Soil organic matter quality exerts a stronger control than stoichiometry on microbial substrate use efficiency along a latitudinal transect

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Abstract

A substantial portion of soil organic matter (SOM) is of microbial origin. The efficiency with which soil microorganisms can convert their substrate carbon (C) into biomass, compared to how much is lost as respiration, thus co-determines the carbon storage potential of soils. Despite increasing insight into soil microbial C cycling, empirical measurements of microbial C processing across biomes and across soil horizons remain sparse. The theory of ecological stoichiometry predicts that microbial carbon use efficiency (CUE), i.e. growth over uptake of organic C, strongly depends on the relative availability of C and nutrients, particularly N, as microorganisms will either respire excess C or conserve C while mineralising excess nutrients. Microbial CUE is thus expected to increase from high to low latitudes and from topsoil to subsoil as the soil C:N and the stoichiometric imbalance between SOM and the microbial biomass decrease. To test these hypotheses, we collected soil samples from the organic topsoil, mineral topsoil, and mineral subsoil of seven sites along a 1500-km latitudinal transect in Western Siberia. As a proxy for CUE, we measured the microbial substrate use efficiency (SUE) of added substrates by incubating soil samples with a mixture of ^{13}C labelled sugars, amino sugars, amino acids, and organic acids and tracing ^{13}C into microbial biomass and released CO_2. In addition to soil and microbial C:N stoichiometry, we also determined the potential extracellular enzyme activities of cellobiohydrolase (CBH) and phenol oxidase (POX) and used the CBH:POX ratio as an indicator of SOM substrate quality. We found an overall decrease of SUE with latitude, corresponding to a decrease in mean annual temperature, in mineral soil horizons. SUE decreased with decreasing stoichiometric imbalance in the organic and mineral topsoil, while a relationship of SUE with soil C:N was only found in the mineral topsoil. However, contrary to our hypothesis, SUE did not increase with soil depth and mineral subsoils displayed lower average SUE than mineral topsoils. Both within individual horizons and across all horizons SUE was strongly correlated with CBH:POX ratio as well as with climate variables. Since enzyme activities likely reflect the chemical properties of SOM, our results indicate that SOM quality exerts a stronger control on SUE than SOM stoichiometry, particularly in subsoils were SOM has been turned over repeatedly and there is little variation in SOM elemental ratios.

Keywords: Carbon use efficiency, Ecological stoichiometry, Extracellular enzymes, Soil carbon, Carbon cycling

1. Introduction

A substantial part of soil organic matter (SOM) is of microbial origin, as both plant inputs and microbial products are cycled through the soil microbial community (Miltner et al., 2012; Rumpel and Kögel-Knabner, 2011; Simpson et al., 2007). The carbon (C) taken up by heterotrophic microorganisms is partitioned between biomass production and respiration (del Giorgio and Cole, 1998). This partitioning is described by the microbial carbon use efficiency (CUE, also referred to as microbial growth efficiency (Six et al., 2006), growth yield efficiency (Thiet et al., 2006), or substrate use efficiency (Schimel and Weintraub, 2003)). High CUE therefore increases the amount of microbial products potentially available for storage in soils (Cotrufo et al., 2013). At the same time, high CUE means that more biomass is produced per unit substrate, which may in turn lead to a larger microbial biomass pool and higher rates of SOM decomposition and C mineralisation (Allison et al., 2010; Wieder et al., 2013). The efficiency with which microorganisms can convert available C substrates into biomass is therefore an important factor in determining soil C storage (Xu et al., 2014), and even small changes in CUE can strongly affect model estimates of soil respiration and soil C storage (Six et al., 2006).

While the importance of soil microbial CUE for understanding and modelling soil C cycling and storage is widely recognised (Schimel, 2013), empirical studies investigating its controls across ecosystems and soil horizons are largely lacking. Many biogeochemical models assume CUE to be constant (Manzoni et al., 2012; Sinsabaugh et al., 2013), although studies on aquatic systems, litter, and soil indicate that CUE varies with substrate stoichiometry and chemistry, as well as with environmental conditions, such as temperature and substrate availability (del Giorgio and Cole, 1998; Manzoni et al., 2012; Roller and Schmidt, 2015).

Based on ecological stoichiometric theory as well as litter decomposition studies, CUE in soils is believed to be strongly controlled by the substrate C:nitrogen (N) ratio (Manzoni et al., 2012, 2010, 2008; Sinsabaugh et al., 2016, 2013). Microorganisms need to maintain the stoichiometry of their biomass within physiological boundaries and thus show limited variability in their C:N ratios, i.e. display elemental homeostasis (Cleveland and Liptzin, 2007; Xu et al., 2013; Zhou and Wang, 2015). Ecological stoichiometric theory predicts that microorganisms adjust their CUE in response to substrate imbalances between microbial biomass and substrate C:N ratios (Mooshammer et al., 2014b; Sterner and Elser, 2002), as given by the mass balance equation:

$$CUE = NUE\frac{C{:}N_{Biomass}}{C{:}N_{Substrate}} \quad (1)$$

where $C:N_{Biomass}$ is the C:N ratio of the microbial biomass, $C:N_{Substrate}$ is the C:N ratio of the substrate and NUE is the microbial N use efficiency. Similarly to CUE, NUE has been shown to vary in response to substrate stoichiometry and can decrease when N is available in excess relative to C (Mooshammer et al., 2014a). Equation (1) suggests that at low substrate C:N ratios homeostatic microbial communities have high CUE (and low NUE) as microorganisms will be C limited and aim to conserve C. Conversely, when substrate C:N ratios are high, CUE will be low (and NUE high) as excess C is respired through overflow respiration (Larsson et al., 1995; Sterner and Elser, 2002).

