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Paul Dijkstra (Paul.Dijkstra@NAU.EDU)
Northern Arizona University
Weichao Wu
Shanghai Ocean University
Michaela Dippold
University of Tuebingen
Egbert Schwartz
Northern Arizona University
Bruce Hungate
Northern Arizona University
Patrick Megonigal
Smithsonian Environmental Research Center (SERC)
Scott Thomas
New York University College of Dentistry
Cale Seymour
University of Nevada
Ayla Martinez
Northern Arizona University

Research Article

Keywords: Maintenance, Soil Microbial, soil ecology, CUE

Posted Date: January 11th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1193625/v1

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Version of Record: A version of this preprint was published at Plant and Soil on April 9th, 2022. See the published version at https://doi.org/10.1007/s11104-022-05382-9.

On Maintenance and Metabolisms in Soil Microbial Communities

Paul Dijkstra[1], Ayla Martinez[1], Scott C. Thomas[2], Cale O. Seymour[3], Weichao Wu[4], Michaela A. Dippold[5], J. Patrick Megonigal[6], Egbert Schwartz[1] and Bruce A. Hungate[1]

[1] Center for Ecosystem Science and Society (ECOSS) and Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona

[2] New York University College of Dentistry, Department of Molecular Pathobiology, New York, New York

[3] School of Life Sciences, University of Nevada, Las Vegas, NV, United States

[4] Shanghai Engineering Research Center of Hadal Science and Technology, College of Marine Science, Shanghai Ocean University, Shanghai, China and Department of Environmental Science, Stockholm University, Sweden

[5] Geo-Biosphere Interactions, University of Tuebingen, Tuebingen and Biogeochemistry of Agroecosystems, University of Goettingen, Goettingen

[6] Smithsonian Environmental Research Center (SERC), Edgewater, Maryland

Abstract

Biochemistry is an essential yet often undervalued aspect of soil ecology, especially in 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 and growth and maintenance energy requirements; important components of Carbon Use Efficiency (CUE).

The biochemical pathways used to metabolize glucose vary from soil to soil, with mostly glycolysis in some soils, and pentose phosphate or Entner-Doudoroff pathways in others. However, notwithstanding this metabolic diversity, glucose use efficiency is high and thus substrate use for maintenance energy and overflow respiration is low in these three soils. These results contradict current dogma based on four decades of research in soil ecology. 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) production of exudates and microbial turnover affect whole-soil CUE more than variation in maintenance energy demand; 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 of non-growing cells to take up tracer substrates, how biosynthesis responds to these substrates, 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 ecophysiology 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.

Introduction

The topic of this Opinion is ^{13}C metabolic flux analysis and its use for the study of soil microbial ecophysiology, 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 within the central C metabolic network using metabolic flux analysis 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, such as 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 declined due to resource limitations, proportionally more substrate will be required for maintenance and 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 stipulated 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 and death occur at the same time (Blagodatskaya and Kuzyakov 2013; Cruz-Paredes et al. 2021; Koch et al. 2018; Purcell et al. 2021; Reischke et al. 2015).

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), or massive cell death in response to starvation that allow a few cells to feed on

their less-fortunate siblings (Aouizerat et al. 2019; Finkel 2006; Jõers 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. Starvation is accompanied by a reduction of 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. 2021). These survival strategies likely strongly affect soil maintenance energy demand.

Price and Sowers (2004) estimated that the maintenance energy cost for a non-growing cell was three orders of magnitude lower for growing cells, while a dormant cell exhibited activity three orders of magnitude lower again. This means that one actively growing cell equaled 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. As a result, we conclude that maintenance energy demand at the level of soil communities is low.

