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1 **Beyond growth: the significance of non-growth anabolism for microbial**
2 **carbon-use efficiency in the light of soil carbon stabilisation**

3

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22 growth metabolism, microbial exudates, soil carbon models

23 **Abstract**

24 Microbial carbon-use efficiency (CUE) in soils captures carbon (C) partitioning between
25 anabolic biosynthesis of microbial metabolites and catabolic C emissions (i.e. respiratory C
26 waste). The use of C for biosynthesis provides a potential for the accumulation of microbial
27 metabolic residues in soil. Recognized as a crucial control in C cycling, microbial CUE is
28 implemented in the majority of soil C models. Due to the models' high sensitivity to CUE,
29 reliable soil C projections demand accurate CUE quantifications. Current measurements of CUE
30 neglect microbial non-growth metabolites, such as extracellular polymeric substances (EPS) or
31 exoenzymes, although they remain in soil and could be quantitatively important. Here, we
32 highlight that disregarding non-growth anabolism can lead to severe underestimations of CUE.
33 Based on two case studies, we demonstrate that neglecting exoenzyme and EPS production
34 underestimates CUE by more than 100% and up to 30%, respectively. Using these values in
35 model simulations, we observed that the model projects up to 34% larger SOC stocks when non-
36 growth metabolites are considered for estimating CUE. Our considerations outlined here
37 challenge the current ways how CUE is measured. Research efforts should focus on (i)
38 advancing CUE estimations by capturing the multitude of microbial C uses, (ii) improving
39 techniques to quantify non-growth metabolic products in soil, and (iii) providing an
40 understanding of dynamic metabolic C uses under different environmental conditions and over
41 time. In the light of current discussion on soil C stabilization mechanisms, we call for efforts to
42 open the 'black box' of microbial physiology in soil and to incorporate all quantitative important
43 C uses in CUE measurements.



44 **1. Introduction**

45 The microbial origin of stabilized soil organic C (SOC) has received increasing attention in
46 recent years (e.g. Domeignoz-Horta et al., 2021; Kallenbach et al., 2016, 2015; Liang et al.,
47 2020, 2017; Miltner et al., 2012). To date, it remains challenging to quantify the contribution of
48 microbial-derived C to stable SOC (Liang et al., 2019), but some findings suggest that microbial-
49 derived C may make up a quarter to more than half of total SOC (Deng and Liang, 2021; Liang
50 et al., 2019; Miltner et al., 2012). Despite quantitative uncertainties concerning microbial-derived
51 stable SOC, the microbial metabolic performance is a key factor in soil C dynamics, because the
52 vast majority of organic C inputs to soil will be eventually processed by soil microorganisms.
53 Soil C inputs will thus largely be subjected to microbial C allocation towards catabolic C
54 emissions (i.e. C waste via respiration) or biosynthesis, with the latter leading to C remaining in
55 soil. Recognized as a crucial control in C cycling, this microbial carbon use efficiency (CUE) is
56 implemented, implicitly or explicitly, in all soil C models (Schimel et al., 2022; Schimel, 2013),
57 which respond highly sensitive to even small changes in CUE (Allison et al., 2010; Bölscher et
58 al., 2020; Frey et al., 2013; Hyvönen et al., 1998). Due to the models' high sensitivity, reliable
59 SOC projections require accurate CUE quantifications, capturing all metabolic C uses within
60 microorganisms.

61 The concept of microbial CUE—as applied in soil ecology—neglects considerable parts of the
62 microbially processed C, because current methods focus on capturing growth/biomass increases
63 and assume non-growth anabolism as quantitatively unimportant (Manzoni et al., 2018; Paul and
64 Clark, 1989). Here, we show that this quantitatively neglected C will affect CUE estimations.
65 The neglected C comprises all extracellular metabolites released from microbial cells into the
66 surrounding soil and potentially intracellular non-growth metabolites (Fig. 1). Which anabolic C



67 uses are exactly ignored depends on definitions and methods applied to measure CUE. Despite
68 its key importance for soil C cycling, CUE remains an ambiguous and poorly defined concept
69 (Schimel et al., 2022). Within soil ecology, two partially different notions of CUE have emerged,
70 the substrate-specific CUE, which measures the incorporation of C-isotope labels into microbial
71 biomass (Geyer et al., 2019; Manzoni et al., 2012; Steinweg et al., 2008), and the substrate-
72 independent CUE, measuring growth via ^{18}O -water incorporation into DNA (Geyer et al., 2019,
73 2016; Spohn et al., 2016). While substrate-specific CUE treats all C incorporated into microbial
74 biomass as efficiently used (and thus remaining in soil when implemented in soil C models),
75 substrate-independent CUE considers only C directed towards growth as efficiently used C. Both
76 CUE methods and underlying concepts neglect microbial non-growth anabolism. Non-growth
77 anabolism includes all extracellular metabolites, such as extracellular polymeric substances
78 (EPS), exoenzymes or nutrient mobilizing compounds (Flemming and Wingender, 2010; Van
79 Bodegom, 2007), and certain intracellular metabolites, such as storage compounds or
80 endoenzymes (Mason-Jones et al., 2023, 2022). Yet, C directed towards the synthesis of such
81 compounds is not ‘inefficiently’ used C, as it serves crucial functions supporting microorganisms
82 to survive and is primordial for microbial life itself. More importantly, this C remains in soil and
83 thus provides a potential for long-term C stabilization. When CUE, based on current methods, is
84 implemented in soil C models, the models paradoxically treat non-growth metabolites as emitted
85 C waste leaving the soil. Neglecting microbial non-growth anabolism may introduce a bias when
86 quantifying microbial CUE.

87 Here, we (i) scrutinize the idea that non-growth anabolism can be ignored for CUE
88 investigations, (ii) suggest adjustments to common CUE approaches, (iii) demonstrate that
89 current assessments of CUE measure only an ‘apparent’ CUE which can significantly



90 underestimate ‘actual’ CUE, (iv) illustrate the potential consequences for SOC projections and
91 (v) outline research needs and potential ways forward.

92 **2. Why is non-growth anabolism disregarded for microbial CUE?**

93 Why do current concepts of microbial CUE in soil ecology neglect non-growth anabolism,
94 despite the respective C remains in soil and serves important microbial survival strategies? The
95 reason may be three-fold: (i) Ideas of CUE evolved parallel in various scientific fields,
96 contributing to the concept’s ambiguity and amorphous definition (Geyer et al., 2016; Manzoni
97 et al., 2018; Schimel et al., 2022); (ii) Non-growth anabolism is considered quantitatively
98 negligible compared to C used for growth (Manzoni et al., 2018); and (iii) Quantification of
99 microbial non-growth anabolism in soil is challenging:

100 (i) Concepts of CUE have been developed in various subfields of biology. While addressing
101 roughly the same idea, specific definitions and conceptualizations of CUE diverge (Geyer et al.,
102 2016; Manzoni et al., 2018). The assignment of C as efficiently used can comprise growth,
103 biomass production or entire biosynthesis (Manzoni et al., 2018, 2012). Depending on the
104 organism and specific situation, these three entities can be almost similar or they differ
105 substantially (Manzoni et al., 2018). The concept of microbial CUE in soil ecology has been
106 largely influenced by the idea of microbial-growth efficiency (often called growth yield; see
107 Supplementary Note for discussion on how neglecting non-growth anabolism affects estimations
108 of growth efficiency). The latter is commonly used in microbiology (e.g. Gommers et al., 1988;
109 Linton, 1991; von Stockar and Marison, 1993) and has influenced the CUE concept in soil
110 ecology (Frey et al., 2001; Herron et al., 2009; Sinsabaugh et al., 2013; Spohn et al., 2016; Thiet
111 et al., 2006). In microbiology, growth efficiencies are, however, frequently measured in pure
112 cultures with (near)optimal conditions for microbial growth. Under such conditions, non-growth



113 anabolism (e.g. EPS, storage compound or osmolyte production) is less important for microbial
114 survival than in harsh soil environments. As such, pure culture studies serve purposes that are
115 often different from investigations in soil ecology (e.g. process advancement in biotechnology
116 with no substrate limitations vs. questions of C stabilization in resource scarce soils).

117 (ii) The absence of non-growth anabolism from CUE calculations has been justified by its
118 presumably low contribution to overall anabolism under aerobic conditions (Manzoni et al.,
119 2018; Paul and Clark, 1989). This assumption can, however, be questioned, because it is based
120 on glucose tracer experiments (Frey et al., 2001; Šantrůčková et al., 2004) in which glucose was
121 applied in quantities well above the range commonly found in soils (i.e. 315-1000 μg glucose-C
122 g^{-1} soil vs. 0.012-216 μg glucose-C g^{-1} soil, respectively; Dijkstra et al., 2015; Frey et al., 2001;
123 Šantrůčková et al., 2004). Previously, high rates of glucose addition have been criticised for
124 distorting insights into microbial metabolism and CUE, because glucose can trigger rapid uptake
125 and intracellular storage and/or favour rapid growth of r-selective microorganisms over more
126 versatile metabolic performance (Blagodatskaya et al., 2014; Dijkstra et al., 2015; Sinsabaugh et
127 al., 2013). Neglecting non-growth anabolism for CUE investigations is thus only justified from
128 experiments favouring growth over non-growth anabolism, investigating CUE under uncommon
129 soil conditions. The criticism of high glucose application rates has led to experiments reflecting
130 more realistic, *in-situ* conditions (e.g. Bölscher et al., 2017; Dijkstra et al., 2015; Jones et al.,
131 2019; Takriti et al., 2018), and promoting the development of methods independent of ^{13}C - or
132 ^{14}C -labelled substrate addition (Blazewicz and Schwartz, 2011; Canarini et al., 2020; Spohn et
133 al., 2016). Yet surprisingly, it did not trigger a critical re-consideration regarding the neglection
134 of non-growth anabolism for CUE.



135 (iii) Although quantifying microbial non-growth anabolism and its metabolites in soils remains
136 challenging, advancements have been made and deserve attention (Banfield et al., 2017; Mason-
137 Jones et al., 2023, 2019; Redmile-Gordon et al., 2015, 2014). In the following, we will
138 demonstrate the need to consider non-growth anabolism for CUE. We will then discuss how
139 CUE investigations can be advanced using readily available methods that quantify at least some
140 products of non-growth anabolism.

141 **3. Advancing the concept and calculation of CUE in soil**

142 Neglecting of non-growth anabolism is not only affecting the concept of CUE (i.e. viewing that
143 non-growth C is ‘inefficiently’ used for synthesis of waste products) but also impacts how CUE
144 in soil is quantified (Fig. 1). Carbon used for non-growth anabolism is virtually absent from
145 common CUE measurements. Approaches either trace (i) ^{13}C or ^{14}C from labelled substrate into
146 microbial biomass and CO_2 -respiration (i.e. substrate-specific CUE; Frey et al., 2001; Geyer et
147 al., 2019; Manzoni et al., 2012) or (ii) ^{18}O from labelled water into microbial DNA. The latter is
148 then used to estimate growth in combination with biomass measurements and complimented with
149 non-isotope-specific measurements of CO_2 -respiration (i.e. substrate-independent CUE;
150 Blazewicz and Schwartz, 2011; Canarini et al., 2019; Geyer et al., 2019; Spohn et al., 2016). In
151 both approaches, the total C metabolized by microorganisms is calculated as the sum of the C
152 used for microbial respiration and growth, with the latter being estimated from DNA and/or
153 biomass measurements (Geyer et al., 2019) (Fig. 1a):

$$154 \quad CUE_{\text{apparent}} = \frac{C_{\text{growth}}}{C_{\text{growth}} + C_{\text{respiration}}} \quad (1)$$

155 Where CUE_{apparent} is the apparent CUE and C_{growth} and $C_{\text{respiration}}$ are C used for growth or
156 respiration, respectively. Here we propose that equation (1) provides only an *apparent* CUE



157 because it does not consider the entire metabolized C by microorganisms as non-growth
158 anabolism is absent from the calculation (Fig. 1a). It illustrates that microbial extracellular
159 metabolites are not quantified for substrate-specific CUE and even all non-growth metabolites
160 (i.e. extra- and intracellular) are not captured when using substrate-independent CUE. For
161 investigating SOC stabilization, we propose that *actual* CUE should be calculated, considering
162 growth and non-growth anabolism (Fig 1b):

$$163 \quad CUE_{actual} = \frac{C_{growth} + C_{non-growth}}{C_{growth} + C_{non-growth} + C_{respiration}} \quad (2)$$

164 Where CUE_{actual} is the actual CUE and $C_{non-growth}$ is C used in non-growth anabolism (Fig. 1b).

165 From equation (2), it becomes clear that not capturing non-growth anabolism could lead to an
166 underestimation of CUE because $C_{non-growth}$ appears in the numerator and denominator of the
167 equation. In the following, we will quantify potential underestimation of CUE when non-growth
168 metabolites are excluded using data from two published studies two examples (i.e. extracellular
169 enzymes, Domeignoz-Horta et al., 2023, and EPS, Olagoke et al., 2022).

