



Beyond Growth: The Significance of Non-Growth Anabolism for Microbial Carbon-Use Efficiency in the Light of Soil Carbon Stabilisation

Tobias Bölscher, Cordula Vogel, Folasade Olagoke, Katharina H.E. Meurer, Anke Herrmann, Tino Colombi, Melanie Brunn, Luiz Domeignoz-Horta

► To cite this version:

Tobias Bölscher, Cordula Vogel, Folasade Olagoke, Katharina H.E. Meurer, Anke Herrmann, et al.. Beyond Growth: The Significance of Non-Growth Anabolism for Microbial Carbon-Use Efficiency in the Light of Soil Carbon Stabilisation. 2023. <hal-04257607>

HAL Id: hal-04257607

<https://hal.science/hal-04257607v1>

Preprint submitted on 25 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC-ND 4.0 - Attribution - Non-commercial use - No Derivative Works - International License



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

Tobias Bölscher ^{a,*}, Cordula Vogel ^b, Folasade K. Olagoke ^b, Katharina H.E. Meurer ^c, Anke M. Herrmann ^c, Tino Colombi ^c, Melanie Brunn ^{d,e}, Luiz A. Domeignoz-Horta ^{a,f}

ORCID: Tobias Bölscher: 0000-0001-5305-0616; Melanie Brunn: 0000-0002-5692-8575; Tino Colombi 0000-0001-8493-4430; Luiz A. Domeignoz-Horta: 0000-0003-4618-6253; Anke M. Herrmann: 0000-0002-6273-1234; Cordula Vogel: 0000-0002-6525-2634; Katharina H.E. Meurer: 0000-0002-8880-9650

^a Université Paris-Saclay, INRAE, AgroParisTech, UMR EcoSys, 91120 Palaiseau, France

^b Soil Resources and Land Use, Institute of Soil Science and Site Ecology, TU Dresden, Dresden, Germany

^c Department of Soil and Environment, SLU Swedish University of Agricultural Sciences, Box 7014, 75007, Uppsala, Sweden

^d Institute for Integrated Natural Sciences, University of Koblenz, Koblenz, Germany

^e Institute for Environmental Sciences, University of Kaiserslautern-Landau (RPTU), Landau, Germany

^f Department of Evolutionary Biology & Environmental Sciences, University of Zurich, 8057 Zurich, Switzerland

* Corresponding author: E-mail: tobias.bolscher@inrae.fr



Keywords: microbial growth efficiency, substrate-use efficiency, microbial physiology, non-growth metabolism, microbial exudates, soil carbon models

Abstract

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



1. Introduction

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

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



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

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



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

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

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

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



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

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



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

3. Advancing the concept and calculation of CUE in soil

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

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

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



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

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

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

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

--- approximate position Figure 1 ---

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

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



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

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

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



--- approximate position Figure 2 ---

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

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



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

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

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

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



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

--- approximate position Figure 3 ---

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



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

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

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

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

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



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

--- approximate position Figure 4 ---

5. Modelling approach: Potential consequences for SOC projections

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

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



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

--- approximate position Figure 5 ---

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

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

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

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



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



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

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

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

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



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

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



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

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

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



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

7. Conclusions

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



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

Declaration of competing interest

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

Acknowledgments

This work was supported by Agence National de la Recherche (ANR-20-IDEES-0002), the Deutsche Forschungsgemeinschaft (DFG, Bonn, Germany under the grant VO 2111/4-1), the Swedish Research Council for Sustainable Development (Formas 2019-01189) and the EU EJP SOIL project *EnergyLink* (“Linking crop diversification to microbial energy allocation and organic carbon storage in soils”) within the European Union Horizon 2020 research and innovation programme (Grant Agreement No. 862695, EJP SOIL). Figure 1 was created with BioRender.com.

References

- Ågren, G.I., Bosatta, E., 1987. Theoretical analysis of the long-term dynamics of carbon and nitrogen in soils. *Ecology* 68, 1181–1189. doi:10.2307/1939202
- Allison, S.D., Vitousek, P.M., 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biology and Biochemistry* 37, 937–944. doi:10.1016/j.soilbio.2004.09.014
- Allison, S.D., Wallenstein, M.D., Bradford, M.A., 2010. Soil-carbon response to warming dependent on microbial physiology. *Nature Geoscience* 3, 336–340. doi:10.1038/ngeo846



- 476 Bahri, H., Rasse, D.P.P., Rumpel, C., Dignac, M.-F.F., Bardoux, G., Mariotti, A., 2008. Lignin
477 degradation during a laboratory incubation followed by ^{13}C isotope analysis. *Soil Biology*
478 *and Biochemistry* 40, 1916–1922. doi:10.1016/j.soilbio.2008.04.002
- 479 Banfield, C.C., Dippold, M.A., Pausch, J., Hoang, D.T.T., Kuzyakov, Y., 2017. Biopore history
480 determines the microbial community composition in subsoil hotspots. *Biology and Fertility*
481 *of Soils* 53, 573–588. doi:10.1007/s00374-017-1201-5
- 482 Blagodatskaya, E., Blagodatsky, S., Anderson, T.-H., Kuzyakov, Y., 2014. Microbial growth and
483 carbon use efficiency in the rhizosphere and root-free soil. *PloS One* 9, e93282.
484 doi:10.1371/journal.pone.0093282
- 485 Blazewicz, S.J., Schwartz, E., 2011. Dynamics of ^{18}O Incorporation from H_2^{18}O into Soil
486 Microbial DNA. *Microbial Ecology* 61, 911–916. doi:10.1007/s00248-011-9826-7
- 487 Bölscher, T., Ågren, G.I., Herrmann, A.M., 2020. Land-use alters the temperature response of
488 microbial carbon-use efficiency in soils – a consumption-based approach. *Soil Biology and*
489 *Biochemistry* 140, 107639. doi:10.1016/j.soilbio.2019.107639
- 490 Bölscher, T., Paterson, E., Freitag, T., Thornton, B., Herrmann, A.M., 2017. Temperature
491 sensitivity of substrate-use efficiency can result from altered microbial physiology without
492 change to community composition. *Soil Biology and Biochemistry* 109, 59–69.
493 doi:10.1016/j.soilbio.2017.02.005
- 494 Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein,
495 M.D., Weintraub, M.N., Zoppini, A., 2012. Soil enzymes in a changing environment:
496 Current knowledge and future directions. *Soil Biology and Biochemistry* 58, 216–234.
497 doi:10.1016/j.soilbio.2012.11.009
- 498 Cai, Y., Ma, T., Wang, Y., Jia, J., Jia, Y., Liang, C., Feng, X., 2022. Assessing the accumulation
499 efficiency of various microbial carbon components in soils of different minerals. *Geoderma*
500 407, 115562. doi:10.1016/j.geoderma.2021.115562
- 501 Canarini, A., Kaiser, C., Merchant, A., Richter, A., Wanek, W., 2019. Root exudation of primary
502 metabolites: Mechanisms and their roles in plant responses to environmental stimuli.
503 *Frontiers in Plant Science* 10, 157. doi:10.3389/fpls.2019.00157
- 504 Canarini, A., Wanek, W., Watzka, M., Sandén, T., Spiegel, H., Šantrůček, J., Schnecker, J.,



2020. Quantifying microbial growth and carbon use efficiency in dry soil environments via ^{18}O water vapor equilibration. *Global Change Biology* 26, 5333–5341.

doi:10.1111/gcb.15168

Chenu, C., 1995. Extracellular Polysaccharides: An Interface Between Microorganisms and Soil Constituents, in: Huang, P.M., Berthelin, J., Bollag, J.-M., McGill, W.B., Page, A.L. (Eds.), *Environmental Impact of Soil Component Interactions: Land Quality, Natural and Anthropogenic Organics*, Volume I. CRC Press, Boca Raton, London, Tokyo, pp. 217–233.

Chenu, C., Stotzky, G., 2002. Interactions between Microorganisms and Soil Particles: An Overview, in: Huang, P.M., Bollag, J.-M., Senes, N. (Eds.), *Interactions between Soil Particles and Microorganisms: Impact on the Terrestrial Ecosystem*. John Wiley and Sons Ltd, New York, NY, USA, pp. 3–40.

