginine fertilizer used is an organic nutrient source, thus considered to be slow-release fertilizer as nutrients are released over time due to complex biological and chemical soil processes (Blessington 2009; Rosen and Allan 2007). It is likely that the reason we did not observe improved NUE in arginine fertilized treatments is due to the fact the nutrients weren't severely limited, but replenished over time by organic matter and microbial turnover (Lipson and Näsholm 2001) and deadsorption from exchange sites to maintain equilibrium with the soil solution (Havlin and Tisdale 2005). This could explain why we see improved or similar nutrient contents in biomass of arginine fertilized *A. fraseri* and *P. resinosa* seedlings relative to the inorganic control (AS100) (Table 3.2 and 3.3), but reduced NUE (Table 3.5).

In a previous study, it was found that amino acid application rates two to three times greater than the inorganic control were necessary to achieve similar growth responses and foliar N concentrations in conifer seedlings, especially when seedlings were establishing in the field; this trend was suggested to be due to soil adsorption and immobilization of nutrients in microbial biomass (Wilson et al. 2012). Our results are in congruence with growth and foliar chemistry responses in the second year of this previous study, where arginine treatments tended to be

similar to or greater than that of the ammonium sulfate control (Wilson et al. 2012). Our results lend further evidence that competition for nutrients is alleviated overtime, but the mechanisms driving this trend, including changes in microbial activity and cation exchange sites, in amino acid systems need to be further understood.

Plant-fertilizer nutrient ratio

For both species, lower rate arginine treatments (AA50 and AA100) tended to have significantly greater PFNR than higher rate arginine treatments (AA200 and AA300) for all nutrients, and the N-PFNR for the inorganic control treatments was similar to the lower rate arginine treatments (Figure 3.2a-h). Despite lower NUE values in amino acid treatments, the improved PFNR in AA50 and AA100 treatments indicates a greater nutrient recovery. In a container study, Scots pine (*Pinus sylvestris* (L)) seedlings had improved N recovery in the growth substrate when fertilized with amino acids (Öhlund and Näsholm 2002). This is similar to trends observed in low-rate amino acid treatments (AA50 and AA100), but not in high rate amino acid treatments (AA200 and AA300), which again, could be related to improved assimilation of intact amino acids under low amino acid concentrations (Sauheitl et al. 2009). Therefore it is hypothesized that soil nutrient dynamics and conifer nutrient physiology depend upon amino acid concentrations applied to soils.

Amino acids in soils bind to exchange sites (Rothstein 2010) and are assimilated by microbes (Jones 1999), but they also can be mineralized then assimilated by plants or microbes, re-adsorbed to soils, or leached below the rootzone (Kielland et al. 2007). In our previous studies of this system, we found greater and in many cases significant NO_3^--N and cation leaching in ammonium sulfate treatments compared to arginine treatments (Wilson et al. 2012;

Nzokou et al. 2012). Because N-PFNR in ammonium sulfate was similar to that of N-PFNR for AA50 and AA100 treatments of both species, and NO_3^--N leaching tended to be greater than in arginine treatments (especially for *A. fraseri*), this indicates that excess nutrients applied in the high rate amino acid treatments (AA200 and AA300) were primarily taken up by the plant, immobilized in microbial biomass, or bound to soil aggregates. The reduced PFNR in higher rate amino acid treatments (AA200 and AA300) (Fig 3.2) in conjunction with nutrient losses being similar to the unfertilized control (Wilson et al. 2012, Nzokou et al. 2012) provides further evidence of the interception of nutrients by non-target biological organisms or adherence to soils (Näsholm et al. 2009). However the results of this study suggest that these biological and chemical soil processes are not limiting nutrient availability to plants.

Conclusions

We hypothesized limitations in nutrient availability under amino acid nutrition would result in allocation of biomass to roots and improved NUE in conifer seedlings. Our results indicate that while nutrients are likely intercepted by non-targeted biological organisms and adhered to soil surfaces, these processes are not limiting to nutrients to the point of severity. This was evidenced by similar RWR, LWR, N/RW, and shoot:root among species, which revealed no biomass allocation patterns. It is suggested that non-severe nutrient limitations interacting with carbon-nitrogen relationships in conifers, nutrient source, and species affected the biomass and nutrient partitioning patterns observed. Nutrient use efficiency was not improved under arginine nutrition, but enhanced for N, P, and Mg for ammonium sulfate treatments for one or both species. We suggested that soil pH changes, nutrient leaching, and N species used in ammonium sulfate treatments were altering N, P, and cation availability and tree

uptake, which improved NUE. It was suggested that arginine fertilized treatments did not show improved NUE because of their organic nature. The slow release of nutrients over time by microbial turnover and equilibrium changes in the soil solution provided a continual nutrient supply. Plant-fertilizer nutrient use was greater in low rate arginine (AA50 and AA100) and ammonium sulfate treatments than in high rate arginine treatments (AA200 and AA300). Relating this result to previous research, gives support to 1) competition for nutrients is alleviated once seedlings have established in the field, and 2) amino acids applied to soils, especially in high rate amino acid treatments are not just assimilated by plants or lost in leachate, but it is likely that significant quantities are present in microbial biomass and bound soil exchange sites. Further research on photochemical processes, soil nutrient dynamics, and plant-microbial interactions are underway to confirm these hypotheses.

Acknowledgements

We would like to give a special thanks to Coretta Kamdem and Ismail Koç for all their hard work in helping with biomass and nutrient analysis. We would also like to thank SweTree Technologies for providing the amino acid fertilizer used in this study.

Tables and Figures

Table 3.1. Climate Data for 2010 growing season

	Max. Air Temperature (°C)	Min. Air Temperature (°C)	Max. Soil (2”) Temperature (°C)	Min. Soil (2”) Temperature (°C)	Monthly Precipitation (mm)
Jan.	-1.4	-8.3	-0.2	-0.5	6.1
Feb.	0	-7.1	-0.4	-0.8	14.48
Mar.	10.9	-2.1	5.1	2.1	13.21
Apr.	18.1	4.4	13	8.5	51.31
May	21.6	10.3	18.3	13.5	103.89
Jun.	25.4	15.4	24.2	19.2	99.57
Jul.	28.9	17.5	28.1	20.9	44.2
Aug.	28	17	24.8	19.1	14.48
Sept.	21.4	11.3	19.8	15.4	88.64
Oct.	17.4	5.5	14.2	10.2	35.82
Nov.	10.2	-0.9	7.5	4.5	41.91
Dec.	-1	-7.3	0.6	0.2	13.71

Table 3.2. Nutrient partitioning in *Abies fraseri* seedlings.

	Abies fraseri			
	Treatment	Root	Stem	Needle
N content	Control	8.85±3.22 a	34.2±8.80 b	56.9±17.2 b
	AA50	6.83±2.24 a	29.1±6.00 b	46.9±9.90 b
	AA100	19.5±4.51 a	110.5±15.2 a	131.1±2.95 a
	AA200	13.8±3.53 a	48.4±10.0 b	77.1±22.9 ab
	AA300	13.4±2.45 a	57.7±13.1 b	77.7±15.9 ab
	AS100	11.7±0.727 a	48.9±9.73 b	78.7±11.9 ab
	p-value	**P=0.133**	**P=0.002**	**P=0.026**
P content	Control	1.67±0.629 b	6.61±1.32 b	8.35±1.11 b
	AA50	1.56±0.618 b	6.58±1.25 b	6.96±1.30 b
	AA100	5.56±0.524 a	21.9±1.71 a	18.4±0.835 a
	AA200	3.00±1.12 ab	9.82±2.28 b	10.9±3.02 ab
	AA300	2.89±0.752 ab	12.1±3.14 b	10.9±1.60 ab
	AS100	2.44±0.482 ab	10.9±2.25 b	11.4±1.44 ab
	p-value	**P=0.021**	**P=0.002**	**P=0.008**
K content	Control	5.76±1.67 ab	23.7±1.50 ab	33.9±0.433 b
	AA50	5.16±2.16 b	22.2±7.41 b	28.3±8.32 b
	AA100	15.6±2.74 a	55.6±2.95 a	80.9±14.0 a
	AA200	10.7±2.43 ab	38.1±6.37 ab	42.2±7.65 ab
	AA300	9.13±2.17 ab	40.3±12.1 ab	42.6±8.29 ab
	AS100	8.37±1.60 ab	36.4±6.33 ab	41.9±5.0 ab
	p-value	**P=0.051**	**P=0.052**	**P=0.011**
Ca content	Control	8.80±2.16 a	16.8±5.22 ab	16.7±3.66 b
	AA50	8.13±2.78 a	10.2±1.11 b	15.8±2.64 b
	AA100	20.3±3.67 a	30.2±3.21 a	45.5±1.59 a
	AA200	15.9±2.03 a	20.7±0.341 ab	10.4±6.02 b
	AA300	13.3±3.86 a	17.8±3.23 ab	20.3±4.10 b
	AS100	13.0±4.34 a	17.2±3.60 ab	15.1±6.88 b
	p-value	**P=0.160**	**P=0.020**	**P=0.017**
Mg content	Control	2.06±0.828 a	7.18±0.789 b	8.07±0.454 b
	AA50	2.14±0.732 a	6.30±1.11 b	7.87±1.65 b
	AA100	5.41±1.19 a	16.9±1.32 a	15.4±1.41 a
	AA200	3.61±0.288 a	9.93±1.03 ab	9.93±1.67 ab
	AA300	3.49±1.08 a	10.4±2.34 ab	9.41±1.26 ab
	AS100	2.79±0.401 a	9.09±1.87 b	7.92±0.729 b
	p-value	**P=0.111**	**P=0.005**	**P=0.010**

Units=mg

Values followed by the same letter are statistically similar according to Tukey's Honestly-Significant-Difference Test (α=0.05).

Treatments: Control= 0 kg N ha^{-1}, AA50= 56 kg N ha^{-1}, AA100=112 kg N ha^{-1}, AA200= 224 kg N ha^{-1}, AA300=336 kg N ha^{-1}, and AS100= Ammonium Sulfate 112 kg N ha^{-1}.

Table 3.3. Nutrient partitioning in *Pinus resinosa* seedlings.

		Pinus resinosa		
	Treatment	Root	Stem	Needle
N content	Control	24.7±3.68 b	64.8±2.49 b	310.8±44.9 b
	AA50	24.3±4.13 b	57.2±11.3 b	276.6±56.9 ab
	AA100	53.2±8.44 a	113.8±13.0 a	498.9±70.5 ab
	AA200	42.0±5.33 ab	92.4±6.73 ab	385.7±78.8 ab
	AA300	46.9±2.21 ab	111.6±11.6 a	538.6±53.8 ab
	AS100	53.5±6.29 a	119.1±6.79 a	653.5±6.25 a
	p-value	**P=0.005**	**P=0.002**	**P=0.004**
P content	Control	5.75±1.27 ab	14.0±1.05 a	38.7±5.57 ab
	AA50	4.12±0.518 b	11.7±3.20 a	32.6±8.34 b
	AA100	9.11±1.21 a	22.5±2.86 a	62.1±10.5 ab
	AA200	7.08±0.423 ab	17.1±0.746 a	45.4±9.77 ab
	AA300	8.48±0.995 a	20.2±2.70 a	64.4±6.77 ab
	AS100	7.98±0.637 ab	20.9±2.60 a	74.4±2.32 a
	p-value	**P=0.019**	**P=0.048**	**P=0.015**
K content	Control	12.1±2.83 ab	45.8±4.81 ab	109.9±18.9 a
	AA50	9.86±0.545 b	36.1±7.73 b	95.2±24.3 a
	AA100	20.9±2.73 a	67.1±9.36 ab	175.6±38.6 a
	AA200	17.4±2.09 ab	52.1±2.16 ab	143.4±39.0 a
	AA300	20.5±2.80 a	65.2±7.74 ab	195.0±21.6 a
	AS100	19.6±0.441 ab	67.5±4.82 a	213.7±22.8 a
	p-value	**P=0.014**	**P=0.023**	**P=0.072**
Ca content	Control	5.40±0.893 a	17.1±1.42 b	73.2±18.3 a
	AA50	6.60±1.11 a	18.0±2.69 b	67.6±21.6 a
	AA100	10.2±2.74 a	32.2±1.98 ab	95.5±16.8 a
	AA200	7.23±0.669 a	27.5±5.63 ab	104.5±34.3 a
	AA300	8.79±1.07 a	30.8±5.76 ab	107.5±6.80 a
	AS100	9.44±0.510 a	37.3±3.42 a	121.0±2.98 a
	p-value	**P=0.190**	**P=0.017**	**P=0.408**
Mg content	Control	3.09±0.522 a	11.8±0.832 ab	34.2±6.04 a
	AA50	3.23±0.721 a	9.56±1.59 b	26.5±8.31 a
	AA100	5.79±0.994 a	18.3±0.850 a	53.9±9.38 a
	AA200	4.49±0.321 a	14.7±0.458 ab	44.7±8.41 a
	AA300	5.10±0.582 a	16.3±2.12 ab	47.9±3.50 a
	AS100	4.97±0.0788 a	18.2±2.0 a	54.7±3.36 a
	p-value	**P=0.049**	**P=0.006**	**P=0.079**

Units=mg

Values followed by the same letter are statistically similar according to Tukey's Honestly-Significant-Difference Test (α=0.05).

Treatments: Control= 0 kg N ha^{-1}, AA50= 56 kg N ha^{-1}, AA100=112 kg N ha^{-1}, AA200= 224 kg N ha^{-1}, AA300=336 kg N ha^{-1}, and AS100= Ammonium Sulfate 112 kg N ha^{-1}.

Table 3.4. Root weight ratio, leaf weight ratio, index of nitrogen availability, and shoot:root for *Abies fraseri* and *Pinus resinosa* seedlings.

