


Short-term carbon input increases microbial nitrogen demand, but not microbial nitrogen mining, in a set of boreal forest soils

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Abstract Rising carbon dioxide (CO₂) concentrations and temperatures are expected to stimulate plant productivity and ecosystem C sequestration, but these effects require a concurrent increase in N availability for plants. Plants might indirectly promote N availability as they release organic C into the soil (e.g., by root exudation) that can increase microbial soil organic matter (SOM) decomposition (“priming effect”), and possibly the enzymatic breakdown of N-rich polymers, such as proteins, into bio-available units (“N mining”). We tested the adjustment of protein depolymerization to changing soil C and N

availability in a laboratory experiment. We added easily available C or N sources to six boreal forest soils, and determined soil organic C mineralization, gross protein depolymerization and gross ammonification rates (using ¹⁵N pool dilution assays), and potential extracellular enzyme activities after 1 week of incubation. Added C sources were ¹³C-labelled to distinguish substrate from soil derived C mineralization. Observed effects reflect short-term adaptations of non-symbiotic soil microorganisms to increased C or N availability. Although C input promoted microbial growth and N demand, we did not find indicators of increased N mobilization from SOM polymers, given that none of the soils showed a significant increase in protein depolymerization, and only one soil showed a significant increase in N-targeting enzymes. Instead, our findings suggest that microorganisms immobilized

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the already available N more efficiently, as indicated by decreased ammonification and inorganic N concentrations. Likewise, although N input stimulated ammonification, we found no significant effect on protein depolymerization. Although our findings do not rule out in general that higher plant-soil C allocation can promote microbial N mining, they suggest that such an effect can be counteracted, at least in the short term, by increased microbial N immobilization, further aggravating plant N limitation.

Keywords Boreal forest · Priming · Microbial N mining · Organic N · Protein depolymerization · N mineralization · Ammonification

Introduction

Plant primary production in temperate, boreal, and arctic ecosystems is often limited by low soil N availability (Vitousek and Howarth 1991). Most soil N is contained within SOM polymers that are too large for immediate uptake by plants or microorganisms, and soil N availability consequently depends on the breakdown of these polymers into smaller units (Schimel and Bennett 2004). This process is catalyzed by extracellular enzymes that are released into the soil by microbial decomposers. Given that proteins alone represent ca. 40% of the soil N pool (Schulten and Schnitzer 1998), the depolymerization of proteins into oligopeptides and amino acids is thought to dominate the production of available N (Jan et al. 2009). Microorganisms take up available N and invest it in growth and enzyme synthesis, and only N in excess is released to the soil solution as ammonium (Schimel and Bennett 2004). This process is referred to as “ammonification” or, more generally, as “N mineralization”, when other mineral N forms are considered.

Plants compete with soil microorganisms for available N forms (e.g., oligopeptides, amino acids, quarternary ammonium compounds, ammonium, nitrate), but might also be able to stimulate their mobilization from SOM. Plant roots release organic compounds into the soil, e.g., via root litter and root exudation (e.g., Jones et al. 2009; Rovira 1969), and thereby increase C availability in their vicinity. Increased C availability can promote the activity of

microbial decomposers and facilitate SOM decomposition (“priming effect”; Kuzyakov et al. 2000), and thus accelerate not only the mineralization of soil organic C (SOC) to CO₂, but possibly the release of available N from SOM. Additionally, plant-derived organic compounds typically have a low N content (e.g., sugars and organic acids; Jones et al. 2009), and it has been hypothesized that these compounds specifically promote the microbial synthesis of extracellular enzymes that target N-rich SOM polymers (“microbial N mining”; Craine et al. 2007). The release of organic C by plants into the soil might therefore not only stimulate SOC mineralization, but also soil N availability, facilitating plant primary production and, overall, ecosystem C sequestration.

Periods of increased C availability in the soil occur on timescales from hours to years, depending on the degradability and frequency of C inputs (“hot moments”; Kuzyakov and Blagodatskaya 2015). Root litter decomposition for instance increases C availability in the surrounding soil for months or even years (Kuzyakov and Blagodatskaya 2015). Root hairs, in contrast, have a life span of a few days (Kuzyakov and Xu 2013) where root exudation (e.g., of glucose) and plant N uptake alter C and N availability in their vicinity. Even on such short time frames, priming effects (Garcia-Pausas and Paterson 2011; Koranda et al. 2013; Wild et al. 2014), as well as changes in N-targeting enzyme activities (Geisseler and Horwath 2009; Koranda et al. 2013) and gross N mineralization rates (Koranda et al. 2013; Landi et al. 2006; Murphy et al. 2015) with C addition have been frequently observed. While these findings suggest that even short-term changes in C availability can induce adaptations of microbial communities that alter SOM decomposition, and thereby N mining, major knowledge gaps remain. The studies cited above include observations of positive, negative and neutral effects of C addition on N-targeting enzymes and gross N mineralization rates, which suggests that the direction and magnitude of the effects depend on initial soil properties such as N availability (Yin et al. 2016). Furthermore, direct evidence linking increases in N-targeting enzyme activities and N mineralization rates to an accelerated breakdown of N-containing SOM polymers is missing. Methods for quantifying gross production rates of oligopeptides or amino sugars by polymer breakdown are lacking, and although a method for gross production rates of amino

acids is available (Wanek et al. 2010), it has rarely been applied. We are not aware of any study showing an adjustment of gross amino acid production by protein depolymerization to soil C or N availability. Considering the frequent N limitation of terrestrial plant productivity and consequently ecosystem C storage, a better mechanistic understanding of how plant-soil C allocation influences the breakdown of N-containing SOM polymers such as proteins is urgently required.

In this study, we focus on short-term increases of C and N availability in soils and provide insights into their effects on gross release rates of amino acids from SOM proteins. We hypothesized that an increase in C availability would facilitate the microbial investment in extracellular enzymes that target N-rich SOM polymers and thus promote protein depolymerization, overall leading to higher soil N availability, but also a higher mineralization of SOC to CO₂. We expected that an increase in N availability would have the opposite effect, and reduce N-targeting enzyme activities and gross protein depolymerization rates. By affecting amino acid and ammonium production rates, these microbial adaptation mechanisms can alter soil N availability for plants. We further hypothesized that the magnitude of the response to C or N addition would depend on the initial balance between C and N availability in the soil, with strongest C effects where C availability was low, and strongest N effects where N availability was low. To describe the balance between C and N availability, we used the C/N ratio of SOM as an indicator. Although C and N within SOM might differ in their chemical and physical stabilization, C/N ratios of SOM have been found to be well correlated with indices of microbial C or N excess in soils (e.g., Mooshammer et al. 2014a; Spohn and Chodak 2015).

We tested our hypotheses in boreal forest soils where such effects are thought to be particularly pronounced (e.g., Fan et al. 2013; Hartley et al. 2012). Low N availability typically limits net primary production in this biome (LeBauer and Treseder 2008) and organic N forms likely dominate plant N uptake (Inselsbacher and Näsholm 2012). Given the mechanistic focus of our study, we tested our hypotheses in a laboratory incubation experiment, by manipulating C and N availability in six types of boreal forest soils that covered a range of SOM contents and decomposition states. We altered soil C

or N availability by adding immediately available forms of C (glucose) or N (ammonium), monitored the release of CO₂ from the soil over the course of 1 week, and in the case of glucose, distinguished between CO₂ derived from added glucose (that was enriched in ¹³C) and from native SOC (that was of natural ¹³C abundance). At the end of the incubation, we determined gross rates of protein depolymerization (the breakdown of proteins into amino acids) and ammonification (the microbial release of excess N as ammonium) using a set of ¹⁵N pool dilution assays, as well as extractable soil C and N pools, microbial biomass, and potential activities of a range of extracellular enzymes involved in the breakdown of different C- and N-rich SOM polymers. Observed effects reflect physiological responses of non-symbiotic soil microorganisms to short-term increases in C or N availability.

