The effect of nitrogen fertilization on the physiology and morphology of *Sphagnum capillifolium* in an ombrotrophic bog

by

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Abstract

Degraded peatlands release 100-200 g-CO_2 eqv. m^{-2} yr^{-1} in net emissions and account for more than 10% of global CO_2 emissions. The success of bog restoration is dependent on creating suitable moisture conditions for the donor material to establish, propagate, and develop a new layer of *Sphagnum* that has hydrophysical and water retention properties similar to natural peatlands. Techniques to improve moisture retention during the transplant process and increase water holding capability of the restored *Sphagnum* layer have been identified as an area of bog restoration that requires more research. Samples were collected from plots fertilized with six different nitrogen treatments at Mer Bleue Bog in Ottawa, Canada. Net CO_2 assimilation, fresh weight, dry weight, water content, and dissolved nutrient measurements were made to determine the potential effectiveness of incorporating nitrogen fertilization into the *North American approach* to peatland restoration. High levels of nitrogen fertilization exerted deleterious effects on individual morphology, growth density, water holding and retention capacity, CO_2 assimilation, and nutrient dynamics and decomposition. Fertilization with 1.6 g m^{-2} yr^{-1} of ammonium has the potential to ameliorate water retention capacity through more robust individual morphology and denser growth patterns and increases carbon assimilation and photosynthetic capacity. The results indicate integrating low levels of ammonium fertilization into bog restoration techniques can potentially increase restoration success.

Keywords: water content; carbon dioxide assimilation; growth density; peatland restoration; ammonium; nitrate

Dedication

To Pat, thank you for attentively listening to me talk about nitrogen fertilization and *Sphagnum* mosses for the past 8 months. Words cannot express how much I appreciate all the support, motivation, belief, advice, work breaks, smiles, and laughter you have provided me throughout this process. I am so incredibly lucky to have you in my life.

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Chapter 1. Introduction and Background

1.1. Peatlands, Ombrotrophic Bogs, and *Sphagnum* moss

Peatlands are organic wetlands that are characterized by the accumulation of over 40 cm of peat and are classified into bog-, fen-, or swamp-type peatlands based on hydrology, chemistry, geomorphology, and biota (National Wetlands Working Group 1997). This paper will focus on ombrotrophic bogs, though peatlands as a whole will be addressed particularly with regards to carbon storage and climate change. Ombrotrophic bogs are peatlands that have a surface that is isolated from groundwater due to peat accumulation, and receive water and nutrient inputs exclusively from precipitation and dry atmospheric deposition (Rydin and Jeglum 2013). These ecosystems are unique wetland types that are generally characterized by bryophyte-dominated vegetation communities and waterlogged, anoxic, acidic, and nutrient-poor biochemical conditions (Tomassen et al. 2004, Bragazza et al. 2004, Knorr et al. 2015).

Sphagnum mosses are the dominant and keystone vegetation genus in ombrotrophic bogs (Rochefort 2000, Rydin and Jeglum 2013). *Sphagnum* species have co-evolved within these ecosystems for millennia to develop physiological, morphological, and organo-chemical adaptations that allow this genus to substantially influence many biological, chemical, and physical properties and ecosystem functions (Van Breemen 1995, Weston et al. 2017). *Sphagnum* are poikilohydric bryophytes that grow in dense carpets of vertical shoots and are spatially distributed across environmental gradients influenced by surface moisture, water chemistry, water table depth, and pH (Titus and Wagner 1984, Hájková and Hájek 2007, Rydin and Jeglum 2013, Weston et al. 2015). During the development of ombrotrophic bogs a microtopographical mosaic of raised hummocks, expansive lawns, and wetter hollow depressions emerges (Titus et al. 1983, Hájek and Vicherová 2014). These microtopographic features and associated water-availability gradients induce plasticity in morphological and physiological functional traits, thus regulating the distribution of species and the role played in ecosystem function (Rydin 1993, Rice et al. 2008, Weston et al. 2015) In general *Sphagnum* species create and maintain the environmental conditions that allow the genus to dominate bogs and outcompete other vegetation species through three main mechanisms: 1) a strong cation-exchange capacity (CEC), 2) an intrinsic decomposition resistance, and 3) a large water-holding capacity.

Sphagnum have unique cell morphology and tissue chemistry whereby polyuronic acids are produced and integrated into the structure of the cell walls (Clymo 1964, Van Breemen 1995, Verhoeven and Liefveld 1997). These cell-wall polysaccharides form a pectin-like polymer known as Sphagnan that can account for 10-30% of the dry mass of *Sphagnum* tissue (Clymo 1963, Stalheim et al. 2009). The Sphagnan polymers are mainly comprised of galacturonic acid and 5-keto-D-mannuronic acid, which are polysaccharides that have a carboxyl group in place of the CH_2OH side chain (Brown and Wells 1990, Van Breemen 1995, Verhoeven and Liefveld 1997). The carboxyl group facilitates the enhanced exchange of protons for monovalent and bivalent cations, which provides *Sphagnum* with a strong CEC (Clymo 1973, Barkman 1992, Van Breemen 1995). This enhanced CEC allows for efficient nutrient acquisition in nutrient-poor ombrotrophic bogs (Rydin and Jeglum 2013). Additionally, the associated proton exchange acidifies the water in the rhizosphere, thus creating unfavourable chemical conditions for vascular vegetation and reducing the availability of nutritional cations for microorganisms and other plant species (Clymo 1964, Stalheim et al. 2009). The excretion of phenolic and uronic compounds by living *Sphagnum* tissue further contributes to the acidification of the bog environment, which limits microbial decomposition of organic material in the acrotelm (Clymo 1964, Painter 1991, Hájek et al. 2011).

Sphagnum do not have cuticles or stomata and therefore cannot actively regulate water uptake and loss (Titus et al. 1983, Hájek and Vicherová 2014). Interspecific variation in capillarity and water-storage capacity traits is significantly associated with the hummock-hollow microtopographic mosaic. However, water transport and water holding capacity mechanisms in *Sphagnum* are regulated by three main morphological adaptations – pendant and spreading branch growth patterns, leaf size and density, and large hyaline cells with thick walls (Figure 1; Rydin and Jeglum 2013, Weston et al. 2017). Water is transported horizontally and vertically via capillary action along the exterior of *Sphagnum* stems (Proctor 1982, Rydin 1993). The horizontal spreading of stem branches facilitates lateral capillary action, while dense pendant branches overlap and grow tight to the shoot stems forming a vertical water-transport structure (Hayward and Clymo 1982, Weston et al. 2017). Therefore, morphological adaptations regulating growth density at the individual and community levels exert significant control on capillarity and water transport within the living surface of ombrotrophic bogs (Thompson and Waddington 2008, Strack and Price 2009). The capillary matrix of stems, branches, and leaves combined with specialized hyaline cells allows *Sphagnum* to effectively trap and store water from precipitation and transport water from the water table via capillary action (Hayward and Clymo 1982, Rice et al. 2008). Hyaline cells are specialized water-holding cells found in the stems and branches of *Sphagnum* individuals that are a morphological adaptation that aids in the ability of *Sphagnum* to buffer evaporation and store water (Hajek and Beckett 2008, Rydin and Jeglum 2013). The phenolic acids produced by *Sphagnum* create polymeric networks that bind cellulose in cell walls providing hyaline cell walls a rigid structure that allows for a large water-holding capacity (Verhoeven and Liefveld 1997). The combination of these mechanisms and adaptations allows *Sphagnum* to passively maintain the moisture conditions required for vital metabolic and physiological functions (e.g., photosynthesis) while also contributing to waterlogged and anoxic conditions in bogs that limit decomposition and promote peat accumulation (Rice and Giles 1996, Rydin and Jeglum 2013, Weston et al. 2015).

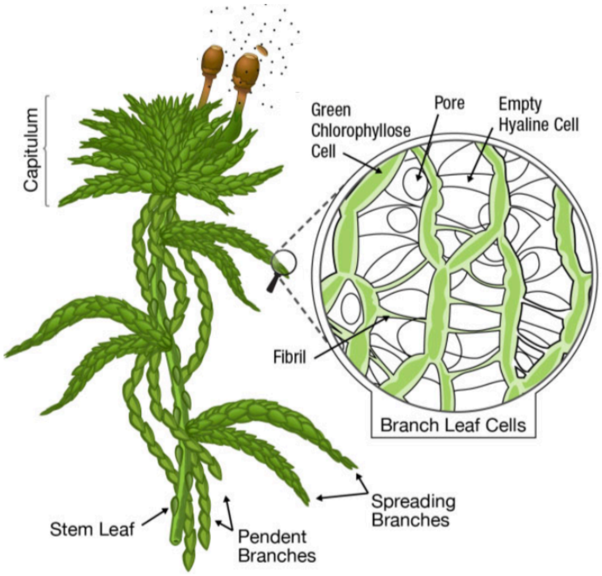

Figure 1. Schematic of external and cellular anatomy and morhpology of an individual *Sphagnum* plant. The pendent and spreading branches and hyaline cells are key morphological adaptations contributing to efficient water retention and transport. Adapted from (Weston et al. 2015).

The acidic and waterlogged conditions reduce decomposition rates in ombrotrophic bogs; however, *Sphagnum* tissue also exhibits an intrinsic resistance to decay associated with an abundance of secondary metabolite (i.e., phenolic compounds) and structural carbohydrate (i.e., cell-wall polysaccharides) organo-chemicals (Clymo 1965, Verhoeven and Liefveld 1997). Soluble phenolic compounds released by living *Sphagnum* tissue and produced during the breakdown of dead tissue in the aerobic acrotelm limit microbial decomposition; however, these phenolics exert limited influence on the carbon-mineralization rate of *Sphagnum* litter (Verhoeven and Liefveld 1997, Hájek et al. 2011). The recalcitrance of *Sphagnum* tissue and suppression of carbon mineralization is mainly attributed to the formation of three-dimensional networks of polymeric phenolic compounds bound to cell-wall polysaccharides and a hydrocarbon and fatty acid lipid coating on the cell-wall surface (Van der Heijden 1994, Van Breemen 1995, Hájek et al. 2011). These stable networks restrict the breakdown of cell-wall components, thus making the majority of cell-wall polysaccharides unavailable for microbial decomposition to such an extent that the bulk of cell-wall polysaccharides can still be present in deep peat layers up to 70,000 years old (Van der Heijden 1994, Verhoeven and Liefveld 1997). *Sphagnum* litter can account for up to 90% of the volume of peat composition in *Sphagnum*-dominated peatlands meaning that a substantial portion of the litter is inherently decomposition resistant, and therefore *Sphagnum* substantially influences many chemical and physical properties and ecosystem functions, including carbon storage and sequestration (Clymo 1963, Kuhry 1997, Clymo et al. 1998, Turetsky 2003).

1.2. Peatlands, Carbon Storage, and Climate Change

Northern hemisphere peatlands play a crucial role in the global carbon cycle. It is estimated that peatland ecosystems account for only 3% (~400 million ha) of the landscape area, but store 25% of the world's soil carbon (~400-600 Pg-C) and sequester approximately 12% of current anthropogenic emissions (Gorham 1991, Clymo et al. 1998, Frolking et al. 2011, Yu 2012). In Canada, peatlands encompass ~12% of the land surface area, store 147-154 Pg of soil carbon, and accumulate 100–400 kg-C ha^{-2} yr^{-1} (Roulet 2000, Bridgham et al. 2006, Frolking et al. 2011). Organic carbon stores are built up over thousands of years and partially-decomposed organic matter forms layers of peat that can be several metres thick, with high carbon densities of 50 to >500 kg-C m^{-2}

(Frolking et al. 2011, Rydin and Jeglum 2013). These vast reserves are vital on a global scale as long-term carbon sinks and storage sites, which is facilitated by a positive ratio of net primary production to ecosystem respiration (Moore 2002, Limpens et al. 2008). Northern peatlands are a significant carbon exchange interface between the organic carbon pool and the atmosphere, and these peatlands are potentially vulnerable to climate warming, changes in hydrological conditions, increased frequency and magnitude of natural disturbance regimes (e.g., wildfires), and anthropogenic impacts (Gorham 1991, Limpens et al. 2008, McGuire et al. 2009). Disturbances to the carbon pool cause increased decomposition due to microbial degradation of existing carbon stores, thus triggering significant impacts on the global climate system (Turetsky et al. 2008, Frolking et al. 2011).

Northern peatland processes impact the global carbon and climate (carbon-climate) systems primarily through the exchange of carbon dioxide (CO_2) and methane (CH_4) gases (Frolking et al. 2006, Frolking and Roulet 2007, Limpens et al. 2008). Undisturbed peatlands are generally net CO_2 sinks (<1000 kg-C ha^{-1} yr^{-1}) and net CH_4 sources (~0-100 kg-CH_4 ha^{-1} yr^{-1}), and both gas exchange processes are highly dependent on hydrologic regimes, vegetation composition, and temperature (Bartlett and Harriss 1993, Limpens et al. 2008, Frolking et al. 2011). On the millennia scale peatlands have been net carbon sinks (averaging 0.02–0.03 kg-C m^{-2} yr^{-1}) based on carbon budgets accounting for CO_2, CH_4, and dissolved organic carbon (DOC), and have contributed a net radiative cooling impact on global climate (Gorham 1991, Frolking and Roulet 2007, Yu 2012). On a shorter timescale, the radiative impact of northern peatlands continues to be a net cooling effect (driven by CH_4 fluxes over the last 50 years and the total organic carbon store in peat), and undisturbed peatlands are likely to continue to act as long-term carbon sinks (Moore et al. 1998, Frolking et al. 2006, Frolking and Roulet 2007). However, the persistence of these ecosystems as long-term carbon sinks becomes uncertain if environmental and anthropogenic impacts shift the carbon balance in peatlands. Therefore, the destabilization of the peat carbon pool and the production and oxidation of CH_4 are consequences of climate change, anthropogenic impacts, and altered disturbance regimes that could have globally significant impacts (Gorham 1991, Moore 2002, Limpens et al. 2008). The vast quantities of organic carbon stored in northern peatlands suggests that even slight changes in the carbon balance of these ecosystems could instigate carbon-climate feedback loops (Frolking et al. 2011, Turetsky et al. 2012, Weston et al. 2015).

Global climate models indicate that without a decrease in atmospheric CO_2 concentrations annual mean surface temperature in Canada is likely to increase by 1.8-3.2°C by 2100, with higher latitudes (40°N to 70°N) experiencing more rapid warming (Moore 2002, Intergovernmental Panel on Climate Change 2014, Environment Canada; Coupled Model Intercomparison Project Phase 5). There is significant variability in precipitation models, but predictions suggest that high latitudes are likely to experience an increase in annual mean precipitation of 7.1-11.6% by the end of the century with much of this increase occurring during the winter months resulting in warmer and drier summers and wetter winters (Moore et al. 1998, Moore 2002, Intergovernmental Panel on Climate Change 2014, Environment Canada; Coupled Model Intercomparison Project Phase 5). Over the next few centuries climatic warming may expand peatland formation northward, with a concomitant degradation of peatlands at lower latitudes (Gorham 1991). However, under these predicted climatic conditions it is unlikely that climate change will drive significant changes in the areal land cover of northern peatlands over the next 100 years because rates of terrestrialization, paludification, and disappearance are too slow to be globally significant (Frolking et al. 2011). As such, the impact of northern peatlands on the carbon-climate system will be the result of destabilization of the carbon pools due to direct climate change impacts, anthropogenic impacts, and indirect impacts via changes in hydrological conditions, vegetation communities, and natural disturbance regimes (Moore et al. 1998, Frolking et al. 2011, Turetsky et al. 2012). The combination of these factors will likely result in a portion of existing undisturbed peatlands exhibiting reduced carbon accumulation and sequestration and/or increased carbon loss. Over the next century northern peatlands will act as a small but continuous source of atmospheric carbon (an estimated net loss of 0.1 Pg-C yr^{-1}) punctuated by significant flux pulses in dry years (Frolking et al. 2011). However, on a global scale these impacts will be small relative to carbon loss and perturbations to the carbon balance in peatlands subjected to land use conversion and pollution loading (i.e., anthropogenic impacts) (Moore 2002, Frolking et al. 2006, 2011).

1.3. Loss and Restoration of Peatlands

Peatlands have historically been lost and degraded by numerous anthropogenic impacts such as peat harvesting for horticultural and energy use, agriculture, forestry, resource extraction, land use conversion (e.g., urban, industrial, and infrastructural use), and increased atmospheric pollution (Moore 2002, Howie et al. 2009, González and Rochefort 2014, Chimner et al. 2017). Since the early 19th century global peatlands have declined by ~50 Mha (10-15%), which accounts for a loss in soil carbon stock of 5.5-7.1 Pg-C, and it is likely that peat formation and accumulation has ceased in the majority of these converted peatlands (Maltby and Immirzi 1993, Bridgham et al. 2006, Frolking et al. 2011). The most common stressor is the disruption of the hydrologic regime through peatland drainage, usually using ditches (Rochefort 2000, Rochefort et al. 2003, Chimner et al. 2017). Drainage lowers the water table and exposes peat stores to oxidation and subsidence, alters the composition of vegetation communities, and increases the threat of burning (Price et al. 2003, Howie et al. 2009, Turetsky et al. 2011, Schimelpfenig et al. 2014). Drained peatlands experience elevated levels of decomposition due to increased heterotrophic respiration releasing CO_2 into the atmosphere while decreasing CH_4 emissions (Frolking et al. 2011). It is estimated that disturbed peatlands release 100-200 g-CO_2 eqv. m^{-2} yr^{-1} in net emissions and account for more than 10% of global CO_2 emissions, which likely surpasses the net uptake by existing undisturbed peatlands (Roulet 2000, Lunt et al. 2010, Frolking et al. 2011). Over the past few decades the restoration of disturbed peatlands has become increasingly prevalent due to the significant role these degraded ecosystems play in the global carbon-climate system.