Chodkowski, J.L., Shade, A., 2020. Exometabolite Dynamics over Stationary Phase Reveal Strain-Specific Responses. *MSystems* 5:e00493-20. doi:10.1128/msystems.00493-20

Chowdhury, P.R., Golas, S.M., Alteio, L.V., Stevens, J.T.E., Billings, A.F., Blanchard, J.L., Melillo, J.M., DeAngelis, K.M., 2021. The Transcriptional Response of Soil Bacteria to Long-Term Warming and Short-Term Seasonal Fluctuations in a Terrestrial Forest. *Frontiers in Microbiology* 12, 666558. doi:10.3389/fmicb.2021.666558

Costerton, J.W., Cheng, K.-J., Geesey, G.G., Ladd, T.I., Nickel, J.C., Dasgupta, M., Marrie, T.J., 1987. Bacterial biofilms in nature and disease. *Annual Review of Microbiology* 41, 435–464.

Craig, M.E., Geyer, K.M., Beidler, K. V., Brzostek, E.R., Frey, S.D., Grandy, A.S., Liang, C., Phillips, R.P., Ridge, O., College, Y.H., Harris, Y., Stuart Grandy, A., Liang, C., Phillips, R.P., 2022. Fast-decaying plant litter enhances soil carbon in temperate forests but not through microbial physiological traits. *Nature Communications* 13, 1–10. doi:10.1038/s41467-022-28715-9

Daniel, R., 2005. The metagenomics of soil. *Nature Reviews Microbiology* 3, 470–478. doi:10.1038/nrmicro1160

De Beeck, M.O., Persson, P., Tunlid, A., 2021. Fungal extracellular polymeric substance matrices – highly specialized microenvironments that allow fungi to control soil organic



- 534 matter decomposition reactions. *Soil Biology and Biochemistry* 159, 108304.
535 doi:10.1016/j.soilbio.2021.108304
- 536 Deng, F., Liang, C., 2021. Revisiting the quantitative contribution of microbial necromass to soil
537 carbon pool: Stoichiometric control by microbes and soil. *Soil Biology and Biochemistry*
538 165, 108486. doi:10.1016/j.soilbio.2021.108486
- 539 Dijkstra, P., Dalder, J.J., Selmants, P.C., Hart, S.C., Koch, G.W., Schwartz, E., Hungate, B.A.,
540 2011. Modeling soil metabolic processes using isotopologue pairs of position-specific ^{13}C -
541 labeled glucose and pyruvate. *Soil Biology and Biochemistry* 43, 1848–1857.
542 doi:10.1016/j.soilbio.2011.05.001
- 543 Dijkstra, P., Martinez, A., Thomas, S.C., Seymour, C.O., Wu, W., Dippold, M.A., Megonigal,
544 J.P., Schwartz, E., Hungate, B.A., 2022. On maintenance and metabolisms in soil microbial
545 communities. *Plant and Soil* 476, 385–396. doi:10.1007/s11104-022-05382-9
- 546 Dijkstra, P., Salpas, E., Fairbanks, D., Miller, E.B., Hagerty, S.B., van Groenigen, K.J., Hungate,
547 B.A., Marks, J.C., Koch, G.W., Schwartz, E., 2015. High carbon use efficiency in soil
548 microbial communities is related to balanced growth, not storage compound synthesis. *Soil*
549 *Biology and Biochemistry* 89, 35–43. doi:10.1016/j.soilbio.2015.06.021
- 550 Domeignoz-Horta, L.A., Pold, G., Erb, H., Sebag, D., Verrecchia, E., Northen, T., Louie, K.,
551 Eloë-Fadrosch, E., Pennacchio, C., Knorr, M.A., Frey, S.D., Melillo, J.M., DeAngelis, K.M.,
552 2023. Substrate availability and not thermal acclimation controls microbial temperature
553 sensitivity response to long-term warming. *Global Change Biology* 29, 1574–1590.
554 doi:10.1111/gcb.16544
- 555 Domeignoz-Horta, L.A., Shinfuku, M., Junier, P., Poirier, S., Verrecchia, E., Sebag, D.,
556 DeAngelis, K.M., 2021. Direct evidence for the role of microbial community composition
557 in the formation of soil organic matter composition and persistence. *ISME Communications*
558 1, 5–8. doi:10.1038/s43705-021-00071-7
- 559 Flemming, H.C., Wingender, J., 2010. The biofilm matrix. *Nature Reviews Microbiology* 8,
560 623–633. doi:10.1038/nrmicro2415
- 561 Flemming, H.C., Wuertz, S., 2019. Bacteria and archaea on Earth and their abundance in
562 biofilms. *Nature Reviews Microbiology* 17, 247–260. doi:10.1038/s41579-019-0158-9



- 563 Frey, S.D., Gupta, V.V.S.R., Elliott, E.T., Paustian, K., 2001. Protozoan grazing affects estimates
564 of carbon utilization efficiency of the soil microbial community. *Soil Biology and*
565 *Biochemistry* 33, 1759–1768. doi:10.1016/S0038-0717(01)00101-8
- 566 Frey, S.D., Lee, J., Melillo, J.M., Six, J., 2013. The temperature response of soil microbial
567 efficiency and its feedback to climate. *Nature Climate Change* 3, 395–398.
568 doi:10.1038/nclimate1796
- 569 Geyer, K.M., Dijkstra, P., Sinsabaugh, R., Frey, S.D., 2019. Clarifying the interpretation of
570 carbon use efficiency in soil through methods comparison. *Soil Biology and Biochemistry*
571 128, 79–88. doi:10.1016/j.soilbio.2018.09.036
- 572 Geyer, K.M., Kyker-Snowman, E., Grandy, A.S., Frey, S.D., 2016. Microbial carbon use
573 efficiency: accounting for population, community, and ecosystem-scale controls over the
574 fate of metabolized organic matter. *Biogeochemistry* 127, 173–188. doi:10.1007/s10533-
575 016-0191-y
- 576 Gommers, P.J.F., van Schie, B.J., van Dijken, J.P., Kuenen, J.G., 1988. Biochemical limits to
577 microbial growth yields: An analysis of mixed substrate utilization. *Biotechnology and*
578 *Bioengineering* 32, 86–94. doi:10.1002/bit.260320112
- 579 Guhra, T., Stolze, K., Totsche, K.U., 2022. Pathways of biogenically excreted organic matter
580 into soil aggregates. *Soil Biology and Biochemistry* 164, 108483.
581 doi:10.1016/j.soilbio.2021.108483
- 582 Herron, P.M., Stark, J.M., Holt, C., Hooker, T., Cardon, Z.G., 2009. Microbial growth
583 efficiencies across a soil moisture gradient assessed using ^{13}C -acetic acid vapor and ^{15}N -
584 ammonia gas. *Soil Biology and Biochemistry* 41, 1262–1269.
585 doi:10.1016/j.soilbio.2009.03.010
- 586 Hobbie, J.E., Hobbie, E.A., 2013. Microbes in nature are limited by carbon and energy: The
587 starving-survival lifestyle in soil and consequences for estimating microbial rates. *Frontiers*
588 *in Microbiology* 4, 1–11. doi:10.3389/fmicb.2013.00324
- 589 Hyvönen, R., Ågren, G.I., Bosatta, E., 1998. Predicting Long-Term Soil Carbon Storage from
590 Short-Term Information. *Soil Science Society of America Journal* 62, 1000–1005.
591 doi:10.2136/sssaj1998.03615995006200040022x



