



# Deep roots of *Carex aquatilis* have greater ammonium uptake capacity than shallow roots in peatlands following permafrost thaw

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## Abstract

**Aims** Climate warming in northern ecosystems is triggering widespread permafrost thaw, during which deep soil nutrients, such as nitrogen, could become available for biological uptake. Permafrost thaw shift frozen

organic matter to a saturated state, which could impede nutrient uptake. We assessed whether soil nitrogen can be accessed by the deep roots of vascular plants in thermokarst bogs, potentially allowing for increases in primary productivity.

**Methods** We conducted an ammonium uptake experiment on *Carex aquatilis* Wahlenb. roots excavated from thermokarst bogs in interior Alaska. Ammonium uptake capacity was compared between deep and shallow roots. We also quantified differences in root ammonium uptake capacity and plant size characteristics (plant aboveground and belowground biomass, maximum shoot height, and maximum root length) between the actively-thawing margin and the centre of each thermokarst bog as a proxy for time-following-thaw.

**Results** Deep roots had greater ammonium uptake capacity than shallow roots, while rooting depth, but not belowground biomass, was positively correlated with aboveground biomass. Although there were no differences in aboveground biomass between the margin and centre, our findings suggest that plants can benefit from investing in the acquisition of resources near the vertical thaw front.

**Conclusions** Our results suggest that deep roots of *C. aquatilis* can contribute to plant nitrogen uptake and are therefore able to tolerate anoxic conditions in saturated thermokarst bogs. This work furthers our understanding of how subarctic and wetland plants respond to warming and how enhanced plant biomass production might help offset ecosystem carbon release with future permafrost thaw.

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## Introduction

Permafrost soils store an estimated  $1035 \pm 150$  Pg of carbon (C) in the upper 3 m of depth (Schuur et al. 2018). This permafrost C stock overlaps with the northern peatland C pool, as approximately 30% of northern peatlands are thought to contain surface permafrost (Lindgren et al. 2018). Permafrost in peatlands can thaw via gradual thickening of the active layer, during which surface permafrost slowly thaws, leading to increases in microbial activity and soil C mineralization (Hong et al. 2014; Osterkamp et al. 2009). Permafrost in peatlands can also thaw via thermokarst, or subsidence and inundation of the ground layer, which occurs more abruptly and affects the entire soil column to greater depth relative to active layer thickening (Rodenhizer et al., 2020; Turetsky et al. 2019). Both active layer thickening and thermokarst result in the release of carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>) to the atmosphere through microbial respiration of formerly frozen permafrost organic matter (Hodgkins et al. 2014; Schuur et al. 2015). Because of these biological responses, permafrost thaw is thought to be one of the most important terrestrial biogeochemical feedbacks to future climate (Hodgkins et al. 2014; Schuur et al. 2015). Empirical and modeling studies to date largely have focused on how decomposition of organic matter from thawing permafrost might increase C emissions to the atmosphere, while fewer studies have addressed whether permafrost thaw could stimulate ecosystem C uptake from the atmosphere by increasing nutrient availability and thus, plant productivity (but see Hewitt et al. 2019, Keuper et al. 2012; Natali et al. 2011; Pedersen et al., 2020; Salmon et al., 2016; Salmon et al., 2018).

A fundamental pattern controlling many terrestrial ecosystems is that primary productivity is generally limited by nitrogen (N) availability (Vitousek and Howarth 1991) and northern peatlands are no exception (Aerts et al., 1992; Finger et al., 2016; Keuper et al., 2017). When permafrost thaws, large stocks of soil N that were previously frozen could be leached or mineralized and become available for biological uptake (Harden et al., 2006; Keuper et al. 2012; Pedersen et al., 2020; Salmon et al. 2016). Given that N is a limiting resource for primary productivity, if plants

and microbes can access this newly-available N, it could fundamentally alter the way northern ecosystems function (Keuper et al. 2012; Natali et al. 2012). When released through permafrost thaw, a substantial fraction of the N stock bound in permafrost soils could potentially be taken up by the deep root systems of plants, particularly if it is released in plant-available forms, either nitrate (NO<sub>3</sub><sup>-</sup>) or ammonium (NH<sub>4</sub><sup>+</sup>) (Finger et al. 2016; Keuper et al. 2012; Pedersen et al., 2020), with the latter likely being the preferred form in the colder, more acidic, and waterlogged soils of northern peatlands (Britto and Kronzucker, 2013). Increases in microbial activity post-thaw can also stimulate net N mineralization throughout the soil column (Keuper et al. 2012), with higher mineralization rates being found in deeper portions of the active layer (Salmon et al., 2018), as well as along the edges of thermokarst bogs (Finger et al., 2016). Thus, we believe that rooting systems extending into areas of thawing permafrost have a large potential for taking up these newly-available sources of N. This would include areas deeper in the peat profile near the vertical thaw front as well as potentially shallow peat close to the lateral thaw front, at the transition between thermokarst bog and permafrost plateau peat.