For equation (1) to be valid, it needs to be assumed that C assimilation is not limited by the chemical composition of the substrate. However, substrates with similar C:N stoichiometry but with different chemical structure may be converted into biomass with different efficiency. In soils, complex substrates are initially broken down by the activity of extracellular enzymeswhich can be substrate specific (hydrolytic enzymes) or unspecific (oxidative enzymes). Complex compounds, including phenolic substances such as lignin and humic substances, which require multiple enzymatic steps for decomposition, may be less efficiently converted into biomass (Bosatta and Ågren, 1999). Also, different compounds are assimilated through different metabolic pathways, which leads to different respiration rates per unit C assimilated (Gommers et al., 1988). Furthermore, C assimilation into biomass is constrained by the chemical energy per unit C, given as the degree of reduction (Manzoni et al., 2012). If the degree of reduction of the substrate is lower than that of the microbial biomass, CUE will remain below a theoretical maximum of approximately 0.8 for the assimilation of individual compounds (Gommers et al., 1988; Roller and Schmidt, 2015). However, Sinsabaugh et al. (2013) have suggested that, when taking the full maintenance costs of microbial metabolism into consideration, the thermodynamic maximum of CUE is around 0.55.

Organic matter chemistry, nutrient status, and productivity of ecosystems are strongly determined by climate and follow latitudinal patterns at a large scale. High latitude ecosystems, such as arctic tundra and boreal forest, display higher soil C:N ratios compared to lower latitudes (Post et al., 1985; Xu et al., 2013). This is attributed to low-quality litter inputs and harsh climatic conditions that limit the activity of microbial decomposers (Hobbie et al., 2000). Substrate properties and nutrient availability also change within soil profiles, since C:N ratios decrease with depth as C is successively respired during decomposition, and the chemical composition of SOM changes from primarily plant-derived to primarily microbial derived compounds (Rumpel and Kögel-Knabner, 2011).

The aim of this study was to investigate changes in microbial CUE in response to changes in C:N stoichiometry across ecosystems as well as within the soil profile. Specifically, we focused on stoichiometric controls of microbial CUE and hypothesized that (i) CUE increases from high to low

latitudes with decreasing soil C:N ratios, (ii) this latitudinal effect is less pronounced in the mineral horizons than in the organic topsoil, as environmental fluctuations are attenuated and substrate properties are less dependent on the vegetation, and (iii) CUE increases with soil depth as the C:N of SOM decreases. To this end, we established a 1500-km latitudinal transect through Western Siberia that corresponded to a threefold decrease in organic topsoil C:N ratios. The transect included seven sampling sites and spanned four major biomes: tundra, taiga, forest steppe, and steppe. Soil samples were collected from the organic topsoil, mineral topsoil and mineral subsoil horizons at each site.

Soil samples were incubated with a mixture of ^{13}C-labelled substrates and ^{13}C incorporation was traced into biomass and CO_2 to estimate microbial CUE. While often reported as CUE, such an approach measures the efficiency of the microbial community to incorporate an added substrate and may not fully capture microbial growth and maintenance respiration. We therefore use the term substrate efficiency (SUE) (Sinsabaugh et al., 2013) instead of CUE throughout the manuscript to highlight that for methodological reasons CUE could not be directly measured. This does not compromise, however, the validity of our hypotheses. In addition, we measured soil and microbial C:N stoichiometry to assess possible stoichiometric constraints on microorganisms, and we assessed the potential activities of cellobiohydrolase and phenol oxidase as indicators of the chemical complexity and recalcitrance of the substrates that microorganisms decompose. We expected that with diminishing substrate quality SUE would decrease.

2. Materials and methods

2.1. Site description and sampling

Samples were taken from seven ecosystems along a 1500-km latitudinal transect in Western Siberia that spans a range of climate and vegetation zones, from arctic tundra, to boreal forest to semiarid steppe (Supplementary Fig. 1; see also Wild et al., 2015). Along the transect, mean annual temperature (MAT) displays a near perfect negative correlation with latitude ($r = -0.99$), that is, MAT increases linearly along the transect from north to south. Mean annual precipitation (MAP) slightly increases from the tundra to the middle taiga and then decreases towards the south (Table 1, climate data were taken from Stolbovoi and McCallum, 2002). Ecosystems sampled were: tundra, northern taiga, middle taiga, southern taiga, forest steppe (forest and meadow sites), and steppe. Forest steppe is a dominant land cover type in the semi-arid south of Siberia, characterized by a mosaic of deciduous forestand grassland patches. Both forest and grassland sites were sampled, hereafter referred to as “forest steppe: forest” and “forest steppe: meadow”. Sites for each ecosystem type were selected based on zonal vegetation and low anthropogenic influence.

Table 1. Basic characterization of sites along the latitudinal transect in Western Siberia. MAT, mean annual temperature (in °C); MAP, mean annual precipitation (in mm), climate data from Stolbovoi and McCallum (2002). Soil types according to World Reference Base for Soil Resources (IUSS Working Group WRB, 2007). Horizon description and sampling depth (in cm) are given for five replicate soil pits at each site.

	Coordinates	MAT	MAP	Dominant plant species	Soil Type	Organic topsoil		Mineral topsoil		Mineral subsoil	
						Horizon	Depth	Horizon	Depth	Horizon	Depth
Tundra	67°16′N 78°50′E	−8.2	455	*Betula nana*, *Cladonia* spp.	Turbic Cryosol	O	0–6	A	2–13	Bg, BCg	6–57
Northern taiga	63°17′N 74°32′E	−5.1	540	*Picea obovata*, *Larix sibirica*	Histic Podzol	Oi, Oe	0–22	AE, EA	8–30	Bg	14–47
Middle taiga	60°09′N 71°43′E	−1.7	540	*Abies sibirica*, *Picea obovata*	Endogleyic Regosol	Oi	0–6	A, AE, EA	6–14	E, EA	12–55
Southern taiga	58°18′N 68°35′E	−0.4	486	*Picea obovata*, *Abies sibirica*	Albic Podzol	Oi	0–7	A, AE	4–18	E, EA	15–59
Forest steppe: forest	56°14′N 70°43′E	0.5	412	*Populus tremula*, *Betula pendula*	Haplic Phaeozem	O, Oa	0–10	A	7–46	B	57–109

Forest steppe: meadow	56°14′N 70°43′E	0.5	412	*Calamagrostis epigeios*, *C. arundinacea*	Luvic Phaeozem	Oa	0–7	A	4–35	Bt	26–84
Steppe	54°41′N 71°38′E	1.5	370	*Stipa capillata*, *Festuca valesiaca*	Calcic Kastanozem	OA	0–12	Ak	8–37	Bk	27–109

Soils were sampled during August 2012, starting near the time of peak summer temperatures and proceeding from north to south in order to sample under phenologically similar conditions. Samples were collected from the top three dominant soil horizons of five replicate soil pits at each site. These horizons are further referred to as organic topsoil (O, OA), mineral topsoil (A, AE, or EA), and mineral subsoil (B, BC, E, or EA) (Table 1). Soil classification follows the World Reference Base for Soil Resources (IUSS Working Group WRB, 2007). The category of organic topsoil thus also includes the steppe uppermost horizons, which qualify as mineral horizons based on their comparatively low C content. Live plant roots were removed (judged by colour and elasticity) and samples were sieved to 2 mm, except for the tundra where samples were too moist for sieving and were homogenized by hand. Before further processing, soil water content was re-adjusted to a minimum of 60% (organic topsoil, except steppe uppermost horizon), 15% (mineral topsoil, including steppe uppermost horizon), or 10% (mineral subsoil) of fresh weight with de-ionized water.

