2016

The role of amino acids in the nitrogen cycle of peatlands

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THE ROLE OF AMINO ACIDS IN THE NITROGEN CYCLE OF PEATLANDS

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A THESIS
Submitted in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE
In Environmental Engineering

MICHIGAN TECHNOLOGICAL UNIVERSITY
2016

This thesis has been approved in partial fulfillment of the requirements for the Degree of MASTER OF SCIENCE in Environmental Engineering.

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Contents

Preface

Chapter 2 is intended for publication as Scarpelli T, Doskey PV, Kane E, Lilleskov E (2016). Amino acids in pore water of peat. The manuscript was written by myself and reviewed by Dr. Paul Doskey. I completed the laboratory work and data analysis. Input and guidance on laboratory work and data analysis was provided by Dr. Paul Doskey. Input on data analysis and design of initial field work was provided by Dr. Evan Kane and Dr. Erik Lilleskov.

Acknowledgments

I would like to thank my advisor, Dr. Paul Doskey, for providing advice and support throughout the duration of my project. I would also like to thank my committee member, Dr. Noel Urban, for providing advice on developing my amino acid model, and my committee member Evan Kane and the research scientists involved with the PEATcosm project who provided me with a wonderful base of knowledge on peatlands.

Funding for the research that went in to developing the thesis was provided in part by the McIntire Stennis Grant 1001685 from the USDA National Institute of Food and Agriculture. The PEATcosm project mentioned in Chapter 2 was funded by NSF DEB grant 1146149. The research for the thesis was also supported in part by the GRO Fellowship Assistance Agreement no. MA91756201-0 awarded by the U.S. Environmental Protection Agency (EPA). It has not been formerly reviewed by the EPA. The views expressed in this publication are solely those of the authors, and EPA does not endorse any products or commercial services mentioned in this publication.

Abstract

Amino acids can provide both a nitrogen and carbon source for peatland ecosystems. The environmental conditions of peatlands often limit nitrogen availability, and the slow decomposition rates lead to large stores of carbon in these ecosystems. Future release of carbon from peatlands in response to climate change may be impacted by nitrogen limitation. Amino acid availability for microbial and plant uptake will also likely be impacted by climate change and may influence the response of the peatland carbon cycle to climate change.

In Chapter 2, the experimental procedures and resulting observations for determination of amino acid and inorganic nitrogen species concentrations in peat mesocosms are provided. The current study considers the role of amino acids as a nitrogen source in peatlands and the possible implications of seasonal amino acid variability. The total free amino acid (TFAA) concentration for peats ranged from 0-2.3 μM, and leucine was the primary contributor for all peats. Free amino acid concentrations showed significant seasonal variation for the three sampling periods considered. The TFAA concentrations were greatest in the spring and least in the fall. The springtime maxima and summer decrease in concentrations observed for the peat mesocosms were captured in the modeling study discussed in Chapter 3 of the thesis; however, the fall minima observed for the peat mesocosms were not captured by the model. The dominance of sedge or ericaceous shrub plant types did not significantly impact the free amino acid pool, possibly indicating that microbial processes rather than plant uptake control the dynamics

of TFAA pool. The observed ammonium concentrations were generally much greater than the amino acid and nitrate concentrations. Amino acids may still be considered an important nitrogen source even at low concentrations if the low concentrations are a result of rapid amino acid turnover.

Chapter 3 presents a Simulink® model of amino acid and ammonium cycling in boreal peatlands. Current literature on amino acid cycling in northern ecosystems was used to track the total amino acid concentration in peatland pore water. Model results show low amino acid concentrations with depletion of amino acids and ammonium in mid-summer, at the peak of the growing season. The model results show greater amino acid concentrations than observed for peat mesocosm pore water. The model was shown to be sensitive to microbial process rates which is likely contributing to model uncertainty. The thesis discusses the need for further research on peatland microbial dynamics and the seasonal cycle of the TFAA pool in order to improve modeling of amino acid cycling in peatlands.

Chapter 1
Introduction

Nitrogen in peatlands

Nitrogen is often a limiting nutrient in wetland environments, so it is important to understand the biogeochemical cycling of nitrogen species in these anaerobic soils. The availability of nitrogen will influence the productivity of the microbial, fungal, and plant communities that will compete for organic and inorganic nitrogen sources. Plants living in these environments have adapted to flooding, and the soils are often anoxic for long periods of time. Generally, as ecosystems develop, they have greater storage of nutrients, and internal cycling increases (Odum and Barrett, 2005). Wetlands at different points of development will have different levels of internal cycling and nutrient storage capacity. The nitrogen turnover in a forested bog was observed at 66 kg ha^{-1} while nitrogen inputs were only 14.6 kg ha^{-1} (Urban and Eisenreich, 1987). Peatland bogs have significant internal nitrogen cycling because they lack large external nitrogen inputs, and environmental conditions like flooded soils and cooler temperatures inhibit microbial processing of organic matter.

Studies of forest soils have shown competition between microbial and plant communities for ammonium (NH_4^+), nitrate (NO_3^-) and organic nitrogen in the form of amino acids. For field and laboratory studies of ombrotrophic bogs with historically low nitrogen deposition, there have been observations of increased bryophyte and vascular plant growth in response to increased atmospheric nitrogen deposition and observations

of increased nitrogen absorption for both plant types at sites with greater nitrogen inputs (Aerts et al., 1992; Tomassen et al., 2003; Bragazza et al., 2004). Inorganic nitrogen is a bioavailable nitrogen source; however, when inorganic nitrogen is limited, organic nitrogen may also provide an additional source of readily available nitrogen. The pool of organic matter in soils has a labile and a recalcitrant fraction, and it is the labile, dissolved organic nitrogen (DON) fraction that is directly accessible by plants and microbes (Neff et al., 2003).

Climate change is expected to affect carbon and nitrogen cycles in peatlands. If warmer temperatures and lower water tables allow an increase in microbial productivity of peatlands, the large pools of carbon that are now stored might be released into the atmosphere. Studies have also shown that water table position impacts plant community composition (Potvin et al. 2015). Nitrogen availability may limit the ability of the plant and microbial communities to respond to changing environmental conditions; however, alteration of peatland plant and microbial communities may also impact the cycling of organic nitrogen, leading to feedback between the carbon and nitrogen cycle. Organic sources of nitrogen may be able to meet increased demands for nitrogen as increased decomposition releases amino acids stored in proteinaceous material.

Major N species

The nitrogen pool of soils is made up of inorganic and organic nitrogen species. The principal inorganic nitrogen species are NH_4^+, NO_3^-, and nitrite (NO_2^-), while proteinaceous material constitutes most of the organic nitrogen pool. The contribution of

inorganic and organic nitrogen to total soil nitrogen varies by climatic region with tropical areas dominated by organic nitrogen and colder regions dominated by NH_4^+ (Sowden et al. 1977).

In waterlogged soils, NH_4^+ is typically found at greater concentrations than NO_3^- because the anoxic conditions inhibit nitrification. Although NO_3^- and NO_2^- are typically at low concentrations in flooded soils, their concentrations may vary depending on wetland hydrology and the location within the soil profile. The soluble nitrogen pool of an Arctic salt marsh was principally composed of NH_4^+ with soluble nitrogen concentrations generally low at the start of the growing season and greater towards the end of the growing season (Henry and Jefferies, 2002). Mean growing season NH_4^+ and NO_3^- concentrations for the salt marsh were 55-160 μM and 10-31 μM, respectively. Levels of NO_3^- were 70-570 mg N m^{-2} in a Minnesota swamp, showing maximum concentrations in spring and fall (Zak & Grigal, 1991). The swamp NH_4^+ concentrations were similar to the observed NO_3^- concentrations, and the maximum NH_4^+ concentrations were observed in mid-summer and early fall. A similar pattern was also observed in a Colorado subalpine fen where NO_3^- dominated the available nitrogen pool from spring to early summer, NH_4^+ dominated later in summer, and amino acids began to dominate in early fall (Raab et al., 1999). In shrub and heath tundra ecosystems, the approximate contributions of NH_4^+, NO_3^-, and amino acids to total available nitrogen were 75%, <3%, and 25%, respectively (Clemmensen et al., 2008). A similar pattern was observed for moist arctic tundra where NH_4^+ dominated with lesser amounts of amino acids and very low NO_3^- contributions to available nitrogen (Nordin et al., 2004).

Peptides and amino acids make up a majority of the organic nitrogen in soils (Friedel and Scheller, 2002). Proteins are made up of amino acids linked in peptide chains, while free amino acids are monomers that can be taken up more easily by microbes and plants as nutrient sources. Organic acids constituted 75-83% of the pool of organic acids, free amino acids, and simple carbohydrates in pore water of an Arctic fen (Ström et al., 2012). Concentrations of free amino acids in alpine and tundra ecosystems have been measured; however, few quantitative measurements have been made of the amino acid pool for peatlands. The average concentration of free amino acids in a grazed Arctic salt marsh was 32-45 μM (Henry and Jefferies, 2002). The seasonal mean concentration of asparagine in pore water of an Arctic fen, which constituted 72-82% of the free amino acid pool, was 2 μM for sites of high and low sedge coverage (Ström et al., 2014). Undetectable amino acid concentrations were found in a subalpine fen at the start of the growing season with concentrations reaching 15-20 μM in late summer and early fall (Raab et al., 1999).

Humic material, which contains insoluble nitrogen and amino acids bound in peptides, is abundant in peatlands; however, the free amino acid pool is relatively small (Isirimah and Keeney, 1973; Sowden et al. 1978; Sposito, 1994). Peptide material contributed 27% of the organic matter at a depth of 650-660 cm in peat (Knicker, 2011). A majority of the amino acids in bogs are associated with humic acids deep within the peat with low to undetectable concentrations of free amino acids in the surface layers (Swain et al. 1959). The wet and cool conditions in peatlands might slow microbial processing of the organic nitrogen pool and limit the concentrations of free amino acids that are an important source of bioavailable nitrogen to the ecosystem.

The amino acids commonly observed in soil organic matter generally reflect the composition of plant litter and were shown to be relatively consistent for different soil types (Friedel and Scheller, 2002). The dominant amino acids making up organic matter for various surface soil horizons, each contributing greater than 5% (molar) of the amino acid pool, were glycine, serine, aspartate, glutamate, threonine, alanine, valine, proline, and leucine (Friedel and Scheller, 2002). Humic material is generally dominated by neutral amino acids bound in peptides (Sposito, 1994). The amino acid composition of peat and humic acids from a Minnesota bog was similar and included glycine, aspartate, glutamate, threonine, alanine, valine, and leucine (Swain et al. 1959). The commonly observed free amino acids in tundra (Kielland, 1995; Nordin et al., 2004; Weintraub and Schimel, 2005), dry meadow (Raab et al. 1999), and northern forest (Yu et al. 2002; Werdin-Pfisterer et al. 2009) soils were glycine, serine, aspartate, glutamate, threonine, alanine, and arginine. Asparagine was the principal free amino acid in an Arctic fen (Ström et al. 2014), and alanine contributions to the free amino acid pool were greatest in an Arctic salt marsh where the composition was similar to feces of geese that grazed in the region (Henry and Jefferies, 2002). Single amino acids were also prevalent during various periods of the growing season in shrub and heath tundra ecosystems (Clemmenson et al., 2008).

External N sources

There is variability in the major nutrient source for wetlands. Nutrient sources are often related to wetland hydrology and most wetlands are characterized in this manner.

Peatlands can be ombrotrophic and receive most of their nutrients from atmospheric deposition or minerotrophic and receive nutrient inputs from inflowing streams or groundwater. Nitrate inputs are often connected with the hydrologic dynamics of wetlands because of its mobility in soils (Jones et al., 2005). Molecular nitrogen is used by nitrogen fixing bacteria in wetlands; however, the process is generally a minor input pathway for nitrogen (Barraquio et al., 1982). Nitrogen fixation in a forested bog was observed at 0.0-0.7 kg ha^{-1} y^{-1} while the influx of nitrogen from atmospheric deposition was 10.4 kg ha^{-1} y^{-1} (Urban and Eisenreich, 1987). Atmospheric inputs and internal cycling typically regulate the nutrient pools in bogs.

Organic nitrogen species have been identified as a major component of nitrogen in precipitation with amino acids and peptides contributing 20-50% of water soluble nitrogen (see, e.g., review by Cornell, 2011). Serine, glycine, and alanine are derived from both anthropogenic and biogenic sources and are the most common amino acids that occur in the atmosphere (Gorzelska and Galloway, 1990; Gorzelska et al., 1992; Neff et al., 2002; Mace et al. 2003; Cape et al. 2011). Various free amino acids have been observed in rainwater and may provide a nitrogen source for wetlands through wet deposition (Gorzelska et al., 1992; Mace et al., 2003; Altieri et al., 2009). Amino acids have also been observed in particles, fog, and clouds, and thus, dry deposition is also likely to be an important atmospheric source (Zhang and Anastasio, 2001; Zhang and Anastasio, 2003). Atmospheric deposition is also an important source of inorganic nitrogen to wetlands. Dry and wet deposition of NO_3^- is spatially variable and often depends on levels of NO_x and photochemical activity (Press et al., 1986). Rates of combined atmospheric deposition of NO_3^-, NH_4^+ and organic nitrogen to North American

wetlands are 0.55- 1.21 g N m^{-2} y^{-1} with the range of reported NO_3^-, NH_4^+, and organic total nitrogen contributions at 0.17-0.96, 0.14-0.40 and 0.05-0.47 g N m^{-2} y^{-1} (see, e.g., review by Morris, 1991). Some specific geographic regions may be prone to greater rates of NO_3^- or NH_4^+ deposition (Morris, 1991).

Internal N cycling

Nitrogen stabilization

Amino acids can be removed from soil through processes that stabilize organic matter from decomposition. The link between protein depolymerization and recondensation might allow a fraction of amino acids derived *from* proteolysis and other monomers to combine into more complex molecules that can resist biodegradation (Stevenson, 1982). Amino acid inputs are minimized when there is an impediment to biodegradation of proteins. Some organic nitrogen compounds are resistant to depolymerization because of their structure, and others may become physically stabilized by adsorption on surfaces (see, e.g., review by Knicker, 2011). Complexation with phenols (Hättenschwiler and Vitousek, 2000) or encapsulation by recalcitrant compounds (Knicker and Hatcher, 1997) in the soil may also lead to the formation of insoluble organic nitrogen compounds that prevents enzymatic decomposition. Yu et al (2002) recognized the presence of dissolved organic nitrogen in the hydrophobic fraction of extracts of forest soil and concluded that proteins were likely complexing with phenols or humic acids that attracted charged amino acids. The organic compounds in soils that cannot be readily processed by

microorganisms will be sequestered and accumulate in peatlands or flow out of systems that are hydraulically connected to surface waters.

Stabilization of free amino acids and inorganic nitrogen species is also an important pathway for sequestering nitrogen. Studies have shown amino acids adsorb to solid phases in alpine dry meadow, mineral soil aggregates and forest soil (Raab et al., 1999; Vieublé Gonod et al., 2006; Rothstein, 2010). Glycine and glutamate showed similar retention (50-72%) in alpine soil [solid-liquid partition coefficient (K_d), 12-24 L/kg] and the retention for NH_4^+ was 30-45% (K_d, 4-17 L/kg) (Raab et al. 1999). Adsorption coefficients for ammonium in sediments of a mangrove forest were 0.04-0.10 g NH_4^+ g^{-1} (Holmboe and Kristensen, 2002). Adsorption coefficients for NH_4^+ in constructed wetlands were 0.1-33 g NH_4^+ kg^{-1}, and negative correlations of NH_4^+ adsorption with organic matter content were observed (Zhu et al., 2011). Adsorption of NH_4^+ in peatlands has been shown to increase after freeze-thaw events and is positively correlated with clay content and cation exchange capacity (Yu et al., 2011; Zhu et al., 2011). The degree of amino acid adsorption with mineral soils is related to amino acid characteristics like polarity and molecular weight (Rothstein, 2010). About 90% and 7.5% of lysine and leucine, respectively, was adsorbed to mineral soil aggregates (Vieblé Gonod et al. 2006). Adsorption affinities and rates of adsorption of amino acids with mineral soils vary greatly, which is attributed to differences in amino acid charge at soil pH (Jones and Hodge, 1999; Vieublé Gonod et al., 2006). Mineralization and bioavailability of adsorbed amino acids is diminished relative to free amino acids in soil solution (Vieublé Gonod et al., 2006).

Biological N cycling

Although wetlands may have external sources for nutrients, the major nutrient cycling pathways in many wetlands involve transfer of nitrogen between biota, sediments, organic matter, and pore water. Amino acids are directly released into the soil through cell lysis and root exudation. Many studies have shown large concentrations of amino acids following snow melt and spring thaw that have been attributed to lysis of microbial cells, root turnover, and litter leachates (Mack, 1963; Kielland, 1995; Lipson et al., 2001). About 67% of the nitrogen associated with microbial biomass of various surface horizons of mineral soils was released as amino acid nitrogen after fumigation (Friedel and Scheller, 2002). Plants also contain high concentrations of free amino acids that can be exuded from roots through passive diffusion, which can provide transient increases in available nitrogen (Jones and Darrah, 1994).