To Measure Maintenance

Carbon Use Efficiency is very important for 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 ^{13}C-labeled tracers in microbial biomass, captures multiple processes, including metabolic inefficiencies and 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 method used and the duration of the experiment (Geyer et al. 2016; Hagerty et al. 2014). We submit that it is unproductive to continue with the current tradition of calculating average CUE values without making an effort to untangle the underlying processes of metabolic efficiency, 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 CO_2 and biosynthesis, but not affect the processes of exudation and turnover that also affect CUE. Therefore, we introduce a new term, Biochemical Efficiency or BE, to characterize the C use efficiency of the biochemical processes (Table 1). We also limit ourselves to experiments using ^{13}C 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 CO_2 will be released by the reactions of the central C metabolic network (glycolysis, TCA cycle, etc.; Fig. 1). More CO_2 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 early response to a glucose addition is mostly limited to the accumulation of C reserves, and thus is not representative of real growth (Sinsabaugh et al. 2013). However, a direct test of this hypothesis using ^{13}C metabolic flux analysis found no evidence for reserve

138 production (Dijkstra et al. 2015). Recent papers by Mason-Jones et al. (2019, 2021) and Manzoni et al.
139 (2021) further detail storage compound formation in soil microbes and its effects on CUE and overflow
140 respiration.
141
142 *^{13}C Metabolic Flux Analysis*
143 ^{13}C-Based Metabolic Flux Analysis studies the incorporation of ^{13}C from position-specific labeled
144 substrates into biosynthesis products (Zamboni et al. 2009). Observed position-specific incorporation is
145 a direct consequence of the flux or activity patterns of central C metabolic network processes. These
146 processes have been studied in depth and appear remarkably similar across all domains of life (Long and
147 Antoniewicz 2019).
148 We adapted the metabolic flux analysis for soil communities using six position-specific ^{13}C
149 labeled glucose isotopomers in parallel incubations (Dijkstra et al. 2011a, 2015), but instead of
150 determining ^{13}C in biosynthesis products, we measure the ^{13}C incorporation into CO_2 for each C atom in
151 the glucose molecule (Dijkstra et al. 2011a,b; Dijkstra et al. 2015; Geyer et al. 2019; Hagerty et al. 2014;
152 van Groenigen et al. 2013). CO_2 is released almost instantaneously after glucose addition, and its isotope
153 composition can be readily measured at low cost using modern laser spectrometers. The position-
154 specific ^{13}C-CO_2 measurements usually take about 40 min at room temperature, although can be done
155 within 5 min (A. Martinez, D. Verdi, and P. Dijkstra unpublished results).
156 The proportion of CO_2 production per C atom from position-specific ^{13}C-labeled glucose directly
157 reflects the rates of the biochemical processes of the central C metabolic network and BE. Theoretical
158 analysis reveals patterns of position-specific CO_2 production that are associated with high activity of one
159 of the three main metabolic pathways (Fig. 2 left): high CO_2 production from positions C3 and C4 is
160 expected when Embden-Meyerhof-Parnass glycolysis (EMP or 'glycolysis') is highly active, whereas high
161 CO_2 production from position C1 and C4 are predicted when the Pentose Phosphate (PP) or Entner-

Doudoroff (ED) pathway activity is dominant. The ED pathway is an evolutionarily ancient glycolytic pathway (Conway 1992; Kopp and Sunna 2020) and is widely 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 CO_2 production between a soil community with high PP or a high ED pathway activity are more subtle; high ED activity results in a slightly higher CO_2 production from position C2 than C3, while high PP activity produces slightly less CO_2 from C2 than C3.

The differences in CO_2 production from different C positions are larger when BE is high (Fig. 3; Dijkstra et al. 2015), because some C atoms are preferentially released as CO_2, while others are incorporated into biosynthesis products. However, as maintenance energy demand increases and BE decreases, CO_2 production per C position 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 CO_2 and position-specific CO_2 production is 16.7% (=1/6) of the total CO_2 production (Fig. 3). Therefore, when differences between C-positions are large, we can conclude that BE is high, and thus maintenance or overflow respiration are low.