170 --- approximate position Figure 1 ---

171 **4. Accounting for microbial non-growth anabolism reveals underestimations of actual** 172 **CUE.**

173 We employed two approaches to quantify potential underestimation of CUE_{actual} when non-
174 growth anabolism is not quantified during CUE measurements (Domeignoz-Horta et al., 2023,
175 Olagoke et al., 2022; section 4.1 and 4.2, respectively). Then, we performed a theoretical
176 exercise assuming various relative allocations of C during metabolism towards non-growth
177 versus growth anabolism (section 4.3). In all cases, CUE_{actual} versus $CUE_{apparent}$ was expressed as
178 a unitless number between 0.00 and 1.00 (i.e. 0-100% efficiency).



179 *4.1. Case study 1: What is the effect of extracellular enzyme production on CUE?*

180 We used data on extracellular enzymes and substrate-independent CUE, measured by
181 Domeignoz-Horta et al. (2023), to compare CUE_{actual} and CUE_{apparent} (for detailed information,
182 see Supplementary Methods): In a first scenario (*enzyme pool maintenance*), we assumed that the
183 microbial community invests C only into non-growth anabolism in order to maintain the existing
184 pool of exoenzymes. In a second scenario (*enzyme pool expansion*), we assume that the
185 microbial community expands the exoenzyme pool by 20%. Similar enzyme pool increases were
186 observed previously following shifts in soil nutrient inputs (Allison and Vitousek, 2005).

187 Depending on the underlying scenario (Fig. 2, Table S1), our analysis demonstrates that
188 exoenzyme production can influence CUE to contrasting degrees. For the first scenario, enzyme
189 pool maintenance, the underestimation of CUE_{actual} is minute, i.e. less than 0.01 differences
190 between CUE_{actual} and CUE_{apparent} (Fig. 2a and b). This result indicates that microbial investments
191 of C into exoenzymes may remain negligible for CUE measurements when merely
192 compensate for enzyme turnover. But, for the enzyme pool expansion scenario, we found
193 substantial underestimation of CUE_{actual} . The assumed 20% increase in the exoenzyme pool
194 caused underestimations of CUE_{actual} between 0.002 and 0.189 (Fig. 2c and d). Here, 40% of the
195 samples would show an underestimation of 0.05 or larger and in 12% of the samples CUE_{actual}
196 would be more than twice as high as CUE_{apparent} . Consequently, even smaller increases than the
197 assumed 20% in the exoenzyme pool (e.g. 5% or 10% increase) could cause considerable
198 underestimation of CUE_{actual} . These findings demonstrate that microorganisms can potentially
199 invest an important fraction of their C budget into the production of enzymes. We therefore
200 consider that microbial C investments into enzymes should not be—a priori—ignored during
201 investigations of CUE.



202 --- approximate position Figure 2 ---

203 The high sensitivity of CUE_{actual} to increased enzyme production calls for a better understanding
204 of enzyme pool dynamics in soils and their effects on CUE. Generally, it can be assumed that the
205 formation and turnover of enzymes, thus the size of the exoenzyme pool, is dynamic in soils
206 (Schimel et al., 2017; Sinsabaugh, 2010; Zuccarini et al., 2023). Both, enzyme production and
207 turnover depend on the environmental conditions, such as organic matter quality, nutrient
208 availability, season, or soil moisture (Allison and Vitousek, 2005; Zuccarini et al., 2023) and
209 change considerably over short time (Allison and Vitousek, 2005). To advance our knowledge
210 whether neglecting exoenzymes affects CUE, we call for measuring exoenzyme pool dynamics
211 over time and in relation to microbial growth. We need to further our understanding of
212 (environmental) conditions when microorganisms (i) merely maintain existing enzyme pools and
213 invest little C into new exoenzyme, and (ii) when they increase their C investment to expand the
214 exoenzyme pools. Especially, it is necessary to take further consideration in relation to growth
215 dynamics, because increased exoenzyme formation is generally followed by accelerated
216 microbial growth (Schimel and Weintraub, 2003). It remains, however, unclear in how far these
217 coupled—yet shifted in time—changes affect the underestimation of CUE_{actual} over time. Yet,
218 due to the time-shift, it can be assumed that CUE_{actual} remains high over longer periods than
219 CUE_{apparent} , because CUE_{apparent} is only affected by accelerated growth while CUE_{actual} increases
220 already with the preceding increase in enzyme formation.

221 In the second scenario, exoenzyme pool expansion, the underestimation of CUE_{actual} was
222 dependent on measured CUE_{apparent} . We found a clear trend that the underestimation of CUE_{actual}
223 decreased with increasing CUE_{apparent} (Fig. 2d). This trend occurs, because the relative
224 distribution of C between growth and non-growth anabolism is of significance for CUE.



225 Assuming a fixed amount of C allocated to enzyme production will have a stronger effect on
226 CUE_{actual} when less C is used for growth compared with a scenario when much C is used for
227 growth. Substrate-independent methods (i.e. ^{18}O tracing techniques) commonly measure
228 CUE_{apparent} for SOM decomposition less than 0.40 (Geyer et al., 2019). As exoenzyme-related
229 underestimations of CUE_{actual} seem to be larger below this value, special considerations should
230 be given to potential effects of exoenzyme pool dynamics on CUE.

231 *4.2. Case study 2: What is the effect of EPS production on CUE?*

232 We used data from a soil microcosms experiment by Olagoke et al. (2022) and compared
233 hypothetical CUE_{apparent} with CUE_{actual} when EPS production was quantified (for detailed
234 information, see Supplementary Methods). For our purpose, we focused on two clay treatments
235 (i.e. +0% and 1+% clay) in combination with three substrate additions (i.e. starch, cellulose and
236 no substrate). Since no CUE was measured in the experiment, we assumed that CUE_{apparent} can
237 range between 0.10 to 0.80 in each sample. We then calculated the respective cumulative
238 respiration and CUE_{actual} based on the measured changes in EPS and microbial biomass C as well
239 as the assumed CUE_{apparent} (see Supplementary Methods). Based on real EPS and microbial
240 biomass C data, this approach provided us with a set of hypothetical CUE_{apparent} and CUE_{actual} for
241 each treatment.

242 Our analysis revealed that the underestimation of CUE_{actual} can be considerable when microbial
243 EPS production is neglected (Fig. 3, Supplementary Table S2 and S3). Depending on the amount
244 of produced EPS relative to microbial biomass, the underestimation of CUE_{actual} can span from
245 virtually 0 (see 0% clay and starch addition treatment, assuming CUE_{apparent} of 0.10; Fig 3b) up
246 to 0.12 (1% clay and cellulose addition treatment, assuming CUE_{apparent} of 0.40 and 0.50; Fig 3b).
247 In the latter case, CUE_{actual} would be 0.52 or 0.62, respectively (Fig. 3a). The implication of these



248 underestimations becomes clearer when we consider the relative differences in the two
249 approaches to calculate CUE. In our case, CUE_{actual} would be 4 to 30% higher than CUE_{apparent} ,
250 which means that up to 30% more C may potentially be stabilized than estimated in the approach
251 neglecting microbial non-growth anabolism.

252 --- approximate position Figure 3 ---

253 Furthermore, in all treatments, underestimations of CUE_{actual} peak between 0.40 to 0.50
254 CUE_{apparent} and decreases towards both ends of the CUE range (i.e. 0.10 to 0.80, Fig. 3b). Thus,
255 CUE_{actual} could be substantially underestimated over the range of usually reported CUEs in soils
256 (Dijkstra et al., 2015; Geyer et al., 2019; Sinsabaugh et al., 2013), the range of CUE usually
257 assumed in soil C models (Parton et al., 1987; Manzoni et al., 2012). Studies using labelled
258 glucose have been criticized for triggering rapid growth and uncommonly high CUE (Geyer et
259 al., 2019; Sinsabaugh et al., 2013). Some soil C models consider therefore lower CUE (e.g. the
260 Q-model assuming CUE of ~ 0.25 ; Ågren and Bosatta, 1987) or substrate-independent methods
261 are adopted (i.e. ^{18}O tracer techniques; Blazewicz and Schwartz, 2011; Canarini et al., 2020;
262 Spohn et al., 2016) resulting in CUEs below 0.40 (Geyer et al., 2019). Yet, even in this lower
263 range, underestimations of CUE_{actual} can remain high and may reach as much as 0.12 (Fig. 3b).
264 Underestimations of CUE_{actual} seem to depend on the metabolized substrate. While we found
265 relatively small underestimations of CUE_{actual} for soil amended with starch (i.e. less than 0.02;
266 Fig. 3b), the underestimations were considerably higher for soils amended with cellulose or
267 unamended soils (i.e. 0.03-0.12 and 0.03-0.08, respectively, respectively; Fig. 3b). Differences
268 were related to the relative production of EPS to biomass (Fig. 3c; Olagoke et al., 2022). Starch
269 is readily available substrate for microbial metabolization, while cellulose breakdown is more
270 complex and control soil were depleted of labile and particulate SOC (Olagoke et al., 2022).



271 Assuming a general bias in substrate-dependent underestimation of CUE_{actual} has two
272 consequences for future CUE investigations:

273 First, underestimations of CUE_{actual} could be less pronounced (i) in the rhizospherewhere roots
274 exudate low-molecular-weight organic compounds, (ii) at locations receiving fresh dissolved
275 organic C via leaching, or (iii) at declining hot-spots of microbial activity with increased cell
276 lysis (Kuzyakov and Blagodatskaya, 2015) Yet, underestimations of CUE_{actual} could be more
277 pronounced if organic C supply for microorganisms is low and/or dominated by complex organic
278 matter. In the latter case, underestimations of CUE_{actual} may not only be caused by C investments
279 into EPS production (analysed here in case study 2), but also by a need to produce exoenzymes
280 (see case study 1, section 4.1).

281 The second consequence for CUE investigations is related to methodology. If the substrate-
282 specific CUE method is applied, addition of readily available substrates (e.g. glucose) could lead
283 to lower underestimations of CUE_{actual} than using more complex substrates (e.g. lignin or phenol;
284 Bahri et al., 2008; Bölscher et al., 2017; Frey et al., 2013) or applying the substrate-independent
285 method (i.e. the ^{18}O -techniques; Blazewicz and Schwartz, 2011; Canarini et al., 2020; Spohn et
286 al., 2016) which measures CUE on native complex SOC.

287 *4.3. Theoretical approach: What is the effect of the relative distribution between non-growth and*
288 *growth anabolism on CUE?*

289 Our aim here was to evaluate the quantity of non-growth metabolites required to cause
290 substantial underestimation of CUE_{actual} . Because quantitative information on non-growth
291 metabolites in soils is limited, we considered a theoretical approach using various relative
292 microbial C allocation ratios between non-growth and growth anabolism (see Supplementary
293 Methods). In general. underestimations of CUE_{actual} increase with increasing amounts of C used



294 for non-growth anabolism relative to growth (Fig. 4; Table S4). They become larger than 0.05
295 when the C used for non-growth anabolism is 50% or more than the C used for growth. The
296 graphs showing the underestimation of CUE_{actual} follow inverse U-shapes peaking around a
297 CUE_{apparent} of 0.35-0.50. At low CUE_{apparent} , most of the total C used is directed towards
298 respiration and $C_{\text{respiration}}$ dominates the CUE calculations (equations (1) and (2)). Adding $C_{\text{non-}}$
299 growth to the calculation has therefore relatively small effect on CUE. At high CUE_{apparent} most of
300 the C is used for growth. As C_{growth} dominates the CUE calculation, adding $C_{\text{non-growth}}$ to the
301 calculation has also a relative small effect. Considering $C_{\text{non-growth}}$ for CUE has the largest effect
302 on CUE values when the distribution of C between respiration, growth and non-growth
303 anabolism is balanced, a range of CUE_{apparent} close to commonly measured in soils and
304 considered in soil C models (Dijkstra et al., 2015; Geyer et al., 2019; Parton et al., 1987;
305 Sinsabaugh et al., 2013). Our theoretical approach demonstrates that non-growth anabolism
306 should not be neglected for CUE in situations when non-growth C is equal or more than 50%
307 growth C and when CUE is expected to be in the medium range of potential CUEs.