Plants and microorganisms can take up NH_4^+ and NO_3^- directly. Water-soluble species of organic nitrogen like amines and amino acids also provide a bioavailable nutrient source for terrestrial and marine biota (Peierls and Paerl, 1997; Seitzinger and Sanders, 1999; Lipson and Nasholm, 2001). Half-lives of amino acids, NH_4^+, and NO_3^- in a salt marsh were 8.2-25.2, 5.6-14.7, and 5.6-15.6 h, respectively (Henry and Jefferies, 2003). Turnover rates of amino acids in soils vary with the specific amino acid and environmental conditions with cooler climates exhibiting slower turnover rates (see, e.g., Kielland, 1995; Vinolas et al., 2001; Jones and Kielland, 2002).

Mycorrhizal fungi can take up NH_4^+, NO_3^-, and amino acids (Finlay et al., 1992). Some species are also able to utilize organic nitrogen species like proteins and peptides

(Finlay et al., 1992; Bajwa and Read, 1985); however, assimilation occurs more slowly for longer peptide chains (Bajwa and Read, 1985). Mycelia of ectomycorrhizal fungi in a shrub and heath tundra ecosystem exhibited a strong preference for uptake of NH_4^+ over NO_3^- (Clemmensen et al., 2008). There was a weaker preference for glycine over NO_3^- in the heath tundra with more than 67% of the uptake of the total labeled glycine occurring as intact glycine molecules for both tundra ecosystems. Nitrogen additions in the heath and tundra ecosystem accelerated biomass growth, which indicated a portion of the nitrogen was transferred to plants (Clemmensen et al., 2008). Laboratory studies have also demonstrated that ectomycorrhizal fungi prefer NH_4^+ to NO_3^-, which is similar to the pattern observed in field studies; however, the preference for nitrogen species varies with species of fungi (Finlay et al., 1992).

Plants in terrestrial ecosystems generally rely on nitrogen, which is released by microorganisms through mineralization of organic nitrogen. Recent studies have shown that plants in various environments can take up amino acids in addition to inorganic nitrogen. Species of shrub and sedge, plants commonly found in peatland ecosystems, have been shown to take up amino acids even when other nitrogen species are present (Raab et al., 1999; Nordin et al., 2004; Clemmensen et al., 2008). Uptake of glycine by grass species in an Arctic salt marsh was not inhibited by the presence of inorganic nitrogen species, and amino acid uptake was estimated to be up to 57% of NH_4^+ uptake and depended on pore water concentrations during the growing season (Henry and Jefferies, 2002). A minimum of 5-11% uptake of amino acids by grass in the Arctic salt marsh grass was attributed to intact amino acids (Henry and Jefferies, 2003). Plants in

other studies of Arctic environments have also been shown to take up intact amino acids (Nordin et al., 2004).

Plants are able to compete with microorganisms for the various nitrogen species (see, e.g., review by Kaye and Hart, 1997). Plants and microbes in a salt marsh showed that plants took up 17, 29, and 19% of added glycine, NH_4^+, and NO_3^-, respectively, and microbes took up 30, 34, and 29%, respectively (Henry and Jefferies, 2003). After two days, heath tundra plants contained 5.6, 7.7, and 9.1% of added glycine, NH_4^+, and NO_3^-, respectively, while shrub tundra plants contained 7.1, 14.3 and 12.5%, respectively, of the nitrogen species; however, the majority of added nitrogen was taken up and assimilated by the microbial community (Clemmensen et al., 2008). Microbial uptake of the various nitrogen species during nutrient addition experiments in moist Arctic tundra in Alaska was 40 and 49% of total added species for non-acidic and acidic sites, respectively, with uptake of NH_4^+ being slightly greater than NO_3^- or amino acids; however, uptake of nitrogen by plants was estimated to be less than 1% for both sites (Nordin et al., 2004). The ability of plant types common to peatland ecosystems to compete with microbes for amino acids varies for different amino acids and with environmental conditions (Schimel and Chapin, 1996; Lipson and Monson, 1998; Lipson et al., 1999b). The bacterial population in an Alpine ecosystem was able to utilize amino acids even at low concentrations, which allowed the microbes to compete with plants throughout the growing season (Lipson et al., 1999a). Studies of competition between plants and microbes in peatland bogs or wetlands for nitrogen species are few; however, the variability observed for similar environments indicates it will be difficult to generalize plant and microbial uptake on a regional scale or on an annual basis.

Microbes are able to utilize the carbon and nitrogen in amino acids as an energy source and for biomass production. Microbial uptake of nitrogen is typically described by Michaelis-Menten kinetics. Maximum uptake rates of amino acid uptake in mineral soils are typically 0.02-0.13 mmol kg^{-1} h^{-1} (Jones and Hodge, 1999; Vinolas et al. 2001); however, the magnitude of the free amino acid pool in wetland soils is likely to be significantly lower than the millimolar levels optimal for growth in mineral soils. Microbial biomass production in mineral soils utilizing amino acids as the carbon source has been estimated at 0.68-0.81 μmol biomass-C per μmol amino acid-C (Jones and Hodge, 1999). Rapid production of microbial biomass in soil aggregates in response to addition of amino acids was attributed to uptake of intact amino acids (Vieblé Gonod et al., 2006). In a field experiment in a tundra ecosystem using labeled glycine, 42-55% of the total nitrogen taken up by soil microbes was intact glycine (Clemmensen et al. 2008). Uptake of intact amino acids indicates that microbes are utilizing the species as a carbon and nitrogen source. Following microbial uptake, the added nitrogen was stabilized in the pool of soil organic nitrogen, which was attributed to the turnover of microbial biomass and exudation of compounds like enzymes (Clemmensen et al., 2008). Microbial biomass was shown to immobilize nitrogen in an alpine ecosystem when soils were colder, and a decrease in biomass was observed after snow melt followed by an increase in soil amino acids and NH_4^+ (Lipson et al., 1999a).

Soil transformations

Extracellular enzymes produced by microorganisms, and some plants, break down peptide chains into free amino acids via proteolysis (see, e.g., review by Vranova et al., 2013). Proteolysis, rather than mineralization, was the slower step in forming available nitrogen in Alpine forest soils (Jones and Kielland, 2002). High proteolytic activity was observed in an Alpine ecosystem after snow melt with very low rates at midsummer followed by a slight increase; however, proteolysis was protein-limited later in the growing season (Lipson et al., 1999a). The initial protein release and subsequent proteolysis, producing amino acids, was a key factor in providing available nitrogen to plants in the Alpine ecosystem. The availability of amino acids in alpine forest, subalpine fen, and shortgrass steppe ecosystems was regulated by protein availability, and protein addition led to increased proteolysis for all ecosystems (Raab et al., 1999).

Microbial respiration transforms organic nitrogen into NH_4^+. Microorganisms take up organic matter for energy and biomass production, and the nitrogen is assimilated to make new proteins or released as NH_4^+ through mineralization. Lipson et al. (2001) estimated that 33% of nitrogen taken up as amino acids is released as NH_4^+ through mineralization. The kinetics of nitrogen mineralization in peatlands are generally fit to a model that separates organic nitrogen into a recalcitrant pool that resists biodegradation and a labile pool containing bioavailable nitrogen (Updegraff et al., 1995; Bridgham et al., 1998). Estimated net mineralization rates for floodplain wetland soils in Belgium were 3.0-18.6 g N m^{-2} y^{-1} (Sleutel et al., 2008) and measured rates for forested swamps in Minnesota averaged 1.5 g N m^{-2} y^{-1} (Zak and Grigal, 1991). Variability in mineralization

rates is related to differences in soil moisture and structure, aeration, seasonal climate, and ecosystem type (Zak and Grigal, 1991; Bridgham et al., 1998; Sleutel et al., 2008). Increased aeration and temperature produced higher mineralization rates in surface peats (Updegraff et al., 1995). Nitrogen mineralization under aerobic conditions, which includes nitrification and ammonium mineralization, is more rapid than the processes under anaerobic conditions; however, differences in the rates vary by ecosystem. Mineralization of organic nitrogen under aerobic conditions was 2.6 and 1.1 times greater than mineralization under anaerobic conditions in a bog and beaver meadow, respectively (Bridgham et al., 1998).

Nitrification is an aerobic process that oxidizes NH_4^+ to NO_3^-, and thus, NO_3^- concentrations remain low in saturated soils. Patrick and Reddy (1976) performed an incubation experiment to demonstrate the zonal nature of nitrification and denitrification due to oxygen penetration in flooded soils. Oxygen penetrates and forms an aerobic layer near the water surface where nitrification occurs and sequentially transforms NH_4^+ into NO_2^- and NO_3^- (Patrick and Reddy, 1976). Autotrophic nitrifying bacteria in the aerobic layer that use oxygen as an electron acceptor to oxidize NH_4^+ to NO_3^- compete with heterotrophic bacteria that use oxygen to oxidize organic matter (Patrick and Reddy, 1976). Vegetation in constructed wetlands can impact the composition of the nitrifying and denitrifying bacterial communities (Ruiz-Rueda et al. 2009), and thus, there are variations in bacterial community composition in various types of wetland ecosystems (see, e.g., review by Bothe et al., 2000). The contribution of nitrification to total aerobic nitrogen mineralization varied greatly (2-98%) between northern wetland sites and indicated a degree of variability in microbial population dynamics (Bridgham et al.,

1998). Nitrification of NH_4^+ in the surface, aerobic layer and NH_4^+ diffusion to the surface from the anaerobic layer were identified as pathways for producing NO_3^- (Patrick and Reddy, 1976). Nitrate transported from the surface aerobic layer deep into the water column where anaerobic conditions persist will undergo denitrification that produces molecular nitrogen and lesser amounts of nitrous oxide (Patrick and Reddy, 1976). Denitrification rates in a forested swamp in Minnesota were most rapid in early spring and late fall and produced nitrous oxide emissions of 8040 and 2525 μg N_2O-N m^{-2} d^{-1}, respectively (Zak and Grigal, 1991). Nitrification was highest in late summer for the swamp with an annual mean of 0.7 g N m^{-2} y^{-1} with denitrification consuming an average of 14% of the nitrified nitrogen daily during the growing season.

Response to global change

Peatlands sequester large stores of carbon, and climate researchers have recognized the sensitivity of these ecosystems to future warming. The response of the peatland nitrogen cycle to seasonal variations in environmental conditions provides insight into the impact of future changes in climate. Amino acid concentrations in an Arctic fen in Greenland were not correlated with temperature or season (Ström et al., 2012). There was also a lack of seasonal variability for amino acid, NH_4^+, and NO_3^- levels in acidic Arctic tundra in Alaska; however the non-acidic site exhibited seasonal variability in the species concentrations (Nordin et al. 2004). Fumigating a forest with CO_2 concentrations above ambient atmospheric concentrations led to an increase in plant nitrogen uptake; however, there was suppression of microbial decomposition of soil organic matter due to greater

competition with plants for available nitrogen (Hu et al. 2001). The complexity of interactions between processes controlling the internal nitrogen cycle of peatlands and coupling of the nitrogen and carbon cycles makes it difficult to observe ecosystem scale changes in the organic nitrogen pool.

The quality and magnitude of the carbon pool, which is highly sensitive to climatic variables, regulates nitrogen availability by determining the rate of decomposition and release of nutrients. Knicker (2011) recognized the complex linkage between soil organic nitrogen and carbon sequestration and highlighted various theories for the capacity of an ecosystem to store carbon in response to increasing nitrogen deposition and CO_2 concentrations. In arctic soils and wetlands, organic matter quality and the size of the labile organic matter pool exert greater control over carbon and nitrogen mineralization rates than temperature or hydrology (Nadelhoffer et al., 1991; Updegraff et al., 1995). Organic nitrogen species hold a unique niche in linking the carbon and nitrogen cycles of peatlands; however, investigations of organic nitrogen dynamics in peatlands are few.

Increased plant and microbial productivity in direct response to warming or indirect response to permafrost thawing and lowering of peatland water tables might release large stores of carbon to the atmosphere (see, e.g., review by Gorham, 1991). Nitrogen is typically a limiting nutrient, and availability of fixed nitrogen exerts some degree of control over microbial and plant productivity in wetlands. Increases in the decomposition of organic matter will release fixed nitrogen and amino acids and lead to an increase in biotic productivity.

Predicting the biotic response of peatlands to indirect and direct effects of climate change is difficult without fully understanding the dynamics of various nitrogen species and the factors controlling availability. In the current study, seasonal variations in the magnitude and composition of free amino acids and inorganic nitrogen in pore water of peat are examined. Relationships between plant functional types are investigated to gain a better understanding of peatland response to climate change in the context of nitrogen availability. It was hypothesized that the free amino acid pool would be variable on a seasonal basis, showing correlation with other nitrogen species in pore water, and would vary depending on the dominant plant functional type present in the peat. Ultra-high pressure liquid chromatography and ion chromatography were used to analyze pore water from peats with differing vegetation cover to determine the magnitude and composition of the free amino acid pool. The current study also includes a basic mechanistic model of amino acid biogeochemical cycling in peatlands which was created in Simulink®. The model was used to show the influence of the dominant removal and addition pathways for amino acids in peatlands. It was hypothesized that microbial processes would be the dominating influence on amino acid pools based on a study of current literature. The results of the current study are discussed in the context of future research directions and the implications for future peatland modeling are considered.

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Chapter 2

Amino acids in pore water of peat[a]

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Abstract Productivity of peatland ecosystems is often limited by dissolved forms of inorganic and organic nitrogen. The magnitude and composition of the free amino acid pool and nitrate and ammonium content of pore water from peat were determined during three seasonal periods for mesocosms with various dominant plant functional types. Leucine dominated the free amino acid pool for all mesocosms, and the range of total free amino acid concentrations was 0-2.3 μM. Plant functional type did not significantly influence the free amino acid pool. Seasonal variations in amino acid concentrations were observed with maximum concentrations in spring. Springtime amino acid concentrations correlated with dissolved organic carbon. Amino acid concentrations tracked ammonium and nitrate concentrations in spring and summer. Amino acid concentrations were the lowest in the November, post-growing season period and showed weak correlations with other chemical species in pore water. The cycling of amino acids in pore water appeared

[a] The material contained in this chapter is in preparation for submission to a scientific journal.

to be regulated by microbial processes, and environmental drivers like temperature indirectly influenced availability of amino acids.

Keywords Amino acids · Nitrogen availability · Organic nitrogen · Peatlands

Acknowledgements Funding for the study was provided in part by the McIntire Stennis Grant 1001685 from the USDA National Institute of Food and Agriculture. Funding was also provided in-kind support from the USDA Forest Service, Northern Research Station (Houghton, MI). Support for the PEATcosm project was provided by NSF DEB grant 1146149. This study was also supported in part by the GRO Fellowship Assistance Agreement no. MA91756201-0 awarded by the U.S. Environmental Protection Agency (EPA). It has not been formerly reviewed by the EPA. The views expressed in this publication are solely those of the authors, and EPA does not endorse any products or commercial services mentioned in this publication. The authors are grateful to David Perram for assistance with laboratory instrumentation. The authors would also like to extend thanks to the members of the PEATcosm project, especially to Lynette Potvin for providing access to samples and project databases.

Introduction

Terrestrial ecosystems are generally considered to be nitrogen limited. Nitrogen fertilization stimulated growth of heterotrophic microbial populations in peatlands and conifer forests (Norton and Firestone 1996; Gilbert et al. 1998). Shaver and Chapin

(1980) observed that tundra plants were initially limited by nitrogen and then by phosphorus. Peatlands hold large stores of carbon and the response of peatland carbon cycle to future climate change will likely be influenced by the availability of limiting nutrients like nitrogen. Free amino acids exist in pore water as monomers rather than as part of a peptide chain, and thus, the total free amino acid content represents the pool of bioavailable organic nitrogen for plants and microbes.

Nitrogen in soils is present in either inorganic or organic form. The dominant inorganic nitrogen species are nitrate (NO_3^-) and ammonium (NH_4^+), which are readily available for uptake by biota. Generally NH_4^+ is the dominant inorganic species in peatlands where nitrification is inhibited by water-saturated soils. Organic nitrogen in soils is generally in the form of peptides and amino acids, with lesser contributions of amino sugars, which are similar to the forms of nitrogen in plant litter (Friedel and Scheller 2002; Knicker, 2011). Sowden et al. (1977) observed that amino acids made up a greater portion of total nitrogen in tropical climates than in temperate or Arctic climates, while NH_4^+ made a greater contribution to soil nitrogen in cooler climates. The organic matter in peatlands is often dominated by humic material, which contains a significant portion of its nitrogen as amino acids bound in peptides. Concentrations range from 0.05 to 0.6 mol m^{-3} with the dominant amino acids being neutral forms like alanine and glycine (Sposito 1994). Studies of peatlands with a high degree of humification in Canada and Wisconsin have shown that the fraction of total soil nitrogen represented by amino acids is low while the fraction represented by insoluble nitrogen is high (Isirimah and Keeney 1973; Sowden et al. 1978).

