Soil Biology and Biochemistry 169 (2022) 108677

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Estimating microbial carbon use efficiency in soil: Isotope-based and enzyme-based methods measure fundamentally different aspects of microbial resource use

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ARTICLE INFO

Keywords:
Carbon use efficiency
Exoenzyme
Stoichiometry

ABSTRACT

How carbon partitions between microbial biomass and CO_2 (carbon use efficiency, CUE) is key in all soil carbon cycling models. Traditional methods to estimate CUE focus on the physiological partitioning of specific substrates, typically labeled with isotopes. However, an alternative approach (Sinsabaugh et al., 2016) is based on community-level resource capture using assays of extracellular enzymes; although this uses the same name (CUE), it measures something distinctly different from the isotopic methods. Rather, it assesses how microbes shift resource use in response to substrate stoichiometry.

In every soil carbon cycle model—whether purely conceptual or elaborately mathematical—a critical parameter is how carbon (C) partitions between reuse vs. waste. Is a substrate converted to some new organic form—including biomass—or is it released as a waste, usually CO_2?

This partitioning has acquired a variety of names, the most common of which in terrestrial ecology is *carbon use efficiency* (CUE; Geyer et al., 2016), or the similar *substrate use efficiency* (Takriti et al., 2018). Analogous terms, however, include *microbial growth efficiency* (Six et al., 2006), *bacterial growth efficiency* (more commonly used in marine systems, Del Giorgio and Cole, 1998), *growth yield efficiency* (Strickland and Rousk, 2010), and just *efficiency* (Sugai and Schimel, 1993).

CUE measures the steady-state proportion of an organic substrate that is converted into new forms relative to the amount respired to CO_2.

$$CUE = C_{micro}/C_{metabolized} \quad \text{Equation 1}$$

Where C_{micro} is the amount of C converted into microbial material, while $C_{metabolized}$ is the amount of C taken up and metabolized. The most common approach to measuring CUE has been to add an isotopically labeled compound (commonly ^{14}C or ^{13}C, but methods also use ^{18}O-water; Pold et al., 2020) and then to follow the added label into new sink pools (biomass, CO_2, etc.).

In such direct, usually short-term, assays the presumption is that they measure actual biochemical efficiency—based on pathways and energetics. Biochemistry constrains how much of a molecule may be reprocessed into new molecules. Some molecules may be efficiently assimilated into new biomass (e.g. glucose or amino acids) while others require substantial, inefficient, reprocessing to do so (e.g. complex phenolics; Sugai and Schimel, 1993).

The challenge with CUE, however, is that it is amorphous and ill-defined. It is framed by the specific context (pure cultures or mixed communities of organisms) and method: which C form was applied, which calculation was used, and even the duration of the experiment (Geyer et al., 2016, 2019). How we measure CUE reflects the assumptions and presumptions we put into the measurement.

One approach to estimating CUE in soils with mixed communities of organisms of unknown composition, and likely varying in their C allocation, is based on activities of extracellular enzymes (Sinsabaugh et al., 2016). This assumes that, despite differences among organisms, microbes growing on plant detritus allocate C to acquire needed resources in the appropriate elemental ratios at the whole community scale. The core equation underlying this approach is:

$$TER_{C:X} / B_{C:X} = A_X / CUE \quad \text{Equation 2}$$

Here, $TER_{C:X}$ is the "threshold element ratio" or the ratio at which microbes shift between being limited by C to being limited by element X—usually either N or P. $B_{C:X}$ is the elemental ratio of C:X in microbial biomass, while A_X is the apparent assimilation efficiency for element X.

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https://doi.org/10.1016/j.soilbio.2022.108677
Received 9 February 2022; Received in revised form 14 April 2022; Accepted 16 April 2022
Available online 20 April 2022

This equation assumes organisms will use as much of the C in a substrate as required to achieve the target elemental concentration in their biomass. Rearranged, the equation states:

$$CUE = (B_{C:X} * A_X) / TER_{C:X} \quad \text{Equation 3}$$

If a microbial community can assimilate all of resource X, then CUE $= B_{C:X}/TER_{C:X}$. Thus, if the TER were twice $B_{C:X}$, then at TER, microbes would use half the C and so CUE would be 50%. This assumes that microbial CUE is defined by a need to match biomass C:X ratio to the available resources. Should microbes consume a substrate richer in C than the TER, they would have to burn off C and so reduce CUE to reach the target $B_{C:X}$.

