

# Unearthing the microbial ecology of soil carbon cycling with DNA-SIP

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## Abstract

We explored the dynamics of microbial carbon (C) decomposition in soil by coupling DNA Stable Isotope Probing (SIP) and high throughput sequencing. Our experiment evaluated the degradative succession hypothesis, described dynamics of C metabolism during organic matter degradation, and characterized bacteria that metabolize labile and structural C in soils. We added a complex amendment representing plant derived organic matter to soil substituting  $^{13}\text{C}$ -xylose or  $^{13}\text{C}$ -cellulose for unlabeled equivalents in two experimental treatments. Xylose and cellulose are abundant components in plant biomass and represent labile and structural C pools, respectively. We assessed  $^{13}\text{C}$  assimilation into DNA for SSU rRNA gene OTUs finding evidence of  $^{13}\text{C}$ -incorporation from  $^{13}\text{C}$ -xylose and  $^{13}\text{C}$ -cellulose in 49 and 63 OTUs, respectively. Microorganisms primarily assimilated xylose-C into DNA on days 1, 3, and 7 and cellulose-C on days 14 and 30. The types of microorganisms that assimilated xylose-C changed with time dominated by *Firmicutes* at day 1 followed by *Bacteroidetes* at day 3 and then *Actinobacteria* at day 7. Temporal dynamics of  $^{13}\text{C}$ -labeling suggests C traveled through different trophic levels within the soil bacterial community. Microbes that metabolized cellulose-C belonged to cosmopolitan soil lineages that remain uncharacterized physiologically including *Spartobacteria*, *Chloroflexi* and *Planctomycetes*. Our study links microorganisms to specific soil C processes revealing the ecological properties of specific microbial lineages and functionally defined groups of microbes within complex communities.

stable isotope probing | structure-function relationships | soil microbial ecology | 16S rRNA gene

Abbreviations: C, Carbon; OTU, Operational Taxonomic Unit; SOM, Soil Organic Matter; BD, Buoyancy Density; SIP, Stable Isotope Probing

## Significance

Soil microorganisms drive C flux through the terrestrial biosphere, and intuitively, accounting for

microbial physiological diversity improves global C models. But, characterizing the ecophysiology of microbes involved with C decomposition in soil has proven difficult due to their overwhelming diversity. We characterized C use of microbial taxa in soil and show different C forms have distinct decomposition dynamics governed by different microbial lineages. For example, we found microbes belonging to uncharacterized but cosmopolitan taxa in soils assimilated cellulose-C into DNA. These microbes may drive cellulose decomposition on a global scale. We identify microbial lineages engaging in labile and structural C decomposition and explore their ecological properties.

## Introduction

Soils worldwide contain 2,300 Pg of carbon (C) which accounts for nearly 80% of the C present in the terrestrial biosphere [1, 2]. C respiration by soil microorganisms produces annually tenfold more  $\text{CO}_2$  than fossil fuel emissions [3]. Despite the contribution of microorganisms to global C flux, many global C models ignore the diversity of microbial physiology [4–6]. Further, predictions of climate change feedbacks on soil C flux improve when biogeochemical models explicitly represent microbial physiology [7]. However, we still know little about the ecophysiology of soil microorganisms, and such knowledge should assist the development and refinement of global C models [8–10]. Cellulose comprises most plant C (30–50%) followed by hemicellulose (20–40%), and lignin (15–25%) [11]. Hemicellulose, being the most soluble,

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degrades in the early stages of decomposition. Xylans are often an abundant component of hemicellulose, and xylans themselves include differing amounts of xylose, glucose, arabinose, galactose, mannose, and rhamnose [12]. Xylose is often the most abundant sugar in hemicellulose, comprising as much as 60–90% of xylan in some plants (e.g. hardwoods) [13], wheat [14], and switchgrass [15]. Microbes that respire sugars proliferate during the initial stages of decomposition [16, 17], and metabolize as much as 75% of sugar C during the first 5 days of decomposition [18]. In contrast, cellulose decomposition proceeds more slowly with rates increasing for approximately 15 days and decomposition continues for 30–90 days [18, 19]. It is hypothesized that different microbial guilds mediate the decomposition of different plant biomass components [19–22]. The degradative succession hypothesis posits that fast growing organisms proliferate in response to the labile fraction of plant biomass such as sugars [23, 24] followed by slow growing organisms targeting structural C such as cellulose [23]. Evidence to support the degradative succession hypothesis comes from observing soil respiration dynamics and characterizing microbes cultured at different stages of decomposition. The degree to which the succession hypothesis presents an accurate model of litter decomposition has been questioned [21, 25, 26] and it’s clear that we need new approaches to dissect microbial contributions to C transformations in soils.

Though microorganisms mediate 80–90% of the soil C-cycle [27, 28], and microbial community composition can account for significant variation in C mineralization [29], terrestrial C-cycle models rarely consider the community composition of soils [30, 31]. We measure rates of soil C transformations without knowledge of the organisms that mediate these reactions [28] leaving undefined the importance of community membership towards maintaining ecosystem function [28, 32, 33]. Variation in microbial community composition can be linked effectively to rates of soil processes when diagnostic genes for specific functions are available (e.g. denitrification [34], nitrification [35–37], methanotrophy [38], and nitrogen fixation [39]). However, the complexity of soil C transformations and the lack of diagnostic genes for describing these transformations has limited progress in characterizing the contributions of individual microbes to the soil C-cycle. Remarkably, we still lack basic information on the physiology and ecology of the majority of organisms that live in soils. For example, contributions to soil processes remain uncharacterized for entire and cosmopolitan bacterial phyla in soil such as *Acidobacteria*, *Chloroflexi*, *Planctomycetes*, and *Verrucomicrobia*. These phyla combined can

comprise 32% of soil microbial communities (based on surveys of the SSU rRNA genes in soil) [40, 41].

