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Soil carbon and nitrogen cycling processes and composition of terpenes five years after clear-cutting a Norway spruce stand: Effects of logging residues

Sylwia Adamczyk*, Veikko Kitunen, Antti-Jussi Lindroos, Bartosz Adamczyk, Aino Smolander

The Natural Resources Institute, Finland, Vantaa Research Unit, PO Box 18, FI-01301 Vantaa, Finland

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ABSTRACT

The aim of this study was to determine the effects of logging residues on C and N cycling in relation to abundant plant secondary compounds, terpenes and tannins in a Norway spruce clear-cut site. The study site was located in Southern Finland. After clear-cutting piles having 0, 10, 40 kg m^{-2} of fresh Norway spruce logging residues were built and as additional treatment commercial wood ash was provided to study the possibilities for recycle of wood ash. Samples were taken from the organic layer and uppermost mineral soil layer five years after the treatments. Logging residues increased net N mineralization, especially net nitrification. Addition of wood ash also increased net nitrification. C mineralization, microbial biomass C and N were less affected by the treatments. Observed changes were more visible in organic layer than in mineral soil. Logging residues did not affect much tannin and terpene concentrations in the organic layer; however, we observed increase in terpene concentration in treatment with the highest amount of logging residues. In conclusions, on this study site logging residues did not affect much soil C cycling. However, logging residues and wood ash seemed to stimulate N cycling processes.

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1. Introduction

The need to replace fossil fuels with renewable energy sources escalated interest in forest biomass for energy production. Therefore, traditional stem-only harvest (SOH) is often replaced by whole-tree harvest (WTH) in which not only tree stems but also logging residues, consisting of branches and stem tops with green needles are removed. Finland and Sweden have become leading countries in EU regarding the use of wood-based energy in 2000s ([Asikainen et al., 2008](#page--1-0)) and the demand for bioenergy from forest is expected to further increase [\(Björheden, 2006; Hakkila, 2006\)](#page--1-0). However, how these forest management practices affect soil N and C cycling, decomposition and forest productivity is still poorly understood.

As one may expect, intensive harvesting decrease soil nutrient pools (e.g. [Saarsalmi et al., 2010\)](#page--1-0) and thus also tree growth may be diminished. In several Nordic Norway spruce and Scots pine thinning experiments logging residues removal resulted in a long-term decrease of tree volume growth [\(Jacobson et al., 2000;](#page--1-0) [Helmisaari et al., 2011; Kaarakka et al., 2014](#page--1-0)). There were also indications of changes in microbial processes as well as organic matter

⇑ Corresponding author. E-mail address: Sylwia.Adamczyk@luke.fi (S. Adamczyk). composition due to logging residues removal ([Smolander et al.,](#page--1-0) [2008, 2010, 2013\)](#page--1-0). Results indicated for example a long-term decrease in one of the key processes in nutrient cycling, the rate of net nitrogen mineralization at some sites. Moreover, it was suggested that logging residues harvest decrease enzymatic activity in the organic layer ([B. Adamczyk et al., 2015\)](#page--1-0). However, the overall response of forest ecosystem to logging residue removal seems to be under control of site-specific effects as some sites showed negligible response [\(Brais et al., 2002; Johnson et al., 2002; Smolander](#page--1-0) [et al., 2013](#page--1-0)). It was stated by [Thiffault et al. \(2011\) and Achat et al.](#page--1-0) [\(2015\)](#page--1-0) that there are no consistent effects of logging residues harvesting from boreal forest soil; however, if the effects are observed they indicate positive role of logging residues on soil nutrient status and microbial activities.

