Are Plants Able to Utilize Nitrogen Released from Thawing Permafrost? Implications for Carbon Cycling and Feedback with the Climate System

by

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ABSTRACT

ARE PLANTS ABLE TO UTILIZE NITROGEN RELEASED FROM THAWING PERMAFROST? IMPLICATIONS FOR CARBON CYCLING AND FEEDBACK WITH THE CLIMATE SYSTEM

by Lucas Jacob Albano
University of Guelph, 2018

Climate warming in high-latitude regions triggers widespread permafrost thaw, releasing massive amounts of carbon and nitrogen that were previously frozen in soil organic matter, through increased microbial activity. Climate warming has motivated extensive research on permafrost carbon release; however, fewer studies have addressed whether plants can access new nitrogen sources, potentially increasing primary productivity.

Two research questions were explored using Carex aquatilis in sites experiencing thaw and thermokarst in interior Alaska: 1) does C. aquatilis ammonium uptake vary with depth and time-since-thaw; 2) does variation in C. aquatilis growth characteristics and ammonium uptake correlate with aboveground primary production? An ammonium uptake experiment was conducted on C. aquatilis roots, determining that deep roots took up equal if not greater amounts of ammonium than shallow roots. I also found that rooting depth was positively correlated with aboveground biomass, providing a plausible mechanism for increased N uptake post-thaw to impact aboveground plant productivity.
To Mom and Dad,

For straying off the pavement with me
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CHAPTER 1: INTRODUCTION

1.1 Overview of permafrost thaw and thermokarst in ice-rich wetlands

Permafrost is soil, rock, and/or aggradation sediment that has remained permanently frozen for a minimum of two consecutive years, and exists beneath a seasonally thawed layer, known as the active layer. However, in recent decades, climate warming as well as changes in disturbances, such as human land use or wildfire, are causing the acceleration of rates of permafrost thaw (Schuur et al., 2015). For example, currently approximately 85% of Alaska is underlain by permafrost (Hong et al., 2014); however, it is estimated that by 2100, near-surface permafrost extent state-wide could be reduced by 16-24% (Pastick et al., 2015). Permafrost thaw at this magnitude has the potential to lead to various changes in both the structure and function of tundra and boreal ecosystems. Using a projection known as the Permafrost Settlement Hazard Index (PSHI), Hong et al. (2014) determined that within the state of Alaska, the Alaskan interior is currently most at risk of the effects of extensive permafrost thaw. This is because the interior is largely dominated by discontinuous permafrost, which consists of areas where only 50-90% of the ground surface is underlain by permafrost (Melvin et al., 2016). In this region, surface ground temperatures often are only slightly below the freezing threshold (often around -1 °C; Osterkamp et al., 2000) and are projected to be likely to rise above that threshold by 2050, leading to their high susceptibility to permafrost thaw (Hong et al., 2014).

When ice-rich permafrost thaws, thermokarst causes localized land subsidence of typically around 1 to 2 m (Osterkamp et al., 2000). The formation of thermokarst terrain is often characterized by shifts in hydrology, whereby standing water is able to accumulate in the subsided areas of the ground surface, while the surrounding areas of higher ground remain drier (Baltzer et al., 2014; Osterkamp et al., 2009). The increased soil moisture of the newly-collapsed thermokarst
features leads to flooding stress of the shallow-rooted woody vegetation that occupies the soil above the stable active layer of permafrost, eventually resulting in mortality of the vegetation community that typically characterizes the boreal forest (Baltzer et al., 2014). These thermokarst features, often referred to as collapse scar bogs or thermokarst bogs, then become dominated by more deeply-rooted herbaceous plants, particularly graminoids like sedge species, as well as hollow-dwelling Sphagnum mosses, which are more equipped to tolerate the anoxic conditions caused by the higher water table in the newly-formed wetland system (Osterkamp et al., 2009; Figure 1). This vegetative community shift brings along a variety of changes in ecosystem functioning, including changes in nutrient cycling (Aerts et al., 1999), but also faster rates of decomposition and greater plant productivity (Turetsky et al., 2007), resulting in a vertical accumulation of decaying peat.

1.2 Introduction to thermokarst and brief overview of impacts to peatland carbon cycling

Currently, peatlands make up nearly 20% of the circumpolar permafrost region in the Northern Hemisphere (Tarnocai et al., 2009). Through peat accumulation over the past 8,000 to 10,000 years, northern peatlands have accumulated an estimated 1035 Pg of C in the first 3 metres of depth (Schuur et al., 2015). In permafrost in particular, the amount of stored carbon is more than twice the amount of C as is contained in the entire atmosphere (Abbott et al., 2016). Because of the strong insulation capability of peat, permafrost is able to persist in organic soils even when mean annual temperatures exceed 0°C (Shur and Jorgenson, 2007). For this reason, “ecosystem-protected” permafrost is strongly associated with the presence of thick organic soils, as is characteristic of northern peatlands (Shur and Jorgenson, 2007). Similar to permafrost thaw in other ecosystems, permafrost in peatlands can thaw from the top down (a deepening of the active layer), causing thawed permafrost organic matter to become exposed to warmer conditions,
leading to increases in microbial activity, which stimulates soil C mineralization (Hong et al., 2014; Osterkamp et al., 2009). The subsequent release of greenhouse gases due to microbial respiration, namely carbon dioxide (CO$_2$) and methane (CH$_4$), then potentially contributes to further climate warming (Schuur et al., 2015; Hodgkins et al., 2014). However, permafrost in peatlands tends to be ice-rich. Thus, thaw also can lead to surface deformation or thermokarst, which results in flooding and the creation of an anaerobic zone for thawed permafrost organic matter (Baltzer et al., 2014; Osterkamp et al., 2009). This leads to slower C mineralization rates overall, but also results in a greater proportion of CH$_4$ production relative to CO$_2$ production (Benstead and Lloyd, 1996; Hodgkins et al., 2014; Turetsky et al., 2007). This is significant because CH$_4$ is approximately 30 times more effective than CO$_2$ as a greenhouse gas over a century time frame (Hodgkins et al., 2014; Schaefer et al., 2014; Yvon-Durocher et al., 2014), a difference which is projected to correspond to a 35% to 48% increase in the warming potential of C released from permafrost over the next 100 years (Schuur et al., 2015).

The climate warming involved with the release of these greenhouse gases has the potential to cause even more extensive permafrost thaw in northern regions, leading to further release of greenhouse gases, and subsequently, further warming. This series of positive interactions is known as the permafrost carbon-climate feedback (Koven et al., 2015; Schuur et al., 2015; Figure 1). Without a consideration of the permafrost carbon-climate feedback, it is possible that Earth system models will underestimate warming on the decade- and century-scales due to the contribution of permafrost C losses to the atmosphere (Schuur et al., 2015). For this reason, it is understandable that most empirical and modeling studies to date have focused on how thawing permafrost organic matter might contribute new emissions of C to the atmosphere. On the other hand, fewer studies have addressed any potential uptake mechanisms that might help to ameliorate the permafrost
carbon-climate feedback. One such effect is whether permafrost thaw could stimulate ecosystem C uptake from the atmosphere through faster plant growth (Keuper et al., 2012; McGuire et al., 2018; Natali et al., 2011). Experimental warming treatments carried out in Alaska have provided some evidence for increased aboveground biomass and net primary productivity, but these increases in the potential for CO$_2$ uptake based solely on higher temperatures will most likely be offset by similar increases in CO$_2$ losses due to plant respiration (Natali et al., 2011; Natali et al., 2012). For this reason, it has been speculated that N availability may have a larger impact over time then just temperature alone (Natali et al., 2011; Natali et al., 2012).

1.3 Implications of thermokarst for peatland nitrogen cycling

A fundamental pattern controlling most terrestrial ecosystems is that primary productivity is limited by N availability (Chapin, 1980). When permafrost thaws, soil N that was previously frozen could be leached or mineralized and become available for biological uptake (Harden et al, 2012; Keuper et al., 2012). Permafrost organic matter stores large stocks of N (Abbott et al., 2016; Harden et al., 2012). Given that N is a limiting resource for primary productivity, if plants and microbes are able to access this newly-available N, it could fundamentally alter the way northern ecosystems function (Keuper et al., 2012; Natali et al., 2012; Salmon et al., 2016). It was shown by Finger et al. (2016) that late in the growing season, dissolved inorganic N concentrations increased in deeper peat layers. This sets up the possibility that, when released through permafrost thaw, a substantial fraction of the stores of N bound in permafrost soils could potentially be taken up by the deep root systems of plants, assuming that this N is released in plant-available forms (either nitrate (NO$_3^-$) or ammonium (NH$_4^+$); Keuper et al., 2012). If this assumption is supported, and if plants are able to take up this N, then its release upon thaw could potentially lead to increases in plant and ecosystem productivity (Keuper et al., 2012). Increases in microbial activity can also
result in a rise in net N mineralization, which involves a conversion of non-plant-available organic forms of N into the aforementioned forms of bioavailable N that can be taken up by plants directly (Keuper et al., 2012). No matter the mechanism, if plants can access these N pools made available by warming or permafrost thaw, increases in plant productivity could stimulate the uptake of C by vascular plants in northern ecosystems (Keuper et al., 2012; Keuper et al., 2017).

