High carbon use efficiency in soil microbial communities is related to balanced growth, not storage compound synthesis

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The efficiency with which microbes use substrate (Carbon Use Efficiency or CUE) to make new microbial biomass is an important variable in soil and ecosystem C cycling models. It is generally assumed that CUE of microbial activity in soils is low, however measured values vary widely. It is hypothesized that high values of CUE observed in especially short-term incubations reflect the build-up of storage compounds in response to a sudden increase in substrate availability and are therefore not representative of CUE of microbial activity in unamended soil.

To test this hypothesis, we measured the $^{13}CO_2$ release from six position-specific ^{13}C-labeled glucose isotopomers in ponderosa pine and piñon-juniper soil. We compared this position-specific CO_2 production pattern with patterns expected for 1) balanced microbial growth (synthesis of all compounds needed to build new microbial cells) at a low, medium, or high CUE, and 2) synthesis of storage compounds (glycogen, tri-palmitoyl-glycerol, and polyhydroxybutyrate).

Results of this study show that synthesis of storage compounds is not responsible for the observed high CUE. Instead, it is the position-specific CO_2 production expected for balanced growth and high CUE that best matches the observed CO_2 production pattern in these two soils. Comparison with published studies suggests that the amount of glucose added in this study is too low and the duration of the experiment too short to affect microbial metabolism. We conclude that the hypothesis of high CUE in undisturbed soil microbial communities remains viable and worthy of further testing.

1. Introduction

Heterotrophic microbes use organic carbon (C) compounds to synthesize cellular compounds while releasing some substrate-C as CO_2. Which compounds are synthesized depends on the physiology of the cells (active growth and division, survival when substrate availability is low, dormancy). It is currently not possible to determine directly the compounds that are produced. It seems plausible that the microbial community consists of cells in all possible physiological states at any time, unless there are synchronizing events, such as a simultaneous depletion of substrate in all soil niches or a sudden increase in substrate availability. The C Use Efficiency (CUE; biomass-C synthesized per substrate-C consumed; mol C/mol C) of the soil microbial community is an important ecosystem variable that influences what proportion of organic C utilized is released to the atmosphere as CO_2 or potentially remains in the soil as organic matter in living cells or dead soil organic matter (Billings and Ballantyne, 2013; Bradford, 2013; Hagerty et al., 2014). Indirectly, CUE also determines whether nutrients such as nitrogen (N) or phosphate are immobilized or mineralized (Manzoni et al., 2012; Sinsabaugh et al., 2013). Consequently, an improved understanding of CUE is important for soil C and N cycling models (Allison et al., 2010; Manzoni et al., 2012; Wieder et al., 2013; Hagerty et al., 2014; Li et al., 2014). The CUE is a

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function of the cellular demand for energy and biosynthesis, and therefore a function of the physiological state and the type of compounds that are being produced. When only energy is required (such as for cell maintenance), CUE is close or equal to zero (Chapman and Gray, 1986; Amthor, 2000).

Because of low C availability in soil and the supposedly recalcitrant nature of soil organic matter, the CUE of the microbial community is often assumed to be low (Anderson and Domsch, 2010; Manzoni et al., 2012; Sinsabaugh et al., 2013; Reischke et al., 2015). The limited substrate available is used to satisfy energy demands for cell maintenance with little left for growth. However, many studies find high values of CUE (0.6 and higher; e.g., Brant et al., 2006; Dijkstra et al., 2011a,b; Frey et al., 2013; van Groenigen et al., 2013; Hagerty et al., 2014; Steinweg et al., 2008; Thiet et al., 2006; Tucker et al., 2013; Ziegler et al., 2005). The average CUE observed in soil is 0.55 (Manzoni et al., 2012; Sinsabaugh et al., 2013). This value is remarkably close to the average maximum value of CUE observed in pure culture studies (~0.6; Blagodatskaya et al., 2014; Roels, 1980; Sinsabaugh et al., 2013), but below the theoretical thermodynamic maximal CUE of growth on glucose (0.88−1.0; Gommers et al., 1988; Heijnen, 2010; Heijnen and van Dijken, 1992; Manzoni et al., 2012; Roels, 1980; Xiao and van Briesen, 2006). The average CUE for soil is much higher than that found in aquatic ecosystems (~0.3; Hobbie and Hobbie, 2013; Manzoni et al., 2012; Sinsabaugh et al., 2013). This large discrepancy in CUE raised concerns (Hobbie and Hobbie, 2013; Sinsabaugh et al., 2013), prompting a critical evaluation of methods used to determine community CUE (Sinsabaugh et al., 2013).

