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Incorporating mass flow strongly promotes N flux rates in boreal forest soils

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ABSTRACT

Large differences in productivity and species composition are characteristic for the boreal forest and nitrogen (N) availability has been deemed the proximate cause of this variation.

We used a modified microdialysis technique to assess N availability through monitoring *in situ* inorganic and organic soil N fluxes in the presence and absence of mass flow in two forest ecosystems of contrasting fertility, a nutrient rich Norway spruce forest and a nutrient poor Scots pine forest. This was enabled by using solutions of different osmotic potentials as perfusates. In the absence of mass flow, amino acids dominated soil N fluxes of both ecosystems representing 62 and 82% of total flux in the nutrient rich and the nutrient poor ecosystem respectively. In the presence of mass flow, N flux increased by nine times in the nutrient rich and four times in the nutrient poor soil and nitrate comprised a greater share of total N flux. Our results suggest that mass flow may be a strong driver for plant N acquisition in boreal forests through delivering higher amounts of amino acids and NO_3^- to plant roots and mycorrhizas. These results points to a strong interaction between water and N availabilities, the former enhancing the supply of the latter through enabling high rates of transpiration.

1. Introduction

Plant roots and mycorrhizal hyphae have the potential to acquire a wide range of nitrogen (N) forms (Nacry et al., 2013). Boreal forest soils often contain large stocks of N (Callesen et al., 2007), but much of this N is not bioavailable to plants, as reflected by numerous fertilization trials which show tree growth and understorey species composition respond strongly to this additional N resource (Hyvönen et al., 2008; LeBauer and Treseder, 2008). The overall availability of N is not reflected by the amount of N in soil solution; rather it might be better reflected by the composition of the N forms in soil solution (Giesler et al., 1998). Nitrification and detectable pools of NO_3^-, rather, appear to be characteristic of high tree productivity while organic N dominates at the low end (Giesler et al., 1998; Ste-Marie and Pare, 1999; Nordin et al., 2001; Oyewole et al., 2016).

One likely cause of nutrient and in particular N limitation of growth is a restriction in nutrient mobility in soils rather than in nutrient contents (Bray, 1954). The factors that affect N mobility; the composition of the soil N pool and water availability would therefore also affect N limitation. In addition to this, model calculations suggest plant nutrient acquisition to be far more dependent on soil nutrient movement to the root surface through diffusive and mass flow fluxes than root uptake capacities (Nye, 1977; Tinker and Nye, 2000), which has also been confirmed by *in situ* studies (Oyewole et al., 2016). Linking mobility of N to plant N acquisition is therefore crucial for our understanding of N limitation in boreal forest ecosystems. Diffusion and mass flow are the main processes governing movement of nutrients through the soil towards roots and mycorrhizal hyphae (Barber, 1995; Comerford, 2005; Tinker and Nye, 2000). While diffusion results from the formation of concentration gradients between root surfaces and the surrounding soil, driven by active root uptake, mass flow results from bulk flow of water, driven by transpiration by needles or leaves. Diffusion is generally believed to be the dominant process in nutrient poor soils and mass flow the principal process in nutrient rich soils (Barber,

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1995; Clarke and Barley, 1968; Comerford, 2005; Cramer et al., 2008; Smethurst, 2000). This differentiation is often insufficient, since it is sometimes hard to determine if a soil is nutrient rich or nutrient poor. For example, even if boreal forest soils are often nutrient poor, Norway spruce forest soils are generally considered to be nutrient rich in comparison to Scots pine forest soils. Thus, a direct measurement of soil N fluxes via diffusion and mass flow is essential.

Still, the role of diffusion and mass flow for plant N has until recently not been possible to assess with the type of indirect methods that have been at hand. A recent study, however, presented a development of a miniaturized dialysis technique (microdialysis) earlier used for studies of diffusion processes in soil (Inselsbacher et al., 2011). In this refined method, transpirationally induced mass flow was simulated using an osmotically active solution as perfusate (Oyewole et al., 2014). Although this technique does not allow for a direct quantification of the contributions of diffusion and mass flow for plant N acquisition, it offers the possibility to study the potential importance of these processes directly in soil (Oyewole et al., 2014, 2016; Inselsbacher et al., 2014; Brackin et al., 2015).

Another important aspect of the microdialysis method, in addition to the opportunity to sample induced diffusive fluxes and mass flow in undisturbed soil directly in the field, is the benefit of the semipermeable membrane that enables instantaneous separation of the sample from potential chemical alteration (enzymes, microbes) during sampling (Rousk and Jones, 2010; Inselsbacher, 2014). The method was developed and evaluated for mass flow measurements in a previous lab study (Oyewole et al., 2014), but considering the apparent problem of the disruption of the natural soil matrix and the natural equilibrium of soil N pools during soil sampling and handling, the full potential of the method should be evaluated *in situ*.