In wetland soils, saturation causes slow oxygen (O<sub>2</sub>) diffusion in the soil water column, triggering anoxia and a much less efficient use of energy (Benstead and Lloyd 1996; Chen and Qualls 2003; Kirk 2004), as well as inhibited nutrient uptake by plants (Baltzer et al. 2014). One common adaptation in wetland plants is the development of specialized root cortical tissue known as aerenchyma (Chen et al. 2002), which allows for intercellular transport of O<sub>2</sub> down a concentration gradient from the atmosphere to deep roots (Kirk 2004). However, radial oxygen loss (ROL), the diffusion of O<sub>2</sub> away from aerenchyma and into the water column, occurs continuously along the length of the root (Colmer 2003). This increases the O<sub>2</sub> requirement for the normal functioning of deeper root cells in order to maintain the typical rate of metabolism and nutrient uptake (Kirk 2004). In ecosystems underlain by permafrost, plant roots are constrained to the surface, seasonally-thawed active layer. As permafrost thaws and N availability increases, plant roots can potentially extend downward into deeper, newly-thawed organic matter to access available N (Keuper et al. 2012). However, it is unclear whether vascular plants in saturated thermokarst systems can overcome the limitations of developing and maintaining deep root systems in a potentially stressful

environment (i.e., decreasing temperature and O<sub>2</sub> availability with increasing depth) in order to take up newly-available deep soil N sufficiently. If plants are able to access the new pools of a limiting nutrient made available by warming or permafrost thaw, it could stimulate plant C uptake and net primary productivity (Keuper et al. 2012; Keuper et al. 2017).

The objective of this study was to understand if vascular plants in thermokarst bogs can access deep N pools made available by permafrost thaw, and if so, whether this influences plant productivity. To address this broad objective, we asked three specific research questions: 1) Does plant N uptake capacity (measured as the depletion of NH<sub>4</sub><sup>+</sup> from porewater samples by excavated roots) differ between shallow and deep roots in thermokarst bogs? 2) How does aboveground plant biomass vary between areas of active versus old permafrost thaw? and 3) Is aboveground plant biomass correlated with rooting depth, root biomass, or root N uptake capacity? Due to a more recent influx of nutrients from thawing permafrost organic matter, we predicted that plant biomass and maximum rooting depth would be greater near the actively-thawing margin compared to areas in the centre of each thermokarst bog, where permafrost in the peat layer thawed decades ago and nutrients are more likely to be depleted. In both the edge and centre sampling locations, we predicted that NH<sub>4</sub><sup>+</sup> uptake capacity would be greater in shallow roots than in deeper roots due to the greater anoxic stress imparted on roots in deeper peat layers, which would inhibit their ability to take up nutrients.

## Materials and methods

### Description of study sites and target species

The main study area is located in a permafrost peatland complex affiliated with the Alaska Peatland Experiment (APEX) near the Bonanza Creek Experimental Forest (64°41' N, 148°19' W), about 30 km southwest of Fairbanks, AK, USA. In the APEX study area, permafrost thaw has created numerous thermokarst bogs, causing a shift from a black spruce-dominated peat plateau to *Carex*- and *Sphagnum*-dominated thermokarst bogs. These thermokarst bogs do not have surface permafrost in the upper 1 m of peat by the end of a typical growing season, and due to thaw of ice-rich permafrost they have experienced 0.5–1.0 m of ground

subsidence relative to the adjacent permafrost peatlands that continue to have intact surface permafrost with an active layer depth of approximately 40 cm (Klapstein et al., 2014). A previous study showed that vegetation in these bogs is N-limited, making them good candidates for exploring the uptake of N post-thaw (Finger et al., 2016). We also included a second, more remote study area located about 50 km southwest of Fairbanks (referred to as Nenana Farms), in which thermokarst leads to bog formation within a birch-dominated peatland complex. These sites have not been previously used for monitoring research and therefore further information on the timing of thaw or depth-age characteristics of peat is limited. Rates of permafrost thaw and nutrient cycling are thought to be greater in birch- than conifer-dominated ecosystems (Lara et al. 2016; Osterkamp et al. 2000). Thus, a comparison between these two study areas allowed some of our results to be more generalizable across interior Alaskan peatlands.

Within each of the APEX and Nenana Farms peatland complexes, we selected three thermokarst bogs experiencing ongoing thaw and subsidence (identified as “sites”). The actively-thawing margins of thermokarst bogs were discerned by the presence of saturated moats and drunken forest or tipping trees, which are indicative of soil inundation and instability caused by permafrost thaw over the past 10–30 years, while the older-thaw areas were characterized by hummocks and hollows that have formed within the last 80–400 years following thaw (Finger et al., 2016; Klapstein et al., 2014; Turner et al., 2020). Within each thermokarst bog, we compared vascular plant growth characteristics between the actively-thawing margin (hereafter referred to as “edge”) and older areas of thaw located in the middle of the bog (hereafter referred to as “centre”).

Here we use the water sedge (*Carex aquatilis* Wahlenb.) as a target species because it was the dominant vascular plant in all of our selected thermokarst bogs, making up >90% of the vascular vegetation cover in both study areas. More broadly, it is a dominant sedge species in the wetlands of interior Alaska and across much of the boreal region of North America (Tande and Lipkin 2003). It is a perennial, herbaceous, aerenchyma-forming plant of the Cyperaceae (sedge) family, that can grow over 1 m in shoot height and over 70 cm in root length (Tande and Lipkin 2003), making it an ideal candidate to study both aboveground and belowground vascular plant responses to permafrost thaw.

