

Early season dynamics of soil nitrogen fluxes in fertilized and unfertilized boreal forests



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ABSTRACT

The supply of soil nitrogen (N) for plant uptake largely controls plant growth and has a major impact on a wide range of biogeochemical processes in terrestrial ecosystems. The soil solution typically contains a large variety of N forms and recent evidence suggests that the share of amino acids to soil N fluxes dominates over inorganic N in boreal forest soils. In this study we applied a microdialysis technique to investigate in-situ induced diffusive fluxes across microdialysis membranes (F_{MD}) in fertilized and non-fertilized boreal forest sites in early spring, at the onset of plant growth. We studied temporal shifts of F_{MD} at short (minutes to hours) and prolonged time-scales (hours to days). We also estimated N pools in soil water and KCl extracts and critically evaluated the significance of results depending on the method chosen. Our results indicate that F_{MD} of boreal forest soil is dominated by amino acids in early spring and that growing roots should encounter the full range of organic and inorganic N forms in these soils. In contrast, soil water and KCl extracts were dominated by NH_4^+ . Some amino acids displayed rapidly declining F_{MD} (<1 h) possibly due to the rapid formation of a depletion zone near the membrane surface but the F_{MD} of most amino acids remained high and unchanged over extended periods of dialysis indicating that these soils provide a continuous supply of amino acids for root uptake. Forest fertilization with NH_4NO_3 led to a significant increase in F_{MD} of NO_3^- and NH_4^+ , with F_{MD} of NH_4^+ but not of NO_3^- remaining high for prolonged time. A separate trial with addition of NO_3^- showed a significantly slower decline of F_{MD} in soils of previously fertilized forests compared to unfertilized forests, suggesting biological immobilization being a major cause of rapid decline of nitrate fluxes. Our results corroborate earlier studies suggesting amino acids to be a significant fraction of plant available N in boreal forests. They also suggest that, besides inorganic N, roots may encounter a wide spectrum of amino acids after intercepting new soil microsites and that most, but not all, amino acids may be constantly replenished at the root surface. Further, from our results we conclude that detailed insights into in-situ N dynamics of soils can be gained through microdialysis.

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1. Introduction

Plant nitrogen (N) nutrition in terrestrial ecosystems is largely dependent on the supply of N from the surrounding soil to the surface of roots and mycorrhizal fungi. The pool of plant available N consists of many N forms, including inorganic N and amino acids (Näsholm et al., 2009), and a large range of other organic N compounds of varying molecular size (Paungfoo-Lonhienne et al., 2008, 2012; Warren, 2013a, b) suggesting that plants may have access to a diverse pool of N for their nutrition. It has been shown that plants

are indeed capable of taking up many of these organic N compounds, particularly amino acids (e.g., Kielland, 1994; Näsholm et al., 1998; Jämtgård et al., 2008; Paungfoo-Lonhienne et al., 2008; Näsholm et al., 2009). Still, plant N acquisition is a complex process and one principal finding, independent of N form studied, is that it is fundamentally controlled by the availability and continuous replenishment of N at the root surface, rather than by root uptake kinetics or bulk soil N concentrations (Nye and Marriott, 1969; Nye and Tinker, 1977; Clarkson and Hanson, 1980; Leadley et al., 1997; Tinker and Nye, 2000). Active roots (and mycorrhizal hyphae) have three principal mechanisms for encountering N: diffusion, mass flow and interception (Chapin et al., 2002) of which diffusion is deemed the dominating process in most low N ecosystems. Root cells induce diffusion towards root surfaces by active

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uptake at cell membranes. The reliable estimation of N supplied for root uptake by diffusion in undisturbed soils is, therefore, crucial for a better understanding of plant growth and performance. Until recently, however, it was not possible to directly measure diffusion with traditional sampling methods commonly used by soil scientists. Further, many methods such as soil extractions are severely disrupting to the natural soil matrix. Additional soil sample treatments further increase the risk of introducing errors such as continuous N transformations, contaminations or losses (Jones and Willett, 2006; Rousk and Jones, 2010; Warren and Taranto, 2010; Inselebacher, 2014). To overcome these problems, several alternative sampling techniques such as soil centrifugation and in-situ perfusion or lysimeter sampling have emerged aiming at reflecting nutrient availabilities and dynamics in undisturbed soils (Giesler and Lundström, 1993; Weihermüller et al., 2007; Chen and Williams, 2013). From a plant nutritional perspective, a suitable sampling method allows estimating induced soil N fluxes thereby enabling for estimation of potential rates of root uptake of N from the soil (Nye, 1979; Shaver and Chapin, 1991; Leadley et al., 1997; Inselebacher and Näsholm, 2012a). One such technique based on passive microdialysis was recently introduced for environmental research (Öhlund, 2004; Sulyok et al., 2005; Miro and Hansen, 2006; Miro et al., 2010). This non-invasive technique induces a diffusive flux over a semi-permeable membrane driven by the concentration gradient between the perfusate solution on the inside of the membrane and the soil solution on the outside (Kehr, 1993; Torto et al., 2001; Seethapathy et al., 2008). When using high-purity deionized (MilliQ) water as perfusate, all free compounds with a molecular size smaller than the molecular cut-off of the membrane (20 kDa in the present study) will diffuse across the membrane into the constant stream of MilliQ water pumped through the system (for a detailed setup of the microdialysis system see section 2.1. in the Material & Methods; see also Inselebacher et al., 2011). A recent study used a solution of low osmotic potential (Dextran 20) instead of MilliQ water as perfusate which induced mass flow of soil water into the perfusate and, therefore, allowed for the simultaneous estimation of N passing the microdialysis membrane via diffusion and via mass flow (Oyewole et al., 2014). However, until now this technique for directly estimating mass flow has not been tested and evaluated under field conditions and was not included in the present study. The small dimension of the microdialysis probes used in the present study (10 mm length and 0.5 mm outer diameter) allows for virtually non-invasive installation in soils and for subsequent monitoring the dynamics of induced diffusive fluxes of N from the soil across the membranes. Hence, in the present study we applied the microdialysis technique to investigate temporal shifts in diffusive N fluxes from soils across the microdialysis membranes at a high temporal resolution (20 min intervals).

In a recent comparative study, this microdialysis approach revealed that amino acids dominated the induced diffusive N fluxes of 15 boreal forest soils across the microdialysis membranes while inorganic N accounted for less than 20% of the total induced diffusive N flux (Inselebacher and Näsholm, 2012a). This was in clear opposition to the results of extracted soil in which NH_4^+ was the major N compound (~80%), a common finding in soil water extracts (e.g., Likens et al., 1969; Robertsson, 1982; Kronzucker et al., 1997; Rothstein, 2009). This difference in results depending on the sampling method chosen was surprising, but also raised a number of questions. First, diffusive fluxes of N across microdialysis membranes installed in boreal forest soils were previously assessed only during the late growing season (Inselebacher and Näsholm, 2012a) but information on N availabilities at the onset of growth in early spring is critical for evaluating the importance of different N forms for plant growth. As boreal forest plants are commonly N-

limited, available soil N pools may be significantly depleted in autumn relative to early spring and hence the composition of plant available N may exhibit larger shares of inorganic N during this period. Second, the significant discrepancy between sampling techniques led to the question, if a similar pattern would be observed when soil N pool composition is expected to be different, as e.g. shortly after freeze-thaw cycles or during drying and re-wetting cycles in early spring. Third, based on the results from a previous laboratory study, induced diffusive fluxes of individual N forms across the microdialysis membrane surface are decreasing over time due to the formation of a depletion zone, similar to depletion zones forming around active roots (Tinker and Nye, 2000; Leitner et al., 2010; Inselebacher et al., 2011). Thus, concentrations of available N close to root surfaces may be significantly dissimilar from those of bulk soil. However, this depletion effect was only shown in homogenized soils, but not in undisturbed soils in-situ. Due to the highly dynamic turnover of N in soils (e.g. Rousk and Jones, 2010) and simultaneous uptake and immobilization of N by plants and soil microbes in natural soils, induced diffusive N fluxes across microdialysis membranes are expected to vary significantly, both on a short-term (within minutes to hours) and a long-term (within several hours to days) basis. Furthermore, a recent review argues that damaging of hyphae and fine roots during microdialysis membrane installation could lead to a short-term pulse of low molecular weight organic N compounds such as amino acids (Hobbie and Hobbie, 2013). In order to avoid an over-estimation of the relative contribution of amino acids due to such methodological artifacts it is therefore necessary to monitor induced diffusive N fluxes across membranes over longer periods of time.