- 592 Joergensen, R.G., Wichern, F., 2018. Alive and kicking: Why dormant soil microorganisms
593 matter. *Soil Biology and Biochemistry* 116, 419–430. doi:10.1016/j.soilbio.2017.10.022
- 594 Jones, D.L., Cooledge, E.C., Hoyle, F.C., Griffiths, R.I., Murphy, D. V, 2019. pH and
595 exchangeable aluminum are major regulators of microbial energy flow and carbon use
596 efficiency in soil microbial communities. *Soil Biology and Biochemistry* 138, 107584.
597 doi:10.1016/j.soilbio.2019.107584
- 598 Kallenbach, C.M., Frey, S.D., Grandy, A.S., 2016. Direct evidence for microbial-derived soil
599 organic matter formation and its ecophysiological controls. *Nature Communications* 7,
600 13630. doi:10.1038/ncomms13630
- 601 Kallenbach, C.M., Grandy, A.S., Frey, S.D., Diefendorf, A.F., 2015. Microbial physiology and
602 necromass regulate agricultural soil carbon accumulation. *Soil Biology and Biochemistry*
603 91, 279–290. doi:10.1016/j.soilbio.2015.09.005
- 604 Kleber, M., Bourg, I.C., Coward, E.K., Hansel, C.M., Myneni, S.C.B., Nunan, N., 2021.
605 Dynamic interactions at the mineral–organic matter interface. *Nature Reviews Earth &*
606 *Environment* 2, 402–421. doi:10.1038/s43017-021-00162-y
- 607 Kleber, M., Eusterhues, K., Keiluweit, M., Mikutta, C., Mikutta, R., Nico, P.S., 2015. Mineral-
608 Organic Associations: Formation, Properties, and Relevance in Soil Environments,
609 *Advances in Agronomy* 130, 1-140. doi:10.1016/bs.agron.2014.10.005
- 610 Kleber, M., Sollins, P., Sutton, R., 2007. A conceptual model of organo-mineral interactions in
611 soils: Self-assembly of organic molecular fragments into zonal structures on mineral
612 surfaces. *Biogeochemistry* 85, 9–24. doi:10.1007/s10533-007-9103-5
- 613 Kuzyakov, Y., Blagodatskaya, E., 2015. Microbial hotspots and hot moments in soil: Concept &
614 review. *Soil Biology and Biochemistry* 83, 184–199. doi:10.1016/j.soilbio.2015.01.025
- 615 Lehmann, J., Hansel, C.M., Kaiser, C., Kleber, M., Maher, K., Manzoni, S., Nunan, N.,
616 Reichstein, M., Schimel, J.P., Torn, M.S., Wieder, W.R., Kögel-Knabner, I., 2020.
617 Persistence of soil organic carbon caused by functional complexity. *Nature Geoscience* 13,
618 529-534. doi:10.1038/s41561-020-0612-3
- 619 Lever, M.A., Rogers, K.L., Lloyd, K.G., Overmann, J., Schink, B., Thauer, R.K., Hoehler, T.M.,
620 Jørgensen, B.B., 2015. Life under extreme energy limitation: A synthesis of laboratory- and



- 621 field-based investigations. *FEMS Microbiology Reviews* 39, 688–728.
622 doi:10.1093/femsre/fuv020
- 623 Liang, C., Amelung, W., Lehmann, J., Kästner, M., 2019. Quantitative assessment of microbial
624 necromass contribution to soil organic matter. *Global Change Biology* 25, 3578–3590.
625 doi:10.1111/gcb.14781
- 626 Liang, C., Kästner, M., Joergensen, R.G., 2020. Microbial necromass on the rise: The growing
627 focus on its role in soil organic matter development. *Soil Biology and Biochemistry* 150,
628 108000. doi:10.1016/j.soilbio.2020.108000
- 629 Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control
630 over soil carbon storage. *Nature Microbiology* 2, 17105 (2017).
631 doi:10.1038/nmicrobiol.2017.105
- 632 Linton, J.D., 1991. Metabolite production and growth efficiency. *Antonie van Leeuwenhoek* 60,
633 293–311. doi:10.1007/BF00430371
- 634 Manzoni, S., Capek, P., Porada, P., Thurner, M., Winterdahl, M., Beer, C., Brüchert, V., Frouz,
635 J., Herrmann, A.M., Lindahl, B.D., Lyon, S.W., Šantrucková, H., Vico, G., Way, D., 2018.
636 Reviews and syntheses : Carbon use efficiency from organisms to ecosystems – definitions,
637 theories, and empirical evidence. *Biogeosciences* 15, 5929–5949. doi:doi.org/10.5194/bg-
638 15-5929-2018
- 639 Manzoni, S., Taylor, P., Richter, A., Porporato, A., Ågren, G.I., 2012. Environmental and
640 stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytologist* 196,
641 79–91. doi:10.1111/j.1469-8137.2012.04225.x
- 642 Mason-Jones, K., Ban, C.C., Breidenbach, A., Dyckmans, J., Dippold, M.A., 2023. Intracellular
643 carbon storage by microorganisms is an overlooked pathway of biomass growth. *Nature*
644 *Communications* 14, 2240. doi:10.1038/s41467-023-37713-4
- 645 Mason-Jones, K., Banfield, C.C., Dippold, M.A., 2019. Compound-specific ¹³C stable isotope
646 probing confirms synthesis of polyhydroxybutyrate by soil bacteria. *Rapid Communications*
647 *in Mass Spectrometry* 33, 795–802. doi:10.1002/rcm.8407
- 648 Mason-Jones, K., Robinson, S.L., Veen, G.F. (Ciska., Manzoni, S., van der Putten, W.H., 2022.
649 Microbial storage and its implications for soil ecology. *ISME Journal* 16, 617–629.



- doi:10.1038/s41396-021-01110-w
- Meurer, K.H.E., Chenu, C., Coucheney, E., Herrmann, A.M., Keller, T., Kätterer, T., Svensson, D.N., Jarvis, N., 2020. Modelling dynamic interactions between soil structure and the storage and turnover of soil organic matter. *Biogeosciences* 17, 5025–5042. doi:10.5194/bg-17-5025-2020
- Miltner, A., Bombach, P., Schmidt-Brücken, B., Kästner, M., 2012. SOM genesis: Microbial biomass as a significant source. *Biogeochemistry* 111, 41–55. doi:10.1007/s10533-011-9658-z
- Noë, J. Le, Manzoni, S., Abramoff, R., Bölscher, T., Bruni, E., Cardinael, R., Ciais, P., Chenu, C., Clivot, H., Derrien, D., Ferchaud, F., Garnier, P., Goll, D., Lashermes, G., Martin, M., Rasse, D., Schimel, J., Wieder, W., Abiven, S., Barré, P., Cécillon, L., Guenet, B., 2023. Soil organic carbon models need independent time-series validation for reliable prediction. *Communications Earth & Environment* 4, 1–8. doi:10.1038/s43247-023-00830-5
- Olagoke, F.K., Bettermann, A., Nguyen, P.T.B., Redmile-Gordon, M., Babin, D., Smalla, K., Nesme, J., Sørensen, S.J., Kalbitz, K., Vogel, C., 2022. Importance of substrate quality and clay content on microbial extracellular polymeric substances production and aggregate stability in soils. *Biology and Fertility of Soils* 58, 435–457. doi:10.1007/s00374-022-01632-1
- Parton, W.J., Schimel, D.S., Cole, C. V., Ojima, D.S., 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. *Soil Science Society of America Journal* 51, 1173–1179.
- Paul, E.A., Clark, F.E., 1989. *Soil Microbiology and Biochemistry*. Academic Press, San Diego. doi:10.1016/C2009-0-02814-1
- Redmile-Gordon, M.A., Brookes, P.C., Evershed, R.P., Goulding, K.W.T., Hirsch, P.R., 2014. Measuring the soil-microbial interface: Extraction of extracellular polymeric substances (EPS) from soil biofilms. *Soil Biology and Biochemistry* 72, 163–171. doi:10.1016/j.soilbio.2014.01.025
- Redmile-Gordon, M.A., Evershed, R.P., Hirsch, P.R., White, R.P., Goulding, K.W.T., 2015. Soil organic matter and the extracellular microbial matrix show contrasting responses to C and N



