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Short communication

Short-term soil mineral and organic nitrogen fluxes during moderate and severe drying–rewetting events

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ABSTRACT

Nitrogen (N) availability to plants in dry soil is limited by diffusive flux of N compounds through the soil solution towards the root surface. Conventional soil extraction procedures only provide information about bulk soil N concentrations, which can be distorted during soil sampling, transport, storage and extraction, and hence are of limited use to detect short-term N dynamics. Soil microdialysis is a new tool to monitor diffusive flux of mineral and organic N compounds *in situ* in high temporal and spatial resolution with minimal disturbance, and is therefore well-suited to determine dynamic fractions of plant-available N in soil microsites.

We investigated N availability and mobilization during a drying–rewetting event in a temperate beech forest using soil microdialysis and soil extractions with water. While water extracts mainly revealed mineral N in the form of NH_4^+ and NO_3^-, diffusive N fluxes *in situ* were dominated by amino acids. Microdialysis showed that rewetting of dry soil led to a fast but short-lived mobilization of NO_3^- and some neutral hydrophilic amino acids (lysine, glutamine, cysteine, glycine), which was not detected in water extracts, and the rewetting N flush was larger with increasing drought duration. Our results suggest that at our temperate forest site plant-available N was dominated by amino acids, a fraction of N that might be missed using conventional soil extraction methods. Considering expected increases in the frequency of extreme climatic events, the observed release of mobile N forms bears the potential of N loss from soil if severe drought is followed by a heavy rain event.

1. Introduction

Almost all ecosystems experience periods of drought followed by rewetting events. An increase in the frequency and severity of extreme weather events due to climate change is expected to aggravate the negative effects associated with drought (IPCC, 2014). One of the most obvious adverse effects is that plants as well as microorganisms may be impacted by severe drought stress. At the same time, the lack of water leads to nutrient limitation since the capacity of soils to supply sufficient nutrients is determined by the availability of water. When soils dry out, diffusion of nutrients through the soil towards root surfaces and soil microorganisms is inhibited by reduced water-filled pore space and increased tortuosity of water films around solid particles (Moldrup et al., 2001). Furthermore, when plants experience drought stress, stomatal conductance and consequently transpiration is reduced, which decreases mass flow of water and dissolved nutrients to the root surfaces, further decreasing the supply of nutrients for plant uptake. With ongoing soil drying, water films are disrupted and roots and microorganisms get physically separated from nutrients. Taken together, these drought effects lead to an accumulation of nutrients in soils during extended dry periods because they are not taken up by plants or immobilized by microorganisms. During rewetting of dry soil these accumulated nutrients can be mobilized rapidly and are prone to leaching. This nutrient flush during rewetting results in temporary pulses of increased microbial activity (Manzoni et al., 2014) and high rates of nutrient turnover (Birch, 1958; Evans et al., 2016).

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Plant nitrogen (N) acquisition is a complex process involving both transport of N in the soil and across root membranes and mycorrhizal hyphae (hereafter referred to as roots) (Leadley et al., 1997; Tinker and Nye, 2000), but several studies indicate that soil N supply rates, not root uptake rates, critically determine plant N acquisition (Clarkson and Hanson, 1980; Lambers et al., 2008; Leadley et al., 1997). Generally, the supply of N by diffusion becomes increasingly important at times when mass flow is low or absent and cannot meet the N demand of plants, as is the case at reduced transpiration rates and low soil water contents (Clarke and Barley, 1968; Comerford, 2005; Gerber and Brookshire, 2014).

Besides soil water content, N diffusion is controlled by a variety of other factors including bulk density, buffering capacity and ion exchange capacity (Jungk and Claassen, 1997; Lipson and Näsholm, 2001; Van Rees et al., 1990). Mobility of different forms of N in the soil solution depends on solute charge because the predominantly negative surface charge of clay minerals and soil organic matter leads to a retention of cations like ammonium (NH_4^+) and basic amino acids, whereas anions like nitrate (NO_3^-) and acidic amino acids or neutral hydrophilic amino acids can move more easily through the soil solution (Rothstein, 2010). On the other hand, hydrophobic compounds have been shown to be more easily adsorbed to soil particles compared to hydrophilic substances (Kaiser and Zech, 2000). Therefore, it is important to not only estimate soil factors such as pH and ion exchange capacity, but also to estimate the relative abundance of individual N compounds in undisturbed soils directly in the field. However, until now, this task remained challenging owing to inadequate soil sampling techniques.

Over the last three decades, several attempts have been made to estimate soil N pools and turn-over rates *in situ*. The most prominent are the use of ion-exchange resin bags or resin columns, soil solution collection by different kinds of lysimeters, soil centrifugation, and *in-situ* water perfusion and extraction (Andersson, 2003; Binkley et al., 1992; Chen and Williams, 2013; Giesler and Lundström, 1993; Raison et al., 1987; Weihermüller et al., 2007). The primary objective of all these studies was to best approximate N under field conditions.

