Foraging the Thaw Front: Increased nutrient uptake at the permafrost surface enhances biomass production of deep-rooting subarctic peatland species

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Abstract

1. Plant production in subarctic peatlands is nitrogen (N)-limited. Climate warming increases N mineralization in superficial peat layers and recent results additionally show that permafrost thawing in these peatlands may substantially increase plant-available N at the thaw front. This might stimulate net primary production and affect species composition. However, the ability of individual peatland plant species to take up N from the permafrost thaw front has never been studied before.

2. We aimed to identify the potential impact of increased N-availability due to thawing permafrost on subarctic peatland plant productivity and species composition. We compared this impact with the effect of increased nutrient availability in shallower layers (e.g. through enhanced N-mineralization due to climatic warming). Therefore, we supplied \(^{15}\)N-labeled nitrogen at the thaw front and performed a 3-year full-factorial belowground fertilization experiment with deep-fertilization at the thaw front at 45 cm depth and shallow-fertilization at 10 cm depth.

3. We found that only particular species (e.g. Rubus chamaemorus) are present with active roots at the thaw front. Further, if supplied with nitrogen at the thaw front, these species had higher aboveground biomass and N-content, whereas this was not the case for shallower-rooting species (e.g. Empetrum hermaphroditum and Andromeda polifolia). Moreover, the effects of increased nutrient availability at the thaw front on total aboveground biomass production were similar in magnitude to the effects of increased nutrient availability in shallower layers. Additionally, nutrient limitation of plant growth in subarctic peatlands appeared to be sufficiently strong for the effects of increased deep and shallow nutrient-availability on biomass production to be additive.

4. Synthesis Altogether, our results show that plant-available N released from thawing permafrost can be considered a true ‘new’ N source for deep-rooting sub-arctic plant species, which will increase their biomass production. As this is not the case for shallow-rooting species, the release of plant-available N from thawing permafrost has the potential to alter species composition on the long-term by benefitting specific deep-rooting species only.

Introduction

Many of the world’s northern peatlands are underlain by permafrost (perennially frozen ground) which is thawing rapidly as a result of climate change (ACIA, 2004, Tarnocai et al., 2009). Recently, we have shown thawing permafrost may release considerable amounts of plant-available nitrogen (N) into subarctic peatlands (up to 0.3 g N m\(^{-2}\) yr\(^{-1}\) in the coming decade) (Keuper et al., 2012). This ‘new’ global-change induced N-source at the permafrost thaw front is additional to the predicted and observed increase in N-availability in shallower soil layers due to atmospheric deposition or increased N-mineralisation rates by higher air temperatures (Rustad et al., 2001, Weedon et al., 2012). Plant production in these northern peatlands is often N-limited (Aerts et al., 1992, Berendse & Jonasson, 1992) and a climate-change induced increase in N availability in shallower soil layers stimulates primary production and alters species composition (Chapin et al., 1995, Van Wijk et al., 2004). A release of stored permafrost N due to thawing may likely have similar effects, if plants are able to access this new permafrost-thaw induced N-source.
So far, little is known about the ability of peatland plants to take up nutrients released at the thaw front, although some studies suggest that specific peatland graminoids and forbs extend their roots to deeper soil layers (up to 60 cm soil depth). This applies in particular to the forb *Rubus chamaemorus*, as observed by Rapp and Steenberg (1977) in (non-permafrost) *Sphagnum fuscum*-dominated peatlands in northern Norway, and the sedge *Eriophorum vaginatum* (Wein, 1973), as observed in peatlands of the British Isles. Moreover, roots of *Eriophorum angustifolium*, *E. vaginatum* and *Arctagrostis latifolia* are able to grow at the frozen soil surface while this recedes down the soil profile during the northern summers (Bliss, 1956, Callaghan *et al.*, 1991) and can resume their growth after having been frozen for several days (Billings *et al.*, 1976). Further, although the moment of maximum thaw depth coincides with the end of the growing season, most perennial sub-arctic species can store the N in belowground structures such as rhizomes and thick roots (Chapin *et al.*, 1990) and use the stored N in the next growing season. Additionally, observations of increased canopy N in Alaskan thermokarst areas suggest that increased plant N-uptake from thawing permafrost is indeed possible in some areas (Schuur *et al.*, 2007). However, so far it has not been tested whether or not species can effectively utilize nutrients released at the permafrost thaw front at the time of maximum thaw depth (end of the growing season) and how much they rely on this uptake for their total nutrient budget relative to their nutrient-uptake from shallower soil layers.

