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REGULAR ARTICLE

Nutrient availability measurement techniques in arctic tundra soils: *in situ* ion exchange membranes compared to direct extraction

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Abstract
Aims The use of ion exchange membranes (IEMs) as an alternative to direct chemical extractions for measuring soil nutrient availability has many theoretical advantages but was not well-investigated in the arctic. We compared the two methods in a low Arctic tundra ecosystem, and investigated the applicability of IEMs to determine experimental treatment effects on soil fertility and plant nutrient acquisition.
Methods We compared IEM incubation and soil sample water-extraction methods to assess the intra-seasonal availabilities of ammonium, nitrate, and phosphate in tundra soils from experimental fertilization treatments. We determined plant species' foliar nutrient concentrations in those treatments to evaluate the effectiveness of the two methods in predicting plant nutrient acquisition. We also incubated IEMs in summer greenhouse warming and snowfence treatment soils and investigated the corresponding plant community biomass responses.
Results NH_4-N accumulations on IEMs across the fertilization treatments were closely correlated to NH_4-N pools obtained by soil extraction. However, the IEM method was more sensitive and so was able to detect effects of low level fertilization that corresponded with increases in plant foliar nutrient concentrations. Furthermore, temporal pattern of IEM nutrient fluxes differed from the nutrient pools. The warming treatment enhanced phosphate but not ammonium fluxes, although plant community biomass increased. The snowfence treatment had no effects on either flux, and the overwinter fluxes of both nutrients were extremely low compared to the growing season.
Conclusions We conclude that the IEM method is a relatively sensitive, simple, and effective method for measuring nutrient availability in tundra soils.

Keywords Nitrogen · Phosphorus · Fertilization · Climate warming · Deepened snow · Plants' response

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Introduction

Soil nutrient availability to plants is a fundamental determinant of terrestrial ecosystem structure and functioning (Chapin III et al. 2011), and therefore, choosing a suitable measuring method to determine this availability is very important. Two major factors determine the availability of nutrient ions to the plant root systems: (1) the concentrations of ions in the soil solution within the plant rhizosphere (i.e. the pool of ions immediately accessible by plants); and (2) supply of additional ions from outside the rhizosphere to the root surface (i.e. ion fluxes from the bulk soil via diffusion and mass flow that replenish the rhizosphere pools), which is affected

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by soil moisture, soil pH, temperature, etc. (Abrams and Jarrell 1992; Barber 1962; Chapin 1980; Yang et al. 1991). A good measurement method should encompass these key factors influencing nutrient availability to plants, and also be relatively easy to apply (Schinner et al. 2012). Additional methodological issues include disturbance and degradation of the habitat caused by sampling, the labor involved in sampling and processing, and logistical issues such as transportation and sample storage (Giblin et al. 1994; Weih 1998).

Direct extraction compared to ion exchange as methods to determine soil nutrient availability

The direct chemical extraction method has been the most commonly used method for estimating soil nutrient availability (Gregorich and Carter 2007; Kuo et al. 1996; Mulvaney et al. 1996). For this method, a representative number of soil samples to a certain depth are taken from an area under investigation. These samples are combined and homogenized to a bulk sample after removing the coarse materials (rocks, roots, etc.). Soil nutrient pools in that bulk sample are determined by extraction with one of a variety of chemical solutions depending on the ion of interest (Gregorich and Carter 2007). Although it has been widely used, there are several problems associated with this method. The first major concern is the interpretation of the results, because data based on this method are operationally defined by the chemical extractant used rather than by nutrient availability to plants. Second, these data reflect static nutrient pools and accordingly can only provide an instantaneous "snapshot" of soil available nutrient status (Abrams and Jarrell 1992; Curtin et al. 1987). Since soil nutrient availability can vary substantially over time scales of days or even hours (Buckeridge and Grogan 2010; Pedersen et al. 2015; Vandecar et al. 2009), it is often inappropriate to extrapolate these data across time periods. Third, for repeated (i.e. time series) measurements, this method introduces the potential error associated with spatial variation among successive samples from the same plot. This error due to spatial heterogeneity can be very significant as soil biogeochemical variables (especially those associated with instantaneous nutrient availability) can vary substantially over distances of only a few centimeters (Frankland et al. 1963; Lechowicz and Bell 1991; Pedersen et al. 2015; Snaydon 1962; Wijesinghe and Hutchings 1997). Fourth, direct soil extraction is a destructive data collection method that removes soil and inevitably causes disturbances to the field sampling area that may be scientifically undesirable (Ehrenfeld et al. 2005; Iversen et al. 2015; Raison et al. 1987; Schinner et al. 2012; Schmidt et al. 2002). Lastly, this method is time-restricted in terms of sample processing because soil biochemical characteristics can significantly change over time scales of hours to days (Pedersen et al. 2015), and such changes may be exacerbated by changes in the physical environment once the sample is removed from its *in situ* field location. Accordingly, soil samples need to be transported and stored at a constant cool temperature (e.g. 4 °C) and processed and extracted as soon as possible (normally within one to two days) after being collected (Gregorich and Carter 2007). However, these requirements may be difficult to achieve routinely when sampling in remote field areas such as tundra. Despite these drawbacks, the direct extraction method is still very widely used, mainly because it has been well established (Bremner and Keeney 1966; Gregorich and Carter 2007).

The ion exchange resin bag (IEB) and the ion exchange membrane (IEM) methods as alternatives for measuring soil nutrient availability have been developed in recent decades, especially in the tundra ecosystems (Abrams and Jarrell 1992; Giblin et al. 1994; Gregorich and Carter 2007; Natali et al. 2012; Norby et al. 2019; Qian and Schoenau 2002; Salmon et al. 2016; Skogley and Dobermann 1996). Most synthetic ion exchange resin bags (IEBs) and ion exchange membranes (IEMs) have similar chemical properties and operating principles, despite their different physical forms (Luqman 2012; Skogley and Dobermann 1996). These ion exchange materials contain a backbone of solid organic hydrocarbon or fluorocarbon polymers to which fixed charge chemical groups (i.e. the "functional groups") are covalently bound. Mobile ions (i.e. the "counterions") are electrostatically bound to these functional groups, and can be exchanged for an equivalent number of target nutrient ions of similar charge from the surrounding medium if the electrostatic and concentration gradients favor counterion displacement (Skogley and Dobermann 1996). Hence, the IEBs and IEMs function in a manner analogous to charged soil colloids (Gregorich and Carter 2007; Luqman 2012; Skogley and Dobermann 1996).

Operationally, the IEB/IEM method differs from the direct extraction method in that a quantified volume of charged resin beads contained in porous nylon fabric

bags, or a specified area of charged membranes (for the IEM method), are incubated in the field for a predetermined amount of time. During incubation, the target nutrient ions in the soil replace some of the initial counterions on the resin beads/membranes. At the end of the incubation, the resin bags/membranes are retrieved from the field and processed by separating the resin beads/membranes from trapped soil particles, eluting the nutrient ions accumulated on the resin beads/membranes using chemical solutions, and quantifying their amounts (Giblin et al. 1994; Skogley and Dobermann 1996). The IEB/IEM method provides a novel way to estimate soil nutrient availability compared to the conventional soil extraction method, and has thus expanded our understanding of soil nutrient dynamics. Most importantly, the IEB/IEM method is sensitive to many critical *in-situ* factors (such as soil water content, soil pH, and soil temperature) that concurrently *and* dynamically affect soil nutrient availability during the period of incubation (Gregorich and Carter 2007; Weih 1998). The ion exchange-derived nutrient flux results therefore reflect *in situ* real dynamic soil differences in: initial soil solution nutrient ion concentrations; nutrient release rates from soil particles and active microbes; and movement of ions toward the resin beads via diffusion and mass flow (Gregorich and Carter 2007; Skogley and Dobermann 1996). The IEB/IEM method also avoids the error of spatial variation for repeated measurements, because the same incubation spot can be used repeatedly. In addition, this method causes minimal soil disturbance since no soil needs to be removed from the sampling area. Lastly, it is much more logistically feasible as the resin bags/membranes are very easy to carry and can be stored indefinitely after removal from the incubation location, prior to chemical analysis.

Compared with the IEB method, the IEM method provides additional advantages associated with the physical shape differences between the membranes and the resin bags. Specifically, compared with the three-dimensional resin bags, the thin, essentially two-dimensional membranes: (1) cause even less soil disturbance during incubation deployment (Duran et al. 2008; Raison et al. 1987; Schmidt et al. 2002); (2) have greater effective surface area contact with the soil solution and plant roots (Castillo-Monroy et al. 2010; Johnson et al. 2005; Schmidt et al. 2002); (3) can be processed more easily as they eliminate the task of separating the resin beads and soil particles; and (4) have an easily defined and stable surface area, a feature that is critical to precision and comparison of values among and between samples (Qian and Schoenau 2002). Overall, compared with the direct soil extraction and the IEB methods, the IEM method is the most sensitive to factors that regulate plant available elements, has the fewest potentially confounding effects, is least destructive to the sampling areas, and is simplest to process.