- 679 availability. *Soil Biology and Biochemistry* 88, 257–267. doi:10.1016/j.soilbio.2015.05.025
- 680 Rumpel, C., Amiraslani, F., Chenu, C., Garcia Cardenas, M., Kaonga, M., Koutika, L.S., Ladha,
681 J., Madari, B., Shirato, Y., Smith, P., Soudi, B., Soussana, J.F., Whitehead, D., Wollenberg,
682 E., 2020. The 4p1000 initiative: Opportunities, limitations and challenges for implementing
683 soil organic carbon sequestration as a sustainable development strategy. *Ambio* 49, 350–
684 360. doi:10.1007/s13280-019-01165-2
- 685 Šantrůčková, H., Pícek, T., Tykva, R., Šimek, M., Bohumil, P., 2004. Short-term partitioning of
686 ¹⁴C-[U]-glucose in the soil microbial pool under varied aeration status. *Biology and*
687 *Fertility of Soils* 40, 386–392. doi:10.1007/s00374-004-0790-y
- 688 Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial Stress-Response Physiology and Its
689 Implications for Ecosystem Function. *Ecology* 88, 1386–1394. doi:10.1890/06-0219
- 690 Schimel, J., Becerra, C.A., Blankinship, J., 2017. Estimating decay dynamics for enzyme
691 activities in soils from different ecosystems. *Soil Biology and Biochemistry* 114, 5–11.
692 doi:10.1016/j.soilbio.2017.06.023
- 693 Schimel, J., Weintraub, M.N., Moorhead, D., 2022. Estimating microbial carbon use efficiency
694 in soil: Isotope-based and enzyme-based methods measure fundamentally different aspects
695 of microbial resource use. *Soil Biology and Biochemistry* 169, 108677.
696 doi:10.1016/j.soilbio.2022.108677
- 697 Schimel, J.P., 2013. Soil carbon: Microbes and global carbon. *Nature Climate Change* 3, 867–
698 868. doi:10.1038/nclimate2015
- 699 Schimel, J.P., Weintraub, M.N., 2003. The implications of exoenzyme activity on microbial
700 carbon and nitrogen limitation in soil: a theoretical model. *Soil Biology and Biochemistry*
701 35, 549–563. doi:10.1016/S0038-0717(03)00015-4
- 702 Schmalenberger, A., Duran, A.L., Bray, A.W., Bridge, J., Bonneville, S., Benning, L.G.,
703 Romero-Gonzalez, M.E., Leake, J.R., Banwart, S.A., 2015. Oxalate secretion by
704 ectomycorrhizal *Paxillus involutus* is mineral-specific and controls calcium weathering
705 from minerals. *Scientific Reports* 5, 1–14. doi:10.1038/srep12187
- 706 Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil*
707 *Biology and Biochemistry* 42, 391–404. doi:10.1016/j.soilbio.2009.10.014



- 708 Sinsabaugh, R.L., Manzoni, S., Moorhead, D.L., Richter, A., 2013. Carbon use efficiency of
709 microbial communities: stoichiometry, methodology and modelling. *Ecology Letters* 16,
710 930–939. doi:10.1111/ele.12113
- 711 Spohn, M., Klaus, K., Wanek, W., Richter, A., 2016. Microbial carbon use efficiency and
712 biomass turnover times depending on soil depth – Implications for carbon cycling. *Soil*
713 *Biology and Biochemistry* 96, 74–81. doi:10.1016/j.soilbio.2016.01.016
- 714 Steinweg, J.M., Plante, A.F., Conant, R.T., Paul, E.A., Tanaka, D.L., 2008. Patterns of substrate
715 utilization during long-term incubations at different temperatures. *Soil Biology and*
716 *Biochemistry* 40, 2722–2728. doi:10.1016/j.soilbio.2008.07.002
- 717 Takriti, M., Wild, B., Schnecker, J., Mooshammer, M., Knoltsch, A., Lashchinskiy, N., Eloy
718 Alves, R.J., Gentsch, N., Gittel, A., Mikutta, R., Wanek, W., Richter, A., 2018. Soil organic
719 matter quality exerts a stronger control than stoichiometry on microbial substrate use
720 efficiency along a latitudinal transect. *Soil Biology and Biochemistry* 121, 212–220.
721 doi:10.1016/j.soilbio.2018.02.022
- 722 Thiet, R.K., Frey, S.D., Six, J., 2006. Do growth yield efficiencies differ between soil microbial
723 communities differing in fungal:bacterial ratios? Reality check and methodological issues.
724 *Soil Biology and Biochemistry* 38, 837–844. doi:10.1016/j.soilbio.2005.07.010
- 725 Van Bodegom, P., 2007. Microbial maintenance: a critical review on its quantification.
726 *Microbial Ecology* 53, 513–23. doi:10.1007/s00248-006-9049-5
- 727 von Stockar, U., Marison, I.W., 1993. The definition of energetic growth efficiencies for aerobic
728 and anaerobic microbial growth and their determination by calorimetry and by other means.
729 *Thermochimica Acta* 229, 157–172.
- 730 Warren, C.R., 2020. Pools and fluxes of osmolytes in moist soil and dry soil that has been re-
731 wet. *Soil Biology and Biochemistry* 150, 108012. doi:10.1016/j.soilbio.2020.108012
- 732 Yang, J.Q., Zhang, X., Bourg, I.C., Stone, H.A., 2021. 4D imaging reveals mechanisms of clay-
733 carbon protection and release. *Nature Communications* 12, 622. doi:10.1038/s41467-020-
734 20798-6
- 735 Zamboni, N., Fendt, S.-M., Rühl, M., Sauer, U., 2009. ^{13}C -based metabolic flux analysis. *Nature*
736 *Protocols* 4, 878–892.



737 Zuccarini, P., Sardans, J., Asensio, L., Peñuelas, J., 2023. Altered activities of extracellular soil
738 enzymes by the interacting global environmental changes. *Global Change Biology* 29,
739 2067–2091. doi:10.1111/gcb.16604
740

