Do growth yield efficiencies differ between soil microbial communities differing in fungal:bacterial ratios? Reality check and methodological issues

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Abstract

Soil communities dominated by fungi such as those of no-tillage (NT) agroecosystems are often associated with greater soil organic matter (SOM) storage. This has been attributed in part to fungi having a higher growth yield efficiency (GYE) compared to bacteria. That is, for each unit of substrate C utilized, fungi invest a greater proportion into biomass and metabolite production than do bacteria. The assumption of higher fungal efficiency may be unfounded because results from studies in which fungal and bacterial efficiencies have been characterized are equivocal and because few studies have measured microbial GYE directly. In this study, we measured microbial GYE in agricultural soils by following ^{13}C-labeled glucose loss, total CO_2–C, and $^{13}CO_2$–C evolution at 2 h intervals for 20 h in two experiments (differing in N amendment levels) in which the fungal:bacterial biomass ratios (F:B) were manipulated. No differences in efficiency were observed for communities with high versus low F:B in soils with or without added inorganic N. When calculated using $^{13}CO_2$–C (in contrast to total CO_2–C) evolution, growth yield efficiencies of soils having high and low F:B were 0.69 ± 0.01 and 0.70 ± 0.01, respectively. When soils were amended with N, soils with high and low F:B had growth yield efficiencies of 0.78 ± 0.01 and 0.76 ± 0.01, respectively. Our experiments do not support the widely held assumption that soil fungi have greater growth efficiency than soil bacteria. Thus, claims of greater fungal efficiency may be unsubstantiated and should be evoked cautiously when explaining the mechanisms underlying greater C storage and slower C turnover in fungal-dominated soils.
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1. Introduction

Fungal:bacterial (F:B) biomass ratios are typically higher in soils from undisturbed grassland ecosystems (Bardgett et al., 1996; Bailey et al., 2002), grasslands lacking long-term fertilization histories (Lovell et al., 1995; Bardgett et al., 1996), and reduced tillage agroecosystems (Beare et al., 1992; Frey et al., 1999a). Fungal-dominated soil communities may enhance C storage and slow soil organic matter (SOM) turnover due both to fungal alteration of soil physical properties and to fungal physiology (Nakas and Klein, 1979; Tisdall and Oades, 1982). Thus, ecosystems with soils dominated by fungi may sequester more C than systems with lower fungal abundance (see Six et al., 2005 for a review).

Increased C storage in fungal-dominated systems is often explained by a hypothesized greater growth yield efficiency (GYE) of fungi compared to bacteria (Holland and Coleman, 1987; Parton et al., 1987; Zak et al., 1996); it has become virtually axiomatic that for each unit of substrate C immobilized, fungi invest a greater proportion into biomass and metabolite production than bacteria. It is unclear where this concept originated as there are few data in the published literature supporting its validity. There is significant overlap in the range of reported values for fungal versus bacterial growth efficiency based on laboratory culture studies (Six et al., 2005). Efficiency

values reported for soils have usually been obtained by amending soil containing a mixed microbial population with a simple substrate, usually glucose. Since most of these studies do not report the relative abundance/biomass of fungi and bacteria, it is impossible to attribute observed differences in GYE to differences in F:B biomass ratios. To our knowledge, direct measurements of the production efficiency of microbial communities differing in F:B ratios have not been made. The idea of greater fungal efficiency, which has been in the literature for decades yet for which there is little supporting evidence, needs rigorous testing.

