Organic nitrogen use by different plant functional types in a boreal peatland

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Abstract

Mineralization has long been thought to be the main driver in providing plant available nitrogen (N). However, slow mineralization rates of northern ecosystems cannot sustain total plant N accumulation and it is now recognized that plants can utilize organic forms of N. N is often a limited nutrient in ombrotrophic bogs and at Mer Bleue peatland nearly 80% of the N in the porewater is in the dissolved organic nitrogen (DON) form. This study determined whether peatland plants can take up organic forms of N and whether there are differences between plant functional types, which dominate bog vegetation. To determine if bog plants take up organic N, 16 plots were selected at Mer Bleue where half remained a control and half received a treatment of isotopically labeled glycine ($^{13}C_2$,^{15}N, 98% atom). The labeled glycine was injected into the rhizosphere at a depth of 0-20cm. After 72 hours the leaves and roots of shrub (*C. calyculata, V. myrtilloides, L. groenlandicum*), sedge (*E. vaginatum*) and moss (*S. magellanicum, S. capillifolium*) in the plots were sampled and analyzed for plant $\delta^{13}C$ and $\delta^{15}N$. Foliar samples showed a significant uptake of ^{15}N across all species and no significant uptake of ^{13}C. Root samples showed greater enrichment in ^{15}N and ^{13}C for both shrub and sedge species; however, sedge uptake of ^{13}C was not found to be significant. Results showed that shrub species took up glycine intact while a significant uptake of glycine was not found for sedge and moss species. This suggests that the mycorrhizal associations of ericaceous shrubs may contribute to organic N uptake at Mer Bleue bog.

Resume

La minéralisation a longtemps semblé être le conducteur principal fournissant l'azote aux plantes. Cependant, les faibles taux de minéralisation des écosystèmes nordiques ne peuvent pas pourvoir l'apport total d'azote des plantes et il est maintenant reconnu que les plantes peuvent utiliser les formes organiques de l'azote. L'azote est souvent un nutriment limitant dans les tourbières ombrotrophes et, à la tourbière Mer Bleue, près de 80% de l'azote dans l'eau interstitielle est sous forme d'azote organique dissous. Cette étude avait pour but de déterminer si les plantes des tourbières peuvent absorber l'azote sous formes organiques et s'il y a des différences entre les types fonctionnels de plantes qui dominent la végétation des tourbières. Pour déterminer si les plantes des tourbières absorbent l'azote organique, 16 parcelles ont été choisies à Mer Bleue, où une moitié a été utilisée comme contrôle et l'autre moitié a reçu un traitement de glycine marquée isotopiquement ($^{13}C_2$, ^{15}N, 98% atomes). La glycine marquée a été injecté dans la rhizosphère à une profondeur de 0-20cm. Après 72 heures, les feuilles et les racines des arbustes (*C.calyculata, V. myrtilloides, L.groenlandicum*), laîches (*E. vaginatum*) et les mousses (*S. magellanicum, S.capillifolium*) dans les parcelles ont été recueillies et analysées pour les plantes $\delta^{13}C$ et $\delta^{15}N$. Les échantillons foliaires ont montré une absorption importante de ^{15}N pour toutes les espèces et aucune augmentation significative de signatures $\delta^{13}C$. Les échantillons de racines ont montré un enrichissement plus grand en $\delta^{15}N$ et $\delta^{13}C$ pour les deux espèces d'arbustes et celle de laîche. Cependant, l'absorption de $\delta^{13}C$ pour espèces de laîche n'a pas été jugée significative. Les résultats ont montré que les espèces d'arbustes ont absorbé la glycine entièrement alors que l'absorption de glycine n'a pas été importante pour les espèces de carex et de mousse, ce qui suggère que les associations mycorhiziennes des arbustes éricacées peut être le facteur déterminant dans l'absorption de l'azote organique à la tourbière Mer Bleue.

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Chapter 1 - Literature Review and Research Objectives

This Chapter synthesizes the relevant literature ranging from a global review of the importance of nitrogen (N) for improving global climate-carbon models to the more detailed biogeochemical processes linking soil, plants, and microbes in the cycling of organic nitrogen. The Chapter ends with a summary of and justification for the research objectives.

1.1 Global importance of N: Understanding ecosystem response to climate change

Climate change is no longer an invisible problem of the future but a reality of our time. Surface temperatures have already risen by 0.74°C in the last hundred years (IPCC 2007) and the Amazon rainforest has recorded an expansive drought, record low water levels in the Rio Negro and an average 2-3°C increase in the average temperature for 2010 since the anomalous drought of 2005 (Tollefson, 2010). One hope has been the uncertain ability of forests and other ecosystems to remain or become sinks of carbon dioxide (CO_2) as determined by climate change forecast models. Yet climate change models still involve some uncertainty in the prediction of the amount of carbon (C) that can be sequestered by vegetation. C models often exclude nutrient constraints, such as N, in their calculations and some scientists (Hungate *et al*., 2003, Nadelhoffer *et al*., 1999) have argued that C sequestration should not be predicted without first looking at N in order to determine how vegetation would react under CO_2 fertilization.

Hungate *et al*. (2003) point out that the C models probably exaggerate the sink potential of the terrestrial biosphere because they would require N accumulation to occur

as well. In N-poor ecosystems, CO_2 fertilization may only have a small effect on vegetation growth as the vegetation will still be N-limited. Thornton *et al*. (2007) was the first to publish results from a coupled C-N model which showed that a C-N model in a scenario of increasing atmospheric CO_2 resulted in lower total C uptake by a factor of 3.8 for the next century versus a C only model. Therefore models that simulate ecosystems around the world, especially forests, to be large sinks of C may be defective in their prediction. A better understanding and modeling of C cycling under both contemporary conditions and in response to disturbances, such as drainage or climate change, is dependent on the link between C and N (Limpens *et al*., 2008).

1.2 Contemporary studies of nitrogen cycling

1.2.1 The overlooked organic N

Although it is well documented that plants take up inorganic forms of N, such as nitrate (NO_3) and ammonium (NH_4), plant uptake of organic N has been historically overlooked in N mineralization studies until the beginning of the 1990's (Schimel and Bennett, 2004). Organic N is often thought to be completely consumed by the more competitive microbial community; however, Schimel and Bennett (2004) suggest a new conceptual understanding of how N is cycled that no longer centers around mineralization to provide plant available N. A diagram of the concept (Figure 1), which was first introduced in Chapin *et al*. (2002), illustrates another "pathway" whereby plants have access to the nutrient N; it involves the depolymerization of N-containing polymers allowing plants to absorb organic N sources, even where high levels of inorganic N exist. Polymers are not normally available for plant uptake because they are too large to enter

root uptake pathways. However, polymers can be broken down by extracellular enzymes, from microbes and mycorrhizae, and become available in monomer forms, such as amino acids, amino sugars, and nucleic acids (Schimel and Bennett, 2004). Depolymerization is proposed as the rate-limiting step in N cycling and the production of plant available N and Schimel and Bennett (2004) formulate a new N cycle paradigm where organic N monomers can be available to plants without the process of microbial mineralization. The conceptual model of depolymerization is a new and interesting view of N cycling that coincides with field observations but remains mostly theoretical, as depolymerization has yet to be quantified.

The premise for Schimel and Bennett's (2004) microsite model (Figure 2) was based on studies that showed N mineralization lacked the ability to explain N uptake in N-poor ecosystems and others where organic N uptake by plants was observed in N-poor ecosystems. In the model, N-poor ecosystems are composed largely of N-poor soil microsites in which both microbes and plants are competing for the products of depolymerization, for example amino acids (Schimel and Bennett, 2004). In this low N system, not enough N is available for mineralization and nitrification, so that organic N appears to be the dominant N pool available to plants, rather than inorganic N (Case A, Figure 2).

The micro-site model does not, however, imply that plant organic N uptake at N-poor sites arises from a decrease in competition from the microbial community. Jones *et al*. (2009) studied labeled amino acid mineralization across different soil types from a wide range of latitudes and found that the microbial community in all soils, irrespective of latitude, had an innate ability to assimilate and mineralize amino acids rapidly. They

suggested that organic N uptake by some plants in N-poor ecosystems are the result of increased root competition or mycorrhizal symbionts rather than a decrease in the microbial sink but stipulate that the processes behind organic N acquisition remain unknown.

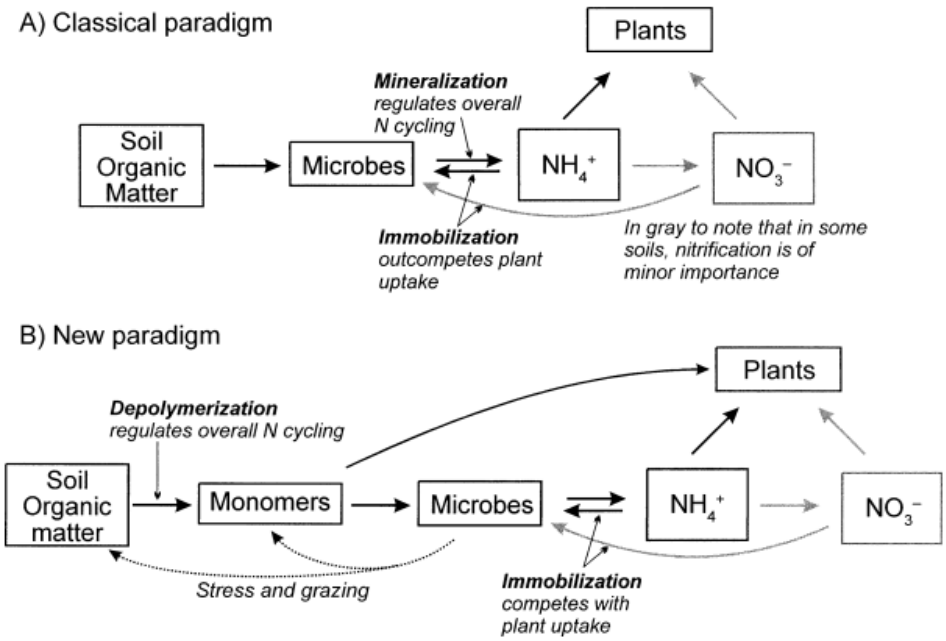

Figure 1. The classical paradigm of how N cycling has been viewed for the last century and the new paradigm (Schimel and Bennett, 2004).

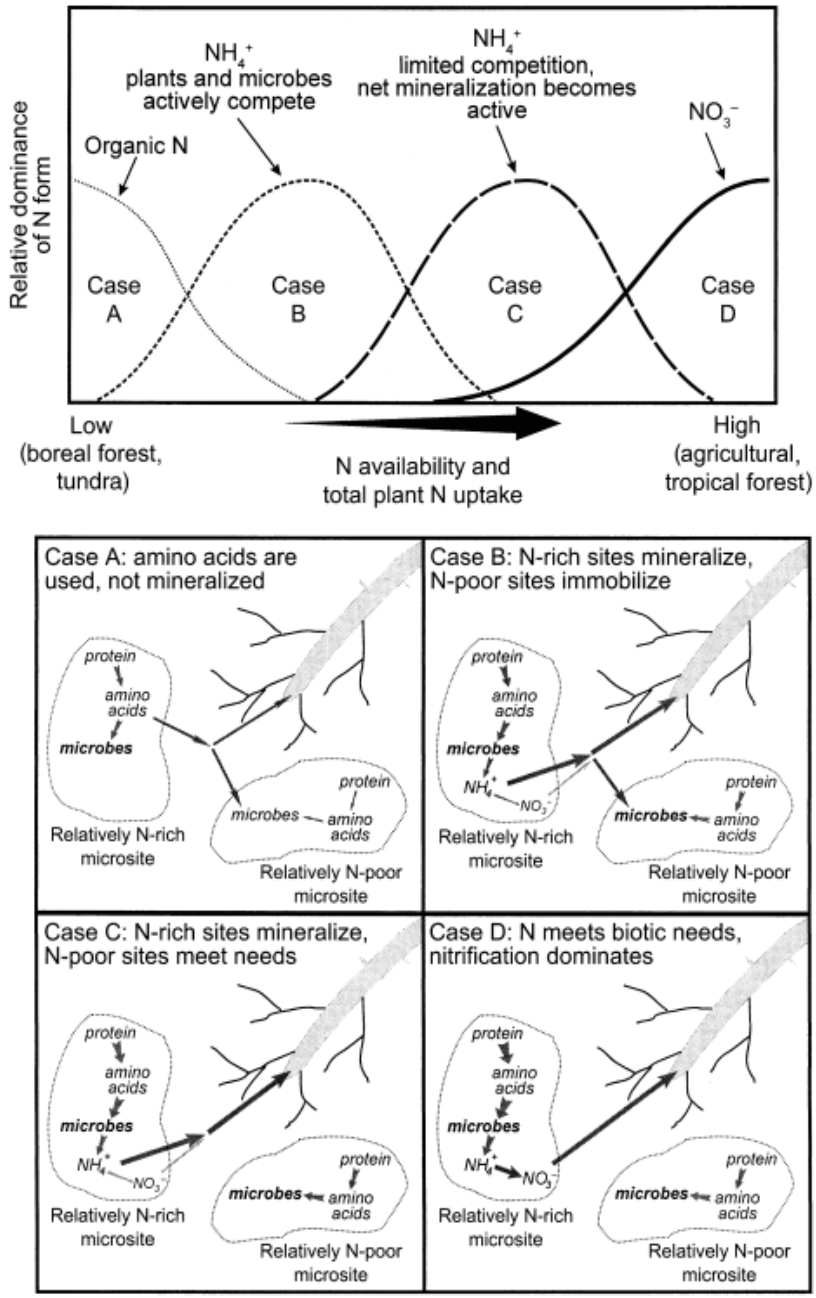

Figure 2. Relative dominance of N form across different cases and the composition of N-rich and N-poor microsites in each case (from Schimel and Bennett, 2004).

1.2.2 Using isotopes to understand the role of organic N

A common methodology to determine whether plants take up organic N is to trace a pulse of ^{13}C and ^{15}N labeled organic N that is injected into the soil (e.g. Chapin *et al*., 1993; Nasholm *et al*., 1998; McFarland *et al*., 2002). Organic N compounds, for example glycine, contain both C and N atoms and it is for this reason that both the ^{13}C and ^{15}N isotopes are used to measure uptake. If glycine has been taken up in its whole form then an increase in concentration of both the heavy C and N isotopes in the plant tissue should be observed. However, if glycine is first mineralized into inorganic forms of N before plant absorption, then the plant tissue would not show an increase in the ^{13}C isotope.

Isotope labeling and recovery is the typical method used among organic N studies, however some weaknesses have been noted for this experimental methodology. Rasmussen and Kuzyakov (2009) critiqued the assumption that the recovery of the C from labeled organic N shows uptake of intact amino acids, pointing out that ^{13}C can enter the plant through $^{13}CO_2$ via photosynthesis or through the absorption of $H^{13}CO_3^-$. They suggested including control treatments where $H^{13}CO_3^-$ and either $^{15}NH_4^+$ or $^{15}NO_3^-$ are added in the same ratios as the dual labeled organic N compounds, to test the uptake of inorganic C and compare values of organic N uptake. Nasholm *et al*. (2009) noted that the soil pH in their study was 3.1 (pH_{CaCl2} was 3.1, equivalent to pH_{H2O} of 4) and from this it can be assumed that the carbonic acid, or $H^{13}CO_3^-$ remains undissociated and quantities of ^{13}C uptake would be minimal. As N-poor soils are generally acid this pathway is unlikely.