2.2. Carbon and nitrogen pools

Bulk organic C and total N content were determined in dried (60 °C) and ground samples with elemental analyzer-isotope ratio mass spectrometry (EA-IRMS; CE Instrument EA 1110 elemental analyzer, coupled to a Finnigan MAT Delta^{Plus} IRMS with a Finnigan MAT ConFlo III Interface). Mineral topsoil and subsoil at both forest steppe sites, as well as all horizons of the steppe site, contained carbonate (0.4%–13.5%). Carbonate was removed from these samples by acidification with HCl before EA-IRMS analysis following Prommer et al. (2014). Extractable organic C (EOC) and total extractable N (TEN) were measured in K_2SO_4extracts (2 g of fresh soil were extracted with 13 mL 0.5 M K_2SO_4) with a TOC/TN analyzer (Shimadzu TOC-V CPH/CPN/TNM-1, Shimadzu, Vienna, Austria). Soil pH was determined in 1 M KCl extracts.

Microbial biomass C and N were estimated using chloroform-fumigation-extraction (Amato and Ladd, 1988; Vance et al., 1987): samples were fumigated with ethanol-free chloroform in a desiccator for 24 h, fumigated and unfumigated samples (2 g each) were extracted with 13 mL 0.5 M K_2SO_4. Microbial biomass C (C_{mic}) and N (N_{mic}) were estimated as the difference in organic C and N in both sets of extracts, as determined by TOC/TN analysis (not corrected for extraction efficiency). C:N ratios of soil and microbial biomass are expressed as mass ratios. Stoichiometric imbalance between resource and microbial biomass (C:N imbalance) was calculated as the ratio of soil C:N over microbial C:N. All measures were calculated on a dry mass basis. In multiple subsoil samples TEN was within measurement uncertainty of K_2SO_4 blanks. TEN and derived measures N_{mic}, microbial C:N, and C:N imbalance in subsoils where thus excluded from further analysis.

2.3. Substrate use efficiency

Samples were incubated with a mixture of uniformly ^{13}C-labelled sugars, amino sugar, organic acids and amino acids (Supplementary Table 1), enriched at 10.4 at% ^{13}C. The overall C:N ratio of the mixture was 20, the overall degree of reduction (γ), a measure of the chemical energy per unit mole of C, was 4.0. The degree of reduction represents the number of available electrons per mole compound (Gary et al., 1995) and was calculated for each compound as:

$$\gamma = 4C + H - 2O - 3N \quad (2)$$

where C, H, O, and N are the number of carbon, hydrogen, oxygen, and nitrogen atoms, respectively. This mixture was chosen to contain low molecular weight compounds available in soils for microbial consumption (van Hees et al. 2005, Manzoni et al. 2012). A mixture of common substrates was chosen over a single substrate, such as glucose, as this may only be accessible to a part of the microbial community. We expected that this would allow microbial communities in different soils which may be adapted to different SOM qualities to use their substrate of choice and therefore the measured SUE to present a better proxy for CUE than with glucose alone. Soil samples (2 g for organic and mineral topsoil, 4 g for mineral subsoil) were placed into glass bottles (250 mL headspace for topsoil and 100 mL headspace for subsoil). The dissolved substrate mixture equivalent to 400 µg C, 40 µg C and 4 µg C was added to organic topsoil, mineral topsoil, and mineral subsoil samples, respectively. Different weights, headspace volumes, and substrate quantities were chosen to account for differences in microbial biomass and respiration rates between soil horizons. The bottles were sealed with gas-tight butyl rubber stoppers (Glasgerätebau Ochs Laborfachhandel e.K. Bovenden, Germany). Using a syringe, 20 mL headspace samples were taken from the bottles and injected into evacuated 12 mL Exetainers® (Labco Ltd. Ceredigion, UK), directly after adding the ^{13}C-labelled mixture. The syringe was purged with ambient air between samples. The air removed from the bottles was replaced from a gas bag with known CO_2 concentration and carbon isotope composition. Samples were incubated at 15 °C for 24 h, after which a second set of gas samples was taken. At the end of the incubation period, soil samples were split into equal portions and C_{mic} was estimated by CFE as described above. Aliquots of fumigated and non-fumigated K_2SO_4 extracts were used to determine $\delta^{13}C$ of EOC, by direct injection (without column, direct mode) on an HPLC (Dionex Corporation, Sunnyvale, CA, USA) connected through a Finnigan LC-IsoLink Interface (Thermo Fisher Scientific, Waltham, MA, USA) to a Finnigan Delta V Advantage Mass Spectrometer (Thermo Fisher, Bremen, Germany). Samples from soil containing carbonate were acidified with H_3PO_4. Biomass incorporation was calculated as the difference between ^{13}C in EOC of chloroform-fumigated and non-fumigated samples. The $\delta^{13}C$ signatures of

CO_2 in air samples was analysed by headspace gas sampler (GasBench II, Thermo Fisher, Bremen, Germany) coupled to an isotope ratio mass spectrometer (Delta V Advantage, Thermo Fisher, Bremen, Germany). CO_2 reference gas was calibrated using ISO-TOP gas standards (Air Liquide) with certified ^{13}C concentrations. SUE was calculated as:

(3)SUE=C13mic(C13mic+C13O2)

where $^{13}C_{mic}$ is the amount of ^{13}C-substrate incorporated into biomass and $^{13}CO_2$ is the cumulative ^{13}C-substrate respired during incubation. Cumulative respiration was corrected for the air replaced at the start of the incubation. Microbial respiration in samples from the mineral subsoil horizon of the steppe was marginal and within measurement uncertainty, samples were therefore excluded from further analysis.