308 --- approximate position Figure 4 ---

309 **5. Modelling approach: Potential consequences for SOC projections**

310 To test how sensitive SOC projections are to varying CUEs, we applied a model introduced by
311 Meurer et al. (2020). Here, we introduced step-wise increase in the CUE model parameter
312 covering the underestimations of CUE_{actual} found in the case studies and theoretical approach (see
313 Supplementary Methods).

314 In the model reference scenario (i.e. $CUE = 0.14$, assuming no underestimation of CUE_{actual}),
315 SOC stocks are modelled with 4.21 kg C m^{-2} . Yet, for the largest difference in CUE (i.e.
316 underestimation of CUE_{actual} being 0.23, non-growth anabolic C equal to 150% of growth C, see



317 red solid line in Fig. 4), SOC stocks are modelled with 5.97 kg C m^{-2} , a SOC stock which is 42%
318 larger than the reference scenario (Fig. 5). Also, we found almost as large discrepancies in
319 modelled SOC stocks when the CUEs from the empirical case studies were applied. In the
320 exoenzyme case, differences in $\text{CUE}_{\text{actual}}$ and $\text{CUE}_{\text{apparent}}$ ranged from no differences to 0.19 (Fig.
321 2) and calculated SOC stocks range from 4.21 kg C m^{-2} (i.e. the reference value) to 5.63 kg C m^{-2} .
322 The latter is 34% higher than the reference scenario (Fig. 5). In the EPS case study, $\text{CUE}_{\text{actual}}$
323 was 0.004 to 0.12 units higher than $\text{CUE}_{\text{apparent}}$ (Fig. 3). Modelled SOC stocks range from 4.22 to
324 5.10 kg C m^{-2} . While the former resulted in only a small discrepancy of 0.2% to the reference
325 scenario, the latter is 21% larger than the reference scenario (Fig. 5b).

326 --- approximate position Figure 5 ---

327 Our modelling approach shows how crucial accurate estimations of CUE are for SOC
328 projections, because an underestimation of CUE as little as 0.03 caused 5% higher SOC stocks,
329 and that non-growth anabolism should not be disregarded from CUE measurements.

330 **6. Perspective on microbial CUE in the light of SOC stabilization**

331 *6.1. Non-growth anabolism consumes likely a major part of microbially processed C*

332 Quantifying microbial non-growth anabolism in soils remains challenging because the complex
333 nature of the soil matrix hampers extraction, separation and analysis of non-growth metabolites.
334 Yet, we show here that non-growth anabolites can make up a substantial part of microbially
335 processed C and thus affect microbial CUE. The findings of our case studies (section 4) are
336 supported by other (semi-)quantitative investigations. First of all, non-growth conditions are
337 expected to dominate in soils, where access to available substrate and nutrients is restricted
338 (Hobbie and Hobbie, 2013; Joergensen and Wichern, 2018; Kuzyakov and Blagodatskaya,



339 2015). While the non-growth state of microorganisms remains largely uncharacterized in respect
340 to metabolite production, recent studies suggest that metabolic activity and production can be
341 substantial without microbial growth (Chodkowski and Shade, 2020; Joergensen and Wichern,
342 2018; Lever et al., 2015). Furthermore, it is widely accepted that the vast majority of bacteria and
343 archaea in soils, as well as certain fungi, are surrounded by an EPS matrix (Costerton et al.,
344 1987; De Beeck et al., 2021; Flemming and Wingender, 2010; Flemming and Wuertz, 2019).
345 This matrix consists of polysaccharides, proteins, lipids and nucleic acids which account for 90%
346 of the EPS matrix, while microbial cells account for less than 10% of its dry mass (Flemming
347 and Wingender, 2010). Chenu (1995) estimated that microbial EPS in soil could be
348 quantitatively equal to microbial biomass, representing up to 1.5% of SOC. Microbial cellular
349 storage compounds are another form of non-growth C which is not accounted for by common
350 organic C and DNA extractions. Mason-Jones et al. (2023) demonstrated recently that storage
351 compounds could be of similar quantity as microbial biomass, even under C-limited conditions,
352 counting for up to 19-46% of the extractable microbial biomass C and a biomass increase as
353 large as 2.8 fold accounted for by DNA-based techniques. Other examples of non-growth
354 anabolites are osmolytes, which can account for 10% or more of microbial biomass (Schimel et
355 al., 2007; Warren, 2020), and oxalic acid, which was released by mineral weathering fungi in
356 quantities equal to 1-20% of the fungi's biomass during a 19 hours incubation experiment
357 (Schmalenberger et al., 2015). These examples represent a glimpse of studies to illustrate that
358 soil microbial communities produce a diverse set of non-growth metabolites, potentially in
359 quantities which can be crucial when estimating CUE values. Yet, the quantities of non-growth
360 metabolites produced *in-situ* remain uncertain. Non-growth metabolites should thus not be
361 forgotten, especially as microbial communities likely synthesise various non-growth metabolites



362 simultaneously. Further attention should be given to improving techniques to quantify non-
363 growth metabolites in soil.

364 *6.2. Non-growth metabolites in soils are likely as stable as residues of microbial growth*

365 Microbial non-growth metabolites may not only be quantitatively important for CUE, but may
366 also be a stable soil C pool. It is suggested that residues of microbial growth make up a major
367 part of SOC (Deng and Liang, 2021; Kallenbach et al., 2015; Liang et al., 2017; Miltner et al.,
368 2012). Although less studied, microbial non-growth metabolites in soils may be as stable as
369 microbial growth residues and foster processes that promote C stabilization, such as soil
370 aggregation and formation of mineral-organic associations (Chenu and Stotzky, 2002; Kleber et
371 al., 2015). Non-growth metabolites, like EPS or exoenzymes, are built of proteins,
372 polysaccharides, lipids, and other polymeric substances (Burns et al., 2012; Flemming and
373 Wingender, 2010). These substances have a high affinity to reactive mineral surfaces. They form
374 strong multiple bonds, due to a diverse set of molecular functional groups, via various
375 mechanisms (Kleber et al., 2021, 2015, 2007; Lehmann et al., 2020), promoting the formation of
376 relatively stable forms of mineral-organic associations (Chenu, 1995; Chenu and Stotzky, 2002;
377 Yang et al., 2021). Additionally, it is widely established that EPS contribute to SOC stabilization
378 via soil aggregation (Guhra et al., 2022). These examples demonstrate that non-growth
379 anabolism does not only serves important functions for microbial survival, but likely contributes
380 to stable SOC in similar ways as residues of microbial growth. Recent findings indicate even
381 that products of non-growth anabolism may be more important for forming stable SOC than
382 products of growth (Craig et al., 2022).

383 *6.3. We need to open the 'black box' of microbial physiology in soil*



384 The concept of CUE is ambiguous (Schimel et al., 2022) and treats microbial physiology as a
385 ‘black box’. While its ambiguity has received attention (e.g. Geyer et al., 2019, 2016; Joergensen
386 and Wichern, 2018; Manzoni et al., 2018; Schimel et al., 2022), its ‘black box’ character has
387 been rarely considered (Dijkstra et al., 2022). Here, we argue for a need to open the ‘black box’
388 of CUE, supporting Dijkstra et al. (2022) in their call to disentangle the underlying metabolic
389 processes, including those of non-growth anabolism. Efforts in this direction seem more
390 promising to advance our knowledge than attempts to overcome the CUE ambiguity via
391 additional efficiency definitions (e.g. Cai et al., 2022; Geyer et al., 2016; Manzoni et al., 2018).
392 Strictly speaking, the latter provides primarily additional ‘black boxes’ of varying sizes rather
393 than furthering our process understanding. To advance our understanding of the microbial
394 physiology’s role in SOC stabilization, we need to disentangle the different pathways of
395 microbial anabolism, improve our abilities to quantify the various products of microbial
396 physiology (i.e. endogenous and exogenous) in soil and understand the environmental influence
397 on their dynamics.

398 How do habitat conditions in combination with microbial life-history strategies influence the
399 microbial metabolic C allocation? It can be assumed that most non-growth metabolites (such as
400 EPS, enzymes and osmolytes) show linked dynamic behaviours, following environmental
401 changes within the microbial habitat (Redmile-Gordon et al., 2015; Schimel et al., 2007;
402 Zuccarini et al., 2023). For example, fresh inputs of complex organic C can trigger an increase in
403 production of exoenzymes (Allison and Vitousek, 2005), followed by a delay in microbial
404 growth (Schimel and Weintraub, 2003), which then is followed by an increase in formation of
405 EPS when substrate becomes scarce (Olagoke et al., 2022). Such associated dynamics of
406 metabolite production have consequences for CUE measurements. In our example, CUE_{apparent}



407 would only increase for a short time with enhanced microbial growth, while CUE_{actual} would
408 remain on a high level for an extended period from the start of enzyme production until EPS
409 formation subsides. To advance our understanding of CUE and metabolite dynamics, we need to
410 establish procedures with repeated measurements of CUE and microbial metabolites over short
411 periods, but also over seasonal shifts throughout the year.

412 The research aims laid out here require that we develop new and advance existing techniques for
413 identification of metabolic C fluxes and quantify endo- and exogenous metabolites in soil. To
414 date, advances have been made to quantify, for instance, microbial EPS (Redmile-Gordon et al.,
415 2015, 2014), storage compounds (Banfield et al., 2017; Mason-Jones et al., 2019) and
416 extracellular enzyme C (this study, see Supplementary Methods). ^{13}C Metabolic Flux Analysis
417 offers a way forward to track C fluxes during metabolism. It measures active metabolic pathways
418 via the incorporation of ^{13}C from position-specific labelled substrate into products of
419 biosynthesis (Zamboni et al., 2009) or CO_2 (Dijkstra et al., 2022, 2015, 2011). ^{13}C Metabolic
420 Flux Analysis may offer great potential for opening the black boxes of soil microbial physiology
421 and CUE, especially in combination with improved extraction procedures, metabolomic
422 approaches and other “omics” technologies (e.g. transcriptomics and genomics; Chowdhury et
423 al., 2021; Daniel, 2005).

424 Capturing entire microbial metabolic C fluxes in soil is currently impossible and it will likely
425 remain a major challenge in the near future. Adopting the ‘black box’ of CUE is advantageous
426 e.g. as a simple indicator for large-scale SOC projections (reducing required input data and
427 computing capacity). But, we will need to understand the underlying processes of microbial
428 physiology to judge when current simpler CUE measurements are sufficient (i.e. equation (1)) or
429 when more inclusive complex CUE measurements are required (i.e. equation (2)). In the end, the



430 development around microbial CUE may go in parallel with the development of soil C models,
431 where complex mechanics models provide process understanding while simple kinetic-based
432 models are commonly used for large-scale SOC projections (Noë et al., 2023). While the
433 complex mechanistic soil C models would profit from an ‘open box’ of soil microbial
434 physiology, simple soil C models would profit from improved measurements of actual CUE,
435 keeping this physiological feature as a ‘black box’.

436 **7. Conclusions**

437 Carbon used for non-growth anabolism is commonly disregarded in estimations of microbial
438 CUE. Thus, CUE values represent only ‘apparent’ CUEs. In the light of SOC stabilization, non-
439 growth anabolism is essential and needs to be quantified to capture entire microbial C use and
440 measure ‘actual’ CUE. Here, we argue for an adjustment of microbial CUE measurements. Using
441 two case studies and a theoretical approach, we demonstrated that measurements of apparent
442 CUE can substantially underestimate actual CUE, especially over a CUE range commonly
443 observed in soils. Considering an exoenzyme pool expansion by 20% will result in doubling of
444 CUE values, while considering EPS production increased CUE by up to 30%. A SOC model
445 reacted highly sensitive when we increased the CUE parameter similarly, projecting up to 34%
446 larger SOC stocks after 64 years. Although quantification of non-growth metabolites in soils
447 remains challenging, efforts should be made to further our understanding of their role in the
448 terrestrial C cycle. Microbial communities may invest substantial amount of metabolized C into
449 non-growth metabolites, which are likely as much stabilized in soils as residues of microbial
450 growth. Both metabolite types are exposed to the same C stabilization mechanisms. We call for
451 efforts to open the ‘black box’ of microbial physiology, represented by CUE, to advance our
452 mechanistic understanding of how microbial physiology contributes to stabilized SOC. Recent



453 advances allow us to quantify, to some degree, non-growth metabolites such as EPS,
454 exoenzymes and storage compounds. Efforts in this direction should continue and acknowledge
455 the dynamic, linked nature of the various microbial C pathways and their dependence on
456 conditions in the microbial habitat, an underexplored research area in terrestrial C cycle.