Distinctive features of the use of IEMs to measure nutrient availability in tundra

Despite all the theoretical benefits of the IEM method outlined above, a side-by-side direct comparison between it and the soil extraction method using experimental nutrient additions is necessary to demonstrate to what extent these two methods provide comparable results in terms of response linearity and sensitivity along a wide soil nutrient gradient. It is important to note that the IEM nutrient flux data are expressed as μg per cm^2 of membrane surface (one-sided) per burial day (i.e. $\mu g/cm^2/day$), whereas the soil extraction nutrient pool data are expressed as μg/g dw soil, and therefore the data are not directly comparable in absolute terms. The IEM data indicate the mass of nutrient ions that in the soil solution that have been adsorbed to the surface of the membrane during a specified incubation period, but the mass or volume of soil that actually contributed to delivering these ions to the membrane is not known (Gregorich and Carter 2007; Skogley and Dobermann 1996; Subler et al. 1995; Ziadi et al. 2006). In contrast, the mass or volume of soil that contributed to the nutrient pool is clearly defined for the soil extraction data. Nevertheless, soil nutrient availability from the two methods should correlate. As far as we know, only one study has specifically tested for such correlation, and only for soil nitrate (NO_3-N) in a temperate ecosystem (Ziadi et al. 2006). Soil NO_3-N concentrations as extracted by water and by KCl solution were positively related to soil NO_3-N fluxes based on the anion exchange membrane (AEM) incubation method (slope = 14.2 with $R^2 = 0.95$, and slope = 2.6 with $R^2 = 0.66$, respectively) along a gradient of fertilization plots supporting forage or corn production (Ziadi et al. 2006). Comparisons for other important soil nutrient elements (e.g. ammonium (NH_4-N) and phosphate (PO_4-P)) in temperate or other ecosystem-types however, have yet to be investigated.

Besides the importance for methodological comparisons, because of global climate warming (IPCC 2013), it is also meaningful to test whether the IEM method is sufficiently sensitive to detect effects of changes in one of the most important environmental factors - temperature - on soil nutrient availabilities. Soil nutrient mineralization, availability, and the resultant plant growth rates are exceptionally low in the arctic compared to other regions, mainly due to low soil temperatures both within the short growing season and during the long non-growing season (Giblin et al. 1994; Semenchuk et al. 2015; Shaver and Chapin 1980; Weintraub 2011). However, warmer air temperatures are raising summer soil temperatures and increasing winter precipitation in many areas across the Arctic in recent decades (IPCC 2013). Deeper snowpack generates greater thermal insulation of the underlying soil, resulting in less soil cooling during winter (Mörsdorf et al. 2019). Both the summer and winter effects are projected to accelerate the organic matter decomposition and nutrient-mobilizing activities of soil microbes, resulting in enhanced availabilities of soil nutrients, which strongly regulate plant growth (Buckeridge and Grogan 2010; Chapin et al. 1995; Edwards and Jefferies 2010; Mörsdorf et al. 2019; Schimel and Bennett 2004; Schinner et al. 2012; Schmidt and Lipson 2004; Semenchuk et al. 2015; Van Der Heijden et al. 2008; Weih 1998). As a result, changes in both the magnitudes and the temporal patterns of soil fertility in arctic tundra due to a warmer environment may profoundly affect ecosystem structure, processes, services and climatic regulation (Chapin and Shaver 1989; Elmendorf et al. 2012; Mörsdorf et al. 2019; Schimel et al. 2004; Zamin et al. 2014), yet they are not precisely investigated so far.

Research objectives and scientific questions

The general objective of this study was to evaluate the potential of IEMs as an alternative and more effective method to direct soil extraction for determining soil nutrient availability and dynamics in arctic tundra. Our research addressed the following specific research questions: (1) Are soil nutrient availabilities based on the IEM and the soil extraction methods positively correlated, and do they reveal the same intra-seasonal patterns? (2) Do the IEM and extraction methods differ in their sensitivity to detect experimental increases in soil nutrient availability that are sufficient to enhance plant species' nutrient concentrations? And (3) Do experimental summer warming and deepened snow treatments alter intra- and inter-seasonal soil nutrient availabilities as determined by the two methods, and if so, are there corresponding effects on aboveground plant community biomass?

Materials and methods

Study site and experimental manipulations

This study was conducted in mesic birch hummock tundra vegetation near the Tundra Ecosystem Research Station at Daring Lake, Northwest Territories, Canada (64° 52' N, 111° 33' W). This ecosystem is characterized by hummocks 10–30 cm high and deciduous dwarf birch (*Betula glandulosa* Michx.) shrubs that are 10–40 cm tall and make up ~ 14% community aboveground biomass (Qian Gu, unpublished data). The remaining cover is a mixture of mostly ericaceous shrubs (*Vaccinium uliginosum* L., *Rhododendron subarcticum* Harmaja [formerly *Ledum decumbens*] (Aiton) Lodd. ex Steud.], *Vaccinium vitis-idaea* L., and *Andromeda polifolia* L.), one graminoid species (*Eriophorum vaginatum* L.), and one forb species (*Rubus chamaemorus* L.). Besides vascular species, there is also a well-developed moss and lichen layer (see (Nobrega and Grogan 2008) and (Zamin et al. 2014) for more details). The soil of this ecosystem-type is well-drained, with no surface water table or evidence of permafrost degradation, and is classified as an orthic dystric turbic cryosol. The top surface horizon (typically 5–12 cm deep) is highly organic and contains most of the root biomass (Churchland et al. 2010), and lies above a mineral deposits that thaws each summer to at least 50 cm (Nobrega and Grogan 2008). Data based on a one-time soil chemical extraction sampling (to 2–5 cm depth) in this ecosystem-type indicate a soil pH of 4.6 (SD = 0.2), a total soil carbon content of 36.2% (SD = 5.5), a total soil nitrogen (N) content of 1.79% (SD = 0.29), a dissolved organic carbon content of 789 mg kg^{-1} (SD = 133), and a dissolved organic N content of 65 mg kg^{-1} (SD = 11.9) (Chu and Grogan 2010).

This study uses three field experimental manipulations: fertilization (at two levels); summer greenhouse warming; and snowfence treatments. All three manipulations were established in 2004 and have been maintained annually ever since (see (Christiansen 2016;

Zamin 2013; Zamin et al. 2014) for full details). The only exception is the low level phosphorus (P) addition treatment, which was established in 2012. For each experimental manipulation, plots of representative and fairly homogenous birch hummock vegetation of similar gentle slope and aspect were selected and then randomly assigned to control and treatment. Briefly, soil nutrient availabilities were enhanced by implementing annual low level N (LN) (1 g N m^{-2} per year), high level N (HN) (10 g N m^{-2} per year), low level P (LP) (0.5 g P m^{-2} per year), and high level P (HP) (5 g P m^{-2} per year) additions to replicate plots (5 m x 7 m each; n = 05) for each nutrient treatment. To enhance summer air temperatures, A-frame greenhouses (1.8 m x 4.7 m each, n = 10) were covered with transparent plastic during each growing season. Triangle vents were cut out of the tops to avoid extreme maximum temperatures within the greenhouses and to reduce humidity differentials. These greenhouses warm the summer mean diel temperature of the air and the soil (from 0 to 10 cm depth) by 2.1–2.4 °C compared to their associated control plots (Zamin et al. 2014). To manipulate winter snow depth, snowfences (15 m long, 1.2 m high each; n = 05) were set up perpendicular to the prevailing winter wind direction, which typically results in deepened snow patches extending out ~ 20 m from both sides of each fence (Christiansen 2016). In comparison to their associated control plots, these snowfences generally increase the ambient snow cover from 0.3 to 1.0 m (Christiansen 2016), and have several environmental impacts, including warmer soil temperatures during winter (Christiansen 2016), and a 1–2 week delay in snowmelt and the start of the growing season (Buckeridge and Grogan 2010; Nobrega and Grogan 2008).

IEM preparation

The IEMs (SUEZ Water Technologies & Solutions, USA) were obtained in large sheet forms (18.25 cm x 40.25 cm) in two different types: cation exchange membranes (CEMs, ID: CR67HMR) and anion exchange membranes (AEMs, ID: AR204SZRA). The CEMs have sulfonic acid as the exchange group and an exchange capacity of 2.1 meq/dry g resin. The AEMs have quaternary ammonium as the exchange group and an exchange capacity of 2.4 meq/dry g resin. The quaternary ammonium has a strong ability to exclude cations and a high resistance to contamination by organic materials (Ziadi et al. 2006). The CEM and AEM sheets were not charged with counterions, so we cut and charged them before applying them in the field. The CEMs and AEMs were prepared separately and charged using different solutions (0.5 *M* HCl and 0.5 *M* $NaHCO_3$ respectively). Our choices of H^+ as the counterions for the CEMs and of HCO_3^- as the counterions for the AEMs have been shown to work well (Qian and Schoenau 2002). The large IEM sheets were cut into 5 cm x 5 cm squares (Fig. S1a) and soaked immediately in distilled water. These IEM squares were then transferred to another large plastic container and filled with sufficient charging solution to ensure that all of the membranes were totally covered by the solution (Fig. S1b). The container was covered with a lid and gently shook on the lab bench for 10 min and then let sit for one hour. Afterwards, the IEM squares were removed and placed in another container containing sufficient distilled water to cover and soak them for 10 min, and then placed in another clean container containing fresh charging solution. This charging process was repeated three times using new charging solution each time and great caution was taken throughout the IEM preparation to avoid chemical contamination from the lab environment. After the final shaking in distilled water, the membrane squares were stored in sealed bags in the freezer.