	Treatment	RWR	LWR	N/RW	Shoot:Root
Abies fraseri	Control	0.233±0.11 a	0.363±0.009 a	23.0±0.263 a	3.31±0.192 a
	AA50	0.209±0.015 a	0.375±0.049 a	20.2±1.96 a	3.85±0.372 a
	AA100	0.254±0.008 a	0.329±0.011 a	16.7±1.00 a	2.94±0.129 a
	AA200	0.246±0.004 a	0.357±0.031 a	19.2±1.62 a	3.07±0.0635 a
	AA300	0.235±0.011 a	0.338±0.032 a	21.7±1.16 a	3.27±0.214 a
	AS100	0.230±0.013 a	0.354±0.026 a	19.2±1.64 a	3.37±0.243 a
	p-value	P=0.149	P=0.892	P=0.173	P=0.160
Pinus resinosa	Control	0.128±0.010 a	0.625±0.025 a	55.2±8.38 a	6.90±0.615 a
	AA50	0.148±0.030 a	0.606±0.026 a	53.7±13.6 a	6.48±1.79 a
	AA100	0.153±0.013 a	0.584±0.017 a	46.8±5.1 a	5.62±0.512 a
	AA200	0.149±0.009 a	0.598±0.043 a	46.1±6.13 a	5.74±0.416 a
	AA300	0.129±0.014 a	0.622±0.030 a	62.9±12.7 a	6.97±0.921 a
	AS100	0.136±0.011 a	0.616±0.004 a	56.5±0.911 a	6.45±0.572 a
	p-value	P=0.797	P=0.880	P=0.769	P=0.858

Root weight ratio (RWR)= (total seedling biomass/root biomass); leaf weight ratio (LWR)= (total seedling biomass/leaf biomass); Index of nitrogen availability (N/RW) = (foliar N/root biomass).

Values followed by the same letter are statistically similar according to Tukey's Honestly-Significant-Difference Test (α=0.05).

Treatments: Control= 0 kg N ha^{-1}, AA50= 56 kg N ha^{-1}, AA100=112 kg N ha^{-1}, AA200= 224 kg N ha^{-1}, AA300=336 kg N ha^{-1}, and AS100= Ammonium Sulfate 112 kg N ha^{-1}.

Table 3.5. Nutrient use efficiency of *Abies fraseri* and *Pinus resinosa* seedlings

	Treatment	N-NUE	P-NUE	K-NUE	Ca-NUE	Mg-NUE
Abies fraseri	Control	104.2±5.7 a	676.8±33.7 a	197.6±2.2 a	259.5±27.2 a	777.1±8.1 bc
	AA50	136.1±9.3 a	676.8±63 a	193.4±14.3 a	324.5±17.2 a	655.6±6.1 d
	AA100	119.5±5.0 a	680.5±33 a	209±16.5 a	327.7±8.6 a	864.8±1.3 a
	AA200	121.1±10.5 a	700.9±52.9 a	203.3±7.8 a	349.0±22.2 a	775.1±17.5 bc
	AA300	121.5±12.2 a	703.3±70.6 a	202±16.1 a	356.4±24.7 a	795.4±16.6 ac
	AS100	129.1±6.3 a	730.9±46.7 a	207.3±8.8 a	328.6±3.7 a	871.3±15.9 a
	p-value	P=0.329	P=0.969	P=0.969	P=0.127	P=0.000
Pinus resinosa	Control	103.7±2.73 c	810.4±28.9 ab	272±15.2 a	568±40.9 a	984.7±16.8 bc
	AA50	107.4±1.74 ac	838.5±26.6 ab	277±9.59 a	369.6±15.3 a	1050.2±45.9 b
	AA100	106.3±0.59 ac	758.7±13.7 ab	274.2±16.4 a	529.2±39.8 a	907.7±31.3 c
	AA200	113±1.41 a	806.4±26 ab	267.3±8.27 a	420.1±32.1 a	932.5±11.6 bc
	AA300	93.1±2.36 b	714.4±20.6 b	249.1±3.14 a	478.9±46.8 a	1038.9±36.1 bc
	AS100	112.7±0.19 ac	852.4±14.4 a	287.4±6.58 a	511.6±20.4 a	1104.5±7.3 a
	p-value	P=0.000	P=0.026	P=0.304	P=0.044	P=0.004

Nutrient use efficiency (NUE)= (seedling biomass/g of nutrient)

Values followed by the same letter are statistically similar according to Tukey's Honestly-Significant-Difference Test (α=0.05).

Treatments: Control= 0 kg N ha^{-1}, AA50= 56 kg N ha^{-1}, AA100=112 kg N ha^{-1}, AA200= 224 kg N ha^{-1}, AA300=336 kg N ha^{-1}, and AS100= Ammonium Sulfate 112 kg N ha^{-1}.

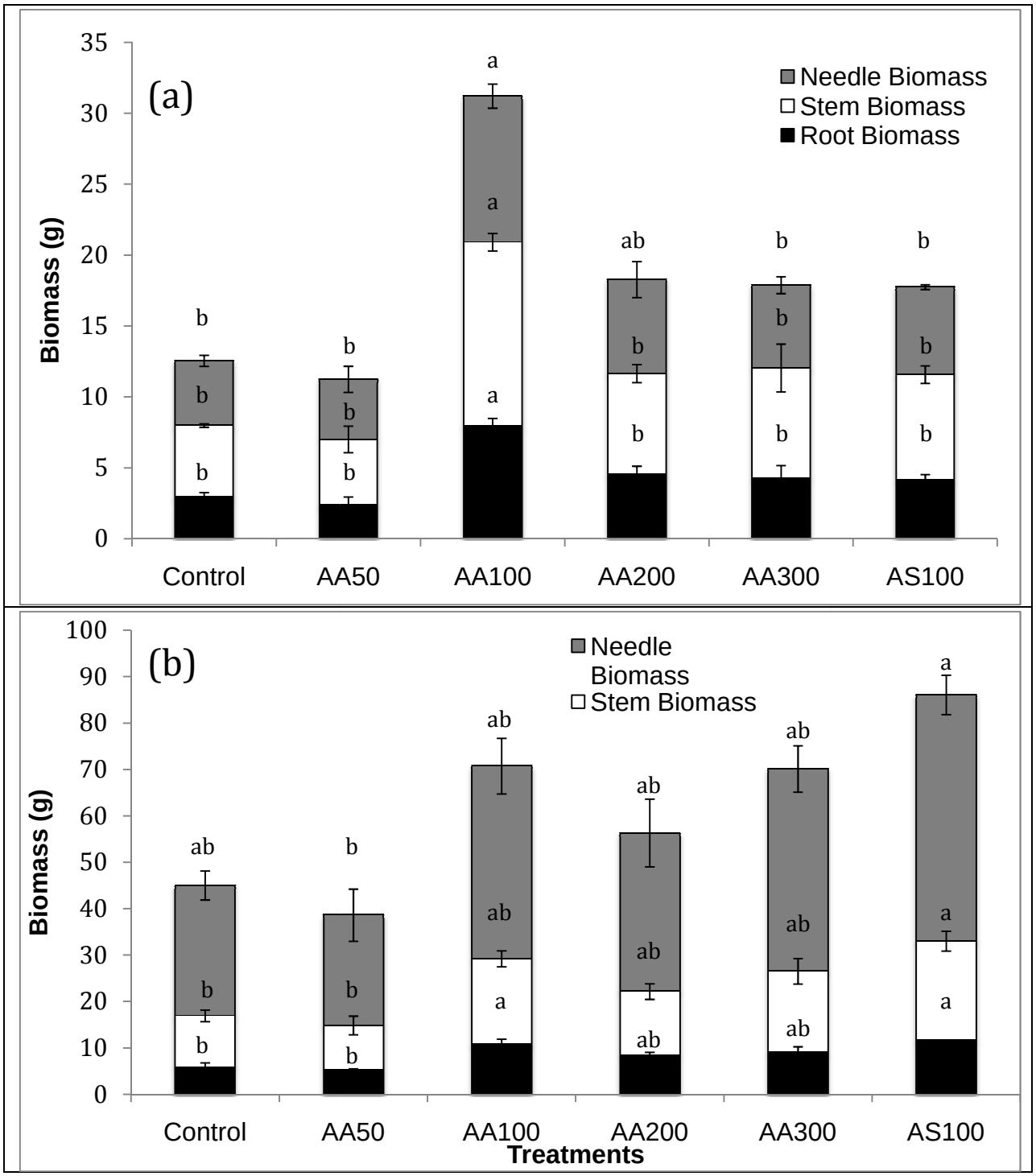

Figure 3.1. Biomass partitioning in conifers in 2010, (a) *Abies fraseri* biomass in roots (p=0.001), stem (p=0.001), and needles (p=0.002). (b) *Pinus resinosa* biomass in roots (p=0.001), stem (p=0.008), and needles (p=0.022). Letters correspond to variation between treatments for each tissue. Treatments: Control= 0 kg N ha^{-1}, AA50= 56 kg N ha^{-1}, AA100=112 kg N ha^{-1}, AA200= 224 kg N ha^{-1}, AA300=336 kg N ha^{-1}, and AS100= Ammonium Sulfate 112 kg N ha^{-1}.

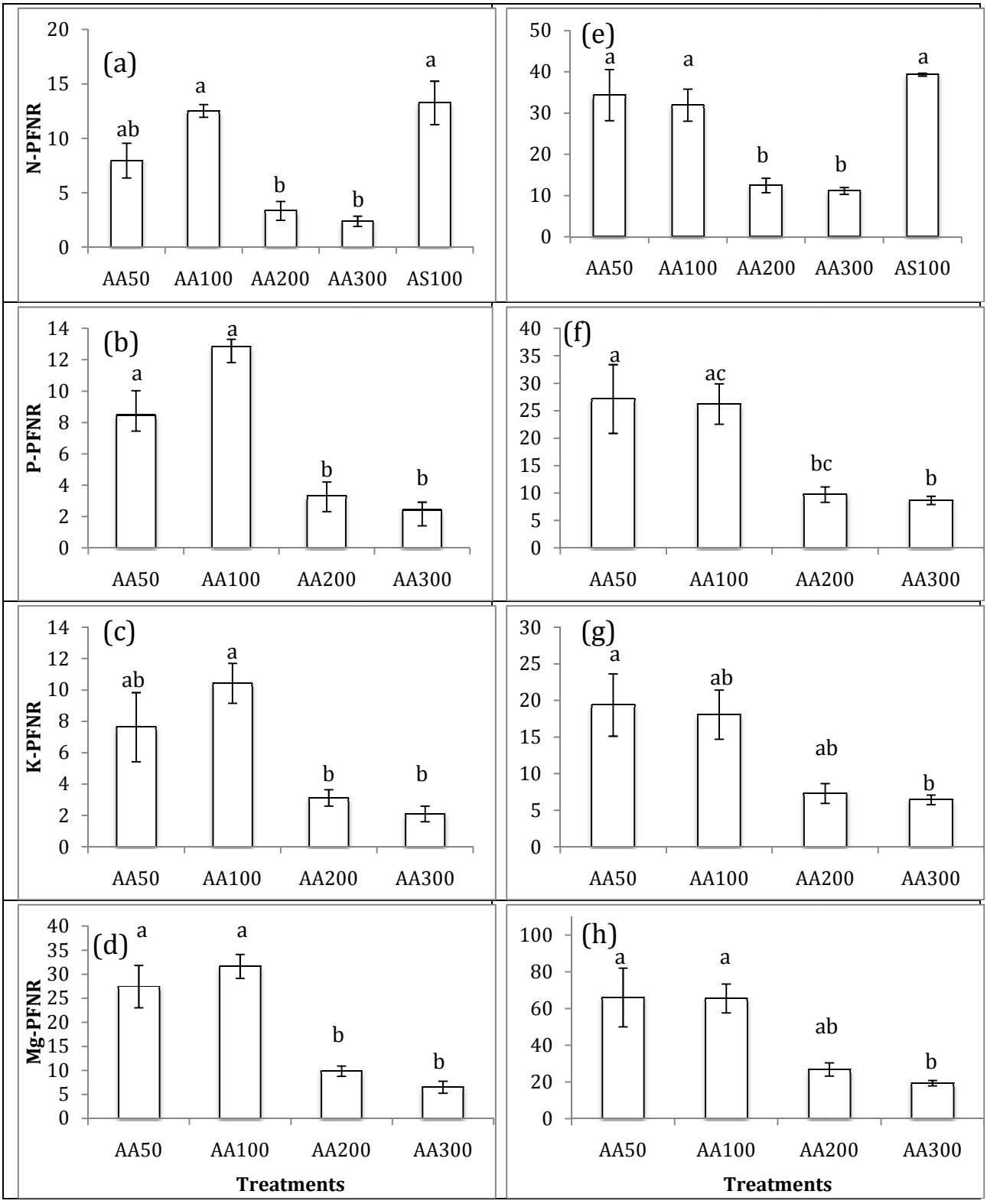

Figure 3.2. Plant fertilizer nutrient ratio (PFNR) for *Abies fraseri* N (a, p=0.000), P (b, p=0.000), K (c, p=0.007), and Mg (d, p=0.000) and *Pinus resinosa* N (e, p=0.000), P (f, p=0.011), K (g, p=0.019), Mg (h, p=0.011). PFNR=plant nutrient biomass (g)/nutrient supplied (g). Treatments with the same letter are statistically similar. Treatments: Control= 0 kg N ha^{-1}, AA50= 56 kg N ha^{-1}, AA100=112 kg N ha^{-1}, AA200= 224 kg N ha^{-1}, AA300=336 kg N ha^{-1}, and AS100= Ammonium Sulfate 112 kg N ha^{-1}.

REFERENCES

REFERENCES

Adesemoye AO and JW Kloepper (2009). Plant-microbes interactions in enhanced fertilizer-use efficiency. Appl. Microbiol. Biotechnol. 85:1-12.

Antikainen R, R Haapanen, R Lemola, JI Nousiainen and S Rekolainen (2008). Nitrogen and phosphorus flows in the Finnish agricultural and forest sectors, 1910-2000. Water Air Soil Pollut. 194:163-177.

Baligar VC, NK Fageria, and ZL He (2001). Nutrient use efficiency in plants. Communications in Soil Science and Plant Analysis 32:921-950.

Blessington, TM, DL Clement, and KG Williams (2009). Organic and inorganic fertilizers. University of Maryland Cooperative Extension, Fact Sheet 837.

