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Evaluating soil microbial carbon use efficiency explicitly as a function of cellular processes: implications for measurements and models

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Abstract

Carbon use efficiency (CUE), the proportion of carbon (C) consumed by microbes that is converted into biomass, is an important parameter for soil C models with explicit microbial controls. While often considered as a single parameter, CUE is an emergent property of multiple microbial processes, including assimilation efficiency, biomass-specific respiration, enzyme production, and respiratory costs of enzyme production. These processes occur over variable time scales and imply different fates for C, and the same emergent CUE value can result when C is allocated in fundamentally different ways (e.g. a high investment in enzyme production vs. a high assimilation cost). We developed a model that represents the individual processes underlying emergent CUE to test how shifts in microbial allocation alter equilibrium soil C pool sizes. We found that an increase in emergent CUE that results from a change in assimilation efficiency, biomass specific respiration, or respiration costs from enzyme production causes soil organic C (SOC) to decline, while the same change in emergent CUE resulting from a change in enzyme production causes SOC to increase.

We also used the model to test the sensitivity of CUE from isotopic C tracer estimates to changes in microbial allocation processes. We found that these estimates do not account for the same microbial processes represented by emergent CUE in models. We propose that considering microbial processes explicitly rather than representing CUE as a single parameter can improve data-model integration. In addition, modeling microbial processes explicitly will account for a wider range of possible outcomes from shifts in microbial C allocation, such as when increased SOC results from increasing CUE.

Introduction

The Earth's soils represent a large carbon (C) reservoir that is vulnerable to climate change (Cox et al. 2000, Jobbágy and Jackson 2000, Schlesinger and Andrews 2000, Jackson et al. 2017). Predicting the dynamics of soil C is therefore critical to understanding the global C cycle (Cox et al. 2000, Davidson and Janssens 2006). However, models that are currently used to predict soil C storage under climate change often fail to match the results from field experiments (Cox et al. 2000, Melillo et al. 2002, Bouskill et al. 2014). For example, models overestimate the magnitude and duration of the response of soil respiration to warming (Bouskill et al. 2014), and also produce estimates of current soil C stocks that can range from 40% to 240% of estimates based on empirical measurements (Todd-Brown et al. 2013).

Better predictions of soil C stocks under dynamic conditions may be possible if models can account for how microbial allocation of C changes with environmental conditions (Conant et al. 2011). In most soil C models, microbial allocation is represented implicitly; a proportion of C is respired as it move between pools (Schimel and Schaeffer 2012a). This partitioning parameter is thought to represent microbial carbon use efficiency (CUE), which is the proportion of C consumed by microbes that is incorporated into the biomass (Allison et al. 2010, Wang et al. 2013, Wieder et al. 2015). Models have also begun to account for microbial processes by including CUE explicitly, so that CUE partitions consumed C between respiration and growth of a microbial biomass pool. Varying this parameter reveals the sensitivity of soil C stocks to changing microbial C allocation with environmental conditions for models that represent microbes both implicitly (Conant et al. 2011) and explicitly. (Allison et al. 2010, Wieder et al. 2013, Li et al. 2014). In microbial-

explicit models, assumptions about the temperature sensitivity of CUE alters not just the magnitude but also the direction of soil C stock changes in response to climate warming (Wieder et al. 2013).

Empirical studies have been unable to reach the consensus necessary to constrain the possible range of CUE in soil or its sensitivity to environmental change (Manzoni et al. 2012). For example, while some theory suggests that CUE should decline with temperature (del Giorgio and Cole 1998, Steinweg et al. 2008, Allison et al. 2010, Sinsabaugh et al. 2013), studies of temperature sensitivity show mixed results. Some studies have found a decrease in CUE with increasing temperature (Steinweg et al. 2008, Tucker et al. 2013), but others have found no change (Dijkstra et al. 2011b, Hagerty et al. 2014), or a response that depends on substrate type (Frey et al. 2013). The average CUE of 0.55 across studies in soil reviewed by Manzoni et al. (2012) is almost double the average of the aquatic and pure culture studies reviewed. In fact, soil studies have measured values across virtually the full range of possible CUE (Sugai and Schimel 1993, Frey et al. 2013, Manzoni et al. 2017). Recent measurements are typically higher—near 0.7—which approaches the theoretical maximum CUE (estimated near 0.8) and surpasses the maximum value of 0.6 from pure culture (Roels 1980, Gommers et al. 1988, Dijkstra et al. 2015).

Much of this inconsistency may arise because CUE itself is a coarse aggregation of microbial processes that are difficult to constrain in a single parameter (Lee and Schmidt 2014, Xu et al. 2014, Geyer et al. 2016, Manzoni et al. 2017). CUE is the proportion of consumed C that is used to produce new biomass, a definition that is ambiguous as to which physiological processes should be counted (Sinsabaugh 2013). At a cellular level, partitioning C between growth and non-growth is expected to represent the balance

between anabolic versus catabolic metabolism (Carlson et al. 2007, Dijkstra et al. 2011b). However when considering the soil microbial community, anabolism and growth are not equivalent. For example, some products of anabolism such as extracellular enzymes and exudates are generated within the cell but then excreted into the soil matrix. When growth is considered over very short time scales (i.e. seconds or minutes), C directed toward exudates may still be within the cell and could be counted as growth. However, over longer timescales, exudation would remove these products and they would not contribute to growth.

The overall CUE of the soil microbial community is an emergent property—the result of integrating multiple microbial biochemical pathways. A single parameter is unlikely to capture the full suite of effects on soil C cycling that result from shifts in each individual pathway. For example, a reduction in microbial CUE could occur if microbes either allocate more C toward exudative products or if they respire more of the C they take up. These two mechanisms would have fundamentally different effects on soil C cycling; an increase in exudative products may increase soil C storage without increasing respiration. Here, we explicitly define CUE as a function of multiple cellular processes to ask the question: is emergent CUE in models able to capture the variable effects of different microbial allocation strategies on soil C stocks? We then evaluate how isotopic C tracer measurements integrate the multiple microbial processes involved in determining emergent CUE in order to assess if and how these measurements can be used to inform microbial model parameters.

Explicitly modeling the processes involved in CUE

Comprehensively modeling the processes involved in determining CUE would require a full biochemical model that would include the metabolic pathways of the entire soil microbial community. This level of process resolution is unrealistic; we therefore aggregate these processes in a way that represents the main microbial C allocation pathways. We define CUE as:

$$\mathrm{CUE} = \frac{\mathrm{U} - \mathrm{R_U} - \mathrm{R_B} - \mathrm{R_E} - \mathrm{EX}}{\mathrm{U}} \quad (1)$$

where U (uptake) is C consumed by the microbial biomass, R_U is respiration that occurs as a function of uptake, R_B is respiration that occurs as a function of the biomass (i.e. biomass-specific respiration), R_E is respiration from the production of extracellular enzymes, and *EX* is the exudation of microbial products including extracellular enzymes. Most microbial soil C models are structured so that growth (C that stays in live microbes) increases the pool of carbon consumers (i.e. microbial biomass) (Wieder et al. 2015). As exudates would not contribute to this pool, we follow the definition of Manzoni et al. (2012) and represent exudation as a loss term in the CUE calculation. Respiration that occurs with uptake (R_U), includes any respiratory cost of transporting substrate into the cell and the minimum cost for catabolism (e.g. dissimilation). This minimum cost depends on where in the metabolic pathway the substrate enters and the complexity of substrate consumed (Hobbie and Hobbie 2013). We define the proportion of C remaining after respiration from uptake as the microbial assimilation efficiency (*ae*) where:

$$ae = \frac{U - R_U}{U} \quad (2)$$

Assimilation efficiency sets the maximal amount of C that is incorporated into the biomass, but it does not reflect all of the energetic costs of the microbial cell (Gommers et al. 1988, Manzoni et al. 2012, Xu et al. 2014). Respiration also occurs as a proportion of the biomass (R_B) through biomass maintenance costs like energy directed toward osmoregulation, cell motility, defense mechanisms, proofreading and turnover of macromolecular compounds, shifts in metabolic pathways, and overflow metabolism (Schimel and Weintraub 2003, van Bodegom 2007). Growth is also reduced by exudation of extracellular products (EX), including exopolysaccharides and enzymes, and by the respiration that results from their production (R_E).

