Received Date : 07-Jan-2016

Revised Date : 01-Jun-2016

Accepted Date : 16-Jun-2016

Article type : Standard Paper

Effects of permafrost thaw on nitrogen availability and plant-soil interactions in a boreal Alaskan lowland

Rebecca A. Finger[1,2*], Merritt R. Turetsky[3], Knut Kielland[2], Roger W. Ruess[2], Michelle C. Mack[4], Eugénie S. Euskirchen[2]

[1] Department of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, AK 99775 USA

[2] Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK 99775 USA

[3]Department of Integrative Biology, University of Guelph, Guelph ON N1G 1G2, Canada

[4]Center for Ecosystem Science and Society, Northern Arizona University, Flagstaff, AZ 86011 USA

*Corresponding author:

Email: rebecca.finger@gmail.com; Fax: (603)646-1682

Current address:

Dartmouth College, Hinman Box 6182, Hanover, NH 03755 USA

Running headline: Permafrost thaw and N availability in a boreal peatland

Summary

1) Increasing rates of permafrost thaw in boreal peatlands are converting conifer forests to waterlogged open wetlands. Permafrost thaw increases soil nitrogen (N) availability, but it is unclear whether such changes are due solely to changes in surface soil N mineralization or N mobilization from thawing permafrost soils at depth.

2) We examined plant species composition and N availability along triplicate permafrost thaw gradients in Alaskan peatlands. Each gradient comprised four community types including: 1) a permafrost peatland with intact permafrost; 2) a drunken forest experiencing active thaw; 3) a moat representing initial complete thaw; and 4) a collapse scar bog representing several decades of post-thaw succession.

3) Concentrations of dissolved organic (DON) and inorganic N (DIN) in the upper 60 cm of soil increased along the permafrost thaw gradients. The drunken forest had the greatest mean concentrations of total dissolved N relative to the other community types, primarily due to greater concentrations of large molecular DON. The moat and collapse bog had significantly greater inorganic N concentrations than the permafrost or drunken forest, suggesting that changes in N availability are not a short-term effect, but can be sustained for decades or centuries. Across all plant community types, DIN and DON concentrations increased with soil depth during maximum seasonal ice thaw (September), suggesting that deeper soil horizons are important reservoirs of N post-thaw.

4) Vegetation responses to permafrost thaw included changes in plant community composition shifting from upland forest species to hydrophilic vegetation with deeper

rooting profiles in the collapse scar bogs, and changes in foliar N and δ^{15}N values. N concentrations in plant foliage and litterfall increased with concentrations of DIN during collapse bog succession, suggesting that plants are utilizing additional mineralized N.

5) *Synthesis*: Our results suggest that the conversion of forest to wetlands associated with permafrost thaw in boreal lowlands increases N availability, at least in part by increasing turnover of deep soil organic matter. Plants appear to utilize these additional deeper N sources over timescales of years to centuries following permafrost thaw.

Key-words: climate change, collapse scar bog, nutrients, peatlands, rooting depth, stable isotopes, subarctic, thermokarst, δ^{15}N

Introduction

Peatlands represent a globally important sink for atmospheric carbon. Despite serving as a large natural source of methane, peatlands throughout the Holocene have had net cooling effect on the climate system due to carbon sequestration in peat (Gorham 1991). In northern peatlands, approximately 30-40% are underlain by permafrost where peat insulates deeper frozen soils from solar radiation. However, since the mid-1800s, warmer climate conditions have caused widespread permafrost thaw across the discontinuous permafrost zone of North America (Chapin *et al*. 2010; Osterkamp *et al.* 2009). Furthermore, many boreal peatlands are underlain by warm permafrost, with mean annual near-surface permafrost temperatures within ~1 ° C of thawing (Jorgenson *et al* 2001), making these ecosystems particularly susceptible to thaw under future climate scenarios.

By altering surface topography and hydrology, permafrost thaw has consequences for peatland vegetation community structure and biogeochemical cycling (Camill 2005; Osterkamp *et al*. 2000, Shuur *et al.* 2015). Thaw of permafrost in boreal lowlands typically causes forested permafrost peatlands to be replaced by inundated collapse scar bogs or fens (Camill 1999; Quinton & Baltzer 2012). Permafrost thaw has been shown to increase decomposition losses of deep peat carbon, which diminishes the carbon sink capacity of peatlands (O'Donnell *et al.* 2011, Euskirchen *et al.* 2014). However, vegetation communities in the post-thaw state are more productive than vegetation in the permafrost state, causing a new surface peat layer to accumulate quickly following thaw (Turetsky *et al*. 2007). Permafrost organic matter also contains elements such as nitrogen and phosphorus, both considered limiting macronutrients for plant productivity, though changes in these nutrient stocks as organic matter moves from a frozen to a thawed state are largely unknown.

Recent studies have shown that 1) permafrost thaw stimulates plant-available N in surface soils (Keuper *et al.* 2012; Salmon *et al.* 2016) and 2) that deeper soils (representing the thawing permafrost soils pool) store large amounts of N (Harden *et al.* 2012). However, the fate of these deeper soil N stocks post-thaw is poorly understood, and it is not known whether plants are able to access deep soil N in collapse scar bogs or fens due to soil saturation. Saturated soil conditions in these wetland ecosystems leads to low redox potential and may impede mineralization in these deeper soil layers and exacerbate N limitations to productivity over long time periods. These saturated conditions may also constrain rooting depth, especially for woody vegetation, given that deep root systems below a water table tend to be costly to plants (Kohzu *et al.* 2003). When permafrost thaws in peatlands, changes in species composition and nutrient supply will have repercussions for plant nutrient acquisition and storage strategies. If permafrost thaw leads to increases in nutrient supply in these ecosystems, it can be generally expected that species adapted to low-nutrient conditions would be outcompeted by species that can readily capitalize on increase nutrient sources (Tilman 1990; Shaver *et al*. 2001). Conversely, saturated soil conditions in collapse scar bogs could constrain rooting depth and nutrient acquisition, thereby further exacerbating nutrient limitations. Changes in plant-nutrient indicators, such as root depth, foliar and litterfall nutrient concentrations, and nutrient resorption efficiencies may help to provide insights into mechanisms underlying changes in plant community composition (Sorrell *et al.* 2011).

Our objectives were to examine how soil and plant N dynamics change with permafrost thaw and how plant traits relate to N uptake and use across a permafrost thaw gradient (Fig. 1). We asked the following questions: Does permafrost thaw increase N availability in deeper soil layers, and, if so, how does this compare to changes in N availability in the historic active layer (surface soils)? Are plants able to utilize these potential sources of N deeper in the soil profile, or is plant uptake of deeper N limited by access and anoxic soil conditions? For this study, we focused on changes to dissolved inorganic nitrogen (DIN) concentrations, total dissolved nitrogen (TDN), dissolved organic nitrogen (DON), and free amino acids (FAA) in soil solution. This study tested three predictions: 1) First, we predicted that DIN concentrations would be low in permafrost forest peat due to temperature constraints on microbial mineralization, and that total N, including DIN, would increase following permafrost thaw. With ecosystem succession following thaw, we expected to see declines in DIN availability due to vertical accumulation of a peat layer and development of ombrotrophic conditions in collapse scar bogs. 2) If changes in N availability following thaw are driven more by the turnover and release of N from thawing permafrost organic matter than by active layer processes, we predicted that deeper soil layers would be associated with the greatest DIN concentrations and that variation in DIN concentrations with depth in the thaw sites would not be strongly related to soil temperature. 3) Driven by these patterns in soil N availability, we predicted that increasing availability of N at depth would cause hydrophilic plants to increase investment in deep roots and would lead to enriched δ^{15}N values in plant tissues as roots extended into deeper soil profiles with δ^{15}N values closer to zero, to access older sources of thawing permafrost organic matter (Kohzu *et al.* 2003).

Materials and methods

SITE DESCRIPTION AND TRANSECT DESIGN

Research was conducted at the Alaskan Peatland Experiment (APEX) sites, located near the Bonanza Creek Experimental Forest adjacent to the Tanana River floodplain approximately 35 km southeast of Fairbanks, AK (64.82º N, 147.87º W). In this region, air temperatures change dramatically across seasons, ranging from -50°C in January to +33°C in July with a mean annual temperature of -2.9°C (1988-2014; BNZ LTER climate database). Growing seasons average 135 days. Atmospheric N deposition is low, averaging 0.2 kg N ha^{-1} y^{-1} (Jones 2005).

The study sites are situated in discontinuous permafrost, where most low-lying peatlands are underlain by ice-rich permafrost. In this Interior Alaska region, permafrost peatlands tend to be forested with sparse black spruce (*Picea mariana*, plant scientific names provided by Johnson, Kershaw, & MacKinnon 1995) cover (mean tree density of <800 trees ha^{-1}; Churchill 2011). The APEX sites include a forested permafrost peatland complex with several internal thermokarst collapse scar bogs that have developed within the past several hundred years (Klapstein *et al.* 2014). The difference in surface elevation between permafrost and collapse bog ranges from about 0.5-1 m. Due to soil destabilization and flooding associated with thermokarst, trees tilt and eventually die, creating "drunken forests" near the active thaw zone. Tilting trees fall into the expanding collapse scar bog and are eventually buried by post-thaw accumulation of moss and peat.

Similar to findings at other collapse bog and fen sites (Camill 1999), the post-thaw community at our sites is dominated by fast-growing aquatic plant species such as sedges and hydrophilic *Sphagnum* mosses (Churchill 2011; Table 1). With increasing time-following-thaw, hummock-hollow microtopography develops in the collapse bogs with woody plants such as leather leaf (*Chamaedaphne calyculata)*, dwarf and bog birch (*Betula nana and B. glandulosa*), larch (*Larix laricina*) and black spruce emerging in larger, drier hummocks (Table 1, See Table S1 for Supporting Information). Hollows remain near the water table and are saturated throughout the growing season. During the winter when ambient air temperatures rarely exceed 0°C, surface soil layers up to about 50 cm in depth will freeze, although deeper thaw peats in the collapse scar bogs that interface with a standing hydric zone remain unfrozen year round. Shifts in plant functional group abundances across the study sites were observed from percent cover estimates (Fig. S1).