457 **Declaration of competing interest**

458 The authors declare that they have no known competing financial interests or personal
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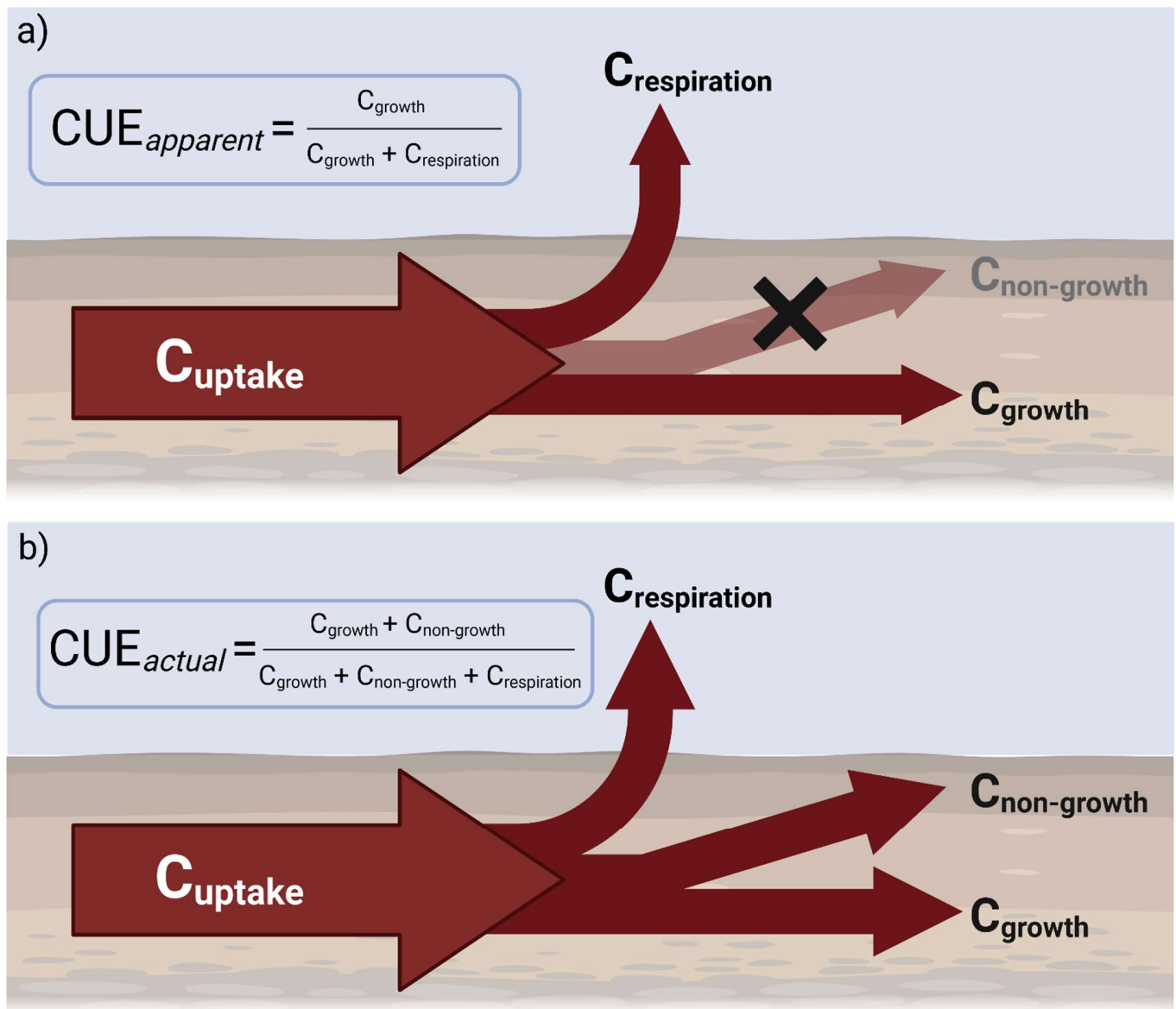


Fig. 1. Schematic illustration of carbon (C) partitioning during microbial metabolism when considering carbon-use efficiency (CUE). **(a)** Current concepts of CUE disregard C used for non-growth anabolism ($C_{non-growth}$). CUE is quantified from C used for growth (C_{growth}) and respiration ($C_{respiration}$) where the entire C uptake (C_{uptake}) is considered as the sum of C_{growth} and $C_{respiration}$ (equation (1)). Current approaches measure therefore an apparent CUE ($CUE_{apparent}$). **(b)** To measure actual CUE (CUE_{actual}), $C_{non-growth}$ should be considered. The latter remains in soil at the time and therefore needs to be included in the numerator and denominator of the CUE equation, if soil C stabilization is of interest (equation (2)).

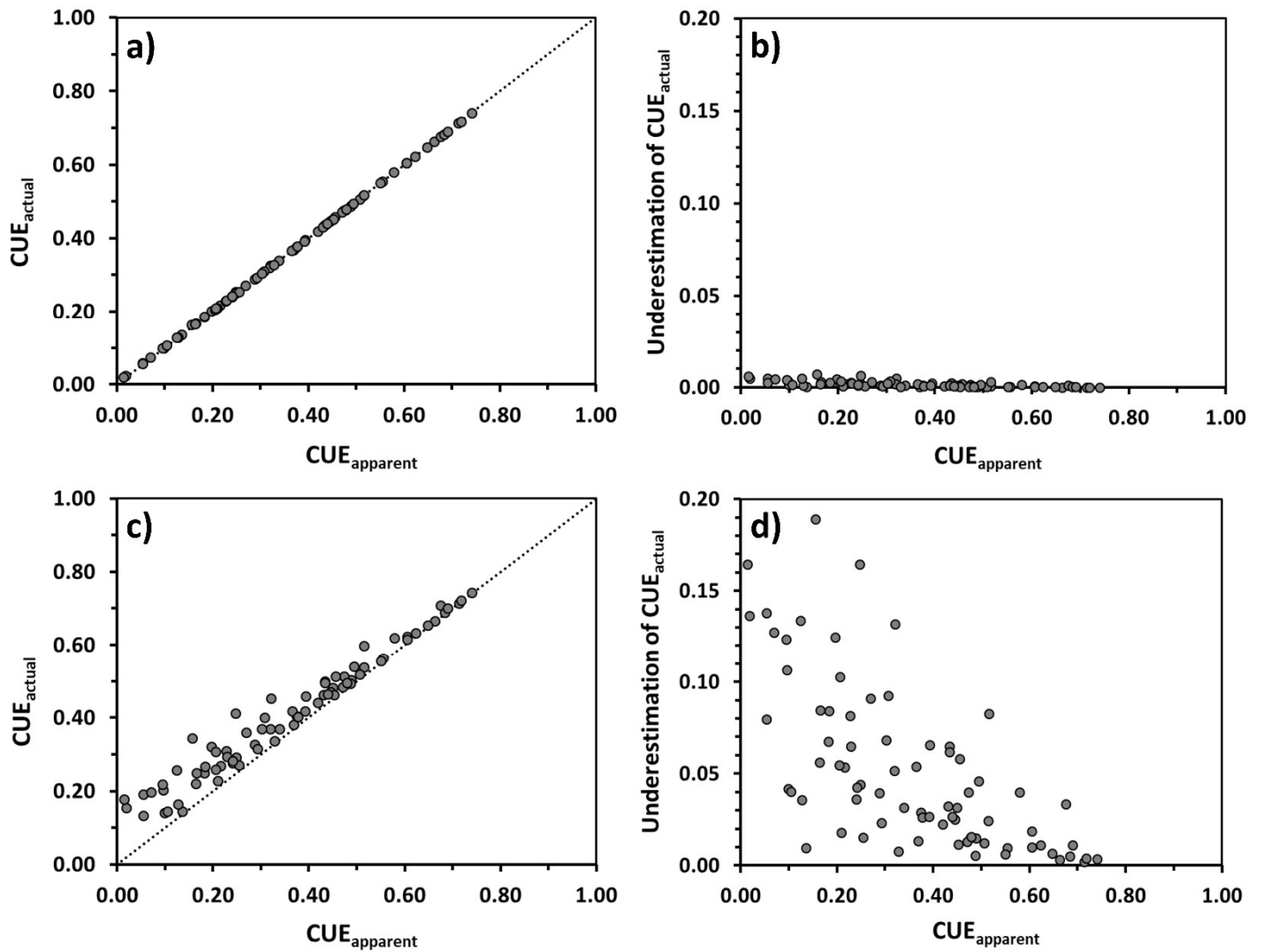


Fig. 2. Actual and apparent carbon-use efficiencies (CUE_{actual} and CUE_{apparent} , respectively) considering extracellular enzyme formation based on data adopted from Domeignoz-Horta et al. (2023). The displayed results consider two scenarios for enzyme formation: First, microbial communities maintaining the existing exoenzyme pool by replacing turned-over exoenzymes (**a**, **b**). Second, an expansion of the exoenzyme pool by 20% (**c**, **d**). Left panels (**a**, **c**) compare CUE_{apparent} (equation (1)) and corresponding CUE_{actual} (equation (2)) for the two scenarios, respectively. The dotted lines indicate the 1:1 ratio of equal CUE_{apparent} and CUE_{actual} . Right panels (**b**, **d**) present the underestimation of CUE_{actual} (i.e. CUE_{actual} minus CUE_{apparent}) plotted as function of assumed CUE_{apparent} for the two scenarios, respectively.

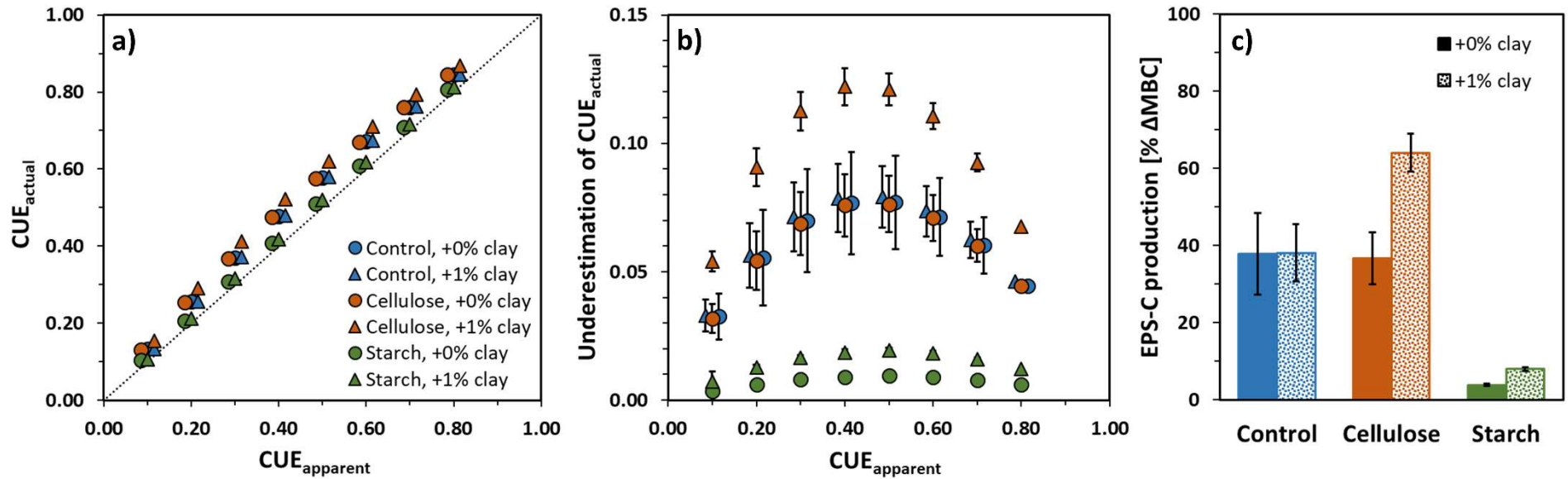


Fig. 3. Actual and apparent carbon-use efficiencies (CUE_{actual} and CUE_{apparent} , respectively) and production of extracellular polymeric substances (EPS) calculated from data of Olagoke et al. (2022). **(a)** Comparison between assumed CUE_{apparent} (equation (1)) and corresponding CUE_{actual} (equation (2)) for soil treated with cellulose, starch or no substrate (i.e. control) in combination with either +0 or +1% clay. The dotted line indicates the 1:1 ratio of equal CUE_{apparent} and CUE_{actual} . **(b)** Underestimation of CUE_{actual} (i.e. CUE_{actual} minus CUE_{apparent}) plotted as a function of assumed CUE_{apparent} . **(c)** Production of EPS carbon (EPS-C) relative to the change in microbial biomass carbon (ΔMBC) after substrate addition. Results are displayed as means and error bars show standard errors ($n = 4$). If no whiskers are visible, standard errors are smaller than the symbol size. Symbols in **(a)** and **(b)** are slightly shifted along the x-axis to improve visibility.