One promising approach, based on soil microdialysis, has recently been established as a novel tool to monitor soil N fluxes *in situ* at high spatial and temporal resolution (Inselsbacher and Näsholm, 2012a; Inselsbacher et al., 2011). In contrast to conventional soil extracts, which have been criticized for altering soil N concentrations during soil sampling, transport, storage, sieving and shaking (Černohlávková et al., 2009; Inselsbacher, 2014; Jones and Willett, 2006; Rousk and Jones, 2010; Warren and Taranto, 2010), monitoring soil N fluxes by microdialysis directly reflects N availability to plant roots (Inselsbacher and Näsholm, 2012a). Because of the small size of the microdialysis membrane (1 cm long with an outer diameter of 0.5 mm), its installation causes minimal disturbance of the soil matrix (Inselsbacher et al., 2011) and allows the continuous measurement of N diffusion from the bulk soil across the membrane surface for hours or days.

In the present study we combined a conventional soil water extraction method with soil microdialysis to detect mobilization of mineral (NH_4^+ and NO_3^-) and organic (amino acids) N in the first 72 h after a rewetting pulse *in situ*. We hypothesized that (i) rewetting of dry soil mobilizes both mineral and organic N and that soil microdialysis reveals short-term N patterns which are not reflected in conventional soil extracts, and (ii) that the size of the mobilization flush is larger when the preceding drought is longer. To this end, a precipitation manipulation experiment in a temperate forest was used, and N mobilization was monitored at high temporal resolution following irrigation of dry soil in the autumn of 2014.

2. Material and methods

2.1. Study site and experimental design

The study was conducted in a temperate beech forest (*Fagus sylvatica* L., stand age 120 years) at the ILTER-site "Rosalia Lehrforst" in Lower Austria (47°42′26.33″N, 16°17′58.15″E, 600 m asl). The mean annual temperature is 6.5 °C and the mean annual precipitation 796 mm. The soil was classified as pseudo-gleyic cambisol over granitic bedrock with 4.1% SOC, 0.18% N, pH 4.0, and 0.595 g cm^{-3} bulk density (Leitner et al., 2016).

At the study site a rainfall manipulation experiment had been set up in May 2013 (Schwen et al., 2014). In short, 8 experimental plots of 2 m × 2 m each were covered with 4 m × 4 m transparent acrylic roofs 1.2 m above the ground surface to exclude rainfall. Two parallel manipulation treatments ($n = 4$) were conducted during the vegetation period (May until October): i) a moderately stressed four-week drought (4WD) treatment, which experienced six drying–rewetting cycles, each consisting of four weeks of precipitation exclusion followed by irrigation with 75 mm decalcified tap water, and ii) a severely stressed eight-week drought (8WD) treatment that received three drying–rewetting cycles, each consisting of eight weeks of precipitation exclusion followed by irrigation with 150 mm decalcified tap water. Both manipulation treatments were repeated in 2013 and 2014. In each plot, soil sensors were buried in 10 cm depth to measure soil volumetric water content (VWC, TDR theta.ML2x probes, UMS, Germany) and temperature (T_{soil}, thermistor Th2-f probes, UMS, Germany).

2.2. Soil analysis

In October 2014 at the end of the experimental rainfall manipulation, soil samples were taken in each plot 1 h before, and 24 h and 72 h after irrigation in triplicates with a steel soil corer (4 cm diameter, 10 cm length) and homogenized into one composite soil sample per plot. Soil was transported to the lab on ice and immediately sieved (<2 mm) and stored at 4 °C over night. On the next day, aliquots of 2.5 g field-moist soil were extracted with 25 ml high-purity deionized water (MilliQ) for 1 h on a rotary shaker, filtered with acid-free filter paper (Whatman Type 40, pore size 8 μm), and stored at −20 °C for further analysis.

To determine *in-situ* N diffusion before and during the first 20 h after irrigation we deployed two microdialysis systems, each consisting of a syringe infusion precision pump (CMA 400) equipped with four gas-tight microsyringes (5 ml, Hamilton, Bonaduz, Switzerland) which provided the perfusate solution. Each syringe was connected via 50 cm FEP tubing to a microdialysis probe with a polyarylethersulphone membrane (CMA 20, 10 mm length, 500 μm outer and 400 μm inner diameter, 20 kDa molecular weight cut-off). Membranes were installed 2 h prior to the irrigation at least 50 cm within the plots to a soil depth of 1.5 cm. In detail, the litter layer was lifted and a guiding channel was prepared by a steel cannula (800 μm outer diameter). The membranes were inserted carefully into the prepared channels and were then left in the soil throughout the experiment. The membranes were perfused with MilliQ water at a flow rate of 5 μl min^{-1} for 9 h, after which the flow rate was switched to 1 μl min^{-1} over night. Samples were collected continuously in 300 μl vials in a refrigerated microfraction collector (6 °C; CMA 470), transported to the lab on ice and stored at −20 °C until analysis. All equipment is commercially available at CMA Microdialysis AB (Solna, Sweden). Membrane calibration and calculation of N diffusion rates based on microdialysis membrane surface and time was done according to Inselsbacher and