To address these questions, we tested the hypotheses that (1) induced diffusive N fluxes across microdialysis membranes installed into fertilized and non-fertilized boreal forest soils will exhibit significantly higher proportions of inorganic N at the onset of plant growth in spring compared to later times of the year, (2) the relative contribution of amino acids to induced diffusive fluxes of total N (sum of inorganic N and amino acids) across microdialysis membranes will be significantly higher than their contribution to the total N pool estimated by standard soil extractions, and (3) diffusive fluxes of individual N compounds across microdialysis membranes will change significantly, both during short (minutes to hours) and prolonged (hours to days) time periods.

2. Material and methods

2.1. The microdialysis system

2.1.1. Setup of the microdialysis system

The microdialysis system was set up as described previously (Inselebacher et al., 2011). In detail, two syringe infusion pumps (CMA 400) were equipped with a total of eight gas-tight microsyringes (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, 0.5 mm outer and 0.4 mm inner diameter) with a 20 kDa molecular weight cut-off. There are many different kinds of microdialysis membranes available and we chose the CMA 20 membranes for our study as they have already been thoroughly evaluated for their suitability to sample inorganic N and amino acids (Inselebacher et al., 2011). The relative and absolute recovery (RR and AR, see below) have been shown to be satisfactory also at higher flow rates and the material of the membranes is not interacting with the target N compounds, as no binding of them to the membrane surface was observed (Inselebacher et al., 2011). Further, the experimentally estimated diffusive flux of a range of N

compounds across the CMA 20 membrane fitted well with theoretical calculations of their diffusion in water based on the Einstein–Stokes equation (Inselsbacher and Näsholm, 2012b). Last but not least, these membranes have already been used in a series of previous studies on N availabilities in soil allowing a direct comparison of results (Inselsbacher et al., 2011; Inselsbacher and Näsholm, 2012a, b; Oyewole et al., 2014). The probes were perfused with MilliQ water and samples (dialysates) were collected with two refrigerated microfraction collectors (CMA 470) in 300 µl glass vials. The temperature in the microfraction collectors was set to 6 °C at all times. All equipment is commercially available at CMA Microdialysis AB (Solna, Sweden).

2.1.2. Calibration of the microdialysis membranes

Passive microdialysis is a membrane-based sampling technique where the sole driving force for sampling the target compounds is the concentration gradient across a semi-permeable membrane (Ungerstedt, 1991; Torto et al., 2001; Seethapathy et al., 2008). This gradient depends on the difference in concentration of the target compounds in the perfusate and the outer medium (soil in the present study). To guarantee the correct performance of each membrane, it is necessary to calibrate them by assessing the relative recovery (RR) of each target compound from an outer medium.

In detail, the calibration of the microdialysis membranes was performed before and after each sampling event in order to guarantee the correct performance throughout the experiments. For calibration microdialysis probes were submerged in a standard solution containing NH_4^+ , NO_3^- and 18 amino acids (AAS 18 standard solution, plus additional glutamine and asparagine; Sigma Aldrich). The solution was 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 perfusion flow rate was set at $5.0 \mu\text{l min}^{-1}$ and dialysates were collected continuously at 30 min intervals for 2.5 h. Ammonium, NO_3^- and amino acids in the dialysates were analysed as described below. The RR, given as percentage of standard recovered in the dialysate, was then calculated based on the microdialysis probe calibration (Bungay et al., 1990; Torto et al., 2001; Nandi and Lunte, 2009):

$$\text{RR}(\%) = 100 * C_{\text{dial}} / C_{\text{std}} \quad (1)$$

Where C_{dial} is the concentration of the measured compound in the dialysate, i.e. that has diffused across the microdialysis membrane and C_{std} is the concentration of the compound in the standard solution.

The RR of NH_4^+ , NO_3^- and individual amino acids depended on the molecular weight of each N compound and ranged from 19 to 37%. These values of RR estimated for the CMA 20 membranes at a perfusion flow rate of $5 \mu\text{l min}^{-1}$ are identical to RR of individual N compounds estimated under the same conditions in previous studies (Inselsbacher et al., 2011; Inselsbacher and Näsholm, 2012a, b; Oyewole et al., 2014).

Similarly, we determined the absolute recovery (AR) of each N compound from the soil by estimating the total amount of N in the dialysate. We then calculated diffusive fluxes across the membrane surface during a given sampling time according to Inselsbacher and Näsholm (2012a):

$$F_{MD} \left(\text{nmol cm}^{-2} \text{h}^{-1} \right) = C_{\text{dial}} / A_{MD} * t \quad (2)$$

Where F_{MD} is the diffusive flux across the probe membrane, A_{MD} is the membrane surface (15.9 mm^2) and t is sampling time.

Further, the mass transfer across the microdialysis probe membrane (and consequently rates of RR) is influenced by

perfusion flow rate, membrane properties, and the resistances of analyte transport across the membrane, through the perfusate and through the soil (Stenken et al., 2001; Plock and Kloft, 2005). Accordingly, the simplified calculation of RR given in equation (1) can be defined in more detail as:

$$\text{RR} = 100 * \{ 1 - \exp[- 1 / (Q_d * (R_d + R_m + R_{\text{ext}}))] \} \quad (3)$$

Where Q_d is the perfusion flow rate, R_d , R_m and R_{ext} are the resistances to the dialysate, membrane and external factors. The term $(R_d + R_m + R_{\text{ext}})$ is also termed the permeability factor (Miro et al., 2010; Miro and Frenzel, 2011).

There is a plentitude of studies addressing microdialysis membrane properties, calibration methods and other factors influencing the performance of microdialysis experiments and we would like to refer the readers to these (some examples are Bungay et al., 1990; Torto et al., 1999; De Lange et al., 2000; Weiss et al., 2000; Stenken et al., 2001; Miro and Frenzel, 2004, 2005; Plock and Kloft, 2005; Nandi and Lunte, 2009; Torto, 2009; 2001, 2002; Miro et al., 2010, Tsai, 2011). However, relatively little is known when applying this technique in soil, especially when working in in-situ environments. One apparent difficulty when working in complex matrixes such as soil is that R_{ext} is expected to be contributing most to the permeability factor but is difficult to calculate (Hsiao et al., 1990; Lafontan and Arner, 1996; Miro and Frenzel, 2011). While microdialysis membranes can be calibrated by estimating the RR of N compounds from a stirred standard solution, it is not possible to directly relate such results to results from in-situ experiments. The main reason for this is that in soil R_{ext} , and consequently FMD, is strongly affected by soil factors such as soil tortuosity, volume fraction accessed by the probe, impedance (for charged molecules), buffer capacity and supply and removal rates from competing biological processes (Plock and Kloft, 2005). Therefore, while it is difficult to get reliable estimates of the in-situ concentration of individual N compounds in the soil surrounding the membrane surface, we submit that removal of solutes by a microdialysis probe exhibit similarities with that of removal (uptake) of solutes by plant roots and as such microdialysis probe recovery of substances from the soil provides information on potential root uptake rates from soil as well as the composition of the N pool that is accessible for uptake.

2.1.3. Change in sample volume during storage in the fraction collector

Additionally, we performed a quality control test to ensure the delivery of the correct amount of dialysate by the pump and the stability of the sample volumes in the microfraction collector. In detail, microdialysis probes were submerged in MilliQ water and MilliQ water was also used as perfusate to guarantee equal water potentials on the inside and outside of the membrane. The perfusion flow rate was set at $1.0 \mu\text{l min}^{-1}$ and dialysates were collected for 2 h resulting in a total sample volume of 120 µl. Samples were weighed on a high-precision scale (AT 460 Delta Range, Mettler Toledo International Inc., Greifensee, Switzerland) immediately after sampling. Samples were then left in the fraction collector with cooling turned on (6 °C) or at room temperature (22 °C) and weighed again at 2 h intervals for 24 h to estimate losses of sample from the vials by evaporation. We found no significant change in sample volume with active cooling during 24 h ($P > 0.05$) but a constant and significant ($P < 0.001$) loss of $0.9\% \text{ h}^{-1}$ of sample volume when samples were left at 22 °C (data not shown). This shows that for long term sampling events the fraction collector should be cooled at all times and that, optimally, vials should be closed directly after each sampling interval and stored refrigerated

or on ice. Hence, all following experiments in this study were performed with cooled (6 °C) fraction collectors.