The deep belowground release of plant-available N from thawing permafrost makes this N-source fundamentally different from other climate-change related increases in N-input, which generally affect nutrient availability in the surface layer of the soil only (e.g., increased mineralisation as a result of higher temperatures or increased atmospheric N deposition) (Rustad *et al.*, 2001, Weedon *et al.*, 2012). Nutrient utilisation in shallow layers (0-15 cm depth) is largely determined by species-specific plant traits related to their competitive ability for nutrient uptake, such as nutrient absorption capacity, specific root length and root allocation (Lambers *et al.*, 1998). Competition for the uptake of nutrients released from thawing permafrost would take the importance of these last two traits to a different level: in areas with thawing permafrost, community responses will first and foremost depend on whether and which specific species can reach this ‘new’ deep N source. Whether species composition in permafrost peatlands is affected by resource partitioning within the rooting zone (Berendse, 1982, Casper *et al.*, 2003, McKane *et al.*, 2002) is largely unknown. However, if such vertical resource partitioning occurs in permafrost peatlands, a release of nutrients at the thaw front will most likely benefit deeper rooting species only.

There may be interactive effects of climate-change induced increases in nutrient supply between shallow and deeper soil layers of northern peatlands, i.e. nutrient-uptake from deeper soil layers may be dampened when the vegetation’s need for nutrients is already satisfied by increased nutrient supply in shallower, more easily accessible layers. However, given the strong nutrient-limitation of plant production in northern peatlands, the effects on plant production of increased nutrient supply in shallow- and deeper soil layers are most likely additive.

In this paper we aim to identify the potential impact of increased N-availability at the permafrost thaw front on subarctic peatland vegetation. We hypothesized that: a) only particular peatland species have roots present and active at the thaw front of subarctic peatlands; and that b) if presented with increased nutrient availability at the permafrost thaw front, only these deep-rooting species will show increased aboveground biomass and N-content. Moreover, we hypothesized that c) the effects on aboveground biomass production of subarctic peatland
vegetation of increased nutrient availability at the thaw front can be similar in magnitude to the effects of an equal increase in nutrient availability in shallower layers and that d) nutrient limitation of plant-growth in subarctic peatlands is strong enough for the effects of increased deep- and shallow-nutrient availability to be additive.

**Methods**

*General approach*

All experiments were performed at two *Sphagnum fuscum* dominated ombrotrophic peatlands in the Stordalen nature reserve in northernmost Sweden (68°21.428’N, 19°03.181’E). We first determined the vertical plant root distribution in the active layer down to the thaw front, which is at a depth of 45-50 cm in these peatlands. Subsequently, we analysed qualitative differences among vascular subarctic peatland species in their capability to take up N from the thaw front between the time of maximum thaw depth (coinciding with the end of the growing season) and the beginning of the next growing season, by injecting $^{15}$N-ammonium at the thaw front. In addition, we performed a below-ground fertilization experiment to test whether increased plant-available nutrient supply at the thaw front can affect net aboveground primary production, plant N-content, and species composition, and to get insight in the relative importance of increased nutrient supply at the thaw front compared to increased nutrient supply in the current (main) rooting zone (5-15 cm) and potential interactions. The below-ground fertilization experiment included full-factorial combinations of fertilization in the active layer (at 10 cm depth) and fertilization at the thaw front (at 45 cm depth). After three growing seasons, we measured treatment effects on aboveground biomass and N-content of the dominant species.

*Root presence down to the thaw front*

Vertical root biomass distribution was determined in five randomly taken cores (measuring 6 x 5 cm, to a depth of 45 cm) which were extracted at the time of maximum thaw depth (second half of September) from the first peatland, where *Rubus chamaemorus*, *Empetrum hermaphroditum* and *Andromeda polifolia* were the key species. Upon extraction, each core was divided into four layers of each 10 cm at subsequent depths (5-15 cm; 15-25 cm; 25-35 cm and 35-45 cm, the latter representing the thaw front). All living roots (> 0.1 mm) were removed from the *Sphagnum* peat and sorted per species by means of visual estimation of differences in colour, diameter and structure. The roots were sorted in the lab, oven dried (48 hrs, 60 °C), weighed and root biomass was expressed as root dry weight per cm$^3$ soil volume.