2.4. Potential enzyme activities

Potential enzyme activities were measured in separate soil aliquots using microplate assays according to Kaiser et al. (2010). Cellobiohydrolase (CBH) was measured fluorimetrically using 4-methyl-umbelliferyl-β-D-cellobioside as a substrate (Marx et al., 2001). Assays were incubated for 140 min at room temperature in a sodium-acetate-buffer (pH 5.5) before measuring (excitation 365 nm, emission 450 nm). Phenol oxidase (POX) was measured photometrically using L-3,4-dihydroxyphenylalanin (L-DOPA) as a substrate. Compared to other oxidative enzyme substrates, L-DOPA has been shown to be useable across a wide range of pH values (Bach et al., 2013). Assays were measured immediately and after incubating for 20 h under same conditions as above (absorbance 450 nm).

CBH catalyses the hydrolytic depolymerisation of cellulose, releasing cellobiose, whereas POX is involved in the decomposition of complex irregular substrates. As the fraction of easily degradable substrates, such as cellulose, decreases, the relative amount of oxidative enzymes is thought to increase (Sinsabaugh and Follstad Shah, 2011). We therefore further calculated the ratio of ln CBH over ln POX (in short CBH:POX), which is used as an indicator of the relative availability of chemically complex or recalcitrant substrate (Sinsabaugh and Follstad Shah, 2011).

2.5. Statistical analysis

In order to assess the effect of site and horizon as well as their interaction on SUE, we performed two-way ANOVA with η^2 as a measure of effect size (analogous to R^2 in regression analysis), followed by Tukey's HSD test to compare individual groups for SUE and soil parameters. If necessary to meet the assumptions for ANOVA, Box-Cox transformations were applied to the data. Differences in parameters between topsoil horizons were tested using t-tests. Linear least squares regression was used to relate SUE and mean annual precipitation, soil C:N, and stoichiometric imbalance (Fig. 2). Spearman's rank correlations were used to investigate relationships between soil parameters (Table 3) after determining that multiple pairs of variables

violated the assumptions of Pearson-product-moment correlation. We used a saturating nonlinear model (Michaelis-Menten type) to describe the relationship between SUE and CBH:POX (Fig. 3). All statistical analysis and visualisation were performed in R version 3.1.0 (R Core Team, 2013), with the additional use of the car (Fox and Weisberg, 2011), heplots (Fox et al., 2013), Hmisc (Harrell, 2014), ggplot2 (Wickham, 2009), and TukeyC (Faria et al., 2014) packages.

3. Results

Soil C:N ratios significantly decreased across all horizons from north to south along the transect ($p \leq 0.001$), with highest values in all horizons observed in the Northern taiga, although there was only a weak trend and little variation in the mineral subsoil (Table 2, Table 3). Stoichiometric imbalance (soil C:N over microbial biomass C:N) decreased from north to south in the organic ($p \leq 0.001$) and mineral topsoil horizons ($p \leq 0.001$) (Table 2, Table 3). Soil C:N decreased significantly with depth (Tukey HSD, $p \leq 0.001$), while microbial C:N increased from organic to mineral topsoil, leading to a significant decrease in C:N imbalance from organic topsoil to mineral topsoil (*t*-test, $p \leq 0.001$, no data available for mineral subsoil). Mean CBH:POX ratios also significantly decreased from organic topsoil (1.49 ± 0.83 mean $\pm$ standard error), to mineral topsoil (1.39 ± 0.55), to mineral subsoil (1.25 ± 0.56, Tukey HSD, $p \leq 0.05$).

Table 2. Basic characterization of sampled soil horizons. All values are means ± standard errors. C:N imbalance is calculated as soil C:N over microbial C:N. Subsoil microbial C:N and C:N imbalance were excluded due to marginal extractable N values.

	C (mg g^{-1}DW)	N (mg g^{-1}DW)	Soil C:N	Cmic (µg g^{-1} DW)	Nmic (µg g^{-1} DW)	Microbial C:N	C:N imbalance	pH
Tundra								
Organic topsoil	308 ± 37	8.81 ± 0.66	34.9 ± 3.5	2290 ± 365	328 ± 40	6.89 ± 0.33	5.08 ± 0.47	3.78 ± 0.09
Mineral topsoil	30.4 ± 3.1	1.83 ± 0.12	16.5 ± 0.73	290 ± 55	30.5 ± 5.5	9.54 ± 0.32	1.73 ± 0.09	3.7 ± 0.03
Mineral subsoil	4.13 ± 0.51	0.37 ± 0.03	11.1 ± 0.63	29.1 ± 6.1	1.7 ± 0.28	n.a.	n.a.	3.86 ± 0.05
Northern taiga								
Organic topsoil	448 ± 7	12.5 ± 0.27	35.9 ± 0.71	2130 ± 52	332 ± 13	6.46 ± 0.24	5.58 ± 0.18	2.76 ± 0.04
Mineral topsoil	37.0 ± 3.1	1.36 ± 0.08	27.4 ± 2.0	201 ± 26	13.7 ± 1.7	14.8 ± 1.3	1.93 ± 0.26	3.06 ± 0.05

Mineral subsoil	8.17 ± 1.7	0.50 ± 0.06	15.7 ± 1.5	133 ± 15	3.43 ± 0.30	n.a.	n.a.	3.72 ± 0.06
Middle taiga								
Organic topsoil	426 ± 25	17.4 ± 1.0	24.5 ± 0.53	3670 ± 382	505 ± 58	7.33 ± 0.38	3.38 ± 0.19	3.66 ± 0.05
Mineral topsoil	74.7 ± 17	3.46 ± 0.65	20.8 ± 1.9	489 ± 116	47.4 ± 13	11 ± 0.88	1.99 ± 0.34	3.32 ± 0.08
Mineral subsoil	16.7 ± 3.8	0.97 ± 0.13	16.3 ± 1.7	136 ± 27	5.43 ± 0.86	n.a.	n.a.	3.48 ± 0.05
Southern taiga								
Organic topsoil	398 ± 18	15.8 ± 0.89	25.4 ± 0.80	3070 ± 652	628 ± 79	4.83 ± 0.68	5.83 ± 1.0	4.26 ± 0.10
Mineral topsoil	43.4 ± 3.6	3.11 ± 0.18	14.0 ± 0.80	302 ± 22	36.3 ± 3.3	8.42 ± 0.56	1.69 ± 0.15	3.62 ± 0.07
Mineral subsoi	4.79 ± 0.30	0.51 ± 0.03	9.38 ± 0.18	62.2 ± 4.9	3.41 ± 0.15	n.a.	n.a.	3.76 ± 0.07