2.2. Site description, soil properties and experimental setup

The experiments were performed at a Scots pine heath forest site at the Rosinedal Research area (cf. Oyewole et al., 2014) in the vicinity of Umeå, Sweden (64°10'20" N, 19°44'30" E). The soil is classified as a sandy glacial till Haplic Podzol (FAO, 1998; WRB, 2006) with 20 g kg⁻¹ silt, 980 g kg⁻¹ sand and 10 g kg⁻¹ gravel. The organic layer is 5–10 cm deep, has a pH (H₂O) of 5.2 and a C to N ratio of 38.7. Average annual precipitation is 587 mm and the annual mean air temperature is 1.9 °C. The tree layer is dominated by *Pinus sylvestris* and the understorey by ericaceous shrubs (*Vaccinium myrtillus*, *Vaccinium vitis-idaea*), mosses (*Pleurozium schreberi*, *Dicranum* sp.) and lichen (*Cladonia rangiferina*). Total wet and dry N deposition in the study area is approximately 2 kg N ha⁻¹ yr⁻¹ (Gundale et al., 2011).

In 2006 a large-scale N addition experiment was established, in which experimental plots (15 ha) received either no fertilizer input or 100 kg N (in the form of NH₄NO₃) ha⁻¹ yr⁻¹. In 2012 fertilizer was applied in the beginning of May, two weeks before the experiments were performed.

2.3. Microdialysis sampling and soil extraction

A series of microdialysis experiments were conducted in the end of May 2012 to estimate diffusive fluxes of individual N compounds from the soil across microdialysis membranes. Four sub-plots (each 9 m²) within each experimental forest (control and fertilized forests) were randomly chosen for the experiments. Within each sub-plot, 4 microdialysis probes were installed in the organic soil layer. In detail, the understory growth and the 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. Thereafter, the probes were carefully inserted into the prepared channel using a special designed split tubing introducer to protect the membrane surface (CMA Microdialysis AB, Solna, Sweden). The soil surrounding the probes was then slightly compressed to ensure full contact of the membrane surface with the surrounding soil layer. As active root N uptake of boreal forest plants in this area generally takes place in the organic soil layer (e.g. Huang and Schoenau, 1997), special care was taken not to insert the membranes into the sand layer underneath. The probes were then left in the soil throughout the experimental period. Soil samples ($n = 5$) were taken from the organic soil layer close to the probes (within 50 cm) and soil water contents were estimated gravimetrically after oven-drying (105 °C for 48 h). Soil water contents ranged between 47 and 57% at times of sampling, and were high enough to guarantee diffusion of N compounds through soil towards the microdialysis membranes. As soil water content is affecting the impedance factor of soil, and consequently diffusion, this variation in soil water contents might have affected the effective diffusion coefficient of different N compounds in soils (Olesen et al., 2001). However, compared to other factors such as ion exchange capacity of soils and rapid microbial turnover of N, the effect of the observed variation in soil water content is expected to be relatively small.

In a first step, short-term dynamics of F_{MD} were estimated. To accomplish this, MilliQ water was used as perfusate, the perfusion flow rate set to 5.0 µl min⁻¹ and samples were collected continuously at 20 min intervals during a total sampling time of 100 min. Samples were stored at -20 °C until chemical analyses (within 2 weeks) as described below. In a second step, we monitored daily shifts of F_{MD} of NO₃⁻ by simulating a small-scale fertilization event and continuously taking samples for 25 days. Two sub-plots within

each forest site (control and fertilized forest) were sprinkled with water (10 l m⁻²) and two other sub-plots within each site were sprinkled with water (10 l m⁻²) containing KNO₃ (1 g N l⁻¹). Solutions were applied 1 day after the initial microdialysis sampling and samples were taken again after 4 h, 1, 4, 10, 18 and 25 days after sprinkling. Samples were collected for a period of 100 min at each time point and kept on ice and analysed for NO₃⁻ concentrations within 2 weeks.

Additionally to microdialysis sampling we took soil samples for standard soil extractions. Soil samples ($n = 5$) were collected from the uppermost organic soil layer (0–10 cm) from all subplots at the same day as the initial microdialysis sampling and immediately stored at 4 °C until further processing. Coarse debris, needles and stones were removed manually and soils were then sieved (<2 mm mesh size) and homogenized. Aliquots of sieved soil (4 g FW) were extracted with 30 ml of either MilliQ water or KCl (2 M), shaken for 90 min and subsequently filtered through ashless filter paper (Whatman filter paper, ashless, Grade 41). Samples were then immediately analysed for NH₄⁺, NO₃⁻ and amino acid concentrations as described below.

2.4. Chemical analyses

Amino acids, NH₄⁺ and NO₃⁻ in microdialysis samples and soil extracts were analysed as described previously (Inselsbacher et al., 2011). Briefly, NH₄⁺ and amino acids were analysed 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 samples (20 µl) were derivatized with a Waters AccQ-Tag Ultra Derivatization kit for amino acid analysis and individual amino acids were separated on an AccQ-Tag Ultra column. Nor-valine was used as internal standard. Nitrate was analysed by the Vanadium (III) chloride (VCl₃) and Griess method as described by Hood-Novotny et al. (2010) based on the technique described by Miranda et al. (2001). MilliQ water and KCl (2M) was analysed for concentrations of NH₄⁺, NO₃⁻ and amino acids for blank corrections. Standards of each N form were analysed and ranged from 0.1 to 100 µmol l⁻¹.

2.5. Calculations and statistical analysis

Statistical analyses were carried out using one-way ANOVA followed by Tukey's honestly significant difference post-hoc test using Statgraphics 5.0 (Statistical Graphics Inc., Rockville, MD, USA). When necessary, data were either square-root of log₁₀-transformed before analysis to meet the assumptions of ANOVA after testing normality using a Kolmogorov–Smirnov test and homogeneity of variances using Bartlett's test. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Diffusive fluxes of N across microdialysis membranes

Average F_{MD} of N in undisturbed forest soils assessed by in-situ microdialysis were dominated by amino acids, contributing 82% and 67% of total N (sum of NH₄⁺, NO₃⁻ and amino acids) flux in the control and fertilized soils, respectively (Fig. 1). Ammonium contributed 17% in the control site and was significantly higher (32%) in the fertilized forest site. F_{MD} of NO₃⁻ was low in both sites contributing only 1.3% to total N fluxes. With the exception of arginine and methionine, all proteinic amino acids were found in the dialysates. In most cases, F_{MD} of amino acids were similar in both control and fertilized soils (Fig. 1). F_{MD} of only 3 out of the 18 amino acids analysed were different in the two sites. In detail, F_{MD}

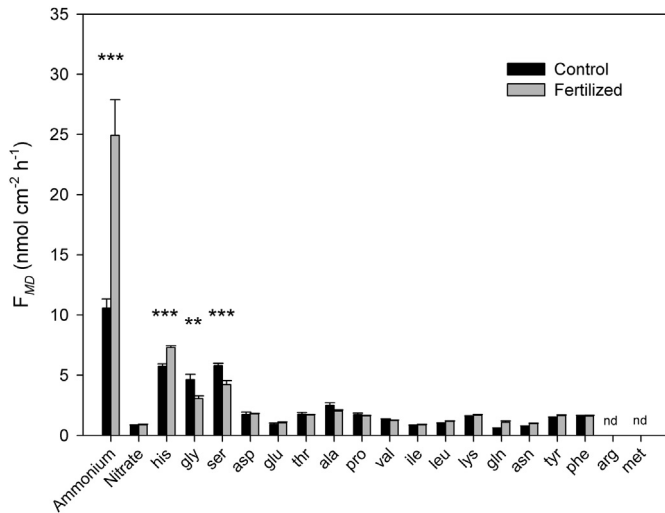


Fig. 1. F_{MD} (diffusive flux across the microdialysis membrane) of ammonium, nitrate and amino acids estimated by microdialysis in control (no N) and fertilized ($100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) boreal forest soils. Bars represent means \pm SE ($n = 40$). Asterisks indicate significant differences between control and fertilized soils. *** $P < 0.001$, ** $P < 0.01$, $P < 0.05$, nd not detected.

of histidine was significantly higher in the fertilized site while F_{MD} of glycine and serine were higher in the control site (Fig. 1).

When extending the microdialysis sampling time and monitoring F_{MD} by taking samples at 20 min intervals we found clear differences in temporal patterns between N forms and soils. In the control site F_{MD} of NH_4^+ , NO_3^- (Fig. 2a) and most amino acids (Fig. 2b) did not change significantly ($P > 0.05$) during the 100 min sampling period. However, phenylalanine was present only at the beginning of the sampling and could not be detected at later time points. Moreover, tyrosine and asparagine declined below detection limit after 40 min and lysine and glutamine after 80 min (Fig. 2c). In the fertilized forest site we found a significant, time-dependent, decrease of F_{MD} of NH_4^+ (Fig. 2d) and 6 amino acids (Fig. 2f). In detail, phenylalanine, tyrosine and glutamine were only found in the first 20 min of sampling and leucine, lysine and asparagine decreased below detection limit after 40 min of sampling. For all other amino acids and NO_3^- no temporal shift was observed during the total 100 min of sampling (Fig. 2d, e).