The large stores of organic matter in peatlands provide ample nitrogen in the form of proteins; however, decomposition of proteins into amino acids is usually required for uptake by biota. Bioavailability of the nitrogen and carbon in amino acids is related to proteolysis of larger proteinaceous polymers and release of amino acids by plant roots and lysis of microbial cells. Peptide chains of amino acids are broken down into monomers by extracellular protease enzymes (see, e.g., review by Vranova et al. 2013). The monomers can then be removed from soil through microbial and plant uptake or adsorption to inorganic or organic particles. Soil microbial populations can utilize amino acids for a carbon and nitrogen source (Lipson and Monson 1998). Lipson et al. (2001) modeled the abiotic and biotic processing of amino acids in soil and predicted that the free amino acid pool in alpine soils was regulated by rates of proteolysis and microbial uptake.

The dominant plant functional groups of northern peatlands are sedge, ericaceous shrub, and *Sphagnum* moss. Studies of non-mycorrhizal sedges (Kielland 1994; Raab et al. 1996; Raab et al. 1999) and mycorrhizal ericaceous shrubs (Stribley and Read 1980; Read and Bajwa 1985; Kielland 1994; Read et al. 2004) have shown plant uptake of amino acids. *Sphagnum* mosses that dominate peatlands have also been shown to take up amino acids (Kielland 1997). Sedge and shrub plants both influence the free amino acid pool through amino acid uptake; however, the shallow rooting structure and poor litter quality of shrub species also indicates the possibility for unique interaction with the free amino acid pool.

Microbial decomposition of labile organic matter in soils, which contributes free amino acids to pore water, is influenced by environmental factors like soil water content and

temperature. Amino acid turnover rates are typically low for cooler climates (Vinolas et al. 2001), and mineralization rates increase with increasing aeration and temperature in surface peats (Updegraff et al. 1995). The magnitude and composition of the free amino acid pool of boreal forests and tundra ecosystems have been shown to vary between seasons and ecosystem types (Kielland 1995; Raab et al. 1999; Jones and Kielland 2002; Yu et al. 2002; Weintraub and Schimel 2005; Werdin-Pfisterer et al. 2009). Seasonal variability in the free amino acid pool may indicate changes in microbial decomposition or plant nitrogen uptake in response to environmental factors. Low concentrations of free amino acids are typically observed in wetland ecosystems (Swain et al. 1959; Ström et al. 2012). The low levels might indicate limited inputs through proteolysis because of large insoluble nitrogen pools and cooler temperatures; however, the low levels of amino acids may also indicate significant removal via biotic and abiotic pathways that fluctuate seasonally.

Carbon and nitrogen mineralization rates in peatlands are strongly influenced by the dynamics of the labile substrate pool (Updegraff et al. 1995). An increase in amino acids, which are a source of carbon and nitrogen for microbes, is likely to increase nitrogen and carbon mineralization rates. Amino acids may provide a source of organic nitrogen to peatland microbes and plants in addition to the inorganic forms of bioavailable nitrogen; however, very little is known about the magnitude, composition, and environmental drivers that affect the free amino acid pool in peatland ecosystems.

Here we examine seasonal variations in the concentrations of free amino acids, NO_3^-, and NH_4^+ in mesocosms of peat dominated by either sedges or ericaceous shrubs. Our hypotheses are the following: (1) The composition of the free amino acid pool will be

related to plant functional type and concentrations will vary with season and (2) Amino acid concentrations in pore water will be low compared to levels in soil from boreal ecosystems due to slow decomposition, and levels will correlate with other carbon and nitrogen species in pore water. Concentrations of various primary amino acids and inorganic nitrogen species were determined using ultrahigh-pressure liquid chromatography (UHPLC) and ion chromatography, respectively. Characteristics of the amino acid pool were compared with pore water chemistry and physical characteristics of the plots.

Materials and methods

Site description

Samples were collected in 2013 as part of the PEATcosm project (see, Potvin et al. 2015). Twenty-four blocks of natural peat were excavated from an oligotrophic peatland in Meadowlands, MN USA and were transported to the USDA Forest Service Northern Research Station in Houghton, MI. Each block of peat was installed in a mesocosm bin which was approximately 1 m^3 with an open top (described in detail by Potvin et al. 2015). The dominant plant functional types were manipulated in sedge bins and ericaceous shrub bins to remove ericaceous shrub and sedge, respectively, while the plant functional types of other bins were not manipulated to serve as a reference condition. Vegetation manipulations began in 2011 with four bins established for each vegetation treatment. *Sphagnum* dominated bryophyte production with annual average production in

2013 considering all bins ranged from 416-688 g m^{-2} (Potvin et al. 2015). Annual average vascular plant production was 12.6 g m^{-2}, 14.7 g m^{-2} and 44.9 g m^{-2} for sedge, shrub and un-manipulated bins, respectively, and the respective standing vascular biomass was 12.6 g m^{-2}, 99.2 g m^{-2} and 97.0 g m^{-2} (Potvin et al. 2015). Precipitation in winter, spring, summer and fall was 21, 28, 27 and 22 cm, respectively based on data collected at a nearby National Atmospheric Deposition Program (NADP) site (MI99) in Chassell, MI (NADP 2015). The seasonal NH_4^+ concentration in precipitation ranged from 8-26 µM while NO_3^- was 10-13 µM (see Table A2.1 in Supplementary Information; NADP 2015).

Sample collection, preparation and storage

Archived samples collected on 09 May, 06 August, and 04 November in 2013 were chosen to represent the pre-growing, growing, and post-growing seasons, respectively. Samples collected from a depth of 20 cm were analyzed for bins (n=4) representing both vegetation treatments. Samples from the 20 cm depth were also analyzed for the reference condition bins (n=4) with un-manipulated vegetation. The range in the depth of the water table below the peat surface was 0-11 cm, 9-18 cm and 6-13 cm for the spring, summer and fall sampling dates, respectively. The height of the water table for each of the sampled bins was based on the 45-year record of average, low variability seasonal water table profiles for the USDA Forest Service Marcell Experimental Forest (Potvin et al. 2015).

Lysimeters were installed in each bin to depths of 20, 40 and 70 cm from which pore water samples were collected and archived every four weeks (see, Romanowicz et al.

2015). Pore water samples were filtered immediately after collection through 0.45 μm nylon filters. Samples to be archived were frozen in Naglene® HDPE amber bottles. It was assumed that freezing samples after filtration would not introduce significant artifacts based on the results of Gorzelska et al. (1992), showing that changes observed in sample amino nitrogen concentration after samples were frozen for a 3 month period were within the level of analytical precision. Prior to analysis, samples were allowed to come to room temperature. Sample bottles were inverted five times to homogenize the sample, and a 2-mL aliquot was removed for analysis and filtered by syringe with a 0.2 μm cellulose acetate membrane filter (VWR International, Batavia, IL). Samples were prepared in batches of 8 to 14, which included a replicate. Water from a MilliQ® system (EMD Millipore, Billerica, MA) was filtered as described and used for method blanks.

Analysis of amino acids

Amino acid analysis was performed using a Dionex Ultimate 3000 ultra high-pressure liquid chromatograph (UHPLC) with fluorometric detector (Thermo Scientific, Sunnyvale CA). The amino acids were separated on a Zorbax Eclipse AAA 3.5 μm, 4.6 x 150 mm, C18 column (Agilent Technologies, Santa Clara, CA). The analytic technique was a modification of several procedures (Dorrestijn et al. 1996; Kutlán et al. 2002; Dionex 2011). A gradient elution at 2 mL min^{-1} was used to separate the amino acids (Table A2.2). Mobile phase A was a borate buffer (pH 7.8) made up with10 mM sodium tetraborate decahydrate and 10 mM sodium phosphate. Mobile phase B was a 45/45/10 (v/v/v) mixture of acetonitrile/methanol/water. Mobile phases were prepared within 24

hours of use and were vacuum filtered through 0.2 µm, 316-stainless-steel filters (Mott Corporation, Farmington, CT) and left capped at room temperature until analysis.

Amino acids were derivatized with *o*-phthaldialdehyde (OPA) and 3-mercaptopropionic acid (MPA; Sigma-Aldrich, St. Louis, MO). The derivatizing reagent consisted of 1 ml of OPA and 2 µl of MPA. The derivatizing reagent was stored for 1-4 d after preparation at 4 °C and was equilibrated to room temperature prior to use. The reagent was left at room temperature for a maximum of 12 h. A 5-µL aliquot of derivatizing reagent was mixed with 5 µl of sample through online derivatization and allowed to react for 60 s prior to injection.

A stock standard at a level of 2.5 mM each for aspartate, glutamate, serine, histidine, glycine, threonine, arginine, alanine, tyrosine, valine, methionine, phenylalanine, isoleucine, leucine, lysine, proline and ammonium chloride and at 1.25 mM for cystine (Sigma-Aldrich) was prepared weekly using MilliQ® water for dilutions. The stock standard was stored at 4 °C and was equilibrated to room temperature prior to preparing serial dilutions for analysis, which remained at room temperature for a maximum of 12 h.

Each batch of samples included a sample of MilliQ® water followed by MilliQ® water mixed with derivatizing reagent (i.e., reagent blank). For each batch, 3-4 reagent blanks were run intermixed with samples. Five serial dilutions of the stock standard that ranged in concentration from 50 to 1000 nM were used for each batch of samples. Working standards, one or two replicates of a working standard, and reagent blanks were intermixed with the pore water samples.

The limit of quantitation was either equal to three times the mean reagent blank or equal to the limit of detection depending on which quantity was greater (see Table A2.3

in Supplementary Information). The limit of quantitation within each batch was used as the minimum reporting detectable limit for each amino acid, or the minimum concentration necessary for that amino acid to be reported as being present in the sample (Armbruster and Pry, 2008). Reagent blanks ranged from 5-40 nM (1.5-8.2 nM standard deviation range), 3-35 nM (0.4-4.8 nM), and 0-31 nM (0.2-3.7 nM). Amino acids identified in reagent blanks and method blanks at the highest concentrations were glycine, serine, alanine, threonine and aspartate. The presence of these amino acids in blanks prevented detection at very low concentrations; however, a limit of quantitation greater than 100 nM was only observed for 3 amino acids for all sample runs. Further details on limits of blanks, limits of detection and limits of quantitation are included in Table A2.3 of the Supplementary Information.

Analysis of ammonium and nitrate

Samples were analyzed for NH_4^+ and NO_3^- using a Dionex ICS-1100 and Dionex ICS-2100, respectively (Thermo Scientific). Samples were thawed, and 5-ml aliquots were filtered through 0.2 μm nylon membrane Acrodisc® syringe filters (Sigma-Aldrich). Samples were then diluted before analysis, using 4 ml of sample and 2 ml of water that had passed through a Barnstead easyPure II UV/UF water system (Thermo Scientific). All 36 samples were run in one batch together with 3 blanks using the dilution water and 3 filtered blanks using MilliQ® water. Further details on the analysis method are included in Table A2.4 of the Supplementary Information.

The filtration methods used for filtration of pore water samples, i.e. nylon filters prior to archiving samples and syringe filtration with cellulose acetate filters prior to UHPLC analysis, were tested as sources of amino acid contamination. The reagent blank mean concentrations were 0-76 nM (standard deviation of 0-6.8 nM, except leucine at 26 nM). The syringe filtration blanks did not contribute additional amino acids above the reagent blank except for glycine (6.1 nM) and threonine (1.1 nM). The pre-archiving method of filtration using nylon filters contributed concentrations of 0.5-63 nM above the reagent blank (standard deviation of 4.5-25 nM, except for four amino acids at 35-45 nM).

Data analysis

All amino acids in the mixed standard were analyzed with the exception of cystine and proline. Cystine was identified in some samples; however, the species could not be reliably measured using the derivatization method. Averaged peak areas for reagent blanks were subtracted from the peak areas for amino acids in the samples. Peak areas were converted to molar concentrations using response factors for the amino acids that were determined from five point calibration curves. Mean concentrations of amino acids that were introduced by the filtration method for processing pore water samples prior to archiving were subtracted from the sample concentrations. Individual amino acids above the limit of quantitation were summed for each sample to determine the total free amino acid (TFAA) concentration.

The influence of season and plant functional type were determined using 2-factor analysis of variance (ANOVA) with replication. Data was log transformed to ensure

normality and similarity of variance. Sampling date and plant functional type were used as the determining factors. TFAA concentrations were compared with various peat characteristics by determining linear correlation coefficients (r).

Results

Free amino acids

The overall average TFAA concentration considering all samples collected was 0.40 μM with a range in TFAA concentration of undetectable to 2.29 μM. Mesocosm bins exhibiting low TFAA concentrations during the first sampling period often had low concentrations during later sampling periods (Fig. 2.1); however, bins with high TFAA concentrations showed greater variability and did not always show the highest concentrations for later sampling periods (e.g., Bins 16, 12, 2). The TFAA concentration in one bin from each plant functional group was above 1 μM for the first sampling period; however, for later sampling periods, only two sedge bins exhibited a TFAA concentration above 0.5 μM. The amino acid that exhibited the highest concentration for all sampling periods and bins was leucine, which constituted 43-100% of the TFAA pool. Leucine made up the entire TFAA pool for a majority of samples. The maximum observed concentration of leucine was 1.47 μM, and the maximum concentration of amino acids when leucine was subtracted from TFAA was 1.31 μM (see Table A2.5 in Supplementary Information). Amino acids that contributed greater than 5% in addition to leucine in at least one sample included aspartate, serine, glycine, arginine, alanine and lysine.

Influence of plant functional type and season

The May sampling date had the highest average TFAA concentration at 0.76 μM while the November sampling date had the lowest average TFAA concentration at 0.18 μM (Table 2.1). There was a large degree of variation in amino acid concentrations for each vegetation treatment. When data were grouped by vegetation treatment, the sedge and shrub bins had similar TFAA averages of 0.51 μM and 0.49 μM, respectively. The average TFAA concentration for the un-manipulated bins was 0.20 μM.

The entire set of samples was considered to examine the significance of the relationships among plant functional type and sampling date and the TFAA concentrations. ANOVA results did not show a significant difference ($p > 0.05$) in TFAA concentrations when data were grouped by plant functional type (Table 2.2). There was a significant difference between samples taken on each sampling date. When plant functional type and sampling date were considered together, there was no significant influence on amino acid concentrations.

Correlation with pore water characteristics

Concentrations of TFFA, NO_3^-, and NH_4^+ exhibited similar trends across bins and for all three sampling dates with the exception of NO_3^-, which did not track the other two species for the November sampling date (Fig. 2.2). Concentrations of NH_4^+ were much higher than levels of NO_3^- and TFAA, which were generally of the same order of

magnitude. All three nitrogen species generally showed the greatest concentrations during the May sampling period (Figure 2.3a). Some individual bins exhibited similar patterns for all 3 sampling periods. Levels in Bin 1 (sedge bin) were consistently high for all species analyzed, and concentrations of NH_4^+ and amino acids in Bins 3 and 8 (un-manipulated bins) were consistently near zero. The contribution of TFAA was less than 8% of the 3 nitrogen species for all samples, and there was no clear sampling period that showed greater TFAA contributions (Fig. 2.3).

Pore water properties [e.g., dissolved organic carbon (DOC), phenolics, NO_3^-, NH_4^+, total dissolved nitrogen (TDN), and water table height (WT)] were compared with TFAA levels to examine correlations between pore water characteristics (Table 2.3). Pore water pH is typically around 4. Water table height (WT) did not exhibit a significant correlation with TFAA concentrations. The correlation with DOC and phenolics was greatest for the early sampling date; however, all other periods exhibited a weak correlation. Levels of TFAA did not show a strong correlation with other characteristics during the November sampling period. The strongest correlations between TFAA and NO_3^-, NH_4^+ and TDN were observed for the August sampling period.

The last three rows of Table 2.3 show comparisons of nitrogen species other than TFAA with each other. The strongest correlations between any pore water characteristics were observed when NH_4^+ and NO_3^- concentrations were compared. Correlations between TDN, NO_3^-, and NH_4^+ were stronger with each other than with TFAA levels.

A statistically significant linear correlation ($p \leq 0.05$) between NH_4^+ and TFAA was observed for every sampling date (Table 2.4). The TFAA relationships with NO_3^- and TDN were only significant for the first two sampling periods. Linear correlations showed

the strongest statistical confidence when all sampling dates were considered as one large data set. Annual average TFAA, NO_3^-, and NH_4^+ concentrations were determined for each bin by taking the mean of all three sampling periods. The annual sphagnum production and vascular standing biomass data, obtained from Potvin et al. (2015), showed weak negative correlations that were not statistically significant when compared with the annual average TFAA concentrations (-0.355 and -0.280, respectively). The concentrations of NH_4^+ and NO_3^- both showed a strong positive correlation ($p \leq 0.001$) with annual average TFAA concentrations.

Discussion

Composition and magnitude of the free amino acid pool

The current study observed low concentrations (undetectable - 2.3 μM) of TFAA in peat mesocosm pore water compared with other studies of peatland environments. Pore waters of a sub-alpine fen in Colorado showed concentrations of free amino acids that ranged from undetectable to 15-20 μM (Raab et al. 1999). Swain et al. (1959) observed that levels of TFAA in extracts of peat from various bogs were very low or negligible. The average pore water concentration of the dominant amino acid in an Arctic fen was 2 μM (Ström et al. 2012). Differences in sampling methods and environmental conditions make it difficult to compare TFAA data from various studies. Release of amino acids from roots that are severed during sampling of soil is a potential source of additional TFAA to soil extracts (Kielland, 1995). Saturated soils of wet meadows in the Alaskan tundra and

fens in alpine Colorado exhibited lower concentrations of TFAA than unsaturated soils of ecosystems in the same climatic region (Kielland 1995; Raab et al. 1999).