An assortment of restoration techniques for a wide variety of disturbance and peatland types are well-documented. However, this paper will focus on the restoration of *Sphagnum*-dominated peatlands, specifically ombrotrophic bogs, that have been subject to peat harvesting/extraction or other land use conversion that has left exposed bare peat surfaces. Harvested, cutover, and mined bogs rarely recolonize with native vegetation or regenerate into functioning peat-accumulating ecosystems without active intervention (Rochefort 2000, Poulin et al. 2005, González and Rochefort 2014). As such, the main goal of restoring ombrotrophic bogs is to re-establish *Sphagnum*-

dominated vegetation cover and peat accumulating functions and processes, which can take decades (Rochefort et al. 2003, Lucchese et al. 2010, Chimner et al. 2017).

The successful restoration of degraded ombrotrophic bogs is dependent on restoring natural hydrologic conditions and water table levels through the blocking or filling of ditches (Rochefort et al. 2003, González and Rochefort 2014). Once suitable hydrologic conditions have been created the *North American approach* (also known as the moss layer transfer method) is used to re-establish *Sphagnum*-dominated vegetation communities and suitable microclimate, water-retention characteristics, and hydro-physical properties for peat accumulating ecosystem functions (Rochefort et al. 2003, Schimelpfenig et al. 2014). The *North American approach* has four main steps:

1) collect the top 5-10 cm of living vegetation at a 1:10 ratio (area collected-to-area to be restored) from healthy donor sites as a source of *Sphagnum* diaspores,

2) spread the donor material over the restoration area in a 1-2 cm thick layer,

3) cover the donor material with straw mulch at a density of 3000 kg ha^{-1}, and

4) Fertilize donor material with granular phosphate at a rate of 150-250 kg ha^{-1} (Rochefort et al. 2003, Sottocornola et al. 2007).

Long-term monitoring programs have been implemented in restored peatlands in eastern Canada, and monitoring data indicates that in ~60% of sites the *North American approach* has successfully re-established *Sphagnum*-dominated vegetation communities within 10 years of restoration (Poulin et al. 2013, González and Rochefort 2014). Peatland restoration plays an important role in reducing peat susceptibility to burning and mitigating carbon fluxes and loss from previously degraded ecosystems (Granath et al. 2016, Chimner et al. 2017). The restoration of ombrotrophic bogs can be made more effective by incorporating scientific knowledge and approaches into restoration research, particularly in the fields of individual, community, and ecosystem ecology, which form the basis for the functions and processes that influence the re-establishment of peat accumulating ecosystems.

1.4. Ecology to Inform Restoration

Over the last 30-plus years the field of ecological restoration has shifted from relying heavily on anecdotal observations and naturalist knowledge to implementing a complimentary approach of synthesizing knowledge of practical restoration and theoretical ecology to promote the establishment of a scientific framework for ecological restoration (Palmer et al. 1997, Török and Helm 2017, Wainwright et al. 2017). In practice, however, theoretical ecology and ecological restoration often remain separated in applied research – ecological theories are not applied to the development of restoration solutions and restoration practitioners rely on logistic and socioeconomic knowledge (Török and Helm 2017, Wainwright et al. 2017). There is strong evidence that when properly integrated both fields have experienced mutualistic benefits of this complimentary approach. Implementing ecological restoration projects within a rigorous scientific framework allows conceptual ecological theories to be empirically tested in natural ecosystems, while restoration treatments that are designed and implemented based on ecological science are more likely to produce replicable data and successful results than “trial-and-error” restoration (Giardina et al. 2007, Wainwright et al. 2017).

Community ecology theory is one of the core ecological concepts most commonly applied, and most relevant, to ecological restoration because it allows for a fundamental understanding of the ecological functions and processes in different ecosystems that influence and support the diversity of, and interactions within, ecological communities that are often the focus of restoration efforts (Palmer et al. 2016, Wainwright et al. 2017). This has led to an increased focus on functional restoration endpoints based on the ecosystem processes, functions, and linkages that contribute to a resilient and self-supporting ecosystem (Palmer et al. 1997, Török and Helm 2017). The integration of community ecology theory into restoration design is vital for restoring ecosystems that are stable and resilient to natural disturbance regimes and future environmental and climatic change (Palmer et al. 2016, Török and Helm 2017). Population-level ecology and individual physiology and morphology become relevant to ecosystem ecological theory and restoration in the case of species that exert a disproportionate influence on functions, processes, and the physical, chemical, and biological properties of the ecosystem, as is the case with *Sphagnum* species (Palmer et al. 1997).

Ombrotrophic bogs cover the most area, store the most organic carbon, and have the greatest total storage capacity of all peatland types in the northern hemisphere (Armentano and Menges 1986, Verhoeven and Liefveld 1997). Therefore, the ecological conditions, processes, and functions of bogs exert a significant role on the global carbon-climate system. In *Sphagnum*-dominated bogs, the CEC, intrinsic decomposition resistance, and water-holding capacity of the genus combine to instigate and perpetuate a positive feedback loop that creates the waterlogged, anoxic, acidic, and nutrient-poor conditions that promote organic carbon accumulation, sequestration, and storage (Van Breemen 1995, Verhoeven and Liefveld 1997, Weston et al. 2015). Additionally, *Sphagnum*-microbial associations are vital to bog ecosystem functions, most importantly carbon and nitrogen cycling (Turetsky 2003, Lindo et al. 2013). *Sphagnum* species are the dominant land cover of more than 1.5 million km^2 in the northern hemisphere, up to 90% of the peat volume in ombrotrophic bogs consists of *Sphagnum* litter, and it is estimated that in total the genus stores ~150 Gt of carbon (Kuhry 1997, Turetsky 2003, Rydin and Jeglum 2013). The role of *Sphagnum* is so vital that researchers have suggested that *Sphagnum* stores more carbon (in both dead and living tissue) and has the greatest impact on global carbon fluxes, and by extension climate, than any other genus of plant (Hayward and Clymo 1982, Van Breemen 1995). As such, research that contributes to the understanding of how fundamental *Sphagnum* traits, adaptations, mechanisms, and trade-offs impact important ecosystem functions (e.g., peat accumulation and carbon storage) provides valuable information and data to increase the understanding of how these ecosystems function and how they might change in response to different environmental variables. This information and data can be accessed and applied by restoration practitioners to inform their restoration activities.

Building this repository of ecological knowledge is especially important, as the changing environmental and climatic conditions will necessitate increased ecological resilience to maintain basic ecological functions in peatlands. Holling defines ecological resilience as the magnitude of disturbance that can be sustained before a change in system control or structure occurs (Holling 1996). In terms of restored bogs, this means the magnitude of disturbance that can be sustained before the ecosystem shifts from a net peat and carbon accumulating system to a net carbon producing system. Therefore, research that contributes knowledge pertaining to the role ecosystem functions and processes play in ecological resilience, and the role *Sphagnum* plays in influencing those functions and processes, will be vital to the success of restoration efforts in ombrotrophic bogs. The success of bog restoration is dependent on creating suitable moisture conditions for the donor material to establish, propagate, and develop a new layer of *Sphagnum* that has hydrophysical and water retention properties similar to natural peatlands (e.g., water table <40 cm below the surface, soil moisture >50%, and soil water pressure >-100 mb), a process that can take decades (Price and Whitehead 2001, Schimelpfenig et al. 2014, González and Rochefort 2014, McCarter and Price 2015, Taylor and Price 2015). Techniques to improve moisture retention during the transplant process and increase water holding capability of the restored *Sphagnum* layer have been identified as an area of bog restoration that requires more research (Chimner et al. 2017). As has been discussed above, fundamental *Sphagnum* traits, adaptations, mechanisms, and trade-offs exert great influence on these properties. Therefore, research that investigates the morphological and physiological adaptations of *Sphagnum* and the effect on ecosystem functions (e.g., primary production, water storage, and nutrient cycling) can inform the development of techniques to consistently achieve hydrophysical and water retention properties required for successful *Sphagnum* regeneration and the restoration of peat accumulating functions.

1.5. Effect of elevated nitrogen deposition

The effects of increased atmospheric deposition of reactive nitrogen on bog structure, function, and processes are one of the most studied impacts in scientific literature due to increased deposition rates observed in the northern hemisphere, especially in Europe. Numerous fertilization and reciprocal-transplant experiments provide an abundance of information and data on the responses to increased nitrogen concentrations of morphological and physiological traits in *Sphagnum* and the ecosystem functions and process in ombrotrophic bogs (e.g., Press et al. 1986, Limpens et al. 2003, Bragazza et al. 2004, Manninen et al. 2011, Granath et al. 2012). This constitutes a vast repository of information that can be examined to gain an understanding of the fundamental biological and ecological processes in *Sphagnum*-dominated bogs, which can form the basis for advances in the science of ecological restoration.

Elevated rates of atmospheric nitrogen deposition potentially pose a significant threat to the ecological resilience of ombrotrophic bogs (Press et al. 1986, Heijmans et al. 2001, Manninen et al. 2011). The reactive nitrogen includes nitrate (NO_3^-) and ammonium (NH_4^+) introduced to the atmosphere via fossil fuel combustion and food production (i.e., fertilizer use), respectively (Manninen et al. 2011). Efficient uptake and assimilation mechanisms enable *Sphagnum* carpets to act as a nitrogen filter in functional ombrotrophic bogs by retaining close to 100% of the N from precipitation almost instantaneously at low levels of deposition (<0.5-1 g-N m^{-2} yr^{-1}) and storing large amounts of N in *Sphagnum* biomass and peat (Jauhiainen et al. 1998, Aldous 2002b). This nitrogen-retention capacity prevents the build-up of nitrogen in the *Sphagnum* layer and substantially reduces the availability of nitrogen further down the peat profile where microbes and vascular plants compete for nutrient availability (Fritz et al. 2014). *Sphagnum* individuals can uptake nutrients along the entire tissue surface, from the apical capitulum and the living portions of the branches and stem, via the adsorption of ions from water held by capillary forces in and around the chlorophyllose and hyaline cells (Jauhiainen et al. 1998). The capitula act as nitrogen-capturing "apical nets" whereby nitrogen retention is substantially greater in the capitula than in stems, and retention decreases exponentially down the vertical stem profile (Aldous 2002b). This "apical net" captures up to 90% of the deposited nitrogen in the capitula and upper branches, and the remainder of the N will leach down through the moss layer towards the peat (Aldous 2002a, 2002b). A "second net" for nitrogen capture occurs further down the shoot due to the combination of the ability to uptake nitrogen throughout the entire living stem profile and specialized translocation mechanisms observed in *Sphagnum* (Aldous 2002a). *Sphagnum* is capable of translocating nutrients from the stem up to the capitulum thereby increasing overall N retention efficiency by returning a portion of the nitrogen to the capitulum for metabolic activity that was not initially retained (Aldous 2002a). As such, concentrations of total nitrogen, amino acids, and soluble proteins are always higher in capitula than the stems (Van der Heijden et al. 2000).

Sphagnum are generally nitrogen-limited in the nutrient-poor conditions of undisturbed ombrotrophic bogs. Evolving in these nutrient-poor environments, *Sphagnum* have developed efficient nitrogen uptake, storage, and translocation mechanisms such that N is taken up very quickly at low levels of deposition (Lamers et al. 2000, Aldous 2002a, 2002b, Kneip et al. 2007). Thus, under slight increases in nitrogen deposition (critical load for N-limitation is 1-1.5 g-N m^{-2} yr^{-1}) *Sphagnum* continues efficient N-assimilation via an increase in growth and biomass production

(Gunnarsson and Rydin 2000, Lamers et al. 2000, Bragazza et al. 2004, Granath et al. 2009). One mechanism by which this occurs is the incorporation of increased glutamine availability to increase the chlorophyll content of chlorophyllose cells, thereby enhancing photosynthetic capacity in the capitulum (Van der Heijden et al. 2000, Limpens and Berendse 2003). As nitrogen deposition increases (>1-1.5 g-N m^{-2} yr^{-1}) nitrogen is no longer limiting, yet *Sphagnum* continues to exhibit high rates of N uptake and assimilation and accumulates excess nitrogen as free amino acids that act as nitrogen storage compounds in *Sphagnum* tissue (Baxter et al. 1992, Limpens and Berendse 2003). The bulk of these accumulated amino acids are the N-rich free amino acids asparagine (mainly responsible for storage in stem tissue), arginine (mainly responsible for storage in capitulum tissue), and glutamine (Baxter et al. 1992, Kahl et al. 1997, Nordin and Gunnarsson 2000).

Along with increased N-rich free amino acid concentrations in plant tissue, *Sphagnum* can adapt other detoxification mechanisms in response to long-term increases in nitrogen deposition at these moderate levels including reduced nitrogen uptake, increased nitrogen tissue content, and reduced nitrate reductase activity (Bragazza et al. 2004, Granath et al. 2009, Bonnett et al. 2010, Fritz et al. 2014). However, toxic saturation (1.3 mg-N g-DW^{-1}) and deposition thresholds (>1.5-2 g-N m^{-2} yr^{-1}) exist at which *Sphagnum* can no longer sequester additional nitrogen and both ammonium and nitrate can have deleterious effects on *Sphagnum* resulting in decreased growth, biomass production, and photosynthetic capacity, and increased nitrogen concentrations in the pore water and rhizosphere and shifts in vegetation community compositions and ecosystem nutrient cycling (e.g., Gunnarsson and Rydin 2000, Lamers et al. 2000, Limpens and Berendse 2003, Wiedermann et al. 2007).

Elevated nitrogen inputs also influence vegetation dynamics in ombrotrophic bogs, and a critical load of 1-1.5 g-N m^{-2} yr^{-1} has been identified at which vegetation composition begins to shift from *Sphagnum*-dominated communities to an increased abundance of vascular plants (Bobbink and Roelofs 1995, Tomassen et al. 2003, Bragazza et al. 2004). Nitrogen fertilization studies have indicated that changes in vegetation composition begin to emerge after 4-8 years of fertilization at high loads (>2 g-N m^{-2} yr^{-1}), resulting in decreasing abundance and cover of *Sphagnum* and a concomitant increase in vascular plants (Limpens et al. 2004, Wiedermann et al. 2007, Bubier et al. 2007, Juutinen et al. 2016). Long-term exposure to high levels of nitrogen deposition increases respiration, decomposition, and nutrient leaching in *Sphagnum* (Lamers et al. 2000, Limpens et al. 2003, Curtis et al. 2005, Manninen et al. 2011, Fritz et al. 2014). However, the carbon and nitrogen cycles in *Sphagnum* may be decoupled to some extent (Manninen et al. 2016). Nitrogen leaching occurs when *Sphagnum* uptake, storage, and translocation mechanisms have become saturated (Lamers et al. 2000, Limpens et al. 2003). Nitrate is leached into the pore water and the rhizosphere at higher levels than ammonium due to the preferential uptake and faster assimilation of ammonium by *Sphagnum* (Živković et al. unpublished manuscript, Jauhiainen et al. 1998, Wiedermann et al. 2009, Fritz et al. 2014).

The majority of research examining the effect of enhanced nitrogen deposition on *Sphagnum* morphology and physiology has used ammonium nitrate (NH_4NO_3) as the fertilization compound (e.g., Bubier et al. 2007, Juutinen et al. 2010, 2016, Larmola et al. 2013, Fritz et al. 2014). Therefore, the effect of nitrate and ammonium as separate forms of nitrogen input are not well documented in the literature. Due to distinctive uptake, assimilation, and storage mechanisms nitrate and ammonium can differentially impact photosynthesis, production, nutrient leaching, decomposition, and water-holding capacity in *Sphagnum* (Press et al. 1986, Jauhiainen et al. 1998, Wiedermann et al. 2009, Fritz et al. 2014, Živković et al. 2017). *Sphagnum* are capable of the active uptake of both ammonium and nitrate, however, the uptake and assimilation mechanisms differ for each compound, respectively. Ammonium is preferentially and more quickly taken up by *Sphagnum* because nitrate must first be converted to ammonium before it can be assimilated (Wiedermann et al. 2009, Fritz et al. 2014). Free NH_4^+ ions are toxic in cells, thus *Sphagnum* have evolved mechanisms whereby ammonium is rapidly assimilated into glutamate, glutamine, and other amino acids (Kahl et al. 1997, Limpens and Berendse 2003). Nitrate is first reduced to ammonium by nitrate reductase enzymes, and then assimilated into amino acids (Press and Lee 1982, Press et al. 1986, Kahl et al. 1997). Nitrate reductase activity is induced without a significant lag phase, meaning that *Sphagnum* can assimilate even relatively short nitrate pulses, though usually only when nitrate is the primary N source (Press and Lee 1982, Woodin et al. 1985). When ammonium and nitrate are available in equal supply, rates of ammonium uptake are an order of magnitude greater than that of nitrate uptake across multiple *Sphagnum* species (Jauhiainen et al. 1998). Additionally, hummock-forming species, including species closely related to *Sphagnum capillifolium* (i.e., *Sphagnum fuscum* and *Sphagnum* *rubellum*), have been observed to be particularly efficient in ammonium uptake compared to other species due to a higher cation-exchange capacity (Clymo 1963, Jauhiainen et al. 1998, Limpens and Berendse 2003, Bragazza et al. 2004).

