Comparison of the impacts of acid and nitrogen additions on carbon fluxes in European conifer and broadleaf forests☆

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ABSTRACT

Increased reactive nitrogen (N) loadings to terrestrial ecosystems are believed to have positive effects on ecosystem carbon (C) sequestration. Global "hot spots" of N deposition are often associated with currently or formerly high deposition of sulphur (S); C fluxes in these regions might therefore not be responding solely to N loading, and could be undergoing transient change as S inputs change. In a four-year, two-forest stand (mature Norway spruce and European beech) replicated field experiment involving acidity manipulation (sulphuric acid addition), N addition (NH_4NO_3) and combined treatments, we tested the extent to which altered soil solution acidity or/and soil N availability affected the concentration of soil dissolved organic carbon (DOC), soil respiration (Rs), microbial community characteristics (respiration, biomass, fungi and bacteria abundances) and enzyme activity. We demonstrated a large and consistent suppression of soil water DOC concentration driven by chemical changes associated with increased hydrogen ion concentrations under acid treatments, independent of forest type. Soil respiration was suppressed by sulphuric acid addition in the spruce forest, accompanied by reduced microbial biomass, increased fungal:bacterial ratios and increased C to N enzyme ratios. We did not observe equivalent effects of sulphuric acid treatments on Rs in the beech forest, where microbial activity appeared to be more tightly linked to N acquisition. The only changes in C cycling following N addition were increased C to N enzyme ratios, with no impact on C fluxes (either Rs or DOC). We conclude that C accumulation previously attributed solely to N deposition could be partly attributable to their simultaneous acidification.

1. Introduction

Human activities have been altering element cycling for several thousand years (Ruddiman, 2003; Shotyk, 1998). Since the Industrial Revolution, emissions of greenhouse gases, nitrogen (N) and sulphur (S) have been increasing (Kopáček and Posch, 2011; Lamarque et al., 2013) and their negative impacts on ecosystems led to the implementation of various international agreements to abate these emissions (United Nations Framework Convention on Climate Change, Convention on Long-range Transboundary Air Pollution, Kyoto Protocol, Paris Agreement). Besides exerting significant negative pressures on global biodiversity (Butchart et al., 2010), atmospheric pollutant deposition has impacted natural biogeochemical cycles of terrestrial ecosystems, including the ecosystem C balance (Hyvönen et al., 2007; Lamersdorf and Borken, 2004; Thomas et al., 2013).

Nitrogen limitation is an inherent feature of many temperate terrestrial ecosystems, thus fertilization by atmospheric N deposition may have increased C pool in biomass and soils of terrestrial ecosystems and thus stimulate the terrestrial CO_2 sink (de Vries et al., 2009). Increased N availability may affect terrestrial C balances via increased net primary productivity (NPP) and/or decreased litter decomposition rates (Janssens et al., 2010; Liu and

Greaver, 2010). The potential of a negative effect of increased N availability on decomposition rates was suggested by many experimental N addition studies (e.g. Berg and Matzner, 1997; Olsson et al., 2005; Hobbie, 2008). Stimulation of litter decomposition is expected at sites with low ambient N deposition and high-quality litter (low lignin), whereas suppression of litter decomposition is suggested at sites with higher ambient N deposition or at sites with low quality (high lignin) litter (Knorr et al., 2005). Despite current studies have focused on the impact of N deposition on C balance, there is clear evidence that acidification (either from S or N deposition) may also affects a range of key processes, including slowdown of litter decomposition (Chen et al., 2015; Liang et al., 2013; Oulehle et al., 2011), as well as mobility and leaching of dissolved organic carbon (Evans et al., 2012). Suppression of litter decomposition at low pH may occur through reduction in the availability of C for microbial community (Persson et al., 1989) and/or by direct effect on the microorganisms themselves (Błońska et al., 2017; 2016; Pennanen et al., 1998; Wei et al., 2013). Input of N to the soil, either from deposition or fertilization, can have an acidifying effect, depending on the fate (immobilization vs. leaching, nitrification) of mineral N in the soil. Thus, it is likely that experimentally induced responses to N fertilization might in fact be due to changes in soil acid-base status (Evans et al., 2008; Chen et al., 2016). A better, integrated understanding of soil C and N responses to changing inputs of both S and N is needed to predict whether soils will in future act as C sinks or C sources as atmospheric pollutant loadings change. Accounting for, that much of Europe and North America have experienced high historic depositions of S, and receive high N inputs, whilst other regions including large parts of Asia continue to receive high depositions of both S and N (Duan et al., 2016; Larssen et al., 2006; Posch et al., 2015), it is likely that recent measurements of C fluxes from these regions might not be in long-term balance with climatic conditions, but represent ecosystems subjected to multiple anthropogenic pressures, and in many cases undergoing transient change as deposition levels decline or rise.