Wardle and Ghani (1995) suggested that qCO_2 (i.e., the amount of CO_2–C respired per unit microbial biomass-C) is a useful measure of microbial efficiency. Observed negative correlations between F:B and qCO_2 (Sakamoto and Oba, 1994; Blagodatskaya and Anderson, 1998) have been taken as indirect evidence that fungal-dominated communities are more efficient than bacterial-dominated ones. However, there are equally plausible explanations for the observed results: (1) fungal-dominated communities may utilize less substrate per unit of measured biomass (thus lower respiration) or (2) biomass may be overestimated in fungal-dominated communities because much of the measured hyphae (e.g. when microscopy is used to estimate biomass) may be inactive (Six et al., 2005). Without knowing how much substrate was utilized by the microbial community for the time period over which respiration and biomass measurements were made, or how much CO_2 was respired per unit *new* biomass, it is impossible to determine the relative partitioning between biomass production and respiration losses, a necessary requisite for calculating GYE.

Quantifying microbial GYE accurately is important for incorporating microbial growth and metabolism parameters into C and nutrient cycling models. For example, the Century SOM model assumes that 55% of substrate C immobilized by microbes is lost as CO_2 when soil organic matter is decomposed, whereas 45% of immobilized C is lost as CO_2 when surface litter is decomposed (Parton et al., 1987). Since fungi are the primary decomposers of surface litter, predicted CO_2 losses assumed in this model center largely on the assumption that fungi are more efficient than bacteria. A sensitivity analysis of the model indicated that small changes in the microbial GYE parameter led to significant changes in predictions of C and N cycling (K. Paustian, Colorado State University, personal communication).

Our objective was to test the hypothesis that fungal-dominated microbial communities have a higher GYE than bacterial-dominated communities. To do this we traced ^{13}C-labeled glucose through soils differing in F:B biomass ratios. We conducted two laboratory experiments with no-tillage (NT) agricultural soils in which microbial community composition was altered using additions of high and low quality wheat residues (Bossuyt et al., 2001).

We expected that adding ^{13}C-labeled glucose, tracking ^{13}C-glucose loss, and coupling ^{13}C-glucose loss with $^{13}CO_2$–C respiration would allow us to ensure that microbes were metabolizing the added substrate rather than background SOM or recycled microbial cells. Growth yield efficiencies of the different microbial communities were calculated using ^{13}C-glucose utilization and $^{13}CO_2$–C evolution data.

2. Materials and methods

Bulk soil was collected from the 0–5 cm depth of NT plots under continuous corn rotation at the Ohio Agricultural Research and Development Center in Wooster, OH, USA. The soil is Wooster silt loam with 18% sand, 56% silt, and 26% clay. Total organic C and particulate organic matter C of this soil (to 20 cm depth) are 4381 and 752 g C m^{-2}, respectively, and bulk density is 1.36 g cm^{-3} (Paustian et al., 1997). Mean annual temperature at the site is 9.5 °C and mean annual precipitation is 733 mm. Bulk soil was air dried, sieved (2 mm), and stored dry until the experiments began.

This study was organized in two experiments (hereafter experiment 1 and experiment 2), differing only in their level of N amendment; soils were amended with inorganic N in experiment 2. Both experiments had two treatments: high and low F:B biomass ratios. For each experiment, microbial community structure was altered by adding low or high quality wheat straw residues. High F:B treatment communities were established in bulk soils by adding 4 mg straw g^{-1} soil of low quality ground (500–850 µm) wheat straw stems (C:N = 142). Low F:B treatment communities were established by adding 4 mg straw g^{-1} soil of high quality ground (500–850 µm) wheat straw leaves (C:N = 56) (Bossuyt et al., 2001). The straw-amended soil was moistened to field moisture capacity (29%) and incubated for 14 d at 25 °C to stimulate development of the different microbial communities.

Following the 14 d incubation, soil from each treatment was divided into 12.7 g field moist (equivalent to 10 g dry) aliquots. All samples were then partially dried for 6 h and rewetted to field moisture capacity at the onset of each experiment using a uniformly labeled ^{13}C-glucose solution (1.99% enrichment) at a rate of 2000 µg glucose g^{-1} soil (equivalent to 800 µg glucose-C g^{-1} soil). We observed low ^{13}C respiration rates in experiment 1 and attributed this to N limitation (Chahal and Wagner, 1965; Smith et al., 1985); thus, the ^{13}C-glucose solution added to soils in experiment 2 was supplemented with 127 µg $(NH_4)_2SO_4$ g^{-1} soil to achieve a substrate C:N of 30. Control soils were rewetted with deionized water only.

