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Clarifying the interpretation of carbon use efficiency in soil through methods comparison

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ABSTRACT

Accurate estimates of microbial carbon use efficiency (CUE) are required to predict how global change will impact microbially-mediated ecosystem functions such as organic matter decomposition. Multiple approaches are currently used to quantify CUE but the extent to which estimates reflect methodological variability is unknown. This limits our ability to apply or cross-compare published CUE values. Here we evaluated the performance of five methods in a single soil under standard conditions. The microbial response to three substrate amendment rates (0.0, 0.05, and 2.0 mg glucose-C g^{-1} soil) was examined using: ^{13}C and ^{18}O isotope tracing approaches which estimate CUE based on substrate uptake and growth dynamics; calorespirometry which infers growth and CUE from metabolic heat and respiration rates; metabolic flux analysis where CUE is determined as the balance between biosynthesis and respiration using position-specific $^{13}CO_2$ production of labeled glucose; and stoichiometric modeling which derives CUE from elemental ratios of microbial biomass, substrate, and exoenzyme activity. The CUE estimates we obtained differed by method and substrate concentration, ranging under *in situ* conditions from < 0.4 for the substrate-nonspecific methods that do not use C tracers (^{18}O, stoichiometric modeling) to > 0.6 for the substrate-specific methods that trace glucose use (^{13}C method, calorespirometry, metabolic flux analysis). We explore the different aspects of microbial metabolism that each method captures and how this affects the interpretation of CUE estimates. We recommend that users consider the strengths and weaknesses of each method when choosing the technique that will best address their research needs.

1. Introduction

Microorganisms exert control over soil organic matter (SOM) decay and stabilization as a consequence of their carbon use efficiency (CUE), the proportion of substrate C that a microbe commits towards growth relative to other processes such as respiration. CUE is thus a critical parameter directing C resources between soil organic matter pools like microbial biomass versus mineralized CO_2, with potential effects on the soil C balance (Frey et al., 2013; Kallenbach et al., 2016).

There is wide variability in reported soil CUE estimates and understanding the sources of this variability is critical to accurately predict soil C dynamics. Variability falls into two general categories: genetic and environmental. Genetic drivers of CUE are perhaps most evident in cultured strains of individual taxa, where inherent species-specific characteristics determine CUE (CUE_P; Geyer et al., 2016). For example, it has been hypothesized that a tradeoff exists among microbes between growth rates and CUE (Pfeiffer et al., 2001; Molenaar et al., 2009). Evidence for this, however, is limited to a small number of culturable species (Keiblinger et al., 2010; Roller and Schmidt, 2015). Saprotrophic bacteria and fungi are subjected to additional environmental factors in soil that influence CUE, including fluctuating temperatures (Steinweg et al., 2008; Frey et al., 2013), substrate quality or quantity (Frey et al., 2013; Sinsabaugh et al., 2013; Blagodatskaya et al., 2014), and interspecific interactions (Maynard et al., 2017). Taken together, genetic sources of variability represent the bounds of potential microbial metabolic and physiological responses (and hence CUE), while environmental sources of variability represent the external conditions under which the former must operate.

An underexplored third source of variability is methodological (Manzoni et al., 2012). Current methods for estimating CUE depend on various assumptions and capture different aspects of microbial metabolism. For example, ^{13}C and ^{18}O labeling approaches measure

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substrate incorporation into biomass (Brant et al., 2006) and DNA (Blazewicz and Schwartz, 2011; Spohn et al., 2016a), respectively, but are deployed over short incubations to avoid microbial exudation effects. Both methods are also influenced by microbial turnover (Hagerty et al., 2014). Calorespirometry measures soil heat rate relative to respiration rate to indicate growth dynamics and CUE. Calculating CUE from this method requires knowledge of the oxidation states of both microbial biomass and substrate C (Hansen et al., 2004; Barros et al., 2016). Metabolic flux analysis (Dijkstra et al., 2011b, 2015) measures patterns of ^{13}C position-specific CO_2 production and from that calculates the partitioning between biosynthesis and respiration rates. This method does not integrate the effects of microbial turnover or account for the fate of exuded compounds. Finally, stoichiometric modeling uses extracellular enzyme activities and the C:N ratio of microbial biomass and bioavailable C to calculate the CUE necessary to satisfy microbial C and N demands (Sinsabaugh et al., 2016). These calculations can be performed using widely available data, but the approach has not been applied in the experimental context used by other methods.

While the range in published CUE estimates may reflect the diversity of soil communities and environmental heterogeneity, methodological differences have not been fully investigated. Here we measured CUE under identical conditions using the five methods described above: ^{13}C incorporation into microbial biomass; ^{18}O incorporation into DNA; calorespirometry; metabolic flux analysis; and stoichiometric modeling. A range of soil glucose amendments was used to elicit a gradient of microbial growth conditions over which methodological performance could be compared. Our goals were to describe methodological variability in CUE, evaluate underlying assumptions of each method, and clarify the interpretation of CUE estimates to inform methods selection for future studies.

2. Methods

2.1. Soil and laboratory conditions

Mineral soil (0–15 cm depth) of the Gloucester series (fine loamy, mixed, mesic, typic dystrochrepts) was collected from the Harvard Forest Long Term Ecological Research (LTER) site in Petersham, MA, USA. Soil was sieved (< 2 mm) in the field and stored at 4 °C in Ziploc bags until analysis. Gravimetric water content (0.47 g g^{-1}) was determined by drying at 60 °C for 48 h. Field capacity (0.90 g g^{-1}) was determined in the same manner from saturated soil after gravity draining for 48 h. Soil organic carbon (5.67% of dry soil), microbial biomass concentrations (1% of SOC), pH (4.2) and C:N ratio (24.0) were typical for these soils (Frey et al., 2014). All soil was pre-incubated for 48 h at 25 °C before experimentation.

At the end of the pre-incubation period (time zero; T0), soils received a glucose amendment (0.0, 0.05, or 2.0 mg glucose-C g^{-1} dry soil) that included nutrients (0.1% $MgCl_2$, 0.2% KH_2PO_4, and 0.1% K_2SO_4) and sufficient sodium nitrate to achieve a C:N ratio = 40 (when glucose was present) (Wadso, 2009). Amendment increased soil moisture to 65% of field capacity. The low glucose concentration (0.05 mg) was chosen specifically to represent a typical substrate amendment rate used in other tracer experiments (Frey et al., 2013) whereas high (2.0 mg) glucose amendment was used to induce microbial growth (Reischke et al., 2014).

2.2. CUE methods

Five methods were chosen for comparison: ^{13}C-glucose tracing, ^{18}O-water tracing, calorespirometry, metabolic flux analysis, and stoichiometric modeling. Measurements were made for the ^{13}C, ^{18}O, and calorespirometry methods at 6, 12, 18, 24, 32, 40, 48, 60, and 72 h after amendment but less frequently with metabolic flux analysis (6, 24, 72 h) and the stoichiometric modeling approach (24, 48, 72 h). All estimates of growth, respiration, and CUE were calculated using published mixing models and equations as described in more detail below for each method. An important distinction among these methods is that calorespirometry infers net growth whereas the ^{13}C, ^{18}O, and metabolic flux analysis methods measure gross growth through short-term isotopic enrichment. Not all processes that are responsible for biomass loss are avoidable, however; we discuss below how these and other processes influence CUE particularly over longer incubations.

2.2.1. ^{13}C-glucose tracing

This method traces the uptake and mineralization of ^{13}C labeled substrates, where growth is inferred from ^{13}C incorporation into microbial biomass (Brant et al., 2006; Frey et al., 2013). For this approach, two amendment solutions were prepared using the nutrient solution described above, differing only in final glucose concentration (0.05 and 2.0 mg glucose-C g^{-1} soil). Universally labeled 99 at% ^{13}C-glucose was diluted with unlabeled glucose to achieve total glucose enrichment of 5 at%. Five replicate soil samples (40 g each) and one soil control (nutrient solution without glucose) were prepared for each combination of glucose concentration and incubation length. Amendments were briefly mixed by spatula into the soil. Specimen cups (60 mL) of soil were parafilm covered and incubated at 25 °C for up to 72 h.