Materials and methods

Soil sampling

We compared six types of boreal forest soils that we collected at two stations of the Swedish Infrastructure for Ecosystem Science (SITES), Svartberget in northern Sweden (64°15'N, 19°47'E) and Skogaryd in south-western Sweden (58°23'N, 12°09'E). Mean annual temperature and precipitation between 1981 and 2010 were 1.8 °C, 614 mm in Svartberget (Laudon et al. 2013), and 6.8 °C, 803 mm in the vicinity of Skogaryd (Vänerns meteorological station of the Swedish Meteorological and Hydrological Institute). These managed forests are typical for the boreal forest biome. They are dominated by *Pinus sylvestris* and *Picea abies* with an understory of *Vaccinium myrtillus* and *Vaccinium vitis-idaea*, and underlain by a patchwork of Podzols and Histosols (following the World Reference Base for Soil Resources classification; IUSS Working Group WRB 2007), with the latter at locations where fens or bogs had been drained and afforested.

Soils were sampled in November 2014 (Svartberget) and December 2014 (Skogaryd) before they started to freeze. We sampled both Histosols and Podzols at each site, and in the case of the Podzols, from both organic layers and mineral B horizons. Histosol samples were from a depth of 0–10 cm, Podzol organic layer samples from 0 to 10 cm, and

Podzol mineral B horizon samples from 15 to 30 cm. Skogaryd Histosol and Podzol organic layer samples were cut from the surface, the other soil samples were retrieved by coring. Each of the six soil categories (3 soil types \times 2 sites) was sampled in five replicates, with 5–10 m distance between individual sampling points. Overall, we thus retrieved 30 soil samples that covered a natural range in SOM quantity and quality, including C/N ratios (Table 1). Immediately after sampling, we removed large roots from samples of Histosols and Podzol organic layers and homogenized the samples by hand. Podzol mineral horizons were sieved to 2 mm. None of the sampled soils contained carbonates. Soil organic C and total N content, as well as C and N isotopic composition were measured in dried and ground samples using elemental analysis— isotope ratio mass spectrometry (EA-IRMS; CE Instrument EA 1110 elemental analyzer, Finnigan MAT ConFlo II Interface, Finnigan MAT DeltaPlus IRMS), and C/N ratios were calculated as mass ratios. Data are presented in Table 1. Samples were stored for a maximum of 7 weeks at 4 °C before the start of the experiment.

Since the aim of our study was not to describe gross protein depolymerization and ammonification rates in situ, but to investigate the microbial adjustment of these processes to changes in C and N availability, we opted for an experimental design that permitted us to control soil C and N availability, reduce variability in abiotic experimental conditions, and dissect mechanisms behind observed patterns. It should, however, be noted that the absence of plant roots leads to an underrepresentation of root associated microorganisms such as mycorrhiza that might contribute to protein depolymerization in situ (Read and Perez-Moreno 2003). Furthermore, sample storage before incubation might have promoted the accumulation of inorganic N especially in the N-rich Skogaryd samples, as indicated by higher dissolved inorganic N concentrations in our control samples than in fresh soils (personal communication, T. Rütting). While these limitations mean that the presented data should not be interpreted as absolute concentrations and process rates that might be observed in the field, they do not constrain our ability to test our hypotheses related to relative effects of C and N addition on microbial processes and their dependency on the initial balance between C and N availability in the soil.

Setup of the incubation experiment and respiration measurements

We subjected the field-moist soils to three treatments by either amending them with immediately available C or N sources (glucose or ammonium), or leaving them unamended as controls. Before the start of the experiment, triplicates of fresh soil were weighed into flasks (60 g of Histosols and Podzol organic layers, 75 g of Podzol mineral horizons) that were then covered with parafilm and pre-incubated at room temperature (19 °C). Total volumes of the flasks were 305 ml, and the headspaces ranged between 225 and 271 ml depending on the sample. After 1 week of pre-incubation, samples received either glucose (uniformly enriched in ^{13}C at a level of 5 atom%, i.e., 5% of all C atoms were ^{13}C ; purchased from Sigma-Aldrich) or ammonium sulphate in aqueous solution. We optimized the solution volume added to each soil in a preliminary experiment, to ensure that samples were homogeneously moistened, but not water saturated after substrate addition (1.5 ml for Histosols, 7.5 ml for Podzol organic layers, and 1.0 ml for Podzol mineral horizons). Substrates were added dropwise to the samples, and samples were shaken immediately after addition. Carbon and N additions varied between soils to account for differences in SOC and total soil N content. Each soil received an amount of C or N that corresponded to 1% of its SOC or total soil N content, respectively. Compared to microbial C in control samples after incubation, C addition represented 150 ± 19 , 138 ± 17 , and $291 \pm 51\%$ of microbial C in the Histosol, Podzol organic layer, and Podzol mineral horizon, respectively, from Svartberget, and 432 ± 53 , 185 ± 53 , and $446 \pm 96\%$ of microbial C in the Histosol, Podzol organic layer, and Podzol mineral horizon from Skogaryd. Control samples received neither glucose nor ammonium sulphate solution, but the equivalent volume of de-ionized water. Flasks were again covered with parafilm and incubated at room temperature for 1 week. Our incubation temperature thus exceeded field temperature (summer temperature at 10 cm depth ca. 15–20 °C in Skogaryd and 15 °C in Svartberget). Given that decades of research have demonstrated predictable increases in e.g., microbial growth, respiration and enzyme activities with temperature, and optima above 30 °C (Bárceñas-Moreno et al. 2009; Schindlbacher et al. 2015), we expect that our

Table 1 Properties of initial soils, as well as C and N pools, fluxes, and potential extracellular enzyme activities of control samples after 1 week of incubation