A Modicum of Metabolisms and High Efficiency

Metabolic flux measurements of soil indicated a large proportion of CO_2 from the first C-atom in glucose (C1). We have interpreted this as an indication of a high PP activity (Dijkstra et al. 2011a,b; Dijkstra et al. 2015; Geyer et al. 2019; Hagerty et al. 2014; van Groenigen et al. 2013), but did not consider the effect of an ED pathway (Fig. 1). However, the observed patterns of CO_2 production per C-atom for three distinct soil ecosystems suggested a greater diversity in glucose metabolism than initially proposed (Fig. 2 right). We expanded our metabolic model (Dijkstra et al. 2011a, 2015) to include reactions of the ED pathway and explored whether differences in position-specific CO_2 production (Fig. 2 right) were associated with significant differences in glycolysis, PP, or ED pathway activity. Three

reactions determined what pathway was used when processing glucose: reaction r2 (glucose metabolized via EMP glycolysis), r10 (PP pathway), and r13 (ED pathway; Fig. 1). Reactions rates exhibited significant differences (Fig. 4 top), with high activity of r2 for the tidal freshwater marsh soil, high activity of r10 for the low elevation cool desert grassland soil, and high activity of r10 and r13 for the high elevation mixed conifer soil. This indicated that glucose was mostly processed via EMP glycolysis in the freshwater marsh soil, via ED and PP pathways in the high elevation mixed conifer soil, and via PP with some contributions from glycolysis and ED pathway for the low elevation desert grassland soil (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 (Edirisinghi 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 ED pathway, concluding that the ED pathway was important in marine ecosystems. It appears from our results that this holds for some soils as well. The ED pathway may provide advantages over the EMP 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 ED (and PP) pathways also produce large quantities of NADPH, thus offering protection against oxidative damage (Dijkstra et al. 2011b; Klingner et al. 2015), potentially a crucial requirement in oxygen-rich soil environments.

High (EMP) glycolysis activity was also observed in a paddy soil and lake sediment (Krumböck and Conrad 1991), and may be characteristic of anoxic environments. Likewise, 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

glycolysis over ED pathway may be critical, while the production of NADPH to fight oxidative stress is less important. It should be noted that measurement of CO_2 production per C atom as described here can be supplemented with 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 had a slightly but significantly lower BE (Fig. 4 bottom). BE 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 (CUE_A).

The Full Maintenance?

It has been argued that tracer experiments do not capture the full maintenance demand in soil ecosystems, but only energy demand 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 the BE measured shortly after adding an isotope enriched substrate is high, the efficiency across the cell's lifecycle, including an extended period as starved, non-growing cells, is much lower (0.32; Hagerty et al. 2018). However, no clear mechanism was proposed that would prevent us from measuring glucose metabolism from 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 limitation 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 (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, the large reduction in 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 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, conducted by Geyer et al. (2019), showed that BE was maintained for at least 72-h after a large glucose addition. This again suggests that a glucose addition, even across a growth pulse, does not result in a community with high maintenance energy demands.

However, it is possible that tracer compounds (for example glucose) are metabolized by growing but not by non-growing cells. This could occur if glucose instantaneously 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 extracellular 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 lag-phase after glucose addition (Anderson and Domsch 1985a; Blagodatskaya et al. 2007, 2014; Reischke et al. 2015). However,

it is possible that an increase in biosynthesis may precede cell replication by several hours. A rapid change in physiology after a glucose addition also undermines the estimates 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. Similarly, it seems likely that dormant spores do not utilize external substrates, as they are inactive with hardly any ATP and enzyme activities (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 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. 2020). We submit that it is to the benefit of C cycling models when the underlying mechanisms are experimentally verified.

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 across soils that utilize different biochemical pathways to process glucose. These results contradict four decades of discussions in soil ecology on the importance of maintenance energy demand in soil communities, and instead point to other factors, such as exudation and microbial turnover, as the cause for variation in CUE. These results 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. 2021; 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.