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Forest steppe: forest

Organic topsoil	293 ± 24	17.7 ± 1.3	16.5 ± 0.31	2500 ± 427	399 ± 67	6.31 ± 0.43	2.66 ± 0.18	6.64 ± 0.37
Mineral topsoil	45.6 ± 4.5	3.57 ± 0.43	12.9 ± 0.25	156 ± 9.4	11.5 ± 0.80	13.6 ± 0.32	0.95 ± 0.03	4.26 ± 0.06
Mineral subsoil	5.16 ± 0.15	0.52 ± 0.03	10.1 ± 0.35	46.9 ± 1.9	2.9 ± 0.13	n.a.	n.a.	4.06 ± 0.04

Forest steppe: meadow

Organic topsoil	202 ± 23	14.0 ± 1.6	14.4 ± 0.16	2590 ± 369	390 ± 30	6.53 ± 0.47	2.26 ± 0.17	5.54 ± 0.25
Mineral topsoil	24.5 ± 1.6	1.88 ± 0.11	13.0 ± 0.13	198 ± 20	14.9 ± 1.6	13.4 ± 0.40	0.98 ± 0.03	4.14 ± 0.02
Mineral subsoil	5.84 ± 0.35	0.55 ± 0.03	10.7 ± 0.22	53.2 ± 4.0	2.72 ± 0.17	n.a.	n.a.	4.02 ± 0.07

Steppe

Organic topsoil	36.9 ± 3.0	3.33 ± 0.25	11.1 ± 0.13	401 ± 73	36.1 ± 7.4	11.3 ± 0.43	0.99 ± 0.03	4.62 ± 0.10
Mineral topsoil	20.1 ± 2.7	1.84 ± 0.21	10.8 ± 0.26	247 ± 38	17.9 ± 2.6	13.9 ± 0.56	0.79 ± 0.04	5.08 ± 0.32
Mineral subsoil	7.16 ± 0.81	0.79 ± 0.10	9.15 ± 0.18	87.9 ± 7.1	5.0 ± 0.80	n.a.	n.a.	7.92 ± 0.41

Horizon mean

Organic topsoil	302 ± 24	12.8 ± 0.89	23.2 ± 1.6	2380 ± 208	374 ± 34	7.09 ± 0.35	3.68 ± 0.33	4.47 ± 0.21
Mineral topsoil	39.4 ± 3.8	2.43 ± 0.18	16.5 ± 0.99	269 ± 25	24.6 ± 2.9	12.1 ± 0.46	1.44 ± 0.10	3.88 ± 0.12
Mineral subsoil	7.42 ± 0.88	0.60 ± 0.04	11.8 ± 0.57	78.3 ± 8.0	3.57 ± 0.27	n.a.	n.a.	4.40 ± 0.25

Table 3. Spearman's rank correlation coefficients for correlations of soil parameters. Measures of C, N, and enzyme activities are calculated g^{-1} DW. Steppe mineral subsoils are excluded from all correlations.

	SUE	C	N	C:N	EOC[a]	TEN[a]	C_{mic}	C:N imbalance[b]	pH	CBH:POX[c]	Latitude
Organic topsoil											
C	−0.61***										
N	−0.35*	0.52**									
C:N	−0.3	0.75***	0.03								
EOC[a]	−0.64***	0.75***	0.75***	0.41*							
TEN[a]	−0.63***	0.68***	0.82***	0.29	0.98***						
C_{mic}	−0.33	0.53**	0.63***	0.32	0.58**	0.58**					
C:N imbalance[b]	−0.50**	0.74***	0.11	0.90***	0.55**	0.45*	0.2				
pH	0.41*	−0.63***	0.17	−0.74***	−0.18	−0.07	−0.18	−0.65**			

CBH:POX[c]	0.62***	−0.70***	−0.18	−0.58***	−0.55***	−0.45**	−0.21	−0.67**	0.70**		
Latitude	−0.21	0.67**	0.04	0.95***	0.34*	0.21	0.32	0.83***	−0.76***	−0.61**	
MAP[d]	−0.63***	0.88***	0.40*	0.73***	0.66**	0.58**	0.55**	0.71***	−0.77***	−0.75**	0.74**
Mineral topsoil											
C	−0.33										
N	0.23	0.70**									
C:N	−0.73***	0.42*	−0.2								
EOC[a]	−0.50**	0.58**	0.1	0.68**							
TEN[a]	−0.37*	0.64**	0.34*	0.48**	0.89**						
C_{mic}	0.01	0.21	0.28	0.12	0.50**	0.52**					
C:N imbalance[b]	−0.60***	0.52**	0.01	0.83***	0.70***	0.53**	0.29				

pH	0.77***	−0.47**	0.14	−0.88***	−0.78***	−0.63***	−0.26	−0.83**			
CBH:POX[c]	0.69***	−0.22	0.26	−0.73***	−0.69***	−0.52**	−0.22	−0.72**	0.77**		
Latitude	−0.70***	0.36*	−0.15	0.82***	0.60**	0.39*	0.19	0.82***	−0.80***	−0.76**	
MAP[d]	−0.73***	0.56**	0.03	0.85***	0.82**	0.73**	0.31	0.81***	−0.93***	−0.78**	0.75**
Mineral subsoil											
C	0.11										
N	0.23	0.86***									
C:N	−0.22	0.56**	0.21								
EOC[a]	−0.19	0.66***	0.43*	0.67***							
TEN[a]	n.a.	n.a.	n.a.	n.a.	n.a.						
C_{mic}	−0.18	0.66***	0.56**	0.55**	0.84***	0.72**					
C:N imbala	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.				

