On maintenance and metabolisms in soil microbial communities

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Abstract
Scope Biochemistry is an essential yet undervalued aspect of soil ecology, especially when analyzing soil C cycling. We assume, based on tradition, intuition or hope, that the complexity of biochemistry is confined to the microscopic world, and can be ignored when dealing with whole soil systems. This opinion paper draws attention to patterns caused by basic biochemical processes that permeate the world of ecosystem processes. From these patterns, we can estimate activities of the biochemical reactions of the central C metabolic network and gain insights into the ecophysiology of microbial biosynthesis, growth, and maintenance energy requirements: important components of Carbon Use Efficiency (CUE).
Observations We show that glucose is processed via the Embden-Meyerhof-Parnas glycolysis in one soil, but via Pentose Phosphate or Entner-Doudoroff pathways in two other soils. However, notwithstanding this metabolic diversity, glucose use efficiency is high and thus substrate use for maintenance energy and overflow respiration is low in these soils. These results contradict current dogma, based on four

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OPINION PAPER
decades of debate in soil ecology, that the maintenance energy demand in soil communities is a quantitative important, although variable, component of soil community energy metabolism.

Conclusions We identify three main shortcomings in our current understanding of substrate use efficiency: 1) in numeric and conceptual models, we lack appreciation of the strategies that microbes employ to quickly reduce energy needs in response to starvation; 2) in order to understand variation in CUE, we need to improve our understanding of the processes of exudation (including all changes in allocation of C from the cell soluble to insoluble fraction and extracellular environment) and microbial turnover; and 3) whether tracer experiments can be used to measure the long-term substrate use efficiency of soil microbial communities depends critically on the ability and speed with which non-growing cells take up tracer substrates and metabolism activates and subsequently de-activates in response to starvation, as well as on how cellular activities scale to the community level. To move the field of soil ecology forward, future research must consider the details of microbial eco-physiology and develop new tools that enable direct measurement of microbial functioning in intact soils. We submit that $^{13}$C metabolic flux analysis is one of those new tools.

Keywords Metabolic flux analysis · Biochemical efficiency · Carbon use efficiency · Maintenance · Exudation · Turnover · Embden-Meyerhof-Parnass glycolysis · Entner-Doudoroff pathway · Pentose phosphate pathway · Marsh · Forest · Grassland

Introduction

The topic of this Opinion is $^{13}$C metabolic flux analysis and its use for the study of soil microbial eco-physiology, specifically maintenance energy demand, overflow respiration, and substrate use efficiency. We briefly review research on maintenance energy demand in soil communities, followed by an introduction to $^{13}$C metabolic flux analysis. We then quantify the process rates of the biochemical pathways of the central C metabolic network for soil from three distinct ecosystems. These biochemical pathways are important as they release most of the CO$_2$, produce the precursors for biosynthesis, and energy for maintenance and growth. Finally, we address the question whether tracer experiments can be used to measure the overall efficiency of substrate use in soil microbial communities, and identify important knowledge gaps.

The Myth of Maintenance

Microbes require energy for cell maintenance and growth. The concepts of cellular energy demand were developed in the middle of the last century (Marr et al. 1963; Payne 1970; Pirt 1965; Roels 1980). These early studies found a linear relationship between microbial growth rate and substrate consumption and a positive intercept (Stouthamer and Bettenhaussen 1973; Tempest and Neijssel 1984). This intercept was interpreted as evidence for a ‘maintenance’ energy cost that was independent of growth rate and did not contribute to biosynthesis (Pirt 1965). Numerous definitions of maintenance have been proposed (see discussion in van Bodegom 2007 and Kempes et al. 2017), but, in this paper, we define maintenance as the processes that require energy, but do not directly contribute to biosynthesis, including maintenance of solute gradients, motility, and protein and RNA turnover (Pirt 1965; Russell and Cook 1995; Schimel and Weintraub 2003; van Bodegom 2007). When growth rates of microbes decline due to resource limitations, proportionally more substrate will be required for cell maintenance, less will be available for biosynthesis, more substrate-C is released as CO$_2$, and Carbon Use Efficiency (CUE, biomass produced per substrate consumed) declines. Researchers during this early period were careful to point out that maintenance energy demand estimated in chemostat experiments referred to growing cells, and suggested that maintenance for non-growing cells was likely lower (Pirt 1965).