Acknowledgements

Contributions from authors were made possible by funding from the following agencies: PD and AM: USDA National Institute of Food and Agriculture Foundational Program [award #2017-67019-26396]; PD, AM, and PM: the DOE Terrestrial Ecosystem Science Program [award #DE-SC0014413]; BAH: US Department of Energy's Biological Systems Science Division Program in Genomic Science DE-SCSC0020172; WW: the European Union's Horizon 2020 Research and Innovation Programme under the Marie Sklodowska-Curie grant No 840240 and the National Natural Science Foundation of China (42106046); and MD "EcoEnergeticS" DFG DI 2136/17-1.

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Figure Legends

Fig. 1. Metabolic model with three glucose metabolizing pathways: Embden-Meyerhof-Parnas (EMP, r2 - red arrow) glycolysis, Pentose Phosphate pathway (r10 - blue), and Entner-Doudoroff (ED, r13 - yellow) pathway. Pathway reactions (r2 ... r15) and biomass reactions (br1 ... br8) are expressed as moles relative to r1 (set at 100 moles). 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).

Fig. 2. Modeled (left) and observed (right) CO_2 production per C-position (means and stdev, n=4). Modeled values are CO_2 production per C atom calculated using a metabolic model with EMP, PP, ED pathway and TCA cycle reactions (Fig. 1; adapted from Dijkstra et al. 2011a, 2015). Modeled CO_2 production per C atom are for BE = 0.6 and maximal activity of EMP, PPP, and ED respectively. Observations are based on measurement of CO_2 production from 6 glucose mono-isotopomers in parallel incubations for soil from the Jug Bay tidal freshwater marsh near Annapolis, Maryland (FM; A. Martinez, P. Megonigal, and P. Dijkstra, in prep), a low elevation cool desert grassland (GL) and a high elevation meadow in mixed conifer forest (MC) near Flagstaff, Arizona (Dijkstra et al. 2007). Theoretical calculations are done for maximum EMP activity by setting r13 at zero, and r10 at the lowest rate that satisfies biosynthesis demand (br8), maximum PP activity by setting r13 and r10 to zero, and maximum ED activity by setting r2 to zero and PP at lowest value that satisfies br2 plus br8 (Fig. 1).

Fig. 3. Relationship between Biochemical Efficiency (BE) and CO_2 production from C-atom 1 in glucose relative to CO_2 production from uniformly-labeled glucose (CU) modeled for a central C metabolic network dominated by PP pathway activity. Results from theoretical calculations using metabolic model (Fig. 1), r2 and r13 set at 0, and varying r10 and br1 to change BE.