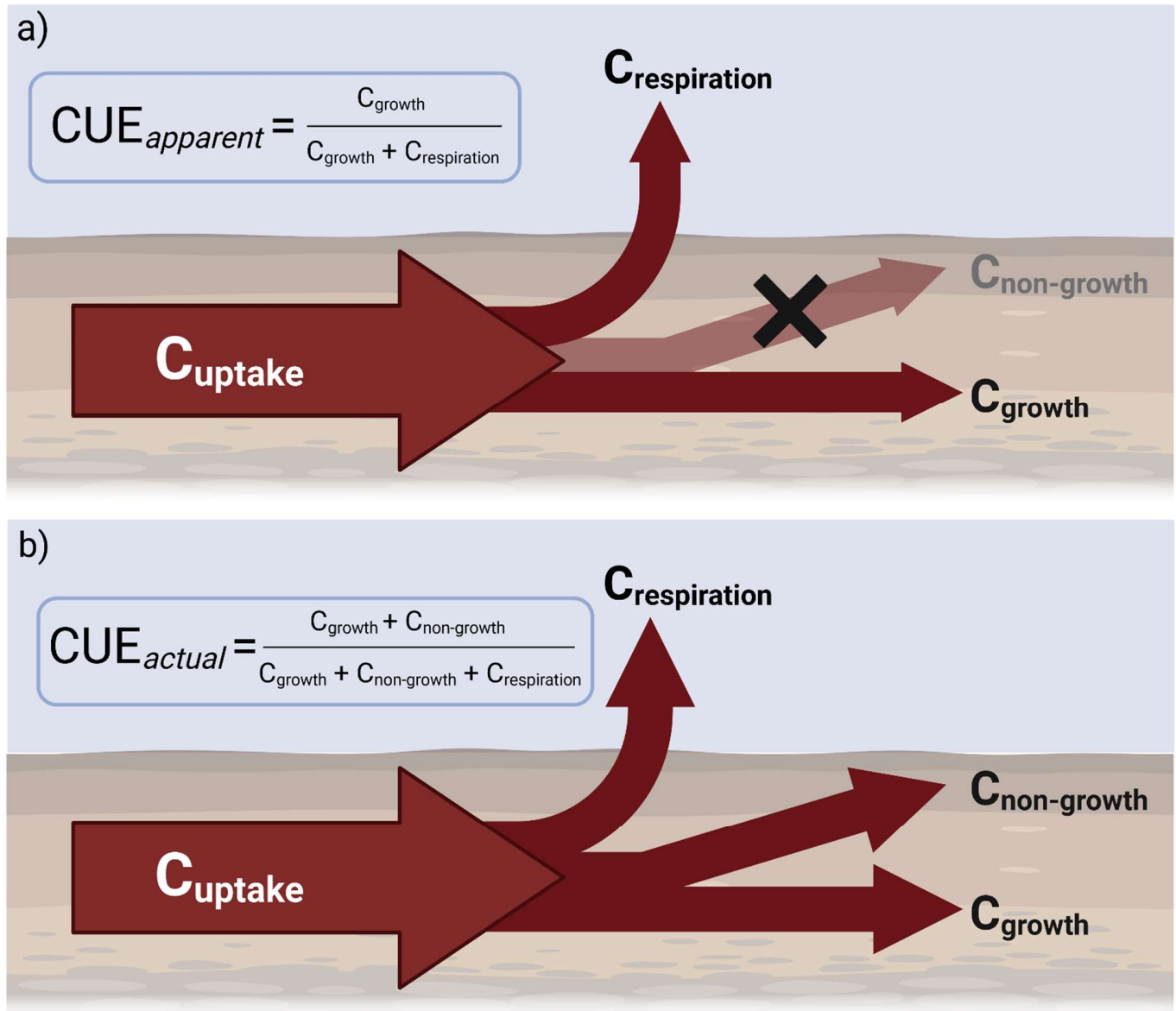


Fig. 1. Schematic illustration of carbon (C) partitioning during microbial metabolism when considering carbon-use efficiency (CUE). **(a)** Current concepts of CUE disregard C used for non-growth anabolism ($C_{non-growth}$). CUE is quantified from C used for growth (C_{growth}) and respiration ($C_{respiration}$) 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)).

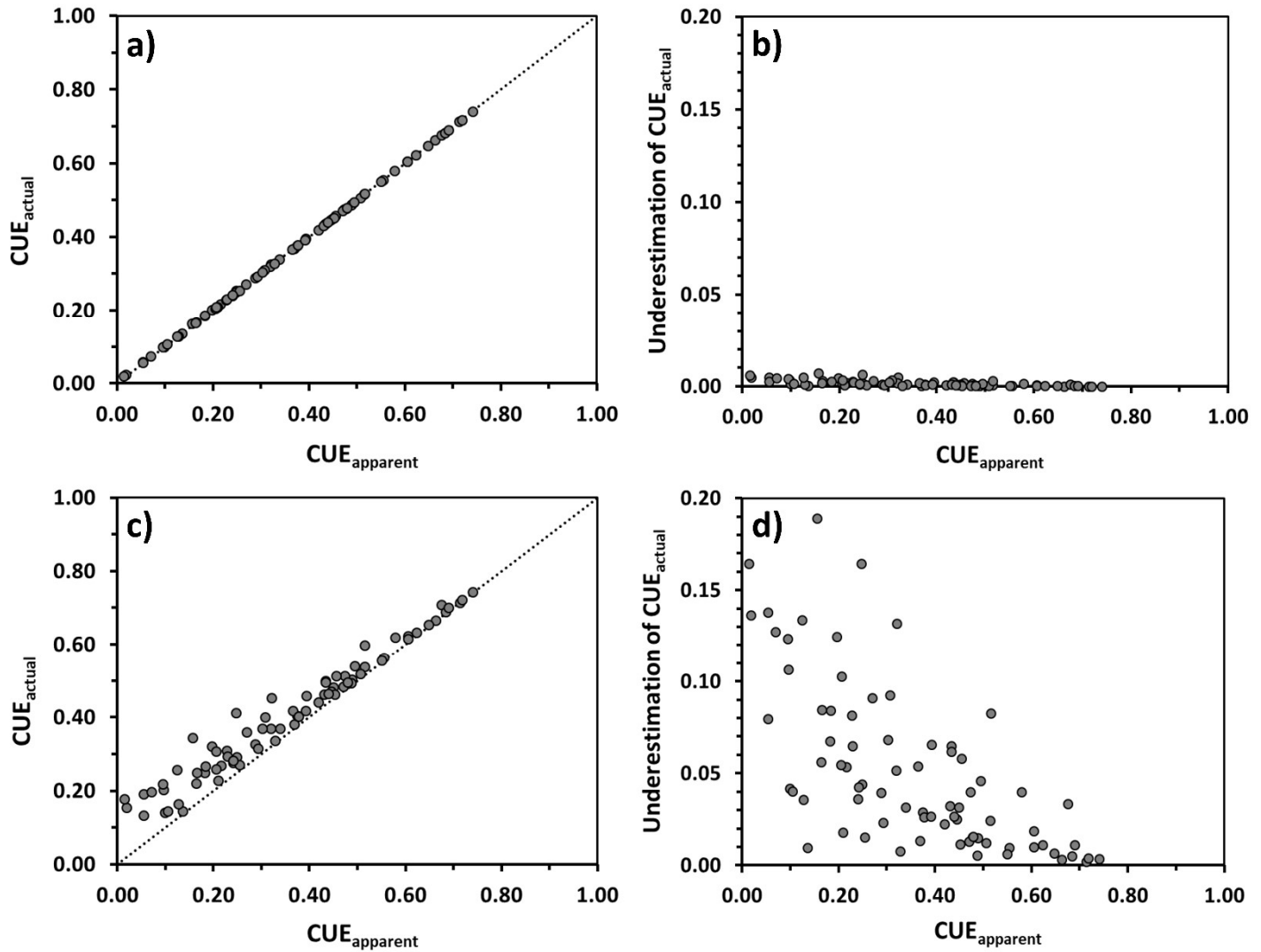


Fig. 2. Actual and apparent carbon-use efficiencies (CUE_{actual} and $CUE_{apparent}$, respectively) considering extracellular enzyme formation based on data adopted from Domeignoz-Horta et al. (2023). The displayed results consider two scenarios for enzyme formation: First, microbial communities maintaining 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.

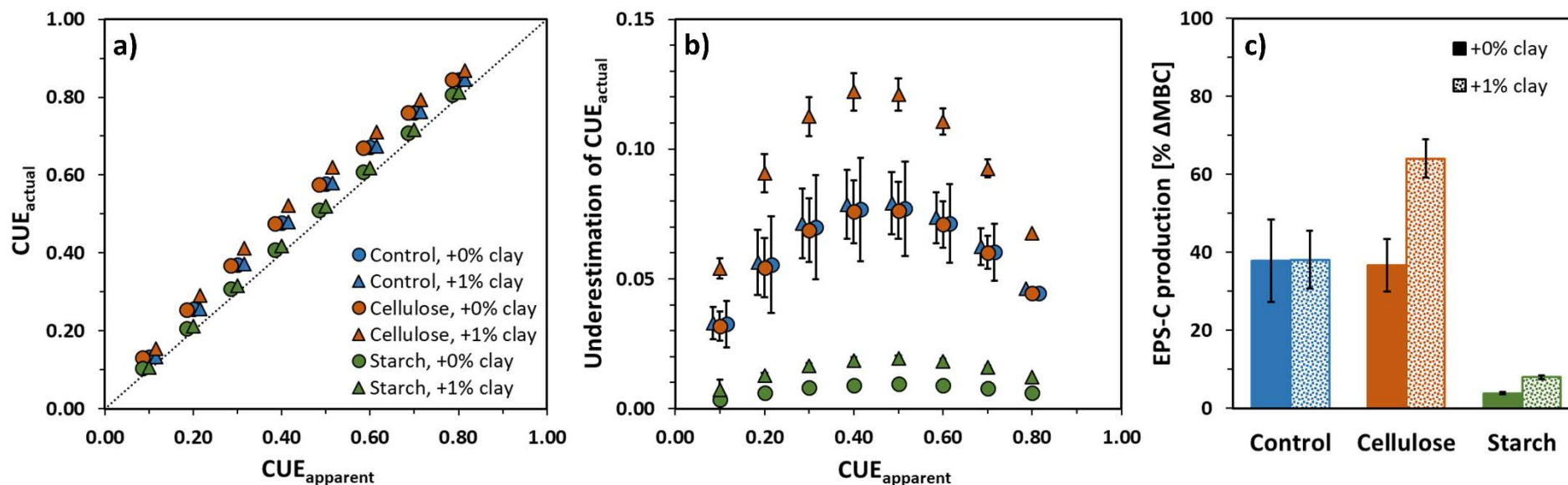


Fig. 3. Actual and apparent carbon-use efficiencies (CUE_{actual} and CUE_{apparent} , respectively) and production of extracellular polymeric substances (EPS) calculated from data of Olagoke et al. (2022). **(a)** Comparison between assumed CUE_{apparent} (equation (1)) and corresponding CUE_{actual} (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.