Soil ecologists applied the ideas of microbial energy metabolism and maintenance energy demand to soil ecosystems beginning in the 1970s (Anderson and Domsch 2010). These first studies compared the maintenance energy demand estimated in chemostat experiments with the annual C and energy available in soil ecosystems (Barber and Lynch 1977) and concluded that maintenance energy demand exceeded annual C inputs. For example, Lynch (1982) concluded that “in
agricultural and forest soils the energy input may not be enough to satisfy even the maintenance energy requirement”. Similarly, Babiuk and Paul (1970) wrote that “considering the amount of available energy, that the individual cells have enough energy to divide only a few times each year”.

Anderson and Domsch, pioneers in soil ecophysiology, determined maintenance energy demand for actively metabolizing (Anderson and Domsch 1985a) and dormant communities (Anderson and Domsch 1985b) using glucose additions. The maintenance demand for actively metabolizing communities was similar to that observed in chemostat experiments, while the maintenance energy requirement estimated for a ‘dormant’ community was two to three orders of magnitude lower. In their studies, Anderson and Domsch assumed that no growth and no death occurred. However, it is now well established that even for soil without substrate amendments, microbial growth does occur (Blagodatskaya and Kuzyakov 2013; Cruz-Paredes et al. 2021; Koch et al. 2018; Purcell et al. 2021; Reischke et al. 2015), while Koch et al. (2018), using quantitative stable isotope probing, showed that growth and death in microbial communities are simultaneous processes.

Microbes have an array of strategies to quickly reduce energy consumption when faced with starvation (Greening et al. 2019; Hoehler and Jorgensen 2013; Lever et al. 2015; Lloyd 2021). These strategies include dormancy and quiescence (LaRowe and Amend 2015; Lennon and Jones 2011; Rittershaus et al. 2013), and massive cell death in response to starvation that enables a few cells to feed on their less-fortunate siblings (Aouizerat et al. 2019; Finkel 2006; Jöers et al. 2020). When faced with starvation in laboratory experiments, microbes do not enter a state of perpetual maintenance, but instead actively transition, often within a few hours, to a different physiological state (for example, Bernhardt et al. 2003; Löffler et al. 2017). Starvation is accompanied by a reduction in cell size, reorganization of cellular membranes, increased C reserves, cell wall modifications, and reduced protein and RNA content (Lennon and Jones 2011; Navarro Llorens et al. 2010; Mason-Jones et al. 2022). These survival strategies likely strongly affect soil maintenance energy demand. Of course, most knowledge on microbial starvation responses is derived from few model organisms under laboratory conditions. However, new insights in microbial responses to feast-famine transitions may become available from metagenome and metatranscriptome studies (for example Chuckran et al. 2021).

Price and Sowers (2004) estimated that the activity of a non-growing cell was three orders of magnitude lower than of growing cells, while a dormant cell exhibited activity three orders of magnitude lower again. This means that one actively growing cell equals the activity of 1,000 starved cells and 1,000,000 dormant cells. According to Blagodatskaya and Kuzyakov (2013), about 50% of microbial cells in soil are dormant, 40–49% are non-growing but metabolically active, and 1–10% are actively growing. Using these numbers, one can easily calculate that most community respiration is associated with actively growing cells and less than 5% of respiration comes from dormant or potentially-active cells. These estimates suggest that respiration in soil communities overwhelmingly represents the small minority of growing cells, not the silent majority of non-growing organisms. Therefore, we conclude that demand of substrate for cellular maintenance energy demand, aggregated at the level of the whole community, is low.

To Measure Maintenance

Carbon Use Efficiency is an important factor that affects soil organic matter production and C cycling (Allison 2014; Cotrufo et al. 2013; Geyer et al. 2016; Hagerty et al. 2018; Manzoni et al. 2018; Schimel and Schaeffer 2012). The concept of CUE – the proportion of substrate-C that is used to make microbial biomass – is straightforward, but surprisingly difficult to measure in soil. Variation in maintenance energy demand affects CUE, suggesting that the measurement of CUE is a good way to estimate maintenance energy demand. However, we now know that the measurement of CUE, for example by measuring the retention of 13C-labeled tracers in microbial biomass, captures multiple processes, including metabolic inefficiencies, C-loss via exudation, and microbial turnover (Geyer et al. 2016; Hagerty et al. 2014, 2018; Manzoni et al. 2018). The relative impact of these processes on CUE depends on the methods used to measure CUE and the duration of the experiment (Geyer et al. 2016; Hagerty et al. 2014). We submit that a concerted effort is needed to separate the underlying processes of CUE: biochemical efficiency, and especially exudation and microbial turnover.
In this opinion, we focus on the processes of respiration and biosynthesis only. Variability in maintenance energy demand will directly affect the partitioning of substrate-C over CO₂ production and biosynthesis by altering the rates of the central C metabolic network processes. Using metabolic flux analysis, we can capture the partitioning of substrate over CO₂ production and biosynthesis and thus measure the efficiency of substrate use by these metabolic pathways. To emphasize this aspect of CUE and set it apart from other components of CUE (Table 1), we introduce a new term, Biochemical Efficiency or BE, to characterize the substrate use efficiency of the biochemical processes (Table 1). We limit this discussion to experiments using ¹³C labeled glucose and thus to glucose use efficiency. This limitation is justified as much of our conceptual understanding of microbial energy metabolism and CUE is based on glucose-addition experiments. Extrapolating this discussion to other organic compounds is straightforward but goes beyond the goal of this Opinion.