Another factor to take into account when using isotope geochemistry is that plants are subject to a natural range of variability of the ^{15}N isotope. One type of variation

occurs within the plant itself. For example, in controlled hydroponic studies, $\delta^{15}N$ in leaves can be 3-7‰ more enriched than in roots resulting from organ-specific loss of N, different patterns of N assimilation and reallocation of N (Evans, 2001). Intra-plant variation of $\delta^{15}N$ in roots and leaves is usually small (less than 3‰) in deciduous forests and tallgrass prairies but as high as 7‰ in cold and warm desert ecosystems (Evans, 2001). Peatlands, wetter than a cold desert but more nutrient limited than prairie and deciduous forests, may lie in the middle range of ecosystem specific intra-plant $\delta^{15}N$ variation. However, the range observed between leaf and root $\delta^{15}N$ for shrub and sedge species at Mer Bleue was also often less than 3%. Nonetheless, the range of intra-plant variation emphasizes the necessity to compare similar plant components to each other and have control plots to compare the $\delta^{15}N$ from labeled organic N for significance.

1.3 Nitrogen cycling and organic N in peatlands

N is a growth-limiting nutrient that influences plant production, decomposition and C cycling in peatlands (Berendese *et al*., 2001). Inputs of N to peatland ecosystems occur mainly through N deposition, N_2 fixation by bacteria or algae, and through inflow from uplands (Limpens *et al*., 2006). In ombrotrophic peatlands such as Mer Bleue, inflow from upland areas does not occur and all of its nutrients and water are derived from atmospheric deposition. N deposition is composed of both inorganic inputs (NO_x and NH_y) and organic inputs (amino acids and organic nitrates) (Neff *et al*., 2002).

No studies have been published where organic N uptake was examined across multiple species in a boreal bog. Karagatzides *et al*. (2009) examined the carnivorous pitcher plant *Sarracenia purpurea* in both bog and tundra ecosystems and found direct

uptake of amino acids, however carnivorous plants are already known to obtain N from sources other than mineralization. Olsrud and Michelsen (2009) found that four dwarf shrubs (*Andromeda polifolia*, *Empetrum hermaphroditem, Vaccinium uliginosum* and *Vaccinium vitis-idaea*) and one forb (*Rubus chamaemorus*) from a subarctic mire near Abisko, Sweden were able to take up intact glycine under normal and shaded conditions. Another study in Abisko examined 11 *Sphagnum* and non-*Sphagnum* moss species and found all species but *Polytrichum commune* were able to take up amino acids in both the lab and at a lesser extent in the field (Krab *et al*., 2008). Several other species that make up or are similar to boreal bog vegetation have been studied in other ecosystems such as in tundra or boreal forests. For example, roots of the sedge *Eriophorum vaginatum* were collected from Toolik Lake in arctic Alaska and were found to absorb several amino acids in a lab experiment (Chapin *et al*., 1993). Stribley and Read (1980) found that *Vaccinium macrocarpon* inoculated with mycorrhizal fungi in a lab setting could utilize various amino acids but that aseptically grown plants could not. Similarly, several studies have been performed in cold, nutrient poor systems such as alpine and arctic tundra (Lipson and Monson, 1998; Kielland, 1994), boreal (McFarland et al., 2002; Nasholm *et al*., 1998) and northern temperate forest ecosystems (Gallet-Budynek et al., 2009) and observed plant uptake of amino acid-N.

Ombrotrophic peatlands, where the water is derived from precipitation, have low nutrient availability and concentrations of NH_4 and NO_3 in the soil solution are small, with approximately 80% of the total N in the porewater below the water table in dissolved organic N (DON) form (Rattle, 2006). Bragazza and Limpens (2004) found similar DON concentrations in from free standing water with DON making 66-82% of

the total dissolved nitrogen (TDN) across several European bogs. Limpens *et al*. (2006) reviewed observed DIN:DON ratios from various peatlands and found it to vary from 1:5 to as low as 1:1.4. DON is a large pool of proteinaceous N of which only a small fraction is in the form of free amino acids. Jones *et al*. (2009) found that soil solutions (n = 40) from the Arctic to Antarctic had a generally constant amino acid concentration of 23±5 µM whereas inorganic N varied dramatically in natural ecosystems from <10 to 70 µM for NH_4^+ and <10 to 2000 µM for NO_3^-. McFarland *et al*. (2010) recognized that free amino acid-N (FAA-N) may be as important as mycorrhizal infection for plant amino acid uptake. Despite the large DON pool at Mer Bleue and other bogs, a better indicator of organic N use by plants would be to examine the FAA-N concentration in the porewater.

Spatial variability of N concentrations in the soil can also be connected to patterned peatlands that show a hummock-hollow topography. Eppinga *et al.* (2008) used N and P content of plant tissue and water chemistry as an indicator of soil nutrient availability and found that ridges had higher nutrient concentrations than hollows in the Great Vasyugan Bog, Siberia. The possibility of an uneven spatial distribution of depolymerization which would lead to 'hotspots' (McClain *et al.*, 2003) of organic N concentrations near increased root activity warrants further investigation (Jones *et al*., 2005). Rietkirk *et al.* (2004) developed a nutrient dynamics theory for the formation of topographical patterns in peatlands that hypothesizes nutrients are diminished from hollows as they move toward and remain concentrated under hummocks. Moore *et al*. (2004), in an examination of N and sulfur (S) accumulation rates across 23 eastern Canadian bogs, showed that N and S accumulation was greater in hummocks than

hollows. This nutrient-dynamics theory of peatland patterning poses some interesting questions about organic N use by plants. If nutrients are concentrated under hummocks then would the preferential uptake of N be in inorganic form and would the depleted hollows adapt by using more organic N? Would increased nutrient concentrations under hummocks reduce the amount of mycorrhizal infections of a species and lead to lower organic N uptake than the same species in a hollow?

1.4 Organic N uptake and links to biogeochemistry

1.4.1 Isotope tracer studies under special environmental conditions

Studies have also been carried out to determine if organic N uptake changes under different environmental conditions in the same ecosystem. Bardgett *et al*. (2003) performed an isotope tracer study in two adjacent grasslands of similar geology, topography and climate in Littledale, Lancashire, United Kingdom. One grassland was a fertilized, "improved" *Lolium perenne*-dominated productive grassland and the other was a less productive, unfertilized, "unimproved" grassland. Intact glycine uptake was observed for both the improved and unimproved grassland however the plant uptake was greater for the unimproved grassland at both spring and fall testing. At the same time the low productivity *Agrostis capillaris–Festuca ovina* grassland also had a soluble N pool dominated by amino acid N and a lower total plant captured N (for both organic and inorganic source of N) compared to other ^{15}N sinks, such as microbes. Despite the immediate and large microbial sink for added N in the unimproved grassland, Bardgett *et al*. (2003) suggest that organic N uptake may be of potentially greater significance to

plant nutrition for the acidic, low productivity grassland because of the larger amino acid N pool and because of the slower accumulation of organic matter.

Lipson and Monson (1998) determined if dry-rewetting and freeze-thaw events affected competition for amino acids between alpine plants and microbes, because these events can disrupt microbial biomass. The freeze-thaw and rewet events did not lead to increased uptake of organic N by the plant *Kobresia myosuroides*. Lipson and Monson (1998) remarked that the alpine microbial community was more resilient to disturbances than was anticipated from previous studies and that the plant *Kobresia myosuroides* can in fact compete well for amino acid N under non-stressed conditions, especially when soils are warm.

Hofmockel *et al*. (2007) performed a tracer experiment at the Duke Forest Free Air CO_2 Enrichment (FACE) site to examine alanine uptake by loblolly pine under normal and elevated CO_2 levels. Intact alanine uptake was observed for loblolly pine under both conditions, but elevated CO_2 did not result in increased amino acid recovery in plant tissue nor did they find any increase in the potential proteolytic enzyme activity in the soil. Furthermore, Hofmockel *et al*. (2007) stated that despite the understanding that ecosystem N demand increases with elevated CO_2 and previous Duke Forest research detecting increased fine root biomass under elevated CO_2, both plants and microorganisms utilized more alanine under ambient CO_2 than in the elevated plots. They concluded that plants will need to acquire additional N through other mechanisms and suggested increased root exploration or increased N use efficiency.

1.4.2 Amino acid flux, sorption and concentration in soil

Amino acid N is the most common form of N in soils but only a small fraction of this N pool is in the form of free amino acids, or "individual amino acids dissolved in the soil solution", which are mainly produced following protein and peptide hydrolysis by extracellular enzymes (Lipson and Nasholm, 2001). Generally, the flux of amino acid N is rapid and great enough to exceed most plant N requirements, but the majority of the amino acid flux is captured by microbial biomass and the soil matrix (Lipson and Nasholm, 2001). Amino acids tend to interact strongly and rapidly with humic substances and Lipson and Monson (1998) found that 43% of added labeled glycine was found in the non-biomass fraction of soil and Kuzyakov (1997) reported that amino acid N has a fast turnover in the humic acid fraction. This tendency of amino acid N to be adsorbed to the soil matrix affects its diffusion through the soil and thus the ability of plants to take up organic N. The consequence is that if acidic and neutral amino acids move more freely in soils than basic amino acids perhaps amino acids may have a greater diffusion in acidic soils than more basic soils. Vinolas *et al*. (2001) found that sorption was greatest for lysine (positive charge, basic), followed by glycine (neutral) and then glutamate (negative charge, acidic), and that sorption retarded mineralization by reducing amino acid bioavailability. Amino acid mineralization was greater in a S-treated acidic soil than in the untreated control soil and lowest in a simulated (top 10cm were previously manually removed) eroded soil (Vinolas *et al*., 2001). Raab *et al*. (1999) also conclude that the high retention of amino acid by the soil matrix may be a constraint to both microbial and plant uptake.

Early studies of organic N use were applied in arctic ecosystems because it was understood that net mineralization was insufficient for plant N demand and that the dominant N form in the soil was organic N. Chapin *et al*. (1993) noted that protease activity was an order of magnitude faster than mineralization in tundra soils, which was the suggested reason for greater water extractable free amino acids than inorganic N. Similar results were seen for alpine meadow sites, which prompted the suggestion that the "limiting factor [to organic N uptake by plants] is likely to reside in the availability of amino acids in the soil, which in turn is controlled either by rates of proteolysis or the exchange of amino acids between the solid and liquid phases" (Raab *et al*., 1999, p. 2415). It is important to note, then, that amino acid flux rather than the size of the amino acid N pool, is proposed to affect plant organic N uptake. Kielland (1994) said early on that he found no significant differences in the absorption capacities of plants "across gradients of soil amino acid concentrations" in his study of organic N uptake by arctic species and since then, little evidence for a correlation of amino acid concentration to absorption capacity has appeared in arctic or other ecosystems. Jones *et al*. (2005b) found no relationship at low concentrations but that at very high concentrations plants become more competitive for amino acids compared to microbes, reasoning that the microbial community's ability to uptake amino acids had become saturated. In contrast, Sauheitl *et al*. (2009) used four amino acids and showed a negative effect of amino acid concentration on direct plant uptake of organic N, concluding that increasing amino acid concentrations led to an increase in mineralized N and therefore a decrease in the direct uptake of amino acids. The evidence provided for this conclusion was that both ^{13}C enrichment and ^{15}N recovered relative to amino acid concentration was lower with higher

amino acid concentrations. Since the negative relationship of higher amino acid concentration on direct organic N uptake by plants was independent of amino acids structure, Sauheitl *et al*. (2009) suggest that an internal plant mechanism controlled by mineralized N is responsible for determining direct uptake of amino acids.

Protease activity has been noted to be high in arctic and alpine environments by Raab *et al*. (1999) and Chapin *et al*. (1993). However, Jones *et al*. (2009) examined amino acid mineralization across several latitudinal gradients and found that microbes in soils had the innate ability to assimilate amino acids rapidly when temperature and moisture constraints were removed, suggesting that preferential uptake of organic N in some ecosystems is a function of increased root activity or mycorrhizal associations rather than reduced microbial competition.

As can be seen from above, there are many diverging conclusions on what is the main control on plant absorption of organic N, from amino acid and soil properties to microbes and mycorrhizal fungi symbiosis. Undoubtedly, amino acid uptake by plants is a result of many factors but the roles of mycorrhizae and microbes to direct uptake of organic N by plants will be addressed in the following sections.

1.4.3 Role of mycorrhizae and roots in organic N uptake

Mycorrhizae play a role in plant $\delta^{15}N$ variation and plant uptake of organic N. The type and degree of infection of mycorrhizae affect the $\delta^{15}N$ signature of plants because mycorrhizae can utilize N with different $\delta^{15}N$ values, have fractionation differences during the uptake of N and have different physiologies from their host plants (Hogberg, 1997; Hogberg *et al*., 1999). Crane *et al*. (2009) compiled data from 11,000 species

from around the globe and found that plants with ericoid mycorrhizal associations had the lowest $\delta^{15}N$ values, followed by ectomycorrhizal, arbuscular mycorrhizal and non-mycorrhizal plants (Figure 3, Craine *et al*., 2009). The main explanation for this trend has revolved around the different N sources that are used by the type of mycorrhizae (Smith and Read, 2008 as cited in Craine *et al*., 2009). It has been shown that ericoid mycorrhizae are able to take up a wider variety of organic N forms and provide enzymes to break down complex organic N sources into more plant-available forms and that arbuscular mycorrhizae lack such proteolytic capabilities and are adapted for the absorption of phosphorus (P) rather than N (Chalot and Brun, 1998; Bolan, 1991). Depleted foliar $\delta^{15}N$ with respect to mycorrhizae has been recorded in boreal and tundra ecosystems where N availability is considered low (Nadelhoffer *et al.*, 1996; Emmerton *et al.*, 2001). The ericaceous shrubs of Mer Bleue peatland also showed depleted foliar $\delta^{15}N$ in comparison to sedge and moss species (Tim Moore, pers. comm.) and the depleted values may indicate the utilization of organic N through ericoid mycorrhizal symbiosis, though many factors play a role in the $\delta^{15}N$ composition of plants and the exact mechanisms are not known.

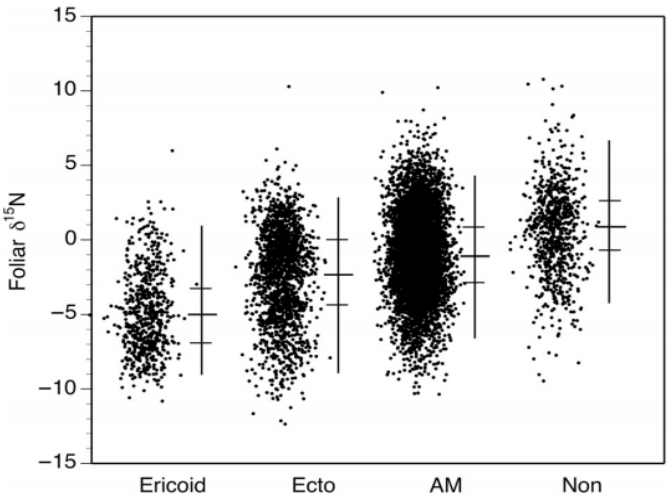

Figure 3. Foliar ^{15}N signatures of plant species by mycorrhizal type (Craine *et al*., 2009).