Through the above mechanisms, microbial C costs are incurred as a proportion of uptake (i.e. assimilation), biomass pool size (i.e. maintenance and overflow costs), or both (i.e. enzyme production and respiration) depending on the process. Processes that occur as a function of substrate uptake can be described as:

$$X = p_X * U \quad (3)$$

where p represents the proportion of uptake directed toward process X, where X is either R_U, R_E, or EX. However, C costs that are proportional to the biomass are incurred as a proportion (t) per unit of biomass C (B):

$$Y = t_y * B \quad (4)$$

where Y represents one of the biomass-specific processes; R_B, R_E, or EX. Given equations 1-4, CUE therefore equivalent to:

$$\mathrm{CUE} = (\mathrm{ae} * \mathrm{U}) - p_{EX} * U - p_{RE} * U - \frac{t_{RB} * B}{U} - \frac{\mathrm{t}_{EX} * \mathrm{B}}{U} - \frac{t_{RE} * B}{U} \quad (5)$$

From this formulation it is clear that biomass specific costs occur as a proportion of the biomass to uptake ratio; thus CUE will only be constant when the biomass pool is at steady state so that this ratio is stable. Under conditions where uptake increases such as from an increase in substrate concentration or microbial uptake affinity, the relative amount of C going toward biomass specific costs would decrease such that CUE increases.

The dynamic nature of CUE has implications for both models and measurements. For microbial models, it means that representing CUE as a constant parameter is unlikely to reflect the true microbial costs of growth under dynamic conditions. For typical isotopic measurements of CUE, estimates are made using a labeled substrate that is out of equilibrium with the biomass and therefore cannot equally account for assimilation efficiency and biomass specific energy costs. This is because the amount of C directed toward biomass-specific costs changes with the ratio of biomass to uptake, whereas costs that occur as a proportion of uptake do not; thus, outside of steady-state biomass conditions, the relative influence of biomass specific costs varies. The extent to which these processes are accounted for in measurements will depend on the abundance of the isotope in the substrate versus biomass pool, which will change over time. When a labeled substrate is first added, the ratio of labeled biomass to C taken up is at its lowest, and so the

influence of biomass specific costs is low. As time proceeds this ratio will increase and so will the influence of biomass specific costs on the CUE estimate; hence, estimated CUE should decline.

Explicitly considering the microbial processes that determine CUE could explain the variation in CUE empirical estimates. Whether microbial exudates are counted as growth has often been determined by methodological constraints. Many CUE estimates require measurements of microbial biomass to quantify growth, and most available methods for estimating microbial biomass exclude exudates (e.g. chloroform fumigation, cell counts, and substrate-induced respiration). However, even recently developed methods for estimating CUE vary in how they account for exudation. While biochemically explicit approaches account for exudation as part of growth (Dijkstra et al. 2011a), CUE estimates using ^{18}O incorporation into DNA likely excludes it from growth completely (Spohn et al. 2016b). Improved theoretical analysis of CUE and its underlying processes could help empiricists address these inconsistencies.

Measurements of CUE

The most widely used method of estimating CUE in soil involves adding an isotopically labeled C-substrate and calculating CUE based on measured concentrations of the label in the remaining substrate, CO_2, and microbial biomass pools after some time period (Frey et al. 2013, Kallenbach et al. 2015, Riggs and Hobbie 2016). For this method, how exudation is counted depends on the assumptions of the equation applied to estimate CUE. We add the subscripts S, C, and B, adopted from Frey et al. (2001) to distinguish the three methods of calculating CUE (i.e. substrate based (CUE_S), concentration based (CUE_C),

biomass based (CUE_B)) with isotopic data from CUE as defined in equation 1 and 5. In the formulation for CUE_S, the substrate-based CUE formulation, all C consumed and not respired is assumed to be growth; the total amount of C consumed is determined as the change in the extractable C pool from the initial label addition:

$$CUE_S = \frac{\Delta Di - CO2i}{\Delta Di} \quad (6)$$

where ΔDi is the change in the concentration of the isotope in the extractable C pool over the incubation period and CO_2i is the amount of isotope released as CO_2. The change in the extractable C pool is often assumed to represent microbial uptake, meaning processes like abiotic sorption of the substrate to soil particles or recycling of products from the biomass back to the extractable C pool are assumed to be negligible. Given this, CUE_S can be expressed in terms of the microbial processes involved:

$$CUE_S = \frac{U - R_U - R_B - R_E}{U} \quad (7)$$

Using this equation, exudation will be counted as growth.

In a second equation for concentration-based CUE (CUE_C), growth is equivalent to the label concentration in the microbial biomass pool (Bi) measured by chloroform-fumigation extraction, which would exclude exudation from growth (Manzoni et al. 2012); consumed C is determined as the change in the extractable C pool (i.e. D_i). CUE_C can be expressed in terms of measured isotopic pools and microbial processes involved in the estimate:

$$CUE_C = \frac{Bi}{\Delta Di} = \frac{U - R_U - R_B - R_E - EX - \tau_B}{U} \quad (8)$$

where τ_B represents C lost from death of the microbial biomass, represented here as a non-respiratory loss of C.

In the biomass-based CUE formulation for CUE_B, growth is the amount of isotopic label in the biomass, and the consumed C is determined as the sum of label respired as CO_2 and label in the biomass:

$$CUE_B = \frac{Bi}{Bi + CO_2 i} = \frac{U - R_U - R_B - R_E - EX - \tau_B}{(U - EX - \tau_B)} \quad (9)$$

This formulation excludes C directed toward exudation from both growth and uptake. It also minimizes the effect of grazing, because like exudates, much of the microbial C that is lost to the food web would not be measured in chloroform extractable fumigation measurements (Frey et al. 2001). The differences between these three equations illustrate that CUE measurements should explicitly consider the processes captured by different methods. Otherwise, measured CUE values will be impossible to interpret, compare, and include in models.

Most models, however, simplify CUE to a single parameter that is not comparable to any empirical estimates. Therefore, we develop a soil C model that explicitly includes biomass specific respiration, assimilation efficiency, and enzyme production/respiration to calculate CUE at steady state. We explore the effect of explicitly modeling these processes

by simulating equilibrium pool sizes under a range of values for each microbial parameter. We simulate an isotopic tracer addition to assess how isotope-based measurements of CUE would compare to actual CUE at steady state. This comparison enables an analysis of how isotopic measurements capture each category of microbial energetic costs.