Characterizing the functions of microbial taxa has relied historically on culturing microorganisms and subsequently characterizing physiology in the laboratory, and on environmental surveys of genes diagnostic for specific processes. However, most microorganisms are difficult to grow in culture [40] and many processes lack suitable diagnostic genes. Nucleic acid stable-isotope probing (SIP) links genetic identity and activity without the need to grow microorganisms in culture and has expanded our knowledge of microbial contributions to biogeochemical processes [42]. However, nucleic acid SIP has notable complications including the need to add large amounts of labeled substrate [43], label dilution resulting in partial labeling of nucleic acids [43–45], the potential for cross-feeding and secondary label incorporation [45–50], and variation in genome G+C content [51–54]. As a result, most applications of SIP have targeted specialized microorganisms such as methanotrophs [43], methanogens [55], syntrophs [56], or microbes that target pollutants [57]. SIP has proved less useful for exploring the soil C-cycle because it has lacked the resolution necessary to pick up the complex signal that results from adding components of plant biomass to microbial communities in soil. High throughput DNA sequencing technology, however, improves the resolving power of SIP [58].

Coupling SIP with high throughput DNA sequencing now enables exploration of microbial C-cycling in soils. SSU rRNA amplicons can be sequenced from numerous density gradient fractions across multiple samples thereby increasing the resolution of a typical nucleic acid SIP experiment [59]. It is now possible to use far less isotopically labeled substrate resulting in more environmentally realistic experimental conditions [58]. We have employed such a high resolution DNA stable isotope probing approach to explore the assimilation of <sup>13</sup>C labeled xylose and/or cellulose into bacterial DNA in an agricultural soil.

Specifically, we added to soil a complex amendment that simulated organic matter derived from fresh plant biomass. All treatments received the same amendment but the identity of isotopically labeled substrates was varied between treatments. We set up a control treatment where all components were unlabeled, a treatment with <sup>13</sup>C-xylose instead of unlabeled xylose, and a treatment with <sup>13</sup>C-cellulose instead of unlabeled cellulose. Soil was sampled at days 1, 3, 7, 14, and 30 and we identified which microorganisms assimilated <sup>13</sup>C into DNA at each point in time. The experiment was designed to provide a test of the degradative succession hypothesis as it applies to soil bacteria, to identify soil bacteria that metabolize xylose and

cellulose, and to characterize temporal dynamics of xylose and cellulose metabolism in soil.

## 200 Results

After adding an organic matter amendment to soil, we tracked the flow of C from xylose or cellulose into microbial DNA over time using DNA-SIP (Figure S1). The amendment consisted of various plant biomass compounds including cellulose, lignin, sugars found in hemicellulose, amino acids, and inorganic salts (see Supplemental Information (SI)). The amendment was added at 2.3 mg C g<sup>-1</sup> soil dry weight (d.w.), and this comprised 16% of the total C in the soil. The cellulose-C (0.88 mg C g<sup>-1</sup> soil d.w.) and xylose-C (0.42 mg C g<sup>-1</sup> soil d.w.) in the amendment comprised 6% and 3% of the total C in the soil, respectively. The soil microbial community respiration 65% of the xylose within one day and 29% of the added xylose remained in the soil at day 30 (Figure S2). In contrast, cellulose-C declined at a constant rate of approximately 18 µg C d<sup>-1</sup> g<sup>-1</sup> soil d.w. and 40% of added cellulose-C remained in the soil at day 30 (Figure S2).

**13C-labeling of OTUs changed with time and substrate.** We assessed assimilation of <sup>13</sup>C into microbial DNA by comparing the SSU rRNA gene sequence composition of SIP density gradient fractions between <sup>13</sup>C treatments and controls (see Methods and SI). All treatments used the same amendment which included xylose and cellulose, but <sup>13</sup>C-xylose or <sup>13</sup>C-cellulose was substituted for its unlabeled equivalent in two amendments. A treatment without isotopically labeled components served as the control. In the gradient density fractions of the control treatment, fraction density represented the majority of the variance in SSU rRNA gene composition (Figure 1). DNA buoyant density correlates positively with G+C content [51] and therefore DNA G+C content influences variation in the SSU rRNA gene composition of density gradient fractions. For the <sup>13</sup>C-cellulose treatment, SSU rRNA gene composition in gradient fractions deviated from control at high density (> 1.72 g mL<sup>-1</sup>) on days 14 and 30 (Figure 1). For the <sup>13</sup>C-xylose treatment, SSU rRNA gene composition in density gradient fractions also deviated from control in high density fractions, but in contrast to the <sup>13</sup>C-cellulose treatment it deviated from control on days 1, 3, and 7 (Figure 1). SSU rRNA gene composition from <sup>13</sup>C-cellulose treatment and <sup>13</sup>C-xylose treatment density fractions differed at high density indicating different microorganisms assimilated C from xylose than cellulose (Figure 1). Further, in the <sup>13</sup>C-cellulose treatment, the SSU rRNA gene sequence composition of high density