Recent studies have shown some conflicting results as to whether increased N availability due to thawing permafrost organic matter could have significant impacts on plant function and ecosystem C balance. Salmon et al. (2016) used experimental warming treatments in interior Alaska to demonstrate a ~20% increase in aboveground biomass of *Eriophorum vaginatum* following thaw (Salmon et al., 2016). These results agreed with those of Natali et al. (2011 and 2012), who speculated that increases in total plant biomass and net primary productivity with thaw were due to greater N availability in the soil. Keuper et al. (2017) used $^{15}$N labeling to confirm that not only are vascular plants able to take up N that is released post-thaw, but that this additional N uptake also translates into increased aboveground plant biomass. Together, these field studies provide some mechanistic links between permafrost thaw, N availability, and plant performance. Conversely, Koven et al. (2015) used a modeling framework to show that any release of new N from thawing permafrost is unlikely to have large effects on plant productivity, in part due to seasonal asynchrony between maximum N supply (fall and winter) and maximum plant N demand (spring and summer).

Overall, whether increases in net primary productivity following permafrost thaw will be large enough to offset permafrost C releases depends on a range of factors, including the level of future warming. Recent models reviewed by Schuur et al. (2015) tended to converge on the
prediction that increases in C taken up by vegetation could offset permafrost C losses for a few decades, but that permafrost C emissions are likely to exceed plant uptake after 2100.

1.4 Wetland plants, adaptations to anoxic soils, and costs versus benefits of deep roots

Wetland ecosystems are unique in that water, which is often a limiting resource for plants in terrestrial ecosystems, is in great supply. In wetland soils, excess availability of water for an extended period of time causes a low rate of oxygen (O\textsubscript{2}) diffusion in the soil (Benstead and Lloyd, 1996), otherwise known as anoxic soil conditions, which results in a much less efficient use of energy by the plant (Chen and Qualls, 2003; Kirk, 2004). More specifically, under anoxic conditions, oxidative phosphorylation and electron transport (key components of aerobic respiration) in root cells are inhibited, so the only production of energy comes from fermentation (anaerobic respiration, which produces far less ATP per molecule of glucose; Chen and Qualls, 2003; Kirk, 2004). Additionally, anoxic conditions cause a disruption of the pH balance between the vacuole and cytosol of roots cells, resulting in a further inhibition of plant metabolism (Taiz and Zeiger, 2014). This inefficient use of energy, along with the overall decreased permeability of root cells under flooding stress (Baltzer et al., 2014), then results in an inhibition of nutrient uptake and transport through the xylem to aboveground plant tissues (Taiz and Zeiger, 2014). Lastly, anoxic stress also has the potential to cause an increase in the plant’s production of reactive oxygen species (Chen and Qualls, 2004; Taiz and Zeiger, 2014), as well as ethylene, a stress hormone that can cause leaf chlorosis and abscission when produced at the high levels synonymous with stress (Chen et al., 2002), along with stomatal closure, which is likely to inhibit transpiration (Taiz and Zeiger, 2014).

One adaptation that is common in wetland plants to overcome the stresses of anoxic soil conditions is the development of specialized root cortical tissue known as aerenchyma (Chen et
Aerenchyma tissue contains a series of interconnected gas-filled chambers that allow for intercellular transport of O$_2$ down a concentration gradient from the atmosphere, via aboveground tissues, to deep roots, allowing for more efficient respiration in below-ground plant cells (Chen et al., 2002; Kirk, 2004). However, the need for continuous transport of O$_2$ to maintain respiration in deep roots is also indirectly proportional to the depth at which the root cells needing O$_2$ are located. This is because the dissolved O$_2$ present in the waterlogged thermokarst feature decreases steadily with depth (Benstead and Lloyd, 1996), while radial oxygen loss to the rhizosphere continuously occurs along the length of the root (Armstrong, 1979; Colmer, 2003). Therefore, the deeper that roots are extended, the more O$_2$ is required to allow for normal functioning of the root cells, allowing metabolism and nutrient uptake to continue at the typical rate (Kirk, 2004).

Waterlogging also causes changes in root architecture in wetland ecosystems, namely because root system construction becomes more likely to be dictated by anoxia in root cells (Colmer, 2003; Moog and Janiesh, 1990), both as a direct stress as well as its indirect effect on nutrient availability, particularly N. This is especially relevant in northern wetlands, where N deposition and O$_2$ availability are both very low, so the main supply of N is through organic matter decomposition, which also occurs slowly (Chapin, 1980). In ecosystems underlain by permafrost, plant roots are constrained to the surface, seasonally thawed layer, but as permafrost thaws and N availability potentially increases (both seasonally and gradually over time), plant roots can potentially extend downward into deeper, newly-thawed organic matter in order to access available N (Keuper et al., 2012; Natali et al., 2012). However, it is unclear whether vascular plants in thermokarst systems are able to overcome the limitations of developing and maintaining root
systems in such a stressful environment in order to benefit significantly (in terms of aboveground productivity) from accessing the newly-formed deep N pools.

1.5 Research Objectives

The overall objective of my study is to understand if and how limitations on vascular plant characteristics shift in response to permafrost thaw and thermokarst, based on the expected accompanying shifts in hydrology and N availability in the system. This objective was examined along the actively-thawing margins of thermokarst bogs that formed within a coniferous-dominated permafrost forest in interior Alaska. Previous studies conducted at the sites involved in this study concluded that vascular plants in the thermokarst bogs are N-limited (Finger et al., 2016), based on an assessment of foliar N:P ratios (Tessier and Raynal, 2003). In this study, I used field- and laboratory-based measurements and experiments to test several hypotheses aimed at linking changes in soil nutrient pools to plant aboveground primary production. My hypotheses were structured according to two general research questions:

**Question 1:** How does plant N uptake vary with depth (root length)?

The following hypothesis will be tested using an *in vivo* N-uptake experiment:

1) Nitrogen uptake by the roots of *C. aquatilis* is related to rooting depth, root biomass, and the accessibility of plant-available forms of N. This hypothesis corresponds to the following predictions:

   a. When accounting for the amount of root biomass present, the depletion of NH$_4^+$ from porewater spiked with (NH$_4$)$_2$SO$_4$ will be greater if the submerged root tips were excavated from peat layers at a lesser depth. This will be due to the more stressful anoxic conditions present in the deeper soil layers of the
thermokarst feature, causing deep roots to be less capable of N uptake
(assuming these root tips are alive at all)

**Question 2:** How does aboveground plant performance vary across different thermokarst features and/or differing locations within the same thermokarst feature and does this correlate with trends in rooting depth or trends in N uptake?

The following hypotheses will be tested using laboratory-based measurements on plants harvested from selected thermokarst features:

2) If plant root functionality (ie. N uptake) varies with depth as a function of soil nutrient availability, then enhanced plant nutrient uptake following thaw should result in increases in aboveground plant production. If this hypothesis is supported, then I predict that:

b. *C. aquatilis* aboveground biomass will be positively correlated to belowground biomass

c. Aboveground primary production (shoot biomass) will be positively correlated to maximum rooting depth

d. Aboveground primary production (shoot biomass) will be greater in areas of active thaw compared to areas of old thaw, due to a more recent influx of nutrients post-thaw

My results have the potential to provide support for the hypothesis established in the empirical literature that increased N availability post-thaw leads to shifts in belowground N acquisition and aboveground primary productivity of vascular plants. I explored for potential correlations between improved aboveground plant performance and several traits related to N uptake. Alternatively, if no differences in aboveground primary production were observed under
Hypothesis 2, it can be inferred that *C. aquatilis* plants are either experiencing sufficient stress (likely due to anoxia) to limit their uptake of N or they are limited in their ability to utilize N once it has been taken up. I assessed whether anoxic conditions served as a potential co-limitation on the function of *C. aquatilis* deep roots by measuring root porosity at regular depth intervals. I used these data to determine if there is a limit in the percentage of root cross section area that is composed of aerenchyma tissue, based on the length of the root. This may indicate that at a certain depth, *C. aquatilis* will experience reduced capacity of O$_2$ transport to belowground tissues, inhibiting the ability of cells in very deep roots to undergo normal metabolic processes (or take up N, for that matter).

This work contributes to a mechanistic understanding of how subarctic plants respond to warming, and will supplement information on the permafrost carbon-climate feedback with an understanding of how ecosystems might help stabilize future warming through enhanced carbon sequestration.

**CHAPTER 2: RESEARCH DESIGN AND METHODS**

**2.1 Description of Study Sites and Target Species**

The main study site is located in a system of thermokarst bogs that is involved in APEX (Alaska Peatland Experiment) in the Bonanza Creek Experimental Forest (64°41' N, 148°19' W), which is found in the Tanana River Floodplain, about 30 km southwest of Fairbanks, Alaska. These sites are representative of patterns of permafrost thaw in ice-rich permafrost, which covers more than 20% of the global permafrost region (Tarnocai et al., 2009). This lowland area is characterized by high variation in seasonal temperature, but overall low soil temperatures, which has the potential to impact the root and nutrient cycling characteristics mentioned earlier. Over the past 1000 years, a series of collapse scar bogs have developed in the existing lowland boreal
forest ecosystem, leading to a shift from a black spruce-dominated peat plateau to Carex- and Sphagnum-dominated bogs (known as the APEX Beta site).

This study also included an additional study site of thermokarst bogs that developed within a birch-dominated forest, because the rate of permafrost thaw is thought to differ between these different site types (Lara et al., 2016). Birch forests typically form on permafrost with a higher ice content than do spruce forests, which results in a faster rate of permafrost thaw, leading to more rapid lateral and (downward) vertical expansion of thermokarst features, as well as faster nutrient cycling (Lara et al., 2016; Osterkamp et al., 2000). This is particularly important to the study because of the hypotheses being tested specifically on the actively-thawing margins of thermokarst features, where permafrost thaw and nutrient influx are occurring. While these hypotheses are more focused on testing mechanisms related to how plants may utilize N released through permafrost thaw, it is also important to be able to compare these mechanisms across thermokarst sites found in these two forest types to ensure that a range of environmental conditions related to thaw are included, thus allowing for a broader inference of results in interior Alaskan ecosystems. This additional site is known as the Nenana Farms site and is located near the APEX Beta site, about 50 km southwest of Fairbanks, Alaska. All measurements for both sites were conducted across the typical growing season in interior Alaska, from May-August, 2016.