Both ammonium and nitrate can be physiologically toxic to *Sphagnum* at high concentrations; however, the effect of ammonium appears to be more detrimental (Press et al. 1986, Gunnarsson and Rydin 2000). This is in part due to the preferential uptake of ammonium over nitrate, but is also influenced by changes in nitrate reductase activity. Nitrate reductase activity is stimulated by pulses of nitrate availability, but ammonium (at high concentrations) is a known inhibitor of the nitrate reductase enzyme (Woodin and Lee 1987). Long-term exposure to high rates of both ammonium and nitrate results in reduced nitrate reductase activity by inhibiting induction and/or enhancing degradation of the enzyme due to the increased NH_4^+ concentrations in *Sphagnum* tissue (Press and Lee 1982, Woodin et al. 1985, Woodin and Lee 1987). Therefore, *Sphagnum* subjected to repeated nitrate fertilization will have reduced nitrogen uptake rates compared to ammonium treatments due to reduced nitrate reductase activity, though this inhibitory effect is minimal at moderate deposition rates (Jauhiainen et al. 1998). A study by Manninen et al. (2011) at the Whim Bog in Scotland is one of the few studies to examine the effects of nitrate and ammonium fertilization separately, and the results suggest that the cross-sectional area of *Sphagnum capillifolium* leaf hyaline cells increased under the nitrate fertilization treatment (as compared to the control and ammonium treatments) without a corresponding negative impact on photosynthetic capability (Figure 2). The hyaline cell cross-sectional area results have not been observed before in the literature.

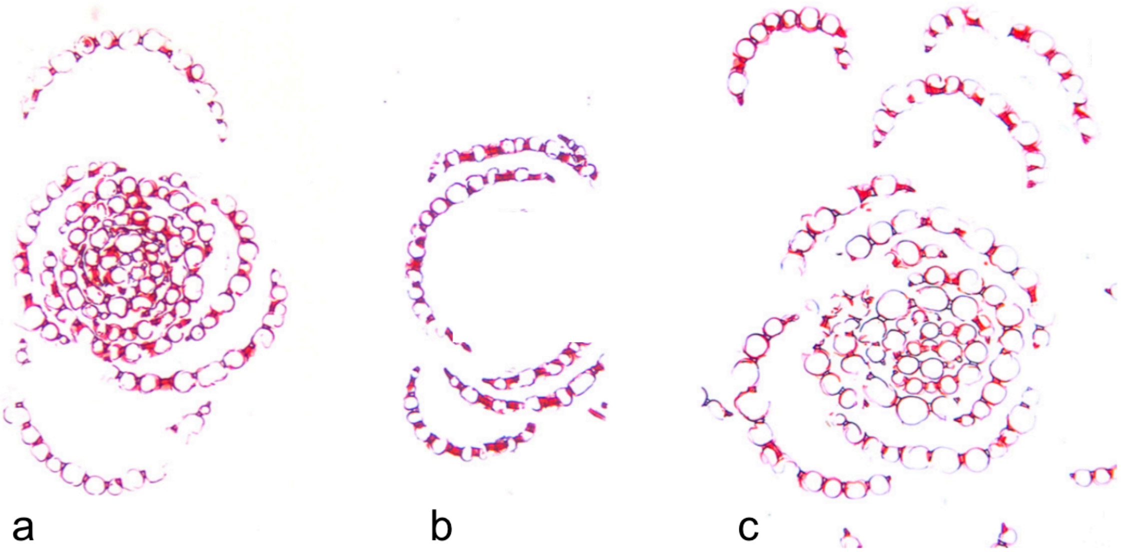

Figure 2. Tissue cross-section samples from microscopic analysis of cellular morphology of *S. capillifolium* at Whim Bog, Scotland. The white spaces within the red tissue demonstrate the differences in hyaline cell cross-sectional areas for a) control, b) ammonium, and c) nitrate fertilization treatments. Adapted from (Manninen et al. 2011).

Manninen et al. (2011) proposed that in response to increased levels of nitrate *S. capillifolium* allocated excess nitrogen into cell-wall proteins resulting in larger hyaline cells. These water-storing cells provide a reservoir of water that acts as a buffering mechanism against drought when precipitation is low (Schipperges and Rydin 1998). As such, larger hyaline cells could provide increased drought resistance and desiccation avoidance. Hyaline cells also host diverse microbial communities through endosymbiotic or passive association, including diazotrophic cyanobacteria and methanotrophic bacteria (Weston et al. 2017). Recent research has revealed that these microbial communities play an important role in both nitrogen and carbon cycling in *Sphagnum* through nitrogen fixation and methane oxidation processes (Kip et al. 2010, Berg et al. 2013, Lindo et al. 2013). Other morphological and physiological responses have been observed in *Sphagnum* species under increased levels of nitrogen fertilization including reduced nitrogen uptake, reduction in *Sphagnum*-associated nitrogen reductase activity, storage of nitrate in vacuoles, and changes in production, growth, and photosynthetic capacity (Press and Lee 1982, Jauhiainen et al. 1998, Granath et al. 2012).

1.6. Objectives

Much of the existing scientific literature focuses on the deleterious effects of enhanced atmospheric nitrogen deposition on *Sphagnum* and bog ecology. However, examination and analysis of the fundamental ecological, physiological, and morphological functions and processes of species and ecosystems can reveal results that can inform non-traditional restoration solutions that may not have been apparent through an exclusively restoration-centric lens (Wainwright et al. 2017). This is the case with the existing scientific literature pertaining to increased nitrogen deposition loads, and particularly the results of Manninen et al. (2011). Nitrogen fertilization has the potential to create favourable moisture conditions in donor *Sphagnum* material by increasing the water holding capacity and drought tolerance in donor layers through changes in growth patterns and morphology. This in turn has the potential to increase ecological resilience and maintain carbon storage and sequestration functions in restored bogs, thus mitigating some of the impacts of degradation and predicated climate change.

However, as discussed above high levels of nitrogen deposition has been shown in the literature to have a toxic effect on *Sphagnum* including reductions in growth, productivity, and photosynthesis and an increase in respiration, decomposition, and leaching of nutrients from the capitula. The effect of nitrogen fertilization on photosynthesis, water holding capacity, and dissolved nutrient dynamics in the literature has mainly been investigated at high concentrations over long periods of time and using ammonium nitrate as the nitrogen source (e.g., Bubier et al. 2007, Manninen et al. 2011, Larmola et al. 2013, Fritz et al. 2014, Juutinen et al. 2016). Therefore, this study aims to determine the differential effects produced by nitrate and ammonium fertilization at different concentrations. The results of the morphology, physiology, and dissolved nutrient dynamics data will be analyzed to examine whether any potential positive morphological and physiological responses (e.g., increases in growth, CO_2 assimilation, or water holding capacity) are offset by negative trade-offs (e.g., reduced photosynthetic capacity or increased leaching of dissolved nutrients). Additionally, a pilot study will incorporate nitrogen fertilization treatments into the North American moss layer transfer peatland restoration methods to examine the effects of the treatments on establishment success, growth density, and vegetation composition. As such, the overarching objective of this study is to:

> Determine the morphological and physiological responses and trade-off mechanisms in *Sphagnum capillifolium* under different forms (nitrate and ammonium) and concentrations of nitrogen fertilization.
>
> Specifically, the two objectives of this study are to:
>
> 1. Examine the effect of nitrate and ammonium fertilization on individual morphology, growth density, water content, CO_2 assimilation, and dissolved nutrient dynamics in *Sphagnum capillifolium.*
> 2. Implement a *Sphagnum*-transplant pilot study to determine the effect of nitrogen fertilization on establishment success, growth density, and vegetation composition in donor sites.

Chapter 2. Methods

2.1. Study site

This study was conducted at the Mer Bleue peatland in Ottawa, Ontario, Canada (46°N, 75.5°W) from June to October 2017. Mer Bleue has a cool continental climate with a mean annual temperature of 6.4 °C and mean annual precipitation of 943.4 mm (Environment Canada; Canadian Climate Normals 1981-2010). Peat formation began ~8500 years and the peat thickness varies from 0.3 m at the margins of the bog to >5 m near the study site (Roulet et al. 2007). A dedicated research site was established in the ombrotrophic bog part of the peatland in 1998 and the surface has a hummock-hollow microtopographic mosaic. The dominant vascular vegetation species are *Chamaedaphne calyculata* Moench*, Ledum groenlandicum* Oeder, *Vaccinium myrtilloides* Michx., and *Kalmia angustifolia* L. The groundcover is dominated by the mosses *Sphagnum capillifolium* (Ehrl.) Hedw., *Sphagnum magellanicum* Brid., and *Polytricum strictum* Brid. (Bubier et al. 2006).

The background atmospheric nitrogen deposition was 0.32-0.8 g-N m^{-2} yr^{-1} in the region (Turunen et al. 2004). The fertilization experiment was implemented in 2012 in a hummock-dominated portion of the research site. Nitrogen, as nitrate ($NaNO_3$) and ammonium (NH_4Cl) separately, was applied to randomly assigned triplicate 1-m × 1-m plots per treatment seven times per year between May and August, dissolved in the equivalent of 2 mm of distilled water. The treatments are summarized in Table 1. The fertilization plots are dominated by *Sphagnum capillifolium* (Ehrl.) Hedw., and the vascular vegetation were clipped throughout each growing season to eliminate interactive effects of shading and competition by vascular species on *Sphagnum* growth and other characteristics and to isolate the effects of the nitrogen addition on the *Sphagnum*. *S. capillifolium* is a member of Sect. *Acutifolia*, and is a hummock-forming species that generally grows in open, drier habitats (Bonnett et al. 2010, Manninen et al. 2011). As a hummock species, *S. capillifolium* tends to grow more robust shoots with longer branches and denser growth clumps to optimize water conservation (Rice et al. 2008, Manninen et al. 2011). *S. capillifolium* often exhibits a distinctive reddish-violet hue that is the result of a secondary cell-wall pigment called sphagnorubin, though individuals growing in more shaded conditions adapt to be green in colour (Gerdol et al.

1996, Bonnett et al. 2010). This red-violet colour and sphagnorubin increases drought tolerance, in part, through mitigation of photo-oxidation (Bonnett et al. 2010, Manninen et al. 2011).

Table 1. Summary of control, nitrate, and ammonium treatments for the long-term fertilization study at the Mer Bleue Bog, Ottawa, Canada. Three replicate plots for each treatment were established in 2012.

Fertilization Treatment	N concentration (g-N m^{-2} yr^{-1})
Control	Distilled water
5NO3	1.6
5NH4	1.6
10NO3	3.2
10NH4	3.2
20NO3	6.4
20NH4	6.4

The summer of 2017 was the wettest growing season on record at Mer Bleue since the establishment of the research site in 1998. Most of the precipitation fell during June (130 mm) and July (249.8 mm), representing precipitation levels 106% higher than the climate normals for those months (Environment Canada; Canadian Climate Normals 1981-2010). Water availability in ombrotrophic bogs exerts substantial influence on *Sphagnum* physiological function (Weston et al. 2015). Thus, it is likely that the atypical precipitation regime over the study period increased variability in the data and certain results may not be indicative of the typical functions and processes observed at the Mer Bleue Bog.

2.2. Morphology and Physiology

Net CO_2 Assimilation and In-situ Water Content

Samples were collected from each plot on five different sampling days (August 4, 11, 14, 25, and 30). On each sampling day, 4-5 *S. capillifolium* shoots were randomly selected from each plot, the capitula apical segments were cut to 1 cm in length, and weighed in the field to determine the fresh weight (FW). Samples were transported back to the lab in a cooler and placed on paper towel saturated with 2 ml of deionized water to keep the samples moist. The order for net CO_2 assimilation measurements was randomized on each day independently. Samples were allowed to acclimate to light conditions under two T5H0 6400K lights at a photosynthetic photon flux density (PPFD) of ~500 μmol m^{-2} s^{-1}, which is around the light saturation point for species closely related to *S. capillifolium* (e.g., *Sphagnum rubellum*) (Marschall and Proctor 2004).

Net CO_2 assimilation measurements were made using an open system infrared gas analyzer (IRGA) equipped with a 2-cm × 3-cm LED light source measurement chamber (Licor 6400XT Portable Photosynthesis System, 6400-02B Red/Blue LED light source). The samples were placed in the chamber with the capitula facing up towards the light source. After the reference and sample IRGAs were matched, the system was allowed to stabilize for 30 seconds and then three measurements were logged spaced 15 seconds apart for each sample to capture the inherent variability in CO_2 assimilation measurements. The mean of these three values was used as the net CO_2 assimilation value for each sample. Chamber CO_2 concentration (380 μmol mol^{-1}), flow rate (μmol s^{-1}), PPFD (500 μmol m^{-2} s^{-1}), fan speed (2 V), and temperature (~25 °C) settings in the IRGA were kept constant for all measurements. Samples were left to air dry for one week, then weighed to determine dry weight (DW). Assimilation measurements were standardized per gram dry weight of each sample. Water content (WC) was calculated as (FW-DW)/DW × 100%.

Maximum WC, Water Loss Rate, and Individual Morphology

After drying, the capitula from all five sampling days were combined for each plot (20-24 capitula) and soaked in 50 ml of deionized water for two hours to allow the samples to reach a saturated water content. Samples were weighed immediately after removal from the water to obtain the water content at hour 0, which represents maximum water content. Samples were left to air dry in the lab and weighed at hours 1, 4, 6, and 50 after soaking, each of these measurements represent the FW used at each time interval for the water loss rate calculation. Samples were then oven-dried for 24 hours at ~60 °C and then weighed to obtain the DW. Water content was calculated as (FW-DW)/DW × 100%. The FW and DW per capitulum were calculated for each plot as (Max WC/number of capitulum) and (DW/number of capitulum), respectively. As the capitula were all cut to the same length (1 cm) for the CO_2 assimilation and WC analyses the FW and DW per capitulum was used to examine whether decreases in FW compared to the Control treatment are accompanied by a decrease in DW in the samples, reflecting changes in individual capitulum morphology.

Microtopography

The microtopography of each plot was surveyed using a HiPer V receiver differential GPS system on August 29 (Topcon Positioning Systems, Inc.; vertical accuracy = 5 mm, horizontal accuracy = 3 mm). A grid of nine measurement points (longitude, latitude, and elevation) was collected for each plot. The mean elevation of each plot was used as a proxy for depth to water table to examine whether differences in microtopography affected in-situ WC in the plots.

2.3. Dissolved Nutrients

Dissolved Nutrients and Growth Density

Samples were collected for the incubation and leachate experiment on July 5. Fertilization plots were divided into four quadrants, and cores (6-cm diameter) comprising the top 4.5 cm of *Sphagnum* growth were extracting from a randomly selected quadrant at each plot. Samples were stored at 5 °C for two days and then all non-*Sphagnum* vegetation was removed and samples were air-dried for a week and weighed to obtain DW. *Sphagnum* samples were incubated for 12 weeks in polypropylene cups (5.8 cm diameter) with flat, perforated bottoms. Cups were lined with approximately 0.5 g of acid-washed and oven-dried glass wool (soaked in 10% HCl for 24 h, rinsed with DI water, dried at 30 °C for 24 h) to prevent loss of *Sphagnum* tissue through the perforations (Magill and Aber 2000). After glass wool and samples were in place the cups were covered with plastic wrap and secured with an elastic band. Samples were stored in the lab with a constant temperature of approximately 20 °C.

Samples were leached with 88 ml of deionized (DI) water on weeks 0, 1, 2, 3, 4, 6, 8, 10, and 12 starting on July 17. Sample cups were attached to a Büchner funnel, which was placed and sealed in a beaker using a rubber gasket. The beaker was attached to a vacuum pump to help pull the leachate through the *Sphagnum* material. The resulting leachate was then filtered through a Whatman sterile membrane 0.45 μm filter and poured into a scintillation vial for analysis. Beakers and funnels were rinsed with DI water between each sample. Immediately after the initial leaching, 20 ml of inoculant was added to each cup. The inoculant was made by thoroughly mixing 1 L of DI water with 200 g of a *Sphagnum* and peat mixture extracted from an area adjacent to the study site. The inoculant was left to settle for 24 h and then filtered through a Whatman 5 (2.5 μm) filter. The volume of leaching water (704 ml over 12 weeks = 88 ml × 8 leachings) was equivalent to the average amount of precipitation expected for July, August, and September in the study site area, adjusted to the surficial area of the cup (Environment Canada; Canadian Climate Normals 1981-2010).

The ultraviolet absorbance (Abs) at 254 nanometers of the leachate samples was measured using a Cary 60 UV-Vis spectrophotometer with 1-cm path length (Agilent Technologies). The specific ultraviolet absorbance ($SUVA_{254}$) was calculated using (Abs/NPOC concentration) × 100 for each sample. Samples were then acidified with 2-3 drops of 10% HCl to prevent further decomposition and to allow for more efficient removal of dissolved inorganic carbon forms through sparging during analysis. Samples were stored in a 5 °C fridge then transported in coolers to McGill University for non-purgeable organic carbon (NPOC) and total nitrogen (TN) analysis. NPOC and TN was measured using a Total Organic Carbon (TOC)-V_{CSH} analyzer (Shimadzu Corporation). Samples were sparged with Ultra-Zero compressed (CO_2 free) air for 5 minutes prior to analysis and then combusted at 720° C using a platinum catalyst. $SUVA_{254}$, NPOC, and TN were standardized per gram dry weight for data analysis.