	Svartberget Histosol	Svartberget Podzol organic	Svartberget Podzol mineral	Skogaryd Histosol	Skogaryd Podzol organic	Skogaryd Podzol mineral
Initial soils						
SOC (%)	36.84 ± 6.02a	45.03 ± 1.99a	3.16 ± 0.33b	42.25 ± 1.78a	38.52 ± 5.55a	4.55 ± 0.42b
N (%)	1.26 ± 0.20b	1.16 ± 0.06bc	0.10 ± 0.02d	2.06 ± 0.11a	1.51 ± 0.22ab	0.20 ± 0.03bd
SOM C/N	29.32 ± 2.16b	39.38 ± 3.20a	29.18 ± 1.70bc	20.67 ± 0.99c	25.58 ± 1.46bc	23.39 ± 1.08bc
δ ¹³ C (‰)	-27.35 ± 0.17cd	-28.00 ± 0.19d	-26.53 ± 0.19a	-27.51 ± 0.10c	-26.93 ± 0.20bc	-25.94 ± 0.10a
δ ¹⁵ N (‰)	2.14 ± 0.58bc	1.00 ± 0.50cd	5.91 ± 0.34a	2.43 ± 0.15bc	-0.85 ± 0.59d	4.83 ± 0.12ab
Control soils after incubation						
Micr. C (mg C g ⁻¹ SOC)	7.11 ± 0.90a	7.47 ± 0.96a	3.85 ± 0.62ab	2.44 ± 0.27b	6.57 ± 1.03a	2.70 ± 0.56b
Micr. N (mg N g ⁻¹ SOC)	1.16 ± 0.29a	1.38 ± 0.21a	0.47 ± 0.10b	0.28 ± 0.05b	1.19 ± 0.20a	0.27 ± 0.05b
Micr. C/N	6.75 ± 0.63abc	5.44 ± 0.13bc	8.50 ± 0.70ab	9.44 ± 0.92a	5.57 ± 0.14c	9.98 ± 1.06a
C/N imbalance	4.67 ± 0.91ab	7.22 ± 0.65a	3.38 ± 0.25bc	2.26 ± 0.22c	4.60 ± 0.26ab	2.43 ± 0.21c
SOC Min. (mg C g ⁻¹ SOC)	4.64 ± 0.94a	6.29 ± 1.05a	3.02 ± 0.51a	0.69 ± 0.12b	3.01 ± 0.65ab	1.26 ± 0.24b
Prot. dep. (μg N g ⁻¹ SOC h ⁻¹)	2.78 ± 0.55ab	8.61 ± 2.23a	3.16 ± 0.75ab	1.13 ± 0.35b	4.25 ± 2.07ab	N.A.
Ammon. (μg N g ⁻¹ SOC h ⁻¹)	0.12 ± 0.09b	0.21 ± 0.13ab	0.51 ± 0.41ab	0.65 ± 0.12ab	1.29 ± 0.46a	1.50 ± 0.60a
DOC (mg C g ⁻¹ SOC)	1.20 ± 0.11c	2.16 ± 0.34b	6.40 ± 1.27a	1.33 ± 0.14c	1.40 ± 0.18bc	3.66 ± 0.34a
TDN (μg N g ⁻¹ SOC)	77.45 ± 5.32c	126.79 ± 5.69bc	259.88 ± 66.03ab	221.85 ± 41.02ab	135.84 ± 21.53bc	426.96 ± 90.17a
DIN (μg N g ⁻¹ SOC)	23.17 ± 14.58bc	16.35 ± 2.79bc	27.86 ± 20.97c	124.64 ± 43.23ab	48.16 ± 12.22abc	239.26 ± 72.41a
CBH (μmol g ⁻¹ SOC h ⁻¹)	0.59 ± 0.19abc	0.61 ± 0.22ab	0.89 ± 0.48a	0.15 ± 0.03bc	0.55 ± 0.15a	0.14 ± 0.05c
BG (μmol g ⁻¹ SOC h ⁻¹)	2.95 ± 0.43a	3.06 ± 1.00ab	4.02 ± 1.51a	0.77 ± 0.12c	2.62 ± 0.86abc	1.01 ± 0.26b
NAG (μmol g ⁻¹ SOC h ⁻¹)	2.93 ± 0.47b	9.02 ± 1.18a	11.22 ± 7.05ab	0.63 ± 0.12c	2.04 ± 0.68bc	2.43 ± 0.79bc
LAP (μmol g ⁻¹ SOC h ⁻¹)	1.97 ± 0.25ab	1.75 ± 0.28b	2.90 ± 0.34a	0.74 ± 0.10c	1.20 ± 0.21b	1.63 ± 0.31b
PHO (μmol g ⁻¹ SOC h ⁻¹)	44.89 ± 4.58a	48.68 ± 8.69a	32.21 ± 4.78a	35.06 ± 4.31a	51.06 ± 7.89a	29.01 ± 10.35a
POX (μmol g ⁻¹ SOC h ⁻¹)	14.90 ± 4.71a	3.40 ± 1.07bc	15.98 ± 2.73a	8.13 ± 0.79ab	1.89 ± 0.69c	13.26 ± 3.76a
PER (μmol g ⁻¹ SOC h ⁻¹)	7.43 ± 1.51ab	N.A.	16.20 ± 4.47a	4.43 ± 0.19b	1.89 ± 0.69c	12.56 ± 2.78a
Enzyme C/N	0.95 ± 0.01ab	0.85 ± 0.04bc	0.83 ± 0.01bc	0.93 ± 0.01ab	0.99 ± 0.04a	0.75 ± 0.03c
PC1	-1.83 ± 0.63b	-2.55 ± 0.80b	-0.59 ± 0.62b	2.60 ± 0.44a	-1.24 ± 0.65b	2.60 ± 0.46a
PC2	0.24 ± 0.42bc	0.99 ± 0.19abc	-2.84 ± 0.45d	1.20 ± 0.20ab	1.99 ± 0.31a	-1.18 ± 0.57cd

C/N ratios of SOM and microbial biomass were calculated on a mass basis, and the C/N imbalance as the ratio of SOM C/N over microbial C/N. The C/N of enzyme target compounds (enzyme C/N) was calculated as LN(CBH + BG)/LN(NAG + LAP). PC1 and PC2 were derived from Principal Component Analysis of control parameters (Fig. 1). Values represent means ± standard errors; significant differences between soils are indicated by different letters (p < 0.05; N.A. = not analyzed). PER was below the detection limit in all Podzol organic layer samples, with one exception at the Skogaryd site of 0.41 μmol g⁻¹ SOC h⁻¹

Micr. C microbial C, *Micr. N* microbial N, *Micr. C/N* microbial C/N, *SOC Min.* cumulative SOC mineralization, *Prot. dep.* gross protein depolymerization, *Ammon.* gross ammonification, *DOC* dissolved organic C, *TDN* total dissolved N, *DIN* dissolved inorganic N, *CBH* cellobiohydrolase, *BG* β-glucosidase, *NAG* N-acetyl-β-D-glucosaminidase, *LAP* leucine-aminopeptidase, *PHO* phosphatase, *POX* phenoloxidase, *PER* peroxidase

comparatively high incubation temperature affected absolute rates of C and N cycling processes, but not relative responses of these processes to changes in C and N availability.

We took the first samples of respired CO_2 1 h after substrate amendment, and then every 24 h. For each sampling, we closed flasks with gas-tight caps with septum, evacuated and refilled four times with air of known CO_2 concentration and isotopic composition (393 ppm, 1.1 atom% ^{13}C), and incubated samples at room temperature for 1 h. We then mixed the headspace with a syringe, transferred aliquots into pre-evacuated gas vials and determined CO_2 concentrations using gas chromatography (Agilent 7597A Headspace Sampler, 7890A GC System with flame ionization detector). For control and C addition samples, we took a second set of gas samples immediately after the first, and measured the ^{13}C content of CO_2 with a trace gas— ^{13}C isotope ratio mass spectrometer (SerCon Automated Nitrogen Carbon Analyser ANCA-TGII and 20-20 isotope ratio mass spectrometer). We corrected respiration rates and ^{13}C contents of the respired CO_2 for the initially present CO_2 , and then distinguished between CO_2 from added glucose and from native SOC using the equations

$$C_{\text{SOC}} = C_{\text{Total}} \times (\text{atom}\%_{\text{Total}} - \text{atom}\%_{\text{Glc}}) / (\text{atom}\%_{\text{SOC}} - \text{atom}\%_{\text{Glc}}) \quad (1)$$

and

$$C_{\text{Glc}} = C_{\text{Total}} - C_{\text{SOC}} \quad (2)$$

C_{Total} , C_{SOC} and C_{Glc} represent total, SOC-derived and glucose-derived CO_2 , and $\text{atom}\%_{\text{Total}}$, $\text{atom}\%_{\text{SOC}}$ and $\text{atom}\%_{\text{Glc}}$ the isotopic compositions (in atom% ^{13}C) of total CO_2 , SOC-derived (i.e., control) CO_2 , and glucose. Throughout the text, we use the term “SOC mineralization” to refer to the mineralization of native, unlabelled SOC, excluding C mineralized from added, ^{13}C -enriched glucose. The development of SOC- and glucose-derived respiration over time is presented in Supplementary Fig. S1. After the last CO_2 sampling 1 week after the start of the incubation, we harvested the soils to measure gross N transformation rates, extractable C and N pools, microbial biomass and potential extracellular enzyme activities. Gravimetric water content was determined in all samples, and amounted to 77.9 ± 2.3 , 73.2 ± 1.0 , and $20.9 \pm 1.1\%$ of fresh weight (mean \pm standard error)

in the Histosol, Podzol organic layer and Podzol mineral horizon, respectively, from Svartberget, and 67.8 ± 1.0 , 70.2 ± 1.3 , and $28.3 \pm 0.9\%$ in the Histosol, Podzol organic layer and Podzol mineral horizon from Skogaryd. Differences between treatments were not significant ($p > 0.1$).

Gross nitrogen transformation rates

We determined gross rates of protein depolymerization and ammonification using ^{15}N pool dilution assays. Briefly, we labelled the product pool of the respective process (amino acids for protein depolymerization, ammonium for ammonification) with ^{15}N , and calculated the flux into the pool from the changes in concentration and isotopic composition between two timepoints (10 and 30 min for protein depolymerization, 4 and 24 h for ammonification, accounting for the different turnover times of amino acids and ammonium).