In a separate study, we monitored the temporal dynamics of F_{MD} of NO_3^- in fertilized and control soil following a pulse-addition of nitrate. We simulated a fertilization event while at the same time leaving microdialysis probes in the soils and taking samples during the following 25 days. When only water was applied to the soils, no shift in F_{MD} of NO_3^- was found at any time in any of the plots ($P > 0.05$; Fig. 3). On the contrary, four hours after applying NO_3^- fertilizer a significant increase in F_{MD} of NO_3^- was observed at both sites, with a 3.2-times higher increase in the fertilized plot (Fig. 3). After 1 day, F_{MD} of NO_3^- in the fertilized plot further increased to $33.1 \pm 5.9 \text{ nmol N cm}^{-2} \text{ h}^{-1}$, while it remained at a constant level in the control plot ($6.9 \pm 0.5 \text{ nmol N cm}^{-2} \text{ h}^{-1}$). After this initial increase, F_{MD} of NO_3^- displayed a continuous decrease during the subsequent days. Ten days after fertilizer application F_{MD} of NO_3^- had returned to background levels again in the control plots ($P > 0.05$) while this process required 18 days in the fertilized plots (Fig. 3). Integrated over the total experimental time period of 25 days, total F_{MD} of NO_3^- in the control and fertilized soils were similar when only water was applied, being 696 and 646 nmol N cm^{-2} , respectively. After application of NO_3^- , total F_{MD} of NO_3^- were significantly higher in both soils, and F_{MD} of NO_3^- in the previously fertilized soil was 3.8 times higher than in the control soils (3730 and 987 nmol cm^{-2}).

3.2. Free and exchangeable N

Concentrations of free N estimated by water extraction of soils were dominated by NH_4^+ at both the control and the fertilized site, contributing on average 93% to the total soil solution N analysed, while NO_3^- and amino acids only amounted for 1 and 6%, respectively. Further, only 6 amino acids were detected in the water extracts, namely serine, glycine, glutamic acid, threonine, alanine and isoleucine (Fig. 4a). While concentrations of inorganic N, glutamic acid, threonine and alanine were similar in control and fertilized plots ($P > 0.05$), concentrations of serine, glycine and isoleucine were significantly higher in the control plot ($P < 0.001$).

A similar pattern was found for the sum of free and exchangeable N estimated by KCl extraction (Fig. 4b). Ammonium was the dominant N form, contributing 94% of the total N pool while NO_3^- accounted for less than 1%. Compared to water extracts the total N pool was 3.3-times and 3.9-times higher in the KCl extracts in the control and fertilized plots, respectively. The concentrations of amino acids were generally higher in KCl than in water extracts and, unlike in water extracts, all but 3 amino acids (histidine, methionine and phenylalanine) were found. This led to a 3.5-fold and 4.9-fold increase of total amino acid N in control and fertilized plots, respectively. With the exception of NO_3^- , which was significantly higher in the fertilized plots ($P < 0.01$), all other N compounds were higher in the control plots, although not in all cases significantly (Fig. 4b).

4. Discussion

4.1. Microdialysis as a tool to estimate soil N supply for root uptake

We set out to test if (1) induced diffusive fluxes of N across microdialysis membranes (F_{MD}) installed in boreal forest soils will exhibit significantly higher proportions of inorganic N at the onset of plant growth in spring compared to later times of the year, (2) the relative contribution of amino acids to F_{MD} of total N (sum of inorganic N and amino acids) will be significantly higher than their contribution to the total N pool estimated by standard soil extractions, and (3) F_{MD} of individual N compounds will change significantly, both during short (minutes to hours) and prolonged (hours to days) time periods.

In our study, a high flow rate ($5 \mu\text{l min}^{-1}$, which is at the higher end of flow rates used in microdialysis experiments) was used to ensure high AR by keeping diffusional gradients between the perfusate and the surrounding soil high. With these settings, membranes act as strong sinks of N, similar to active plant roots which continuously deplete soil N through active uptake. Clearly, there are some significant differences between the purely passive and non-selective sampling of N compounds by microdialysis compared to the active and selective uptake of N by roots, but there are several apparent similarities stressing the advantage of using microdialysis over other sampling techniques. First, the most important feature of microdialysis is that it allows estimation of F_{MD} of N in virtually undisturbed soils, a capacity which cannot be achieved by any other sampling technique. Second, the small dimensions and the cylindrical form of the microdialysis membranes used in this study are similar to plant fine roots and, therefore, allow for sampling available N at scales relevant for plants. Third, the constant flow of perfusate through the microdialysis system induces the diffusion of N compounds across the membrane with a known surface area. Similarly, continuous uptake of N by plant roots creates a diffusive gradient between N concentrations at the root surface and in the surrounding soil. And last but not least, F_{MD} depends on the constant replenishment of N at the membrane surface which can be estimated by consecutive sampling, as was

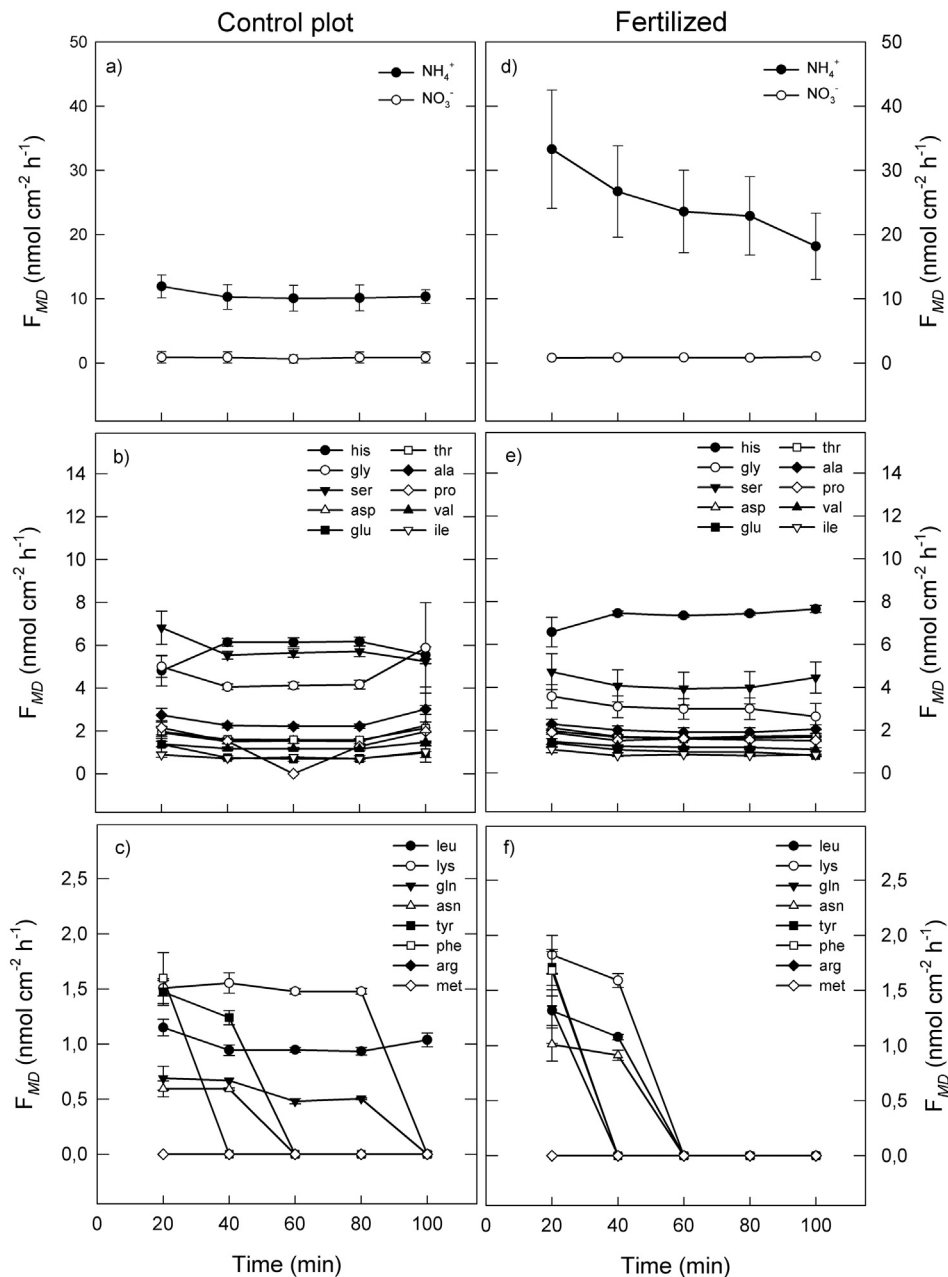


Fig. 2. Time dependency of F_{MD} (diffusive flux across the microdialysis membrane) of a) and d) ammonium and nitrate, b) and e) histidine, glycine, serine, aspartic acid, glutamic acid, threonine, alanine, proline, valine and isoleucine, and c) and f) leucine, lysine, glutamine, asparagine, tyrosine, phenylalanine, arginine and methionine in control (a, b, c) and fertilized ($100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$; d, e, f) boreal forest soils. Symbols represent means \pm SE ($n = 16$).

done in the present study. Similarly, roots rely to a great part on the replenishment of N at the root surface via diffusion, mass flow, ion-exchange and new input through metabolism by exoenzymes and through exudation and leakage from soil organisms. Consequently, from a plant perspective microdialysis has great potential to simulate a root and, by estimating F_{MD} , giving unique insights into the dynamics of individual N forms at the membrane surface.