The incubation samples were extracted using cores of uniform dimensions ($V=127.24\ cm^3$) with all non-*Sphagnum* vegetation removed. As such, the DW of these samples can be used as a proxy for biomass density in the top 4.5 cm of *Sphagnum* growth (DW/core volume). Biomass density was used to examine the interactive effects of treatment, WC, and growth density.

2.4. *Sphagnum*-Transplant Pilot Study

As a compliment to the morphological and physiological results, and to evaluate the potential integration of nitrogen fertilization into peatland restoration treatments, a small-scale *Sphagnum*-transplant pilot study was designed to evaluate the effect of nitrogen on establishment success, growth density, and vegetation composition. Three separate transplant plots (2.44 m × 1.2 m) were established in previously disturbed areas where *Sphagnum* growth no longer occurred. The top layer of vegetation and peat was removed to emulate a mined peat surface. The *North American approach* was used for the transplant process. Donor material was collected from ten 25-cm × 25-cm quadrats at a ratio of 1:10 on July 6, homogenized in a large bucket, and spread in 1-2 cm thick layers over the transplant plots. Donor material was covered in 916 g of straw per transplant plot (3000 kg ha^{-1}). A randomized block design was to be implemented with 5 blocks (0.25 m × 0.6 m) within each transplant plot. Fertilization treatments are summarized in Table 2, and nitrogen treatments were to be applied every three weeks in the equivalent of 2 mm of distilled water while phosphate applications were to be a one-time application. However, due to the extreme precipitation during the summer of 2017 the transplant plots were flooded (6-12 cm of water) for the entire summer and the fertilization treatments could not be implemented.

Table 2. Summary of fertilization treatments for the proposed *North American approach Sphagnum*-transplant pilot study at Mer Bleue. A randomized block design containing all five treatments will be applied to three transplant plots.

Fertilization Treatment	Nutrient concentration
Control	Distilled water
NA method	2.6 g m^{-2} of available P_2O_5
2.5NO3	0.8 g m^{-2} yr^{-1} NO_3; 2.6 g m^{-2} available P_2O_5
5NO3	1.6 g m^{-2} yr^{-1} NO_3; 2.6 g m^{-2} available P_2O_5
5NH4	1.6 g m^{-2} yr^{-1} NH_4; 2.6 g m^{-2} available P_2O_5

2.5. Statistical Analyses

The FW per capitulum, DW per capitulum, biomass density, microtopography, in-situ WC, maximum WC, net CO_2 assimilation, NPOC, TN, and SUVA data were analyzed using a one-way ANOVA and post-hoc Tukey's Honestly Significant Difference (HSD) test (when appropriate) to determine significant differences between treatments. Water loss rates were determined using a log transformation of the Time variable followed by simple linear regression analysis. Differences between water loss slopes and intercepts were analyzed using ANCOVA followed by a pairwise comparison of trends using the Tukey adjustment method of estimated marginal means. All results were considered significant at $p < 0.05$. The high variability of the data and the relatively small sample sizes (n=3 per treatment) resulted in reduced statistical power in the data analysis. Treatment means are presented ± the standard error (SE). All statistical analyses were performed using RStudio 1.1.383 (RStudio Inc.).

Chapter 3. Results

3.1. Morphology

Individual Morphology

Fertilization treatment had a significant effect on sample FW per capitulum (n=21, df=6,14, F=5.88, p=0.0030). The only significant differences in mean FW per capitulum were between 20NH4 and Control (p=0.0038), 5NO3 (p=0.018), and 5NH4 (p=0.0044), respectively. However, there was a distinct negative trend observable in the FW data as the nitrogen concentration increases (Figure 3). There was also a significant treatment effect on sample DW per capitulum by the fertilization treatments (n=21, df=6,14, F=3.99, p=0.015). But, the only significant pairwise difference was between the 10NH4 and 20NO3 treatments (p=0.025). Though not significant, the 10- and 20-level NH4 treatments had DWs 33% (p=0.063) and 28% (p=0.13) lighter, respectively, than the corresponding NO3 treatments (Figure 3). Except for 10NH4 and 20NH4, the data suggests a positive correlation between DW per capitulum and nitrogen deposition concentration.

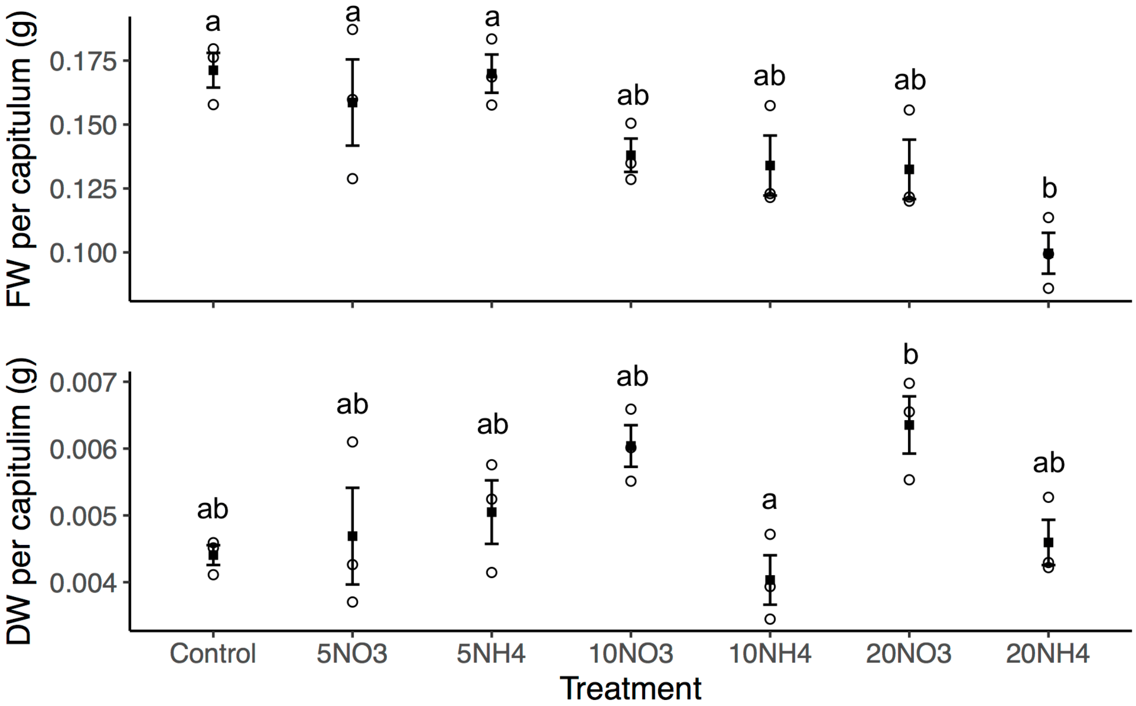

Figure 3. **The effects of fertilization treatment on FW and DW per capitulum (g) of combined capitulum samples for each plot. Black squares and error bars represent the mean values for each treatment ± SE (n=3). White circles represent the measured values for each replicate plot within the treatment. Letters represent the results of Tukey's HSD *post-hoc* test, treatments with the same letter are not significantly different ($p<0.05$).**

Beyond statistical analysis, the differences in individual morphology between fertilization treatments was apparent through visual observation. While not quantified, a qualitative assessment of the samples indicated that all the 10- and 20-level treatments were physiologically damaged, if not already dead. The 10/20NH4 treatments appeared to have more damage and thinner and frailer pendent branches compared to 10/20NO3 treatments (Figure 4). The Control and 5-level treatments looked relatively similar, though it appeared that 5NO3 and 5NO4 had larger and denser capitula.

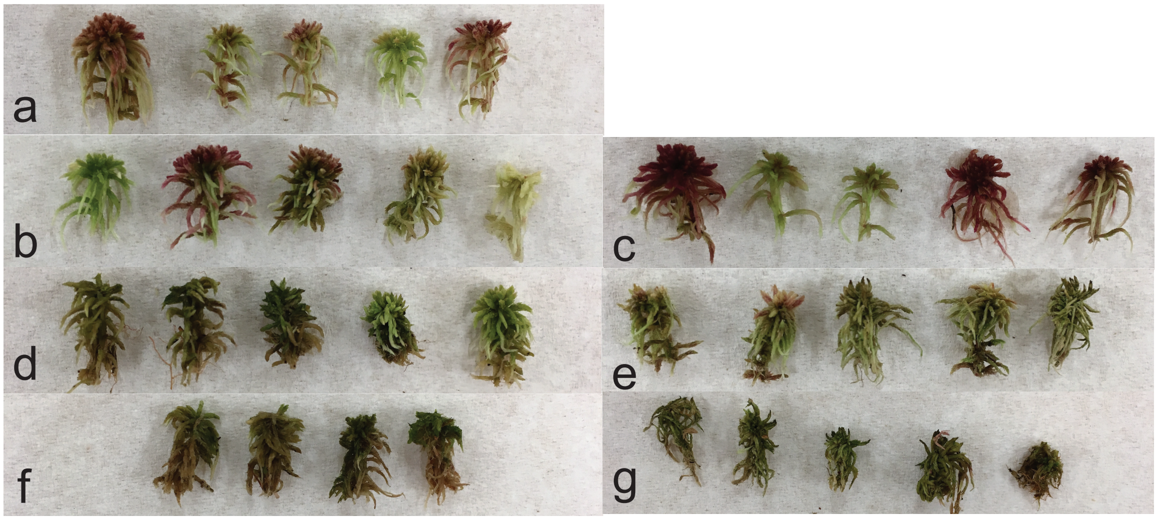

Figure 4. **Examples of samples collected for net CO_2 assimilation analysis on August 14. Photos like this were used to qualitatively compare the individual morphology of a) Control, b) 5NO3, c) 5NH4, d) 10NO3, e) 10NH4, f) 20NO3, and g) 20NH4 treatments (Photos are not to scale).**

Growth Density

Due to high variability in the data treatment did not have a significant effect on biomass density (n=21, df=6,14, F=1.48, p=0.26). The data suggests that there was a negative correlation between biomass density and increasing nitrogen load above the 5-level treatments (Figure 5). The difference in mean biomass densities between the Control treatment (0.015 g-DW cm^{-3}) and the 5NH4 treatment (0.016 g-DW cm^{-3}) appeared inconsequential; however, the difference represented an increase in biomass density equivalent to an additional ~9000-10000 capitula within the top 5 cm of the *Sphagnum* layer in the 1-m x 1-m plots.

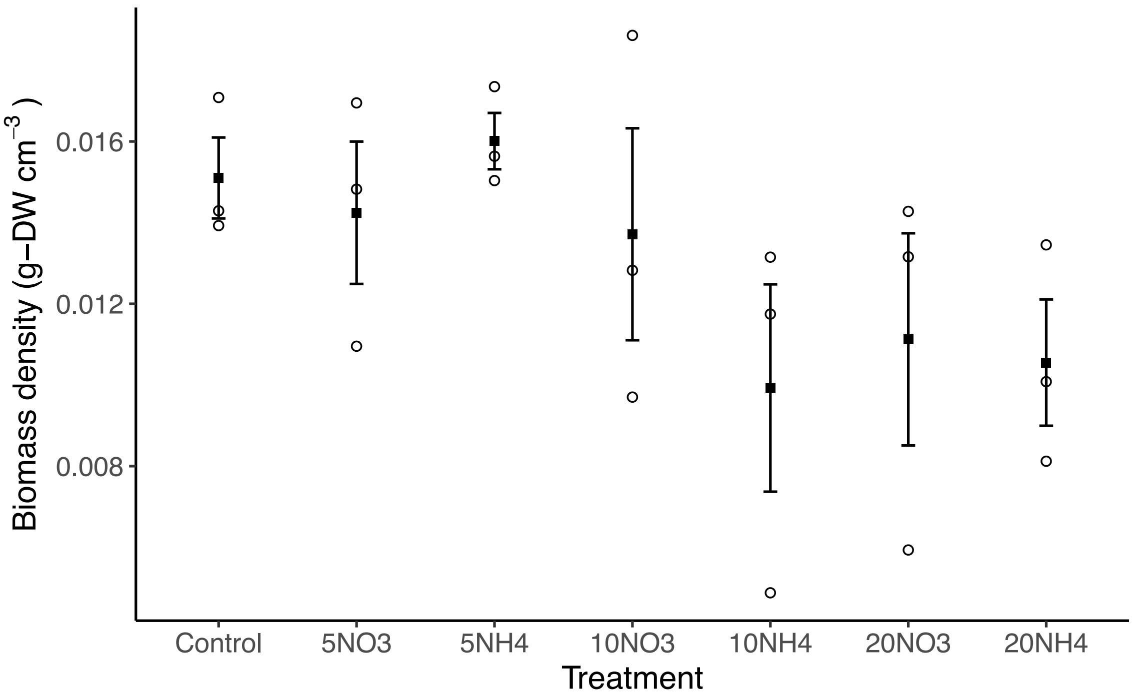

Figure 5. **The effects of fertilization treatment on biomass density (g-DW cm^{-3}). Black squares and error bars represent the mean values for each treatment ± SE (n=3). White circles represent the measured values for each replicate plot within the treatment. One-way ANOVA reported no significant differences between fertilization treatments ($p<0.05$).**

Microtopography

There was no significant difference in elevations between the fertilization treatments (n=21, df=6,14, F=1.34, p=0.30). This is due to substantial inter-plot variability, particularly in the Control, 5NO3, and 10NO3 treatments (Figure 6). The data suggests that differences in elevation were not treatment-dependent, and rather a result of the natural hummock-hollow microtopography. However, the differences in mean elevation between the Control plots and the 5NO3 (5.26 cm), 5NH4 (4.76 cm), and 10NH4 (6.06 cm) plots were substantial enough to potentially affect water availability in the *Sphagnum* layer.

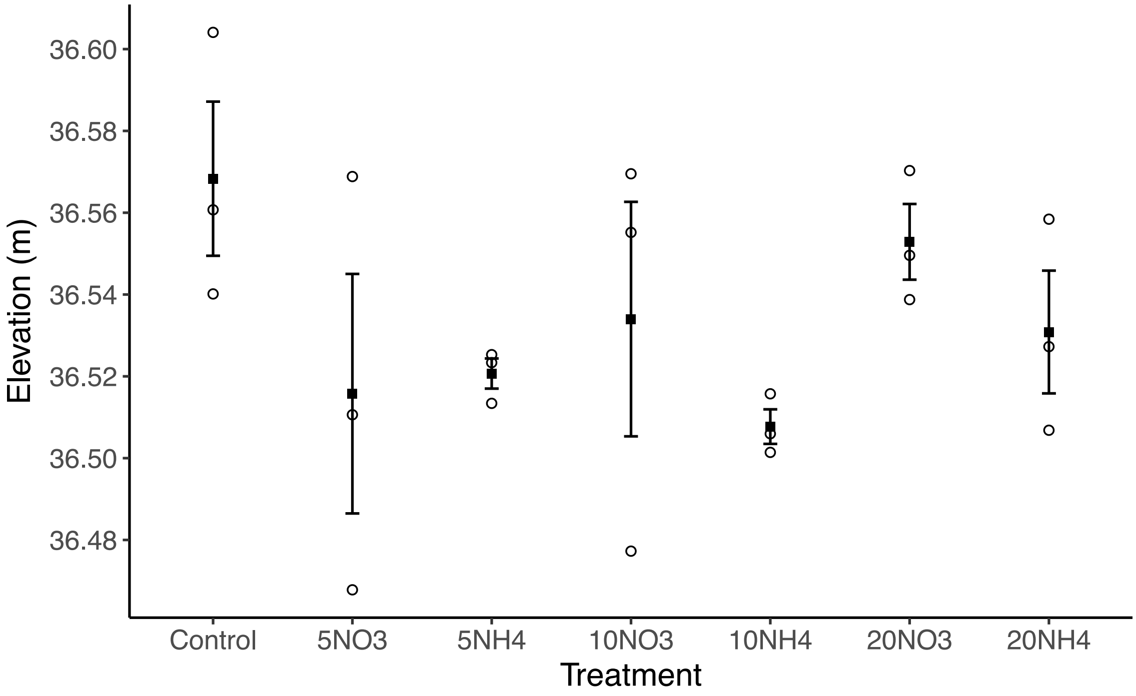

Figure 6. **The elevation (m) of plots within each treatment. It is likely that natural microtopographical variation is responsible for observed differences between treatments, rather than treatment effects. Black squares and error bars represent the mean values for each treatment ± SE (n=3). White circles represent the measured values for each replicate plot within the treatment. One-way ANOVA reported no significant differences between fertilization treatments ($p<0.05$).**

3.2. Physiology

Water Content

The in-situ WC data resulted in large standard errors due to the variable climatic and precipitation conditions that occurred at the research site during the study period. Fertilizer treatment did not have a significant effect on WC (Table 3; n=21, df=6,14, F=2.29, p= 0.095). While the variability in the data reduced the statistical power of the results, the mean in-situ WC for the 10NO3 treatment (314.38% ± 40.70) was substantially lower than the Control treatment (501.94% ± 51.31). Outside of the 10NO3 treatment, the results suggest that fertilization treatment did have a significant influence on in-situ WC (Figure 7), which could be due to the overriding effects of plot-level variables such as microtopography and water table depth.