The large contribution of leucine to the pool of TFAA in peatland pore water has not been observed in studies of TFAA in soils. Other studies have found that glycine, alanine, arginine, serine, threonine, aspartate and glutamate are the typical amino acids that have been observed in soils from the Alaskan tundra (Kielland, 1995; Weintraub and Schimel, 2005), a Colorado dry meadow (Raab et al. 1999), an Alaskan boreal forest (Werdin-Pfisterer et al. 2009), and a northern California forest (Yu et al., 2002). Kielland (1995) reported leucine in an Alaskan tundra soil. Leucine has also been reported as one of the most abundant free amino acids in California forest soils (Yu et al. 2002), Arctic salt marsh soils (Henry and Jefferies 2002), and leachates from a Haplic Luvisol (Fischer et al. 2007). The dominance of a single amino acid has been observed in other studies, including a study of a sub-alpine fen in Colorado where aspartate was found at concentrations greater than 10 μM (TFAA concentrations, 15-20 μM) and a study of Arctic wetlands showing asparagine constituting 72-82% of amino acids in pore water (Raab et al. 1999; Ström et al. 2012). Unique characteristics of the peat in the study and the unique properties of leucine might have contributed to the persistence of leucine in pore water.

Based on current knowledge of the amino acid composition of proteinaceous material across various soil types and climatic regions it is unlikely that the prevalence of leucine in the TFAA pool was a result of unique precursor organic material. Studies have shown that the amino acid composition of humic materials does not vary between climatic regions (Sowden et al. 1977). The composition of the TFAA pool across successional

stages with differing precursor material in an Alaskan forest was also shown to be relatively constant (Werdin-Pfisterer et al., 2009).

Physicochemical properties of leucine might inhibit removal by biotic and abiotic processes if leucine is not introduced with unique precursor material. Leucine possesses a greater hydropathy (i.e., hydrophobicity of the side chain) than other amino acids that are commonly found in soils (Rothstein, 2010). Hydropathy is negatively correlated with abiotic sorption and assimilation into microbial biomass (Rothstein, 2010). Leucine was also shown to have a weak affinity for adsorption by soil aggregates. Vieublé Gonod et al. (2006) observed that only 7.5% of added leucine was adsorbed to soil and 7.8% remained in solution 6 h after addition. In comparison, there was complete removal of lysine from solution, which might indicate that soluble leucine is able to weakly resist initial removal by soil microorganisms. Non-polar amino acids and amino acids without N in side-chains, which are properties of leucine, generally have lower mineralization rates (Rothstein, 2010). Amino acids with more hydrophobic side chains like leucine tend to be located toward the inside of globular proteins (Kyte and Doolittle 1982). The placement of leucine within the protein structure may play a role in proteolysis and amino acid decomposition dynamics.

Influence of season, plant functional type and plant productivity

Although the composition of the free amino acid pool was not significantly influenced by differences in dominant plant functional type or season, the total magnitude of the free amino acid pool exhibited significant seasonal differences. The lowest and highest TFAA

concentrations were observed during the November and May sampling periods, respectively. Leucine dominated the TFAA pool for all sampling periods; however, other studies reported seasonal trends for specific amino acids (Kielland 1995). Alaskan tundra soils showed the lowest TFAA concentrations in July during the peak of the growing season and maxima for tussock and shrub sites were observed in June after thaw and in August before freeze, respectively (Kielland, 1995). Dry meadow soils from Colorado exhibited high glutamate concentrations in spring and early summer and low levels in late summer and fall (Lipson et al. 2001). Weintraub and Schimel (2005) showed that levels of TFAA and NH_4^+ were near the detection limits in July for Alaskan tussock, shrub and wet sedge environments. The pattern of maximal levels of TFAA after snow melt followed by minima in concentrations has been attributed to release of amino acids related to the turnover of microbial biomass in spring followed by an increase in microbial and plant demand as the growing season progresses and temperatures increase (Kielland 1995; Lipson et al. 2001). Activity of the heterotrophic bacterial community of a drained peatland was greatest in summer and least in spring (Gilbert et al. 1998). Proteolysis rates were maximized in spring after thaw for Alpine soils with proteolysis limited by protein availability later in the growing season (Lipson et al. 1999). If amino acids are a significant source of bioavailable nitrogen, then microbial and plant drawdown of the free amino acid pool is expected to be greatest in summer. Thus, seasonal differences observed in the current study are likely influenced by microbial community dynamics.

Differences in nutrient acquisition pathways, litter quality, and root structure of the two plant functional types were expected to influence TFAA concentrations. Decomposition

of the litter of ericoid shrubs by microbial communities is slow and inhibited by phenolic compounds associated with ericoid litter (Jalal et al. 1982; Cornelissen et al. 2001). Ericoid mycorrhizal fungi produce extracellular acid proteases that enhance protein decomposition and produce free amino acids and small peptides (Bajwa et al. 1985). Many species of the sedge family have been shown to take up amino acids directly (Kielland 1994; Raab et al. 1996; Raab et al. 1999). Sedge aerenchyma facilitates transport of oxygen to the rhizosphere, which would accelerate microbial decay rates. Studies have shown differences in TFAA concentrations between shrub- and sedge-dominated ecosystems; however, the differences were not statistically significant (Kielland 1995; Weintraub and Schimel 2005). Mycorrhizal associations and shallow rooting structure of ericaceous shrubs uniquely influence the biogeochemical cycle of nutrients; however, the observed similarity in free amino acid concentrations between vegetation treatments may indicate that plants exert little direct control over the free amino acid pool. *Sphagnum* mosses have also been shown to absorb amino acids (Kielland 1997) and contribute to litter production. The dominance of *Sphagnum* moss in terms of biomass production for sedge and shrub mesocosm bins may also in part explain observed similarities in the TFAA pool between bins with different vegetation treatments; however, the lack of roots predominantly restricts *Sphagnum* moss amino acid absorption to atmospheric deposition, snowmelt, and direct absorption from the TFAA pool at periods when the water table was at the surface of the peat, so it is likely that *Sphagnum* litter inputs rather than amino acid uptake would lead to greater similarities in the TFAA pool between bins.

Bioavailability of free amino acids is also influenced by adsorption, proteolysis, and microbial uptake (Kaye and Hart 1997; Raab et al. 1999). Lipson et al. (2001) concluded that fluctuations in the free amino acid pool of alpine soils could be predicted by protease and microbial uptake rates, which indicated a lack of plant control. However, a model of the ecosystem suggested a missing sink during the growing season that might be attributed to uptake by plants. Uptake of inorganic nitrogen by heterotrophic microorganisms appears to be unaffected by plants (see, e.g., Kaye and Hart 1997). Competition between plants and microbes for organic nitrogen may follow a similar pattern. *In situ* uptake of amino acids by microbes in an Arctic salt marsh was greater than plant uptake (Henry and Jefferies 2003). However, plants were able to incorporate a small portion of amino acid nitrogen as intact amino acids, which indicated a portion of amino acid nitrogen incorporated by plants was initially processed by microbes. The degree of amino acid uptake has been observed to vary with the species of sedge or ericaceous shrub and the amino acid species (Kielland 1994; Raab et al. 1999). Some plants require high concentrations of amino acids in soil for effective uptake (Kielland 1994; Schimel and Chapin 1996; Jones et al. 2005). The microbial community dominated amino acid uptake in agricultural grasslands and in tundra soils with low amino acid concentrations in soil (Schimel and Chapin 1996; Owen and Jones 2001; Bardgett et al. 2003). The specific plant species and low amino acid concentrations that were observed in pore water in the current study may be responsible for low uptake rates that may account for the observed lack of plant influence on the TFAA pool. However, oxidative and anaerobic deamination of amino acids, which produces carboxylic acids and ammonia (NH_3), might represent additional biotic loss mechanisms, allowing plants to

capture amino acid nitrogen after transformation into ammonia. Uptake of amino acids by microbes might dominate removal of amino acids for the spring, summer, and fall seasons in peatlands; however, studies with greater sampling frequency during these seasonal periods would be needed to determine if plants have a transient influence.

The correlation between above ground plant biomass and amino acid concentrations was insignificant, which also implies amino acid removal was not strongly influenced by plant uptake. A study of an Arctic wetland, which showed differences in shrub coverage and growth did not influence amino acid concentrations (Ström et al. 2012), supports the results of the current study. Evaluating the influence of plant type on amino acid cycling was hindered by extreme variations in concentrations of amino acids in pore water between bins with the same plant type on a single sampling date. Other studies have shown similar variations within an ecosystem over small spatial scales. A study by Kielland (1995) showed coefficients of variation ranging from 29% to 89% for amino acid concentrations for various types of tundra soils. Four dry meadow sites, where sedge are commonly found, were shown to have variable maximum amino acid concentrations with a range of 13 to 158 μM (Raab et al. 1999). The high degree of variation might be due to the rapid turnover times for amino acids (see, e.g., Kielland 1995; Jones and Kielland 2002). Unique properties of each plant functional type might have obscured the small variations in pore water amino acid concentrations. TFAA concentrations represent the net effect of plants and microbes on the TFAA pool, so it may be difficult to observe the influence of plant uptake when greater biomass production is accompanied by both increased nitrogen uptake and greater litter production. Peatland shrub and sedge species have the ability to take up amino acids; however, the presence or absence of a specific

plant functional type and the above ground plant productivity do not appear to significantly influence the concentration or composition of the free amino acid pool.

Comparison of TFAA concentrations and pore water characteristics

Amino acids can react with phenolic species to form humic substances, leading to abiotic removal that may affect the free amino acid pool. Soils with high organic matter content have been demonstrated to retain some amino acids (Raab et al. 1999). There was a weak negative correlation between TFAA concentrations and phenolics when all dates were considered, which might imply removal of free amino acids; however, the relationship was not observed when sampling dates were considered individually. The poor correlation might be due to the large contribution of leucine to the TFAA pool. Leucine is a non-polar amino acid with a neutral side chain, properties that influence the abiotic sorption of amino acids in soils (Rothstein, 2010). Phenolics and DOC exhibited a positive correlation with TFAA for the May sampling period; however, the correlation was poor for the August and November sampling dates. The seasonal correlation might indicate that amino acid concentrations in spring are more strongly regulated by soil organic matter content and decomposition, and later in the season they are influenced by interaction with phenolic compounds.

Strong correlations were observed between the various nitrogen species, a similarity between TFAA and NO_3^- concentrations, and high levels of NH_4^+ relative to the other nitrogen species. The results may indicate there are different regulators for the various nitrogen species. Studies of alpine and Arctic ecosystems found concentrations of the

various nitrogen species to be the same order of magnitude or NH_4^+ at lower concentrations than TFAA (Raab et al. 1999; Kielland, 1995; Henry & Jefferies, 2002; Weintraub & Schimel, 2005). The high levels of NH_4^+ and low levels of NO_3^- are related to the anoxic conditions of wetland that hinder nitrification. High levels of NH_4^+ relative to amino acids might indicate biotic deamination of the amino acids. However, nitrogen mineralization was shown to be unrelated to amino acid consumption in Arctic tundra soil, which exhibited very low NH_4^+ concentrations compared to amino acids (Kielland, 1995). Correlations between NH_4^+ concentrations and DON in Alaskan taiga forest soils were significant, and ammonification rates were related to amino acid decomposition (Jones and Kielland, 2002). The correlations indicated amino acid mineralization rates in taiga forest were greater than protein turnover rates with rapid uptake of amino acids by soil microbes. Bioavailability of NH_4^+ and amino acids was limited by proteolysis. Regulation of nitrogen mineralization rates by proteolysis may explain the links between amino acids and other nitrogen species. The correlation between amino acid concentrations and the levels of other nitrogen species in pore waters of the current study may indicate that nitrogen mineralization in peatlands is related to amino acid consumption, which leads to low amino acid concentrations and high NH_4^+ concentrations when amino acids are available.

Levels of NO_3^- and NH_4^+ were more closely linked with amino acids in the spring and mid-summer, respectively. The TFAA concentrations were more closely correlated with NO_3^- during all sampling periods. A similar pattern was observed in Alaskan tundra soils where patterns in TFAA and NH_4^+ concentrations were similar after mid-July in wet sedge environments and TFAA and NO_3^- levels were well correlated for all ecosystems

(Weintraub and Schimel 2005). The correlation between NO_3^- and TFAA concentrations in spring might indicate that oxygen transport to the rhizosphere was responsible for accelerating decomposition and nitrification. However, the correlation might also indicate microbial uptake of amino acids and NO_3^-, which represent carbon and nitrogen sources, respectively. Colder temperatures might inhibit microbial processing of the nitrogen species and were likely responsible for the weak correlation between nitrogen species in November.

Conclusions

Free amino acids were present in pore water of peat at concentrations ranging from undetectable to 2.3 μM. Leucine dominated the free amino acid pool; however, more highly time-resolved sampling from snow-melt to freeze-up may provide a more robust free amino acid composition. Maximum concentrations of the TFAA pool were observed in spring; however, plant functional type did not influence the magnitude or composition of the TFAA pool. Inhibition of nitrification under the anoxic conditions of the saturated peat soils was likely responsible for the high levels of NH_4^+ relative to the other nitrogen species. Deamination of amino acids might also play a role in maintaining NH_4^+ concentrations high relative to amino acids. The correlation between the various nitrogen species indicates common transformation pathways between free amino acids and inorganic nitrogen in peat. Amino acids may play a significant role in alleviating nitrogen limitations in peatland ecosystems when ammonium concentrations are depleted. The seasonal variations observed in the free amino acid pool indicate that environmental

conditions like temperature and oxygen level influence amino acid bioavailability as a carbon and nitrogen source. Thus, understanding the response of peatlands to climatic change will require further study of the cycling of amino acids in peatlands.

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Table 2.1 Average (Avg) TFAA concentrations and variance (Var) by sampling date and plant functional type

	Sedge Bins (μM)		Shrub Bins (μM)		Un-manipulated Bins (μM)		All Bins (μM)	
	Avg[a]	SD[b]	Avg	SD	Avg	SD	Avg	SD
5/9/2013	0.77	0.56	1.09	0.80	0.42	0.61	0.76	0.67
8/6/2013	0.48	0.51	0.22	0.18	0.110	0.16	0.27	0.33
11/4/2013	0.29	0.19	0.16	0.10	0.09	0.13	0.18	0.16
All Dates	0.51	0.46	0.49	0.62	0.20	0.37		

[a] Average (mean) concentration

[b] Standard Deviation

Table 2.2 Results from two factor ANOVA with replication after log transformation of TFAA concentration data

	F	p-value
Date[a]	6.79	0.004
Vegetation[b]	3.19	0.057
Interaction	0.67	0.620

[a] Data grouped by sampling date (09 May, 06 August, 04 November)

[b] Data grouped by vegetation treatment (sedge, shrub and un-manipulated)

Table 2.3 Correlation coefficients (r) for comparison of TFAA concentrations and various pore water characteristics (WT = water table height)

	5/9/2013	8/6/2013	11/4/2013	All Dates
NH_4-TFAA[a]	0.635*	0.857*	0.571	0.704*
NO_3-TFAA	0.758*	0.821*	-0.091	0.770*
WT-TFAA	-0.246	0.190	-0.063	-0.296
DOC-TFAA	0.624*	0.320	-0.050	0.297
TDN-TFAA	0.714*	0.732*	-0.039	0.649*
Phenolics-TFAA	0.651*	0.395	0.040	-0.112
NH_4-NO_3	0.753*	0.977*	0.329	0.814*
TDN-NO_3	0.805*	0.930*	0.865*	0.812*
TDN-NH_4	0.881*	0.949*	0.589*	0.840*

[a] Dash stands for comparison (e.g. NH_4-TFAA represents linear correlation between NH_4^+ and TFAA concentrations)

* Statistically significant ($p \leq 0.05$)

Table 2.4 Regression analysis results, assuming a linear regression between TFAA and various N species with the last four rows showing results for annual averages

	5/9/2013		8/6/2013		11/4/2013		All Dates	
	Slope[a]	p-value	Slope	p-value	Slope	p-value	Slope	p-value
NH_4	26.7	0.0264	82.2	3.72E-04	56.9	0.0521	38.5	1.65E-06
NO_3	0.9	4.30E-03	1.4	1.07E-03	-0.1	0.779	0.9	4.10E-08
TDN	1.0	9.08E-03	1.4	6.77E-03	-0.1	0.905	1.0	1.82E-05
NH_4 avg	-	-	-	-	-	-	56.3	3.01E-04
NO_3 avg	-	-	-	-	-	-	1.1	9.87E-04
B. Prod[b]	-	-	-	-	-	-	-106	0.258
V. Bio[c]	-	-	-	-	-	-	-39.4	0.378

[a] Slopes for regressions between nitrogen species are in units of (μM N species)·(μM TFAA)$^{-1}$ except for B. Prod and V. Bio which are in units of (g m^{-2})·(μM TFAA)$^{-1}$

