

<https://helda.helsinki.fi>

---

Microbial carbon use efficiency and priming of soil organic matter mineralization by glucose additions in boreal forest soils with different C:N ratios

Karhu, Kristiina

2022-04

---

Karhu , K , Alaei , S , Li , J , Merilä , P , Ostonen , I & Bengtson , P 2022 , ' Microbial carbon use efficiency and priming of soil organic matter mineralization by glucose additions in boreal forest soils with different C:N ratios ' , Soil Biology and Biochemistry , vol. 167 , 108615 . <https://doi.org/10.1016/j.soilbio.2022.108615>

---

<http://hdl.handle.net/10138/341778>

<https://doi.org/10.1016/j.soilbio.2022.108615>

---

cc\_by

publishedVersion

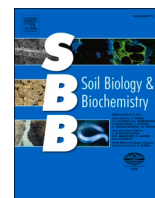
---

*Downloaded from Helda, University of Helsinki institutional repository.*

*This is an electronic reprint of the original article.*

*This reprint may differ from the original in pagination and typographic detail.*

*Please cite the original version.*



# Microbial carbon use efficiency and priming of soil organic matter mineralization by glucose additions in boreal forest soils with different C:N ratios

Kristiina Karhu<sup>a,e,\*</sup>, Saeed Alaei<sup>b,1</sup>, Jian Li<sup>b</sup>, Päivi Merilä<sup>c</sup>, Ivika Ostonen<sup>d</sup>, Per Bengtson<sup>b</sup>

<sup>a</sup> Department of Forest Sciences, University of Helsinki, P.O. Box 27, FI, 00014, Finland

<sup>b</sup> Department of Biology, Microbial Ecology Group, Lund University, Sölvegatan 37, 223 62, Lund, Sweden

<sup>c</sup> Natural Resources Institute Finland, Paavo Havaksentie 3, 90570, Oulu, Finland

<sup>d</sup> Institute of Ecology and Earth Sciences, University of Tartu, Vanemuise 46, 51014, Tartu, Estonia

<sup>e</sup> Helsinki Institute of Life Science (HiLIFE), P.O. Box 63 (Haartmaninkatu 8), 00014, University of Helsinki, Finland

## ARTICLE INFO

### Keywords:

Priming effect  
Carbon use efficiency  
N mining  
PLFA  
13C  
D

## ABSTRACT

During the last decade it has been increasingly acknowledged that carbon (C) contained in root exudates can accelerate decomposition of soil organic matter (SOM), a phenomenon known as rhizosphere priming effect (RPE). However, the controlling factors and the role of different soil microorganisms in RPE are not yet well understood. There are some indications that the response of the soil microbial decomposers to labile C input in the rhizosphere depends on microbial demand of nutrients for growth and maintenance, especially that of C and nitrogen (N). To test this hypothesis, we assessed SOM decomposition induced by <sup>13</sup>C-glucose additions during one week in forest soils with different C:N ratios (11.5–22.2). We estimated SOM respiration, the potential activity (concentration) of a range of extracellular enzymes, and incorporation of <sup>13</sup>C and deuterium (D) in microbial phospholipid fatty acids (PLFAs).

Glucose additions induced positive priming (a 12–52% increase in SOM respiration) in all soil types, but there was no linear relationship between priming and the soil C:N ratio. Instead, priming of SOM respiration was positively linked to the C:N imbalance, where a higher C:N imbalance implies stronger microbial N limitation. The total oxidative enzyme activity and the ratio between the activities of C and N acquiring enzymes were lower in soil with higher C:N ratios, but these findings could not be quantitatively linked to the observed priming rates. It appears as if glucose addition resulted in priming by stimulating the activity rather than the concentration of oxidative enzymes. Microbial incorporation of D and <sup>13</sup>C into PLFAs demonstrated that glucose additions stimulated both fungal and bacterial growth. The increased growth was mainly supported by glucose assimilation in fungi, while the increase in bacterial growth partly was a result of increased availability of C or N released from SOM. Taken together, the findings suggest that the soil C:N ratio is a poor predictor of priming and that priming is more dependent on the C:N imbalance, which reflects both microbial nutrient demand and nutrient provision.

## 1. Introduction

Terrestrial ecosystems assimilate C through primary production and mainly lose C as CO<sub>2</sub> through respiration. At the global scale, respiratory soil C emission is an order of magnitude greater than anthropogenic C emissions (IPCC, 2000). It is estimated that more than a half of the soil C respiration is caused by microbial decomposition of soil organic matter

(SOM) (Hanson et al., 2000). Therefore, even small alterations in the microbial decomposition of SOM could have a profound effect on the net C exchange between soil and atmosphere, and thus impact on the global C cycle.

Organic C enters the soil C pool mainly through above and below-ground plant litter input and root exudation (Rumpel and Kögel-Knabner, 2011). The labile C content of plant root exudates has

\* Corresponding author. Department of Forest Sciences, University of Helsinki, P.O. Box 27, FI-00014, Finland.

E-mail address: [kristiina.karhu@helsinki.fi](mailto:kristiina.karhu@helsinki.fi) (K. Karhu).

<sup>1</sup> These two authors contributed equally to this manuscript.

<https://doi.org/10.1016/j.soilbio.2022.108615>

Received 16 November 2021; Received in revised form 16 February 2022; Accepted 17 February 2022

Available online 21 February 2022

0038-0717/© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

been commonly observed to accelerate the microbial decomposition of SOM, a phenomenon known as the rhizosphere priming effect (RPE; Helal and Sauerbeck, 1984; Cheng et al., 2003; Cheng et al., 2014). The magnitude and even direction of the RPE is highly variable in different studies (Cheng et al., 2003, 2014; Kuz'yakov et al., 2000), but the factors behind this variability are still largely unknown. There are some indications that changes in the microbial decomposition of SOM due to labile C input in the rhizosphere depends on the availability of nutrients needed for microbial growth and maintenance, i.e. C and nitrogen (N) (Wild et al., 2014; Mooshammer et al., 2014). Therefore, differences in the quality and quantity of bioavailable C and N among soils, which determine the microbial demand for these nutrients, could be a possible reason for varying extent of priming between different studies.

Most soil N is bound in complex SOM and only becomes available for plant and microbial uptake after depolymerization into smaller units, e.g. amino acids and ammonium (Schimel and Bennett, 2004). It has, therefore, been suggested that the RPE is a microbial N-mining response in soils with low N availability (Craine et al., 2007; Fontaine et al., 2011), where soil microbes use labile C to produce enzymes in order to acquire N from SOM. According to this theory, the RPE results in enhanced microbial access to N, and at the same time in increased respiration of SOM-C (priming of C mineralization). However, increased decomposition of SOM may not always be manifested as increased SOM-C respiration. For instance, in soils with high N availability labile C input could fuel microbial decomposition activities targeted at releasing bioavailable C from SOM. This could potentially result in increased C use efficiency (CUE) accompanied by a concurrent release of excess SOM derived N (priming of N mineralization). In such a case N mineralization rather than SOM-C respiration would be the best indicator of RPE on SOM decomposition.

Soil microorganisms require C and N in a specific proportion that match their energy and nutrient requirements, i.e. a balanced C:N ratio of resources. This ratio is determined by the C:N ratio of the soil microorganisms and their CUE. For example, growth of a soil microbe with a C:N ratio of 10 and a CUE of 0.5 would theoretically be constrained by C at soil C:N < 20, and by N at soil C:N > 20, and its decomposition activities targeted at releasing the limiting nutrient. Therefore, in soils where labile C input results in balanced C:N availability, microbial SOM decomposition activities might be reduced. In contrast, labile C input into a soil with an imbalanced C:N ratio may increase the abundance of microbial decomposers with the ability of acquiring C or N by decomposing SOM.

In this study, we investigated the effect of the soil C:N ratio on the priming effect (PE) by carrying out a laboratory incubation experiment including six boreal forest soils with different C:N ratios. We hypothesized that (1) Priming of C mineralization is stronger in soils with low N availability (high C:N ratio), (2) Priming of SOM decomposition is accompanied by an increase in the activity of C targeting extracellular enzymes in C-poor soils and with an increase in the activity of N targeting extracellular enzymes in N-poor soils. We further hypothesized that (3) Labile C input in soils with unbalanced C:N ratio (i.e. dissimilar to the microbial C and N requirements) increases the abundance of microbes that decompose complex SOM. In contrast, labile C input in soil with a balanced C to N ratio (i.e. matching the microbial C and N requirements) increases the abundance of microbes that preferentially grow on monomers and other labile compounds, so-called "cheaters" that do not decompose SOM.

The hypotheses were tested in a one-week incubation experiment, where microcosms received semi-continuous additions of  $^{13}\text{C}$ -labelled glucose. The first hypothesis was tested by continuously monitoring the respiration rate of SOM and  $^{13}\text{C}$ -labelled glucose using a Picarro analyzer. The second hypothesis was tested by measuring the potential activity of a range of oxidative enzymes and C and N targeting extracellular enzymes at the end of the incubation. The third hypothesis was tested using MT2 microplates to quantify the abundance of different functional groups growing on substrates of varying recalcitrance. As a

complementary method, we dissolved the added glucose in deuterium-labelled deionized water ( $\text{D}_2\text{O}$ ). This enabled us to carry out a novel double labelling approach for microbial community analysis ( $^{13}\text{C}$  and D incorporation into biomarker PLFAs) to assess to which extent different microbial groups rely on labile C input ( $^{13}\text{C}$ -labelled) and on SOM. We expect microbes that assimilate deuterium (D) without assimilating  $^{13}\text{C}$  in their PLFA to be responsible for the PE.

## 2. Materials and methods

### 2.1. Site description, soil sampling, and soil basic properties

Soil samples were collected in three field replicates from three Norway spruce forest (*Picea abies* (L.) Karst.) sites and from three white birch forest (*Betula pendula* Roth.) sites, covering a wide range of C:N ratios (Table 1) (Ostonen et al., 2017). The sites Alatskivi (birch forest, 58°33'N 27°05'E) and Tõravere (spruce forest, 58°27'N 26°46'E) are located in Estonia. The sites Punkaharju (birch forest, 61°48'N 29°18'E), and Tammela (spruce forest, 60°38'N 23°48'E) are located in southern Finland. The northern sites Kivalo birch (birch forest, 66°20'N 26°40'E), and Kivalo spruce (spruce forest, 66°20'N 26°38'E) are located in the southern part of Finnish Lapland. Due to the differences in latitudes among the sites, samples were collected between May to July 2017 in three different batches (two sites in each batch), moving from south to north to keep the sampling time between the sites at the same season. Due to an exceptionally cold spring, sampling at Kivalo sites was not possible before the beginning of July. We collected the soil samples from Alatskivi and Tõravere on May 23, from Punkaharju and Tammela on May 31, and from Kivalo-birch and Kivalo-spruce on July 3.