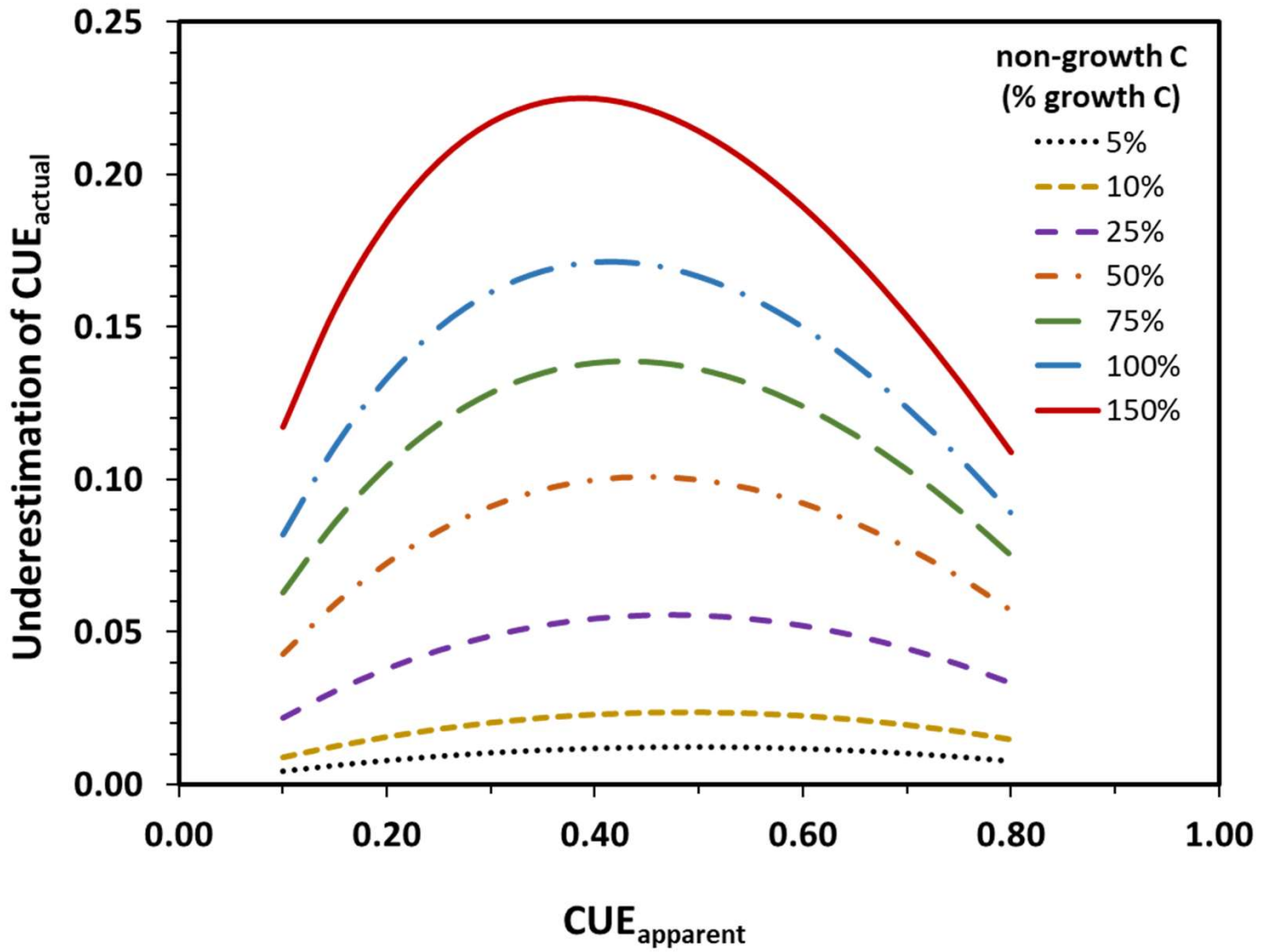
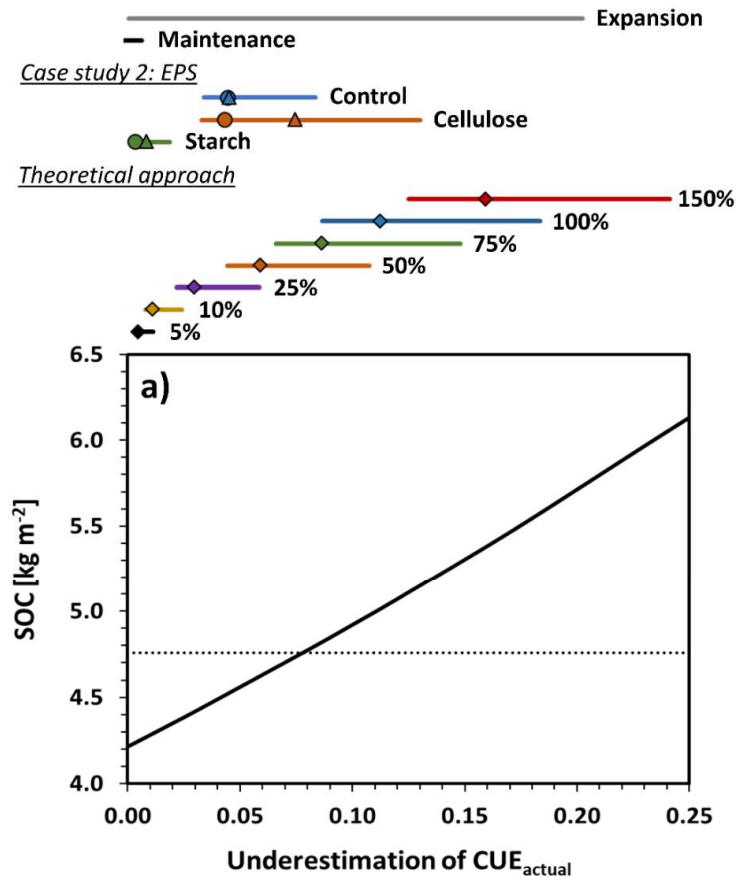


Fig. 4. Underestimation of actual CUE (CUE_{actual}) in relation to microbial carbon (C) allocation between non-growth and growth anabolism. Underestimation of CUE_{actual} (i.e. CUE_{actual} minus $CUE_{apparent}$) is plotted as function of assumed apparent CUE ($CUE_{apparent}$). $CUE_{apparent}$ was assumed to range between 0.10 and 0.80. CUE_{actual} was calculated for seven scenarios with C used for non-growth anabolism relative to C used for growth ranging from 5 to 150% of C used for growth.

Case study 1: Enzymes



Case study 1: Enzymes

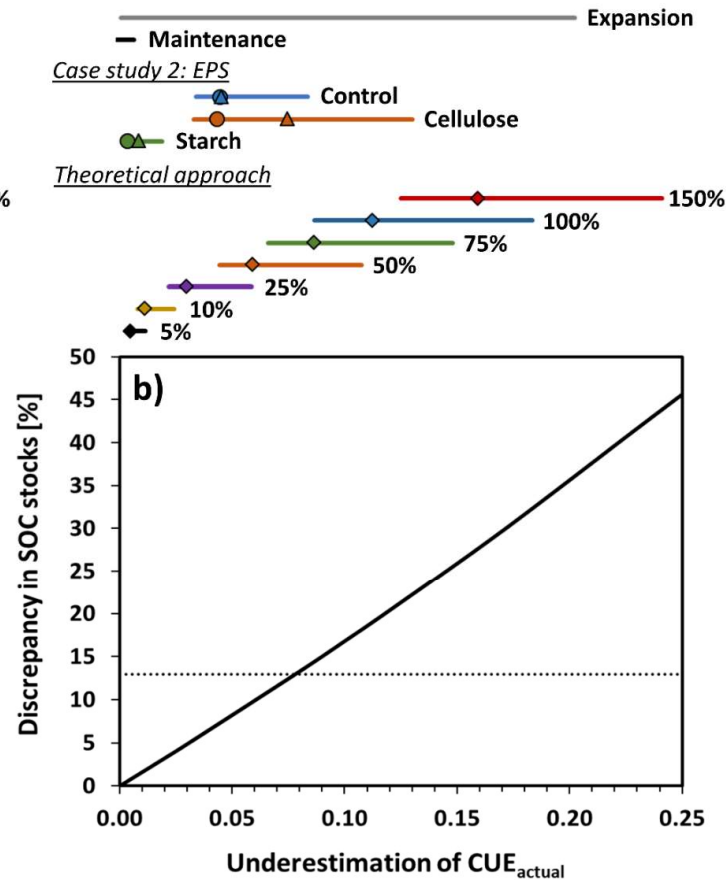


Fig. 5. Results from the modelling approach showing soil organic carbon (SOC) stocks calculated for the Green Manure treatment of the Ultuna Long-Term Soil Organic Matter Experiment (Herrmann and Witter, 2008; Persson and Kirchmann, 1994) in the year 2020. The SOC stocks were modelled assuming CUEs in the range from 0.14 (i.e. the model reference value) to 0.37, a similar range as observed in the two case studies and theoretical approach (section 4). **(a)** On the left, calculated SOC stocks are shown, **(b)** while differences in SOC stocks to the reference (i.e. no underestimation of CUE_{actual}) are shown on the right. Horizontal solid lines above the graphs show the range of the underestimation of CUE_{actual} found in the case studies and theoretical approach. For the case study of extracellular polymeric substances (EPS) and the theoretical approach, markers are placed on the lines where the value is located for an assumed CUE_{apparent} of 0.14 (i.e. the model reference CUE). In the EPS case, round markers are used for the +0% clay treatment and triangular markers for the +1% clay treatment. The dotted horizontal line in the graphs represents **(a)** the SOC stock or **(b)** difference to the reference SOC stock assuming an annual increase by 4 ‰ (Rumpel et al., 2020) until 2020, relative to the initial stocks in 1956.

Supplementary Materials for

Beyond growth: the significance of non-growth anabolism for microbial carbon-use efficiency in the light of soil carbon stabilisation

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This file includes:

Supplementary Methods.

Supplementary Note. Consequences of neglecting non-growth anabolism for investigating microbial growth efficiency.

Fig. S1. Comparison between actual and apparent microbial growth efficiency.

Fig. S2. Maximal formation of total extracellular polymeric substances (EPS) carbon (EPS-C) relative to maximal production of microbial biomass carbon (MBC) over the entire 80 days incubation.

Table S1. Microbial carbon use efficiencies (CUE) calculated from the data by Domeignoz-Horta et al. (2023) (case study 1).

Table S2. Microbial biomass carbon (MBC) and extracellular polymeric substances (EPS) formation (case study 2).

Table S3. Microbial carbon use efficiencies (CUE) calculated from the data by Olagoke et al. (2022) (case study2).

Table S4. Microbial carbon use efficiencies (CUE) calculated based on the relative allocation of carbon (C) between non-growth and growth metabolites (theoretical approach).

Supplementary References.

SUPPLEMENTARY METHODS

Case study 1: What is the effect of extracellular enzyme production on CUE?