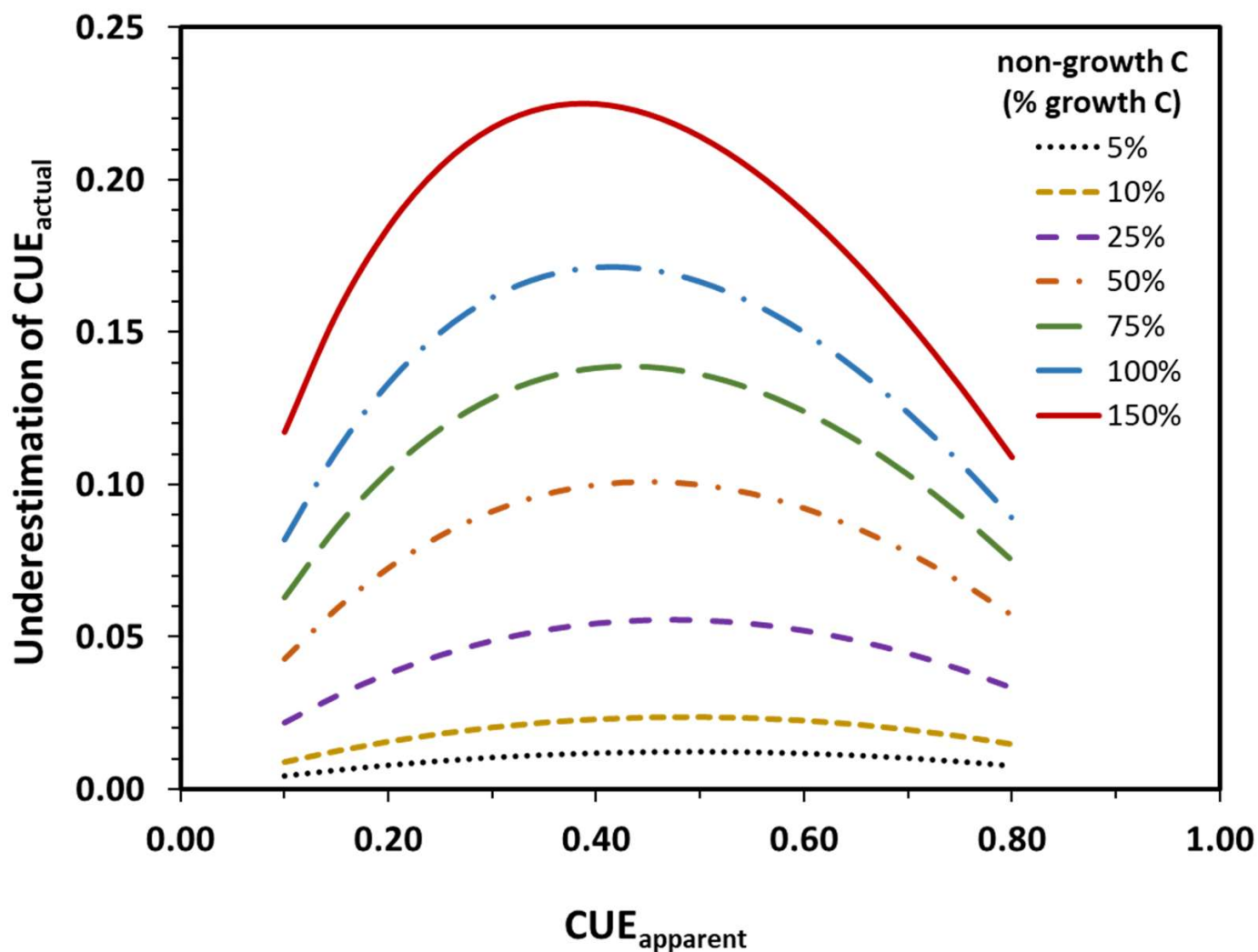
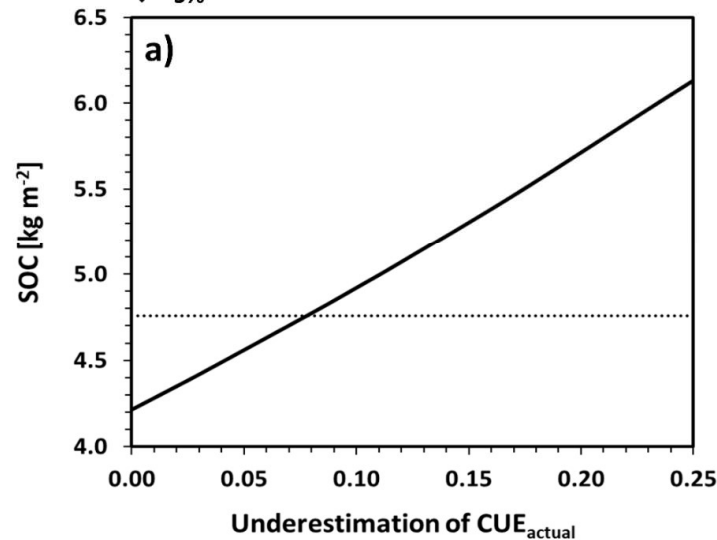
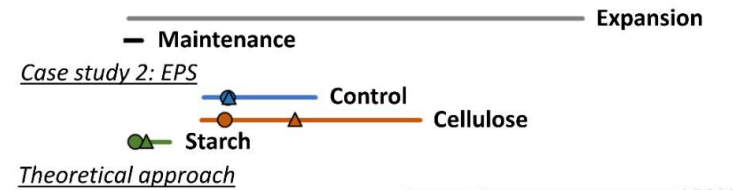


Fig. 4. Underestimation of actual CUE (CUE_{actual}) in relation to microbial carbon (C) allocation between non-growth and growth anabolism. Underestimation of CUE_{actual} (i.e. CUE_{actual} minus $CUE_{apparent}$) is plotted as function of assumed apparent CUE ($CUE_{apparent}$). $CUE_{apparent}$ was 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

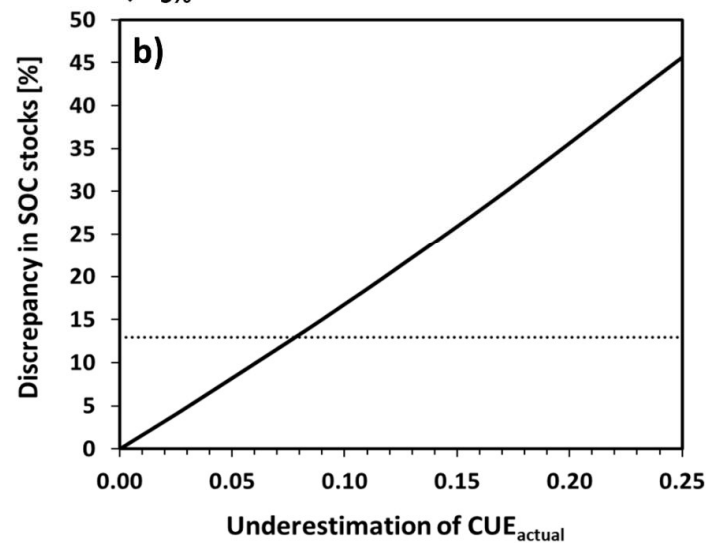
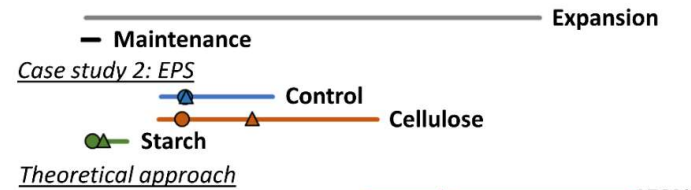


Fig. 5. Results from the modelling approach showing soil organic carbon (SOC) stocks calculated for the Green Manure treatment of the Ultuna Long-Term Soil Organic Matter Experiment (Herrmann and Witter, 2008; Persson and Kirchmann, 1994) in the year 2020. The SOC stocks were modelled assuming CUEs in 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 $\text{CUE}_{\text{actual}}$) are shown on the right. Horizontal solid lines above the graphs show the range of the underestimation of $\text{CUE}_{\text{actual}}$ 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 $\text{CUE}_{\text{apparent}}$ 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