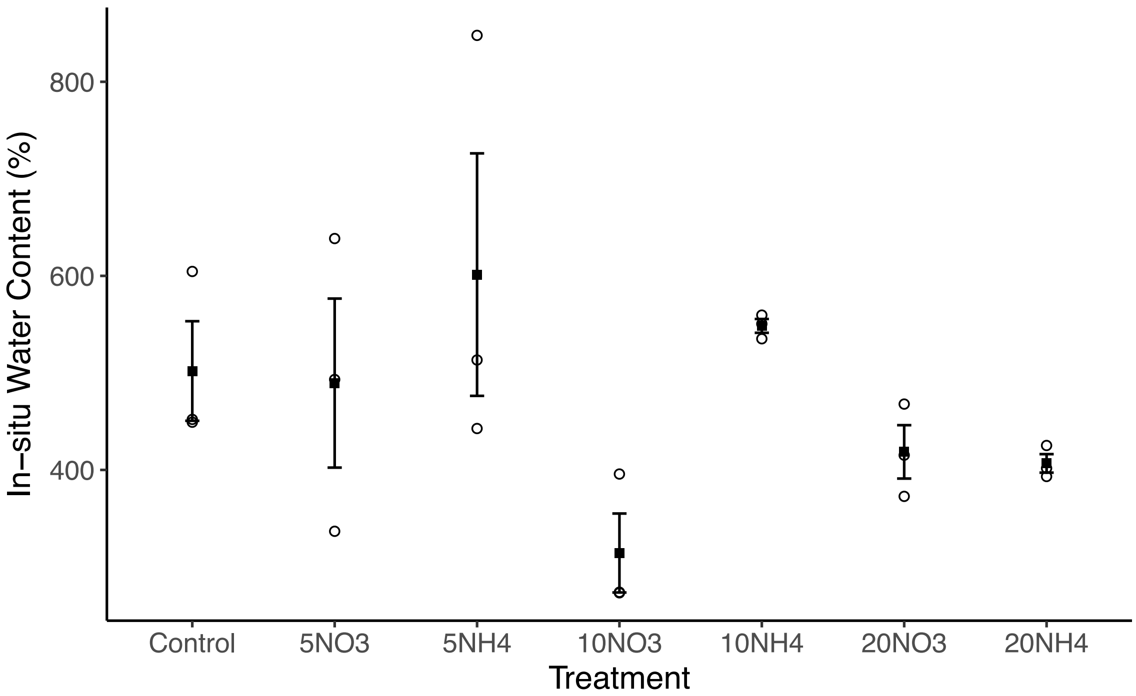

Figure 7. **The effects of fertilization treatment in-situ WC (%). Black squares and error bars represent the mean values for each treatment ± SE (n=3). White circles represent the measured values for each replicate plot within the treatment. One-way ANOVA reported no significant differences between fertilization treatments ($p<0.05$).**

The Control treatment had the highest maximum WC and fertilizer treatment had a significant effect on mean maximum WC (n=21, df=6,14, F=7.79, $p<0.001$). The mean WCs of 10NO3 (p=0.012), 20NO3 (p=0.0044), and 20NH4 (p=0.0065) treatments were significantly lower than the Control (Table 3, Figure 8). Even in a controlled lab setting, the mean maximum WC for 10NO3 was discernably lower than 5NO3 (p=0.10), 5NH4 (p=0.088), and 10NH4 (p=0.13), which is unexpected considering the results of Manninen et al. (2011).

Table 3. **Summary of the effects of fertilization treatment on key physiological variables. Values are treatment means ± SE. Bold values indicate results significantly different from the Control treatment ($p<0.05$).**

Treatment	Net CO_2 Assimilation (μmol s^{-1} g^{-1})	In-situ WC (%)	Maximum WC (%)	Cumulative NPOC (mg L^{-1} g^{-1})	Cumulative TN (mg L^{-1} g^{-1})
Control	4.14 ± 0.17	501.94 ± 51.31	3784.64 ± 23.90	40.59 ± 2.33	2.57 ± 0.31
5NO3	5.099 ± 0.74	489.48 ± 87.11	3331.54 ± 197.53	46.89 ± 8.74	4.057 ± 1.47
5NH4	7.98 ± 2.81	601.29 ± 124.96	3359.48 ± 502.23	44.33 ± 2.21	6.058 ± 1.014
10NO3	1.29 ± 0.55	314.38 ± 40.70	**2189.45 ± 89.63**	34.39 ± 3.53	7.088 ± 2.14
10NH4	4.88 ± 0.79	548.44 ± 7.12	3281.27 ± 421.75	75.43 ± 13.65	**10.64 ± 2.082**
20NO3	-0.058 ± 0.19	418.62 ± 27.51	**1987.70 ± 127.71**	41.36 ± 4.40	9.60 ± 1.88
20NH4	-0.52 ± 0.26	406.74 ± 9.55	**2069.31 ± 80.036**	43.30 ± 7.53	4.76 ± 0.59

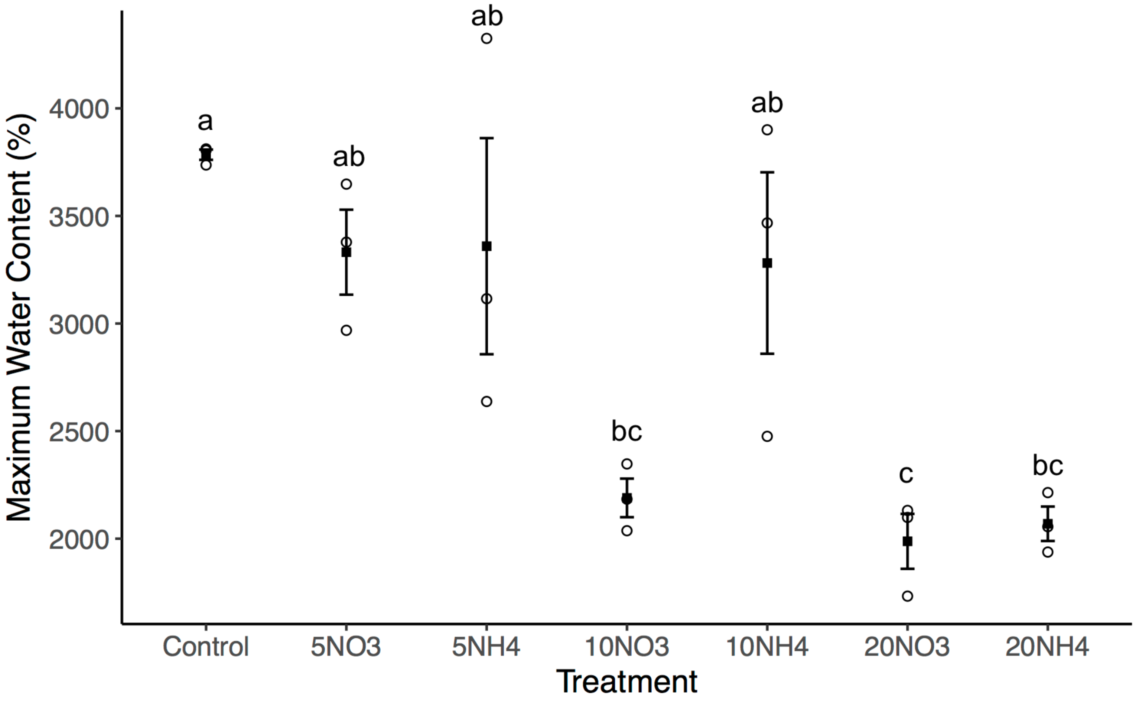

Figure 8. **The effects of fertilization treatment on maximum WC (%). Black squares and error bars represent the mean values for each treatment ± SE (n=3). White circles represent the measured values for each replicate plot within the treatment. Letters represent the results of Tukey's HSD *post-hoc* test, treatments with the same letter are not significantly different ($p<0.05$).**

Water Loss Rate

The water loss rates (decrease in WC over time) of all treatments were well described by linear relationships when time (hours) was log-transformed (Table 4, Figure 9). Time (log-transformed), treatment, and the interaction all had a statistically significant effect on water loss rate (F=822.50,30.98,7.95; p<0.001), which means that there were significant differences in slopes (water loss rates) between some treatments. The water loss rates were strongly influenced by maximum WC, as the treatments that had the lowest maximum water contents also had the slowest water loss rates, and vice versa (Table 3). As such, mirroring the maximum WC data the only treatments that had water loss rates significantly slower than the Control treatment were 10NO3 (p=0.0010), 20NO3 (p<0.001), and 20NH4 (p<0.001). Though the Control treatment had the fastest observed water loss rate, the treatment maintained the highest WC over the course of the entire experiment.

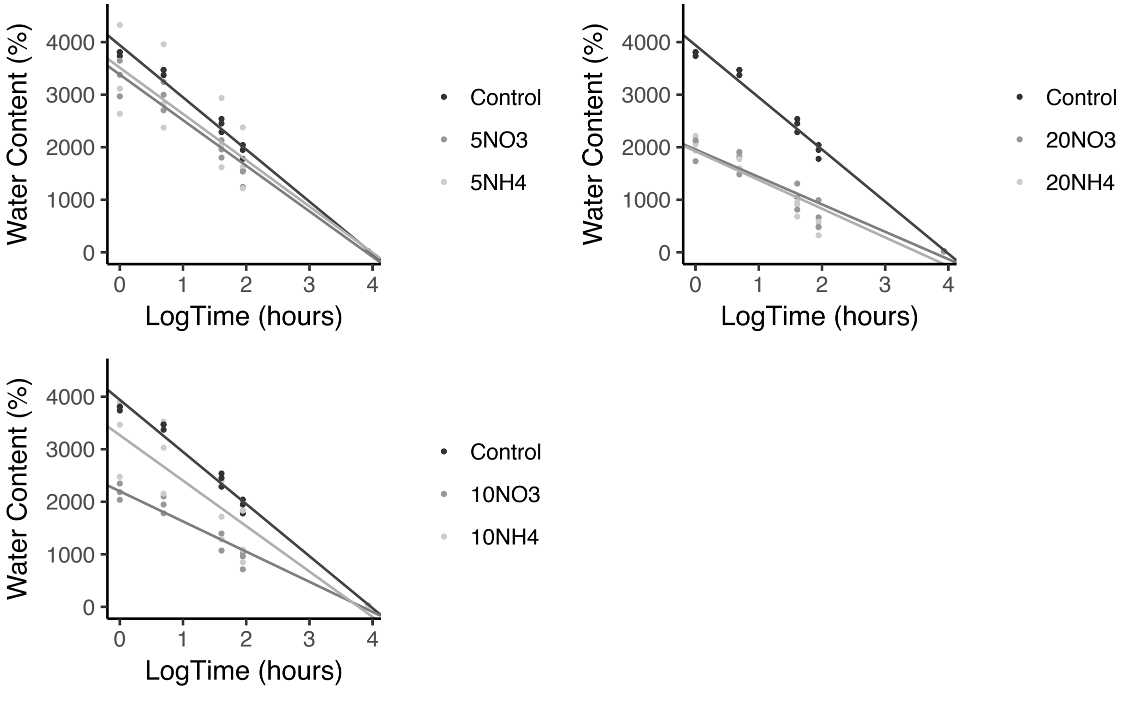

Figure 9. **The log-transformed water loss rate over time of fertilization treatment samples compared to the Control treatment samples. Data points represent individual sample measurement from each plot at each time. The lines represent mean water loss rates based on simple linear regression.**

Table 4. Summary of the effects of fertilization treatment on water loss rate based on simple linear regression analysis. Slope values represent the treatment mean waterloss rate ± 95% CI. Bold values indicate results significantly different from the Control treatment (p<0.05).

Treatment	Slope	Intercept	R^2	p-value
Control	-991.77 ± 63.11	3940.29 ± 133.33	0.9888	<0.001
5NO3	-876.40 ± 103.92	3382.0 ± 219.55	0.9623	<0.001
5NH4	-880.20 ± 249.003	3513.40 ± 526.098	0.8177	<0.001
10NO3	**-574.47 ± 71.91**	2200.08 ± 151.92	0.9582	<0.001
10NH4	-865.0 ± 223.88	3267.20 ± 473.062	0.8427	<0.001
20NO3	**-521.67 ± 98.34**	1955.13 ± 207.75	0.9099	<0.001
20NH4	**-546.57 ± 122.46**	1922.63 ± 258.72	0.8773	<0.001

Net CO_2 Assimilation

Fertilization treatment had a significant effect on net CO_2 assimilation in samples (n=21, df=6,14, F=7.15, p=0.0012). Assimilation appeared to respond positively to increased nitrogen concentrations (for both NO_3 and NH_4), reaching an optimum level for photosynthetic capacity at the 5-level treatments before higher concentrations substantially reduced assimilation rates (Figure 10). The 20-level treatments produced negative mean assimilation rates (i.e., producing net CO_2), but due to high variability in the data the differences were not significantly different compared to the Control (Table 3). The 10NO3 treatment was 296% lower than 5NO3 assimilation rate (p=0.30). The assimilation rate for the 5NH4 treatment was significantly higher than 10NO3 (p=0.016), 20NO3 (p=0.0035), and 20NH4 (p=0.021). The 5NH4 treatment had substantial variation; however, 5NH4 had a 92% higher mean assimilation rate than the Control (p=0.30).

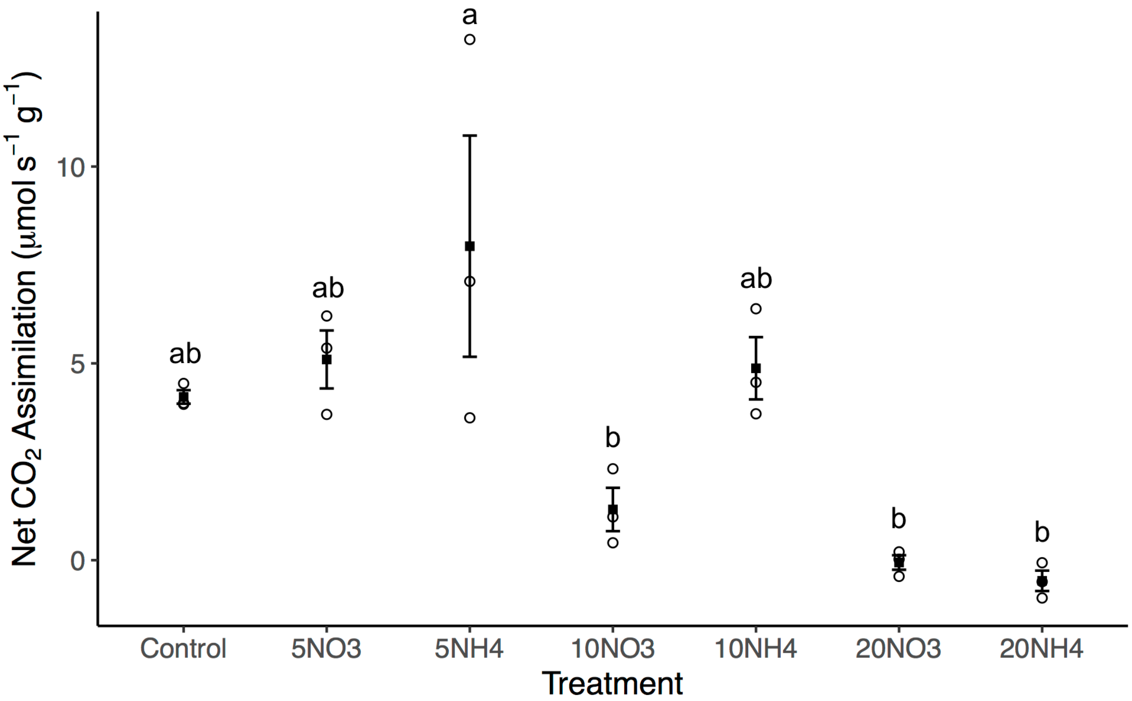

Figure 10. **The effects of fertilization treatment on net CO_2 assimilation rate (μmol s^{-1} g^{-1}). Black squares and error bars represent the mean values for each treatment ± SE (n=3). White circles represent the measured values for each replicate plot within the treatment. Letters represent the results of Tukey's HSD *post-hoc* test, treatments with the same letter are not significantly different ($p<0.05$).**

In addition to the direct effects of increased N availability, photosynthesis and gas exchange in *Sphagnum* species are highly dependent on capitulum water content (Schipperges and Rydin 1998). As such, the in-situ WC of the samples likely explained some of the variation in mean CO_2 assimilation rates between treatments, which can be examined by looking at the relationship between in-situ WC and assimilation rates for each treatment (Figure 11). The effect of WC on assimilation rates is most marked for the 10NO3 treatment, which (other than the 20-level treatments) had substantially lower assimilation rates and water contents than the other treatments. The expected relationship between WC and CO_2 assimilation is a unimodal optimum curve with maximum assimilation rates occurring between ~700-1300% (Schipperges and Rydin 1998). A visual examination of Figure 11 indicates that 5NH4, and to a small extent Control, are the only treatments in which assimilation rates increase as WCs approach optimum levels. The 5NO3 and 10NH4 treatments do not exhibit any increases in assimilation rate with increasing in-situ WC, while 10NO3, 20NO3, and 20NH4 are limited by in-situ WCs below optimum levels (<500%).