Ninety minutes prior to harvest, cups were uncovered and sealed within 1 L jars fitted with rubber septa. Jars were flushed for 15 min with CO_2-free air. At the time of harvest, 15 mL of headspace was sampled via syringe and injected into evacuated exetainers (12 mL). Jars were then opened and the soil immediately extracted for microbial biomass by chloroform fumigation extraction (Vance et al., 1987). Briefly, 15 g of each sample was fumigated with ethanol-free chloroform for 24 h, after which the sample was extracted in 0.05M K_2SO_4. A paired 15 g sample was immediately extracted without fumigation. Total dissolved organic C (DOC) extracts were frozen at −20 °C and shipped, along with headspace samples, to the University of California (Davis) Stable Isotope Facility (Davis, CA, USA) for total C and ^{13}C quantification. The difference in total DOC concentration between fumigated and non-fumigated samples was used to calculate microbial biomass C (extraction efficiency $K_{EC} = 0.45$). Remaining soil was frozen at −20 °C and later dried and finely ground for determination of total soil C and N, and ^{13}C quantification at the University of New Hampshire Stable Isotope Facility (Durham, NH, USA).

Total microbial growth (^{13}MBC; μg C g^{-1} soil) was calculated as the product of total microbial biomass (*F DOC – NF DOC*; μg C g^{-1} soil) and the percent of total microbial biomass labeled (*% ^{13}MBC*):

$$at\%\ MBC = \frac{[(at\%\ F\ DOC \times F\ DOC) - (at\%\ NF\ DOC \times NF\ DOC)]}{(F\ DOC - NF\ DOC)} \quad (1)$$

$$\%\ ^{13}MBC = \frac{(at\%\ MBC_t - at\%\ MBC_c)}{(at\%\ sol - at\%\ MBC_c)} \times 100 \quad (2)$$

$$^{13}MBC = (F\ DOC - NF\ DOC) \times \%\ ^{13}MBC \div 100 \quad (3)$$

CUE was calculated as follows:

$$CUE = \frac{^{13}MBC}{(^{13}MBC + {}^{13}R)} \quad (4)$$

where *at% F DOC*, *F DOC*, *at% NF DOC*, and *NF DOC* represent the atom % and total C concentrations (μg C g^{-1} soil) of fumigated (F) and non-fumigated (NF) K_2SO_4 extracts, respectively. *At% MBC_t* and *at% MBC_c* are the atom % of sample treatments and natural abundance controls, and *at% sol* is the atom % of amendment solution (5 at%). *^{13}R* is the cumulative respiration derived from added glucose (μg $^{13}C0_2$—C g^{-1} soil) using a CO_2 flux curve assembled from all respiration rates gathered from the nine samplings over 72 h.

2.2.2. ^{18}O-water tracing

This method uses the incorporation of ^{18}O-labeled water into DNA to measure gross growth (Blazewicz and Schwartz, 2011; Spohn et al., 2016b). Pre-incubated soil (3 g) was weighed into 26 mL glass vials. Two amendment solutions were prepared using the nutrient solution referenced above, differing only in final glucose concentration (0.05 or 2.0 mg glucose-C g^{-1} soil). Enriched (~97 at%) ^{18}O-water was diluted with unlabeled deionized water to achieve total amendment enrichment of 5 at% (2.0 mg glucose) or 50 at% otherwise. Five replicate soil treatments and one soil control (nutrient solution without glucose) were prepared for each combination of glucose concentration (0.05 and 2.0 mg) and incubation length (same as with ^{13}C method). Five additional soil controls with no glucose (0.0 mg) were each harvested at 24, 48, and 72 h. Amendments were briefly mixed by spatula into the soil. Each vial of soil was covered with parafilm and incubated at 25 °C for up to 72 h.

Three hours prior to harvest, vials were sealed with rubber septa and flushed for 5 min with CO_2-free air to remove all CO_2. At the time of harvest, ~3 mL of vial headspace (26 mL total) was sampled via syringe and injected directly into a benchtop CO_2 detector to estimate respiration rate (LI-COR 6252, Lincoln, NE USA). Two grams of soil was subsampled from each vial and flash frozen to arrest further microbial activity. Soils were kept frozen at −80 °C until DNA extraction. DNA extraction followed the manufacturer's protocol (Qiagen DNeasy PowerSoil Kit, Venlo, Netherlands) with the following modifications to quantitatively estimate total soil DNA concentrations: 1) no supernatant was discarded during extraction, 2) centrifugation time was extended to 1 min after bead beating to facilitate separation of DNA from co-extracted materials, 3) all solution volumes were adjusted to maintain the ratio of extractant:solution detailed in the manufacturer's protocol, and 4) an additional rinse with 600 µL of 96% ethanol was performed after rinsing the spin filter. DNA extracts were dried overnight in silver encapsulation tins at 60 °C. Diluted salmon sperm DNA (1.0 µg $µL^{-1}$) was spiked into samples to bring total oxygen mass within the detectable range, dried overnight, and sent to the UC Davis Stable Isotope Facility for $\delta^{18}O$ quantification.

Calculations for this method were based on Spohn et al. (2016a). Atom % of soil DNA oxygen (*at% O_{soil}*) was found using a two-pool mixing model where *at% $O_{soil+ss}$*, *$O_{soil+ss}$*, *at% O_{ss}*, *O_{ss}*, and *O_{soil}* represent the atom % and oxygen mass (µg) of extracted soil DNA and salmon sperm DNA pools:

$$at\%\ O_{soil} = \frac{[(at\%\ O_{soil+ss} \times O_{soil+ss}) - (at\%\ O_{ss} \times O_{ss})]}{O_{soil}} \quad (5)$$

Atom % excess of soil DNA oxygen (*APE O_{soil}*) was calculated as the difference between atom % of oxygen in the presence of ^{18}O water (*at% $O_{soil\ t}$*) and paired control samples in the presence of natural abundance water (*at% $O_{soil\ c}$*):

$$APE\ O_{soil} = at\%\ O_{soil\ t} - at\%\ O_{soil\ c} \quad (6)$$

Total microbial growth (*^{18}O*; µg O) was estimated as the product of soil DNA oxygen content (*O_{soil}*; µg O) and *APE O_{soil}*. *At% Total*, the final soil water enrichment after amendment, provides a correction for the diluting effect of native soil moisture:

$$^{18}O = (O_{soil} \times APE\ O_{soil} \div 100) \times \frac{100}{at\%\ Total} \quad (7)$$

Microbial growth was scaled to C (*^{18}MBC*; µg C g^{-1} soil) by applying the conversion factors of 0.31 (oxygen:DNA mass ratio) and the average ratio of MBC:DNA for all replicates at each harvest (where MBC represents chloroform fumigated biomass), assuming that only water-derived oxygen is used in biosynthesis.

$$^{18}MBC = {}^{18}O \times \frac{1}{0.31} \times \frac{MBC_{soil}}{DNA_{soil}} \times \frac{1}{soil\ mass} \quad (8)$$

CUE was calculated as follows:

$$CUE = \frac{^{18}MBC}{(^{18}MBC + R)} \quad (9)$$

R is the cumulative respiration (µg $C0_2$–C g^{-1} soil) measured at the time of harvest.

2.2.3. Calorespirometry

Soil heat rate is proportional to net microbial growth (Wadso, 2009; Barros et al., 2016) and the ratio of heat rate to respiration rate (calorespirometric ratio) is related to CUE (Hansen et al., 2004). In this study, heat rate was estimated simultaneously from the 3 g subsample used for the ^{18}O method (see above). Sample vials were flushed with CO_2-free air 3 h prior to harvest, then placed in an isothermal microcalorimeter (CSC 4400, Lindon, UT USA) to equilibrate at 25 °C for 30 min. Two vials of treated soil (0.0, 0.05, or 2.0 mg glucose-C g^{-1} soil) were analyzed per run, along with one soil control (nutrient solution without glucose). After equilibration, heat rate was recorded for 150 min. At the time of harvest, vials were removed from the calorimeter and respiration rate estimated by injecting 3 mL of headspace gas (26 mL total) into a CO_2 detector (LI-COR 6252, Lincoln, NE USA). Microcalorimeter capacity permitted only two estimates per time point.