*$^{15}$N-uptake at the thaw front*

To test for differences among subarctic peatland species in their capability to take up nitrogen from the thaw front between the time of maximum thaw depth and the beginning of the next growing season, a $^{15}$N-pulse-labeling at the thaw front was performed. Six peatland plots were selected based on mutual presence of the vascular species *E. hermaphroditum*, *R. chamaemorus*, *Vaccinium uliginosum*, *A. polifolia*, and if possible *Eriophorum vaginatum* and *Betula nana*, and an active layer thickness of approximately 45 cm. At the time of maximum soil thaw (September 15th, 2008), 28 mg $^{15}$N-ammonium chloride (98 atom% $^{15}$N, Isotec, Miamisburg, OHIO, USA) dissolved in 60 ml deionized water, was injected at the thaw front in triangular grids, using three metal tubes (10 cm apart, 20 ml per tube) with small openings at the bottom of the tube. The
N-ammonium solution was inserted by connecting a syringe to the airtight tubes and forcing the liquid to exit the tube at the bottom under pressure (See picture, Supplementary material Fig. SI 1). Six control plots were selected and all plots were at least one meter apart from each other. At the start of the following growing season (June 12, 2009), leaf material of all vascular species present within the triangle was collected. The leaves were stored in separate paper bags, oven dried (at 60 °C) and ground. Additionally, on September 20, 2009, one year after the pulse labeling and at the time of maximum thaw, soil cores were extracted from the centre of every 15N-plot and of four control plots to check for potential undesired vertical redistribution of the labeled nitrogen. Subsamples were taken at three depths per soil core (15, 30 and 45 cm depth) and roots were removed. Soil samples were then oven dried (48 hrs, 70 °C) and ground. Before further analysis of the plant material, all soil samples were analyzed for the abundance of 15N to check if 15N-enrichment had not spread to layers other than the deepest layer (either through vertical redistribution via plant root litter or exudates, capillary rise or methodological contamination), using a cut-off value of 3.75 mg 15N gN⁻¹. Because we had no means to distinguish between the natural redistribution processes or methodological contamination, only plant samples from plots without any vertical redistribution of the labeled nitrogen were used in subsequent analyses, resulting in n = 5 for most species. Unfortunately, for both E. vaginatum and B. nana only two replicates remained. Atom percentages of 15N of all plant and soil samples were determined with an elemental analyser (NC2500, ThermoQuest Italia, Rodano, Italy) coupled online to a stable isotope ratio mass spectrometer (DeltaPlus, ThermoQuest Finnigan, Bremen, Germany).

Three-year full-factorial belowground fertilization experiment

To quantitatively assess the relative importance of deep N availability vs. shallow N availability, a full-factorial belowground fertilization experiment was performed with factors ‘thaw front (deep)-fertilization’ (+/-) and ‘current rooting zone (shallow)-fertilization’ (+/-). This thus resulted in four treatments: ‘shallow-fertilized’ (S), ‘deep-fertilized’ (D), ‘shallow- plus deep-fertilized’ (SD) and a control treatment (C). Plots of 60 x 60 cm were randomly chosen within an ombrotrophic peatland dominated by E. hermaphroditum and R. chamaemorus (78% and 12% of total biomass, respectively), and each experimental treatment was replicated eight times. Maximum active layer thickness was measured at the time of maximum thaw depth (second half of September) in each plot by probing a steel rod into the soil until refusal, and was around 45 cm. In early June 2008, 11.8 g slow release fertilizer grains (NPK 17:3:11, with the 17% N consisting of 8.9% NH₄-N and 8.1% NO₃-N), equivalent to 8 gN m⁻² (to be released slowly during the three years after insertion) were added in the shallow-fertilization treatment (10 cm depth). The grains were inserted through an aluminium tube (diameter 10 mm) in grids of nine points (3 x 3 design with insertion points 20 cm apart, see Supporting Information, Fig. SI 2). Control plots were equally grid-wise perforated, but did not receive fertilizer. As the deeper soil layers are hard-frozen at the beginning of the growing season, an equal amount of slow release fertilizer was inserted in the same grid of the deep-fertilization treatments (D and SD) at a depth of 45 cm at the time of maximum thaw depth prior to the shallow addition (September 2007). In this way, we synchronized the potential availability of shallow and deep N into the same growing season.

To verify treatment effects on nutrient availability, plant root simulator (PRS™) soil probes (Western Ag Innovations Inc., Saskatoon, Canada), commercially manufactured ion exchange resins, were inserted in four plots per treatment and at two depths (10-15 cm and 40-45 cm).
two years after the start of the experiment. The shallow- and the deep- inserted probes showed higher N-values at the depths where the fertilizer grains had been inserted but not at other depths (Supporting Information, Fig. SI 3). There were no interaction effects, showing that our treatments indeed had the intended effect on soil nutrient availability.