Soil samples used for assessment of CO_2 evolution were sealed in Mason jars with luer lock fittings and maintained at 25 °C for 24 h. In both experiments, starting 8 h after

^{13}C-glucose addition a 12 ml gas sample was collected every 2 h from the Mason jar headspace (see below) of each of four replicates per F:B treatment (plus controls: no manipulation of the microbial community) to measure total and $^{13}CO_2$–C gas production. In addition, four samples per treatment were destructively sampled at the time of each CO_2–C measurement and assayed for the ^{13}C-labeled glucose concentration. The experiments were terminated 20 h after ^{13}C-glucose addition, as prior tests of glucose loss in these soils demonstrated that glucose was entirely utilized by 16 h. Glucose concentrations were determined spectrophotometrically using the hexokinase-glucose-6-phosphate dehydrogenase enzymatic assay (Frey et al., 1999b).

The gas-tight syringe (Hamilton Gastight #1025, Reno, NV) used for gas collection was purged with ambient air between samples, and the CO_2 in the headspace of each jar was mixed thoroughly before sampling by plunging the syringe three times into the headspace through the luer lock fitting. The jar headspaces were not aired out in the 2 h intervals between gas sample collections. Each 12 ml gas sample was injected into a 12 ml glass vial (LabCo Exetainer, UK) and stored for up to 14 d until $^{13}CO_2$–C analyses were performed. Gas samples were analyzed for total and $^{13}CO_2$–C with a trace gas isotope ratio mass spectrometer (TGII-IRMS, PDZ Europa, UK) at the University of California Davis Stable Isotope Facility.

Fungal and bacterial biomass were determined by direct microscopy (Frey et al., 1999a) on a subset of conditioned high and low F:B soils ($n=2$ for each treatment) to ensure that communities were sufficiently distinct. Fungal:bacterial biomass ratios in high and low F:B treatments averaged 7.5 and 1.0, respectively. Microbial growth yield efficiency (GYE) was calculated as:

$$GYE = (d^{13}S_C - \Sigma^{13}CO_2 - C)/d^{13}S,$$

where $d^{13}S_C$ is the change in added ^{13}C-labeled substrate (i.e. added ^{13}C-labeled glucose that is utilized) and $\Sigma^{13}CO_2$–C is the cumulative $^{13}CO_2$–C lost during respiration (Frey et al., 2001). We assumed that all utilized substrate-C not oxidized was incorporated into microbial biomass or metabolites. For comparison with other studies that have reported microbial efficiencies using only total CO_2 evolution, growth yield efficiencies of high and low F:B communities were also calculated using the same equation but without the ^{13}C label; i.e. GYE was also calculated using total (unlabeled) CO_2–C evolution. Glucose utilization in experiments 1 and 2 approached 90% at 14 and 10 h, respectively (Figs. 1 and 2). Thus, for statistical comparisons of community GYE in both experiments we used GYE values calculated for total and $^{13}CO_2$–C evolution at 14 and 10 h, respectively, to avoid measuring respiration from the recycling of microbial cells as they die and are used as substrate by living cells (cryptic
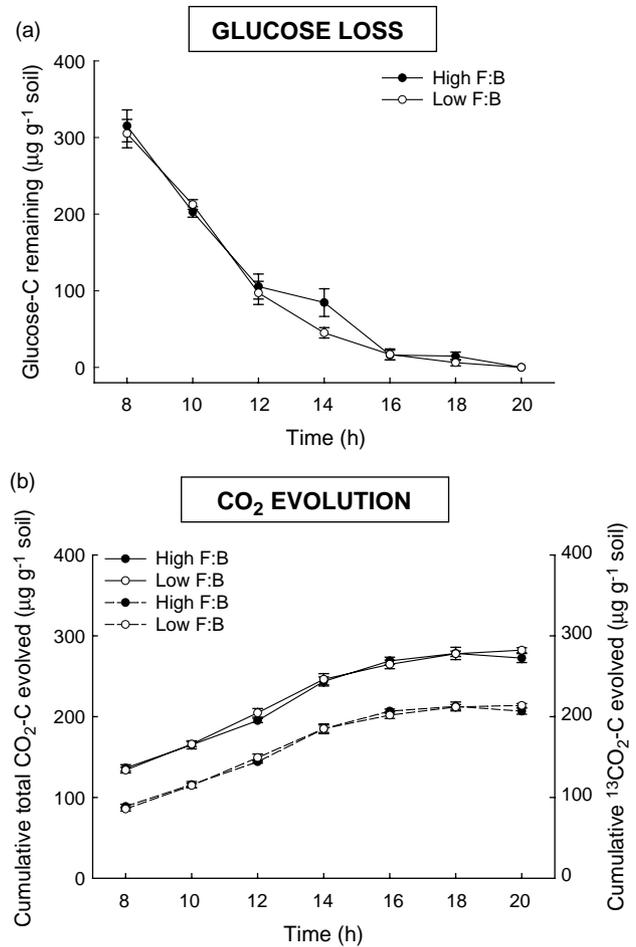