At each of the two sites involved in my study (APEX Beta and Nenana Farms), I selected three collapse scar bogs that are experiencing active thaw and subsidence (Table 1). This is generally indicated by the presence of “drunken forest” along one or more margins of the thermokarst feature (known as the actively-thawing margin), and each site involved comparisons of vascular plant growth characteristics between the actively-thawing margin (hereafter referred
to as the “edge”) and areas that have thawed previously in the middle of the thermokarst feature (hereafter referred to as the “centre”; Figure 2a).

Earlier in the 2016 growing season, the plant community was visually assessed at each of the three thermokarst features selected at the APEX Beta site, in order to determine the dominant species of herbaceous vegetation (Figure 2b). This was determined to be the water sedge (Carex aquatilis Wahlenb.) at all three selected thermokarst bogs at both study sites (Mark Winterstein, personal communication), leading to its use as the target species for both comparisons of biomass within and between sites, as well as NH$_4^+$ uptake at the APEX Beta site. This species is often considered to be the dominant sedge species in the wetlands of interior Alaska (Tande and Lipkin, 2003). Its distribution in wetland systems is also consistent across much of the boreal region of North America, allowing for potentially more relevant applications of the results to areas outside of Alaska as well (Tande and Lipkin, 2003). It is a perennial herbaceous plant of the Cyperaceae (sedge) family, that can grow over 1 m in height aboveground, as well as greater than 70 cm in root length (Tande and Lipkin, 2003). This results in the species being an ideal candidate to study both aboveground and belowground vascular plant responses to permafrost thaw.

2.2 Nitrogen Uptake Experiment

Throughout the 2016 field season, a number of trials for a NH$_4^+$ uptake experiment were carried out at the APEX Beta sites, in order to ensure that the method that would provide the most accurate representation of NH$_4^+$ uptake as possible was used during the final run of the experiment. These trials are described in more detail in Appendix A.

The biomass of C. aquatilis is generally at its peak in late July to early August before it begins to senesce in the fall, so therefore, August 1, 2016 was determined to be the ideal date to carry out the experiment. On this day, porewater was collected using a sipper and syringe from
the edge of Bog B6 at 40 cm in depth and homogenized in a large container. Aliquots of 40 mL of homogenized porewater were transferred to 60 mL HDPE bottles. For the full experiment across all three selected bogs, each bottle was spiked with a liquid ammonium sulfate – (NH$_4$)$_2$SO$_4$ – stock solution to a final NH$_4^+$ concentration of 14.17 mg/L in each 60 mL HDPE bottle (Rennenberg et al., 1996; McFarlane and Yanai, 2006). Through preliminary experiments (Appendix A), this concentration of NH$_4^+$ was determined to be sufficient enough to allow for some NH$_4^+$ to still be remaining in all samples after 8 hours (allowing for accurate measurement over the entire time period), but not so excessive that discernable differences in NH$_4^+$ uptake could not be determined. An additional trial was conducted in concert with the above experiment, but only involving Bog B6, whereby the initial concentration of (NH$_4$)$_2$SO$_4$ was halved to 7.09 mg/L in each 60 mL HDPE bottle, allowing for additional replication and comparisons. Three of the filled HDPE bottles for each concentration level were immediately set aside in a cooler on ice to represent the initial concentrations of NH$_4^+$ in the spiked porewater. Individuals of C. aquatilis were haphazardly selected and removed from each sampling location (the edge and the centre of each of the three selected thermokarst features at the APEX Beta site) by simply pulling each plant, along with its entire intact root system, out of the feature by hand. Any excess peat attached to the root system was shaken off or removed by rinsing the roots briefly with deionized water. These excavated roots were separated by length (a proxy for their depth within the vertical peat profile) based on two categories: shallow (less than 20 cm in length) and deep (greater than 40 cm in length). There were extremely few, if any, roots with lengths between these two categories, which led to the distinction (Lucas Albano, personal observation). Ten to twelve roots falling into one of the two depth categories were placed in each HDPE bottle, ensuring that the root tips were fully submerged in the spiked porewater. The bottles were then covered with plastic wrap to avoid
contamination and allowed to incubate for a period of 8 hours in the field at ambient conditions. Aliquots of 20 mL in volume were removed after 4 hours, transferred to additional 60 mL HDPE bottles, and immediately set aside in a cooler on ice in order to provide an intermediate data point for NH$_4^+$ uptake over time. After 8 hours, the excavated root tips were removed from the spiked porewater and the submerged portion of each root tip was excised in order to allow each NH$_4^+$ uptake value to be corrected for the amount of root biomass actually present within the volume of spiked porewater from which NH$_4^+$ was being taken up. All root samples and HDPE bottles were then placed in a cooler on ice, removed from the field site, and then immediately frozen. After shipping back to the University of Guelph (again in a cooler on ice to limit any potential thawing and decomposition), the excised root tip samples were dried in an oven at 65°C and then weighed. The (NH$_4$)$_2$SO$_4$-spiked porewater samples from each 60 mL HDPE bottle were filtered using syringes with inserted GVS Life Sciences PreSep Glass (G15) Membrane Disks (25 mm in diameter with 0.5 µm pore spaces) and the NH$_4^+$ concentration was measured using an ion chromatograph (Dionex ICS-1600) and the accompanying computer software (Chromeleon Version 7.1). These concentration values were then used to determine the depletion of NH$_4^+$ from the original spiked porewater solution by the excavated _C. aquatilis_ root tips after 4 and 8 hours, and these depletion values were used to determine NH$_4^+$ uptake over time. Each NH$_4^+$ uptake value was then corrected for the amount of root tip dry biomass actually submerged in its respective HDPE bottle.

### 2.3 Relationships to Plant Performance

On August 1, 2016, a number of additional individual _C. aquatilis_ plants were haphazardly selected and removed from each selected thermokarst bog at the APEX Beta site, stored in a cooler on ice for the duration of the NH$_4^+$ uptake experiment, removed from the field, and then frozen
and shipped to the University of Guelph, in order to determine root porosity, plant size characteristics, and above- and belowground biomass. On August 4, 2016, the same process was repeated for the Nenana Farms site. However, since the NH$_4^+$ uptake experiment was not conducted, all plants were removed from the field as soon as sampling was complete.

Root porosity (%) was determined by taking digital images of root cross sections at 5 cm depth intervals, starting where the root meets the stem (labelled as 0 cm), using the longest root of 3 harvested plants from each selected thermokarst bog. The root cross sections were cut using a scalpel, aided by a dissecting microscope (Leica EZ4 D), while images were captured using a compound microscope (Leica DM750) along with the accompanying computer software (Leica Application Suite, Version 1.7.0). These digital images were converted to binary (black and white), allowing for the pore spaces to be quantified with respect to the total area of the cross section using the thresholding feature of the ImageJ software (Version 1.48), following the protocol of Purnobasuki and Suzuki (2004), outlined in greater detail in Figure 3.

Harvested individuals of *C. aquatilis* from each site were also measured for maximum shoot height and maximum root length, and then the biomass of both shoots and roots was dried in an oven at 65°C, and then weighed, in order to provide a cumulative estimate of not only belowground, but also aboveground primary production per each sampled plant across the entire growing season.

### 2.4 Environmental Measurements

A number of environmental measurements were recorded to characterize key differences affecting plant performance between the field sites. Porewater was sampled at a depth of 40 cm at the edge of each selected thermokarst bog at each study site (the same depth and location at which the porewater was sampled for use in the NH$_4^+$ uptake experiment) and used to measure
dissolved oxygen (DO) and pH using a Hach probe in the lab. Additional porewater samples were taken at depths of 20 cm and 40 cm at both the edge and centre of each selected thermokarst bog and used to measure oxidation-reduction potential (ORP) and porewater temperature in the field (according to standard procedure for most experiments conducted at the APEX sites; Romanowicz et al., 2015) using a Hach ORP probe (Hach Co., Loveland, Co., USA, IntelliCAL MTC301). ORP is a measure of the redox capacity of the soil, and lower values generally specify a higher demand for O2 in the soil (Pezeshki and DeLaune, 2012).

2.5 Statistical Analysis

In order to describe NH4+ uptake over time for the samples taken at all three thermokarst bogs with the original spike of 14.17 mg/L of (NH4)2SO4, a linear mixed effect model was constructed using the “lme” function in R package “nlme” (Version 3.1-131), with NH4+ uptake as the response variable. This model included time, root depth (shallow versus deep), plant location within the bog (edge versus centre), and two-way interactions (time x root depth, time x plant location) as fixed effects, as well as sample ID as a random effect variable. An additional model was constructed using the same functions and variables for the samples collected at Bog B6 that received the halved (NH4)2SO4 spike. The slopes of uptake over 8 hours for each spike level were also compared using a three-way ANOVA (R function “aov”), including spike level (low versus high), sampling location (edge versus centre), and root depth (shallow versus deep), as well as interactions between each variable, included as predictors. I used Tukey’s HSD Tests (R function “TukeyHSD”) for post hoc analysis of differences between means for all ANOVAs in my study.

I also explored variation in plant traits (shoot dry biomass, root dry biomass, maximum shoot height, maximum root length, and root:shoot biomass ratio) using two-way ANOVAs (R
function “aov”), with study site (APEX Beta versus Nenana Farms), sampling location (edge versus centre of each thermokarst bog), and the site x sampling location interaction as predictors. A general linear model (R function “lm”) was used to predict shoot dry biomass, an estimate of aboveground primary production, using root dry biomass, maximum shoot height, and maximum root length as predictor variables.