Methods

Steady-State Model

We used the Allison-Wallenstein-Bradford (AWB; Allison et al. 2010) model with three additional fluxes to explicitly represent microbial biomass-specific respiration (R_B), uptake driven enzyme production (F_{UE}), and respiration from the production of enzymes (R_E; Figure 1). We also added a maintenance respiration flux that is a constant proportion of the biomass (R_B):

$$R_B = r * B \quad (10)$$

where r is the biomass specific respiration rate (Table 1) and B is the concentration of C in the biomass. The uptake-driven enzyme production flux occurs as a proportion (es) of assimilated C:

$$F_{UE} = \left(\frac{V_U * B * D}{K_U + D}\right) * ae * es \quad (11)$$

where ae is the assimilation efficiency and D is the concentration of C in the DOC pool. Microbial C uptake is calculated with a Michaelis-Menten function where V_U is the

maximum DOC uptake rate and K_U is the DOC uptake half-saturation constant. Enzymes are also produced constitutively as a proportion of the biomass:

$$F_E = ep * B \quad (12)$$

where ep is the enzyme production rate. Respiration from enzyme production is then:

$$R_E = ze * \left(\left(\frac{V_U * B * D}{K_U + D} \right) * ae * es + ep * B \right) \quad (13)$$

where ze is the respiration C cost per enzyme-C produced. Microbial biomass increases with uptake from the DOC pool (F_U):

$$F_U = \frac{V_U * B * D}{K_U + D} \quad (14)$$

The amount of C that is incorporated into the biomass is a function of assimilation efficiency (ae). We use the default value of 0.7 for assimilation efficiency, which is close to the value usually measured in instantaneous C partitioning (Dijkstra et al 2011) but below the maximum assimilation efficiency estimated for glucose in Grommers et al (1988). By using this assimilation efficiency value, we are simulating a highly labile tracer (e.g. glucose). Assimilation efficiency will vary across substrate types and would be lower for more complex substrates (e.g. phenol; (Frey et al. 2013). Respiration proportional to uptake (R_U) can then be calculated as:

$$R_U = F_U * (1 - ae) \quad (15)$$

A proportion of the microbial biomass carbon (MBC) is also lost from the biomass pool by microbial death in the AWB model (F_X).

$$F_X = \tau_B * B \quad (16)$$

where τ_B is the biomass death rate. The biomass C lost from death is split between the SOC and DOC pools by the parameter α_{BS}. The differential equation of the biomass pool is therefore:

$$\frac{dB}{dt} = F_U - R_U - R_B - R_E - F_X - F_E - F_{UE} \quad (17)$$

The SOC pool increases with inputs (I_S), and decreases as C is decomposed, moving from the SOC into the DOC pool (F_S). Decomposition of the SOC pool follows Michaelis-Menten kinetics:

$$F_S = \frac{V \times E \times S}{K + S} \quad (18)$$

where V is the maximum decomposition rate, E is the concentration of C in the enzyme pool, and K is the half-saturation constant for the reaction. The SOC pool also increases by a proportion of microbial death (F_X). The differential equation for the SOC pool is:

$$\frac{dS}{dt} = I_S - F_S + F_X * \alpha_{BS} \quad (19)$$

The DOC pool decreases with microbial uptake (F_U) and increases with decomposition of the SOC pool (F_S), a proportion of microbial death, and with decay of the enzyme pool, which occurs as a constant proportion of the enzyme pool (F_L).

$$F_L = \tau_L * E \quad (20)$$

where τ_L is the enzyme pool decay rate. The differential equation for the DOC pool is:

$$\frac{dD}{dt} = I_D + F_S - F_U + F_X * (1 - \alpha_{BS}) + F_L \quad (21)$$

The enzyme pool increases with microbial enzyme production (F_E and F_{UE}) and decreases with enzyme decay (F_L). The differential equation for the enzyme pool is:

$$\frac{dE}{dt} = F_E + F_{UE} - F_L \quad (22)$$

We used the AWB model to calculate CUE under steady state conditions. The steady-state equations for the model are:

$$B = -\frac{ae(es * ze + es - 1)(I_D + I_S)}{-(ae * es * r) + ae * es * \tau_B * ze - ae * \tau_B - ae * ep + r + \tau_B + ep * ze + ep} \quad (23)$$

$$D = -\frac{K_U * (r + \tau_B + ep * ze + ep)}{(ae * es * V_U * ze + ae * es * V_U - ae * V_U + r + \tau_B + ep * ze + ep)} \quad (24)$$

$$E = -\frac{(ae * (I_D + I_S) * (es * (r + \tau_B) + ep)}{\tau_L * (r * (ae * es - 1) + \tau_B * (ae * (-es) * ze + ae - 1) + ep(ae - ze - 1))} \quad (25)$$

$$S = -\frac{K*\tau_L(\alpha_{BS}*ae*Id*\tau_B*X_1+Is(X_2*r+ep*X_3+\tau_B(-1+ae(1-es*ze+\alpha_{BS}*X_1))))}{ae*Id((es(r+\tau_B)+re)*V+\alpha_{BS}*\tau_B*\tau_L*X_1)+Is*(mr(X_2*rl+ae*es*V)+ep(ae*V+\tau_L*X_3)+\tau_B(ae*es*V+\tau_L(-1+ae(1-es*ze+\alpha_{BS}*X_1))))} \quad (26)$$

where; $X_1 = (-1 + es + es * ze)$, $X_2 = (-1 + ae * es)$, and $X_3 = (-1 + ae - ze)$.

The emergent steady-state CUE can be calculated from equation 1 where $EX = F_E + F_{UE}$ and U = F_U

$$\text{CUE} = \frac{F_U - R_U - R_B - R_E - F_E - F_{UE}}{F_U} \quad (27)$$

Sensitivity analyses

We tested the local sensitivity of steady-state CUE to the parameter values for assimilation efficiency, biomass-specific respiration rate, enzyme production per unit biomass, enzyme production per unit substrate consumed, and the respiration cost of enzyme production (*ae*

r, *ep, es,* and *ze* respectively). We calculated the parameter sensitivity value using the equation from Allison et al (2010):

$$\frac{|log_{10}(High\ output) - log_{10}(Low\ output)|}{|log_{10}(High\ parameter) - log_{10}(Low\ parameter)|} \quad (28)$$

For the sensitivity analysis, we increased *r* and *ep* by one order of magnitude over the reference values (Table 1), for *ze* we doubled the default, for *ae* we reduced the default value by half, and for *es* we increased the value from 0.0 to 0.0001 or 0.001. Sensitivity values were calculated for steady-state CUE for all output over the course of 150 simulated hours.

Assessing significance of explicitly modeling processes involved in CUE

In order to assess the significance of the mechanism behind CUE changes and its effect on modeled soil C pools, we calculated the emergent CUE and the equilibrium values for the SOC, DOC, biomass, and enzyme pools across a range of values for each microbial allocation parameter (i.e. *ae*, *r*, *ep*, *es*, and *ze*). For each parameter we simulated the range of values where SOC was between 0 and 100 mg C g^{-1} soil, all other C pools were greater than 0.001 mg C g^{-1} soil, and CUE was greater than 0.1.