Fig. 1. (a) Glucose loss and (b) cumulative total CO_2–C (solid lines) and $^{13}CO_2$–C (dashed lines) evolution in high and low F:B microbial communities in soils with no added inorganic N (experiment 1). Data are mean ± one standard error ($n=4$).

growth). The data were analyzed by analysis of variance after being tested for normality and homogeneity of variances (PROC GLM; SAS Institute 1996). All assumptions of parametric techniques were met. Statistical significance was determined at $P \leq 0.05$.

3. Results

3.1. Experiment 1 (no N amendment)

3.1.1. C mineralization

In experiment 1, 90–95% of the added glucose was utilized by both high F:B and low F:B communities within 14 h of the initial glucose amendment, and the rate and level of glucose utilization were not significantly different for the two communities (Fig. 1a). Total CO_2–C evolution by high and low F:B communities proceeded at approximately the same rate and did not differ significantly between the two communities. Microbial $^{13}CO_2$–C evolution was
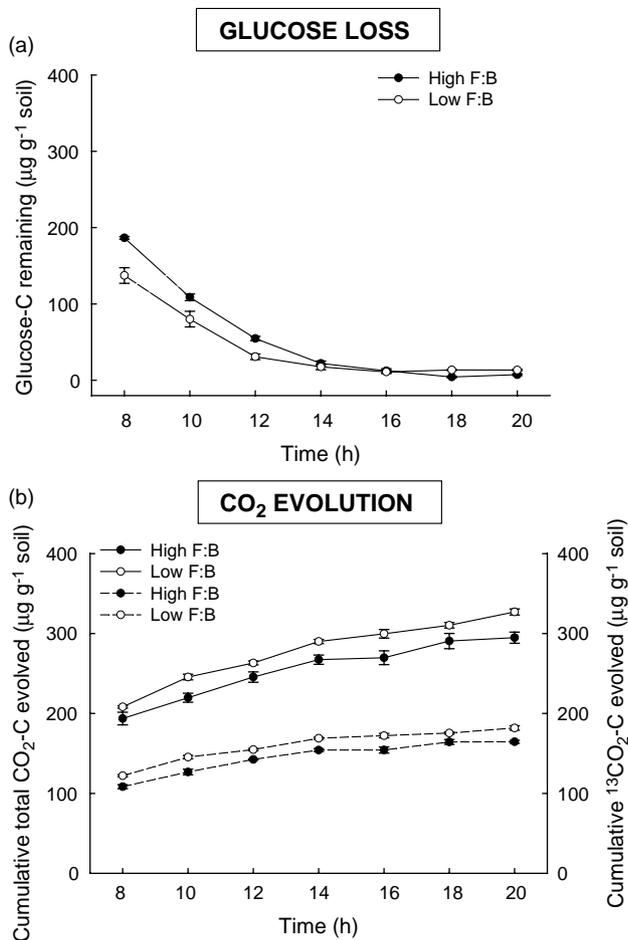
(a)