Mineral soil was collected from 10 to 20 cm depth (B-horizon), using a soil core sampler with a diameter of 6 cm. On the Alatskivi site there was one permanent plot (15 m × 16 m) used for aboveground stand measurements in previous studies (Varik et al., 2015). Three sub-plots were established within this area for the purpose of soil sampling, and five soil cores were randomly sampled from each plot, and pooled to form a composite sample (n = 3). The Tõravere site (50 m × 50 m) is an ICP Forests Level II plot, and pooled replicate samples (each consisting of 5 soil cores) were collected from three sub-plots established within the larger experimental area. The Punkaharju birch, Tammela spruce, and Kivalo spruce sites belong to the long-term UN-ECE ICP Forests Level II intensive monitoring network (<http://icpforests.net/>; Ferretti and Fischer, 2013), and no soil sampling inside the three replicate 30 × 30 m field plots in each site was allowed. Therefore the soil samples were taken right outside the plots, one soil core from each side of the plot, and these four cores were pooled to form each field replicate (n = 3). For Kivalo birch site, this same sampling scheme was followed. After removing coarse roots, the soil samples were sieved through a 4 mm mesh and stored for a maximum of 4 days at 4 °C before the start of the incubation experiment. SOM content was measured by loss on ignition (12 h at 600 °C). Total C and N content were measured in dried (65 °C) and ground soil using a Flash 2000 elemental analyzer (Thermo Scientific Inc., Bremen Germany). Soil pH was measured in 1:4 soil to deionized water slurry. We extracted the soil inorganic N content by adding 50 ml 1 M KCl (Merck, Darmstadt, Germany) to 10 g fresh soil in a urine beaker (125 ml). The beakers were shaken over a horizontal shaker for 1 h after which the extracts were filtered through GF/F- filter paper (VWR, Leuven, Belgium) placed in a ceramic funnel. The effluents were collected in a new set of beakers. The concentrations of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  were then determined using flow injection analyser (FIAS 5000 Analyzer, Foss Tecator, Sweden). Microbial C and N content were estimated using chloroform fumigation-extraction assay as in Vance et al. (1987) where we fumigated 8 g (dry weight) soil with ethanol-free chloroform for 24 h. Fumigated and non-fumigated (control) soils where extracted by 40 ml 0.5 M  $\text{K}_2\text{SO}_4$  and C and N content of extracts were determined using Shimadzu TOC/TN analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA). Microbial C and N were then estimated

**Table 1**

The C:N ratio, soil organic matter (SOM) content, pH, NH<sub>4</sub>-N, NO<sub>3</sub>-N, as well as the total extractable inorganic N (TIN) in the different soils. Values within brackets represent standard error of the mean. Asterisks indicate significance level of Anova test (\*p < 0.05, \*\*p < 0.01, and \*\*\*p < 0.001). Different letters represent significant differences of Fisher's Least Significant Difference (LSD) between sites (p < 0.05).

Site	Forest type	Soil C:N**		SOM %***		pH*		NH <sub>4</sub> -N *** (µg N g SOM <sup>-1</sup> )		NO <sub>3</sub> -N (µg N g SOM <sup>-1</sup> )		TIN** (µg N g SOM <sup>-1</sup> )	
Alatskivi	Birch	11.5	(0.3)a	4.5	(0.1)a	5.9	(0.0)ab	31.1	(3.7)ab	3.7	(0.6)a	34.8	(4.0)a
Töravere	Spruce	13.5	(1.2)ab	3.4	(0.2)b	6.1	(0.4)a	27.3	(1.3)ac	4.3	(0.8)a	31.6	(1.8)ac
Punkaharju	Birch	15.1	(0.4)ac	3.3	(0.3)b	5.6	(0.0)ac	38.4	(3.3)b	4.6	(3.1)a	43.0	(6.0)a
Kivalo-birch	Birch	17.6	(1.5)bc	3.7	(0.4)ab	5.6	(0.1)ac	17.6	(2.1)c	2.4	(0.2)a	20.0	(2.3)c
Tammela	Spruce	18.0	(0.7)cd	4.6	(0.5)a	5.0	(0.1)c	19.8	(1.0)c	3.0	(2.2)a	22.8	(2.8)c
Kivalo-spruce	Spruce	22.2	(2.7)d	6.9	(0.3)c	5.3	(0.1)bc	13.4	(3.6)c	2.7	(0.1)a	16.1	(4.5)bc

from the differences between values in fumigated and the control samples. Characteristics of the sites and the soils are summarized in Table 1.

## 2.2. Experimental design

The microcosms consisted of 12 PVC chambers (5.6 cm × 12.8 cm (R × H), net V: 1130 cm<sup>3</sup>) filled with 77 g soil (dry weight). PTFE tubes were used to connect each microcosm to a Picarro analyzer (G2131-i, Picarro Inc., Santa Clara, CA, USA) equipped with two 16-port manifolds that enabled recycling and automatic shifting between microcosms. The microcosms were equipped with a removable gas-tight lid fitted with a rubber septum.

We incubated the soil samples in three batches, where each batch comprised 12 microcosms (three replicates from two sites × two treatments). Soil samples from each replicate plot were distributed into two microcosms. One of these microcosms received daily addition of 5 atom % <sup>13</sup>C labelled glucose (50 µg glucose C g<sup>-1</sup> soil day<sup>-1</sup>) dissolved in 5 atom % D-labelled water during six days, and the other received a daily addition of 5 atom % D labelled water (control treatment). The water content was kept around 50% WHC and temperature was kept at 18 °C during the six days incubation period.

## 2.3. Soil respiration and priming

Respiration was measured using the closed measurement system described above. To prevent accumulation of high concentrations of CO<sub>2</sub> in the headspace of the microcosms; we flushed them with CO<sub>2</sub>-free air for 2 min immediately after the daily substrate additions. The respiration rate was derived from four daily measurements (one every 6 h) of the concentration of <sup>12</sup>CO<sub>2</sub> and <sup>13</sup>CO<sub>2</sub> in the headspace of the microcosms. Before each measurement, residual air from the previous measurement remaining in the tubing and analyser cavity was removed by flushing with CO<sub>2</sub> free air for 2 min. During each measurement, the concentration and <sup>13</sup>C/<sup>12</sup>C ratio of respired CO<sub>2</sub> in each microcosm were continuously measured for 27 min. The total respired CO<sub>2</sub> in the headspace was separated into respired SOM and respired glucose using a two end-member isotope-mixing model (Eqs. (1) and (2)).

$$f_{\text{glucose}} = (\text{at}\% \text{ } ^{13}\text{C}_{\text{CO}_2} - \text{at}\% \text{ } ^{13}\text{C}_{\text{SOM}}) / (\text{at}\% \text{ } ^{13}\text{C}_{\text{glucose}} + \text{at}\% \text{ } ^{13}\text{C}_{\text{SOM}}) \quad (1)$$

$$f_{\text{SOM}} = 1 - f_{\text{glucose}} \quad (2)$$

Where  $f_{\text{glucose}}$  and  $f_{\text{SOM}}$  represent the fraction of CO<sub>2</sub> derived from respiration of glucose and SOM, respectively, and atom % <sup>13</sup>C<sub>glucose</sub> and atom % <sup>13</sup>C<sub>SOM</sub> represent the atom % <sup>13</sup>C in glucose and in SOM, respectively. We then calculated respiration of soil organic matter (R<sub>SOM</sub>) and respiration of glucose (R<sub>glucose</sub>) according to Eqs. (3) and (4), where R<sub>total</sub> represent the total respiration (R<sub>SOM</sub> + R<sub>glucose</sub>).

$$R_{\text{SOM}} = R_{\text{total}} \times f_{\text{SOM}} \quad (3)$$

$$R_{\text{glucose}} = R_{\text{total}} - R_{\text{SOM}} \quad (4)$$

Priming was then determined as the difference between the SOM respiration in the samples that received glucose (R<sub>SOM glucose</sub>) and the

SOM respiration in the control samples that did not receive glucose (R<sub>SOM control</sub>) according to Eq. (5).

$$\text{Priming} = R_{\text{SOM glucose}} - R_{\text{SOM control}} \quad (5)$$

## 2.4. Quantification of microbial functional groups

At the end of the priming experiment, we determined the abundance of microbes growing on substrates of varying recalcitrance using MT2 microplates (Biolog, Inc., Hayward, CA, USA), where colorimetric changes of a tetrazolium violet dye contained in the plate-wells indicate microbial utilization of the provided substrate. We used three technical replicates for each soil sample on the microplates. Five different substrates were used, including sugars (50% glucose and 50% sucrose), C-polymers (as a mix of equal portions of carboxymethylcellulose sodium salt, starch, and pectin), Bovin Serum Albumin (BSA), kraft lignin (Sigma Aldrich-370959), and amino acids. Amino acids were a mix of equal amounts of twenty L-amino acids consisting of glycine, cysteine, histidine, isoleucine, threonine, glutamic acid, leucine, methionine, glutamine, hydroxyproline, proline, lysine, serine, cysteine, arginine, tryptophan, tyrosine, valine, phenylalanine, and asparagine. Each well received 0.3 mg of the respective substrate dissolved in 15 µl Milli-Q water. The microplates were then placed in the oven at 45 °C for 1 h, after which they were kept at 4 °C until inoculation.

At the end of the microcosm incubation, we placed 4 g of soil from each replicate into a falcon tube and prepared a 1:10 suspension of soil and water by adding 40 ml Milli-Q water. The tubes were then shaken on a Heidolph Multi Reax shaker (Schwabach, Germany) at maximum speed for 10 min. After 5 min of sedimentation, we added 1 ml of the solution to 9 ml Milli-Q water in a 15 ml falcon tube. From this final dilution, we inoculated 125 µl into the microplate wells in triplicates. The plates were then incubated at 23 °C in the dark for three weeks to ensure detection of any potential microbial utilization of the substrates. The well colour development was quantified using a microplate reader (Fluostar Omega, BMG Labtech) at optical density of 590 nm. The data were processed using the data analyzer software (MARS Data Analysis 3.01 R.2). Measurements were repeated three times on the first two days, two times a day on the second to seventh day, followed by once a day measurements from day eight until the end of the third week. There was a minimum of 3 h between each time point and the next measurement. Well color development was then calculated as described in Eq. (6).

$$OD_{590\text{real}} = OD_{590\text{well}} - \text{avr}OD_{590\text{blank}} \quad (6)$$

Where  $OD_{590\text{real}}$  and  $OD_{590\text{well}}$  represent the actual and the observed well color development, respectively, and  $\text{avr}OD_{590\text{blank}}$  is the average of the values of control replicates for each sample (soil inoculate with no substrate) at each time point. We fitted the well color development data points with modified version of Gompertz sigmoidal growth curve model (Zwietering et al., 1990) using a least squares non-linear regression algorithm (Eq. (7)).



$$y(t) = A \times \exp \{-\exp [\mu_{\max} \times e / A \times (\lambda - t) + 1]\} \quad (7)$$

Where,  $y$  is  $OD_{590\text{real}}$ , corresponding to the relative microbial population size or  $\ln(N/N_0)$ ,  $A$  is the asymptotic described as the plateau phase of color development. Also,  $e$  is a constant value equal to 2.71, and  $\lambda$  is the lag phase defined as the time needed to reach a microbial density high enough for color change to occur (hours),  $t$  is time (hours), and  $\mu_{\max}$  is the maximum rate of well color development ( $\text{hour}^{-1}$ ). Microbial growth need to reach to a density threshold in order for color development to occur (Garland and Mills, 1991; Garland and Lehman, 1999). Therefore, the duration of the lag phase depends on the microbial growth rate and initial microbial density of the inoculum. We could therefore calculate the relative microbial density in the inoculum at time zero ( $N_{0\text{rel}}$ ) from  $\mu_{\max}$  (defined as the tangent in the inflexion point) and  $\lambda$  (defined as the x-axis intercept of this tangent) as described in Eq. (8).

$$y = b + (\mu_{\max} \times \lambda) \quad (8)$$

Where, the intercept with y-axis ( $b$ ) is negatively correlated with the inoculate density at time zero due to its dependence on  $\mu_{\max}$  and  $\lambda$ . Therefore,  $b$  is an approximation of  $N_{0\text{rel}}$  (Eq. (9)). Finally, we multiplied  $N_{0\text{rel}}$  values by  $A$  to compensate for the underestimation of  $\mu_{\max}$  in the wells containing substrates with a low maximum color development.