Here, we present a set of replicated acidity/N availability manipulation experiments in adjacent mature broadleaved (beech forest) and coniferous (spruce forest) sites. Spruce and beech represent the dominant tree species in the Central European forests. Both stands are influenced by similar abiotic conditions (climate, deposition, bedrock, original soil type) but differ in vegetation cover as a result of long-term differences in land-use. Furthermore, long-term ambient monitoring from spruce site provided evidence of C soil loss as a consequence of acidification recovery (Oulehle et al., 2011) and both sites were intensively studied to assess the rates of acidification recovery in formerly highly polluted areas (Oulehle et al., 2006). We examined whether there has been a shift in the ecosystem C fluxes in either (dissolved) organic or (gaseous) inorganic forms, as a function of altered acidity and N availability. *In situ* decomposition experiments and measurements of microbial characteristics and enzyme activity provided further insights into C metabolism under changing acidity and N availability.

2. Material and methods

2.1. Sites and experimental design

In 2013 two parallel acidity and N availability manipulation experiments were established, at two adjacent forests (50.59 N, 13.26 E) selected to represent major European forest types — mature Norway spruce forest (*Picea abies* (L.) Karst.) and mature European beech forest (*Fagus sylvatica* L.). The spruce stand consists of Norway spruce monoculture planted in the early 1930s, and the

beech stand consists of European beech monoculture (Oulehle et al., 2016). The distance between stands is approximately 1 km and both stands are underlined by gneiss bedrock. The dominant soil type is dystric cambisol (Dambrine et al., 1993). Soils at both sites were acidified by acid deposition during the 20th century, which resulted in low soil pH and low base saturation. Despite similar bulk S deposition, total deposition is higher in the spruce stand compared to the beech stand. Throughfall fluxes of inorganic nitrogen are similar in both stands (Table 1; Oulehle et al., 2011, 2016). Ground cover vegetation in the spruce stand (60%) is formed mainly by *Avenella flexuosa* L. Drejer with admixture of *Vaccinium myrtillus* L., *Dryopteris dilatata* (Hoffm.) A. Gray, *Calamagrostis villosa* (Chaix) J. F. Gmel. and *Galium saxatile* L. The ground cover vegetation in the beech stand (1.5%) comprises mainly of *Calamagrostis villosa* (Chaix) J. F. Gmel. and *Avenella flexuosa* L. Drejer.

At each forest stand, soil acidity and N availability were manipulated for 3 years by systematic addition of S and N treatment solutions. At each experimental site, sixteen 3 × 3 m plots were assigned to control (Ctrl), nitrogen (N), acid (S) and acid + nitrogen (S + N) treatments in a randomized blocked design, thus four replicates per treatment were available. In October 2012 plots were established (mineral soil lysimeter installation), and in April 2014 treatments began following a period of pre-treatment measurements (April 2013–March 2014). Treatments consists of monthly additions (April–November) of sulphuric acid (H_2SO_4) and ammonium nitrate (NH_4NO_3), mixed with rainwater collected at sites and applied using 15 l watering cans evenly across the plots, followed by addition of extra 15 l rainwater to properly seep in soil all added chemicals. The treatment dose was equivalent to 50 kg S ha^{-1} $year^{-1}$, while the additional water addition was equivalent to 2.5% of average annual rainfall. Nitrogen treatments gave an input of 50 kg N ha^{-1} $year^{-1}$. NH_4NO_3 addition, albeit ammonium is a weak acid, did not significantly alter pH of added solution (applied solution pH was ≈5.4).

2.2. Soil solution sampling and analysis

Every 3–5 weeks during the snow/ice free period (usually April–November) samples were taken, ideally after rainfall when soil moisture conditions permitted collection of soil water. In every case soil water was collected before treatment application. Soil water samples were collected using Rhizon® suction samplers (Rhizosphere Research Products, Wageningen, NL), comprising 10 cm long, 2.5 mm diameter porous membranes attached to 50 ml syringes. Four to six Rhizon samplers were inserted randomly into the forest floor of each plot, to give one composite sample per plot. In the mineral soil, Prenart Super quartz standard suction samplers (Prenart Equipment, Frederiksberg, DK) were inserted into 30 cm soil depth. Soil solution was collected by applying suction into a 2 l collecting bottle.

Samples were stored at 4 °C, and analysed for pH using Radiometer TTT-85 with a combination electrode, nitrate (NO_3^-) by high-performance liquid chromatography (Knauer 1000); ammonium (NH_4^+) was determined by indophenol blue colorimetry. DOC (dissolved organic carbon) was determined by a Tekmar-Dohrman Apollo 9000 analyzer (Tekmar Dohrmann, OH, USA). Samples for DOC determination were filtered (0.4 μm glass fiber Macherey-Nagel GF 5) before analysis.

2.3. Soil CO2 flux measurements

Soil CO_2 efflux (Rs) was measured monthly between 2013 and 2016 during the snow-free period (April–November/December) using a LiCor infrared gas analyzer LI-8100A (LiCor Biosciences, NE, USA) attached to a LiCor Survey Chamber (8100–103 20 cm survey