If maintenance energy demand is high, more CO₂ will be released by the reactions of the central C metabolic network (Fig. 1). More CO₂ will also be released if the energy cost for biosynthesis or membrane transport processes increases (Du et al. 2018; Stouthamer 1973), or if high C availability causes inefficiencies in ATP production and overflow respiration (Manzoni et al. 2012). The value of BE must be measured over a short period of time to minimize the effects of metabolite recycling and turnover. It has been suggested that the early response to a glucose addition results mostly in accumulation of C reserves, and thus is not representative of real growth (Sinsabaugh et al. 2013). However, a direct test of this hypothesis using ¹³C metabolic flux analysis found no evidence for a dominant role of reserve production (Dijkstra et al. 2015).

¹³C Metabolic Flux Analysis

¹³C-Based Metabolic Flux Analysis studies the incorporation of ¹³C from position-specific labeled substrates into biosynthesis products (Zamboni et al. 2009). Observed position-specific incorporation is a direct consequence of the flux or activity patterns of central C metabolic network processes. These processes have been studied in depth and appear remarkably similar across all domains of life (Long and Antoniewicz 2019).

We adapted the metabolic flux analysis for soil communities using parallel incubations of six position-specific ¹³C labeled glucose isotopomers (Dijkstra et al. 2011a, 2015), but instead of determining ¹³C in biosynthesis products, we measured the ¹³C incorporation into CO₂ (Dijkstra et al. 2011a,b, 2015; Geyer et al. 2019; Hagerty et al. 2014; van Groenigen et al. 2013). CO₂ is released very quickly after glucose addition, and its isotope composition can be readily measured at low cost using modern laser spectrometers. The position-specific ¹³C-CO₂ measurements usually take about 40 min at room temperature, although they can be done within 5 min (A. Martinez, D. Verdi, and P. Dijkstra unpublished results).

The proportion of CO₂ production per C atom from position-specific ¹³C-labeled glucose directly reflects the rates of the biochemical processes of the central C metabolic network and Biochemical Efficiency (BE). Theoretical analysis (for details, see Supplemental Table 1 Terminology and definitions. U = Uptake, R = respiration, EX = exudation, T = microbial turnover. First column contains names of variables used in this paper, with mathematical representation in second column. Last three columns contain corresponding variable names used by Geyer et al. (2016), Hagerty et al. (2018), and Manzoni et al. (2018). It must be noted that, if the chloroform-fumigation extraction method is used to measure microbial biomass, any shift of C from the soluble cellular fraction to the insoluble fraction and extracellular environment must be included in the definition of EX.

| Variable                  | Equation          | Geyer et al. 2016* | Hagerty et al. 2018† | Manzoni et al. 2018$ |
|---------------------------|-------------------|-------------------|----------------------|----------------------|
| Biochemical efficiency (BE) | (U-R)/U           | CUE<sub>C</sub>   | CUE<sub>E</sub>      | CUE                  |
| Biomass Yield             | (U-R-EX)/U        | CUE<sub>C</sub>; CUE<sub>P</sub> | CUE                 | GGE                  |
| CUE (apparent)            | (U-R-EX-T)/U      | CUE<sub>Ei</sub>  | CUE<sub>C</sub>      | CUE<sub>A</sub>      |

