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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.

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; Inselsbacher, 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; Inselsbacher 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 Inselsbacher 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 instalment 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 (Inselsbacher 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 (Inselsbacher 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; Inselsbacher 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 overestimation 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 (Inselsbacher 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 (Inselsbacher 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 (Inselsbacher et al., 2011). Further, the experimentally estimated diffusive flux of a range of N