[b] B. Prod – Average annual *Sphagnum* (bryophyte) production

[c] V. Bio – Annual average vascular plant standing biomass

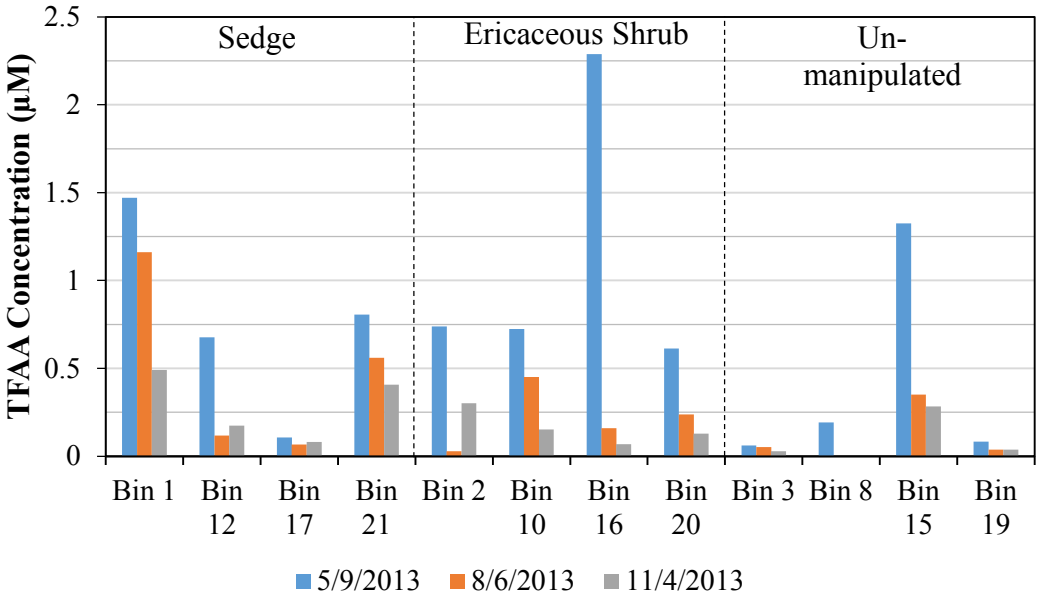

Fig. 2.1 Total free amino acid concentrations for each bin at various sampling dates

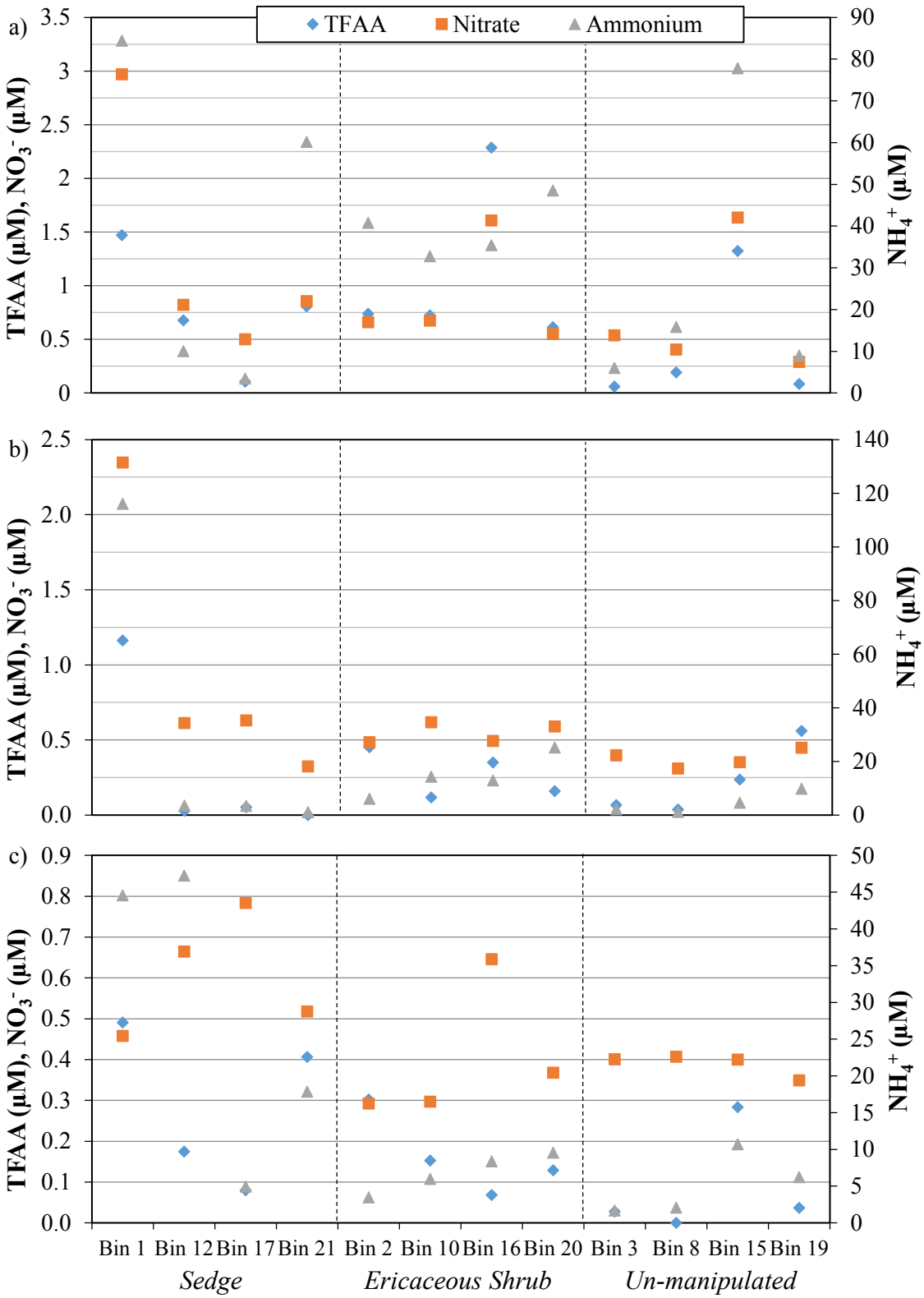

Fig. 2.2 TFAA, NO_3^- and NH_4^+ concentrations for a) 5/9/2013, b) 8/6/2013, and c) 11/4/2013

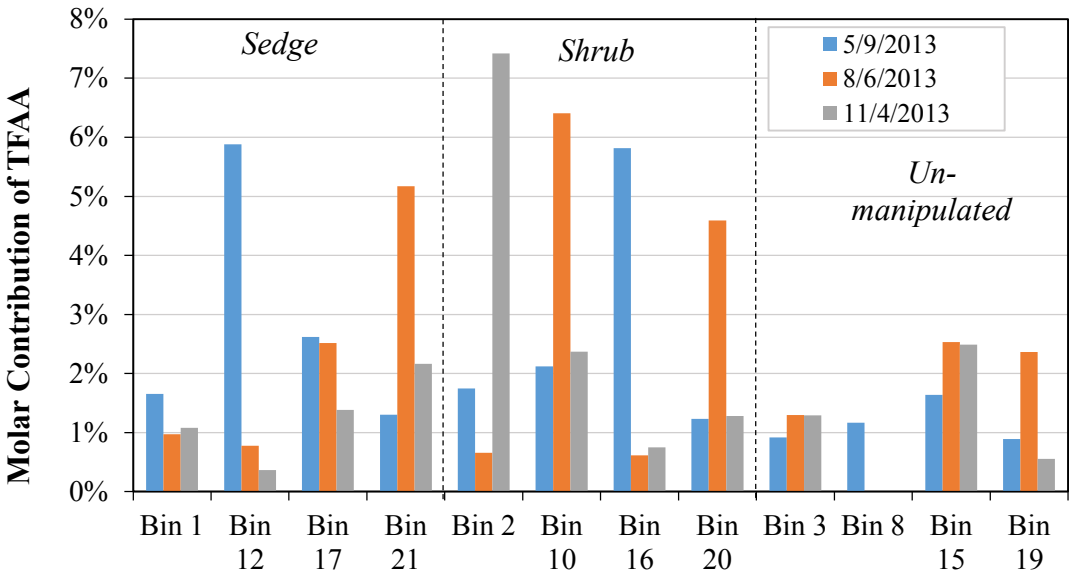

Fig. 2.3 Contribution of TFAA to the sum of NH_4^+, NO_3^- and TFAA for various sampling dates

Amino acids in pore water of peat

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Supplementary Information

Site description

Table A2.1 Mean NH_4^+ and NO_3^- concentrations for seasonal precipitation for NADP site MI99 in Chassell, MI (NADP 2015)

Season	Start Date	End Date	NH_4^+ (μM)	NO_3^- (μM)	Precipitation (cm)
Winter	11/27/2012	2/26/2013	8	11	21
Spring	2/26/2013	5/28/2013	26	13	28
Summer	5/28/2013	8/29/2013	24	12	27
Fall	8/29/2013	12/2/2013	17	10	22

Notes on methods

The peak areas for each individual amino acid were averaged for all reagent blanks run in each batch. The method blanks were included in this average (filtered reagent blanks) because they were not significantly different from the reagent blanks with the exception of the glycine peak area. For glycine, the method blanks and reagent blanks were kept separate for the two batches that included a method blank. The first batch (5/9/13

samples) did not include a method blank, but the glycine peak areas in the reagent blanks were significantly higher than peak areas in the other two batches (due to differences in derivatization reagent age) and of the same magnitude observed for method blanks, so reagent blanks were used in place of method blanks for this batch. Amino acid peak areas for reagent blank averages were subtracted from standard peak areas and linear standard curves were created for each amino acid. If replicate standards were run, peak areas were averaged for the standard before the reagent blank peak areas were subtracted.

Table A2.2 Elution gradient for UHPLC amino acid analysis

Elapsed Time (min)	Mobile Phase B
0	1%
1.9	1%
15	47%
15.5	100%
18.7	100%
19.6	1%
22.4	1%

Table A2.3 Various analytical limits for UHPLC analysis of amino acids

	LoB[a, b]			LoD[a, b]			3·(Reagent Blank)[b]			LoQ[a, b]		
	B1[c]	B2	B3	B1	B2	B3	B1	B2	B3	B1	B2	B3
Asp	38.8	24.1	23.0	38.8	24.1	23.0	78.0	61.8	63.3	78.0	61.8	63.3
Glu	15.6	5.6	6.9	15.6	5.6	6.9	24.3	15.1	12.5	24.3	15.1	12.5
Ser	46.9	24.5	18.8	46.9	24.5	18.8	120.8	59.2	43.8	120.8	59.2	43.8
His	33.8	20.2	17.5	33.8	20.2	17.5	60.6	51.2	40.5	60.6	51.2	40.5
Gly	29.1	11.6	20.5	29.1	11.6	20.5	74.8	64.1	87.3	74.8	64.1	87.3
Thr	43.8	17.8	36.5	43.8	17.8	36.5	97.8	40.3	92.9	97.8	40.3	92.9
Arg	16.4	1.4	0.0	16.4	1.4	0.0	19.9	1.0	0.0	19.9	1.4	0.0
Ala	51.3	42.6	27.8	51.3	42.6	27.8	121.0	103.9	65.0	121.0	103.9	65.0
Tyr	11.2	4.4	4.6	11.2	4.4	4.6	16.5	9.9	10.3	16.5	9.9	10.3
Val	22.2	12.1	7.8	22.2	12.1	7.8	34.7	26.7	17.9	34.7	26.7	17.9
Met	13.4	4.9	2.8	13.4	4.9	2.8	18.3	8.8	5.2	18.3	8.8	5.2
Phe	13.4	5.1	3.5	13.4	5.1	3.5	18.4	9.8	7.0	18.4	9.8	7.0

Iso	13.3	4.6	1.8	13.3	4.6	1.8	22.4	8.4	4.6	22.4	8.4	4.6
Leu	14.7	8.5	5.4	14.7	8.5	5.4	30.7	20.0	10.9	30.7	20.0	10.9
Lys	9.5	6.9	3.0	9.5	6.9	3.0	20.9	13.1	2.3	20.9	13.1	3.0

[a] LoB – Limit of Blank [LoB = Mean $_{\text{Reagent Blank}}$ + 1.645·(Standard Deviation $_{\text{Reagent Blank}}$)], LoD – Limit of Detection [LoD = LoB + 1.645·(Standard Deviation $_{\text{Low Standard}}$)], LoQ – Limit of Quantitation, see Armbruster & Pry (2008) for a description of each limit. The LoQ was chosen to equal the greater of the LoD or 3·(Reagent Blank) and was used to determine if amino acid concentrations were reportable.

[b] Values are in nanomolar concentration (nM)

[c] Each numbered B refers to a separate blank (e.g. B1 – blank one)

Table A2.4 Ion chromatography instrument and method

	Anions (Dionex ICS-2100)	Cations (Dionex ICS-1100)
Sample Loop Volume	25 µL	25 µL
Analytical Columns	Dionex IonPac AS-11 HC	Dionex IonPac CS12A
Guard Columns	Dionex IonPac AG-11 HC	Dionex IonPac CG12A
Self-Regenerating Suppressor	Dionex AERS 500 4 mm	Dionex CERS 500 4 mm
Eluent	30 mM potassium hydroxide (KOH)	20 mM methanesulfionic acid (MSA)
Eluent Flow Rate	1.5 ml/min	1 ml/min
Run Time	15.5 min	15.5 min
Column Heater	Yes (35°C)	No
Conductivity Cell (Detector)	DS6 Heated Conductivity Cell	DS6 Heated Conductivity Cell
Autosampler	Dionex AS-DV Autosampler	Dionex AS-DV Autosampler

Supplemental Data

Table A2.5 Contribution of leucine (Leu) and other amino acids to the TFAA pool

	5/9/13		8/6/13		11/4/13[a]
	Leu (µM)	Other AA (µM)	Leu (µM)	Other AA (µM)	Leu (µM)
Bin 1 (S[b])	1.47	-	1.09	0.07[f]	0.49
Bin 2 (E)	0.68	0.06[c]	0.03	-	0.30
Bin 3 (U)	0.06	-	0.05	-	0.03
Bin 8 (U)	0.19	-	-	-	-
Bin 10 (E)	0.72	-	0.32	0.13[g]	0.15

Bin 12 (S)	0.68	-	0.12	-	0.17
Bin 15 (U)	1.21	0.11[d]	0.35	-	0.28
Bin 16 (E)	0.98	1.31[e]	0.16	-	0.07
Bin 17 (S)	0.11	-	0.07	-	0.08
Bin 19 (U)	0.08	-	0.04	-	0.04
Bin 20 (E)	0.61	-	0.24	-	0.13
Bin 21 (S)	0.81	-	0.56	-	0.41

[a]Only leucine was present for all samples

[b]Vegetation treatments: S - sedge, E - ericaceous shrub, U - un-manipulated

[c]Arginine (8%)

[d]Glycine (8%)

[e]Arginine (18%), Alanine (12%), Lysine (9%), Aspartate (5%), less than 5% of Tyrosine, Valine, Methionine, Phenalanine, Isoleucine

[f]Phenalanine (3%), Isoleucine (3%)

[g]Serine (15%), Glycine (15%)

Chapter 3

Process-scale model of seasonal free amino acid cycling in a peatland bog

Abstract A process-scale model was developed to estimate seasonal amino acid concentrations in the pore water of peatland bogs. Model results using Simulink® show seasonal variation in the concentration of amino acids for peatland pore water. Maximum concentrations are attained in spring and fall and amino acids are depleted from pore water in late summer. Microbial processes dominate the flux of amino acids for a majority of the year; however, plant uptake makes a contribution to the flux for a short period after ammonium is depleted and before amino acids are depleted from pore water. The summertime amino acid and ammonium pool depletions predicted by the model were not observed in pore water samples from peat mesocosms, and the fall increase in amino acid and ammonium concentrations was not seen in pore water samples, indicating that the model is not able to fully capture the seasonal dynamics of peatlands observed in peat mesocosms. For simplicity, the model did not incorporate a limitation on plant nitrogen uptake at low nitrogen species concentrations, atmospheric nitrogen inputs or variability in the soil organic matter pool. The simplifications may in part account for the discrepancies between the model and pore water samples. Greater plant uptake of amino acids results in a longer period of amino acid depletion and lower maximum concentrations. Predictions of amino acid concentrations show the greatest sensitivity to decreases in proteolysis and microbial uptake rates, which decrease and increase amino acid concentrations, respectively. Further research on microbial processes in peatlands and plant preference for various nitrogen species are required to improve modeling of

seasonal amino acid concentrations and to advance understanding of nutrient limitation in peatland bogs.

Introduction

Peatlands are a type of wetland with large stores of carbon relative to land surface area that occupy 3-5% of the land surface with 0.5% of the land surface identified as peatland bogs, fens and mires (Dahl and Zoltai, 1997; Lehner and Doll, 2004). Many peatland ecosystems are located in Northern latitudes where growing seasons are short due to colder temperatures and where seasonal patterns in temperature and precipitation are common. Ombrotrophic peatland bogs have saturated soils for extensive periods of the year and receive almost all nutrients from atmospheric inputs and internal cycling (Gorham, 1957). Sphagnum moss often dominates the vegetation in bogs. Large stores of carbon in water-saturated soils make peatlands particularly sensitive to climate change.