Calorespirometry uses a ratio of heat production (*R_q*; kJ g^{-1}) and respiration (*R_{CO2}*; mol CO_2–C g^{-1}) known as the calorespirometric ratio (CR; kJ mol^{-1} CO_2–C) to infer CUE through the following relationship (Hansen et al., 2004):

$$\frac{R_q}{R_{CO_2}} = 469\left(1 - \frac{\gamma_s}{4}\right) - 115(\gamma_s - \gamma_{MB})\left[\frac{CUE}{(1 - CUE)}\right] \quad (10)$$

where 469 (kJ mol^{-1} O_2) is the oxycaloric equivalent for aqueous glucose combustion, γ_s - γ_{MB} is the difference between C oxidation states in substrate (glucose = 0; Barros et al., 2016) and microbial biomass (−0.3; von Stockar and Liu, 1999), and 115 is the average energy loss (kJ) per change in oxidation state of C during the conversion of substrate to microbial biomass (Kemp, 2000). Derivation of Eq. (10) depends on oxidative production of CO_2 via catabolic or anabolic processes that result in R_q/R_{CO2} estimates in the range of 250–469 kJ mol^{-1} C. Values below this range indicate anaerobic respiration, whereas values above may indicate metabolism of highly reduced substrates other than carbohydrates, or incomplete oxidation of substrate to CO_2 (Hansen et al., 2004). Heat and respiration rates were integrated over time to calculate calorespirometric ratios such that estimates were comparable to the cumulative CUE estimates of other methods; these did not vary significantly from instantaneous rates. See Supplementary Material for a rearrangement of Eq. (10) solving for CUE.

2.2.4. Metabolic flux analysis

Metabolic flux analysis measures CO_2 production for individual C atoms using position-specific ^{13}C-labeled substrates (Dijkstra et al., 2011a). After 48 h pre-incubation, soil (15 g) was amended with one of three solutions as described above. At 6 and 72 h (0.0 mg glucose) or 6, 24, and 72 h (0.05 or 2.0 mg glucose) after amendment, 1 mL of 1–^{13}C and U–^{13}C glucose and 1–^{13}C and 2,3–^{13}C pyruvate was added (8.57 µg C g^{-1} soil) in parallel incubations (n = 4). Before the isotopomer solution was added, the headspace atmosphere was refreshed, 5 mL of pure CO_2 was added, and a sample of the headspace was taken. Twenty, 40 and 60 min after isotopomer addition, additional headspace samples were taken. All gas samples were analyzed on a CO_2 isotope spectrometer (Picarro G2201-*i*). Samples were diluted with CO_2-free air to ensure enough volume for 4–5 min of measurement. We calculated the slope of the $^{13}CO_2$ concentration over time (40 min) and the ratios of slopes of U–^{13}C/1–^{13}C glucose and 1–^{13}C/2,3–^{13}C pyruvate $^{13}CO_2$ production. CUE was estimated after matching observed isotopomer

ratios with ratios produced by a metabolic flux model (Dijkstra et al., 2011b), as

$$CUE = \frac{6 \times v1 - \Sigma CO_2}{6 \times v1} \quad (11)$$

with *v1* being the rate of uptake of glucose-C in the model and ΣCO_2 the sum of all C lost as CO_2 by pyruvate dehydrogenase, gluconate dehydrogenase, isocitrate dehydrogenase and alpha-ketoglutarate dehydrogenase. The model itself consists of glycolysis, TCA cycle, pentose phosphate pathway and anaplerotic reactions (Dijkstra et al., 2011a).

2.2.5. Stoichiometric modeling

Stoichiometric modeling of CUE is based on the difference between the elemental C, nitrogen (N), and phosphorus (P) composition of microbial biomass and the elemental composition of the labile substrate pool (Sinsabaugh et al., 2016). Substrate uptake is related to the activities of indicator enzymes, whose activities are modulated in relation to substrate availability by feedbacks that regulate enzyme synthesis at the transcription level. The relative distribution of activities at the community scale is assumed to reflect optimum resource allocation in relation to substrate availability and growth requirements (Sinsabaugh and Shah, 2012; Sinsabaugh et al., 2015). The indicator enzymes most commonly used are β-1,4-glucosidase (BG), leucine aminopeptidase (LAP), β-1,4-N-acetylglucosaminidase (NAG), and acid (alkaline) phosphatase (AP) which mediate the acquisition of C (BG), N (LAP, NAG) and P (AP) from the largest environmental substrate pools. CUE is calculated as a scalar ratio ($S_{C:X}$) fitted to a Michaelis-Menten model.

$$S_{C:X} = \frac{B_{C:X}}{L_{C:X}} \times \frac{1}{EEA_{C:X}} \quad (12)$$

$$CUE_{C:X} = CUE_{max} \times \frac{S_{C:X}}{(S_{C:X} + K_X)} \quad (13)$$

where $B_{C:X}$ is the molar C:X ratio of microbial biomass (X = N or P), $L_{C:X}$ is the molar C:X ratio of labile substrate, and $EEA_{C:X}$ is the ratio of BG/(LAP + NAG) when X = N, and BG/AP when X = P. CUE_{max} is set at 0.6 based on thermodynamic constraints (Roels, 1980); K_X is the half-saturation constant set at 0.5.

For this study, soils were prepared and amended with 0.0, 0.05 or 2.0 mg glucose-C g^{-1} soil, and microbial biomass C and N were estimated by chloroform fumigation and extraction, as described above. Labile substrate availability for C and N was measured as the quantity of DOC and N extracted from non-fumigated control samples in chloroform fumigation analyses. Indicator enzyme activities were measured at pH = 5 using fluorescent substrate analogs (Sinsabaugh and Shah, 2012). Five replicates of CUE were estimated after 24, 48, and 72 h.

2.3. Statistical analysis

Pearson correlation was used to examine the relationships among results of methods over time for each glucose treatment. Analysis of variance (ANOVA) was used to test for significant two-way effects of glucose concentration (e.g., glucose × duration interaction) and method (e.g., method × duration interaction) on responses (e.g., growth, CUE) modeled with generalized least squares analysis. Models including glucose concentration were often dominated by the effect of the high glucose treatment, and so we also examined the main effects of time for response variables individually. Piecewise regression of log-transformed growth was used to distinguish growth phases with significantly different slopes using the SEGMENTED R package (Muggeo, 2008). Sensitivity analyses were performed using the RELAIMPO R package (Gromping, 2006). We considered CUE undefined for the isotopic methods when average growth (and thus CUE) was negative (see Supplementary Material for further discussion). This occurred only within the first ~24 h after high (2.0 mg) glucose amendment when MBC estimates exhibited large standard deviations. All analyses were performed with R (R Development Core Team, 2008).

3. Results

Microbial growth (e.g., ^{13}C- or ^{18}O-labeled biomass) and CUE estimates were affected by the method used and glucose amendment rate. In general, CUE at 24 h of incubation ranged from ~0.20 (^{18}O method) to ~0.70 (^{13}C method and metabolic modeling) when glucose amendment was low. Increasing the glucose amendment generally reduced growth and CUE according to the ^{13}C, ^{18}O, and stoichiometric modeling approaches. However, CUE was insensitive to glucose treatment when estimated by metabolic flux analysis. Specific results for each method are detailed below. All measured and calculated data can be found in Supplemental Table 1.

3.1. ^{13}C-glucose tracing

Microbial growth (^{13}MBC) and CUE estimated by this method were significantly affected by the interaction of glucose treatment and incubation duration. Under low (0.05 mg) glucose amendment, ^{13}C enriched biomass remained constant over time suggesting that new

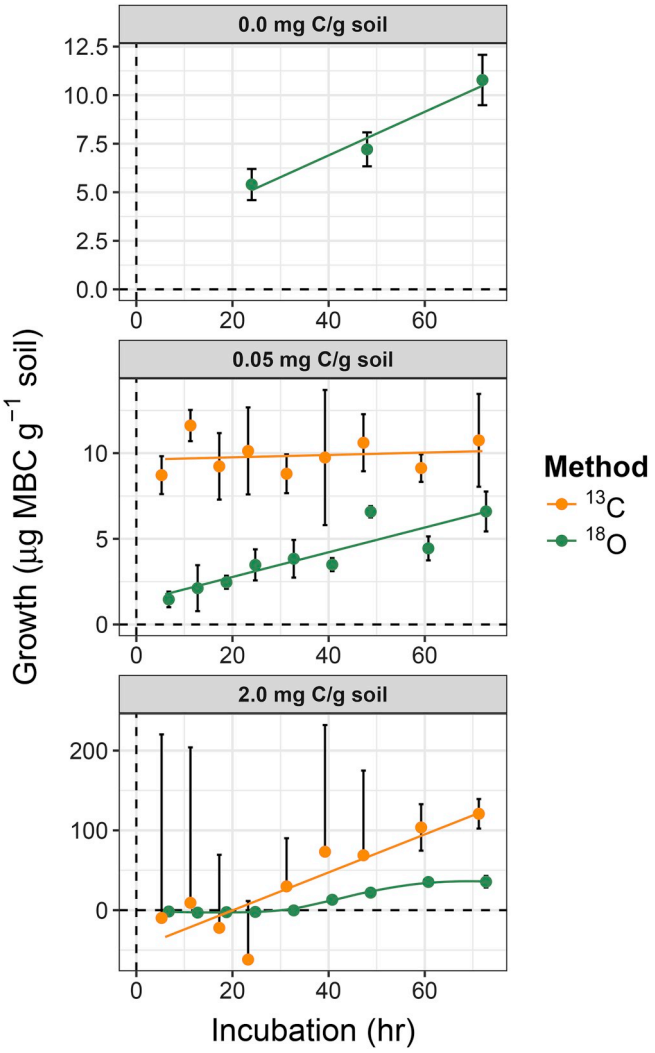

Fig. 1. Growth of enriched microbial biomass over 72 h following glucose amendment of 0.0, 0.05, or 2.0 mg C g^{-1} soil, as measured by the ^{13}C and ^{18}O techniques. Means are dodged to prevent overlapping data. Error bars represent one standard deviation; bars are one-sided for the ^{13}C method from 0 to 48 h in order to avoid distortion on the y-axis. N = 5.