Again, it has to be noted that there are still several issues related to plant N nutrition that cannot be resolved with the application of microdialysis. This technique does not simulate active root uptake behaviour through ion-channels and transporters. Further, microdialysis probes are installed at fixed positions in soil while plant roots explore new patches of soil through constant growth. Plant roots are also able to gain access to N bound to soil particles by ion-

exchange with compounds commonly exuded by roots. Microdialysis may theoretically have the potential to simulate root exudation (Miro and Frenzel, 2011) but until now there is no experimental proof for this approach.

4.2. Availability of individual N forms for plant uptake

Plant growth and biomass production greatly depend on the availability of N at the root surface and the replenishment of N following root uptake. The most important mechanisms responsible for this are the depolymerization of proteins to peptides and amino acids, one of the bottlenecks in the N cycle of N-limited ecosystems (Schimel and Bennett, 2004) and the continuous supply of plant available N from the bulk soil to the root surface. Thus,

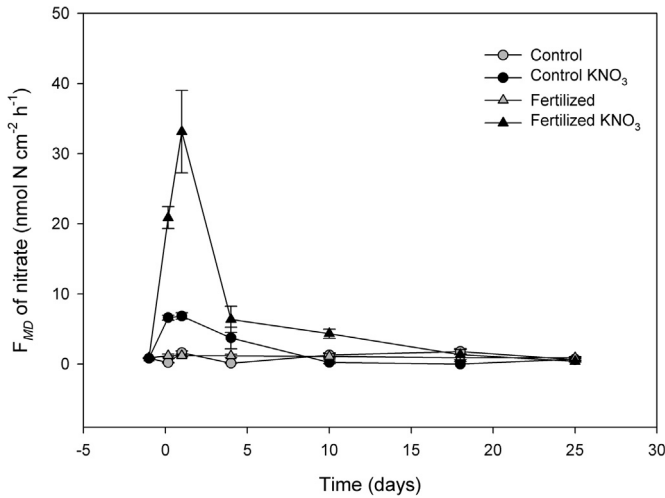


Fig. 3. Response of F_{MD} (diffusive flux across the microdialysis membrane) of nitrate to a single pulse of nitrate addition. Samples were taken 1 day before and 4 h, 1, 4, 10, 18, and 25 days after application of water (10 l m^{-2} , grey symbols) or KNO_3 (1 g N l^{-1} ; black symbols) to control (circles) and fertilized ($100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$; triangles) boreal forest soils. Symbols represent means \pm SE ($n = 8$).

estimates of diffusion and mass flow, the two main drivers of soil N supply are better indicators of N availability than potential N turnover rates or soil solution N concentrations (Nye, 1979; Clarkson and Hanson, 1980; Shaver and Chapin, 1991; Marschner, 1995; Leadley et al., 1997; Tinker and Nye, 2000; Comerford, 2005; Lambers et al., 2008).

In the present study, we estimated F_{MD} of individual N forms in boreal forest soils applying the microdialysis technique (Inselsbacher et al., 2011; Inselsbacher and Näsholm, 2012a, b) and free and exchangeable soil N concentrations by extracting soils.

Our results show that amino acids accounted for the majority of F_{MD} in undisturbed soils in spring (Fig. 1). Further, among the 18 amino acids analysed all but two (arginine and methionine) were found in dialysates and were, therefore, potentially available for plant uptake. On the contrary, NH_4^+ dominated the pool of free N estimated in soil water extracts and only 5 amino acids were detected (Fig. 4a), a pattern which was found at these sites at the

end of the growth period in autumn (Inselsbacher and Näsholm, 2012a) and is in agreement with previous studies (e.g., Likens et al., 1969; Robertsson, 1982; Kronzucker et al., 1997; Rothstein, 2009). Higher concentrations and a significantly higher diversity of amino acids (15 individual amino acids) were recovered when extracting soils with KCl (Fig. 4b), indicating that plant roots would mainly encounter NH_4^+ and only a negligible share of amino acids, which are bound to soil particles and accessible for plant uptake only after exchange with other ions, such as those commonly found in root exudates. Our in-situ measurements of F_{MD} , however, gave a strikingly opposite result. We found that amino acids contributed approximately 80% of N available at the membrane (and therefore also potentially root) surface and that plant roots in the field are exposed to the full range of protein amino acids instead of only a few dominant ones (Fig. 1). As boreal forest plants were shown to have the capacity to absorb all amino acids present at the root surface (Persson and Näsholm, 2001) this implies that the share of amino acids for plant nutrition may be higher than previously believed and that this share is high also at the period of rapid plant growth in the boreal forest. This significant discrepancy of results depending on the technique used for estimating free available N is striking and corroborates previous findings (Inselsbacher and Näsholm, 2012a). As has been highlighted before (Thomsen and Schjonning, 2003; Johnson et al., 2005; Miro and Frenzel, 2011), our results once again highlight that results from soil extractions may not reflect N availabilities in undisturbed soils and that low-invasive sampling methods such as the microdialysis technique should be preferred for future assessment of plant-available N.

4.3. Early season diffusive N fluxes

This study investigated F_{MD} of individual N forms at the onset of plant growth in early spring, a period of critical importance for plant growth in the boreal biome. We expected that F_{MD} would differ significantly in spring compared to at later times of the growing season, not least because spring is a period of intense growth in boreal ecosystems and of frequent freeze-thaw cycles affecting soil N turn-over (Ivarson and Sowden, 1966; Edwards and Cresser, 1992; Lipson and Monson, 1998). However, our results indicate that the relative shares of NH_4^+ , NO_3^- and amino acids to F_{MD} of total N are largely similar at both seasons in the control plot

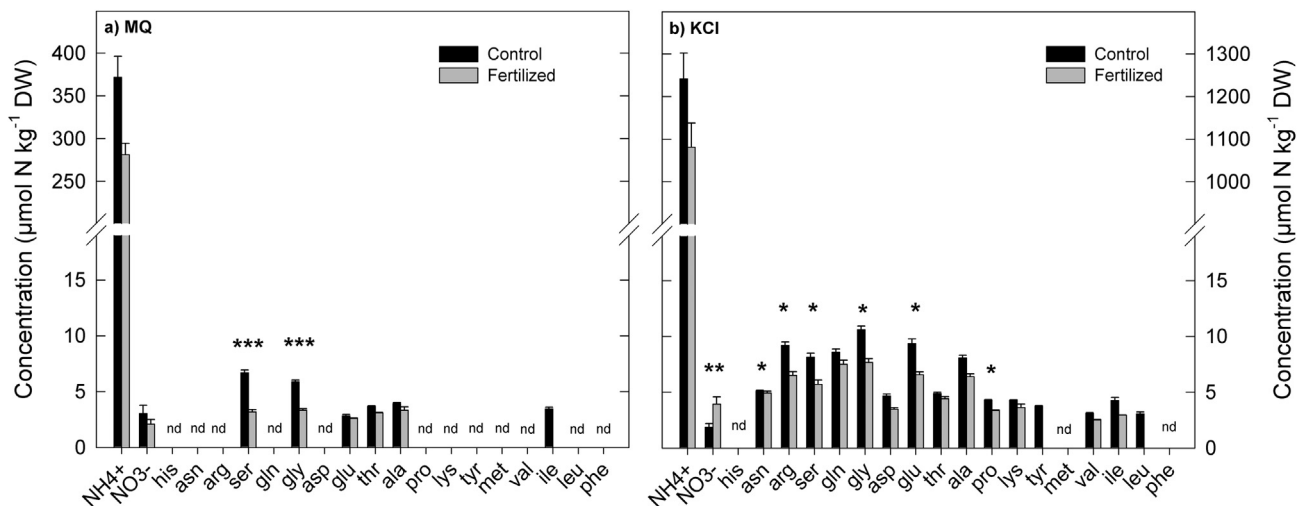


Fig. 4. Nitrogen forms in boreal forest soil as determined by conventional extraction procedures. Concentrations of a) free and b) free and exchangeable ammonium, nitrate and amino acids estimated by extracting boreal forest soils from control (no N) and fertilized ($100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) sites with water or KCl (2 M), respectively. Bars represent means \pm SE ($n = 5$). Asterisks indicate significant differences between control and fertilized soils. *** $P < 0.001$, ** $P < 0.01$, $P < 0.05$, nd not detected.

