

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

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33 Keywords: carbon use efficiency, ecological stoichiometry, extracellular enzymes,

34 soil carbon, carbon cycling

35 **Abstract**

36 A substantial portion of soil organic matter (SOM) is of microbial origin. The
37 efficiency with which soil microorganisms can convert their substrate carbon (C)
38 into biomass, compared to how much is lost as respiration, thus co-determines the
39 carbon storage potential of soils. Despite increasing insight into soil microbial C
40 cycling, empirical measurements of microbial C processing across biomes and
41 across soil horizons remain sparse. The theory of ecological stoichiometry predicts
42 that microbial carbon use efficiency (CUE), i.e. growth over uptake of organic C,
43 strongly depends on the relative availability of C and nutrients, particularly N, as
44 microorganisms will either respire excess C or conserve C while mineralising
45 excess nutrients. Microbial CUE is thus expected to increase from high to low
46 latitudes and from topsoil to subsoil as the soil C:N and the stoichiometric
47 imbalance between SOM and the microbial biomass decrease. To test these
48 hypotheses, we collected soil samples from the organic topsoil, mineral topsoil,
49 and mineral subsoil of seven sites along a 1,500-km latitudinal transect in Western
50 Siberia. As a proxy for CUE, we measured the microbial substrate use efficiency
51 (SUE) of added substrates by incubating soil samples with a mixture of ^{13}C labelled
52 sugars, amino sugars, amino acids, and organic acids and tracing ^{13}C into
53 microbial biomass and released CO_2 . In addition to soil and microbial C:N
54 stoichiometry, we also determined the potential extracellular enzyme activities of
55 cellobiohydrolase (CBH) and phenol oxidase (POX) and used the CBH:POX ratio
56 as an indicator of SOM substrate quality. We found an overall decrease of SUE
57 with latitude, corresponding to a decrease in mean annual temperature, in mineral
58 soil horizons. SUE decreased with decreasing stoichiometric imbalance in the
59 organic and mineral topsoil, while a relationship of SUE with soil C:N was only

60 found in the mineral topsoil. However, contrary to our hypothesis, SUE did not
61 increase with soil depth and mineral subsoils displayed lower average SUE than
62 mineral topsoils. Both within individual horizons and across all horizons SUE was
63 strongly correlated with CBH:POX ratio as well as with climate variables. Since
64 enzyme activities likely reflect the chemical properties of SOM, our results indicate
65 that SOM quality exerts a stronger control on SUE than SOM stoichiometry,
66 particularly in subsoils where SOM has been turned over repeatedly and there is
67 little variation in SOM elemental ratios.

68 **1 Introduction**

69 A substantial part of soil organic matter (SOM) is of microbial origin, as both plant
70 inputs and microbial products are cycled through the soil microbial community
71 (Miltner et al., 2012; Rumpel and Kögel-Knabner, 2011; Simpson et al., 2007). The
72 carbon (C) taken up by heterotrophic microorganisms is partitioned between
73 biomass production and respiration (del Giorgio and Cole, 1998). This partitioning
74 is described by the microbial carbon use efficiency (CUE, also referred to as
75 microbial growth efficiency (Six et al., 2006), growth yield efficiency (Thiet et al.,
76 2006), or substrate use efficiency (Schimel and Weintraub, 2003)). High CUE
77 therefore increases the amount of microbial products potentially available for
78 storage in soils (Cotrufo et al., 2013). At the same time, high CUE means that more
79 biomass is produced per unit substrate, which may in turn lead to a larger microbial
80 biomass pool and higher rates of SOM decomposition and C mineralisation (Allison
81 et al., 2010; Wieder et al., 2013). The efficiency with which microorganisms can
82 convert available C substrates into biomass is therefore an important factor in
83 determining soil C storage (Xu et al., 2014), and even small changes in CUE can
84 strongly affect model estimates of soil respiration and soil C storage (Six et al.,
85 2006).

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

93 Giorgio and Cole, 1998; Manzoni et al., 2012; Roller and Schmidt, 2015).
94 Based on ecological stoichiometric theory as well as litter decomposition studies,
95 CUE in soils is believed to be strongly controlled by the substrate C:nitrogen (N)
96 ratio (Manzoni et al., 2012, 2010, 2008, Sinsabaugh et al., 2016, 2013).
97 Microorganisms need to maintain the stoichiometry of their biomass within
98 physiological boundaries and thus show limited variability in their C:N ratios, i.e.
99 display elemental homeostasis (Cleveland and Liptzin, 2007; Xu et al., 2013; Zhou
100 and Wang, 2015). Ecological stoichiometric theory predicts that microorganisms
101 adjust their CUE in response to substrate imbalances between microbial biomass
102 and substrate C:N ratios (Mooshammer et al., 2014b; Sterner and Elser, 2002), as
103 given by the mass balance equation:

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

104 where $C:N_{Biomass}$ is the C:N ratio of the microbial biomass, $C:N_{Substrate}$ is the C:N
105 ratio of the substrate and NUE is the microbial N use efficiency. Similarly to CUE,
106 NUE has been shown to vary in response to substrate stoichiometry and can
107 decrease when N is available in excess relative to C (Mooshammer et al., 2014a).
108 Equation (1) suggests that at low substrate C:N ratios homeostatic microbial
109 communities have high CUE (and low NUE) as microorganisms will be C limited
110 and aim to conserve C. Conversely, when substrate C:N ratios are high, CUE will
111 be low (and NUE high) as excess C is respired through overflow respiration
112 (Larsson et al., 1995; Sterner and Elser, 2002).
113 For equation (1) to be valid, it needs to be assumed that C assimilation is not
114 limited by the chemical composition of the substrate. However, substrates with
115 similar C:N stoichiometry but with different chemical structure may be converted

116 into biomass with different efficiency. In soils, complex substrates are initially
117 broken down by the activity of extracellular enzymes which can be substrate
118 specific (hydrolytic enzymes) or unspecific (oxidative enzymes). Complex
119 compounds, including phenolic substances such as lignin and humic substances,
120 which require multiple enzymatic steps for decomposition, may be less efficiently
121 converted into biomass (Bosatta and Ågren, 1999). Also, different compounds are
122 assimilated through different metabolic pathways, which leads to different
123 respiration rates per unit C assimilated (Gommers et al., 1988). Furthermore, C
124 assimilation into biomass is constrained by the chemical energy per unit C, given
125 as the degree of reduction (Manzoni et al., 2012). If the degree of reduction of the
126 substrate is lower than that of the microbial biomass, CUE will remain below a
127 theoretical maximum of approximately 0.8 for the assimilation of individual
128 compounds (Gommers et al., 1988; Roller and Schmidt, 2015). However,
129 Sinsabaugh et al. (2013) have suggested that, when taking the full maintenance
130 costs of microbial metabolism into consideration, the thermodynamic maximum of
131 CUE is around 0.55.