Within the APEX permafrost peatland, we established three transects encompassing the transition from permafrost forest to established collapse scar bog. Transects were selected such that the thaw bogs were distinct, hydrologically and spatially separate systems. Within each transect, we established sampling plots in four different plant community types: 1) the forested permafrost peatland, 2) the actively thawing forest edge (drunken forest), 3) the area of most recent complete thaw where the forest is replaced by the new semi-aquatic community (moat), and 4) the established collapse scar bog where a new peat layer is actively accumulating following thaw (collapse bog). Our study design relied on a qualitative space for time substitution that allowed for the examination of the impacts of permafrost thaw on decadal to century time scales, with the drunken forest and moat plots representing the impacts of thaw on annual to decadal time scales, and the collapse scar bogs representing the impacts of thaw over 100-300 years (M. Jones *unpublished data;* Klapstein et al. 2014). Our main transects encompassing each of these 4 community types ranged from 50 to 100 m. Within 5 m of each of these transects, we established replicate 1 m^2 plots within each community type (n=4 for the permafrost forests and bogs; n=3 for the drunken forests and moats due to limited space). Plots were chosen to represent the full range of variation in vegetation and water table conditions.

PORE WATER CHEMISTRY AND ENVIRONMENTAL VARIABLES

We quantified dissolved organic and inorganic N concentrations in soil pore water with depth in each sampling plot along each of the four permafrost transects. In mid-June 2013, 10-cm microlysimeter Rhizon SMS moisture samplers (Eijkelkamp Agrisearch Equipment, the Netherlands) were installed within each 1 m^2 plot along the three transects (for a total of 114 samplers). Before insertion into the soil, each Rhizon sampler was taped to a 60 cm piece of PVC pipe in order to insert samplers at desired depth intervals: 5-15 cm, 20-30 cm, and 50- 60 cm beneath the live moss surface. However, since the permafrost table in the forest had an average depth of around 40 cm, samplers in the permafrost forest were buried at 5-15 cm and 20-

30 cm depths only using 30 cm length PVC pipe. Using a hand drill to core through seasonal ice, 2.5 cm diameter holes were drilled into the soil at each sampling plot in the forested sites. PVC pipes were then inserted into pre-drilled holes until the top of the pipe was flush with the ground surface to insure proper Rhizon placement. All samples were left in place for two weeks prior to the first collection to allow for equilibration in the soil column.

Soil pore water samples were collected every three to four weeks from late June to late September, 2013 for a total of five sampling events. The collection of samples at depth was limited by the presence of seasonal ice or unsaturated soil conditions in some cases. Prior to sampling for chemical analysis, a small amount of pore water was suctioned into a 20 mL syringe and used as a pre-rinse for the Rhizon, syringe, and sampling bottle to remove sampler tubing head space and mitigate contamination. Because the Rhizon samplers have a 1 μm pore size membrane, no additional filtration was required prior to collection. Between 10-20 mL of soil pore water sample was extracted by syringe and ejected into acid-rinsed bottles. After extraction, samples were placed on ice packs in a cooler and immediately transported to the University of Alaska-Fairbanks. Samples were split into three parts for three different analyses: (1) 5 mL was added to 20 mL of deionized water and analysed for total dissolved nitrogen (TDN) using a TOC-V Analyzer with TDN module (Shimadzu Scientific Instruments, Columbia, MD USA), (2) 5 mL was refrigerated prior to NO_3^- and NH_4^+ analysis (dissolved inorganic nitrogen; DIN) using a Technicon II Autoanalyzer (NH_4^+ + NO_3^-) (Whitledge *et al.* 1981), and (3) 0.5 mL was frozen at -20°C prior to amino acid analysis using a modified ninhydrin reaction (Rosen 1957). Absorbance at 570 nm was measured on a Perkin-Elmer UV/Vis Lambda 25 spectrometer. Amino acid concentrations were determined by comparison to a leucine standard analysed at the same time as the soil samples. We calculated dissolved organic N (DON) concentrations as the difference between TDN and DIN concentrations.

Environmental variables, including soil temperature at 10 cm depth, soil volumetric moisture content at 10 cm depth, and seasonal ice depth, were also recorded manually during the pore water sampling events at each sampling plot. Soil temperature was measured using a 10 cm ThetaProbe thermometer (Delta-T Devices, Cambridge, England) that was inserted into the soil at each sampling plot. Soil moisture was also measured manually using a ThetaProbe soil moisture sensor (Delta-T Devices, Cambridge, England) inserted 10 cm vertically into the soil surface. Seasonal ice depth was measured using a 1.5 m long metal probe. If no ice was encountered upon complete insertion of the metal probe into the ground, then sites were considered to be completely thawed.

ESTIMATES OF NITROGEN STOCKS ACROSS THE THAW GRADIENT

In addition to examining plant traits and soil pore water chemistry along the gradient, we also estimated changes in N stocks along the thaw gradient for soils, peat, and aboveground subcanopy biomass. Plant biomass and soil cores were collected in late July 2013 to coincide with peak seasonal biomass production and maximum seasonal thaw. Additionally, tree basal diameter and density from the forested sites were collected in August 2015 to include tree N content based on allometric equations developed to estimate N stocks(Van Cleve *et al.* 1983; Alexander *et al.* 2012). Three plots from the four transects and four community types were designated for plant and soil sampling (n = 3 transects by 3 plots by 4 community types = 36). Using a 20 cm × 20 cm frame placed adjacent to the lysimeter plots, destructive harvests of vascular vegetation down to the moss surface and the top 3 cm of nonvascular plants were collected from within the frame. All harvested plant materials were dried at 60° C for 3 days and weighed. Belowground N pools were estimated using the same soil cores that were collected for root abundance and depth (see section below). For each 5 cm core increment, wet weights were taken for bulk density and gravimetric moisture content calculations. After soils were homogenized and dead coarse materials (>5 mm) were removed, soil subsamples, including fine roots, were dried at 60° C for 3 days and then reweighed. Plant tissues, soils, and peat were then ground using a ball-mill, packed into combustion tins, and analysed for % C and N on an elemental analyser COSTECH ECS 4010 CHNS-O analyser (Costech Analytical Technologies Inc., Valencia, CA, USA). N stocks for aboveground biomass were calculated by multiplying tissue % N concentration by the biomass per area (g/m^2). Gravimetric moisture content, bulk density, average maximum core depth, and N concentration were used to calculate N stocks in belowground pools of soil, peat, and pore water based on allometric equations from Alexander *et al.* (2012).

PLANT NITROGEN-USE TRAITS

To determine whether plants across our permafrost thaw gradients were primarily N or P limited, we sampled 177 plants across gradients encompassing variation in dominant species. We randomly selected 50 of these plant tissue samples and analysed tissue P concentrations colorometrically using perchloric acid digests and analysis on a Technicon autoanalyser (Technicon Instruments Corporation, Tarrytown, New York, USA). These P concentrations were compared to measurements of %N, as described above.

We measured rooting depth and relative root abundance using soil cores collected in late July and early August of 2013. Soil cores were collected using a 5 cm diameter metal core barrel adjacent to each 1 m^2 sampling plot along the three transects. Maximum core depths varied across the community types, but were around 30 cm in the permafrost forest, and between 45-60 cm in the drunken forest, moat, and collapse bog plots. Cores were divided into 5 cm depth increments in the field, placed on ice, and transported immediately to the laboratory. Each 5 cm depth increment was visually inspected for up to 10 minutes and then given a root abundance score based on the number of apparent live roots and the size of the roots found in that increment (adapted for root analysis from Harden 1982). Root relative size was classified by root diameter and divided into four categories: very fine (<1 mm), fine (1-5 mm), medium (5-10 mm), and coarse (>10 mm). The number of roots within a size class was then approximated and given an abundance index score where few (<10 roots) was assigned an index of 10, common (10-100 roots) was assigned an index of 20, and many (<100 roots) was assigned an index of 30. Indices for each size class per depth increment were then added together for the total root abundance score for that soil increment.

Stable isotope analysis of ^{15}N natural abundances were performed for plant species that were found in at least three or more locations along our thaw gradients, which included *Carex aquatilis*, *Betula glandulosa*, and *Chamaedaphne calyculata*. These species also vary in mychorrhizal status, ranging from the non-mycorrhizal sedge, *C. aquatilis*, to the shrubs *B. glandulosa* and *C. calyculata,* associated with ectomycorrhizae and ericoid mycorrhizae respectively (Bartsch 1994). For ^{15}N analysis, green leaves were collected from each species (if present) in late July adjacent to three sampling plots in each community type along all three transects, and brought to the lab for drying. Plant leaves were dried at 60 °C and ground using a ball mill to prepare for analysis. Prepared samples were sent to the University of Alaska Fairbanks Stable Isotope Facility for processing using a ratio mass spectrometer and combustion analyser.

Chamaedaphne calyculata was the only species observed across all four permafrost thaw community types. We used this species to quantify how foliar chemistry and N-resorption efficiency varies with time-following-thaw. Previous fertilization studies have shown that *C. calyculata* increases biomass, shoot lengths, and foliar N concentrations in response to increased nutrient availability (Bartsch 1994; Bubier *et al.* 2011), suggesting that this species would likely be responsive to potential changes in soil N availability with thawing permafrost organic matter. Leaf litter traps were placed underneath *C. calyculata* shrubs in early June and checked periodically throughout the season. Plants were revisited during periods of peak biomass in late-July, and during senescence and leaf fall in late-September for green leaf foliage and litter collection, respectively. After collection, foliage samples and leaf litter were dried at 60 °C and ground for analysis of total N using a COSTECH ECS 4010 CHNS-O analyser (Costech Analytical Technologies Inc., Valencia, CA, USA). Nutrient-resorption efficiency (RE) was then calculated as the change in foliar mass-based N pools between green and senescent leaves [$RE=(N_{Green}-N_{Senecsed})/N_{Green}$ x 100; Aerts *et al.* 1999].

STATISTICAL ANALYSES

Statistical analyses were performed using R version 3.0.2 (R Development Core Team, 2013). For all analyses, diagnostic plots and visual assessments of normality and homogeneity of variation were used to ensure no major statistical assumptions were violated with regards to residual homogeneity, independence, and normality. Statistical significance was assessed using $\alpha=0.05$.