GLUCOSE LOSS

(b)

CO$_2$ EVOLUTION

Fig. 2. (a) Glucose loss and (b) cumulative total CO_2–C (solid lines) and $^{13}CO_2$–C (dashed lines) evolution in high F:B and low F:B microbial communities in soils amended with inorganic N (experiment 2). Data are mean ± one standard error ($n=4$).

consistently lower than total CO_2–C efflux throughout the experiment (Fig. 1b). $^{13}CO_2$–C flux accounted for only 64–77 and 63–77% of total CO_2–C respired from high and low F:B soils, respectively. High and low F:B soils did not differ in $^{13}CO_2$–C evolution (Fig. 1b).

3.1.2. Growth yield efficiencies

Microbial efficiencies of high and low F:B communities in experiment 1 were not significantly different when calculated using either total CO_2–C or $^{13}CO_2$–C evolution

Table 1
Effect of fungal:bacterial biomass ratio (F:B) on growth yield efficiency (GYE) in soil with or without added inorganic N

Community	Experiment 1 (no N added)		Experiment 2 (N added)	
	Total CO_2–C	$^{13}CO_2$–C	Total CO_2–C	$^{13}CO_2$–C
High F:B	0.59 (0.02)	0.69 (0.01)	0.62 (0.01)	0.78 (0.01)
Low F:B	0.61 (0.01)	0.70 (0.01)	0.60 (0.01)	0.76 (0.01)

Values represent mean GYE values and one standard error of four replicates.

(Table 1). When calculated using total mineralized C by 14 h, the GYE values of high versus low F:B communities were not different (0.59 ± 0.02 and 0.61 ± 0.01, respectively). When microbial efficiencies were calculated using $^{13}CO_2$–C evolution at 14 h, GYE values of high versus low F:B communities were also not different, but were higher than the GYE values based on total mineralized C (Table 1).

3.2. Experiment 2 (N amendment)

3.2.1. C mineralization

In experiment 2, 87–90% of the added glucose was used within 10 h of glucose + N amendment, and low F:B communities used significantly more glucose than high F:B communities until 14 h (Fig. 2a). However, $^{13}CO_2$–C evolution was similar between high and low F:B communities. Nitrogen amendment in this experiment significantly increased microbial respiration in both high and low F:B soils until 16 h, well after the added glucose had been utilized. Low F:B communities mineralized significantly more total C than high F:B communities throughout the experiment (Fig. 2).

As in experiment 1, microbial $^{13}CO_2$–C respiration was substantially lower than total CO_2–C respiration, but the difference was especially pronounced in these N-amended soils (Fig. 2). Although total CO_2–C respiration increased slightly with N addition, the percentage of ^{13}C mineralized was consistently lower than in experiment 1 and rarely exceeded 60% of the total CO_2–C evolved. Only 52–61% of total CO_2–C evolved was $^{13}CO_2$–C in high F:B communities, and $^{13}CO_2$–C evolution accounted for only 55–60% of total CO_2–C from low F:B communities (Fig. 2b). Although there were no differences in $^{13}CO_2$–C evolution between low and high F:B soils in experiment 1, when soils were amended with N low F:B communities mineralized significantly more ^{13}C than high F:B communities (Fig. 2b).