Domeignoz-Horta et al. (2023) measured substrate-nonspecific carbon-use efficiency (CUE) and extracellular enzymes in soil samples from two soil warming experiments, running since 13 and 28 years (i.e. Soil Warming and Nitrogen Addition Study and Prospect Hill, respectively), at the Harvard Forest Long-Term Ecological Research sites in Petersham MA USA (42°30'30"N, 72°12'28"W). For a detailed description, we refer to the original study (Domeignoz-Horta et al., 2023). In short, soil was sampled from soil warming (ambient +5 °C) and control plots (ambient; n = 5, both) in July and October 2019. Soil was separated in organic and mineral soil by layer, sieved on site (2 mm) and stored at 15 °C. Activities of beta-glucosidase (cellulose-degrading enzyme) and N-acetylglucosaminidase (chitin- and peptidoglycan-degrading enzyme) were assayed at several temperatures between 4 and 30 °C within four days of sampling (Domeignoz-Horta et al., 2023). Here, we focused our analysis on CUE and enzyme data measured at 20 °C, representing a mid-temperature within the growing season's temperature range (Domeignoz-Horta et al., 2023). To quantify the carbon (C) investments associated to extracellular enzymatic activity, we divided the C allocation into investments associated to *maintenance* and *expansion* of the enzymatic pool. To estimate the C investment related to maintenance of the enzymatic pool, we considered first the enzyme turnover rates to be 0.004 d⁻¹ for beta-glucosidase and 0.0085 d⁻¹ and N-acetylglucosaminidase (Schimel et al., 2017). We then estimated the mean protein sizes to be made of 700 and 500 amino-acids for beta-glucosidase and N-acetylglucosaminidase, respectively, based on the NCBI protein database (NCBI, 2023). Finally, we considered the average C fraction by amino-acid in the proteins for each enzyme. To estimate the C investments related to the expansion of the enzymatic pool, we considered an expansion of the enzymatic pool by 20%. Comparable enzyme pool expansions have been found previously following shifts in soil nutrient inputs (Allison and Vitousek, 2005). Substrate-nonspecific CUE (i.e. CUE_{apparent}, ¹⁸O-water technique) was measured by Domeignoz-Horta et al. (2023) two days after sampling using the method described by Spohn et al. (2016).

We used the CUE values measured by Domeignoz-Horta et al. (2023) as CUE_{apparent} (equation (1)) and calculated CUE_{actual} (equation (2); see section 3 in main paper), assuming that the measured exoenzymes represented C_{non-growth} (i.e. we neglected all other non-growth metabolites here, but acknowledge that they may be quantitatively important).

Case study 2: What is the effect of EPS production on CUE?

Olagoke et al. (2022) investigated EPS production and changes in microbial biomass C (MBC) over time. For a detailed description, we refer to the original study (Olagoke et al., 2022). Briefly, a sandy soil received clay addition using montmorillonite after the removal of labile and particulate organic carbon. For our purpose, we concentrated on the +0% and +1% clay treatments (i.e. 4.4% and 5.4% final clay content). Soil microcosms were incubated at 20 °C over a period of 80 days after receiving either cellulose, starch or no substrate (i.e. rather ‘recalcitrant’, readily available or no C, respectively) to stimulate microbial activity. Microcosms were harvested at several time points during the incubation (including 0 and 3 days) and analysed for EPS-proteins, EPS-polysaccharides (Frølund et al., 1996; Olagoke et al., 2022; Redmile-Gordon et al., 2014) and MBC (Olagoke et al., 2022; Vance et al., 1987; Wu et al., 1990).

Here, we focus on changes in EPS and MBC between days 0 and 3 to calculate CUE_{apparent} and CUE_{actual} (equation (1) and (2), respectively). Focusing on the first three days of incubation reduced the risk of confounding CUE values due to recycling of microbial metabolites (Hagerty et al., 2014). Increases in MBC were considered as growth (i.e. C_{growth}), while increases in EPS were considered as C used non-growth anabolism (i.e. $C_{\text{non-growth}}$). Total EPS production was calculated by adding increases in EPS-proteins and EPS-polysaccharides. In one case (i.e. cellulose, +0% clay) where EPS-polysaccharides decreased, we assumed their production as zero. We derived the C used for respiration (i.e. $C_{\text{respiration}}$) from changes in MBC (i.e. C_{growth}), as cumulative respiration was not measured (Olagoke et al., 2022). Therefore, we used equation (1) and assumed a set of specific CUE_{apparent} values between 0.10 and 0.80. In other words, we solved equation (1), knowing C_{growth} from the MBC change and an assumed CUE value, to calculate $C_{\text{respiration}}$. For example, if C_{growth} was 10 $\mu\text{g C g}^{-1}$ soil and we assumed CUE_{apparent} to be either 0.10, 0.50 or 0.80, we derived $C_{\text{respiration}}$ to be 90, 10 and 2.5 $\mu\text{g C g}^{-1}$ soil, respectively (i.e. the total microbial C uptake—numerator in equation (1)—would be 100, 20, and 12.5 $\mu\text{g C g}^{-1}$ soil, respectively). The highest assumed CUE value of 0.80 represents the approximate theoretical upper maximum of CUE (Hagerty et al., 2014).

Theoretical approach: What is the effect of the relative distribution of C between non-growth and growth anabolism on CUE?

For the theoretical approach, we first set the total amount of C metabolized to 100 units of C. We then calculate CUE_{apparent} (equation (1)), assuming that the 100 units of C are allocated between respiration (i.e. $C_{\text{respiration}}$) and growth (i.e. C_{growth}) and that no C is used for non-growth anabolism. Here, we assumed that CUE_{apparent} varies between 0.10 and 0.80, similar to the approach used in case study 2. Based on the assumed CUE_{apparent} , the 100 units of C would be divided accordingly between $C_{\text{respiration}}$ and C_{growth} . For example, if CUE_{apparent} was set to 0.30 or 0.80, C_{growth} would be 30 or 80 units of C, respectively. Accordingly, $C_{\text{respiration}}$ would be 70 or 20 units of C, respectively. We then assumed that a certain proportion of C is used to synthesize non-growth metabolites (i.e. $C_{\text{non-growth}}$) relative to the amount of C_{growth} . For instance, we would assume that $C_{\text{non-growth}}$ would be equal to 10% of C_{growth} . This means, that $C_{\text{non-growth}}$ would then be 3 or 8 units of C, respectively, in our previous example. Here, the total amount of C remaining in soil would be 33 or 88 units of C (i.e. C_{growth} plus $C_{\text{non-growth}}$) rather than 30 or 80 units of C (i.e. C_{growth} alone). In a last step, we calculated CUE_{actual} (equation (2)). For our example, the values would be 0.32 or 0.81, as the total amount of C metabolized (i.e. denominator of equation (2)) would be 103 or 108 units of C rather than 100 units of C initially assumed, neglecting non-growth anabolism.

We assumed quantities of C used for non-growth anabolism equal to 5 to 150% of C used for growth. These numbers include the range of maximum biomass and EPS formation measured in the experiment by Olagoke et al. (2022) (Fig. S2). We further assumed that in certain situations more C is used for non-growth anabolism than for growth, addressing the different life-history strategies of microorganisms in soil. Especially K-selective (or oligotroph) microorganisms may, under resource scarcity, rather invest C into non-growth-based survival strategies, such as biofilm formation, than using C for growth (Fierer et al., 2007; Flemming et al., 2016). We captured such a scenario by assuming $C_{\text{non-growth}}$ equals 150% of C_{growth} . Recently, it was found that storage compound formation—a commonly unaccounted type of non-growth anabolism—can be as large as almost 300% of C_{growth} (Mason-Jones et al., 2023).

Modelling approach: Potential consequences for SOC projections

To test how sensitive SOC projections are to the differences in CUE found in our case studies, we applied the model introduced by Meurer et al. (2020) and used data from the Green Manure treatment of the Ultuna Frame Trial (Sweden, started in 1956; Herrmann and Witter, 2008; Persson and Kirchmann, 1994). For this treatment, the model fitted best with a CUE of 0.14. In the modelling approach, we step-wise increased the CUE parameter from 0.14 to 0.37 to cover the range of differences between CUE_{actual} and CUE_{apparent} that we found in the case studies and theoretical approach (i.e. underestimations of CUE_{actual} of up to 0.23). The model was used to calculate the changes in SOC and bulk density from 1956 (start of the field trial) to 2020 in the Green Manure treatment. The modelled SOC content and bulk density were then used to calculate SOC stocks to a depth of 20 cm (i.e. the sampling depth during yearly field campaigns at the site; Persson and Kirchmann, 1994) for the year 2020.

SUPPLEMENTARY NOTE

Consequences of neglecting non-growth anabolism for investigating microbial growth efficiency

Microbial growth efficiency (MGE) is often used synonymously for carbon use efficiency (CUE; Schimel et al., 2022; Sinsabaugh et al., 2013). Here, we call for a distinction of MGE and CUE in the light of soil carbon (C) stabilization. By distinguishing, CUE would assign all microbially metabolized C remaining in soil as efficiently used (i.e. all C used for biosynthesis), while MGE would only assign C used for growth as efficiently used. In both cases, neglecting non-growth anabolism during quantification of efficiency can lead to incorrect values of efficiency. The entire microbial C use is not captured, because non-growth is ignored. Measured efficiencies are thus *apparent* rather than *actual* efficiencies. While the neglect of non-growth anabolism causes underestimations of actual CUE (see main article), it leads to overestimations of actual MGE.

Commonly, MGE is calculated as (del Giorgio and Cole, 1998):

$$MGE_{apparent} = \frac{C_{growth}}{C_{growth} + C_{respiration}} \quad (S1)$$

Where $MGE_{apparent}$ is the *apparent* MGE and C_{growth} and $C_{respiration}$ are C used for growth and respired, respectively. To fully capture all metabolized C, C used for biosynthesis of non-growth anabolites needs to be added to the numerator of equation (S1). This provides us with the *actual* MGE (MGE_{actual}):

$$MGE_{actual} = \frac{C_{growth}}{C_{growth} + C_{non-growth} + C_{respiration}} \quad (S2)$$

Where, $C_{non-growth}$ is the C used for non-growth anabolism.

Comparing equations (S1) and (S2) demonstrates that $MGE_{apparent}$ tends to overestimate the actual MGE. The degree of overestimation will depend on the amount of C used for non-growth biosynthesis (i.e. $C_{non-growth}$) relative to the amount of C used for growth (i.e. C_{growth}). In Fig. S1, we plotted the difference between MGE_{actual} and $MGE_{apparent}$, depending on $MGE_{apparent}$ as well as the relative amount of C used for non-growth anabolism (similar to our approach used for CUE; see section 4.3 of main article and Supplementary Methods). The results show that overestimations

of MGE increase with higher MGE and relatively larger amounts of C used for non-growth anabolism (Fig. S1). The further demonstrate that overestimations of MGE can be quite substantial, especially when large amounts of non-growth metabolites are produced by soil microorganisms. In situations where microorganisms receive large quantities of readily decomposable substrate – such as glucose, using substrate-specific $^{13/14}\text{C}$ tracer techniques –, microorganisms are assumed to produce very little non-growth metabolites (Blagodatskaya et al., 2014; Dijkstra et al., 2015). Here, overestimates of MGE remain relatively small (≤ 0.03 for non-growth C equalling 5 % of growth-C) even at high MGE. But, when larger amounts of non-growth metabolites are produced, MGE can be considerably overestimated even in the range of MGE commonly measured using substrate-nonspecific approaches (i.e. $\text{MGE}_{\text{apparent}} < 0.40$, using ^{18}O tracer techniques; Blazewicz and Schwartz, 2011; Canarini et al., 2020; Geyer et al., 2019; Spohn et al., 2016).