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

141 derived compounds (Rumpel and Kögel-Knabner, 2011).

142 The aim of this study was to investigate changes in microbial CUE in response to
143 changes in C:N stoichiometry across ecosystems as well as within the soil profile.
144 Specifically, we focused on stoichiometric controls of microbial CUE and
145 hypothesized that (i) CUE increases from high to low latitudes with decreasing soil
146 C:N ratios, (ii) this latitudinal effect is less pronounced in the mineral horizons than
147 in the organic topsoil, as environmental fluctuations are attenuated and substrate
148 properties are less dependent on the vegetation, and (iii) CUE increases with soil
149 depth as the C:N of SOM decreases. To this end, we established a 1,500-km
150 latitudinal transect through Western Siberia that corresponded to a threefold
151 decrease in organic topsoil C:N ratios. The transect included seven sampling sites
152 and spanned four major biomes: tundra, taiga, forest steppe, and steppe. Soil
153 samples were collected from the organic topsoil, mineral topsoil and mineral
154 subsoil horizons at each site.

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

166 complexity and recalcitrance of the substrates that microorganisms decompose.

167 We expected that with diminishing substrate quality SUE would decrease.

168 **2 Materials and methods**

169 **2.1 Site description and sampling**

170 Samples were taken from seven ecosystems along a 1,500-km latitudinal transect
171 in Western Siberia that spans a range of climate and vegetation zones, from arctic
172 tundra, to boreal forest to semiarid steppe (Supplementary Fig. 1; see also Wild et
173 al., 2015). Along the transect, mean annual temperature (MAT) displays a near
174 perfect negative correlation with latitude ($r = -0.99$), that is, MAT increases linearly
175 along the transect from north to south. Mean annual precipitation (MAP) slightly
176 increases from the tundra to the middle taiga and then decreases towards the
177 south (Table 1, climate data were taken from Stolbovoi and McCallum, 2002).
178 Ecosystems sampled were: tundra, northern taiga, middle taiga, southern taiga,
179 forest steppe (forest and meadow sites), and steppe. Forest steppe is a dominant
180 land cover type in the semi-arid south of Siberia, characterized by a mosaic of
181 deciduous forest and grassland patches. Both forest and grassland sites were
182 sampled, hereafter referred to as “forest steppe: forest” and “forest steppe:
183 meadow”. Sites for each ecosystem type were selected based on zonal vegetation
184 and low anthropogenic influence.
185 Soils were sampled during August 2012, starting near the time of peak summer
186 temperatures and proceeding from north to south in order to sample under
187 phenologically similar conditions. Samples were collected from the top three
188 dominant soil horizons of five replicate soil pits at each site. These horizons are
189 further referred to as organic topsoil (O, OA), mineral topsoil (A, AE, or EA), and
190 mineral subsoil (B, BC, E, or EA) (Table 1). Soil classification follows the World
191 Reference Base for Soil Resources (IUSS Working Group WRB, 2007). The
192 category of organic topsoil thus also includes the steppe uppermost horizons,

193 which qualify as mineral horizons based on their comparatively low C content. Live
194 plant roots were removed (judged by colour and elasticity) and samples were sieved
195 to 2 mm, except for the tundra where samples were too moist for sieving and were
196 homogenized by hand. Before further processing, soil water content was re-
197 adjusted to a minimum of 60% (organic topsoil, except steppe uppermost horizon),
198 15% (mineral topsoil, including steppe uppermost horizon), or 10% (mineral subsoil)
199 of fresh weight with de-ionized water.

200

201 **2.2 Carbon and nitrogen pools**

202 Bulk organic C and total N content were determined in dried (60°C) and ground
203 samples with elemental analyser-isotope ratio mass spectrometry (EA-IRMS; CE
204 Instrument EA 1110 elemental analyzer, coupled to a Finnigan MAT Delta^{Plus} IRMS
205 with a Finnigan MAT ConFlo III Interface). Mineral topsoil and subsoil at both forest
206 steppe sites, as well as all horizons of the steppe site, contained carbonate (0.4 % to
207 13.5 %). Carbonate was removed from these samples by acidification with HCl before
208 EA-IRMS analysis following Pommer et al. (2014). Extractable organic C (EOC) and
209 total extractable N (TEN) were measured in K₂SO₄ extracts (2 g of fresh soil were
210 extracted with 13 mL 0.5 M K₂SO₄) with a TOC/TN analyzer (Shimadzu TOC-V
211 CPH/CPN /TNM-1, Shimadzu, Vienna, Austria). Soil pH was determined in 1 M KCl
212 extracts.

213 Microbial biomass C and N were estimated using chloroform-fumigation-extraction
214 (Amato and Ladd, 1988; Vance et al., 1987): samples were fumigated with ethanol-
215 free chloroform in a desiccator for 24 h, fumigated and unfumigated samples (2 g
216 each) were extracted with 13 mL 0.5 M K₂SO₄. Microbial biomass C (C_{mic}) and N
217 (N_{mic}) were estimated as the difference in organic C and N in both sets of extracts,

218 as determined by TOC/TN analysis (not corrected for extraction efficiency). C:N
219 ratios of soil and microbial biomass are expressed as mass ratios. Stoichiometric
220 imbalance between resource and microbial biomass (C:N imbalance) was
221 calculated as the ratio of soil C:N over microbial C:N. All measures were calculated
222 on a dry mass basis. In multiple subsoil samples TEN was within measurement
223 uncertainty of K₂SO₄ blanks. TEN and derived measures N_{mic}, microbial C:N, and
224 C:N imbalance in subsoils were thus excluded from further analysis.