growth was counterbalanced by losses of ^{13}C from the biomass pool (Fig. 1). CUE, however, declined over time ($-0.003\ h^{-1}$; $p < 0.001$) from ~0.75 to ~0.50 (Fig. 2) because of increasing cumulative ^{13}C respiration ($0.076\ \mu g\ ^{13}CO_2$–C $g^{-1}\ h^{-1}$; $p < 0.001$). With high glucose amendment, growth increased linearly over time at a constant rate ($2.37\ \mu g$ MBC $g^{-1}\ h^{-1}$; $p = 0.008$) but ^{13}MBC was not significantly different from zero until 60 h. Microbial biomass was difficult to detect in the presence of high background DOC (i.e., unused glucose) until substantial microbial utilization began after ~24 h of incubation. As a result, average CUE estimates through 24 h were negative and considered undefined. CUE became significantly higher than zero (~0.15) at 32 h.

The ^{13}C data was used to estimate a C budget including respiration, microbial biomass, DOC, and soil C pools (see Supplementary Material). A majority of ^{13}C remaining in soil (~72%) was unextractable within 6 h following low glucose amendment (Supp. Fig. 1). This C was ostensibly in the form of mineral stabilized, extracellular microbial products and necromass since we assume any unused glucose was fully extractable. With the high glucose amendment, all ^{13}C was indeed extractable from soil early in the incubation (0–24 h) before microbial uptake, but a majority (~86%) was again unextractable by 72 h after microbial growth began, corroborating the above assumption.

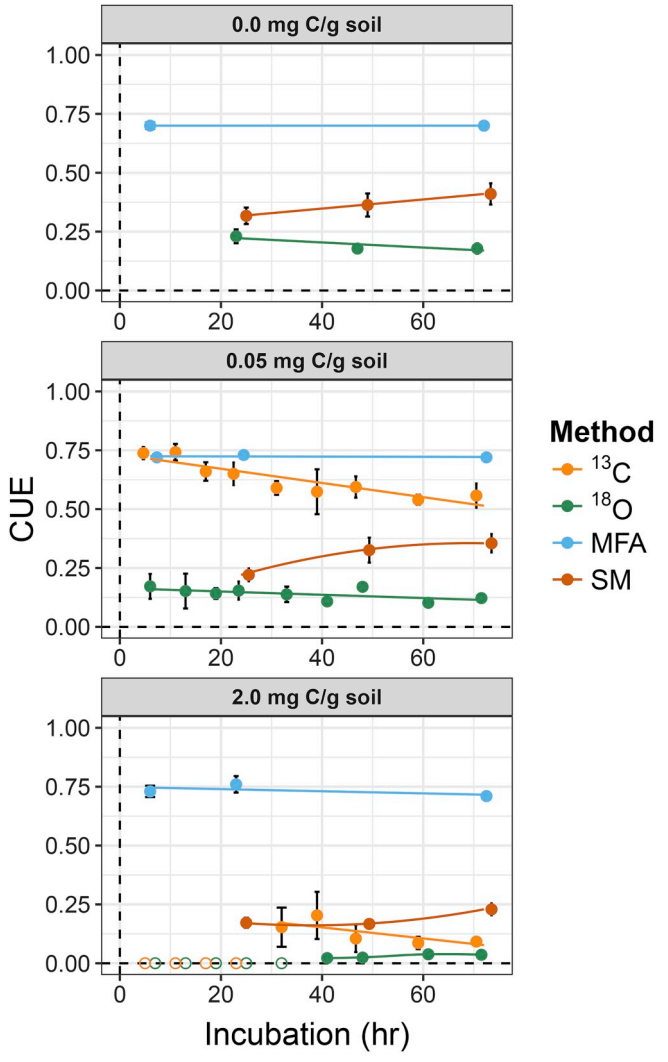

Fig. 2. Carbon use efficiency over 72 h following glucose amendment of 0.0, 0.05, or 2.0 mg C g^{-1} soil using the ^{13}C, ^{18}O, metabolic flux analysis (MFA), and stoichiometric modeling (SM) methods. Open circles indicate when CUE was undefined because average estimates were negative. Means are dodged to prevent overlapping data. Error bars are one standard deviation. N = 5 for all methods but MFA (N = 4).

3.2. ^{18}O-water tracing

Microbial growth (^{18}MBC) and CUE estimated by the ^{18}O-water method were significantly influenced by the interaction of glucose treatment and incubation duration ($p < 0.001$). Growth increased at a constant rate ($0.11\ \mu g$ MBC $g^{-1}\ h^{-1}$) through 72 h of incubation for the no glucose amendment (Fig. 1) yet no change in total microbial biomass (isotopically labeled and unlabeled) occurred. CUE significantly declined from 0.23 to 0.18 in the absence of glucose addition ($-0.001\ h^{-1}$; $p = 0.01$; Fig. 2). With low glucose amendment, growth again increased linearly at a constant rate ($0.07\ \mu g$ MBC $g^{-1}\ h^{-1}$) and CUE declined from 0.17 to 0.12 ($-0.0007\ h^{-1}$; $p = 0.01$; Fig. 2). Both growth and CUE were significantly reduced by low glucose amendment compared to no amendment ($p = 0.0001$; 2-way ANOVA).

High glucose amendment induced logistic growth typical of cell proliferation under pure culture conditions. Three distinct growth phases were evident: a lag phase with minimal and undetectable growth from 0 to 30 h, a significant exponential growth phase from 30 to 56 h ($p < 0.001$), and a stationary growth phase from 56 to 72 h. Growth was negative during the lag phase, causing negative CUE estimates through 32 h that were considered undefined. As with the ^{13}C method, this was caused by an inability to accurately measure microbial biomass in the presence of high background DOC. CUE rose above zero during log growth but only reached ~0.05 by the end of the incubation.

3.3. Calorespirometry

Calorespirometric heat rate and ratio (R_q/R_{CO2}) were influenced by glucose amendment and incubation duration. Soil heat rate was low ($< 10\ \mu J\ s^{-1}\ g^{-1}$) and did not change over time after no or low glucose amendment, suggesting no net microbial growth (Fig. 3). The average

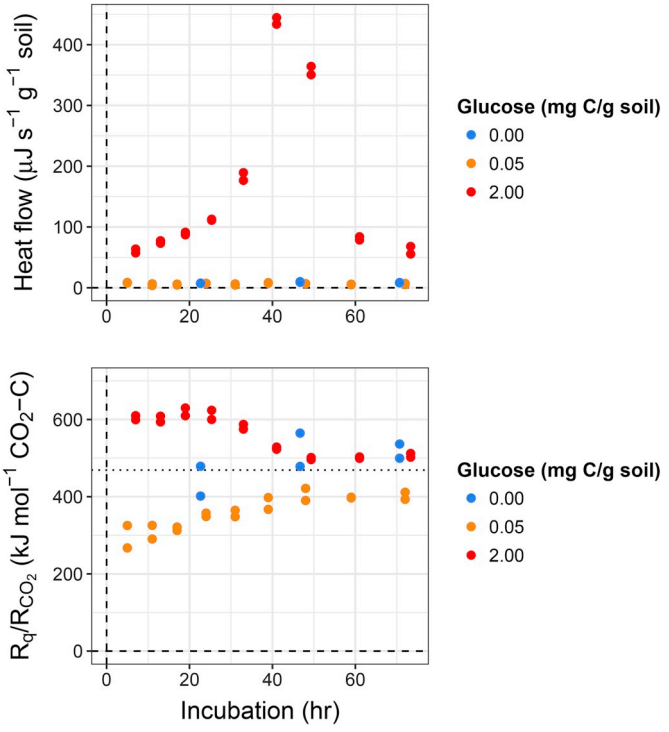

Fig. 3. Heat rate (a) and calorespirometric ratio (b) over 72 h following glucose amendment of 0.0, 0.05, or 2.0 mg C g^{-1} soil. The dotted line (3b) is the expected calorespirometric ratio upon complete oxidation of glucose ($469\ kJ\ mol^{-1}\ CO_2$–C). N = 2.