Here, we set out to study the potential importance of diffusion and mass flow for plant N nutrition in boreal forest soils. This was the first time the microdialysis method was used on site in undisturbed soil for directly and simultaneously estimating the delivery of soil N by diffusion and mass flow. Measurements were carried out in two boreal forest ecosystems representing contrasts in the spectrum in nutrient availabilities in this biome: a nutrient-poor heath forest dominated by Scots pine (*Pinus sylvestris* L.) and a nutrient-rich forest dominated by Norway spruce (*Picea abies* L. Karst). This difference in ecosystem fertility was manifested both by contrasting tree productivities and by clear differences in dominating understorey plant species. The aim of the study was to assess the relative importance of the two main processes delivering N to plant roots and mycorrhizas in these two ecosystems. A second, related aim was to unveil the potential impact of plant transpiration on N availabilities.

2. Materials and methods

2.1. Study sites

Microdialysis field experiments were conducted in a Scots pine (*Pinus sylvestris* (L.)) heath forest in Krogheden near Umeå, Sweden (63°52′22″ N, 20°11′51″ E) and in a Norway spruce (*Picea abies* (L.)) -dominated forest in Kulbäcksliden near Vindeln, Sweden (64°11′29″ N, 19°34′9″ E). At both sites, the annual average precipitation is 587 mm and annual mean air temperature is 1.9 °C. The forest soils are classified as Haplic podzols (FAO, 2006). Total wet and dry N depositions are approximately 2 kg N ha^{-1} yr^{-1}. At the site in Krogheden, the soil organic layer is approx. 5 cm deep, has a C/N ratio of 43.5, pH (H_2O) of 3.8 and the soil moisture content at sampling time was 0.9 g g^{-1} DW (Table 1). The organic content of the organic horizon was 68.5% (g g^{-1} soil DW). The tree layer is dominated by Scots pine and the understorey vegetation by *Vaccinium myrtillus*, *Vaccinium vitis-idaea*, *Pleurozium schreberi*, *Hylocomium splendens* and *Cladonia* spp. This site is similar to the experimental research site Rosinedal which has been used frequently in previous studies (e.g. Hasselquist et al., 2012). At the Norway spruce site in Kulbäcksliden, the soil organic layer is mixed with the mineral horizon (A-horizon) resulting in a low organic content of the soil (14.5%) in comparison to the pine forest soil. The O/A horizon is approximately 5–15 cm deep, has a C/N ratio of 22.1, pH (H_2O) of 5.4 and the soil moisture content was 1.2 g g^{-1} DW (Table 1). The tree layer is dominated by old Norway spruce (>170 years) and the understorey vegetation is dominated by *Pleurozium schreberi*, *Hylocomium splendens* (L.) *Vaccinium myrtillus* (L.), *Deschampsia flexuosa* (L.) Trin. the tall herbs *Geranium sylvaticum* (L.), *Cicerbita alpina* (L.) Wallr. and low herbs (e.g. *Gymnocarpium dryopteris* (L.) Newman, *Maianthemum bifolium* (L.) F. W. Schmidt, *Trientalis europaea* (L.), *Moneses uniflora* (L.) A. Grey) (Lidén et al., 2004).

2.2. Microdialysis system and set up

The microdialysis system was set up as described previously (Inselsbacher et al., 2011). Briefly, it consisted of two syringe infusion pumps (CMA 400), equipped with eight gas-tight glass syringes (2.5 ml, Hamilton, Bonaduz, Switzerland) which provided the perfusate solution. Each syringe was connected to a microdialysis probe (CMA 20) with a polyarylethersulphone membrane (10 mm long; 500 μm outer and 400 μm inner diameter; molecular weight cut-off of 20 kDa). The probes were perfused with high-purity distilled (MilliQ) water and effluxes from the probes (dialysates) were collected with two refrigerated microfraction collectors (CMA 470) in 300 μl vials. Microfraction collector temperature was kept at 6 °C throughout the experiments. All equipment is commercially available at CMA Microdialysis AB (Solna, Sweden).

2.2.1. Calibration of the microdialysis probes

Each microdialysis probe was calibrated before and after each sampling event according to the general calibration method (Bungay et al., 1990; Torto et al., 2001; Nandi and Lunte, 2009) and as described for low molecular weight N compounds by Inselsbacher et al. (2011), in order to ensure uniform performance of all probes throughout the experiments. Briefly, microdialysis probes were submerged in a standard solution containing NH_4^+, NO_3^- and 19 amino acids (AAS 18, Amino acid standard solution, plus additional glutamine and asparagine; Sigma Aldrich). The standard solution was kept at a constant temperature of 22 °C and stirred with a magnetic stirrer throughout the calibration period to prevent the formation of a depletion zone around the probe surface (Inselsbacher et al., 2011). The probes were perfused with MilliQ water at a constant flow rate of 1.0 μl min^{-1} for 8 h. Dialysates were collected continuously at 2 h intervals and were immediately prepared for chemical analysis as described below. The relative recoveries of the individual N compounds by each probe were calculated as given in equation (1):

$$\text{Relative recovery (\%)} = C_{dial}/C_{std} \times 100 \quad (1)$$

where C_{dial} is the concentration of the measured N compound in the dialysate and C_{std} is the concentration of the compound in the standard solution.