Biotic processes regulate the cycling of carbon between the atmosphere and carbon reservoirs in peatlands. Biological growth and decay are dependent on the availability of nutrients within the ecosystem with nitrogen often limiting primary production. The major nitrogen species available for biological uptake are nitrate (NO_3^-), ammonium (NH_4^+), and organic nitrogen. Permanent soil saturation limits nitrification and favors denitrification, and thus, concentrations of NO_3^- in pore water are often low. Amino acids are a water soluble form of bioavailable organic nitrogen (Lipson and Nähsolm, 2001). Amino acids that are not associated with protein polymers are considered a reactive form of organic matter that is readily available to biota (Cowie and

Hedges, 1993). Microbial communities, mycorrhizal fungi, and plants have been shown to take up amino acids directly from soil (see, e.g., Jones and Darrah, 1993; Jones et al., 2005a and 2005b; Krab et al., 2008). Alpine plant functional types, like sedges and *Sphagnum* mosses, have also been shown to take up amino acids (Kielland, 1994; Leadley et al., 1997; Lipson and Monson, 1998). Thus, understanding the role of amino acids as nutrients for plant and microbial communities of bog ecosystems is required to identify the processes that regulate the cycle of amino acids in water-saturated soils.

The biogeochemical cycle of nitrogen species in peatlands can be used to predict future changes in the response of peatlands to environmental factors like increasing temperature or elevated CO_2. The response of vascular plants to CO_2 enrichment was limited by nutrient availability that prevented increased carbon sequestration (Langley and Megonigal, 2010). Increases in vascular plant biomass in response to CO_2 enrichment were accompanied by a decrease in sphagnum, which would have implications for internal cycling of nitrogen and decomposition of peat due to differences in litter quality of the various plant functional types (van Breemen, 1995; Berendse et al., 2001; Freeman et al., 2001). Seasonal changes in amino acid availability might play a role in limiting or increasing nitrogen availability, and thus, amino acids may impact growth of vascular plants and decomposition rates in response to increased CO_2. Increasing temperatures might lower water tables in peatland bogs and other peatland ecosystems and accelerate the release of CO_2 from decomposition of the extensive carbon reservoirs (Yu et al., 2003). Examining the sensitivity of peatlands to future climate change scenarios requires advances in understanding of nutrient limitations of ecosystem-scale processes, particularly the processes that link the nitrogen and carbon cycles.

Project definition

A literature review was conducted and a process-scale model of amino acids was developed to improve understanding of amino acid cycling in peatland bogs. The model was first used to identify the dominant processes in regulation of free amino acid concentrations in bog pore water. Secondly, the model was used to assess the seasonal changes in amino acid concentrations. Various model parameters were then altered to determine model sensitivity to each parameter. Model uncertainty was considered in the context of current scientific knowledge.

Methods

Literature review

A collection of journal articles was compiled to identify the basic biogeochemical processes that influenced amino acids in peatland bogs. The literature review focused on regions in which amino acid cycling studies had been performed that included boreal forests and tundra and peatlands in alpine and Arctic environments. Articles on plant or microbial uptake of amino acids were collected; however, studies of bog ecosystems were few, and thus, findings from investigations of forest ecosystems were used in the model development. The body of literature on measurement of amino acid concentrations in peatlands is limited; however, amino acid concentrations in alpine tundra ecosystems

have been determined. If impacts of soil saturation and organic matter content on cycling of amino acids are accurately represented in the model, the magnitude of amino acid concentrations in peatlands can be estimated for various types of ecosystems. Ecosystem-scale models of amino acid cycling in boreal forest and tundra ecosystems (Zhu and Zhuang, 2013) and in alpine soils (Lipson et al., 2001) were used to guide model development. A diagram of the biogeochemical cycle of amino acids in a bog ecosystem, which identifies reservoirs and fluxes between reservoirs, is shown in Fig 3.1. The plant functional types for bogs included shrubs, sedges (graminoids), and bryophytes, which are identified as blue, orange, and green, respectively. Atmospheric inputs of amino acids were not considered to simplify the model because of the lack of regional data and the episodic nature of atmospheric deposition.

Model development & application

The major reservoirs of amino acids included species (1) dissolved in pore water, (2) adsorbed to dissolved organic matter (DOM), (3) associated with plant biomass, and (4) incorporated in soil organic matter and microbial biomass. The cycle of free amino acids in pore water was the focus of the modeling experiment. Fungi were not included to simplify the model. A sinusoidal curve was used to represent variations in plant nitrogen uptake. The microbial biomass and soil organic matter pools were not tracked by the model for simplicity, so it was assumed that proteolysis rates would not be limited by protein availability. The concentration of amino acids adsorbed to DOM was assumed to

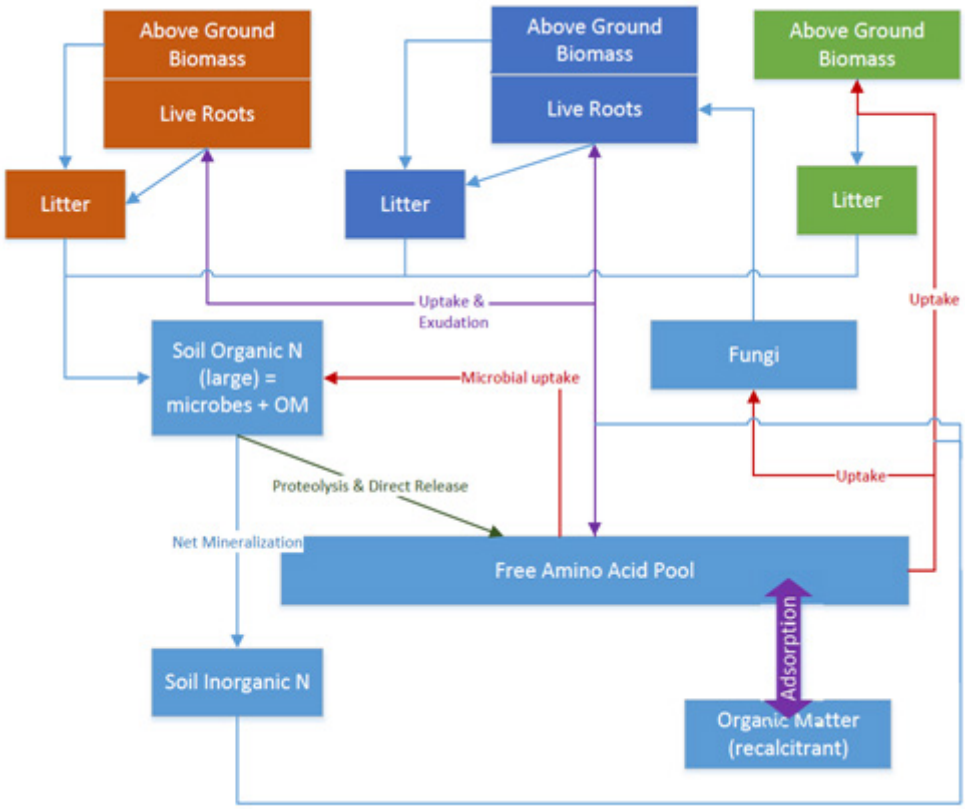


Fig. 3.1 Biogeochemical cycling of amino acids in peatland bogs

be 7% of the free amino acids in pore water (Rothstein, 2010). Major sources and sinks are identified in Table 3.1. Atmospheric inputs of NH_4^+ were not included because of the episodic nature of deposition events; however, it should be noted that seasonal NH_4^+ concentrations in precipitation for the National Atmospheric Deposition Program site (MI99) in Chassell, MI, ranged from 8-26 µM in 2013 (NADP 2015).

Proteolysis rate constants were developed from measurements of proteolysis during various seasons in alpine soils (Lipson et al., 1999a; Table 3.2). Inputs from the soil organic matter pool to the free amino acid pool of pore water via proteolysis were not adjusted for fluctuations in microbial biomass. Instead, a constant proteolysis rate was designated for specific seasonal periods based on changes in microbial biomass (see

Table 3.1 Sources and sinks for amino acids in bog pore water

Sources	Flux	Modeled	Sinks	Flux	Modeled
Proteolysis	$P(t)\cdot\theta^{T-Tref}$	yes	Plant Uptake	F(t) based on plant needs	yes
	$\theta=(1-k_p)$				
Root release	constant	no	Microbe Uptake	$[FAA]\cdot R(T)\cdot\theta^{T-Tref}$	yes
				$\theta=(1-k_R)$	
Cell lysis	constant	no	Adsorption		yes
Desorption		yes			

column 5 and 7 of Table 3.2). A first order decay rate was used to represent microbial uptake of amino acids. Similar to proteolysis, the rate constants for microbial uptake were developed for seasonal periods. Respiration and proteolysis rates in Table 3.2 are laboratory measurements at corresponding reference temperatures and were adjusted for field temperatures. Rate constants for seasonal periods were separated based on changes in observed rates. Rates for proteolysis and respiration were corrected for temperature using k_P, 0.068 h^{-1}, and k_R, 0.094 h^{-1}, respectively (Lipson et al., 2001). Net mineralization was assumed to be 33% of microbial nitrogen uptake (Lipson et al., 2001).

Table 3.2 Proteolysis and microbial uptake rates used in model and from literature

Date ranges used for model (days after Jan. 1)	Modelled P nmol g^{-1} h^{-1}	Modelled R (h^{-1})	Dates for measured P	P nmol AA g^{-1} h^{-1}	Ref Temp (°C)	Microbial Biomass µg C/g soil	Source
0-152, 274-365	55	0.19	May	55	5	650	*Lipson et al. (1999) Alpine Soil*
152-182	14	0.209	June	14	5	500	
182-213	1	0.43	July	1	5	300	
213-274	17	0.427	August	14	5	500	
			Sept	20	5	500	
			Dates for measured R	R (h^{-1})	Ref Temp (°C)	Source	
			7-May	0.16	5	*Lipson et al. (2001) Alpine Soil*	
			31-May	0.222	5		
			26-Jun	0.209	16		
			28-Jul	0.43	16		
			27-Aug	0.427	16		
			23-Sep	0.427	16		

Uptake of nitrogen by bryophytes and vascular plants was estimated separately using sinusoidal curves. The change in total biomass nitrogen for each plant type is calculated by subtracting root and litter nitrogen losses from total nitrogen requirements that are based on plant production. Fluxes for litter nitrogen and root nitrogen are then added back to the biomass nitrogen flux to obtain the initial plant nitrogen uptake required for plant production. Plant production is independent of plant nitrogen uptake in the subject model, and thus, subtracting and then adding the same fluxes is unnecessary. However, the subtraction and addition was included to incorporate nutrient-dependent plant production in future model adaptations. Average bryophyte and vascular (stem and roots) plant production rates in the model were 4.3 mg N m^{-2} d^{-1} and 1.2 mg N m^{-2} d^{-1}, respectively, and tissue nitrogen content was 8 mg N g^{-1} (Gunnarsson, 2005; Limpens et al., 2006). The amplitude of the vascular plant production curve was determined by assuming a minimum production of zero, which resulted in a maximum plant production rate within estimated ranges and prevented zero values for plant production nitrogen requirements (Limpens et al., 2006). The amplitude of the bryophyte plant production curve was determined from the maximum whole ecosystem production rate adjusted to 20% moss production, which resulted in a maximum rate of 0.0061 g N m^{-2} d^{-1} (Limpens et al., 2006). Plants were assumed to prefer NH_4^+ to meet nitrogen requirements with uptake of amino acids following depletion of NH_4^+. Michaelis-Menten kinetics (Kielland 1994; Kielland, 1997) were used to represent NH_4^+ uptake. Plant growth was not adjusted when plant nitrogen uptake was greater than available nitrogen. Requirements for plant nitrogen were set equal to uptake rates when nitrogen requirements for plant growth were greater than the nitrogen uptake rates that were based on pore water NH_4^+ and amino acid

concentrations. The simplification neglected the consequences of nitrogen limited plant growth; however, proteolysis was not limited by substrate (i.e., litterfall) availability, and thus, impacts were not expected to be significant. Expressions for plant production and uptake were used to determine the output fluxes of amino acids and NH_4^+ from pore water.

General units for fluxes and pore water concentrations are g N m^{-2} d^{-1} and mg L^{-1}, respectively. Peat density was assumed to be 0.0295 g cm^{-3} and was based on averages of measured densities of 0-10 and 10-20 cm deep layers of a Minnesota peat bog (Potvin et al., 2014). The surface to 0.2 m deep layer of peat was considered in the model and an average water saturation of 50%, which is typical for saturated wetland soils, was assumed (Schlesinger and Bernhardt, 2013). The pattern of seasonal temperature was represented by a sinusoidal function with a range of -13 °C to 24 °C (Potvin et al., 2014).

The seasonal biogeochemical cycle of amino acids for peatland bogs was modelled using Simulink® software. A one-year period in units of days was considered using time as the independent variable, and amino acid (mg L^{-1} pore water) and NH_4^+ (mg L^{-1} pore water) concentrations were allowed to vary with time. The integrator block in Simulink® was used to simultaneously solve the system of differential equations (using variable-step solver with automatic solver selection). A diagram of the model is shown in Fig. 3.2. The left-and right-hand sides include estimations of the differential equations for NH_4^+ (dy dt^{-1}) and amino acids (dx dt^{-1}), respectively. The model was run for multiple years to determine initial concentrations of NH_4^+ and amino acids. Input concentrations were adjusted at the beginning of each year to match the final winter time concentrations for the previous year. Initial concentrations were established when the input and final

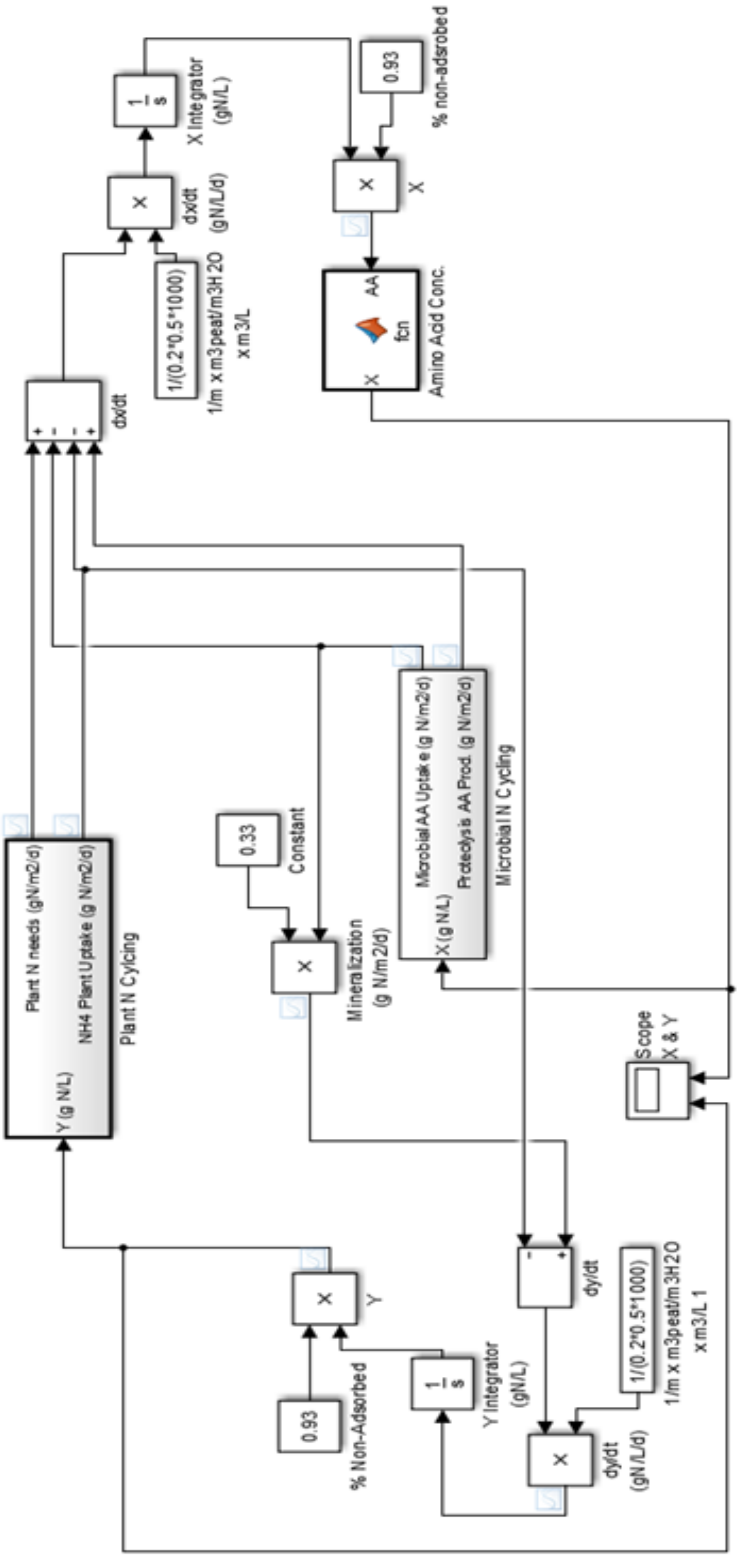


Fig. 3.2 Simulink ® model of amino acid and ammonium cycling in peatland bog

concentrations converged on the same value. Initial concentrations of NH_4^+ and amino acids for the baseline model were 9000 μg N L^{-1} and 200 μg N L^{-1}, respectively. After a 1-year baseline simulation, model parameters, including proteolysis rate, microbial uptake rate, NH_4^+ concentrations and plant uptake rate, were altered to determine impact on model output. Each parameter was adjusted individually for simplicity. Results for the baseline simulation and the adjusted simulations are reported in the following section.