but that NH_4^+ contributed considerably more to total N in the fertilized plot in early season (32% of total N compared to 15% in autumn). The latter probably reflects that this forest was fertilized with NH_4NO_3 two weeks before sampling. Contrary to NH_4^+ , however, we found no increase in F_{MD} of NO_3^- at the fertilized sites compared to the control plots (Fig. 1). This was surprising, as we would have expected an increase of both inorganic N forms after applying large amounts of NH_4NO_3 . The reason for this is not known but is in line with the longer retention of NH_4^+ in the soil due to its higher capacity to bind to soil particles while NO_3^- is comparably mobile in soil (Chapin et al., 2002). Similarly, rapid decline of F_{MD} of NO_3^- was also found in our experiments simulating fertilization and subsequent monitoring of F_{MD} of NO_3^- . Thus, NO_3^- was available for plant uptake during the first days after application but not longer than 2 weeks (Fig. 3). While we did not follow the fate of applied NO_3^- into plants, soil microbes or leachate our results show that fertilizer applied as NO_3^- is available in the upper soil layer for a very limited time period only. As active root N uptake of boreal forest plants in this area generally takes place in the organic soil layer (e.g. Huang and Schoenau, 1997), NO_3^- would potentially contribute to plant N nutrition only infrequently at times of high NO_3^- inputs. The finding that previously fertilized soils display a higher, and more extended peak of F_{MD} of NO_3^- (Fig. 3) suggests rates of immobilization of added NO_3^- are lower for these soils, a result that is corroborated by studies using the stable isotope ^{15}N showing that in boreal forest soils N immobilization is much more rapid in N-poor than in N-rich sites (Nordin et al., 2001; Högborg et al., 2011).

Further, with the exception of arginine and methionine, all amino acids were found to diffuse across the microdialysis membranes in both studies. In the present study, however, F_{MD} of most N forms was significantly higher than observed in autumn. F_{MD} of NH_4^+ were higher by an order of magnitude and F_{MD} of amino acids ranged from 0.6 to 5.8 $\text{nmol cm}^{-2} \text{h}^{-1}$ compared to 0.1–1.6 $\text{nmol cm}^{-2} \text{h}^{-1}$ in autumn in the previous study (Inselsbacher and Näsholm, 2012a). On a total N basis, and assuming that F_{MD} is a measure of N availability at the root surface (see discussion above), this implies that in early spring soils potentially provided 33 $\text{nmol N cm}^{-2} \text{h}^{-1}$ for root uptake, which is 3 times more than in autumn (Inselsbacher and Näsholm, 2012a). These differences in F_{MD} between the two seasons suggest that boreal forest soils provide significantly more N for plant uptake at the onset of plant growth than later during the season. This difference may be due to the lysis of microbial and plant cells during freeze–thaw and/or drying–rewetting cycles in early spring generally leading to an increased release of N to the soil and consequently to increased soil N supply rates (Ivarson and Sowden, 1966; Edwards and Cresser, 1992; Lipson and Monson, 1998). Further, soil N pools are progressively depleted during the growth season, which could at least partly explain lower F_{MD} of N in autumn (Inselsbacher and Näsholm, 2012a) compared to spring (present study). We also detected some differences in the composition of plant-available N between the two seasons. F_{MD} of histidine, glycine and serine were highest among all amino acids (Fig. 1), which was expected for glycine and serine but not for histidine. In autumn (Inselsbacher and Näsholm, 2012a), histidine was not detected in most unfertilized boreal forest soils and in the fertilized plots F_{MD} of histidine were still significantly lower than those of glycine and serine. A possible reason for higher F_{MD} of histidine in the present study is the slightly higher pH (>6.0) in soil microsites of microdialysis sampling which could have led to the deprotonation of histidine and thereby increasing its mobility in soil. Another surprising observation was that glutamine and asparagine contributed relatively little to F_{MD} of total amino acid in these early-season measurements (Fig. 1). While F_{MD} of both amino acids were in range of

previously reported values, this indicates that the relative share of individual amino acids might differ significantly at different times of the season.

4.4. Short term dynamics in F_{MD}

F_{MD} of amino acids not only varied considerably at different times of the year, but at short time scales as well. In a laboratory-based microdialysis study it was shown that F_{MD} of all N forms added to a sieved and homogenized soil decreased significantly within 2 h (Inselsbacher et al., 2011). The depletion of N could have been caused by several factors, especially by the formation of a so-called diffusion shell around the root (or, in this case, the microdialysis membrane) surface, microbial degradation, adsorption to soil particles or, most likely, a combination of these processes (cf. Nye, 1979; Tinker and Nye, 2000; Leitner et al., 2010; Inselsbacher et al., 2011). As microdialysis acts as a sink for all free-moving N forms, we expected a similar rapid depletion of various N forms when monitoring F_{MD} in-situ. On the other hand, in the field N is turned over rapidly (Jones, 1999; Lipson et al., 2001; Owen and Jones, 2001; Jones and Kielland, 2002; Rousk and Jones, 2010) and the constant depolymerization of proteins and peptides to amino acids, as well as the subsequent mineralization to NH_4^+ and nitrification to NO_3^- may replenish the amounts of N removed from the soil. In the present study this was indeed the case for all but 6 N forms (leucine, lysine, glutamine, asparagine, tyrosine, phenylalanine), as F_{MD} were not decreasing during the course of the 100 min sampling (Fig. 2). This suggests that soil N pools in the field may, at least to some extent, be in a steady state, even when N is taken up by plants or immobilized by soil microbes. However, the decrease of F_{MD} was dependent on the individual N form as F_{MD} of some amino acids remained at a steady state throughout the sampling period while F_{MD} of others could not be detected already 40 min after installing the membranes into the soil (Fig. 2). Interestingly, the decrease of F_{MD} was thereby correlated to the initial F_{MD} (estimated during the first 20 min after membrane installation), i.e. F_{MD} of amino acids with low initial F_{MD} were generally more affected and declined more rapidly than those with higher initial F_{MD} . One exception to this pattern was F_{MD} of NH_4^+ in the fertilized soils (Fig. 2d); although initial F_{MD} was significantly higher than of any other N form, F_{MD} of NH_4^+ declined rapidly during the sampling period. As no simultaneous increase in F_{MD} of NO_3^- was observed, nitrification was probably not responsible for the observed decrease of NH_4^+ . Similarly, microbial immobilization or root uptake of NH_4^+ were not causing this effect, as both would have led to lower initial F_{MD} of NH_4^+ already before the sampling start. Therefore, the most likely reason for the decrease of F_{MD} of NH_4^+ was the formation of a diffusion shell, i.e. insufficient replenishment of NH_4^+ by diffusion from the bulk soil to the membrane surface. These results suggest that, in the field, depolymerization was most likely the limiting step for the replenishment of some, but not all amino acids, and that mineralization of amino acids to NH_4^+ could not replenish the pool of NH_4^+ in the fertilized soils.

Further, a recent review suggests that most soil sampling methods, including microdialysis, are grossly overestimating the concentration of amino acids, measuring mostly amino acids released from severed fine roots and mycorrhizal hyphae (Hobbie and Hobbie, 2013). In support for this, Hobbie and Hobbie also argued that glutamine/glutamic acid and asparagine/aspartic acid are the most abundant amino acids in fine roots and mycorrhizal hyphae and these compounds commonly dominate in soil water analyses as well.