The induced diffusive fluxes of N compounds during each sampling time were calculated as described previously (Inselsbacher and Näsholm, 2012a,b; Inselsbacher et al., 2014;

Table 1
Soil properties of the two sites and temperature conditions during the measurements.

Site	Nutrient poor Scots pine forest	Nutrient rich Norway spruce forest
Organic soil layer (cm)	5	5
C%	36.8	6.9
N%	0.8	0.3
C/N	43.5	22.1
Soil organic matter (%)	68.5	14.5
Moisture content (g g^{-1} DW)	0.9	1.2
pH $_{H2O}$	3.8	5.4
pH $_{KCl}$	2.7	4.1
Soil temp (°C)	13.0	9.0
Air temp (°C)	19.0	13.0

Oyewole et al., 2014), equation (2):

$$\text{Diffusive flux (nmol m}^{-2}\text{ s}^{-1}) = (C_{dial} \times V_{pump}) / (A_{probe} \times t) \quad (2)$$

where V_{pump} is the volume provided at each individual pump flow rate (1), A_{probe} is the membrane surface area (15.9×10^{-6} m^2) and t is the sampling time (s).

The total induced flux rate of total amino acids (TotAA) and inorganic N compounds (IN) was calculated as the sum of induced flux rates of TotAA and IN compounds.

2.2.2. Estimation of diffusive and mass flow fluxes of soil N

Microdialysis field samplings for estimation of diffusive fluxes and mass flow fluxes of individual N compounds in soils were conducted in the Scots pine heath forest on June 10–11, 2014, and in the Norway spruce forest on June 17–18, 2014. Mean temperatures during the week prior to each sampling occasion were 15.9 and 11.7 °C (Scots pine, Norway spruce) and the total precipitation 17.5 and 3.5 mm (Scots pine, Norway spruce) (Reference climate monitoring program at SLU experimental forests and SITES Svartberget). At each study site, four plots (each 0.04 m^2) were chosen randomly. In each plot, four microdialysis probes were inserted into the organic soil layer for diffusive flux measurements and another four dialysis probes were inserted for mass flow measurements. Briefly, understorey growth and moss layer were carefully lifted, and a guiding channel was prepared by vertically inserting a steel needle (0.5 mm outer diameter) to a depth of 5 cm into the soil organic layer (Table 1). The probes were carefully inserted into the channel and the soil surrounding the probe was slightly compressed to ensure full contact of the membrane surface with the surrounding soil. The probes were perfused with either MilliQ water or 10% (w/v) Dextran 40 (corresponding to an osmotic potential of −0.04 MPa) at a flow rate of 1 μl min^{-1} (for further details on the choice of perfusate see Oyewole et al., 2014). Dialysates were collected twice from each probe when inserted into the soil in each plot at 1.5 h intervals (t_1, t_2), at each study site. Dialysates were immediately put on ice and stored at −20 °C until chemical analyses as described below. Mass flow of soil solution across the microdialysis membrane (V_F) was estimated gravimetrically according to Oyewole et al. (2014) and radial fluxes were subsequently calculated as given in equation (3):

$$\text{Radial flux (m s}^{-1}) = V_F / (A_{probe} \times t) \quad (3)$$

where V_F [m^3] is the volume of soil solution that passed the microdialysis membrane as a result of induced mass flow. For the probes perfused with MilliQ water the sampled volumes were on average 81% of the theoretical volume. This could be attributed to the difference in osmotic potential between probe and soil, but also to the accuracy of the system at lower temperatures than room temperature in the field. Therefor measured volumes were used in the calculations. For analyses, samples from each membrane and plot were pooled for each time point (t_1, t_2) so the number of replicates for each time point and perfusate was N = 4. In detail, we took equal volumes of the dialysates from each of the four vials at each sampling time and pooled them into a fresh vial (to serve as the representative sample) for each plot and time point. This was done to guarantee sufficient sample volume for chemical analyses.