Logging residues are harvested from both, clear-cuttings and thinning stands. In Finland, 0.12 Mha of forests are clear-cut annually, mostly with stem-only harvest [\(Peltola, 2014](#page--1-0)); in 2012 logging residues were harvested from about 30% of the clear-cuts ([Asikainen et al., 2014\)](#page--1-0). Studies showed that logging residues left on a site after clear-cutting can potentially increase soil C and N stocks; however, actual increases are rather negligible [\(Piirainen](#page--1-0) [et al., 2015\)](#page--1-0). The effects of logging residues removal after clear-cutting on soil decomposition processes are not wellknown. Net nitrification and $NO₃$ -N leaching are usually negligible

in undisturbed boreal forest soil, but after clear-cutting nitrification may increase [\(Smolander et al., 2001; Smolander and](#page--1-0) [Kitunen, 2011](#page--1-0)). At clear-cut site extraordinary high $NO₃$ -N concentrations in percolate water were associated with high amounts of conifer logging residues [\(Rosen and Lundmark-Thelin, 1987;](#page--1-0) [Wall, 2008; Ring et al., 2015; Lindroos et al., 2016\)](#page--1-0).

Logging residues with green branches are not only nutrientrich, but also abundant in plant secondary metabolites, phenolic compounds and terpenes [\(Obst, 1998\)](#page--1-0). Plant secondary compounds are considered as important mediators in microbial processes related to soil C and N transformations (see for review [Kraus et al., 2003; Smolander et al., 2012\)](#page--1-0). Both, tannins and terpenes may alter the quantity and forms of N available to plant and microbes, inhibit microorganisms ([Smolander et al., 2012; S.](#page--1-0) [Adamczyk et al., 2013\)](#page--1-0) and decrease soil enzyme activities, e.g. acid phosphatase, chitinase, beta-glucosidase ([Adamczyk et al., 2009, S.](#page--1-0) [Adamczyk et al., 2015; Triebwasser et al., 2012](#page--1-0)).

Our aim was to study the effects of logging residues on soil microbial processes related to C and N cycling 5 years after clearcutting of a Norway spruce stand. Moreover, we studied changes in soil tannin and terpene concentrations as these groups of plant secondary compounds affect decomposition. Large amount of ash is produced as a byproduct of burning wood for energy in power plants. It contains all plant nutrients except of nitrogen. We studied also how recycling of this waste product would affect soil properties and whether wood ash can counteract the effects of decreased amounts of nutrients due to logging residues harvest.

2. Materials and methods

2.1. Study site and soil sampling

The study site was a Norway spruce stand in Anjalankoski, Southern Finland (60 \textdegree N, 26 \textdegree E), having podzolized soil, humus type mor, glaciofluvial parent material and soil texture sorted sand. After clear-cutting, piles having 0, 10, 40 kg m^{-2} of fresh logging residues were built corresponding to 0, 5, 20 kg m^{-2} dry matter, on 1 m² plots. In an additional treatment, 0.3 kg m⁻² of commercial wood ash was added to soil; this amount was based on the recommendation of the wood ash fertilization for the Finnish forested mineral soils. For element concentrations in the wood ash typical for Finnish forestry see [Saarsalmi et al. \(2004\).](#page--1-0) There were 4 replicate plots per treatment.

Five years after the treatments, five soil cores (diameter 58 mm) were systematically taken from each plot and divided into organic layer (Ofh) and mineral soil (uppermost 5 cm). Samples were combined to one composite organic layer sample and one composite mineral soil sample per plot. Samples were moved from the field to laboratory in a cold box and stored at $+2-3$ °C. On the following day samples were sieved through a 4 mm sieve to homogenize the samples and to remove larger roots.

2.2. Determination of soil characteristics

Soil water-holding capacity, pH $(H₂O)$, dry matter, and total C and N were measured as described in [Priha and Smolander](#page--1-0) [\(1999\).](#page--1-0) Organic matter content was measured as loss on ignition (+550 \degree C, 4 h). Microbial biomass and activities were measured from the fresh, sieved organic layer samples. The microbial biomass C and N were determined using the chloroform fumigation-extraction method as described earlier [\(Smolander](#page--1-0) [et al., 1994\)](#page--1-0). Briefly, soil samples were fumigated for 24 h at 28 \degree C with ethanol-free chloroform vapor. C and N flushes from the microbial biomass were calculated by subtracting K2SO4-extractable organic C and N, respectively, in unfumigated control samples from those in fumigated samples. C and N flushes were converted to microbial biomass with the formulas of [Martikainen and Palojarvi \(1990\)](#page--1-0).