Bown HE, MS Watt, PW Clinton, and EG Mason (2010). Influence of ammonium and nitrate supply on growth, dry matter partitioning, N uptake and photosynthetic capacity of *Pinus radiata* seedlings. Trees 24:1097-1107.

Dannenmann M, Simon J, Gasche R, Holst J, Naumann PS, Kogel-Knabner I, Knicker H, Mayer H, Schloter M, Pena R, Polle A, Rennenberg H, Papen H (2009) Tree girdling provides insight on the role of labile carbon in nitrogen partitioning between soil microorganisms and adult European beech. Soil Biol Biochem 41: 1622-1631.

Elliot KJ and AS White (1994). Effects of light, nitrogen, and phosphorus on Red pine seedling growth and nutrient use efficiency. Forest Science 40: 47-58.

Ericsson T (1995). Growth and shoot: root ratios of seedlings in relation to nutrient availability. Plant and Soil 168-169:205-214.

Gallet-Budynek A, E Brozostek, VL Rodgers, JM Talbot, S Hyzy, and AC Finzi (2009). Intact uptake by northern hardwood conifer trees. Oecologica 160:129-138.

Ge T, Song S, Roberts P, Jones DL, Huang C, Iwasaki K (2009) Amino acids as a nitrogen source for tomato seedlings: The use of dual-labeled (^{13}C, ^{15}N) glycine to test for direct uptake by tomato seedlings. Environ Exp Bot 66: 357-361.

Goodrich, J.A., B.W. Lykins Jr., and R.M. Clark (1991). Drinking water from agriculturally contaminated groundwater. Journal of Environmental Quality 20:707-717.

Gonod, L.V., D.L. Jones, and C. Chenu. (2006). Sorption regulates fate of the amino acids lysine and leucine in soil aggregates. Eur. J. Soil Sci. 57: 320-329.

Havlin, JL and SL Tisdale (2005). *Soil Fertility and Fertilizers*. Upper Saddle River: Pearson Education, Inc. (7^{th} edition).

Jagus, A. and M. Rzetala (2011). Influence of agricultural anthropopression in water quality of the dam reservoirs. Ecological Chemistry and Engineering. 18: 359-367.

Jones DL (1999) Amino acid biodegradation and its potential effects on organic nitrogen capture by plants. Soil Biol Biochem 31: 613-622.

Jones DL, Shannon D, Junvee-Fortune T, Farrar JF (2005) Plant capture of free amino acids is maximized under high soil amino acid concentrations. Soil Biol & Biochem 37: 179-181.

Kaakinen S, A Jolkkonen, S Iivonen, and E Vapaavuori (2004). Growth, allocation and tissue chemistry of *Picea abies* seedlings affected by nutrient supply during the second year of growth. Tree Physiology 24:707-719.

Kielland K (1995) Patterns of free amino acids in arctic tundra soils. Biogeochemistry 31: 85-98.

Kielland K, McFarland JW, Ruess RW, Olson K (2007) Rapid cycling of organic nitrogen in taiga forest ecosystems. Ecosystems 10: 360-368.

Linder S and DA Rook (1984). Effects of mineral nutrition on carbon dioxide exchange and partitioning in trees. In *Nutrition of Plantation Forests*. GD Bowen and EKS Nambiar (Eds). Pp.211-236. Academic Press, London.

Lipson D, Näsholm T (2001) The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. Oecologica 128: 305-316.

Metcalfe, RJ, J. Nault, and BJ Hawkins (2011). Adaptations to nitrogen form: comparing inorganic nitrogen and amino acid availability and uptake by four temperate forest plants. Can J For Res 41:1626-1637.

Näsholm T, A Ekblad, A Nordin, R Giesler, M Högber, and P Högber (1998). Boreal forest plants take up organic nitrogen. Nature 392:914-916.

Näsholm T, Huss-Danell K, Hogberg P (2000) Uptake of organic nitrogen in the field by four agriculturally important plant species. Ecology 81: 1155-1161.

Näsholm, T., K. Kielland, and U. Ganeteg (2009). Uptake of organic nitrogen by plants. New Phytologist 182: 31-48.

Nzokou P and BM Cregg (2010). Growth, biomass, and nitrogen use efficiency of containerized Fraser fir (*Abies fraseri*) as related to irrigation and nitrogen fertilization. HortScience 45:946-951.

Nzokou P, AR Wilson, D Güney and S Kulaç (2012) Amino acid fertilization of Fraser fir (*Abies fraseri*), red pine (*Pinus resinosa*), and hybrid poplar: Effect on foliar chemistry and nutrient dynamics. Submitted to HortScience.

Öhlund J, Näsholm T (2001) Growth of conifer seedlings on organic and inorganic nitrogen sources. Tree Physiology 21: 1319-1326.

Öhlund J, Näsholm T (2002) Low nitrogen losses with a new source of nitrogen for cultivation of conifer seedlings. Environ Sci Technol 36: 4854-4859.

Persson J, Näsholm T (2001) Amino acid uptake: a widespread ability among boreal forest plants. Ecology Letters 4: 434-438.

Poorter H, KJ Niklas, PB Reich, J Oleksyn, P Poot, and L Mommer (2012). Biomass allocation to leaves, stems, and roots: meta-analyses of interspecific variation and environmental control. New Phytologist 193: 30-50.

Proe MF and P Millard (1994). Relationships between nutrient supply, nitrogen partitioning and growth in young Sitka spruce (*Picea sitchensis*). Tree Physiology 14:75-88.

Raab TK, Lipson DA, Monson RK (1996) Non-mycorrhizal uptake of amino acids by roots of the alpine sedge *Kobresia myosuroides*: implications for the alpine nitrogen cycle. Oecologia 108: 488-494.

Reeve, J.R., J.L. Smith, L. Carpenter-Boggs, and J.P. Reganold. (2008). Soil-based cycling and differential uptake of amino acids by three species of strawberry (*Fragaria* spp.) plants. Soil Biology and Biochemistry 40: 2547-2552.

Rosen CJ and DL Allan (2007). Exploring the benefits of organic nutrient sources for crop production and soil quality. HortTechnology 17:422-430.

Rothstein, D.E. (2010). Effects of amino-acid chemistry and soil properties on the behavior of free amino acids in acidic forest soils. Soil Biology and Biochemistry 42: 1743-1750.

Rothstein DE, Cregg BM (2005) Effects of nitrogen form on nutrient uptake and physiology of Fraser fir (*Abies fraseri*). For Ecol Manage 219: 69-80.

Sauheitl L, Glaser B, Weigelt A (2009) Uptake of intact amino acids by plants depends on soil amino acid concentrations. Environ Exp Bot 66: 145-152.

Schobert C, Kockenberger W, Komor E (1988) Uptake of amino acids by plants from soil: A comparative study with castor bean seedlings grown under natural and axenic soil conditions. Plant Soil 109: 181-188.

Sheriff DW, HA Margolis, MR Kaufmann, and PB Reich (1995). Resource use efficiency. In WK Smith and TM Hinckley (Eds.), *Resource physiology of conifers: Acquisition, allocation, and utilization* (pp. 143-178). San Diego: Academic Press.

Thomas RB, JD Lewis, and BR Strain (1994). Effects of leaf nutrient status on photosynthetic capacity in loblolly pine (*Pinus taeda* L.) seedlings grown in elevated atmospheric CO_2. Tree Physiology 14: 947-960.

Wilson, AR, P Nzokou, D Güney, and S Kulaç (2012). Growth response and nitrogen use physiology of Fraser fir (*Abies fraseri*), red pine (*Pinus resinosa*), and hybrid poplar under amino acid nutrition. New Forests, doi: 10.1007/s11056-012-9317-9.

CHAPTER FOUR: AMINO ACID NUTRITION IN SHORT-ROTATION TREE PRODUCTION: THE EFFECTS ON NUTRIENT DYNAMICS, MICROBIAL INTERACTIONS, AND PHOTOSYNTHESIS

Submitted to Tree Physiology

Abstract

Previous studies of amino acid nutrition in short rotation tree production have suggested the occurrence of nutrient limitations in the rhizosphere due to immobilization of the organic nitrogen (N) in microbial biomass and binding to soils. We report on a study that aimed to understand the effects of amino acid fertilization on nutrient dynamics, microbial interactions, and photosynthesis of short rotation trees. Two conifer species—Fraser fir (*Abies fraseri* [Pursh Poir.]) and red pine (*Pinus resinosa* Aiton)—and one hardwood (hybrid poplar) were grown in a nursery bed and treated with varying rates (0, 112, and 336 kg N ha^{-1}) of amino acid fertilizer containing arginine and other plant essential nutrients. Ammonium sulfate (21% N) was applied at 112 kg N ha^{-1} as positive control treatment. Parameters monitored included tree growth response, cation exchange capacity, microbial respiration, mycorrhizal infection, foliar nutrient concentrations, and photosynthetic parameters. We did not observe enhanced microbial respiration or cation exchange capacities in arginine treatments and suggested this to be due to the short duration of the study and/or microsite variability in environmental conditions including soil moisture and temperature. Ectomycorrhizae root colonization was significantly greater in arginine and unfertilized control treatments indicating that arginine has the potential to increase the abundance of beneficial microbes. We observed correlations between microbial respiration and photosynthetic rate, but due to similar microbial respiration among treatments, we suggest that foliar nutrient status, N form taken up by plants, and limitations of photosynthetic biochemical processes had a greater effect on the observed photosynthetic parameters. Based on the results of from this study, we suspect that a significant proportion of applied arginine is remaining in soils and future research should elucidate the mechanisms by which this is occurring.

Introduction

Research in the past few decades has challenged the theory of inorganic nitrogen (N) being the only N supply used by plants by demonstrating that plants can use organic N and compete well with microbes, depending on the N status of the microsite (Schimel and Bennett 2004). Among organic N sources are amino acids, which are important to the N nutrition of plants growing in arctic tundra (Kielland 1995), boreal (Näsholm et al. 1998; Persson and Näsholm), alpine (Raab et al. 1996), and temperate (Gallet-Budynek et al. 2009; Metcalfe et al. 2011) ecosystems. These principles are becoming increasingly relevant applications in production and have been tested in agricultural (Jones and Darrah 1994; Yamagata and Ae 1996; Näsholm et al. 2000) and forestry production systems (Wilson et al. 2012; Nzokou et al. 2012; Wilson and Nzokou 2012).

It is well established that plants are able to assimilate amino acids intact (Öhlund and Näsholm 2001; Näsholm et al. 2000; Ge et al. 2009), although their ability to do so declines under high amino acid concentrations (Sauheitl et al. 2009). Wilson and Nzokou (2012) found conifer seedlings grown in the field had improved nutrient content, biomass production, and plant nutrient content relative to fertilizer nutrients applied when arginine was applied at lower rates (100 lbs N ac^{-1}). Amino acids used as a nutrient source in controlled container studies have been shown to improve fine root growth of tree seedlings (Öhlund and Näsholm 2001), enhance N recovery in plant tissues and growth substrate (Öhlund and Näsholm 2002), and be assimilated at rates similar to ammonium (NH_4^+-N) and nitrate (NO_3^--N) (Öhlund and Näsholm 2001). In a field study, amino acid nutrition did not tend to increase root growth of conifers, but nutrient

content of conifer tissues tended to have similar or greater nutrient contents as compared to the inorganic control (Wilson and Nzokou 2012).

Amino acids in soils can rapidly mineralize due to their short half lives (Jones 1999); they also can bind to soil exchange sites (Rothstein 2010) and are taken up by microbes until saturation occurs (Jones 1999), regulating the rate at which they become available for mineralization (Reeve et al. 2008; Gonod et al. 2006). These processes reduce nutrient losses through leaching, but also reduce amino acid availability to plants (Näsholm et al. 2009). Previous studies of conifer and hybrid poplars grown under amino acid nutrition in production soils found that amino acid applications did not increase NO_3^--N (Wilson et al. 2012) or cation (Nzokou et al. 2012) content in leachate. Wilson et al. (2012) found amino acid applications two to three times greater than the inorganic control were necessary to achieve similar growth and foliar N, especially during seedling establishment, and suggested this to be due to nutrient limitations from immobilization in microbial biomass and binding to cation exchange sites. However, it has also been suggested that because of its organic nature, amino acids would become available over time, thus reducing the severity of nutrient limitations (Wilson and Nzokou 2012).

It has been reported that initial competition between plants and microbes exists for amino acids in soils (Andresen et al. 2009), due to the carbon input associated with the organic N source (Schobert et al. 1988). However, challenges faced by plants in accessing amino acids can be overcome. When amino acids are present in high concentrations, plants are the favored competitors (Jones et al. 2005). Mycorrhizal fungi have also been proven to aid in the assimilation of amino acids in soils (Näsholm et al. 2009; Dannenmann et al. 2009). The nature

of plant-microbial interactions and interactions between amino acids and soils must be understood to make interpretations on the implications for plant physiological processes.

Little research has been conducted on organic nutrient sources, especially amino acids, influence on photosynthesis. A study using poultry/cow manure and barley mulch as organic N sources combined with mineral nutrition found increased stomatal conductance and photosynthetic rate in sweet maize when compared to inorganically fertilized treatments (Efthimiadou et al. 2010). The high correlations between these parameters, leaf area, and dry biomass, were attributed to a greater N availability. Improved nutrient assimilation can lead to increases in photosynthetic capacity because of the intimate relationship of photosynthesis and leaf N status (Mae 1997). Enhanced photosynthesis was observed in mycorrhizal *Ipomea carnea* spp. *fistulosa*, but was attributed to greater nutrition (Amaya-Carpio et al. 2009). Topically applying glutamic acid to hawthorn has been demonstrated to increase photosynthetic capacity by improving N metabolism and N content in foliage (Yu et al. 2010), however, this study does not address the effects plant-microbial interactions in the soil will have on nutrient assimilation and consequently plant metabolic activity.

More research is needed to understand the availability of arginine for plant nutrition based on arginine's chemical properties and its influence on microbial communities and how these factors interact with nutrient acquisition and tree physiological processes. We hypothesized that 1) arginine would bind to cation exchange sites and affect microbial activity, thus resulting in transient nutrient limitations to trees, 2) tree-microbial interactions and foliar nutrient status would have a significant effect on photosynthesis, and 3) because arginine can be assimilated directly, improved photosynthesis will occur in arginine fertilized trees. The objectives of this study were to determine the nutrient limitations imposed under arginine

nutrition by evaluating cation exchange capacity and microbial respiration, evaluate the tree-microbial interactions occurring, and determine the effect of arginine nutrition on tree photosynthesis.