fractions at days 14 and 30 was similar indicating similar microorganisms had <sup>13</sup>C-labeled DNA in <sup>13</sup>C-cellulose treatments at days 14 and 30. In contrast, in the <sup>13</sup>C-xylose treatment, the SSU rRNA gene composition of high density fractions varied between days 1, 3, and 7 indicating that different microbes had <sup>13</sup>C-labeled DNA on these days. In the <sup>13</sup>C-xylose treatment, the SSU gene composition of high density fractions was similar to control on days 14 and 30 (Figure 1) indicating that <sup>13</sup>C was no longer detectable in bacterial DNA on these days for this treatment.

**255 Temporal dynamics DNA <sup>13</sup>C incorporation of OTUs.** We monitored the soil microcosm microbial community over the course of the experiment by surveying SSU rRNA genes in non-fractionated DNA from the experimental soil. The SSU rRNA gene composition of the non-fractionated DNA changed with time (Figure S3, P-value = 0.023, R<sup>2</sup> = 0.63, Adonis test [60]). In contrast, the non-fractionated DNA SSU rRNA gene composition showed no statistical evidence for changing with treatment (P-value 0.23) (Figure S3). The latter result demonstrates the substitution of <sup>13</sup>C-labeled substrates for unlabeled equivalents could not be shown to alter the soil microbial community composition. Twenty-nine OTUs exhibited sufficient statistical evidence (adjusted P-value < 0.10) to conclude they changed in relative abundance over the course of the experiment (Figure S4). When SSU rRNA gene abundances were combined at the taxonomic rank of “class”, the classes that changed in abundance (P-value < 0.10) were the *Bacilli* (decreased), *Flavobacteria* (decreased), *Gamma proteobacteria* (decreased), and *Herpetosiphonales* (increased) (Figure S5). Of the 29 OTUs that changed in relative abundance over time, 14 putatively incorporated <sup>13</sup>C into DNA (Figure S4). OTUs that likely assimilated <sup>13</sup>C from <sup>13</sup>C-cellulose into DNA tended to increase in relative abundance with time whereas OTUs that assimilated <sup>13</sup>C from <sup>13</sup>C-xylose tended to decrease (Figure S6). OTUs that responded to both substrates did not exhibit a consistent relative abundance response over time as a group (Figure S4 and S6).

**260 OTUs that assimilated <sup>13</sup>C into DNA.** If an OTU exhibited strong evidence for assimilating <sup>13</sup>C into DNA, we refer to that OTU as a “responder” (see Methods for our operational definition of “responder”). The SSU rRNA gene sequences produced in this study were binned into 5,940 OTUs and we assessed evidence of <sup>13</sup>C-labeling from both <sup>13</sup>C-cellulose and <sup>13</sup>C-xylose for each OTU. Forty-one OTUs responded to <sup>13</sup>C-xylose, 55 OTUs responded to <sup>13</sup>C-cellulose, and 8 OTUs responded

to both xylose and cellulose (Figure 2, Figure 3, 310 Figure S7, Table S1, and Table S2). The number of xylose responders peaked at days 1 and 3 and declined with time. In contrast, the number of cellulose responders increased with time peaking at days 14 and 30 (Figure S8).

315 The phylogenetic composition of xylose responders changed with time and 86% of xylose responders shared > 97% SSU rRNA gene sequence identity with bacteria cultured in isolation (Figure 2 and 4). On day 1, *Bacilli* OTUs represented 320 84% of xylose responders and the majority of these OTUs were closely related to cultured representatives of the genus *Paenibacillus* (Table S1, Figure 3). For example, “OTU.57” (Table S1), annotated as *Paenibacillus*, had a strong signal of 325 <sup>13</sup>C-labeling at day 1 coinciding with its maximum relative abundance in non-fractionated DNA. The relative abundance of “OTU.57” declined until day 14 and did not appear to be <sup>13</sup>C-labeled after day 1 (Figure S9). On day 3, *Bacteroidetes* 330 OTUs comprised 63% of xylose responders (Figure 4) and these OTUs were closely related to cultured representatives of the *Flavobacteriales* and *Sphingobacteriales* (Table S1, Figure 3). For example, “OTU.14”, annotated as a flavobacterium, 335 had a strong signal for <sup>13</sup>C-labeling in the <sup>13</sup>C-xylose treatment at days 1 and 3 coinciding with its maximum relative abundance in non-fractionated DNA. The relative abundance of “OTU.14” then declined until day 14 and did not show evidence 340 of <sup>13</sup>C-labeling beyond day 3 (Figure S9). Finally, on day 7, *Actinobacteria* OTUs represented 53% of the xylose responders and these OTUs were closely related to cultured representatives of *Micrococcales* (Table S1, Figure 3). For example, “OTU.4”, annotated as *Agromyces*, had signal for <sup>13</sup>C-labeling 345 in the <sup>13</sup>C-xylose treatment on days 1, 3 and 7 with the strongest evidence of <sup>13</sup>C-labeling at day 7 and did not appear <sup>13</sup>C-labeled at days 14 and 30. “OTU.4” relative abundance in non-fractionated 350 DNA increased until day 3 and then declined until day 30 (Figure S9). *Proteobacteria* were also common among xylose responders at day 7 where they comprised 40% of xylose responder OTUs. Notably, *Proteobacteria* represented the majority (6 355 of 8) of OTUs that responded to both cellulose and xylose (Figure S7).