225

226 **2.3 Substrate use efficiency**

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

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

233 where C , H , O , and N are the number of carbon, hydrogen, oxygen, and nitrogen
234 atoms, respectively. This mixture was chosen to contain low molecular weight
235 compounds available in soils for microbial consumption (van Hees et al. 2005,
236 Manzoni et al. 2012). A mixture of common substrates was chosen over a single
237 substrate, such as glucose, as this may only be accessible to a part of the
238 microbial community. We expected that this would allow microbial communities in
239 different soils which may be adapted to different SOM qualities to use their
240 substrate of choice and therefore the measured SUE to present a better proxy for
241 CUE than with glucose alone. Soil samples (2 g for organic and mineral topsoil, 4 g

242 for mineral subsoil) were placed into glass bottles (250 mL headspace for topsoil
243 and 100 mL headspace for subsoil). The dissolved substrate mixture equivalent to
244 400 $\mu\text{g C}$, 40 $\mu\text{g C}$ and 4 $\mu\text{g C}$ was added to organic topsoil, mineral topsoil, and
245 mineral subsoil samples, respectively. Different weights, headspace volumes, and
246 substrate quantities were chosen to account for differences in microbial biomass
247 and respiration rates between soil horizons. The bottles were sealed with gas-tight
248 butyl rubber stoppers (Glasgerätebau Ochs Laborfachhandel e.K., Bovenden,
249 Germany). Using a syringe, 20 mL headspace samples were taken from the
250 bottles and injected into evacuated 12 mL Exetainers® (Labco Ltd., Ceredigion,
251 UK), directly after adding the ^{13}C -labelled mixture. The syringe was purged with
252 ambient air between samples. The air removed from the bottles was replaced
253 from a gas bag with known CO_2 concentration and carbon isotope composition.
254 Samples were incubated at 15 °C for 24 h, after which a second set of gas
255 samples was taken. At the end of the incubation period, soil samples were split
256 into equal portions and C_{mic} was estimated by CFE as described above.
257 Aliquots of fumigated and non-fumigated K_2SO_4 extracts were used to determine
258 $\delta^{13}\text{C}$ of EOC, by direct injection (without column, direct mode) on an HPLC
259 (Dionex Corporation, Sunnyvale, CA, USA) connected through a Finnigan LC-
260 IsoLink Interface (Thermo Fisher Scientific, Waltham, MA, USA) to a Finnigan Delta
261 V Advantage Mass Spectrometer (Thermo Fisher, Bremen, Germany). Samples
262 from soil containing carbonate were acidified with H_3PO_4 . Biomass incorporation
263 was calculated as the difference between ^{13}C in EOC of chloroform-fumigated and
264 non-fumigated samples. The $\delta^{13}\text{C}$ signatures of CO_2 in air samples was analysed
265 by headspace gas sampler (GasBench II, Thermo Fisher, Bremen, Germany)
266 coupled to an isotope ratio mass spectrometer (Delta V Advantage, Thermo Fisher,

267 Bremen, Germany). CO₂ reference gas was calibrated using ISO-TOP gas
268 standards (Air Liquide) with certified ¹³C concentrations. SUE was calculated as:

$$SUE = \frac{{}^{13}C_{mic}}{({}^{13}C_{mic} + {}^{13}CO_2)} \quad (3)$$

269 where ¹³C_{mic} is the amount of ¹³C-substrate incorporated into biomass and ¹³CO₂ is
270 the cumulative ¹³C-substrate respired during incubation. Cumulative respiration
271 was corrected for the air replaced at the start of the incubation. Microbial
272 respiration in samples from the mineral subsoil horizon of the steppe was marginal
273 and within measurement uncertainty, samples were therefore excluded from further
274 analysis.

275

276 **2.4 Potential enzyme activities**

277 Potential enzyme activities were measured in separate soil aliquots using
278 microplate assays according to Kaiser et al. (2010). Cellobiohydrolase (CBH)
279 was measured fluorimetrically using 4-methyl-umbelliferyl-β-D-cellobioside as a
280 substrate (Marx et al., 2001). Assays were incubated for 140 min at room
281 temperature in a sodium-acetate-buffer (pH 5.5) before measuring (excitation
282 365 nm, emission 450 nm). Phenol oxidase (POX) was measured
283 photometrically using L-3,4-dihydroxyphenylalanin (L-DOPA) as a substrate.
284 Compared to other oxidative enzyme substrates, L-DOPA has been shown to
285 be useable across a wide range of pH values (Bach et al., 2013). Assays were
286 measured immediately and after incubating for 20 hours under same conditions
287 as above (absorbance 450 nm).

288 CBH catalyses the hydrolytic depolymerisation of cellulose, releasing
289 cellobiose, whereas POX is involved in the decomposition of complex irregular

290 substrates. As the fraction of easily degradable substrates, such as cellulose,
291 decreases, the relative amount of oxidative enzymes is thought to increase
292 (Sinsabaugh and Follstad Shah, 2011). We therefore further calculated the
293 ratio of ln CBH over ln POX (in short CBH:POX), which is used as an indicator
294 of the relative availability of chemically complex or recalcitrant substrate
295 (Sinsabaugh and Follstad Shah, 2011).