nce[b]											
pH	0.35	−0.39*	−0.36	−0.54**	−0.61***	−0.53**	−0.70***	n.a.			
CBH:POX[c]	0.65***	0.13	0.22	−0.29	−0.23	−0.15	−0.26	n.a.	0.58**		
Latitude	−0.71***	−0.12	−0.33	0.43*	0.15	0.25	0.1	n.a.	−0.51**	−0.81***	
MAP[d]	−0.45*	0.39*	0.3	0.55**	0.61**	0.65**	0.73**	n.a.	−0.87***	−0.65**	0.60**
All horizons[e]											
C	0.06										
N	0.19	0.95***									
C:N	−0.21*	0.79***	0.62***								
EOC[a]	−0.20*	0.83***	0.76***	0.80***							
TEN[a]	−0.49***	0.88***	0.85***	0.63***	0.97***						
C_{mic}	0.11	0.91***	0.93***	0.71***	0.83***	0.85***					

C:N imbalance[b]	−0.53***	0.85**	0.66***	0.88**	0.88**	0.82**	0.72**				
pH	0.44***	−0.04	0.19	−0.43***	−0.17	−0.04	0.06	−0.32*			
CBH:POX[c]	0.63***	0.32*	0.46***	−0.11	0.03	−0.06	0.33*	−0.23	0.66**		
Latitude	−0.50***	0.1	−0.11	0.54**	0.27*	0.26*	0.03	0.61***	−0.72***	−0.69**	
MAP[d]	−0.60***	0.21*	0.02	0.56**	0.45**	0.48**	0.14	0.59***	−0.82***	−0.72**	0.71**

Levels of significance: ***, $p \leq 0.001$; **, $p \leq 0.01$; *, $p \leq 0.05$.

a. EOC: extractable organic carbon; TEN: total extractable nitrogen.

b. C:N imbalance: soil C:N over microbial C:N.

c. CBH:POX: ln cellobiohydrolase over ln phenol oxidase.

d. MAP: mean annual precipitation.

e. Correlations with TEN and C:N imbalance are based on data from topsoils only.

Microbial SUE varied across both sites and soil horizons, ranging from 0.42 in the southern taiga organic topsoil to 0.84 in the steppe mineral topsoil (Fig. 1). Two-way ANOVA showed that site had a larger effect on SUE than horizon (F (6,78) = 19.98, $p \leq 0.001$, $\eta^2 = 0.41$, and F (2,78) = 16.65, $p \leq 0.001$, $\eta^2 = 0.11$, respectively), with a significant interaction between site and horizon (F (11,79) = 5.59 $p \leq 0.001$, $\eta^2 = 0.21$). SUE did not increase with soil depth, even though soil C:N decreased and C:N imbalance decreased at least from the organic to the mineral topsoil. In fact, mineral subsoils exhibited significantly lower mean SUE than mineral topsoils (Fig. 1b and c).

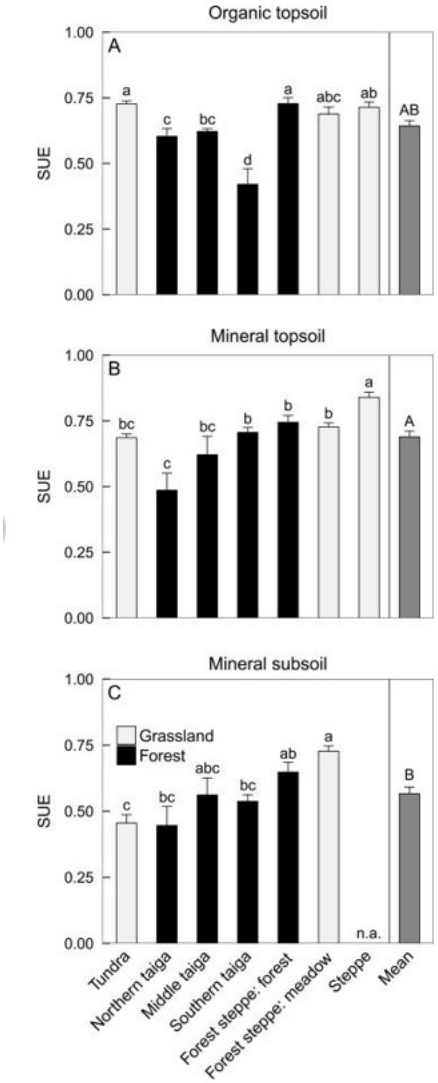

Fig. 1. Microbial substrate use efficiency (SUE) in the top three dominant soil horizons of seven sites along a latitudinal transect through Western Siberia. SUE was calculated as assimilated substrate over total substrate uptake. Steppe mineral subsoil was excluded due to marginal microbial respiration. Bars represent means ± standard errors. Different letters above bars indicate significant differences between sites (lowercase) and horizons (uppercase) (Tukey HSD test, $p \leq 0.05$).