$$N_{0\text{rel}} = 11 / \mu_{\max} \times \lambda \quad (9)$$

## 2.5. PLFA extraction and $^{13}\text{C}$ -D<sub>2</sub>O PLFA analysis

At the end of incubation, soils were freeze-dried and homogenized by grinding in a ball-mill. From these samples, phospholipid fatty acids (PLFAs) were extracted, purified and derivatized to their corresponding fatty acid methyl esters (FAMES), as described in Ehtesham and Bengtsson (2017). After derivatization, the resulting FAMES were dissolved in 100  $\mu\text{l}$  hexane and vortexed (5 s), after which 30  $\mu\text{l}$  were transferred to inner assembly of GC vials (filled with 100  $\mu\text{l}$  hexane to minimize evaporation before analysis). Quantification and incorporation of  $^{13}\text{C}$  and deuterium into fatty acid methyl esters (FAMES) was done at the stable isotope facility at the Department of Biology, Lund University, Sweden, using a Delta IV Plus Isotope Ratio Mass Spectrometer (IRMS). The IRMS is coupled to a Trace GC Ultra gas chromatograph via the GC Isolink II preparation device and ConFlow IV interface (Thermo Scientific Inc., Bremen Germany). The GC is equipped with a HP-5MS UI column (60 m  $\times$  0.25 mm I.D., 0.25  $\mu\text{m}$  thick stationary phase, Hewlett Packard®), allowing for chromatographic separation of FAMES. The inlet port temperature was set at 250  $^{\circ}\text{C}$  and the flow of Helium carrier gas at 1.5  $\text{ml min}^{-1}$ . The operating condition of the GC was as follows: splitless injection with initial temperature of 50  $^{\circ}\text{C}$  held for 1 min; ramped at 15  $^{\circ}\text{C}/\text{min}$  to 160  $^{\circ}\text{C}$ ; followed by ramping at 2  $^{\circ}\text{C}/\text{min}$  to

$$CUE_{\text{glucose}} = \frac{\text{growth on } ^{13}\text{C glucose}}{\text{uptake of } ^{13}\text{C glucose}} = \frac{\text{fungal + bacterial growth on } ^{13}\text{C glucose}}{\text{fungal + bacterial growth on } ^{13}\text{C glucose} + R_{\text{glucose}}} \quad (10)$$

200  $^{\circ}\text{C}$  held for 10 min; ramping to 230  $^{\circ}\text{C}$  at 3  $^{\circ}\text{C}/\text{min}$  and ramping further at 20  $^{\circ}\text{C}/\text{min}$  to the final temperature of 300  $^{\circ}\text{C}$  and held for 4 min. This ramping program resulted in good separation between different FAMES. FAMES were quantified and identified based on their peak area and retention time relative to the internal standard nonadecanoic acid (19:0), as previously established by means of GC-MS. Quantification was done using the mass-detector, and the  $^{13}\text{C}$  and D incorporation estimated by multiplying the total C and H content with the fraction  $^{13}\text{C}$  and D excess (of background). PLFA 18:2 $\omega$ 6,9 was

selected as the fungal biomarker and PLFAs i15:0, a15:0, i16:0, 16:1 $\omega$ 5, 16:1 $\omega$ 7c, 10Me18:0, 18:1 $\omega$ 7c, cy19:0 were selected as bacterial PLFAs representatives (Frostegård et al., 1993; Frostegård and Bååth, 1996; Ruess and Chamberlain, 2010).

## 2.6. Bacterial and fungal carbon use efficiency (CUE)

Bacterial and fungal CUE of SOM and glucose were calculated from respiration rates of SOM and glucose, and the incorporation of glucose derived  $^{13}\text{C}$  and D<sub>2</sub>O derived D into biomarker PLFAs. Fungal and bacterial biomass C production supported by growth on glucose and SOM were calculated from the  $^{13}\text{C}$  and D incorporation, respectively. To achieve this we used conversion factors adopted from Frostegård and Bååth (1996) and Klamer and Bååth (2004), with some modifications. Frostegård and Bååth (1996) found that each mg of bacterial biomass C contains 0.7  $\mu\text{mole}$  bacterial PLFAs. Their conversion factor was based on analysis of 12 bacterial PLFAs, while our analysis was based on quantification of 8 PLFAs, containing on average 16.75 C atoms and 32.25 H atoms. To account for these differences we used the conversion factors 7.82  $\text{mmol}$  bacterial PLFA-C per gram bacterial biomass C, and 15.05  $\text{mmol}$  bacterial PLFA-H per gram bacterial biomass C. In order to convert the fungal biomarker PLFA 18:2 $\omega$ 6,9 to fungal biomass C, we used the conversion factor from Klamer and Bååth (2004), suggesting that 212.4  $\mu\text{mol}$  PLFA-C of the fungal biomarker 18:2 $\omega$ 6,9 corresponds to 1 g fungal biomass C, and following from this, that 365.8  $\mu\text{mol}$  PLFA-H of the fungal biomarker 18:2 $\omega$ 6,9 corresponds to 1 g fungal biomass C.

By using the conversion factor above the combined bacterial growth on glucose and SOM (expressed as mg biomass C) was calculated from the amount of D ( $\mu\text{mol}$ ) incorporated into the bacterial PLFAs i15:0, a15:0, i16:0, 16:1 $\omega$ 5, 16:1 $\omega$ 7c, 10Me18:0, 18:1 $\omega$ 7c, and cy19:0, divided by the fraction D excess (i.e. atom% D excess/100) in the soil water after addition of D<sub>2</sub>O. Similarly, fungal growth on glucose and SOM was calculated from the amount of D ( $\mu\text{mol}$ ) incorporated into the fungal PLFA 18:2 $\omega$ 6,9, divided by the fraction D excess in the soil water after addition of D<sub>2</sub>O. In samples that received  $^{13}\text{C}$ -labelled glucose the total bacterial and fungal growth was separated into growth on glucose and growth on SOM by calculating the growth on glucose (expressed as mg biomass C) from the amount of  $^{13}\text{C}$  ( $\mu\text{mol}$ ) incorporated into bacterial PLFAs, divided by the fraction  $^{13}\text{C}$  excess (i.e. atom%  $^{13}\text{C}$  excess/100) in the added glucose (5 atom%). Fungal growth on glucose was calculated from the amount of  $^{13}\text{C}$  ( $\mu\text{mol}$ ) incorporated into the fungal PLFA 18:2 $\omega$ 6,9, divided by the fraction  $^{13}\text{C}$  excess in the added glucose. Bacterial and fungal growth on SOM was then calculated by subtracting the growth on glucose from the total growth on SOM and glucose combined, calculated from the D incorporation into bacterial and fungal PLFA's, as described above. The carbon use efficiency (CUE) of glucose was calculated as:

and CUE of SOM was calculated as:

$$CUE_{\text{SOM}} = \frac{\text{growth on SOM}}{\text{uptake of SOM}} = \frac{\text{fungal + bacterial growth on SOM}}{\text{fungal + bacterial growth on SOM} + R_{\text{SOM}}} \quad (11)$$

## 2.7. Activities of extracellular enzymes

We measured the potential activity (i.e. concentration) of hydrolytic

extracellular enzymes including cellobiosidase (CB, targeting cellulose),  $\beta$ -glucosidase (BG, targeting cellulose), N-acetyl- $\beta$ -D-glucosaminidase (NAG, targeting chitin and peptidoglycan), and leucine-aminopeptidase (LAP, targeting proteins and peptides) as well as the potential activity (i. e. concentration) of two oxidative extracellular enzymes including phenoloxidase (POX) and peroxidase (PER) using fluorometric and photometric methods modified after Kaiser et al. (2010). We transferred 2 g of fresh soil to a 100 ml urine cup, and added 50 ml sodium acetate buffer (100 Mm, pH 5.5) to the soil. The suspension was mixed using an Omni Macro-homogenizer (model No. 17505, Marietta, GA, USA) for 30 s. The potential activity of hydrolytic enzymes was measured fluorometrically by adding fluorometrically labelled substrates to one set of aliquots in four replicates for each soil and enzyme. We used 4-methylumbelliferyl- $\beta$ -D-cellobiosidase for cellobiosidase, 4-methylumbelliferyl- $\beta$ -D-glucopyranoside for  $\beta$ -glucosidase, 4-methylumbelliferyl-N-acetyl- $\beta$ -D-glucosaminidase for N-acetyl  $\beta$ -D-glucosaminidase, and L-leucine-7-amido-4-methylcoumarin for leucine-aminopeptidase. We transferred 200  $\mu$ l of the suspensions to corresponding wells of microplates together with 50  $\mu$ l of the respective substrates. After 140 min of incubation at room temperature (20 °C) the fluorescence of samples as well as standards were measured (extinction 360 nm and emission 460 nm). To measure the potential activity of phenoloxidase 1 ml of suspension received 1 ml (20 mM) L-3, 4-dihydroxyphenylalanine (DOPA). For peroxidase, DOPA was added together with 10  $\mu$ l of H<sub>2</sub>O<sub>2</sub> (0.3%) as substrate. Thereafter, triplicate samples and blanks were shaken for 10 min followed by centrifugation (at 10000 g for 5 min). Wells received 250  $\mu$ l of the suspension together with the corresponding substrates. The absorbance was measured photometrically at 450 nm immediately after substrate addition and after 20 h of incubation at room temperature (20 °C). The potential C-acquiring enzymes activities were calculated as the sum of the activities of CB and BG and the potential N-acquiring enzymes activities were calculated as the sum of the activities of NAG and LAP. We also calculated the ratio between C and N acquiring enzymes as the ratio between natural logarithm of C and N acquiring enzymes as in Sinsabaugh et al. (2008).

## 2.8. Statistical analysis

Our experiment was designed to test the influence of the soil C:N ratio separately for spruce and birch stands. However, due to unexpectedly similar C:N ratios at some of the sites this was not possible. We

therefore analysed the influence of soil C:N ratio for all sites combined, meaning that any relationship between soil C:N ratio and the other estimated parameters and processes supersedes the influence of tree species differences on the same parameters and processes. In order to avoid the interference of site differences in SOM content, we present our results per gram SOM.

Curve fitting and statistical analyses were performed using Statistica 13, except for Figs. 7 and 8, which were drawn using Excel, and linear regression analysis presented in these Figures were conducted using SPSS 25. In the regression analysis average values for each site were used as input for the explanatory variable (for Figs. 1–5 and 7 to 8). For the independent variable the values of individual replicates within a site were used, to account for its variation. For the data presented in Fig. 6 values of individual replicates are used both for y- and x-axis data. We tested significance of the differences between sites and additions using factorial ANOVA (difference with  $p < 0.05$  was considered significant). Pairwise differences among sites were further tested using Fisher's least significant difference (LSD) test and differences were considered to be significant if  $p < 0.05$ .

## 3. Results

### 3.1. Total respiration, respiration of SOM, respiration of glucose, and priming of C mineralization

The total respiration was negatively correlated with the soil C:N ratio in both the control ( $r^2 = 0.56$ ,  $p < 0.001$ ) and glucose treatment ( $r^2 = 0.57$ ,  $p < 0.001$ ; Fig. 1a). We observed the same pattern for SOM respiration in both treatments (Fig. 1b). In the control treatment cumulative SOM respiration varied between 578–1245  $\mu$ g CO<sub>2</sub>-C g SOM<sup>-1</sup> (Table 2). The soil samples with the lowest C:N ratios (Alatskivi and Töravere) showed higher SOM respiration than the other soil samples (Table S1, Table 2). Glucose addition increased SOM respiration by 83–421  $\mu$ g C g SOM<sup>-1</sup>, equivalent to 12–52% priming (Table 2), but there was no linear relationship between priming of SOM respiration and the soil C:N ratio. Priming in soil samples from the Kivalo-birch site, which was ranked in the mid-range of C:N ratios (C:N = 17.6), was significantly higher than in the other sites (Table 2).