IEM incubation in field

We developed a simple method for the rapid placement, retrieval, and handling of IEMs in the field. Prior to field deployment, we tagged one corner of each IEM with a plastic cloth barb (~ 10 cm long) using a clothes tag gun (Tech-It, USA) so that it could be easily located in the vegetation (Fig. S1c). Tagging of the IEMs in this way should not affect the membrane exchange capacity (Cain et al. 1999; Duran et al. 2008). In the field, we cut a vertical slit into the soil with a serrated knife and placed the IEM square at a uniform depth within the surface organic soil horizon (i.e. extending vertically from 2 to 7 cm underneath the soil surface measured relative to the green-brown transition in the moss/vegetation ground cover layer) at each incubation location (Figs. S1d & S1e). Vertical deployment of the IEMs avoids the confounding effect of ion accumulation on the membranes via gravitational solution flow. Within each sampling area of the replicate treatment plots, CEMs and AEMs were placed in pairs about 2 cm apart in inter-tussock microsite locations. The slits were then

closed by gently pressing the soil surface on either side to ensure good contact between the membrane and the soil (Fig. S1f). Afterwards, we tied a piece of flagging tape to the cloth barb of each membrane as an easily visible marker to facilitate finding them later, and as a way to write a label for each (Fig. S1c). The membranes were left to incubate in the soil for a certain period (see below for specific time periods), and were then removed and replaced with new membranes in the same slits. One concern about using the same soil incubation spot is that repeated deployment of membranes may cause ion depletion to below ambient levels in the incubation spot over time. For example, NO_3-N ion uptake rates by AEMs over seven days decreased significantly in a laboratory experiment using homogenized agricultural soil (Subler et al. 1995). However, a comparable field study on tundra soil found negligible ion depletion effects (Giblin et al. 1994), perhaps because ongoing nutrient supply in the *in situ* field soil sufficiently replenished the soil solution pools in the incubation spot.

After removal, the IEM membranes in our study were immediately rinsed free of visible soil particles using a spray bottle containing distilled water (Fig. S1g), the cloth barb was cut off (Fig. S1h). Each group of anion or cation exchange membranes from within the same treatment sub-plot (i.e. the unit of experimental replication) were placed together in a clean labelled plastic bag (Fig. S1i), and transported to a freezer for storage prior to elution. Blank membranes were prepared and processed in exactly the same way except without being incubated in the soil (i.e. the only difference was that the blank membranes were stored in the refrigerator during the incubation periods).

IEM incubation, and soil sampling and analyses from the experimental fertilization plots

Each of the five experimental fertilization plots (5 m x 7 m) was visually divided into two subplots, generating 10 replicate subplots in total. Within each subplot, the IEM incubations and soil collections described below were conducted in a single marked sampling area (~ 40 cm x 40 cm) that was deliberately located at least two meters away from the corresponding marked sampling area in the other subplot, in order to minimize the likelihood of any possible spatial dependency in terms of soil nutrient availability between them. Within each sampling area of the replicate treatment subplots, CEMs and AEMs were placed in pairs about 2 cm apart in inter-tussock microsite locations. Four IEM pairs were used for each incubation, with each pair 20–30 cm apart from each other. Three 14-day incubations were conducted sequentially throughout the main growing season in 2016. Our selection of three 14-day incubations was a tradeoff between logistical practicalities and an effort to encompass the main growing season. These individual incubation periods were: Round 1 (R1) from July 10/11 to July 24/25, R2 from July 24/25 to Aug 7/8, and R3 from Aug 7/8 to Aug 21/22. Soil samples were also collected three times (R1 on July 4, R2 on July 16, and R3 on August 1) within the same growing season. Note that the soil sampling dates did not always match with the IEM incubation periods, due to logistical restrictions associated with conducting these two field experiments at the same time. Three organic soil samples (0–7 cm depth; 5 cm x 5 cm in diameter) that were about 10 cm distant from the IEM incubation spots were cut out from inter-tussock microsites in each replicate marked sampling area and composited into a single sample. All aboveground plant materials were cut off, and then all coarse roots (> 2 mm in diameter) and occasional stones were removed from each sample. The soil was then homogenized by hand and subsampled (10 g fresh weight each) to determine soil moisture and nutrient pools. Soils were kept at field moisture in plastic bags at 4 C° for no more than two days before processing and extraction at the field laboratory.

Three soil nutrient pools (NH_4-N, NO_3-N, and PO_4-P) were determined using the traditional extraction and chemical analyses procedure (Gregorich and Carter 2007). Each soil sample was placed in a specimen cup with 50 mL of distilled water and shaken manually every 15 min over the next hour, allowed to sit for another hour, and then vacuum-filtered through glass fibre filters (Fisher G4; 1.2 μm pore space). All extracts were kept frozen until analysis. NH_4-N, NO_3-N, and PO_4-P concentrations in the extracts were determined colorimetrically using automated flow analysis (Bran-Leubbe Autoanalyzer III, Norderstadt, Germany) and the indophenol, sulphanilamide (Mulvaney et al. 1996), and molybdate – ascorbic acid methods (Kuo et al. 1996), respectively. Oven-dry weight conversion factors were determined by heating 10 g of fresh soil at 60 C° to a constant weight. These factors were used to correct the measured N and P concentrations in the extracts for dilution associated with initial soil moisture content in each individual sample, and to calculate the

concentrations on a dry soil weight basis (μg N/P g^{-1} dry weight soil).

IEM incubations for the summer greenhouse warming and snowfence experiments

Six sequential sets of IEM incubations throughout the growing season and one single set during the following non-growing season were deployed in the summer greenhouse warming and associated control plots (n = 10). The growing season incubations were conducted in 2017, with individual incubation periods varying from nine to 17 days. Specifically, Round 1 (R1) was from June 25 to July 4, R2 July 4 – July 13, R3 July 13 – July 22, R4 July 22 – July 31, R5 July 31 – August 17, and R6 August 17 - August 26. The single long-term non-growing season incubation followed immediately after the R6 incubation from August 26, 2017 until June 29, 2018 (307 days in total). Three paired membranes of each type were installed within each replicate sampling area during each incubation for both the growing season and non-growing season measurements, except for the last incubation round in the growing season (i.e. the R6 incubation), for which only two paired membranes were used due to a shortage of membranes.

For the snowfence and associated control plots (n = 05), we first conducted a single long-term non-growing season incubation from August 17, 2016 until June 24, 2017 at which time the soil had completely thawed (311 days in total), followed by five sequential sets of incubations throughout the growing season in 2017. Dates of these five sequential sets correspond to the first five incubations in the greenhouse warming treatments as described above. Six paired membranes were installed in each replicate plot for the non-growing season incubation, whereas three paired membranes were installed for each incubation during the growing season measurements.

IEM elution and chemical analyses

The IEM membranes from within the same replicate sampling areas from each incubation round in each experimental manipulation were placed together for elution and chemical analyses. Each pooled sample was placed in a single 50 mL petri dish (Fig. S1j). Membranes were then extracted with 2 *M* NaCl in 0.1 *M* HCl solution (Giblin et al. 1994) by shaking for 2 h on a shaker table (Fig. S1k). The specific amounts of elution solution used varied according to the particular replicate numbers of the membranes and the specific experimental treatments from which these membranes were retrieved. As a rule, 5 mL of elution solution were used for each replicate group of membranes. However, for the extractions in each of the fertilization treatments (i.e. the LN, LP, HN, and HP treatments), we used 10 mL of elution solution (and larger containers) to avoid potential saturation of the elution solution (i.e. ion exchange equilibrium when the rate of target nutrient ions leaving and returning the membrane polymer becomes equal). Moreover, for the membranes collected from the high level fertilization plots (i.e. the HN and HP plots), we repeated the elution process two to three times until concentrations in the last elutant solutions were negligible. See "Fig. S2" in Supportive Information for the IEM elution efficiency equations for NH_4-N (Fig. S2a), NO_3-N (Fig. S2b), and PO_4-P (Fig. S2c), respectively. Elutant solutions were then vacuum-filtered through glass fibre filters (Fisher G4; 1.2 μm pore space) and subsequently stored in the freezer until chemical analyses. Tests of the blank membranes (i.e. those not incubated in soil) indicated contaminations of PO_4-P and NO_3-N on AEMs, and NH_4-N on CEMs, were below our detection limits.

IEM methodological sensitivity characterization

Since soil nutrient mineralization rates and dissolved soil N and P pools are exceptionally low in Arctic tundra (Chapin and Shaver 1981; Chapin III et al. 1978; Giblin et al. 1991; Jonasson et al. 1993), we characterized the sensitivity of the IEMs in estimating low level mineral elemental fluxes in the laboratory before applying the IEM method in the field. Fresh pre-charged IEM membrane squares (n = 02 per individual solution) were immersed in a range of NH_4-N, NO_3-N or PO_4-P replicate solutions of known concentrations for 1 h (n = 06 for each NH_4-N concentration, and n = 03 for each NO_3-N concentration and each PO_4-P concentration). These membranes were then eluted with 2 *M* NaCl in 0.1 *M* HCl solution for 2 h. Ion recoveries from the membranes into the eluted solutions were compared with the known concentrations in the initial immersion solutions. These sensitivity tests indicated that the membranes can detect NH_4-N fluxes as low as 0.25 μg/cm^2 (0.5 mg/L) with an accuracy of ± 7%, NO_3-N fluxes as low as 0.05 μg/cm^2 (0.1 mg/L) with an accuracy of ± 13%, and PO_4-P fluxes as low as 0.05 μg/cm^2 (0.1 mg/L) with an accuracy of ±

20%, and furthermore that the accuracies for all three ions improve substantially for larger fluxes (see Fig. S3 and Table S1 in Supportive Information for full data). These minimum detection limits were well below the actual amounts accumulated on the field-incubated membranes for NH_4-N and PO_4-P. However only one-third of the membranes across all the field treatments accumulated NO_3-N ions at rates above the minimum detection limit, and these were mostly from the later season incubations from the experimental fertilization settings. NO_3-N flux only accounted for 5.9% -14.3% of total inorganic N (NH_4-N + NO_3-N) measured during the growing season in the control plots associated with fertilization treatment. This percentage range is similar to a buried bag soil incubation experiment in the tussock tundra (Nadelhoffer et al. 1991). Therefore, we excluded this variable from our main analysis and only reported the results of sensitivity test (Table S1) and elution efficiency from the HN addition plots (Fig. S2b). On the other hand, the fact that we did detect reliable NO_3-N fluxes from the ambient soils provides evidence that a low soil pH (4.6 at our study site) does not prevent nitrification by soil microbes, which is most effective at a higher pH of ~ 7.5 to 8.0 (Pietri and Brookes 2008). Overall, based on the above sensitivity tests, we conclude that all the NH_4-N, NO_3-N, and PO_4-P flux data reported in this study are robust.