The permafrost forest pore water collection was limited by the permafrost table, preventing measurements deeper than 60 cm. Therefore, the pore water dataset was not full rank, preventing us from analysing the community type x depth interaction on pore water chemistry. Consequently, all possible community type and depth combinations were combined to examine interactions on pore water N chemistry. To assess N concentrations in pore water for DIN, DON, TDN, and FAA separately by date, community type, and depth, we constructed generalized linear mixed effects model using stepwise procedures as outlined by Zuur *et al.* (2009) and Bolker *et al.* (2009). Mixed effects models were created using the package 'nlme' in R (Pinheiro *et al*. 2014). To address repeated seasonal measurements at sampling plots, a nested random factor of sampling date within plot ID was included as a random effect in the model. To reduce heterogeneity in the data, a multiple variance structure for transect location was added to the model to account for inter-transect variability across the study area. Fixed effects and possible interactions were determined using a log likelihood ratio test. The final models for pore water N chemistry analyses included community, lysimeter depth, and sampling date as fixed effects, and transect as a nested random effect. Tukey HSD *post hoc* comparisons of means tests were conducted using the package 'multcomp'(Hothorn *et al.* 2008).

We used multiple regression models to predict variation in dissolved N concentrations using both community type (permafrost forest, drunken forest, moat, thaw bog) and environmental data (soil temperature, seasonal ice depth) as predictors. Model parameters were eliminated using backwards stepwise regression, incorporating Akaike Information Criteria (AIC) to determine the best fit (i.e., most parsimonious) models. Interaction terms were not included in the final model since they did not significantly alter the slopes of the independent variable coefficients.

Linear regression and analysis of variance (ANOVA) models, followed by Tukey HSD *post hoc* comparisons, were used to examine controls on relative root abundance, plant foliage chemistry, stable isotope abundance, and N stocks.

Nitrogen stock estimates were calculated for TDN, DIN, soils, and aboveground vegetation. For aboveground vegetation, N stocks were calculated as the product of the dry weight of biomass for a given plot (g/m^2) and the mean N concentration. Tree N contributions were calculated from stem density measurements, and scaled up to an ecosystem level using allometeric equations and estimates from Van Cleve *et al.* (1983) and Alexander *et al.* (2012), We added tree N and understory N stocks to quantify aboveground N stocks. Soils N stocks were calculated as the product of mean soil bulk density (g/m^3) to a depth of 30 cm, a standardized depth across all community types, and the mean N concentration. For the permafrost peatland forest and drunken forest sites, pore water N stocks were calculated as the product of mean TDN and DIN concentration estimates (from the mixed effects model output) for each community type and volumetric moisture content of the soil to a depth of 30 cm. For the moat and collapse bog sites, we assumed that that soil pores were completely filled with water, and therefore calculated soil porosity (P_S) as:

$$P_S=1-[\text{bulk density}(g\times cm^{-3})/\text{particle density } (g\times cm^{-3})]$$

where particle density was considered a constant at 1.5 g×cm^{-3} (Rühlmann, Körschens & Graefe 2006; Redding & Devito 2006). Saturated pore water N stocks were then calculated as the product of mean TDN and DIN concentrations (from the mixed effects model output) to a depth of 30 cm and the P_S constant.

Results

Mean growing season soil temperature at 10 cm depth ($F_{[3,38]}$=32.89, p<0.001), volumetric moisture content within the top 10 cm ($F_{[3,38]}$=59.62, p<0.001), and maximum active layer depth ($F_{[3,38]}$=768.9, p<0.001) increased along the thaw gradients (Table 1). The coldest and driest soils were located in the permafrost forest while the moat was associated with the warmest and wettest soils.

EFFECTS OF PERMAFROST THAW ON N AVAILABILITY AND ECOSYSTEM N POOLS

Between 80-90% of TDN was in the form of DON, resulting in strong relationships between TDN and DON concentrations across sampling events (Fig. 3, Fig. 4). Both TDN and DON concentrations varied by a depth and sampling date. Averaged across community types, TDN concentrations varied with soil depth during all sampling events, except for July 24 (Fig. 3a).

All community types had very similar concentrations of TDN and DON across depths (Fig. 3). However, DON concentrations in the drunken forest at 30 and 60 cm depths were greater than DON concentrations in the bog at 10 and 60 cm (Fig. 3b). When concentrations were averaged by depth across community types, we observed the greatest concentrations of TDN and DON in the drunken forest, and the smallest concentrations in the collapse bog (Fig.

4c,d). The forested sites (permafrost forest and drunken forest) had the greatest variation in N concentrations and therefore did not differ significantly from the other two community types.

Despite large contributions of TDN consisting of DON, FAA did not differ either among community types or depth (Fig. 3d, Fig. 4f). However, amino acids concentrations, as well as DON, did steadily decline throughout the growing season with the smallest concentrations found in September (Fig. 3d). Generally FAA constituted between 8-16% of total DON across sampling dates and depths, with the exception of the August 20th sampling date where FAA comprised an average 27% of DON at 30 cm sampling depths. This was opposite to seasonal trends in DON concentrations, suggesting that lighter, more labile compounds dominate pore water DON late in the season.

Inorganic nitrogen in pore water was predominately composed of NH_4^+-N across sampling events, depths, and community types (mean NH_4^+-N approximately 92% of total DIN, mean NO_3^--N approximately 8% of total DIN, data not shown). NO_3^--N concentrations were consistently very low. There was some effect of community type on DIN concentrations, as the moat and collapse scar bog had greater concentrations of DIN than the permafrost forest, while the drunken forest sites had intermediate values (Fig. 3e). Concentrations of DIN had an interaction between sampling event and depth (Fig. 3c). In early July, DIN availability appeared to be largely confined to shallower soils at 10 and 30 cm. With increases in seasonal thaw depth over time, deeper soils contributed more to DIN availability. Analysis of community type interactions by depth did not indicate a significant difference in mean DIN concentrations ($F_{[9,239]}$=1.42, p=0.180). When N stocks were scaled to a meter-squared basis, the moat and the bog had the greater dissolved stocks but smaller vegetation and soil stocks than the other sites along the permafrost thaw gradients (Table 2). However, because dissolved N pools in the saturated moat and bog were calculated under the assumption that the entire soil pore space was filled with water, these estimates must be cautiously interpreted as this is likely an over-estimation of dissolved pools due to the presence of some gas bubbles trapped under the water table that were unable to be adequately quantified by this study.

PLANT RESPONSES TO PERMAFROST THAW AND CHANGING N AVAILABILITY

Foliar tissue of dominant species from our sites consistently had high P concentrations and low N:P (Fig. 2), suggestive of N limitation across our thaw gradients. Across 16 species and functional types present across the thaw gradients, 92% of samples had N:P < 13.5, suggestive of N limitation (Güsewell & Koerselman 2002). Only 4% of samples had N:P ≥ 13.5 and ≤16, which is suggestive of N and P co-limitation. The lowest N:P ratios were dominated by the composite samples from the functional groups of sedge, *Sphagnum*, and evergreen shrubs (Fig. 2).

Comparative multiple regression models were used to examine controls on dominant plant functional groups (evergreen shrubs, deciduous shrubs, sedges, and *Sphagnum*), and species found across three or more community types (*C. calyculata, B. glandulosa,* and *C. aquatilis*). Of the four functional groups selected for model construction, only sedge communities failed to converge with an adequate predictive model based on N availability. At a functional type level, evergreen shrub cover was positively related to concentrations of DON, and to mean soil temperature (Table 3). Deciduous shrub cover was correlated with seasonal ice depth and DON and DIN availability. *Sphagnum* coverage was largely correlated with changes in soil moisture across the gradient. At the species level, N availability in pore water did not correspond to species abundance for any of the species of interest. Instead, environmental variables served as stronger predictors; seasonal ice depth as the strongest predictor of both *C. aquatilis* and *C. calyculata* abundance, and soil temperature as a significant positive predictor of *Betula glandulosa* abundance.

The root abundance index varied by a community type x depth interaction ($F_{4,331}$=27.67, $p < 0.001$). Within the top 30 cm of the soil profile, permafrost forest cores had greater root abundance than the other communities (Fig. 5). In the drunken forest, there was a slight, nonlinear pattern in rooting density as rooting depths extended into the deeper, unfrozen soils (Fig. 5). In the moat and collapse bog communities, root abundance increased with depth in the upper 60 cm of soil.

N concentrations of *C. calyculata* foliage varied by a community type x sampling date interaction ($F_{7,54}$=56.17, p<0.001). Along the gradient from the forest to the collapse bog, both the concentration of midsummer foliar N and litterfall N percentage increased (summer: $F_{3,27}$=4.383, p =0.012; fall:$F_{3,27}$=5.444, p=0.004). At peak biomass, foliar N averaged 1.2% in the permafrost forest, and was significantly lower than the other community types, in which mean foliar N ranged 1.4-1.5%. Litterfall N concentrations were significantly higher in the collapse bog than in the other three community types (Fig. 6b). Resorption efficiencies (RE) were fairly consistent across the gradient, and were lower in the collapse bog plants than in moat plants (Fig. 6c).

Summertime and litterfall foliage N concentrations were positively correlated with DIN pore water concentrations (summer: R^2=0.18, p<0.001; litter:R^2=0.38, p<0.001; 7a,b), but there was no significant relationship between RE and DIN concentrations (p=0.17; 7c). We also found no relationships between DON or TDN and leaf chemistry (data not shown).

Stable isotope values for all three species studied were variable (Fig. 8). Foliar δ^{15}N values for *C. calyculata* were the least depleted in the drunken forest, with intermediate values found in the moat and the most depleted in the permafrost forest and the thaw bog (ANOVA $F_{3,37}$=7.91, p<0.001; Fig. 8). *C. aquatilis* also experienced a small, non-significant (ANOVA $F_{2,24}$=2.90, p=0.074), but consistent depletion in δ^{15}N along the drunken forest-bog gradient (not present in the drier permafrost forest). *B. glandulosa* only experienced a significant increase in δ^{15}N between the forest and the moat communities (ANOVA $F_{3,10}$=4.21, p=0.04), though we had a small sample size for this species.

