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Decomposition of trembling aspen leaf litter under long-term nitrogen and sulfur deposition: Effects of litter chemistry and forest floor microbial properties

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ABSTRACT

Litter decomposition rates are affected by abiotic and biotic factors such as climate, soil physico-chemical properties, litter chemistry, nitrogen (N) availability, and activities of soil organisms. Elevated N and sulfur (S) deposition originated from oil sands mining and upgrading activities can change soil microbial properties, litter chemistry, and litter decomposition rates in the surrounding forest ecosystems in northern Alberta. We studied (1) the effect of long-term N and S deposition on litter chemistry and soil microbial properties, and (2) the effect of changed litter chemistry and soil microbial properties on litter decomposition (CO_2 emission) in a 100-day laboratory incubation experiment using trembling aspen (*Populus tremuloides*) leaf litter and forest floor collected from a mixedwood boreal forest that has been subject to simulated N and S deposition for 10 years. Litter chemistry (lignin, total carbon (C) and N, and calcium (Ca), aluminum (Al), manganese (Mn), and magnesium (Mg) concentration) and forest floor microbial properties (microbial biomass C and N, and extracellular enzyme activities) were analyzed. Ten years of N and S addition increased N ($P < .05$ unless otherwise stated) and decreased lignin concentrations resulting in lower C/N and lignin/N ratios in the litter. In addition, N and S addition increased forest floor microbial biomass ($P < .01$) and enzyme activities. Cumulative CO_2 emission (C_{cum}) from litter was greater from the N and/or S addition treatments than that from the control, probably due to decreased C/N and lignin/N ratios in litter from the N and S addition treatments; meanwhile, C_{cum} from litter was not affected by soil microbial activity. The results indicate that N and S deposition enhances decomposition of aspen leaf litter by decreasing C/N and lignin/N ratios, suggesting that long-term exposure to high levels of N and S deposition can significantly change C (and associated nutrients) cycling in forest ecosystems in the oil sands region.

1. Introduction

Litter decomposition plays a critical role in carbon (C) and nutrient cycling in forest ecosystems (Melillo et al., 1982) as carbon dioxide (CO_2) and nutrients are released through litter decomposition in the soil (Berg and McClaugherty, 2003; Wood et al., 2006). Litter decomposition is affected by abiotic and biotic factors such as climate, soil physico-chemical properties, litter chemistry, nitrogen (N) availability, and activities of soil organisms (Berg, 2000; Lavelle et al., 1993; Swift et al., 1979). Among those, the effect of N availability on litter decomposition has received particular interest as increased N deposition alters litter decomposition pattern through changed litter chemistry (Berg and Matzner, 1997) as well as changed soil microbial community composition and enzyme activities (Berg, 1986; DeForest et al., 2004; Fog, 1988). For example, N deposition has been shown to increase N concentration of litter, leading to decreasing C to N (C/N) and lignin to N (lignin/N) ratios, which are key parameters associated with litter decomposability; i.e., litter with low lignin/N ratio is known to decompose faster than those with high lignin/N ratios (Berg and Matzner, 1997; Carreiro et al., 2000). Studies have also shown that N deposition can increase microbial biomass and enzyme activities and enhance decomposition by increasing soil N availability (Lv et al., 2013; Sinsabaugh et al., 2002).

Many studies reported that N addition increased litter decomposition rates (Carreiro et al., 2000; Hobbie, 2000; Hobbie and Vitousek, 2000; Hunt et al., 1988). However, lack of effect (Hunt et al., 1988; Pastor et al., 1987; Prescott, 1995) or suppressive effects of N addition on litter decomposition (Carreiro et al., 2000; Magill and Aber, 2000)