Methods

Site description and management

The study site is located in a nursery bed at the Tree Research Center (TRC) on Michigan State University's campus (42.65ºN and 84.42ºW). The soils in the nursery are classified as sandy with a gross mean cation exchange capacity of 3.7 meq/100g soil (tested in 2010). Weeds were controlled by hand removal throughout the growing season to avoid competition with trees. Rainfall was monitored by rain gauges, which were placed evenly throughout the field and irrigation was applied in the absence of adequate rainfall.

Plant material

Plant materials selected for this study included two conifer species, Fraser fir (*Abies fraseri* [Pursh] Poir.) and Red pine (*Pinus resinosa* Aiton), and one hardwood hybrid, (*Populus nigra* L. x *Populus maximowiczii* A. Henry 'NM6'). *P. resinosa* seeds were sown and grown in a greenhouse in 2008 and plug seedlings were transplanted into the nursery bed on May 22, 2009. Bare-root *A. fraseri* plug 2-3 seedlings were transplanted into the nursery bed on May 4, 2011 (Peterson's Riverview Nursery). *A. fraseri* roots were pruned to approximately 30 cm. The hybrid poplar was grown from cuttings obtained from trees of our previous study and placed in the field on April 29, 2011.

Nutrient source

The nutrient source used in this study was arGrow complete®, which contains the amino acid arginine (SweTree Technologies, Umea, Sweden). arGrow complete® contains 70 g/L of N and many other plant-essential nutrients including: phosphorus (P) (12 g/L), potassium (K) (49 g/L), magnesium (Mg) (4 g/L), sulfur (S) (10 g/L), boron (B) (0.24 g/L), copper (Cu) (0.03 g/L), iron (Fe) (1.2 g/L), manganese (Mn) (0.6 g/L), molybdenum (Mo) (0.05 g/L), and zinc (0.18 g/L). arGrow complete® was applied in diluted form (1/200 dilution) over 12 weeks from June 1, 2011 to August 19, 2011. Ammonium sulfate (21-0-0-14) was used as the inorganic nutrient source in positive control treatments and was hand-broadcasted on June 1, 2011.

Experimental Design

The experiment was a randomized block design (4x4) with three species and four treatments replicated four times. For both conifer species, there were ten trees per plot, and the hybrid poplar had 16 trees per plot. Trees were spaced 1 ft (30.48 cm) from neighboring trees. There was an 8 ft (243.8 cm) buffer zone between each hybrid poplar plot to prevent roots from invading other treatments. The four treatments included arGrow complete® applied at 0 (Control), 100 (AA100), and 300 (AA300) lb N ac^{-1} (0, 112, and 356 kg N ha^{-1}, respectively) and ammonium sulfate applied at 100 lb N ac^{-1} (112 kg N ha^{-1}).

Tree Growth Response

Initial height and root collar diameter (RCD) were measured prior to fertilization on May 16, 2011. Following termination of fertilization (August 30, 2011), the same parameters were re-

measured. Height growth was measured on the east side of each tree, to prevent confounding results from uneven soil. RCD measurements were taken in the morning to prevent measuring diurnal stem shrinkage due to water stress and the same axis was measured for both dates. Growth response was calculated by subtracting the initial measurement from the final measurement for each parameter. Initial height and RCD for the hybrid poplar was considered to be zero.

Cation Exchange Capacity

Soil samples were collected on May 21, 2011 and September 13, 2011 for determination of cation exchange capacity (CEC). Four samples were collected from a depth of 0-15 cm using a soil auger. Composite samples were thoroughly mixed and stored in a cooler at 4º C until they were shipped for analysis at Midwest Laboratories (Omaha, Nebraska).

Microbial Respiration

Microbial respiration was measured *in situ* prior to fertilization (May 24, 2011), mid-season (July 7, 2011), and following termination of fertilization (September 13, 2011). Microbial respiration was also monitored when photosynthesis was measured. Two soil rings reaching a depth of 15 cm were installed in each plot at the beginning of the season (May 20, 2011) and situated between trees. At least 24 h prior to taking soil CO_2 efflux measurements, all foliage was removed from the soil ring. Microbial respiration was measured using the soil CO_2 flux chamber attachment for the LI-6400 Portable Photosynthesis System (LI-COR Biosciences, Lincoln, Nebraska). Before measurements were taken, the chamber was allowed to equilibrate with ambient air CO_2. The fan speed was set to low to improve the quality of the readings.

Three cycles of soil CO_2 efflux were measured to determine changes in CO_2 over time and microbial respiration was determined. Soil temperature and moisture were monitored with soil CO_2 flux measurements.

Ecto- and Arbuscular Mycorrhizae Evaluation

Root systems were collected on November 1, 2011. Because the soils of the study site were sandy, it was possible to collect nearly the entire root system. Root samples were rinsed in de-ionized water and frozen until mycorrhizal colonization was determined.

For determination of percent root tip colonization of ectomycorrhizae (EcM), *A. fraseri* and *P. resinosa* roots were cut into 1-7 cm segments similar to Karpati et al. (2011) and analyzed on a dissecting microscope at 7.5-35 X. Root tips were examined for presence or absence of EcM structures, even if EcM structures spanned more than one root tip, similar to Dickie and Reich (2005). A minimum of 600 root tips was evaluated for each treatment. Percent root tip colonization of EcM was determined by dividing the infected root tips by the total number of root tips examined.

Arbuscular mycorrhizae (AM) infection was evaluated for the hybrid poplar as described by Koske and Gemma (1989) using the line-intersect method as described by Giovannetti and Mosse (1980).

Photosynthesis

Photosynthetic rate (A), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were measured throughout the growing season using the LI-6400 XT Portable

Photosynthesis System (LI-COR Biosciences). All measurements were taken between 11 am and 2 pm and only on clear, sunny days on two trees in each treatment plot. The CO_2 concentration was fixed at 400 µmol (except in June when ambient CO_2 was used) and the fan was maintained at high speed to disrupt the boundary layer. Photosynthesis was measured on the top whorl of conifers using the 6400-22L Lighted Conifer Chamber (LI-COR Biosciences) and PAR (photosynthetically active radiation) was set to ambient light in the field. *A. fraseri* measurements were taken on June 30, July 26, and August 17, 2011 and *P. resinosa* measurements were taken on June 29 and July 30, 2011. Hybrid poplar photosynthesis was measured at similar heights on the tree using the broadleaf chamber (LI-COR Biosciences), which used natural light. Hybrid poplar measurements were taken June 25, July 31, and August 15, 2011.

Following photosynthetic measurements, the measured shoot was harvested and stored in a cooler at 4ºC until nutrient analysis. Projected needle area of conifers was determined by arranging all of the harvested biomass on a scanner and analysis using ImageJ (Bethesda, MD).

Nutrient Analysis

Following photosynthetic measurements and leaf area determination of conifers, harvested biomass was analyzed for N, P, K, Ca, Mg, and Mn concentrations. Foliar tissues were dried in an oven at 65ºC for at least 48 h then ground into a fine powder. Approximately 0.3 g of dried tissue was acid digested with 4.5 mL sulfuric acid and 1.5 mL hydrogen peroxide in a 75 mL digestion tube. Samples were pre-digested overnight and run on a heating program to 340±10ºC on a digestion block (AIM600 Block Digestion System). Samples were then diluted to 75 mL on a vortex and thoroughly mixed before aliquots were used for N and P determination

on a SAN ++ segmented flow analyzer (Skalar, Inc., Buford, GA) and K, Ca, Mg, and Mn concentrations on an atomic absorption spectrometer (Aanalyst 400, Perkin Elmer, Waltham, MA).

Data analysis

All statistical analysis was performed using Systat 13 (Systat Software Inc., Chicago, Illinois). Effects of treatment on growth parameters, CEC, microbial respiration, percent mycorrhizal root colonization, photosynthetic parameters, and foliar nutrient concentrations were evaluated using Analysis of Variance for each sampling date. Tukey's Honestly-Significant-Different Test was used in pair-wise analysis. ANOVA for repeated measures test was used to analyze interactions between treatments and CEC and microbial respiration throughout the season. We did not conduct repeated measures analysis on photosynthetic parameters due to differences in sampling methods. The significance threshold was α=0.05. Pearson's correlations were used to evaluate the relationship between microbial respiration and photosynthetic rate.

Results

Growth Response

A. fraseri and *P. resinosa* seedlings had similar height and RCD growth responses among treatments (Table 4.1). The hybrid poplar had a significantly greater height growth response in trees fertilized with arginine (AA100 and AA300) or ammonium sulfate (AS100) as compared to the unfertilized control (Table 4.1). RCD growth response of hybrid poplars was similar among treatments (Table 4.1).

Cation exchange capacity

Cation exchange capacity in *A. fraseri* and *P. resinosa* was similar among treatments for both sampling dates and there were no significant differences in CEC from May to September for either species (p=0.154 and p=0.456, respectively) (Table 4.2). CEC of hybrid poplar treatment plots was similar among treatments in May (Table 4.2). In September, AA100 treatments had significantly lower CEC than AA300 treatments (Table 4.2), but similar to the ammonium sulfate and unfertilized control treatments. CEC was similar over time in hybrid poplar treatment plots (p=0.205).

Microbial respiration and mycorrhizal evaluation

Treatment affected microbial respiration in *A. fraseri* plots on June 29 (p=0.011), with the unfertilized control having significantly lower microbial respiration than both arginine treatments (AA100 and AA300) (Figure 4.1a). Microbial respiration did not vary among treatments over time (p=0.671).

Microbial respiration was similar among *P. resinosa* plots for all sampling dates (Figure 4.1b) and was similar throughout the growing season (p=0.361).

In hybrid poplar plots, microbial respiration tended to increase and then decrease throughout the season (Figure 4.1c) and respiration varied among treatments throughout the growing season (p=0.008). On May 24, microbial respiration was significantly greater in the unfertilized control than AA100 treatments, but similar to the remaining treatments. Microbial respiration was significantly greater in ammonium sulfate treatments (AS100) on July 7 than both the unfertilized control and AA100 treatment (Figure 4.1c). Respiration did not vary among treatments on the remaining sampling dates.

A. fraseri seedlings in unfertilized control and arginine (AA100 and AA300) treatments had greater root colonization of EcM than seedlings in the ammonium sulfate treatment (AS100) (Figure 4.2a). This trend was also observed in *P. resinosa* seedlings (Figure 4.2b). There were no differences among treatments on the percent root colonization of AM for the hybrid poplars (Figure 4.2c).

Photosynthesis

Fertilization affected photosynthetic rate (A) of *A. fraseri* seedlings on the first sampling date (Jun 29) only (Table 4.3). Seedlings in unfertilized control treatments had higher photosynthetic rates than seedlings fertilized with arginine at a rate of 112 kg N ha^{-1} (AA100), but similar to seedlings in AA300 and AS100 treatments. Stomatal conductance (g_s) varied among treatments only on the final sampling date (Aug 17), with seedlings in AA300 treatments having significantly greater g_s than seedlings in ammonium sulfate treatments (AS100) (Table 4.3). Intercellular CO_2 concentration (C_i) was significantly greater in seedlings of AA100 treatments than AS100 treatments on Jun 29, but similar to seedlings in remaining treatments (Table 4.3). On July 26, C_i was significantly greater in seedlings in unfertilized control treatments than in ammonium sulfate treatments (AS100), but similar to seedlings in arginine treatments. Seedlings in AA300 treatments had significantly greater C_i than seedlings in AS100 treatments on August 17, though similar to seedlings in the unfertilized control and AA100 treatments.

Photosynthetic rate varied among treatments in *P. resinosa* seedlings on June 29, with greater A in seedlings of ammonium sulfate treatments (AS100) than of seedlings in AA100 treatments (Table 4.4). Photosynthetic rate was similar among treatments on July 30, and stomatal conductance and C_i were similar among treatments for both sampling dates (Table 4.4).

Fertilization affected photosynthetic rate of hybrid poplars on July 31, with greater A in hybrid poplars in AA100 treatments than in ammonium sulfate treatments (AS100) (Table 4.5). Stomatal conductance and C_i were similar among hybrid poplar treatments for all sampling dates.

Nutrient Analysis

Foliar N concentrations of *A. fraseri* seedlings remained similar throughout the growing season ($p=0.534$) (Table 4.6). On July 26, seedlings growing in AS100 treatments had significantly greater foliar N concentrations than seedlings in all other treatments. In August, seedlings in AA300 treatments had significantly greater foliar N concentrations than seedlings in the unfertilized control, but similar to seedlings in AA100 and AS100 treatments. *A. fraseri* foliar K concentrations were significantly greater in seedlings in ammonium sulfate treatments (AS100) than seedlings in arginine treatments (AA100 and AA300) on July 26. Seedling foliar K concentrations were similar among treatments on June 26 and August 17, and no significant differences were observed in foliar K concentrations throughout the sampling period ($p=0.560$). *A. fraseri* foliar P, Ca, Mg, and Mn concentrations were similar among seedlings in all treatments for all sampling dates (Table 4.6). No significant differences were observed throughout the growing season in foliar P ($p=0.173$), Ca ($p=0.453$), Mg ($p=0.755$), or Mn ($p=0.802$) concentrations.