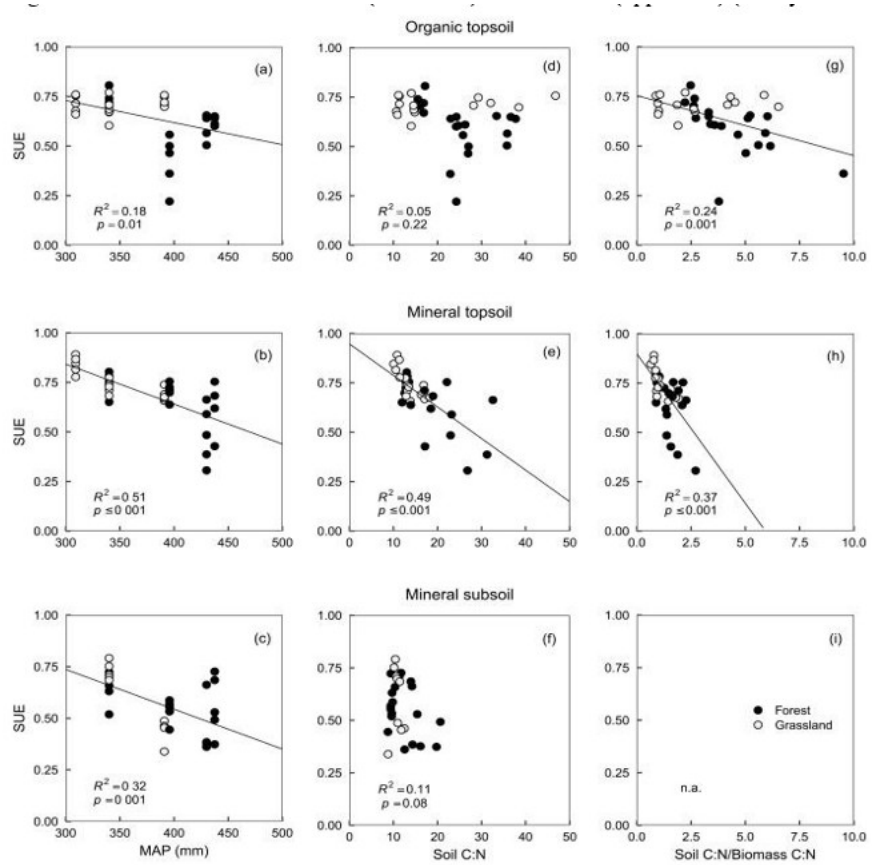

Fig. 2. Ordinary least squares regression of microbial SUE on (a–c) mean annual precipitation (MAP), (d–f) soil C:N ratio, and (g–i) stoichiometric C:N imbalance (soil C:N over microbial biomass C:N) in three soil horizons. Subsoil C:N imbalance was excluded due to marginal extractable N values.

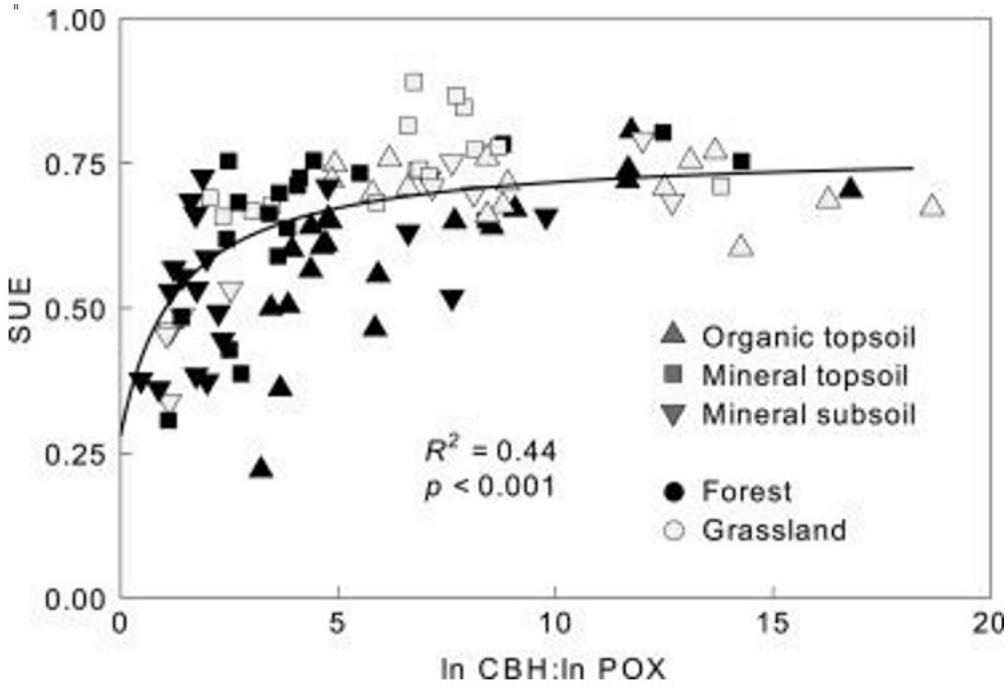

Fig. 3. Relationship of microbial SUE and ln(cellobiohydrolase) to ln(phenol oxidase) (CBH:POX) ratio in three soil horizons. CBH:POX is an indicator for substrate complexity or recalcitrance. The relationship is described by a saturating non-linear model with the following parameters: SUE = 0.77 × (CBH:POX)/[0.82 + (CBH:POX)].

SUE was negatively correlated with latitude (and positively correlated with MAT) in the mineral horizons, while there was no clear pattern in the organic topsoil (Table 3). SUE was negatively related to MAP in all horizons (Fig. 2a–c, Table 3). In organic and mineral topsoils, SUE was negatively related to C:N imbalance, as well as to soil C:N in the mineral topsoil. There was no significant relationship between soil C:N and SUE in the mineral subsoil horizons. In organic topsoils, SUE showed a strong negative correlation with EOC and TEN, as well as with soil C content. In mineral topsoils, SUE was negatively correlated with pH and EOC.

Across all horizons, SUE was positively correlated with pH, and negatively correlated with CBH:POX, latitude, and MAP, as well as showing weak negative correlations with soil C:N and EOC (Table 3). It is important to note that some of the correlations shown in Table 3 may be the result of confounding environmental processes. The strong correlation between SUE and CBH:POX, an indicator for substrate complexity or recalcitrance, in all three individual horizons and across all horizons was the most consistent pattern observed and the best predictor for SUE among all variables examined, followed by MAP. The relationship was described by a non-linear saturation model, that approaches a maximum SUE of 0.77 as CBH:POX increases (Fig. 3).

4. Discussion

In line with ecological stoichiometric theory, we expected to find a decrease in SUE with increasing soil C:N and stoichiometric C:N imbalance as the relative availability of N is considered to control the partitioning of C between microbial growth and respiration (Manzoni et al., 2012). While our hypothesis was generally supported by the results for organic and mineral topsoil horizons, we found no relationship between SUE and soil C:N in mineral subsoil, while subsoil C:N imbalance could not be assessed and may explain part of the observed variation in SUE. This absence of a significant relationship may be due to the low variability in subsoil C:N as with progressing organic matter decomposition C is lost at a higher rate than N and soil C:N values are expected to converge towards the C:N ratio of the microbial biomass (Fig. 2f). Under conditions of excess N, microbes may also reduce their NUE to adjust to stoichiometric imbalances. While Mooshammer et al. (2014a) have not found a relationship between NUE and C:N stoichiometry within organic horizons, NUE in their study did decrease from litter to subsoil. However, the decrease in SUE from mineral topsoil to subsoil suggests that any potential stoichiometric effects between the horizons were outweighed by changes in other soil parameters. It has to be considered though, that a large proportion of SOM in mineral horizons is associated with soil minerals (Kögel-Knabner et al., 2008) and thereby

protected from decomposition (Kalbitz et al., 2005; Mikutta et al., 2007). Such mineral-associated organic matter can have lower elemental ratios than the bulk soil (Kirkby et al., 2011), indicating that the stoichiometry of bioavailable compounds may diverge from bulk soil stoichiometry.