Isotope model

To simulate a labeled substrate moving through the soil system over time, we created a parallel isotope model that simulates fluxes of carbon between isotopically labeled SOC, DOC, biomass, and enzyme pools (Si, Di, Bi, and Ei respectively). The isotope

model is similar in structure to the steady-state model described above but differs in three ways. First, in the isotope model there are no inputs into the SOC or DOC pools over time; the initial conditions of the DOC isotope (Di) pool are 0.1% of the equilibrium DOC pool, simulating a tracer addition of isotopically labeled labile substrate like glucose. Because we are modeling a tracer level addition over a short time period (150 hours), we assume recycling of the isotopic substrate from the SOC pool back into the DOC pool is negligible, so we do not include a decomposition flux (F_S) in the isotopic model. Finally, the model assumes that the isotopic label is completely assimilated in a half hour, with no abiotic sorption of the tracer, as seen in a tracer study of ^{14}C glucose (Glanville et al. 2016). The equations for microbial death (F_X), enzymatic production (F_E), enzymatic decay (F_L), respiration proportional to uptake (R_U), biomass-specific respiration (R_B), respiration from enzyme production (R_E) remain the same, however we substitute the steady-state pools S, D, B, and E with the isotopically labeled pools Si Di, Bi, and Ei respectively. Given these divergences the differential equation for the SOC isotopic pool is:

$$\frac{dSi}{dt} = F_X * \alpha_{BS} \quad (29)$$

and change in the isotopic DOC pool (Di) is modeled as:

$$\frac{dDi}{dt} = -V_{DI} * Di + F_X * (1 - \alpha_{BS}) + F_L \quad (30)$$

where V_{DI} is the uptake rate of the labeled DOC pool (Table 1). The differential equation for the isotopically labeled biomass pool is then:

$$\frac{dBi}{dt} = V_{DI} * Di - R_U - R_B - F_X - F_E - F_{UE} - R_E \quad (31)$$

and the isotopically labeled enzymatic pool is

$$\frac{dEi}{dt} = F_E + F_{UE} - F_L \quad (32)$$

We also tracked accumulation of isotopically labeled CO_2 over time; the differential equation is written as:

$$\frac{dCO_2 i}{dt} = R_U + R_B + R_E \quad (33)$$

We used the simulated amount of the label in the CO_2i, Bi, and Si pools over the course of 150 hours and estimated CUE_S, CUE_C, CUE_B (eqns. 6, 8, and 9 respectively.) We performed a parameter sensitivity analysis for each of these estimates under the same conditions as the sensitivity analysis for the steady-state CUE.

Results

At steady state the soil microbial CUE was 0.32 (Figure 2). Steady-state CUE was most sensitive to assimilation efficiency (*ae*); it was less sensitive to biomass-specific respiration (*r*) enzyme production per unit biomass (*ep*), respiration from enzyme

production (*ze*) and enzyme production per assimilated C (*es*) (Sensitivity values: 1.00, 0.70, 0.27, 0.12, and 0.004, respectively; Figure 3a).

Simulation of variation in mechanisms underlying CUE

The assimilation efficiency (*ae*) parameter was positively related to the MBC and enzyme-C pools as well as to the emergent CUE; it was negatively related to the DOC and SOC pools. As the value of *ae* decreased from 1 to 0.34 the microbial costs of uptake increased (Figure 4), and CUE decreased linearly from 0.45 to 0.15. Over this range of *ae* values, MBC decreased from 0.48 to 0.10 mg C g^{-1} soil and enzyme-C decreased from 2.67 x10^{-3} mg C g^{-1} soil to 5.8 x10^{-4}. In both the SOC and DOC pools, the C concentration increased nonlinearly as *ae* decreased; DOC increased from 0.02 to 0.06 mg C g^{-1} and SOC increased from 22 to 99 mg C g^{-1}.

The biomass-specific respiration (*r*) parameter had a positive relationship with the DOC and SOC pool and a negative relationship with enzyme-C, MBC, and CUE. As the value of *r* increased from 0 to 9.4 x10^{-4} mg C mg^{-1} C h^{-1}, CUE decreased from 0.57 to 0.15 and the rate of decline was highest at lower values of *r* (Fig. 4). Over this range of *r* values, MBC declined from 0.79 to 0.10 mg C g^{-1} soil and enzyme-C declined from 0.004 to 0.0005 mg C g^{-1} soil. The concentration of C in the DOC and SOC both increased with *r*, from 16 to 99 mg C g^{-1} soil in the SOC pool and 0.01 to 0.06 mg C g^{-1} soil in the DOC pool.

The enzyme production rate per unit biomass (*ep*) was varied from 2.32 x 10^{-6} to 1.26 x 10^{-4} mg C mg^{-1} C h^{-1}. Over this range of *ep* values, the DOC and enzyme C pools increased while SOC, MBC, and CUE declined. CUE declined from 0.33 to 0.10. The MBC pool declined from 0.29 to 0.07 mg C g^{-1} soil, and SOC declined exponentially from 99 to 4.8 mg C

g^{-1} soil. Enzyme-C and DOC concentration increased from 0.0007 to 0.0085 mg C g^{-1} soil in the enzyme pool and from 0.02 to 0.10 mg C g^{-1} soil in the DOC pool.

When the respiratory cost of enzyme production (*ze*) was increased from 0 to 46, the SOC and DOC pools increased while MBC, enzyme C, and CUE declined. CUE declined from 0.35 to 0.24, enzyme C declined from 0.002 to 0.001 mg C g^{-1} soil, and MBC decreased from 0.31 to 0.18 mg C g^{-1} soil. SOC and DOC both increased slightly as *ze* increased from 0.02 to 0.03 mg C g^{-1} soil in the DOC pool and from 32 to 53 mg C g^{-1} soil in the SOC pool.

We increased the proportion (*es*) of assimilated C that goes toward enzyme production from 0 to 0.061, which resulted in an increase in enzyme-C and DOC, but a decrease in SOC, MBC, and CUE. Over this range of *es* values, CUE declined from 0.32 to 0.10 mg C mg^{-1} C and MBC decreased linearly from 0.26 to 0.07 mg C g^{-1} soil. The SOC concentration decreased from 36.0 to 4.8 mg C g^{-1} soil, with the rate of decline decreasing as *es* increased. The enzyme C concentration increased from 0.001 to 0.008 mg C g^{-1} soil. DOC increased from 0.03 to 0.10 mg C g^{-1} soil and the rate of change increased as *es* increased.

Comparison between steady-state CUE and simulated isotopic measurements

The isotopic estimations of CUE were initially more than twice the steady-state CUE; all the isotopic estimates were 0.70 at the first hour but they declined over time (Figure 2). The formulation used to calculate CUE from the isotopic simulation affected the time sensitivity of the measurement: calculated CUE declined fastest over time when using the CUE_C equation, followed by the CUE_B and CUE_S equations. The differences, however, were small (0.65, 0.66, and 0.64 for CUE_B, CUE_S, and CUE_C respectively; Figure 2).

The isotopic estimations of CUE also diverged from the steady-state CUE in sensitivity to the processes involved. The isotopic estimates had comparable sensitivity to *ae* and *es* as the steady-state CUE (Figure 3). For the parameters: *r, ep,* and *ze,* the parameter sensitivities to the isotopic calculations of CUE were notably lower than the sensitivities of the steady-state CUE to the same parameter (Fig. 3a-d). While the sensitivities to these parameters increased slightly with incubation time, even at the end of the simulated time the isotopic measurement of CUE was 4 times lower than the steady-state CUE for *r*, 8 times lower for *ep,* and 10 times lower for *ze* (Figure 3).