Results

Model outputs

Inputs of amino acids to pore water include proteolysis and outputs are represented by plant and microbial uptake (Fig. 3.3). Model results indicate that fluxes of amino acids via microbial uptake are generally much greater than those associated with plants. Plant nitrogen requirements are always met by NH_4^+ uptake except for a short period in June and August when NH_4^+ is depleted. Plant uptake is the dominant pathway for removal of amino acids during periods in June and August after NH_4^+ depletion. Plant requirements for nitrogen are greater than microbial production rates via proteolysis during the depletion period. Proteolysis rates increase in fall, followed by uptake of amino acids by microbes, which is accompanied by an increase in NH_4^+ due to net mineralization. Microbial processes regulate amino acid concentrations during a majority of the year and plant processes dominate for a short period of the summer.

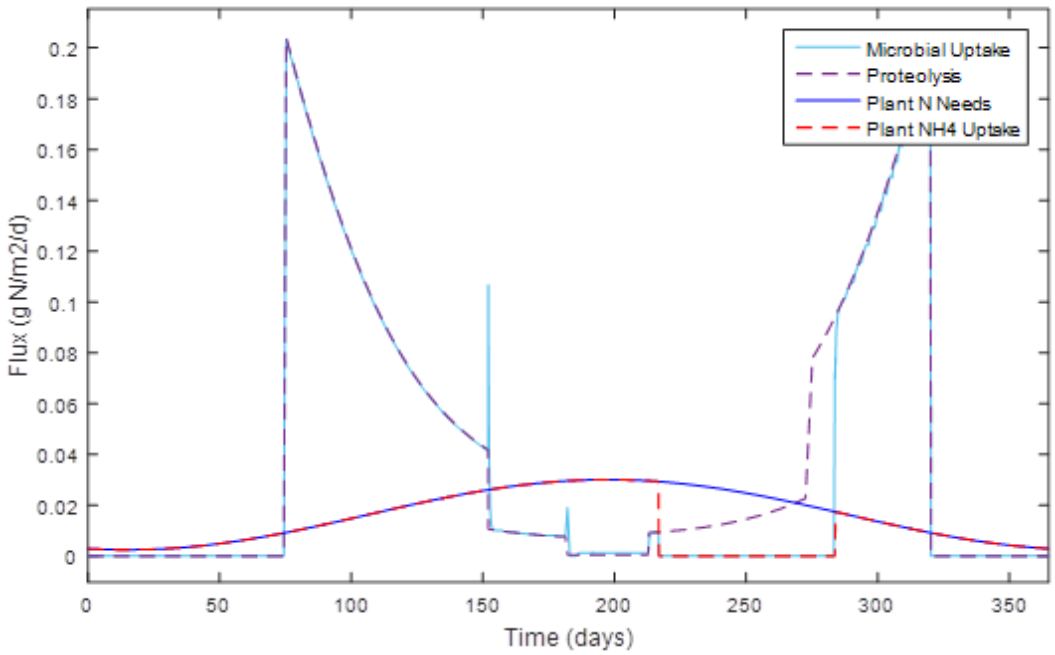
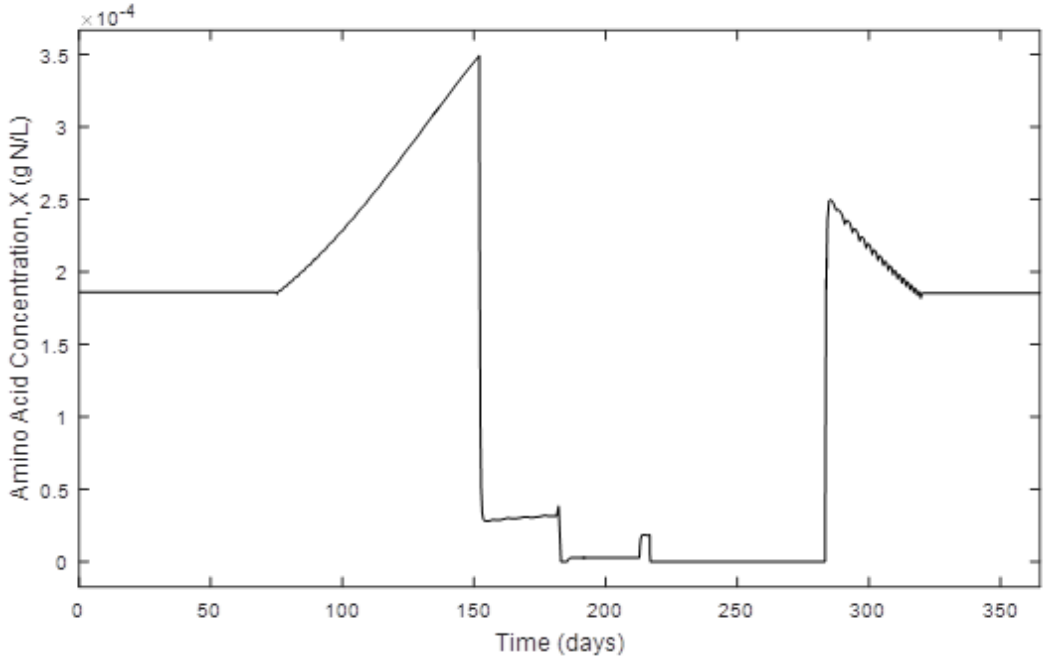
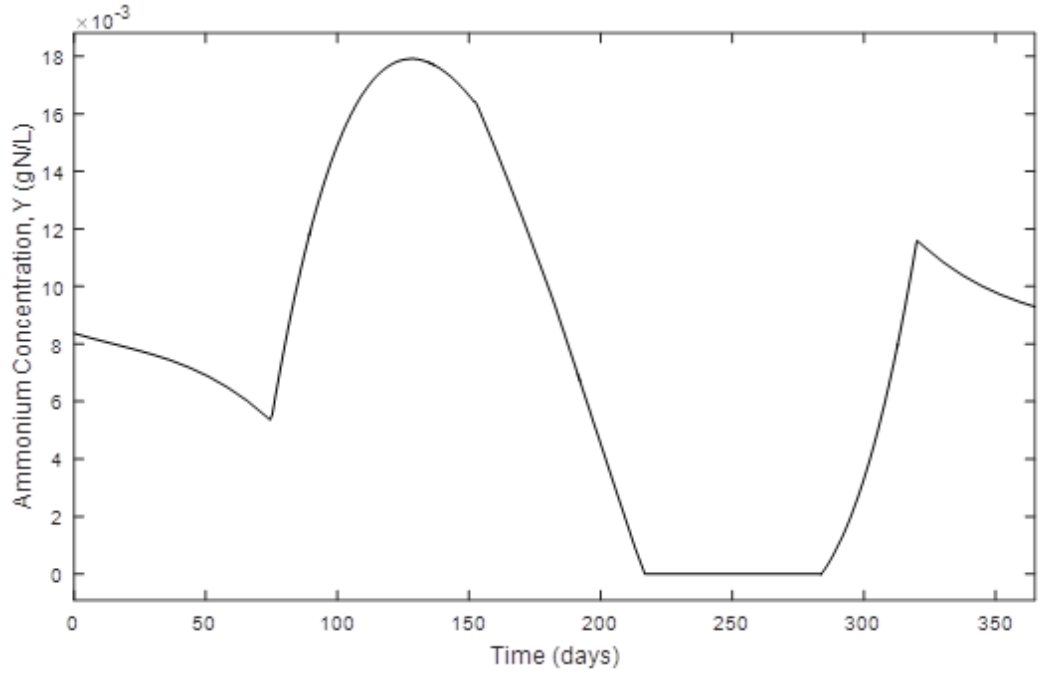


Fig. 3.3 Nitrogen fluxes for amino acid concentration in pore water (starting Jan. 1)

Concentrations of amino acids in pore water reached a maximum in the late spring and mid fall and were at a minimum between the 2 periods (Fig. 3.4). The pattern in the NH_4^+ concentrations is similar to the amino acids; however, maximum levels are reached earlier in spring and later in fall. Depletion of NH_4^+ occurs before the amino acids due to preferential uptake of NH_4^+ by plants. The level of amino acids was 0-350 μg N L^{-1} and remained constant during the winter period (200 μg N L^{-1}). A slight increase in amino acid concentrations was observed in June; however, levels quickly diminished due to uptake by plants in response to NH_4^+ depletion.



(a)



(b)

Fig. 3.4 Amino acid (a) and ammonium (b) concentrations in pore water (starting Jan. 1)

Manipulation of model parameters

The concentration of amino acids decreased when proteolysis rates were decreased by a factor of 10, showing depletion for a majority of the year. The concentration of amino acids significantly increased when microbial uptake rates were increased by a factor of 10 (Fig. 3.5). The initial concentration of the baseline study was retained for both simulations; however, neither simulation resulted in a similar winter amino acid concentration to the baseline simulation. Amino acid concentrations reached a maximum (3500 μg N L^{-1}) in the simulation of reduced microbial uptake with a concentration of 2000 μg N L^{-1} during winter. The maximum amino acid concentration for the simulation of diminished proteolysis was 25 μg N L^{-1}. After the maximum was reached the amino acids remained depleted for the remainder of the year. Adjustment of both microbial process rates generally resulted in drastic changes to amino acid concentrations; however, all 3 simulations showed depletion of amino acids in late summer.

Simulations in which NH_4^+ concentrations were set to zero, and in which plant uptake of amino acids was held constant at 50%, predicted similar amino acid concentrations (Fig. 3.6); however, the simulation in which NH_4^+ concentrations were held constant at 300 μg N L^{-1} were similar to the baseline simulation. Amino acids were quickly depleted and were only greater than zero in spring and fall for the simulations with NH_4^+ concentrations held at zero and with the level of plant uptake at 50% amino acids. Predicted levels of amino acids only deviated from results of the baseline simulation in summer and early fall when NH_4^+ was held constant at 300 μg N L^{-1}, which corresponded to the period when NH_4^+ was depleted in the baseline simulation. Amino

acid concentrations were below the levels of baseline simulation in spring and fall in the two simulations where plant uptake of NH_4^+ was reduced, and concentrations were greater than baseline levels in summer and early fall in the simulation where NH_4^+ concentrations met plant nitrogen requirements.

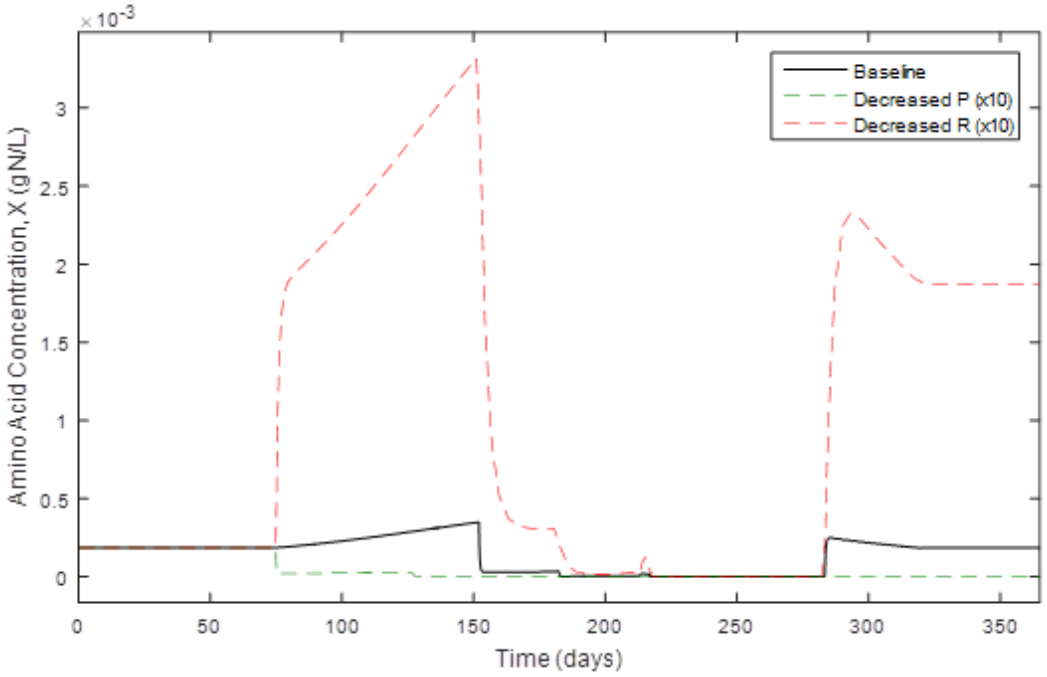


Fig. 3.5 Amino acid concentrations for decreased proteolysis and microbial uptake

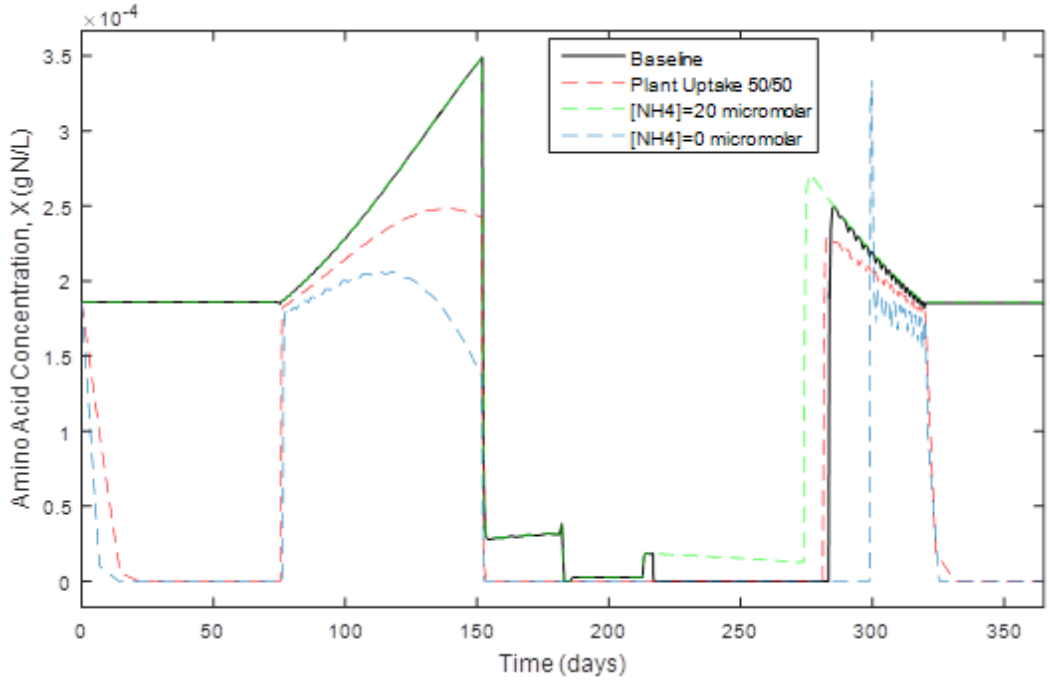


Fig. 3.6 Amino acid concentrations for set NH_4^+ concentrations and adjusted plant uptake

Discussion & implications

Dominant pathways

Model simulations of the contribution of the various amino acid removal pathways to total removal can be compared to studies of nitrogen uptake in the literature. Assuming 7% adsorption of amino acids to dissolved organic matter (DOM), microbial and plant uptake were 13.4 (89%) and 550 mg N m^{-2} y^{-1} (4%), respectively. In arctic tundra soils, 1-12% and 41-68% of added glycine was shown to be taken up by plants and microbes, respectively (Lipson and Monson, 1998); however, in alpine tundra soils approximately 3% and 20%, of added amino acid nitrogen was taken up by plants by microbes,

respectively (Lipson et al. 1999b). Table 3.3 shows amino acid flux partitioning for various ecosystems. Contributions of plant uptake of added amino acid nitrogen to the total amino acid uptake are low, which is in agreement with the model simulations that indicate microbial uptake dominates amino acid nitrogen removal from soil pore water. The model generally captured the dominance of microbial uptake of amino acids and the small contribution of plants to amino acid removal from pore water; however, more highly time resolved measurements of amino acid concentrations in peatland bogs is lacking, which makes it difficult to determine model accuracy.