2.3. Chemical analyses

Amino acids, NH_4^+ and NO_3^- in microdialysis samples were analyzed as described previously (Inselsbacher et al., 2011). Briefly, NH_4^+ and individual amino acids were analyzed by reversed-phase liquid chromatography using a Waters (Milford, USA) Ultra High Performance Liquid Chromatography (UPLC) system with a Waters Tunable UV (TUV) detector. Aliquots of sample (20 μl) were derivatized with a Waters AccQ-Tag™ Ultra Derivatization kit for amino acid analyses. Individual amino acids were separated on an AccQ-Tag™ Ultra column by elution with a mixture of 0.1% formic acid (solution A) and 10% acetonitrile (solution B) using the following gradient: 0–5.74 min isocratic 99.9% solution A, declining to 90.9% solution A from 5.74 to 7.74 min, to 78.8% solution A at 8.24 min and then to 40.4% solution A at 8.74 min, before re-equilibration with 99.9% solution A from 8.74 to 9.54 min. The flow rate was 0.6 ml min^{-1} and the column temperature was 55 °C. Nitrate was analyzed by the Vanadium (III) chloride (VCl_3) and Griess method as described by Hood-Nowotny et al. (2010) based on the technique described by Miranda et al. (2001).

2.4. Calculations and statistical analyses

Mann-Whitney test was conducted on induced flux rates measurements from both Pine and Spruce sites for differences in induced flux rates between perfusates within each sampling time point, in Minitab 17.1.0 (Minitab Inc. PA, USA). Differences in induced flux rates between sampling times (t_1 and t_2), within each perfusate were tested with Wilcoxon signed rank test (OriginPro, 2016; Originlab Corp. MA, USA) for non-normally distributed data and with Paired *t*-test for normally distributed data (Minitab 17.1.0). Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Induced flux rates of water and N in boreal forest soils

Using Dextran 40 at a concentration of 10% (w/v) as perfusate resulted in radial fluxes of soil solution over the probe membranes at an average velocity of $2.5 \times 10^{-7} \pm 0.4 \times 10^{-7}$ m s^{-1} and $2.0 \times 10^{-7} \pm 0.2 \times 10^{-7}$ m s^{-1} in the soils of the Scots pine and Norway spruce forests, respectively.

Diffusive fluxes of the sum of measured N compounds were similar in the two forests, being 6.4 ± 1.1 and 5.6 ± 0.7 nmol m^{-2} s^{-1} for the poor Scots pine and the rich Norway spruce soil, respectively (Fig. 1a and b; Table 2). In the presence of mass flow, fluxes of the sum of measured N compounds were significantly higher than in the absence of mass flow, being 24.5 ± 7.2 nmol m^{-2} s^{-1} for the poor Scots pine soil and 50.0 ± 20.9 nmol m^{-2} s^{-1} for the Norway spruce soil. Thus, mass flow increased flux rates of the measured compounds by four times in the nutrient poor soil and by nine times in the nutrient rich soil (Fig. 1a and b).

Mass flow did not lead to a significant increased rate of NH_4^+ flux in either the nutrient poor Scots pine forest soil, or the nutrient rich Norway spruce forest soil (Table 2). In contrast, the flux rates of NO_3^-

were 35 and 40 times higher in the presence of mass flow in the Scots pine and Norway spruce soils, respectively (Table 2). Additionally, flux rates of total amino acids were three and five times (Scots pine, Norway spruce soils) higher in the presence of mass flow (Table 2).

3.2. Effects of mass flow on the composition of N fluxes

Mass flow had a strong effect on the composition of N flux (Fig. 2). While amino acids dominated the diffusive fluxes of measured N compounds in both the Scots pine soil and the Norway spruce soil, in the presence of mass flow the share of NO_3^- increased substantially; from 3 to 28% and from 14 to 63% in the Scots pine and Norway spruce soils, respectively (Fig. 2). While NO_3^- showed the largest increase in both soils, total amino acids also increased in presence of mass flow and dominated the fluxes in presence of mass flow in the Scots pine soil. Furthermore, the composition of total amino acids-flux changed significantly in response to mass flow (Table 3). 14 of the 19 amino acids analyzed were detected in the samples (Table 3). In the Scots pine soil mass flow resulted in increased flux rates of all measured amino acids except Ser, Glu, Ala and Leu while in the Norway spruce soil the effect of mass flow was significant for all measured compounds except Ser, Ala, Pro and Val (Table 3).

3.3. Effects of mass flow on time-dependent decreases in N flux rates

In general, total flux rates of measured N were lower during the second sampling phase (1.5–3 h) compared to the first phase (0–1.5 h; Table 2) for both perfusates and for both soil types. In the absence of mass flow total flux rates (AA + IN) decreased to 54 and 55% of the initial rates for the Scots pine and Norway spruce forests respectively. In the presence of mass flow, AA + IN fluxes during the second sampling phase were 54 and 71% of the initial rates in the Scots pine and Norway spruce forests respectively. The effect of sampling time on AA + IN fluxes was, however, not significant (Table 2). Even so, the observed difference in time dependence of total N fluxes was due to decreases in AA without changes in NH_4^+ nor NO_3^-. Thus, in the N poor soil of the Scots pine forest and the rich soil of the Norway spruce forest the decrease in total amino acid flux was statistically significant in the absence of mass flow (Table 2). All individual amino acids present at the first sampling phase in absence of mass flow decreased significantly during the second sampling phase in the Norway spruce forest soil (Table 3.) The same pattern was found in the Scots pine forest soil although not with significant differences.