Discussion

Changes in N availability following permafrost thaw

The results from this study supported our predictions about changing N availability with permafrost thaw. In support of our first prediction, TDN generally increased with thaw and this was true of concentrations in both surface and deeper soils. The permafrost forest tended to have the lowest mean concentrations of TDN, DIN, and DON, particularly in comparison to the drunken forest and moat (Fig. 4, Table 2). We found that mean concentrations of both TDN and DON (but not DIN) were greater in the drunken forest plots relative to the permafrost forest plots. Over longer periods of time, years to decades, additional thaw further destabilizes the forest, leading to the formation of open semi-aquatic ecosystems (Fig. 1, Table 1). The moat plots were characterized by open, high light conditions, and warm and wet soils relative to the permafrost peatland or drunken forest plots. Our results showed that the moats were associated with the highest concentrations of DIN, suggesting that changing environmental conditions rather than immediate release of N from actively thawing permafrost has the greatest impact on the availability of DIN. This is in contrast to previous studies showing large increases in DIN upon the onset of thaw (Keuper *et al*. 2012). One explanation for this discrepancy is that permafrost SOM at our sites is recalcitrant due to a history of decomposition prior to being frozen into the permafrost table (Coolen *et al.* 2011), resulting in high lignin content of historic SOM (Rovira & Vallejo 2002) and thus low levels of gross N mineralization following thaw. Release of DIN from thawing permafrost SOM is likely to be taken up immediately by soil microorganisms or plants, possibly leading to low DIN concentrations in soil pore water despite faster N turnover rates in the drunken forests. This ‘immediate uptake’ hypothesis is supported by an increase in belowground N stocks on a m^2 basis in the drunken forest (Table 2), where increasing DIN availability may have been utilized and immobilized by vegetation (peat and root accumulation) or microbes. Therefore further investigation is needed to draw conclusions about highly labile N potentially being released from thawing permafrost.

With ecosystem succession occurring with time-following-thaw, a new peat layer in collapse scar bogs and fens accumulates vertically above the water table, progressively "burying" the previously frozen permafrost soil pool. Due to increasing thickness of this post-thaw peat layer, our deepest sampling points (60 cm) were no longer in direct contact with permafrost SOM, but instead reflected SOM that had accumulated post-thaw. Comparing trends in N availability in the collapse scar bogs relative to the other community types thus allows for an assessment of how aspects of N cycling change as a result of post-thaw succession over many decades to centuries. In general, we observed greater DIN concentrations in the collapse scar bog compared to the permafrost forest, though concentrations were lower than in the moat (Fig. 4). This suggests that although DIN availability increases during the forest-to-wetland state change associated with permafrost thaw, autogenic succession and peat accumulation over centuries timescales causes N availability to decline as soil conditions become relatively drier, colder, and more acidic. Another confounding factor is that trapped gas bubbles in the saturated peat layers of the moat and collapse scar bog may complicate our calculations of DIN as we assumed that pore space was 100% filled by water. Previous studies in this area have found that younger bog features have higher levels of ebullition (Klapstein *et al.* 2014), therefore we may have over-reported DIN concentrations in the moat and collapse scar bogs where trapped gas bubbles from ebullition would have occupied soil pore space.

If thawing permafrost organic matter stimulates the release of labile dissolved organic matter (Gorham 1991; Grosse *et al.* 2011), it is reasonable to assume that this N pool would include simple, monomeric organic N molecules that can be taken up by both plants and microbes. Whereas concentrations of DON were greatest in the drunken forest and moat community types, we did not observe a similar increase in relative amino acid abundance in soil pore water (Fig. 4). Therefore, despite increases in pore water DON upon the onset of thaw, our results do not support the conclusion that thaw increases the availability of plant-available DON. Methodological issues may be partial responsible for our failure to detect increases in plant-available DON following permafrost thaw, as Rhizon tension microlysimeters may preferentially collected amino acids already in solution and confined within saturated pore-spaces and exclude loosely-bound molecules from sampling uptake (Inselsbacher *et al.* 2011). While our results showed no consistent changes in amino acid concentrations along the thaw gradient or with depth, amino acids concentrations tended to decline throughout the growing season, suggesting either biological uptake of amino acids, or a reduction in amino acid production. Our data also suggest that mineralization of the DON pool affected DIN concentrations. For example, increases in DIN concentrations during our August sampling events coincided with decreases in

DON concentrations (Fig. 3). This suggests that increased microbial mineralization activity may be occurring during late-summer when soil temperatures are at their warmest. An alternative explanation is that DON is lost from soils due to leaching as the growing season progresses, though this does not explain the strong relationship to patterns of DIN concentrations.

Our results partially supported our second prediction that changes in N availability following thaw would result in deeper soil layers being associated with the greatest concentration of DIN. Early in the growing season, larger DIN concentrations were associated with near-surface soils, as N turnover in deeper soils was constrained by the presence of seasonal ice. However, at maximum thaw depth in September, increases in DIN concentrations post-thaw were attributed to deeper soil horizons (Fig. 3c), given that late-season DIN concentrations in surface soils declined. This pattern was consistent across the drunken forest, moat, and thaw bog communities. Thus, our results indicate that thaw of deeper soil layers may lead to increasing concentrations of dissolved N concentrations.

Changing vegetation and plant N uptake following thaw

We were interested in quantifying variation in plant traits related to N uptake and use across the permafrost thaw gradients. We found that foliar and litter N concentrations were positively related to DIN concentrations across community types (Fig. 7).We found that dissolved N concentrations were not predictors of variation in individual species abundance, suggesting that plant density/abundance may be controlled more strongly by other factors such as changing soil temperature and moisture conditions (or indirectly via changes in seasonal ice conditions) than by changes in nutrient supply (Table 3). However, our results did show

Supporting on third hypothesis, we found that total root abundance increased with depth following permafrost thaw. Deeper rooting species, especially sedges, often have belowground aerenchymotous tissue that can assist with nutrient uptake under anoxic conditions by facilitating oxygen transport to belowground biomass, thereby lowering energy requirements for cellular respiration (Cronk & Fennessy 2001; Rydin & Jeglum 2006). Our results showed that the bulk of roots found at depth were classified as fine and very fine roots (data not shown), indicating that plants may be modifying root architecture to increase nutrient acquisition strategies by investing in faster growing, deeper penetrating roots (Jackson *et al.* 2008). Previous studies in our research sites have shown that permafrost thaw stimulates understory primary productivity, in part due to these shifts in community composition (Churchill 2011), though this does not always translate into higher ecosystem productivity post-thaw (Euskirchen *et al.* 2014). Strong competition for available N by microbes may also lead to lack of relationships between DIN availability and foliar N concentrations.

Previous research in arctic tundra and boreal forests has linked rooting depth and changing soil N availability with variations in foliar δ^{15}N values (Garten 1993; Nadelhoffer *et al.* 1996). Our results showed foliar ^{15}N enrichment in the drunken forest and moat relative to other communities, suggesting that plants in these recently thawed environments receive nutrients from different sources, depths or through different mechanisms than either the permafrost forest or the collapse scar bog. Nadelhoffer *et al.* (1996) compared δ^{15}N values of sedges and ericaceous shrubs in the arctic plant communities, and found more enriched δ^{15}N values in sedge species and deeper rooting profiles. Similar results have been observed in boreal forests (Kielland 2001). In contrast, more depleted δ^{15}N values tend to be measured in plants that rely primarily on nutrients from shallower, near surface soils and these species are often associated with mycorrhizae. This, in general, is consistent with our results. We found that *C. aquatilis*, a deep rooting non-mycorrhizal plant, had more enriched $\delta^{15}N$ values on average than the other two shrub species investigated (Fig. 8). Additionally, coincident with increasing root abundance at depth in the drunken forest (Fig. 5), both shrub species showed foliar $\delta^{15}N$ enrichment. However, all three species had more depleted foliar $\delta^{15}N$ values in the collapse bog compared to the other community types (Fig. 8). This latter result points to the importance of changing sources of plant N uptake rather than deeper rooting as the main determinant of higher $\delta^{15}N$ values post-thaw. For example, both the moat and the collapse bog had similar rooting profiles and root abundance at depth (Fig. 5), but differed in $\delta^{15}N$ values (Fig. 8).

Changes in mycorrhizal ecology are also likely impacting $\delta^{15}N$ values. Generally, plants with mycorrhizal associations have more depleted plant signatures relative to N sources due to substrate fractionation by the fungal partner which passes on a highly depleted fraction to the host plant (Evans, 2001; Hobbie *et al.* 2009, Sorrell *et al.* 2011). Conversely, non-mycorrhizal plants can become slightly ^{15}N enriched as NH_4^+ increases due to microbial preferences for the lighter isotope, resulting in $\delta^{15}N$ enrichment with peat depth (Kohzu *et al*. 2003). Soil saturation following permafrost thaw may disrupt rhizome fungal communities (Muthukumar *et al.* 2004), suggesting that thermokarst and moat development may lead to more enriched foliar $\delta^{15}N$. With increased time following thaw, drying associated with peat accumulation above the water table may allow mycorrhizae to reinvade the roots of ericaceous shrubs in developing hummocks, which would explain the more depleted $\delta^{15}N$ values of plants sampled from the collapse bog plots. Together, these results support the conclusion that plants alter rooting abundance and patterns of plant-N uptake in response to permafrost thaw.

While this study focused on patterns of N "release" upon permafrost thaw and potential mechanisms of plant N uptake, thawing permafrost organic matter is also likely to influence the availability of other key nutrients in soils as well, including phosphorus (P). While a focus on P availability was beyond the scope of this study, we note that our survey of foliar tissue across our plots showed slight increases in N:P with thaw, suggesting perhaps that N availability increases more so than P upon thaw of permafrost peat. Additionally, disruption of mycorrhizal communities with wet conditions post-thaw also will have consequences for P uptake by plants. Therefore as N availability increases following permafrost thaw, lowland boreal ecosystems could become more P limited unless sufficient amounts of P also are "released" from thawing soils. In general, P is thought to be recycled more efficiently in peat than is N (Wang *et al.* 2015) though it is unclear how the processes controlling N:P stoichiometry might be affected by additional sources of nutrient inputs to peat caused by thawing permafrost.

Found in all four community types along the thaw gradients, *C. calyculata* is an evergreen plant known to store nutrients as simple organic compounds within plant tissues (Bartsch 1994, Bubier *et al.* 2011). We used this species as a "model plant" to better understand how changes in N availability alters plant N uptake or N-use patterns in a single plastic species. Across the thaw gradients, we found increasing N concentrations in *C. calyculata* summertime foliage and litterfall that were positively correlated with increasing DIN availability (Fig 6a, b; Fig. 7a, b). Despite changes in foliage chemistry, we only observed a slight reduction in resorption efficiency in the bog (Fig. 6c), suggesting that either N availability may not significantly exceed the N demands of vegetation in collapse scar bogs, or that increasing N availability may further P co-limitation in these sites (Wang, Murphy, & Moore 2014). Therefore, considering plant and ecosystem N demands and ecosystem stoichiometry may be critically important when considering the magnitude and significance of increasing N from thawing permafrost.