296

297 **2.5 Statistical analysis**

298 In order to assess the effect of site and horizon as well as their interaction on SUE,
299 we performed two-way ANOVA with η^2 as a measure of effect size (analogous to
300 R^2 in regression analysis), followed by Tukey's HSD test to compare individual
301 groups for SUE and soil parameters. If necessary to meet the assumptions for
302 ANOVA, Box-Cox transformations were applied to the data. Differences in
303 parameters between topsoil horizons were tested using t-tests. Linear least
304 squares regression was used to relate SUE and mean annual precipitation, soil
305 C:N, and stoichiometric imbalance (Fig. 2). Spearman's rank correlations were
306 used to investigate relationships between soil parameters (Table 3) after
307 determining that multiple pairs of variables violated the assumptions of Pearson-
308 product-moment correlation. We used a saturating nonlinear model (Michaelis-
309 Menten type) to describe the relationship between SUE and CBH:POX (Fig. 3). All
310 statistical analysis and visualisation were performed in R version 3.1.0 (R Core
311 Team, 2013), with the additional use of the car (Fox and Weisberg, 2011), heplots
312 (Fox et al., 2013), Hmisc (Harrell et al., 2014), ggplot2 (Wickham, 2009), and
313 TukeyC (Faria et al., 2014) packages.

314

315 **3 Results**

316 Soil C:N ratios significantly decreased across all horizons from north to south along
317 the transect ($p \leq 0.001$), with highest values in all horizons observed in the
318 Northern taiga, although there was only a weak trend and little variation in the
319 mineral subsoil (Tables 2 and 3). Stoichiometric imbalance (soil C:N over microbial
320 biomass C:N) decreased from north to south in the organic ($p \leq 0.001$) and mineral
321 topsoil horizons ($p \leq 0.001$) (Tables 2 and 3). Soil C:N decreased significantly with
322 depth (Tukey HSD, $p \leq 0.001$), while microbial C:N increased from organic to
323 mineral topsoil, leading to a significant decrease in C:N imbalance from organic
324 topsoil to mineral topsoil (t-test, $p \leq 0.001$, no data available for mineral subsoil).
325 Mean CBH:POX ratios also significantly decreased from organic topsoil ($1.49 \pm$
326 0.83 mean \pm standard error), to mineral topsoil (1.39 ± 0.55), to mineral subsoil
327 (1.25 ± 0.56 , Tukey HSD, $p \leq 0.05$).

328 Microbial SUE varied across both sites and soil horizons, ranging from 0.42 in the
329 southern taiga organic topsoil to 0.84 in the steppe mineral topsoil (Fig. 1). Two-way
330 ANOVA showed that site had a larger effect on SUE than horizon ($F(6,78) = 19.98$,
331 $p \leq 0.001$, $\eta^2 = 0.41$, and $F(2,78) = 16.65$, $p \leq 0.001$, $\eta^2 = 0.11$, respectively), with
332 a significant interaction between site and horizon ($F(11,79) = 5.59$ $p \leq 0.001$, $\eta^2 =$
333 0.21). SUE did not increase with soil depth, even though soil C:N decreased and
334 C:N imbalance decreased at least from the organic to the mineral topsoil. In fact,
335 mineral subsoils exhibited significantly lower mean SUE than mineral topsoils (Fig.
336 1b, c).

337 SUE was negatively correlated with latitude (and positively correlated with MAT)
338 in the mineral horizons, while there was no clear pattern in the organic topsoil
339 (Table 3). SUE was negatively related to MAP in all horizons (Fig. 2a-c, Table

340 3). In organic and mineral topsoils, SUE was negatively related to C:N imbalance,
341 as well as to soil C:N in the mineral topsoil. There was no significant relationship
342 between soil C:N and SUE in the mineral subsoil horizons. In organic topsoils, SUE
343 showed a strong negative correlation with EOC and TEN, as well as with soil C
344 content. In mineral topsoils, SUE was negatively correlated with pH and EOC.
345 Across all horizons, SUE was positively correlated with pH, and negatively
346 correlated with CBH:POX, latitude, and MAP, as well as showing weak negative
347 correlations with soil C:N and EOC (Table 3). It is important to note that some of
348 the correlations shown in Table 3 may be the result of confounding environmental
349 processes. The strong correlation between SUE and CBH:POX, an indicator for
350 substrate complexity or recalcitrance, in all three individual horizons and across all
351 horizons was the most consistent pattern observed and the best predictor for SUE
352 among all variables examined, followed by MAP. The relationship was described
353 by a non-linear saturation model, that approaches a maximum SUE of 0.77 as
354 CBH:POX increases (Fig. 3).

355

356 **4 Discussion**

357 In line with ecological stoichiometric theory, we expected to find a decrease in SUE
358 with increasing soil C:N and stoichiometric C:N imbalance as the relative
359 availability of N is considered to control the partitioning of C between microbial
360 growth and respiration (Manzoni et al., 2012). While our hypothesis was generally
361 supported by the results for organic and mineral topsoil horizons, we found no
362 relationship between SUE and soil C:N in mineral subsoil, while subsoil C:N
363 imbalance could not be assessed and may explain part of the observed variation in
364 SUE. This absence of a significant relationship may be due to the low variability in
365 subsoil C:N as with progressing organic matter decomposition C is lost at a higher
366 rate than N and soil C:N values are expected to converge towards the C:N ratio of
367 the microbial biomass (Fig. 2f). Under conditions of excess N, microbes may also
368 reduce their NUE to adjust to stoichiometric imbalances. While Mooshammer et al.
369 (2014a) have not found a relationship between NUE and C:N stoichiometry within
370 organic horizons, NUE in their study did decrease from litter to subsoil. However,
371 the decrease in SUE from mineral topsoil to subsoil suggest that any potential
372 stoichiometric effects between the horizons were outweighed by changes in other
373 soil parameters. It has to be considered though, that a large proportion of SOM in
374 mineral horizons is associated with soil minerals (Kögel-Knabner et al., 2008) and
375 thereby protected from decomposition (Kalbitz et al., 2005; Mikutta et al., 2007).
376 Such mineral-associated organic matter can have lower elemental ratios than the
377 bulk soil (Kirkby et al., 2011), indicating that the stoichiometry of bioavailable
378 compounds may diverge from bulk soil stoichiometry.
379 Soil microorganisms decompose SOM to acquire soluble substrates for assimilation
380 through the production of extracellular enzymes whose activities have repeatedly