Root porosity was assessed using a general linear model (R function “lm”) that included percentage of the total root length at which the cross section was cut, location within the bog at which the plant was harvested (edge versus centre), and the depth class of the root (in this case, shallow consisted of roots less than 40 cm in length, while deep consisted of roots greater than 40 cm in length) as predictor variables. I also included the interactions between percentage of root length and either location within the bog or depth class of the root in this model.

Porewater temperature and ORP were analyzed using two-way ANOVAs (R function “aov”) with sampling location (edge versus center), depth (20 cm versus 40 cm), and the sampling location x depth interaction as predictors. These same temperature and ORP samples were also assessed using one-way ANOVAs (R function “aov”) in order to compare between the APEX Beta and Nenana Farms study sites. Additional two-way ANOVAs were used to analyze for differences between the APEX Beta and Nenana Farms sites in DO and pH samples collected from a depth of 40 cm at the edge of each selected thermokarst bog at each site.

All statistical analyses were conducted in RStudio, Version 1.1.383 (2017).

CHAPTER 3: RESULTS

3.1 Nitrogen Uptake Experiment

The mean accumulation of NH₄⁺ at all three APEX Beta thermokarst bogs demonstrated similar patterns of NH₄⁺ uptake over time (Figure 4). While there were no differences in NH₄⁺
uptake rates between shallow (less than 20 cm in length) and deep (greater than 40 cm in length) roots (df = 58, t = -0.244, p = 0.808), or between roots collected at the edge versus the center of each collapse scar bog (df = 34, t = -0.125, p = 0.901), my results did tend to indicate greater uptake rates in deep roots compared to shallow roots (non-significant but consistent across all sites and sampling locations). There were also no significant interactions between time and either root depth or sampling location.

For the additional trial involving the halved initial concentration of (NH$_4$)$_2$SO$_4$ at Bog B6, a similar pattern was observed for centre plants, with the results tending to indicate greater uptake in deep roots compared to shallow roots (Figure 5a). This also occurred in edge plants, but only after 8 hours, with shallow roots demonstrating greater uptake after 4 hours (Figure 5b). However, statistically there were still no significant differences in NH$_4^+$ uptake between shallow and deep roots (df = 8, t = 0.000, p = 1.000) or between roots collected at the edge versus the center of Bog B6 (df = 8, t = 0.000, p = 1.000). On average across sites, the slope of NH$_4^+$ uptake over 8 hours for C. aquatilis root tips receiving the high concentration of (NH$_4$)$_2$SO$_4$ was significantly greater than the slope for C. aquatilis root tips receiving the low concentration of (NH$_4$)$_2$SO$_4$ ($F_{1,15} = 7.126, p < 0.05$; Figure 6). Conversely, there were no significant differences in the slope of NH$_4^+$ uptake based on sampling location ($F_{1,15} = 1.415, p = 0.2527$) or root depth ($F_{1,15} = 3.948, p = 0.0655$), and all two-way and three-way interactions were not significant (all p values > 0.01).

3.2 Relationships to Plant Performance

There were no significant differences in mean C. aquatilis shoot dry biomass, root dry biomass, maximum root length, or the root:shoot biomass ratio between sampling locations (edge versus center; Table 2) or by a sampling location x site interaction (all p values > 0.1). I found that maximum shoot height did not vary between sampling locations ($F_{1,74} = 0.328, p > 0.1$), or by a
sampling location x site interaction \((F_{1,74} = 0.705, p > 0.1)\), but shoot heights were significantly larger at the Nenana Farms site \((F_{1,74} = 9.744, p < 0.01; \text{ Table 2})\).

Based on the linear model constructed to predict \(C. \text{ aquatilis}\) shoot dry biomass \((df = 34, r^2 = 0.614)\), used as an estimate of aboveground primary production, root dry biomass was not a significant predictor \((p = 0.9970)\). Maximum shoot height was a near-significant predictor of shoot dry biomass \((p = 0.0539)\), whereby a taller shoot results in a greater shoot dry biomass, and maximum root length was a significant predictor \((p = 0.0450)\), whereby longer maximum root length results in greater shoot dry biomass (Figure 7).

An additional model was constructed to predict \(C. \text{ aquatilis}\) shoot dry biomass at the Nenana Farms site \((df = 28, r^2 = 0.499)\), identical to the model constructed for the APEX Beta sites. Similarly, root dry biomass was not a significant predictor \((p = 0.619)\). However, contrary to the results at the APEX Beta site, at the Nenana Farms site, maximum shoot height \((p = 0.358)\) and maximum root length \((p = 0.689)\) were also not significant predictors of shoot dry biomass (Figure 7).

I used a general linear model to analyze variation in root porosity \((df = 245, r^2 = 0.028)\), incorporating the percentage of the total root length at which each root cross section was cut, as well as location within each bog from which the plant was harvested (edge versus centre) and the depth class of the root (less than 40 cm versus greater than 40 cm) as predictor variables. Neither the percentage of the total root length \((p = 0.966)\), the location within the bog \((p = 0.399)\), nor the depth class of the root \((p = 0.493)\) were significant predictors of root porosity. Interaction terms among these main effects were also not significant (all \(p\) values > 0.1). However, I found a consistent trend of root porosity percentage with increasing depth, whereby the initial value taken where the root meets the stem is quite low, and the final value, where pore spaces begin to close
as the root reaches its apex, but otherwise the porosity values remain quite consistent. This trend was consistent regardless of total root length, sampling location, or study site (Figure 8 and Figure 9).

3.3 Environmental Measurements

Mean porewater temperature and ORP were measured at two different depths at the edge and centre of each of the three selected thermokarst bogs at the APEX Beta and Nenana Farms sites (Table 4). Overall, porewater samples from the APEX Beta site were warmer and had higher mean ORP than the Nenana Farms porewater samples (temperature: $F_{1,70} = 9.254, p < 0.005$; ORP: $F_{1,70} = 107.5, p < 0.0001$). Across regions, mean porewater temperature varied by sampling location ($F_{1,68} = 16.658, p < 0.0005$), as porewater sampled from the centre was consistently warmer than porewater sampled from the edge of a given thermokarst feature. There were no significant differences between sampling depths of 20 cm versus 40 cm ($F_{1,68} = 3.434, p = 0.068$), nor was there a sampling location x depth interaction ($F_{1,68} = 0.025, p = 0.876$). Mean porewater ORP showed similar trends, with variation between sampling locations ($F_{1,68} = 17.067, p < 0.0005$), whereby porewater samples taken from the centre had significantly greater ORP than those taken from the edge, with no effects of depth ($F_{1,68} = 1.436, p = 0.235$) or a sampling location x depth interaction ($F_{1,68} = 0.501, p = 0.482$).

Mean porewater pH and DO were also measured at a depth of 40 cm at the edge of each of the six total selected thermokarst bogs across the two study sites. I found that porewater samples taken from the APEX Beta site were significantly more acidic ($F_{5,12} = 10.89, p < 0.005$), with all pH values falling within a range of 5.30 to 5.69 across both sites. The APEX Beta site also contained significantly greater ($F_{5,12} = 23.01, p < 0.0001$) levels of DO (ranging from 3.01 to 4.45 mg/L) than those taken from the Nenana Farms site (ranging from 0.53 to 2.57 mg/L)
CHAPTER 4: DISCUSSION

4.1 Can plants acquire N from thawing permafrost organic matter?

A key objective of this study was to improve our understanding of how vascular plants respond to permafrost thaw and thermokarst, particularly concerning shifts in factors limiting their primary production. In most boreal ecosystems, plants are generally limited by N availability (Chapin, 1980). This is particularly true in northern peatland ecosystems, where cold, acidic, and waterlogged conditions lead to very low decomposition rates, reducing the amount of plant-available forms of N present in the system (Chapin, 1980). More specifically, Finger et al. (2016) demonstrated consistent N limitation in vascular plants growing in thermokarst bogs at the APEX Beta site, through the analysis of foliar N:P ratios (Tessier and Raynal, 2003). However, in the context of how northern ecosystems may respond to warming, increasing N availability may occur through two main mechanisms: 1) via the initial thawing of permafrost, whereby plant-available forms of N that were previously bound in frozen soil layers become available in the newly-thawed water column of the thermokarst feature, and 2) via increased levels of microbial activity, both in the newly-thawed soil layers as well as the consequence of the soil column experiencing warming, resulting in a more long-term net mineralization of N, converting more complex organic material into the inorganic forms of plant-available N (NH$_4^+$ and NO$_3^-$). In this context, “plant-available N” refers to the plant’s ability to take up NH$_4^+$ and NO$_3^-$, if these molecules are present next to a root cell with sufficient capacity for the uptake and transport of inorganic forms of N. However, if these NH$_4^+$ and NO$_3^-$ molecules are primarily being released in deep layers of peat, it is unknown whether or not the roots of vascular plants in northern peatland ecosystems will be able to overcome the anoxic stress present in order to extend roots deep enough to access this N. Therefore, even if permafrost thaw is increasing the availability of “plant-available N”, a key
question is whether or not this N can actually be acquired and utilized by vascular plant roots. Previous studies confirm that deep roots of wetland vascular plants are under sufficient limitation to inhibit their ability to take up significant amounts of N, mainly through anoxic stress. For example, Moog and Janiesch (1990) performed an experiment involving simulated omission of DO from a hydroponic solution, in which Carex species were grown. It was shown that O\(_2\) deficiency reduced the root length of *C. remota* by 47\%, demonstrating the potential for a reduced ability (or reduced benefit) in vascular plants developing deep roots under anoxic conditions (Moog and Janiesh, 1990).