The phylogenetic composition of cellulose responders did not change with time to the same extent as the xylose responders. Also, in contrast to xylose responders, cellulose responders often were not closely related (< 97% SSU rRNA gene sequence identity) to cultured isolates. Both the relative abundance and the number of cellulose responders increased over time peaking at 360 days 14 and 30 (Figure 2, Figure S8, and Figure S6). Cellulose responders belonged to the *Pro-*

*teobacteria* (46%), *Verrucomicrobia* (16%), *Planctomycetes* (16%), *Chloroflexi* (8%), *Bacteroidetes* (8%), *Actinobacteria* (3%), and *Melanobacteria* (1 OTU) (Table S2). The majority (86%) of cellulose responders in the *Proteobacteria* were closely related (> 97% identity) to bacteria cultured in isolation, including representatives of the genera: *Cellvibrio*, *Devosia*, *Rhizobium*, and *Sorangium*, which are all known for their ability to degrade cellulose (Table S2). Proteobacterial cellulose responders belonged to *Alpha* (13 OTUs), *Beta* (4 OTUs), *Gamma* (5 OTUs), and *Delta-proteobacteria* (6 OTUs).

The majority (85%) of cellulose responders outside of the *Proteobacteria* shared < 97% SSU rRNA gene sequence identity to bacteria cultured in isolation. For example, 70% of the *Verrucomicrobia* cellulose responders fell within unidentified *Spartobacteria* clades (Figure 3), and these shared < 85% SSU rRNA gene sequence identity to any characterized isolate. The *Spartobacteria* OTU “OTU.2192” exemplified many cellulose responders (Table S2, Figure S9). “OTU.2192” increased in non-fractionated DNA relative abundance with time and evidence for <sup>13</sup>C-labeling of “OTU.2192” in the <sup>13</sup>C-cellulose treatment increased over time with the strongest evidence at days 14 and 30 (Figure S9). Most *Chloroflexi* cellulose responders belonged to an unidentified clade within the *Herpetosiphonales* (Figure 3) and they shared < 89% SSU rRNA gene sequence identity to any characterized isolate. Characteristic of *Chloroflexi* cellulose responders, “OTU.64” increased in relative abundance over 30 days and evidence for <sup>13</sup>C-labeling of “OTU.64” in the <sup>13</sup>C-cellulose treatment peaked days 14 and 30 (Figure S9). Cellulose responders found within the *Bacteroidetes* fell within the *Cytophagales* in contrast with *Bacteroidetes* xylose responders that fell instead within the *Flavobacteriales* or *Sphingobacteriales* (Figure 3). *Bacteroidetes* cellulose responders included one OTU that shared 100% SSU rRNA gene sequence identity to species of *Sporocytophaga*, a genus that includes known cellulose degraders.

#### Characteristics of cellulose and xylose responders.

Cellulose responders, relative to xylose responders, tended to have lower relative abundance in non-fractionated DNA, demonstrated signal consistent with higher atom % <sup>13</sup>C in labeled DNA, and had lower estimated *rrn* copy number (Figure 5). In the non-fractionated DNA, cellulose responders had lower relative abundance ( $7e^{-4}$  (s.d.  $2e^{-3}$ )) than xylose responders ( $2e^{-3}$  (s.d.  $4e^{-3}$ )) (Figure 4, P-value = 0.00028, Wilcoxon Rank Sum test). Six of the ten most common OTUs observed in the non-fractionated DNA responded to xylose,

and, eight of the ten most abundant responders  
425 to xylose or cellulose in the non-fractionated DNA were xylose responders.

DNA buoyant density (BD) increases in proportion to the atom %  $^{13}\text{C}$  of the DNA. Hence, the extent of  $^{13}\text{C}$  incorporation into DNA can be evaluated by the difference in BD between  $^{13}\text{C}$ -labeled and unlabeled DNA. We calculated for each OTU its mean BD weighted by relative abundance to determine its “center of mass” within a given density gradient. We then quantified for each OTU the difference in center of mass between control gradients and gradients from  $^{13}\text{C}$ -xylose or  $^{13}\text{C}$ -cellulose treatments (see SI for the detailed calculation). We refer to the change in center of mass position for an OTU in response to  $^{13}\text{C}$ -labeling as  $\Delta\hat{BD}$ .  
440  $\Delta\hat{BD}$  can be used to compare relative differences in  $^{13}\text{C}$ -labeling between OTUs.  $\Delta\hat{BD}$  values, however, are not comparable to the BD changes observed for DNA from pure cultures which generate molecules uniform in isotopic labeling, in part  
445 because  $\Delta\hat{BD}$  is based on relative abundance in density gradient fractions (and not DNA concentration) and in part because all members of an OTU may not respond uniformly to the isotopic label. Cellulose responder  $\Delta\hat{BD}$  ( $0.0163 \text{ g mL}^{-1}$   
450 (s.d.  $0.0094$ )) was greater than that of xylose responders ( $0.0097 \text{ g mL}^{-1}$  (s.d.  $0.0094$ )) (Figure 5, P-value =  $1.8610\text{e}^{-6}$ , Wilcoxon Rank Sum test).