If post-thaw changes in nutrient availability increase primary productivity and peat accumulation rates, this could serve to at least partially offset permafrost C release (Zimov *et al.* 2006; Harden *et al*. 2012; Jones *et al.* in review). Taken together, our results clearly showed that plants are altering patterns of N-use along these thaw gradients. Coincident with increases in dissolved N availability, we observed increasing rooting depth, increasing foliar nitrogen concentrations, and altered foliar δ^{15}N values. As deeper frozen soil layers are impacted by permafrost thaw in a warmer climate, large soil N stocks will be exposed. However, if increasing N shifts plants towards P limitations to productivity (Wang, Murphy, & Moore 2014), than collapse scar ecosystem will remain nutrient limited and primary productivity will likely not increase. Whether increases in dissolved N availability at depth following permafrost thaw can actually enhance plant productivity (versus shifting ecosystems towards P limitation), and how increases in plant C uptake compare to permafrost C losses, remain important questions for future studies.

AKNOWLEDGEMENTS

This work was made possible by assistance from J. Barta, C. Gibson, and L. Oliver. We also thank Evan Kane and David Olefeldt for comments on earlier versions of this manuscript. Research was funded by the National Science Foundation (DEB-0425328, DEB-0724514, and DEB-0830997) the Bonanza Creek Long-Term Ecological Research program (funded jointly by NSF Grant DEB-0620579, an USDA Forest Service Pacific Northwest Research Grant PNW01-JV11261952-231), a Society of Wetland Scientists Student Research Grant, and a UAF Center for Global Change Student Research Grant from the Alaska Climate Science Center. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

DATA ACCESSIBILITY

Data collected in this study are archived with the Bonanza Creek LTER at the University of Alaska Fairbanks.

-Soil pore water nitrogen data: doi: 10.6073/pasta/ef745c1a7e83a746e39a05346731e407

-Root abundance: doi: 10.6073/pasta/55a2b554347fdf76cb8be82d31bf7c72

-Plant vs nitrogen availability: doi: 10.6073/pasta/aed4e22fc766b9dda5bec6b3f7c97b2b

-Isotope data: doi: 10.6073/pasta/318543ce17386618a69d869084c14677

References

Aerts, R. (1996) Nutrient resorption from senescing leaves of perennials: are there general patterns? *Journal of Ecology*, **84**, 587–608.

Aerts, R. & Chapin, F.S.I. (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research*, **30**, 1–67.

Aerts, R., Verhoeven, J.T.A. & Whigham, D.F. (1999) Plant-mediated controls on nutrient cycling in temperate fens and bogs. *Ecology*, **80**, 2170–2181.

Alexander, H.D., Mack, M.C., Goetz, S., Beck, P.S.A. & Belshe, E.F. (2012) Implications of increased deciduous cover on stand structure and aboveground carbon pools of Alaskan boreal forests. *Ecosphere*, **3**.

Baltzer, J.L., Veness, T., Chasmer, L.E., Sniderhan, A.E. & Quinton, W.L. (2014) Forests on thawing permafrost: fragmentation, edge effects, and net forest loss. *Global change biology*, **20**, 824–34.

Bartsch, I. (1994) Effects of fertilization on growth and nutrient use by *Chamadaephne calyculata* in a raised bog. *Canadian Journal of Botany*, **72**, 323–329.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H. & White, J.-S.S. (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution*, **24**, 127–35.

Bubier, J.L., Smith, R., Juutinen, S., Moore, T.R., Minocha, R., Long, S. & Minocha, S. (2011) Effects of nutrient addition on leaf chemistry, morphology, and photosynthetic capacity of three bog shrubs. *Oecologia*, **167**, 355–68.

Camill, P. (1999) Patterns of boreal permafrost peatland vegetation across environmental gradients sensitive to climate warming. *Canadian Journal of Botany*, 721–733.

Chapin, F.S.I. (1980) The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics*, **11**, 233–260.

Churchill, A. (2011) *The Response of Plant Community Structure and Productivity to Changes in Hydrology in Alaskan Boreal Peatlands*. University of Alaska Fairbanks.

Van Cleve, K., Oliver, L., Schlentner, R., Viereck, L.A. & Dyrness, C. (1983) Productiviey and nutrient cycling in taiga forest ecosystems. *Canadian Journal of Forest Research*, **13**, 747–766.

Coolen, M.J.L., van de Giessen, J., Zhu, E.Y. & Wuchter, C. (2011) Bioavailability of soil organic matter and microbial community dynamics upon permafrost thaw. *Environmental Microbiology*, **13**, 2299–314.

Cronk, J.K. & Fennessy, M.S. (2001) *Wetland Plants: Biology and Ecology,* CRC Press, Cleveland, Ohio.

Euskirchen, E.S., Edgar, C.W., Turetsky, M.R., Waldrop, M.P. & Harden, J.W. (2014) Differential response of carbon fluxes to climate in three peatland ecosystems that vary in the presence and stability of permafrost. *Journal of Geophysical Research*, **119**, 1576–1595.

Evans, R.D. (2001) Physiological mechanisms influencing plant nitrogen isotope composition. *TRENDS in Plant Science*, **6**, 121–6.

Farrell, M., Hill, P.W., Farrar, J., DeLuca, T.H., Roberts, P., Kielland, K., Dahlgren, R., Murphy, D. V., Hobbs, P.J., Bardgett, R.D. & Jones, D.L. (2013) Oligopeptides Represent a Preferred Source of Organic N Uptake: A Global Phenomenon? *Ecosystems*, **16**, 133–145.

Finger, R.A. (2015) Effects of permafrost thaw on nitrogen availability and plant-soil interactions in a boreal Alaskan lowland-Isotope Data 2013. Bonanza Creek LTER - University of Alaska Fairbanks. **BNZ:598**, http://www.lter.uaf.edu/data/data-detail/id/598.

Finger, R.A. (2015) Effects of permafrost thaw on nitrogen availability and plant-soil interactions in a boreal Alaskan lowland-Nitrogen Data 2013. Bonanza Creek LTER - University of Alaska Fairbanks. **BNZ:595**, http://www.lter.uaf.edu/data/data-detail/id/595.

Finger, R.A. (2015) Effects of permafrost thaw on nitrogen availability and plant-soil interactions in a boreal Alaskan lowland-Plant vs Nitrogen Data 2013. Bonanza Creek LTER - University of Alaska Fairbanks. **BNZ:597**, http://www.lter.uaf.edu/data/data-detail/id/597.

Finger, R.A. (2015) Effects of permafrost thaw on nitrogen availability and plant-soil interactions in a boreal Alaskan lowland-Root Abundance 2013. Bonanza Creek LTER - University of Alaska Fairbanks. **BNZ:596**, http://www.lter.uaf.edu/data/data-detail/id/596.

Garten, C.T. (1993) Variation in foliar 15N abundance and the availability of soil nitrogen on

Walker Branch Watershed. *Ecology*, **74**, 2098–2113.

Gorham, E. (1991) Northern peatlands: role in the carbon cycle and probable response to climatic warming. *Ecological Applications*, **1**, 182–195.

Grosse, G., Harden, J., Turetsky, M., McGuire, A.D., Camill, P., Tarnocai, C., Frolking, S., Schuur, E.A.G., Jorgenson, T., Marchenko, S., Romanovsky, V., Wickland, K.P., French, N., Waldrop, M., Bourgeau-Chavez, L. & Striegl, R.G. (2011) Vulnerability of high-latitude soil organic carbon in North America to disturbance. *Journal of Geophysical Research*, **116**, 1–23.

Güsewell, S. & Koerselman, W. (2002) Variation in nitrogen and phosphorus concentrations of wetland plants. *Perspectives in Plant Ecology, Evolution and Systematcis*, **5**, 37–61.

Harden, J.W. (1982) A quantitative index of soil development from field descriptions: Examples from a chronosequence in central California. *Geoderma*, **28**, 1–28.

Harden, J.W., Koven, C.D., Ping, C.-L., Hugelius, G., McGuire, A.D., Camill, P., Jorgenson, T., Kuhry, P., Michaelson, G.J., O'Donnell, J.A., Schuur, E.A.G., Tarnocai, C., Johnson, K. & Grosse, G. (2012) Field information links permafrost carbon to physical vulnerabilities of thawing. *Geophysical Research Letters*, **39**, 1-6.

Hobbie, J.E., Hobbie, E.A., Drossman, H., Conte, M., Weber, J.C., Shamhart, J. & Weinrobe, M. (2009) Mycorrhizal fungi supply nitrogen to host plants in Arctic tundra and boreal forests: 15N is the key signal. *Canadian Journal of Microbiology*, **55**, 84–94.

Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, **50**, 346–363.

Inselsbacher, E. & Näsholm, T. (2012) The below-ground perspective of forest plants: soil provides mainly organic nitrogen for plants and mycorrhizal fungi. *The New Phytologist*, **195**, 329–34.

Inselsbacher, E., Öhlund, J., Jämtgård, S., Huss-Danell, K., Näsholm, T. (2011) The potential of microdialysis to monitor organic and inorganic nitrogen compunds in the soil. *Soil Biology and Biochemistry,* **43**, 1321-1332.

Jackson, L.E., Burger, M. & Cavagnaro, T.R. (2008) Roots, nitrogen transformations, and ecosystem services. *Annual Review of Plant Biology*, **59**, 341–63.

Johnson, D., Kershaw, L., MacKinnon, A. (1995) *Plants of the Western Boreal Forest and Aspen Parkland.*

Jonasson, S. & Shaver, G.R. (1999) Within-stand nutrient cycling in arctic and boreal wetlands. *Ecology*, **80**, 2139–2150.

Jones, J.B. (2005) Nitrogen loss from watersheds of interior Alaska underlain with discontinuous permafrost. *Geophysical Research Letters*, **32**, 1–4.

Jones, D.L. & Kielland, K. (2002) Soil amino acid turnover dominates the nitrogen flux in permafrost-dominated taiga forest soils. *Soil Biology and Biochemistry*, **34**, 209–219.

Jorgenson, M.T., Racine, C.H., Walters, J.C. & Osterkamp, T.E. (2001) Permafrost degradation and ecological changes associated with a warming climate in central alaska. *Climate*

Change, **48**, 551–579.