calorespirometric ratio without glucose addition was consistently ~100 kJ mol^{-1} C higher than that of the low glucose treatment. This would suggest that relatively reduced SOM was metabolized in the absence of glucose (e.g., where R_q/R_{CO2} was > 469 kJ mol^{-1} C), but that glucose metabolism occurred under low amendment (e.g., where R_q/R_{CO2} was < 469 kJ mol^{-1} C). Both heat rate and calorespirometric ratio were strongly influenced by high glucose amendment. Heat rate increased exponentially to a peak at 40 h but then declined to background levels within 72 h, indicating that net growth occurred through 40 h. The calorespirometric ratio under this treatment exhibited a pattern similar to that of ^{18}O growth with no significant change for the first 22 h, a significant linear decline from 22 to 46 h ($p < 0.001$), and no significant change after 46 h. Calorespirometric ratios were directly used in all comparisons to other methods because the conversion to CUE using Eq. (10) produced estimates outside of the 0–1 convention. An exception to this occurred in the low (0.05 mg) glucose treatment, where CUE determined using Eq. (10) linearly declined from 0.84 to 0.66 ($p < 0.001$) through 72 h.

3.4. Metabolic flux analysis

CUE measured by metabolic flux analysis was not significantly affected by glucose treatment and remained nearly constant over time, varying within the range of 0.68–0.77 (Fig. 2). These CUE estimates most closely matched those of the ^{13}C method under low glucose amendment, but only within the first 24 h.

3.5. Stoichiometric modeling

Stoichiometric modeling estimates of CUE were significantly influenced by the main effects of glucose treatment and incubation duration, but not their interaction. CUE increased significantly during incubation (0.0025 h^{-1}; $p < 0.004$) with no (0.32–0.41) or low (0.22–0.35) glucose amendment because the C:N ratio of microbial biomass and exoenzyme activity increased over time for both treatments. Low glucose amendment significantly reduced CUE relative to the no glucose treatment ($p = 0.0001$, 2-way ANOVA), as observed with the ^{18}O method. High glucose amendment caused a decline in CUE early in the incubation relative to other treatments, after which CUE increased significantly over time (0.001 h^{-1}; $p = 0.004$) until reaching ~0.25 at 72 h.

3.6. Correlations in CUE over time and sensitivity analysis

The strongest correlations over time occurred under low glucose amendment between the ^{13}C and calorespirometric estimates of CUE ($r = -0.92$, $p < 0.001$) (Table 1). Due in part to their low sample sizes, metabolic flux analysis and stoichiometric modeling results remained uncorrelated with those of the other techniques. Low sample size was also a limitation under high glucose amendment because this treatment resulted in frequent negative (undefined) CUE estimates.

Sensitivity analyses conducted for each method indicated that growth and respiration were often of equal importance for predicting CUE. In a few cases, CUE was more strongly related to the variable that changed more rapidly over time (e.g., $^{13}CO_2$ relative to ^{13}MBC after 0.05 mg glucose addition). For metabolic flux analysis, flux pattern solutions obtained using the model exactly matched observations in all but three (of eight) cases. In these cases, the nearest solution for the model was used where flux through the pentose phosphate pathway was maximal and all fluxes were positive. Lack of model solution was interpreted to be the result of random variation associated with $^{13}CO_2$ production for individual isotopomers and replicates. For example, in one case a U–^{13}C/1–^{13}C ratio was observed to be 1.86, while a ratio of 1.90 would have yielded a valid model solution with a CUE that was nearly identical to the CUE of the unmatched solution (0.692 vs. 0.693).

Table 1
Pearson correlation results for CUE techniques across incubation duration following glucose amendment (0.0, 0.05, and 2.0 mg glucose-C g^{-1} soil). Correlation coefficient (r) is listed when sample number (N, in parentheses) ≥ 3. ^{13}C-glucose tracing (^{13}C), ^{18}O-water tracing (^{18}O), calorespirometric ratio (CR), metabolic flux analysis (MFA), stoichiometric modeling (SM).

0.0 mg	^{18}O	CR	MFA	SM	
CR	−0.99* (3)	1.00			
MFA	NA (1)	NA (1)	1.00		
SM	−0.86 (3)	0.84 (3)	NA (1)	1.00	
0.05 mg	**^{13}C**	**^{18}O**	**CR**	**MFA**	**SM**
^{18}O	0.69* (9)	1.00			
CR	−0.92*** (9)	−0.50 (9)	1.00		
MFA	0.01 (3)	0.11 (3)	0.04 (3)	1.00	
SM	−0.98 (3)	−0.32 (3)	0.96 (3)	NA (2)	1.00
2.0 mg	**^{13}C**	**^{18}O**	**CR**	**MFA**	**SM**
^{18}O	−0.69 (4)	1.00			
CR	0.53 (5)	−0.39 (4)	1.00		
MFA	NA (1)	NA (1)	0.91 (3)	1.00	
SM	NA (2)	NA (2)	−0.38 (3)	NA (2)	1.00
0.05 mg	**^{13}C**	**^{18}O**	**CR**	**MFA**	**SM**
^{18}O	0.69* (9)	1.00			
CR	−0.92*** (9)	−0.50 (9)	1.00		
MFA	0.01 (3)	0.11 (3)	0.04 (3)	1.00	
SM	−0.98 (3)	−0.32 (3)	0.96 (3)	NA (2)	1.00
2.0 mg	**^{13}C**	**^{18}O**	**CR**	**MFA**	**SM**
^{18}O	−0.69 (4)	1.00			
CR	0.53 (5)	−0.39 (4)	1.00		
MFA	NA (1)	NA (1)	0.91 (3)	1.00	
SM	NA (2)	NA (2)	−0.38 (3)	NA (2)	1.00

* < 0.05; ** < 0.01; *** < 0.001.

4. Discussion

We observed substantial methodological variability in CUE estimates. CUE was lower under *in situ* conditions for the substrate-nonspecific ^{18}O and stoichiometric modeling methods that do not use C tracers (< 0.4) compared to the substrate-specific ^{13}C, calorespirometry, and metabolic flux modeling approaches that trace glucose use (> 0.6). Furthermore, the methods were differentially sensitive to the variable growth conditions elicited by glucose amendment. To understand these results, we examine the different aspects of microbial metabolism that each method captures and the assumptions on which they each depend (Table 2).

Although similar in concept, the ^{13}C and ^{18}O methods differ in critical ways (Fig. 4). Microbial uptake of glucose can occur quickly (< 1 min (Hill et al., 2008); resulting in peak ^{13}C-labeling of chloroform-extracted biomass within a short period of time. In comparison, enrichment of DNA-based biomass with ^{18}O is slower because synthesis of DNA, and not simply uptake of substrate, must occur. CUE estimated by the ^{13}C method may consequently be near unity within seconds of isotope amendment whereas CUE estimated by the ^{18}O method may be near zero. ^{13}C-CUE should decline thereafter if the concentration of labeled biomass has already peaked shortly after amendment but $^{13}CO_2$ losses continue to accrue. Our results indeed suggest declining ^{13}C-CUE over time. In fact, growth was constant between 6 and 72 h presumably because additional C gains (e.g., uptake of recycled ^{13}C-labeled necromass) were in equilibrium with losses (e.g., biomass turnover). ^{18}O-CUE was comparatively less variable over time because growth and cumulative respiration more closely covaried. Standardizing incubation length seems particularly important for comparing results obtained by the ^{13}C approach; however, this poses challenges when soils with

Table 2
Characteristics and assumptions associated with the five CUE methods evaluated and their anticipated consequences for CUE estimation. ^{13}C-glucose tracing (^{13}C), ^{18}O-water tracing (^{18}O), calorespirometric ratio (CR), metabolic flux analysis (MFA), stoichiometric modeling (SM).