Soil represents extremely complex environments and acquiring accurate information on pool sizes, fluxes and transformations from soils presents a great challenge. We argue that microdialysis is a

less invasive technique for studying soils than conventional excavation-extraction based techniques. This infers that if damaging of roots and hyphae is the main source of amino acids, microdialysis should detect significantly lower relative shares of amino acids than the conventional techniques, not a higher relative contribution of amino acids as is obvious from this study and from earlier studies using the same technique (Inselsbacher and Näsholm, 2012a). Further, if damaging of fine roots and hyphae was a major source of amino acids, significantly lower concentrations of these compounds should be recorded at extended times of dialysis. In contrast, in this study a majority of amino acids exhibited relatively stable F_{MD} over time (Fig. 2). Furthermore, histidine, glycine, serine and alanine dominated among the detected amino acids while the amides (glutamine and asparagine) and their respective acids (glutamic and aspartic acid) contributed less (Fig. 1), results that do not corroborate the claim that severed tissues would be the major source of soil amino acids.

5. Conclusions

Here we show that F_{MD} of N in boreal forest soils is dominated by amino acids in early spring. Further, fertilization with NH_4NO_3 of a boreal coniferous forest significantly increased F_{MD} of NH_4^+ but not of NO_3^- which is rapidly lost from the soil organic layer either due to leakage, microbial and plant uptake or both. A rapid decrease in F_{MD} of NO_3^- following NO_3^- addition corroborates this suggestion. Moreover, a significantly greater and more prolonged response in F_{MD} of NO_3^- in previously fertilized soils suggests immobilization by microbes and plants to be the main driver of decreased F_{MD} . The continuous supply of N from the bulk soil to the membrane surface strongly depended on N form, as F_{MD} of half of all amino acids remained stable over time, while several others could not be detected in dialysates after a short (<1 h) time period. This implies that growing plant roots may encounter the full range of N forms after intercepting new soil microsites but significantly less N forms and/or at lower concentrations after a short time due to the rapid formation of a depletion zone around the root surface. Our results indicate that studies aiming at understanding the role of different N forms for plant nutrition should consider their availabilities at the root surface in soils over short (hours) and longer (days) time-periods (Clarke and Barley, 1968). The current study also suggests high F_{MD} of amino acids are not resulting from damaging of roots and hyphae during installation of dialysis probe membranes but to be an inherent characteristic of boreal forest soils.

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References

- Bungay, P.M., Morrison, P.F., Dedrick, R.L., 1990. Steady-state theory for quantitative microdialysis of solutes and water in vivo and in vitro. *Life Sciences* 46, 105–119.
- Chapin, F.S., Matson, P.A., Mooney, H.A., 2002. *Principles of Terrestrial Ecosystem Ecology*. Springer, New York, NY, USA.
- Chen, J., Williams, D.G., 2013. A novel in situ water perfusion and extraction method for soil amino acid quantification. *Soil Biology & Biochemistry* 59, 86–88.
- Clarke, A.L., Barley, K.P., 1968. The uptake of nitrogen from soils in relation to solute diffusion. *Australian Journal of Soil Research* 6, 75–95.
- Clarkson, D.T., Hanson, J.B., 1980. The mineral nutrition of higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 31, 239–298.
- Comerford, N.B., 2005. Soil factors affecting nutrient availability and uptake by plant roots: soil nutrient bioavailability. In: Bassirirad, H. (Ed.), *Nutrient Acquisition by Plants. An Ecological Perspective, Series: Ecological Studies*, vol. 181. Springer, Heidelberg, Germany, pp. 1–14.
- De Lange, E.C.M., De Boer, A.G., Breimer, D.D., 2000. Methodological issues in microdialysis sampling for pharmacokinetic studies. *Advanced Drug Delivery Reviews* 45, 125–148.
- Edwards, A.C., Cresser, M.S., 1992. Freezing and its effect on chemical and biological properties of soil. In: Stewart, B.A. (Ed.), *Advances in Soil Science*, vol. 18. Springer, Berlin Heidelberg New York, pp. 59–76.
- FAO, 1998. *World Reference Base for Soil Resources*. World Soil Resources Reports, Rome, Italy.
- Giesler, R., Lundström, U., 1993. Soil solution chemistry – effects of bulking soil samples. *Soil Science Society of America Journal* 57, 1283–1288.
- Gundale, M.J., DeLuca, T.H., Nordin, A., 2011. Bryophytes attenuate anthropogenic nitrogen inputs in boreal forests. *Global Change Biology* 17, 2743–2753.
- Hobbie, J.E., Hobbie, E.A., 2013. Microbes in nature are limited by carbon and energy: the starving-survival lifestyle in soil and consequences for estimating microbial rates. *Frontiers of Microbiology* 4, 1–11.
- Högberg, P., Johannisson, C., Yarwood, S., Callesen, I., Näsholm, T., Myrold, D.D., Högberg, M.N., 2011. Recovery of ectomycorrhiza after 'nitrogen saturation' of a conifer forest. *New Phytologist* 189, 515–525.
- Hood-Novotny, R., Hinko-Najera Umana, N., Inselsbacher, E., Oswald-Lachouani, P., Wanek, W., 2010. Alternative methods for measuring inorganic, organic, and total dissolved nitrogen in soil. *Soil Science Society of America Journal* 74, 1018–1027.
- Hsiao, J.K., Ball, B.A., Morrison, P.F., Mefford, I.N., Bungay, P.M., 1990. Effects of different semipermeable membranes on invitro and invivo performance of microdialysis probes. *Journal of Neurochemistry* 54, 1449–1452.
- Huang, W.Z., Schoenau, J.J., 1997. Seasonal and spatial variations in soil nitrogen and phosphorus supply rates in a boreal aspen forest. *Canadian Journal of Soil Science* 77, 597–612.
- Inselsbacher, E., 2014. Recovery of individual soil nitrogen forms after sieving and extraction. *Soil Biology & Biochemistry* 71, 76–86.
- Inselsbacher, E., Näsholm, T., 2012a. The below-ground perspective of forest plants: soil provides mainly organic nitrogen for plants and mycorrhizal fungi. *New Phytologist* 195, 329–334.
- Inselsbacher, E., Näsholm, T., 2012b. A novel method to measure the effect of temperature on diffusion of plant-available nitrogen in soil. *Plant and Soil* 354, 251–257.
- Inselsbacher, E., Öhlund, J., Jämtgård, S., Huss-Danell, K., Näsholm, T., 2011. The potential of microdialysis to monitor organic and inorganic nitrogen compounds in soil. *Soil Biology & Biochemistry* 43, 1321–1332.
- Ivarson, K.C., Sowden, F.J., 1966. Effect of freezing on the free amino acids in soil. *Canadian Journal of Soil Science* 46, 115–120.
- Jämtgård, S., Näsholm, T., Huss-Danell, K., 2008. Characteristics of amino acid uptake in barley. *Plant and Soil* 302, 221–231.
- Johnson, D.W., Verburg, P.S.J., Arnone, J.A., 2005. Soil extraction, ion exchange resin, and ion exchange membrane measures of soil mineral nitrogen during incubation of a tallgrass prairie soil. *Soil Science of America Journal* 69, 260–265.
- Jones, D.L., 1999. Amino acid biodegradation and its potential effects on organic nitrogen capture by plants. *Soil Biology & Biochemistry* 31, 613–622.
- Jones, D.L., Kielland, K., 2002. Soil amino acid turnover dominates the nitrogen flux in permafrost-dominated taiga forest soils. *Soil Biology & Biochemistry* 34, 209–219.
- Jones, D.L., Willett, V.B., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. *Soil Biology & Biochemistry* 38, 991–999.
- Kehr, J., 1993. A survey on quantitative microdialysis – theoretical models and practical implications. *Journal of Neuroscience Methods* 48, 251–261.
- Kielland, K., 1994. Amino acid absorption by arctic plants: implications for plant nutrition and nitrogen cycling. *Ecology* 75, 2373–2383.
- Kronzucker, H.J., Siddiqi, M.Y., Glass, A.D.M., 1997. Conifer root discrimination against soil nitrate and the ecology of forest succession. *Nature* 385, 59–61.
- Lafontan, M., Arner, P., 1996. Application of in situ microdialysis to measure metabolic and vascular responses in adipose tissue. *Trends in Pharmacological Sciences* 17, 309–313.
- Lambers, H., Chapin, F.S., Pons, T.L., 2008. *Plant Physiological Ecology*. Springer, New York, USA.
- Leadley, P.W., Reynolds, J.F., Chapin, F.S.A., 1997. A model of nitrogen uptake by *Eriophorum vaginatum* roots in the field: ecological implications. *Ecological Monographs* 67, 1–22.
- Leitner, D., Klepsch, S., Ptashnyk, M., Marchant, A., Kirk, G.J.D., Schnepf, A., Roose, T., 2010. A dynamic model of nutrient uptake by root hairs. *New Phytologist* 185, 792–802.
- Likens, G.E., Borman, F.H., Johnson, N.M., 1969. Nitrification – importance to nutrient losses from a cutover forested ecosystem. *Science* 163, 1205–1206.
- Lipson, D.A., Monson, R.K., 1998. Plant-microbe competition for soil amino acids in the alpine tundra: effects of freeze-thaw and dry-rewet events. *Oecologia* 113, 406–414.
- Lipson, D., Raab, T.K., Schmidt, S.K., Monson, R.K., 2001. An empirical model of amino acid transformations in an alpine soil. *Soil Biology & Biochemistry* 33, 189–198.
- Marschner, H., 1995. *Mineral Nutrition of Higher Plants*. Academic Press Limited, London, UK.
- Miranda, K.M., Espey, M.G., Wink, D.A., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide* 5, 62–71.