Discussion

Mechanisms of CUE change are significant for models

We simulated steady-state SOC, DOC, MBC, enzyme C pools and the emergent CUE under a range of values for each of the microbial parameters: assimilation efficiency (*ae*), biomass specific respiration rate (*r*), enzyme production rate (*ep*), respiratory cost of enzyme production (*ze*), and enzyme production per unit of assimilated C (*es*). The relationship between CUE and the soil C pools (i.e. SOC, DOC, MBC, and Enzyme-C) depended on the mechanism setting the CUE value. For example, if CUE decreases due to a decline in assimilation efficiency (*ae*) the C concentration in the SOC pool increases. However, if CUE decreases because enzyme production (*ep*) increases, then SOC pool size declines (Figure 4). CUE determines the relationship between the substrate and biomass pools, and consequently the DOC pool size is the same across all mechanisms at any given CUE value. However, the sizes of the biomass, enzyme, and SOC pools can vary even when CUE is the same, depending on the microbial allocation strategy (i.e. how the C is

partitioned between assimilation losses, enzyme production, and maintenance respiration).

In the scenarios where assimilation efficiency (*ae*), biomass-specific respiration (*r*), or respiratory cost of enzyme production (*ze*) varied, the relationship between CUE and each of the four C pools was the same (Figure 4). In fact, at any given value of CUE the SOC, DOC, MBC, and Enzyme-C pool sizes are each identical across the three scenarios. In these scenarios, when CUE increased, the biomass pool size also increased because more C was available for growth. Enzyme production and concentration increased in proportion to the biomass with an increase in CUE. With more enzymes, there was greater decomposition resulting in a lower SOC pool size. The effects on the soil C pools from reduced assimilation efficiency, increased biomass-specific respiration, or increased respiratory cost of enzyme production are similar to previous studies that reduced CUE (Li et al. 2014).

A different pattern emerged when microbial C costs increased due to an increase in enzyme production, such as when enzyme production per unit biomass (*re*) or per assimilated C (*es*) increased (Figure 4). Under these scenarios, the enzyme pool increased while CUE declined, and the larger enzyme pool accelerated SOC decomposition. Although the MBC pool size also declined with lower CUE, the rate of decline was less than in the scenarios in which changes in *ae*, *r*, or *ze* caused the CUE decline. These simulation results demonstrate that at steady state, the relationship between CUE and the soil C pools is dependent on the pathway of microbial C costs (i.e. enzyme production or respiration).

Comparing isotopic estimates vs. steady-state CUE

In the isotopic simulations, the isotope did not equilibrate between the biomass and substrate pools. The isotope was assimilated completely at the start of the simulation, so all CO_2 respired from assimilation occurred immediately. However, the biomass-specific processes only began after the isotope had been assimilated. Thus, compared to steady state, the amount of CO_2 originating from biomass-specific processes was initially lower than the amount from assimilatory costs. Because the total amount of labeled CO_2 is used for isotopic estimates of CUE, these estimates were initially dominated by assimilation efficiency, with only minimal contribution from biomass-specific processes such as enzyme production and biomass-specific respiration (Figure 3). The sensitivity of the isotopic estimates to enzyme production as a function of uptake was low because the C flux was small relative to the other microbial C costs. Isotopic estimate sensitivity to biomass-specific costs increased with time because these costs continue to increase the absolute amount of labeled C that is respired and decrease the amount of label in the biomass.

These simulation results explain why isotopic measurements of CUE in soil often report high values (Frey et al. 2013, Kallenbach et al. 2015). They disproportionately weight the assimilatory C costs relative to the biomass-specific C costs (e.g. maintenance respiration, endogenous metabolism, respiratory costs of enzyme production). In addition, the increasing influence of biomass-specific costs over time may help explain the variability in CUE measurements across studies (Tucker et al. 2013, Hagerty et al. 2014). Soil CUE measurements show greater variability and higher average values than CUE estimates in systems where growth is calculated from the increase in cell abundance (Manzoni et al. 2012), as can be done in aquatic and pure culture studies (Eiler et al. 2003, James et al. 2017). Isotopic measurements consider all the C incorporated into the biomass to be

growth; however in studies where growth is estimated in a way that more closely approximates cell doubling, such as through label assimilation into DNA (as opposed to just biomass), values of CUE are lower because more energetic costs are incorporated, including the maintenance costs of the existing cells, production of exudates, and the energetic demand of cell division (Spohn et al. 2016a, Spohn et al. 2016b).

Our model simulations demonstrate that when CUE accounts for the total energetic costs of cell growth (i.e. including both assimilatory and biomass-specific costs) a CUE of 0.3 is possible even while the isotopic measurement yields an apparent CUE of 0.7 (Figure 2). Because isotopic measurements are made using a single addition of labeled substrate, the biomass and substrate pool are not in equilibrium. After assimilation, biomass-specific C costs will continue to reduce the isotopic concentration in the biomass—reducing apparent CUE—with the rate of this decline dependent on the magnitude of the biomass-specific costs. The apparent CUE will at one point in time be equivalent to the emergent CUE; however, biomass-specific processes will continue to reduce isotopic concentrations in the biomass until apparent CUE eventually reaches zero. Our results suggest that isotopic measurements of CUE made quickly after label addition actually quantify the assimilatory costs of microbial C uptake rather than emergent CUE.

Implications for models and measurements

In order to accurately represent microbial growth and the role of microbes in soil C dynamics, models need to represent microbial allocation processes (Schimel and Schaeffer 2012b). CUE is an emergent property resulting from these processes, so using a single CUE parameter in models is a major simplification, if a mathematically appealing one. In most

soil C models a CUE parameter partitions C between respiration and biomass (i.e. microbial explicit models) or another soil C pool (i.e. microbial implicit models). These formulations assume that CUE changes are predominantly a result of changing respiratory costs associated with uptake. Our simulations show that varying these model parameters, as they are currently formulated, is unlikely to account for effects on soil C stocks that result from a change in CUE via non-respiratory pathways (e.g. enzyme production).

In soil C models with explicit microbial controls, representing microbial allocation processes is critical for capturing dynamics of the microbial biomass pool. For example, most of these models represent CUE as assimilation efficiency; if biomass specific respiration is substantial and not represented separately, then the models will overestimate microbial growth. Microbial growth may also be overestimated if exudation products are not modeled as losses from the biomass. When the size of the microbial biomass pool determines decomposition rates (Allison et al. 2010), it is important to accurately capture total exudate loss. Additionally, if extracellular enzymes drive SOM or detritus breakdown, extracellular enzyme production must be considered separately from other types of microbial exudation. Microbes produce a wide variety of extracellular products such as antibiotics (Tyc et al. 2016), extracellular polysaccharides (Basler et al. 2015), and extracellular enzymes (Sinsabaugh and Shah 2012), but C costs aside from extracellular enzymes have been ignored in most models. The distinction between enzymes and other exudates is critical; extracellular enzyme exudation will accelerate decomposition, while non-enzyme exudation will not directly affect decomposition but may increase long-term soil C storage (Liang et al. 2017). It may be important to distinguish constitutive enzyme production, which may be biomass-specific, from inducible production

that is a function of substrate uptake, as these two fluxes likely have different controls and responses to changing environmental conditions or shifts in available substrates (Burns 1982, Allison and Vitousek 2005). Quantifying and modeling non-respiratory losses of C from the microbial pool (i.e. death and exudation) is likely the greatest challenge for incorporating microbial allocation processes into models, as these products are difficult to measure in situ and have variable effects on soil C dynamics.