A problem is that this model's estimates of CUE are distinct from a biochemically defined CUE, which only measures the partitioning of a particular molecule between biomass and CO_2. This stoichiometrically-defined CUE actually embeds the biochemical CUE within it, but then adds the *additional* assumption that when microbes are N- or P-limited, they must reduce C-assimilation to convert C-rich substrates into biomass (Fig. 1). Thus the two definitions of CUE diverge. In the stoichiometric case, when organisms take up a C-rich molecule such as glucose, which they can assimilate efficiently based on biochemistry, they might still need to either leave it unmetabolized or respire it away to reduce the C-content, and so bring the stoichiometric balance to the right level for synthesizing biomass. Respiring away C to achieve stoichiometric balance is what Schimel and Weintraub (2003) called "overflow metabolism," or what Russell (2007) called "energy spilling."

In the stoichiometric formulation of CUE, TER varies with biochemical efficiency and other factors controlling CUE (Eq. (1))—depending on the nature the substrate, and the community's ability to assimilate it into biomass, TER would vary. For example, if microbes were growing on amino acids, the $TER_{C:N}$ would be low—cells could readily assimilate much of the material into biomass. If, however, cells were metabolizing complex condensed polyphenolics, more of the C would have to be respired to provide energy to fuel metabolism, and so the $TER_{C:N}$ would necessarily be higher. This is also analogous to the A_X term (in Equation (2)), which deals directly with how efficiently a cell assimilates resource X from an available resource.

Thus, although the approaches use the same term of *carbon use efficiency*, in fact the metabolic and stoichiometric conceptions of CUE differ from each other. Despite the importance of assimilation efficiency in both estimates, there is not enough direct overlap between how they are conceived or formulated to accurately describe them with the same term. That creates an ambiguity in its conceptual definition, which can be a problem!

That problem grows with the next step in the Sinsabaugh et al. (2016) paper, which states:

> "Sinsabaugh and Follstad Shah (2012) extended this model by proposing that the $TER_{C:X}/B_{C:X}$ term, which is difficult to estimate directly, was proportional to the term $EEA_{C:X}/(B_{C:X}/L_{C:X})$"

This allowed them to derive their enzyme-based estimate for CUE:

$$CUE_{C:X} = CUE_{max} * S_{C:X} / (S_{C:X} + K_X) \quad \text{Equation 4}$$

where $S_{C:X} = (1 / EEA_{C:X}) * (B_{C:X}/L_{C:X})$

Here, K_X is a simple half-saturation constant, $EEA_{C:X}$ is the ratio of activities of enzymes that target C vs. X acquisition, and $L_{C:X}$ is the elemental composition of the substrate (but is typically measured on bulk organic matter). This formulation introduces an additional critical assumption into the argument and the equation: that microbes are processing *polymeric* material that requires cells to use extracellular enzymes to fragment the substrate polymers before they can take them up and metabolize them. For soil microbes that don't process polymeric detritus, however, this assumption is invalid, and at the community level, the acquisition of resources that do not require extracellular enzymes can skew estimates of $CUE_{C:X}$ (eq. (4)).

Many, perhaps most, soil microbes are not plant litter degraders and do not produce extracellular enzymes on their own; rather, they rely on a guild of primary decomposers to produce extracellular enzymes that fragment polymers, and then opportunistically take up the released oligomers and monomers (Moorhead and Sinsabaugh, 2006; Bailey et al., 2002; Frey et al., 1999). In the Sinsabaugh et al. (2016) model, the calculated CUE represents the overall value for such an integrated community—both the enzyme producers *and* the "cheaters" (Allison, 2005; Kaiser et al., 2014). Thus, the whole-community assumption is invalid for specialized communities that are not dependent on plant detritus and exoenzyme-producing primary decomposers, such as rhizosphere communities, which rely mostly on root exudates (Vives-Peris et al., 2020). Similarly, microbial communities in mineral soils may depend on small molecules released by microbial death and lysis, or that are freed from mineral protection (Kleber et al., 2007); these compounds may have originated with plant detritus, but far removed in time. Moreover, acquiring mineral nutrients (N, P) that may not require exoenzymes can similarly skew estimates of $CUE_{C:X}$.