In the current study, individuals of the dominant vascular plant species (*C. aquatilis*) of interior Alaskan thermokarst bogs were excavated from two locations (varying in time-since-thaw) within each selected thermokarst feature at the APEX Beta study site. Roots excavated from different depths at each of these locations were assessed for their level of NH\(_4^+\) (the preferential form of plant-available N for *C. aquatilis*) uptake over a period of 8 hours. My results showed that, across all sampling sites and locations, deep roots were able to acquire NH\(_4^+\) at levels and rates similar to those of the shallow roots; in fact, my results suggest deep roots, if anything, trend towards greater NH\(_4^+\) acquisition than shallow roots. Although these results contrast with the notion that deep roots in these saturated thermokarst bog systems may be under sufficient levels of stress to severely limit their ability to take up nutrients, a major finding of this study remains that the deep roots of *C. aquatilis* are capable of overcoming the presumed more stressful conditions present in the deep soil layers, and that the deep root cells are adapted with sufficient machinery for NH\(_4^+\) uptake.

One logical assumption to explain this relatively efficient uptake of NH\(_4^+\) roots is that the roots are not actually experiencing stress. This is entirely possible in the natural environment in
which *C. aquatilis* thrives but based on the conditions present in the thermokarst features used in this study it is also possible that deeper roots are at least experiencing less favourable environmental conditions to shallow roots. In most locations within each feature, the water table is at or very close to the peat surface, which would mean that in order for the deep roots of *C. aquatilis* to avoid anoxic stress, O$_2$ would have to diffuse over 40 cm in depth through the water column, which is extremely unlikely. In fact, Benstead and Lloyd (1996) measured DO concentrations at a number of dates in peat cores taken from wetland hollows and found that generally, DO was undetectable at depths greater than 5 cm. However, as mentioned above, *C. aquatilis* plants do not rely on the diffusion of DO through the water column, because they are able to develop aerenchyma tissue in order to diffuse O$_2$ downward through its root system. This allows belowground tissue to continue to conduct aerobic respiration in anoxic conditions. However, although aerenchyma is a vital adaptation for some wetland plants, it is not an infallible one. Root systems in waterlogged conditions are also subject to radial oxygen loss, which is the diffusion of O$_2$ from aerenchymatous tissue through the root cortex and into the water column, where it can no longer be used by the plant to combat anoxic stress (Armstrong, 1979; Colmer, 2003). Radial oxygen loss has been shown to deplete O$_2$ availability in root aerenchymatous tissue by up to 30-40%, significantly reducing the capacity of deep roots to conduct aerobic respiration (Colmer, 2003). Therefore, at the very least, the deep roots of *C. aquatilis* present in thermokarst bogs are experiencing similar levels of O$_2$ availability to the shallow roots, and are in fact likely to be experiencing lower availability based on radial oxygen loss; conversely, in this study, the deep roots of *C. aquatilis* are at least demonstrating NH$_4^+$ uptake similar to that of shallow roots, and are in fact demonstrating a consistent trend toward greater NH$_4^+$ uptake.
Considering that deep roots indeed are likely experiencing lower \( \text{O}_2 \) availability, how can they be contributing equally, if not more than shallow roots, to N uptake potential of the wetland plant? This can be partially explained by the morphology of \textit{C. aquatilis} roots. It has been shown that \textit{C. aquatilis} invests 5- to 6-times greater root biomass per unit root length than other wetland graminoids, such as \textit{Eriophorum angustifolium} (Billings et al., 1978). This statistic is generally used to discern differences in root longevity between the two species; however, it could also explain why \textit{C. aquatilis} is able to extend its roots to lengths of greater than 70 cm, while \textit{Eriophorum} species are generally considered to be shallow-rooted wetland graminoids (Keuper et al., 2017). The consumption of \( \text{O}_2 \) by roots cells along the radial path through which \( \text{O}_2 \) diffuses from aerenchymatous tissue to the rhizosphere is one of the key determining factors of radial oxygen loss (Armstrong, 1979; Colmer, 2003). Therefore, it is possible that additional investment in root biomass by \textit{C. aquatilis} is somewhat able to limit radial oxygen loss, allowing for a reduced likelihood of anoxic stress at greater depths within the water column. This could increase the potential benefit to the plant to extend roots deeper in search of nutrients, despite the large additional cost of root biomass.

Support for potentially greater uptake by deeper roots can also be provided by my root porosity results. Neither the percentage of total root length from which the cross sections was cut, location within the bog, nor depth class of the root were significant predictors of root porosity, and there were no significant interactions between any of the predictor variables. This demonstrates that since the trend of root porosity with depth below the peat surface graphically follows a similar shape regardless of the depth class or sampling location of the root, the point at which pore spaces begin to close (which would limit the diffusion of \( \text{O}_2 \) to root cells) does not differ depending on
the total length of the root, and instead the pore spaces simply close as each root reaches its apex (Figure 8 and Figure 9).

Although a more comprehensive study on the cross-sectional structure of *C. aquatilis* roots, as well as their function, with respect to both the diffusion and loss of O$_2$ would lead to more definitive conclusions, some speculations can be made based on results from past studies and those of the current study. While radial oxygen loss is certainly occurring along the length of the root, it may not be occurring at such a high rate, based on the significant biomass investment in *C. aquatilis* roots compared to other wetland vascular plants (Armstrong, 1979; Billings et al., 1978; Colmer, 2003). The results of Ryser et al. (2011) also show some evidence of differences in the allocation of root biomass by plants under waterlogging treatment, in order to limit O$_2$ losses, and therefore maximize rooting depth. Furthermore, the deepest root cells still contain sufficient pore spaces for the diffusion of O$_2$ from the shoots, suggesting that the flow of O$_2$ to these cells is not restricted by root (cross-section) architecture and it is possible that O$_2$ availability is similar in deep and shallow roots. This then leads to the inference that the availability of nutrients is a greater contributor to a potential trend towards greater NH$_4^+$ uptake by deeper roots than is the decrease in DO availability in deeper soil layers.

The results of my NH$_4^+$ uptake experiment agree with a recent study that used $^{15}$N fertilization to demonstrate that vascular plants are indeed able to take up the newly-available N present following permafrost thaw in deeper soils (Keuper et al. 2017). This study found that *Rubus chamaemorus* was particularly efficient in acquiring N from depth (Keuper et al., 2017), and therefore agrees with my results that plants are able to invest in deep root systems to acquire a limiting resource. However, in the ice-rich peatlands of Alaska, forbs such as *R. chamaemorus* are more typical of plant communities in peat plateaus prior to thaw and comprise less than 1%
cover of the understory once peat plateaus have undergone thermokarst and are converted into collapse scar bogs (Finger et al., 2016). Thus, the Keuper et al. (2017) results alone do not provide generalized insights into the fate of thawed permafrost N, as permafrost peatlands experience thermokarst, flooding, and replacement of plateau vegetation with wet collapse scar bog vegetation. Formerly frozen permafrost peat can be rapidly converted into saturated margins that typically have standing water for at least several decades post-thaw. This area within a thermokarst bog, with its characteristic high water table, is therefore conducive to more deeply-rooted Carex species that are more resilient to water stress (and more ubiquitously dominant in wetland ecosystems), rather than forbs, such as R. chamaemorus. Therefore, the results of my study and Keuper et al. (2017) together show that different families of plants that thrive post-thaw under very different hydrologic conditions can consistently demonstrate greater N uptake by deeper roots than that of shallow roots.

Admittedly, it is possible that the NH$_4^+$ uptake experiment could have introduced conditions that may have improved the performance of deep roots. The concentration of NH$_4^+$ was artificially high, and high N availability is known to induce the expression of inorganic N transporters in root cells (Duan et al., 2016). This is supported by my comparison of the slopes of NH$_4^+$ uptake over 8 hours, which were significantly lower when the starting concentration of NH$_4^+$ was halved, meaning that uptake rates are affected by initial N supply, which should be expected. Additionally, because the C. aquatilis roots were excavated and the entire plants remained intact, the surface of the porewater samples in which the root tips were submerged was exposed to the atmosphere, and the depth of the porewater sample was less than 5 cm, meaning that O$_2$ could have diffused throughout the porewater sample, increasing the O$_2$ availability to the roots (Benstead and Lloyd, 1996). Goel and Singh (2015) showed the downregulation of the expression of a number
of NH$_4^+$ transporters in the root cells of *Brassica juncea*, when subjected to abiotic stressors, such as drought, heat, cold, and osmotic stress, for periods of 1 hour or 24 hours, depending on the specific transporter. Similarly, Duan et al. (2016) also demonstrated the downregulation of NH$_4^+$ transporters in the root cells of *Triticum aestivum* plants experiencing drought stress. However, it was also shown that one specific NH$_4^+$ transporter was also upregulated in its expression after exposure to drought stress (Duan et al., 2016). Therefore, there is a lack of definitive understanding in how the expression of NH$_4^+$ transporters respond to abiotic stressors, particularly through anoxic stress, or at least variation in O$_2$ availability, which to my knowledge has not been directly studied at such a fine scale. Although the *C. aquatilis* plants in my study did still display a consistent trend toward greater and faster uptake by deeper roots, regardless of sampling location or initial concentration of NH$_4^+$, without conducting an additional study specifically with the goal of measuring how levels of NH$_4^+$ transporters respond to increase and decreases in O$_2$ availability, it is difficult to definitively state whether or not the artificial conditions of the NH$_4^+$ uptake experiment in this study introduced confounding factors that may have quickly and significantly influenced NH$_4^+$ uptake by *C. aquatilis*, which is possible based on the speed at which expression changed in both the study by Goel and Singh (2015) and the study by Duan et al. (2016).