Method	Characteristics and assumptions	Consequences for CUE estimation	References
^{13}C	Organic tracer is required, which may lower microbial growth	Underestimation of *in situ* CUE	This study
	Tracer uptake can be rapid (< 6 h) then gradually mineralized over time	High initial CUE that declines over time; CUE is sensitive to incubation duration	This study; Ziegler et al., 2005
	Tracer can be rapidly lost from biomass (< 6 h) and become mineral stabilized as microbial products	Underestimation of microbial uptake as (MBC + R) alone; overestimation of CUE	This study; Creamer et al., 2016
	Affected by microbial exudation and turnover	May reduce CUE	Frey et al., 2001
	Assumes glucose metabolism = SOM metabolism	Overestimation of CUE if native SOM is metabolized less efficiently than glucose	
	Soil environment may interfere with microbial biomass and DNA extraction	Inability to determine growth under high DOC concentrations, high clay content, etc.	This study; Cai et al., 2006
^{18}O	No organic tracer is required	*In situ* CUE is measured	
	Tracer uptake and growth is gradual and covaries with respiration	CUE relatively stable over time	This study
	Affected by microbial exudation and turnover	May reduce CUE	Frey et al., 2001
	Assumes water is only source of oxygen for growth	Underestimation of CUE	Hungate et al., 2015
	Assumes DNA content of newly formed cells is same as that of mature cells	Over/underestimation of CUE	
	Soil environment may interfere with microbial biomass and DNA extractions	Inability to determine growth under high DOC concentrations, high clay content, etc.	This study; Cai et al., 2006
CR	Knowledge of substrate C oxidation state is needed to estimate CUE	Substrate amendment necessary to verify y_S for Eq. (10), or use calorimetry to estimate combustion enthalpy of SOM	LaRowe and Van Cappellen, 2011; Bölscher et al., 2017
	Knowledge of microbial biomass C oxidation state is needed to estimate CUE	Active microbial community must conform to y_{MB} estimate for Eq. (10).	von Stockar and Liu, 1999
	CUE can only be determined using Eq. (10) under oxidative conditions	CUE cannot be determined using Eq. (10) during anaerobic or fermentative growth	Hansen et al., 2004
	Not limited by soil extractions	CUE can be determined in diverse soil environments	
MFA	Uses short incubation periods that do not capture microbial exudation or turnover	CUE is relatively high	This study; Dijkstra et al., 2015
	Integrates metabolic processes more than environmental conditions	CUE is consistent among soil types and environmental conditions	This study; Dijkstra et al., 2011c
	Assumes glucose metabolism = SOM metabolism	Overestimation of CUE if native SOM is not metabolized as efficiently as glucose	
	Not limited by soil extractions	CUE can be determined in diverse soil environments	
SM	CUE is determined by stoichiometry of 'bulk' pools in soil	Stoichiometry may not reflect actively cycling pools (e.g., total microbial biomass vs. active biomass)	Manzoni et al., 2017
	Utilizes common soil measurements	CUE can be calculated using pre-existing datasets	
	Assumes maximum CUE of 0.6 due to thermodynamics constraints	CUE range is smaller than for other methods	Sinsabaugh et al., 2016

dissimilar microbial communities or growth conditions are to be compared.

Calorespirometric heat rate and R_q/R_{CO2} captured growth and metabolic dynamics in our soils, but CUE could only be calculated under certain conditions. Monitoring heat rate revealed exponential growth following high glucose addition, which was also observed with the ^{18}O method. R_q/R_{CO2} values, while useful for determining the type of C being metabolized, could only be used to calculate CUE (of 0–1 range) for the low glucose treatment. CUE estimation failed without glucose amendment likely because the assumption of glucose utilization (i.e., that $\gamma_S = 0$) was incorrect. The relatively high R_q/R_{CO2} estimates we observed under this scenario suggest instead that substrate more reduced than glucose was being metabolized (e.g., lignin, $\gamma_S = -0.6$). This limitation may be overcome by finding the combustion enthalpy of SOM via bomb calorimetry (Bölscher et al., 2017). CUE estimation failed under high glucose amendment because reduced SOM (rather than glucose alone) was being utilized, or perhaps incomplete oxidation of substrate to CO_2 was occurring. The former explanation seems unlikely given that respiration was dominated by glucose. Incomplete oxidation, however, yielding fermentation products rather than CO_2 may have occurred in response to the high glucose availability. We can assess this by examining the cumulative heat produced over the incubation per total glucose-C amended; values for this are 250 kJ mol^{-1} C, much less than the expected 469 kJ mol^{-1} C if complete oxidation of substrate had occurred. CUE was successfully calculated from data obtained from the low glucose amendment because limitations associated with an unknown substrate being metabolized, and incomplete oxidation in the presence of high DOC, did not apply.

CUE estimates obtained by metabolic flux analysis were constant for all treatments, suggesting that biochemical processing of substrate continued unaltered. Given this, we conclude that high glucose amendment does not necessarily decrease biochemical efficiency by overflow respiration (Russell and Cook, 1995; Manzoni et al., 2017). Metabolic flux analysis has also yielded relatively constant CUE of a similar magnitude as observed in our study in response to temperature manipulation (Dijkstra et al., 2011c; Hagerty et al., 2014). Because this method does not discern the fate of microbial products (e.g., biomass vs. exoenzyme production), estimated efficiency could be high even if microbial growth was not apparent. For example, high soil glucose concentrations may result in relatively greater microbial exudation or cell turnover, both of which would lead to lower ^{13}C and ^{18}O estimates of CUE while not affecting metabolic flux estimates. The metabolic flux perspective is distinct from that of the other methods, more akin to a physiological-scale assessment of CUE (*sensu* CUE_P; Geyer et al., 2016).

Stoichiometric modeling estimates of CUE consistently increased over time for all treatments. CUE determined by this method may have

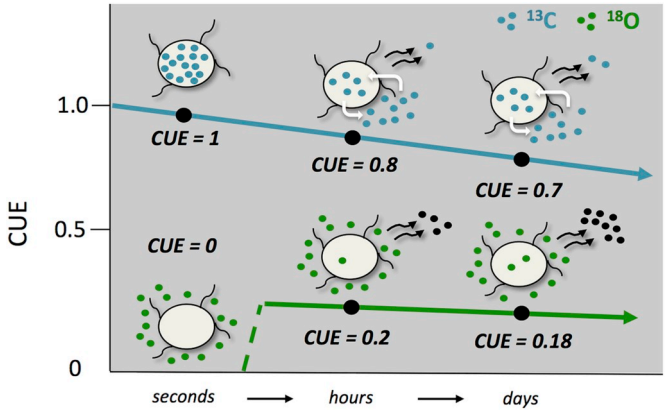

Fig. 4. Conceptual depiction of ^{13}C and ^{18}O dynamics during typical incubation conditions for a soil CUE assay. Immediately after ^{13}C amendment, enrichment of microbial biomass peaks because of rapid tracer uptake, resulting in a CUE near unity. Microbial uptake and biosynthesis of ^{18}O into DNA is comparatively slower causing near zero CUE immediately after ^{18}O amendment. Over time, CUE must decline by the ^{13}C method as enrichment of biomass declines from its maximum and ^{13}C–CO_2 losses accrue (black arrows). CUE will likely change less over time by the ^{18}O method because growth and respiration (black arrows) more closely covary. A majority of ^{13}C is extracellular during incubation and likely available for exchange with the cell (e.g., recycling, white arrows) even after short incubation times (e.g., 6 h in this study).

begun low due to possible nutrient limitation, as suggested by relatively high DOC C:N at the start of incubation (Supplemental Table 1). The subsequent decline in this parameter indicates that nutrient limitation was alleviated over time and CUE may have increased as a result. These conclusions are supported by a decline in the C:N ratio of exoenzyme activity over time (i.e., BG/(LAP + NAG), which indicate a microbial shift towards N acquisition. Although this method has been primarily used to estimate CUE from pre-existing data, stoichiometric modeling appears suited for use in experimental incubations given the trends in stoichiometry and CUE we observed over 72 h.