We predicted the *rrn* gene copy number for responders as described [61]. The number of *rrn*  
455 gene copies a microorganism has is correlated to its ability to proliferate after rapid nutrient influx [62]. Cellulose responders possessed fewer estimated *rrn* copy numbers ( $2.7$  ( $1.2$  s.d.)) than xylose responders ( $6.2$  ( $3.4$  s.d.)) (Figure 5 and Figure S10; P =  $1.878\text{e}^{-9}$ ). Furthermore, the estimated *rrn* gene copy number for xylose responders was inversely related to the day of first response (P =  $2.02\text{e}^{-15}$ , Wilcoxon Rank Sum test, Figure S10, Figure 5).

460 We assessed phylogenetic clustering of  $^{13}\text{C}$ -responsive OTUs with the Nearest Taxon Index (NTI) and the Net Relatedness Index (NRI) [63]. We also quantified the average clade depth of cellulose and xylose responders with the consenTRAIT metric [64]. Briefly, the NRI and NTI evaluate phylogenetic clustering against a null model for the distribution of a trait in a phylogeny. The NRI and NTI values are z-scores or standard deviations from the mean and thus the greater the magnitude of the NRI/NTI, the stronger the evidence for clustering (positive values) or overdispersion (negative values). NRI assesses overall clustering whereas the NTI assesses terminal clustering [65]. The consenTRAIT metric is a measure of the average clade depth for a trait in a phylogenetic tree. NRI values indicate that cellulose responders

clustered overall and at the tips of the phylogeny (NRI: 4.49, NTI: 1.43) while xylose responders clustered terminally (NRI: -1.33, NTI: 2.69). The consenTRAIT clade depth for xylose and cellulose responders was 0.012 and 0.028 SSU rRNA gene sequence dissimilarity, respectively. As reference, the average clade depth is approximately 0.017 SSU rRNA gene sequence dissimilarity for arabinose (another five C sugar found in hemicellulose) utilization as inferred from genomic analyses, and was 0.013 and 0.034 SSU rRNA gene sequence dissimilarity for glucosidase and cellulase genomic potential, respectively [64, 66]. These results indicate xylose responders form terminal clusters dispersed throughout the phylogeny while cellulose responders form deep clades of terminally clustered OTUs.  
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## Discussion

500 We identified microorganisms participating in soil C cycling using a nucleic acid SIP approach. Specifically, we observed assimilation of  $^{13}\text{C}$  from either  $^{13}\text{C}$ -xylose or  $^{13}\text{C}$ -cellulose into DNA for 104 OTUs in an agricultural soil. We found  $^{13}\text{C}$  from 505  $^{13}\text{C}$ -xylose appeared to move into and then out of groups of related OTUs over time. By coupling nucleic acid SIP to high throughput sequencing we could diagnose OTU activity even when OTUs were at low relative abundance in non-fractionated DNA (e.g. on three occasions we did not detect 510  $^{13}\text{C}$ -responders in the non-fractionated DNA). Our results support the degradative succession hypothesis, elucidate ecophysiological properties of soil microorganisms, reveal activity of widespread uncultured soil bacteria, and begin to piece together the microbial food web in soils.

The degradative succession hypothesis predicts an ecological transition in activity during the decomposition of plant matter from microbes that 515 decompose labile plant biomass C to those that decompose structural more recalcitrant C. Our results concur with the degradative succession hypothesis. Microorganisms consumed xylose-C before cellulose-C and assimilated xylose-C into DNA faster than to cellulose-C. Xylose is major constituent of hemicellulose and is a labile component of fresh plant biomass. The phylogenetic composition of xylose responders changed between days 1, 3 and 7 and few OTUs appeared  $^{13}\text{C}$ -labeled in the  $^{13}\text{C}$ -xylose treatment after day 7. In the  $^{13}\text{C}$ -cellulose treatment, few OTUs were  $^{13}\text{C}$ -labeled in the beginning of the experiment but  $^{13}\text{C}$  labeled OTUs increased at day 14 and maintained  $^{13}\text{C}$ -labeling through day 30. Finally, 520 few (8 of 104) OTUs appeared to metabolize both xylose and cellulose meaning over 30 days cellulose responders succeeded xylose responders in activ-

ity. In addition to agreeing with the degradative succession hypothesis, our results suggest complex interactions between microbes occur during labile C decomposition.

Correlations between community composition and environmental characteristics often indirectly reveal microorganisms that belong to ecologically consistent groups [67]. In this experiment, we directly identified ecological groups as a function of *in situ* metabolism and inferred group ecological properties through phylogenetic affiliation, temporal dynamics of  $^{13}\text{C}$ -assimilation, and the extent of  $^{13}\text{C}$ -labeling per OTU. Xylose responders grew faster than cellulose responders and appeared to assimilate C from multiple sources. Xylose responders assimilated xylose-C into DNA within 24 hours and had low  $\Delta\hat{BD}$  relative to cellulose responders suggesting xylose was not the sole C source used for growth. Xylose represented 20% of the amendment and 3.5% of total soil C. Xylose responders often included the most abundant OTUs within the non-fractionated DNA and had high estimated *rrn* copy number relative to cellulose responders. However, to some degree, high *rrn* gene copy number may inflate observed xylose responder relative abundance. Notably, the majority of xylose responder SSU rRNA genes (86%) matched SSU rRNA genes from cultured isolates at high sequence identity (> 97%).