Gross protein depolymerization rates were determined following Wanek et al. (2010), as modified by Wild et al. (2013). Duplicates of fresh soil were amended with a $2 \mu\text{g ml}^{-1}$ solution of ^{15}N -labeled amino acids that contained 12% lysine; 9% leucine and glutamic acid; 8% aspartic acid; 6% arginine and alanine; 5% glycine, proline, glutamine, and asparagine; 4% phenylalanine, serine, valine, and threonine; 3% isoleucine, tyrosine, cysteine, and tryptophane; and 1% histidine and methionine with 96–98 atom% ^{15}N (Cambridge Isotope Laboratories). For Histosols and Podzol organic layers, we added 1.0 ml of the amino acid mixture to 2 g fresh soil, and for Podzol mineral horizons 0.5 ml to 4 g fresh soil. These additions corresponded to $10.5 \text{ nmol N g}^{-1}$ fresh soil (Histosols and Podzol organic layers) and $2.6 \text{ nmol N g}^{-1}$ fresh soil (Podzol mineral horizons). Samples were shaken vigorously immediately after additions to ensure a homogenous distribution of ^{15}N amino acids, incubated for 10 or 30 min at room temperature, extracted with 20 ml of a 10 mM CaSO_4 solution containing 3.7% formaldehyde, filtered through synthetic wool and GF/C filters (Whatman), and loaded on pre-cleaned cation exchange cartridges (OnGuard II H 1 cc cartridges, Thermo). Amino acids were eluted from the cartridges using 10 ml 3 M NH_3 , amended with an internal standard mixture (norvaline, nor-leucine and para-chloro-phenylalanine, Sigma-Aldrich), and dried under reduced pressure.

Samples were derivatized with ethyl-chloroformate (Wanek et al. 2010) and analyzed with gas chromatography—mass spectrometry (GC–MS; Thermo Trace GC and DSQ I; OV1701 column, PTV injection, 1.4 ml min⁻¹ helium as carrier, temperature program: 60 °C for 1.5 min, first ramp 29 °C min⁻¹ to 130 °C, second ramp 3 °C min⁻¹ to 180 °C, third ramp 15 °C min⁻¹ to 280 °C, 280 °C for 5 min). One set of blanks and external amino acid standards (Standard Set 1; one concentration level) was processed together with the samples throughout the entire procedure to account for background concentrations of amino acids and for incomplete recovery of amino acids from the cation exchange cartridges. A second set of external amino acid standards (Standard Set 2; eight concentration levels) was amended with the internal standards, derivatized, analyzed together with the samples, and used for calibration of alanine, glycine, leucine, proline, serine, valine, aspartate and asparagine concentrations. Asparagine and aspartate were not distinguished as formaldehyde causes the deamination of asparagine to aspartate (Wanek et al. 2010). Concentrations of individual amino acids were corrected using recovery values calculated from Standard Set 1 (alanine: 71%, glycine: 73%, leucine: 27%, proline: 43%, serine: 93%, valine: 27%, asparagine and aspartate: 19%). Nitrogen isotopic compositions were calculated by comparing the peak areas of amino acid fragments containing ¹⁴N or ¹⁵N (Wanek et al. 2010). Since this GC–MS based method of determining ¹⁵N isotopic values is sensitive to ¹³C enrichment such as in the samples amended with ¹³C-labeled glucose, we conducted a parallel incubation to this treatment where we amended samples with glucose that was not enriched in ¹³C. These non-enriched glucose-amended samples were treated identically to the enriched ones and used to determine gross rates of protein depolymerization and also ammonification without interference by ¹³C.

Gross ammonification rates were measured following Kaiser et al. (2011), by amending duplicates of fresh soil with a 62.5 μM (¹⁵NH₄)₂SO₄ solution (25 atom% ¹⁵N, Cambridge Isotope Laboratories; Histosols and Podzol organic layers: 1.0 ml for 2 g soil; Podzol mineral horizon: 0.5 ml for 4 g soil). Samples were incubated for 4 or 24 h at room temperature, extracted with 15 ml 2 M KCl, and filtered through ash-free cellulose filters (Whatman). Ammonium was isolated with acid traps (Sørensen and Jensen 1991)

and analyzed with EA-IRMS (SerCon Europa EA-GSL, 20-22 Stable Isotope Mass Spectrometer). All gross rates were calculated using the equations in Kirkham and Bartholomew (1954).

Extractable and microbial carbon and nitrogen pools

We determined ammonium and nitrate concentrations photometrically in 1 M KCl extracts, following Kandel and Gerber (1988) for ammonium and Miranda et al. (2001) for nitrate. Dissolved organic C as well as total dissolved N were measured in 0.5 M K₂SO₄ extracts with a Shimadzu TOC-V CPH equipped with a TN unit. Since nitrification, i.e., the conversion of ammonium to nitrate, was not a focus of this study, we calculated dissolved inorganic N concentrations as sums of ammonium and nitrate concentrations.

Microbial C and N pools were determined with chloroform-fumigation-extraction following Kaiser et al. (2011), by fumigating soils for 2 days with ethanol-free chloroform and extracting them with 0.5 M K₂SO₄. Dissolved organic C and total dissolved N in extracts of fumigated and non-fumigated soils were determined as described above (Shimadzu TOC-V CPH with TN unit), and microbial C and N were calculated from the difference between fumigated and non-fumigated soils. We note that C and N released by chloroform-fumigation-extraction are mostly derived from microbial cytoplasm. As correction factors in the literature are highly variable (e.g., Brookes et al. 1985), we refrained from applying a correction but present values as measured. Microbial C/N was calculated as a mass ratio. We further calculated the stoichiometric C/N imbalance between SOM and microbial biomass following Mooshammer et al. (2014a), as the ratio of SOM C/N over microbial biomass C/N. High C/N imbalance thus reflects C excess of SOM compared to the microbial biomass.

Extracellular enzyme activities

We measured potential activities of the hydrolytic enzymes cellobiohydrolase (CBH, targeting cellulose), β-glucosidase (BG, targeting cellulose and cellobiose), N-acetyl-β-D-glucosaminidase (NAG, targeting chitin and peptidoglycan), leucine-aminopeptidase (LAP, targeting proteins and peptides), and phosphatase (PHO, targeting phosphate

groups), as well as of the unspecific oxidative enzymes phenoloxidase (POX) and peroxidase (PER) using a combination of fluorometric and photometric assays modified after Kaiser et al. (2010) and Schindlbacher et al. (2015). Briefly, 1 g of soil was suspended in 100 ml sodium acetate buffer (100 mM, pH 5.5) and ultrasonicated. For the hydrolytic enzymes, one set of aliquots was amended with fluorescence labelled substrates in five replicates (4-methylumbelliferyl- β -D-cellobioside for cellobiosidase, 4-methylumbelliferyl- β -D-glucopyranoside for β -glucosidase, 4-methylumbelliferyl-N-acetyl- β -D-glucosaminide for N-acetyl- β -D-glucosaminidase, L-leucine-7-amido-4-methylcoumarin for leucine-aminopeptidase, 4-methylumbelliferyl-phosphate for phosphatase). A second set of aliquots was amended with different concentrations of the respective fluorescence marker (4-methylumbelliferone or 7-amido-4-methylcoumarin) to provide standards for calibration that thus accounted for possible quenching of the fluorescence signal by the soil suspension. Fluorescence of samples and standards was measured once after 140 min of incubation at room temperature (excitation 365 nm, emission 450 nm). To measure the oxidative enzymes phenoloxidase and peroxidase, aliquots of the soil suspensions received L-3,4-dihydroxyphenylalanine (DOPA) as substrate, and samples for peroxidase additionally H_2O_2 . Samples were measured photometrically (absorbance 450 nm) immediately after substrate addition and after 20 h of incubation at room temperature. Potential phenoloxidase activities were calculated from the change in absorbance after DOPA addition, and potential peroxidase activities from the change in absorbance after DOPA + H_2O_2 addition, subtracting the change after DOPA addition alone. We compared the microbial investment into C- versus N-acquiring hydrolytic enzymes (“enzyme C/N”) based on Sinsabaugh et al. (2008), by calculating the ratio of the natural logarithm of (CBH + BG) over the natural logarithm of (NAG + LAP).