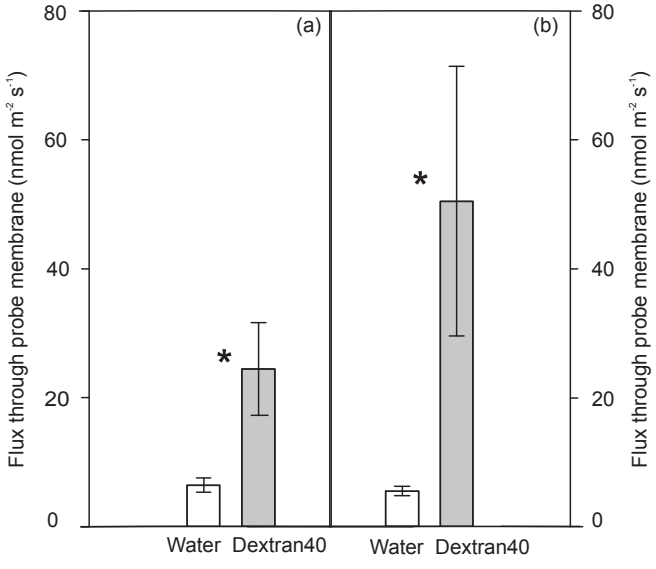

Fig. 1. Induced flux rates (nmol m^{-2} s^{-1}) of inorganic nitrogen and total amino acids (AA + IN) across microdialysis probe membranes installed in (a) Pine forest soil and (b) Spruce forest soil using MilliQ water (white bars) or Dextran 40 (grey bars) as perfusate. A solution of 10% (w/v) Dextran 40 corresponding to osmotic potential of −0.04 MPa was used to drive mass flow into the probes, and a pump flow rate of 1 μl min^{-1} was used for both types of perfusate. Values represent means ± SE (n = 4) from the first sampling time point (t_1). * denotes differences between perfusate types at $P \leq 0.05$ (Mann-Whitney test).

Table 2
Induced flux rates (nmol m^{-2} s^{-1}) of soil nitrogen across the dialysis probe membrane when the probes were perfused with MilliQ water or Dextran 40 at sampling times t_1 (0–1.5 h) and t_2 (1.5–3 h) respectively in the Scots pine and Norway spruce forest sites. Values represent means ± SE (n = 4). Capital letters denote statistically significant differences in induced fluxes of N compounds between perfusates within each sampling time point; absence of letter denotes no significant difference (Mann-Whitney test, $P \leq 0.05$). Lower case letters denotes statistically significant differences in induced flux rates between the sampling times (t_1 and t_2), within each perfusate (Paired *t*-test, $P \leq 0.05$). Total amino acids (TotAA) and inorganic N compounds (IN).

	Water		Dextran	
	t_1	t_2	t_1	t_2
Scots pine				
NH_4^+	1.0 ± 0.2	1.1 ± 0.2	2.0 ± 0.5	1.2 ± 0.5
NO_3^-	0.2 ± 0.1	0.4 ± 0.3	6.9 ± 6.9	4.8 ± 2.8
TotAA	5.3 ± 1.0^{Aa}	2.0 ± 0.2^{Ab}	15.7 ± 3.8^{B}	7.2 ± 1.4^{B}
AA + IN	6.4 ± 1.1^{A}	3.5 ± 0.3^{A}	24.5 ± 7.2^{B}	13.3 ± 4.6^{B}
Norway spruce				
NH_4^+	1.3 ± 0.4	1.0 ± 0.2	2.2 ± 0.7	2.0 ± 1.3
NO_3^-	0.8 ± 0.5	0.4 ± 0.2	32.0 ± 20.2	23.3 ± 18.5
TotAA	3.5 ± 0.3^{Aa}	1.7 ± 0.0^{Ab}	16.3 ± 4.7^{B}	10.4 ± 2.3^{B}
AA + IN	5.6 ± 0.7^{A}	3.0 ± 0.3^{A}	50.5 ± 20.9^{B}	35.6 ± 16.5^{B}

4. Discussion

Large variations in productivity and species composition are found in the vast boreal forest biome and N availability has been deemed the principal underpinning of this spectrum (LeBauer and Treseder, 2008). Soils of these forests are characterized by relatively large stocks of N (Callesen et al., 2007) implying N limitation to be a function of availability rather than of amount. The current study set out to investigate the importance of diffusion and mass flow for plant N nutrition in boreal forests. This was carried out in two widely contrasting forests representing endpoints of a broad spectrum of typical boreal forests: an unproductive, dry, Scots pine forest and a high-productive, moist, Norway spruce forest. The two forest types have similar soil types (Haplic podzol), but differ in several soil parameters (Table 1) and vegetation cover, and may be considered representative for unproductive water recharge and productive discharge areas respectively (Giesler et al., 1998). (Giesler et al., 1998; Högberg et al., 2006). Thus, in the current study we aimed at assessing if, independent of forest type, potential plant N acquisition would benefit from transpirationally induced mass flow.