* CUE<sub>C</sub> is community-scale CUE; CUE<sub>E</sub> is ecosystem-scale CUE (Geyer et al. 2016). † CUE<sub>E</sub> is substrate-based CUE; CUE<sub>C</sub> is concentration-based CUE (Hagerty et al. 2018). ‡ GGE is Gross Growth Efficiency; CUE<sub>A</sub> is apparent CUE (Manzoni et al. 2018).
Information) reveals the patterns of position-specific CO₂ production that are associated with high activity of each of the three main metabolic pathways (Fig. 2 left): high CO₂ production from positions C3 and C4 is expected when Embden-Meyerhof-Parnas glycolysis is highly active, whereas high CO₂ production from position C1 and C4 is predicted when either the Pentose Phosphate or Entner-Doudoroff pathway activity is dominant. The Entner-Doudoroff pathway is an evolutionarily ancient glycolytic pathway (Conway 1992; Kopp and Sunna 2020) and is widely

![Fig. 1](image-url) Metabolic model with three glucose metabolizing pathways: Embden-Meyerhof-Parnas glycolysis (EMP, r2—red arrow), Pentose Phosphate pathway (PP, r10—blue), and Entner-Doudoroff pathway (ED, r13—yellow). Pathway (r2 … r15) and biomass reactions (br1 … br8) are expressed as moles relative to r1 (set at 100 mol). Abbreviations: AcCoA, acetyl-CoA; αKG, α-ketoglutarate; E4P, erythrose-4P; F6P, fructose-6P; G3P, glyceraldehyde-3P; G6P, glucose-6P; KDPG, 2-keto-3-deoxy-6-phosphogluconate; OAA, oxaloacetate; PYR, pyruvate; RU5P, ribulose-5P; S7P, sedoheptulose-7P. Additional details in Dijkstra et al. (2011a). Molar balance equations are available in Table S2 of the Supplemental Information.

![Fig. 2](image-url) Modeled (left) and observed (right) CO₂ production per C-atom (means and stdev, n=4). Modeled CO₂ production per C atom are calculated using a metabolic model (Fig. 1; adapted from Dijkstra et al. 2011a, 2015). Modeled CO₂ production per C atom are for BE=0.6 and maximal activity of Embden-Meyerhof-Parnas glycolysis, Pentose Phosphate pathway, and Entner-Doudoroff pathway, respectively. Observations are based on measurement of CO₂ production from 6 glucose isotopomers in parallel incubations for a soil from a high elevation meadow in mixed conifer forest (F) and a low elevation cool desert grassland (G) (Dijkstra et al. 2006), and a soil from the Jug Bay tidal freshwater marsh (M) (A. Martinez, P. Megonigal, and P. Dijkstra, in prep). Arrows indicate the characteristic similarities between the theoretical and observed position-specific CO₂ production patterns, while * indicates significant differences between C2 and C3 (P<0.05; NS is not significant). For details of soil characteristics, experimental procedures and modeling, see Supplemental Information.)
distributed among Proteobacteria (Conway 1992; Edirisinghe et al. 2016; Kopp and Sunna 2020), but also found in Archaea, diatoms and plants (Chen et al. 2016). The differences in position-specific CO₂ production between a soil community with high Pentose Phosphate or a high Entner-Doudoroff pathway activity are more subtle: high Entner-Doudoroff pathway activity results in a slightly higher CO₂ production from position C2 than C3, while high Pentose Phosphate pathway activity produces slightly less CO₂ from C2 than C3 (Fig. 2 left).

The expected differences in CO₂ production per C atom are larger when BE is high (Fig. 3; Dijkstra et al. 2015), because some C atoms are preferentially released as CO₂, while others are incorporated into biosynthesis products. However, as maintenance energy demand increases and BE decreases, CO₂ production per C atom becomes more similar. At BE = 0, expected for non-growing cells using substrate only to satisfy maintenance energy demand, all glucose-C is released as CO₂ and position-specific CO₂ production is 16.7% (= 1/6) of the total CO₂ production for all C positions (Fig. 3). Therefore, when differences between C-positions are large, we can conclude that BE is high, and thus maintenance and overflow respiration are low.

A Modicum of Metabolisms and High Efficiency

Metabolic flux measurements of soil in the past indicated a large proportion of CO₂ from the first C-atom in glucose (C1; Dijkstra et al. 2011a,b, 2015; Geyer et al. 2019; Hagerty et al. 2014; van Groenigen et al. 2013). We interpreted this as an indication of a high Pentose Phosphate pathway activity, but did not consider the possibility of the Entner-Doudoroff pathway (Fig. 1). However, the observed patterns of CO₂ production per C-atom for three distinct soil ecosystems suggested a greater diversity in glucose metabolism than initially imagined (Fig. 2 right).