Vegetation sampling and analyses

We determined foliar N and P concentrations for the seven principal vascular plant species in the experimental fertilization treatments. Healthy and mature leaf samples (~ 20 g dry weight) for each plant species were collected on July 27/28 in 2016. These foliar samples were first air-dried, and later fully dried at 60 °C in a fan-assisted oven for 24 h before grinding mechanically (IKA MF 10 Microfine grinder, Staufen, Germany) or with a pestle and mortar, depending on the texture of the specific samples. Total N concentrations (% of dry mass) were analysed by combustion and gaseous N detection (Elementar,Hanau, Germany). Total P concentrations were determined using the sulfuric acid/hydrogen peroxide/lithium sulfate/selenium digestion method (Parkinson and Allen 1975). We also measured aboveground biomass of all vascular plant species in the summer greenhouse warming, the snowfence, and their associated control plots during the growing season in 2017 using the non-destructive point framing method (Alatalo et al. 2015; Jonasson 1988; Schuur et al. 2007; Walker 1996; Zamin 2013; Zamin et al. 2014). This method records plant species' hit data and we use previously established regressions from our site (see Gu and Grogan (in review), and Zamin et al. (2014) for details) to infer shoot biomass.

Statistical analyses

Separate linear mixed models were used to determine the regression relationships between IEM fluxes and pools across levels of fertilization treatments for each of the two nutrients (NH_4-N and PO_4-P), with IEM fluxes as the dependent variable, extractable nutrient pools as the independent variable, and treatment as the grouping factor (i.e. random effect), for the averaged datasets (i.e. the average daily IEM nutrient flux across the full 42 days calculated by summing the flux values of the three 14-day incubations; and the average of the three extraction nutrient pool data sets for each individual replicate subplot). Note that because the date of the first soil sample collection did not overlap with that of the first IEM incubation, we did not include those data in the regression test. The 'lmer()' function from the 'lme4' package was used for these analyses, and we calculated the corresponding R^2 values using the 'r.squaredGLMM()' function from the 'MuMIn' package, and report both the marginal R^2 (R^2m, variance explained by fixed factors only), and the conditional R^2 (R^2c, variance explained by both fixed and random factors) values (Nakagawa and Schielzeth 2013).

Separate one-way repeated measure ANOVAs were used to test for overall effects of the individual fertilizer additions (LN, LP, HN, and HP additions), of the summer greenhouse warming, and of the snowfence treatments, on the time series of IEM NH_4-N and PO_4-P fluxes/pools across the whole growing season. Specifically, the 'lme()' function from the 'nlme' package was used for this purpose, and the 'glht()' function from the 'multcomp' package was used to perform post-hoc pairwise tests between incubation sampling periods if the one-way repeated measures ANOVA indicated significant differences. To address the potential for temporal autocorrelation within the series of incubations, we performed the ANOVA analyses for each nutrient both with and without considering the temporal autocorrelation, and compared the AICs and the corresponding P-values between these two competing models. In most cases, these two models yielded AICs that were not

significantly different, indicating that NH_4-N and PO_4-P fluxes at a specific soil location were generally highly variable over time (Cain et al. 1999; Lee et al. 1983). However, in two cases (i.e. the snowfence effect on PO_4-P flux and the greenhouse warming effect on NH_4-N flux) the model that included temporal autocorrelation had significantly lower AICs than the model without. Accordingly, all results reported in this study are based on the more conservative statistics (i.e. the model that includes consideration of temporal autocorrelation).

Student's t-tests were used to examine the impacts of: (a) individual fertilization treatments on IEM nutrient fluxes/pools for individual incubation/sampling periods; (b) the summer greenhouse warming and snowfence treatments on IEM nutrient fluxes for individual incubation periods; (c) individual fertilization treatment effects on plant species' foliar N and P concentrations; and (d) the summer greenhouse warming and snowfence on community aboveground biomass. Data were natural log transformed when necessary to meet the assumptions of constant variance and normality, and a Wilcoxon test was performed when the assumptions for the t test were not met even after transformation. All statistically significant results ($P \leq 0.05$) and trends ($P \leq 0.10$) for all analyses are reported directly in the text.

Results

Comparison between IEM incubation and direct extraction nutrient measurements

IEM nutrient fluxes and water-extractable nutrient pools across the whole growing season were closely and positively correlated for NH_4-N (equation: log (flux NH_4-N) = 0.91 x log (extractable NH_4-N) − 2.38; Table 1; Fig. 1a), but not for PO_4-P (Table 1; Fig. 1b). The lower value for R^2m compared to R^2c indicates that the strength of this overall regression was substantially influenced by the fertilization treatments (Table 1; Fig. 1a; see Materials and methods for full explanation). Moreover, when considering the lower end data only (i.e. data from the control and the low level fertilization plots) - which are expected to be more realistic of the normal natural range of nutrient availabilities in tundra ecosystems, there was a weaker but still significant regression for NH_4-N (equation: log (flux NH_4-N) = 1.34 x log (extractable NH_4-N) − 3.05; Table 1; Fig. 1a), but not for PO_4-P (Table 1; Fig. 1b). Furthermore, the identical R^2m and R^2c values indicate that the fertilization treatments did not affect the regression of these low fertility range data (Table 1; Fig. 1a). Finally, similar patterns were also observed within the individual incubation datasets for NH_4-N (Table 1, Fig. S4; note that to restrict the analysis to temporally overlapping datasets, only two of the three individual datasets were examined - see 'Materials and methods' for specific data collection dates).

Although we found close regressions between the two methods in estimating soil NH_4-N availabilities, the patterns of both the fertilization effects and temporal changes differed between these two methods (Fig. 2; Tables S1, S2). For the fertilization effects, the IEM method was able to detect both high and low level fertilizer addition effects on soil nutrient availability, whereas the soil extraction method was only able to detect the high level addition effects. Specifically, the IEM data indicated that soil NH_4-N fluxes were not just significantly elevated by the HN treatment (151.6-fold), but also by the LN (3.1-fold) and the HP (1.9-fold) treatments (Fig. 2a; Table S2). By comparison, the soil NH_4-N extraction data also indicated a significant HN treatment effect (57.9-fold) and a statistical trend toward a positive LN effect (3.0-fold), but no significant HP treatment effect (Fig. 2b; Table S3).

The soil PO_4-P IEM fluxes were significantly or nearly significantly increased by the HP (202.4-fold), LP (4.2-fold), and HN (2.3-fold) treatments (Fig. 2c; Table S2). However, although the soil PO_4-P extraction data indicated corresponding significant HP and HN effects, it did not show a significant LP treatment effect (Fig. 2d; Table S3). For temporal changes, both datasets indicated significant within-seasonal changes for the ambient soils in the control plots but not for the fertilized soils. However, the directions and magnitudes in changes for PO_4-P differed between these datasets (Fig. 2). Specifically, the IEM PO_4-P data indicated a significant decrease over time (2.8–6.9 fold), whereas the soil extraction data indicated that soil PO_4-P pools increased over time (1.2–2.3 fold) (Fig. 2c and d; Tables S2 & S3). For soil NH_4-N, both datasets indicated a consistent decrease over time of similar magnitude (1.7–2.2 fold for the IEM dataset, whereas a constant 1.5-fold decrease for the soil extraction dataset; Fig. 2a and b; Tables S2-S3).

Table 1 Results from the linear mixed models for estimating the relationships between soil IEM nutrient fluxes and water-extractable nutrient pools across the whole growing season and for individual incubation datasets

Model	Fixed effects					Random effects	
	Estimate	t value	*P* value	R^2m	R^2c	Treatment	Residual
NH_4-N_average_full range[1]	0.91 (0.18)	5.00	0.05	0.63	0.80	0.63 (0.79)	0.76 (0.87)
NH_4-N _average_lower end[2]	1.34 (0.25)	5.34	< 0.01	0.60	0.60	0.00 (0.00)	0.60 (0.77)
NH_4-N _earlier season_full range[3]	1.12 (0.16)	6.89	< 0.01	0.71	0.78	0.34 (0.59)	1.04 (1.02)
NH_4-N _earlier season_lower end[4]	1.13 (0.31)	3.63	< 0.01	0.36	0.39	0.07 (0.26)	1.11 (1.05)
NH_4-N _later season_full range[5]	1.14 (0.17)	6.55	< 0.01	0.75	0.84	0.56 (0.75)	0.98 (0.99)
NH_4-N _later season_lower end[6]	1.19 (0.39)	3.05	< 0.01	0.27	0.37	0.24 (0.49)	1.51 (1.23)
PO_4-P_average_full range[1]	0.24 (0.16)	1.45	0.16	0.06	0.93	3.73 (1.93)	0.32 (0.57)
PO_4-P _average_lower end[2]	0.02 (0.29)	0.07	0.94	< 0.01	0.79	1.38 (1.17)	0.36 (0.60)
PO_4-P _earlier season_full range[3]	-0.07 (0.17)	-0.41	0.68	< 0.01	0.83	4.62 (2.15)	0.96 (0.98)
PO_4-P _earlier season_lower end[4]	-0.35 (0.25)	-1.37	0.18	0.04	0.36	0.53 (0.73)	1.04 (1.02)
PO_4-P _later season_full range[5]	0.24 (0.16)	1.46	0.15	0.04	0.91	4.04 (2.01)	0.42 (0.65)
PO_4-P _later season_lower end[6]	-0.04 (0.23)	-0.19	0.85	< 0.01	0.80	1.69 (1.30)	0.41 (0.64)