Keuper, F., Bodegom, P.M., Dorrepaal, E., Weedon, J.T., Hal, J., Logtestijn, R.S.P. & Aerts, R. (2012) A frozen feast: thawing permafrost increases plant-available nitrogen in subarctic peatlands. *Global Change Biology*, **18**, 1998–2007.

Kielland, K. (1994) Amino acid absorption by arctic plants : implications for plant nutrition and nitrogen cycling. *Ecology*, **75**, 2373–2383.

Kielland, K. (1997) Role of free amino acids in the nitrogen economy of actic Cryptogams. *Ecoscience*, **4**, 75–79.

Kielland, K. (2001) Stable isotope signatures of moose in relation to seasonal forage composition: a hypothesis. *Alces*, **37**, 329–337.

Kielland, K., McFarland, J. & Olson, K. (2006) Amino acid uptake in deciduous and coniferous taiga ecosystems. *Plant and Soil*, **288**, 297–307.

Klapstein, S.J., Turetsky, M.R., McGuire, A.D., Harden, J.W., Czimczik, C.I., Xu, X., Chanton, J.P. & Waddington, J.M. (2014) Controls on methane released through ebullition in peatlands affected by permafrost degradation. *Journal of Geophysical Research: Biogeosciences*, **119**, 418–431.

Kohzu, A., Matsui, K., Yamada, T. & Sugimoto, A. (2003) Significance of rooting depth in mire plants : Evidence from natural 15 N abundance. *Ecological Research*, **18**, 257–266.

McFarland, J.W., Ruess, R.W., Kielland, K., Pregitzer, K., Hendrick, R. & Allen, M. (2010) Cross-ecosystem comparisons of in situ plant uptake of amino acid-N and NH4 +. *Ecosystems*, **13**, 177–193.

Muthukumar, T., Udaiyan, K. & Shanmughavel, P. (2004) Mycorrhiza in sedges--an overview. *Mycorrhiza*, **14**, 65–77.

Nadelhoffer, K.J., Shaver, G.R., Fry, B., Giblin, A., Johnson, L. & McKane, R. (1996) 15N natural abundances and N use by tundra plants. *Oecologia*, **107**, 386–394.

Näsholm, T., Ekbald, A., Nordin, A., Giesler, R., Hogberg, M. & Hogberg, P. (1998) Boreal forest plants take up organic nitrogen. *Nature*, **392**, 914–916.

O'Donnell, J.A., Jorgenson, M.T., Harden, J.W., McGuire, A.D., Kanevskiy, M.Z. & Wickland, K.P. (2011) The effects of permafrost thaw on soil hydrologic, thermal, and carbon dynamics in an Alaskan peatland. *Ecosystems*, **15**, 213–229.

Osterkamp, T.E., Jorgenson, M.T., Schuur, E.A.G., Shur, Y.L., Kanevskiy, M.Z. & Vogel, J.G. (2009) Physical and ecological changes associated with warming permafrost and thermokarst in interior Alaska. *Permafrost and Periglacial Processes*, **256**, 235–256.

Pinheiro, J., Bates, D., DebRoy, S., Sarakar, D. & Team, R.C. (2014) nlme: Linear and nonlinear mixed effects models. R package version 3.1–117.

Quinton, W.L. & Baltzer, J.L. (2012) The active-layer hydrology of a peat plateau with thawing permafrost (Scotty Creek, Canada). *Hydrogeology Journal*, **21**, 201–220.

Read, D.J., Leake, J.R. & Perez-Moreno, J. (2004) Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. *Canadian Journal of Botany*, **82**, 1243–1263.

Redding, T.E. & Devito, K.J. (2006) Particle densities of wetland soils in northern Alberta, Canada. *Canadian Journal of Soil Science*, **86**, 57–60.

Rosen, H. (1957) A modified ninhydrin colorimetric analysis for amino acids. *Archives of Biochemistry and Biophysics*, **67**, 10–15.

Rovira, P. & Vallejo, V.R. (2002) Labile and recalcitrant pools of carbon and nitrogen in organic matter decomposing at different depths in soil: An acid hydrolysis approach. *Geoderma*, **107**, 109–141.

Rühlmann, J., Körschens, M. & Graefe, J. (2006) A new approach to calculate the particle density of soils considering properties of the soil organic matter and the mineral matrix. *Geoderma*, **130**, 272–283.

Rydin, H. & Jeglum, J.K. (2006) *The Biology of Peatlands*. Oxford University Press, Oxford.

Salmon, V.G., Soucy, P, Mauritz, M. , Celis, G., Natali, S.M., Mack, M., & Schurr, E.G. (2016) Nitrogen availability increases in a tundra ecosystem during five years of experimental permafrost thaw. *Global Change Biology,* **22**, 1927-1941.

Schimel, J.P. & Bennett, J. (2004) Nitrogen mineralization: challenges of a changing paradigm. *Ecology*, **85**, 591–602.

Schurr, E.A.G., Crummer, K.G., Vogel, J.G. & Mack, M.C. (2007) Plant species composition and productivity following permafrost thaw and thermokarst in Alaska tundra. *Ecosystems*, **10**, 280–292.

Schurr, E.A.G., A. D. McGuire, A.D., Scha, C., Grosse, G., Harden, J.W., Hayes, D.J., Hugelius, G., Koven, C.D., Kuhry, P., Lawrence, D.M., Natali, S.M., Olefeldt, D., Romanovsky, V.E., Schaefer, K., Turetsky, M.R., Treat, C.C., & Vonk, J.E. (2015) Climate change and the permafrost carbon feedback. *Nature,* **520**, 171-179.

Shaver, G.R., Bret-Harte, M.S., Jones, M.H., Johnstone, J., Gough, L., Laundre, J. & Chapin, F.S.I. (2001) Species compostition interacts with fertilizer to control long-term change in tundra productivity. *Ecology*, **82**, 3163–3181.

Sorrell, B.K., Chagué-Goff, C., Basher, L.M. & Partridge, T.R. (2011) N:P ratios, δ15N fractionation and nutrient resorption along a nitrogen to phosphorus limitation gradient in an oligotrophic wetland complex. *Aquatic Botany*, **94**, 93–101.

Tilman, D. (1990) Constraints and tradeoffs: toward a predictive theory of competition and succession. *Oikos*, **1**, 3–15.

Turetsky, M.R., Wieder, R.K., Vitt, D.H., Evans, R.J. & Scott, K.D. (2007) The disappearance of relict permafrost in boreal north America: Effects on peatland carbon storage and fluxes. *Global Change Biology*, **13**, 1922–1934.

Wang, M., Murphy, M.T. & Moore, T.R. (2014) Nutrient resorption of two evergreen shrubs in response to long-term fertilization in an ombrotrophic peatland. *Oecologia* **174**: 365–377.

Wang M., Moore, T.R., Talbot, J. and Riley, J.L. (2015) The stoichiometry of carbon and nutrients in peat formation. *Global Biogeochemical Cycles*, **29,** 113–121.

Whitledge, T., Malloy, S., Patton, C. & Wirick, C. (1981) *Automated Nutrient Analysis in Seawater*.

Zimov, S.A., Schuur, E.A.G. & Chapin, F.S.I. (2006) Permafrost and the global carbon budget. *Science*, **312**, 1612–1613.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A. & Smith, G.M. (2009) *Mixed Effects Models and Extensions in Ecology with R,* Springer Publishing, New York.

TABLES

Table 1: Environmental characteristics for each community type along the thaw gradients. Data are growing season means ± one standard error. Letters indicate post-hoc test differences between community types at a 95% confidence interval.

Community Type	**Dominant Species**	**Soil temperature (°C)**	**Soil moisture (% VMC)**	**Maximum seasonal thaw depth (cm)**
Forest	*Picera mariana, Rhododendron* spp., *Vaccinium* spp., *Rubus chamaemorus*, Feather moss spp., *Sphagnum* spp.	$4.3 \pm 0.3_a$	$24.2 \pm 3.5_a$	$50.6 \pm 1.9_a$
Drunken Forest	*Carex* spp., *Eriophorum* spp., *Chamaedaphne calyculata, Rubus chamaemorus, Sphagnum* spp.	$6.1 \pm 0.4_b$	$53.1 \pm 5.0_b$	$70.3 \pm 3.7_b$
Moat	*Carex* spp., *Eriophorum* spp., *Chamaedaphne calyculata, Andromeda polifolia, Sphagnum riparium*	$9.7 \pm 0.6_c$	$79.4 \pm 2.3_c$	$> 150_c$
Bog	*Carex* spp., *Eriophorum* spp., *Chamaedaphne calyculata, Betula glandulosa, Larix laricina, Sphagnum* spp.	$8.8 \pm 0.5_c$	$72.5 \pm 1.9_c$	$> 150_c$

Table 2 Estimates of nitrogen stocks ± one standard error in pore water total dissolved nitrogen (TDN) and dissolved inorganic N (DIN), unfrozen peat and soils (Belowground N stocks), and aboveground vegetation, including understory and trees, (Aboveground N stocks). Soil and vegetation samples for analysis were collected during peak biomass within a two week time period between in late July/early August, 2013. Letters indicate post-hoc test differences between community types at a 95% confidence interval.

Community Type	**TDN (mg TDN m^{-2})**	**DIN (mg DIN m^{-2})**	**Belowground N stocks (g N m^{-2})**	**Aboveground vegetation N (g N m^{-2})**	**C:N of aboveground vegetation**
Forest	137 ± 14 **c**	12 ± 2 **c**	261 ± 56 **a**	10.0 ± 0.3 **a**	51.3 ± 3.0 **a**
DF	299 ± 14 **b**	38 ± 2 **b**	318 ± 31 **a**	5.9 ± 0.8 **b**	49.1 ± 3.5 **ab**
Moat	441± 5 **a**	98± 2 **a**	104 ± 30 **b**	1.8 ± 0.3 **b**	41.5 ± 3.8 **b**
Bog	481± 7 **a**	98 ± 3 **a**	137 ± 21 **b**	3.1 ± 0.5 **b**	45.7 ± 2.3 **ab**

Table 3: Best fit models from multiple regressions predicting vegetation abundance (% cover) using mean soil temperature at 10 cm (Temp), mean soil moisture at 10 cm (Moist), mean seasonal ice depth (Ice), mean total dissolved nitrogen (TDN), mean dissolved inorganic nitrogen (DIN), and mean dissolved organic nitrogen (DON) as predictors. Potential models were compared using backwards regression and AIC values; only the most parsimonious models based on AIC are shown.