381 been linked to substrate chemistry (Carreiro et al., 2000; Chávez-Vergara et al.,
382 2016; Grandy et al., 2009, 2008, 2007). Oxidative enzymes act rather unspecifically
383 and can catalyse the break-down of complex irregular substrates (Baldrian, 2006).
384 Bach et al. (2013) suggest that soil oxidative activity represents a soil property that
385 depends on a combination of both biotic and abiotic factors. As such, we here use
386 the CBH:POX ratio as an indicator of soil and substrate chemistry rather than a
387 measure of specific enzyme concentrations. Ratios of hydrolytic to oxidative enzyme
388 activity have repeatedly been used as indicators of chemical recalcitrance in both
389 terrestrial and aquatic systems (Hill et al., 2014; Sinsabaugh et al., 2012;
390 Sinsabaugh and Follstad Shah, 2011). The increase in SUE with CBH:POX in all
391 three horizons indicates that the assimilation efficiency of substrates increases with
392 substrate quality (Table 3). Across all horizons, SUE increased with CBH:POX, and
393 approached a maximum of around 0.77 (Fig. 3). This suggests that, as the fraction of
394 recalcitrant C decreases, its effect on substrate assimilation diminishes and SUE
395 approaches its theoretical maximum of c. 0.8 (Gommers et al., 1988), presumably
396 because microorganisms will preferentially acquire nutrients and energy from easily
397 decomposable C sources. This interpretation is supported by findings from a litter
398 decomposition model that shows constant CUE during decomposition up to the point
399 where the exhaustion of a C fraction that provides a net energy gain drives
400 microorganisms to decompose a C fraction that requires a net energy investment in
401 order to access biochemically shielded resources, at which point CUE starts to
402 decline (Moorhead et al., 2013).

403 Although the labelling method we employed does not directly capture the utilization
404 of SOM-C, but rather reflects the current physiological state of the microbial
405 community, the results of our SUE measurements can be linked to enzyme

406 activities and SOM composition in several ways: First, decomposition of complex
407 substrates by oxidative enzymes may entail a low yield of C and energy (Sinsabaugh
408 and Follstad Shah, 2011). When easily available substrates are added, such as is
409 done in our method, C and/or energy limited microorganisms may allocate a higher
410 proportion of these substrates to respiration, resulting in lower SUE. This is
411 consistent with models that predict slower microbial growth when substrate
412 complexity increases as the efficiency of enzymatic decomposition decreases
413 (Moorhead and Sinsabaugh, 2006).

414 Second, microbes decompose complex substrates not only to acquire C, but also to
415 gain access to nutrients (Moorhead and Sinsabaugh, 2006). High oxidative enzyme
416 activity may reflect nutrient mining in response to nutrient limitation by the microbial
417 community. However, Wild et al. (2015) used N transformation rates of the same
418 transect as indicators of N limitation and found that N limitation decreases with soil
419 depth while there was no latitudinal trend along the transect. While this suggests that
420 the observed patterns in SUE and enzyme activity are not the result of microbial N
421 limitation, an effect of other nutrients, such as phosphorus, cannot be ruled out.

422 Finally, SUE and extracellular enzyme activities are both characteristics of the
423 microbial community composition, which reflects the complex interplay between
424 microbes, their resources, edaphic, and climatic conditions. In the same transect,
425 Schneckner et al. (2015) found pronounced differences in microbial community
426 composition (based on phospholipid fatty acid analysis) between horizons and
427 significant correlations between community composition and enzyme patterns within
428 horizons. Similarly to SUE, variations in community composition and enzyme
429 patterns were highest in mineral subsoils, and despite the fact that the physical
430 distance between horizons increased from north to south (Table 1), differences

431 between horizons in community composition, enzyme patterns and SUE decreased,
432 suggesting a link between these factors (Fig. 1 in Schnecker et al., 2015).

433 The observed patterns in SUE broadly followed climate trends across all horizons
434 and particularly in the mineral horizons (Fig. 2a-c, Table 3), with generally higher
435 SUE in more southern, warmer, and in drier climates. This may be due to higher
436 chemical quality and lower C:N ratios of litter inputs, as well as more favourable
437 environmental conditions which both increase decomposition rates (Aerts, 1997;
438 Allison, 2005; Jobbágy and Jackson, 2000) and may also positively affect SUE
439 (Cotrufo et al., 2013). While microbial physiology will respond to proximate controls
440 such as short term changes in temperature, moisture or O₂ availability, these are
441 also subject to state factors like climate, which regulate interconnected ecosystem
442 properties such as vegetation type, productivity, as well as the physical and
443 chemical properties of soils, including, pH and chemical composition of SOM.

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

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

471 **Acknowledgements**

472 This work was funded by the Austrian Science Fund as part of the International
473 Program CryoCARB – Long-term Carbon Storage in Cryoturbated Arctic Soils
474 (FWF – I370-B17). M.M. was supported by the dissertation completion fellowship
475 2014 of the University of Vienna. We thank three anonymous reviewers whose
476 insightful comments greatly improved the manuscript.

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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 & 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	<i>Betula nana</i> , <i>Cladonia</i> spp.	Turbic Cryosol	O	0-6	A	2-13	Bg, BCg	6-57
Northern taiga	63°17'N 74°32'E	-5.1	540	<i>Picea obovata</i> , <i>Larix sibirica</i>	Histic Podzol	Oi, Oe	0-22	AE, EA	8-30	Bg	14-47
Middle taiga	60°09'N 71°43'E	-1.7	540	<i>Abies sibirica</i> , <i>Picea obovata</i>	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	<i>Picea obovata</i> , <i>Abies sibirica</i>	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	<i>Populus tremula</i> , <i>Betula pendula</i>	Haplic Phaeozem	O, Oa	0-10	A	7-46	B	57-109
Forest steppe: meadow	56°14'N 70°43'E	0.5	412	<i>Calamagrostis epigeios</i> , <i>C. arundinacea</i>	Luvic Phaeozem	Oa	0-7	A	4-35	Bt	26-84
Steppe	54°41'N 71°38'E	1.5	370	<i>Stipa capillata</i> , <i>Festuca valesiaca</i>	Calcic Kastanozem	OA	0-12	Ak	8-37	Bk	27-109