We expanded our metabolic model (Dijkstra et al. 2011a, 2015) to include reactions of the Entner-Doudoroff pathway (Fig. 1). With this expanded model, we explored whether observed patterns in position-specific CO₂ production (Fig. 2 right) were associated with differences in Embden-Meyerhof-Parnas glycolysis, Pentose Phosphate or Entner-Doudoroff pathway activities (Fig. 2 left). The model was used to calculate all reaction rates in the metabolic network (r2-r15; br1-br8) and BE (Table S3 Supplemental Information). Two branch points in the metabolic network determine the relative activity of the three metabolic pathways: the branch between Embden-Meyerhof-Parnas glycolysis and Pentose Phosphate/Entner-Doudoroff pathway (r2 versus r9), and between Pentose Phosphate and Entner-Doudoroff pathways (r10 versus r13; Fig. 1).

Reaction r2, r10, and r13 exhibited significant differences (Fig. 4 top), with high activity of r2 for the tidal freshwater marsh soil (M), high activity of r10 for the low elevation cool desert grassland soil (G), and high activity of r10 and r13 for the high elevation mixed conifer soil (F). This indicated that glucose was mostly processed via Embden-Meyerhof-Parnas glycolysis in the freshwater marsh soil (M), via both Entner-Doudoroff and Pentose Phosphate pathways in the high elevation mixed conifer soil (F), and via Pentose Phosphate pathway with some contributions from Embden-Meyerhof-Parnas glycolysis and Entner-Doudoroff pathway for the low elevation desert grassland soil (G; Fig. 4 middle).
Why soil communities utilize different metabolic pathways to catabolize the same substrate is unknown. Further research is needed to determine the geographic distribution of these patterns, and test whether these patterns are the result of genetic differences in community composition (Edirisinghe et al. 2016) or a physiological change in response to soil or environmental conditions (Bore et al. 2017; Thomas et al. 2019). Klingner et al. (2015) analyzed 25 strains of marine bacteria and found that most utilized the Entner-Doudoroff pathway, concluding that this pathway was important in marine ecosystems. It appears from our results that this holds for some soils as well. The Entner-Doudoroff pathway may provide advantages over the Embden-Meyerhof-Parnas glycolysis because of lower protein cost, and more favorable thermodynamic characteristics, thus compensating for the slightly lower ATP yield under aerobic conditions (Conway 1992; Flamholz et al. 2013; Klingner et al. 2015). The Entner-Doudoroff (and Pentose Phosphate) pathway also produce large quantities of NADPH, thus offering protection against oxidative damage (Dijkstra et al. 2011b; Klingner et al. 2015), potentially a critical requirement in oxygen-rich soil and marine environments.

High Embden-Meyerhof-Parnas glycolysis activity was also observed in a paddy soil and lake sediment (Krumböck and Conrad 1991), and may be characteristic of anoxic environments. Likewise, Embden-Meyerhof-Parnas glycolysis was the dominant pathway for glucose metabolism in a hot spring at 60 °C, but not at higher temperatures (up to 90 °C; Thomas et al. 2019). Under anoxic conditions, assuming fermentation only, the higher ATP yield of Embden-Meyerhof-Parnas glycolysis over Entner-Doudoroff pathway may be critical, while the production of NADPH to fight oxidative stress is less important. It should be noted that measurement of CO₂ production per C atom as described here can be supplemented with an analysis of position-specific isotope incorporation into biosynthesis products, for example phospholipid fatty acids, thus revealing additional details of soil C metabolism (Apostel et al. 2015; Dippold et al. 2019; Wu et al. 2020).

Biochemical Efficiency was high in all three soils, although the tidal freshwater marsh soil (M) had a slightly, but significantly, lower BE (Fig. 4 bottom). Biochemical Efficiency was high even at high glucose concentrations (2 mg glucose C g⁻¹ soil; Geyer et al. 2019), suggesting inefficiencies associated with overflow respiration were minimal. We conclude that, although soil communities process glucose through...
different biochemical pathways, glucose is metabolized with high efficiency, and therefore a high maintenance energy demand or overflow respiration can be excluded. Of course, exudation, especially under anoxic or high C conditions, may result in a low Biomass Yield, while changes in microbial turnover will affect apparent CUE ($\text{CUE}_A$).

The Full Maintenance?