Foliar N concentrations of *P. resinosa* seedlings were similar among treatments on June 29, but on July 30, seedlings in AA100 treatments had significantly greater foliar N concentrations than seedlings in AS100 treatments (Table 4.7). Foliar N concentrations of *P. resinosa* did not change significantly over time (p=0.569). *P. resinosa* seedlings' foliar P and K concentrations were similar among treatments for both sampling dates and no differences were observed in foliar P and K concentrations throughout the growing season (p=0.241 and p=0.307, respectively). *P. resinosa* seedlings had similar foliar Ca concentrations on June 29, but seedlings in AA100 treatments had significantly greater foliar Ca concentrations than seedlings in ammonium sulfate treatments (AS100) on July 30. Foliar Ca concentrations of seedlings did not change significantly throughout the sampling period (p=0.142). On June 29, *P. resinosa* seedlings in AA100 treatments had significantly greater foliar Mg concentrations than the unfertilized control, but seedling foliar Mg concentrations were similar among treatments on July 30. Foliar Mg concentrations of seedlings significantly decreased throughout the season (p=0.008). Foliar Mn concentrations of seedlings in AS100 treatments were significantly greater than seedlings in AA100 treatments on June 29, and significantly greater than AA100 and unfertilized control seedlings on July 30. *P. resinosa* seedlings' foliar Mn concentrations significantly decreased over time (p=0.618).

Foliar N and P was significantly greater in hybrid poplars in AA300 and AS100 treatments than hybrid poplars in AA100 and the unfertilized control treatments on June 25, but were similar on July 31 and August 15 (Table 4.8). Foliar N concentrations changed significantly throughout the growing season (p=0.004), tending to increase from June to July and decrease from July to August. Foliar P concentrations of hybrid poplars did not change significantly throughout the growing season (p=0.076). Hybrid poplars had similar foliar K concentrations

among treatments on June 25 and July 31 but on August 15, hybrid poplars in unfertilized control treatments had significantly lower foliar K concentrations than hybrid poplars fertilized with arginine or ammonium sulfate at a rate of 112 kg N ha^{-1} (AA100 and AS100, respectively). Significant changes throughout the growing season in foliar K concentrations of hybrid poplars were not observed (p=0.295). Hybrid poplar foliar Ca concentrations were similar among treatments and throughout the growing season (p=0.721). Foliar Mg concentrations were significantly greater in hybrid poplars in unfertilized control treatments than AA300 and AS100 treatments on June 25. Hybrid poplar foliar Mg concentrations throughout the growing season were significant (p=0.000), with Mg concentrations tending to decrease from June to July and then slightly increase from July to August. On June 25, hybrid poplars in AA300 treatments had significantly lower foliar Mn concentrations than all other treatments. Foliar Mn concentrations were similar among hybrid poplars in all treatments on July 31, but significantly greater in unfertilized control treatments than in ammonium sulfate treatments on August 15. Changes in foliar Mn concentrations of hybrid poplars throughout the growing season were significant (p=0.000) and tended to decrease over time in hybrid poplars of all treatments except the unfertilized control, which had increased Mn concentrations from July to August.

Microbial Respiration and Photosynthesis

Microbial respiration had a weak negative correlation with *A. fraseri* photosynthesis on June 25 and July 26, but a fairly strong positive correlation between A and microbial respiration was observed on August 17 (Table 4.9). Microbial respiration had a somewhat strong negative correlation with A of *P. resinosa* seedlings on June 29, with the opposite trend observed on July 30 (Table 4.9). On June 25, microbial respiration in hybrid poplar treatments had a fairly strong

positive correlation with A of seedlings (Table 4.9). On July 31, microbial respiration and A had an incredibly weak negative correlation, and on August 15, the correlation was weakly positive.

Discussion

Growth response

Growth responses (height and RCD) were similar among treatments for all species with the exception of height growth response in hybrid poplars (Table 4.1). A previous field study of hybrid poplar grown from cuttings found amino acid nutrition had a significant effect on height growth in the establishment year and attributed it to nutrient limitations in the rhizosphere (Wilson et al. 2012). In our study, we only observed reduced height growth of hybrid poplars in unfertilized control treatments relative to hybrid poplars in ammonium sulfate and arginine treatments (Table 4.1). Because shoot elongation occurs earlier in the growing season, this could be due to the effect of greater microbial activity in hybrid poplar unfertilized control treatments on May 24 (Figure 4.1c), which could have reduced nutrient availability. On June 25, hybrid poplars in unfertilized control and AA100 treatments had significantly lower foliar N and P concentrations (Table 4.8). The greater height growth at the end of the season in hybrid poplars in AA100 treatments as compared to the control, despite similar foliar N and P concentrations in June, could have been due to greater photosynthetic rates mid-season (Table 4.5). Fertilization of hybrid poplars significantly increased height growth regardless of the nutrient source applied.

Similarities in growth responses among treatments of the two conifer species is likely due to a combined effect of tree age and stage of establishment. The lack of significant differences between seedlings in *P. resinosa* treatments can likely be attributed to seedlings being well established in the field (transplanted in 2009). Growth responses of conifer plug seedlings

following transplant were significantly different under amino acid nutrition and was suggested to be a result of nutrient limitations, however, in the season following transplant, growth responses among treatments were more similar and nutrient limitations were suggested to be overcome (Wilson et al. 2012). Similarly, lack of significant differences among *A. fraseri* seedlings under different treatments is likely due to transplants being plug 2-3 and therefore having greater root mass at planting or due to differences in planting techniques (bare-root versus plug).

Because diameter expansion occurs later in the growing season, it is likely that water availability had a greater impact on RCD growth response than nutrient availability as suggested in other studies (Nikiema et al. 2011; Wilson et al. 2012). This factor in conjunction with the reasons suggested previously likely resulted in lack of a treatment effect on RCD growth responses.

Cation exchange capacity and microbial respiration

Soil organic matter amendments improve cation exchange capacity of soils by increasing organic carbon in soils (Rice et al. 2007). Thus, due to the carbon input associated with arginine application, we would expect to see increases in CEC over time in amino acid fertilized treatments. We did not observe changes in CEC throughout the growing season, likely due to the short duration of the study (Table 4.2) and due to the fact that organic matter (OM) changes were very small. This is in congruence with previous short-term studies using organic fertilizer sources, which found no changes in soil chemical properties, including CEC and OM contributions (Schiavoni et al. 2011; Gasparatos et al. 2011). In rice-wheat cropping systems where soils were amended with OM, it was found that organic carbon accumulates after 15-20 years (Tirol-Padre et al. 2007).

It is well established that amino acids can bind to anion and cation exchange sites (Rothstein 2010) and soil aggregates (Jones 1999), which can limit availability of amino acids to plants (Näsholm et al. 2009). We hypothesized that arginine availability to trees would be limited in part due to binding to cation exchange sites in the soils. However, because of the low cation exchange capacities observed (Table 4.2) relative to the amount of arginine applied, it is unlikely that a significant portion of arginine is being rendered unavailable to trees or stored in the system by this soil chemical mechanism alone.

Cation exchange capacity is calculated based on results of soil analysis for exchangeable cations and bases (Ross 1995), thus this method may be underestimating the potential proportion of amino acids bound to soils because arginine is not being factored in as an exchangeable cation. Also of consideration is that arginine can undergo conformation changes, which displace the charge of its side chains. In the case that this is occurring by loss of a hydrogen ion, a negatively charged site would allow the molecule to bind to anion exchange sites.

Previous studies using arginine as a nutrient source have found no contributions to mineral N pools and similar contents of nitrate (NO_3^--N) (Wilson et al. 2012) and cations (Nzokou et al. 2012) in leachate relative to unfertilized control treatments, even when applied at application rates two to three times greater than inorganic control treatments. This indicates that arginine is not significantly mineralizing and therefore arginine is either being 1) immobilized in microbial biomass, 2) assimilated immediately by plants, or 3) binding to soils. Because we do not see improved microbial respiration in amino acid fertilized treatments (Figure 4.1a-c), this mechanism alone cannot be considered a sink for the arginine applied. We did not experiment with labeling in this study, but seedlings in arginine fertilized treatments tended to have similar foliar nutrient concentrations as ammonium sulfate seedlings (Table 4.6, 4.7, 4.8). This

observation is similar to a previous study that found similar or improved biomass nutrient content of conifers under amino acid nutrition relative to inorganic control treatments (Wilson et al. 2012). However, because foliar nutrient status (Table 4.6, 4.7, 4.8) and photosynthetic parameters (Table 4.3, 4.4, 4.5) are not greatly enhanced under amino acid nutrition, this too cannot be considered as a sole explanation for the trends observed. Therefore, we hypothesize that amino acids are in fact binding to soils despite low CEC. Although we did not observe improved CEC under amino acid nutrition, our results may be confounded by the interaction of strong positively charged arginine molecules occupying CEC sites while their associated carbon input is creating exchange sites for other cations to bind. We suggest that future research explore all possible mechanisms by which amino acids are binding to soils, including the quantification of net soil amino acid concentrations, to gain a greater understanding of its distribution in production systems.

It was also hypothesized that we would observe increased microbial activity under arginine nutrition because of the carbon input associated with organic N sources (Schobert et al. 1988), but this was only observed in *A. fraseri* on June 30, 2011 (Figure 4.1a). A previous study found that organic amendments did not improve soil microbial biomass C or respiration (Tirol-Padre et al. 2007). It has also been found that microbial biomass C and N is greater after 41 years of organic fertilization versus after 3 years, and organic fertilization effects on microbial biomass are not significant until after the ninth year (Friedel and Gabel 2001). Lack of significant treatment effects on microbial respiration could be attributed to the short duration of the study.

The greater microbial respiration observed in hybrid poplar unfertilized control treatments on May 24 and in ammonium sulfate treatments on July 7 (Figure 4.1c) may be due in

part to the effects of microsite variability in soil moisture and temperature which are known to greatly influence on microbial activity (Zak et al. 1999), though mycorrhizal respiration has been found to be less influenced by soil temperature (Moyano et al. 2008). Additionally, hybrid poplars could have had greater AM colonization at these discrete sampling times, thus increasing soil CO_2 efflux on these sampling dates. Plants mediate their relationship with AM fungi by altering root growth and digesting arbuscles (Brundrett 2009), thus AM-plant interactions are dynamic and could be the reason we observed significant differences in soil CO_2 efflux throughout the growing season (Figure 4.1c.).

In a previous study of container grown and bare-root nursery conifer seedlings, fertilization versus no fertilization had no effect on EcM root colonization (Khasa et al. 2001) while greater occurrences of AM fungi have been found under organically fertilized conditions than under mineral fertilization (Gryndler et al. 2006). In our study, we observed this trend in conifer seedlings, which had significantly greater EcM root colonization under amino acid nutrition or no fertilization than ammonium sulfate treatments (Figure 4.2 a,b), but did not observed difference in AM root colonization among treatments of hybrid poplars (Figure 4.2c). Because AM-plant interactions are so dynamic, the timing of sampling may have confounded our results and explain why we observed similar AM root colonization among hybrid poplar treatments.

Photosynthesis, Tree-Microbial Interactions, and Foliar Nutrient Status

We hypothesized that arginine nutrition and tree-microbial interactions would have an effect on photosynthesis. On June 30, *A. fraseri* seedlings treated with arginine had significantly different photosynthetic rates among treatments (Table 4.3) and had an inverse trend with

microbial respiration (Fig. 4.1a). Arginine treatments tended to have significantly lower A with greater microbial activity, however the correlation for this relationship was weak (r^2= -0.099) (Table 4.9). Additionally, for this sampling date, *A. fraseri* seedling foliar nutrient concentrations were similar among treatments for all nutrients (Table 4.6), indicating that nutrient limitations were likely not causing differences in photosynthetic rates of arginine-fertilized seedlings. C_i of *A. fraseri* seedlings on June 30 was also significantly greater in arginine treatments despite similar g_s among treatments (Table 4.3), which should increase photosynthetic rate. Therefore it is likely that photosynthesis for this sampling date was limited internally by another biochemical factor. Loblolly pine (*Pinus taeda* L.) grown under varying N, P, and CO_2 had limited photosynthetic capacity under all conditions and was attributed to limitations imposed by interactions of biochemical factors including depressed carboxylation efficiency, electron transport, and regeneration of phosphate (Thomas et al. 1994).

While photosynthesis was similar among treatments on July 26 and August 17, C_i of seedlings in arginine and control treatments tended to be greater than seedlings in ammonium sulfate (Table 4.3), indicating the potential to increase photosynthesis was limited by a factor other than CO_2. On July 26, *A. fraseri* seedlings fertilized with ammonium sulfate had significantly greater foliar N concentrations than all other treatments and significantly greater foliar K concentrations than arginine treatments, which may have limited A in spite of greater C_i. It is unlikely that microbial activity had a strong effect on this trend, as there were no significant differences among treatments (Figure 4.1a) and microbial respiration correlated weakly with A (r^2= -0.074) (Table 4.9). On August 17, *A. fraseri* seedlings in AA300 treatments had

significantly greater g_s and C_i than seedlings in ammonium sulfate treatments, despite the similar A (Table 4.3) and foliar N concentrations (Table 4.6). This result suggests that photosynthesis was limiting by another biochemical factor. For this sampling date, microbial respiration had a positive correlation with A (r^2=0.491) (Table 4.9), which may have contributed to the observed results.

On June 29, *P. resinosa* seedlings in ammonium sulfate treatments had significantly greater photosynthetic rates than seedlings in AA100 treatments, despite similar g_s and C_i, (Table 4.4). While microbial respiration was similar among treatments on this sampling date, it had a negative relationship with A (r^2= -0.285) (Table 4.9). This factor in conjunction with ammonium sulfate seedlings having significantly greater foliar Mn concentrations than AA100 (Table 4.7) could explain the reduced photosynthesis observed in *P. resinosa* seedlings in AA100 treatments. Mn is essential in the "water-splitting" step in Photosystem II, which liberates an electron for transfer through the thylakoid membrane (Epstein and Bloom 2005), thus Mn limitations may have reduced electron transport in *P. resinosa* AA100 seedlings. Although it was not measured in this study, it is unlikely that chlorophyll was limiting to photosynthesis in AA100 seedlings because N and Mg, the key components of chlorophyll (Epstein and Bloom 2005), concentrations in foliage were similar to that of seedlings in ammonium sulfate treatments (Table 4.7).

Similarities observed in photosynthetic parameters of *P. resinosa* seedlings on July 30 and hybrid poplars on June 25 and August 17 (Table 4.4, 4.5) in spite of the differences observed in foliar nutrient concentrations (Table 4.7, 4.8) among treatments indicate that foliar nutrients were not limiting to photosynthesis. The strong somewhat strong correlations between A of *P.*

resinosa seedlings and microbial respiration on July 30 (r^2=0.250) and the fairly strong correlation between these parameters for hybrid poplars on June 25 (r^2=0.436) may have contributed to these results (Table 4.9).

