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Altered microbial communities and nitrogen availability in temperate forest edges

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ABSTRACT

Due to forest fragmentation, forest edges have become dominant features in landscapes around the world. Forest edges are exposed to a different microclimate and to higher atmospheric nitrogen (N) deposition compared to the forest interior. It is still unclear how both factors affect N cycling at temperate forest edges. In this study, the microbial community structure was mapped using phospholipid fatty acids (PLFA) in forest edge (0–5 m) and interior (64 m) in two oak (*Quercus robur*) stands, two pine (*Pinus nigra*) stands and one spruce (*Picea sitchensis*) stand in northern Belgium and Denmark. Nitrogen mineralization, nitrification and immobilization rates were obtained via the *in situ* ^{15}N pool dilution technique in the forest edge and interior, and linked to the microbial community structure. Furthermore, we assessed ^{15}N recovery in simulated throughfall via the ^{15}N tracing method in edge and interior as a proxy for the long-term fate of mineral N. Microbial biomass was higher at the forest edges compared to the forest interiors and was associated to the higher gross mineralization rates. Gross nitrification rates were not increased at the edge, hereby preventing NO_3^- leaching losses. Gross and net nitrification rates differed between the forest types, where the oak stands were characterized by higher nitrification rates than the pine and spruce stands. The oak stand retained $^{15}NO_3^-$ in the mineral soil at the edge, while in the pine stand the polyphenol-rich litter layer retained more $^{15}NO_3^-$ in the forest interior. Overall, our results indicated that the specific characteristics of the forest edge (atmospheric deposition, microclimate, pH of mineral soil and C/N ratio of the forest floor) increased microbial biomass and gross mineralization rates. Given the omnipresence of forest edges, more research should be conducted to validate our observations for other forest and soil types.

1. Introduction

Central and Western Europe are characterized by small forest remnants resulting from a long-term history of land-use change (Decocq et al., 2016; Hofmeister et al., 2013). Consequently, forest edges have become important features in the landscape (Harper et al., 2005). Forest edges differ substantially from forest interior zones, via an increase in air and soil temperature, light availability, and wind speed, but often a decrease in soil moisture (e.g. Marchand and Houle, 2006). Microclimatic gradients of soil temperature and moisture may cause altered decomposition and mineralization rates, as for instance Bol et al. (2003) and Ritter (2005) found increased mineralization rates at higher soil temperatures. Secondly, forest edges receive more atmospheric deposition of eutrophying, acidifying and basic compounds, due to obstruction of the wind profile causing local advection and turbulent exchange (Devlaeminck et al., 2005; Draaijers and Erisman, 1993; Wuyts et al., 2008). The edge effect on atmospheric deposition spans ca. 15 m to more than 100 m from the edge to the forest's interior and causes an up to five-fold increase in throughfall deposition (De Schrijver et al., 2007). The magnitude and depth of edge effects depend on several forest characteristics, including tree species, stand density and stand structure (De Schrijver et al., 1998; Devlaeminck et al., 2005; Draaijers and Erisman, 1993; Wuyts et al., 2008, 2009).

Most forests of mid to high latitudes on the northern hemisphere were N limited until the 1950s, but due to a high atmospheric N load during the last decades this has changed considerably (Duprè et al., 2010). For example, in Europe, very high N deposition values (> 35 kg N ha^{-1} yr^{-1}) are observed in intensive livestock breeding

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areas (De Vries et al., 2011; MIRA, 2011). Characteristics of N limitation are strong N recovery and efficient recycling of available N (Perakis et al., 2005). However, when forests become N saturated, excess N can be lost from the ecosystem via leaching and denitrification (Templer et al., 2012). Besides eutrophication, other harmful effects of increased N inputs include soil acidification, i.e. loss of exchangeable cations and the mobilization of aluminum and other potentially toxic metals (Wilpert et al., 2000), pollution of groundwater reserves (Dise et al., 2009) and biodiversity loss (De Schrijver et al., 2011). However, in forest edges, higher N deposition does not always lead to enhanced N losses, since Spangenberg and Kölling (2004) and Wuyts et al. (2011) found higher N deposition but lower inorganic N leaching in the first 30 m of forest edges of oak, birch, beech, spruce and pine monocultures compared to the forest interior. Moreover, Remy et al. (2017) showed that gaseous N losses of nitric oxide (NO) were lower at the forest edge compared to the interior. Therefore, improved understanding of how ecosystem N pools and fluxes respond to increased N deposition is needed (Lu et al., 2011).

The ^{15}N tracing method is used to study the fate and recovery of N input (Schlesinger, 2009). By labelling N input with ^{15}N, the distribution of this N to the different ecosystem pools can be traced over time (Dörr et al., 2012). Furthermore, the ^{15}N pool dilution technique has been widely used to quantify gross N transformation rates (Dannenmann et al., 2006; Staelens et al., 2012). The principle is based on the dilution of a product pool that has been labelled with ^{15}N (Hart et al., 1994). However, many pool dilution experiments have been conducted via laboratory incubations, altering the *in situ* N transformation rates, as soil disturbance promotes gross N mineralization (Booth et al., 2006). Here, we used the *in situ* ^{15}N soil-labelling method, developed by Rütting et al. (2011b), called the 'virtual soil core' injection, which enables the study of undisturbed soils with live roots and their associated microbial communities. Soils are only disrupted at sampling, insuring that soil temperature, water and gas exchange, as well as plant root and microbial activity remain under field conditions during the experiment.