3.2.2. Growth yield efficiencies

There were no differences in the growth yield efficiencies of high and low F:B communities in N-amended soils (Table 1). When calculated using total C mineralization by 10 h, GYE values of high and low F:B communities in N-amended soils were 0.62 ± 0.01 and 0.60 ± 0.01, respectively. When ^{13}C mineralization by 10 h was used to calculate GYE, growth yield efficiencies of high and low F:B communities were higher than when calculated based on total mineralized C, but again were not different from each other (Table 1).

4. Discussion

We conducted this study to directly examine whether fungal-dominated soils are more efficient at using C than soils with lower F:B biomass ratios. Other researchers have measured total C mineralization of added glucose to

determine microbial growth yield efficiencies (e.g. Bremer and Kuikman, 1994; Frey et al., 2001). This approach has been criticized for its inability to ensure that microbes are metabolizing only the added glucose and not native soil organic matter, which makes it impossible to determine the relative proportion of glucose C allocated to biomass and metabolite production versus that respired. We attempted to address this criticism by using a label to trace substrate utilization through the microbial community in order to more effectively quantify the relative allocation of substrate C to biomass versus respiration.

4.1. Growth yield efficiency

Our findings do not support the hypothesis that soil microbial communities with higher F:B have greater growth yield efficiencies than communities with lower F:B. Instead, growth yield efficiencies of high and low F:B communities were comparable in both experiments. Microbial growth yield efficiency increased with N addition independent of the F:B; however, the increase was detected only when $^{13}CO_2$–C was used to calculate efficiency.

We observed that high and low F:B communities behaved similarly in soils not amended with N; however, when N was added, low F:B communities utilized glucose-C faster and showed higher overall C mineralization than high F:B communities. This is consistent with other studies that show soil fungi to be more sensitive to mineral N additions than bacteria (Bardgett et al., 1996; Bardgett et al., 1999; Bardgett and McAlister, 1999; Bossuyt et al., 2001; Frey et al., 2004). For example, Bardgett et al. (1996) observed a rapid shift in an upland grassland soil in the UK from bacterial dominance toward fungal dominance after N fertilization was ceased. In a later study, Bardgett et al. (1999) characterized the microbial community using phospholipid fatty acid (PLFA) analysis and reported that the ratio of fungal:bacterial PLFA decreased along a fertilization gradient in grazed temperate grasslands, being lowest in fertilized plots. Frey et al. (2004) observed that chronic N additions to both hardwood and pine forest stands in central Massachusetts significantly decreased active fungal biomass while active bacterial biomass did not change. These studies point to broad shifts in microbial community structure with increased N inputs, to which fungi respond less favorably than bacteria.

The differential response of fungi and bacteria to nutrient amendments raises interesting questions about whether N fertilization causes shifts in fungal and bacterial C utilization efficiency. When calculated using ^{13}C respiration, we observed an increase in growth yield efficiency with N amendment that was independent of the relative abundance of bacteria and fungi. Results from aquatic systems suggest that microbial efficiency is limited by the availability of C substrate or inorganic nutrients (del Giorgio and Cole, 1998). Studies of aquatic bacteria have shown that anabolic and catabolic processes are not always closely coupled, especially where resources limit cell growth. Bacteria consume considerable energy in processes not directly related to growth when substrate or nutrient limitation limits biosynthesis (Tempest, 1978; Russell and Cook, 1995; del Giorgio and Cole, 1998), and bacterioplankton may increase substrate consumption without increasing their net growth even when nutrients are added (Benner et al., 1998; del Giorgio and Cole, 1998). Both processes decrease microbial efficiency. Few studies have reported microbial efficiencies under different N levels in terrestrial systems. Lekkerkerk et al. (1990) reported that the efficiency of two species of brown and white rot fungi increased when substrates with higher N content were degraded. However, Horwath and Elliott (1996) reported that total C mineralization declined when N was added to perennial ryegrass straw decomposing at two incubation temperatures. More work examining nutrient effects on soil fungal and bacterial efficiencies is needed.