Cellulose responders, on the other hand, incorporated  $^{13}\text{C}$  into DNA after xylose responders and appeared to specialize in using cellulose as a C source. Cellulose responders grew over a span of weeks and had high  $\Delta\hat{BD}$  indicating cellulose remained the dominant C source for cellulose responders even though multiple sources of C were present (cellulose represented 6% of total C present in soil at the start of the experiment). Cellulose responders were also lower in relative abundance on average within the non-fractionated DNA and had lower estimated *rrn* copy number than xylose responders. The majority of cellulose responders were not close relatives of cultured isolates although a number of cellulose responders shared high SSU rRNA gene sequence identity with cultured *Proteobacteria* (e.g. *Cellvibrio*). We identified cellulose responders among phyla such as *Verrucomicrobia*, *Chloroflexi*, and *Planctomycetes* – phyla whose functions within soil communities remain unknown.

*Verrucomicrobia* made up 16% of the cellulose responders. *Verrucomicrobia* are cosmopolitan soil microbes [68] that can make up to 23% of SSU rRNA gene sequences in soils [68] and 9.8% of soil SSU rRNA [69]. Genomic analyses and laboratory experiments show that various isolates within the *Verrucomicrobia* are capable of methanotrophy, diazotrophy, and cellulose degra-

dation [70, 71]. Moreover, *Verrucomicrobia* have been hypothesized to degrade polysaccharides in many environments [72–74]. However, only one of the 15 most abundant verrucomicrobial phylotypes in globally distributed soil samples shared > 93% SSU rRNA gene sequence identity with a cultured isolate [68] and hence the role of soil *Verrucomicrobia* in global C-cycling remains unknown. The majority of verrucomicrobial cellulose responders belonged to two clades that fall within the *Spartobacteria* (Figure 3). *Spartobacteria* outnumbered all other *Verrucomicrobia* phylotypes in SSU rRNA gene surveys of 181 globally distributed soil samples [68]. Given their ubiquity and abundance in soil as well as their demonstrated incorporation of  $^{13}\text{C}$  from  $^{13}\text{C}$ -cellulose, *Verrucomicrobia* lineages, particularly *Spartobacteria*, may be important contributors to cellulose decomposition on a global scale.

Other notable cellulose responders include OTUs in the *Planctomycetes* and *Chloroflexi* both of which have previously been shown to assimilate  $^{13}\text{C}$  from  $^{13}\text{C}$ -cellulose added to soil [75]. *Planctomycetes* are common in soil [40], comprising 4 to 7% of bacterial cells in many soils [76, 77] and 7% ± 5% of SSU rRNA [78]. Although soil *Planctomycetes* are widespread, their activities in soil remain uncharacterized. *Planctomycetes* represented 16% of cellulose responders and shared < 92% SSU rRNA gene sequence identity to their most closely related cultured isolates. *Chloroflexi* are known for metabolically dynamic lifestyles ranging from anoxygenic phototrophy to organohalide respiration [79] and are among the six most abundant bacterial phyla in soil [40]. Recent studies have focused on *Chloroflexi* roles in C cycling [79–81] and several *Chloroflexi* isolates use cellulose [79–81]. Four of the five *Chloroflexi* cellulose responders belong to a single clade within the *Herpetosiphonales* (Figure 3).

Finally, a single cellulose responder belonged to the *Melainabacteria* phylum (95% shared SSU rRNA gene sequence identity with *Vampirovibrio chlorellavorus*). The phylogenetic position of *Melainabacteria* is debated but *Melainabacteria* have been proposed to be a non-phototrophic sister phylum to *Cyanobacteria*. An analysis of a *Melainabacteria* genome [82] suggests the genomic capacity to degrade polysaccharides though *Vampirovibrio chlorellavorus* is an obligate predator of green alga [83]. The *Melainabacteria* cellulose responder did not respond to xylose so if it is predatory, it may prey specifically on structural C degraders (i.e. cellulose responders).

Responders did not necessarily assimilate  $^{13}\text{C}$  into DNA directly from  $^{13}\text{C}$ -xylose or  $^{13}\text{C}$ -cellulose. In many ways, knowledge of secondary C degradation and/or microbial biomass turnover

may be more interesting with respect to the soil C-cycle than knowledge of primary degradation. The response to xylose suggests xylose-C moved through different trophic levels within the soil bacteria food web. The *Bacilli* degraded xylose first (65% of the xylose-C had been respired by day 1) representing 84% of day 1 xylose responders. *Bacilli* also comprised about 6% of SSU rRNA genes present in non-fractionated DNA on day 1. However, few *Bacilli* remained <sup>13</sup>C-labeled by day 3 and their abundance declined reaching about 2% of soil SSU rRNA genes by day 30. Members of the *Bacillus* [84] and *Paenibacillus* in particular [59] have been previously implicated as labile C decomposers. The decline in relative abundance of *Bacilli* could be attributed to mortality and/or sporulation coupled to mother cell lysis. Concomitant with the decline in relative abundance and loss of <sup>13</sup>C-label of *Bacilli*, *Bacteroidetes* OTUs appeared <sup>13</sup>C-labeled at day 3. Finally, *Actinobacteria* appeared <sup>13</sup>C-labeled at day 7 as *Bacteroidetes* xylose responders declined in relative abundance and became unlabeled. Hence, it seems reasonable to propose that *Bacteroidetes* and *Actinobacteria* xylose responders became <sup>13</sup>C-labeled via the consumption <sup>13</sup>C-labeled microbial biomass as opposed to primary degradation of <sup>13</sup>C-xylose.