Priming of SOM respiration was negatively linked to the microbial C:N ratio ( $r^2 = 0.26$ ,  $p < 0.05$ ; Fig. 2a). We also calculated the soil C:N imbalance, defined as the soil C:N ratio divided by the microbial C:N ratio, to investigate the interactive effect of differences in soil C:N and

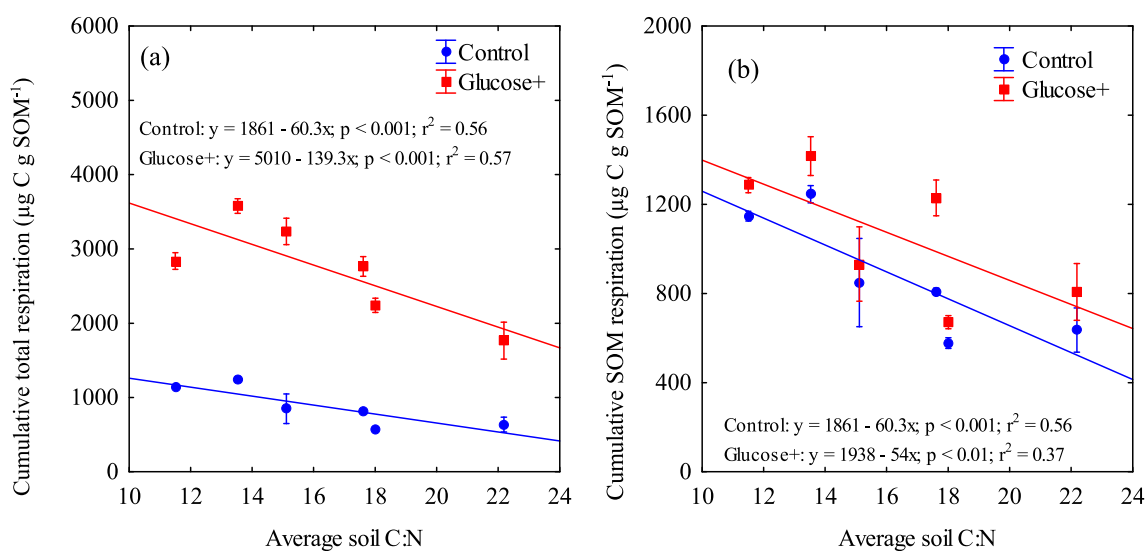
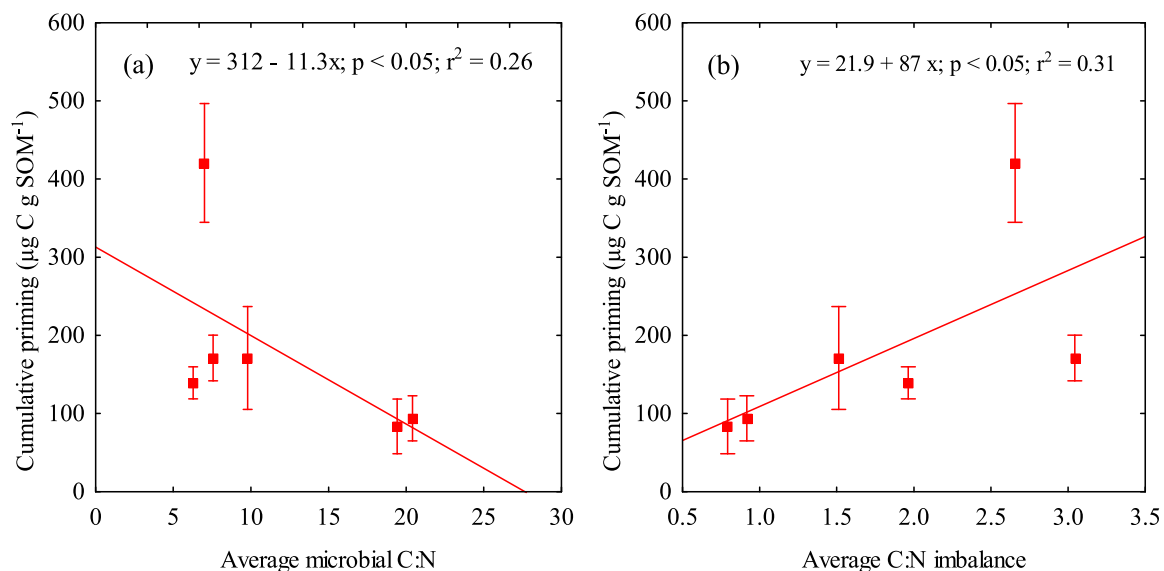


Fig. 1. The relationship between the soil C:N ratio and cumulative rates of total respiration and SOM respiration.

**Table 2**

Cumulative total respiration, SOM respiration, glucose respiration, and priming during one week of incubation in control treatments and after glucose addition. Values within brackets represent standard error of the mean. Different letters represent significant differences between sites ( $p < 0.05$ ) for each treatment. B = Birch site, S = Spruce site.

Sites	C:N	Treatment	Total respiration		SOM respiration		Glucose respiration		Priming		Priming %	
			( $\mu\text{g C g SOM}^{-1}$ )	( $\mu\text{g C g SOM}^{-1}$ )	( $\mu\text{g C g SOM}^{-1}$ )	( $\mu\text{g C g SOM}^{-1}$ )	( $\mu\text{g C g SOM}^{-1}$ )	( $\mu\text{g C g SOM}^{-1}$ )				
Alatskivi	11.5	Control	1147	(22)a	1147	(22)a						
Töravere	13.5		1245	(38)a	1245	(38)a						
Punkaharju	15.1		849	(198)b	849	(198)b						
Kivalo-birch	17.6		808	(15)b	808	(15)b						
Tammela	18.0		578	(24)b	578	(24)b						
Kivalo-spruce	22.2		636	(99)b	636	(99)b						
Alatskivi	11.5	Glucose	2838	(113)b	1286	(34)a	1552	(85)b	139	(20)a	12.1	(1.7)a
Töravere	13.5		3578	(99)b	1416	(87)a	2161	(128)a	171	(66)a	13.6	(5.0)a
Punkaharju	15.1		3236	(177)ab	933	(167)bc	2303	(69)a	83	(35)a	12.8	(7.1)a
Kivalo-birch	17.6		2763	(133)b	1229	(80)ac	1534	(189)b	421	(76)b	52.0	(9.1)b
Tammela	18.0		2240	(96)c	671	(29)b	1569	(94)b	94	(29)a	16.5	(5.1)a
Kivalo-spruce	22.2		1766	(248)d	807	(127)b	959	(133)c	171	(29)a	26.9	(1.8)a



**Fig. 2.** The relationship between the microbial C:N ratio and cumulative priming and (a) and between the C:N imbalance and cumulative priming (b).

microbial C:N ratios on priming. The calculations showed that priming of SOM respiration was positively related to the C:N imbalance, ( $r^2 = 0.31$ ,  $p < 0.05$ ; Fig. 2b).

### 3.2. Extracellular enzyme activities

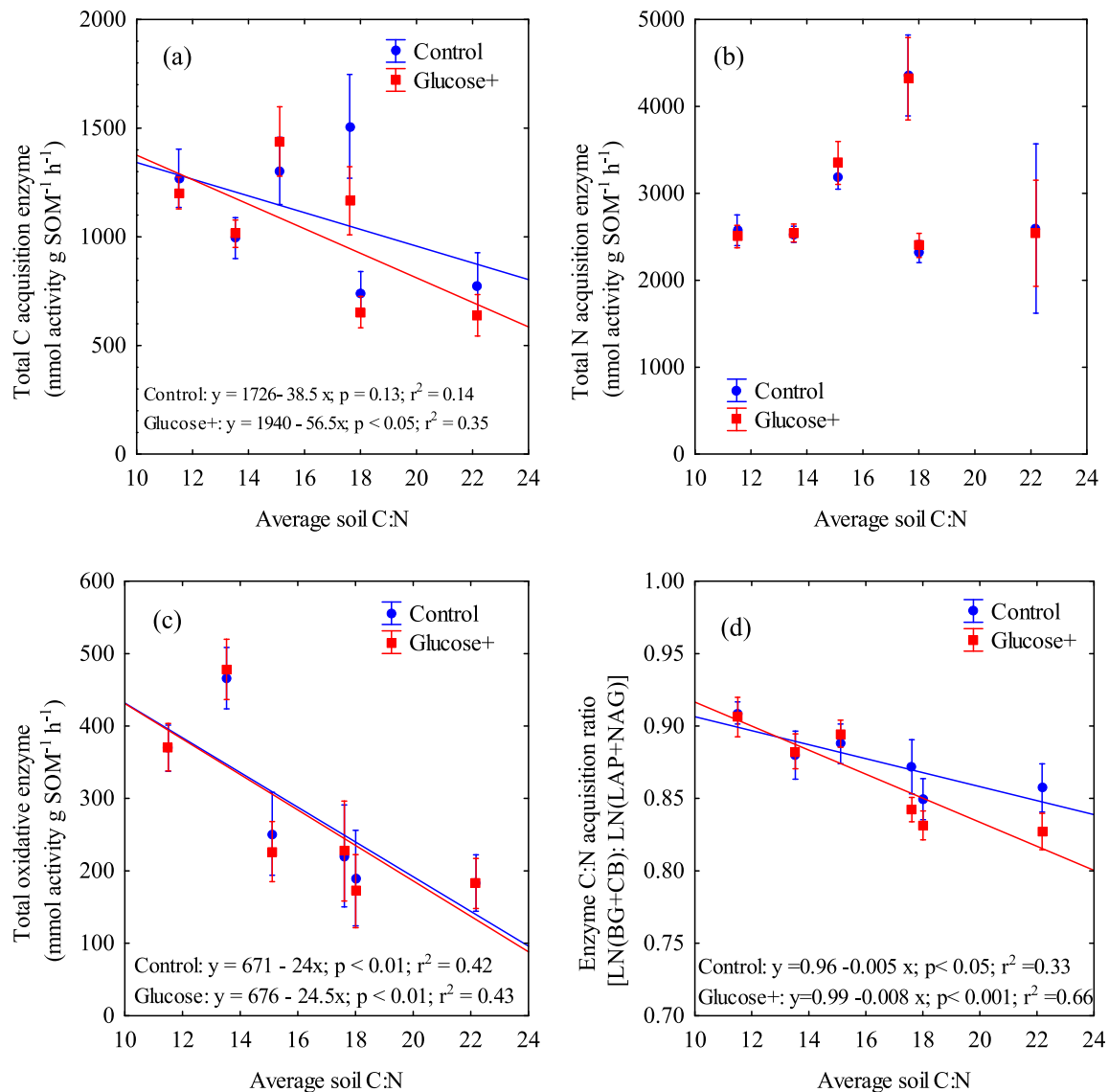
Glucose addition did not have any effect on the potential activities of total C-acquiring enzymes (CB + BG), total N-acquiring enzymes (NAG + LAP), total oxidative enzymes (PER + POX), or on the ratio between potential activity of the C-acquiring enzymes and N-acquiring enzymes [LN (BG + CB): LN (LAP + NAG)] (Table S2, Fig. 3). The potential activity of C-acquiring enzymes was negatively correlated to the soil C:N ratio in the glucose treatment ( $r^2 = 0.35$ ,  $p < 0.05$ ; Fig. 3a). The same pattern was observed for total oxidative enzymes in both the glucose and control treatments (Control:  $r^2 = 0.42$ ,  $p < 0.01$ ; Glucose:  $r^2 = 0.43$ ,  $p < 0.01$ ; Fig. 3c). The total potential activity of N-targeting enzymes did not show any linear correlation with the soil C:N ratio (Fig. 3b). On the other hand, the ratio between the potential activity of C-and N-acquiring enzymes were negatively linked to the soil C:N ratio in both the glucose and control treatment (Control:  $r^2 = 0.33$ ,  $p < 0.05$ ; Glucose:  $r^2 = 0.66$ ,  $p < 0.001$ ; Fig. 3d).