Effects of belowground fertilization on vegetation

Three growing seasons after insertion of the fertilizer, at the peak of the growing season 2010 (July 26th), total aboveground vascular biomass was harvested from two 20 x 20 cm subplots per plot (Supporting Information, Fig. SI 2) which were combined for further analysis. The samples were divided into leaves of *R. chamaemorus*, *E. hermaphroditum* (together 90% of total aboveground biomass) and the remaining species (*A. polifolia*, *Vaccinium microcarpum*, *V. uliginosum*). These species, which were all shallow-rooting, were grouped because of generally low abundance and because none of these remaining species were present in all plots. Leaves were oven dried (48 hours at 60 °C), weighed, ground and nitrogen concentrations were determined by dry combustion with a Flash EA1112 elemental analyser (Thermo Scientific, Rodana, Italy).

Additionally, to test whether roots at the thaw front are actively ‘foraging’ when presented with increased nutrient supply, changes in *R. chamaemorus* root biomass at the thaw front were determined by comparing root biomass in five randomly selected control (C) plots with eight deep-fertilization (D) plots at the time of maximum thaw depth in the last year of the experiment. In every plot one core (of 6 x 5 cm and a depth of 10 cm) was extracted at 35-45 cm (due to practical constraints not all treatments and depths were sampled). Roots (> 0.1 mm) were sorted in the lab, oven dried (48 hrs, 60 °C), weighed and root biomass was expressed as root dry weight per cm$^3$ (fresh) soil volume.

Statistical analysis

All data were tested for normality and homogeneity of residual variances by visual estimation of residual plots and normal probability plots. Log-transformation improved the homogeneity of residual variances for the $^{15}$N labeling experiment data, as well as for the aboveground biomass, the N-content and the root biomass data of the full-factorial belowground fertilization experiment.

Due to the high number of zero-values, the ambient root biomass distribution data did not render enough power for credible significance-testing. We therefore chose to present the vertical distribution of mean root biomass and presence-absence data only graphically. $^{15}$N-contents of plant leaves in the $^{15}$N-labeling experiment were analysed with a repeated-measures (RM)-ANOVA with within-subject factor ‘species’ (*A. polifolia*, *V. uliginosum*, *E. hermaphroditum* and *R. chamaemorus*) and as between-subject factor $^{15}$N-treatment’. The differences among species were subsequently analysed by paired t-tests. Aboveground biomass and N-content of the two dominant species (*R. chamaemorus*, *E. hermaphroditum*) in the full-factorial belowground fertilization experiment were analysed with a RM-ANOVA with ‘species’ (*R. chamaemorus*, *E. hermaphroditum*) as the within-subject factor, and ‘shallow-fertilization’ and ‘deep-fertilization’ as the between-subject factors. We performed the analyses on aboveground biomass and N-content data in the full-factorial belowground fertilization experiment on the two key species only (*R. chamaemorus* and *E. hermaphroditum*, together 90% of total biomass) because of many near-zero values in the ‘rest-group’. Differences in root biomass at the thaw front in control plots vs. deep-fertilization plots were analysed with a one-way ANOVA with ‘deep-fertilization’ as an independent factor.
All analyses were performed with SPSS 15.0 for Windows.

**Results**

*Roots at the thaw front*

On the five randomly chosen locations where *Rubus chamaemorus*, *Empetrum hermaphroditum* and *Andromeda polifolia* were mutually present, we found strong interspecific differences in vertical root distribution patterns. At the thaw front, only roots of *R. chamaemorus* were present (Fig. 1). Root biomass of *R. chamaemorus* was relatively evenly distributed over the soil profile, with the deepest layer an average root biomass of 0.8 ± 0.1 (SE) gDW cm\(^{-3}\) (n = 5). Roots of the other species were largely confined to the upper soil layer (up to 15 cm depth) and were scarcely (at 15-25 cm) or not at all found below 25 cm depth (Fig. 1). Thus the bulk of root biomass of *A. polifolia* or *E. hermaphroditum* occurred at 5-15 cm soil depth, with a steep vertical decrease in root biomass (e.g. for *Empetrum* 1.5 gDW cm\(^{-3}\) at 5-15 cm and only 0.15 gDW cm\(^{-3}\) at 15-25 cm) (Fig. 1).

*N-uptake at the thaw front*

*R. chamaemorus*, *E. hermaphroditum*, *A. polifolia* and *Vaccinium uliginosum* differed in their mean \(^{15}\)N content as a result of uptake from the thaw front between the time of maximum thaw depth and the beginning of the following growing season (‘species’ \(F = 15.92; P < 0.005\); ‘species’x’treatment’ \(F = 15.84; P < 0.005\) and the between-subject factor ‘treatment’ \(F = 16.59; P < 0.005\)). The significant interaction indicates \(^{15}\)N uptake by some species in the treatment only. Paired t-tests and Fig. 2 show that the significant differences among species were due to the significantly higher \(^{15}\)N content in *R. chamaemorus* compared to each of the other species. Visual