## Nitrogen uptake experiment

*C. aquatilis* generally reaches peak biomass in our study areas in late July to early August before it begins to senesce. We conducted our N uptake experiment on August 1, 2016 in the three APEX thermokarst bogs. We were not able to conduct this experiment at the Nenana Farms thermokarst bogs given the remoteness of those sites. Porewater was collected using a sipper and syringe from the actively-thawing margin of one of the three selected thermokarst bogs at 40 cm 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, conducted across all three bogs, each bottle was spiked with a liquid ammonium sulfate –  $(\text{NH}_4)_2\text{SO}_4$  – stock solution to a final  $\text{NH}_4^+$  concentration of 14.17 mg/L in each 60 mL HDPE bottle (McFarlane and Yanai 2006; Rennenberg et al. 1996). Through preliminary experiments (data not shown), this concentration of  $\text{NH}_4^+$  was determined to be sufficient to allow for some  $\text{NH}_4^+$  to still be remaining after 8 h, but not so excessive that discernable differences in  $\text{NH}_4^+$  uptake could not be determined. An additional set of bottles allotted to only one thermokarst bog received a halved  $(\text{NH}_4)_2\text{SO}_4$  spike (7.09 mg/L), in order to confirm the consistency of our experimental design across multiple initial  $\text{NH}_4^+$  concentrations (Fig. S1). Three of the filled HDPE bottles were immediately set aside in a cooler on ice to represent the initial concentration of  $\text{NH}_4^+$  in the spiked porewater. Individuals of *C. aquatilis* were haphazardly selected from each sampling location (the edge and centre of each thermokarst bog) by pulling each plant, along with its entire intact root system, out of the bog gently by hand. This was possible because of the loose, unconsolidated nature of peat in these thermokarst bogs. Any excess peat attached to the root system was shaken off or removed by rinsing the roots briefly with deionized water. Excavated plants were categorized by root length (a proxy for their depth within the vertical peat profile) into two groups for our experiment: plants with shallow roots <20 cm in length versus plants with deep roots >40 cm in length. Ten to twelve roots from the shallow or deep root length categories, still intact with their complete plant system (i.e., excavated but not excised), were placed in each HDPE bottle, ensuring that the root tips were fully submerged in the spiked porewater, while the remainder of each plant was laid on the ground in open air. We analyzed triplicate samples

for each sampling location (edge versus centre) and each root length category (shallow versus deep) within each thermokarst bog for a total of 36 sample bottles. The bottles were then covered with plastic wrap to avoid contamination and allowed to incubate for a period of 8 h in the field at ambient conditions (temperatures between 18 and 20 °C). Aliquots of 20 mL in volume were removed from each bottle after 4 h, transferred to an additional HDPE bottle, and immediately placed in a cooler on ice to provide an intermediate data point for  $\text{NH}_4^+$  uptake over time.

After 8 h, the excavated root tips were removed from the spiked porewater and the submerged portion of each root tip was excised to allow each  $\text{NH}_4^+$  uptake value to be corrected for the amount of root biomass actually present within the volume of spiked porewater. All root samples and HDPE bottles were then placed in a cooler on ice, removed from the field site, and frozen. After shipping back to the University of Guelph, the excised root tip samples were dried in an oven at 65 °C for 48 h and weighed. The  $(\text{NH}_4)_2\text{SO}_4$ -spiked porewater samples from each HDPE bottle were filtered using syringes with inserted PreSep Glass (G15) Membrane Disks (25 mm in diameter with 0.5 µm pore spaces; GVS Life Sciences, Sanford, ME, USA) and the  $\text{NH}_4^+$  concentration was measured using an ion chromatograph (Dionex ICS-1600; Thermofisher Scientific, Mississauga, ON, Canada) with a Dionex IonPac CS12A Cation-Exchange column (4 × 250 mm dimensions, 8.5 µm particle size; Thermofisher Scientific, Mississauga, ON, Canada) and Chromeleon software (Version 7.1; Thermofisher Scientific, Mississauga, ON, Canada). We analyzed these concentrations against incubation time to determine the depletion of  $\text{NH}_4^+$  from the original spiked porewater solution by the excavated *C. aquatilis* root tips. Each  $\text{NH}_4^+$  uptake value was standardized by the root tip dry biomass that was submerged in each respective HDPE bottle (mg/g of root dry biomass). Four samples were excluded from all statistical analyses due to repeated failure in ion chromatography.

## Plant traits and water chemistry

We harvested six *C. aquatilis* plants from the actively-thawing margin and six plants from the older-thaw centre of each thermokarst bog in each study area, then shipped them frozen to the University of Guelph. These plants were used to determine maximum shoot height

and maximum root length. We then measured the biomass of shoots and roots by weighing after oven drying at 65 °C for 48 h. These biomass measurements were assumed to represent estimates of belowground and aboveground primary production for the 2016 growing season. Porewater samples were taken at depths of 20 cm and 40 cm at both the edge and centre of each thermokarst bog in each study area and used to measure oxidation-reduction potential (ORP) and porewater temperature in the field (Megonigal and Rabenhorst 2013; Romanowicz et al. 2015) using a Hach ORP probe (IntelliCAL MTC301, Hach Co., Loveland, CO, USA).