Net N mineralization, C mineralization and net nitrification were determined in a 4-week soil incubation experiment ([Smolander et al., 1995](#page--1-0)). Soil (20 ml of organic layer, equal to 8 g, and 20 ml of mineral soil, equal to 14.7 g) was weighted to 125 ml bottles, covered with foil and incubated at constant moisture (60%WHC) and temperature (14 \degree C). For the initial values, some of the bottles were stored at -20 °C. Ammonium (NH₄-N) and nitrate ($NO₂ + NO₃ - N$) were measured with a flow injection analyzer (FIA Star 5020, Tecator). The $CO₂$ evolution (C mineralization) was measured by sampling the headspace and analyzing the amount of $CO₂$ by a gas chromatograph (Hewlett Packard HP 6890 series, GC System) according to [Kanerva and Smolander \(2007\).](#page--1-0)

2.2.1. Determination of terpenes and tannins in soil and plant material

Monoterpenes were measured from the organic layer as described by [Asensio et al. \(2008\)](#page--1-0), modified by [Smolander et al.](#page--1-0) [\(2013\).](#page--1-0) Immediately after sieving soil samples were crushed in a mortar with liquid nitrogen and extracted with pentane spiked with internal standard (chlorodecane), shaken in planar shaker for 1 h and centrifuged (15 min, 10,000 rpm at 5 $°C$) [\(Asensio et al., 2008\)](#page--1-0). Extraction was repeated and combined supernatant was stored in freezer until gas-chromatography-mass spectrometry (GC-MS) analysis, which was based on authentic reference compounds.

Sesqui-, di-, and triterpenes were determined as described previously [\(Smolander et al., 2008](#page--1-0)); briefly, soil samples and plant samples were dried at $+40$ °C, ground (0.5 mm sieve) and stored in -20 °C until analyzed. Concentrations of these terpenes were determined from samples after acetone extraction by GC-MS. Identification of the terpenes was based on authentic reference compounds, mass spectrometric data and the literature ([Pohjola,](#page--1-0) [1993\)](#page--1-0).

Concentration of condensed tannins was determined from 0.5 g of dried and ground sample (for plant material 0.1 g) using modified acid-butanol assay (proanthocyanidin assay) ([Terrill et al.,](#page--1-0) [1992; Waterman and Mole, 1994; Ossipova et al., 2001](#page--1-0)) as described previously [\(Smolander et al., 2005](#page--1-0)). The method involves HCl-catalyzed depolimerization of condensed tannins in butanol to yield a pink-red anthocyanidin product, the absorbance of which was measured spectrophotometrically. As a standard we used CT extracted and purified from Norway spruce needles (for extraction and characterization see [B. Adamczyk et al., 2013](#page--1-0)).

2.3. Measurement of potential nitrification in soil suspension

Nitrification was studied in soil suspension experiments at constant shaking and with excess of NH4-N according to the method described by [De Boer et al. \(1992\).](#page--1-0) The soil was incubated for 14 days in three replicate 600 ml glass bottles on a rotary shaker (150 rev min⁻¹) in the dark at $+20$ °C. The suspensions included 50 ml of fresh soil (organic layer – 20 g, mineral soil – 35 g of fresh material) in 150 ml of mineral solution (KH_2PO_4 , CaCl₂·2H₂O, MgSO₄· $6H₂O$, (NH₄)₂SO₄). The bottles were sealed with aluminium foil. Every day the pH was adjusted with 0.1 M $Na₂CO₃$ or 1 M $H₂SO₄$ to the original pH 3.5–4 and to pH 6. To ensure an excess of NH_4-N , 1 ml of 2.5 M $(NH_4)_2SO_4$ was added every second day. After 7 and 14 days, 50 ml of soil suspensions were filtered (S&S 5893). NH₄-N and $(NO₂ + NO₃) – N$ were measured using a flow-injection analyzer (FIA Star 5020, Tecator) ([Paavolainen and Smolander, 1998](#page--1-0)).

2.4. Statistics

Results were expressed on organic matter basis to describe the quality of organic matter. To compare organic layer and mineral soil, some results were expressed also on soil volume basis.

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