There was no linear relationship between the potential activity of C- or N-acquiring enzymes and cumulative SOM respiration (Fig. 4a and b). In contrast, there was a positive relationship between the potential activity of total oxidative enzymes and the cumulative SOM respiration in both treatments (glucose and control, Fig. 4c), but the same enzyme concentration resulted in higher SOM respiration in the glucose treatment. Cumulative SOM respiration was also positively related to the ratio between C and N acquiring enzymes (Control:  $r^2 = 0.48$ ,  $p < 0.01$ ; Glucose:  $r^2 = 0.28$ ,  $p < 0.05$ ; Fig. 4d), but we did not find any significant relationship between the C:N imbalance and the potential activity of oxidative, C acquiring or N acquiring enzymes (data not shown).

The potential activity of N-acquiring enzymes was positively linked to cumulative priming of SOM respiration ( $r^2 = 0.45$ ,  $p < 0.01$ , Fig. 5a), but there were no linear relationships with the potential activity of total C-acquiring enzymes (Fig. 5b), oxidative enzymes (Fig. 5b), or the enzyme C:N acquisition ratio (Fig. 5d).

### 3.3. Microbial functional groups

The abundance of microbes growing on amino acids varied among sites (significant effect of site, in the main factorial ANOVA results,  $p <$



**Fig. 3.** The relationship between soil C:N ratio and total potential activity of C acquisition enzymes (CB + BG), N acquisition enzymes (NAG + LAP), and oxidative enzymes (POX + PER) as well as the ratio between potential C and N acquisition enzyme (a–d) activities at the end of the one week incubation period.

0.01) (Table S3) with the highest abundance found in the Kivalo-birch site (see Table 3, even though the between groups comparisons/post-hoc tests according to LSD were not statistically significant). The abundance of these microbes was reduced by glucose addition ( $p < 0.05$ , Table S3). In contrast, we could not find any significant difference in the abundance of microbes growing on sugars, C-polymers, BSA, and lignin among sites or between different treatments (Table S3). After glucose additions, abundance of microbes growing on BSA were at a comparably higher level according to LSD in the Kivalo-birch and Tammela soils, when compared to other soils, even though the difference to Törevä site was not statistically significant (Table 3). Also, there was no link between C:N ratio of the soil and the abundance of microbes growing on any of the tested substrates (data not shown).

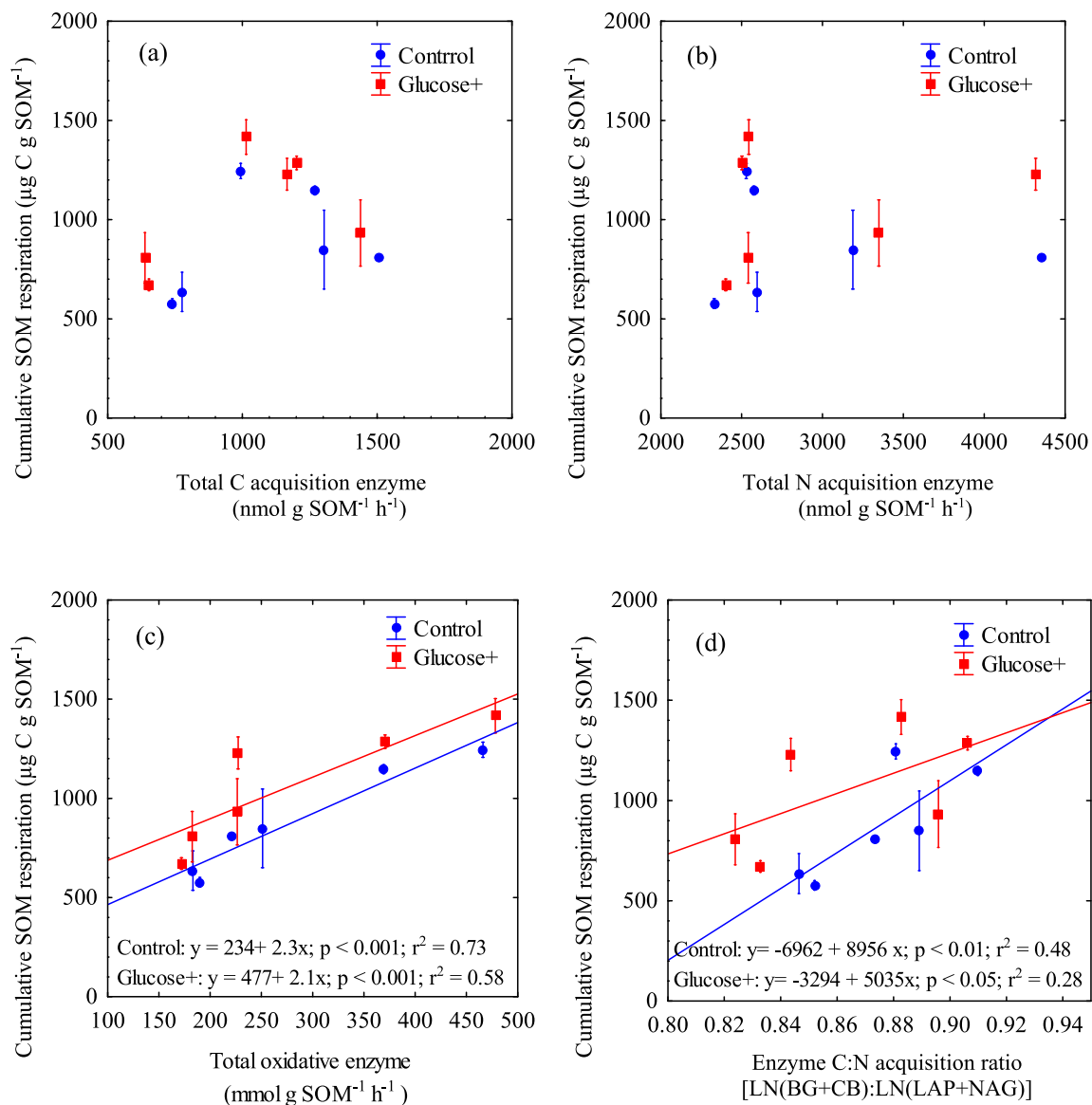
### 3.4. <sup>13</sup>C and D incorporation into microbial PLFAs

Glucose addition increased D incorporation into fungal and bacterial PLFAs in soil from all sites ( $p < 0.001$ ; Table S4). The highest <sup>13</sup>C incorporation into fungal PLFAs was observed in Kivalo-birch site, while incorporation of <sup>13</sup>C into bacterial PLFAs was highest in the Alatskivi

and Törevä sites, which had the lowest C:N ratios (Table 4). The three sites with lowest C:N ratios had a higher incorporation of D into total and bacterial PLFAs than the three sites with high C:N ratio (Table 4). In glucose amended samples the Kivalo-birch site had the highest ratio between fungal and bacterial PLFA-D incorporation, as well as the highest ratio between fungal and bacterial PLFA-<sup>13</sup>C incorporation (Table 4). There was a positive correlation between the incorporation of D and <sup>13</sup>C into fungal ( $r^2 = 0.77$ ,  $p < 0.001$ ; Fig. 6a) and bacterial PLFAs ( $r^2 = 0.60$ ,  $p < 0.001$ ; Fig. 6b).

The magnitude of priming effect (PE%) was positively correlated with the ratio of fungal to bacterial growth on glucose and on SOM (Fig. 7a), and glucose CUE and PE (%) also correlated positively (Fig. 7b). There was a negative correlation between bacterial growth on glucose and the average soil C:N ratio (Fig. 8a). Total bacterial growth was also greater in low C:N soils. There was a positive correlation between soil C:N and F:B ratios (Fig. 8b), as well as between total CUE and fungal to bacterial biomass ratio (Fig. 8c). When fungal growth was high relative to bacterial growth, CUE was also consistently higher, irrespective of whether it was the total CUE ( $p < 0.05$ , Fig. 8d), CUE of SOM decomposition (positive trend,  $p = 0.054$ , Fig. 8e), or CUE of glucose use





**Fig. 4.** The relationship between total potential activity of C acquisition enzymes (CB + BG), N acquisition enzymes (NAG + LAP), oxidative enzymes (POX + PER), C:N acquisition ratio and SOM respiration (a–d) after one week of glucose additions.

( $p < 0.05$ , Fig. 8f).

#### 4. Discussion

Soil microorganisms require a specific proportion of C and N to produce biomass and to maintain their activities. A major part of the soil C and N is in the form of polymers, and microbial access to these C and N sources depends on the release and depolymerization of these compounds from SOM (Schimel and Bennett, 2004; Jan et al., 2009). Previous studies have demonstrated that input of labile C might prime the decomposition of SOM (Hamer and Marschner, 2005), and it has been suggested that the soil C:N ratio might be an important regulator of the priming effect (Wang et al., 2015; Qiao et al., 2016). In this experiment glucose addition primed SOM decomposition by 12–52%, but the PE was independent on the soil C:N ratio. This is in contradiction to our hypothesis that the PE on SOM respiration is stronger in soils with low N availability (high C:N ratio). However, even if we did not find a relationship between soil C:N ratio and the PE, priming of SOM respiration was positively related to the microbial C:N ratio and the C:N imbalance

(the soil C:N ratio divided by the microbial C:N ratio), where a higher C:N imbalance implies stronger microbial N limitation (Fig. 2). These results support the view that SOM decomposition is regulated by stoichiometric imbalances between terrestrial decomposer communities and their resources (Mooshammer et al., 2014), and suggest that the soil C:N ratio could be a poor predictor of priming unless the microbial C and N demand is also taken into account. This is in line with previous reports concluding that the soil C:N ratio is a poor predictor of microbial C and N mineralization (Bengtsson et al., 2003). The findings further suggest that priming of SOM respiration is at least in part regulated by microbial N deficiency, which supports the microbial N-mining theory – a hypothesis which suggests that microbial communities use labile C as a source of energy needed to decompose recalcitrant SOM in order to release bioavailable N (Craine et al., 2007; Fontaine et al., 2011).