Statistical analyses

All statistical analyses were performed using R 3.1.3 (R Development Core Team 2015) with the additional package GenABEL (Aulchenko et al. 2007). We present all C and N pools and fluxes normalized by unit SOC, and thus focussed not on differences in SOM quantity between soil samples, but in SOM

quality (see Supplementary Tables S3 and S4 for values related to unit SOC, and Supplementary Tables S5 and S6 for values related to unit dry soil). We tested for significant differences between initial soils, and between unamended control samples using ANOVA, after log- or rank-transformation where necessary. In some cases conditions for ANOVA could not be met and we applied Mann–Whitney-U tests. We further described differences between control samples using Principal Component Analysis (PCA) of C and N pools and fluxes as well as of extracellular enzyme activities. Values for microbial C and N, dissolved organic C, total dissolved N, dissolved inorganic N, cumulative SOC mineralization, and potential activities of individual extracellular enzymes were normalized by unit SOC, transformed where necessary to meet the assumption of normal distribution, scaled and centered, before applying the `prcomp` function of the stats package in R 3.1.3. Peroxidase activity was below the detection limit in nine of ten samples of Podzol organic layers, and set to zero for PCA and correlations. Gross rates of protein depolymerization and ammonification are missing for some samples where amino acid or ammonium concentrations were too low for ^{15}N determination, or where rates were below the detection limit. We therefore could not include gross N transformation rates in the PCA, but tested for correlation with Principal Components.

We tested for significant differences between controls and treatments using Welch’s paired *t* test in individual soils, and thus accounted for the high variability in control values and treatment responses between and even within soil categories. Data were log- or rank-transformed where necessary. Treatment effects are displayed as response ratios in tables and figures, calculated as treatment over control value for the respective parameter. We further used Spearman’s rank sum correlations to test for monotonous relationships between parameters, and present Spearman’s rho to describe the closeness of this relationship. Differences and correlations were considered significant at $p < 0.05$; for treatment effects and correlations, we also indicate effects with $p < 0.1$.

Results

Carbon and nitrogen dynamics in control samples

We found high variabilities in C and N pools, fluxes and potential extracellular enzyme activities among unamended control samples, even when values were normalized by SOC content (Table 1). Microbial C and N, cumulative SOC mineralization and gross protein depolymerization rates, as well as the activities of several hydrolytic enzymes were significantly and positively correlated with each other (Supplementary Table S1), with lower values in the Histosol and Podzol mineral horizon from Skogaryd than in the other soils (Table 1). In contrast, gross ammonification rates and concentrations of total dissolved N and dissolved inorganic N were negatively correlated with cumulative SOC mineralization, but positively with each other (Supplementary Table S1), and were highest in the Skogaryd soils (Table 1). This pattern was reflected in a Principal Component Analysis, where Principal Component 1 (PC1) separated Skogaryd Histosol and Podzol mineral horizon samples from the other soils. This separation was due to low microbial biomass, SOC mineralization, and hydrolytic enzyme activities as well as high concentrations of total dissolved N and dissolved inorganic N in the Skogaryd Histosol and Podzol mineral horizon samples (Fig. 1, Table 1). Principal Component 2 (PC2) separated Podzol mineral horizons from Podzol organic layers and Histosols irrespective of location, and this separation was driven by higher concentrations of dissolved organic C, total dissolved N, as well as activities of oxidative (phenoloxidase, peroxidase) and N-acquiring hydrolytic enzymes (N-acetyl- β -D-glucosaminidase, leucine-aminopeptidase) in mineral horizons when normalized by SOC content. Gross protein depolymerization rates showed a negative correlation with PC1 (Spearman's $\rho = -0.69$, $p < 0.01$), whereas gross ammonification rates were not significantly correlated with either PC.

Given that these opposing patterns were observed after normalization by SOC content, they reflect differences not in quantity, but in quality of SOM. We found that PC1 was negatively correlated with the C/N ratio of SOM, as well as with the C/N imbalance between SOM and microbial biomass ($\rho = -0.67$ and -0.84 , $p < 0.001$ in both cases). Accordingly, parameters showing a strong negative loading on PC1

(microbial C and N, cumulative SOC mineralization, activities of cellobiohydrolase, β -glucosidase, N-acetyl- β -D-glucosaminidase and leucine-aminopeptidase), as well as gross protein depolymerization rates, were significantly and positively correlated with SOM C/N and C/N imbalance (ρ between 0.35 and 0.88; Supplementary Table S1). Parameters with a strong positive loading on PC1 (total dissolved N, dissolved inorganic N), as well as gross ammonification rates were negatively correlated with C/N (ρ between -0.37 and -0.55), and the former two also with C/N imbalance ($\rho = -0.51$ and -0.39 , respectively).

Principal Component 2 did not show a correlation with C/N ratio or C/N imbalance at $p < 0.1$, but was positively correlated with SOC and total N content ($\rho = 0.75$ and 0.73 , $p < 0.001$ in both cases; Supplementary Table S2), even though pools, fluxes, and enzyme activities were normalized by SOC before PCA. This correlation is in line with the separation of mineral and organic horizons along PC2.

Changes in carbon and nitrogen dynamics induced by carbon and nitrogen addition

The addition of glucose affected C and N pools and processes in all soils, but to a different extent. The Skogaryd soils showed the strongest responses, with increases in microbial C (but not N), as well as decreases in gross ammonification rates and concentrations of total dissolved N and dissolved inorganic N (Fig. 2, Table 2). Svartberget Histosol and Podzol organic layer samples were less responsive, and showed no significant effect of glucose addition on microbial C. Gross ammonification rates increased in the Svartberget Podzol organic layer ($p < 0.1$), whereas dissolved inorganic N concentrations decreased in both soils ($p < 0.05$). All soils showed an increase in microbial C/N (not significant in the Skogaryd Histosol).

Although the increase in microbial C and the reduction in ammonification and dissolved inorganic N suggest that glucose addition had promoted microbial growth and N demand at least in the Skogaryd soils, we did not find indications for a stimulation of microbial N mining. The ratio of C- over N-acquiring enzymes decreased in all Skogaryd soils (significant only in the Skogaryd Histosol), but this effect was rather due to a decrease in C- than an increase in

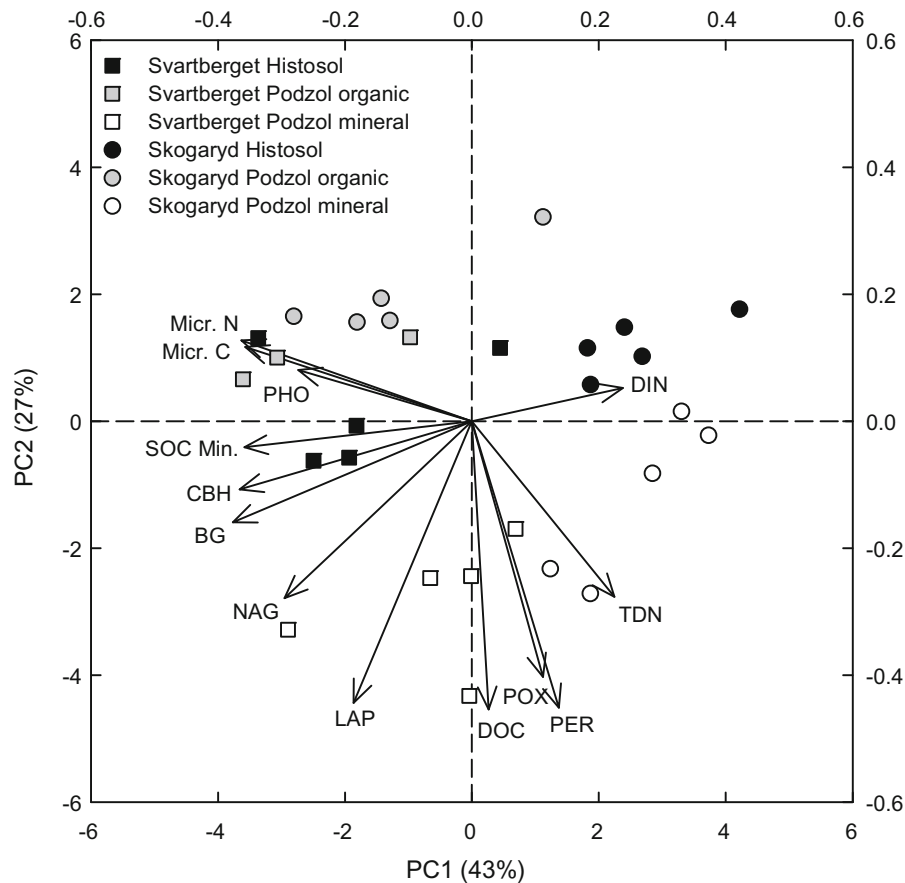


Fig. 1 Plot of Principal Components 1 and 2 from Principal Component Analysis of C and N pools, fluxes, and potential extracellular enzyme activities in control samples after 1 week of incubation (*Micr. C* microbial C, *Micr. N* microbial N, *SOC Min.* cumulative SOC mineralization, *DOC* dissolved organic C, *TDN* total dissolved N, *DIN* dissolved inorganic N, *CBH* cellobiohydrolase, *BG* β -glucosidase, *NAG* N-acetyl- β -D-glucosaminidase, *LAP* leucine-aminopeptidase, *PHO* phosphatase, *POX* phenoloxidase, *PER* peroxidase). Data were normalized by