Tobias Bölscher^{a,*}, Cordula Vogel^b, Folasade K. Olagoke^b, Katharina H.E. Meurer^c, Anke M. Herrmann^c, Tino Colombi^c, Melanie Brunn^{d,e}, Luiz A. Domeignoz-Horta^{a,f}

^a Université Paris-Saclay, INRAE, AgroParisTech, UMR EcoSys, 91120 Palaiseau, France

^b Soil Resources and Land Use, Institute of Soil Science and Site Ecology, TU Dresden, Dresden, Germany

^c Department of Soil and Environment, SLU Swedish University of Agricultural Sciences, Box 7014, 75007, Uppsala, Sweden

^d Institute for Integrated Natural Sciences, University of Koblenz, Koblenz, Germany

^e Institute for Environmental Sciences, University of Kaiserslautern-Landau (RPTU), Landau, Germany

^f Department of Evolutionary Biology & Environmental Sciences, University of Zurich, 8057 Zurich, Switzerland

* Corresponding author: E-mail: tobias.bolscher@inrae.fr

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 Field 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

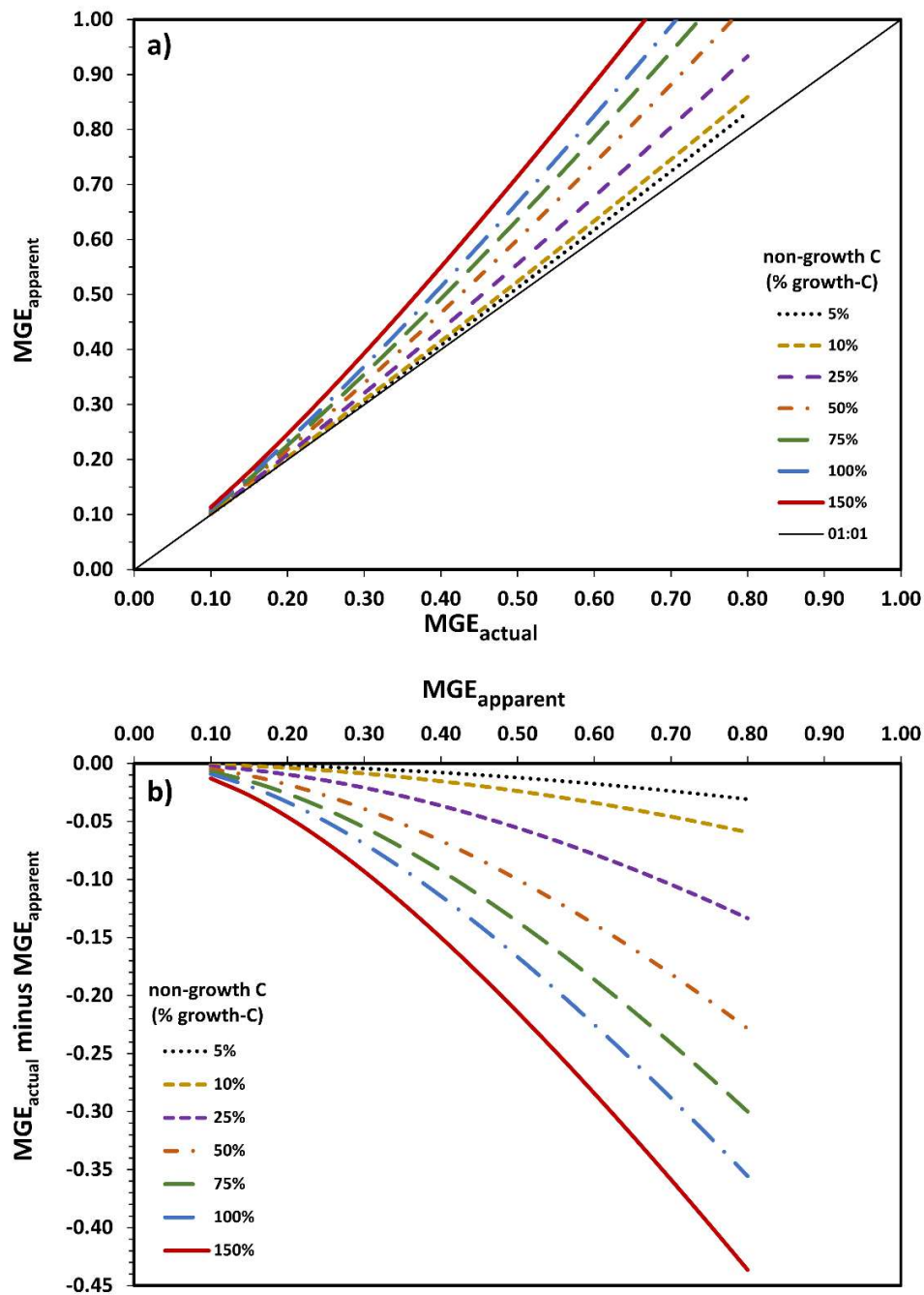


Fig. S1. Comparison between actual and apparent microbial growth efficiency (MGE_{actual} and $MGE_{apparent}$, respectively) depending on the amount carbon (C) used for non-growth to growth anabolism. (a) Direct comparison between MGE_{actual} and $MGE_{apparent}$ demonstrating the overestimation of 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 $MGE_{apparent}$.