Developing soil C models that can account for microbial C allocation pathways will require redirecting empirical research toward quantifying rates and sensitivities of microbial assimilation efficiency, biomass-specific respiration, enzyme production, and exudation, and away from lumping distinct processes into a single CUE value. This presents a considerable challenge, as these processes cannot yet be directly measured in soil; studies have likely focused on CUE because it is seemingly easier to measure than the individual processes involved (Ballantyne and Billings 2018). However we have shown here that most measurements of CUE are not capturing the same processes being modeled. In addition our results demonstrate that knowing CUE without some insight into the processes involved is not enough to confidently predict the resulting changes in soil C stocks. At identical emergent CUE values, changes in respiration cost of enzyme production, biomass-specific respiration rate, or assimilation efficiency produce the same effect on modeled C stocks, but emergent CUE is not equally sensitive to changes in these parameters. Because of this variable sensitivity, model predictions for soil C pools will be less responsive to temperature-induced shifts in maintenance respiration compared to changes in assimilation efficiency or the respiratory cost from enzyme production even if these parameters have the same fractional change per degree Celsius. Assuming

assimilation efficiency is temperature sensitive rather than maintenance respiration would cause models to overestimate the response of soil C stocks to temperature. In addition, Ballantyne and Billings (2018) found that whether respiration is modeled as function of biomass or as a function of uptake can also produce different transient responses in modeled SOC stocks, suggesting differences between these two processes might be even larger under dynamic environmental conditions like those modeled at the Earth system level. Currently most of the data on soil microbial CUE comes from studies using the C isotopic tracer method, which mainly reflects microbial assimilation efficiency. The potential response of other microbial allocation processes to environmental change is therefore underexplored and the consequent impacts on model predictions are poorly constrained.

Conclusions

Earlier model simulations have demonstrated that assumptions about CUE can have major effects on SOC predictions (Allison et al. 2010, Wieder et al. 2013, Li et al. 2014). We found that the relationship between CUE and SOC is dependent on the particular microbial allocation strategy and is sensitive to the methods used to measure it—our short-term isotopic modeling gave values that were up to twice the steady-state microbial CUE.

To generate accurate model predictions at large scale under dynamic conditions, the concept of CUE should be updated to reflect an emergent property that accounts for microbial growth and C allocation strategy. Modelers and empiricists should therefore focus on constraining the environmental sensitivity of the individual processes involved in microbial CUE to predict microbial physiology effects on soil C stocks.

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Table 1 Default parameter description, units, and default values.

Fig. 1 Schematic of the Allison Wallenstein Bradford model with our modifications of adding a biomass specific respiration rate (R_M), enzyme production respiration (R_E), and uptake driven enzyme production (F_{UE}).

Fig. 2 Steady-state CUE and calculations of isotopic CUE simulated over 150 hours. Isotopic CUE calculated with three different formulations (i.e. CUE_B, CUE_C, CUE_S) using the simulated concentrations of label in the biomass pool (Bi), respired (CO2i), or the change in label concentration in the extractable C pool from the start of the incubation to the time of measurement.

Fig 3 Sensitivity of the a) steady-state CUE and isotopic calculations; b) CUE_B, c) CUE_C, and d) CUE_S to changes in the model parameters. Larger sensitivity values indicate greater sensitivity of the CUE value to changes in the specified parameter.

Fig. 4 The response of modeled equilibrium concentrations for SOC, MBC, DOC, enzyme-C pools and emergent CUE in response to changing microbial parameter values; assimilation efficiency (*ae*), biomass-specific respiration (*r*), enzyme production rate (*ep*), respiratory cost of enzyme production (*ze*), and proportion of assimilated C to enzyme production (*es*). A decline in *ae* increases microbial C costs while for all other parameters increasing parameter value also increases microbial C costs.

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Fig. 1

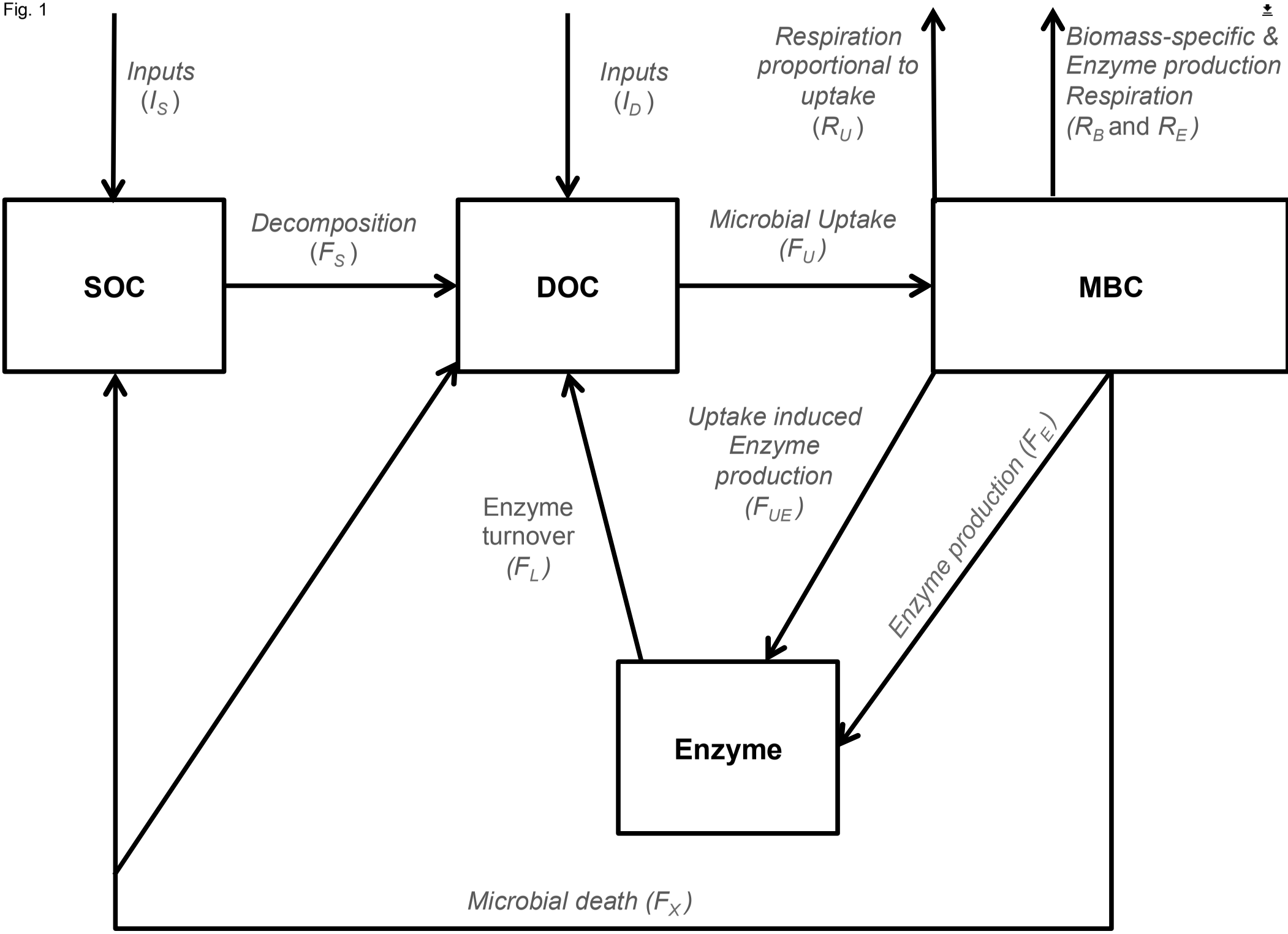
Inputs (I_S)
Inputs (I_D)
Respiration proportional to uptake (R_U)
Biomass-specific & Enzyme production Respiration (R_B and R_E)
SOC
Decomposition (F_S)
DOC
Microbial Uptake (F_U)
MBC
Uptake induced Enzyme production (F_{UE})
Enzyme turnover (F_L)
Enzyme production (F_E)
Enzyme
Microbial death (F_X)