SOC content, scaled and centered before analysis. Bottom and left axes apply to plotted data points, top and right axes to plotted input parameters. Gross rates of protein depolymerization and ammonification were not included due to missing data points. Protein depolymerization was negatively correlated with PC1 (Spearman's $\rho = -0.69$, $p < 0.01$), and ammonification was not significantly correlated with either PC. Correlations between individual control parameters are shown in Supplementary Table S1

N-targeting enzymes (Table 2). A significant increase in N-targeting enzymes was only observed in the Podzol mineral horizon from Skogaryd, where the proteolytic enzyme leucine-aminopeptidase increased by 18%, the chitinolytic enzyme N-acetyl- β -D-glucosaminidase by 47%, and phosphatase, phenoloxidase and peroxidase by 95, 44 and 25%, respectively (Table 2). Gross protein depolymerization rates could not be measured in these samples due to low amino acid concentrations. In the other soils, we found no significant increase in enzyme activities or gross protein depolymerization rates. Cumulative SOC

mineralization increased by 31% in the Svartberget Podzol mineral horizon ($p < 0.1$) and by 46% in the Skogaryd Histosol ($p < 0.05$; Fig. 2).

In line with our hypothesis, we found that for parameters affected by C addition, the magnitudes of the responses depended on the initial balance between C and N availability in the soil. Strongest increases in microbial C and cumulative SOC mineralization, and strongest decreases in total dissolved N and dissolved inorganic N were thus observed in soils of low C/N ratio. These relationships were indicated by negative correlations between C/N and response ratios of

microbial C ($\rho = -0.45$, $p < 0.05$) and cumulative SOC mineralization ($\rho = -0.44$, $p < 0.05$), and by positive correlations between C/N and response ratios of total dissolved N ($\rho = 0.37$, $p < 0.1$) and dissolved inorganic N ($\rho = 0.45$, $p < 0.05$; Fig. 2, Table 2). No correlations with C/N were found for gross rates of protein depolymerization and ammonification.

The addition of ammonium strongly increased concentrations of total dissolved N and dissolved inorganic N as well as gross ammonification rates, but had little other effect on microbial biomass and microbial processes (Table 2, Fig. 2). Cumulative SOC mineralization increased by 37% in the Svartberget Podzol mineral horizon and decreased by 11% in the Skogaryd Podzol organic layer (both $p < 0.1$). Response ratios of SOC mineralization were negatively correlated with SOC content and PC2 ($\rho = -0.51$ and -0.55 , $p < 0.01$ in both cases; data not shown), reflecting a more positive response of mineral and a more negative response of organic

horizons. Although ammonium addition significantly decreased the activity of the proteolytic enzyme leucine-aminopeptidase by 34% in the Svartberget Podzol organic layer, by 20% in the Skogaryd Histosol, and by 45% in the Skogaryd Podzol organic layer (Table 2), we did not find a concomitant reduction in protein depolymerization rates. In contrast, N addition stimulated protein depolymerization, but this effect was not statistically significant for any of the individual soils (Fig. 2). We did not find any significant correlations between C/N ratios of initial soils and response ratios to N addition at $p < 0.1$ (Fig. 2, Table 2).

Discussion

Our findings suggest that microorganisms in six boreal forest soils adjusted to short-term variations in C and N availability not by altering the breakdown of SOM polymers, but rather, by altering the release of excess

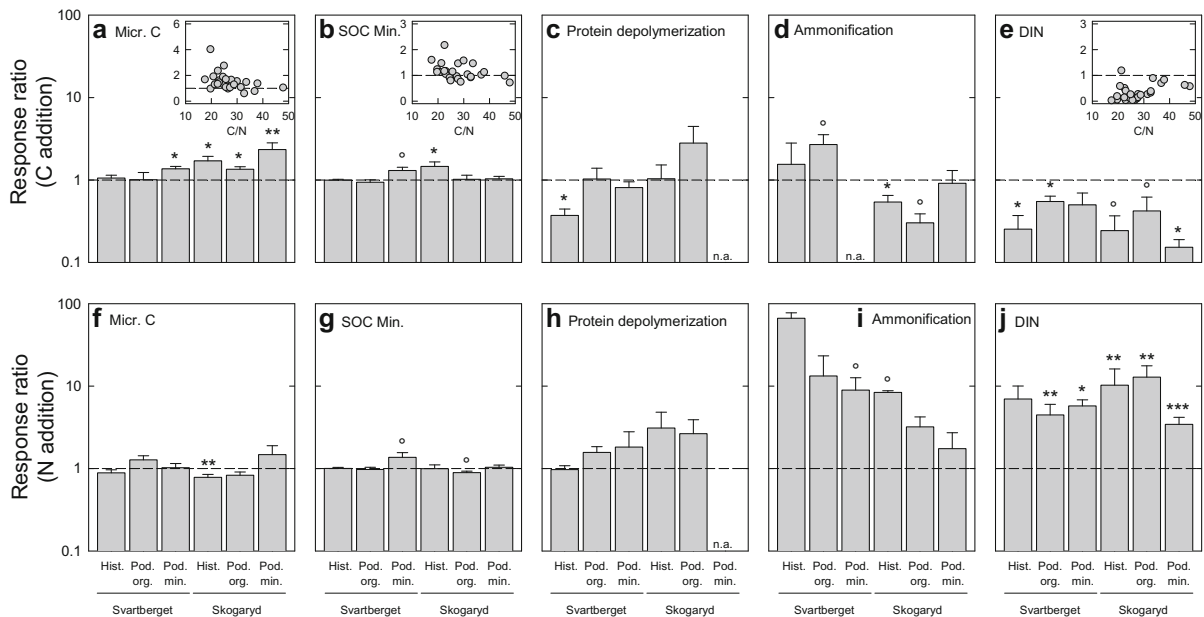


Fig. 2 Response ratios of key parameters to C (a–e) or N (f–j) addition (*Micr. C* microbial C, *SOC Min.* cumulative SOC mineralization, *DIN* dissolved inorganic N) in six boreal forest soils (*Hist.* Histosol, *Pod. org.* Podzol organic layer, *Pod. min.* Podzol mineral B horizon). Response ratios were calculated as treatment over control values, and significant differences between treatment and control values are indicated (Welch's paired *t* tests between control and treatment; *** $p < 0.001$;

** $p < 0.01$; * $p < 0.05$; ° $p < 0.1$). Note the logarithmic scaling. Response ratios of microbial C, SOC mineralization and dissolved inorganic N to C addition were significantly correlated with C/N ratios of initial soils (plotted in inserts; Spearman's $\rho = -0.45$ for microbial C, $\rho = -0.44$ for SOC mineralization, and $\rho = 0.45$ for dissolved inorganic N, $p < 0.05$ in all cases). See Table 2 for response ratios and correlations of other measured parameters