Additionally, ORP values can be used to speculate if the results of the NH$_4^+$ uptake experiment carried out at the APEX Beta site would also apply at the Nenana Farms site. ORP values at Nenana Farms were significantly lower than at APEX Beta, indicating that the demand for O$_2$ in the waterlogged layers of peat will be greater at Nenana Farms, so any available O$_2$ will be taken up extremely quickly, and vascular plants are likely to be outcompeted by microbes. Lower ORP values have also been shown to increase radial oxygen loss from the aerenchymatous tissue of vascular plants (Pezeshki and DeLaune, 2012), potentially exacerbating the anoxic stress
on the roots of vascular plants. This increase in radial oxygen loss contradicts the results for DO, which were also significantly lower at the Nenana Farms site, but these could also be explained by the available O₂ being taken up extremely quickly, due to the high demand indicated by the ORP values. Based on these results, it can be speculated that the results of the NH₄⁺ uptake experiment may not apply to the Nenana Farms site, and therefore may not apply ubiquitously across all thermokarst systems following permafrost thaw.

4.2 Can permafrost N release boost aboveground primary production?

My second research goal was to explore whether or not the increase in plant-available forms of N post-thaw translates into a significant increase in aboveground primary production of vascular plants in thermokarst bogs. This would allow more substantial conclusions to be drawn concerning the relationship between climate and the permafrost carbon-climate feedback, given that increased plant productivity has the potential to offset releases of permafrost C to the atmosphere. I predicted that aboveground biomass of *C. aquatilis* would be greater in plants growing closer to the actively-thawing margin of a thermokarst feature because of new N sources created by thawing permafrost (Finger et al., 2016). I predicted that as thaw progresses, this source of N released from thawing permafrost would be taken up either by plants or microbes, but also would move deeper into the soil profile due to surface peat accumulation, and thus would become more difficult for plant uptake with greater time-since-thaw. However, my results did not support this hypothesis, as there were no significant differences in plant size characteristics or investment in root biomass versus shoot biomass based on the comparison between *C. aquatilis* plants growing at the edge versus the centre of each selected thermokarst bog. This could be partially due to the high amount of variability within each plant size measurement, but may also be explained more fully by a lack of difference in the release of N between the two locations. Additionally, even if
N availability is higher at the edge of a thermokarst bog, I demonstrated less favourable environmental conditions, such as lower ORP and temperature values, at the edge, which could mitigate the ability of *C. aquatilis* to use the available N pool.

An additional comparison involved in the NH$_4^+$ uptake experiment was made between *C. aquatilis* plants growing at the edge of each thermokarst feature versus those growing at the centre. I predicted that plants growing at the edge (i.e., actively-thawing margin) would exhibit greater NH$_4^+$ uptake, based on a more recent influx of N through permafrost thaw. However, this prediction was not supported by the results of the experiment, because NH$_4^+$ uptake rates did not differ between the locations within each bog. It is therefore not surprising that *C. aquatilis* primary production also did not differ between the edge and centre of each thermokarst bog. Additionally, there were no significant differences in the root:shoot biomass ratio of *C. aquatilis* between the edge and centre at any of the thermokarst features, suggesting that there is no change in biomass investment based on time-since-thaw. These results are similar to those of Finger et al. (2016), who compared additional plant characteristics between the edge and the centre of collapse scar bogs. Using *Chamaedaphne calyculata* as a target species, they demonstrated that neither foliar C:N ratio nor foliar N concentration (%) differed between the edge and the centre of a collapse scar bog (Finger et al., 2016). This corresponded to the result that dissolved inorganic nitrogen (DIN) also did not differ between the edge and the centre, which is contrary to the prediction made in my study, though dissolved organic nitrogen (DON) and total dissolved nitrogen (TDN) were significantly greater at the edge than the centre (Finger et al., 2016). This could potentially be explained through the so called “immediate uptake” hypothesis, whereby the initial supply of DIN released post-thaw is taken up extremely quickly by vascular plants (and microbes), due to their status as being severely N-limited, and indeed continuing to be N-limited despite the additional
release of N from permafrost (Finger et al., 2016). Therefore, regardless of DIN supply, continuous N limitation or less favourable environmental conditions could be preventing any significant increases in biomass by the edge plants in this system, or an additional step in N metabolism could be limiting the benefit of additional uptake. For example, although uptake is increased, plants could be storing N rather than utilizing it immediately, they could be limited in their ability to utilize the amount of N that is being taken up, or they could potentially become co-limited with another factor or nutrient, such as phosphorus (Finger et al., 2016). An isotopic labelling study, or more in-depth analysis of N uptake and utilization of wetland plants post-thaw from a molecular level, could lead to greater insight into the how plants may continue to experience limitations in their productivity, despite an influx of their limiting nutrient.

My results suggest that aboveground primary production is not higher in areas of recent thaw, which corresponds more closely with the results of Koven et al. (2015), who concluded that although increases in N availability post-thaw can potentially occur, these would not offset the effects of C losses from the same systems through increases in plant biomass, likely due to seasonal asynchrony between maximum N supply and maximum N demand for vascular plants growing in these systems. Accounting for seasonal differences was beyond the scope of this study, because the \( \text{NH}_4^+ \) uptake experiment (and subsequent harvesting of biomass) was focused on early August, in order to ensure the encapsulation of the effects of rooting depth and time-since-thaw during the period of peak biomass. However, while I did not find significant differences in biomass, and therefore aboveground primary production, based on time-since-thaw, this does not necessarily negate the importance of deep N sources for plants growing in thermokarst bogs. I used a general linear model with plant traits (root dry biomass, maximum shoot height, and maximum root length) to predict variation in aboveground primary production (shoot dry biomass). While maximum
shoot height and root dry biomass were not significant predictors, maximum root length was a significant predictor of aboveground primary production. This could explain why I found no differences in root:shoot biomass ratio with time-since-thaw, given that maximum root length seems to be a more sensitive variable along this ecological thaw gradient. However, this finding also highlights the potential importance of deep N sources for plant function. Plants that invested in deeper root lengths were associated with greater aboveground primary production.

Conversely, this result was not seen for the Nenana Farms site, with none of maximum root length, root dry biomass, or maximum shoot height being significant predictors of shoot dry biomass. This result was contrary to my predictions, and therefore unexpected, but could be due to the high amount of variability with each of the size measurements taken, but is more likely due to reduced capacity of *C. aquatilis* to take up nutrients released at the Nenana Farms site.

My results, therefore, along with the findings of Salmon et al., 2016 and Keuper et al., 2017 do demonstrate that enhanced N availability during the growing season can affect plant N uptake and overall plant performance. However, whether increases in net primary production following permafrost thaw would be large enough to offset permafrost C releases depends on a range of factors, including the level of future warming and the characteristics of the thermokarst system from which C is being released, and there is still debate within the literature for how effective increased primary production will actually be in terms of increased C uptake from the atmosphere. Most recently, however, McGuire et al. (2018) showed that under aggressive climate change scenarios, northern permafrost regions would not become a net source of C to the atmosphere, in large part due to C uptake by plants, while under the RCP4.5 climate change projection, it is possible that northern permafrost regions could still remain a net sink for carbon even after 2100.
4.3 What is the potential dominant source of N for plant productivity in post-thaw peatlands?

It was beyond the scope of this study to identify the source of N, and thus, it is unknown whether the sampled plants were acquiring N “released” from thawing permafrost organic matter versus more surface, active layer sources. The results of Finger et al. (2016) did demonstrate a negative correlation between DIN and DON concentrations in August, 2013, suggesting that mineralization of N by the microbial community is high when temperature is at its seasonal peak, and also coinciding with peak vascular plant biomass. It was also shown that this increase in DIN availability correlated with an increase in foliar N concentrations in C. calyculata (Finger et al., 2016), which suggests that N mineralization is occurring following permafrost thaw, and that vascular plants in these systems are able to utilize this as a significant source of N. As expected, I found that deeper soil temperatures were similar to those in shallower layers of peat. Taken together with the results for ORP and root porosity, it is unlikely then, that temperature or O2 supply would result in greater NH4+ uptake by deeper roots. Therefore, if deeper roots are trending towards greater NH4+ uptake, it is more likely due to a greater supply of newly-available N through thawing permafrost organic matter in deeper peat layers, compared to the supply of N in the shallow layers, rather than through a faster N mineralization rate in deeper peat layers.

CHAPTER 5: CONCLUSION

The permafrost carbon-climate feedback has been the subject of many studies involving potential shifts in ecosystem C losses due to thawing permafrost. However, it has also been shown that permafrost thaw can lead to increases in soil N availability. Fewer studies have addressed how permafrost thaw will affect N cycling, particularly with respect to how vascular plants will respond to these increases in N availability following permafrost thaw. One of the main questions regarding N cycling following permafrost thaw is whether or not vascular plants can access or
meaningfully utilize the increased N supply created from thawing permafrost organic matter. I addressed this issue using an $\text{NH}_4^+$ uptake experiment, in which $C. \text{ aquatilis}$ root tips were excavated from shallow and deep peat layers of three thermokarst bogs, and from the edge and centre of each bog (two locations which differ in time-since-thaw, so it was expected that N supply would differ as well). I showed that there was a non-significant trend toward greater $\text{NH}_4^+$ uptake by deep roots (or at the very least, uptake equal to that of shallow roots) despite a suspected decrease in $\text{O}_2$ availability for deeper roots. Conversely, no significant differences were quantified between plants growing in active margins (edge) versus older centers in terms of $\text{NH}_4^+$ uptake rates, plant size characteristics, relative investment of biomass in shoots and roots, or root porosity. Therefore, no direct link between increased N supply/uptake and increased aboveground primary production was shown. However, I found that maximum root length (as opposed to maximum shoot height or root dry biomass) was the only significant predictor of shoot dry biomass at the APEX Beta site, indicating that there is the potential for a link between increased N uptake by deep roots, due to a greater supply of N post-thaw, and increased aboveground primary production.