It has been argued that tracer experiments do not capture the full maintenance energy demand in soil ecosystems, but only the maintenance during a short period that is dominated by active growth immediately after tracer addition (Sinsabaugh et al. 2013; Hagerty et al. 2018). Carbon cycle model calculations suggest that, even if BE, measured shortly after adding an isotope enriched substrate, is high, the efficiency across the cell’s lifecycle, including an extended period as a starved, non-growing cell, is much lower (0.32; Hagerty et al. 2018). However, no clear mechanism was proposed that would prevent us from measuring glucose metabolism for growing and non-growing cells all at once using $^{13}$C labeled tracers.

Models are, by necessity, a simplified or conceptualized representation of reality, and rarely consider the dynamics and speed of microbial responses to substrate limitations and the large differences in maintenance energy requirement between growing and non-growing cells. Instead, models often assume a perpetual state of maintenance and low activity. Although such a state of low activity is possible (with difficulty) in well-mixed chemostat and retentostat experiments (e.g., Bisschops et al. 2017), it is unlikely that such activity can be maintained in soil where substrates are strongly localized and depletion zones quick to develop. Moreover, low energy demand of non-growing cells (Price and Sowers 2004) comprises only a small fraction of whole-community activity (see above). Additionally, the initial burst of cell growth after glucose addition is likely rapidly followed by a secondary surge of activity by microbial predators or grazers (e.g., Hungate et al. 2021), thereby reducing the number of non-growing cells with high maintenance cost and maintaining a high community BE.

One way to test the hypothesis of temporally separated growth and maintenance after a tracer addition is to add a large pulse of unlabeled tracer, followed by small tracer additions to probe BE using $^{13}$C metabolic flux analysis across this feast-famine event. In fact, such an experiment showed that BE was maintained for at least 72 h after adding 2 mg glucose-C g$^{-1}$ soil (Geyer et al. 2019). This again suggests that even a large glucose addition does not result in a community with high maintenance energy demand.

However, it is possible that tracer compounds (for example glucose) are metabolized by growing but not by non-growing cells. If this were the case, maintenance energy demand of soil communities will be underestimated. This could occur if glucose, within minutes, stimulates a physiological transition from non-growing to growing cells, or if non-growing cells do not have the transporters and metabolic capabilities to utilize the added substrates. Added substrate is thought to stimulate growth, an artefact often assigned to tracer experiments (e.g., Sinsabaugh et al. 2013). However, such a near instantaneous transition from non-growing to growing contradicts the idea of a sustained lag-phase after glucose addition (Anderson and Domsch 1985a; Blagodatskaya et al. 2007, 2014; Reischke et al. 2015). A rapid change in physiology after a glucose addition also undermines the estimate of maintenance energy demand based on work by Anderson and Domsch (1985a,b; 2010). At present, experimental evidence for or against a rapid stimulation of microbial growth in response to glucose is lacking.

It seems likely that dormant spores do not utilize external substrates, as they are inactive with hardly any ATP or enzyme activity (Keijser et al. 2007; Setlow 2008). This may be true for other survival strategies as well. Results from laboratory studies suggest that large reductions in uptake capacity occur in response to starvation (Casey and Follows 2020; Chubukov and Sauer 2014; Ferenci 2001; Löffler et al. 2017; Navarro Llorens et al. 2010). However, the precise dynamics of glucose transport capabilities await experimental verification under in situ soil conditions. Such experimental evidence may be obtained using metatranscriptomes or metaproteomes (Chuckran et al. 2021). We submit that it is to the benefit of C cycling models when the underlying mechanisms are experimentally verified.

Conclusions

In conclusion, $^{13}$C metabolic flux analysis shows that BE is high under conditions where we expect low
efficiencies associated with high maintenance energy demand or overflow respiration. This appears to be broadly true, even across soils that utilize different biochemical pathways to metabolize glucose. These results contradict four decades of debate in soil ecology arguing that maintenance energy demand in soil communities is high (although variable), and instead point to other factors, such as exudation and microbial turnover, as the main explanation for variation in CUE. These conclusions require that soil C cycling models incorporate a greater spectrum of microbial traits, including growth and survival strategies, and cell death through predation and grazing. Some of this rethinking of model mechanisms is already happening (e.g., Hagerty et al. 2018; Mason-Jones et al. 2022; Manzoni et al. 2021). New tools, including $^{13}$C metabolic flux analysis, will need to play an important role to verify that mechanisms hypothesized in models are supported by experimental evidence.

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Declarations

Competing Interests Authors declare no competing interests.

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