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have also been reported. The inconsistent results are ascribed to the variations in litter type, the chemical composition of N added (NH_4^+ or NO_3^-), the N addition rate, and the experiment duration (Thirukkumaran and Parkinson, 2000). In addition to the effect of N deposition, the effect of sulfur (S) deposition on litter decomposition has also been investigated; S deposition may inhibit (McKinley and Vestal, 1982; Traaen, 1980), enhance (Lee and Weber, 1983; Roberts et al., 1980), or have no effect (Killham et al., 1983) on litter decomposition. A recent study has also found that the reduction of S deposition can limit C sequestration in Europe and the USA (Fernandez-Martinez et al., 2017). However, how the co-occurrence of N and S deposition might affect litter decomposition by altering litter chemistry and soil microbial activities (e.g., enzyme activities) is poorly understood. This is an important research question as forest areas with heavy industrial activities are often subject to elevated levels of deposition of both N and S.

This study investigated the effect of N and S addition on changes in litter chemistry and soil microbial properties and the subsequent effect of changed litter chemistry and soil microbial properties on the rate of litter decomposition. We hypothesized that (1) external N addition will decrease litter C/N and lignin/N ratios by increasing litter N concentration in a N-limited forest; (2) external N and S addition will increase soil microbial activity as N and S are required macronutrients for microorganisms; and (3) lowered C/N and lignin/N ratios and increased soil microbial activity will enhance litter decomposition.

2. Materials and methods

2.1. Research site and experimental design

To study the effect of N and S deposition on forest ecosystems in the oil sands region in northern Alberta, research plots were established in 2006 in a mixedwood boreal forest stand (56.1° N 110.9° W), located about 100 km southeast of Fort McMurray, a major city in the Athabasca oil sands region (AOSR) in western Canada (Jung and Chang, 2012). The background N and S deposition rates in the study site were measured between September 2014 and August 2016, with precipitation samples collected every two months during each growing season. The mean total inorganic N bulk deposition rates were 1.0 kg N ha^{-1} yr^{-1}, with 0.7 and 0.3 kg N ha^{-1} yr^{-1} for NH_4^+ and NO_3^-, respectively, and deposition rate of S was 0.5 kg S ha^{-1} yr^{-1} (Kwak et al., 2018a). Such N and S deposition rates are similar to Fenn et al. (2015) and Hsu et al. (2016), who measured N and S deposition rates in AOSR from May 2008 to May 2012 and from 2005 to 2013, respectively. The climate of the region is continental boreal, with a mean annual temperature of 1 °C and mean annual precipitation of 419 mm from 1981 to 2010 (Environment Canada, 2010). The main canopy tree species were approximately 60-year old *Populus tremuloides* (trembling aspen, accounting for 71% of the total tree stems) and 25–55 years old *Picea glauca* (white spruce, accounting for 22% of the total tree stems) (Jung and Chang, 2012). Soils were classified as Gray Luvisols based on the Canadian system of soil classification (Soil Classification Working group, 1998) or Boralf in Soil Taxonomy (Soil Survey Staff, 1994).

The research plots were set up in a randomized complete block design with two factors. One factor was N addition (with two levels, 0 and 30 kg N ha^{-1} yr^{-1} as NH_4NO_3) and the other one was S addition (with two levels, 0 and 30 kg S ha^{-1} yr^{-1} as Na_2SO_4) to simulate elevated levels of N and S deposition in the oil sands region, resulting in four treatments: control (CK), N addition (+N), S addition (+S), and N and S addition (+NS). Three blocks were set up and four plots 20 × 20 m in size were established in each block and the four treatments were randomly assigned to the plots. The granule forms of NH_4NO_3 and Na_2SO_4 were applied using a hand spreader. Sodium sulfate, instead of H_2SO_4, was used to reflect the natural form of S deposition in the AOSR, which comes with base/dust deposition (Kwak et al., 2018b). Nitrogen and S were applied once a year from 2006 to 2008 in early summer. From 2009, the N and S were applied three times (in May, July, and September) each summer on an equal split to better simulate the dynamics nature of N and S deposition.