For each linear mixed model, parameter estimates (SE) of the fixed effects, and the corresponding t values, P values, R^2 m (marginal R^2, variance explained by fixed factor only), and R^2 c (conditional R^2, variance explained by both fixed and random factors); and variance (SD) of the random effects are reported

[1] Model 'NH_4-N_average_full range' indicates the regression for NH_4-N (or PO_4-P) IEM flux and water-extractable pools across the whole growing season and across all the fertilization treatments (Control, LN, HN, LP, and HP additions)

[2] Model 'NH_4-N_average_lower end' indicates the regression for NH_4-N (or PO_4-P) IEM flux and water-extractable pools across the whole growing season and across the lower fertilization treatments (Control, LN, and LP additions)

[3] Model 'NH_4-N_earlier season_full range' indicates the regression for NH_4-N (or PO_4-P) IEM flux and water-extractable pools over the earlier growing season and across all the fertilization treatments (Control, LN, HN, LP, and HP additions)

[4] Model 'NH_4-N_earlier season_lower end' indicates the regression for NH_4-N (or PO_4-P) IEM flux and water-extractable pools over the earlier growing season and across the lower fertilization treatments (Control, LN, and LP additions)

[5] Model 'NH_4-N_later season_full range' indicates the regression for NH_4-N (or PO_4-P) IEM flux and water-extractable pools over the later growing season and across all the fertilization treatments (Control, LN, HN, LP, and HP additions)

[6] Model 'NH_4-N_later season_lower end' indicates the regression for NH_4-N (or PO_4-P) IEM flux and water-extractable pools over the later growing season and across the lower fertilization treatments (Control, LN, and LP additions)

Plant foliar nutrient concentrations across the fertilization treatments in relation to the IEM and extractable soil nutrient availability measures

As expected, plant foliar nutrient concentrations dramatically increased under the high level fertilization treatments (i.e. the HN and HP plots) (Table 2), and this response corresponded with the patterns of soil nutrient enhancement reported above for each method (Fig. 2). Furthermore, foliar N concentrations for all three evergreen shrubs (*R. subarcticum*, *V. vitis-idaea*, and *A. polifolia*) were significantly increased (1.1- to 1.4-fold) by the LN treatment (Table 2). These increases matched the enhanced available soil N in these LN addition plots as detected in both the IEM data (a 3.1-fold increase) and the soil extraction data (a 3.0-fold increase) (Fig. 2a and b). Foliar P concentrations for all but the graminoid plant species were significantly increased (1.3- to 2-fold) by the LP treatment (Table 2). However, although these increases matched the enhanced available soil P as detected in the IEM data (a 4.2-fold increase) (Fig. 2c, Table S2), they conflicted with the soil PO_4-P extraction data which indicated no corresponding significant LP effect on available P (Fig. 2d, Table S3).

Effects of the summer greenhouse warming and snowfence treatments on seasonal soil nutrient availability, and on aboveground vascular plant community biomass

The effects of the long-term summer greenhouse warming treatment on soil intra-seasonal NH_4-N fluxes differed from the PO_4-P fluxes. Perhaps surprisingly,

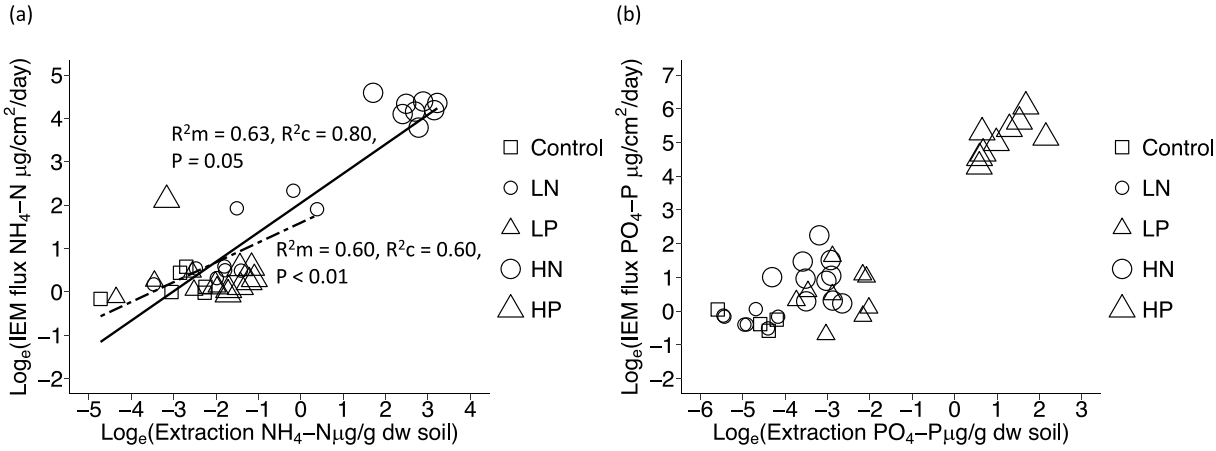

Fig. 1 Correlations between the IEM and direct water-extraction method determinations of average growing season soil NH_4-N (**a**) and PO_4-P (**b**) availability in the various experimental fertilization treatment plots (n = 04–10). The solid line is a linear regression based on data from all the experimental plots (Control, LN addition, LP addition, HN addition, and HP addition), whereas the dashed line is a linear regression based on data from control, LN addition, and LP addition plots only (the corresponding PO_4-P regressions were not statistically significant). Linear mixed models were used with fertilization treatment as a grouping factor (random effect), and both marginal R^2 (R^2m), conditional R^2 (R^2c), and P values for the fixed effects are reported (Nakagawa and Schielzeth 2013). Note the log-scale of the X- and Y-axes

the greenhouses did not alter soil NH_4-N fluxes during any of the individual incubations within the growing season (Table 3; Fig. 3a). Since there was no treatment effect, we pooled the data from the greenhouse warming and associated control plots to further examine the intra-seasonal patterns. Soil NH_4-N fluxes increased steadily (although not statistically significantly) for the first four incubations from late June to the end of July (from 0.12 to 0.17 µg/cm^2/day), and then declined significantly (3.8-fold) for the following incubation up until mid-August, and remained low into late August (Fig. 3a). In contrast, the greenhouse warming treatment increased the average growing season soil PO_4-P flux by 4.0-fold, and the individual incubations by 1.3- to 7.6-fold (Table 3; Fig. 3b). In addition, intra-seasonal changes in PO_4-P flux were slightly different between control and greenhouse warming treatment plots. Specifically, the PO_4-P fluxes in the control plots remained unchanged for the first three incubations, then decreased slightly for the fourth incubation, and gradually recovered afterwards (R5 to R6) (Fig. 3b). PO_4-P fluxes in the greenhouse warming plots showed a generally similar intra-seasonal pattern as those in the control plots, although there were much larger variabilities among replicate plots, especially for the first incubation period from late June to early July (Fig. 3b). Finally, the PO_4-P fluxes during the non-growing season were not significantly affected by the summer greenhouse warming treatment (Table 3; unfortunately the corresponding NH_4-N samples were lost due to lab equipment malfunction), and were extremely low compared with those for the summer season (Table 3).

Contrary to the summer greenhouse warming effects, no snowfence treatment effects on either intra-seasonal soil NH_4-N fluxes or PO_4-P fluxes were observed (Table 3; Fig. 3c and d). When using the pooled data to examine the intra-seasonal patterns for the growing season, we found that soil NH_4-N fluxes first increased from R1 to R3 (from 0.14 to 0.16 µg/cm^2/day), and then decreased during the R4 incubation (Table 3; Fig. 3c). By contrast, soil PO_4-P fluxes first increased from R1 to R2, and then decreased for the following two incubations (R2 to R4), followed by a small increase (Table 3; Fig. 3d). Similarly, the snowfence treatment also did not affect either the NH_4-N flux or the PO_4-P flux for the non-growing season, with both fluxes very low compared with those for the summer season (Table 3).