4.2. Methodological issues

4.2.1. Priming effect

We operated under the assumption that we would observe a close coupling between ^{13}C-glucose uptake and $^{13}CO_2$–C evolution, and that the balance between uptake and respiration would be incorporated into microbial biomass. Instead, while ^{13}C-glucose uptake proceeded rapidly, ^{13}C evolution was consistently lower than total CO_2–C evolution, accounting for only up to 60% (experiment 2) or 77% (experiment 1) of the total C metabolized. Two mechanisms may account for this. First, adding a labile substrate may have primed microbes to mineralize recalcitrant, unlabeled SOM (the 'priming effect' *sensu* Bingeman et al., 1953). Several researchers have reported priming effects after additions of fresh but complex organic substrates (Bingeman et al., 1953; Dalenberg and Jager, 1989; Wu et al., 1993; Shen and Bartha, 1997; Magid et al., 1999; Bell et al., 2003). Magid et al. (1999) observed a 15% increase in native SOM turnover in a loamy sand amended with labeled *Lolium* shoots, but suggested this 'priming effect' was due to increased microbial turnover rather than to an actual increase in the rate of soil organic matter decomposition. We used a label to account for a possible priming effect in our study, and we attempted to avoid biasing GYE values due to microbial recycling by calculating GYE using respiration measurements just before glucose was totally depleted. Therefore, if the low ^{13}C evolution was caused only by the use and/or priming of native SOM decomposition, we would be able to calculate a correct absolute value of the GYE.

4.2.2. Endogenous metabolism

Second, our observation of a discrepancy between total C and ^{13}C respiration may be explained by endogenous metabolism, whereby microbes mineralize C (primarily ^{12}C) already present in their cells when added labile C compounds are assimilated (Ribbons and Dawes, 1963; Pirt,

1975; Jenkinson et al., 1985; Smith et al., 1985; Bell et al., 2003). Ribbons and Dawes (1963) and Pirt (1975) reported that the addition of highly labile substrates to stable microbial populations increased endogenous metabolism substantially. Smith et al. (1985) attributed their observed discrepancy between total C and labeled-^{14}C evolution in glucose and glucose+nutrient broth addition studies to microbial endogenous metabolism. Jenkinson et al. (1985) noted for isotopes of N that priming can be mimicked by the process of 'isotope displacement', in which added labeled N displaces native unlabeled N already present in a 'bound' pool. If we can extrapolate this phenomenon to C isotopes in our study, it appears that ^{13}C displaced ^{12}C in already-existing microbial tissue (a 'bound' pool), and that this immobilization of ^{13}C resulted in consistently greater [12]CO_2 than [13]CO_2 evolution. Therefore, endogenous metabolism leads to observations of apparent (not real) priming effects.

4.2.3. Rate of ^{13}C-glucose metabolism

Another trend in our data related to the discrepancy between ^{12}C and ^{13}C evolution is important to note. We observed a substantial lag time between ^{13}C-substrate uptake and ^{13}C metabolism. Despite that over 90% of added glucose was depleted by 10–14 h in both experiments, the isotopic signatures of [13]CO_2 samples remained high and did not show substantial dilution by ^{12}C for the entire 20 h duration of either experiment (Table 2). Concerned that this was an artifact of not airing out the Mason jars between sampling times, we calculated the differences in CO_2 signatures between 2 h sampling intervals. Delta values of CO_2 collected between 14 (when 90% of glucose was depleted) and 20 h (when the experiment was terminated) in experiment 1 (no N amendment) declined by only 12% and 20% in high and low F:B treatments, respectively. In experiment 2 (N amendment), delta values of CO_2 collected from the high F:B treatment declined by 14%, but delta values from low F:B treatments actually increased 2% between 10 h (when 90% of glucose was depleted) and 20 h. If ^{13}C-glucose was taken up and mineralized in short order as we originally expected, we would have observed greater dilution of the [13]CO_2 present in the jar headspace than we actually

observed. This suggests that ^{13}C continued to be respired well beyond the time it was utilized by microbes. The high delta values we observed well beyond microbial uptake of ^{13}C-glucose in both experiments support the hypothesis of endogenous metabolism to explain the discrepancy between [12]CO_2 and [13]CO_2 evolution.