Mycorrhizal fungi have been noted as playing a key role in the breakdown of organic sources of N but they are often at the frontline of organic N uptake. Ericaceous shrubs often form symbiotic relationships with mycorrhizal fungi which can improve the ability of plants to take up organic N compounds (Stribley and Read, 1980; Chalot and Brun, 1998). In a study of ten Arctic species, the amino acid uptake was significantly greater for mycorrhizal than non-mycorrhizal species under natural concentrations (Kielland, 1994). Interestingly, mycorrhizal fungi not only improve plant absorption of organic N but also provide the enzymes needed to break down macromolecular N (Stribley and Read, 1980; Chalot and Brun, 1998). Hobbie and Hobbie (2008) describe a model whereby the mycorrhizal hyphae release the enzymes that break down N-containing polymers so that the products of degradation remain close to the hyphal tips and therefore mycorrhizal fungi can out-compete soil microbes for the products before they diffuse away. The mycorrhizal associations of the ericaceous shrubs at Mer Bleue (Meaghan Murphy, pers. comm.) may provide an advantage in organic N uptake over the

mosses and sedges. The roles played by mycorrhizal fungi emphasize their importance to the N cycling dynamics in many ecosystems, especially in those that tend to be N-limited.

The uptake of organic N compounds by non-mycorrhizal plants has also been observed therefore showing plant roots also have the ability to absorb organic N. In a study of Arctic plants Kielland (1994) found that both mycorrhizal and non-mycorrhizal (or weakly infected) plants absorbed amino acids and Chapin *et al*. (1993) observed that a non-mycorrhizal sedge could not only utilize organic N but that it accumulated more N and biomass when grown on a solution of amino acids rather than inorganic N. Paungfoo-Lonhienne *et al*. (2008) used a fluorescent protein to show that *Hakea actites* and *Arabidopsis thaliana*, which do not form mycorrhizae, can use protein as a N source. They showed that *Arabidopsis* grown on a mixture of protein and low amount of inorganic N "grew significantly better than plants grown with either nitrogen source individually and produced the same dry weight as plants grown with a high amount of inorganic nitrogen" (Paungfoo-Lonhienne *et al*., 2008, p. 4524). Two mechanisms by which roots can access protein were identified; one is through the exudation of proteolytic enzymes and the second is by endocytosis whereby protein is taken up intact. Uptake of two amino acids was also observed for the non-mycorrhizal, carnivorous *Sarracenia purpurea*, at three bogs across North America and Karagatzides *et al*. (2009) suggested that the plants' versatility in their N acquisition is a means to balance the unpredictable N obtained by a seasonally reconstructed foodweb. Therefore, these cases of organic N use by plants may be driven by plant N-demand with or without advantageous mycorrhizal associations.

1.4.4 Role of microbes

Microbes play more than one role in organic N uptake by plants. Firstly, they share the role of producing extracellular enzymes with mycorrhizae, which are required for the proteolysis of proteins into individual amino acids. Schimel and Bennett (2004) consider this process, which they call depolymerization, as the rate limiting step to providing available N to plants and later N mineralization. Rates of proteolysis are generally several orders of magnitude greater than net mineralization rates (Lipson and Nasholm, 2001). Vinolas *et al*. (2001) found that amino acid mineralization by microbes is rapid even at low temperatures (1°C) but that it increases with temperature. They also found that mineralization rates varied among amino acids: glutamate > glycine > lysine, explained by microbes having amino acid-specific metabolism as well as by amino acid sorption differences in soils.

A secondary role of microbes, one that is also necessary to their functioning, is to consume amino acids, making them a competitor to plants for this source of nutrition. Thus, amino acids are considered a better nutritional substrate for microbes because they contain C and microbes require C as well as N for their growth (Nordin *et al*., 2004). The accumulation of N and other nutrients from the soil substrate and solution into the soil microbial biomass is known as immobilization (Schlesinger, 1994) and the sink for both organic and inorganic N is quite large. For example, Nordin *et al*. (2004) supplied aspartic acid, glycine, NH_4^+ and NO_3^- to field sites in a combination of equal parts, where only one form was labeled (^{15}N for all forms with included ^{13}C label for the amino acids) at a time, and found that the microbial biomass was able to incorporate about half of the added N irrespective of N form. Bardgett *et al*. (2003) also found the bulk of added ^{15}N

was recovered in the microbial biomass for both the organic (glycine) and inorganic (NH_4^+) form and Hofmockel *et al*. (2007) found ~25% and ~35% recovery of added ^{15}N in the microbial biomass for alanine and NH_4^+ respectively. Therefore microbes compete directly with plants for both the inorganic and amino acid N pools.

Microbes are not only a sink but also a reservoir of amino acids along with decaying plant tissues. Their cell walls are made up of polymers containing amino acids, such as glycine, lysine, alanine and D and L-isomers of glutamic acid (Stanier *et al*., 1986 as cited in Lipson and Nasholm, 2001) which are released, along with NH_4^+ (Schlesinger, 1994), upon microbial death. Microbial biomass N turns over several times a year and is partly responsible for replenishing the large protein and peptide N pool, of which free amino acids are only a small fraction (Lipson and Nasholm, 2001).

1.5 Summary and rationale

Our understanding is advanced enough to know that we must include N cycles in ecosystem response models to climate change and increased atmospheric CO_2. However, our representation of the N cycle is undergoing a paradigm shift whereby mineralization, and its production of mineral N, is no longer the rate-limiting step that can be used to determine plant available N.

Studies from the last two decades have shed light on the ability of plants to access the organic N pool for nutrition. The earliest experiments supported Schimel and Bennet's (2004) paradigm whereby the likeliest users of organic N would exist at sites with poor mineralization, such as in arctic and tundra systems. More recently, organic N use has been shown across many ecosystems and plant types. Plants that have shown the

greatest ability to use organic N have often been known to have mycorrhizal associations. In ombrotrophic peatland ecosystems such as at Mer Bleue, the dissolved organic N pool is large and the inorganic N pool is small and ^{15}N natural abundance data suggest that organic use may vary across plant functional type. Thus, the research pursued in this thesis attempts to merge the literature on organic N use by plants and the N cycle in peatlands by undertaking an isotope tracer field study at Mer Bleue peatland.

Section 1.6 Research objectives

Objective 1 – Determine if peatland plants can utilize organic N in the field by applying a dual labeled amino acid and examining the change in $\delta^{13}C$ and $\delta^{15}N$ isotope signatures of the plant tissues.

Objective 2 – Identify differences in organic N uptake between the plant functional types: moss, sedge and shrub, which dominate bog vegetation.

Objective 3 – Quantify the size and composition of the organic N pool of the porewater solution from the unsaturated rooting zone at Mer Bleue peatland.

Chapter 2: Methods and Materials

2.1 Research site

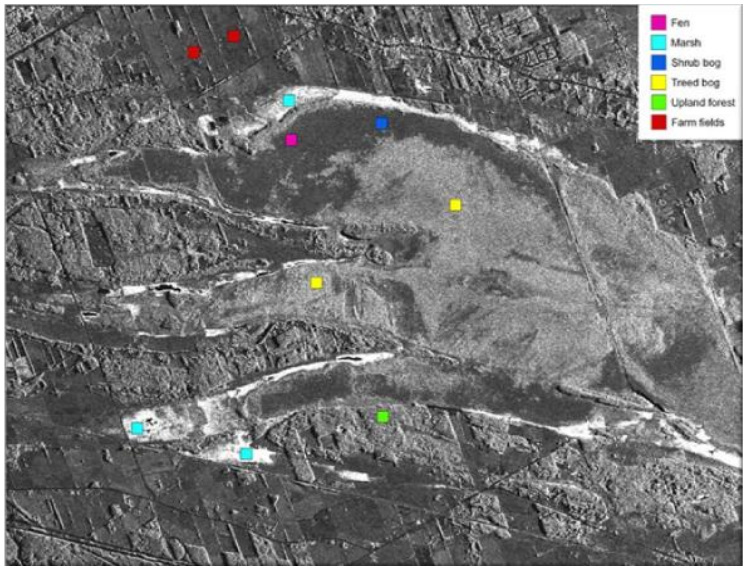

Figure 4. Convair-580 HH SAR image of Mer Bleue peatland, from Touzi *et al.* (2009).

Fieldwork was carried out at the Mer Bleue peatland, typical of boreal and cool temperate bogs. Mer Bleue, located approximately 10 km east of Ottawa, Ontario in the Ottawa River Valley (45.40°N, 75.50°W), is a large dome shaped ombrotrophic bog with a hummock-lawn-hollow topography. The peat depth of the bog ranges from >5 m at the center to about 2 m at the edge of the bog which is bordered by beaver ponds (Bubier *et al*., 2006). The vegetation is dominated mostly by ericaceous shrubs, both evergreen (*Ledum groenlandicum*, *Kalmia angustifolia* and *Chamaedaphne calyculata, Vaccinium oxycoccus*) and deciduous (*Vaccinium myrtilloides*,), tussock sedge (*Eriophorum*

vaginatum) and carpeted by bryophytes (*Polytrichum strictum, Sphagnum magellanicum, Sphagnum capillifolium*) (Bubier *et al*., 2006). Trees occur less frequently in the bog but the most common species are *Larix laricina* and *Betula populifolia* (Bubier *et al*., 2006). At Mer Bleue the majority of the root production occurs in the top 20 cm of the soil (Murphy and Moore, 2010).

Section 2.2 Experimental design

The uptake of organic N at Mer Bleue peatland was determined by injecting aqueous solution of the low molecular weight amino acid glycine, containing ^{13}C and ^{15}N isotopes, into the peat. I then traced the $\delta^{13}C$ and $\delta^{15}N$ changes in the roots and leaves of shrub (*C. calyculata, V. myrtilloides, L. groenlandicum),* sedge (*E. vaginatum*) and moss (*S. magellanicum, S. capillifolium*) tissues. Sixteen 20 x 50 cm experimental plots were chosen in the Blue Dome area of the bog along two boardwalks. The plots were situated no closer than 20 cm from the boardwalk edge and at least 2 m apart from each other. The plots were selected so that half were on drier hummocks and half were located on wetter lawns or hollows and contained as many of the six dominant species mentioned above. Whenever other species were found in the plots, they were sampled along with the six dominant species. *K. angustifolia* occurred consistently across plots, however other species (*V. oxycoccus, Kalmia polifolia, Sphagnum angustifolium* and *L. laricina*) were infrequently present and *P. strictum* only occurred in the drier hummock plots. Plots were categorized as either a treatment or control.

2.2.1 Sampling Porewater

Drawing porewater from the rhizosphere proved difficult unless performed in a wet lawn or after a rain event. Porewater samples were drawn from four of the eight control plots using Macrorhizon Soil Moisture Samplers (Eijkelkamp). Depending on the depth of the water table, or rather whether the plot was a hummock or lawn, the soil moisture samplers were inserted at an angle to draw porewater from 5-10, 10-15 and 15-20 cm below the peat surface. The porewater samples (~50 mL) were filtered (0.45 µm), preserved with a drop of HCl and stored at ~4°C. The porewater samples were then divided among labs to analyze N and C content. TDN and DOC were analyzed using a Shimadzu TOC-Vcsn Total Organic Carbon/Total Nitrogen analyzer in the Biogeochemistry Lab of Department of Geography, McGill University. NO_3 and NH_4 were analyzed at Macdonald Campus (Environmental Chemistry and Soil Nutrient Lab, Natural Resource Sciences, Helen Lalande). DON was determined by subtracting the inorganic fraction from the total porewater N content, or NO_3 and NH_4 from the TDN. The rest of the porewater samples were sent to Luc Tremblay at Université de Moncton for amino acid analysis in order to determine the quantity and composition of amino acids in the soil solution from the rooting zone.

2.2.2 Tracer study

The experiments took place over five days, from July 14-18, 2010, which were consistently warm, sunny days with occasional light rain in the afternoons. The initial injections of treatment and control experiments were staggered over two days. On each day, the experiment for four control and four treatment plots was initiated. The treatment

plots received isotopically labeled glycine and the controls received distilled water. The leaves of the vegetation within the plot were sampled at 3, 24, and 72 hours after injection. The plots were then excavated to a depth of 20 cm, stored in a walk-in fridge (-4ºC) and later sampled for shrub and sedge roots. The experimental design yielded 86 leaf samples (dominant species) and 30 root samples (shrub and sedge). Additional data collection was done for the water table depth at each plot and porewater sampling for four of the eight control plots.

The tracer study involved injecting an isotopically labeled glycine treatment to half of the 16 plots and subsequently tracing the change in $\delta^{13}C$ and $\delta^{15}N$ in the plant tissue. Following Nasholm *et al.* (1998), who used 250 mL 1.2 mM labeled glycine for a plot with surface area of 0.05 m^2, I applied the same concentration in my plots which have a surface area size of 0.1 m^2 (50 x 20 cm). Therefore 374.4 mg of glycine ($^{13}C_2$,^{15}N, 98% atom, Sigma Aldrich) was dissolved in 4 L of distilled water at the field site. The control plots received 500 mL of distilled water. The glycine treatment received 500 mL of 1.2 mM labeled glycine. The control and glycine treatments were injected using a sideport needle that was attached to a 60 mL syringe. The treatments were injected so that 62.5 mL was injected at eight stratified injection points within the 50 x 20 cm plot. The sideport needle was inserted at a depth of 20 cm and then slowly withdrawn to the surface while expelling the solution into the peat. Therefore the rooting zone of the vascular plants as well as the moss at the peat surface was targeted to receive treatment. No attempts were made to stop the solution from moving further downward or laterally into the peat. The control and treatment injections took place at 10 am on July 14 and July 15 2010.

For further reference, the concentration of glycine solution used in my study was equivalent to 10.59 μg N g^{-1} soil. Mer Bleue has a peat density of 0.0412 gcm^{-3} which is much lower than the 1.3 gcm^{-3} soil density from Nasholm *et al.* (1998) making my injection of glycine about eight times as much as was used in his study (1.343 μg N g^{-1} soil) but within the range used by others: Ohlund and Nasholm (2001) (15.76 μg N g^{-1}), Vinolas *et al*. (2001a) (0.26, 0.63, 1.25, 2.55, 4.95, 12.45, 25.05 μg N g^{-1}), Weigelt *et al*. (2005) (10 μg N g^{-1}), Jones *et al*. (2005b) (0.00015, 0.0015, 0.015, 1.5, 15 μg N g^{-1}), Harrison *et al*. (2008) (7.5 μg N g^{-1}).

2.2.3 Tissue Sampling

Plant tissue samples were collected at 3, 24 and 72 hour intervals after glycine addition, similar to previous tracer studies (Nasholm *et al.*, 1998; Nasholm *et al*., 2000; Karagatzides *et al*., 2009; McFarland *et al*., 2010; Sauheitl *et al*., 2009). New growth from the 2010 growing season of the plant was collected with respect to the leaves of the shrubs, the blades of the sedge and the capitula (top 3-4 cm) of the mosses. Approximately four to five leaves of the shrubs, five to ten capitula and eight sedge blades were sampled at each interval in order to produce a dry weight of ~100 mg for analysis. Roots were collected from the extracted peat, after 72 hours, and organized into sedge and shrub roots, because shrub roots could not be differentiated into species. The quantity and size of sedge roots varied, depending on whether the core was from a lawn or hummock, because the majority of fine root production for sedges occurs at a depth of 30-40 cm (Murphy and Moore, 2010) and there was not a large quantity to sample from within the top 20 cm. However, the sedge roots collected were considered as performing

the nutrient uptake function of a fine root rather than a structural or transport function of larger older roots (Meaghan Murphy, pers. comm.).