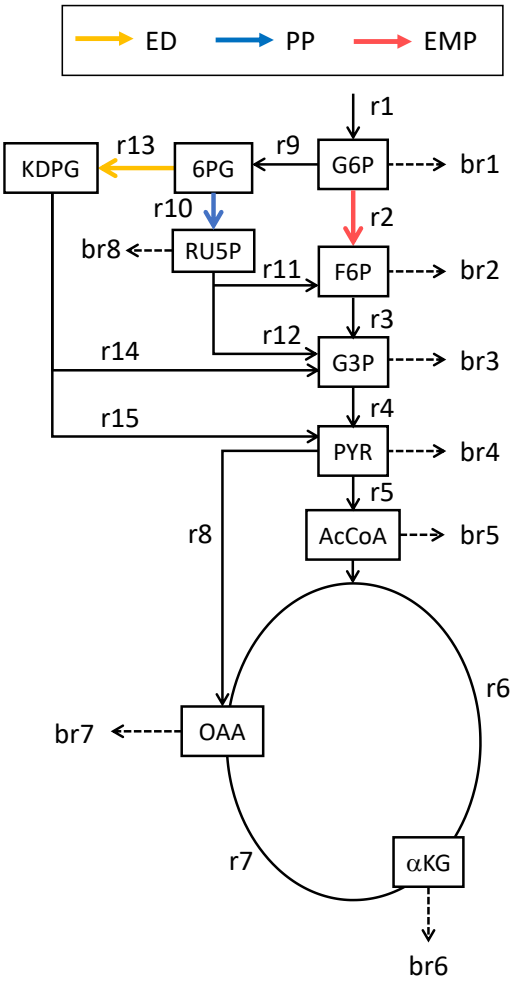
Fig. 4. Differences in activity for initial reactions of EMP glycolysis (r2), PP (r10) and ED (r13) pathways (upper panel), proportion of activity of the three main glucose metabolizing pathways (middle), and Biochemical Efficiency (lower) of a high elevation meadow in mixed conifer forest (MC), low elevation cold desert grassland (GL) and a tidal freshwater marsh (FM). BE is calculated as $\frac{6 \times r1 - \sum CO_2}{6 \times r1}$*. Means and 95% confidence intervals are calculated using 1,000 random samples from the observed distribution of CO_2 production per C atom (Fig. 2 right) and solving the metabolic model (Fig. 1) using a Generalized Reduced Gradient non-linear optimization method with 100 restarts (Solver – Excel; Dijkstra et al. 2011a).*

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.

Variable	Equation	Geyer et al. 2016[#]	Hagerty et al. 2018[*]	Manzoni et al. 2018[$]
Biochemical efficiency (BE)	(U-R)/U	CUE_C	CUE_S	CUE
Biomass Yield	(U-R-EX)/U	$CUE_C = CUE_P$	CUE	GGE
Apparent CUE (CUE_A)	(U-R-Ex-T)/U	CUE_E	CUE_C	CUE_A

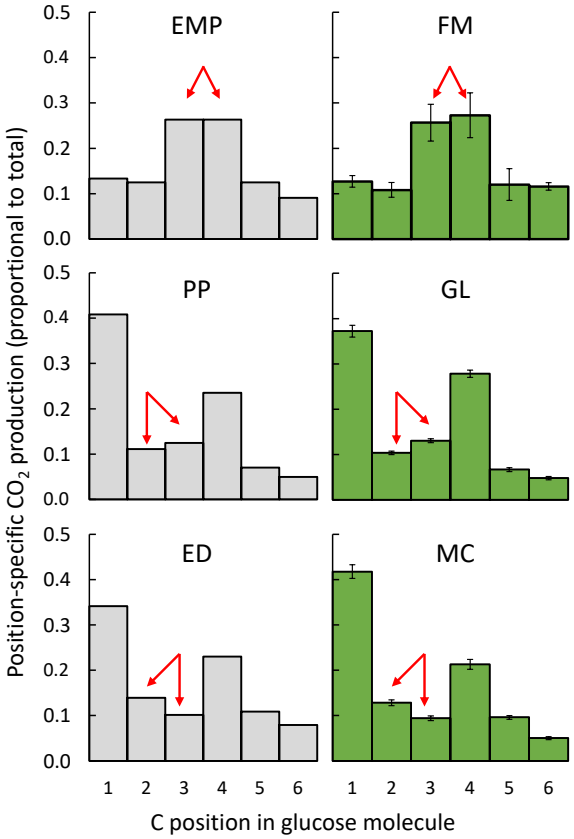
[#]CUE_C is community-scale CUE; CUE_E is ecosystem-scale CUE (Geyer et al. 2016). [*]CUE_S is substrate-based CUE; CUE_C is concentration-based CUE (Hagerty et al. 2018). [$]GGE is Gross Growth Efficiency; CUE_A is apparent CUE (Manzoni et al. 2018).