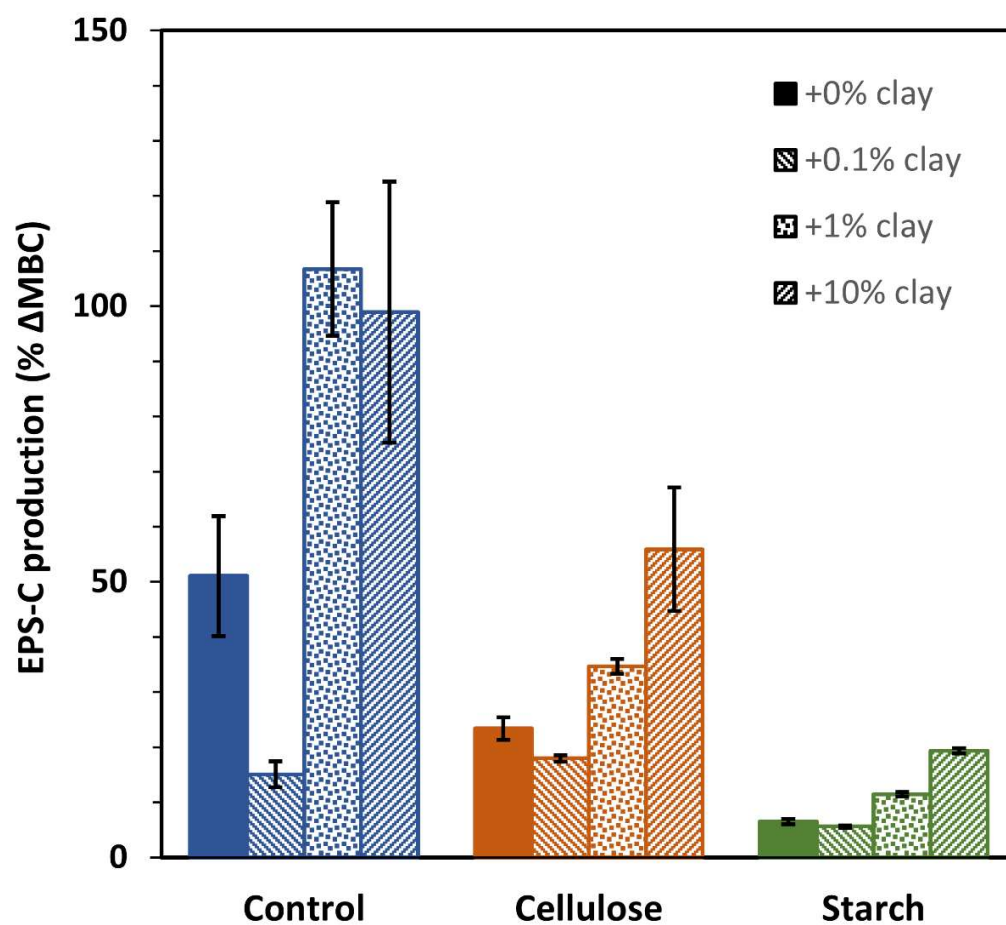


Fig. S2. Maximal production of extracellular polymeric substances (EPS) carbon (EPS-C) relative to maximal production of microbial biomass carbon (MBC) over the entire 80 days incubation. EPS-C and MBC were measured in soil amended with cellulose or starch or controls soils receiving no substrate. All 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 ¹⁸O-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 (μg C g ⁻¹ soil)	Growth (μg C g ⁻¹ soil)	Enzyme pool maintenance (μg C g ⁻¹ soil)	Enzyme pool expansion (μg 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 ($\mu\text{g C g}^{-1} \text{ soil}$)	Growth ($\mu\text{g C g}^{-1} \text{ soil}$)	Enzyme pool maintenance ($\mu\text{g C g}^{-1} \text{ soil}$)	Enzyme pool expansion ($\mu\text{g C g}^{-1} \text{ soil}$)	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 ($\mu\text{g C g}^{-1} \text{ soil}$)	Growth ($\mu\text{g C g}^{-1} \text{ soil}$)	Enzyme pool maintenance ($\mu\text{g C g}^{-1} \text{ soil}$)	Enzyme pool expansion ($\mu\text{g C g}^{-1} \text{ soil}$)	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

SUPPLEMENTARY REFERENCES

- Allison, S.D., Vitousek, P.M., 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biology and Biochemistry* 37, 937–944.
doi:10.1016/j.soilbio.2004.09.014
- Blagodatskaya, E., Blagodatsky, S., Anderson, T.-H., Kuzyakov, Y., 2014. Microbial growth and carbon use efficiency in the rhizosphere and root-free soil. *PloS One* 9, e93282.
doi:10.1371/journal.pone.0093282
- Blazewicz, S.J., Schwartz, E., 2011. Dynamics of ^{18}O Incorporation from H_2^{18}O into Soil Microbial DNA. *Microbial Ecology* 61, 911–916. doi:10.1007/s00248-011-9826-7
- Canarini, A., Wanek, W., Watzka, M., Sandén, T., Spiegel, H., Šantrůček, J., Schnecker, J., 2020. Quantifying microbial growth and carbon use efficiency in dry soil environments via ^{18}O water vapor equilibration. *Global Change Biology* 26, 5333–5341.
doi:10.1111/gcb.15168
- del Giorgio, P.A., Cole, J.J., 1998. Bacterial growth efficiency in natural aquatic systems. *Annual Review of Ecology and Systematics* 29, 503–541.
doi:10.1146/annurev.ecolsys.29.1.503
- Dijkstra, P., Salpas, E., Fairbanks, D., Miller, E.B., Hagerty, S.B., van Groenigen, K.J., Hungate, B.A., Marks, J.C., Koch, G.W., Schwartz, E., 2015. High carbon use efficiency in soil microbial communities is related to balanced growth, not storage compound synthesis. *Soil Biology and Biochemistry* 89, 35–43. doi:10.1016/j.soilbio.2015.06.021
- Domeignoz-Horta, L.A., Pold, G., Erb, H., Sebag, D., Verrecchia, E., Northen, T., Louie, K., Eloë-Fadrosch, E., Pennacchio, C., Knorr, M.A., Frey, S.D., Melillo, J.M., DeAngelis, K.M., 2023. Substrate availability and not thermal acclimation controls microbial temperature sensitivity response to long-term warming. *Global Change Biology* 29, 1574–1590.
doi:10.1111/gcb.16544
- Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. *Ecology* 88, 1354–64. doi:10.1890/05-1839
- Flemming, H.C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S.A., Kjelleberg, S., 2016. Biofilms: An emergent form of bacterial life. *Nature Reviews Microbiology* 14, 563–575.
doi:10.1038/nrmicro.2016.94
- Frølund, B., Palmgren, R., Keiding, K., Nielsen, P.H., 1996. Extraction of extracellular polymers from activated sludge using a cation exchange resin. *Water Research* 30, 1749–1758.
doi:10.1016/0043-1354(95)00323-1
- Geyer, K.M., Dijkstra, P., Sinsabaugh, R., Frey, S.D., 2019. Clarifying the interpretation of carbon use efficiency in soil through methods comparison. *Soil Biology and Biochemistry* 128, 79–88. doi:10.1016/j.soilbio.2018.09.036
- Hagerty, S.B., van Groenigen, K.J., Allison, S.D., Hungate, B.A., Schwartz, E., Koch, G.W., Kolka, R.K., Dijkstra, P., 2014. Accelerated microbial turnover but constant growth efficiency with warming in soil. *Nature Climate Change* 4, 903–906.
doi:10.1038/NCLIMATE236
- Herrmann, A.M., Witter, E., 2008. Predictors of gross N mineralization and immobilization during decomposition of stabilized organic matter in agricultural soil. *European Journal of*

- Soil Science 59, 653–664. doi:10.1111/j.1365-2389.2008.01023.x
- Mason-Jones, K., Ban, C.C., Breidenbach, A., Dyckmans, J., Dippold, M.A., 2023. Intracellular carbon storage by microorganisms is an overlooked pathway of biomass growth. *Nature Communications* 14, 2240. doi:10.1038/s41467-023-37713-4
- Meurer, K.H.E., Chenu, C., Coucheney, E., Herrmann, A.M., Keller, T., Kätterer, T., Svensson, D.N., Jarvis, N., 2020. Modelling dynamic interactions between soil structure and the storage and turnover of soil organic matter. *Biogeosciences* 17, 5025–5042. doi:10.5194/bg-17-5025-2020
- NCBI, 2023. National Library of Medicine – National Center for Biotechnology Information, Protein. <http://www.ncbi.nlm.nih.gov/protein/>, Accessed: 2023-05-10.
- Olagoke, F.K., Bettermann, A., Nguyen, P.T.B., Redmile-Gordon, M., Babin, D., Smalla, K., Nesme, J., Sørensen, S.J., Kalbitz, K., Vogel, C., 2022. Importance of substrate quality and clay content on microbial extracellular polymeric substances production and aggregate stability in soils. *Biology and Fertility of Soils* 58, 435–457. doi:10.1007/s00374-022-01632-1
- Persson, J., Kirchmann, H., 1994. Carbon and nitrogen in arable soils as affected by supply of N fertilizers and organic manures. *Agriculture, Ecosystems and Environment* 51, 249–255. doi:10.1016/0167-8809(94)90048-5
- Redmile-Gordon, M.A., Brookes, P.C., Evershed, R.P., Goulding, K.W.T., Hirsch, P.R., 2014. Measuring the soil-microbial interface: Extraction of extracellular polymeric substances (EPS) from soil biofilms. *Soil Biology and Biochemistry* 72, 163–171. doi:10.1016/j.soilbio.2014.01.025
- Schimel, J., Becerra, C.A., Blankinship, J., 2017. Estimating decay dynamics for enzyme activities in soils from different ecosystems. *Soil Biology and Biochemistry* 114, 5–11. doi:10.1016/j.soilbio.2017.06.023
- Schimel, J., Weintraub, M.N., Moorhead, D., 2022. Estimating microbial carbon use efficiency in soil: Isotope-based and enzyme-based methods measure fundamentally different aspects of microbial resource use. *Soil Biology and Biochemistry* 169, 108677. doi:10.1016/j.soilbio.2022.108677
- Sinsabaugh, R.L., Manzoni, S., Moorhead, D.L., Richter, A., 2013. Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. *Ecology Letters* 16, 930–939. doi:10.1111/ele.12113
- Spohn, M., Klaus, K., Wanek, W., Richter, A., 2016. Microbial carbon use efficiency and biomass turnover times depending on soil depth – Implications for carbon cycling. *Soil Biology and Biochemistry* 96, 74–81. doi:10.1016/j.soilbio.2016.01.016
- Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring microbial biomass C. *Soil Biology and Biochemistry* 19, 703–707. doi:10.1016/0038-0717(87)90052-6
- Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass C by fumigation-extraction - an automated procedure. *Soil Biology and Biochemistry* 22, 1167–1169. doi: 10.1016/0038-0717(90)90046-3