The summer greenhouse warming treatment tended to increase aboveground vascular plant community biomass after 12 years (by ~1.6 fold; t = 1.8, P = 0.09) (Fig. 4), consistent with significantly enhanced soil fertility as confirmed by the IEM soil PO_4-P flux data (although not by the soil NH_4-N fluxes which were unaffected) (Fig. 3a and b). By contrast, the snowfence treatment did not significantly affect the aboveground vascular plant community biomass (Fig. 4), which was

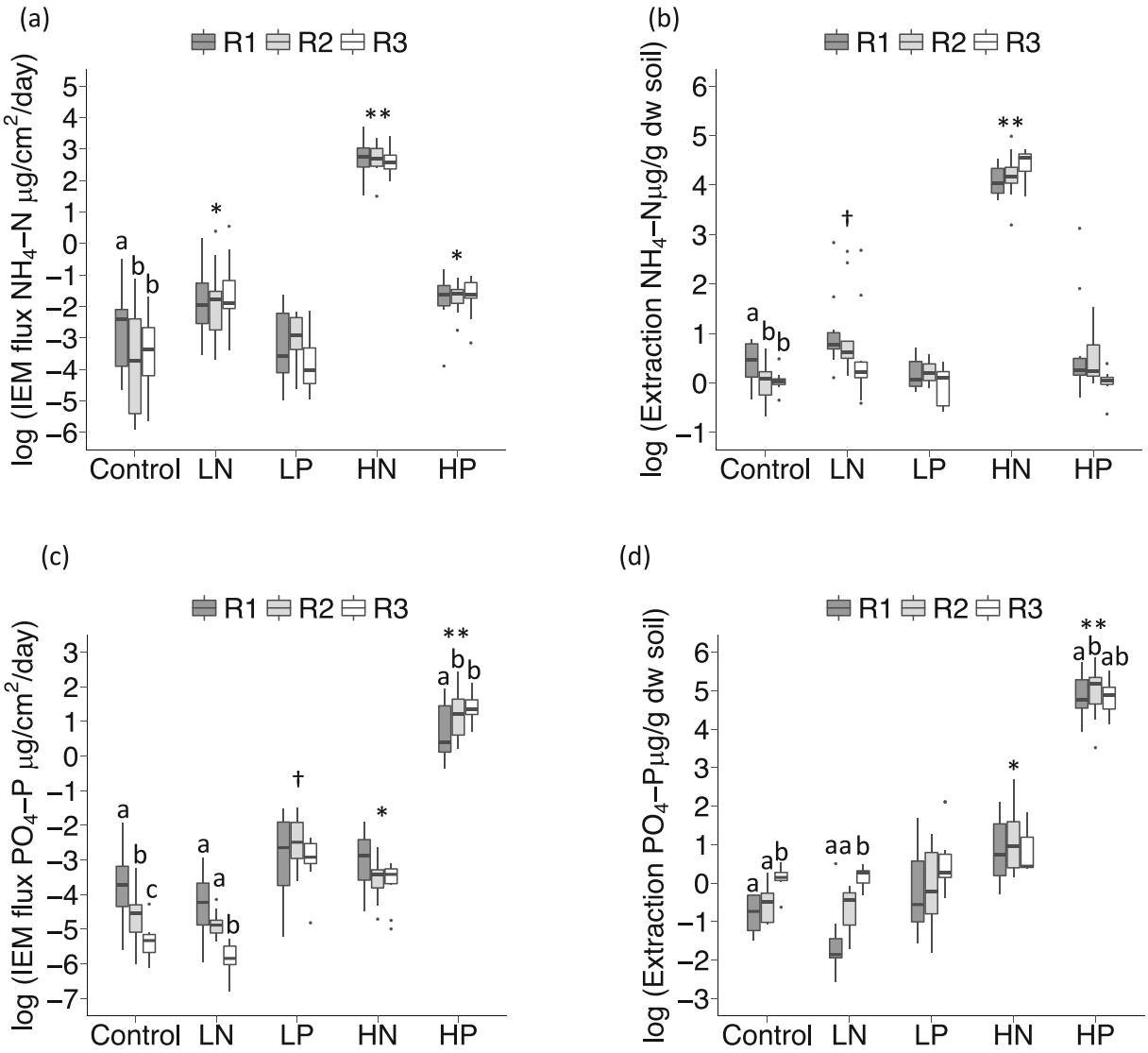

Fig. 2 Fertilization effects on soil NH_4-N IEM fluxes (**a**) and water-extractable NH_4-N pools (**b**), and on soil PO_4-P IEM fluxes (**c**) and water-extractable PO_4-P pools (**d**) during three sequential incubations over the growing season (R1-R3). 'LN' is low level nitrogen addition, 'HN' is high level nitrogen addition, 'LP' is low level phosphorus addition, and 'HP' is high level phosphorus addition. Symbols indicate the statistical significance levels: $p \leq 0.1$†, $p \leq 0.05$*, $p \leq 0.01$**. Different labels (a, b, and c) indicate significant differences among the three incubation rounds (for the IEM data) or serial collections (for the extraction data) at level of $\alpha = 0.05$ (n = 06–10). Note the log-scale of the Y-axes

consistent with the IEM data indicating generally negligible effects on soil fertility (Fig. 3c and d).

Discussion

Relationships between soil nutrient fluxes and pools

Significant regressions between IEM fluxes and water-extractable pools were only observed for NH_4-N, but not for PO_4-P. Fertilization treatments has a greater influence on soil PO_4-P availability than on NH_4-N availability. PO_4-P ions are less mobile and more readily adsorbed to soil particles than NH_4-N ions (Chapin 1980; Duran et al. 2008; Jones and Jacobsen 2005), and therefore their mobility may have been more strongly regulated by microscale soil conditions than the NH_4-N ions. The weaker and more inconsistent regressions for the lower fertility range data than for the full range data for NH_4-N

Table 2 Foliar N and P concentrations, and N:P ratios for the seven principal vascular plant species in the control, low level nitrogen addition (LN), high level nitrogen addition (HN), low level phosphorus addition (LP), and high level phosphorus addition (HP) plots; as well as individual fertilization effects on foliar N and P concentrations, and N:P ratios, in mesic birch hummock tundra vegetation after 12 years of experimental manipulations (except for the LP treatment which was established four years before the 2016 sampling)

Species		Nutrient concentration (%)					Fertilization effect			
		Control	LN	LP	HN	HP	LN	LP	HN	HP
Betula glandulosa	N%	2.35 (0.18)	2.32 (0.26)	**2.18** (0.17)	**5.35** (0.41)	2.39 (0.31)	-0.33	-2.07 ↓*	23.40 ↑**	0.36
	P%	0.16 (0.04)	**0.13** (0.02)	**0.25** (0.07)	0.24 (0.11)	**1.77** (0.31)	-2.07 ↓*	3.53 ↑**	2.00 ↑†	14.56 ↑**
	N:P	15.24 (4.54)	18.38 (4.56)	**9.23** (2.59)	**26.99** (11.23)	**1.38** (0.17)	1.50	-3.64 ↓**	3.06 ↑**	-9.64 ↓**
Vaccinium uliginosum	N%	1.84 (0.42)	1.95 (0.19)	1.59 (0.39)	**2.74** (0.12)	1.89 (0.17)	0.69	-1.27	3.54 ↑**	0.31
	P%	0.09 (0.02)	0.08 (0.02)	**0.14** (0.03)	0.11 (0.03)	**0.94** (0.16)	-0.44	4.32 ↑**	1.31	15.54 ↑**
	N:P	21.29 (5.99)	24.10 (6.00)	**11.39** (3.50)	26.96 (8.68)	**2.07** (0.44)	0.99	-4.09 ↓**	1.29	-9.60 ↓**
Rhododendron subarcticum	N%	1.55 (0.17)	**1.70** (0.09)	1.63 (0.17)	**2.52** (0.28)	**1.77** (0.23)	W = 22 ↑*	1.16	9.23 ↑**	2.52 ↑*
	P%	0.11 (0.01)	0.11 (0.01)	**0.15** (0.01)	**0.15** (0.03)	**0.33** (0.06)	1.09	7.32 ↑**	3.90 ↑**	11.44 ↑**
	N:P	14.26 (2.18)	16.10 (1.76)	**11.20** (1.17)	**17.31** (1.89)	**5.52** (0.60)	2.02 ↑†	-3.87 ↓**	3.26 ↑**	-11.63 ↓**
Vaccinium vitis-idaea	N%	0.80 (0.09)	**1.02** (0.19)	0.82 (0.04)	**1.65** (0.31)	**1.19** (0.12)	3.32 ↑**	W = 42	W = 0 ↑**	7.59 ↑**
	P%	0.07 (0.01)	0.08 (0.01)	**0.14** (0.03)	**0.10** (0.01)	**0.28** (0.04)	0.81	6.33 ↑**	3.88 ↑**	14.12 ↑**
	N:P	10.94 (0.93)	**13.40** (2.69)	**6.13** (1.14)	**16.11** (3.90)	**4.25** (0.65)	2.59 ↑*	-9.25 ↓**	2.91 ↑*	W = 56 ↓**
Andromeda polifolia	N%	1.09 (0.11)	**1.51** (0.21)	1.04 (0.08)	**2.15** (0.07)	**1.45** (0.26)	5.28 ↑**	-1.04	21.61 ↑**	3.58 ↑**
	P%	0.08 (0.02)	0.09 (0.01)	**0.14** (0.03)	**0.11** (0.01)	**0.55** (0.13)	W = 18	W = 4 ↑**	W = 4 ↑**	W = 0 ↑*
	N:P	13.51 (2.02)	**16.68** (2.92)	**7.67** (2.04)	**19.14** (1.65)	**2.66** (0.27)	2.53 ↑*	W = 63 ↓**	5.56 ↑**	-14.84 ↓**
Rubus chamaemorus	N%	2.17 (0.16)	2.09 (0.14)	2.15 (0.16)	**2.57** (0.33)	2.09 (0.18)	-1.03	-0.26	3.45 ↑**	-1.09
	P%	0.12 (0.02)	0.13 (0.03)	**0.15** (0.02)	0.14 (0.04)	**1.11** (0.36)	0.51	3.46 ↑**	1.04	8.72 ↑**
	N:P	18.16 (2.33)	17.18 (4.47)	**14.16** (2.20)	19.85 (3.89)	**2.20** (1.19)	-0.58	-3.95 ↓**	1.18	W = 100 ↓**
Eriophorum vaginatum	N%	1.82 (0.13)	1.84 (0.12)	1.80 (0.11)	**2.27** (0.20)	1.81 (0.15)	0.32	-0.30	5.55 ↑**	-0.19
	P%	0.14 (0.03)	0.14 (0.04)	0.14 (0.01)	0.14 (0.03)	**0.47** (0.12)	W = 59	0.33	0.18	8.61 ↑**
	N:P	13.66 (2.98)	14.30 (3.97)	12.76 (1.38)	16.81 (4.59)	**4.03** (0.76)	0.37	-0.79	1.67	-8.91 ↓**