- Miro, M., Frenzel, W., 2004. Implantable flow-through capillary-type microdialyzers for continuous in situ monitoring of environmentally relevant parameters. *Analytical Chemistry* 76, 5974–5981.
- Miro, M., Frenzel, W., 2005. The potential of microdialysis as an automatic sample-processing technique for environmental research. *TrAC Trends in Analytical Chemistry* 24, 324–333.
- Miro, M., Frenzel, W., 2011. Microdialysis in environmental monitoring. pp 509–530. In: Tsai, T.H. (Ed.), *Applications of Microdialysis in Pharmaceutical Science*. Wiley & Sons, Inc., Hoboken, New Jersey, USA.
- Miro, M., Hansen, E.H., 2006. Recent advances and perspectives in analytical methodologies for monitoring the bioavailability of trace metals in environmental solid substrates. *Microchimica Acta* 154, 3–13.
- Miro, M., Fitz, W.J., Swoboda, S., Wenzel, W.W., 2010. In-situ sampling of soil pore water: evaluation of linear-type microdialysis probes and suction cups at varied moisture contents. *Environmental Chemistry* 7, 123–131.
- Nandi, P., Lunte, S.M., 2009. Recent trends in microdialysis sampling integrated with conventional and microanalytical systems for monitoring biological events: a review. *Analytica Chimica Acta* 651, 1–14.
- Näsholm, T., Ekblad, A., Nordin, A., Giesler, R., Högborg, M., Högborg, P., 1998. Boreal forest plants take up organic nitrogen. *Nature* 392, 1155–1161.
- Näsholm, T., Kielland, K., Ganeteg, U., 2009. Uptake of organic nitrogen by plants. *New Phytologist* 182, 31–48.
- Nordin, A., Högborg, P., Näsholm, T., 2001. Soil nitrogen form and plant nitrogen uptake along a boreal forest productivity gradient. *Oecologia* 129, 125–132.
- Nye, P.H., 1979. Diffusion of ions and uncharged solutes in soils and soil clays. *Advances in Agronomy* 31, 225–272.
- Nye, P.H., Marriott, F.H., 1969. A theoretical study of distribution of substances around roots resulting from simultaneous diffusion and mass flow. *Plant and Soil* 30, 459–472.
- Nye, P.H., Tinker, P.B., 1977. *Solute Movement in the Soil-root System*. Blackwell Scientific Publications, Oxford, UK.
- Öhlund, J., 2004. Organic and inorganic nitrogen sources for conifer seedlings. *Acta Universitatis Agriculturae Sueciae. Silvestria* 1401–6230, 312.
- Olesen, T., Moldrup, P., Yamaguchi, T., Rolston, D.E., 2001. Constant slope impedance factor model for predicting the solute diffusion coefficient in unsaturated soil. *Soil Science* 166, 89–96.
- Owen, A.G., Jones, D.L., 2001. Competition for amino acids between wheat roots and rhizosphere microorganisms and the role of amino acids in plant N acquisition. *Soil Biology & Biochemistry* 33, 651–657.
- Oyewole, A.O., Inselebacher, E., Näsholm, T., 2014. Direct estimation of mass flow and diffusion of nitrogen compounds in solution and soil. *New Phytologist* 201, 1056–1064.
- Paungfoo-Lonhienne, C., Lonhienne, T.G.A., Rentsch, D., Robinson, N., Christie, M., Webb, R.I., Gamage, H.K., Carroll, B.J., Schenk, P.M., Schmidt, S., 2008. Plants can use protein as a nitrogen source without assistance from other organisms. *Proceedings of the National Academy of Sciences of the United States of America* 105, 4524–4529.
- Paungfoo-Lonhienne, C., Visser, J., Lonhienne, T.G.A., Schmidt, S., 2012. Past, present and future of organic nutrients. *Plant and Soil* 359, 1–18.
- Persson, J., Näsholm, T., 2001. Amino acid uptake: a widespread ability among boreal forest plants. *Ecology Letters* 4, 434–438.
- Plock, N., Kloft, C., 2005. Microdialysis – theoretical background and recent implementation in applied life-sciences. *European Journal of Pharmaceutical Sciences* 25, 1–24.
- Robertsson, G.P., 1982. Factors regulating nitrification in primary and secondary succession. *Ecology* 63, 1561–1573.
- Rothstein, D.E., 2009. Soil amino-acid availability across a temperate forest fertility gradient. *Biogeochemistry* 92, 201–215.
- Rousk, J., Jones, D.L., 2010. Loss of low molecular weight dissolved organic carbon (DOC) and nitrogen (DON) in H₂O and 0.5 M K₂SO₄ soil extracts. *Soil Biology & Biochemistry* 42, 2331–2335.
- Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. *Ecology* 85, 591–602.
- Seethapathy, S., Gorecki, T., Li, X.J., 2008. Passive sampling in environmental analysis. *Journal of Chromatography A* 1184, 234–253.
- Shaver, G.R., Chapin, F.S., 1991. Production-biomass relationships and element cycling in contrasting arctic vegetation types. *Ecological Monographs* 61, 1–31.
- Stenzen, J.A., Chen, R., Yuan, X.N., 2001. Influence of geometry and equilibrium chemistry on relative recovery during enhanced microdialysis. *Analytica Chimica Acta* 436, 21–29.
- Sulyok, M., Miro, M., Stinger, G., Koellensperger, G., 2005. The potential of flow-through microdialysis for probing low-molecular weight organic anions in rhizosphere soil solution. *Analytica Chimica Acta* 546, 1–10.
- Thomsen, I.K., Schjonning, P., 2003. Evaluation of a non-destructive technique for inorganic soil N measurement. *Geoderma* 113, 147–160.
- Tinker, P.B., Nye, P.H., 2000. *Solute Movement in the Rhizosphere*. Oxford University Press, Inc., New York, USA.
- Torto, N., 2009. A review of microdialysis sampling systems. *Chromatographia* 70, 1305–1309.
- Torto, N., Majors, R.E., Laurell, T., 2001. Microdialysis sampling – challenges and new frontiers. *LC GC North America* 19, 462–475.
- Torto, N., Mikeladze, E., Gorton, L., Csoregi, E., Laurell, T., 1999. Maximising microdialysis sampling by optimising the internal probe geometry. *Analytical Communications* 36, 171–174.
- Torto, N., Mwatseteza, J., Sawula, G., 2002. A study of microdialysis sampling of metal ions. *Analytica Chimica Acta* 456, 253–261.
- Tsai, T.H., 2011. *Applications of Microdialysis in Pharmaceutical Science*. Wiley & Sons, Inc., Hoboken, New Jersey, USA.
- Ungerstedt, U., 1991. Microdialysis – principles and applications for studies in animals and man. *Journal of Internal Medicine* 230, 365–373.
- Warren, C.R., 2013a. High diversity of small organic N observed in soil water. *Soil Biology & Biochemistry* 57, 444–450.
- Warren, C.R., 2013b. Quaternary ammonium compounds can be abundant in some soils and are taken up as intact molecules by plants. *New Phytologist* 198, 476–485.
- Warren, C.R., Taranto, M.T., 2010. Temporal variation in pools of amino acids, inorganic and microbial N in a temperate grassland soil. *Soil Biology & Biochemistry* 42, 353–359.
- Weihermüller, L., Siemens, J., Deurer, M., Knoblauch, S., Rupp, H., Göttlein, A., Pütz, T., 2007. In situ soil water extraction: a review. *Journal of Environmental Quality* 36, 1735–1748.
- Weiss, D.J., Lunte, C.E., Lunte, S.M., 2000. In vivo microdialysis as a tool for monitoring pharmacokinetics. *TrAC-Trends in Analytical Chemistry* 19, 606–616.
- WRB, 2006. IUSS Working Group WRB. World Reference Base for Soil Resources 2006. World Soil Resources Reports No. 103, second ed. FAO, Rome.