We hypothesized that priming of SOM respiration is accompanied by an increase in the activity of C targeting extracellular enzymes in C-poor soils (low C:N ratio) and with an increase in the activity of N targeting extracellular enzymes in N-poor soils (high C:N ratio). Contrary to these hypotheses, glucose addition did not influence the potential activities of

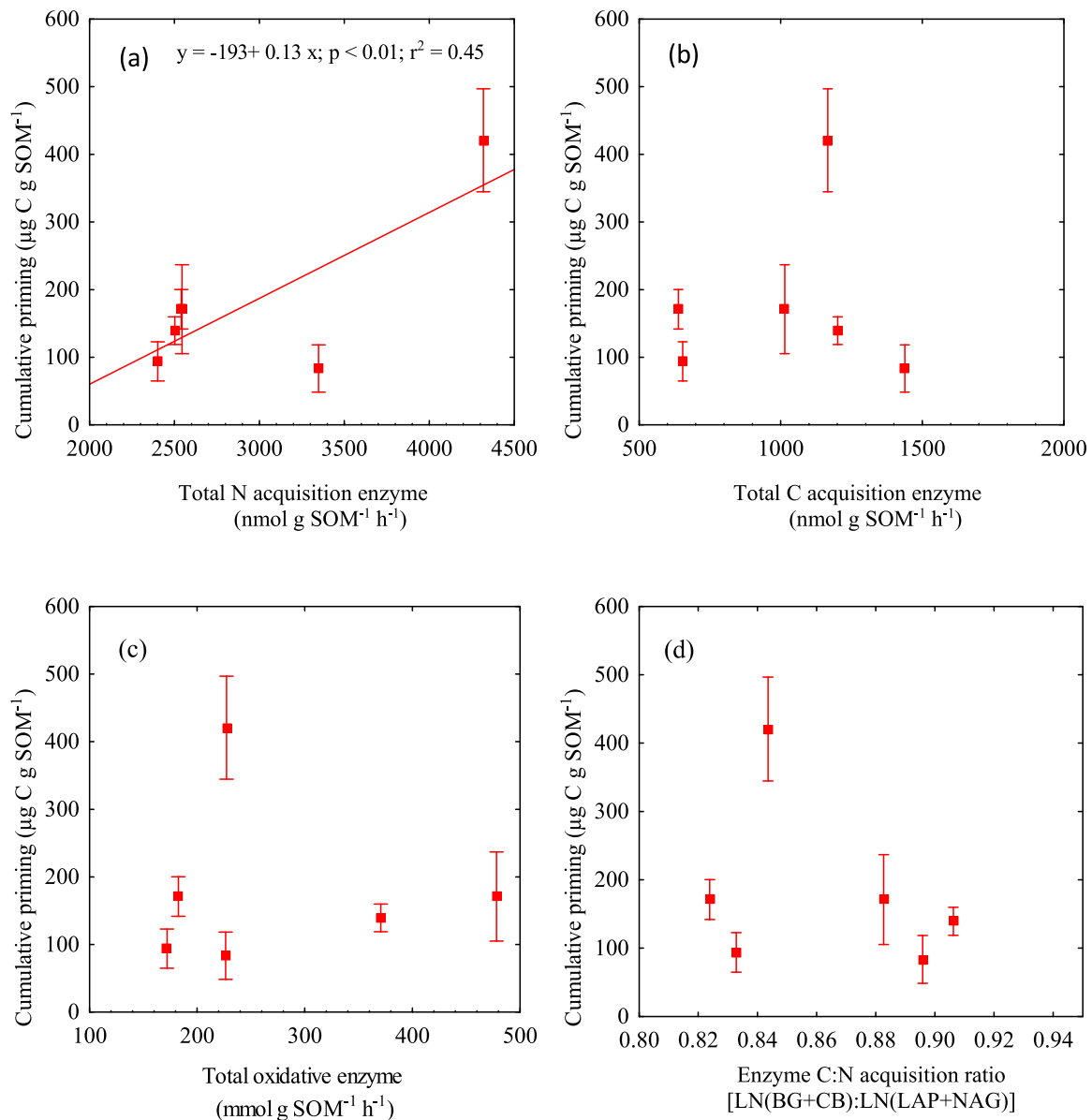
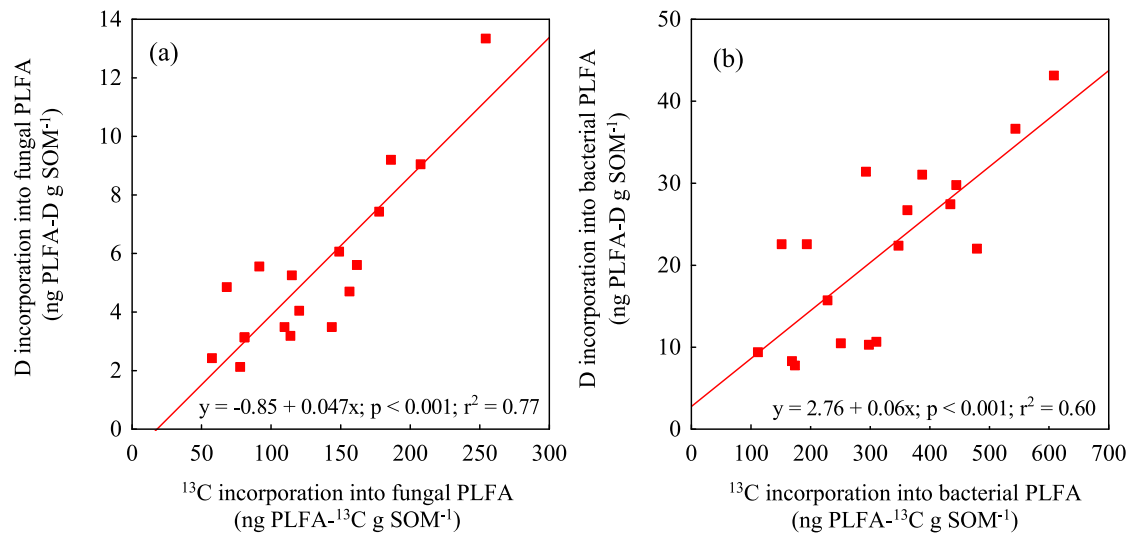


Fig. 5. The relationship between potential activity of C acquisition enzymes (CB + BG), N acquisition enzymes (NAG + LAP), oxidative enzymes (POX + PER), C:N acquisition ratio and priming (a–d) after one week of glucose additions.

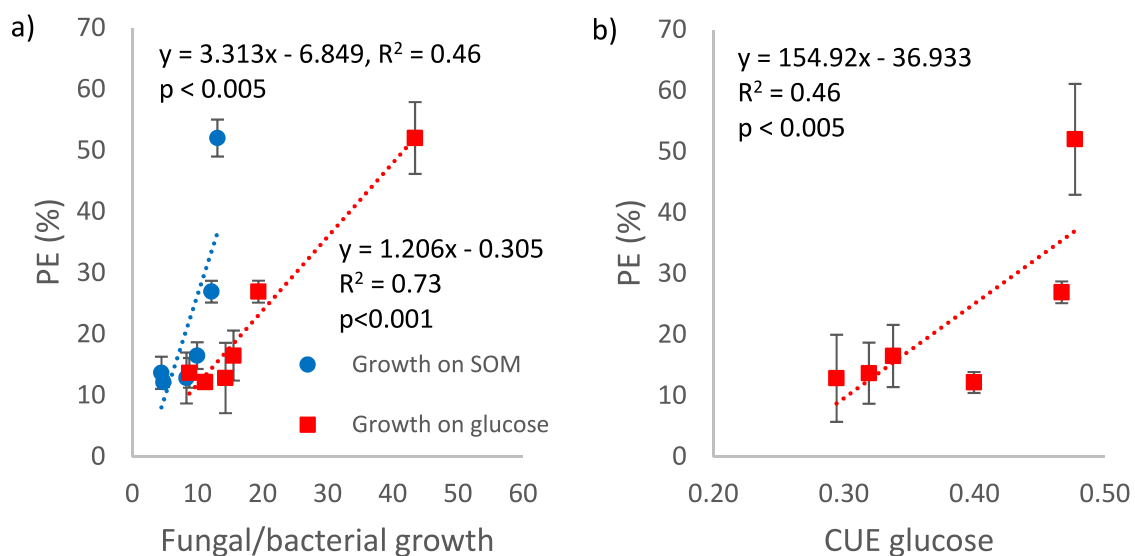
C-acquiring, N-acquiring or oxidative enzymes (Table S2), which implies that glucose addition did not influence the enzyme concentration in soil. It thus appears as if there is no direct connection between the concentration of soil enzymes and the extent of priming, which contradicts theories suggesting that priming is the result of increased enzyme production fueled by labile C inputs (Hamer and Marschner, 2005; Blagodatskaya and Kuzyakov, 2008). However, the fact that glucose additions did not influence the concentration of enzymes does not exclude the possibility that labile C input causes a PE by stimulating enzymatic decomposition of SOM. The activity of oxidative enzymes is commonly considered as the regulatory step in the decomposition of recalcitrant SOM (Perez et al., 2002; Hofrichter et al., 2010). A proposed explanation to priming is that labile C (in this case glucose) stimulates the activity rather than the concentration of such enzymes (Bengtson et al., 2012; Rousk et al., 2015). Accordingly, we found a positive relationship between the potential oxidative enzyme activity and SOM respiration in both the glucose and control treatment. Interestingly, even if the slope of the linear relationship between the potential oxidative

enzyme activity and SOM respiration was the same in both treatments, respiration of SOM was consistently higher in glucose amended samples (Fig. 4c). These observations combined suggest that oxidative enzymes limit decomposition of SOM, and that glucose additions caused priming by increasing the activity of these enzymes. A possible explanation to these findings is that the activity of oxidative enzymes is limited by regeneration of H<sub>2</sub>O<sub>2</sub>, and that labile C substrates like glucose could function as a substrate for H<sub>2</sub>O<sub>2</sub> production (Ander and Marzullo, 1997; Halliwell and Gutteridge, 1999).

Even if the PE appears to be regulated by the stimulatory effect of labile C on oxidative enzyme activity, our results also suggest that the concentration of hydrolytic C- and N-acquiring enzymes might influence observations of priming by moderating the way the increased decomposition is manifested. As expected, the enzyme C:N acquisition ratio decreased with increasing soil C:N ratio, while priming of SOM respiration was stronger at low C:N acquisition ratios (Fig. 4d). The latter result reinforces the conclusion that priming of SOM respiration is a result of microbial N mining (Craine et al., 2007; Fontaine et al., 2011).



**Fig. 6.** The ratio between D incorporation into fungal PLFA to the  $^{13}\text{C}$  incorporation into fungal PLFA (a), and the ratio between  $^{13}\text{C}$  D incorporation into bacterial PLFA to the  $^{13}\text{C}$  incorporation into bacterial PLFA (b) after one week of incubation.