Table 2 Response ratios to C and N addition after 1 week of incubation

	Svartberget Histosol	Svartberget Podzol organic	Svartberget Podzol mineral	Skogaryd Histosol	Skogaryd Podzol organic	Skogaryd Podzol mineral	Corr. with C/N
C addition							
Microbial N	0.81 ± 0.13	0.78 ± 0.17	0.89 ± 0.07	0.85 ± 0.12	0.83 ± 0.05*	1.09 ± 0.14	N.S.
Microbial C/N	1.45 ± 0.23°	1.31 ± 0.03**	1.55 ± 0.03***	2.33 ± 0.67°	1.63 ± 0.06**	2.22 ± 0.21*	N.S.
DOC	1.03 ± 0.06	1.00 ± 0.16	1.13 ± 0.07	1.77 ± 0.43	0.89 ± 0.05	1.17 ± 0.16	N.A.
TDN	0.98 ± 0.04	0.94 ± 0.14	1.11 ± 0.12	0.61 ± 0.18°	0.69 ± 0.13	0.50 ± 0.11°	0.37°
CBH	1.26 ± 0.37	1.60 ± 0.55	0.76 ± 0.11°	0.75 ± 0.08°	0.93 ± 0.21	1.19 ± 0.32	N.S.
BG	1.00 ± 0.10	1.63 ± 0.58	0.96 ± 0.08	0.80 ± 0.06*	0.82 ± 0.15	1.00 ± 0.09	0.40*
NAG	0.97 ± 0.05	1.05 ± 0.11	0.90 ± 0.07	0.90 ± 0.09	0.95 ± 0.14	1.47 ± 0.27°	N.S.
LAP	0.96 ± 0.06	0.93 ± 0.05	1.05 ± 0.03	1.02 ± 0.10	0.91 ± 0.06	1.18 ± 0.04**	N.S.
PHO	0.98 ± 0.05	1.06 ± 0.10	0.81 ± 0.10	1.12 ± 0.12	0.95 ± 0.04	1.95 ± 0.35*	N.S.
POX	1.27 ± 0.29	1.10 ± 0.19	0.92 ± 0.02*	0.78 ± 0.20	1.03 ± 0.55	1.44 ± 0.17°	N.S.
PER	1.24 ± 0.20	N.A.	0.92 ± 0.09	0.85 ± 0.22	N.A.	1.25 ± 0.10*	N.S.
Enzyme C/N	1.00 ± 0.01	1.04 ± 0.04	1.00 ± 0.02	0.96 ± 0.01*	0.98 ± 0.04	0.95 ± 0.03	0.47**
N addition							
Microbial N	0.99 ± 0.13	0.97 ± 0.11	1.14 ± 0.08	1.09 ± 0.11	0.99 ± 0.07	1.12 ± 0.22	N.S.
Microbial C/N	0.95 ± 0.10	1.36 ± 0.28	0.90 ± 0.08	0.73 ± 0.05**	0.84 ± 0.03**	1.27 ± 0.09*	N.S.
DOC	0.94 ± 0.10	0.91 ± 0.07	1.04 ± 0.05	1.08 ± 0.05	0.90 ± 0.05	0.95 ± 0.07	N.S.
TDN	2.20 ± 0.44°	1.71 ± 0.29°	1.72 ± 0.17*	3.35 ± 0.51***	4.31 ± 0.77**	1.98 ± 0.20***	N.A.
CBH	1.53 ± 0.33	1.65 ± 0.69	1.00 ± 0.21	0.75 ± 0.05*	1.02 ± 0.11	1.68 ± 0.33	N.S.
BG	1.09 ± 0.11	1.39 ± 0.38	0.92 ± 0.08	0.87 ± 0.16	0.88 ± 0.19	1.07 ± 0.14	N.S.
NAG	1.02 ± 0.11	1.02 ± 0.10	1.04 ± 0.17	0.88 ± 0.07	1.01 ± 0.12	1.48 ± 0.47	N.S.
LAP	0.97 ± 0.13	0.66 ± 0.04*	1.03 ± 0.05	0.80 ± 0.04**	0.55 ± 0.03***	1.08 ± 0.07	N.S.
PHO	1.13 ± 0.08	1.09 ± 0.15	0.88 ± 0.19	0.89 ± 0.03°	0.93 ± 0.04	1.46 ± 0.23°	N.S.
POX	0.97 ± 0.05	1.13 ± 0.27	0.94 ± 0.07	0.85 ± 0.23	1.32 ± 0.68	1.09 ± 0.10	N.S.
PER	0.97 ± 0.06	N.A.	0.93 ± 0.12	0.77 ± 0.19	N.A.	1.18 ± 0.11	N.S.
Enzyme C/N	1.02 ± 0.01	1.04 ± 0.04	0.99 ± 0.02	0.99 ± 0.02	1.01 ± 0.04	0.98 ± 0.03	N.S.

The C/N ratio of the microbial biomass was calculated on a mass basis, and the C/N of enzyme target compounds (enzyme C/N) as LN (CBH + BG)/LN(NAG + LAP). Response ratios were calculated as treatment over control values, means ± standard errors are presented. Significant differences between treatment and control values are indicated (Welch's paired *t* tests between control and treatment). The right column shows results of Spearman's correlations between response ratios across all soils and C/N ratios of SOM; Spearman's rho for correlations significant at $p < 0.1$ are shown (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ° $p < 0.1$; *N.S.* not significant, *N.A.* not analyzed). Correlations of DOC for C addition, and of TDN for N addition were not tested as soils received different amounts of C or N to account for variability in initial SOC and total N contents. See Fig. 2 for response ratios of selected key parameters (microbial C, SOC mineralization, gross protein depolymerization, ammonification, and dissolved inorganic N concentrations)

DOC dissolved organic C, *TDN* total dissolved N, *CBH* cellobiohydrolase, *BG* β-glucosidase, *NAG* N-acetyl-β-D-glucosaminidase, *LAP* leucine-aminopeptidase, *PHO* phosphatase, *POX* phenoloxidase, *PER* peroxidase

C and N via mineralization to CO₂ or ammonium. The predominance of this adaptation mechanism was supported both for the natural gradient in C and N availability across our soils, and for short-term experimental increases in C or N availability. Our findings thus contrast previous suggestions that increased soil C availability, e.g., due to increased plant C input, stimulates the microbial synthesis of

enzymes targeting N-rich SOM polymers and thus accelerates the mobilization of available N.

We found that microbial C and N transformation processes in unamended soils, as well as their responses to short-term C input, depended on properties of the initial SOM that we qualified using SOM C/N ratios. The observed dependency on SOM C/N ratios is in line with a threshold elemental ratio (TER)

where microorganisms switch between C and N limitation (e.g., Manzoni et al. 2012; Moorhead et al. 2012; Mooshammer et al. 2014a, b; Sinsabaugh et al. 2013). We found that soils of high C/N ratio showed high SOC mineralization, but low ammonification rates, as well as low concentrations of dissolved inorganic N (normalized by SOC content; Supplementary Table S1), pointing at an excess of C, but a shortage of N for microorganisms. These findings are in line with stoichiometric theory which predicts high C mineralization and low ammonification at high C/N, and vice versa (e.g., Mooshammer et al. 2014b). However, C/N ratios were also positively correlated with microbial biomass, activities of C- and N-acquiring hydrolytic enzymes, as well as protein depolymerization rates. Our data thus suggest a second, overlapping mechanism that links SOM C/N ratios to SOM decomposition. As C/N ratios decrease during SOM decomposition, high C/N ratios coincide with SOM that is predominantly plant-derived, still rather poorly decomposed, and not protected by association with soil minerals (Rumpel and Kögel-Knabner 2011; von Lützow et al. 2006). Such easily degradable SOM can support a large and highly active microbial community, further facilitating positive correlations of C/N with SOC mineralization, but also with microbial biomass, enzyme activities, and protein depolymerization, as observed in our study. Taken together, we suggest that C/N ratios served as a proxy for relative C availability across our samples, and reflect both SOM stoichiometry (i.e., C versus N availability) and, indirectly, SOM degradability. In soils of high C/N, C availability exceeded microbial C demand also without C addition, as indicated by high C/N imbalances even when considering the additional C demand for maintenance respiration (Sinsabaugh et al. 2013). In line with our hypothesis, these soils did not show a strong response to short-term C input (Fig. 2, Table 2).