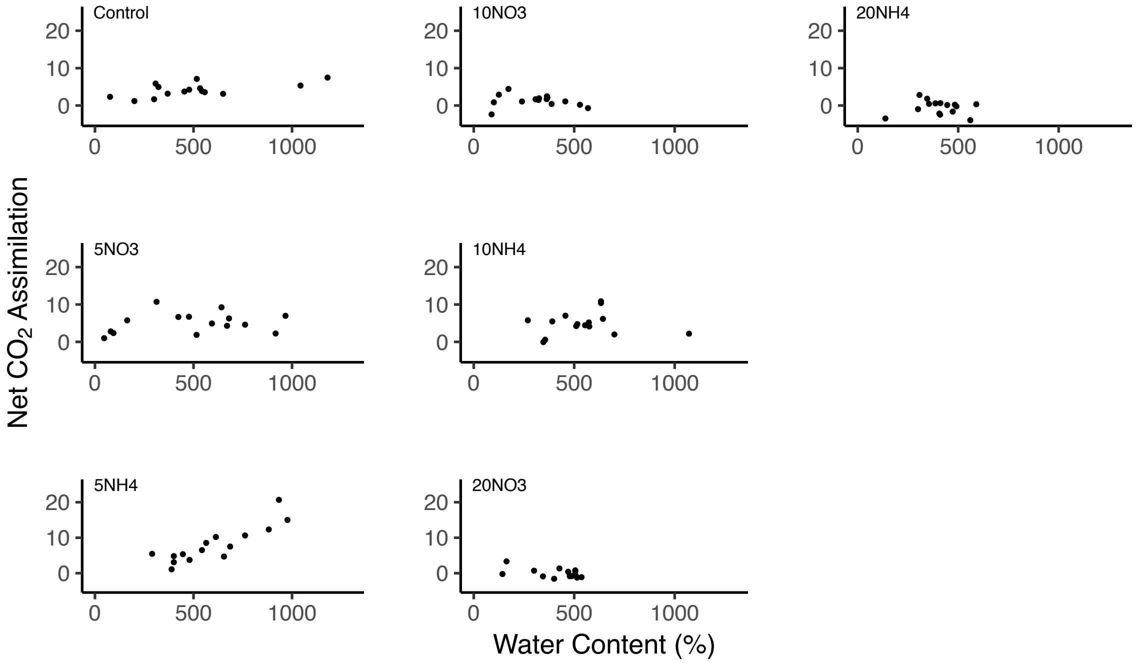

Figure 11. **Scatter plots of the net CO_2 assimilation values and associated in-situ WCs for individual observation points for each fertilization treatment. The visual relationship of the data points demonstrates the interactive effects of treatment and WC on CO_2 assimilation rates.**

3.3. Dissolved Nutrients

Total Nitrogen and NPOC

As would be expected under artificially increased nitrogen deposition rates the cumulative TN concentrations generally increased with increasing treatment concentrations (Figure 12); however, most of these differences were not significant (n=20, df=6,13, F=3.63, p=0.025). The sole significant difference in mean TN concentrations in the leachate was the 10NH4 treatment, which had concentrations 314% higher than the Control treatment (p=0.032) (Table 3). Interestingly, cumulative TN concentrations peaked in the 10NH4 treatment, with both 20-level treatments exhibiting lower concentrations. The 20NH4 mean TN concentration was markedly lower than the 20NO3 treatment, and even the 10NO3 and 10NH4 treatments. Additionally, one sample of the 5NH4 treatment from week 2 was lost due to equipment failure in the lab, thus for this treatment only two replicates were used in analysis.

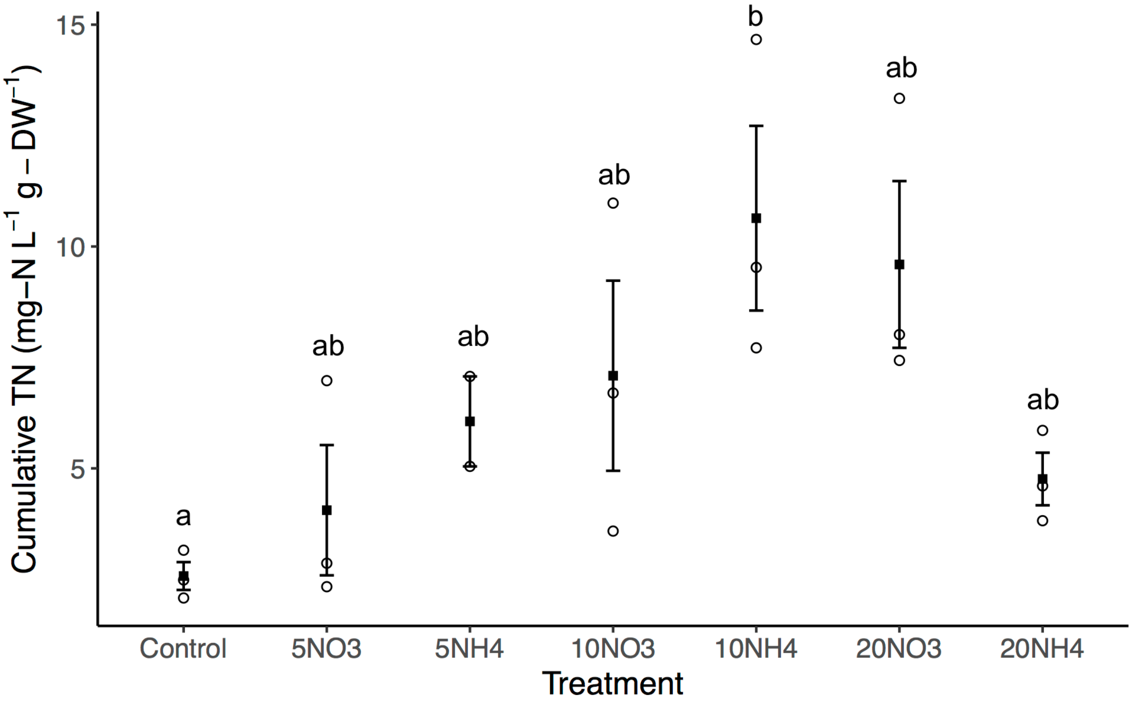

Figure 12. **The effects of fertilization treatment on cumulative TN concentration (mg-N L^{-1} g-DW^{-1}) in leachate samples. Black squares and error bars represent the mean values for each treatment ± SE (n=3). White circles represent the measured values for each replicate plot within the treatment. Letters represent the results of Tukey's HSD *post-hoc* test, treatments with the same letter are not significantly different (p<0.05). Note that 5NH4 only has two replicates due to an error in the lab.**

The fertilizer treatments had little effect on the NPOC present in the leachate samples with all mean cumulative NPOC concentrations observed within ~12 mg L^{-1} g^{-1} of each other (n=20, df=6,13, F=3.20, p=0.037). The exception is the 10NH4 treatment, which had a mean NPOC concentration 89% greater than the Control (p=0.063), though this is the result of one replicate in this treatment having a concentration 60.11 mg L^{-1} g^{-1} higher than the next closest replicate (Table 3). The only significant difference was between the 10NH4 and 10NO3 treatments (Figure 13). As NPOC and TN analyses were run on the same samples, only two replicates were used in the analysis of the NH4 treatment.

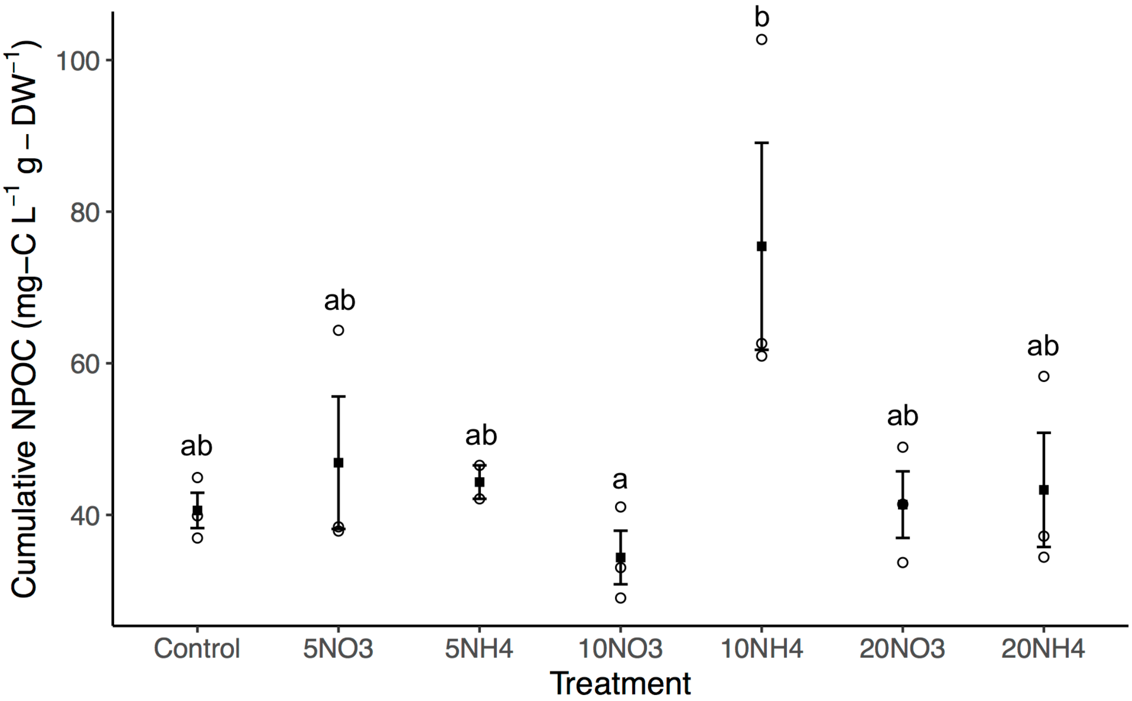

Figure 13. **The effects of fertilization treatment on cumulative NPOC concentration (mg-C L^{-1} g-DW^{-1}) in leachate samples. Black squares and error bars represent the mean values for each treatment ± SE (n=3). White circles represent the measured values for each replicate plot within the treatment. Letters represent the results of Tukey's HSD *post-hoc* test, treatments with the same letter are not significantly different ($p<0.05$). Note that 5NH4 only has two replicates due to an error in the lab.**

SUVA$_{254}$

SUVA$_{254}$ provides the average absorptivity for all the molecules comprising the dissolved organic carbon (DOC) fraction of the leachate sample, and is used as a proxy measurement of DOC aromaticity (Weishaar et al. 2003, Hansen et al. 2016). The SUVA$_{254}$ measurements from the end of the incubation experiment (week 12) were used to compare treatment effects. Fertilization treatment did not have a significant effect on SUVA$_{254}$ of the leachate samples (n=21, df=6,14, F=1.58, p=0.23). There was an apparent negative trend in SUVA$_{254}$ values as nitrogen concentration increased to the 10- and 20-level treatments for both NO3 and NH4, though the variability in the data is quite high making it difficult to ascertain a treatment effect. The 5NH4 treatment had the highest mean SUVA$_{254}$, and therefore highest aromaticity, representing a 19% and 27% increase relative to the Control and 5NO3 treatments, respectively.

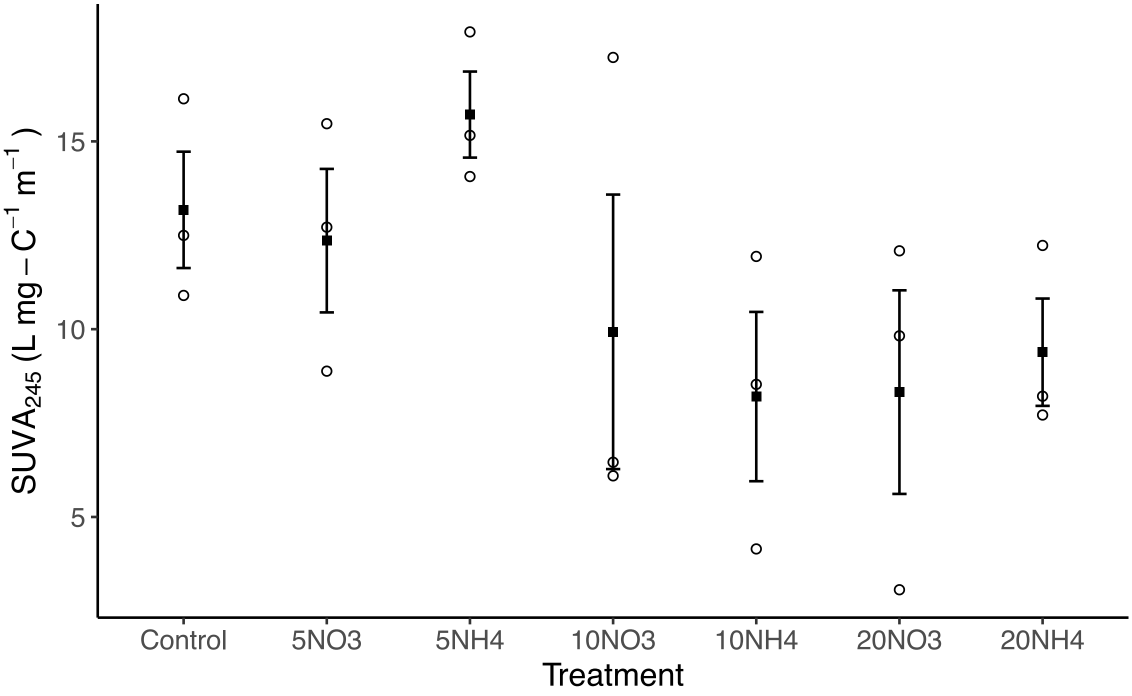

Figure 14. The effects of fertilization treatment on SUVA$_{254}$ (mg-N L^{-1} g-DW^{-1}) of leachate samples. SUVA$_{254}$ is a positively correlated proxy measure of DOC aromaticity. Black squares and error bars represent the mean values for each treatment ± SE (n=3). White circles represent the measured values for each replicate plot within the treatment. Note that 5NH4 only has two replicates due to an error in the lab. One-way ANOVA reported no significant differences between fertilization treatments (p=0.05).

Chapter 4. Discussion and Implications for ER

4.1. Discussion

Morphology

The nitrogen treatments had distinctive effects on the individual and community morphology of *S. capillifolium* in the fertilization plots at Mer Bleue, most markedly at the 10- and 20-level treatments. The FW and DW per capitula data reflects changes in morphology at the level of individual capitula. For both nitrate and ammonium treatments the general trend of decreasing FW per capitulum was associated with an increase in DW per capitulum as nitrogen deposition concentrations increased. Van der Heijden et al. (2000) hypothesized that in response to long-term elevated nitrogen deposition rates a reduction in tissue FW without an associated decrease in DW indicated adverse effects on capitulum morphology via a reduction in hyaline-to-chlorophyllose cell volume ratio. This suggests that the increased available nitrogen at the 10- and 20-levels would reduce the size of hyaline cells, which play an important role in water storage. However, Manninen et al. (2011) concluded that one mechanism by which *S. capillifolium* responded to long-term nitrate fertilization at high concentrations (5.6 g m^{-2} yr^{-1}) was by allocating excess nitrogen to cell-wall proteins, resulting in larger hyaline cells. In this study, 10NO3 and 20NO3 had reduced FWs per capitulum compared to the Control, 5NO3, and 5NH4 (though not statistically significant), while also exhibiting the highest DWs per capitulum of all the treatments. This suggests that contrary to the findings of Manninen et al. (2011), the nitrogen from nitrate uptake and assimilation was having an adverse effect on the cell morphology-associated water storage in the samples. In the context of the supporting literature, the FW and DW trends indicate that additional available nitrogen was used for biomass production, but in areas of the individual that play a limited role in water retention and storage (Limpens and Berendse 2003). However, without analysis of cellular structure in the Mer Bleue samples, changes in cell morphology cannot be directly observed.

While not a quantifiable measure of individual morphology, a qualitative observation of the samples indicated that 10NO3 and 20NO3 appeared to produce thicker stems but shorter spreading and pendent branches, whereas samples from

Control and both 5-level treatments had larger and more numerous branches. This suggests that the nitrogen deposition level of 10NO3 (3.2 g-N m^{-2} yr^{-1}) exceeded the load at which *Sphagnum* can sequester and incorporate the available nitrogen into the capitulum and photosynthetic apparatus, and that the excess nitrogen is being shifted to biomass production and N-rich free amino acid storage in the stem. This shift in nitrogen allocation has been observed in the scientific literature and is posited as a metabolic adaptation mechanism to prevent damaging accumulation of nitrogen in the capitulum (Baxter et al. 1992, Limpens et al. 2003, Bonnett et al. 2010). This corresponds to the findings of Nordin and Gunnarsson (2000) who observed that NH_4NO_3 fertilization at loads ≥ 3 g-N m^{-2} yr^{-1} resulted in increased amino acid concentrations, including asparagine – an N-rich free amino acid mainly responsible for nitrogen storage in stem tissue, in three *Sphagnum* species.

Numerous studies have shown that *Sphagnum* growth and morphology is negatively affected at critical loads >1.5 g-N m^{-2} yr^{-1} (e.g., Gunnarsson and Rydin 2000, Lamers et al. 2000, Bragazza et al. 2004). *Sphagnum* tissue has been shown to reach a saturation concentration threshold for toxic damage at ~13 mg-N g-DW^{-1} and a maximum saturation concentration of ~20 mg-N g-DW^{-1}, corresponding to long-term deposition rates of 1.8 and 3 g-N m^{-2} yr^{-1}, respectively (Lamers et al. 2000, Berendse et al. 2001). Van der Heijdan et al. (2000) proposed a tissue nitrogen concentration of 15 mg-N g-DW^{-1} as a bioindicator for nitrogen pollution stress. The storage of excess nitrogen in N-rich free amino acids takes energy and carbon resources that could otherwise be applied to growth, which suggests a possible threshold at which free amino acid concentrations will negatively impact growth (Baxter et al. 1992). Another hypothesized mechanism for the toxic effect on *Sphagnum* at high nitrogen deposition rates is that in response to a significant increase in loading rates the accumulation of toxic free NH_4^+ ions in cells could occur due to the uptake rate exceeding the ammonium assimilation rate, thus leading to morphological damage and decreased growth (Limpens and Berendse 2003).