On July 31, A was significantly lower in ammonium sulfate fertilized hybrid poplars than in hybrid poplars in AA100 treatments. On this sampling date, weak correlations of A with microbial respiration were observed (r^2= -0.006) (Table 4.9) along with similar foliar nutrient concentrations (Table 4.8). This could be due to differences in N invested in photosynthetic structures between treatments. Bown et al. (2010) found reduced photosynthesis of *Pinus radiata* seedlings fertilized with NH_4^+-N despite greater foliar N concentrations and attributed this to reduced investment of N in Rubisco. We suggest that future research addresses the biochemical mechanisms involved in photosynthesis to better understand the influence of arginine nutrition of photochemical processes.

Conclusions

We conclude that lack of significant differences in growth responses among species was likely due to tree age and stage of establishment. We hypothesized that there would be nutrient limitations in the rhizosphere as a result of microbial activity and increased binding of amino acids to cation exchange sites. We did not observe enhanced microbial activity or differences in cation exchange capacity in arginine treatments and suggest this is a result of the short duration of the study. Based on the results of this study and findings of previous research, we hypothesize that significant proportions of arginine are remaining bound in soils and that future studies should aim to elucidate the mechanisms by which this is occurring. Although microbial

respiration tended to be similar among treatments, greater ectomycorrhizal root colonization was observed in conifer seedlings in arginine and unfertilized control treatments, indicating that organic fertilization has the potential to increase the occurrence of beneficial microbes. Lack of significant differences in arbuscular mycorrhizae root colonization of hybrid poplars is suggested to be due to the dynamic tree-mycorrhizae relationship. Although correlations between microbial activity and photosynthetic rate were observed, it was suggested that foliar nutrient status, N form taken up by plants, and photosynthetic biochemical processes had a greater effect on the observed photosynthetic parameters. We suggest that future research address the biochemical limitations of photosynthesis under amino acid nutrition.

Acknowledgements

We would like to thank SweTree Technologies for providing the amino acid fertilizer used in this study. We would also like to thank Peterson's Riverview Nursery for donating the Fraser fir seedlings.

Tables and Figures

Table 4.1. Height and root collar diameter (RCD) growth response of *A. fraseri*, *P. resinosa*, and hybrid poplar under amino acid nutrition.

	Treatment	Height Growth (cm)	RCD Growth (mm)
Abies fraseri	Control	4.63±0.273 a	3.04±0.249 a
	AA100	4.69±0.427 a	2.78±0.239 a
	AA300	4.64±0.277 a	2.56±0.197 a
	AS100	4.15±0.270 a	2.62±0.203 a
	p-value	**P=0.905**	**P=0.423**
Pinus resinosa	Control	13.0±0.398 a	5.75±0.240 a
	AA100	12.8±0.474 a	6.34±0.285 a
	AA300	13.8±0.484 a	6.94±0.436 a
	AS100	14.3±0.411 a	6.38±0.192 a
	p-value	**P=0.060**	**P=0.055**
Hybrid poplar	Control	213.1±5.80 b	22.5±0.487 a
	AA100	235.6±4.55 a	23.7±0.546 a
	AA300	233.4±4.89 a	24.0±0.621 a
	AS100	241.0±4.82 a	23.7±0.460 a
	p-value	**P=0.001**	**P=0.169**

Values followed by the same letter are statistically similar according to Tukey's Honestly-Significant-Difference Test (α=0.05).

Treatments: Control= 0 kg N ha^{-1}, AA100=112 kg N ha^{-1}, AA300=336 kg N ha^{-1}, and AS100= Ammonium Sulfate 112 kg N ha^{-1}.

Table 4.2. Cation exchange capacity (CEC) (meq/100 g soil) of *A. fraseri*, *P. resinosa*, and hybrid poplar treatment plots.

	Treatment	May 21, 2011	September 13, 2011
Abies fraseri	Control	3.7±0.2 a	3.9±0.2 a
	AA100	4.3±0.5 a	3.8±0.3 a
	AA300	3.9±0.3 a	3.8±0.1 a
	AS100	3.9±0.2 a	3.6±0.2 a
	p-value	**P=0.632**	**P=0.791**
Pinus resinosa	Control	4.0±0.2 a	4.0±0.2 a
	AA100	4.0±0.2 a	4.4±0.1 a
	AA300	4.0±0.2 a	3.6±0.3 a
	AS100	4.0±0.3 a	3.8±0.7 a
	p-value	**P=1.00**	**P=0.459**
Hybrid poplar	Control	4.3±0.2 a	3.7±0.3 ab
	AA100	4.7±0.9 a	2.9±0.1 b
	AA300	4.2±0.2 a	3.9±0.2 a
	AS100	4.0±0.3 a	3.7±0.1 ab
	p-value	**P=0.774**	**P=0.032**

Values followed by the same letter are statistically similar according to Tukey's Honestly-Significant-Difference Test (α=0.05).

Treatments: Control= 0 kg N ha^{-1}, AA100=112 kg N ha^{-1}, AA300=336 kg N ha^{-1}, and AS100= Ammonium Sulfate 112 kg N ha^{-1}.

Table 4.3. Photosynthetic rate (A), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) of *Abies fraseri* in 2011.

		Abies fraseri		
	Treatment	30-Jun	26-Jul	17-Aug
A	Control	1.13±0.11 a	3.53±0.26 a	3.95±0.41 a
	AA100	0.538±0.13 b	3.41±0.39 a	5.21±0.62 a
	AA300	0.856±0.16 ab	3.49±0.45 a	5.25±0.55 a
	AS100	1.01±0.14 ab	3.12±0.36 a	3.81±0.50 a
	p-value	**P=0.042**	**P=0.774**	**P=0.123**
g_s	Control	0.0416±0.004 a	0.0360±0.003 a	0.0423±0.006 ab
	AA100	0.0288±0.004 a	0.0296±0.008 a	0.0629±0.012 ab
	AA300	0.0334±0.004 a	0.0352±0.004 a	0.0687±0.008 a
	AS100	0.0305±0.002 a	0.0281±0.004 a	0.0352±0.005 b
	p-value	**P=0.079**	**P=0.486**	**P=0.034**
C_i	Control	140.9±2.03 ab	190.8±3.0 a	189.6±7.3 ab
	AA100	160.3±4.6 a	190.1±7.7 ab	189.1±7.7 ab
	AA300	146.8±4.4 ab	179.8±4.6 ab	208.9±10.1 a
	AS100	130.8±6.6 b	171.7±4.6 b	169.5±5.9 b
	p-value	**P=0.003**	**P=0.040**	**P=0.024**

Units: Photosynthetic rate ($\mu mol\ m^{-2}\ s^{-1}$); Stomatal conductance ($mol\ H_2O\ m^{-2}\ s^{-1}$); Intercellular CO_2 concentration ($\mu mol\ CO_2\ mol\ air^{-1}$).

Values followed by the same letter are statistically similar according to Tukey's Honestly-Significant-Difference Test (α=0.05) within each sampling date.
Treatments: Control= 0 kg N ha^{-1}, AA100=112 kg N ha^{-1}, AA300=336 kg N ha^{-1}, and AS100= Ammonium Sulfate 112 kg N ha^{-1}.

Table 4.4. Photosynthetic rate (A), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) of *Pinus resinosa* in 2011.

		Pinus resinosa	
	Treatment	29-Jun	30-Jul
A	Control	1.97±0.26 ab	6.21±0.38 a
	AA100	1.64±0.17 b	5.54±0.39 a
	AA300	1.82±0.20 ab	5.44±0.33 a
	AS100	2.52±0.12 a	5.47±0.37 a
	p-value	**P=0.026**	**P=0.448**
g_s	Control	0.148±0.023 a	0.304±0.033 a
	AA100	0.157±0.023 a	0.227±0.021 a
	AA300	0.144±0.026 a	0.248±0.022 a
	AS100	0.202±0.011 a	0.252±0.018 a
	p-value	**P=0.246**	**P=0.166**
C_i	Control	134.8±2.6 a	237.6±3.3 a
	AA100	136.0±2.2 a	231.6±3.4 a
	AA300	131.7±1.4 a	233.2±1.3 a
	AS100	130.5±1.2 a	233.7±2.7 a
	p-value	**P=0.204**	**P=0.511**

Units: Photosynthetic rate ($\mu mol\ m^{-2}\ s^{-1}$); Stomatal conductance ($mol\ H_2O\ m^{-2}\ s^{-1}$); Intercellular CO_2 concentration ($\mu mol\ CO_2\ mol\ air^{-1}$).

Values followed by the same letter are statistically similar according to Tukey's Honestly-Significant-Difference Test (α=0.05) within each sampling date.
Treatments: Control= 0 kg N ha^{-1}, AA100=112 kg N ha^{-1}, AA300=336 kg N ha^{-1}, and AS100= Ammonium Sulfate 112 kg N ha^{-1}.

Table 4.5. Photosynthetic rate (A), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) of Hybrid poplar in 2011.

		Hybrid Poplar		
	Treatment	25-Jun	31-Jul	15-Aug
A	Control	5.94±0.83 a	11.7±0.93 ab	12.9±0.75 a
	AA100	6.23±0.82 a	13.2±0.79 a	10.1±0.82 a
	AA300	4.07±0.51 a	11.9±1.1 ab	9.16±1.4 a
	AS100	6.51±0.53 a	8.46±0.84 b	9.65±1.2 a
	p-value	**P=0.066**	**P=0.009**	**P=0.062**
g_s	Control	0.237±0.021 a	0.343±0.023 a	0.315±0.032 a
	AA100	0.210±0.021 a	0.365±0.030 a	0.229±0.041 a
	AA300	0.222±0.022 a	0.353±0.052 a	0.215±0.047 a
	AS100	0.198±0.012 a	0.343±0.032 a	0.224±0.036 a
	p-value	**P=0.538**	**P=0.963**	**P=0.214**
C_i	Control	331.2±7.5 a	304.8±7.6 a	287.8±7.3 a
	AA100	325.4±7.7 a	291.5±6.7 a	276.7±5.2 a
	AA300	340.2±5.8 a	300.7±7.5 a	291.5±8.5 a
	AS100	320.0±6.1 a	317.9±6.6 a	291.0±6.0 a
	p-value	**P=0.183**	**P=0.083**	**P=0.433**

Units: Photosynthetic rate (µmol m^{-2} s^{-1}); Stomatal conductance (mol H_2O m^{-2} s^{-1}); Intercellular CO_2 concentration (µmol CO_2 mol air^{-1}).

Values followed by the same letter are statistically similar according to Tukey's Honestly-Significant-Difference Test (α=0.05) within each sampling date.
Treatments: Control= 0 kg N ha^{-1}, AA100=112 kg N ha^{-1}, AA300=336 kg N ha^{-1}, and AS100= Ammonium Sulfate 112 kg N ha^{-1}.

Table 4.6. Foliar nutrient concentrations (mg/g) of *Abies fraseri* in 2011.

		Abies fraseri		
	Treatment	30-Jun	26-Jul	17-Aug
N	Control	22.3±0.90 a	14.3±0.50 b	12.2±1.5 b
	AA100	24.5±1.6 a	15.1±0.31 b	14.7±0.68 ab
	AA300	21.1±0.94 a	14.9±0.36 b	16.6±0.71 a
	AS100	24.5±1.6 a	16.8±0.23 a	15.0±1.1 ab
	p-value	**P=0.244**	**P=0.002**	**P=0.050**
P	Control	3.31±0.35 a	2.49±0.20 a	1.42±0.22 a
	AA100	3.77±0.30 a	2.36±0.22 a	1.59±0.14 a
	AA300	2.82±0.33 a	2.12±0.11 a	1.80±0.15 a
	AS100	3.53±0.24 a	2.06±0.22 a	1.85±0.25 a
	p-value	**P=0.223**	**P=0.368**	**P=0.370**
K	Control	12.1±1.0 a	10.7±0.58 ab	5.72±0.22 a
	AA100	13.0±0.90 a	10.4±0.58 b	6.62±0.50 a
	AA300	13.6±2.2 a	9.57±0.88 b	6.81±0.47 a
	AS100	14.8±1.4 a	12.7±0.47 a	6.41±0.56 a
	p-value	**P=0.608**	**P=0.014**	**P=0.396**
Ca	Control	1.0±0.13 a	1.52±0.13 a	0.95±0.12 a
	AA100	0.92±0.09 a	1.52±0.10 a	1.0±0.06 a
	AA300	0.90±0.14 a	1.80±0.11 a	1.21±0.11 a
	AS100	0.87±0.13 a	1.48±0.09 a	0.84±0.11 a
	p-value	**P=0.892**	**P=0.158**	**P=0.104**
Mg	Control	1.52±0.12 a	1.82±0.14 a	0.92±0.13 a
	AA100	1.36±0.15 a	1.88±0.10 a	0.96±0.06 a
	AA300	1.50±0.14 a	1.62±0.14 a	1.04±0.11 a
	AS100	1.15±0.15 a	1.49±0.14 a	0.70±0.05 a
	p-value	**P=0.231**	**P=0.166**	**P=0.120**
Mn	Control	0.39±0.04 a	0.39±0.04 ab	0.27±0.03 a
	AA100	0.40±0.05 a	0.46±0.04 a	0.33±0.04 a
	AA300	0.50±0.08 a	0.44±0.02 a	0.33±0.03 a
	AS100	0.40±0.05 a	0.30±0.04 b	0.22±0.02 a
	p-value	**P=0.470**	**P=0.230**	**P=0.144**

Values followed by the same letter are statistically similar according to Tukey's Honestly-Significant-Difference Test (α=0.05) within each sampling date.

Treatments: Control= 0 kg N ha^{-1}, AA100=112 kg N ha^{-1}, AA300=336 kg N ha^{-1}, and AS100= Ammonium Sulfate 112 kg N ha^{-1}.

Table 4.7. Foliar nutrient concentrations (mg/g) of *Pinus resinosa* in 2011.