The most highly correlated CUE estimates over time occurred for the ^{13}C method and calorespirometry after low glucose amendment, although this may not have been for the same reason. ^{13}C CUE declined because of accruing $^{13}CO_2$ losses over time while labeled biomass concentrations (i.e., growth) remained unchanged presumably because of an equilibrium exchange with recyclable necromass. The mechanism for declining calorespirometric CUE is less clear but may reflect exhaustion of amended glucose and a shift towards more reduced substrates (e.g., native SOM or recycled necromass) over time. These two methods have previously been found to correlate with a third: thermodynamic efficiency, defined as the proportion of initial substrate heat remaining in soil despite metabolic processing (Bolscher et al., 2016, 2017). We used thermodynamic efficiency to search for further insights into our dataset, particularly when methods were hindered by poor estimation of microbial biomass after high glucose amendment. Thermodynamic efficiency declined after high glucose amendment from 0.9 to 0.5 suggesting the soil community efficiently conserved heat initially (e.g., during lag growth) until microbial growth accelerated later in the incubation. These results must be interpreted cautiously, however, as the method does not distinguish growth from non-growth functions (Harris et al., 2012). Thermodynamic efficiency could thus appear high even when little C is being dedicated to growth; metabolic flux analysis has a similar interpretation.

Several mechanisms may explain why CUE values determined by the substrate-specific methods (^{13}C, calorespirometry, and metabolic flux analysis) are higher than those of the other approaches. First, CUE determined by glucose tracing may be inflated by intracellular ^{13}C storage in the form of glycogen or other compounds (Nguyen and Guckert, 2001; Lennon and Jones, 2011), although this mechanism has been contested (Dijkstra et al., 2015). Second, it is assumed for the ^{18}O method that oxygen used in DNA biosynthesis is derived entirely from water. Water has been determined to be the source of 33% of oxygen in DNA (Hungate et al., 2015), although only for pure cultures of *E. coli*. If this holds true for most soil microorganisms, the microbial growth estimates we obtained using this method would approximately triple and CUE estimates would double from ~0.2 to ~0.4. These estimates would still be below those obtained from the substrate-specific approaches, however. Third, the ^{18}O method assumes that the actively growing portion of the microbial community has the same DNA:MBC ratio as the broader community. If DNA were relatively less concentrated in new cells, for instance, then the biomass associated with new growth would be underestimated leading to higher CUE estimates. Fourth, the accumulation of ^{13}C in unextractable forms within 6 h of amendment, presumably as mineral-stable microbial products, suggests that microbial uptake is underestimated as the sum of only growth and respiration. Accounting for these extracellular products in the uptake term (*sensu* CUE_E; Geyer et al., 2016) would reduce our estimates of ^{13}C CUE under low glucose amendment to ~0.2. Whether this accounting would also apply to the ^{18}O method depends on whether native SOM undergoes the same rapid stabilization as glucose. Fifth, organic tracers may be preferentially used by a subset of the microbial community that exhibits higher efficiency (Mau et al., 2015). Finally, the CUE of glucose utilization may be naturally higher than that for other substrates in the SOM pool. Several studies have shown CUE to vary with substrate identity (Gommers et al., 1988; Frey et al., 2013). Substrate-nonspecific estimates of CUE (e.g., ^{18}O tracing) likely represent a weighted average of substrate-specific efficiencies. Glucose-based methods may thus approximate *in situ* CUE only in soils where simple carbohydrates dominate the actively cycling portion of SOM, a condition that is site dependent (Schmidt et al., 2011; Gleixner, 2013).

Although the focus of this study was methodological variability, our results offer insight into environmental effects on CUE. We observed microbial growth in unamended soil with the ^{18}O method, as noted by Reischke et al. (2014, 2015) and Dijkstra et al. (2015). Glucose treatment at both low (0.05 mg C g^{-1}) and high (2.0 mg C g^{-1}) levels reduced microbial growth and CUE according to the ^{13}C method, stoichiometric modeling, and most notably the ^{18}O method. Low amendment reduced both ^{18}O growth and CUE by ~40% relative to soils receiving no glucose treatment, and high amendment resulted in a distinct lag phase (0–30 h) with even lower growth and CUE. These observations suggest that the low concentrations of glucose used in ^{13}C tracing experiments can alter *in situ* microbial growth and CUE. It is possible that growth occurred during the lag phase but was not detected if 1) cell turnover increased such that net ^{18}O incorporation was not apparent or 2) growth occurred without ^{18}O incorporation into DNA, for example due to increasing cell size without replication. Although we cannot exclude these alternatives, reduced growth is the most likely explanation for reductions in CUE after high glucose amendment given the prevailing understanding of lag phase dynamics where microbial growth is reduced (Tempest and Neijssel, 1992; Reischke et al., 2014).

5. Conclusions

The method-specific differences in CUE estimates we obtained likely contribute, at least in part, to the high uncertainty in soil CUE values reported in the literature (Six et al., 2006; Manzoni et al., 2012). It is vital that future attempts to cross-compare CUE estimates recognize these methods capture different aspects of microbial metabolism, and thus growth and CUE will inherently differ. Results of the ^{13}C method require standardization of incubation length to account for temporal dynamics and may overestimate the CUE of native SOM when glucose is used as a tracer. The ^{18}O method overcomes this latter challenge by being substrate-nonspecific, but assumptions about the source of oxygen for DNA synthesis, for example, need to be refined. Substrate availability frequently dictated the success of methods: microbial

biomass was difficult to measure in the presence of high background DOC (i.e., unused glucose) limiting application of ^{13}C and ^{18}O methods under these conditions; whereas, CUE could only be inferred from calorespirometric results after low glucose amendment. We recommend that users balance the strengths and weaknesses of available methods and select the method that best addresses their specific research needs. In addition, we suggest simultaneous application of multiple techniques as a means to address novel questions. For example, dual labeling approaches have been recently used to investigate microbial priming by differentiating taxa that grow on amended substrates (both ^{18}O and ^{13}C incorporation) from those which preferentially use native SOM (^{18}O incorporation only) (Mau et al., 2015). Choosing to combine approaches in this way is a potentially powerful way to explore microbial CUE.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2018.09.036.

References

Barros, N., Hansen, L.D., Pineiro, V., Perez-Cruzado, C., Villanueva, M., Proupin, J., Rodriguez-Anon, J.A., 2016. Factors influencing the calorespirometric ratios of soil microbial metabolism. Soil Biology and Biochemistry 92, 221–229.

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, 9.

Blazewicz, S.J., Schwartz, E., 2011. Dynamics of O-18 incorporation from H (2) (18) O into soil microbial DNA. Microbial Ecology 61, 911–916.

Bolscher, T., Wadso, L., Borjesson, G., Herrmann, A.M., 2016. Differences in substrate use efficiency: impacts of microbial community composition, land use management, and substrate complexity. Biology and Fertility of Soils 52, 547–559.

Bölscher, T., Paterson, E., Freitag, T., Thornton, B., Herrmann, A.M., 2017. Temperature sensitivity of substrate-use efficiency can result from altered microbial physiology without change to community composition. Soil Biology and Biochemistry 109, 59–69.

Brant, J.B., Sulzman, E.W., Myrold, D.D., 2006. Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation. Soil Biology and Biochemistry 38, 2219–2232.

Cai, P., Huang, Q., Zhang, X., Chen, H., 2006. Adsorption of DNA on clay minerals and various colloidal particles from an Alfisol. Soil Biology and Biochemistry 38, 471–476.

Creamer, C.A., Jones, D.L., Baldock, J.A., Rui, Y.C., Murphy, D.V., Hoyle, F.C., Farrell, M., 2016. Is the fate of glucose-derived carbon more strongly driven by nutrient availability, soil texture, or microbial biomass size? Soil Biology and Biochemistry 103, 201–212.

Dijkstra, P., Blankinship, J.C., Selmants, P.C., Hart, S.C., Koch, G.W., Schwartz, E., Hungate, B.A., 2011a. Probing carbon flux patterns through soil microbial metabolic networks using parallel position-specific tracer labeling. Soil Biology and Biochemistry 43, 126–132.

Dijkstra, P., Dalder, J.J., Selmants, P.C., Hart, S.C., Koch, G.W., Schwartz, E., Hungate, B.A., 2011b. Modeling soil metabolic processes using isotopologue pairs of position-specific C-13-labeled glucose and pyruvate. Soil Biology and Biochemistry 43, 1848–1857.

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.

Dijkstra, P., Thomas, S.C., Heinrich, P.L., Koch, G.W., Schwartz, E., Hungate, B.A., 2011c. Effect of temperature on metabolic activity of intact microbial communities: evidence for altered metabolic pathway activity but not for increased maintenance respiration and reduced carbon use efficiency. Soil Biology and Biochemistry 43, 2023–2031.

Frey, S., Lee, J., Melillo, J.M., Six, J., 2013. The temperature response of soil microbial efficiency and its feedback to climate. Nature Climate Change 3, 395–398.