Methodological difficulties have earlier hindered direct assessment of diffusion and mass flow processes in soil. The current study accomplished this through the use of a microdialysis method where different perfusates simulated soil N fluxes in the absence and presence of mass flow. In this study direct measurements of mass flow with this technique was carried out for the first time *in situ* in undisturbed soil. A constant flow of pure water inside the microdialysis probe results in diffusion of compounds from

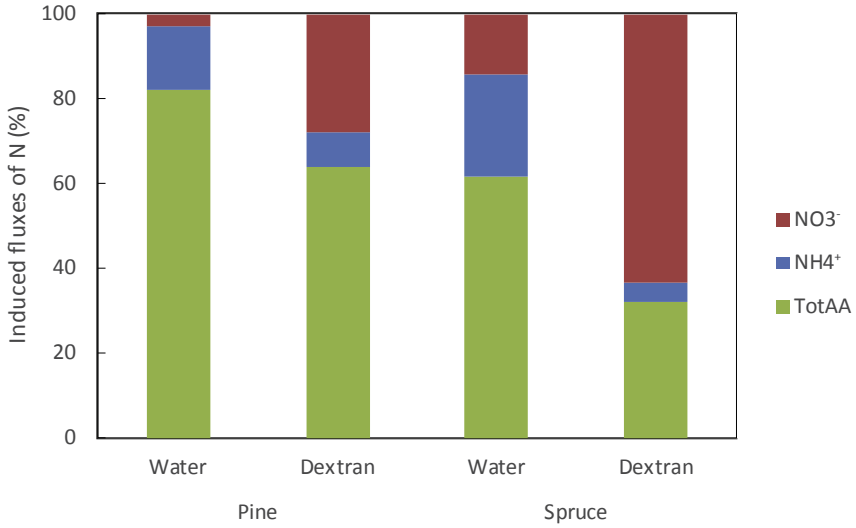

Fig. 2. Composition of induced fluxes of nitrogen (% of AA + IN) in the Scots pine (a, b) and Norway spruce (c, d) forest soils; in the absence (a, c) and presence (b, d) of mass flow at sampling time point 1 (1.5 h) (n = 4).

Table 3
Induced flux rates (nmol m^{-2} s^{-1}) of soil nitrogen compounds across the dialysis probe membrane when the probes were perfused with MilliQ water and Dextran 40 at sampling times t_1 (0–1.5 h) and t_2 (1.5–3 h) respectively. Values represent means ± SE (n = 4). Capital letters denote statistically significant differences in induced fluxes of N compounds between perfusates within each sampling time point; absence of letter denotes no significant difference (Mann-Whitney test, $P \leq 0.05$). Lower case letters denotes statistically significant differences in induced flux rates between the sampling times (t_1 and t_2), within each perfusate (Paired *t*-test, $P \leq 0.05$). Total amino acids (TotAA) and inorganic N compounds (IN). nd not detected.