Mean nutrient concentration/ratio values with standard deviations in parentheses are presented in the first five data columns. Statistics of student's t-tests of fertilization effects on nutrient concentrations/ratios are presented in the last four columns. Data were natural log transformed when necessary to meet the assumptions of constant variance and normality, and a Wilcoxon test was performed when the assumptions for the t test were not met even after transformation. The arrows illustrate the directions of the treatment effects, and symbols following arrows indicate the significance levels: $P > 0.1$: no symbol, $P \leq 0.1$†, $P \leq 0.05$*, $P \leq 0.01$**. Means that are statistically significant different ($p \leq 0.05$) from controls are indicated in bold type

Table 3 Soil NH_4-N and PO_4-P fluxes during sequential incubations in the summer greenhouse warming and snowfence experiments during the growing season of 2017, and during a single incubation during the non-growing season, in mesic birch hummock tundra vegetation. Flux data were collected using the ion exchange membrane (IEM) *in situ* incubation method. A total of six incubations (R1-R6) were conducted on the greenhouse warming and their control plots, and a total of five incubations (R1-R5) were conducted on the snowfence and their control plots, with each incubation for nine to 17 days

		Incubation round	Control for warming (μg/cm^2/d)	Warming (μg/cm^2/d)	*F* (warming effect)	Control for snowfence (μg/cm^2/d)	Snowfence (μg/cm^2/d)	*F* (snowfence effect)
NH_4-N flux	Growing season	R 1	0.120 (0.06)	0.124 (0.10)	0.02	0.139 (0.02)	0.143 (0.03)	0.06
		R 2	0.141 (0.07)	0.145 (0.07)	0.04	0.150 (0.03)	0.151 (0.02)	0.02
		R 3	0.146 (0.08)	0.145 (0.07)	< 0.01	0.163 (0.01)	0.163 (0.01)	< 0.01
		R 4	0.172 (0.05)	0.172 (0.05)	< 0.01	0.141 (0.01)	0.152 (0.02)	1.16
		R 5	0.065 (0.09)	0.030 (0.03)	0.44	B.D.	B.D.	
		R 6	0.032 (0.03)	0.026 (0.02)	0.19	N.D.	N.D.	
		Average	0.113 (0.05)	0.102 (0.04)	0.22	0.134 (0.03)	0.114 (0.03)	0.77
	Non-growing season		S.L.	S.L.		B.D	B.D.	
PO_4-P flux	Growing season	R 1	0.050 (0.07)	0.380 (0.89)	1.54	0.023 (0.01)	0.038 (0.01)	10.38 *
		R 2	0.043 (0.05)	0.070 (0.13)	0.02	0.053 (0.04)	0.060 (0.02)	0.64
		R 3	0.029 (0.06)	0.127 (0.29)	1.29	0.028 (0.02)	0.027 (0.01)	0.14
		R 4	0.007 (0.01)	0.035 (0.08)	4.81*	0.022 (0.02)	0.020 (0.01)	0.01
		R 5	0.009 (0.003)	0.012 (0.01)	1.52	0.042 (0.05)	0.031 (0.02)	0.03
		R 6	0.021 (0.02)	0.079 (0.15)	4.07	N.D.	N.D.	
		Average	0.026 (0.03)	0.103 (0.20)	9.16*	0.034 (0.03)	0.034 (0.01)	1.36
	Non-growing season		0.003 (0.004)	0.007 (0.01)	0.12	0.006 (0.01)	0.003 (0.001)	< 0.01

Mean values with standard deviations in parentheses are presented. Degrees of freedom = 1, 18 (warming effect) and 1, 8 (snowfence effect). Data were natural log transformed when necessary to perform statistics to achieve the assumptions of constant variance and normality.

Symbols following F values indicate the significance levels: $P > 0.1$: no symbol, $P \leq 0.1$†, $P \leq 0.05$*. S.L.: sample lost; B.D.: below detection; N.D: no data collected.

have important implications. It indicates that within the natural range of nutrient availabilities in tundra ecosystems, the direct comparison/replacement between soil NH_4-N fluxes and pools has to be made with caution. However, over an extensive (i.e. fertilization) soil nutrient gradient, it is relatively robust to use one of these indicators to inform the other. Finally, both the fluxes and pools of NO_3-N in this study were mostly below our minimum detection limits (see Materials and methods), as has been frequently reported for direct extraction NO_3-N data in many other tundra studies (Edwards et al. 2006; Giblin et al. 1991). Our results suggest that it is not just the NO_3-N pool but also the flux into that pool is very small in tundra soils, presumably as a result of low temperature, low soil NH_4-N supply, rapid uptake by plants (Liu et al. 2018), and denitrifier activity, especially in moist and organic-rich surface horizons (Högberg et al. 2006).

Soil nutrient availability based on the IEM method is more sensitive than the soil extraction method for predicting plant nutrient concentration responses to low level fertilization

It is not surprising that both the IEM and soil extraction methods detected significant positive effects of high level fertilization treatments on soil N and P availabilities, and that these effects were also reflected in tundra plant species' foliar N and P concentrations (Semenchuk et al. 2015). What's more interesting, however, was that the IEM method captured both the low level N and low level P fertilization effects whereas the soil extraction

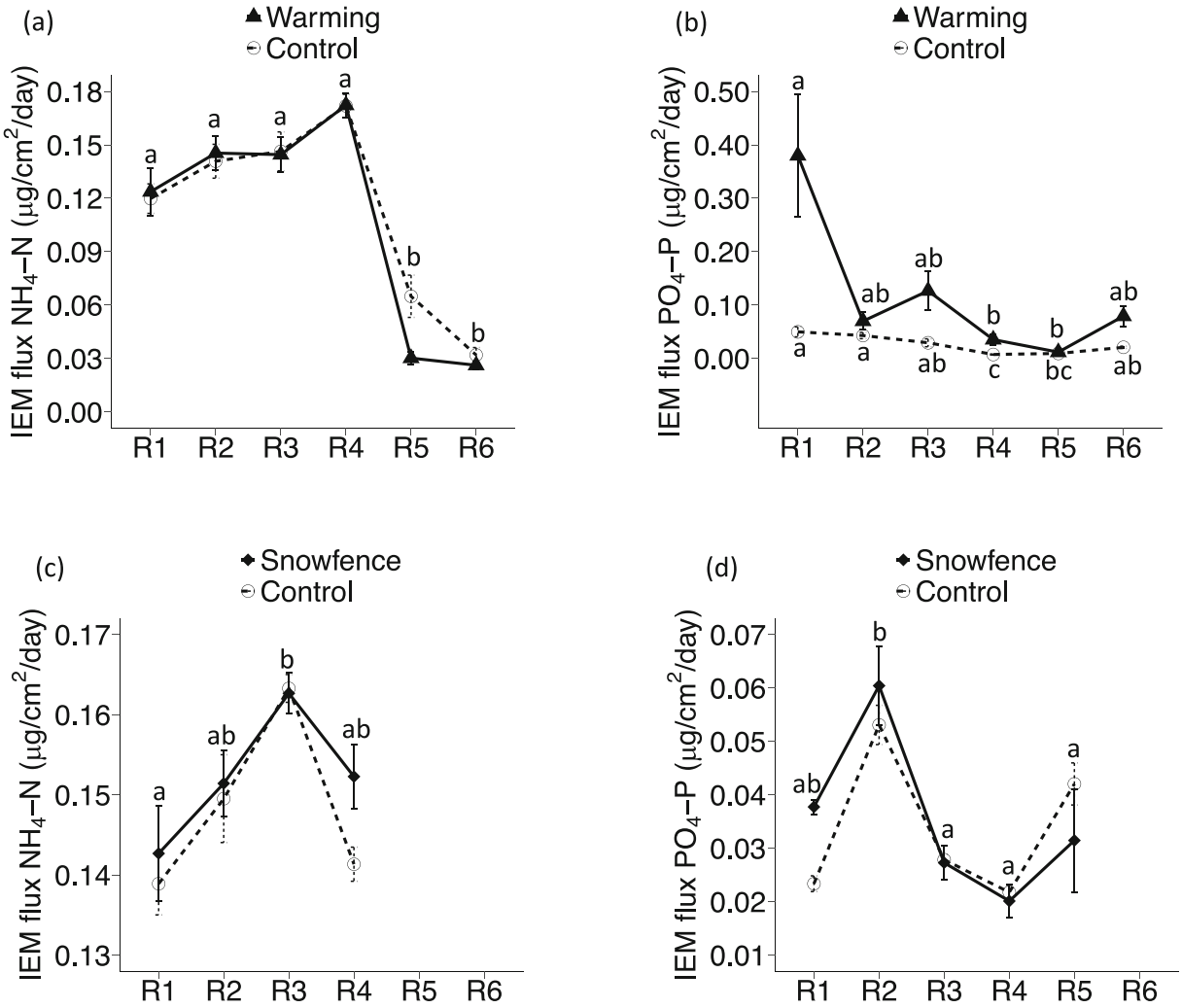

Fig. 3 Greenhouse warming and snowfence treatment effects on temporal soil NH_4-N fluxes (**a, c**) and PO_4-P fluxes (**b, d**) within the 2017 growing season (note the varying Y axis flux scales used for the different figures). R1 indicates the first incubation round (June 25 – July 4), R2 (second incubation July 4 – July 13), R3 (third incubation July 13 – July 22), R4 (fourth incubation July 22 – July 31), R5 (fifth incubation July 31 – August 17), and R6 (sixth incubation August 17 – August 26). Different labels (a, b, and c) indicate significant differences among incubation rounds at level of $\alpha = 0.05$ (n = 20 for a, and n = 10 for b, c and d). Error bars are standard errors

method did not. Together, the plant and soil data consistently indicate that the IEM method was more sensitive than the extraction method in detecting small, yet biologically meaningful, differences in soil NH_4-N and PO_4-P availability. Therefore, we conclude that the IEM method is a superior approach to measuring soil nutrient availability in typical ambient fertility environments not just in temperate mineral-soil dominated ecosystems (Duran et al. 2008; Ziadi et al. 2006), both also in the typical highly organic surface soil of many tundra ecosystems.