Soils of low C/N ratio, in contrast, showed low SOC mineralization, but high ammonification rates, and high concentrations of dissolved inorganic N (Supplementary Table S1), suggesting that microorganisms experienced an excess of N, and a shortage of C. We found that these N-rich soils responded most strongly to short-term C addition, and showed increases in microbial C and SOC mineralization (Fig. 2). Given the short incubation time, part of the increase in SOC mineralization might not have been

caused by accelerated SOM decomposition (“real priming”), but by the replacement of non-labelled, SOC-derived microbial C by labelled, glucose-derived C (“apparent priming”; Blagodatskaya and Kuzyakov 2008). In any case, the positive priming effect was not connected to a stimulation of microbial N mining, as indicated by minor effects on gross protein depolymerization rates and N-acquiring enzyme activities. We instead observed a reduction in gross ammonification rates and dissolved inorganic N concentrations (Fig. 2). Although these findings support a stimulation of microbial growth and microbial N demand by C addition in soils of low C availability, they also suggest that microorganisms had no need to increase the release of available N from SOM to meet their increased N demand, but rather reduced the initially high release of excess N by ammonification. Such changes in internal microbial C and N metabolism leave SOM breakdown rates unaffected, and have rarely been taken into account in the context of ecosystem C and N storage. A number of studies, however, have recently highlighted the importance of microbial C and N use efficiencies for ecosystem C and N cycling. Low C and N use efficiencies (i.e., high mineralization compared to uptake rates) thus correspond to large ecosystem C and N losses (e.g., as CO₂, N₂O or nitrate), whereas high C and N use efficiencies promote the build-up of microbial biomass, which is the main precursor for SOM (Manzoni et al. 2012; Mooshammer et al. 2014a; Sinsabaugh et al. 2013). Such microbially derived organic matter can account for more than 50% of SOM, and for more than 80% of total soil N (Simpson et al. 2007). We consequently argue that changes in the microbial allocation of C and N to mineralization, such as observed in our 1-week incubation study, can significantly affect the ecosystem C and N balance, by altering not the breakdown, but the formation of SOM polymers (see also Schmidt et al. 2011). In our soils, the observed decrease in gross ammonification rates and dissolved inorganic N concentrations with C addition corresponds to a decrease in soil N availability that reduces the potential for plant N uptake, but also for soil N losses, e.g., by nitrate leaching and denitrification.

In contrast to short-term C addition that hardly affected extracellular enzyme activities, short-term N addition significantly reduced potential activities of the proteolytic enzyme leucine-aminopeptidase in three of the six studied soils (Table 2), but not actual

gross protein depolymerization rates. Such a decoupling between leucine-aminopeptidase activity and gross protein depolymerization rates has been previously observed in a litter decomposition study (Wanek et al. 2010), and suggests that either leucine-aminopeptidase is not representative for the multitude of proteolytic enzymes active in soils (Vranova et al. 2013), or that protein depolymerization is not limited by enzyme activity, but by substrate availability, e.g., due to protection of soil proteins (Knicker 2011). While the analysis of potential extracellular enzyme activities provides valuable information about the microbial investment into enzymes targeting different compounds, it cannot substitute the measurement of actual gross transformation rates.

Although we did not find support for a stimulation of microbial N mining by increased C availability over the course of 1 week, we emphasize that our findings do not rule out that plants can promote microbial N mining and at least partly compensate for the N they take up from the soil. Support for a positive effect of plants on N mining is provided by a range of studies that show higher activities of N-targeting enzymes, gross ammonification rates and soil N losses in rhizosphere than in non-rhizosphere soil (Brzostek et al. 2013; Holz et al. 2016; Kieloaho et al. 2016; Koranda et al. 2011; Weintraub et al. 2007; Zhu et al. 2014), a stimulation of N-targeting enzymes by artificial root exudates (Meier et al. 2017), and positive correlations between rates of root exudation, SOC and (gross or net) N mineralization in experiments with intact plants (Bengtson et al. 2012; Yin et al. 2013, 2014). The strongest support for a stimulation of microbial N mining by increased plant C input comes from experiments where plants were exposed to elevated CO₂ concentrations. These studies report an increase in fine root biomass and turnover, plant-soil C allocation, activities of N-targeting enzymes and tree N uptake with elevated CO₂ and suggest that a stimulation of microbial N mining by increased plant-soil C transfer can help to sustain high plant productivity in a future climate (Drake et al. 2011; Phillips et al. 2011, 2012; Zak et al. 2011). Such a feedback mechanism could be crucial for the C balance of terrestrial ecosystems. A recent modelling study found that ecosystem C storage depended on soil N and P availability, and emphasized that an increase in net primary production, as predicted with rising atmospheric CO₂ concentrations, cannot be realized unless

there is a concurrent increase in the release of available N from SOM (Wieder et al. 2015). However, while experiments on living plants realistically describe plant effects on soil N dynamics under natural conditions, they hardly permit the dissection of individual underlying mechanisms as required for predicting plant effects across ecosystems and global change scenarios.

By isolating effects of C and N availability on soil processes, we show that effects of living roots on soil N dynamics do not simply result from increased soil C availability, but might require specific environmental conditions or a specific form of C input. We here discuss four potential requirements: (1) Nitrogen mining might take place only after long exposure to increased C availability. While root hairs have a life span of a few days (Kuzyakov and Xu 2013) that is comparable to our 1-week incubation, root litter decomposition might result in a longer lasting enhancement of C availability, and might induce different adaptation mechanisms in the soil microbial community. Similarly, N mining might take place only after repeated exposure to increased C availability. Nevertheless, a previous study found weaker priming when the same amount of C was added in small, repeated doses than in one single dose (Qiao et al. 2014), (2) it has been suggested that N mining is induced only by organic compounds in polymeric form that might promote a microbial community specialized on the breakdown of SOM polymers, whereas monomeric compounds such as glucose might promote a copiotrophic microbial community of limited depolymerization capacity (Fontaine et al. 2003). Also in this case, microbial N mining would be connected to root litter production rather than to root exudation, which is dominated by monomeric compounds such as glucose (Jones et al. 2009), (3) a stimulation of microbial N mining might also be restricted to the input of compounds that contain not only C, but also N. Although N addition often reduces SOC mineralization (which has been interpreted as a reduction in microbial N mining; Craine et al. 2007; Phillips et al. 2011), some studies show a stronger stimulation of SOC mineralization when both C and N are supplied (Chen et al. 2014; Wild et al. 2016), possibly since the added N can be used for enzyme synthesis (Allison et al. 2009; Drake et al. 2013). However, a stimulation of enzyme synthesis by N was not observed in this study (Table 2), and (4) finally,

microbial N mining might depend on the association of plants with mycorrhizal fungi, an effect that we would have underestimated in our laboratory experiment. Ectomycorrhiza—the dominant mycorrhiza in boreal forests—have been linked to the release of available N from SOM polymers (Lindahl et al. 2007; Talbot et al. 2013), and to increased N mining under elevated CO₂ (Drake et al. 2011; Terrer et al. 2016). Microbial N mining might thus depend not on plant-soil C allocation in general, but specifically on the allocation of C to certain mycorrhiza. However, recent studies in boreal forests suggest that a high transfer of C from plants to ectomycorrhiza can also reduce plant N uptake as ectomycorrhiza keep a larger proportion of the available N for themselves (Hasselquist et al. 2016; Näsholm et al. 2013). Our laboratory experiment showed a similar stimulation of N sequestration by C input in non-symbiotic soil microorganisms and suggests that increased C availability can at least in the short term also reduce N availability for plants.

Conclusions

We provide insights into the regulation of protein depolymerization under changing C and N availability during a time frame of 1 week. In contrast to our hypothesis, we found that non-symbiotic soil microorganisms adjusted to a short-term increase in C availability not by accelerating the mobilization of N from SOM polymers such as proteins, but rather by using the already available N more efficiently. Considering that (1) C effects on microbial processes are typically restricted to soils of initially low C availability (e.g., Bengtson et al. 2012; Wild et al. 2016), and that (2) these soils are typically characterized by an excess of N, and by low N use efficiency (e.g., Mooshammer et al. 2014a; Wild et al. 2015; see also Supplementary Table S1), an increase in microbial N use efficiency might be a common adjustment to enhanced soil C availability and microbial N demand. Although our findings do not rule out that an increase in plant-soil C allocation, such as expected with rising atmospheric CO₂ concentrations and temperatures, can promote the release of available N from SOM polymers, they show that such an effect can be counteracted at least in the short term by an increase in microbial N use efficiency, reducing soil N availability and aggravating plant N limitation, but also mitigating

soil N losses, e.g., by nitrate leaching and denitrification.

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