	Water		Dextran	
	t_1	t_2	t_1	t_2
Scots pine				
Arg	nd	nd	0.5 ± 0.1	0.3 ± 0.0
Ser	0.6 ± 0.2	0.1 ± 0.1^{A}	2.4 ± 1.0	0.9 ± 0.3^{B}
Gln	1.0 ± 0.1^{A}	0.7 ± 0.1^{A}	3.4 ± 0.3^{B}	2.9 ± 0.1^{B}
Gly	1.0 ± 0.1^{A}	0.7 ± 0.0^{A}	3.0 ± 0.9^{B}	1.6 ± 0.2^{B}
Asp	0.5 ± 0.1^{A}	nd	1.5 ± 0.5^{B}	0.4 ± 0.2
Glu	0.6 ± 0.2	nd	0.9 ± 0.1	0.2 ± 0.2
Thr	0.3 ± 0.1^{A}	nd	1.2 ± 0.1^{B}	0.6 ± 0.2
Ala	0.7 ± 0.1	0.5 ± 0.0	0.2 ± 0.2	nd
Pro	0.2 ± 0.1^{A}	0.1 ± 0.1	1.0 ± 0.2^{B}	0.2 ± 0.2
Tyr	nd	nd	nd	nd
Val	0.2 ± 0.1^{A}	nd	0.9 ± 0.2^{B}	0.2 ± 0.2
Ile	nd	nd	0.2 ± 0.2	nd
Leu	0.2 ± 0.1^{A}	nd	0.4 ± 0.2	nd
Phe	nd	nd	nd	nd
Norway spruce				
Arg	nd	nd	0.5 ± 0.1	0.3 ± 0.0
Ser	0.1 ± 0.1	nd	2.5 ± 1.6	1.0 ± 0.5
Gln	0.9 ± 0.1^{Aa}	0.8 ± 0.0^{Ab}	3.5 ± 0.4^{B}	3.4 ± 0.5^{B}
Gly	0.8 ± 0.0^{Aa}	0.6 ± 0.0^{Ab}	3.6 ± 1.0^{B}	2.2 ± 0.6^{B}
Asp	0.4 ± 0.0^{A}	nd	1.7 ± 0.5^{B}	1.8 ± 0.8
Glu	0.4 ± 0.0^{A}	nd	1.0 ± 0.2^{B}	0.2 ± 0.2
Thr	0.1 ± 0.1^{A}	nd	1.2 ± 0.3^{B}	0.8 ± 0.0
Ala	0.6 ± 0.0^{a}	0.4 ± 0.0^{b}	0.3 ± 0.3	nd
Pro	0.1 ± 0.1	nd	0.6 ± 0.3	0.4 ± 0.2
Tyr	nd	nd	0.2 ± 0.2	nd
Val	0.1 ± 0.1	nd	0.6 ± 0.4	0.2 ± 0.2
Ile	nd	nd	0.2 ± 0.2	nd
Leu	nd	nd	0.3 ± 0.3	nd
Phe	nd	nd	0.2 ± 0.2	nd

surrounding soil solution into the perfusate. A flow of an osmotically active solution, in our case Dextran 40, results in simultaneous convection of soil solution and diffusion of soil solutes into the perfusate. The velocity of radial flux of water across the dialysis probe membranes in the current study was *c.* 2×10^{-7} m s^{-1} in both the Scots pine and Norway spruce forest soil. This rate is similar to those observed earlier, in a lab study of boreal forest soil (Oyewole et al., 2014). Estimates of water velocities perpendicular to root surfaces suggest a range of $0–10^{-7}$ m s^{-1} (Tinker and Nye, 2000); thus, rates of mass flow in the current study are in the high end of velocities reported for roots.

Significant increases in induced flux rates of N compounds were found for both forest types when sampling was done in the presence of mass flow (Fig. 1). This result is in agreement with the previous lab study although the relative increase was four and nine times in the two different soils which is two to five times higher compared to the lab study (Oyewole et al., 2014). This effect of mass flow resulted from increased flux rates of total amino acids and to an even greater extent of NO_3^- (Fig. 2; Table 2). Surprisingly, the effect of mass flow on amino acids was highly significant, while we found no effect on NH_4^+ fluxes. This was unexpected, since based on the results from the previous lab study we expected a strong effect of mass flow on NH_4^+ fluxes as well (Oyewole et al., 2014). This discrepancy in results between the two studies could be related to the effect of soil sampling and handling leading to potential underestimation of amino acids and overestimations of NH_4^+ in homogenized soils (Rousk and Jones, 2010; Inselsbacher and Näsholm, 2012a; Inselsbacher, 2014). Therefore, our study further underlines the crucial importance of studying soil processes *in situ*.

The dominance of total amino acids in the diffusive fluxes (82 and 63% of total analyzed N in the Scots pine and Norway spruce forests, respectively; Table 2) is in agreement with earlier reports from analyses of soil solutions from similar forest sites in the area (Giesler et al., 1998; Nordin et al., 2001), largely conforming to results from other studies in boreal and arctic regions (Kielland et al., 2006; Werdin-Pfisterer et al., 2009; Kranabetter et al., 2007; Rothstein, 2010; LeDuc and Rothstein, 2010) and are also in line with the results from previous microdialysis studies (Inselsbacher and Näsholm, 2012a; Inselsbacher et al., 2014; Oyewole et al., 2016; but see Likens et al., 1969 and Kronzucker et al., 1997). These results suggest that, in the absence of transpirationally induced mass flow, amino acids may have a significant role in plant N nutrition in both the unproductive Scots pine forest and the

productive Norway spruce forest.

Soil pH and C/N ratios of the organic layer have in earlier studies been shown to correlate with nitrification rates and abundance of NO_3^- (Vitousek et al., 1989; Stark and Hart, 1997; Giesler et al., 1998). In future studies, microdialysis could be combined with measurements of N transformations (e. g. N mineralization and gross nitrification rates) to evaluate if *in situ* soil N fluxes would correlate to N transformation rates and, in turn, to soil pH and C/N ratios. Here, the latter factors indicate that the nutrient rich Norway spruce forest soil should exhibit substantial nitrification rates (Table 1) while the nutrient poor Scots pine forest should not. A greater share of NO_3^- in the diffusive N fluxes of the nutrient rich soil (Fig. 2) is in line with these differences in soil characteristics but the contrast between the nutrient rich and nutrient poor site was small, and amino acids dominated the diffusive flux of both sites. It is quite remarkable that despite the quite large differences in soil properties between the two sites the effect of diffusion and mass flow are so similar.