Soil microorganisms decompose SOM to acquire soluble substrates for assimilation through the production of extracellular enzymes whose activities have repeatedly been linked to substrate chemistry (Carreiro et al., 2000; Chávez-Vergara et al., 2016; Grandy et al., 2009, 2008, 2007). Oxidative enzymes act rather unspecifically and can catalyse the break-down of complex irregular substrates (Baldrian, 2006). Bach et al. (2013) suggest that soil oxidative activity represents a soil property that depends on a combination of both biotic and abiotic factors. As such, we here use the CBH:POX ratio as an indicator of soil and substrate chemistry rather than a measure of specific enzyme concentrations. Ratios of hydrolytic to oxidative enzyme activity have repeatedly been used as indicators of chemical recalcitrance in both terrestrial and aquatic systems (Hill et al., 2014; Sinsabaugh et al., 2012; Sinsabaugh and Follstad Shah, 2011). The increase in SUE with CBH:POX in all three horizons indicates that the assimilation efficiency of substrates increases with substrate quality (Table 3). Across all horizons, SUE increased with CBH:POX, and approached a maximum of around 0.77 (Fig. 3). This suggests that, as the fraction of recalcitrant C decreases, its effect on substrate assimilation diminishes and SUE approaches its theoretical maximum of c. 0.8 (Gommers et al., 1988), presumably because microorganisms will preferentially acquire nutrients and energy from easily decomposable C sources. This interpretation is supported by findings from a litter decomposition model that shows constant CUE during decomposition up to the point where the exhaustion of a C fraction that provides a net energy gain drives microorganisms to decompose a C fraction that requires a net energy investment in order to access biochemically shielded resources, at which point CUE starts to decline (Moorhead et al., 2013).

Although the labelling method we employed does not directly capture the utilization of SOM-C, but rather reflects the current physiological state of the microbial community, the results of our SUE measurements can be linked to enzyme activities and SOM composition in several ways: First, decomposition of complex substrates by oxidative enzymes may entail a low yield of C and energy (Sinsabaugh and Follstad Shah, 2011). When easily available substrates are added, such as is done in our method, C and/or energy limited microorganisms may allocate a higher proportion of these substrates to respiration, resulting in lower SUE. This is consistent with models that predict slower microbial growth when substrate complexity increases as the efficiency of enzymatic decomposition decreases (Moorhead and Sinsabaugh, 2006).

Second, microbes decompose complex substrates not only to acquire C, but also to gain access to nutrients (Moorhead and Sinsabaugh, 2006). High

oxidative enzyme activity may reflect nutrient mining in response to nutrient limitation by the microbial community. However, Wild et al. (2015) used N transformation rates of the same transect as indicators of N limitation and found that N limitation decreases with soil depth while there was no latitudinal trend along the transect. While this suggests that the observed patterns in SUE and enzyme activity are not the result of microbial N limitation, an effect of other nutrients, such as phosphorus, cannot be ruled out.

Finally, SUE and extracellular enzyme activities are both characteristics of the microbial community composition, which reflects the complex interplay between microbes, their resources, edaphic, and climatic conditions. In the same transect, Schnecker et al. (2015)found pronounced differences in microbial community composition (based on phospholipidfatty acid analysis) between horizons and significant correlations between community composition and enzyme patterns within horizons. Similarly to SUE, variations in community composition and enzyme patterns were highest in mineral subsoils, and despite the fact that the physical distance between horizons increased from north to south (Table 1), differences between horizons in community composition, enzyme patterns and SUE decreased, suggesting a link between these factors (Fig. 1 in Schnecker et al., 2015).

The observed patterns in SUE broadly followed climate trends across all horizons and particularly in the mineral horizons (Fig. 2a–c, Table 3), with generally higher SUE in more southern, warmer, and in drier climates. This may be due to higher chemical quality and lower C:N ratios of litter inputs, as well as more favourable environmental conditions which both increase decomposition rates (Aerts, 1997; Allison, 2005; Jobbágy and Jackson, 2000) and may also positively affect SUE (Cotrufo et al., 2013). While microbial physiology will respond to proximate controls such as short term changes in temperature, moisture or O_2availability, these are also subject to state factors like climate, which regulate interconnected ecosystem properties such as vegetation type, productivity, as well as the physical and chemical properties of soils, including pH and chemical composition of SOM.

Contrary to our hypotheses, SUE showed no latitudinal trend in the organic topsoil and showed only a weak relationship with MAP, which might be due to small scale variation in vegetation and microclimatic conditions. However, the relationship between climate and SUE appeared to be stronger in lower soil horizons, where organic matter has been turned over repeatedly and soil conditions may be more reflective of long term climate conditions. This would indicate that in deeper soil, which is rarely investigated compared to topsoil, microbial physiology is controlled by ecosystem properties that follow climate patterns on a large scale. These results are in overall agreement with Sinsabaugh et al. (2017) who found, using a stoichiometric model, that CUE increases from high to low latitude in response to MAT in both organic and mineral soils.

In conclusion, our results provide limited support for a solely stoichiometric control on microbial C cycling on a large spatial scale since changes in microbial SUE across soil horizons could not be explained by soil C:N stoichiometry. Instead, SUE was strongly linked to the ratio of hydrolytic to oxidative enzymes in all horizons, suggesting that microbial C assimilation, even from labile substrates, is affected by SOM quality. Even though the specific mechanisms remain unclear, our results indicate that unfavourable substrate chemistry or environmental conditions cause low SUE. These findings caution against the common use of bulk soil C:N ratios as a convenient predictor of microbial C assimilation in biogeochemical models, particularly in subsoils, where the complexity of the soil environment may be poorly captured by bulk elemental ratios. Instead, extracellular enzyme activities, which are widely used in ecological studies, may provide a feasible means to better constrain microbial SUE. Furthermore, our findings provide empirical evidence for the utility of climate variables in predicting soil microbial physiology on continental scales and we thus recommend the use of climate data in biogeochemical models to constrain microbial C cycling.

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