### Statistical analyses

All statistical analyses were conducted in R Studio, Version 1.3.1073 (2020). We analyzed  $\text{NH}_4^+$  uptake capacity at the APEX sites using a linear mixed effects model with the “lme” function in R package “nlme” (Version 3.1–150). Each thermokarst bog was considered to be an independent replicate in our design, given that bogs are isolated from one another by a large expanse of surface permafrost. We analyzed  $\text{NH}_4^+$  uptake capacity using root depth (shallow versus deep), sampling location (edge versus centre) and sampling time (4 h versus 8 h) as fixed effects as well as all two- and three-way interactions. We explored models with different structures of random effects to account for repeated measurements over the duration of the  $\text{NH}_4^+$  uptake experiment and the three replicate bogs in our experiment. Akaike’s information criterion (AIC) was used to determine that sample (or bottle) ID nested within thermokarst bog ID was the most appropriate to include in our modeling. We then evaluated bivariate relationships using likelihood-ratio chi-squared tests and removed all nonsignificant effects to achieve the most parsimonious model. The final model included time and depth as fixed effects, with sample ID nested within thermokarst bog ID as a random intercept. An identical model was constructed to assess  $\text{NH}_4^+$  uptake capacity in the additional set of samples receiving the halved spike of  $(\text{NH}_4)_2\text{SO}_4$  (Fig. S1), but model reduction was not conducted because all predictors and interactions were not significant. All significant differences were assessed using pairwise comparisons with Tukey’s HSD tests in R package “emmeans” (Version 1.5.2–1). The slopes of  $\text{NH}_4^+$  uptake capacity over 8 h at each spike level was compared using a three-way ANOVA (R function “aov”), including spike level (low versus

high), root depth, and sampling location, as well as interactions between each variable as predictors.

We also examined 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 area (APEX versus Nenana Farms), sampling location, and the study area  $\times$  sampling location interaction as predictors. A general linear model (R function “lm”) was constructed for each study site to predict shoot biomass, an estimate of aboveground primary production, using root biomass, maximum shoot height, and maximum root length as predictor variables.

Porewater temperature and ORP were analyzed using two-way ANOVAs (R function “aov”) with sampling location, depth (20 cm versus 40 cm), and the sampling location  $\times$  depth interaction as predictors. Temperature and ORP samples were also assessed using one-way ANOVAs (R function “aov”) to compare between the APEX and Nenana Farms study areas. For all significant effects in ANOVAs, we used Tukey’s HSD Tests (R function “TukeyHSD”) post hoc analysis of means.

## Results

### Nitrogen uptake experiment

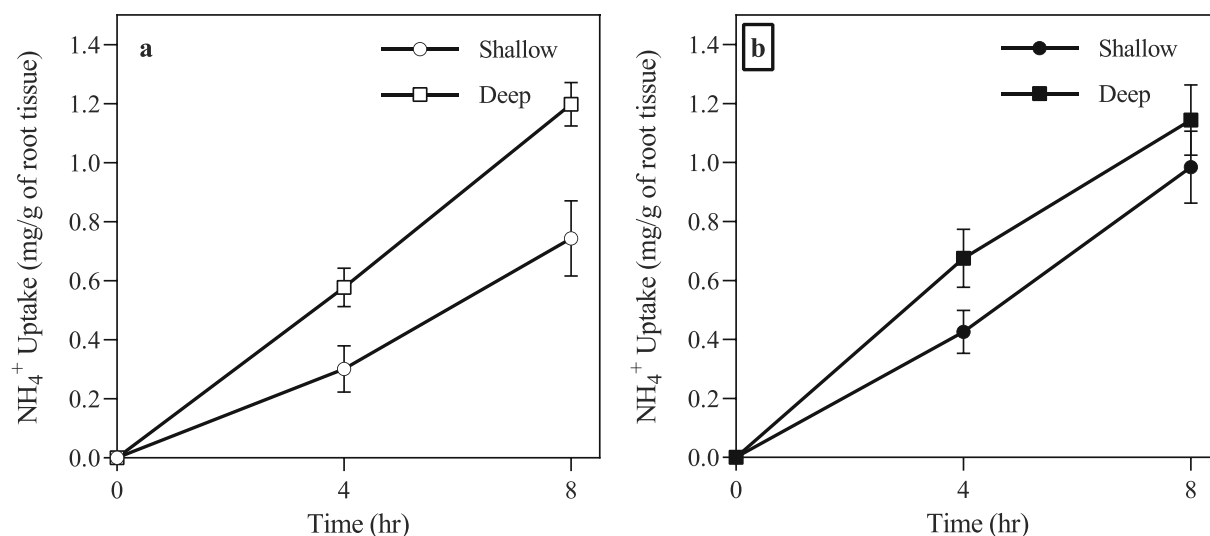
As expected, ammonium uptake consistently increased with incubation time ( $F_{1,32} = 102.298$ ,  $p < 0.0001$ ,  $r^2 = 0.504$ ). Contrary to our hypotheses, deep roots exhibited significantly greater  $\text{NH}_4^+$  uptake capacity than shallow roots during our incubation experiment ( $F_{1,31} = 13.089$ ,  $p < 0.001$ ; Fig. 1). There were no differences in  $\text{NH}_4^+$  uptake capacity between edge and centre roots and no interactions between fixed effects (all  $p$  values  $> 0.1$ ).