	Terms	Coefficients	*p*	R^2_{adj}	$F_{(num\ df,\ den\ df)}$
Evergreen shrubs			**0.005****	**0.263**	**6.35** $_{2,28}$
	(Intercept)	-20.79	0.156		
	DON	21.84	0.004**		
	Temp	2.31	0.053		
Deciduous shrubs			**0.007****	**0.279**	**4.88** $_{3,27}$
	(Intercept)	4.11	0.622		
	DON	-8.17	0.074		
	DIN	18.17	0.033*		
	Ice	0.07	0.084		
Sedge			**0.202**	**0.023**	**1.70** $_{1,29}$
	(Intercept)	31.05	0.006*		
	Moist	-0.20	0.202		
***Sphagnum* moss**			**<0.001**	**0.429**	**12.26** $_{2,28}$
	(Intercept)	15.89	0.207		
	DON	12.39	0.176		
	Moist	0.85	<.001***		
Chamaedaphne calyculata			**0.011**	**0.223**	**5.32** $_{2,28}$
	(Intercept)	10.03	0.033		
	Ice	-0.14	0.003**		
	Moist	0.23	0.021*		
Betula glandulosa			**0.051**	**0.125**	**4.16** $_{1,29}$
	(Intercept)	-6.68	0.167		
	Temp	1.16	0.051		
Carex aquatilis			**0.008****	**0.219**	**8.15** $_{1,29}$
	(Intercept)	-2.17	0.460		
	Ice	0.07	0.008**		

***p<0.001, **p<0.01, *p<0.05

FIGURES

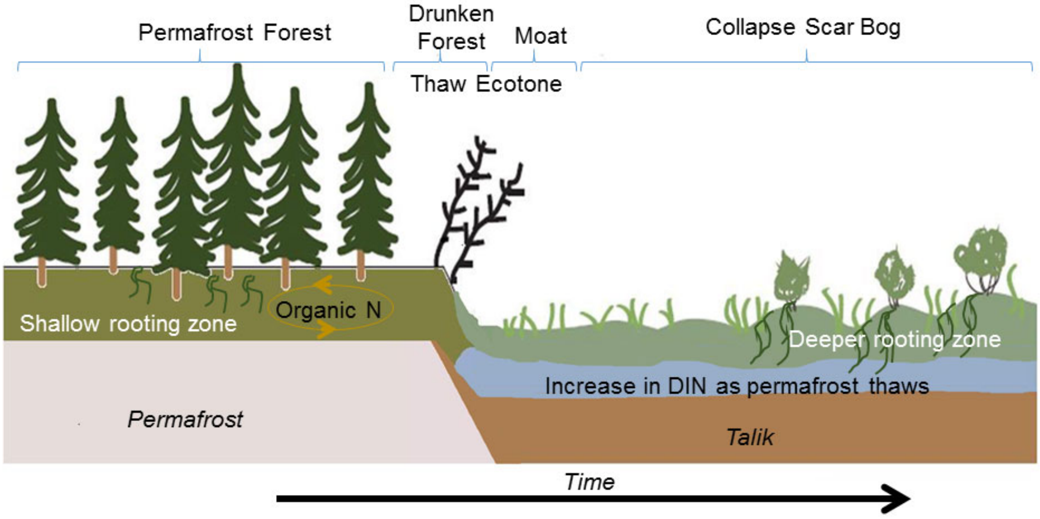

Fig. 1. Conceptual framework examining changes in nitrogen dynamics and plant-soil interactions along a thaw gradient. As permafrost thaws, DIN sources are predicted to increase resulting in changes to plant foliar chemistry, rooting stratigraphy, and mycorrhizal associations. Within the drunken forest, permafrost is actively thawing, while moat sites form within one decade of thaw. The collapse scar bog is likely within 100-300 years post thaw (Klapstein *et al.* 2014).

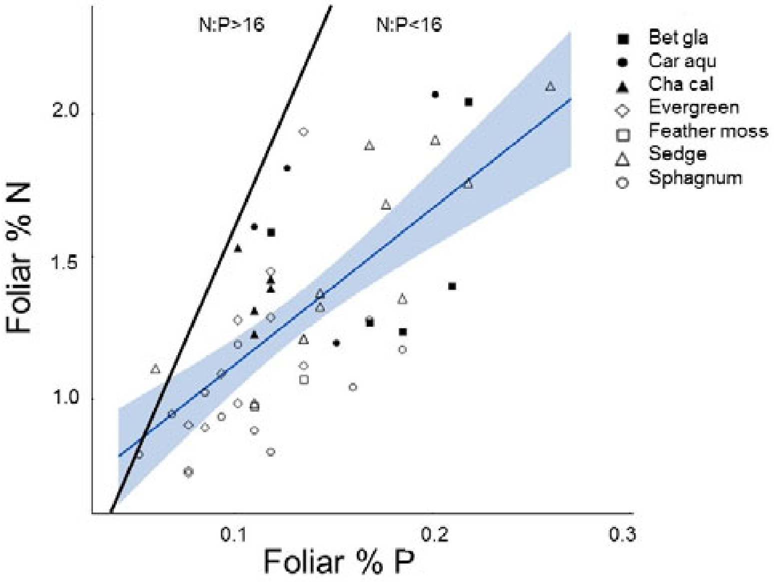

Fig. 2: Relationship between foliar N and P concentrations from 49 samples from APEX boreal lowlands experiencing ongoing permafrost thaw, collected during the summer of 2013. Solid

symbols indicate N:P concentrations for samples from individual species *Betula glandulosa* (Bet gla), *Carex aquatalis* (Car aqu), and *Chamaedaphne calyculata* (Cha cal), while open symbols indicate N:P concentrations for samples from different dominant plant functional types. N:P≥16 indicates plants are P limited while N:P<16 are indicative of plants that are either N limited or N and P co-limited (Güsewell & Koerselman 2002). Blue line and blue shading represent a linear model regression and confidence band respectively of the scatterplot data.

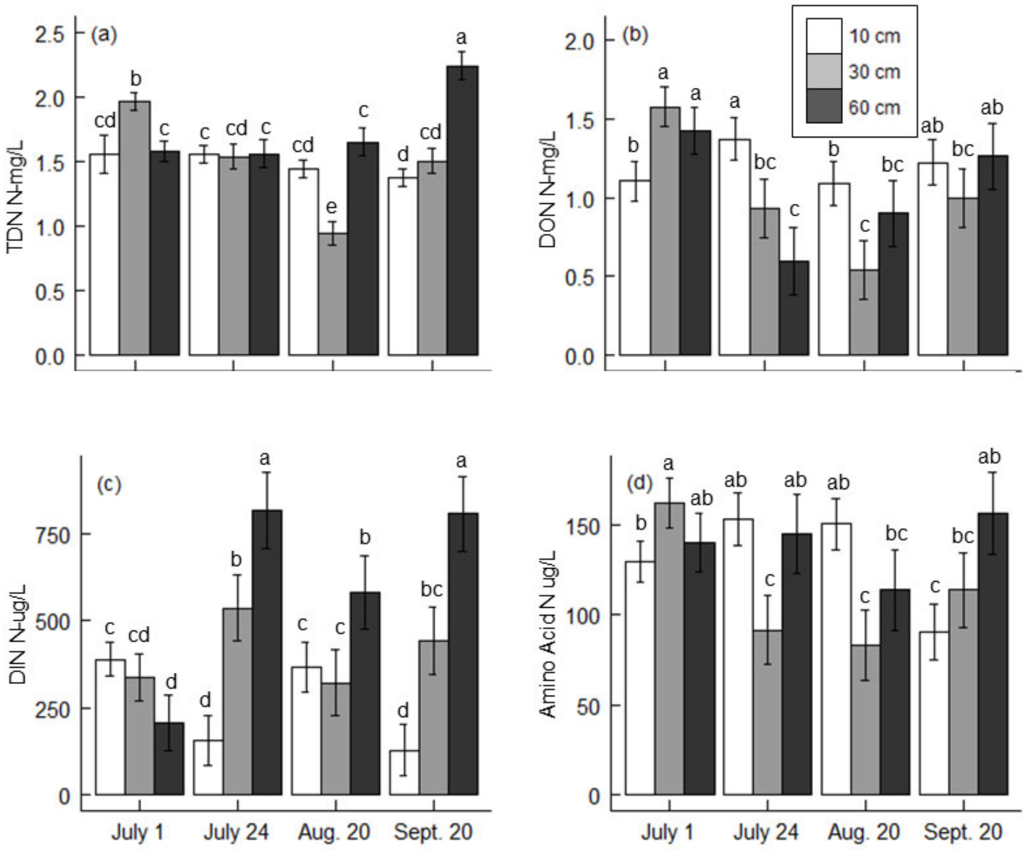

Fig. 3. Seasonal changes in concentrations of **(a)** total dissolved N, **(b)** dissolved organic N, **(c)** dissolved inorganic N, and **(d)** free amino acids by depth averaged across the four community types. Data are mean ± SE, n=363. Same letters above the means indicate non-significant differences within each graph according to Tukey post-hoc comparison of means tests.