Fig. 2

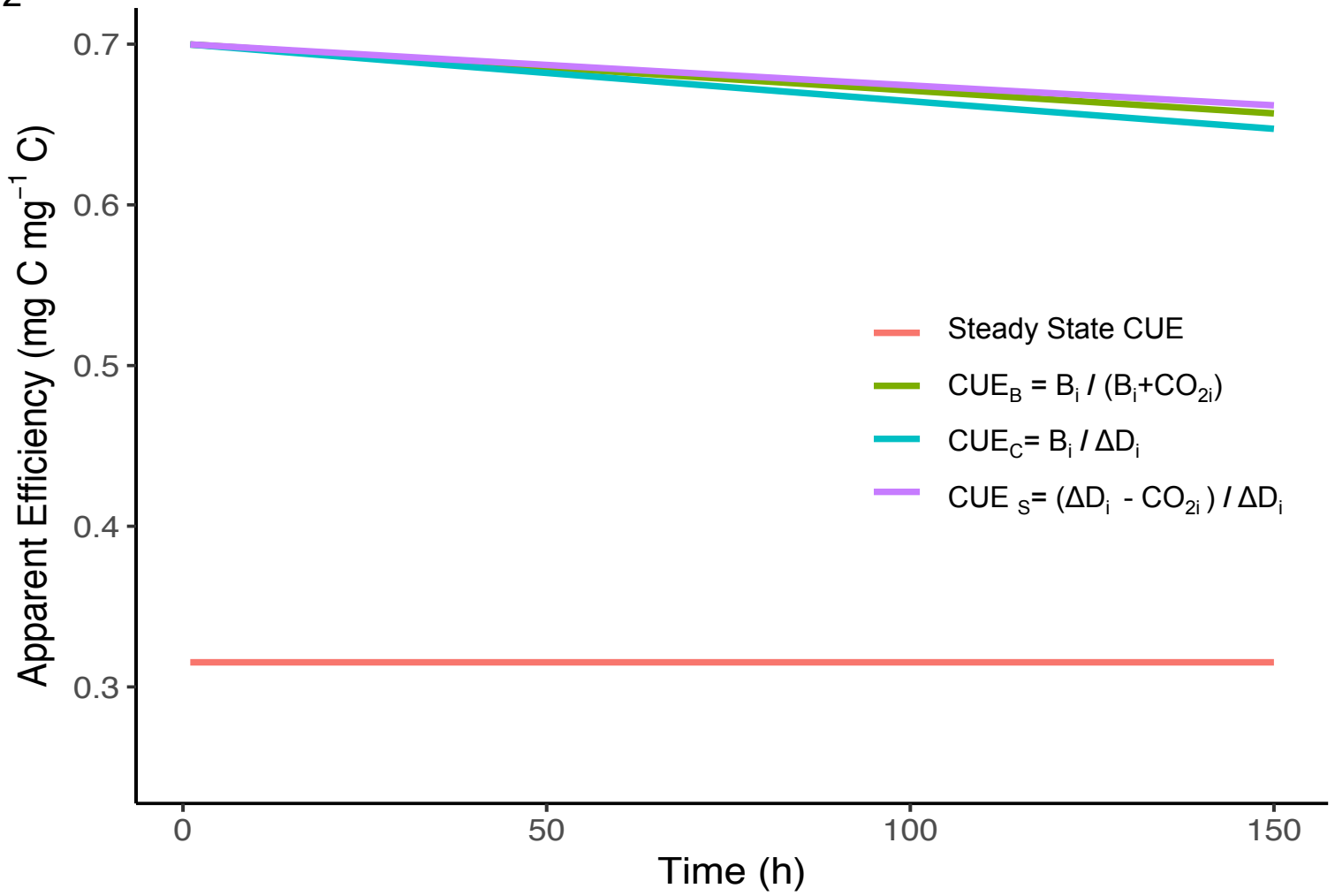
0.7
0.6
0.5
0.4
0.3
Apparent Efficiency (mg C mg^{-1} C)
0
50
100
150
Time (h)
Steady State CUE
$CUE_B = B_i / (B_i+CO_{2i})$
$CUE_C = B_i / \Delta D_i$
$CUE_S = (\Delta D_i - CO_{2i}) / \Delta D_i$

Fig. 3

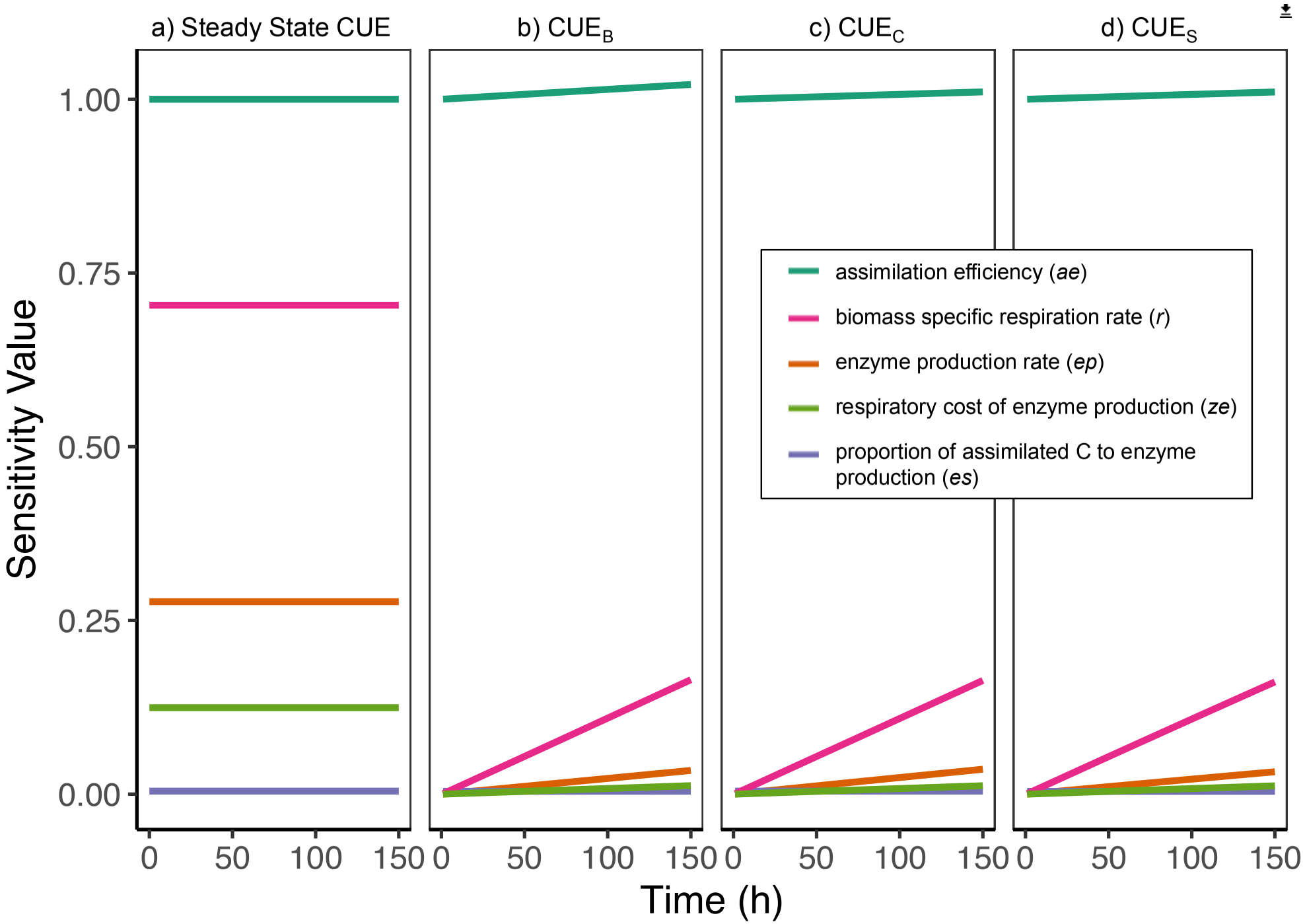
a) Steady State CUE
b) CUE$_B$
c) CUE$_C$
d) CUE$_S$
Sensitivity Value
1.00
0.75
0.50
0.25
0.00
0
50
100
150
Time (h)
assimilation efficiency (*ae*)
biomass specific respiration rate (*r*)
enzyme production rate (*ep*)
respiratory cost of enzyme production (*ze*)
proportion of assimilated C to enzyme production (*es*)