Both moss and root plant tissue samples from the glycine treatment plots were rinsed in 0.5 mM $CaCl_2$ to remove any residual glycine adsorbed to the surface of the samples. Leaf and root samples were oven-dried at 60° C for 48 hours and then ground into fine particles with a Laboratory Wiley Mill (Arthur H. Thomas model, 1725 rpm) and size 40 mesh sieve. Samples were then stored at room temperature in coin paper envelopes until weighed for isotope analysis.

2.3 Isotope analysis

The leaf and root samples from the 72 hour sampling have been analyzed at the GEOTOP stable isotope laboratory at Université de Québec à Montréal (UQAM). The ground plant tissue of the samples were weighed out into tin caps and analyzed for %C and %N concentrations and $\delta^{13}C$ and $\delta^{15}N$ at GEOTOP (UQAM), using **a** Micromass Isoprime**™** continuous flow mass spectrometer coupled to an Elementar Vario Micro Cube™ elemental analyzer. Due to the high C concentrations of plant material, the $\delta^{13}C$ analysis was adjusted to include extra carrier gas (helium) to dilute the samples. Isotope standard references were leucine ($\delta^{13}C$ = -28.50‰; $\delta^{15}N$ = -0.26‰) and DORM-2 ($\delta^{13}C$ = -17.35‰; $\delta^{15}N$ = +14.36‰) and the standards for enriched $\delta^{15}N$ samples were USGS 26 = 53.72‰ ±0.24 (air), IAEA 310A = 47.2‰ ±1.3 (air), and IAEA 310B = 244.6 ‰ ±0.8 (air).

2.4 Data Analysis

The differences in $\delta^{13}C$ and $\delta^{15}N$ between control and treated samples were used as evidence for the quantification of organic N uptake. A simple t-test was used for samples that upheld a normal distribution. Not all samples assumed a Gaussian distribution between control and glycine treated plots for each species due to the spatial variability in sampling within the plot area. Therefore, the non-parametric Mann-Whitney test, for unequal sample size and unequal variance was used to determine if the glycine treated samples were statistically different from the control samples for each species. Means and standard error were calculated for both control and glycine treated samples. Excess ^{13}C was plotted against ^{15}N to compare uptake to the C:N ratio of glycine for each species, plant functional type and microtopographic feature.

Equations to calculate atom percent and excess from delta values

(1) Atm%: $$\frac{100 * AR * ((Delta/1000)+1)}{(1+(AR*((Delta/1000)+1)))}$$

Where AR is the absolute ratio of mol fractions. For ^{15}N this is 0.0036782 ($\pm 1.5*10^{-6}$) (De Bievre *et al.*, 1996) and for ^{13}C is 0.0112372 (VPBD).

The Atm% equation is to determine the atom percentage of the heavy isotope from delta values.

(2) # of mols: $(Atm\%/100)*\left[(X/100*1)/Atw\right]$

Where symbol X is the content of N or C (in percentage) of the sample. To calculate the number of moles in (an arbitrary) 1 gram of dry plant material I took the atom percent and applied it to the amount of mols in 1 gram of material.

(3) Atw: $(Atm\%/100)*B+(1-Atm\%/100)*b$

Where symbol B is the number of neutrons in the heavy/rare isotope and symbol *b* is the number of neutrons in the lighter/common isotope. I calculated the atomic weight based on the Atm% of each sample.

Chapter 3: Results

3.1 Plot characteristics

The 16 plots were assigned to either hummock or lawn and either control (distilled water) or treatment (glycine) categories (Table 1).Water table depth was recorded to verify the visual classification of hummock and lawn when choosing sites. At the Blue Dome in Mer Bleue where my plots were located, the term 'lawn' was chosen as the proper term for a flat microtopography over the more commonly used term 'hollow'. Plot 5 could be considered to be in an actual hollow, however the other plots are better considered as lawns. The average water table depth of my hummock sites on the day of injection was 50.6 cm and for lawns 31.0 cm. The water table depth for hummocks on the day of injection was lower than the growing season average by 2.8 cm. *In situ* measurements of water table depth at each plot were adjusted heuristically using tower data.

From the presence of species in each plot, six were analyzed as these are considered dominant in the bog (Table 1). The three shrubs were deciduous *V. myrtilloides,* evergreen *C. calyculata* and evergreen *L. groenlandicum*. Shrubs that were present but not analyzed included *K. angustifolia,* an evergreen shrub that had a good representation at all plots, *K. polifolia* (data not shown) and *V. oxycoccus,* both of which were rarely found. The two *Sphagnum* species were analyzed as they are the dominant bryophyte species at Mer Bleue whereas the moss, *P. strictum,* was only present in hummock sites. *E. vaginatum* was analyzed as it was the only sedge present. *V. myrtilloides* had the lowest presence at 11, and therefore sample size, of the species tested in the tracer experiment.

Plot label C = control T = treatment	Site	WTD (cm)		Presence (1) /absence (0) of Species by Functional Type								
				Sedge	Moss			Shrub				
		18 July	GS*	*E.v.*	*S.c.*	*S.m.*	*P.s.*	*C.c.*	*L.g.*	*V.m.*	*V.o.*	*K.a.*
1 C	Lawn	28.5	27.0	1	1	1	0	1	1	1	0	1
2 C	Hummock	33.5	37.0	0	1	0	0	1	1	0	1	1
3 T	Lawn	32.5	36.8	1	1	1	0	1	0	1	0	1
4 T	Lawn	27.5	31.0	1	1	1	1	1	1	0	0	1
5 C	Lawn	19.0	22.5	1	1	1	0	1	1	0	0	1
6 C	Hummock	57.5	53.8	1	1	1	0	1	1	1	0	0
7 T	Hummock	67.5	60.0	1	1	1	0	1	1	1	0	1
8 T	Hummock	37.5	39.7	0	1	1	1	1	1	1	1	1
9 T	Lawn	49.5	39.7	1	1	1	0	1	1	1	0	1
10 C	Hummock	45.5	49.0	1	1	1	0	1	1	1	0	1
11 C	Lawn	30.5	33.0	1	1	1	0	1	1	1	0	1
12 T	Hummock	42.5	45.3	1	1	0	1	1	1	0	0	0
13 T	Hummock	44.5	38.7	1	1	1	1	0	1	1	0	1
14 C	Hummock	76.5	59.3	1	0	1	1	1	1	1	0	1
15 C	Lawn	N/A	31.2	1	1	1	0	0	1	0	1	1
16 T	Lawn	29.5	27.7	1	1	1	0	1	1	1	0	1
Sum total				14	15	14	5	14	15	11	3	14
Avg hummock WTD		50.6	47.8									
Avg lawn WTD		31.0	31.1									

Table 1. Microtopographic feature, treatment, water table depth (WTD) and plant species distribution at each plot. GS* denotes the May to October 2010 growing season average WTD at each plot. Plant species observed include *Eriophorum vaginatum* (*E.v.*), *Sphagnum capillifolium* (*S.c.*), *Sphagnum magellanicum* (*S.m.*), *Polytrichum strictum* (*P.s.*), *Chamaedaphne calyculata* (*C.c.*), *Ledum groenlandicum* (*L.g.*), *Vaccinium myrtilloides* (*V.m.*), *Vaccinium oxycoccus* (*V.o.*), and *Kalmia polifolia* (*K.p.*).

Porewater samples were taken from the top 20 cm of four control plots using Macrorhizon Soil Moisture Samplers. Total dissolved N ranged from 0.99 to 1.35 mg L^{-1}. DIN content was low at 0.058 to 0.039 mg L^{-1} with little NO_3^--N detected (Table 2). DON was calculated as the difference between Total N and DIN and the proportion of

DON in the rhizosphere porewater was 95.7-97.0% of the Total N. The DOC concentration at the plots ranged from 45.0 to 70.8 mg L^{-1}.

Amino acid composition of the porewater was also analyzed. The percent of organic C which was in the form of amino acid C (AA-C) ranges from 1.40 to 1.88% across the four plots (Table 2). Figure 5 shows the individual amino acid composition of total AA-C. The top five most abundant amino acids in the porewater at Mer Bleue are Glycine, Glutamic Acid, Phenylalanine, Thr+His and Aspartic Acid. Glycine was the most abundant in the porewater at 14.9 to 24.9% of total AA-C followed by Glutamic Acid with a range from 7.7 to 13.9%. Although Threonine and Histidine could not be separated during analysis, Threonine is generally 2-5 times more abundant than Histidine (Luc Tremblay, pers. comm.). Non-protein amino acid yields, as denoted by G-ABA and B-ALA, were considered high (Luc Tremblay, pers. comm.) and as they are the result of amino acid breakdown (from old, degraded samples) the proportion of some individual amino acid could be underestimates.

Plot	2	5	14	15
Total dissolved nitrogen (mg L^{-1})	1.35	1.32	1.29	0.99
NH_4-N (mg L^{-1})	0.04	0.038	N/A	N/A
NO_3-N (mg L^{-1})	0.018	0.001	N/A	N/A
Estimated DON (mg L^{-1})	1.292	1.281	N/A	N/A
Percent of Total N as DON (%)	95.70	97.05	N/A	N/A
DOC (mg L^{-1})	61.50	50.10	70.80	45.00
% of C as AA-C (AA C/100 mg OC)	1.88	1.85	1.60	1.40

Table 2. Concentrations of total N, NH_4-N, NO_3-N. DON, % TDN as DON, DOC and amino acids (AA) as a percentage of DOC in porewater collected from the top 20 cm from four plots.

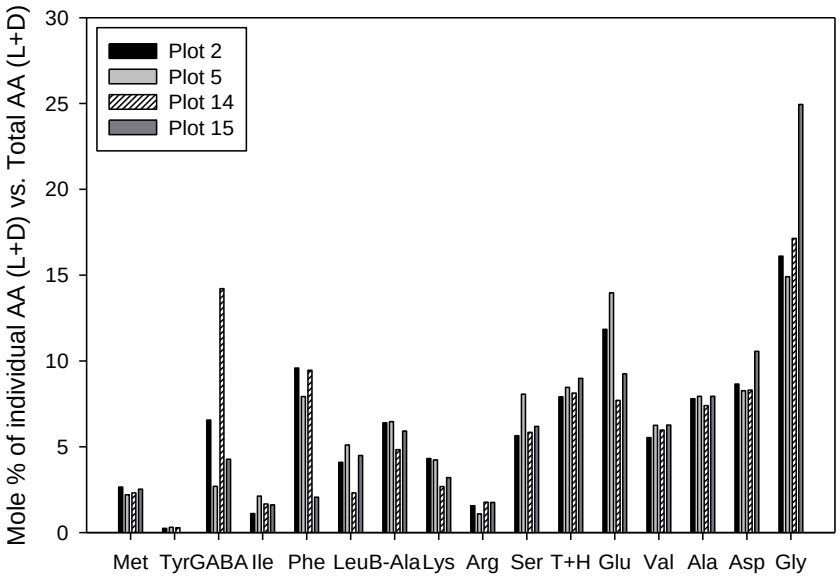

Figure 5. Composition, as mole %, of 16 amino acids of the total AA- C. T+H denotes L-Threonine and L-Histidine combined as they could not be separated during analysis.

3.2 Natural abundances of ^{15}N and ^{13}C in peatland plants

From the control plots, average foliar ^{15}N signatures ranged from 1.34‰ for *E. vaginatum* to -6.88‰ for *L. groenlandicum*, with other species between -2.42 and -4.99‰ (Fig. 6), similar to those observed earlier by Tim Moore (pers. comm.). The foliar $\delta^{15}N$ pattern of different plant functional types is also similar with an order of sedge>moss>shrub for increasingly depleted $\delta^{15}N$.

Variations in ^{13}C abundances are related to differences in plant C metabolism and CO_2 recycling and generally vary less than ^{15}N abundances. The natural ^{13}C abundances (Figure 7) for the six species range from -27.4‰ for *E. vaginatum* to -30.9‰ for *V. myrtilloides*, which is a smaller range in variation compared to foliar ^{15}N abundances, where the variation was as high as ~8‰ across species (Figure 6). No clear plant functional type pattern can be discerned.

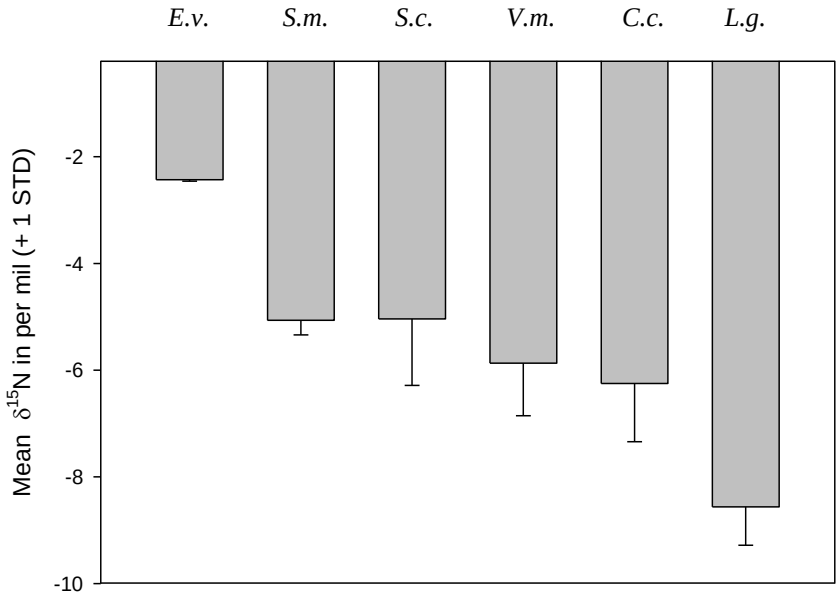

Figure 6. Mean foliar ^{15}N abundances with one standard deviation of six dominant bog species.

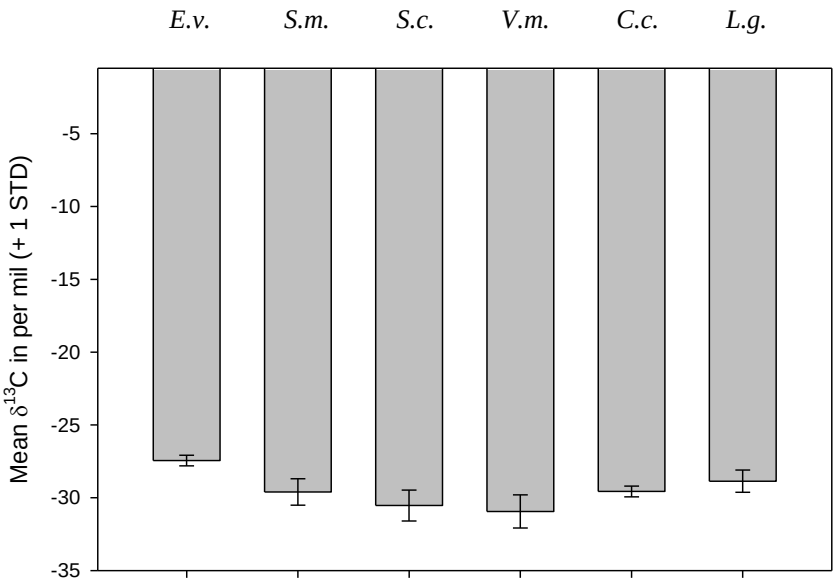

Figure 7. Mean foliar ^{13}C abundances with one standard deviation of six dominant bog species.