The inferred physiology of *Actinobacteria* and *Bacteroidetes* xylose responders provides further evidence that the activity dynamics represent C transfer between microbes by saprotrophy and/or predation. Most of the *Actinobacteria* xylose responders that appeared <sup>13</sup>C-labeled at day 7 were members of the *Micrococcales* (Figure 3) and the most abundant <sup>13</sup>C-labeled *Micrococcales* OTU at day 7 (OTU.4, Table S1) is annotated as belonging in the *Agromyces*. *Agromyces* are facultative predators that feed on the gram-positive *Luteobacter* in culture [85]. Additionally, certain types of *Bacteroidetes* can assimilate <sup>13</sup>C from <sup>13</sup>C-labeled *Escherichia coli* added to soil [86]. However, it is also possible that *Bacilli*, *Bacteroidetes*, and *Actinobacteria* are adapted to use xylose at different concentrations and that the observed activity dynamics resulted from changes in xylose concentration over time. If trophic transfer caused the activity dynamics, at least three different ecological groups exchanged C in 7 days. Models of the soil C cycle often exclude trophic interactions between soil bacteria (e.g. [87]), yet when soil C models do account for predators and/or saprophytes, trophic interactions are predicted to have significant effects on the fate of soil C [88].

**Implications for soil C cycling models.** Functional niche characterization for soil microorganisms is necessary to predict whether and how biogeochem-

ical processes vary with microbial community composition. Functional niches are defined by soil microbiologists and have been successfully incorporated into biogeochemical process models (E.g. [88, 89]). In some C models ecological strategies such as growth rate and substrate specificity are parameters for functional niche behavior [88]. The phylogenetic breadth of a functionally defined group is often inferred from the distribution of diagnostic genes across genomes [66] or from the physiology of isolates cultured on laboratory media [64]. For instance, the wide distribution of the glycolysis operon in microbial genomes is interpreted as evidence that many soil microorganisms participate in glucose turnover [10]. However, the functional niche may depend less on the distribution of diagnostic genes across genomes and more on life history traits that allow organisms to compete for a given substrate as it occurs in the soil. For instance, fast growth and rapid resuscitation allow microorganisms to compete for labile C which may often be transient in soil. Hence, life history traits may constrain the diversity of microbes that metabolize a given C source in the soil under a given set of conditions.

Biogeochemical processes mediated by a broad array of taxa are assumed to be less influenced by community change than narrow processes that involve a single, specific chemical transformation by a narrow suite of microbial participants [10, 90]. In addition, the diversity of a functionally defined group engaged in a specific C transformation is expected to correlate with C lability [10]. However, the diversity of active labile C and recalcitrant C decomposers in soil has not been directly quantified. We found comparable numbers of OTUs responded to <sup>13</sup>C-cellulose and <sup>13</sup>C-xylose (63 and 49, respectively). Cellulose responders were phylogenetically clustered suggesting that the ability to degrade cellulose is phylogenetically conserved. The clade depth of cellulose responders, 0.028 SSU rRNA gene sequence dissimilarity, is on the same order as that observed for glycoside hydrolases which are diagnostic enzymes for cellulose degradation [66]. Xylose responders clustered in terminal branches indicating groups of closely related taxa used xylose but xylose responders also clustered phylogenetically with respect to time of first response (Figure 3, Figure 4). For example, xylose responders on day 1 are dominated by members of *Paenibacillus*. Thus, microorganisms that degraded labile C and structural C were both limited in diversity. Although the genes for xylose metabolism are likely widespread in the soil community, it's possible limited groups of organisms had the ecological characteristics required to degrade xylose under the experimental conditions. Therefore it's possible few phylogenetically coherent

ent taxa actually participate in the metabolism  
<sup>770</sup> of labile C-sources under a given set of conditions, and hence changes in community composition may alter the dynamics of structural and labile C-transformations in soil.

Broadly, we observed labile C use by fast growing  
<sup>775</sup> generalists and structural C use by slow growing specialists. These results agree with the MIMICS model which simulates leaf litter decomposition by modeling microbial decomposers as two functionally defined groups, copiotrophs or oligotrophs [89]. Including these functional types improved predictions of C storage in response to environmental change relative to models that did not consider any microbial physiological diversity. We identified microbial lineages engaged in labile and structural C decomposition or simply, copiotrophs and oligotrophs. We also observed potentially greater turnover – and at the very least rate differences in turnover – for copiotroph biomass relative to oligotroph biomass which may be important to consider when modeling microbial turnover input to SOM. It's also clear that there may be more than two vital functional types mediating C-cycling in soil. That is, soil-C may travel through many bacterial trophic levels where each C transfer represents an opportunity for C stabilization in association with soil minerals or C loss by respiration.