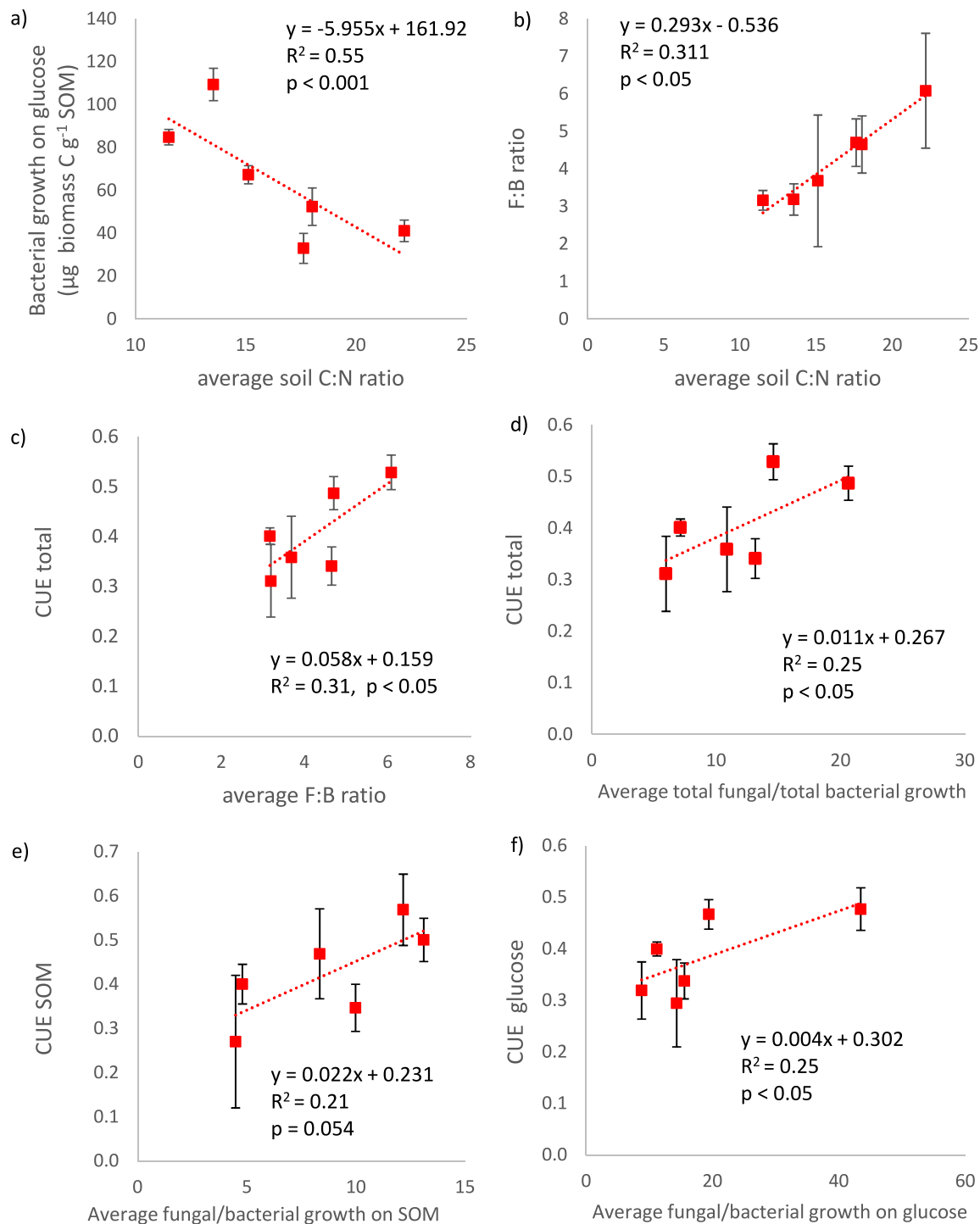
**Fig. 7.** The relationship between a) fungal/bacterial growth on SOM and glucose and the magnitude of priming, b) CUE of glucose and the magnitude of priming.

When organic N compounds produced by enzymes such as NAG and LAP are taken up, the N contained in these compounds is assimilated while excess C is respired away. We used this fact to also estimate gross N mineralization rates using the  $^{15}\text{N}$  pool dilution technique, to investigate if the opposite would be true at low soil C:N and high C:N enzyme acquisition ratios. However, due to low ammonium concentrations and rapid dilution of the  $^{15}\text{N}$  label in glucose-amended samples, this was not possible.

Water is essential for maintaining microbial growth and activities. We used this fact to investigate the relationship between microbial growth and glucose utilization through application of  $^{13}\text{C}$  glucose dissolved in deuterium (D) labelled water. Glucose addition increased D incorporation into fungal and bacterial PLFAs in all sites (Table 4). The incorporation of  $^{13}\text{C}$  or D into fungal PLFA biomarker did not differ significantly among the sites, while incorporation of  $^{13}\text{C}$  into bacterial PLFAs was higher in soil from the Alatskivi and Töreväke sites, i.e. the

sites with the lowest C:N ratios (Table 4). In fact, the incorporation of both  $^{13}\text{C}$  and D into bacterial PLFAs decreased with increasing C:N (Table 4), suggesting that low N availability reduced bacterial growth and  $^{13}\text{C}$  uptake. The reason behind the reduced bacterial growth at high C:N ratios could be caused by bacteria being more susceptible to N deficiency than fungi (Mueller et al., 2015), which have the ability to explore the soil via their extended hyphae (Simard and Durall, 2004). Fungi also have higher biomass C:N ratio (Wallenstein et al., 2006) and C use efficiency (Six et al., 2006) than bacteria, which could also make them less susceptible to N deficiency.

The highest priming of C mineralization occurred in the Kivalo-birch soil, where glucose addition increased SOM respiration by 52%. This soil also had the highest ratio between fungal and bacterial PLFA-D incorporation (Table 4), as well as the highest ratio between fungal and bacterial PLFA- $^{13}\text{C}$  incorporation (Table 4). The  $^{13}\text{C}$  incorporation into fungal PLFAs exceeded the  $^{13}\text{C}$  incorporation into bacterial PLFAs, while



**Fig. 8.** The relationship between average soil C:N ratio and a) bacterial growth on glucose, and b) fungal-to-bacterial biomass (F:B) ratio, c) between average F:B ratio and CUE, and between fungal/bacterial growth and d) total CUE, e) CUE of SOM, and f) CUE of glucose.

the D incorporation into bacterial PLFAs was almost two times higher than D incorporation into fungal PLFAs. These results suggest that fungi assimilated a particularly high proportion of the added glucose at this site, as also found by Rinnan and Bååth (2009) in tundra soil. Even if this also resulted in the highest fungal to bacterial D incorporation ratio, the D incorporation into bacterial PLFAs was still almost two times higher than the D incorporation into fungal PLFAs. It thus seems as if glucose additions stimulated fungal growth by providing a labile C substrate, possibly resulting in increased fungal decomposition of SOM to acquire

N. These N-mining activities seem to have benefited the growth of bacteria, which incorporated more D into their PLFAs than fungi even if they assimilated much less of the added glucose. It therefore seems as if bacterial growth and respiration were dependent on SOM derived C to a greater extent than fungi. A possible explanation to the high priming rate at this site could therefore be that enhanced fungal decomposition of SOM to release N, fueled by glucose, also resulted in release of bioavailable C or N that stimulated bacterial growth and respiration of SOM derived C. Our findings that there were positive correlations

**Table 3**

The abundance of microbes ( $N_0$ ) growing on sugars (glucose and sucrose), C-polymers (cellulose, starch and pectine), BSA, lignin and amino acids. Values within brackets represent standard error of the mean. Different letters represent significant differences between sites ( $p < 0.05$ ) for each treatment.

Site	C:N	Treatment	Sugar mix		C-polymers		BSA		Lignin		Amino acids	
Alatskivi	11.5	Control	17.4	(3.9)a	61	(27)a	21	(5)a	159	(58)a	8.5	(2.5)a
Tõravere	13.5		22.6	(6.4)a	65	(25)a	120	(55)a	96	(22)a	16.6	(6.1)a
Punkaharju	15.1		24.9	(6.7)a	82	(12)a	229	(130)a	129	(13)a	20.4	(1.9)a
Kivalo-birch	17.6		27.7	(7.3)a	101	(36)a	151	(57)a	173	(29)a	44.8	(16.1)a
Tammela	18.0		23.1	(2.8)a	49	(4)a	32	(6)a	63	(15)a	18.1	(3.2)a
Kivalo-spruce	22.2		15.7	(3.9)a	56	(10)a	95	(18)a	67	(8)a	14.2	(1.0)a
Alatskivi	11.5	Glucose	17.9	(3.1)a	74	(31)a	17	(2)b	211	(120)a	3.6	(0.7)a
Tõravere	13.5		25.8	(2.5)a	83	(6)a	97	(56)ab	22	(21)a	12.2	(1.5)ab
Punkaharju	15.1		16.7	(5.7)a	77	(42)a	36	(9)b	71	(29)a	11.5	(4.4)ab
Kivalo-birch	17.6		25.9	(5.0)a	85	(51)a	223	(80)a	143	(52)a	18.8	(4.1)b
Tammela	18.0		31.6	(8.6)a	138	(29)a	202	(29)a	99	(15)a	15.4	(3.1)bc
Kivalo-spruce	22.2		14.4	(2.2)a	40	(27)a	51	(26)b	88	(44)a	8.4	(1.6)ac

**Table 4**

$^{13}\text{C}$  incorporation into total, fungal, and bacterial PLFAs (ng PLFA- $^{13}\text{C}$  g SOM $^{-1}$ ), D incorporation into total, fungal, and bacterial PLFAs (ng PLFA-D g SOM $^{-1}$ ), the ratio between  $^{13}\text{C}$  incorporation into fungal PLFA and  $^{13}\text{C}$  incorporation into bacterial PLFA, and the ratio between D incorporation into fungal PLFA and D incorporation into bacterial PLFA. Values within brackets represent standard error of the mean. Different letters represent significant differences ( $p < 0.05$ ) among the sites.

Sites	C:N	Treatment	Microbial PLFA-D		Fungal PLFA-D		Bacterial PLFA-D		Fungal PLFA-D/Bacterial PLFA-D		Microbial PLFA $^{-13}\text{C}$		Fungal PLFA $^{-13}\text{C}$		Bacterial PLFA $^{-13}\text{C}$		Fungal PLFA- $^{13}\text{C}$ /Bacterial PLFA- $^{13}\text{C}$	
Alatskivi	11.5	Control	32.3	(1.3)a	0.8	(0.1)	18.9	(0.1)	0.04	(0.01)	902	(44)	128	(10)	422	(18)	0.30	(0.02)
Tõravere	13.5		42.2	(2.8)b	1	(0.2)	24.8	(2.0)	0.04	(0.01)	1133	(110)	129	(38)	544	(38)	0.24	(0.07)
Punkaharju	15.1		27.2	(5.2)a	0.4	(0.4)	17	(3.1)	0.02	(0.02)	753	(113)	134	(60)	335	(21)	0.39	(0.16)
Kivalo-birch	17.6		18.4	(0.9)c	0.4	(0.0)	10.8	(0.6)	0.04	(0.00)	639	(87)c	182	(14)	164	(35)	1.18	(0.16)
Tammela	18		13.5	(1.6)c	0.3	(0.1)	7.9	(0.9)	0.03	(0.01)	600	(61)c	100	(11)	261	(43)	0.42	(0.11)
Kivalo-spruce	22.2		12.1	(2.4)c	0.3	(0.1)	7	(1.4)	0.05	(0.01)	522	(71)c	110	(24)	205	(25)	0.52	(0.05)
Alatskivi	11.5	Glucose	60.6	(2.5)	5.1	(0.6)	29.4	(1.0)	0.17	(0.02)	902	(44)	128	(10)	422	(18)	0.30	(0.02)
Tõravere	13.5		70.4	(13.7)	5	(2.1)	34	(6.3)	0.15	(0.04)	1133	(110)	129	(38)	544	(38)	0.24	(0.07)
Punkaharju	15.1		59.2	(6.8)	7.1	(3.2)	26.9	(2.6)	0.26	(0.12)	753	(113)	134	(60)	335	(21)	0.39	(0.16)
Kivalo-birch	17.6		44.2	(8.1)	7.4	(1.0)	15.9	(3.8)	0.50	(0.09)	639	(87)c	182	(14)	164	(35)	1.18	(0.16)
Tammela	18		24.6	(1.3)c	2.9	(0.4)	9.6	(0.9)	0.32	(0.07)	600	(61)c	100	(11)	261	(43)	0.42	(0.11)
Kivalo-spruce	22.2		32.3	(7.8)c	4.5	(0.7)	13.9	(4.5)	0.36	(0.06)	522	(71)c	110	(24)	205	(25)	0.52	(0.05)

between the incorporation of D and  $^{13}\text{C}$  into fungal and bacterial PLFAs across sites, but that the slope of the regression line was steeper for bacteria than fungi (Fig. 6), support the conclusion that bacterial growth and respiration were less dependent on glucose derived C than fungal growth and respiration. The importance of fungi for initialising a PE is further supported by a positive relationship between the PE and the ratio of fungal to bacterial growth on glucose and on SOM (Fig. 7a).