The soil microbial community plays an essential role in the regulation of N cycling (Balser and Firestone, 2005). Scheu and Parkinson (1994) showed that fungi dominate in acid coniferous forest soils, although a shift to bacterial dominance may occur under the influence of high N deposition (Kjøller et al., 2012; Nilsson et al., 2007). Phospholipid fatty acids (PLFA) can be used to determine the relative contribution of bacteria and fungi to the production of microbial derived organic matter (Frey et al., 2004; Nilsson et al., 2007). PLFA are primarily derived from cell membranes (Zelles, 1999) and can quantify presence and relative abundance of Gram-bacteria, Gram + bacteria, actinobacteria and fungi in soil communities (Zogg et al., 1997). PLFA are found in living organisms and decompose quickly after cell death, reflecting the current community structure (Zelles, 1999).

The specific aims of this study were (i) to link soil microbial community structure in forest edges and interiors to N cycling rates, (ii) to quantify mineralization and nitrification rates, and ^{15}N recovery in function of distance to the forest edge, and (iii) to interpret the ^{15}N recovery as an indicator for N retention, where low recoveries indicate an open N cycle. We investigated the structure and abundance of the microbial community by extracting PLFA from mineral soil in oak, pine and spruce stands, situated in agricultural landscapes in Belgium and Denmark. We used analytical pool dilution equations, originally developed by Kirkham and Bartholomew (1954), to quantify gross and net mineralization and nitrification rates in forest edges and interiors. Finally, we estimated N retention by following the fate of ^{15}N in simulated throughfall and measuring the percentage of added ^{15}N that was recovered over a period of 10 months in the organic and mineral soil layers. Due to the higher N deposition and contrasting microclimate (warmer) at the forest edge, we hypothesized: (i) dominance of bacteria over fungi and (ii) higher mineralization and nitrification rates at the forest edge. Furthermore, (iii) as lower inorganic N leaching has been observed at the forest edge, ^{15}N recovery might be increased at the forest edge.

2. Material and methods

2.1. Study sites

The five selected forest edges comprised tree species relevant for their respective region. Four forest stands were situated in Belgium: a 98-year old pedunculate oak (*Quercus robur* L.) stand in Wortegem, West Flanders (Qr1), a 76-year old pedunculate oak stand in Ravels, Antwerp (Qr2), a 73-year old Austrian pine (*Pinus nigra* ssp. *nigra* Arnold) stand in Zedelgem, West Flanders (Pn1), and a 51-year old Corsican pine (*P. nigra* ssp. *laricio* Maire) stand in Ravels, Antwerp (Pn2). One spruce stand (Ps, *Picea sitchensis* (Bong.) Carr.), was situated in Denmark on the peninsula of Jutland (Sonder Omme). The microbial community was mapped in all five stands, while N cycling and N recovery were examined in one oak (Qr2), one pine (Pn2) and one spruce stand (Ps). The mean annual air temperature and precipitation in 1981–2010 are 10.5 °C and 800 mm in Belgium and 7.4 °C and 900 mm on the peninsula of Jutland (Denmark; data obtained from the nearest weather station operated by the Royal Meteorological Institute of Belgium and the Danish Meteorological Institute respectively for the Belgian and Danish forest stands). Monospecific forest stands of pedunculate oak (*Quercus robur* L.), Corsican pine (*P. nigra* ssp. *laricio* Maire) and Sitka spruce (*Picea sitchensis* (Bong) Carr.) were selected with similar soil type, stand history and edge orientation. All stands are even-aged monocultures growing on acid, quartz-dominated Podzols with a low base saturation. Previous land use was heathland before afforestation in last century. Edge-to-interior transects were established perpendicular to the forest edge and facing the locally prevailing wind direction (west to southwest), which creates the steepest throughfall deposition gradients (Draaijers et al., 1988). pH-KCl values of the fermentation and humus (FH) layer and upper mineral soil layer (0–5 cm) ranged between 2.8 and 3.5 (Wuyts et al., 2013; Ginzburg, 2014). Yearly N throughfall deposition and soil leaching fluxes were available from previous studies in the same forest stands at exactly the same distances from the edge (Wuyts et al., 2008; Ginzburg, 2014, Table 1). The understory vegetation is composed of ferns (*Dryopteris dilatata* and *Dryopteris carthusiana*) and grasses (*Molinea caerulea* and *Holcus* sp.) in the pine and spruce stands. The understory vegetation in the edge of the oak stands is characterized by brambles (*Rubus fruticosus* agg.).

2.2. Soil microbial community

For the PLFA analysis, three replicate samples in all five forest stands were taken (September 2015) with a soil auger from the upper 10 cm of the mineral soil, at the forest edge (0–5 m) and in the interior (64 m). Soil samples were frozen until further analysis. Eight g of mineral soil from each sample was sieved (2 mm) in order to homogenize and remove the root fragments and stones. Afterwards, PLFA were extracted following a procedure explained in detail by Moeskops et al. (2010). The PLFA i15:0, a15:0, 15:0, i16:0, 17:0, i17:0 and a17:0 were indicators of Gram + bacteria, while PLFA 16:1w7c, 16:1w9c, cy17:0 and cy19:0 were indicators of Gram-bacteria. Fungal PLFA were 18:1w9c, 18:2w6c and 18:3w6c.

2.3. Nitrogen transformation in mineral soil

2.3.1. Plot installation and N addition

In Qr2, Pn2 and Ps, ten 1 × 1 m^2 plots were selected at the edge (0–5 m) and interior (64 m) with an inter-distance of 8 m. One week prior to ^{15}N addition, the fresh litter layer (L) and fermentation and humus layer (FH) were carefully removed over an area of 40 × 40 cm^2. Two separate nylon meshes (12 × 20 cm^2, 1 mm mesh size), indicating the two injection and sampling locations were put on top of the mineral