The measurement of CUE often involves adding (^{13}C-enriched) substrates. It is suggested that high substrate additions alter CUE, either increasing (Sinsabaugh et al., 2013; van Groenigen et al., 2013) or decreasing it (van Groenigen et al., 2013; Russell, 2007). Specifically for short-term experiments, it is hypothesized that high CUE values may not represent microbial balanced growth (that is, the synthesis of all compounds needed to build new cells), but instead may be the result of rapid uptake of substrate followed by synthesis of storage compounds (Nguyen and Guckert, 2001; Hill et al., 2008; Sinsabaugh et al., 2013; Blagodatskaya et al., 2014; Reischke et al., 2014, 2015). Although this still represents an increase in biomass, for a sound understanding of C cycling in soil ecosystems, it is important to distinguish between CUE during long-term microbial activity and that where microbes temporarily allocate C to storage synthesis associated with a sudden and temporary increase in substrate availability (Sinsabaugh et al., 2013). Microbial cells can store substrate as starch, glycogen, trehalose, extracellular polysaccharides (Wilson et al., 2010), polyhydroxyalkanoates and storage lipids (Olsson and Johansen, 2000; Lu et al., 2009). However, measurements of storage synthesis in soil have not been made.

In this study, we evaluate four mutually exclusive hypotheses: 1) the microbial community uses substrate for maintenance only (CUE = 0); 2) the microbial community exhibits balanced growth but an overall low CUE (CUE = 0.3 as suggested by Sinsabaugh et al., 2013), 3) the microbial community exhibits a high CUE but "unbalanced" growth where biosynthesis is limited to storage compound production (glycogen, lipids, or polyhydroxybutyrate), and 4) the microbial community exhibits balanced growth at high CUE (0.6; close to the maximal CUE in pure culture studies).

We conducted an incubation experiment with six position-specific ^{13}C-labeled glucose isotopomers and two soils from northern Arizona, USA. We compared the observed pattern of position-specific CO_2 production with patterns predicted for balanced microbial growth at varying CUE (CUE = 0, 0.3, or 0.6) and storage synthesis (glycogen, tri-palmitoyl-glycerol − TPG −

and polyhydroxybutyrate - PHB). By comparing our experimental methods and results with published studies of responses of microbial growth to substrate addition, we tested a fifth hypothesis that the increase in substrate availability changed the CUE of the microbial community. We show that the observed position-specific CO_2 production resembles patterns expected for balanced growth at high CUE, and does not match CO_2 production patterns of any combination of low or medium CUE and storage compound synthesis. According to currently published research results, these results were not affected by the change in substrate availability.

2. Materials and methods

2.1. Experimental procedures

We collected soil (0−10 cm depth) from two locations along the C. Hart Merriam Elevation Gradient (www.nau.edu/Ecoss/) near Flagstaff, Arizona in the fall of 2012. The highest site (2340 m elevation, mean annual temperature (MAT) 8 °C, mean annual precipitation (MAP) 660 mm) was a small open area in a ponderosa pine (*Pinus ponderosa*) stand covered with blue grama (*Bouteloua gracilis*) grass. Soil was a Mollic Eutroboralf (C content 1.5%, N content 0.11%; Dijkstra et al., 2006). The second site (2020 m elevation, MAT 10 °C, MAP 380 mm) was an intercanopy space in a piñon-juniper stand (*Pinus edulis, Juniperus monosperma*) also covered with blue grama grass. Soil type was a Calcic Haplustand (C content 1.7%, N content 0.16%; Dijkstra et al., 2006). Soil was sieved (2 mm mesh) and stored at 4 °C until used.

We weighed 40 g of sieved soil into a specimen cup and placed it in a Mason jar (473 ml) equipped with an airtight lid and septum (n = 4). Soil moisture content was adjusted to field capacity (0.272 and 0.300 g water g^{-1} soil dry weight for respectively ponderosa pine and piñon-juniper soil) and soil was incubated overnight in the dark at room temperature (21 °C). The next morning, jars were opened, headspace atmosphere was replaced with lab air, and, after closing the jar, 10 ml of pure CO_2 was added to the headspace. This addition of pure CO_2 was needed to have enough CO_2 in 10 ml headspace gas samples for the Picarro 2101-*i* CO_2 isotope spectrometer (Picarro Inc, Sunnyvale, CA) to measure isotope ratios within the calibrated range of concentrations (Dijkstra et al., 2011a). After 30 min and before glucose isotopologue addition, a 10 ml headspace gas sample was taken (time zero).

We used glucose (^{13}C-labeled in C$_1$, C$_2$, C$_3$, C$_4$, C$_5$, C$_6$ and uniformly (U) labeled) as the metabolic tracer (99 atom fraction %; Cambridge Isotope Laboratories, Andover, Massachusetts). Two ml of a 1.79 mM glucose isotopomer solution was added to each specimen cup (0.536 μmol glucose-C g^{-1} soil; n = 4). Because of the large number of isotopologues, replicates, each consisting of seven glucose isotopologue incubations, were done on successive days. Ten ml headspace gas samples were taken 20, 40, and 60 min after tracer addition and analyzed for isotope composition with the Picarro CO_2 isotope analyzer. The isotope composition of headspace CO_2 was expressed as atom fraction excess (%; Coplen, 2011) and plotted against time. We determined the slope of atom fraction excess (calculated as the difference between the atom fraction at t = 1 and the atom fraction at t = 0) for the period that the CO_2 production rate was constant (40 min, Fig. 3A) and calculated the ratio of position-specific CO_2 production rates as follows:

$$\frac{C_x}{C_U} = \frac{^{13}CO_2 \ production \ from \ x - {}^{13}C \ glucose}{^{13}CO_2 \ production \ from \ U - {}^{13}C \ glucose} \tag{1}$$