Even though soil C:N ratio was not directly correlated with PE it seemed to affect PE through the differences in microbial community composition, their C:N imbalance and functioning. Bacterial growth on glucose, was also negatively correlated with the soil C:N ratio (Fig. 8a). This could indicate a better CUE of bacteria growing on glucose in a low C:N ratio soil, which is in line with ecological stoichiometry. Total bacterial growth was also greater in low C:N soils. Separating CUE of bacteria and fungi was not possible based on our data, but generally our results support the idea of higher CUE in fungal-dominated communities

(Fig. 8c). High C:N ratio soils were fungal dominated (Fig. 8b). When fungal growth was high relative to bacterial growth CUE was consistently higher. Our results indicate that if fungal dominated communities can efficiently grow on the added glucose, they will have excess resources for decomposing N containing recalcitrant SOM.

We also tested the hypothesis that glucose addition would increase the abundance of microbes that decompose recalcitrant compounds in soils with unbalanced C:N ratio, while labile C input in soil with a balanced C:N ratio would increase the abundance of microbes that preferentially grow on monomers, using MT2 plates. We could not find any evidence for this hypothesis, possibly since the method does not capture the abundance of fungi, which are often considered superior decomposers of recalcitrant compounds compared to bacteria (Boer et al., 2005). We did however, find that glucose additions reduced the abundance of microbes with the ability of growing on amino acids, but the effect was independent on the soil C:N ratio. We can only speculate



why this was the case. A possible reason is that labile C input increases gross N mineralization rates (Bengtson et al., 2012; Rousk et al., 2016; Kristensen et al., 2018). Since  $\text{NH}_4^+$  is commonly a preferred N source (Merrick and Edwards, 1995) and might suppress microbial uptake of amino acids (Geisseler and Horwath, 2014), this might be the cause.

In conclusion, our findings suggest that the soil C:N ratio alone is a poor predictor of priming and microbial decomposition of SOM. We instead propose that in order to predict priming and SOM decomposition, both nutrient availability and microbial nutrient demand should be taken into account. This is in line with microbial N mining hypothesis, which suggest that priming is the result of microbial decomposition of SOM to acquire N under N-limited conditions. Our results further suggest that priming of SOM decomposition is the result of stimulated activity of oxidative enzymes, rather than increased enzyme production, and that fungal decomposition of SOM to release N might result in increased bacterial SOM derived C assimilation and respiration.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Acknowledgements

This work was supported by grants from the Swedish Research Council and the Knut and Alice Wallenberg Foundation, and the Academy of Finland (grant numbers 267183, 316401). We thank Kristiina Regina and Ilkka Sarikka for their help in soil sampling in Tammela, Kaie Kriiska for help in sampling in Töravere, and Heljä-Sisko Helmisaari and Aino Smolander for help in site selection and providing background information on the Kivalo-birch site. Pekka Närhi and Jukka Lahti from Natural Resources Institute Finland are acknowledged for soil sampling in Kivalo sites.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2022.108615>.

### References

- Ander, P., Marzullo, L., 1997. Sugar oxidoreductases and veratryl alcohol oxidase as related to lignin degradation. *Journal of Biotechnology* 53, 115–131.
- Bengtson, P., Barker, J., Grayston, S., 2012. Evidence of a strong coupling between root exudation, C and N availability, and stimulated SOM decomposition caused by rhizosphere priming effects. *Ecology and Evolution* 2, 1843–1852.
- Bengtsson, G., Bengtson, P., Månsson, K.F., 2003. Gross nitrogen mineralization-, immobilization-, and nitrification rates as a function of soil C/N ratio and microbial activity. *Soil Biology and Biochemistry* 35, 143–154.
- Blagodatskaya, E., Kuzyakov, J., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. *Biology and Fertility of Soils* 45, 115–131.
- Boer, W.D., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. *FEMS Microbiology Reviews* 29, 795–811.
- Cheng, W., Johnson, D., Fu, S., 2003. Rhizosphere effects on decomposition. *Soil Science Society of America Journal* 67, 1418–1427.
- Cheng, W., Parton, W., Gonzalez-Meler, M., Phillips, R., Asao, S., McNickle, G., Brzostek, E., Jastrow, J., 2014. Synthesis and modeling perspectives of rhizosphere priming. *New Phytologist* 201, 31–44.
- Craine, J., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. *Ecology* 88, 2105–2113.
- Ehteshami, E., Bengtson, P., 2017. Decoupling of soil carbon and nitrogen turnover partly explains increased net ecosystem production in response to nitrogen fertilization. *Scientific Reports* 7, 46286.
- Forest monitoring: methods for terrestrial investigations in Europe with an overview of north America and Asia forest monitoring. In: Ferretti, M., Fischer, R. (Eds.), 2013. *Developments in Environmental Science*, vol. 12. Elsevier, Amsterdam.
- Fontaine, S., Henault, C., Aamor, A., Bdioui, N., Bloor, J.M.G., Maire, V., Mary, B., Revallot, S., Maron, P.A., 2011. Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. *Soil Biology and Biochemistry* 43, 86–96.
- Frostegård, Å., Bååth, E., Tunlid, A., 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. *Soil Biology and Biochemistry* 25, 723–730.
- Frostegård, Å., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biology and Fertility of Soils* 22, 56–65.
- Geisseler, D., Horwath, W., 2014. Investigating amino acid utilization by soil microorganisms using compound specific stable isotope analysis. *Soil Biology and Biochemistry* 74, 100–105.
- Garland, J.L., Lehman, M.R., 1999. Dilution/extinction of community phenotypic characters to estimate relative structural diversity in mixed communities. *FEMS Microbiology Ecology* 30, 333–343. The Oxford University Press.
- Garland, J.L., Mills, A.L., 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level-sole-carbon-source-utilization. *Applied and Environmental Microbiology* 57, 2351–2359.
- Halliwell, B., Gutteridge, J.M.C., 1999. The chemistry of free radicals and related 'reactive species'. In: *Free Radicals in Biology and Medicine*. Oxford University Press, Oxford, pp. 36–104.
- Hamer, U., Marschner, B., 2005. Priming effects in soils after combined and repeated substrate additions. *Geoderma* 128, 38–51.
- Hanson, P.J., Edwards, N.T., Garten, T.C., Andrews, J.A., 2000. Separating root and soil microbial contributions to soil respiration: a review of methods and observations. *Biogeochemistry* 48, 115–146.
- Helal, H.M., Sauerbeck, D.R., 1984. Influence of plant roots on C and P metabolism in soil. *Plant and Soil* 76, 175–182.
- Hofrichter, M., Ullrich, R., Pecyna, M., Liers, C., Lundell, T., 2010. New and classic families of secreted fungal heme peroxidases. *Applied Microbiology and Biotechnology* 87, 871–897.
- IPCC, 2000. *Land Use, Land Use Change, and Forestry. Summary for Policymakers*. Cambridge University Press, Cambridge.
- Jan, M.T., Roberts, P., Tonheim, S.K., Jones, D.L., 2009. Protein breakdown represents a major bottleneck in nitrogen cycling in grassland soils. *Soil Biology and Biochemistry* 41, 2272–2282.
- Kaiser, C., Koranda, M., Kitzler, B., Fuchsluger, L., Schneckner, J., Schweiger, P., Rasche, F., Zechmeister-Boltenstern, S., Sessitsch, A., Richter, A., 2010. Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil. *New Phytologist* 187, 843–858.
- Klamer, M., Bååth, E., 2004. Estimation of conversion factors for fungal biomass determination in compost using ergosterol and PLFA 18:2 $\omega$ 6,9. *Soil Biology and Biochemistry* 36, 57–65.
- Kristensen, J.A., Metcalfe, D.B., Rousk, J., 2018. The biogeochemical consequences of litter transformation by insect herbivory in the Subarctic: a microcosm simulation experiment. *Biogeochemistry* 138, 323–336.
- Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. *Soil Biology and Biochemistry* 32, 1485–1498.
- Merrick, M.J., Edwards, R.A., 1995. Nitrogen control in bacteria. *Microbiology and Molecular Biology Reviews* 59, 604–622.
- Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S., Richter, A., 2014. Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources. *Frontiers in Microbiology* 5, 22.
- Mueller, R.C., Belnap, J., Kuske, R., 2015. Soil bacterial and fungal community responses to nitrogen addition across soil depth and microhabitat in an arid shrubland. *Frontiers in Microbiology* 6, 891.
- Ostonen, I., et al., 2017. Adaptive root foraging strategies along a boreal-temperate forest gradient. *New Phytologist* 215, 977–991.
- Perez, J., Munoz-Dorado, J., de la Rubia, T., Martinez, J., 2002. Biodegradation and biological treatments of cellulose, hemicellulose, and lignin: an overview. *International Microbiology* 5, 53–63.
- Qiao, N., Xu, X., Hu, Y., Blagodatskaya, E., Liu, Y., Schaefer, D., Kuzyakov, Y., 2016. Carbon and nitrogen additions induce distinct priming effects along an organic-matter decay continuum. *Scientific Reports* 6, 19865.
- Rinnan, R., Bååth, E., 2009. Differential utilization of carbon substrates by bacteria and fungi in tundra soil. *Applied and Environmental Microbiology* 75, 11.
- Rousk, J., Hill, P., Jones, D., 2015. Priming of the decomposition of ageing soil organic matter: concentration dependence and microbial control. *Functional Ecology* 29, 285–296.
- Rousk, K., Michelsen, A., Rousk, J., 2016. Microbial control of soil organic matter mineralisation responses to labile carbon in subarctic climate change treatments. *Global Change Biology* 22, 4150–4161.
- Rumpel, C., Kögel-Knabner, I., 2011. Deep soil organic matter—a key but poorly understood component of terrestrial C cycle. *Plant and Soil* 338, 143–158.
- Ruess, L., Chamberlain, P.M., 2010. The fat that matters: soil food web analysis using fatty acids and their carbon stable isotope signature. *Soil Biology and Biochemistry* 42, 1898–1910.
- Simard, S.W., Durall, D.M., 2004. Mycorrhizal networks: a review of their extent, function, and importance. *Canadian Journal of Botany* 82, 1140–1165. <https://doi.org/10.1139/b04-116>.
- Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M. P., Wallenstein, M.D., Zak, D.R., Zeglin, L., 2008. Stoichiometry of soil enzyme activity at global scale. *Ecology Letters* 11, 1252–1264.
- Schimel, J., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. *Ecology* 85, 591–602.

- Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Science Society of America Journal* 70, 555–569.
- Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry* 19, 703–707.
- Varik, M., Kukumägi, M., Aosaar, J., Becker, H., Ostonen, I., Lõhmus, K., Uri, V., 2015. Carbon budgets in fertile silver birch (*Betula pendula* Roth) chronosequence stands. *Ecological Engineering* 77, 284–296.
- Wallenstein, M.D., McNulty, S., Fernandez, I.J., Boggs, J., Schlesinger, W.H., 2006. Nitrogen fertilization decreases forest soil fungal and bacterial biomass in three long-term experiments. *Forest Ecology and Management* 222, 459–468.
- Wang, H., Boutton, T.W., Xu, W., Hu, G., Jiang, P., Bai, E., 2015. Quality of fresh organic matter affects priming of soil organic matter and substrate utilization patterns of microbes. *Scientific Reports* 5, 10102.
- Wild, B., Schnecker, J., Alves, R., Barsukov, P., Bárta, J., Čapek, P., Gentsch, N., Gittel, A., Guggenberger, G., Lashchinskiy, N., Mikutta, R., Rusalimova, O., Šantrůčková, H., Shibistova, O., Urich, T., Watzka, M., Zrazhevskaya, G., Richter, A., 2014. Input of easily available organic C and N stimulates microbial decomposition of soil organic matter in arctic permafrost soil. *Soil Biology and Biochemistry* 75, 143–151.
- Zwietering, M.H., Jongenburger, I., Rombouts, F.M., Vantriet, K., 1990. Modelling of the bacterial growth curve. *Applied and Environmental Microbiology* 56, 1875–1881.