The average rate of  $\text{NH}_4^+$  uptake across 8 h was greater for roots receiving the higher concentration than the lower concentration of  $(\text{NH}_4)_2\text{SO}_4$  ( $F_{1,39} = 8.221$ ,  $p < 0.01$ ; Fig. 2). Again, deeper roots exhibited greater  $\text{NH}_4^+$  uptake rates than shallow roots ( $F_{1,15} = 9.615$ ,  $p < 0.01$ ). There was no difference in  $\text{NH}_4^+$  uptake rates between edge and centre roots and no interactions between fixed effects (all  $p$  values  $> 0.1$ ).

### Plant size characteristics

*Carex aquatilis* shoot biomass, root biomass, root length, and root:shoot biomass ratio did not vary

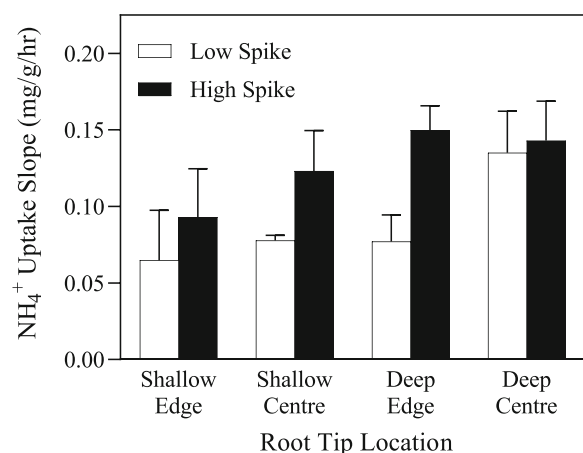




**Fig. 1** Mean  $\text{NH}_4^+$  uptake capacity by *C. aquatilis* root tips harvested from triplicate thermokarst bogs after 4 and 8 h of incubation in  $(\text{NH}_4)_2\text{SO}_4$ -spiked porewater (14.17 mg/L, high spike). Root tips were excavated from the edge (a) and centre

(b) of triplicate APEX thermokarst bogs at depths of <20 cm (shallow) or >40 cm (deep). Error bars are  $\pm$  one standard error for each mean value

between sampling locations or study areas (APEX versus Nenana Farms; Tables 1, S1) nor did they vary by a sampling location  $\times$  study area interaction (all  $p$  values >0.1). Shoot height did not vary between sampling locations ( $F_{1,74} = 0.328$ ,  $p = 0.568$ ), or by a sampling location  $\times$  study area interaction ( $F_{1,74} = 0.705$ ,  $p = 0.404$ ); however, shoots were significantly taller at Nenana Farms than at APEX ( $F_{1,74} = 9.744$ ,  $p < 0.01$ ; Tables 1, S1).



**Fig. 2** Slopes of *C. aquatilis* root  $\text{NH}_4^+$  uptake capacity per hour over an 8-h incubation. Treatments included two initial concentrations of  $(\text{NH}_4)_2\text{SO}_4$  (high spike: 14.17 mg/L, low spike: 7.09 mg/L), shallow versus deep roots, and edge versus centre sampling locations. Error bars are  $\pm$  one standard error for each mean value

We constructed a linear model to predict *C. aquatilis* shoot biomass at the APEX sites ( $\text{df} = 34$ ,  $r^2 = 0.534$ ). Maximum root length was positively related to shoot biomass ( $p < 0.05$ , Fig. 3). Shoot height was also positively related to shoot biomass at near-significant levels ( $p = 0.054$ ) while root biomass was not related to shoot biomass ( $p = 0.997$ ; Fig. S2). We used the same approach to predict *C. aquatilis* shoot biomass at the Nenana Farms sites ( $\text{df} = 28$ ,  $r^2 = 0.373$ ), but found no significant relationships with shoot biomass (root biomass:  $p = 0.619$ ; shoot height:  $p = 0.358$ ; root length:  $p = 0.689$ ; Figs. 3, S2).

### Environmental measurements

Mean porewater temperature and ORP were measured at two different depths at the edge and centre of each selected thermokarst bog in the APEX and Nenana Farms study areas (Table 2). Overall, porewater from the APEX sites was warmer and had higher mean ORP than at the Nenana Farms sites (temperature:  $F_{1,70} = 9.254$ ,  $p < 0.01$ ; ORP:  $F_{1,70} = 107.5$ ,  $p < 0.0001$ ). In both regions, porewater temperature was warmer and ORP was greater at the centre than at the edge of thermokarst bogs (temperature:  $F_{1,68} = 16.658$ ,  $p < 0.001$ ; ORP:  $F_{1,68} = 17.067$ ,  $p < 0.001$ ), while there were no differences between depths (temperature:  $F_{1,68} = 3.434$ ,  $p = 0.068$ ; ORP:  $F_{1,68} = 1.436$ ,  $p =$

**Table 1** Shoot dry biomass, root dry biomass, maximum shoot height, maximum root length, and root:shoot biomass ratio for *C. aquatilis* plants at the triplicate APEX and Nenana Farms thermokarst bogs. Values represent the mean  $\pm$  one standard error