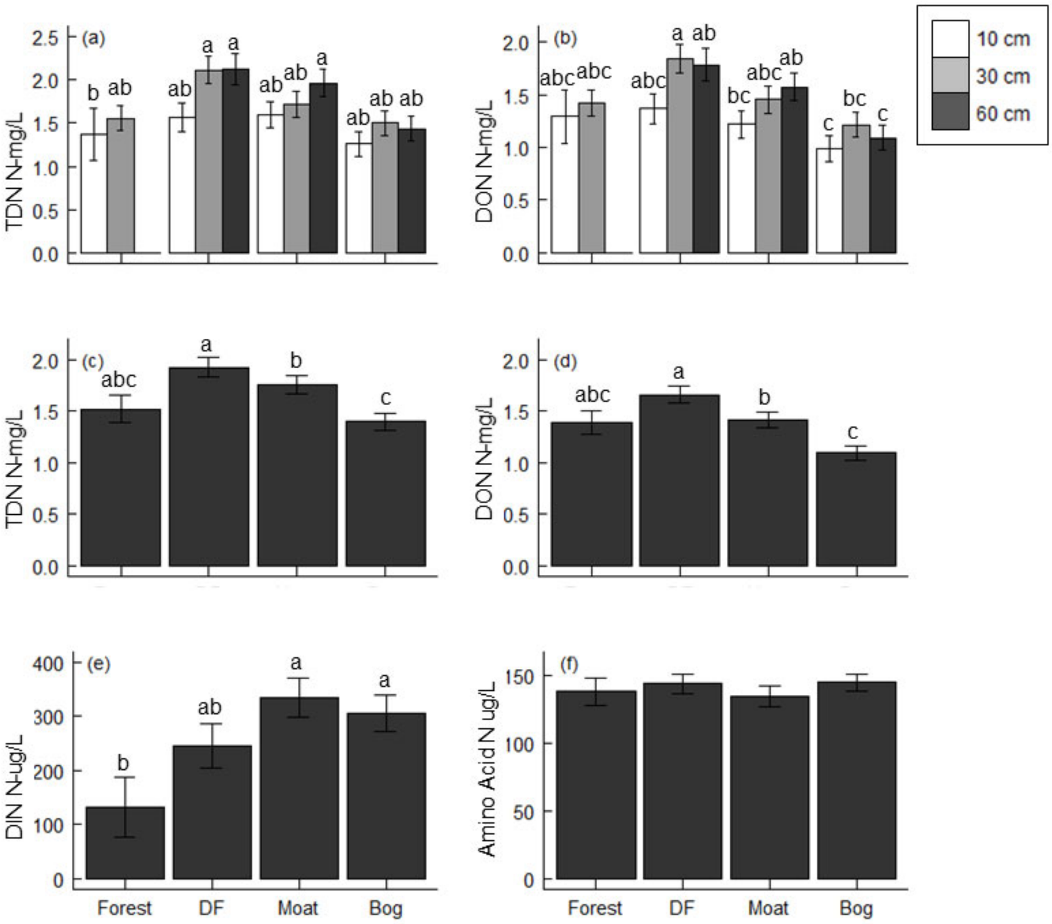

Fig. 4. Concentrations of **(a)** total dissolved N (TDN), and **(b)** dissolved organic N (DON) by depth and community type, and mean concentrations averaged across depths for **(c)** TDN, **(d)** DON, **(e)** dissolved inorganic N (DIN), and **(f)** free amino acids (AA) along permafrost thaw gradients comprising a permafrost forest (Forest), drunken forest with active permafrost collapse (DF), Moat representing recent permafrost thaw, and collapse scar bog (Bog). Data are mean ± SE, n=363. Same letters above the means indicate non-significant differences within each graph according to Tukey post-hoc comparison of means tests between community type × depth interactions. There were no significant differences in DIN or amino acid concentrations at the community type × depth level.

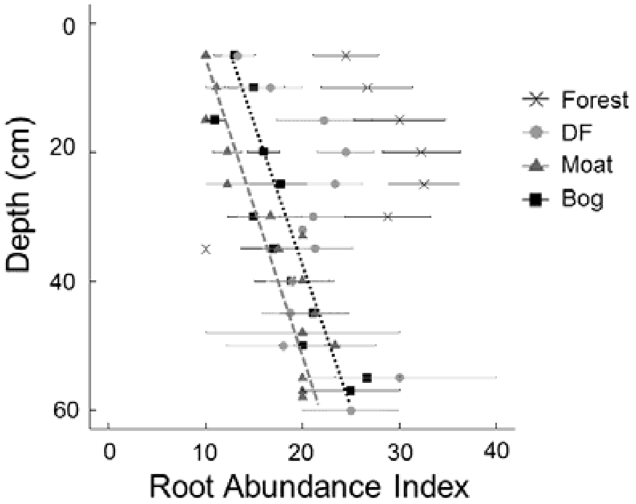

Fig. 5. Root abundance index value by depth across thaw gradient community types. Moat and bog regressions with depth are shown with dotted lines and are significant at a 95% confidence interval (Moat R^2=0.31, p-value<00.1; Bog R^2=0.12, p-value<0.001).

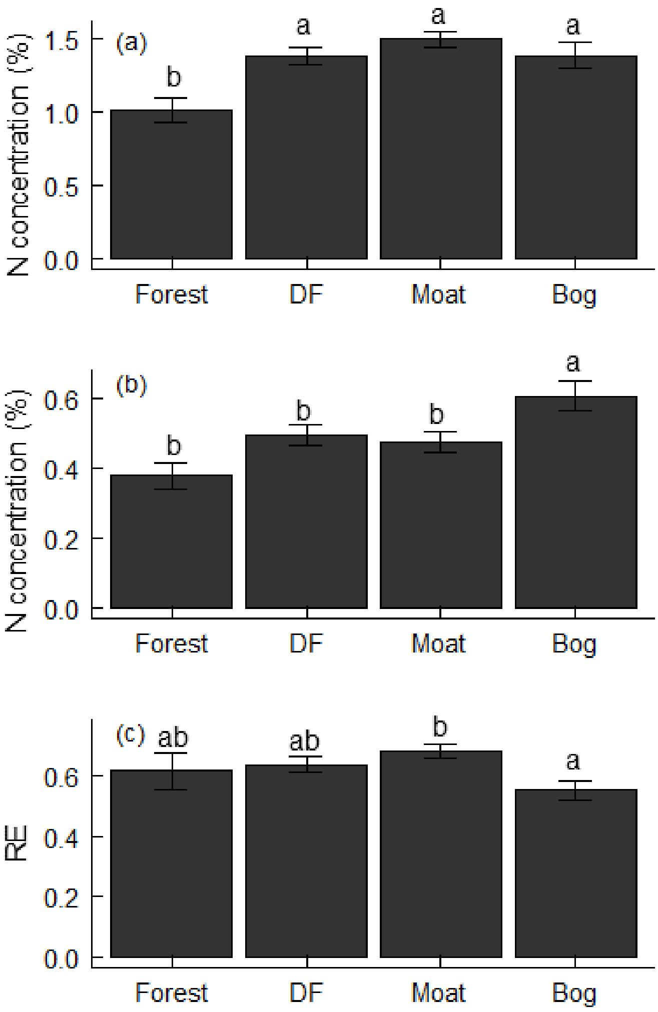

Fig. 6. Foliar N concentrations of *C. calyculata* (Mean± SE, n=36) from (**a**) peak biomass; and (**b**) senesced foliage collected from litter traps in autumn. Nutrient resorption efficiency (RE) **(c)** has been calculated as the change in summer foliage N and senesced foliage N percentages. Letters within each graph indicate post-hoc test differences between community types.

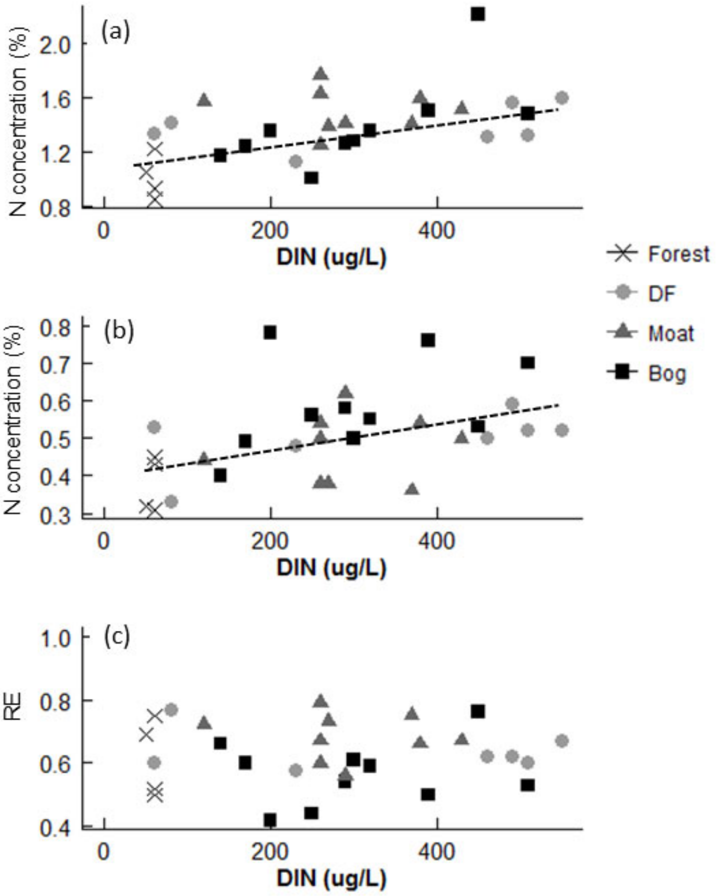

Fig. 7: Scatterplot of foliage N concentrations (%) in *C. calyculata* versus DIN pore water concentrations for (**a**) summer foliage, (**b**) litterfall, and (**c**) resorption efficiency. Lines indicate significant linear regressions at the 95 % confidence level. Summer foliage vs DIN: R^2=0.296 $F_{(1,29)}$=11.8, p=0.002. Litterfall vs DIN: R^2=0.22, $F_{(1,29)}$=7.9, p=0.009.

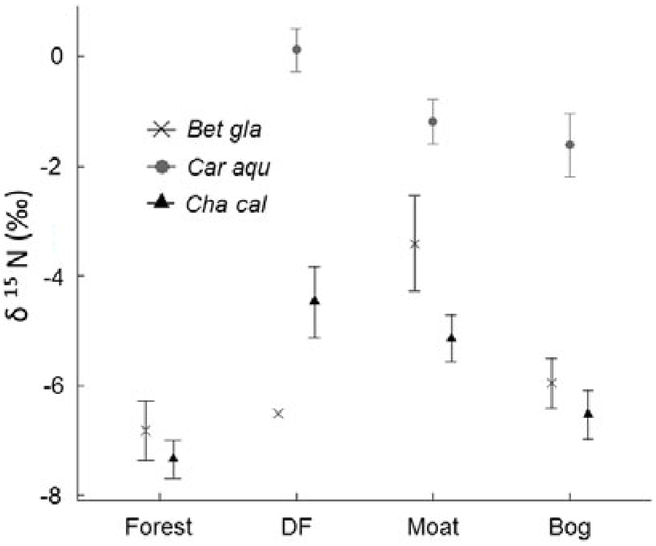

Fig. 8: Foliage δ^{15} N values across thermokarst community types for three commonly found species: *Betula glandulosa* (*Bet gla*), *Carex aquatalis* (*Car aqu*), and *Chamaedaphne calyculata* (*Cha cal*) (Mean ± SE, n=82).