Table 2

Basic characterization of sampled soil horizons. All values are means \pm 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	C _{mic} (μ g g ⁻¹ DW)	N _{mic} (μ g g ⁻¹ DW)	Microbial C:N	C:N imbalance	pH
Tundra								
Organic topsoil	308 \pm 37	8.81 \pm 0.66	34.9 \pm 3.5	2290 \pm 365	328 \pm 40	6.89 \pm 0.33	5.08 \pm 0.47	3.78 \pm 0.09
Mineral topsoil	30.4 \pm 3.1	1.83 \pm 0.12	16.5 \pm 0.73	290 \pm 55	30.5 \pm 5.5	9.54 \pm 0.32	1.73 \pm 0.09	3.7 \pm 0.03
Mineral subsoil	4.13 \pm 0.51	0.37 \pm 0.03	11.1 \pm 0.63	29.1 \pm 6.1	1.7 \pm 0.28	n.a.	n.a.	3.86 \pm 0.05
Northern taiga								
Organic topsoil	448 \pm 7	12.5 \pm 0.27	35.9 \pm 0.71	2130 \pm 52	332 \pm 13	6.46 \pm 0.24	5.58 \pm 0.18	2.76 \pm 0.04
Mineral topsoil	37.0 \pm 3.1	1.36 \pm 0.08	27.4 \pm 2.0	201 \pm 26	13.7 \pm 1.7	14.8 \pm 1.3	1.93 \pm 0.26	3.06 \pm 0.05
Mineral subsoil	8.17 \pm 1.7	0.50 \pm 0.06	15.7 \pm 1.5	133 \pm 15	3.43 \pm 0.30	n.a.	n.a.	3.72 \pm 0.06
Middle taiga								
Organic topsoil	426 \pm 25	17.4 \pm 1.0	24.5 \pm 0.53	3670 \pm 382	505 \pm 58	7.33 \pm 0.38	3.38 \pm 0.19	3.66 \pm 0.05
Mineral topsoil	74.7 \pm 17	3.46 \pm 0.65	20.8 \pm 1.9	489 \pm 116	47.4 \pm 13	11 \pm 0.88	1.99 \pm 0.34	3.32 \pm 0.08
Mineral subsoil	16.7 \pm 3.8	0.97 \pm 0.13	16.3 \pm 1.7	136 \pm 27	5.43 \pm 0.86	n.a.	n.a.	3.48 \pm 0.05
Southern taiga								
Organic topsoil	398 \pm 18.3	15.8 \pm 0.89	25.4 \pm 0.80	3070 \pm 652	628 \pm 79	4.83 \pm 0.68	5.83 \pm 1.0	4.26 \pm 0.10
Mineral topsoil	43.4 \pm 3.6	3.11 \pm 0.18	14.0 \pm 0.80	302 \pm 22	36.3 \pm 3.3	8.42 \pm 0.56	1.69 \pm 0.15	3.62 \pm 0.07
Mineral subsoil	4.79 \pm 0.30	0.51 \pm 0.03	9.38 \pm 0.18	62.2 \pm 4.9	3.41 \pm 0.15	n.a.	n.a.	3.76 \pm 0.07
Forest steppe: forest								
Organic topsoil	293 \pm 24	17.7 \pm 1.3	16.5 \pm 0.31	2500 \pm 427	399 \pm 67	6.31 \pm 0.43	2.66 \pm 0.18	6.64 \pm 0.37
Mineral topsoil	45.6 \pm 4.5	3.57 \pm 0.43	12.9 \pm 0.25	156 \pm 9.4	11.5 \pm 0.80	13.6 \pm 0.32	0.95 \pm 0.03	4.26 \pm 0.06
Mineral subsoil	5.16 \pm 0.15	0.52 \pm 0.03	10.1 \pm 0.35	46.9 \pm 1.9	2.9 \pm 0.13	n.a.	n.a.	4.06 \pm 0.04
Forest steppe: meadow								
Organic topsoil	202 \pm 23	14.0 \pm 1.6	14.4 \pm 0.16	2590 \pm 369	390 \pm 30	6.53 \pm 0.47	2.26 \pm 0.17	5.54 \pm 0.25
Mineral topsoil	24.5 \pm 1.6	1.88 \pm 0.11	13.0 \pm 0.13	198 \pm 20	14.9 \pm 1.6	13.4 \pm 0.40	0.98 \pm 0.03	4.14 \pm 0.02
Mineral subsoil	5.84 \pm 0.35	0.55 \pm 0.03	10.7 \pm 0.22	53.2 \pm 4.0	2.72 \pm 0.17	n.a.	n.a.	4.02 \pm 0.07
Steppe								
Organic topsoil	36.9 \pm 3.0	3.33 \pm 0.25	11.1 \pm 0.13	401 \pm 73	36.1 \pm 7.4	11.3 \pm 0.43	0.99 \pm 0.03	4.62 \pm 0.10
Mineral topsoil	20.1 \pm 2.7	1.84 \pm 0.21	10.8 \pm 0.26	247 \pm 38	17.9 \pm 2.6	13.9 \pm 0.56	0.79 \pm 0.04	5.08 \pm 0.32
Mineral subsoil	7.16 \pm 0.81	0.79 \pm 0.10	9.15 \pm 0.18	87.9 \pm 7.1	5.0 \pm 0.80	n.a.	n.a.	7.92 \pm 0.41
Horizon mean								
Organic topsoil	302 \pm 24.3	12.8 \pm 0.89	23.2 \pm 1.6	2380 \pm 208	374 \pm 34	7.09 \pm 0.35	3.68 \pm 0.33	4.47 \pm 0.21
Mineral topsoil	39.4 \pm 3.8	2.43 \pm 0.18	16.5 \pm 0.99	269 \pm 25	24.6 \pm 2.9	12.1 \pm 0.46	1.44 \pm 0.10	3.88 \pm 0.12
Mineral subsoil	7.42 \pm 0.88	0.60 \pm 0.04	11.8 \pm 0.57	78.3 \pm 8.0	3.57 \pm 0.27	n.a.	n.a.	4.40 \pm 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 imbalance ^b	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.				
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 [†]											
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: ***, $p \leq 0.001$; **, $p \leq 0.01$; *, $p \leq 0.05$.