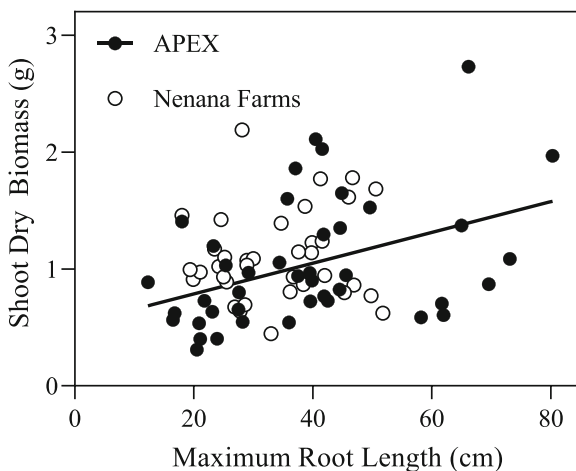
Site	Location	Shoot Dry Biomass (g)	Root Dry Biomass (g)	Max. Shoot Height (cm)	Max. Root Length (cm)	Root:Shoot Biomass Ratio
APEX	Edge	0.99 $\pm$ 0.10	0.38 $\pm$ 0.12	65.0 $\pm$ 3.0	37.8 $\pm$ 3.8	0.32 $\pm$ 0.06
	Centre	1.07 $\pm$ 0.13	0.44 $\pm$ 0.08	60.3 $\pm$ 3.8	39.8 $\pm$ 3.7	0.44 $\pm$ 0.06
Nenana Farms	Edge	1.04 $\pm$ 0.09	0.33 $\pm$ 0.05	73.1 $\pm$ 3.9	29.9 $\pm$ 1.9	0.33 $\pm$ 0.05
	Centre	1.17 $\pm$ 0.09	0.32 $\pm$ 0.03	74.2 $\pm$ 3.3	38.0 $\pm$ 2.3	0.29 $\pm$ 0.03

0.235), nor was there a sampling location  $\times$  depth interaction (temperature:  $F_{1,68} = 0.025$ ,  $p = 0.876$ ; ORP:  $F_{1,68} = 0.501$ ,  $p = 0.482$ ).

## Discussion

Do *Carex aquatilis* roots maintain N uptake capacity with increasing depth?

We used a short-term experiment to compare  $\text{NH}_4^+$  uptake capacity in excavated roots of *C. aquatilis* from thermokarst bogs in interior Alaska. Consistently across sites and sampling locations, deep roots demonstrated greater  $\text{NH}_4^+$  uptake capacity than shallow roots. These results are contrary to our prediction that deep roots would have limited nutrient uptake due to anoxic stress.



**Fig. 3** Relationship between *C. aquatilis* maximum root length and aboveground biomass for plants harvested from the APEX ( $p < 0.05$ ,  $df = 34$ ,  $r^2 = 0.153$ ,  $y = 0.013x + 0.523$ ) and Nenana Farms ( $p = 0.689$ ) study areas. Results for the full model at the APEX study area are presented in the main text. The equation and line present in the figure refer only to the effect of maximum root length on aboveground biomass, with no effect of other predictors

Our results indicate that deep roots are at least similar in function if not better equipped to take up N post-thaw than shallow roots, indicating it is likely that deep rooting systems of *C. aquatilis* in these sites are not experiencing greater anoxic stress, as we had originally hypothesized. It is typical of root systems in water-logged conditions to be subjected to ROL, which can potentially reduce  $\text{O}_2$  availability in aerenchyma by 30–40% (Colmer 2003). However, the consumption of  $\text{O}_2$  by root cells along the radial path through which  $\text{O}_2$  diffuses from aerenchymatous tissue to the rhizosphere is one of the key limiting factors of ROL (Armstrong et al. 2000; Colmer 2003). It has been shown that *C. aquatilis* invests 5–6 times greater root biomass per unit root length than other wetland graminoids, such as *Eriophorum angustifolium* (Billings et al. 1978). Therefore, additional investment in biomass per unit length by *C. aquatilis* could be limiting ROL, allowing for more consistent  $\text{O}_2$  availability between shallow and deep root cells (Ryser et al. 2011). This may explain why *C. aquatilis* extends its roots to lengths of greater than 70 cm, while *Eriophorum* species are generally considered to be shallow-rooted wetland graminoids (Keuper et al. 2017). The potential benefit of deeper rooting for nutrient acquisition could outweigh the high additional cost of root biomass. In turn, we speculate that this might explain why we found greater  $\text{NH}_4^+$  uptake in deep roots than originally expected.