The fate of added ^{13}C in this experiment is important to consider in determining whether using a tracer to quantify C allocation to biomass/metabolite and energy production is totally effective. We observed two discrepancies: one between total and ^{13}C mineralization, and one between ^{13}C-substrate uptake and ^{13}C metabolism. Both are evidence of real priming or endogenous microbial metabolism (apparent priming) or both. We expected that using a label in our study would allow us to account for any priming effects by permitting us to differentiate native versus glucose-C respiration. However, because our data show strong evidence for endogenous metabolism, we cannot measure the relative importance of priming to explain the discrepancies between ^{12}C and ^{13}C mineralization. Further, it is unlikely that the priming effect of glucose addition would be 23–40% (Kuzyakov et al., 2000), as our data would suggest if priming were the only mechanism explaining the discrepancy between [13]CO_2–C and total CO_2–C evolution. Thus, the principal methodological problem we encountered in attempting to address concerns with prior microbial efficiency studies was the occurrence of endogenous metabolism.

These methodological issues present a challenge for using labeled substrates to measure microbial GYE. Due to the lag time between microbial uptake of ^{13}C-glucose and actual microbial mineralization of the substrate, calculating GYE values as we did based on [13]CO_2–C respiration at the time of near-complete ^{13}C-glucose uptake may result in biased GYE values because microbes are not metabolizing only the labeled substrate. However, calculating GYE based upon [13]CO_2–C respiration several hours *after* near-complete ^{13}C-glucose loss, when lower isotopic signatures evidence the cessation of ^{13}C mineralization, risks biasing GYE values because we cannot be sure that CO_2 evolved at that time is solely from ^{13}C-glucose mineralization and not also from microbial turnover.

Table 2
Delta values for [13]CO_2–C evolved from soil communities with high and low fungal:bacterial biomass ratios (F:B) between 8 and 20 h after ^{13}C-glucose additions in soils with or without inorganic N amendment

Time (h)	Experiment 1 (no N added)		Experiment 2 (N added)	
	High F:B	Low F:B	High F:B	Low F:B
8	1150.33 (6.96)	1131.61 (5.08)	989.91 (28.96)	1035.36 (10.59)
10	1242.12 (5.73)	1222.28 (6.61)	1018.80 (25.66)	1045.32 (11.25)
12	1307.36 (4.47)	1290.04 (6.83)	1025.50 (24.08)	1038.10 (7.31)
14	1344.97 (6.86)	1330.50 (6.26)	1019.00 (21.41)	1027.90 (6.48)
16	1361.42 (5.61)	1350.44 (3.44)	1010.48 (20.42)	1014.12 (5.87)
18	1353.44 (7.15)	1350.80 (3.51)	999.41 (20.66)	997.59 (5.56)
20	1344.00 (7.15)	1341.76 (3.88)	985.51 (20.89)	980.63 (6.50)

Values represent the means and one standard error of four replicates.

Despite the methodological problems we uncovered, these issues may only bias the absolute GYE values, not the relative differences in GYE between high and low F:B communities under similar experimental conditions. Thus, since the goal of this study was to evaluate the relative effect of F:B biomass ratios on GYE, our observations of the relative differences in GYE between high and low F:B communities are valid.

4.3. Conclusions

In summary, our use of a label to follow substrate-C through high and low F:B communities did not solve the methodological problems with prior microbial efficiency studies, and may have resulted in biased absolute GYE values. Regardless, valid comparisons of relative GYE values between high and low F:B communities can be made, and our results do not support the prevalent hypothesis that soil fungi have higher GYE than soil bacteria. Thus, care should be taken in invoking higher fungal efficiency as a mechanism for higher C storage in fungal-dominated soil communities.

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