![Figure 1. Mean (± SE) root dry weight for three subarctic peatland species at increasing soil depths, down to the thaw front, at the time of maximum thaw depth. Five cores were sampled; the number of cores in which roots were present is indicated in parentheses.](image-url)
interpretation suggest that leaves of *Eriophorum vaginatum* were most likely also $^{15}$N-enriched in response to insertion of labeled $^{15}$N ammonium at the thaw-front. None of the shallow-rooting species had taken up the labeled nitrogen (Fig. 2). This could not be tested statistically due to the lack of power as a result of the low number of remaining replicates ($n = 2$; see Methods). As a control, an analysis in which all plots and all species were included (not accounting for potential contamination) showed the same pattern (‘species’ $F = 25.70; P < 0.005$; ‘species’ x ‘treatment’ $F = 25.75; P < 0.005$; ‘treatment’ $F = 98.38; P < 0.005$) but with both the deep rooting species *R. chamaemorus* and *E. vaginatum* significantly different from the other species.

**Figure 2.** $^{15}$N-content of early spring-leaves after insertion of $^{15}$N-label at the thaw front at the time of maximum thaw depth in the previous year (end of the growing season). Data are means ± SE and $n = 5$ for all species except *Eriophorum vaginatum* and *Betula nana* ($n = 2$), which were excluded from the statistical analysis.

**Table 1.** Results of a RM-ANOVA for the effects of between-subject factors shallow-fertilization and deep-fertilization and within-subject factor species (*R. chamaemorus* vs. *E. hermaphroditum*) on aboveground biomass and N-content of two subarctic peatland key-species (*Rubus chamaemorus* and *Empetrum hermaphroditum*). Data were log-transformed, * depicts $P \leq 0.05$; ** $P \leq 0.01$ and *** $P \leq 0.001$.
**Full-factorial belowground fertilization experiment: aboveground biomass and N-content**

Deep-fertilization caused significantly different responses of the two dominant species in both mean biomass and N-content (significant ‘deep-fertilization x species’ interaction, Table 1). Only the deep-rooting _R. chamaemorus_ showed higher biomass and N content in response to fertilization at the thaw front, whereas this was not the case for the shallow-rooting _E. hermaphroditum_ (Fig. 3). In contrast, shallow fertilization had the same positive effect on biomass and N-content of both species (i.e. no ‘shallow-fertilization’ x ‘species’ interaction, Table 1). Similar results were obtained when including the biomass data of the ‘other species’ as a third level in the within-subject factor (within-subject factor ‘species’ $F = 107.8$, $P < 0.001$; ‘shallow-fertilization x species’ $F = 1.3$, $P = ns$; ‘deep-fertilization x species’ $F = 4.0$, $P < 0.05$). Moreover, for all analyses, there was no interaction between the treatment effects: thus, the effects of the shallow- and deep-fertilization on mean aboveground biomass and N-content were additive (Fig. 3, Table 1).

**Root biomass at the thaw front in response to increased nutrient supply**

Despite an up to 100% increase of root biomass of _R. chamaemorus_ at the thaw front in the deep-fertilized plots compared to the control plots (1.6 ± SD 0.8 mg cm$^{-3}$ in the deep-fertilized plots vs. 0.8 ± SD 0.3 mg cm$^{-3}$ in the control plots) this difference was only marginally significant (‘deep-fertilization’ $F = 4.6$; $P = 0.056$). This lack of significance despite the large difference was most likely due to the large spatial variability that is characteristic for root distribution patterns.

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**Figure 3.** Mean (± SE) aboveground biomass (a) and canopy N-content (b) of the two key species _Rubus chamaemorus_ and _Empetrum hermaphroditum_ in control (C), shallow-fertilized (S), deep-fertilized (D) and shallow- plus deep-fertilized (SD) plots of the full-factorial belowground fertilization experiment ($n = 8$, See Table 1 for RM-ANOVA statistics).
Discussion

Thawing permafrost is often mentioned as one of the main climate change induced threats to northern peatlands (ACIA, 2004). Recently, we showed that permanently frozen soils of subarctic peatlands contain considerable amounts of plant-available N (Keuper et al., 2012), which may be released upon thawing. Changes in nutrient availability due to climate change are likely to have considerable influence on the vegetation composition and biomass of subarctic peatlands as these ecosystems are usually strongly N-limited (Dormann & Woodin, 2002, Limpens et al., 2011). Here, we show for the first time that vascular peatland plants are able to utilize N at the permafrost thaw front to increase their biomass and N content. Moreover, our results show that increased nitrogen availability at the thaw front may induce a shift in species composition in the longer term because of a differential ability of peatland species with different rooting depths to take advantage of this new nitrogen source.

