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

Olusegun Ayodeji Oyewole [a, 1], Erich Inselsbacher [c], Torgny Näsholm [a, b], Sandra Jämtgård [a, *]

[a] Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden
[b] Department of Forest Genetics and Plant Physiology, Umeå Plant Science Centre, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden
[c] Department of Geography and Regional Research, University of Vienna, 1010 Vienna, Austria

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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,

* Corresponding author.
E-mail address: sandra.jamtgard@slu.se (S. Jämtgård).
[1] Current address: Department of Biology, University of Sherbrooke, J1K 2R1 Sherbrooke, QC, Canada.

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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;