Although a direct comparison of $\text{NH}_4^+$ uptake was not made, a number of environmental characteristics and plant size characteristics were measured at the study site at which the $\text{NH}_4^+$ uptake experiment was conducted (which is surrounded by a coniferous-dominated forest) and an additional study site (which is surrounded by a deciduous-dominated forest) in order to speculate how the results of the experiment would apply elsewhere. It was found that although comparisons in plant size characteristics between $C. \text{ aquatilis}$ plants growing at the edge and centre of the selected thermokarst bogs at each study did not meaningfully differ, there were significant differences in DO and ORP. These differences indicate that deeper roots at the deciduous-dominated site are likely to be under more substantial anoxic stress, and therefore could potentially
be less capable of utilizing the increased supply of N in deeper peat layers than are the deeper roots at the coniferous-dominated site. Therefore, although a potential link between increased N supply post thaw and increased aboveground primary production was shown, it may not apply to all areas experiencing thaw and thermokarst, based on differences in abiotic factors such as ORP.

Recent models (McGuire et al., 2018) have highlighted the importance of continuing the study of connections between the permafrost carbon-climate feedback and vegetation in order to accurately represent the impact that northern regions will have on future climate. Future research directions could include the continued study of N uptake (potentially to a finer scale, making use of techniques such as a $^{15}$N tracer, which was beyond the scope of this study) by a dominant vascular plant species, such as *C. aquatilis*, across a number of different thermokarst environments and across a much larger geographic range, in order to provide more definitive evidence of a potential mitigating effect of the permafrost carbon-climate feedback, based on increases in N uptake by vascular plants following permafrost thaw.
REFERENCES


Billings WD, Peterson KM, Shaver GR (1978) in Vegetation and Production Ecology of an Alaskan Arctic Tundra – Chapter 18: Growth, turnover, and respiration rates of roots and tillers in tundra graminoids. Springer-Verlag, Inc. New York, USA.


Figure 1. Conceptual diagram of a permafrost peat plateau experiencing thermokarst. The localized land subsidence is caused by the deepening of the active layer of permafrost, and leads to waterlogged conditions, resulting in the death of shallow-rooted, non-flooding-tolerant species, such as deciduous and coniferous trees, and the success of flooding-tolerant species, such as C. aquatilis, which are more able to utilize the newly-formed deep rooting zone. This zone is also characterized by the onset of microbial activity, and therefore an increase in N mineralization and an increase in the release of CO₂ and CH₄ to the atmosphere. These greenhouse gases increase the mean global temperature, resulting in further permafrost thaw and therefore further warming, perpetuating the cycle known as the permafrost carbon-climate feedback. However, could subsequent increases in plant productivity due to greater N availability help mitigate the effects of this positive feedback mechanism?
Figure 2. Photographs of APEX Beta Bog B4. a) taken from the actively thawing margin facing toward the centre of the feature; b) depicting the environment (high water table) and plant community commonly found at the actively thawing margin.
Figure 3. Images depicting method for *C. aquatilis* root cross sections. Root cross sections were cut at 5 cm intervals of root length and processed to determine root porosity (%), a measure of the amount of aerenchyma tissue present within a root, and therefore a proxy for the root’s capacity to transport oxygen. The steps of the procedure are as follows: a) remove all dark pixels from around the root cross section, b) use the thresholding tool in ImageJ to reduce the image to binary and calculate the number of dark pixels (identified by the yellow outline), c) fill the entire cross section in black, d) use the thresholding tool again to calculate the total area of the cross section, in number of pixels. Then the number of dark pixels in b) is subtracted from the number of dark pixels in d) to determine the number of light pixels in b), which is then divided by the number of dark pixels in d) to determine the percentage of light pixels in the original cross section.
Figure 4. $\text{NH}_4^+$ uptake experiment (high spike). Mean $\text{NH}_4^+$ uptake by $C. \text{aquatilis}$ root tips after 4 and 8 hours of incubation in $(\text{NH}_4)_2\text{SO}_4$-spiked porewater (14.17 mg/L spike). Root tips were excavated from the centre (a) and edge (b) of Bog B2, the centre (c) and edge (d) of Bog B4, and the centre (e) and edge (f) of Bog B6, at depths of < 20 cm (shallow) or > 40 cm (deep). Neither sampling location nor depth from which root tips were excavated were significant predictors at any of the selected thermokarst bogs. Error bars represent the standard error of each mean value.
Figure 5. NH$_4^+$ uptake experiment (low spike). Mean NH$_4^+$ uptake by *C. aquatilis* root tips after 4 and 8 hours of incubation in (NH$_4$)$_2$SO$_4$-spiked porewater (7.09 mg/L spike). Root tips were excavated from the centre (a) and edge (b) of Bog B6 at depths of 20 cm or less (shallow) or 40 cm or more (deep). Neither sampling location nor depth from which root tips were excavated were significant predictors at any of the selected thermokarst bogs. Error bars represent the standard error of each mean value.
Figure 6. Comparison of linear slopes between the low and high spikes of (NH$_4$)$_2$SO$_4$. The slope of NH$_4^+$ uptake over the entire 8-hour period of the uptake experiment is compared between the two initial concentrations of (NH$_4$)$_2$SO$_4$ provided to C. aquatilis root tips, as well as between the locations from which root tips were excavated (including both the edge versus centre comparison and the shallow versus deep comparison for the purposes of depiction). There were no pairwise significant differences in comparisons of depth or location within each thermokarst bog, but averaged across all sampling locations and root depths, slopes for plants receiving the high spike were significantly greater than slopes for plants receiving the low spike. Error bar represent the standard error of each mean value.
Figure 7. Relationship between C. aquatilis root length and aboveground biomass. Based on the general linear models constructed to predict shoot dry biomass (g), an estimate of aboveground primary production, maximum root length (cm) was a significant predictor at the APEX Beta site ($p < 0.05$), as depicted by the solid black line with the accompanying equation and $r^2$ value, but was not a significant predictor at the Nenana Farms site ($p = 0.689$).

\[ y = 0.0132x + 0.5227 \]

$r^2 = 0.1738$
Figure 8. Comparison of root cross sections at different root lengths. Typical cross sections cut from the longest root (38 cm in length) of a *C. aquatilis* plant harvested from the edge of Bog N2 at the Nenana Farms site. The cross section in a) was cut at a root length of 20 cm, and therefore the pore spaces that are characteristic of aerenchymatous tissue are visible. The cross section in b) was cut at a root length of 35 cm, much closer to the root apex, and therefore the pore spaces are mostly closed. This is typical of the last 5 cm of a root (Purnobasuki and Suzuki, 2004) but generally does not occur at a particular (shallower) depth, regardless of the total length of the root.
Figure 9. Trend of root porosity with depth. Raw data for root porosity (%) as a function of the length of *C. aquatilis* root at which a cross section was cut. Three *C. aquatilis* plants were harvested from the centre (a) and edge (b) of Bog B6 from the APEX Beta site and from the centre (c) and edge (d) of Bog N2 from the Nenana Farms site, and the longest root of each plant was excised for analysis of root porosity, using root cross sections cut at 5 cm intervals of root length. Of these, the shortest and longest roots from each sampling location and site mentioned above have been selected for display.
### Table 1. Location of selected thermokarst bogs

The northing and easting geographic coordinates of *C. aquatilis* and porewater sampling points in the areas of old thaw (centre) and the areas of active thaw (edge) within each thermokarst bog selected for the study along with its respective ID.

<table>
<thead>
<tr>
<th>Study Site</th>
<th>Bog ID</th>
<th>Centre Coordinates</th>
<th>Edge Coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>APEX Beta</td>
<td>B2</td>
<td>64.69925°N, 148.31940°W</td>
<td>64.69925°N, 148.31920°W</td>
</tr>
<tr>
<td></td>
<td>B4</td>
<td>64.69810°N, 148.31943°W</td>
<td>64.69822°N, 148.31915°W</td>
</tr>
<tr>
<td></td>
<td>B6</td>
<td>64.69560°N, 148.32120°W</td>
<td>64.69622°N, 148.32013°W</td>
</tr>
<tr>
<td>Nenana Farms</td>
<td>N1</td>
<td>64.63488°N, 149.06586°W</td>
<td>64.63489°N, 149.06567°W</td>
</tr>
<tr>
<td></td>
<td>N2</td>
<td>64.63596°N, 149.06525°W</td>
<td>64.63589°N, 149.06429°W</td>
</tr>
<tr>
<td></td>
<td>N3</td>
<td>64.63661°N, 149.06482°W</td>
<td>64.63655°N, 149.06482°W</td>
</tr>
</tbody>
</table>
Table 2. Plant size characteristics. Shoot dry biomass, root dry biomass, maximum shoot height, maximum root length, and root:shoot biomass ratio for C. aquatilis plants growing at the APEX Beta site and the Nenana Farms site. Comparisons in ANOVA are made between plants growing at the edge and at the centre of each selected bog and between the cumulative means of the three selected bogs at each site. The mean values are separated here by bog to demonstrate inconsistencies in trends between the replicate sites. Values represent the mean ± standard error.