550 Fig. 1


ED
PP
EMP
r1
G6P
br1
r9
6PG
r13
KDPG
r10
RU5P
br8
r2
F6P
br2
r11
r3
G3P
br3
r12
r14
r4
PYR
br4
r15
r5
AcCoA
br5
r8
r6
OAA
br7
r7
αKG
br6

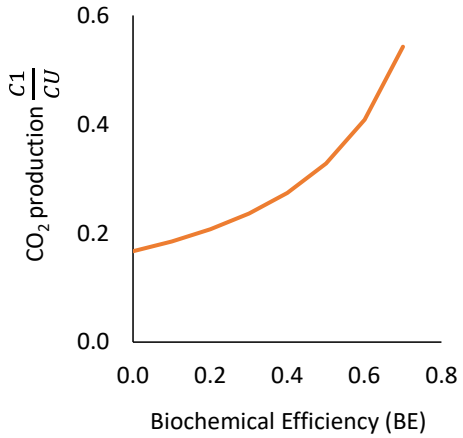
551

552 Fig. 2


EMP
FM
PP
GL
ED
MC
0.5
0.4
0.3
0.2
0.1
0.0
1 2 3 4 5 6
Position-specific CO_2 production (proportional to total)
C position in glucose molecule

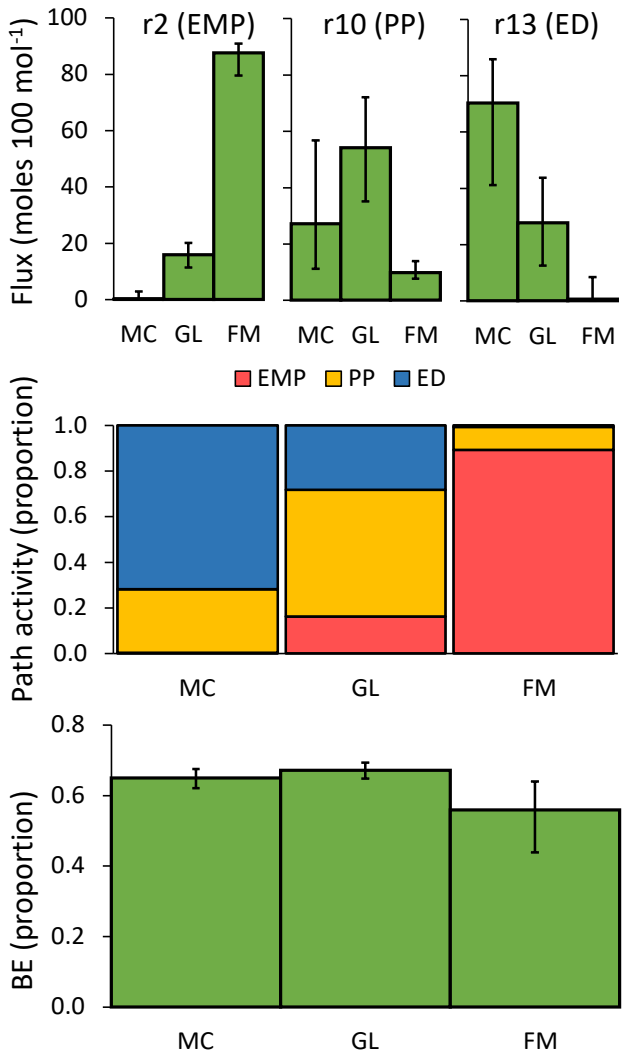
553

554 Fig. 3.


0.6
0.4
0.2
0.0
CO_2 production $\frac{C1}{CU}$
0.0
0.2
0.4
0.6
0.8
Biochemical Efficiency (BE)

555

556 Fig. 4


r2 (EMP)
r10 (PP)
r13 (ED)
Flux (moles 100 mol-1)
100
80
60
40
20
0
MC GL FM MC GL FM MC GL FM
EMP PP ED
Path activity (proportion)
1.0
0.8
0.6
0.4
0.2
0.0
MC GL FM
BE (proportion)
0.8
0.6
0.4
0.2
0.0
MC GL FM

557