The root abundances are presented in Figure 8. The mean shrub root $\delta^{15}N$ from control samples is -3.72‰ whereas the sedge root mean $\delta^{15}N$ is 2.03‰. The average $\delta^{15}N$ for all root samples is ~2.3‰ less depleted compared to the average $\delta^{15}N$ for foliar samples (mean root $\delta^{15}N$ = 1.0‰, mean foliar $\delta^{15}N$ = -3.3‰). This intra-plant $\delta^{15}N$ variation is quite common in natural abundances literature and can be attributed to differences in source N among other reasons as will be discussed in Chapter 4. The root ^{13}C abundances are also slightly less depleted with a difference of ~1.5‰ between foliar and root samples (mean root $\delta^{13}C$ = -27.9‰, mean foliar $\delta^{13}C$ = -29.4‰).

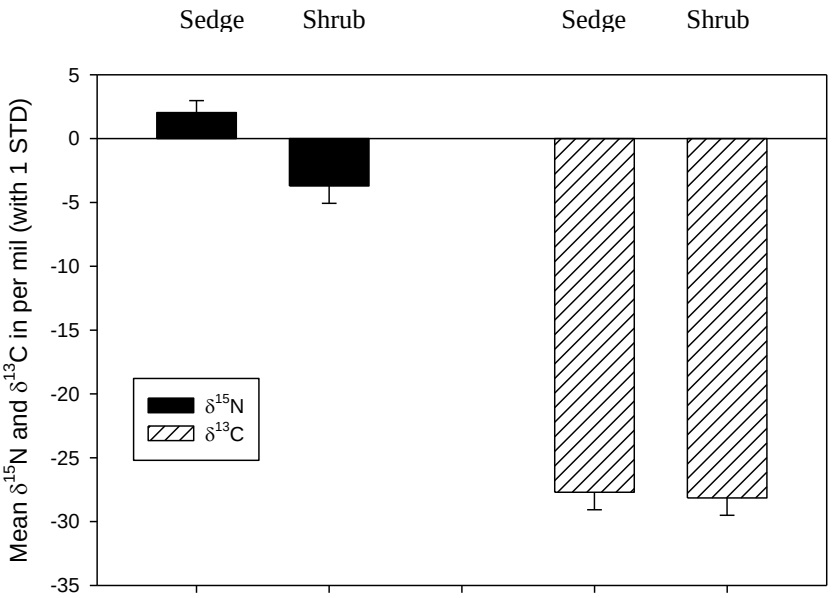

Figure 8. Mean root ^{15}N and ^{13}C signatures with one standard deviation of sedge and shrub control samples.

Mean foliar and root ^{15}N abundances from control plots showed variations between the samples taken from lawn and hummock plots (Figure 9). All species except for *S. magellanicum* and *V. myrtilloides* tend to have more depleted foliar $\delta^{15}N$, though statistically non-significant, when sampled from hummocks over lawns. Such a pattern may be an example of the varying use of N sources by species in hummocks and lawns in the bog, however further analysis with a larger dataset would be required to assess the consistency of such a pattern. A similar trend of more depleted values among hummock plots was also visible for the samples of sedge and shrub root type. The $\delta^{13}C$ between hummock and lawn (data not shown) do not seem to adhere to any one pattern, despite expectations that water table generally affects plant photosynthesis and therefore CO_2 metabolism.

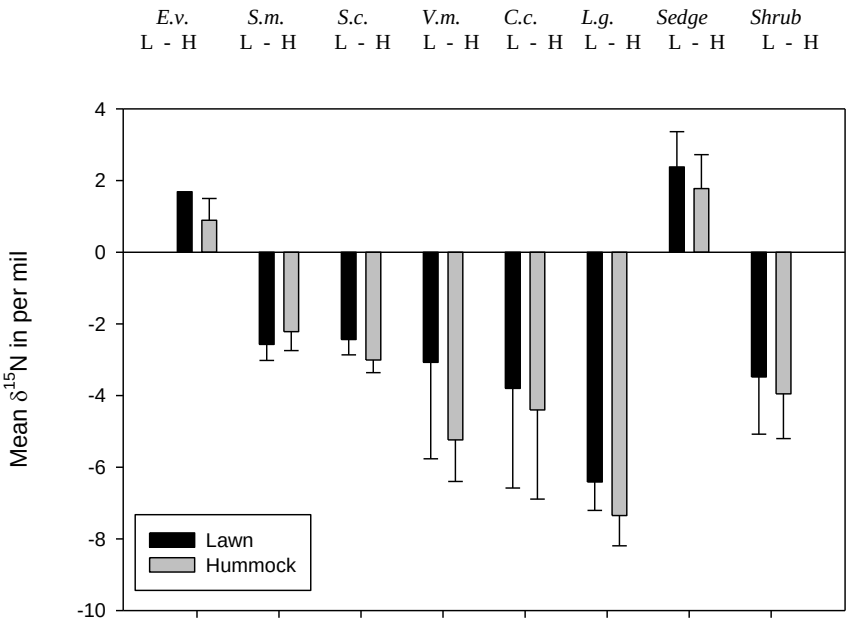

Figure 9. Mean foliar ^{15}N signatures with one standard deviation of six dominant bog species and sedge and shrub roots sampled from lawn (L) and hummock (H) plots.

3.3 Foliar uptake of ^{15}N and ^{13}C after glycine injection

The difference in foliar $\delta^{15}N$ between glycine treated and control sample means are significant for all species using a t-test or Mann-Whitney (non-parametric used for *S. magellanicum, S. capillifolium* and *V. myrtilloides*) statistical test ($P < 0.05$). Glycine treated samples are significantly enriched in ^{15}N but the amount of enrichment varies across each species (Figure 10). The sedge *E. vaginatum*, for example, had the least amount (1.92‰) of enrichment between control and glycine treated samples. The *Sphagnum* mosses showed strong enrichment (*S. magellanicum* = 143.02‰, *S. capillifolium* = 42.5‰) but overall had a highly variable glycine treated dataset and this is

discussed further in Chapter 4. The shrub species responses ranged from 6.22‰ enrichment for *C. calyculata* to 17.54 and 34.09 ‰ for *L. groenlandicum* and *V. myrtilloides*, respectively. *V. myrtilloides* showed the strongest enrichment as well as the most variability within the glycine treated shrub samples.

The mean foliar $\delta^{15}N$ in glycine treated plots ranges from 2.07‰ to a highly enriched 140.60‰ (Figure 10). Across plant functional types, *Sphagnum* mosses had the most enriched $\delta^{15}N$, followed by the shrubs and the sedge *E. vaginatum* had a mean foliar $\delta^{15}N$ of 1.34‰ in the glycine treated samples. Among the glycine treated samples, a large variability was observed in the $\delta^{15}N$ both within the shrub and moss plant functional type as well as within species (Figure 10).

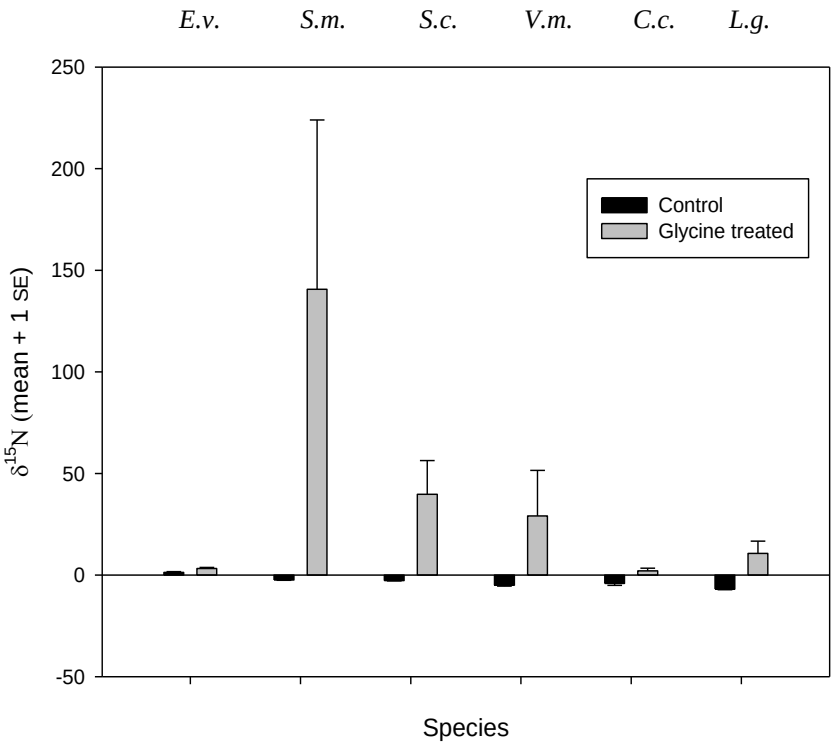

Figure 10. Mean foliar $\delta^{15}N$ (+1 SE) of six dominant species within the control and glycine treated plots 72 hours after injection (n = 6-8).

	Control		Glycine treated		
Species	Mean $\delta^{15}N$	SEM	Mean $\delta^{15}N$	SEM	*P*-value
E.v.	1.34	0.32	3.26	0.50	0.008
S.m.	-2.42	0.18	140.60	83.37	0.005[a]
S.c.	-2.72	0.17	39.78	16.57	0.036[a]
V.m.	-4.99	0.42	29.10	22.42	0.015[a]
C.c.	-4.15	0.91	2.07	1.29	0.002
L.g.	-6.88	0.32	10.66	5.99	0.026

Table 3. Mean foliar $\delta^{15}N$, standard error, and statistical *P*-value ([a] denotes non-parametric Mann-Whitney U-test, otherwise t-test was used) between control and glycine treated samples of each species.

Although significant differences in the $\delta^{15}N$ of the glycine treated samples for each species suggests that $\delta^{15}N$ has been taken up by plants, we cannot identify what form of ^{15}N was taken up, such as intact glycine or a mineralized form such as $^{15}NH_4$ or $^{15}NO_3$.

Glycine is an amino acid with a C:N ratio of 2:1 and therefore if it was taken up intact by plants then an enrichment of ^{13}C should be detected in plant tissue. Unlike $\delta^{15}N$, the change in the $\delta^{13}C$ of the leaves between control and glycine treated plots was not significant across all species (Figure 11). The variation in the $\delta^{13}C$ of control plots was greater than the variation of samples from the glycine treated plots for *S. capillifolium, V.*

myrtilloides and *L. groenlandicum* (Table 1) which points to the insignificance of the slight increase in the $\delta^{13}C$ means from control to glycine treated plots.

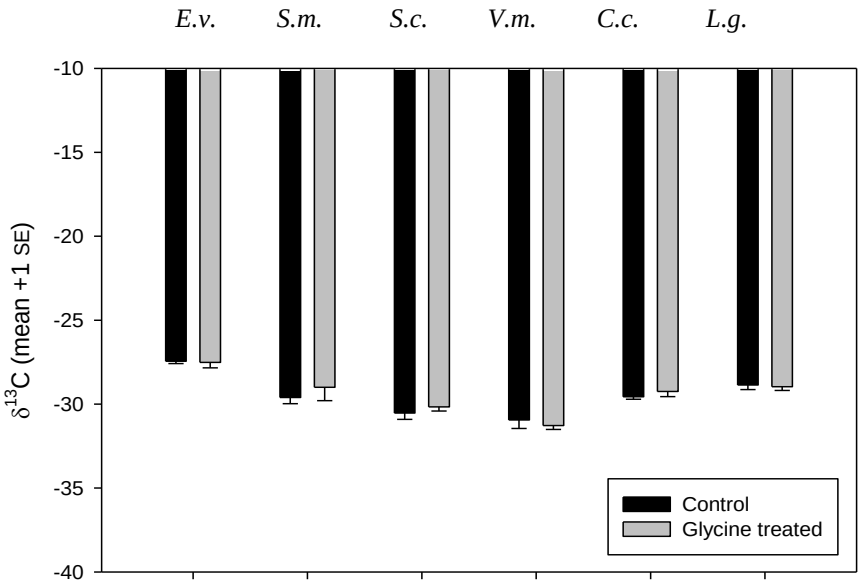

Figure 11. Mean foliar $\delta^{13}C$ (+1 SE) of six dominant species within the control and glycine treated plots 72 hours after injection (n = 6-8).

The difference in root $\delta^{15}N$ between control and glycine treated samples was significant ($P < 0.05$) for both sedge and shrub roots (Table 4) and the increase in root ^{15}N was greater than in leaves. Sedge and shrub roots had a mean ^{15}N enrichment of 38.47‰ and 153.24‰, respectively.

The change in root $\delta^{13}C$ between control and glycine samples was not significant for sedges (P-value = 0.134) but significant for shrub roots (P-value = 0.008). However, four out of six individual sedge samples recorded enrichment over the control average,

ranging from 1.0-8.6‰, larger than the maximum enrichment (0.65‰) between the control and glycine-treated shrub root samples.

	Root type	Control		Glycine treated		
		Mean	SEM	Mean	SEM	*P*-value
^{15}N	*E.v.*	2.03	0.35	40.50	15.67	0.003
	Shrubs	-3.72	0.48	149.52	25.35	0.001
^{13}C	*E.v.*	-27.70	0.52	-25.52	1.36	0.134
	Shrubs	-28.15	0.13	-27.84	0.09	0.008

Table 4. Mean root $\delta^{15}N$ and $\delta^{13}C$ with standard error of the mean (SEM) for the sedge *E. vaginatum* and mixed ericaceous shrub species.

3.4 Atom percent enrichment and excess ^{15}N and ^{13}C in leaves and roots

The atom percent was calculated from the delta values and the absolute ratio of ^{15}N and ^{13}C. By subtracting the control mean we can see the atom percent enrichment (APE) between glycine and control treatments. A comparison of the APE (Figure 12 and 13) shows that the ^{13}C enrichment is quite small in comparison to the ^{15}N enrichment but the increase in ^{13}C APE of root tissue shows that glycine can be taken up in an intact

form. As atom percent is solely based on ^{15}N and ^{13}C delta values it does not represent the actual number of moles in the plant samples, which often has ~50 times more C content than N content. Therefore, excess, which is calculated with the sample N and C content, is used in order to compare the change in plant $\delta^{15}N$ and $\delta^{13}C$ to the molar ratio of glycine of 2:1.

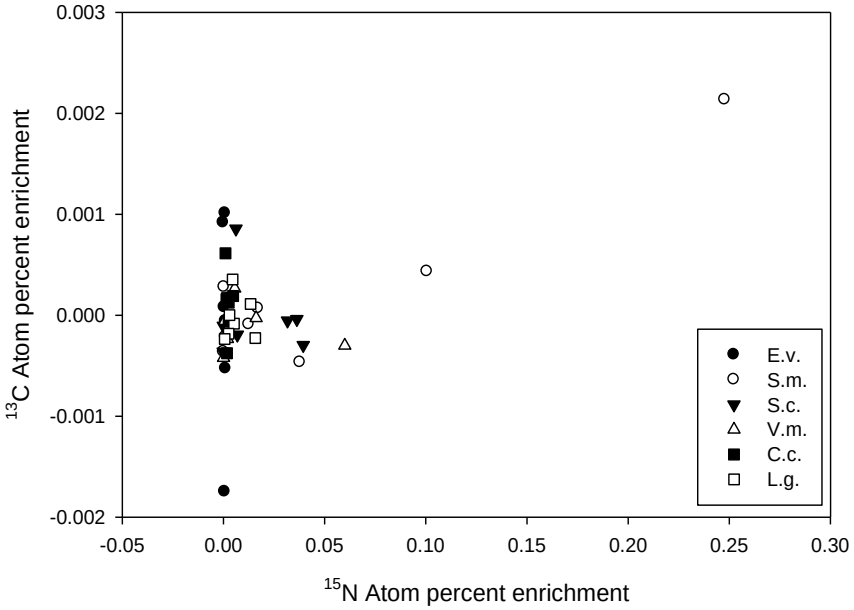

Figure 12. Foliar atom percent enrichment of six dominant species (*n*= 7-8).