Roots at the thaw front: present and actively taking up nutrients

We found large differences in rooting depth among species and only one of our species, Rubus chamaemorus, had roots at the thaw front (hypothesis a; Fig. 1). These findings are consistent with earlier observations of deep-rooting R. chamaemorus (Rapp & Steenberg, 1977) and demonstrate that indeed only particular species have roots at the thaw front. Moreover, our labeling experiment showed that nutrients can be taken up by R. chamaemorus at the end of the growing season (Fig. 2). Given the asynchronicity of the plant growing season and the period in which the deeper layers are unfrozen, this species is most likely capable of storing nutrients that are acquired late in the growing season in rhizomes (Chapin et al., 1990, Taylor, 1971), ready for quick use in early spring of the next growing season. Although our data do not allow for strong conclusions about other potentially deep-rooting species (but see Fig. 2), it is likely that other deep-rooting rhizomatous species like E. vaginatum or Calamagrostis lapponica (Callaghan et al., 1991, Chapin et al., 1990, Venhuizen, 2009) are also capable of utilizing nutrients released from thawing permafrost.

Impacts of differential uptake of nutrients from the thaw front on community composition

As hypothesized, both deep- and shallow-rooting species were responsive to shallow fertilization. Many studies have been performed on the effects of increased nutrient supply in shallow soil layers, usually with fertilizer applied to the soil surface (Haugwitz & Michelsen, 2011, Parsons et al., 1995, Press et al., 1998b, Shaver et al., 2001). Such experimentally increased nutrient supply tends to result in an increased abundance of fast-growing, competitive species (mostly graminoids, deciduous shrubs and herbaceous species) at the expense of slow growers (mostly evergreen shrubs and mosses and lichens) (e.g. Aerts et al., 2006b). Our results suggest that fertilizer applied deep-belowground affects a different combination of species than shallow fertilization, due to differences in vertical root distribution (Fig 1, 2), indicating that in this system, spatial niche differentiation seems to be more important than in other systems (de Kroon et al., 2012). The additional N-uptake at the thaw front clearly provided the deep-rooting R. chamaemorus with a competitive advantage as it significantly increased its aboveground biomass and N-content in the belowground fertilization experiment after three years of treatment (Fig. 3; Table 1). Moreover, the impacts of shallow- and deep-fertilization on aboveground biomass of this deep-rooting species were remarkably similar in magnitude, given that the moment of maximum thaw depth coincides with the end of the growing season (hypothesis c, Fig. 3). The
importance of these observations is illustrated by the results of fertilizer experiments performed on bogs with a similarly low nutrient status in northern Norway, where R. chamaemorus responses to fertilizer applied to the surface of the soil were suppressed by strong responses to the same treatment of competitive deciduous dwarf shrubs in the bog (Taylor, 1971). The discrepancy between our results and the observations of Taylor (1971) demonstrate that competitive species interactions controlled by resource partitioning (niche differentiation between different soil layers) (Berendse, 1982, Casper et al., 2003, McKane et al., 2002) may be affected differentially by increased nutrient supply in shallower layers than by increased nutrient supply at the permafrost thaw front. Hence, increased nutrient supply at the thaw front, in combination with strong spatial niche differentiation caused by differential vertical rooting patterns, may lead in the long term to a species composition shift in favour of deep-rooting species in these nutrient-limited ecosystems.

Additive effects of increased shallow- and thaw-front-nutrient supply

There was no interaction between effects of the shallow-fertilization and the deep-fertilization treatments on aboveground biomass; the effects were additive (hypothesis d; Fig 3a,b; Table 1). Hence, shallow fertilization did not dampen uptake from the thaw front, which indicates that nutrient limitation in these peatlands is strong enough to avoid nutrient saturation, at least until other nutrients such as phosphorus become limiting (Aerts et al., 2001). The amount of nutrients applied in this experiment was relatively large (an initial supply of 8 gN m$^{-2}$) compared to the amount that might be released from thawing permafrost of subarctic peatlands (up to 0.3 g N m$^{-2}$ yr$^{-1}$ in the coming decade) (Keuper et al., 2012) or to the estimated increase in nutrient supply in shallower layers (e.g. climate-warming in the range of 0.3 - 6.0 °C would induce an estimated 46% increase of the 0.8 g N m$^{-2}$ yr$^{-1}$ net N mineralisation in northern peatland ecosystems) (Rosswall & Granhall, 1980). Hence, it seems likely that the effects of ‘natural’ sources of increased N-supply in northern peatlands will also be additive.