		Pinus resinosa	
	Treatment	29-Jun	30-Jul
N	Control	13.2±0.47 a	11.7±0.24 ab
	AA100	13.9±0.30 a	12.8±0.31 a
	AA300	14.0±0.45 a	12.5±0.27 ab
	AS100	14.0±0.53 a	11.5±0.40 b
	p-value	**P=0.492**	**P=0.011**
P	Control	2.66±0.12 a	1.33±0.05 a
	AA100	2.89±0.07 a	1.32±0.03 a
	AA300	2.73±0.11 a	1.29±0.03 a
	AS100	2.93±0.14 a	1.32±0.04 a
	p-value	**P=0.285**	**P=0.828**
K	Control	10.2±0.42 a	7.86±0.22 a
	AA100	10.4±0.43 a	7.67±0.17 a
	AA300	9.37±0.28 a	7.93±0.24 a
	AS100	9.97±0.45 a	7.80±0.23 a
	p-value	**P=0.351**	**P=0.850**
Ca	Control	0.44±0.03 a	0.62±0.03 ab
	AA100	0.43±0.04 a	0.70±0.01 a
	AA300	0.47±0.02 a	0.64±0.03 ab
	AS100	0.54±0.03 a	0.56±0.03 b
	p-value	**P=0.101**	**P=0.011**
Mg	Control	1.04±0.05 b	0.92±0.03 a
	AA100	1.25±0.06 a	0.89±0.02 a
	AA300	1.19±0.03 ab	0.87±0.02 a
	AS100	1.18±0.04 ab	0.85±0.01 a
	p-value	**P=0.024**	**P=0.147**
Mn	Control	0.09±0.01 ab	0.042±0.01 b
	AA100	0.08±0.00 b	0.028±0.01b
	AA300	0.10±0.01 ab	0.045±0.01 ab
	AS100	0.13±0.01 a	0.084±0.01 a
	p-value	**P=0.043**	**P=0.003**

Values followed by the same letter are statistically similar according to Tukey's Honestly-Significant-Difference Test (α=0.05) within each sampling date.

Treatments: Control= 0 kg N ha^{-1}, AA100=112 kg N ha^{-1}, AA300=336 kg N ha^{-1}, and AS100= Ammonium Sulfate 112 kg N ha^{-1}.

Table 4.8. Foliar nutrient concentrations (mg/g) of Hybrid poplar in 2011.

		Hybrid Poplar		
	Treatment	25-Jun	31-Jul	15-Aug
N	Control	26.0±1.5 b	48.4±2.3 a	42.9±3.1 a
	AA100	28.7±1.1 b	46.0±2.7 a	40.1±2.9 a
	AA300	35.7±1.3 a	43.2±1.6 a	41.4±1.4 a
	AS100	40.3±1.5 a	43.2±2.3 a	40.6±2.4 a
	p-value	**P=0.000**	**P=0.262**	**P=0.878**
P	Control	4.56±0.29 b	6.28±0.20 a	6.28±0.42 a
	AA100	4.37±0.21 b	5.95±0.35 a	6.38±0.40 a
	AA300	5.58±0.32 a	5.46±0.32 a	6.42±0.30 a
	AS100	5.79±0.24 a	5.86±0.21 a	6.77±0.25 a
	p-value	**P=0.000**	**P=0.243**	**P=0.796**
K	Control	31.1±1.4 a	33.1±1.8 a	32.6±0.42 b
	AA100	30.5±1.0 a	33.4±1.0 a	34.4±0.77 a
	AA300	28.7±0.82 a	32.1±1.1 a	35.4±0.38 ab
	AS100	32.0±0.78 a	33.3±0.62 a	36.6±0.80 a
	p-value	**P=0.123**	**P=0.871**	**P=0.002**
Ca	Control	2.59±1.0 a	8.42±0.42 a	3.27±0.34 a
	AA100	2.99±0.64 a	6.92±0.62 a	3.25±0.36 a
	AA300	4.20±0.54 a	7.86±0.32 a	3.22±0.60 a
	AS100	4.23±0.75 a	7.50±0.71 a	3.52±0.31 a
	p-value	**P=0.444**	**P=0.298**	**P=0.964**
Mg	Control	6.95±0.44 a	3.71±0.14 a	3.58±0.10 a
	AA100	6.16±0.42 ab	3.57±0.16 a	3.76±0.10 a
	AA300	4.42±0.24 c	3.80±0.17 a	3.86±0.11 a
	AS100	5.41±0.37 bc	3.57±0.13 a	3.95±0.11 a
	p-value	**P=0.000**	**P=0.665**	**P=0.111**
Mn	Control	0.10±0.02 a	0.02±0.01 a	0.08±0.02 a
	AA100	0.14±0.01 a	0.05±0.02 a	0.03±0.02 ab
	AA300	0.04±0.009 b	0.02±0.01 a	0.01±0.01 ab
	AS100	0.10±0.01 a	0.02±0.01 a	0.01±0.01 b
	p-value	**P=0.000**	**P=0.056**	**P=0.025**

Values followed by the same letter are statistically similar according to Tukey's Honestly-Significant-Difference Test (α=0.05) within each sampling date.
Treatments: Control= 0 kg N ha^{-1}, AA100=112 kg N ha^{-1}, AA300=336 kg N ha^{-1}, and AS100= Ammonium Sulfate 112 kg N ha^{-1}.

Table 4.9. Pearson's correlation between microbial respiration and photosynthetic rate for *Abies fraseri*, *Pinus resinosa*, and Hybrid poplar.

	Sampling Dates		
Species	**June**	**July**	**August**
Abies fraseri	-0.099	-0.074	0.491
P-value	**P=0.716**	**P=0.792**	**P=0.053**
Pinus resinosa	-0.285	0.250	N/A
P-value	**P=0.370**	**P=0.351**	**N/A**
Hybrid Poplar	0.436	-0.006	0.073
P-value	**P=0.092**	**P=0.981**	**P=0.788**

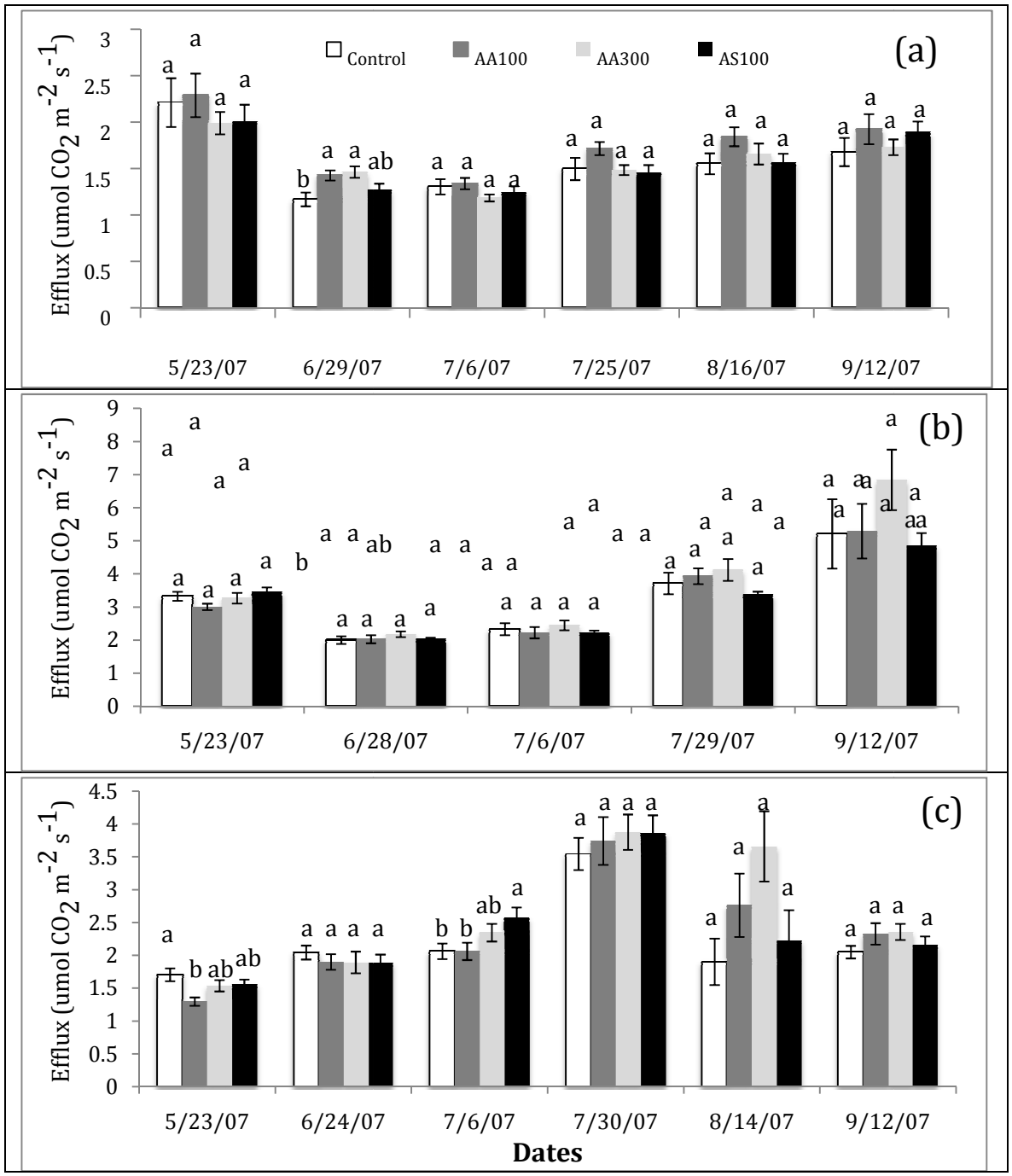

Figure 4.1. Microbial respiration in treatment plots of (a) *Abies fraseri* (p=0.671), (b) *Pinus resinosa* (p=0.361), and (c) Hybrid poplar (p=0.008).

Values followed by the same letter are statistically similar according to Tukey's Honestly-Significant-Difference Test (α=0.05) within each sampling date.
Treatments: Control= 0 kg N ha^{-1}, AA100=112 kg N ha^{-1}, AA300=336 kg N ha^{-1}, and AS100= Ammonium Sulfate 112 kg N ha^{-1}.

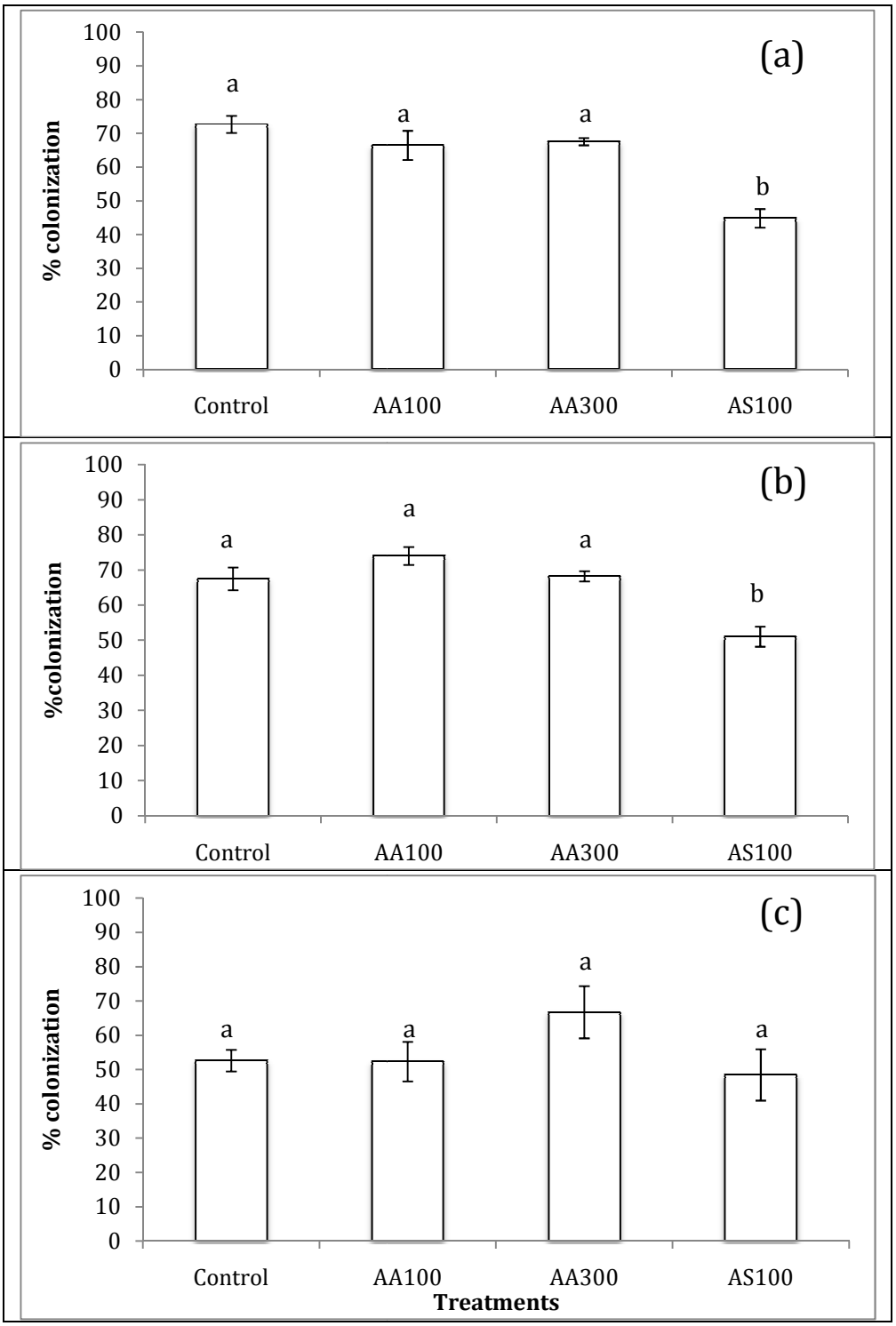

Figure 4.2. Percent colonization of ectomycorrhizae on roots of (a) *Abies fraseri* (p=0.001) and (b) *Pinus resinosa* (p=0.000) and of arbuscular mycorrhizae on roots of (c) Hybrid poplar (p=0.255). Values followed by the same letter are statistically similar according to Tukey's Honestly-Significant-Difference Test (α=0.05). Treatments: Control= 0 kg N ha^{-1}, AA100=112 kg N ha^{-1}, AA300=336 kg N ha^{-1}, and AS100= Ammonium Sulfate 112 kg N ha^{-1}.