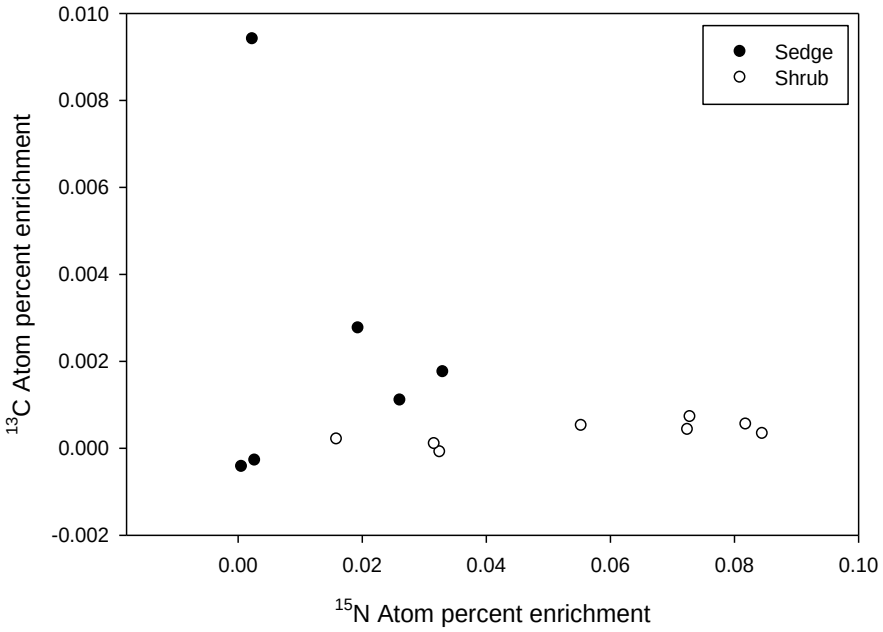

Figure 13. Root atom percent enrichment of six dominant species (*n* = 6-8)

In a study on organic N uptake Nasholm *et al*. (1998) used a metric termed 'excess' to compare the changes in $\delta^{13}C$ and $\delta^{15}N$ in plant tissue. Excess here is calculated as the difference in the total number of mols of the heavy isotope between the control average and glycine-treated samples. When excess ^{13}C is plotted against excess ^{15}N we can see the uptake ratio of ^{13}C to ^{15}N. If glycine was taken up intact as an amino acid the ratio of excess ^{13}C to excess ^{15}N would be 2:1 as glycine has two C atoms and one N atom. Due to the insignificant change in $\delta^{13}C$ in the leaves of all plant species, a 2:1 excess of ^{13}C to ^{15}N is not clearly seen in the following diagrams. In fact, excess ^{13}C is often seen as a negative value, as the variability within control ^{13}C values was sometimes greater than the change between control and glycine treated leaf samples.

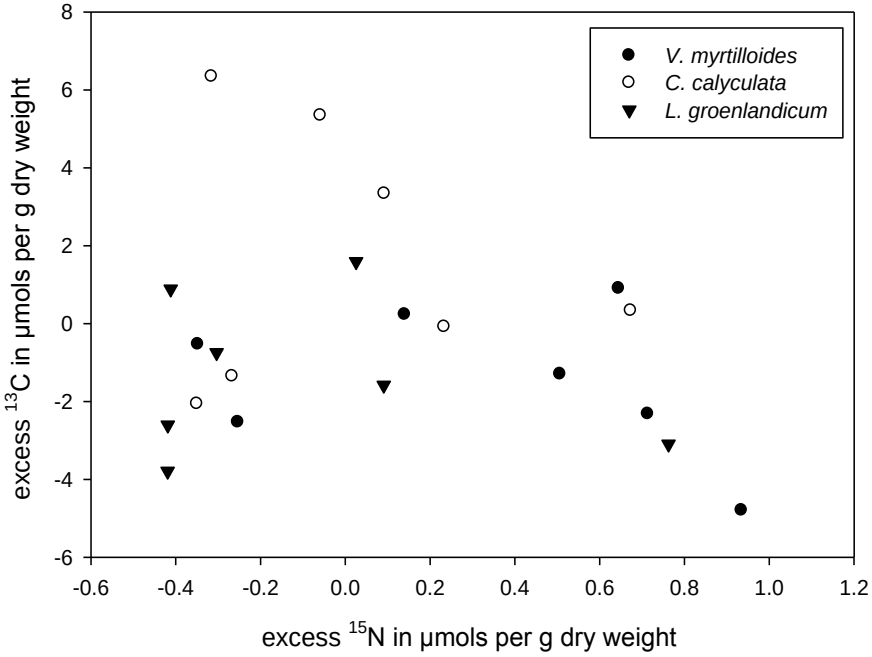

Figure 14. The relationship between excess ^{13}C and excess ^{15}N for shrub species, sampled 72 hours after injection. Values represent the excess of ^{13}C and ^{15}N of glycine treated samples over the mean of control samples (n = 21).

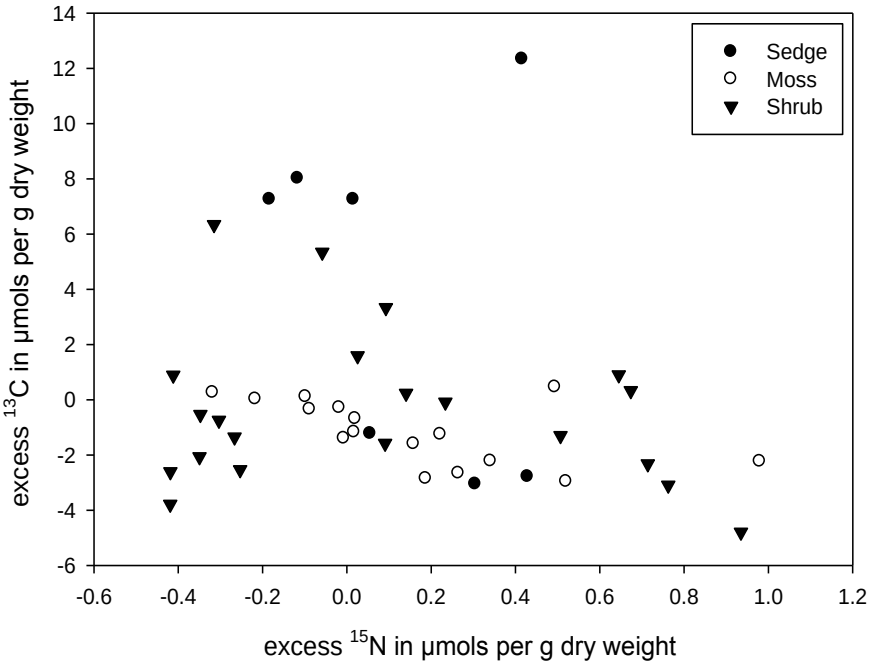

Figure 15. The relationship between excess ^{13}C and excess ^{15}N by plant functional type, sampled 72 hours after injection. Values represent the excess of ^{13}C and ^{15}N of glycine treated samples over the mean of control samples (*n* = 7-21).

Foliar excess ^{13}C and excess ^{15}N, especially a positive excess of ^{13}C, in moss species would be the only indicator of plant uptake of glycine. The moss capitula, which was the only tissue sampled from the mosses as they are bryophytes, were collected as foliar samples. Some individual samples showed positive excess ^{13}C, about half of which showed an increase in ^{15}N uptake, however, unfortunately, the spatial variability of treatment injection precludes being able to detect any significant pattern. As for sedge, it can be seen that the C:N ratio is higher than 2:1 and often the excess δ^{15}N was negative (mean values shown in Figure 17). As a plant functional type, the shrubs were quite

variable (Figure 14) and this may be due to differences in mycorrhizal infection among the species.

As stated earlier, glycine has a C:N ratio of 2:1 and no species really showed a clear 2:1 uptake of ^{13}C and ^{15}N. Both sedge and shrub root samples showed a similar pattern to that seen in the leaves. For sedge, excess ^{13}C was detected but the excess ^{15}N was mostly negative. For shrubs, about half the root dataset showed both a positive excess of ^{15}N and ^{13}C and half showed an excess of ^{15}N only (Figure 16).

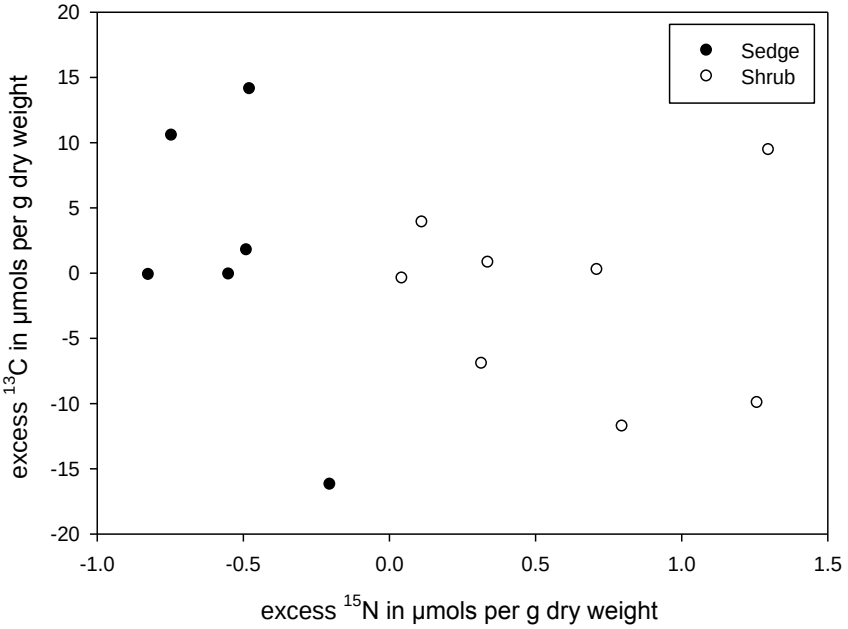

Figure 16. The relationship between excess ^{13}C and excess ^{15}N in roots by plant type sampled 72 hours after injection. Values represent the excess of ^{13}C and ^{15}N of glycine treated samples over the mean of control samples (n = 6-8).

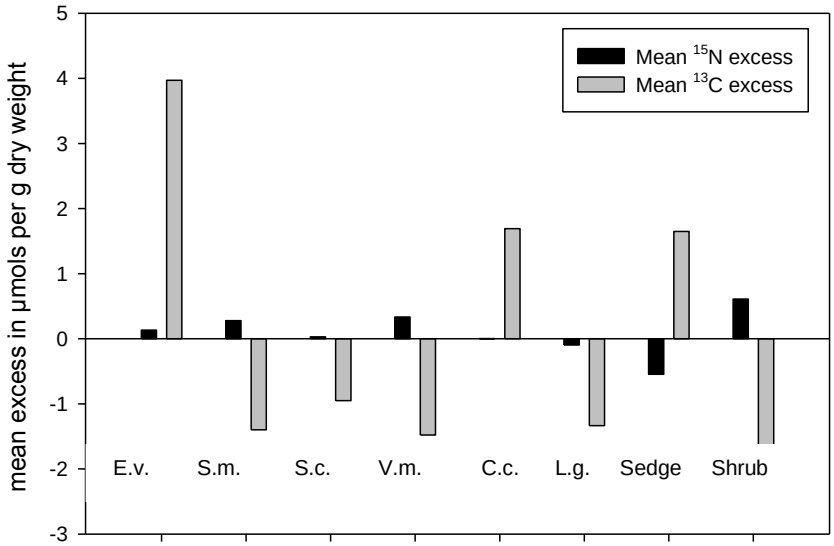

Figure 17. Mean excess ^{15}N and ^{13}C of foliar and root samples.

Chapter 4: Discussion and conclusions

Section 4.1 Inorganic and organic N composition of Mer Bleue porewater

The ratio of DOC to DON in the rhizosphere porewater was approximately 43:1 which is similar to plant tissue C:N ratios. The porewater analysis of Mer Bleue results confirmed earlier work (Basiliko *et al*., 2005; Rattle, 2006) showing low inorganic N content and high organic N content. The percent of total N in DON form in the porewater was 95-97% which is higher than that found for porewater from the saturated zone (~80%) (Rattle, 2006), indicating that the percentage of DON is greater in the rooting zone of Mer Bleue peatland than below the water table. Furthermore, Rattle (2006) found that the percent of N in DON form decreased with depth, supporting a larger percentage of DON above the water table. This is probably a result of fresh organic matter added to the surface while microbial recycling would lead to more inorganic N at greater depths below the surface. The instantaneous assessment of DON is complicated by the fact that the rhizosphere porewater is the most transient N pool. It varies with time and depth, with a steep increase in N concentrations below 25 cm (Limpens *et al*., 2006). Another complication is that some soil solution extraction methods or centrifugal drainage would cause roots and fungal hyphae tips to release DON by mechanical disturbance (Jones and Willet, unpublished as cited in Jones *et al*., 2005b). In my study, the use of macrorhizon soil moisture samplers was the least disruptive method available, especially over centrifugal extraction but as it functions via the use of suction from an attached syringe, it may have led to an overestimate of DON. Surface peat is often coarse and therefore dual porosity is not a problem for suction samplers (Blodau and Moore, 2002) but the coarse, loose property of surface peat also provides little contact with suction sampler tubes and

little water is actually obtained. Nonetheless, the porewater measurements from the unsaturated layer of peat showed that the concentration of inorganic N is very low, with most of the N in DON form.

By determining the DON's individual components, the proportion of high molecular weight organic substances or polymers versus the low molecular weight organic substances or monomer fraction will be known. In the review by Jones *et al*. (2005), the authors state that the DON pool in some ecosystems may be large because it is composed of polymer N that is of a recalcitrant nature that neither plants nor microbes can use. DOC content of the porewater ranged from 45.0-70.8 mg L^{-1}. The amino acid analysis showed that 1.4-1.88% percent of organic C was in the amino acid form making its average concentration in the porewater about around 0.96 mg L^{-1}. Taking the average C:N ratio of the amino acids, the AA-N concentration at Mer Bleue is estimated at 0.21 mg L^{-1} and the AA-N:DIN ratio ~4.8. This is higher than the F-AA:DIN content found in McFarland *et al*. (2010) where the highest ratio is ~2.88. Despite a greater concentration of AA-N compared to inorganic N in the peatland porewater, Jones *et al*. (2005) suggested that measuring the flux of the amino acid pool would be more useful in predicting the bioavailability of amino acids. Similarly, other evidence that would strengthen the suggestion that organic N is an important N source includes the measurement of DON's constituents, the measurement of root exudation and recapture of amino acids, the proportion of absorbed DON to plant total N, and the amount of DON consumed by microbial competitors (Jones *et al*., 2005).

Amino acid composition showed that glycine and glutamic acid were the most abundant amino acids composing from 23-39% of the total AA-C. As glycine has a low

C:N ratio (2:1) it is considered easy to take up by plants whereas glutamic acid has a slightly higher C:N ratio of 5:1. Nonetheless, Schimel and Bennet's (2004) theory of depolymerization implies that extracellular enzymes can break down even recalcitrant or polymer organic N so that it becomes bioavailable. For example, the mycorrhiza known as *Hymenoscyphus ericae* has the ability to degrade and assimilate chitin, which is a complex N polymer (Kerley and Read, 1995).