Potential longer-term effects

So far, observational records on species composition shifts upon thawing permafrost exist only for two systems, with well documented thaw-histories (our study area: Malmer et al., 2005, Alaska: Schuur et al., 2007). These records show mixed results: in Alaska, a shift from graminoid-dominated tundra in the least disturbed site to shrub-dominated tundra in the oldest most subsided site was observed (Schuur et al., 2007), while in a subarctic permafrost peatland site in our study area, dwarf-shrub-dominated hummock sites receded and gave way to wet sites dominated by graminoids (Malmer et al., 2005). This illustrates that long-term vegetation responses to thawing permafrost are most likely not only due to changes in nutrient availability, but also to multiple related factors such as co-occurring changes in hydrology or soil temperature, which were not included in our experimental design. Therefore, we do not address these related factors in depth, but instead confine this discussion to potential long-term responses to permafrost-thaw-induced changes in nutrient availability.

Long-term plant-responses to permafrost-thaw-induced increases in nutrient availability can be affected by several factors. Firstly, vertical transport of nitrogen via root and leaf litter of deep rooting species from the thaw front to shallower layers may occur. Thus, permafrost-N can, in the longer term, become a new N-source also for shallower-rooting species, who might utilize this N after it has been redistributed through the soil layers. Secondly, microbial biomass is a
strong sink for nutrients in arctic soils (Jonasson et al., 1996) and the competitive strength of the microbial community could increase in response to increased N-supply. However, within the scope of this experiment the large increases in vascular plant biomass in response to fertilization at the thaw front were not matched by similar increases in mean microbial biomass N. Instead, microbial biomass was not affected by the deep fertilization treatment and only slightly by the shallow fertilization treatment (Supporting Information, Fig. SI 4). This suggests that the microbial community benefits less than the vegetation from the additional deep N-source. This may increase the discrepancy between vegetation responses to climate-change-induced shallow-versus deep increases in N-supply in the longer-term. Thirdly, longer-term plant responses may differ from the results of this study due to changes in vertical root biomass distribution in response to increased deep nutrient supply (Hodge, 2004). In this experiment, root biomass at the thaw front tended to increase (up to 100%), but only for the deep-rooting species. This is similar to results obtained for crops where deep root biomass increased in response to localized belowground fertilization (Drew, 1975), and in line with plant ‘foraging’ as described by Callaghan et al. (1991) and McNickle et al. (2009). The observed altered root biomass distribution in response to a relatively short-term (three year) experiment suggests that, over the longer-term, the effects on plant productivity of increased nutrient supply at the thaw front may aggravate due to increased root biomass and thus uptake capacity at the thaw front. Overall, although plant responses to thawing permafrost in subarctic peatlands are affected by multiple factors, the experimental results presented in this study suggest that, in the longer term, a release of nutrients from thawing permafrost could accelerate N-cycling in these nutrient-deprived ecosystems.

In conclusion, we have shown for the first time that nitrogen released from thawing permafrost can be taken up by deep-rooting plant species and can result in increased biomass production of such species. Hence, we show that plant-available N from thawing permafrost can be considered a real plant-available ‘new’ N input. Moreover, our results show that the effect of a deep, belowground release of nutrients on subarctic peatland vegetation is fundamentally different from the effect of a shallow increase in nutrient availability. In the longer term, this climate change-induced new N source may lead to an accelerated N cycle and changes in species composition of subarctic peatlands.

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Supporting Information

Figure SI 1. Injection of $^{15}$N-ammonium solution through a metal tube into the thaw-front.

Figure SI 2. A plot of the thaw-front-fertilization experiment: the white dots represent the 9-points slow-release fertilizer insertion grid; the two large rectangles depict the biomass harvest areas; and the smaller rectangle is the root biomass and microbial biomass N soil-sampling site.
**Thaw-front fertilization experiment: effects on N-supply**

To verify treatment effects, plant root simulator (PRS™) soil probes (Western Ag Innovations Inc., Saskatoon, Canada), also known as ion exchange resins were inserted in four plots per treatment and at two depths: 5-15 cm and 35-45 cm deep, two years after the start of the experiment. The PRS probes were inserted at the time of maximum thaw depth (2nd half of September) and removed exactly one year later. After extraction from the soil, the probes were carefully cleaned with deionized water and analysed to obtain inorganic N exchange rates during the incubation period.

![Figure SI 3. Mean PRSTM-probe N-exchange rate (± SE) data after a year-round burial in the thaw-front fertilization experiment at two depths (surface = 5-15 cm, deep = 35-45 cm; n = 4). Treatments are: control (C); shallow-fertilized (at 10 cm depth; S); deep-fertilized (at 45 cm deep; D); deep- and shallow-fertilized (DS).](image)

**Statistics (effects on N-supply)**

Log-transformed N-exchange rates for the shallow and deep inserted probes, respectively, were analysed with two separate two-way ANOVAs to test for the effects of the four different treatments (control, shallow, deep, shallow-and-deep) which were separated by a Tukey’s HSD post-hoc test. For the shallow-inserted probes, treatment effects were significant (F-value 16.92 and P < 0.001) and S>DS>C>D. For the deep-inserted probes treatment effects were significant as well (F-value 4.65 and P < 0.05) and DS>D>C>S, showing that our treatment had indeed the desired effect on soil nutrient availability (Fig. SI 3).