SUPPLEMENTARY FIGURES

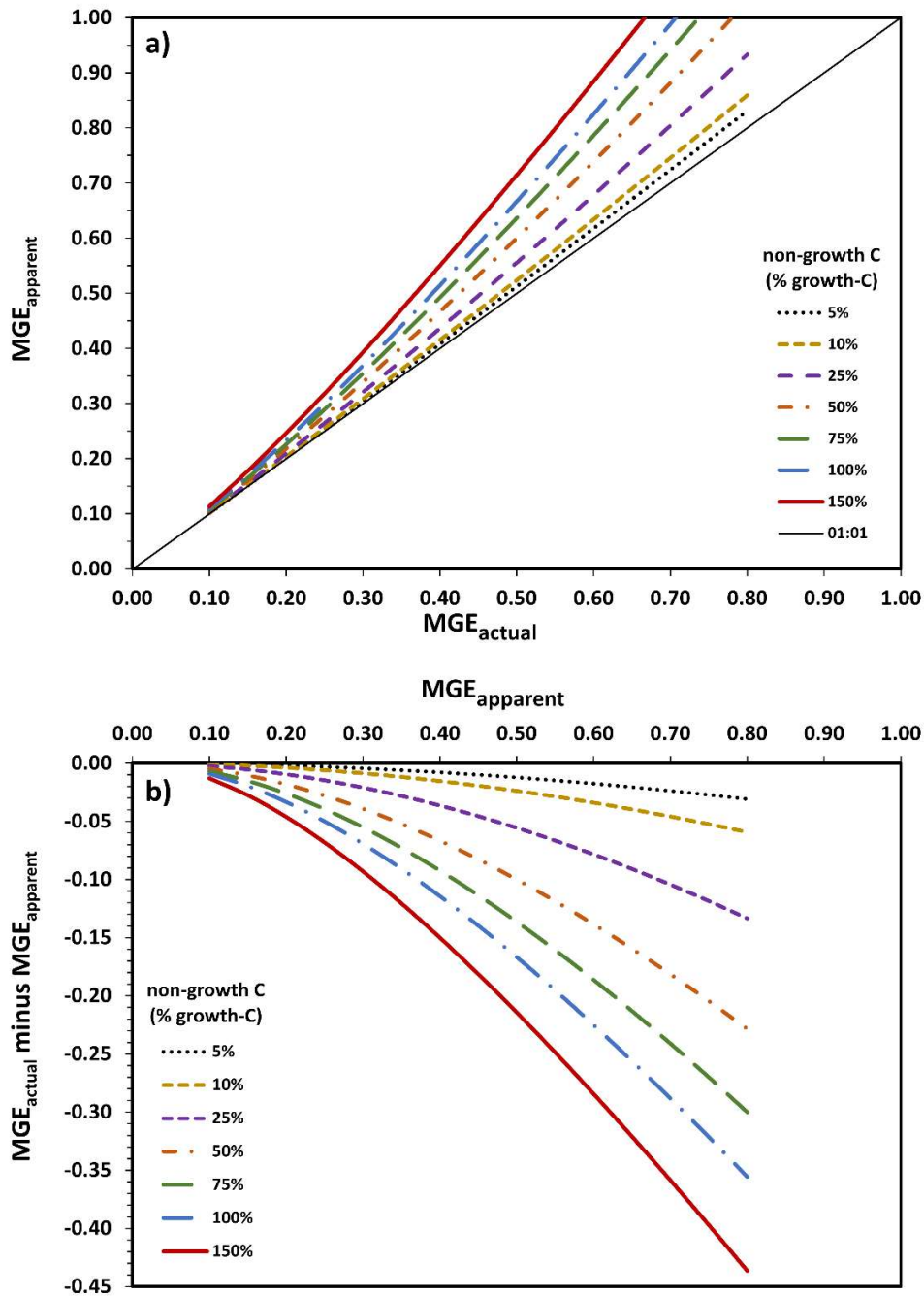


Fig. S1. Comparison between actual and apparent microbial growth efficiency (MGE_{actual} and $MGE_{apparent}$, respectively) depending on the amount carbon (C) used for non-growth to growth anabolism. (a) Direct comparison between MGE_{actual} and $MGE_{apparent}$ demonstrating the overestimation of MGE_{actual} . The solid black line indicates the 1:1 ratio where MGE_{actual} equals $MGE_{apparent}$. (b) Differences between MGE_{actual} and $MGE_{apparent}$ as function of assumed $MGE_{apparent}$.

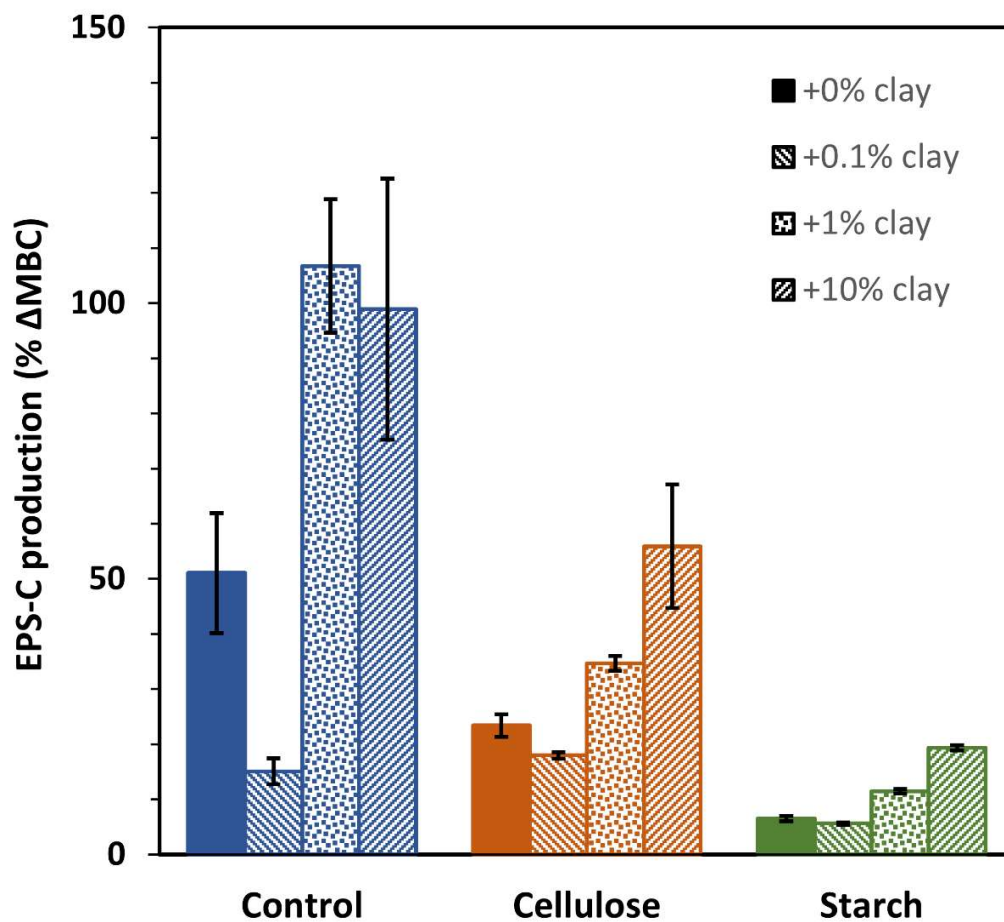


Fig. S2. Maximal production of extracellular polymeric substances (EPS) carbon (EPS-C) relative to maximal production of microbial biomass carbon (MBC) over the entire 80 days incubation. EPS-C and MBC were measured in soil amended with cellulose or starch or controls soils receiving no substrate. All soils were amended with +0, +0.1, +1 or +10% clay. Calculations based on data from Olagoke et al. (2022). See Supplementary Methods for details.

SUPPLEMENTARY TABLES

Table S1. Microbial carbon use efficiencies (CUE) calculated from the data by Domeignoz-Horta et al. (2023). Apparent CUE (CUE_{apparent}) were measured using the ^{18}O -DNA technique and calculated from respiration and growth. Carbon (C) used for maintaining and expanding the enzyme pool was calculated as described in the Supplementary Methods. Actual CUE (CUE_{actual}) were calculated using equation (2) for the enzyme pool maintenance and enzyme pool expansion scenario, respectively (see section 3 and 4.1 of main article and Supplementary Methods). ID = sample ID, PH = Prospect Hill, SWaN = Soil Warming and Nitrogen Addition Study.

ID	Site	Soil warming	Horizon	Time point	Respiration ($\mu\text{g C g}^{-1}$ soil)	Growth ($\mu\text{g C g}^{-1}$ soil)	Enzyme pool maintenance ($\mu\text{g C g}^{-1}$ soil)	Enzyme pool expansion ($\mu\text{g C g}^{-1}$ soil)	CUE_{apparent}	CUE_{actual} (maintenance)	CUE_{actual} (pool expansion)
1	PH	control	Mineral	July	0.230	0.109	0.002	0.081	0.320	0.325	0.452
27	PH	control	Mineral	July	0.554	0.259	0.002	0.066	0.319	0.321	0.370
29	PH	control	Mineral	July	0.378	0.120	0.001	0.025	0.240	0.242	0.277
33	PH	control	Mineral	July	0.237	0.078	0.003	0.088	0.247	0.254	0.412
40	PH	control	Mineral	July	0.324	0.019	0.002	0.058	0.054	0.059	0.192
41	PH	control	Mineral	October	0.351	0.205	0.000	0.012	0.369	0.369	0.382
43	PH	control	Mineral	October	0.439	1.092	0.000	0.011	0.713	0.713	0.715
48	PH	control	Mineral	October	0.561	0.536	0.001	0.033	0.489	0.489	0.504
55	PH	control	Mineral	October	0.464	0.913	0.000	0.014	0.663	0.663	0.666
79	PH	control	Mineral	October	0.542	0.391	0.001	0.037	0.419	0.420	0.441
15	SWaN	control	Mineral	July	0.304	0.075	0.002	0.070	0.197	0.201	0.322
18	SWaN	control	Mineral	July	0.414	0.372	0.002	0.064	0.473	0.475	0.513
25	SWaN	control	Mineral	July	0.274	0.570	0.003	0.097	0.675	0.676	0.709
45	SWaN	control	Mineral	October	0.383	0.476	0.001	0.019	0.554	0.555	0.564
54	SWaN	control	Mineral	October	0.706	2.013	0.001	0.036	0.740	0.741	0.744
69	SWaN	control	Mineral	October	0.199	0.088	0.001	0.044	0.307	0.311	0.400
74	SWaN	control	Mineral	October	0.598	0.731	0.001	0.019	0.550	0.550	0.556
76	SWaN	control	Mineral	October	0.235	0.249	0.001	0.026	0.515	0.515	0.539
7	PH	control	Organic	July	1.906	0.630	0.005	0.157	0.248	0.250	0.292
14	PH	control	Organic	July	1.607	0.030	0.008	0.264	0.018	0.023	0.155
24	PH	control	Organic	July	3.197	0.712	0.011	0.351	0.182	0.184	0.250
31	PH	control	Organic	July	2.598	0.582	0.011	0.365	0.183	0.186	0.267

ID	Site	Soil warming	Horizon	Time point	Respiration ($\mu\text{g C g}^{-1}$ soil)	Growth ($\mu\text{g C g}^{-1}$ soil)	Enzyme pool maintenance ($\mu\text{g C g}^{-1}$ soil)	Enzyme pool expansion ($\mu\text{g C g}^{-1}$ soil)	CUE _{apparent}	CUE _{actual} (maintenance)	CUE _{actual} (pool expansion)
36	PH	control	Organic	July	2.369	0.956	0.006	0.195	0.287	0.289	0.327
46	PH	control	Organic	October	3.600	1.487	0.006	0.174	0.292	0.293	0.316
59	PH	control	Organic	October	5.147	0.805	0.002	0.067	0.135	0.136	0.145
61	PH	control	Organic	October	2.231	0.238	0.010	0.330	0.096	0.100	0.203
67	PH	control	Organic	October	3.460	1.498	0.016	0.538	0.302	0.304	0.370
68	PH	control	Organic	October	2.485	2.362	0.002	0.053	0.487	0.487	0.493
2	SWaN	control	Organic	July	2.721	1.631	0.007	0.211	0.375	0.376	0.404
3	SWaN	control	Organic	July	1.706	0.469	0.005	0.159	0.215	0.217	0.269
8	SWaN	control	Organic	July	1.053	0.880	0.007	0.230	0.455	0.457	0.513
22	SWaN	control	Organic	July	2.816	0.725	0.008	0.261	0.205	0.207	0.259
23	SWaN	control	Organic	July	3.116	4.779	0.012	0.396	0.605	0.606	0.624
52	SWaN	control	Organic	October	2.465	2.528	0.004	0.127	0.506	0.507	0.519
62	SWaN	control	Organic	October	1.228	3.133	0.002	0.063	0.718	0.718	0.722
63	SWaN	control	Organic	October	2.876	1.651	0.013	0.421	0.365	0.366	0.419
66	SWaN	control	Organic	October	1.584	0.541	0.002	0.045	0.255	0.255	0.270
72	SWaN	control	Organic	October	1.843	3.977	0.003	0.094	0.683	0.684	0.688
5	PH	heated	Mineral	July	0.254	0.248	0.002	0.050	0.494	0.496	0.540
11	PH	heated	Mineral	July	0.311	0.092	0.001	0.048	0.227	0.230	0.309
17	PH	heated	Mineral	July	0.293	0.402	0.002	0.073	0.579	0.580	0.619
28	PH	heated	Mineral	July	0.269	0.175	0.002	0.054	0.393	0.396	0.459
39	PH	heated	Mineral	July	0.168	0.002	0.001	0.034	0.014	0.020	0.179
42	PH	heated	Mineral	October	0.618	0.164	0.001	0.018	0.210	0.210	0.228
57	PH	heated	Mineral	October	0.383	0.350	0.001	0.022	0.477	0.478	0.493
71	PH	heated	Mineral	October	0.494	0.403	0.002	0.055	0.449	0.450	0.481
78	PH	heated	Mineral	October	0.360	0.218	0.001	0.025	0.377	0.378	0.403
4	SWaN	heated	Mineral	July	0.288	0.221	0.002	0.066	0.434	0.436	0.499
19	SWaN	heated	Mineral	July	0.285	0.218	0.002	0.062	0.433	0.436	0.495
21	SWaN	heated	Mineral	July	0.296	0.659	0.001	0.036	0.690	0.690	0.701
34	SWaN	heated	Mineral	July	0.237	0.044	0.002	0.081	0.156	0.163	0.345