Section 4.2 Natural ^{15}N and ^{13}C abundances

Section 4.2.1 Foliar ^{15}N abundances of Mer Bleue control samples

The ^{15}N signatures of the species at Mer Bleue range from 1.3‰ for *E. vaginatum* to the more depleted value of -6.88‰ for *L. groenlandicum.* As such, the ^{15}N signatures for plant functional types were in the order of sedge>moss>shrubs towards the most depleted $\delta^{15}N$. The depleted shrub values correspond to the literature as ericaceous shrubs often form mycorrhizal associations which have been observed to have depleted ^{15}N signatures. The mycorrhizal types correlate to increasing foliar $\delta^{15}N$ in the order of ericoid, ectomycorrhiza, arbuscular and non-mycorrhizal plants (Craine *et al*., 2009). One explanation for why ericoid mycorrhizal associations can lead to depleted foliar ^{15}N signatures is that they are able to access a wider variety of N than other fungal forms. Ericoid mycorrhizae can utilize organic N forms and at the same time produce enzymes that break down larger forms of N, such as proteins. On the other hand arbuscular mycorrhizae lack such proteolytic capabilities and are adapted for the absorption of phosphorous (P) sources rather than for N uptake (Chalot and Brun, 1998; Bolan, 1991).

Therefore one of the indicators that shrubs at Mer Bleue may be able to take up organic forms of N was that they have such depleted ^{15}N signatures.

In addition to source N type, another factor involved in the pattern of ^{15}N signatures seen in the species of Mer Bleue is the depth at which plants access their source N. Studies have shown that ^{15}N content increased with increasing depth of the soil profile (Nadelhoffer *et al.*, 1996; Asada *et al*., 2005; Kohzu *et al*., 2003). For example, the surface peat in the Alaskan tundra had a $\delta^{15}N$ of -2.5‰ but at a depth of 30cm, the $\delta^{15}N$ was an enriched 2.9‰ (Nadelhoffer *et al.*, 1996). The change in $\delta^{15}N$ in the peat at Mer Bleue from the surface to a depth of 30 cm is approximately 4-5‰ (Tim Moore, pers. comm.). The non-mycorrhizal sedge, *E. vaginatum*, had the most enriched ^{15}N signature of all species as well as being the deepest rooted of the examined species, with the bulk of root production occurring 40 cm below the surface (Murphy and Moore, 2010). *Sphagnum* mosses did not show the least depleted values based on depth, however N-fixation from atmospheric N_2 would shift the plant ^{15}N signatures toward zero.

The root samples of sedge and shrub species were more enriched in ^{15}N than the corresponding foliar samples, a common finding (Evans, 2001) as intra-plant variation mostly exists between the leaves and roots. This can be due to kinetic isotope fractionation from plant metabolic processes leading to a product having a higher proportion of the lighter isotope and the substrate having a higher proportion of the heavier isotope (Sulzman, 2007). Kinetic isotope fractionation occurs in unidirectional or irreversible reactions such as evaporation, diffusion, and enzymatic affects (Sulzman, 2007). As the plant roots metabolize N (NH_4^+ for example is metabolized in plant roots, whereas NO_3^- can be metabolized in roots and leaves), the lighter isotope will be

translocated to the leaves for photosynthesis and the heavier isotope will remain behind. This would also explain why there was little difference between root and foliar ^{13}C abundances as leaves obtain their C requirements for photosynthesis from the air and do not rely on roots to metabolize C sources (but isotopic fractionation does occur between the atmosphere and leaves).

Though the pattern is contestable, an analysis of foliar samples from hummock and hollows showed that the hummocks had a tendency toward more depleted $\delta^{15}N$. This may be an indicator of differing N source acquisition by plants or N pool size under hummocks and hollows. It has been suggested that nutrient concentration could be the reason for topographical patterning in peatlands such as the formation of hummocks and hollows (Rietkirk *et al.*, 2004; Eppinga *et al.,* 2009). The pattern shown by abundances reports slightly more depleted values in the foliar tissue of plants grown on hummocks however further investigation would be required to ascertain whether this pattern is present ecosystem wide.

Section 4.2.2 Natural foliar ^{13}C abundances in Mer Bleue control samples

The small variations in the $\delta^{13}C$ of plants also make the detection of a change in $\delta^{13}C$ from a pulse of glycine, to the rhizosphere at Mer Bleue, easier. As can be seen in the results the variation of $\delta^{13}C$ across all species is 3.495‰ and the highest standard deviation is 1.14‰ for *V. myrtilloides*. Therefore, even what appears to be a small change in $\delta^{13}C$ between control and glycine treated plots would be significant.

Even though the variation in ^{13}C abundances for all species and plant functional types is small, *E. vaginatum* stands out with the most enriched ^{13}C signature. When foliar

^{13}C abundances were split by hummock and hollow some variability was exposed, which may show sensitivity to a deeper water table but there was no pattern across all species values. Loisel *et al.* (2009) found that ^{13}C signatures of *Sphagnum* mosses correlated more to their microtopographical position among hummock and hollows (water resistance to CO_2 diffusion) than by species-specific features. Results showed that ^{13}C-depleted values were related to a low water table while ^{13}C-enriched values were found when the water table was close to the surface. Since water table depth has an influence over $\delta^{13}C$ because it affects plant CO_2 uptake, that may explain the variations between hummock and hollows and the deeply rooted *E. vaginatum*. However as the variability is not consistent among species or plant functional types there may also be physiological factors in CO_2 metabolism playing a role in defining the ^{13}C abundances.

Section 4.3 Detecting organic N uptake through changes in $\delta^{15}N$ and $\delta^{13}C$ between control and glycine treated samples

The following section discusses the findings for the main objective which was to determine if peatland plants can utilize organic N in the field by examining the $\delta^{13}C$ and $\delta^{15}N$ changes in leaf and root tissue samples between control plots and experimental plots that received a pulse of dual-labeled glycine ($^{13}C_2$,^{15}N, 98% atom). Along with testing the difference between control and glycine treated plant tissue samples, a comparison of excess ^{13}C to excess ^{15}N in the samples was also performed in order to compare it to the ^{13}C:^{15}N ratio of glycine, which is 2:1. If the excess ^{13}C to excess ^{15}N ratio in plant tissue samples was found to be near that of 2:1 then it could be said that glycine was taken up in an intact form (Nasholm *et al.*, 1998).

Estimates of recovered label cannot be calculated as biomass measurements for the plot were not collected. In order to determine the amount of label recovered the plant above and belowground biomass would have to be known as well as the soil and microbial biomass of the plot injected. It would require a large time and resource commitment that was beyond the scope of this project.

Section 4.3.1 Changes in $\delta^{15}N$ and $\delta^{13}C$ of leaf tissue samples

Results showed that a significant enrichment of ^{15}N was detected in the leaf samples of all six species, however, no ^{13}C enrichment was detected. This is similar to findings in previous studies and is further explained in Section 4.3.2, but does not disprove intact organic N uptake. Regardless of the lack of $\delta^{13}C$ found, the foliar enrichment in $\delta^{15}N$ shows that the label from the injection of glycine was taken up by plants. The uptake of ^{15}N varies across species and within plant functional types and warrants an examination.

For the *Sphagnum* species, the increase in $\delta^{15}N$ in glycine treated samples was greatest, however so was the variation within the dataset. The standard error within the glycine treated samples for the *Sphagnum* species is much higher than for the control species, which is due to the large variability in the $\delta^{15}N$ of the glycine treated moss samples. Within these samples the value of $\delta^{15}N$ ranges from 0.26‰ to what I suspect is an outlier at 677.86‰. This large variability in $\delta^{15}N$ could be a result of the spatial variability in the ^{15}N injection, which was done with a sideport needle at eight points within the 50 x 20 cm plot rather than spread evenly among the surface (for example by using a watering can). However a needle was necessary to ensure the targeted rooting

zone of 0-20cm was being supplied with the glycine treatment. As mosses do not have roots and acquire water laterally from the surface it is possible that moss capitula were collected from areas in between injection sites where access to the glycine solution was spatially out of reach.

For the three shrub species that were examined, *V. myrtilloides* had the highest ^{15}N enrichment and variability in the glycine treated samples whereas *C. calyculata* had the lowest enrichment and also the smallest standard error. The enrichment in ^{15}N by *C. calyculata* closely followed expectations with respect to the uptake seen by *V. myrtillus* by Nasholm *et al*. (1998). *L. groenlandicum* fell in between these two extremes. One reason for the difference in ^{15}N label recovered by *C. calyculata* and *V. myrtilloides* is the degree of mycorrhizal infection. It has been shown that a higher degree of infection of mycorrhizal fungi in plant roots result in a larger and wider range of organic N uptake for *Vaccinium* (Stribley and Read, 1980). Despite glycine being the only organic N tracer applied in my experimental study, perhaps the difference in uptake and variability in $\delta^{15}N$ across the shrub species may have been caused by differences in mycorrhizal infection. This would require a more in-depth analysis of degree and type of mycorrhizal infection of the shrubs at Mer Bleue. A preliminary look at ^{15}N abundances show that *V. myrtilloides* has less depleted signatures than *C. calyculata* and *L. groenlandicum,* which is consistent for an ectomycorrhizal association as seen in Craine *et al.* (2009).

The only sedge species examined was *E. vaginatum* and foliar samples also showed a significant increase in the $\delta^{15}N$. It has been shown in a lab experiment that *E. vaginatum* can absorb intact glycine even though it is considered a non-mycorrhizal plant species (Chapin *et al.*, 1993). Later on, Kielland (1994) performed a lab experiment on

field grown plants and showed that *E. vaginatum* roots were able to absorb glycine as well as ammonium. The same study also revealed that deciduous shrubs had the greatest uptake capacity for glycine, followed by evergreen shrubs and graminoids (Kielland, 1994). I have found a similar pattern where the deciduous shrub: *V. myrtilloides* showed greater uptake than the evergreen shrubs, *C. calyculata* and *L. groenlandicum* and the graminoid *E. vaginatum*. Kielland (1994) noted that deciduous shrubs tend to be ectomycorrhizal (Miller, 1982) and have the highest rates of amino acid absorption whereas the evergreen shrubs, with intermediate rates, have ericoid mycorrhizae.

Section 4.3.2 Explanation for a lack of ^{13}C enrichment in leaf tissue

My results show that there was no significant increase in the $\delta^{13}C$ of leaves of the plant species sampled while there was a significant increase in the $\delta^{15}N$ of leaves. Other studies have come across similar results. Lipson and Monson (1998) found that aboveground plant tissues, leaves and stem bases, were not significantly enriched with ^{13}C whereas roots were significantly enriched with ^{13}C and a significant increase in ^{15}N was seen in both the aboveground and belowground plant tissues. Schimel and Chapin (1996) detected no ^{13}C in any of the plant material of *E. vaginatum* while ^{15}N could be detected in the shoots and roots of the plant, with 80% of the ^{15}N recovered in the roots after a 24 h sampling period. The isotopic label in these two studies is quite different. Lipson and Monson (1998) used a ^{13}C-[2]-glycine meaning that only the methylene carbon atom of glycine was labeled. Schimel and Chapin (1996) used a ^{13}C-[1]-glycine where only the carboxyl carbon atom of glycine was labeled, which Lipson and Monson (1998) point out as being "more readily respired and lost from plant tissues" than the

methylene carbon (p.413). In the present study, I used yet another label of glycine where the label is applied to both carbon atoms in glycine, with the formula $H_2{}^{15}N{}^{13}CH_2{}^{13}CO_2H$ (Sigma Aldritch, 489522-CONF), which has been used in more recent organic N uptake studies (Nasholm *et al*., 1998; Mcfarland *et al*., 2002). Nasholm *et al*. (1998) similarly show ^{13}C and ^{15}N increases in plant roots, but only report the ^{15}N values for the shoots of the species examined. In their study they used an excess ^{13}C to excess ^{15}N ratio of 2:1 to represent intact glycine uptake as they used glycine labeled at both C positions. However, they also mention that the loss of the carboxyl group could lead to the underestimation of organic N uptake as well as possibly be responsible for differences in organic N uptake between species, if they have different rates of decarboxylation (Nasholm *et al*., 1998).

A lack of ^{13}C enrichment in leaves also rules out the incorporation of ^{13}C in leaves via photosynthesis. Rasmussen and Kuzyakov (2009) suggested that enrichment via $^{13}CO_2$ was a possible and likely pathway to explain the findings from another study (Harrison *et al.*, 2008) whereby higher ^{13}C than ^{15}N enrichment was found in the shoots of grasses after a phenylalanine tracer. A more reasonable explanation, which was not speculated by Rasmussen and Kuzyakov (2009), is that phenylalanine is a larger amino acid with a C:N of 9:1 than glycine and serine, which were also examined, and therefore could produce the stronger ^{13}C enrichment that was found.

Section 4.3.3 Changes in $\delta^{15}N$ and $\delta^{13}C$ in root tissue samples

Uptake of ^{15}N was significant for both sedge and shrub roots and the magnitude of enrichment was greater than seen in the leaf samples. Contrary to leaf tissue analysis, there was an enrichment of ^{13}C in roots. Root samples were collected for the sedge *E.*

vaginatum and for shrubs, where shrub species are mixed as it was not possible to tell them apart by the naked eye. Significance was only shown for the change in $\delta^{13}C$ between control and glycine treated shrub roots. As the roots of shrubs could not be distinguished into individual species, no comparison can be made in differences of uptake between the deciduous shrub *V. myrtilloides* and evergreen shrubs *C. calyculata* and *L. groenlandicum*.

Despite previous studies showing that *E. vaginatum* can uptake glycine in an intact form, my findings could not provide the evidence of this ability. However both Chapin *et al.* (1993) and Kielland (1994) performed controlled experiments in a lab setting. The variability in the glycine treated samples was larger than in the control dataset with some individual samples showing depleted ^{13}C and enriched ^{13}C. Any future research would have to consider the methodological difficulties with using a control dataset that is not derived from the same plant that was given a pulse label. Another factor involved may be the inability to collect the finest roots of *E. vaginatum* within the first 20 cm depth from the peat surface. Murphy and Moore (2010) reported that the bulk of fine root production for *E. vaginatum* occurs at 40 cm depth and it may be that the roots I was able to collect at 20 cm were acting as transport roots.

Shrub roots showed both a significant enrichment for ^{13}C and ^{15}N. The observed increase in ^{13}C in the plant roots shows that the C from glycine was taken up by shrub roots. This supports the literature that shrubs should be able to take up more organic N over moss and sedge because it has advantageous mycorrhizal associations. The ability of shrubs to take up organic N as amino acids may supplement their N nutrition at Mer Bleue peatland where inorganic N content is low. How much amino acid was recovered

and what percent of plant N is supplied by organic N cannot be identified from this study and would require further study.

Section 4.3.4 Using atom percent enrichment and excess ^{15}N and ^{13}C to determine intact uptake.

Atom percent enrichment (APE) was calculated from the atom percent of the heavy isotope of each sample compared to the control average as was used in McFarland *et al*. (2010). As with excess, results showed that foliar ^{15}N APE was positive for glycine-treated samples whereas the APE for ^{13}C was close to zero. The root samples reported a positive APE for both ^{15}N and ^{13}C, however, the ^{15}N APE was often an order of magnitude greater than ^{13}C APE for both shrub and sedge species. The positive APE values for ^{15}N and ^{13}C in the root samples show that plants may have the ability to take up glycine in an intact form, however, the much larger APE of ^{15}N suggests most of the uptake of the pulse added was in a mineralized form.