Our results are consistent with a  $^{15}\text{N}$  fertilization study finding that *Rubus chamaemorus* was particularly efficient in acquiring N from deep peat layers (Keuper et al. 2017). *Rubus chamaemorus* is a dominant species in many sites experiencing active layer thickening but is not common in newly formed thermokarst bogs (Finger et al. 2016). Lamit et al. (2017) determined that *Carex oligosperma* consistently extended roots up to 1 m in depth and measured porewater  $\text{NH}_4^+$  concentrations that were approximately half those of treatments lacking

**Table 2** 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 triplicate APEX and Nenana Farms thermokarst bogs. Values represent the mean  $\pm$  one standard error

Site	Location	Depth (cm)	Temperature ( $^{\circ}$ C)	ORP (mV)
APEX	Edge	20	17.1 $\pm$ 1.1	107.8 $\pm$ 5.8
		40	16.1 $\pm$ 1.1	85.6 $\pm$ 6.1
	Centre	20	21.8 $\pm$ 1.3	134.9 $\pm$ 5.6
		40	19.5 $\pm$ 1.7	139.4 $\pm$ 9.4
Nenana Farms	Edge	20	15.9 $\pm$ 0.8	10.5 $\pm$ 11.0
		40	14.3 $\pm$ 0.4	-13.6 $\pm$ 11.9
	Centre	20	17.6 $\pm$ 0.4	66.9 $\pm$ 7.3
		40	16.9 $\pm$ 0.5	50.3 $\pm$ 4.8

*Carex*, demonstrating the ability of *Carex* roots to take up large amounts of  $\text{NH}_4^+$ . Furthermore, Salmon et al. (2016) found that after five years of experimental warming in an interior Alaskan peatland, the active layer deepened by 15 cm, and resulted in a 23% increase in aboveground *Eriophorum* biomass and a 49% increase in the foliar N pool. Taken together with our results, the ability to use deeper roots to acquire newly available N appears to be a common plant trait in ecosystems experiencing permafrost thaw.

A distinct advantage of the bottle incubation approach to addressing our research questions is the ability to control for environmental variables that we expected to differ between shallow and deep peat layers, namely temperature,  $\text{O}_2$  availability, ORP, and nutrient availability. Thus, any differences in  $\text{NH}_4^+$  uptake between shallow and deep roots can be inferred to be due to biological differences between roots of different lengths. We speculate that increased  $\text{NH}_4^+$  transporter expression and/or activity in deeper roots is responsible for the observed increase in  $\text{NH}_4^+$  uptake. It has been previously shown in numerous plant systems that high-affinity  $\text{NH}_4^+$  transporters are downregulated in response to greater  $\text{NH}_4^+$  availability (Glass et al., 2002), while the activity of low-affinity  $\text{NH}_4^+$  transporters increases in response to greater  $\text{NH}_4^+$  availability (Howitt and Udvardi, 2000). Currently, it is unknown whether the expression or activity of high- and low-affinity  $\text{NH}_4^+$  transporters are likely to differ between shallow and deep roots in thermokarst systems, while it is also unclear whether the sensing of artificially high  $\text{NH}_4^+$  concentrations by the root tips in our experiment triggered more complex plant processes that may have affected  $\text{NH}_4^+$  uptake (Liu and von Wirén, 2017). To our knowledge, an investigation of  $\text{NH}_4^+$  uptake and assimilation

at a molecular level has not been conducted for vascular plants in permafrost peatlands. Therefore, without further experimentation, it is difficult to make conclusions about the acclimatization of deep roots to greater  $\text{NH}_4^+$  availability based solely on our  $\text{NH}_4^+$  uptake experiment; however, we do conclude that deeper roots appear to be better equipped to take up available  $\text{NH}_4^+$ . Our bottle incubation also introduced *C. aquatilis* root tips to unrealistic environmental conditions, namely higher temperature and  $\text{O}_2$  availability, which could have affected our results. However, a recent study at sites similar to ours involved  $^{15}\text{N}$  fertilization at the thaw front and demonstrated similar results under natural conditions, with deeper rooting plants being associated with greatest immediate enrichment with  $^{15}\text{N}$  (Hewitt et al., 2019). A future  $^{15}\text{N}$  enrichment study monitoring root function at multiple depths would provide a more definitive understanding of deep and shallow root activity and relative contributions to overall plant N uptake, as well as key microclimate or seasonal controls in northern peatlands following permafrost thaw.

At the deciduous-dominated Nenana Farms sites, ORP values were lower than at the APEX thermokarst bogs. This agrees with the notion that rates of nutrient cycling, and therefore rates of microbial activity, are generally greater in thermokarst bogs surrounded by birch-dominated forest than in those surrounded by conifer-dominated forest (Lara et al. 2016). It also suggests that the demand for  $\text{O}_2$  in waterlogged peat layers is greater at Nenana Farms, so any available  $\text{O}_2$  would be taken up extremely quickly, while increased microbial activity drives further ROL from vascular plant roots (Laskov et al. 2006). Lower ORP values have also been shown to directly increase ROL from aerenchymatous tissue (Laskov et al. 2006), potentially



exacerbating O<sub>2</sub> demand in vascular plant roots, and therefore inhibiting metabolic processes in root cells, including nutrient uptake (Pezeshki and DeLaune 2012). It thus seems unlikely that the results of our NH<sub>4</sub><sup>+</sup> uptake experiment conducted at the APEX study area would apply to Nenana Farms. This highlights the need to understand not only the composition of surrounding plant communities, but also spatial and temporal variation in plant functional ecology across diverse thawing permafrost ecosystems.