We might therefore ask, how constrained is the Sinsabaugh et al. (2016) model constrained for estimating CUE? In brief, the approach is constrained by the model's framework and parameters. It is a theoretical framing that inherently emphasizes detritus breakdown, rather than all soil microbial community functions, and it emphasizes stoichiometry over other processes that regulate cellular metabolism. The method facilitates broad cross-system comparisons because measuring enzyme

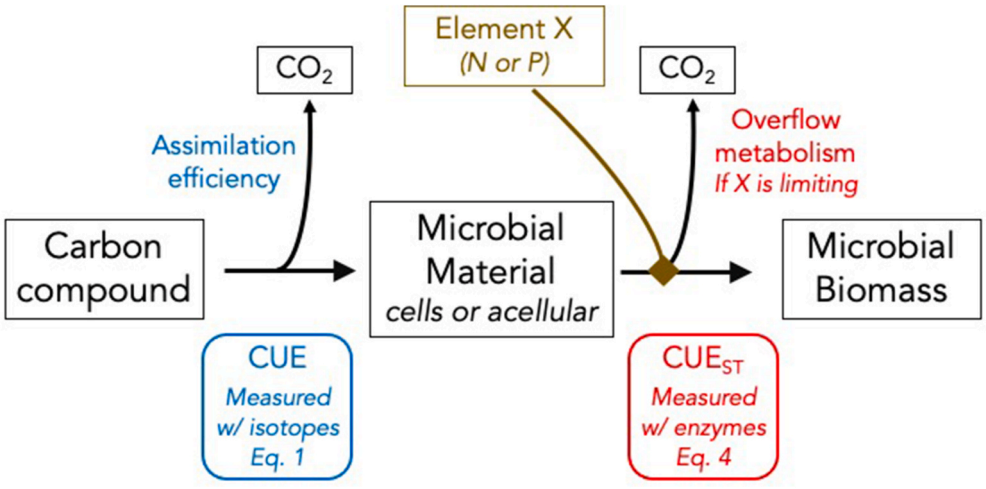

Fig. 1. Flows of C and how the different measures of CUE relate to them.

activities is rapid, relatively inexpensive, and can readily be done at large scale (unlike isotopic tracer studies); enzyme measures may also integrate patterns over longer periods of time, whereas isotope measures are limited to an immediate snapshot of current conditions. This facilitates evaluating how the stoichiometric conceptualization of substrate use relates to large-scale general drivers of microbial function (e.g. soil and microbial biomass C/N ratios). But there is enormous scatter in measured data around the curves the model generates (Sinsabaugh et al., 2016), and it is sensitive to assumptions in key parameters, including $B_{C:X}$ (Supplemental Tables 1 and 2). That doesn't change the overall patterns of large-scale analyses in Sinsabaugh et al. (2016), but it could be a problem if one wished to apply the method to measure CUE at a limited number of sites or experimental conditions.

Carbon use efficiency remains a critical concept in describing C cycling in soil, but it also remains one of the most challenging to define methodologically (Geyer et al., 2019). Sinsabaugh et al. (2016), add a semantic challenge by using the term CUE in a fundamentally different way than the traditional definition based on the processing of specific organic substrates as they are taken up and metabolized, controlled by the constraints of molecular pathways and energetics. Using one term for two fundamentally different phenomena generates miscommunication, confusion, and scientific error.

The Sinsabaugh et al. (2016) extracellular enzyme-based, stoichiometric model, while easy to apply, should not be considered a precise measurement of biochemical C partitioning in any specific study. First it does not directly capture the short-term interplay of factors that regulate the specific C-partitioning that defines biochemically defined CUE; for example, exoenzymes may persist for months and so would not reflect experimental manipulations (Roller and Schmidt 2015). Second, and more important, it relies on different mathematics capturing a broader, integrative framework (Eq. (1) vs. Eq. (4)), and so should be distinguished from traditional CUE measures. Methods based on estimating actual cell growth can reasonably be called *microbial growth efficiency* (MGE). Methods based on following material into biomass or new organic forms can reasonably be called *carbon use efficiency* (CUE; Fig. 1), although methods that rely on a single isotopically-labeled substrate might better be referred to as *substrate use efficiency* (SUE). The Sinsabaugh et al. (2016) approach, on the other hand, is based on enzymatic breakdown of detrital polymers, should, perhaps should more accurately be named *Carbon-use Efficiency from Stoichiometry Theory* (CUE_{ST}) to reflect the approach's emphasis. In using this approach, it must be recognized that some estimate of fundamental biochemical carbon use efficiency (CUE) is embedded *within* the $TER_{C:X}$ term in the model and within CUE_{ST}. However, CUE_{ST} is a fundamentally distinct way of viewing resource use efficiency than classical CUE.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2022.108677.

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