The variability in flux rates of measured N between sampling points was generally much greater in the presence of mass flow and this variability was largely an effect of increased variability in NO_3^- fluxes (Table 2). The setup for sampling aimed at minimizing the fine-scale (mm) variability by pooling samples from four different probes but even so, the variation in NO_3^- fluxes was high. This opens up for future studies using the high spatial resolution that this method holds which could in detail investigate the patchiness in the occurrence of nitrification and hence in NO_3^- production at scales of millimetres.

Roots mine soil for N, which results in depletion of N around active roots. This depletion of N leads to a strong dependence of N uptake on root growth; roots must explore new soil to acquire nutrients (Tinker and Nye, 2000). Mass flow brings N into the depletion zone created by active uptake and hence reduces the need for roots to explore new soil volumes. We analyzed flux rates of N compounds at two time intervals in the absence and presence of mass flow (Table 2). Independent of forest type, total N fluxes were generally lower both in the absence and presence of mass flow at the second time interval compared to the first time interval but the declines were only significant (and in relative terms also greater for total amino acids) in the absence of mass flow. These results point to a potential importance of mass flow not only for short term N acquisition but also for a more efficient exploration of soil by roots: a larger soil volume can be explored, and the need for additional root growth can be reduced, in the presence of mass flow (Tinker and Nye, 2000).

The most striking result of the current study was the more than 30-fold increase in NO_3^- fluxes in the presence of mass flow (Table 2, Fig. 2). This shift is in line with results from a laboratory study (Oyewole et al., 2014) and in general in agreement with the notion that NO_3^- acquisition is to a larger degree than acquisition of other N sources linked to mass flow (Nye and Marriott, 1969; Marschner et al., 1991; Marschner, 1995). Also total amino acids increased and were dominating N fluxes in presence of mass flow in the nutrient poor site. In general, a greater number of amino acid species was detected in the presence of mass flow in comparison to the diffusive fluxes. This was especially prominent for the acidic and neutral amino acids but also the basic amino acid arginine was detected in presence of mass flow but not in diffusive fluxes. Basic amino acids are generally believed to occur at low concentrations in the soil solution due to strong binding to the soil's negatively charged surfaces similar to NH_4^+. This difference in responsiveness to mass flow between cations and acidic, neutral amino acids and NO_3^- therefore was expected. Rates of diffusion are strongly dependent on molecular weight where larger compounds like amino acids have lower diffusive flux rates compared to inorganic N (Inselsbacher et al., 2011). However, mass flow rates would not be affected by molecular weight. These differences suggest that mass flow is resulting in differences in composition of plant available N forms and the amount of total N at both sites, corroborating the findings in the laboratory study (Oyewole et al., 2014).

Model calculations (Nye, 1977; Tinker and Nye, 2000) as well as field estimations (Oyewole et al., 2016) have pointed to diffusive fluxes rather than root uptake capacities as the limiting step to tree N nutrition. The current study shows that transport rates of N, and especially of nitrate, from bulk soil to plant roots may be significantly enhanced in the presence of mass flow. We speculate that transpirationally induced mass flow of N may, under some circumstances, override root N uptake capacities and that this would be especially relevant for nitrate. Higher rates of mass flow towards roots compared to root uptake rates of nitrate would lead to the formation of accumulation, rather than depletion zones around roots. In this scenario, root growth, and thus carbon costs for plant N acquisition would be significantly reduced and plant optimization of N acquisition would be fundamentally different from a scenario in which diffusion dominates root N supply. A further exploration of how plants optimize for capturing N through diffusion and mass flow, respectively, seems warranted.

5. Conclusions

Estimating the relative contribution of diffusion and mass flow to plant N nutrition is inherently difficult. Here, we applied a setup of the microdialysis technique earlier developed for this purpose and tested in the laboratory. Our approach enables sampling of soil in the presence and absence of mass flow to study soil N fluxes for the first time *in situ,* in two boreal forest ecosystems. Thus, our study was designed to assess the potential rather than the actual importance of mass flow in the boreal forest. In the absence of mass flow, N fluxes were dominated by amino acids in both soils. In the presence of mass flow, NO_3^- had a more prominent share of the total N flux in both soils and was even the dominating form of N in the nutrient rich soil. Our results suggest mass flow, and hence transpiration, may be a strong driver of N acquisition for boreal forest plants.

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