#### Are plant belowground traits related to aboveground biomass?

Results of our NH<sub>4</sub><sup>+</sup> uptake experiment indicate the potential for deep roots to contribute to plant N uptake, but do not indicate whether deep root nutrient uptake impacts aboveground primary productivity. To explore this descriptively, we measured plant size characteristics for *C. aquatilis* harvested from the actively-thawing margin and older-thaw centre of each thermokarst bog to test the prediction that aboveground *C. aquatilis* biomass would be greater soon after thaw because of N released from thawing permafrost organic matter. Our results did not support this hypothesis, as there were no differences in NH<sub>4</sub><sup>+</sup> uptake capacity, plant size characteristics, or investment in root biomass versus shoot biomass between sampling locations. Salmon et al. (2018) showed seven-fold greater dissolved inorganic nitrogen (DIN) release from thawed permafrost soils than shallow active layer soils, indicating the potential for greater NH<sub>4</sub><sup>+</sup> uptake and plant productivity with more recent thaw. However, neither foliar C:N ratio nor foliar N concentration (%) differed between the actively-thawing margin and the older-thaw centre of a thermokarst bog located close to our selected bogs within the APEX study area. This finding indicates that vascular plants within these systems are unlikely to be benefitting from luxury consumption of N, as is common in *Carex* species in N-limited soils (Güsewell 2005), and may be further limited in their utilization of N released post-thaw (Finger et al. 2016). Finger et al. (2016) also found that DIN did not differ at these sites with time-following-thaw, while the majority of dominant plant species exhibited low foliar N:P ratios across the entire thaw gradient. These findings indicate that N is maintained as a strong limiting factor post-thaw, which could explain why there were no differences in NH<sub>4</sub><sup>+</sup> uptake capacity or plant traits between sampling locations.

Soil environmental conditions may constrain biological responses to thawing permafrost organic matter. We measured lower mean temperature and ORP values at the actively-thawing margins than at the centres. This suggests that recent lateral thaw is associated with greater ROL from aerenchymatous roots and overall less favourable environmental conditions for plant metabolic processes, including NH<sub>4</sub><sup>+</sup> uptake, which could limit the potential for plants to capitalize on new N sources from permafrost at the actively-thawing margins. Conversely, we did not find any differences in ORP values between sampling depths. This indicates that ROL from *C. aquatilis* roots is more likely to be dictated by lateral proximity to the thaw front as opposed to vertical, which complements our finding that deep roots are well equipped for NH<sub>4</sub><sup>+</sup> uptake, and therefore likely not experiencing significant inhibition of NH<sub>4</sub><sup>+</sup> uptake capacity compared to shallow roots due to anoxic stress. Furthermore, experimental water table lowering has been shown to be associated with greater decomposition rates (Hribljan et al. 2014), nutrient availability (Munir et al. 2017), and plant biomass (van Dijk et al. 2012), all of which are indicators that anoxia in waterlogged environments, such as those likely to be found at the actively-thawing margin, can directly inhibit the success of vascular plants.

We explored the effects of plant traits on aboveground biomass and determined that only maximum root length was a significant factor. While our measurements did not show direct connections between actively-thawing permafrost and plant responses, this finding highlights that plants investing in deeper roots tend to be associated with greater aboveground primary production. On the other hand, similar results were not observed in the Nenana Farms study area, as neither root length, root biomass, nor shoot height were significant predictors of shoot biomass. Based on our ORP results at each study area, we speculate that this is attributable to a reduced capacity of *C. aquatilis* to take up nutrients released in the more anoxic Nenana Farms sites. Taken together, our measurements of soil environmental conditions and plant size characteristics indicate that any potential connections between the release of N post-thaw and vascular plant productivity still require further investigation. This is crucial to improving our understanding of how important plant C uptake could be in offsetting permafrost C losses under future warming and permafrost thaw.

## Conclusion

One of the main questions regarding N cycling following permafrost thaw is whether or not vascular plants can access and meaningfully utilize the increased N supply created by thawing permafrost organic matter. We addressed this issue through an  $\text{NH}_4^+$  uptake experiment using *C. aquatilis* root tips excavated from thermokarst bogs, demonstrating  $\text{NH}_4^+$  uptake capacity by deep roots greater than that of shallow roots, suggesting that deep roots are likely to be effective in taking up N released from permafrost thaw despite anoxic conditions in saturated peat layers. However, no significant differences were quantified between plants growing in actively-thawing margins versus older-thaw centres in terms of  $\text{NH}_4^+$  uptake capacity, plant size characteristics, or biomass investment in shoots versus roots. Therefore, we did not establish a direct link between increased N supply/uptake due to permafrost thaw and increased aboveground primary production. However, a positive relationship between maximum root length and aboveground biomass is suggestive of a relationship between N supply in deeper peat layers post-thaw, N uptake by deep roots, and increased aboveground primary production. Relationships between these processes are likely to be impacted by environmental conditions such as temperature and  $\text{O}_2$  availability, which also varied between the thermokarst sites included in this study. Further investigation of plant N uptake patterns and how they vary across species, thermokarst environments, N sources, and biochemical uptake pathways will be useful for ecosystem models simulating the biogeochemical coupling of C and N under a changing climate.

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**Data accessibility** The datasets generated and analyzed during this study are available in the Bonanza Creek Long-Term Ecological Research program (BNZ LTER) Data Catalog. Data for the  $\text{NH}_4^+$  uptake experiment at the APEX study area can be accessed at <http://www.lter.uaf.edu/data/data-detail/id/735>. Data for plant size characteristics at the APEX and Nenana Farms study areas can be accessed at <http://www.lter.uaf.edu/data/data-detail/id/736>.

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