^aEOC: extractable organic carbon; TEN: total extractable nitrogen.

^bC:N imbalance: soil C:N over microbial C:N.

^cCBH:POX: ln cellobiohydrolase over ln phenol oxidase.

^dMAP: mean annual precipitation.

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

Figure 1

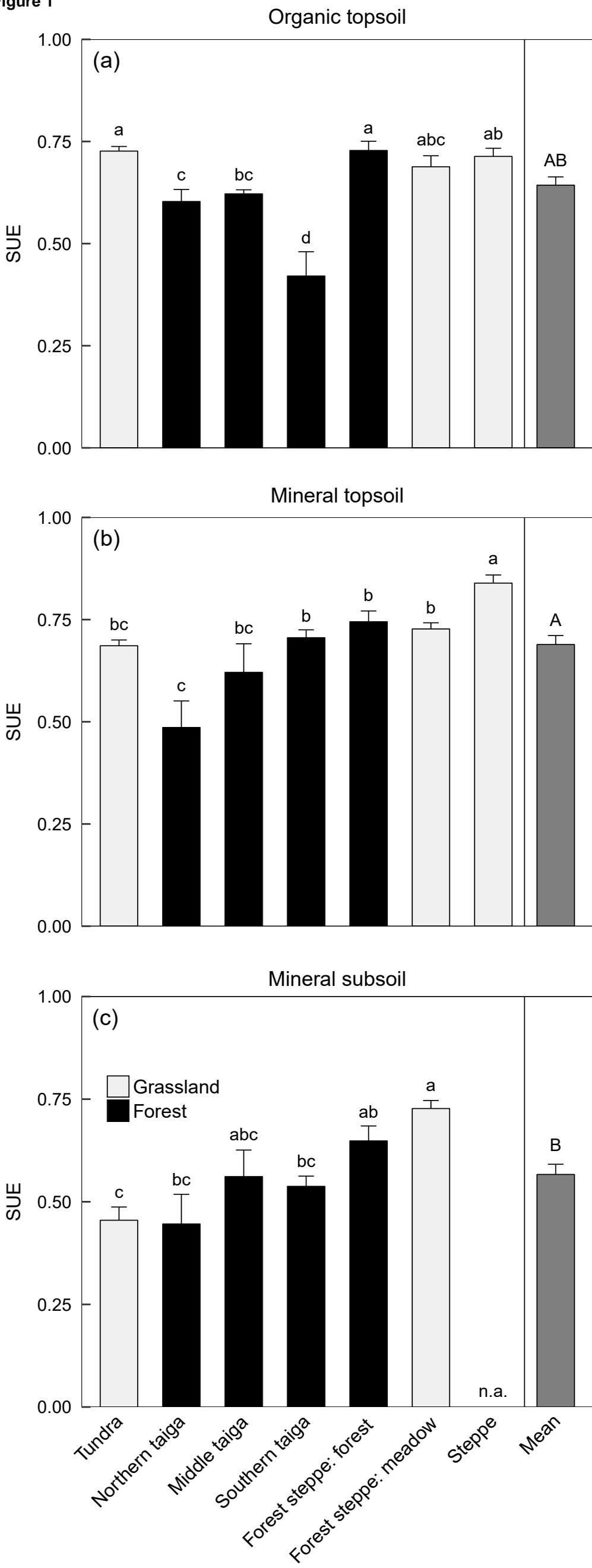


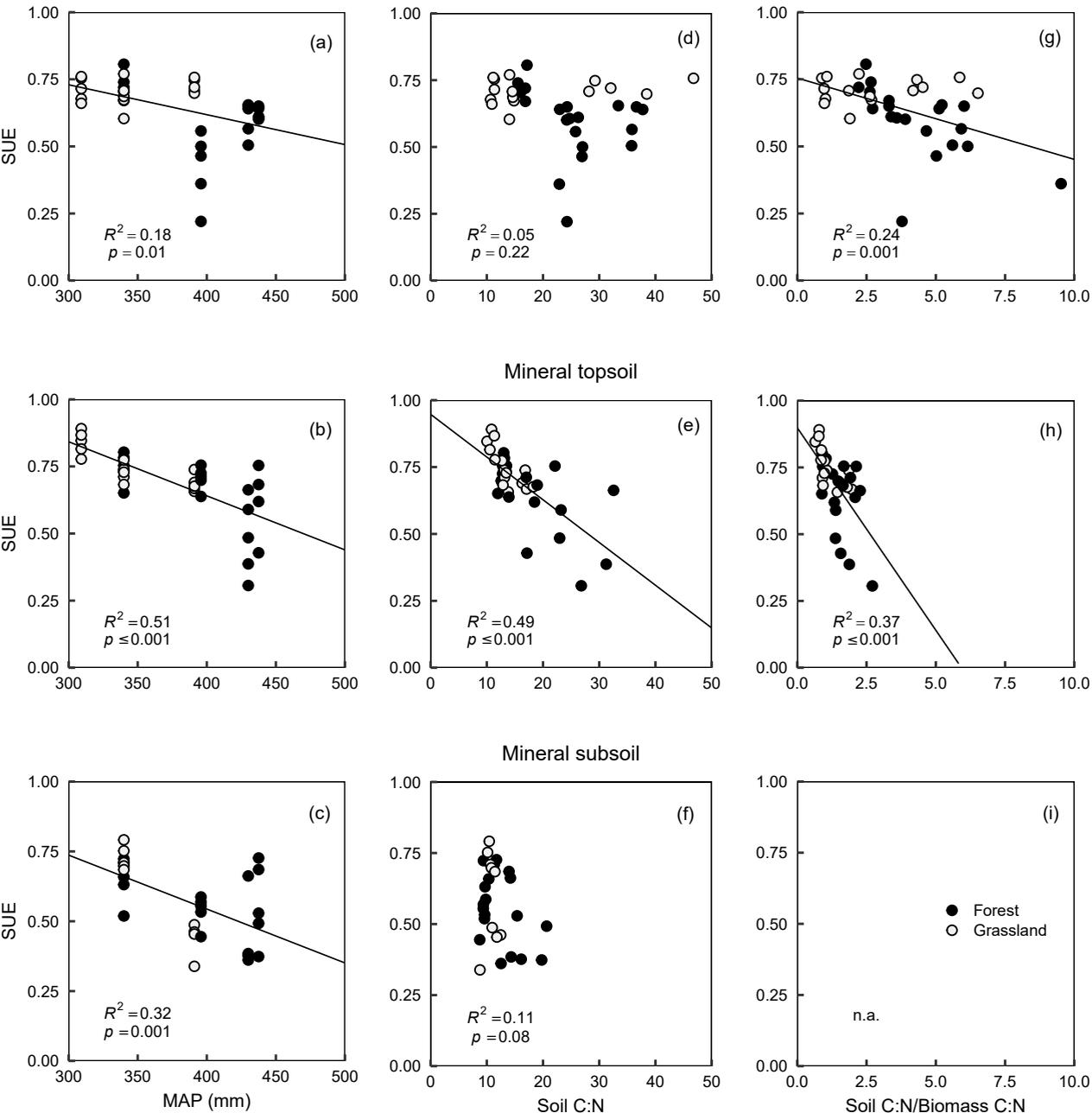
Figure 2

Figure 3

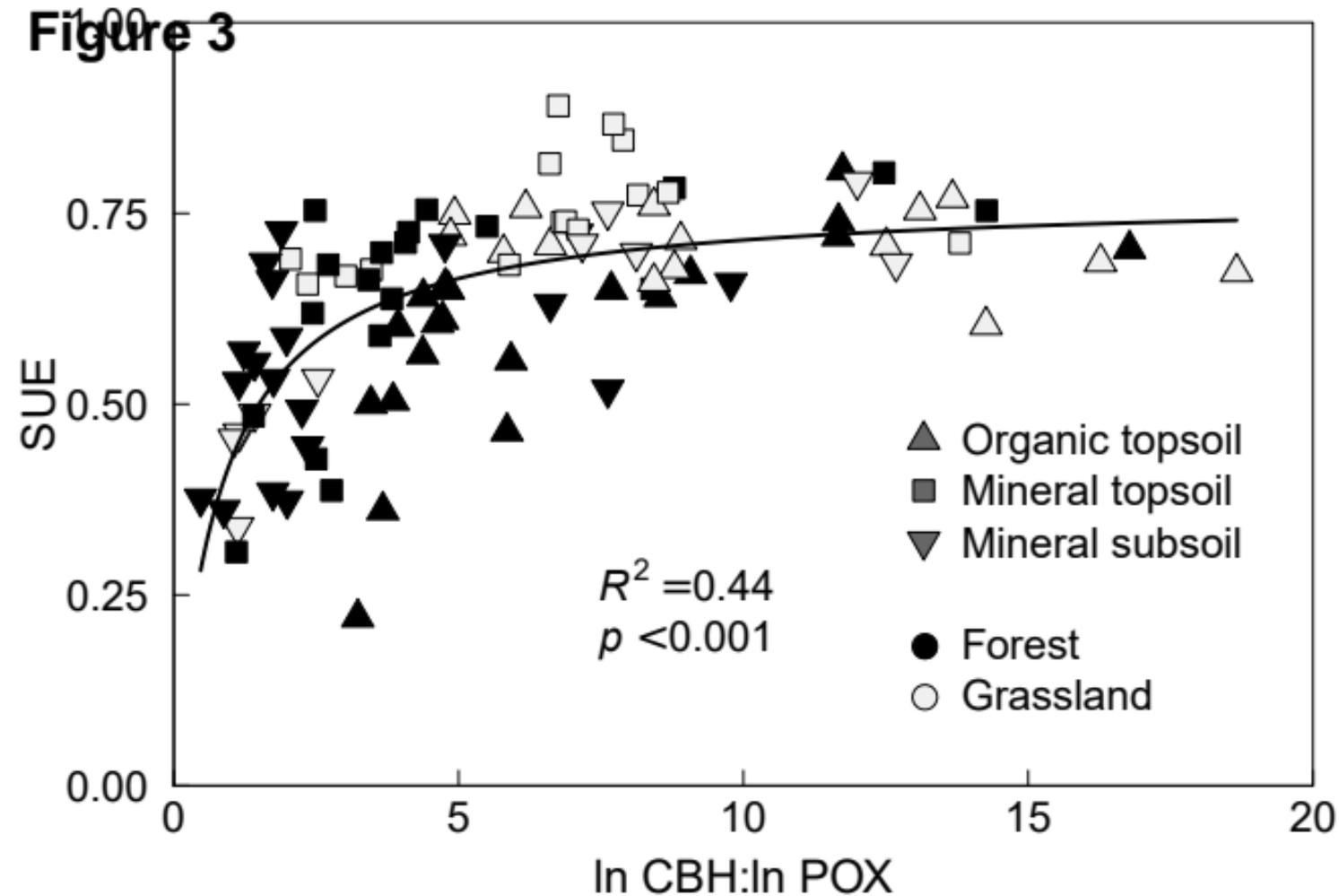


Fig. 1. Microbial substrate use efficiency (SUE) in the top three dominant soil horizons of seven sites along a latitudinal transect through Western Siberia. SUE was calculated as assimilated substrate over total substrate uptake. Steppe mineral subsoil was excluded due to marginal microbial respiration. Bars represent means \pm 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 $\ln(\text{cellobiohydrolase})$ to $\ln(\text{phenol oxidase})$ (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: $\text{SUE} = 0.77 \times (\text{CBH:POX}) / [0.82 + (\text{CBH:POX})]$.

Supplementary Material for online publication only

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