ID	Site	Soil warming	Horizon	Time point	Respiration ($\mu\text{g C g}^{-1}$ soil)	Growth ($\mu\text{g C g}^{-1}$ soil)	Enzyme pool maintenance ($\mu\text{g C g}^{-1}$ soil)	Enzyme pool expansion ($\mu\text{g C g}^{-1}$ soil)	CUE _{apparent}	CUE _{actual} (maintenance)	CUE _{actual} (pool expansion)
38	SWaN	heated	Mineral	July	0.408	0.327	0.001	0.035	0.445	0.446	0.470
47	SWaN	heated	Mineral	October	0.521	0.267	0.001	0.040	0.339	0.340	0.370
53	SWaN	heated	Mineral	October	0.566	0.062	0.001	0.030	0.099	0.100	0.140
65	SWaN	heated	Mineral	October	0.320	0.491	0.001	0.021	0.605	0.605	0.615
70	SWaN	heated	Mineral	October	0.299	0.088	0.001	0.036	0.228	0.230	0.293
75	SWaN	heated	Mineral	October	0.482	0.236	0.000	0.009	0.328	0.328	0.336
6	PH	heated	Organic	July	2.279	0.333	0.004	0.112	0.128	0.129	0.163
10	PH	heated	Organic	July	1.340	0.140	0.007	0.234	0.095	0.099	0.218
13	PH	heated	Organic	July	1.790	0.464	0.010	0.334	0.206	0.209	0.308
26	PH	heated	Organic	July	1.844	0.216	0.003	0.097	0.105	0.106	0.145
37	PH	heated	Organic	July	1.450	0.083	0.004	0.141	0.054	0.057	0.134
44	PH	heated	Organic	October	1.750	1.126	0.004	0.131	0.392	0.392	0.418
51	PH	heated	Organic	October	1.683	1.388	0.002	0.066	0.452	0.452	0.463
58	PH	heated	Organic	October	1.522	0.483	0.003	0.119	0.241	0.242	0.283
60	PH	heated	Organic	October	1.113	2.047	0.002	0.061	0.648	0.648	0.654
73	PH	heated	Organic	October	2.743	2.435	0.004	0.129	0.470	0.471	0.483
9	SWaN	heated	Organic	July	1.421	0.524	0.008	0.276	0.269	0.272	0.360
12	SWaN	heated	Organic	July	1.139	0.225	0.005	0.153	0.165	0.168	0.249
30	SWaN	heated	Organic	July	1.097	0.082	0.006	0.187	0.070	0.074	0.197
32	SWaN	heated	Organic	July	1.528	1.155	0.005	0.161	0.431	0.432	0.463
35	SWaN	heated	Organic	July	1.525	0.217	0.009	0.314	0.125	0.129	0.258
49	SWaN	heated	Organic	October	1.925	1.774	0.003	0.116	0.480	0.480	0.495
56	SWaN	heated	Organic	October	1.672	1.308	0.004	0.148	0.439	0.440	0.465
64	SWaN	heated	Organic	October	1.347	2.221	0.003	0.110	0.623	0.623	0.634
77	SWaN	heated	Organic	October	0.642	0.683	0.008	0.272	0.515	0.518	0.598
80	SWaN	heated	Organic	October	5.544	1.087	0.014	0.478	0.164	0.166	0.220

Table S2. Microbial biomass carbon (MBC) and extracellular polymeric substances (EPS) formation over the first three days of the incubation experiment by Olagoke et al. (2022). ΔMBC is change microbial biomass carbon. $\Delta EPS-C_{total}$, $\Delta EPS-C_{polysacc.}$ and $\Delta EPS-C_{proteins}$ is change in total, polysaccharide and protein EPS carbon, respectively. $\Delta EPS-C_{total}$ is expressed as concentration per gram soil (second column) as well as relative to change in MBC (last column). E and MBC were measured in soil amended with cellulose or starch or controls soils receiving no substrate. All soils were amended with +0 or +1% clay. Values are means ($n = 4$) \pm one standard error.

Treatment	ΔMBC ($\mu\text{g g}^{-1}$ soil)	$\Delta EPS-C_{total}$ ($\mu\text{g g}^{-1}$ soil)	$\Delta EPS-C_{polysacc.}$ ($\mu\text{g g}^{-1}$ soil)	$\Delta EPS-C_{proteins}$ ($\mu\text{g g}^{-1}$ soil)	$\Delta EPS-C_{total}$ (% ΔMBC)
Control, +0% clay	24.3 \pm 3.6	8.3 \pm 1.8	0.0 \pm 0.0	8.3 \pm 1.8	38 \pm 11
Control, +1% clay	39.2 \pm 2.2	14.7 \pm 2.0	0.3 \pm 0.3	14.4 \pm 2.2	38 \pm 7
Cellulose, +0% clay	71.5 \pm 3.1	26.0 \pm 3.9	5.7 \pm 3.3	20.2 \pm 1.4	37 \pm 7
Cellulose, +1% clay	75.7 \pm 6.0	47.5 \pm 1.2	24.5 \pm 2.0	23.0 \pm 1.6	64 \pm 5
Starch, +0% clay	864.2 \pm 15.4	33.3 \pm 1.5	14.8 \pm 0.8	18.5 \pm 0.9	4 \pm 0
Starch, +1% clay	758.4 \pm 15.7	60.4 \pm 2.7	28.3 \pm 0.7	32.4 \pm 2.5	8 \pm 1

Table S3. Microbial carbon use efficiencies (CUE) calculated from the data by Olagoke et al. (2022). Apparent CUEs ($CUE_{apparent}$) are assumed and based on equation (1). Actual CUE (CUE_{actual}) as calculated using equation (2) (see section 3 of main article and Supplementary Methods). Values are means ($n = 4$) \pm one standard error.

Treatment		$CUE_{apparent}$	0.10	0.20	0.30	0.40	0.50	0.60	0.70	0.80
Control, +0% clay	CUE_{actual}		0.13 \pm 0.01	0.26 \pm 0.02	0.37 \pm 0.02	0.48 \pm 0.02	0.58 \pm 0.02	0.67 \pm 0.02	0.76 \pm 0.02	0.84 \pm 0.01
	CUE_{actual} minus $CUE_{apparent}$		0.03 \pm 0.01	0.06 \pm 0.02	0.07 \pm 0.02	0.08 \pm 0.02	0.08 \pm 0.02	0.07 \pm 0.02	0.06 \pm 0.02	0.04 \pm 0.01
Control, +1% clay	CUE_{actual}		0.13 \pm 0.01	0.26 \pm 0.01	0.37 \pm 0.01	0.48 \pm 0.01	0.58 \pm 0.01	0.67 \pm 0.01	0.76 \pm 0.01	0.85 \pm 0.01
	CUE_{actual} minus $CUE_{apparent}$		0.03 \pm 0.01	0.06 \pm 0.01	0.07 \pm 0.01	0.08 \pm 0.01	0.08 \pm 0.01	0.07 \pm 0.01	0.06 \pm 0.01	0.05 \pm 0.01
Cellulose, +0% clay	CUE_{actual}		0.13 \pm 0.01	0.25 \pm 0.01	0.37 \pm 0.01	0.48 \pm 0.01	0.58 \pm 0.01	0.67 \pm 0.01	0.76 \pm 0.01	0.84 \pm 0.01
	CUE_{actual} minus $CUE_{apparent}$		0.03 \pm 0.01	0.05 \pm 0.01	0.07 \pm 0.01	0.08 \pm 0.01	0.08 \pm 0.01	0.07 \pm 0.01	0.06 \pm 0.01	0.04 \pm 0.01
Cellulose, +1% clay	CUE_{actual}		0.15 \pm 0.00	0.29 \pm 0.01	0.41 \pm 0.01	0.52 \pm 0.01	0.62 \pm 0.01	0.71 \pm 0.01	0.79 \pm 0.00	0.87 \pm 0.00
	CUE_{actual} minus $CUE_{apparent}$		0.05 \pm 0.00	0.09 \pm 0.01	0.11 \pm 0.01	0.12 \pm 0.01	0.12 \pm 0.01	0.11 \pm 0.01	0.09 \pm 0.00	0.07 \pm 0.00
Starch, +0% clay	CUE_{actual}		0.10 \pm 0.00	0.21 \pm 0.00	0.31 \pm 0.00	0.41 \pm 0.00	0.51 \pm 0.00	0.61 \pm 0.00	0.71 \pm 0.00	0.81 \pm 0.00
	CUE_{actual} minus $CUE_{apparent}$		0.00 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00
Starch, +1% clay	CUE_{actual}		0.11 \pm 0.00	0.21 \pm 0.00	0.32 \pm 0.00	0.42 \pm 0.00	0.52 \pm 0.00	0.62 \pm 0.00	0.72 \pm 0.00	0.81 \pm 0.00
	CUE_{actual} minus $CUE_{apparent}$		0.01 \pm 0.00	0.01 \pm 0.00	0.02 \pm 0.00	0.02 \pm 0.00	0.02 \pm 0.00	0.02 \pm 0.00	0.02 \pm 0.00	0.01 \pm 0.00

Table S4. Microbial carbon use efficiencies (CUE) calculated in the theoretical approach based on the relative allocation of carbon (C) between non-growth and growth anabolism. Apparent CUEs ($CUE_{apparent}$) are assumed and represent equation (2). Actual CUE (CUE_{actual}) as calculated using equation (2) (see section 3 of main article and Supplementary Methods).

Non-growth C									
(% growth C)	$CUE_{apparent}$	0.10	0.20	0.30	0.40	0.50	0.60	0.70	0.80
5%	CUE_{actual}	0.104	0.208	0.310	0.412	0.512	0.612	0.710	0.808
	CUE_{actual} minus $CUE_{apparent}$	0.004	0.008	0.010	0.012	0.012	0.012	0.010	0.008
10%	CUE_{actual}	0.109	0.216	0.320	0.423	0.524	0.623	0.720	0.815
	CUE_{actual} minus $CUE_{apparent}$	0.009	0.016	0.020	0.023	0.024	0.023	0.020	0.015
25%	CUE_{actual}	0.122	0.238	0.349	0.455	0.556	0.652	0.745	0.833
	CUE_{actual} minus $CUE_{apparent}$	0.022	0.038	0.049	0.055	0.056	0.052	0.045	0.033
50%	CUE_{actual}	0.143	0.273	0.391	0.500	0.600	0.692	0.778	0.857
	CUE_{actual} minus $CUE_{apparent}$	0.043	0.073	0.091	0.100	0.100	0.092	0.078	0.057
75%	CUE_{actual}	0.163	0.304	0.429	0.538	0.636	0.724	0.803	0.875
	CUE_{actual} minus $CUE_{apparent}$	0.063	0.104	0.129	0.138	0.136	0.124	0.103	0.075
100%	CUE_{actual}	0.182	0.333	0.462	0.571	0.667	0.750	0.824	0.889
	CUE_{actual} minus $CUE_{apparent}$	0.082	0.133	0.162	0.171	0.167	0.150	0.124	0.089
150%	CUE_{actual}	0.217	0.385	0.517	0.625	0.714	0.789	0.854	0.909
	CUE_{actual} minus $CUE_{apparent}$	0.117	0.185	0.217	0.225	0.214	0.189	0.154	0.109

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