**Microbial biomass N in the full-factorial below-ground fertilization experiment**

Additionally to differential below-ground plant-species interactions at the thaw front and in shallower soil layers, below-ground plant-microbe interactions might also differ among these soil layers. For example, if less microbial immobilization of nutrients would occur at the thaw front due to a less active microbial community, nutrients released there would be relatively more plant-available than in shallower soil layers (Keuper et al., 2012).
Because the microbial community can be a strong competitor for nutrients in subarctic ecosystems, we also determined microbial biomass N (N_{\text{mic}}) in soil samples taken both in shallow soil layers (5-15 cm depth) as well as at the thaw front (35-45 cm depth) in the full-factorial belowground fertilization experiment in order to gain insight in potential changes in competitive strength of the microbial community in response to increased belowground nutrient supply.

We hypothesized that low initial microbial biomass would lead to no significant increase of the N immobilized by the microbial community at the thaw front, whereas we did expect an increase in N_{\text{mic}} in the shallower soil layers (at 5-15 cm depth) in response to shallow below ground fertilization.

**Methods N_{\text{mic}} determination**

After the final vegetation biomass harvest and at the time of maximum thaw-depth, soil samples for N_{\text{mic}} determination were taken in the full-factorial belowground fertilization experiment. One core per plot was extracted and two samples per core (5-15 cm and 35-45 cm) were transported to the lab in a cooling box with icepacks and subsequently frozen at -18 °C until further analysis. Subsamples of 3 x 3 x 3 cm were taken in the field from each sample for determination of fresh weight per soil volume (bulk density). For determination of the N_{\text{mic}} pool, two subsamples of each 5 gram fresh soil were taken, one of which was chloroform-fumigated for 24 hours at ~20 °C in a darkened desiccation jar. Both subsamples were then extracted after two hours shaking in 25.0 ml 0.5 M K_{2}SO_{4}, and oxidized by potassium persulfate digestion for determination of total extractable N (Cabrera & Beare, 1993). N_{\text{mic}} pool sizes were calculated as the differences between fumigated and non-fumigated extracts, using correction factor KEN = 0.40, representative for organic soils, to account for microbial tissue N that is not released by exposure to chloroform (Jonasson et al., 1996). Values were expressed as µg N_{\text{mic}} cm^{-3} fresh soil.

![Figure SI 4. Mean (± SE) microbial biomass N pool sizes at two depths in the thaw-front fertilization experiment at the time of maximum thaw. Treatments are: control (C); shallow-fertilized (S); deep-fertilized (D) and shallow- plus deep-fertilized (SD). Only shallow-fertilization had a small positive effect (P < 0.1) on microbial biomass N (rm-ANOVA with ‘sampling depth’ as the within-subject factor and the two treatments ‘shallow-fertilization’ and ‘deep-fertilization’ as the between-subject factors, n = 8).](image-url)
Statistical analysis $N_{\text{mic}}$ determination in the full-factorial below-ground fertilization experiment

Microbial biomass N ($N_{\text{mic}}$) data were log(x+1) transformed to improve the homogeneity of residual variances. The $N_{\text{mic}}$ data from the belowground fertilization experiment were analysed with an (RM)-ANOVA with ‘sampling depth’ (shallow/deep) as the within-subject factor and ‘shallow-fertilization’ and ‘deep-fertilization’ as the between-subject factors.

Results $N_{\text{mic}}$ in the full-factorial belowground fertilization experiment

The mean microbial biomass N ($N_{\text{mic}}$) in the shallow rooting zone was more than twice as large as mean $N_{\text{mic}}$ at the thaw front (‘sampling depth’ $F = 28.0; P < 0.001$). There were no significant interactions between the sampling depth and the treatments (‘sampling depth x shallow-fertilization’ $F = 0.04; P > 0.1$; ‘sampling depth x deep-fertilization’ $F = 0.16; P > 0.1$) and no significant effect of the deep fertilization treatment on microbial biomass N ($F = 0.13; P > 0.1$) but there was a positive trend of shallow fertilization on $N_{\text{mic}}$ ($F = 3.9; P < 0.1$) (Fig. SI 4).
Peatland-plants can utilize thawing permafrost N