2.2. Leaf litter and forest floor sampling and analysis

In September 2015, before sampling the forest floor, newly fallen aspen litter was collected from the ground surface of the research plots. Litter from the same treatment plot was composited and the litter samples were then oven-dried at 60 °C for 48 h until constant weight once they were brought back to the laboratory. A portion of each litter sample was crushed and passed through a 2 mm sieve to obtain a homogenized sample. A subsample (5 g) of each crushed litter sample was further ground using a ball mill (MM 200, REtsch GmbH, Haan, Germany) and used for chemical analyses. Total C, N and S concentrations were determined using an elemental analyzer (Carlo Erba NA1500, Carlo Erba Instruments, Milano, Italy). Lignin concentration was analyzed by measuring absorbance at 280 nm following acetyl bromide digestion (Morrison, 1972a, 1972b). Other elemental concentrations including calcium (Ca), magnesium (Mg), aluminum (Al), and manganese (Mn) were determined using an ICP-MS (Elan 6000 quadrupole, Perkin-Elmer, Inc., Shelton, CT) after digestion with nitric acid and hydrogen peroxide (Jones, 2001).

Forest floor F (fragmented, partially decomposed litter material) and H (humified, very well decomposed material) layer samples (approximately 10 kg) were then collected in July 2016 from three randomly selected locations in each plot, after removing the litter layer, approximately 10 days after N and S addition. The forest floor was approximately 5–10 cm thick and had a 0.1 g cm^{-3} bulk density (Cheng et al., 2011). Forest floor samples were then placed in a cooler, transported to the laboratory, and stored in a refrigerator at 4 °C. The samples were sieved through a 4-mm sieve to remove stones, roots, and debris. The forest floor samples were stored at 4 °C until further analysis and the incubation experiment was conducted.

The moisture content of the forest floor samples was determined with a portion of the sample (~10 g) in a forced air oven at 60 °C (temperature for oven-drying organic matter) for 48 h until constant weight. Another portion (~50 g) of the forest floor sample was air-dried at room temperature until constant weight for chemical analysis. The pH (1:10 w:w soil to water ratio) was measured using a pH meter (Orion, Thermo Fisher Scientific Inc., Beverly, MA, USA). Total C and N concentrations were analyzed using the elemental analyzer described above. Exchangeable cations, including sodium (Na^+), potassium (K^+), Mg^{2+}, Ca^{2+}, and Al^{3+}, were determined using the ICP-MS described above after extracting a 2 g sample with 100 mL of 1 mol L^{-1} NH_4Cl (Shuman and Duncan, 1990).

Another fresh forest floor subsample was used to determine microbial biomass C (MBC) and N (MBN) and extracellular enzyme activities. The MBC and MBN concentrations were determined using a fumigation-extraction method (Brookes et al., 1985). The concentrations of C and N in the fumigated and un-fumigated samples extracted with 0.5 mol L^{-1} K_2SO_4 (Brookes et al., 1985) were determined with a TOC-V_{CSN} (Shimadzu, Kyoto, Japan). Activities of extracellular enzymes including β-1, 4-glucosidase (GLU, enzyme classification (EC) 3.2.1.21), β-1, 4-N-acetylglucosaminidase (NAG, EC 3.2.1.14), and arylsulfatase (ARS, EC 3.1.6.1) that are involved in C, N, and S cycling, respectively, were analyzed. The NAG and GLU were measured with a fluorimetric method (Sinsabaugh et al., 2002). Briefly, one gram of fresh forest floor sample was placed in a 250 mL Nalgene HDPE bottle, 125 mL of sodium acetate buffer (50 mmol L^{-1}, pH 5) was then added, and shaken for 30 min on an end-over-end shaker at room temperature. Then 200 μL of soil suspension and 50 μL of 200 μmol L^{-1} of each substrate were pipetted into black 96 well plates. Reference standard and quench controls were added to each reference and quench well in each plate. The plates were incubated at 20 °C in the dark for 3 h. After incubation, a 20 μL of 0.5 mol L^{-1} sodium hydroxide solution was added to each well to stop