Soil NH_4-N and PO_4-P fluxes changed greatly over short periods within the growing season, while summer greenhouse warming significantly increased PO_4-P flux but not NH_4-N flux

Soil mineral nutrient fluxes from the summer greenhouse warming plots, the snowfence plots, and their associated control plots were highly variable over short periods within the growing season. These big fluctuations most likely reflected the combined consequences of changes in weather conditions (especially temperature and moisture) (Cassman and Munns 1980; Ellert

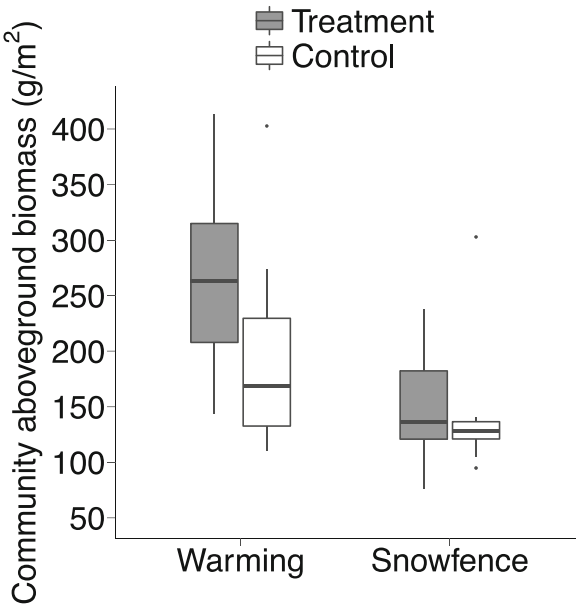

Fig. 4 Effects of greenhouse warming treatment (n = 20) and snowfence treatment (n = 10), respectively, on aboveground vascular plant community biomass in 2017. Mean aboveground plant community biomass was 197 g/m^2 (SD = 93) in the controls for greenhouse warming plots, 262 g/m^2 (SD = 86) in the greenhouse warming plots, 142 g/m^2 (SD = 58) in the controls for snowfence plots, and 151 g/m^2 (SD = 54) in the snowfence plots

and Bettany 1992; Ziadi et al. 2006), plant uptake (Chapin and Shaver 1989), and soil microbial immobilization/mineralization rates that are all closely interlinked.

The significant increases in soil PO_4-P fluxes (but not in NH_4-N fluxes) under the summer warming treatment suggest that the warmer greenhouse soil temperatures stimulated net P mineralization. The magnitude of increase for PO_4-P fluxes (by 3- to 7.6-fold) was similar to another tundra study using exchange resin bags to estimate the effects of elevated temperature on soil nutrient availability (Chapin et al. 1995). Soil microbial biomass is one of the largest reservoirs of potentially available soil P (but not for soil N) in tundra soils (Giblin et al. 1991; Jonasson et al. 1996). In addition, large amounts of exchangeable P are occluded in tundra soil organic matter (Chapin III et al. 1978; Walker and Syers 1976; Weintraub 2011). Accordingly, P mineralization and remineralization mediated by soil microbes is responsible for replenishing most or all of the soil solution PO_4-P (Chapin III et al. 1978; Giblin et al. 1991). As a result, the increases in PO_4-P fluxes in the greenhouse warming plots may be largely due to an increase in soil microbial net P mineralization in response to a warmer environment. Similarly, experimental warming increased net P mineralization, but not net N mineralization, in soils at a fellfield heath tundra site (Schmidt et al. 1999). However, it is important to note that our study yields fundamentally different data to the fellfield study because the IEM method inherently includes the impact of plant uptake on net fluxes, whereas the older buried bag method physically excludes roots. Hence, the greenhouse warming-enhanced PO_4-P flux that we observed is a net flux to the IEMs that occurred in the presence of potential competition by roots for P uptake. Therefore, even though we saw no corresponding *net* increase in NH_4-N flux to the IEMs, it is quite feasible that the greenhouses significantly enhanced soil fluxes of both PO_4-P and NH_4-N, but that the plant community (-whose growth was significantly stimulated by the warming treatment (Fig. 4), and whose growth demand for N is relatively high compared to P) outcompeted the IEMs for the latter.

Plant biomass responses in the greenhouse warming and snowfenced experimental treatments corresponded well with soil nutrient status indicated by the IEM flux data

The fact that aboveground plant community biomass was 1.6-fold higher, combined with earlier measurements indicating that plant community biomass N and P pools were 1.6-fold and 1.7-fold higher respectively in these same greenhouse warming and control plots (Zamin et al. 2014), indicates that the greenhouse treatment enhanced the availabilities of both these soil nutrients. Our IEM results here confirm an increase in soil PO_4-P fluxes. Furthermore, as explained above, although we did not detect a *net* increase in soil NH_4-N fluxes to the IEMs, this result may be because enhanced greenhouse plant NH_4-N uptake was of sufficient magnitude to fully account for a warming-induced increase in soil NH_4-N production. In addition, or alternatively, the greenhouse treatment may have enhanced supply of other soil N forms such as dissolved organic N which is readily taken up by a variety of tundra plant species (McKane et al. 2002). Finally, the snowfence results were consistent in that neither plant community biomass nor soil nutrient fluxes were significantly affected by the treatment. Taken together, these observations all suggest that the IEM method provided reliable insights on experimental treatment effects on soil nutrient status that

were biologically meaningful in predicting plant community responses.

High temporal variation in soil NH_4-N and PO_4-P availability

Our NH_4-N and PO_4-P IEM fluxes were highly temporally variable: the lack of a temporal autocorrelation effect on each of the soil nutrient fluxes (see "Statistical analyses" for details) indicates that microsite areas of relatively high nutrient availability did not persist over periods of weeks within a single growing season. Thus, we conclude that this mesic birch hummock tundra ecosystem contains a highly localized soil nutrient environment in which the duration of "hotspots" of plant available N and P were dynamically changing within weeks, and therefore difficult to predict. This conclusion is important in that it implies dramatic impacts on plants' foraging behavior (Hutchings and de Kroon 1994) and their overall fitness (Wijesinghe and Hutchings 1997).

Future research priorities for the IEM method

The IEMs provide useful information on soil nutrient bioavailability because of their unique ability to simulate biological ion sinks in relatively undisturbed conditions, but their data should be interpreted with caution. However, because of their preferential selectivity for various ions, it is important to know the relative affinity of the IEM for each target ion in the soil (Qian and Schoenau 2002; Skogley and Dobermann 1996). One particular concern is the possible underestimation of soil PO_4-P flux. The AEM does not function particularly well as a sink for phosphate ions, because membrane affinity for HPO_4-P is lowest relative to the principal other competing soil anions (Skogley and Dobermann 1996). Although we overcame this problem to some degree by charging the AEM initially with relatively low affinity counterions (HCO_3^-) (instead of with high affinity counterions such as Cl^-, as adopted by some other studies (e.g. Skogley and Dobermann 1996), our results may still be an underestimation of actual soil PO_4-P flux, and further research is required.

In addition to inorganic ions, the IEM method could also be used to determine dissolved ionically-charged organic nutrient forms (e.g. certain amino acids), which constitute a significant portion of the dissolved soluble N pool that can be taken up directly by plants in ecosystems where mineralization rates are low, such as arctic and alpine tundra (McKane et al. 2002; Nordin et al. 2004; Raab et al. 1999), boreal forest (Näsholm et al. 1998), and low productivity grasslands (Bardgett et al. 2003). Further studies are needed to explore the potential for IEMs to measure soil dissolved organically-bound ionically charged N and P forms.

Conclusions

This is the first report that we know of in which IEM membranes were used to measure soil nutrient dynamics within the growing season, and overwinter, in a tundra ecosystem. Our study contributes two important conclusions to applications of the IEM method in low arctic tundra ecosystems where information on soil nutrient flux dynamics is extremely lacking (Weintraub 2011). First, the results from our fertilization experiments strongly suggest that the IEM method provides a more sensitive and biologically meaningful approach than direct extraction for determining NH_4-N and PO_4-P availabilities in unamended (ambient) and low-moderately fertilized soils. Second, the IEM method is effective at characterizing changes in soil nutrient conditions subjected to warmer environments. The measurement protocol described and tested here provides a foundation for more realistic measurement of soil nutrient availabilities to plants, and hence should enable collection of more meaningful data in future biogeochemical and ecosystem-level studies and associated modelling initiatives.

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Data availability The data from this study will shortly be available on the Polar Data Catalogue website (https://www.polardata.ca/).

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