Table 3.3 Amino acid partitioning between microbial, plant and sorption fluxes in various ecosystems

Ecosystem	Amino Acid	Microbial Uptake	Plant Uptake	Sorption	Source
Acidic Forest Soils	Various	7-45%, Assimilation 6-48% Mineralization	n/a	4-15%	Rothstein (2010)
Alpine Tundra Soils Microcosm	Glycine Glycine	5.0% summer 9.1% fall	3.5% summer 0.8% fall	n/a	Lipson and Monson (1998)
Arctic Tundra Soil	Glycine	41-68%	1-12%	n/a	Schimel and Chapin (1996)
Alpine Tundra Soil Microcosm	Glutamate Glycine	19.70% 22.58%	1.66% 3.98%	n/a n/a	Lipson et al. (1999)

The model currently assumes that all plant nitrogen requirements will be met by NH_4^+ if concentrations are sufficiently high to meet the demand, which is regulated by Michaelis-Menten kinetics. Levels of NH_4^+ in the model are sufficiently high to meet plant requirements for a majority of the year, and plant uptake of amino acids only occurs for a short period of time in mid-summer. Rates of uptake for glutamate and glycine by plants were found to be 22.6 and 73.4 nmol g^{-1} day^{-1}, respectively, in an alpine tundra soil mesocosm experiment (Lipson et al., 1999b). Uptake rates for NH_4^+ were 103-185 μmol g^{-1} day^{-1} for *Eriophorum vaginatum* (tundra sedge) and 48-720 μmol g^{-1} day^{-1} for various

taiga species (Chapin et al., 1986; Marion and Kummerow, 1990). Observed rates of NH_4^+ uptake are greater than the rates for uptake of amino acids, which implies plants prefer NH_4^+ to amino acids to meet nutritional requirements; however, the difference between uptake rates of individual amino acids indicates that some amino acids may be taken up even when NH_4^+ is available. Kielland (1994) determined kinetic parameters of amino acid uptake for various species of Arctic tundra plants and found that glycine was taken up at much higher rates than aspartate and glutamate. Deciduous shrubs, evergreen shrubs and graminoids exhibited the greatest, intermediate, and least capacity for glycine uptake, respectively. Average uptake rates (and the contribution to plant uptake) based on observed species concentrations and kinetic parameters for NH_4^+, glycine, aspartate and glutamate were estimated to be 17.52 (40.8%), 23.04 (53.6%), 1.44 (3.4%) and 0.96 μmol g^{-1} day^{-1} (2.2%), respectively (Kielland, 1994).

The rapid depletion of amino acid concentrations in the baseline model simulation does not agree with the observations by Kielland (1994), which showed a large contribution of amino acids to plant nitrogen uptake. Microbial kinetic parameters in the simulation were not specific to the ecosystem, which might have led to the discrepancy. Glycine was the only amino acid that exhibited significant uptake by Arctic tundra plants; however, the model did not distinguish between specific amino acids. Free amino acid concentrations were diminished in model simulations in which plants competed for amino acids and NH_4^+, and thus, future modeling experiments should include simulations of molecular-specific uptake. The depletion of NH_4^+ and amino acids was also not observed in the mesocosm study of peat pore water; concentrations in the summer and fall were low for peat pore water. The remaining low concentrations may indicate there is

a minimum concentration below which plants simply are not able to take up these nitrogen species; however, the majority of the mesocosm peat pore water samples only showed leucine present, possibly indicating that the amino acids plants prefer to take up were depleted for this period.

Seasonality & magnitude of the free amino acid pool

Maximum concentrations of amino acids were simulated in late spring and mid-fall, and minima were forecast by the model for late summer with a total range in concentration of amino acids of 0-350 μg N L^{-1} (0-24 mg N kg^{-1} peat). Ranges of total free amino acid concentrations in northern environments are lower than the predicted range (Table 3.4). Ström et al. (2012) found the average asparagine concentration was 28 μg N L^{-1} in an Arctic fen, and the contribution of asparagine to the free amino acid pool was 72-82%. In the current study, concentrations of free amino acids in pore water of peat were 0-32 μg N L^{-1}. Levels of free amino acids in waters of a Minnesota bog were very low (Swain et al. 1959). Higher concentrations of amino acids are observed in drier alpine and Arctic environments than fens, wet meadows, and bogs. Thus, using microbial kinetic parameters for forest soils in simulations of bog ecosystems may have contributed in part to the discrepancy in TFAA concentrations. Myccorhizal fungi, which may compete with bacteria and plants for available nitrogen, were not considered in the model. Myccorhizal fungi have the ability to take up various inorganic and organic nitrogen species (Finlay et al., 1992; Clemmensen et al., 2008) and sometimes exhibit a preference for amino acids

(Read et al., 2004); therefore, it will also be important to consider fungi in future models of peatlands.

In the current study of peat mesocosm pore water, leucine dominated the free amino acid pool. Single amino acids have also been observed in an Artic fen (Ström et al. 2012), Artic salt marsh (Henry and Jefferies, 2002) and shrub and heath tundra (Clemmenson et al., 2008). The dominance of a single amino acid may indicate rapid turnover of the additional amino acids contributing to the TFAA pool. The depletion observed in the model may not have been observed in peat mesocosm pore water because leucine, which contributed most of the TFAA pool, is not cycled as rapidly as other amino acids.

Predicted concentrations of NH_4^+ (0-18 mg N L^{-1}; 0-1.2 g N kg^{-1} peat) were greater than field observations. In the current study, levels of NH_4^+ in pore water of peat were 15-1600 μg N L^{-1}, which is an order of magnitude less than the model predictions. Net mineralization was assumed to be a percentage of amino acid uptake, and thus, over predicting amino acid concentrations by an order of magnitude is likely responsible for the over prediction of NH_4^+ concentrations. The higher concentrations of NH_4^+ simulated in model compared to field observations may also in part be attributed to an under prediction of biotic uptake due to the absence of fungi in the model. Variations in the seasonality of NH_4^+ concentrations that were observed in various studies are significant. Model simulations of the seasonal pattern of NH_4^+ concentrations were similar to the observations of Raab et al. (1999) and Henry and Jefferies (2002). Elevated concentrations of NH_4^+ in spring were also observed in a Minnesota bog (Urban and Eisenreich, 1988); however, the elevated spring NH_4^+ concentrations were attributed to

snow melt preventing uptake of NH_4^+ by biota, and the model did not captured this process. The model's discrepancy with observations of low NH_4^+ concentrations in summer for peat pore water may be explained to some degree by the lack of atmospheric inputs in the model.

The seasonal pattern observed in the model simulation is similar to the pattern observed by Kielland (1995); however, Weintraub and Schimel (2005) and Raab et al. (1999) observed maxima in spring or fall. Measured concentrations of total free amino acids and NH_4^+ in pore waters of peat in the current study were generally greater in spring and lower in summer and fall. Observed concentrations in spring were similar to the predicted levels; however, the increase in post-growing season concentrations of amino acids was not observed in the current study of pore water of peat. The simulated post-growing season increase in predicted amino acid concentrations is due to an increase in proteolysis rates, and thus, model discrepancies during the period are likely due to an inaccurate representation of proteolysis or an additional removal mechanism. The model did not track the soil organic matter pool; however, the discrepancy between the model and pore water observations may indicate that proteolysis is substrate limited in the fall, preventing the build-up of the TFAA pool.

Table 3.4 Total free amino acid (TFAA) and dissolved inorganic nitrogen (DIN) concentrations

Environment	Total Free Amino Acids	Maximum TFAA	NH_4^+ (mg/L)[1]	NO_3^- (mg/L)[1]	Maximum DIN
Oligotrophic Peatland[3]	n/a	n/a	~ 0.20	~ 0.20	July
Artic Salt Marsh[4]	25 - 47 µM-N	Varied	53 - 203 µM-N	8 - 37 µM-N	Spring & late GS[2]
Minnesota Bog (streamflow)[5]	n/a	n/a	< 0.40 - 0.80 mg N/L	< 0.10 - 0.70 mg N/L	During snow melt
Artic Fen[6]	2 µM (asparagine avg)	n/a	n/a	n/a	n/a
Alaskan Boreal Forest[7]	0.386 - 5.24 mg/kg soil	Constant	n/a	n/a	n/a
N. California Forest (Oa horizon)[8]	0.033-0.475 mg/L (mid April)	n/a	0.03-0.94		n/a
Alaskan Arctic Tundra[9]					
Tussock	0-28 mg/kg soil	Snow melt-mid July	< 3 mg N/kg (avg)	< 4 mg N/kg (avg)	Mid-July
Intertussock	0-7 mg/kg soil	Both ends of GS[2]			Mid-June (NH_4)
Wet Sedge	<1 -20 mg/kg soil	Mid-July			Mid-July
Shrub	0-3 mg/kg soil	Late July			Mid-June/Aug
Alaskan Arctic Tundra[10]	(average)				
Dry Heath	2.19 mg/kg soil	August	0.34 mg/kg soil	n/a	n/a
Wet Meadow	1.57 mg/kg soil	June	0.38 mg/kg soil	n/a	n/a
Tussock Tundra	8.29 mg/kg soil	July	0.84 mg/kg soil	n/a	n/a
Shrub Tundra	2.88 mg/kg soil	August	0.64 mg/kg soil	n/a	n/a
Colorado Alpine Soils[11]					
Alpine Dry Meadow	~ 10-158 µM	July	~ 10-45 µM	~ 10-50 µM	May

Subalpine Fen	~ undetectable-20 µM	September	~ 0-35 µM	~10-65 µM	July (NH_4) & June (NO_3)
Shortgrass Steppe	~ undetectable-40 µM	September	~ undetectable-20 µM	~0-150 µM	May

[1] Units are in mg/L unless otherwise stated
[2] GS - Growing Season
[3] Gilbert et al. (1998)
[4] Henry & Jefferies (2002)
[5] Urban & Eisenreich (1988)
[6] Ström et al. (2012)
[7] Werdin-Pfisterer et al. (2009)
[8] Yu et al. (2002)
[9] Weintraub & Schimel (2005)
[10] Kielland (1995)
[11] Raab et al. (1999), estimated from graphic

Microbial kinetics

Modeling of amino acid cycling in peatland bogs is hindered by the lack of kinetic parameters for microbial processes and molecular- and vegetation-specific rates of plant nitrogen uptake. Model simulations predicted total amino acid production through proteolysis was 15 g N m^{-2} for a one-year period. Soil protein turnover via proteolysis was estimated to be 42 g N m^{-2} for a dry meadow ecosystem in Colorado (Raab et al., 1999). Microbial kinetic parameters were not available for peatland bogs, and thus, the discrepancy between model results and the observations indicate that environmental factors likely influence proteolysis. Addition of NH_4^+ to low nitrogen environments can inhibit proteolysis as microbes allocate less carbon and nitrogen to protease synthesis (Geisseler and Horwath, 2008). The large contribution of NH_4^+ to total available nitrogen

may limit proteolysis rates in nitrogen limited bogs and allow microbes to save resources that are used for enzyme production. Microbial synthesis of protease enzymes also decreases in response to the addition of both glucose and proteins (Geisseler and Horwath, 2008). Inputs of labile carbon and proteins to peatland bogs may occur during the same period, and if NH_4^+ is readily available, microbes may process the labile carbon to meet carbon requirements before processing the proteinaceous matter. Model output was most sensitive to changes in proteolysis and microbial respiration rates, which indicates that using rates representative of the environmental conditions of peatland bogs is required to reduce model uncertainty. The rates have been shown to vary between ecosystem types and spatially within the same ecosystem. Nitrogen mineralization has been shown to be strongly dependent on soil characteristics in forest soils and wetlands (Rothstein, 2010; Updegraff et al. 1995). Reducing model uncertainties requires investigation of kinetic parameters for microbial processing of inorganic and organic nitrogen in peatlands.

Process-scale models of the cycling of amino acids would be improved by incorporating molecular-specific information on microbial and plant uptake. Sedge and shrub species have been shown to take up glycine at faster rates than other amino acids and glycine has been shown to be a poor substrate for microbial growth (Lipson et al. 1999b; Kielland, 1994). Plants may have an advantage over microbes in terms of organic nitrogen acquisition specific to glycine. Molecular-specific properties like net charge, hydrophobicity, and side-chain chemistry may also influence partitioning of amino acids into DOM and adsorption to particles. Sorption and biotic uptake may vary greatly with the physicochemical properties of the amino acids. Sorption of amino acids to temperate

forest soils was correlated to the charge on the amino acid and mineralization was greater for polar amino acids (Rothstein, 2010). Assimilation also varied by species of amino acid, which indicates that model predictions would be improved by using molecular-specific, microbial uptake rates. Gonod et al. (2006) showed that there was increased competition between adsorption and microbial assimilation for lysine in mineral soils compared to leucine due to strong adsorption to soil aggregates. Future modeling experiments should include simulations of individual amino acids.

The impact of short-term changes in environmental factors or ecosystem characteristics on the free amino acid pool should also be considered in future studies. Lipson and Monson (1998) observed an increase in the removal of added glycine by plants in alpine tundra soil after freeze-thaw events. Sphagnum peatlands dominated by vascular plants and non-sphagnum bryophytes showed greater short-term variability in environmental factors than sites with dense sphagnum cover (Sullivan and Booth, 2011). Changes in testate amoeba community were related to environmental factors like depth of the water table and pH, which could significantly impact amino acid cycling in peatlands where testate amoebae dominate the microbial biomass. The half-life of amino acids in forest soils has also been shown to correlate with pH and soil fertility (Jones and Kielland, 2002). The current study only considered three sampling periods which would not have been able to capture the effects of episodic events on the free amino acid pool. The range of concentrations observed in field studies may not be able to fully capture the range of amino acid concentrations. Further studies of the relationship between environmental factors and seasonal variations in amino acid concentrations is required to reduce model uncertainties; however, the use a high sampling rate would be needed to

understand the influence these events have and how to incorporate them into a process scale model.

Conclusions

The process-scale model developed in the current study was able to simulate seasonal variations in amino acid concentrations, with maxima in spring and fall and minima in June and August. The seasonal variability of microbial processes was responsible for the seasonal variations observed in amino acid concentrations. Plant nitrogen requirements were assumed to be met by NH_4^+ for a majority of the year and amino acids were soon depleted when NH_4^+ was unavailable. Uptake of amino acids by plants only played a significant role for a short period of the summer. The period of amino acid depletion was lengthened beyond summer when plant nitrogen uptake was assumed to consist of 50% amino acids. However, the range of predicted amino acid concentrations was of the same order of magnitude regardless of adjustments to the preference of the plants for the various nitrogen species. Amino acid concentrations exhibited an order of magnitude change when microbial processes were altered by an order of magnitude, which indicates a direct response of the model to alteration of microbial processing rates. Model sensitivity to rates of microbial processes highlights a need for further study of microbial nitrogen processing in peatlands. The carbon and nitrogen cycles of peatlands are coupled and regulated by biotic processes and environmental factors. Thus, improvements in process-scale models of amino acids in peatlands are required to predict feedbacks to the climate system. Increasing temperatures and levels of CO_2 in the atmosphere may

accelerate inputs of amino acids to pore water by increasing decomposition rates. The influx of available nitrogen may stimulate plant growth and offset currently observed nutrient limitations to sequestration of carbon in peatland soils.

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Chapter 4
Conclusions and directions for further research

The labile organic matter pool, which includes amino acids as a key component, regulates ecosystem mineralization rates. Nitrogen availability is apparently related to the cycling of amino acids in nitrogen-poor environments. Amino acids in pore water of peat were shown to respond to seasonal changes in environmental conditions, which may indicate that shifts in climate will lead to changes in amino acid availability in peatlands. Predicting the response of the cycling of amino acids in peatlands to climate change and the impact on ecosystem productivity is problematic without a fundamental understanding of the biogeochemical cycle of amino acids.

The current study demonstrated that NH_4^+ is the dominant form of nitrogen in pore water of peat and amino acids were present at low concentrations (undetectable-2.3 μM, average 0.40 μM). Previous studies have shown that when amino acids and NH_4^+ are both available biota do not exhibit a preference for the chemical species, and thus, despite higher NH_4^+ concentrations, amino acids may still provide a source of nitrogen to peatland plants and microorganisms. Correlations between the inorganic nitrogen species and amino acids in the pore water of peat indicate the biogeochemical cycles of nitrogen species in peatlands are linked. Levels of amino acids were also correlated to dissolved organic carbon species. Predicting the release of carbon from peatlands in response to lowering of water tables related to increases in temperature will require a fundamental understanding of the coupling of the carbon and nitrogen cycles in peatlands and feedbacks to the climate system.

Plant productivity and the dominance of various plant functional types did not appear to influence free amino acid concentrations in the pore water of peat; however, simulations of amino acid cycling in a peatland bog with a process-scale model were sensitive to changes in proteolysis and mineralization rates. Microbial processing of amino acids likely regulates the amino acid concentrations. Investigations of microbial amino acid uptake and proteolysis rates in wetland soils are lacking. Future studies of the peatland nitrogen cycle should focus on microbial dynamics and the influence of substrate quality on microbial nitrogen uptake. There is also a need for further characterization of the free amino acid pool in wetland environments. The dominance of a single amino acid like leucine, which was observed in the current study, may indicate that individual amino acids cycle through unique addition or removal pathways. Greater sampling frequency will also be required to understand the influence of episodic events on seasonal variability of the free amino acid pool. Future field studies of the seasonal variation of the free amino acid pool in pore waters of peat and evaluations of the relationships to microbial and plant processing and environmental factors are required to guide process-scale modeling experiments.