<table>
<thead>
<tr>
<th>Bog ID</th>
<th>Location</th>
<th>Shoot Dry Biomass (g)</th>
<th>Root Dry Biomass (g)</th>
<th>Max. Shoot Height (cm)</th>
<th>Max. Root Length (cm)</th>
<th>Root:Shoot Biomass Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.8083 ± 0.1167</td>
<td>0.1232 ± 0.0297</td>
<td>66.4 ± 3.6</td>
<td>22.6 ± 2.5</td>
<td>0.1454 ± 0.0176</td>
</tr>
<tr>
<td>B2</td>
<td>Edge</td>
<td>1.3503 ± 0.2117</td>
<td>0.4699 ± 0.0464</td>
<td>76.3 ± 5.8</td>
<td>49.2 ± 4.8</td>
<td>0.4196 ± 0.0902</td>
</tr>
<tr>
<td></td>
<td>Centre</td>
<td>1.0505 ± 0.1177</td>
<td>0.2507 ± 0.0360</td>
<td>55.0 ± 5.3</td>
<td>40.0 ± 5.0</td>
<td>0.2585 ± 0.0457</td>
</tr>
<tr>
<td>B4</td>
<td>Edge</td>
<td>1.0291 ± 0.2901</td>
<td>0.3528 ± 0.0971</td>
<td>52.2 ± 5.2</td>
<td>43.0 ± 7.8</td>
<td>0.3804 ± 0.1069</td>
</tr>
<tr>
<td></td>
<td>Centre</td>
<td>1.1166 ± 0.2454</td>
<td>0.7576 ± 0.3163</td>
<td>73.6 ± 4.3</td>
<td>27.2 ± 3.0</td>
<td>0.5527 ± 0.1215</td>
</tr>
<tr>
<td>B6</td>
<td>Edge</td>
<td>0.8439 ± 0.1754</td>
<td>0.5112 ± 0.2138</td>
<td>52.3 ± 4.4</td>
<td>50.8 ± 6.8</td>
<td>0.5259 ± 0.1314</td>
</tr>
<tr>
<td></td>
<td>Centre</td>
<td>1.0519 ± 0.0797</td>
<td>0.4202 ± 0.0996</td>
<td>85.0 ± 3.7</td>
<td>23.5 ± 1.0</td>
<td>0.4234 ± 0.1146</td>
</tr>
<tr>
<td>N1</td>
<td>Edge</td>
<td>1.462 ± 0.1308</td>
<td>0.3237 ± 0.0888</td>
<td>69.9 ± 6.7</td>
<td>29.1 ± 3.4</td>
<td>0.2702 ± 0.0529</td>
</tr>
<tr>
<td></td>
<td>Centre</td>
<td>0.8489 ± 0.0955</td>
<td>0.2157 ± 0.0685</td>
<td>63.6 ± 4.2</td>
<td>32.8 ± 2.3</td>
<td>0.2356 ± 0.0533</td>
</tr>
<tr>
<td>N2</td>
<td>Edge</td>
<td>1.3606 ± 0.1620</td>
<td>0.3613 ± 0.0440</td>
<td>84.1 ± 3.1</td>
<td>43.8 ± 3.2</td>
<td>0.2747 ± 0.0367</td>
</tr>
<tr>
<td></td>
<td>Centre</td>
<td>1.2150 ± 0.2168</td>
<td>0.3646 ± 0.0535</td>
<td>70.5 ± 8.9</td>
<td>33.5 ± 4.2</td>
<td>0.3406 ± 0.0658</td>
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<tr>
<td>N3</td>
<td>Edge</td>
<td>1.0156 ± 0.1792</td>
<td>0.2799 ± 0.0442</td>
<td>68.7 ± 5.3</td>
<td>41.1 ± 2.9</td>
<td>0.3307 ± 0.0838</td>
</tr>
<tr>
<td></td>
<td>Centre</td>
<td>1.1462 ± 0.1308</td>
<td>0.3237 ± 0.0888</td>
<td>69.9 ± 6.7</td>
<td>29.1 ± 3.4</td>
<td>0.2702 ± 0.0529</td>
</tr>
</tbody>
</table>
Table 3. Environmental characteristics at APEX Beta and Nenana Farms. Porewater temperature and oxidation-reduction potential (ORP) for samples drawn from depths of 20 cm and 40 cm and from the edge and centre of each of the three selected thermokarst bogs at each study site. Values represent the mean ± standard error.

<table>
<thead>
<tr>
<th>Bog ID</th>
<th>Location</th>
<th>Depth (cm)</th>
<th>Temperature (°C)</th>
<th>ORP (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B2</td>
<td>Edge</td>
<td>20</td>
<td>13.1 ± 0.6</td>
<td>87.0 ± 4.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>12.2 ± 0.1</td>
<td>71.6 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>Centre</td>
<td>20</td>
<td>17.0 ± 0.8</td>
<td>146.0 ± 3.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>14.3 ± 0.2</td>
<td>141.3 ± 2.2</td>
</tr>
<tr>
<td>B4</td>
<td>Edge</td>
<td>20</td>
<td>18.2 ± 0.5</td>
<td>123.8 ± 4.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>16.6 ± 0.5</td>
<td>76.2 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>Centre</td>
<td>20</td>
<td>25.3 ± 0.4</td>
<td>113.0 ± 3.5</td>
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<tr>
<td></td>
<td></td>
<td>40</td>
<td>18.3 ± 1.0</td>
<td>170.7 ± 0.5</td>
</tr>
<tr>
<td>B6</td>
<td>Edge</td>
<td>20</td>
<td>20.1 ± 0.6</td>
<td>112.7 ± 0.7</td>
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<td></td>
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<td>19.5 ± 0.3</td>
<td>108.9 ± 3.7</td>
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<td>0.8 ± 1.7</td>
</tr>
<tr>
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<td>14.0 ± 0.8</td>
<td>-33.4 ± 1.2</td>
</tr>
<tr>
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<td>49.7 ± 4.8</td>
</tr>
<tr>
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<td>15.9 ± 0.2</td>
<td>42.8 ± 3.3</td>
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<td>Edge</td>
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<td>-21.3 ± 4.8</td>
</tr>
<tr>
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<td></td>
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<td>15.0 ± 0.5</td>
<td>-41.1 ± 3.1</td>
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<td>18.6 ± 0.6</td>
<td>57.1 ± 2.7</td>
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<td>18.9 ± 0.6</td>
<td>39.2 ± 2.2</td>
</tr>
<tr>
<td>N3</td>
<td>Edge</td>
<td>20</td>
<td>16.2 ± 0.7</td>
<td>52.1 ± 1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>14.0 ± 0.5</td>
<td>33.5 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>Centre</td>
<td>20</td>
<td>16.9 ± 0.3</td>
<td>93.9 ± 6.4</td>
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<td>40</td>
<td>16.0 ± 0.2</td>
<td>69.0 ± 0.9</td>
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</tbody>
</table>
APPENDIX A

A number of trial runs for an NH$_4^+$ uptake experiment were conducted earlier in the 2016 field season, with the goal of optimizing the methodology for an experiment that has not previously been run at the APEX sites or, to my knowledge, for a graminoid species, such as *C. aquatilis*. The first two of these trial runs were conducted solely with the goal of determining NO$_3^-$ uptake, because at the time, with the ion chromatograph available from a colleague in Fairbanks, Alaska, only the anion system was functional. For these trial runs, *C. aquatilis* root tips were submerged in a simulated nutrient solution based on the solution used by MacFarlane and Yanai (2006). As it was later confirmed that *C. aquatilis* preferentially takes up NH$_4^+$ over NO$_3^-$, this data is not shown.

The third trial run was conducted on July 6, 2016, and involved the measurement of both NO$_3^-$ and NH$_4^+$ uptake after 0.5, 2, and 4 hours, by root tips submerged in either the simulated nutrient solution or in porewater extracted from the edge of Bog B6 at a depth of 40 cm. Either one, two, or three root tips that were either excised or excavated were submerged in 40 mL of homogenized porewater in each 60 mL HDPE bottle. From this experiment (data not shown), it was determined that *C. aquatilis* preferentially takes up NH$_4^+$ over NO$_3^-$, which could have potentially been influenced by the increased supply of NH$_4^+$ in the natural porewater at the APEX Beta site. Similarly, it was also determined that using natural porewater as the medium from which NH$_4^+$ would be taken up was more successful than using a simulated nutrient solution, again potentially based on the increased supply of NH$_4^+$ but additionally, moving the root tips out of their native porewater and into the simulated solution could also have imparted some stress on the roots which inhibited their uptake in earlier trials, so all further trials were conducted using porewater as the medium for NH$_4^+$ uptake.
The fourth trial run was conducted on July 16, 2016, and contained similar methods to those of the third trial run. In this run, the uptake of NH$_4^+$ was not nearly as significant as the previous run, so it simply served to indicate that a maximum of three root tips was likely not sufficient to obtain meaningful results in the full experiment (data not shown).

The fifth and final trial run was conducted on July 20, 2016, and also contained both excised and excavated root tips. Samples included either 3 or 10 root tips, submerged for 8 hours in either native porewater or porewater spiked with (NH$_4$)$_2$SO$_4$ to a target NH$_4^+$ concentration of 7 mg/L, with additional aliquots removed from each HDPE bottle at 1 hour and 4 hours as well. The results of this trial run can be found in Figure A.1. From this experiment, it was determined that 10 root tips took up a greater amount of NH$_4^+$ at all time intervals, as expected, while excavated root tips took up a greater amount of NH$_4^+$ than did excised root tips. It was also determined that the concentration of NH$_4^+$ in the natural porewater was not nearly enough to allow for the exact depletion of NH$_4^+$ to be measured after 8 hours, and even the (NH$_4$)$_2$SO$_4$-spiked porewater was depleted by a much more significant amount that anticipated. For this reason, the target concentration of NH$_4^+$ in the full experiment was increased to 14 mg/L, along with the use of 10-12 excavated _C. aquatilis_ root tips for a period of 8 hours.
Figure A1. Preliminary NH₄⁺ uptake experiment. NH₄⁺ uptake by C. aquatilis root tips after 1, 4, and 8 hours of incubation in native porewater (a) or (NH₄)₂SO₄-spiked porewater (b). Root tips were excavated from the edge of Bog B6, were excised if necessary, and then submerged in the porewater solution for the full incubation period of 8 hours. Each treatment only received one replicate, so therefore the points do not represent means and no error bars are present.