Frey, S.D., Gupta, V., Elliott, E.T., Paustian, K., 2001. Protozoan grazing affects estimates of carbon utilization efficiency of the soil microbial community. Soil Biology and Biochemistry 33, 1759–1768.

Frey, S.D., Ollinger, S., Nadelhoffer, K., Bowden, R., Brzostek, E., Burton, A., Caldwell, B.A., Crow, S., Goodale, C.L., Grandy, A.S., Finzi, A., Kramer, M.G., Lajtha, K., LeMoine, J., Martin, M., McDowell, W.H., Minocha, R., Sadowsky, J.J., Templer, P.H., Wickings, K., 2014. Chronic nitrogen additions suppress decomposition and sequester soil carbon in temperate forests. Biogeochemistry 121, 305–316.

Geyer, K.M., Kyker-Snowman, E., Grandy, A.S., Frey, S.D., 2016. Microbial carbon use efficiency: accounting for population, community, and ecosystem-scale controls over the fate of metabolized organic matter. Biogeochemistry 127, 173–188.

Gleixner, G., 2013. Soil organic matter dynamics: a biological perspective derived from the use of compound-specific isotopes studies. Ecological Research 28, 683–695.

Gommers, P.J.F., Vanschie, B.J., Vandijken, J.P., Kuenen, J.G., 1988. Biochemical limits to microbial growth yields - an analysis of mixed substrate utilization. Biotechnology and Bioengineering 32, 86–94.

Gromping, U., 2006. Relative importance for linear regression in R: the package relaimpo. Journal of Statistical Software 17, 1–27.

Hagerty, S., 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 1–4.

Hansen, L.D., Macfarlane, C., McKinnon, N., Smith, B.N., Criddle, R.S., 2004. Use of calorespirometric ratios, heat per CO2 and heat per O-2, to quantify metabolic paths and energetics of growing cells. Thermochimica Acta 422, 55–61.

Harris, J.A., Ritz, K., Coucheney, E., Grice, S.M., Lerch, T.Z., Pawlett, M., Herrmann, A.M., 2012. The thermodynamic efficiency of soil microbial communities subject to long-term stress is lower than those under conventional input regimes. Soil Biology and Biochemistry 47, 149–157.

Hill, P.W., Farrar, J.F., Jones, D.L., 2008. Decoupling of microbial glucose uptake and mineralization in soil. Soil Biology and Biochemistry 40, 616–624.

Hungate, B.A., Mau, R.L., Schwartz, E., Caporaso, J.G., Dijkstra, P., van Gestel, N., Koch, B.J., Liu, C.M., McHugh, T.A., Marks, J.C., Morrissey, E.M., Price, L.B., 2015. Quantitative microbial ecology through stable isotope probing. Applied and Environmental Microbiology 81, 7570–7581.

Kallenbach, C.M., Frey, S.D., Grandy, A.S., 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nature Communications 7, 10.

Keiblinger, K.M., Hall, E.K., Wanek, W., Szukics, U., Hammerle, I., Ellersdorfer, G., Bock, S., Strauss, J., Sterflinger, K., Richter, A., Zechmeister-Boltenstern, S., 2010. The effect of resource quantity and resource stoichiometry on microbial carbon-use-efficiency. FEMS Microbiology Ecology 73, 430–440.

Kemp, R.B., 2000. "Fire burn and cauldron bubble" (W. Shakespeare): what the calorimetric-respirometric (CR) ratio does for our understanding of cells? Thermochimica Acta 355, 115–124.

LaRowe, D.E., Van Cappellen, P., 2011. Degradation of natural organic matter: a thermodynamic analysis. Geochimica et Cosmochimica Acta 75, 2030–2042.

Lennon, J.T., Jones, S.E., 2011. Microbial seed banks: the ecological and evolutionary implications of dormancy. Nature Reviews Microbiology 9, 119–130.

Manzoni, S., Capek, P., Mooshammer, M., Lindahl, B.D., Richter, A., Santruckova, H., 2017. Optimal metabolic regulation along resource stoichiometry gradients. Ecology Letters 20, 1182–1191.

Manzoni, S., Taylor, P., Richter, A., Porporato, A., Agren, G.I., 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytologist 196, 79–91.

Mau, R.L., Liu, C.M., Aziz, M., Schwartz, E., Dijkstra, P., Marks, J.C., Price, L.B., Keim, P., Hungate, B.A., 2015. Linking soil bacterial biodiversity and soil carbon stability. The ISME Journal 9, 1477–1480.

Maynard, D.S., Crowther, T.W., Bradford, M.A., 2017. Fungal interactions reduce carbon use efficiency. Ecology Letters 20, 1034–1042.

Molenaar, D., van Berlo, R., de Ridder, D., Teusink, B., 2009. Shifts in growth strategies reflect tradeoffs in cellular economics. Molecular Systems Biology 5, 10.

Muggeo, V.M.R., 2008. Segmented: an R package to fit regression models with broken-line relationships. R News 8, 20–25.

Nguyen, C., Guckert, A., 2001. Short-term utilisation of C-14 U glucose by soil micro-organisms in relation to carbon availability. Soil Biology and Biochemistry 33, 53–60.

Pfeiffer, T., Schuster, S., Bonhoeffer, S., 2001. Cooperation and competition in the evolution of ATP-producing pathways. Science 292, 504–507.

R Development Core Team, 2008. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Reischke, S., Kumar, M.G.K., Baath, E., 2015. Threshold concentration of glucose for bacterial growth in soil. Soil Biology and Biochemistry 80, 218–223.

Reischke, S., Rousk, J., Baath, E., 2014. The effects of glucose loading rates on bacterial and fungal growth in soil. Soil Biology and Biochemistry 70, 88–95.

Roels, J.A., 1980. Application of macroscopic principles to microbial metabolism. Biotechnology and Bioengineering 22, 2457–2514.

Roller, B.R., Schmidt, T.M., 2015. The Physiology and Ecological Implications of Efficient Growth. International Society for Microbial Ecology.

Russell, J.B., Cook, G.M., 1995. Energetics of bacterial growth - balance of anabolic and catabolic reactions. Microbiological Reviews 59, 48–62.

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Koegel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature (London) 478, 49–56.

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.
Sinsabaugh, R.L., Shah, J.J.F., 2012. Ecoenzymatic stoichiometry and ecological theory. Annual Review of Ecology, Evolution and Systematics 43 (43), 313–343.
Sinsabaugh, R.L., Shah, J.J.F., Findlay, S.G., Kuehn, K.A., Moorhead, D.L., 2015. Scaling microbial biomass, metabolism and resource supply. Biogeochemistry 122, 175–190.
Sinsabaugh, R.L., Turner, B.L., Talbot, J.M., Waring, B.G., Powers, J.S., Kuske, C.R., Moorhead, D.L., Shah, J.J.F., 2016. Stoichiometry of microbial carbon use efficiency in soils. Ecological Monographs 86, 172–189.
Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal 70, 555–569.
Spohn, M., Klaus, K., Wanek, W., Richter, A., 2016a. Microbial carbon use efficiency and biomass turnover times depending on soil depth - implications for carbon cycling. Soil Biology and Biochemistry 96, 74–81.
Spohn, M., Potsch, E.M., Eichorst, S.A., Woebken, D., Wanek, W., Richter, A., 2016b. Soil microbial carbon use efficiency and biomass turnover in a long-term fertilization experiment in a temperate grassland. Soil Biology and Biochemistry 97, 168–175.
Steinweg, J.M., Plante, A.F., Conant, R.T., Paul, E.A., Tanaka, D.L., 2008. Patterns of substrate utilization during long-term incubations at different temperatures. Soil Biology and Biochemistry 40, 2722–2728.
Tempest, D.W., Neijssel, O.M., 1992. Physiological and energetic aspects of bacterial metabolite overproduction. FEMS Microbiology Letters 100, 169–176.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass-C. Soil Biology and Biochemistry 19, 703–707.
von Stockar, U., Liu, J.S., 1999. Does microbial life always feed on negative entropy? Thermodynamic analysis of microbial growth. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1412, 191–211.
Wadso, I., 2009. Characterization of microbial activity in soil by use of isothermal microcalorimetry. Journal of Thermal Analysis and Calorimetry 95, 843–850.
Ziegler, S.E., White, P.M., Wolf, D.C., Thoma, G.J., 2005. Tracking the fate and recycling of C-13-labeled glucose in soil. Soil Science 170, 767–778.