In 2015, Živković et al. (unpublished manuscript) analyzed tissue N concentrations for samples from the same fertilization plots at Mer Bleue used in this study. The tissue N concentrations in the fertilization plots for 20NH4 (16 mg-N g-DW^{-1}) and 20NO3 (13 mg-N g-DW^{-1}) are at or above the saturation threshold for toxic damage in *Sphagnum* (Lamers et al. 2000, Van der Heijden et al. 2000, Berendse et al. 2001).

The evidence for toxic damage in these treatments was clear from visual inspection of samples, as well as the net CO_2 assimilation and WC results presented in this study. The 20NH4 (-0.52 ± 0.26 μmol s^{-1} g^{-1}) and 20NO3 (-0.058 ± 0.19 μmol s^{-1} g^{-1}) treatments had negative net CO_2 assimilation rates and significantly reduced maximum WC, suggesting the much of the *S. capillifolium* in these plots was severely degraded, if not dead and decomposing. Deposition rates of this magnitude substantially and deleteriously affected the physiology and morphology of the *Sphagnum* at this site. Therefore, because the focus of this research is the potential application of nitrogen fertilization to improve ombrotrophic bog restoration success, these treatments will largely be excluded from the remainder of the discussion.

The 10NH4 and 20NH4 treatments, which were subjected to the same amount of available nitrogen as the corresponding NO3 treatments, had substantially lower DW per capitula, and exhibited more visual physical damage. The more toxic effect of ammonium on *Sphagnum* compared to nitrate is well documented in the scientific literature (e.g., Press et al. 1986, Gunnarsson and Rydin 2000). The disparate effects of NO3 and NH4 at the 10- and 20-levels was likely a result of the inhibitory effect of ammonium on the nitrate reductase enzyme responsible for the reduction of nitrate to ammonium for uptake by the plant tissue (Press and Lee 1982, Woodin and Lee 1987). As such, the FW and DW results per capitulum and qualitative observations of the samples suggest that the 10NO3 and 20NO3 levels of nitrate deposition have resulted in a sufficient concentration of ammonium in the tissue to inhibit nitrate reductase activity and reduce nitrogen uptake rates compared to the 10NH4 and 20NH4 treatments. This is supported by the fact that at both the 10- and 20-level treatments, tissue N concentrations were significantly lower for NO3 than NH4, whereas there was no difference between the 5NH4 and 5NO3 treatments (Živković et al. unpublished manuscript). These results support the evidence in the scientific literature for reduced uptake rates due to the inhibition of nitrate reductase enzymes at only high levels of nitrate availability compared to ammonium (Jauhiainen et al. 1998).

Tissue N-concentrations for 10NO3 and 10NH4 were lower, but not significantly, than the respective 20-level treatments (Živković et al. unpublished manuscript), and the 10-level plots did not exhibit the same level of toxic damage as the 20-level plots in this study. Tissue concentrations were significantly increased in the 5NH4 (9.4 mg-N g-DW^{-1}) and 5NO3 (9.8 mg-N g-DW^{-1}) treatments compared to the Control (7.1 mg-N g-DW^{-1}), but were well below the toxic damage threshold (Živković et al. unpublished manuscript). The 5-level (1.6 g-N m^{-2} yr^{-1}) and 10-level (3.2 g-N m^{-2} yr^{-1}) fertilization treatments are at or just above the critical loads for *Sphagnum* growth discussed above, and therefore the physiological and morphological responses to these treatments are the most pertinent.

The effects of increased nitrogen availability on morphology and growth extend beyond the individual level and influences population density and growth patterns (Bragazza et al. 2004, Bubier et al. 2006, Wiedermann et al. 2009). The high variability in the data and relatively small sample sizes reduced the statistical power of the data analysis in this study. However, the observed trends in biomass density data in this study (though not statistically significant) are supported by the identification of critical thresholds of nitrogen tissue concentration and deposition rates in the scientific literature above which additional nitrogen ceases to be beneficial and begins to impart deleterious effects on *Sphagnum*. The biomass density data suggest a negative correlation between biomass density and nitrogen load. Notably, 5NH4 had a greater mean biomass density than the Control treatment. If the difference in density between 5NH4 and Control could be identified as a significant treatment effect through further analysis with greater statistical power, the change would have biologically significant implications as an additional 9000-10000 capitula in a 1 m^2 area would affect the water-retention capability in these plots. Nonetheless, at worst the 5NH4 treatment is exerting a neutral effect on biomass density compared to the Control. As such, the trends in the biomass density data indicate that an ammonium load of 1.6 g-N m^{-2} yr^{-1} does not exceed the critical load for nitrogen sequestration in *S. capillifolium* at Mer Bleue. The reduction of biomass density at the 10- and 20-level treatments corresponds to the findings of Bragazza et al. (2004), who observed that increased nitrogen deposition (>1 g-N m^{-2} yr^{-1}) decreased the stem volumetric density in hummock *Sphagnum* species in ombrotrophic bogs in Europe. The fact that the biomass density and DW per capitulum trends are inversely related in response to increasing nitrogen levels (i.e., DW increases while biomass density decreases) suggests that changes in biomass per unit volume is dictated by changes in growth density rather than increased or decreased DW associated with changes in individual morphology. As such, in the context of the supporting literature the trends in the data suggest that increased levels of nitrogen deposition influenced *Sphagnum* growth and morphology on an individual and population level.

The effect of treatment on growth and morphology did not extend to observed differences in elevations of the fertilization plots as there was substantial variability in the data. Mer Bleue, like most ombrotrophic bogs, is characterized by a hummock-hollow microtopographical mosaic, and it is likely that any differences in elevations were a result of this natural variability (Titus et al. 1983, Rydin and Jeglum 2013, Hájek and Vicherová 2014). Additionally, the plots were not surveyed before or during the fertilization experiment and therefore any changes in response to the fertilization that may have occurred cannot be attributed to treatment effects. Regardless of treatment effect, the natural variation in microtopography likely exerts an influence on physiological functions through separation from the water table and the creation of water-availability gradients (Rydin 1993, Rice et al. 2008, Weston et al. 2015).

The nitrogen treatments influenced the individual morphology and growth density of *S. capillifolium* in the fertilization plots. The 10- and 20-level treatments, both NO3 and NH4, resulted in deleterious effects on capitulum morphology and biomass density compared to the Control due to increased tissue N concentrations and over-saturation of nitrogen assimilation mechanisms. While the variability in the data is large, the 5-level treatments appeared to have a neutral effect on individual and community morphology. In fact, the results suggest that the 5NH4 treatment may positively affect *Sphagnum* growth through increased growth densities. As such, it appears that a nitrogen load of 1.6 g-N m^{-2} yr^{-1} does not exceed the critical threshold for nitrogen saturation or morphological damage at the study site. The results of this study suggest that increased nitrogen availability creates morphological responses in *S. capillifolium* at Mer Bleue. However, irrespective of nitrogen availability, morphological characteristics exert an overriding influence on *Sphagnum* physiology mainly via changes in water availability in the *Sphagnum* layer (Hayward and Clymo 1982, Jauhiainen et al. 1998). Therefore, to properly investigate physiological responses of *S. capillifolium* in this study (i.e., water content and net CO_2 assimilation) the examination of the interactive effects nitrogen fertilization treatments and morphology on physiological function is necessary due to the overarching effects of morphology-associated water dynamics.

Physiology

All nitrogen treatments decreased max WC compared to the Control, suggesting that any level of increased nitrogen decreases the ability of *Sphagnum* tissue to hold water. However, the large variability in the data means that the differences in max WC between Control and the 5-level treatments were not significant. The maximum WC and water loss rate results can be linked to changes in individual morphology independent of plot-level confounding variables because these analyses were performed on groups of separated individual capitula in a lab setting. The decreases in max WC with increasing nitrogen loads supports the conclusions drawn from the individual morphology data that increased available nitrogen exerts a toxic effect on individuals and is incorporated into increased biomass in the stems, which do not play a significant role in water retention and storage. This is particularly evident for the 10NO3 treatment that had high DWs per capitulum and the lowest observed max WC (outside of the 20-level treatments). Along with hyaline cells, spreading and pendant branch length and density are the individual morphological traits that most influence water transport and storage and drought resistance in the *Sphagnum* layer (Hayward and Clymo 1982, Weston et al. 2017). As such, it is likely that the larger and more numerous branches observed in Control and 5-level samples overlap and form dense networks allowing for more effective water retention, storage, and transportation resulting in larger max WCs.

The water stored in and around *Sphagnum* tissue is divided into three water fractions: 1) external water, 2) cytoplasmic water in chlorophyllose cells, and 3) apoplastic water bound within cell walls, and water is lost sequentially from each fraction (i.e., the second fraction does not begin to lose water until the first fraction has been depleted) (Hajek and Beckett 2008). The first fraction (external water) can be subdivided into excess water held in the capillary spaces between branches and water held in hyaline cells, and it can comprise upwards of 70% of the total water content in hummock species (Hajek and Beckett 2008). The water loss rate in this study was directly correlated to the associated max WC for each treatment, which implies that the observed differences in max WC were due to increases in the holding capacity of external water (i.e., the most readily available for loss). However, without sophisticated analysis techniques it is often difficult to distinguish whether the increase in external water content is a result of more water being held in capillary spaces or in the hyaline cells (Hajek and Beckett 2008). The results of this study are likely a result of an increase in both sub-fractions. This evidence also suggests the slower water loss rates observed at the 10- and 20-level treatments do not indicate a better ability to retain stored water, but rather a reduced ability to hold water in the external fraction resulting in less water available for loss. The individual morphologies of the Control, 5NO3, and 5NH treatments exhibited the structural characteristics necessary to regulate capillarity, which is the most important mechanism to replace water lost to evaporation at the surface (Thompson and Waddington 2008, Strack and Price 2009).

It is difficult to isolate the effect of treatment on changes to in-situ WC in the fertilization plots due to potential plot-scale confounding variables such as growth density and microtopography (i.e., depth to water table), both of which can in turn be affected by nitrogen fertilization (Bragazza et al. 2004, Fritz et al. 2014). Three of the four treatments with the highest in-situ water contents (Control, 5NO3, and 5NH4) also have the highest biomass densities, suggesting that for these treatments in-situ WC was influenced by higher growth densities. It is well established in the scientific literature that decreased growth density decreases the density of the stem and branch matrix that creates capillarity in the *Sphagnum* layer, and therefore reduces the effectiveness of vertical and horizontal water transport, resulting in decreased water contents in the capitula (Bragazza et al. 2004, Thompson and Waddington 2008, Strack and Price 2009). The available data conforms to patterns observed in the scientific literature suggesting a correlation between nitrogen deposition rates and growth density, and a concomitant link between growth density and in-situ WC. For the Control, 5NO3, and 5NH4 treatments the combination of fertilization treatment and growth density suggests a positive synergistic effect on water retention, storage, and availability.

Surface moisture availability is increased when the water table is closer to the surface of the *Sphagnum* layer because conditions are ameliorated for capillary transport via increased pore-water pressure (Thompson and Waddington 2008). Increased nitrogen deposition can affect microtopography via changes in growth rates; however, without pre-fertilization data it cannot be determined whether the observed variation in microtopography is due to natural variability or to the fertilization treatments (Nordin and Gunnarsson 2000, Granath et al. 2009). The high in-situ WC and low biomass density of the 10NH4 treatment is evidence that growth density was not the sole variable controlling water availability in the *Sphagnum* layer. The 5NO3, 5NH4, and 10NH4 treatments had the lowest elevations, indicating increased proximity to the water table, and relatively high in-situ WCs suggesting that microtopography was a controlling factor for these treatments, and a substantial controlling factor for 10NH4 overriding the effect of low biomass density. Conversely, the higher elevations in the Control plots compared to the 5NO3, 5NH4, and 10NH4 plots indicates that individual morphology, growth density, and capillary transport were able to retain and transport water and maintain sufficient surface moisture availability in the Control plots. The data suggest that in-situ WC was influenced by both growth density and microtopography, independently and through combined effects. Interestingly, it appears that neither growth density nor microtopography can explain the low in-situ WC of the 10NO3 treatments, suggesting it was the observed changes in individual morphology controlling water dynamics.

The results of this study indicate that at nitrogen deposition levels above the 5-level treatments the direct (i.e., nitrogen toxicity) and indirect effects (i.e., changes in morphology) of increased nitrogen availability negatively impacted the water dynamics of *Sphagnum* through a reduced ability to retain, store, and transport water through capillary and cellular mechanisms. Growth size and density, branching structure, and thick-walled hyaline cells are species-specific morphological adaptations that control water dynamics within *Sphagnum* carpets (Schipperges and Rydin 1998, Weston et al. 2017). Water dynamics (e.g., content, availability, transport, and loss) play an important role in photosynthesis, carbon assimilation, desiccation avoidance and tolerance, metabolism, biomass production, and nutrient uptake, assimilation, retention, and translocation mechanisms (Schipperges and Rydin 1998, Aldous 2002a, 2002b, Limpens and Berendse 2003, Hajek and Beckett 2008, Fritz et al. 2014). Morphological adaptations maintain sufficient water content in the capitula necessary for photosynthesis, as water availability is critical for carbon assimilation and other physiological functions in *Sphagnum* (Schipperges and Rydin 1998, Limpens and Berendse 2003).

The net CO_2 assimilation rate results in the fertilization plots at Mer Bleue corresponded to the unimodal optimum curve trend observed in the scientific literature for CO_2 assimilation response to increased nitrogen deposition loads (Van der Heijden et al. 2000, Granath et al. 2009, 2012). Similar to other studies, the 5NO3 (5.099 μmol s^{-1} g^{-1} ± 0.74) and 5NH4 (7.98 μmol s^{-1} g^{-1} ± 2.81) treatments had increased CO_2 assimilation rates compared to the Control (4.14 μmol s^{-1} g^{-1} ± 0.17), which was likely a response to the increased available nitrogen being incorporated into the photosynthetic apparatus (Granath et al. 2009). It is well documented that under slightly elevated nitrogen deposition levels *Sphagnum* continues efficient nitrogen assimilation via an increase in growth and biomass production (Gunnarsson and Rydin 2000, Lamers et al. 2000, Bragazza et al. 2004, Granath et al. 2009). One mechanism by which this occurs is the incorporation of increased glutamine availability to increase the chlorophyll content of chlorophyllose cells, thereby enhancing photosynthetic capacity in the capitula (Van der Heijden et al. 2000, Limpens and Berendse 2003). Meanwhile, the observed assimilation rates were lower for the 10-level treatments, suggesting that the deposition load of 3.2 g m^{-2} yr^{-1} exceeded the critical load at this site and increased toxic free NH_4^+ ion and/or N-rich free amino acid concentrations have impaired physiological functions (Baxter et al. 1992, Nordin and Gunnarsson 2000, Limpens and Berendse 2003). The observed increased CO_2 assimilation rate of 5NH4 is well supported in the literature, and suggests a positive direct effect of fertilization at this level of deposition (Van der Heijden et al. 2000, Granath et al. 2009, 2012). However, the photosynthetic rate of *Sphagnum* is highly dependent on tissue water content, meaning that the in-situ WC of the samples likely influenced assimilation rates independent of treatment (Schipperges and Rydin 1998, Granath et al. 2009).

Schipperges and Rydin (1998) demonstrated that the response of CO_2 assimilation rate to water content was a unimodal optimum curve for four *Sphagnum* species, including *S. fuscum*. The in-situ WC of the majority of samples for all treatments in this study fell below the optimal WC range for CO_2 assimilation (719-1300%) observed by Schipperges and Rydin (1998), and therefore WC likely exerted an influence on net CO_2 assimilation rates irrespective of treatment. Assimilation rates initially increase with increasing WC because sufficient water availability is necessary to facilitate CO_2 assimilation and maintain cell turgidity, whereas assimilation rates at higher water contents begin to decrease as cells saturate and the diffusion resistance increases for CO_2 (Schipperges and Rydin 1998, Hajek and Beckett 2008). The low in-situ WC observed for 10NO3 likely exerted a substantial influence on assimilation rates. However, at similar water contents (400-500%) 10NO3 assimilation rates were lower than Control, 5NH4, and 5NO3 rates, which suggests that an interaction of fertilization treatment and WC was influencing assimilation rates.

Conversely, interaction between treatment and WC exhibited a positive synergistic effect on 5NH4 assimilation rates. In addition to having the highest overall rate of net CO_2 assimilation, 5NH4 also had higher assimilation rates at water contents approaching optimum levels (>500%) compared to the Control and 5NO3. The substantial differences between CO_2 assimilation rates of 5NH4 and 5NO3 at higher WCs (500-1000%) suggests that the type of nitrogen imparted differential impacts on photosynthetic physiology. The tissue N concentrations for 5NH4 and 5NO3 were essentially identical, indicating that the observed differences are not due to differential rates of uptake nor a reduction in nitrate reductase activity. Rather, it is possible that the assimilation mechanisms differentially allocated nitrogen to different tissue areas within the two treatments and that more nitrogen was allocated to the photosynthetic apparatus in 5NH4. There have been studies that have examined differences in uptake and assimilation rates between ammonium and nitrate, but these studies have not involved separate application of one nitrogen type without the other (e.g., Jauhiainen et al. 1998, Fritz et al. 2014). Therefore, the preferential uptake of ammonium over nitrate (of an order of magnitude greater) when both are available has been observed, but potential differences in allocation mechanisms when ammonium and nitrate are isolated remain undocumented (Woodin and Lee 1987, Jauhiainen et al. 1998, Fritz et al. 2014). Overall, it is likely nitrogen deposition rate exerted a direct effect (i.e., changes in N-tissue concentrations) and an indirect effect (i.e., changes in WC due to treatment-associated changes in morphology) on assimilation rates across all treatments (Van der Heijden et al. 2000).