Fig. 4

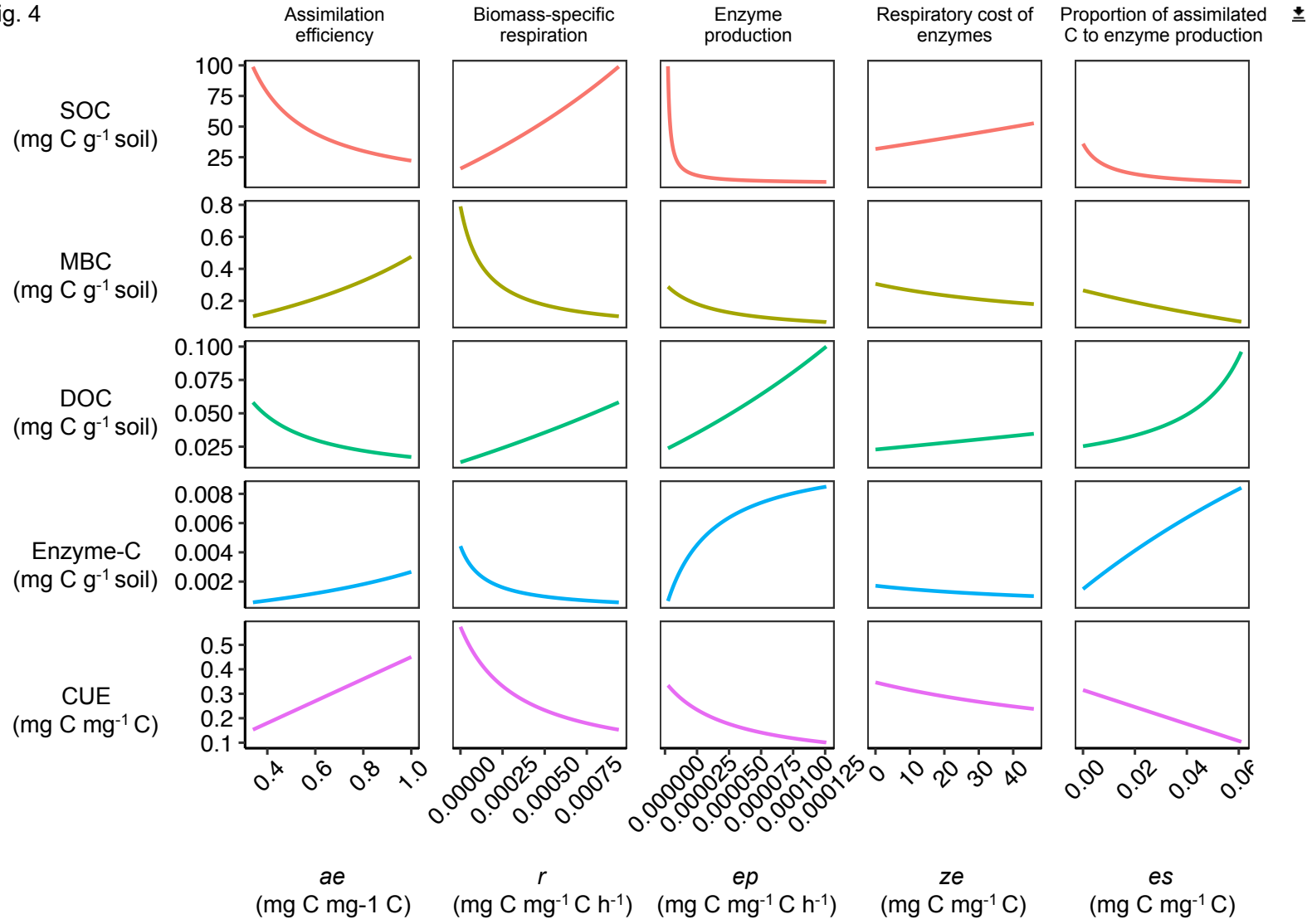
Assimilation efficiency
Biomass-specific respiration
Enzyme production
Respiratory cost of enzymes
Proportion of assimilated C to enzyme production
SOC (mg C g-1 soil)
MBC (mg C g-1 soil)
DOC (mg C g-1 soil)
Enzyme-C (mg C g-1 soil)
CUE (mg C mg-1 C)
100
75
50
25
0.8
0.6
0.4
0.2
0.100
0.075
0.050
0.025
0.008
0.006
0.004
0.002
0.5
0.4
0.3
0.2
0.1
0.4
0.6
0.8
1.0
0.00000
0.00025
0.00050
0.00075
0.000000
0.000025
0.000050
0.000075
0.000100
0.000125
0
10
20
30
40
0.00
0.02
0.04
ae (mg C mg-1 C)
r (mg C mg-1 C h-1)
ep (mg C mg-1 C h-1)
ze (mg C mg-1 C)
es (mg C mg-1 C)

Table 1

Parameter	Description	Value	Unit
I_S	SOC input rate	0.00015	mg C g^{-1} soil h^{-1}
I_D	DOC input rate	0.00001	mg C g^{-1} soil h^{-1}
V	SOC D V_{max}	1	mg C mg^{-1} C h^{-1}
K	SOC K_M	250	mg C g^{-1} soil
V_U	DOC uptake V_{max}	0.01	mg C mg^{-1} MBC h^{-1}
K_U	DOC uptake K_M	0.26	mg C g^{-1} soil
ae	Assimilation efficiency	0.7	
r	Biomass-specific respiration rate	0.00028	mg C mg^{-1} C h^{-1}
τ_B	Microbial death rate	0.00028	mg C mg^{-1} C h^{-1}
$_{ep}$	Enzyme production rate per unit biomass	0.0000056	mg C mg^{-1} C h^{-1}
τ_L	Enzyme loss rate	.001	mg C mg^{-1} C h^{-1}
α_{BS}	Fraction of dead MBC partitioned to SOC	0.5	
V_{DI}	Uptake rate of isotopic tracer	2	mg C mg^{-1} C h^{-1}
ze	Respiration per unit enzyme-C produced	10	mg C mg^{-1} C
es	Enzyme production per unit assimilated C	0	mg C mg^{-1} C