Excess ^{13}C was plotted against excess ^{15}N to determine if the change in heavy isotopes in the plant tissue resembled the glycine C:N ratio of 2:1. Nasholm *et al*. (1998) stated that if the excess plots resembled a C:N ratio of 2:1 then glycine was taken up in an intact form. Similarly, Nasholm *et al*. (2000) was able to use the regressions of his excess plots to propose the percent of labeled glycine-N that was taken up in an intact form. It was my intention to replicate such a methodology however I was unable to do so due to the fact that calculations returned obscure values. The analysis did not reveal a 2:1 ratio of excess ^{13}C against ^{15}N for my samples and instead often showed negative values of excess. Such a result is hard to explain as negative excess values were found even when

a significant positive change in the delta had occurred. One possibility for this is that the variability in the control samples that were averaged to make a control value hid the small changes in $\delta^{13}C$ and $\delta^{15}N$. Another explanation could arise from changes in the N content of the samples, however no significant differences were detected in the N content of control and glycine-treated samples. My thoughts are that a combination of using an average control value instead of a time-0 control value and slight changes in N content may have produced negative excess values. For example, a quick test revealed that the positive excess seen from a 5‰ increase in $\delta^{15}N$ can be cancelled out by a ~0.0084 decrease in percent of N content in a sample. This problem may be overcome with strengthening the experimental methodology which is discussed below.

Section 4.3.5 Differences in uptake between plant functional types and microtopographic forms

The second objective of this study was to determine if there was any difference in uptake of organic N between plant functional types. In our study, no uptake of ^{13}C was detected by the plant functional types of moss and sedge while uptake of ^{15}N was found. This shows that moss and sedge roots may have only taken up the ^{15}N label from glycine in a mineralized form after it was metabolized by soil microbes. Uptake of glycine in an intact form was found for the roots of mixed shrub species. Therefore shrubs had greater uptake of organic N over sedge and moss plant functional types, which did not show uptake in an intact form.

As plots were evenly split among hummocks and lawns an analysis was done in order to discern if there were any differential patterns in glycine uptake among

microtopographic forms. From the natural abundances it looked like there may be N niche differentiation between hummock and lawns, however no pattern was seen among foliar samples of excess ^{13}C to excess ^{15}N, not surprisingly as no pattern of intact uptake was found either. Where uptake was detected with shrub roots, hummock samples tended toward a slightly higher excess ^{13}C enrichment than lawn plots. Among root samples for the sedge *E. vaginatum*, too few samples were collected from hummock plots to notice a pattern.

Section 4.4 Strengthening experimental design and future research

One of the most significant findings from doing this masters thesis is that nature is variable and it is important to develop a strong experimental methodology that can transcend the "noise" to understand biogeochemical patterns. Now that this thesis is near completion I can analyze methodological weaknesses and propose solutions for future research. First and foremost is improving the tracer experiment design. Some aspects of my experiment could not overcome the natural variability of the Mer Bleue ecosystem. In a new experiment I would change the establishment of a baseline to a time-0 sampling within the same plant while still maintaining control and treated sub-samples. A t-0 sample collection would reduce the inter-plant variability I saw within control datasets by allowing me to see the change within an individual plant rather than trying to make a comparison across groups of plants that can be affected by a wide range of different factors. In a similar vein it would be best to design the experiment so that root samples and leaf samples are taken from the same individual plants that have access to absorb the injected amino acid. In the case of *Sphagnum* mosses in my study, it is my belief that

using a needle to inject the labeled organic N in the peat surface only allowed the capitula near the injection points access to the label. This was a problem for the random sampling I later did within the whole plot area as I was collecting moss capitula in between the injection points where the needle was inserted. A better way to ensure moss species can access the label would be to collar a smaller area of moss and ensure the injection of label can diffuse within the collar. A quick lab test can be done to determine the diffusion rate within peat mosses at Mer Bleue. Krab *et al*. (2008) performed a lab and field experiment on arctic mosses whereby they injected the label halfway down a collar at about the transition from green to brown parts, an area the authors believed important to natural amino acids production as they are the result of organic matter breakdown. Their results showed that 10 of 11 arctic mosses were capable of taking up glycine and aspartic acid.

When it comes to future research to better understand organic N uptake at Mer Bleue bog there are research opportunities to complement the findings from this thesis. I think the main area of research would be to single out individual plants to sample and perform the experiment again to collect a more dependable and unvarying dataset but also to determine the type and level of mycorrhizal association a plant may have. The findings from my work have shown that mycorrhizae may be the main factor in the ability to take up organic N for bog plants.

Lastly, understanding the role of the microbial biomass in the turnover of amino acids and competition for amino acid uptake with plants is another research opportunity beyond this masters thesis. Research on microbial competition with plants for amino acid N exists and despite being too large in time and scope for my master's thesis the

following proposal has been written to allow others the opportunity to undertake such a research project at Mer Bleue.

Future research can be carried out to determine the amount of ^{15}N and ^{13}C that can be recovered in the soil microbial biomass. Such an undertaking was performed by Nordin *et al*. (2004) and Lipson and Monson (1998) in arctic and alpine tundra respectively. However, the ratios of amino acid recovered in the soil microbial biomass compared to plants that each study found were inconsistent. Lipson and Monson (1998) reported values were 5.0% and 3.5% for microbes and plants respectively, however it was also stated that the bulk of the ^{15}N and ^{13}C from the added glycine was found in the soil, none in the water-extractable portion and only a small fraction in the microbial biomass. Nordin *et al*. (2004) found that 40-49% of the experimental N was found in the soil microbial biomass with less than 1% recovered in plants which was more consistent with Schimel and Chapin's (1996) study whose results showed the microbial biomass contained 41.3-60.8% of the added ^{15}N labeled glycine after a 5-day incubation, while plants contained 1.0-12.1%.

Lipson and Monson (1998) and Nordin *et al*. (2004) both followed the same method of chloroform fumigation-extraction as described by Brookes *et al*. (1985) with a modification introduced by Lipson and Monson (1998) whereby water instead of K_2SO_4 was used in extraction to avoid a salty residue left behind after evaporation which interferes with stable isotope analysis. The method can be used to extract both C and N though Lipson and Monson (1998) found that using water led to a 15-20% drop in extractability of N (no difference for C extractability) whereby they adjusted for this by using a lower K_{en} factor than used by Brookes *et al*. (1985).

Following a tracer field experiment where distilled water and glycine are applied to control and experimental plots respectively soil samples would be cored to a depth of 20 cm from both control and glycine treated plots and kept cool until brought to the lab. Bryophytes and dead plant material would be removed first by hand and then by passing the soil through a 2 mm sieve. Separate samples from each plot would be kept for ^{13}C and ^{15}N analysis. The subsamples should not be less than 12 g following Nordin *et al*. (2004). Furthermore, control subsamples can undergo a persulfate digestion to compare the C and N results to the chloroform-water extracted subsamples to ensure reasonable results from replacing K_2SO_4 with water in extraction. The soil subsamples will be fumigated with alcohol-free chloroform ($CHCl_3$) for 24 h then extracted with water without removing the fumigant vapour. The extractants can then be stored at 4°C until sent for analysis of ^{13}C and ^{15}N at the GEOTOP stable isotope lab, UQAM. Analysis of ^{13}C and ^{15}N values of soil microbes would be calculated as the difference between the control and label-injected soil samples

Section 4.5 Conclusions

My research was carried out in order to determine if the plants of Mer Bleue are able to utilize organic N, as NH_4 and NO_3 are limited in the environment. In conclusion, all plants took up ^{15}N from the labeled organic N, but only shrubs showed uptake of both ^{15}N and ^{13}C. Detection of ^{15}N alone would imply that glycine was mineralized prior to plant uptake but the presence of increased ^{13}C in the roots of shrub species points to the uptake of intact glycine. Therefore the shrubs at Mer Bleue bog are able to compete with microbes and access the organic N pool and supplement the established mineral N nutrition. Ericaceous shrub roots can form a symbiotic relationship with mycorrhizal fungi, thus providing shrubs with increased competitiveness for nutrient acquisition. Some mycorrhizae are able to produce enzymes that breakdown large organic matter polymers and are able to absorb a wide range of organic N forms. Intact uptake was not detected for *Sphagnum* moss or the sedge *E. vaginatum*, both of which are considered non-mycorrhizal. However further experimentation that is tailored specifically to moss and sedge plants is needed in order to reduce the variability or noise seen in the data collected and to obtain conclusive results. There are many opportunities for future research to advance the understanding of organic N cycling at Mer Bleue. Projects should be undertaken to examine the cycling of amino acids through the peat and microbial biomass at Mer Bleue as well as quantify the proportion of plant N that is supplied by organic N uptake.

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Appendix 1: N/C content (%) and $\delta^{15}N$ and $\delta^{13}C$ (‰) for plant tissue samples

	Control					Glycine-treated			
	N	C	$\delta^{15}N$	$\delta^{13}C$		N	C	$\delta^{15}N$	$\delta^{13}C$
	E. vaginatum								
Plot #					Plot #				
1	1.78	47.48	0.433	-27.45	3	1.71	46.17	4.20	-27.93
2					4	1.65	47.14	1.04	-26.61
5	1.95	45.16	2.164	-27.33	7	1.85	45.97	2.28	-27.37
6	1.61	46.20	0.312	-27.31	8				
10	1.83	46.71	0.836	-27.58	9	1.69	47.10	3.99	-27.50
11	1.56	47.10	1.702	-27.45	12	1.84	47.61	3.50	-26.52
14	1.51	45.00	1.522	-26.89	13	1.61	47.10	4.95	-27.65
15	1.57	46.33	2.439	-28.10	16	1.80	46.02	2.83	-29.04
Mean	1.69	46.28	1.34	-27.45		1.74	46.73	3.26	-27.52
	S. magellanicum								
1	0.66	43.01	-2.39		3	0.75	42.73	0.26	-30.19
2					4	0.85	43.41	31.98	-29.85
5	0.69	43.26	-3.21	-28.99	7	0.72	42.40	101.11	-30.88
6	0.80	43.49	-1.86	-28.91	8	0.64	42.24	677.86	-23.76
10	0.71	43.11	-2.83	-29.95	9	0.67	42.84	44.15	-29.42
11	0.69	43.22	-2.19	-30.68	12	0.78	42.87	-2.36	-30.60
14			-1.97	-30.53	13	0.60	42.23	273.34	-28.42
15	0.59	43.39	-2.52	-28.56	16	0.65	43.25	-1.58	-28.84
Mean	0.69	43.25	-2.42	-29.61		0.71	42.75	140.60	-29.00
	S. capillifolium								
1	0.66	43.10	-2.07	-30.82	3	0.73	42.15	-2.87	-30.82
2	0.70	43.24	-3.11	-30.68	4	0.79	41.87	84.02	-30.68
5	0.68	42.36	-2.69	-30.01	7	0.73	42.36	8.03	-30.01
6	0.84	42.69	-3.44	-30.64	8	0.63	42.50	96.61	-30.64
10	0.82	42.17	-2.62	-31.53	9	0.65	42.66	-3.14	-31.53
11	0.74	42.19	-2.91	-31.06	12	0.71	42.54	16.04	-31.06

14			-2.87	-31.34	13	0.84	41.65	105.35	-31.34
15	0.68	42.54	-2.08	-28.19	16	0.60	42.60	14.17	-28.19
Mean	0.73	42.61	-2.72	-30.53		0.71	42.29	39.78	-30.53

V. myrtilloides

1	1.12	49.33	-4.18	-29.11	3	1.35	48.36	10.27	-30.21
2					4				
5					7	1.04	47.98	39.09	-31.02
6	1.13	49.30	-5.90	-32.24	8	1.23	48.97	-4.90	-32.10
10	1.36	48.03	-3.90	-30.93	9	1.32	47.25	159.25	-31.76
11	1.27	49.22	-5.04	-31.13	12	1.45	48.08	0.27	-31.58
14	1.02	48.30	-5.91	-31.30	13	1.04	48.67	2.93	-31.19
15					16	1.42	49.15	-3.18	-31.10
Mean	1.18	48.84	-4.99	-30.94		1.26	48.35	29.10	-31.28

C. calyculata

1	1.20	51.57	-4.62	-30.14	3	1.21	54.06	0.49	-29.11
2	1.12	53.24	-5.89	-29.38	4	1.48	52.36	8.92	-29.05
5	1.31	52.10	-6.08	-29.57	7	1.33	52.23	-0.01	-29.14
6	1.26	52.73	-5.34	-29.55	8	1.11	54.40	3.02	-29.22
10	1.26	53.88	-0.69	-29.22	9	1.27	53.46	0.73	-30.60
11	1.24	51.44	-0.71	-29.15	12	1.11	51.49	-1.51	-27.89
14	1.31	51.00	-5.70	-29.97	13				
15					16	1.13	51.83	2.83	-29.73
Mean	1.24	52.28	-4.15	-29.57		1.24	52.83	2.07	-29.25

L. groenlandicum

1	1.69	53.69	-7.26	-29.25	3				
2	1.39	55.19	-8.18	-28.10	4	1.74	52.87	36.18	-29.48
5	1.63	53.85	-6.60	-28.48	7	1.34	52.62	7.29	-29.09
6	1.30	53.36	-7.67	-27.98	8	1.55	53.38	0.00	-29.36
10	1.55	54.20	-7.36	-29.49	9	1.36	53.04	-5.22	-29.51
11	1.55	53.57	-6.46	-28.57	12	1.48	54.40	29.72	-28.56
14	1.29	54.72	-6.19	-30.25	13	1.35	54.13	5.43	-27.90
15	1.79	52.47	-5.35	-28.77	16	1.39	53.63	1.21	-28.86
Mean	1.52	53.88	-6.88	-28.86		1.46	53.44	10.66	-28.97

Sedge Root

1	0.48	47.78	1.47	-28.54	3	0.43	46.68	73.63	-26.70
2	1.36	45.72	0.65	-29.35	4	0.46	47.69	8.45	-19.10
5	0.70	45.61	3.42	-25.42	7	0.33	47.63	92.60	-26.10
6	0.43	47.08	1.54	-28.99	8				
10	0.35	46.78	2.94	-26.77	9	0.32	46.40	55.13	-25.18
11	0.54	47.42			12	0.44	46.54	3.68	-28.09
14	0.77	45.62	1.96	-27.38	13				
15	0.54	46.26	2.25	-27.43	16	0.56	44.73	9.53	-27.96
Mean	0.65	46.53	2.03	-27.70		0.42	46.61	40.50	-25.52

Shrub Root

1	0.78	48.26	-4.73	-28.17	3	0.84	47.87	228.02	-27.85
2	0.60	49.30	-5.55	-28.47	4	1.01	48.07	194.96	-27.77
5	0.64	49.74	-4.32	-28.05	7	0.67	49.61	147.96	-27.68
6	0.66	49.69	-4.32	-27.98	8	0.71	49.14	39.89	-27.97
10	0.82	49.02	-3.16	-27.62	9	0.69	48.40	220.71	-27.65
11	0.64	49.55	-3.71	-27.83	12	1.02	50.22	196.04	-27.50
14	0.68	48.38	-2.79	-28.73	13	0.92	49.23	85.52	-28.24
15	1.00	49.65	-1.17	-28.37	16	0.79	49.28	83.03	-28.07
Mean	0.73	49.20	-3.72	-28.15		0.83	48.98	149.52	-27.84