REFERENCES

REFERENCES

Amaya-Carpio L, FT Davies Jr, T Fox, and C He (2009) Arbuscular mycorrhizal fungi and organic fertilizer influence photosynthesis, root phosphatase activity, nutrition, and growth of *Ipomoea carnea* spp. *fistulosa*. Photosynthetica 47: 1-10.

Andresen LC, A Michelsen, S Jonasson, C Beier, and P Ambus (2009) Glycine uptake in heath plants and soil microbes respond to elevated temperature, CO_2, and drought. Acta Oecologica 35: 786-796.

Bown HE, MS Watt, PW Clinton, EG Mason (2010) Influence of ammonium and nitrate supply on growth, dry matter partitioning, N uptake and photosynthetic capacity of *Pinus radiata* seedlings. Trees 24: 1097-1107.

Brundrett MC (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320:37-77.

Dannenmann M, J Simon, R Gasche, J Holst, PS Naumann, I Kogel-Knabner, H Knicker, H Mayer, M Schloter, R Pena, A Polle, H Rennenberg, and H Papen (2009) Tree girdling provides insight on the role of labile carbon in nitrogen partitioning between soil microorganisms and adult European beech. Soil Biology and Biochemistry 41: 1622-1631.

Dickie IA and PB Reich (2005) Ectomycorrhizal fungal communities at forest edges. Journal of Ecology 93: 244-255.

Efthimiadou, A, D Bilalis, A Karkanis, and B Froud-Williams (2010) Combined organic/inorganic fertilization enhance soil quality and increased yield, photosynthesis and sustainability of sweet maize crop. Aust. J. Crop Sci. 4: 722-729.

Epstein E and A Bloom (2005) Mineral Nutrition of Plants: Principles and Perspectives. Sunderland: Sinauer Associates, Inc. (2^{nd} edition).

Friedel JK and D Gabel (2001) Nitrogen pools and turnover in arable soils under different durations of organic farming I: Pool sizes of total soil nitrogen, microbial biomass nitrogen, and potentially mineralizable nitrogen. J. Plant Nutr. Soil Sci. 164:415-419.

Gallet-Budynek A, E Brozostek, VL Rodgers, JM Talbot, S Hyzy, and AC Finzi (2009) Intact uptake by northern hardwood conifer trees. Oecologica 160:129-138.

Gasparatos D, PA Roussos, E Christofilopoulou and C Haidouti (2011) Comparative effects of organic and conventional apple orchard management on soil chemical properties and plant mineral content under Mediterranean climate conditions. Journal of Soil Science and Plant Nutrition 11:105-117.

Ge T, S Song, P Roberts, DL Jones, D Huang, and K Iwasaki (2009) Amino acid as a nitrogen source for tomato seedlings: The use of dual-labeled (^{13}C, ^{15}N) glycine to test for direct uptake by tomato seedlings. Environ. Exp. Bot. 66: 357-361.

Giovannetti M and B Mosse (1980) An evaluation of techniques for measuring vesicular arbuscular vesicular mycorrhizal infection in roots. New Phytologist 84: 489-500.

Gonod LV, DL Jones, and C.Chenu. (2006) Sorption regulates fate of the amino acids lysine and leucine in soil aggregates. Eur. J. Soil Sci. 57: 320-329.

Gryndler M, J Larsen, H Hrselova, V Rezavoa, H Grynderlerova, and J Kubat (2006) Organic and mineral fertilization, respectively, increase and decrease the development of external mycelium of arbuscular mycorrhizal fungi in a long-term field experiment. Mycorrhiza 16:159-166.

Jones DL (1999) Amino acid biodegradation and its potential effects of organic nitrogen capture by plants. Soil Biology and Biochemistry 31: 613-622.

Jones DL and PR Darrah (1994) Amino-acid influx at the soil-root interface of *Zea mays* L. and its implications in the rhizosphere. Plant and Soil 163:1-12.

Jones DL, D Shannon, T Junvee-Fortune and JF Farrar (2005) Plant capture of free amino acids is maximized under high soil amino acid concentrations. Soil Biology and Biochemistry 37: 179-181.

Karpati AS, SN Handel, J Dighton, and TR Horton (2011) *Quercus rubra*-associated ectomycorrhizal fungal communities of disturbed urban sites and mature forests. Mycorrhiza 21:537-547.

Khasa PD, L Sigler, P Chakravarty, BP Dancik, L Erickson and D McCurdy (2001) Effect of fertilization on growth and ectomycorrhizal development of container-grown and bare-root nursery conifer seedlings. New Forests 22: 179-197.

Kielland K (1995) Landscape patterns of free amino acids in arctic tundra soils. Biogeochemistry. 31: 85-98.

Koske RW and JN Gemma (1989) A modified procedure for staining roots to detect VA mycorrhizas. Mycor. Res. 92: 485-505.

Mae T (1997) Physiological nitrogen efficiency in rice: Nitrogen utilization, photosynthesis, and yield potential. Plant and Soil 196: 201-210.

Metcalfe RJ, J. Nault, and BJ Hawkins (2011) Adaptations to nitrogen form: comparing inorganic nitrogen and amino acid availability and uptake by four temperate forest plants. Can J For Res 41:1626-1637.

Moyano FE, WL Kutsch, and C Rebmann (2008) Soil respiration fluxes in relations to photosynthetic activity in broad-leaf and needle-leaf forest stands. Agriculture and Forest Meteorology 148: 135-143.

Näsholm T, A Ekblad, A Nordin, R Giesler, M Högber, and P Högber (1998) Boreal forest plants take up organic nitrogen. Nature 392:914-916.

Näsholm T, K Huss-Danell, and P Hogberg (2000) Uptake of organic nitrogen in the field by four agriculturally important plant species. Ecology 81: 1155-1161.

Näsholm T, K Kielland, and U Ganeteg (2009) Uptake of organic nitrogen by plants. New Phytol. 182: 31-48.

Nikiema P, P Nzokou, and D Rothstein (2011) 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, United States of America. New Forests. DOI 10.1007/s11056-011-9274-8.

Nzokou P, AR Wilson, D Güney and S Kulaç (2012) Amino acid fertilization of Fraser fir (*Abies fraseri*), red pine (*Pinus resinosa*), and hybrid poplar: Effect on foliar chemistry and nutrient dynamics. Submitted to HortScience.

Öhlund J and T Näsholm (2001) Growth of conifer seedlings on organic and inorganic nitrogen sources. Tree Physiology 21: 1319-1326.

Öhlund J and T Näsholm (2002) Low nitrogen losses with a new source of nitrogen for cultivation of conifer seedlings. Environ. Sci. Technol. 36: 4854-4859.

Persson J and T Näsholm (2001) Amino acid uptake: a widespread ability among boreal forest plants. Ecology Letters 4: 434-438.

Raab TK, DA Lipson, and RK Monson (1996) Non-mycorrhizal uptake of amino acids by roots of the alpine sedge *Kobresia myouroides*: implications for the alpine nitrogen cycle. Oecologica 108: 488-494.

Reeve JR, JL Smith, L Carpenter-Boggs, and JP Reganold (2008) Soil-based cycling and differential uptake of amino acids by three species of strawberry (*Fragaria* spp.) plants. Soil Biology and Biochemistry 40: 2547-2552.

Rice CW, K Fabrizzi, and P White (2007) Benefits of soil organic carbon to physical, chemical, and biological properties of soil. In JM Kimble, CW Rice, and D Reed (Eds), *Soil carbon mananagement: Economic, environmental and societal benefits* (pp. 155-162). Boca Raton: CRC Press.

Ross D (1995) Recommended methods for determining soil cation exchange capacity. P. 62–69. In J.T. Sims and A. Wolf (ed.) Recommended soil testing procedures for the northeastern United States. Northeast Regional Bull. 493. Agric. Exp. Stn., Univ. of Delaware, Newark, DE.

Rothstein DE (2010) Effects of amino-acid chemistry and soil properties on the behavior of free amino acids in acidic forest soils. Soil Biology and Biochemistry 42: 1743-1750.

Sauheitl L, B Glaser, and A Weigelt (2009) Uptake of intact amino acids depends on soil amino acid concentrations. Environ. Exp. Bot. 66: 145-152.

Schiavoni EA, MC Alves, ZM de Souza, FG Costa (2011) Influence of organic-mineral fertilization of an oxisol on soil chemical properties and *Bracharia brizantha* production. Revista Braseileira de Ciêcia do Solo 35: 2219-2226.

Schimel JP and J Bennett (2004) Nitrogen mineralization: Challenges of a changing paradigm. Ecology 85: 591-602.

Schobert C, W Kockenberger, and E Komor (1988) Uptake of amino acids by plants from soil: A comparative study with castor bean seedlings grown under natural and axenic soil conditions. Plant Soil 109: 181-188.

Thomas RB, JD Lewis, and BR Strain (1994) Effects of leaf nutrient status on photosynthetic capacity in loblolly pine (*Pinus taeda* L.) seedlings grown in elevated atmospheric CO_2. Tree Physiology 14: 947-960.

Tirol-Padre A, JK Ladha, AP Regmi, AL Bhandari, and K Inbushi (2007) Organic amendments affect soil parameters in two long-term rice-wheat experiments. Soil Soc. Am. J. 71:442-452.

Wilson AR, P Nzokou, D Güney and S Kulaç (2012) Growth response and nitrogen use physiology of Fraser fir (*Abies fraseri*), red pine (*Pinus resinosa*), and hybrid poplar under amino acid nutrition. New Forests doi: 10.1007/s11056-012-9317-9.

Wilson AR and P Nzokou (2012) Biomass allocation and nutrient use efficiency of Fraser fir and Red pine seedlings in response to amino acid fertilization. Submitted to Tree Physiology.

Yamagata M and N. Ae (1996) Nitrogen uptake response of corns to organic nutrition. Soil Sciences and Plant Nutrition 42:389-394.

Yu, C, DG Lv, SJ Qin, L Yang, HY Ma, and GC Liu (2010) Changes in photosynthesis, fluorescence, and nitrogen metabolism of hawthorn (*Crataegus pinnatifida*) in response to exogenous glutamic acid. Photosynthetica 48: 339-347.

Zak DR, WE Holmes, NW MacDonald, and KS Pregitzer (1999) Soil temperature, matric potential, and the kinetics of microbial respiration and nitrogen mineralization. Soil Sci. Soc. Am. J. 63:575-584.

CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH

CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH

This research studied the effects of amino acid nutrition on soil nutrient dynamics, tree-microbial interactions, and tree physiology of short rotation trees grown in production systems. During the establishment phase, amino acids appeared to be limiting to trees as evidenced by the fact that arginine applications two to three times greater than that of ammonium sulfate were necessary to achieve similar growth and foliar N concentrations. This result was attributed to nutrient limitations induced by arginine binding to soil or being immobilized in microbial biomass. Arginine applications did not contribute to soil mineral N pools and nitrate content in leachate tended to be similar to that of unfertilized control treatments, suggesting that rapid mineralization of arginine was not occurring. Another possible explanation is the rapid assimilation of inorganic nitrogen by plants and microbes or binding of arginine to soil cation exchange sites.

Subsequent studies confirmed that arginine was not severely limiting to trees. This was evidenced by similar biomass allocation and nutrient partitioning patterns among conifer seedlings under different nutrient applications (varying rates of arginine versus ammonium sulfate versus unfertilized control). Biomass partitioning ratios also supported this hypothesis. Nutrient use efficiency of seedlings under ammonium sulfate fertilization tended to be greater than that of arginine treatments. This finding was suggested to be due in part by nutrient limitations in ammonium sulfate treatments caused by nutrient leaching and reduced cation availability due to pH changes with ammonium uptake, but also attributed to different physiological effects of N form assimilated. Results suggested that arginine was functioning as a slow-release fertilizer as chemical and biological soil properties were regulating nutrient release

over time, thus nutrient limitations experienced by the tree were merely transient. This study also supported the hypothesis that significant proportions of arginine are being maintained in soils.

The last portion of this research aimed to address this hypothesis, but found no enhancement of cation exchange capacity or microbial respiration in amino acid fertilized treatments. It is suggested that the duration of the study was too short to observe these changes and that cation exchange sites are likely underestimating that amount of arginine bound to soils. We did observe improved ectomycorrhizal colonization of conifers under arginine nutrition, indicating that this nutrient source has potential to promote beneficial soil microbes. Similar arbuscular mycorrhizae colonization of hybrid poplar roots was attributed to the dynamic symbiotic relationship. While microbial activity had somewhat strong correlations with photosynthetic rate, it is unlikely that this is the only factor influencing photochemical processes. It is suggested that this factor in conjunction with nutrient availability and especially limitations in biochemical processes yielded these results.

We conclude that arginine has potential to be a viable organic N source used in short rotation tree production systems. However, careful management is needed during the season of transplantation because arginine has to be applied at rates two to three times greater than the inorganic control to achieve similar growth and foliar N. We suggest that further research continue to elucidate the chemical and biological factors influencing the fate of arginine in soils to understand the proportion being maintained in the production system. Our results indicated that it is likely that some of the applied arginine exists in soils in a form that we were unable to detect. We also propose that experimentation with labeling arginine would be beneficial in confirming the form of N that is used by trees. We also suggest that greater understanding of

arginine nutrition on photosynthetic biochemical processes would lend great insight and predictability in evaluating its potential to improve tree productivity because it would provide a knowledge-base of its affects on physiological processes. Long-term studies evaluating the affects of amino acids on soil biological and chemical characteristics would be beneficial for understanding the benefits of long-term amino acid applications. Evaluating long-term effects would aid in determining the economic viability of amino acid fertilizers by providing the missing link needed to conduct cost-benefit analysis.

The research conducted greatly contributes to the existing body of knowledge on organic fertilization in production systems as it addresses key physiological responses and soil dynamics under organic fertilization that have not been extensively studied.