Nutrient Dynamics

In addition to influencing short-term nutrient uptake, assimilation, and allocation mechanisms, it is well established that morphological characteristics and the associated water storage and availability in the *Sphagnum* layer play a substantial role in longer-term nutrient retention, sequestration, and cycling in ombrotrophic bogs (Clymo 1963, Jauhiainen et al. 1998, Aldous 2002a, 2002b, Rice et al. 2008). The incubation experiment results provide insight into the immobilization and retention during tissue senescence and decomposition of the nitrogen captured and assimilated by the “apical” and “secondary” nets of the *Sphagnum* layer (Jauhiainen et al. 1998, Aldous 2002a, 2002b, Bragazza et al. 2012). There was a substantial increase in TN concentrations in leachate samples as nitrogen deposition levels and tissue concentrations increased across the Control, 5-level, and 10-level treatments. The increasing trend in leachate TN concentrations suggests that only a relatively small portion of assimilated nitrogen can be immobilized, and that increases in tissue N concentration (e.g., from Control (7.1 mg-

N g-DW^{-1}) to 5NH4 (9.4 mg-N g-DW^{-1})) resulted in increases in nitrogen release during decay processes. Nitrogen fertilization (3 g-N m^{-2} yr^{-1}) resulted in N release whereas nitrogen was immobilized in a control treatment during the first year in a study by Bragazza et al. (2012), which supports the trends observed in this study.

The 10NH4 treatment released the highest concentrations of TN in the leachate and had the lowest biomass density, suggesting that growth density also affected the amount of nitrogen released and lost during precipitation events (simulated by leaching in this study). It is well established that nitrogen can be translocated from senescing tissue and re-adsorbed in upper parts of the individual, but these mechanisms require sufficient water availability and retention for uptake processes to occur (Jauhiainen et al. 1998, Aldous 2002a, 2002b). However, decreased growth density decreases the water retention capacity per unit area, resulting in precipitation and extracted dissolved nitrogen moving through the *Sphagnum* layer more quickly and reducing the exposure time required for the re-adsorption of the nitrogen (Jauhiainen et al. 1998, Bragazza et al. 2004, Thompson and Waddington 2008). The increased release and loss of stored nitrogen during tissue decomposition increases the existing pool of nitrogen available in the *Sphagnum* layer and rhizosphere that is the result of the leaching of excess nitrogen that is not taken up or assimilated by *Sphagnum* tissue once saturation thresholds have been exceeded. At higher concentrations, deposited nitrate is particularly susceptible to leaching compared to ammonium due to the inhibition of the nitrate reductase enzyme and reduced uptake rates, as evidenced by the lower tissue concentrations of the NO3 treatments at the 10- and 20-level (Živković et al. unpublished manuscript, Woodin and Lee 1987, Jauhiainen et al. 1998). Increased available nitrogen in the *Sphagnum* layer and rhizosphere can result in changes in nutrient limitation, nutrient cycling, microbial communities, and vegetation composition (Limpens et al. 2003, 2004, Bragazza et al. 2004, 2012, Bubier et al. 2007).

The vascular vegetation in the fertilization plots at Mer Bleue is clipped during the growing season meaning that potential changes in vascular vegetation abundance due to increased nitrogen availability cannot be quantified. However, the literature suggests that long-term levels of increased nitrogen deposition (>1.5-2 g-N m^{-2} yr^{-1}) and availability in the rhizosphere reduces nitrogen limitation for vascular plants, resulting in increased abundances of vascular vegetation and an associated decline in *Sphagnum* density and cover due to direct effects of nitrogen toxicity and the indirect effect of shading by vascular plants (Tomassen et al. 2004, Wiedermann et al. 2007, Bubier et al. 2007, Juutinen et al. 2016). The consensus in the scientific literature identifying deposition thresholds for changes in vegetation composition suggests that in the absence of manual clipping the 10- and 20-level treatments would result in increased vascular plant abundance in the fertilization plots, in part due to changes in nutrient limitation and cycling.

Increased nitrogen availability changes the nutrient stoichiometry and cycling mechanisms in *Sphagnum* tissue, porewater, and peat (Koerselman and Meuleman 1996, Bragazza et al. 2012, Fritz et al. 2012, Kox et al. 2016). Živković et al. (unpublished manuscript) determined that the nitrogen-to-phosphorus (N:P) ratio in *Sphagnum* tissue increased from <14 (N-limited) in the Control plots to >16 (P-limited) for each of the fertilization treatments, with the ammonium treatments having a more marked effect. The increases in N:P ratios were accompanied by a decrease in N_2-fixation rates to ecologically irrelevant amounts, even at the 5-level treatments, due to changes in *Sphagnum*-associated diazotrophic microbial activity (Živković et al. unpublished manuscript). Increased concentrations of ammonium and glutamate in *Sphagnum* tissue inhibits the expression of the nitrogenase enzyme, the catalyst for biological N_2 fixation (Živković et al. unpublished manuscript, Berg et al. 2013, Kox et al. 2016). Despite the virtual elimination of N_2 fixation, the net primary production (NPP) of the 5NH4 treatment did not exhibit a significant decrease relative to the Control (Živković et al. unpublished manuscript). This suggests that the deposited nitrogen replaced the fixed nitrogen as a source to maintain metabolic and physiological requirements for primary production, a process that is supported in the literature (Živković et al. unpublished manuscript, Berg et al. 2013, Vile et al. 2014, Kox et al. 2016). However, the NPP of the 5NO3 and 10NH4 treatments were significantly reduced compared to the Control, and NPP was strongly correlated to increases in N:P ratio (Živković et al. unpublished manuscript). 10NH4 released the highest concentration of leachate nitrogen in this study, and had the highest N:P ratio and lowest NPP of all treatments, which suggests that the leached nitrogen from *Sphagnum* tissue was associated with changes in nutrient limitation, nitrogen cycling pathways, and primary production processes in the fertilization plots (Živković et al. unpublished manuscript).

Increased N also affects nutrient stoichiometry and microbial activity associated with carbon cycling and decomposition processes. The NPOC concentrations in the leachate samples were similar except for the 10NH4 treatment, which was substantially higher than all the other treatments. The singularity of the 10NH4 NPOC results combined with this treatment releasing the highest concentration of TN, suggests the existence of a threshold above which the additional availability of nitrogen resulted in stimulated decomposition processes. Increased NPOC loss under high levels of N deposition (2-3 g m^{-2} yr^{-1}) has been attributed to the enhanced metabolic activity of microbes and changes in the carbon-to-nitrogen (C:N) ratio in *Sphagnum* tissue and peat (Bragazza et al. 2006, 2012). Increased nitrogen availability ameliorates tissue and peat chemistry for degradation by decreasing the C:N ratio, thereby reducing the recalcitrance of stored carbon and increasing respiration (Gorham 1991, Limpens and Berendse 2003, Bragazza et al. 2006, Gerdol et al. 2007, Manninen et al. 2011, Bragazza et al. 2012). The increase in available nitrogen alleviates microbial N-limitation and stimulates bacterial biomass compared to fungi, thus enhancing the metabolic production of hydrolytic enzymes and enhancing decomposition and nutrient cycling (Bragazza et al. 2006, 2012). However, there is some evidence in the literature that the carbon and nitrogen cycling processes are decoupled and that chemical and morphological characteristics (e.g., water availability, pH, and ratio of metabolic-to-structural carbohydrates) are the primary driving factors for decomposition in ombrotrophic bogs (Turetsky et al. 2008, Manninen et al. 2016).

The SUVA data indicate that increased nitrogen deposition and availability may result in increased lability of the NPOC released during leaching events. Though there is substantial intra-treatment variability, the 10- and 20-level treatment appeared to decrease the SUVA in leaching samples compared the Control and 5-level treatments. SUVA is an identified proxy measure for aromaticity of dissolved organic compounds, exhibiting a positive linear correlation (Weishaar et al. 2003, Hansen et al. 2016). As such, lower SUVA values indicate reduced aromaticity, which in turn indicates increased lability of the NPOC due to higher oxidative degradation (Weishaar et al. 2003, D'Andrilli et al. 2010, Hansen et al. 2016). This is consistent with the findings of Bragazza et al. (2012) who observed a significant decrease in SUVA for plots fertilized with 3 g-N m^{-2} yr^{-1} compared to control plots. Interestingly, 5NH4 had a higher SUVA (15.71 ± 1.15 L mg-C^{-1} m^{-1}) than both the Control (13.18 ± 1.55 L mg-C^{-1} m^{-1}) and 5NO3 (12.36 ± 1.91 L mg-C^{-1} m^{-1}). If the repeatability of this result can be determined with greater statistical power, it would suggest that low levels of increased ammonium availability can increase the recalcitrance of the carbon being released during the decomposition process making it less available to microbes. Overall, the incubation results suggest that increased nitrogen deposition increased both the amount and lability of NPOC released above a threshold nitrogen concentration, which indicates an increased decomposition rate at the tissue N concentration and deposition load associated with the 10NH4 treatment.

4.2. Implications for ER

The results of this study, as well as insights from the supporting literature, demonstrate that the 10- and 20-level treatments (both NO3 and NH4) exceeded the critical deposition threshold at the Mer Bleue bog for *S. capillifolium* tissue saturation and toxic effects. The results suggest that these fertilization treatments exerted deleterious effects on individual morphology, growth density, water holding and retention capacity, CO_2 assimilation, and nutrient dynamics and decomposition. While these results provide interesting insight into the differential effects of ammonium and nitrate on biological and physiological mechanisms and contribute to the repository of information for theoretical ecology, the results for the higher levels of nitrogen deposition are not useful from a restoration perspective. The 5-level treatments appeared to have, at worst, a neutral effect on individual morphology and growth density. As such, the results suggest that a nitrogen load of 1.6 g-N m^{-2} yr^{-1} was either at or below the critical threshold for nitrogen saturation or toxic damage at Mer Bleue. The lack of significant differences between the Control and 5NO3 treatments for any of the morphological or physiological parameters examined in this study makes it difficult to extract any beneficial effects that may lead to increased capillarity and water retention in the *Sphagnum* layer. However, the observed increase in hyaline cell cross-sectional area and unique assimilation mechanisms reported in the literature justifies further research to clarify the effects of lower levels of nitrate fertilization on *Sphagnum* morphology and physiology.

The 5NH4 treatment exhibited the greatest potential for integration into ombrotrophic bog restoration actions due to potential beneficial morphological and physiological responses. The results of this study and similar observations in the literature suggest that fertilization with 1.6 g m^{-2} yr^{-1} of ammonium ameliorates water retention capacity through more robust individual morphology and denser growth patterns. Though the maximum WC of 5NH4 in the lab was lower than the Control, the biomass density and in-situ WC results for 5NH4 provides evidence for the formation of morphological growth characteristics conducive to maintaining water availability in the field. The synergistic effects of WC and increased nitrogen tissue concentration in the photosynthetic apparatus resulted in substantially higher CO_2 assimilation rates compared to the Control, without the trade-off of significant (statistically or biologically) increases in nitrogen leaching or NPOC loss. In fact, the SUVA data suggests that the NPOC released by 5NH4 fertilized *Sphagnum* may be more recalcitrant than that of the Control. While the combination of these results indicates beneficial morphological and physiological responses to low levels of ammonium fertilization, it is difficult to draw definitive conclusions due to high variability in the data. Nonetheless, the available literature supports the conclusion that low levels of increased nitrogen deposition (<1-1.5 g m^{-2} yr^{-1}) increases *Sphagnum* growth, productivity, photosynthetic capacity, and stem density, all of which create morphological and physiological characteristics suitable for increasing restoration success (Gunnarsson and Rydin 2000, Van der Heijden et al. 2000, Limpens and Berendse 2003, Granath et al. 2009, 2012).

The *North American approach* for the restoration of harvested and cut-over ombrotrophic bogs relies on the creation of suitable hydrologic conditions for the re-establishment of peat-accumulating functions and *Sphagnum*-dominated vegetation communities (Rochefort et al. 2003). The blocking of ditches and other drainage structures is a prerequisite for moss layer transfer to restore suitable water table levels (Rochefort et al. 2003, Schimelpfenig et al. 2014). Once that is accomplished restoration success hinges on creating suitable water retention and storage properties in the *Sphagnum* donor layer to prevent desiccation and provide sufficient water availability for vital metabolic and physiological functions (Rochefort et al. 2003, González and Rochefort 2014, Taylor and Price 2015). Current monitoring data indicates that *the North American approach* successfully re-established *Sphagnum*-dominated vegetation communities within 10 years of restoration in ~60% of sites, with insufficient moisture availability identified as a primary factor in sites that did not re-establish a viable *Sphagnum* layer (Rochefort et al. 2003, Poulin et al. 2013, González and Rochefort 2014). As such, there is a need for methods that can improve water retention and hydrophysical properties in the donor layer to increase restoration success and create more suitable conditions in a wider variety of sites. Small capillary pore sizes in the donor *Sphagnum* layer are essential for water retention, transport, and storage via increases in soil water pressure, capillary forces, and connectivity to the water table

(Price and Whitehead 2001, Lucchese et al. 2010, Taylor and Price 2015). Individual morphology and population growth patterns are key factors in creating the dense matrices of stem and branch material necessary for sufficient capillarity and restoration success (Hayward and Clymo 1982, Rice et al. 2008) .

Integrating low levels of ammonium fertilization (≤ 1.6 g-N m^{-2} yr^{-1}) into the *North American method* has the potential to create the morphological and hydrophysical characteristics necessary to improve water retention in donor *Sphagnum* layers, which is the main driver of bog restoration success. One potential long-term negative effect of low levels of nitrogen fertilization is the impact on N_2 fixation and diazotrophic cyanobacteria activity, which can account for up to 35% of all nitrogen assimilated by *Sphagnum* (Berg et al. 2013, Kox et al. 2016). As such, the elimination of N_2-fixating microbial activity at even the 5NH4 level of deposition and the reliance on deposited nitrogen for metabolic and physiological processes indicates a potential nitrogen deficit for *Sphagnum* once the fertilization treatment ceases. However, the evidence indicates that diazotrophic activity quickly reacts to changes in nitrogen availability, stimulating N_2 fixation once deposition levels decrease and nutrient cycling mechanisms soon return to a pre-deposition state (Živković et al. unpublished manuscript, Limpens and Heijmans 2008). The stimulation of growth and production of *Sphagnum* in response to increased nitrogen deposition has been observed to substantially decrease after two years of treatment (Gunnarsson and Rydin 2000, 2000, Bonnett et al. 2010). As such, one to two years of ammonium fertilization of the donor material has the potential to create a base layer that kick starts peat accumulating processes and prevents further destabilization of the peat carbon pool by creating sufficient water availability for metabolic and physiological functions, increasing CO_2 assimilation and biomass production, and limiting decomposition and carbon losses in restoration sites. The increased drought avoidance associated with a greater water-retention capacity would increase the ecological resilience of the restored areas allowing more rapid establishment of *Sphagnum*-dominated vegetation communities and the continuance of peat and carbon accumulating functions even during periods of stress and disturbance due to low water availability, increasing the chances of successfully restoring a net carbon accumulating ombrotrophic bog ecosystem.

More research is needed to determine the replicability of the results of this study and the viability and feasibility of incorporating ammonium fertilization into the *North*

American approach for several reasons. The results of this study are based off sample and data collection of one species from one site during one growing season in an anomalous summer for precipitation. Consequentially, there was high variability in all the data making it difficult to interpret differences between treatments and distinguish trends. A series of systematic, multi-year studies are necessary to adequately determine treatment effects on *Sphagnum* morphology and physiology at several different sites for comparisons of results and trends. Additionally, the morphological and physiological responses of *Sphagnum* to increased nitrogen loading are species specific, and therefore further studies on different species are necessary (Granath et al. 2012). Hummock species, particularly members of *Sect. Acutifolia*, are most suitable for restoration donor material due to an increased ability to colonize bare surfaces and increased intrinsic decay resistance due to the preferential allocation of resources to structural carbohydrates (Rochefort 2000, Turetsky et al. 2008). Compared to hollow species, hummock species (like *S. capillifolium*) generally have more efficient capillary transport and denser growth patterns resulting in increased water retention, water availability, and desiccation avoidance (Schipperges and Rydin 1998, Hajek and Beckett 2008, Weston et al. 2015). Finally, more research is needed on the effect of ammonium fertilization on *Sphagnum* establishment success, growth density and production, vascular vegetation abundance, and the feasibility of fertilizer delivery methods in large-scale restoration transplant studies.

This study provides a stepping point to instigate further research of the incorporation of ammonium fertilization into ombrotrophic bog restoration treatments. Increasing restoration success and the ecological resilience of restored sites is crucial for mitigating carbon loss and CO_2 emission from converted and degraded peatlands, which currently account for more than 10% of global CO_2 emissions (Roulet 2000, Lunt et al. 2010, Frolking et al. 2011). The establishment of resilient donor base layers during the restoration process that have increased water-retention capacities and drought-avoidance capabilities will be especially important for instigating and maintaining peat accumulating functions and processes in the face of warmer and drier summer conditions predicted by climate change models (Intergovernmental Panel on Climate Change 2014, Environment Canada; Coupled Model Intercomparison Project Phase 5). This study also provides an example of theoretical ecology research and results providing insight and knowledge pertaining to fundamental ecological processes and functions, and how the application of this knowledge can help move the science of restoration forward.

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