**Conclusion.** Microorganisms sequester atmospheric C and respire SOM influencing climate change on a global scale but we do not know which microorganisms carry out specific soil C transformations. In this experiment microbes from physiologically uncharacterized but cosmopolitan soil lineages participated in cellulose decomposition. Cellulose responders included members of the *Verrucomicrobia* (*Spartobacteria*), *Chloroflexi*, *Bacteroidetes* and *Planctomycetes*. *Spartobacteria* in particular are globally cosmopolitan soil microorganisms and are often the most abundant *Verrucomicrobia* responder in soil [68]. Fast-growing aerobic spore formers from *Firmicutes* assimilated labile C in the form of xylose. Xylose responders within the *Bacteroidetes* and *Actinobacteria* likely became <sup>13</sup>C-labeled by consuming <sup>13</sup>C-labeled biomass that was labeled itself via the primary degradation of <sup>13</sup>C-xylose. Our results suggest that cosmopolitan *Spartobacteria* may degrade cellulose on a global scale, labile plant biomass C may travel through a trophic cascade within the bacteria that includes predators and/or saprotrophs, and life history traits may act as a filter constraining the diversity of active microorganisms relative to those with the genomic potential for a given metabolism.

## Methods

<sup>825</sup> Additional information on sample collection and analytical methods is provided in Supplemental Materials and Methods.

Twelve soil cores (5 cm diameter x 10 cm depth) were collected from six sampling locations within <sup>830</sup> an organically managed agricultural field in Penn Yan, New York. Soils were sieved (2 mm), homogenized, distributed into flasks (10 g in each 250 ml flask, n = 36) and equilibrated for 2 weeks. We amended soils with a mixture containing 5.3 mg C <sup>835</sup> g<sup>-1</sup> soil dry weight (d.w.) and brought experimental soil to 50% water holding capacity. The amendment contained 38% cellulose, 23% lignin, 20% xylose, 3% arabinose, 1% galactose, 1% glucose, and 0.5% mannose by mass, with the remaining <sup>840</sup> 13.5% mass composed of an amino acid (in-house made replica of Teknova C0705) and macronutrient mixture (Murashige and Skoog, Sigma Aldrich M5524). This mixture approximates the molecular composition of switchgrass biomass with hemicellulose replaced by its constituent monomers [91]. We set up three parallel treatments varying the isotopically labeled component in each treatment. The treatments were (1) a control treatment with all unlabeled components, (2) a treatment with <sup>845</sup> <sup>13</sup>C-cellulose instead of unlabeled cellulose (synthesized as described in SI), and (3) a treatment with <sup>13</sup>C-xylose (98 atom% <sup>13</sup>C, Sigma Aldrich) instead of unlabeled xylose. Other details relating to substrate addition can be found in Supplemental Methods. Microcosms were sampled destructively at days 1 (control and xylose only), 3, 7, 14, and 30 and soils were stored at -80°C until nucleic acid extraction. In the manuscript figures, the abbreviation 13CXPS refers to the <sup>13</sup>C-xylose treatment <sup>850</sup> (<sup>13</sup>C Xylose Plant Simulant), 13CCPS refers to the <sup>13</sup>C-cellulose treatment, and 12CCPS refers to the control treatment.

We used DESeq2 (R package), an RNA-Seq differential expression statistical framework [92], to <sup>855</sup> identify OTUs that were enriched in high density gradient fractions from <sup>13</sup>C-treatments relative to corresponding density fractions from control treatments (for review of RNA-Seq differential expression statistics applied to microbiome OTU count data see (30)). We define “high density gradient fractions” as gradient fractions whose density falls between 1.7125 and 1.755 g ml<sup>-1</sup>. Briefly, DESeq2 includes several features that enable robust estimates of standard error in addition to reliable ranking of logarithmic fold change (LFC) (i.e. gamma-Poisson regression coefficients) in relative abundance even with low count groups where LFC can often be noisy. Further, statistical evaluation of LFC can be performed with user-selected <sup>860</sup> thresholds as opposed to the often default null hypothesis that differential abundance for an OTU is

exactly zero enabling the most biologically interesting OTUs to be identified for subsequent analyses. We calculated LFC and corresponding standard errors for enrichment in  $^{13}\text{C}$  treatments relative to control (high density fractions only) for each OTU. Subsequently, a one-sided Wald test was used to statistically assess LFC values (using corresponding standard errors). The user-defined null hypothesis for the Wald test was that LFC was less than one standard deviation above the mean of all LFC values. P-values were corrected for multiple comparisons using the Benjamini and Hochberg method [93]. We independently filtered OTUs on the basis of sparsity prior to correcting P-values for multiple comparisons. The sparsity value that yielded the most adjusted P-values less than 0.10 was selected for independent filtering by sparsity. Briefly, OTUs were eliminated if they failed to appear in at least 45% of high density gradient fractions for a given  $^{13}\text{C}$ /control treatment pair. These sparse OTUs are unlikely to have sufficient data to allow for the determination of statistical significance. We selected a false discovery rate of 10% to denote statistical significance.

See Supplemental Information for DNA extraction, PCR, DNA sequence quality control, OTU ecological characteristic calculations, and SIP density gradient fractionation methods.

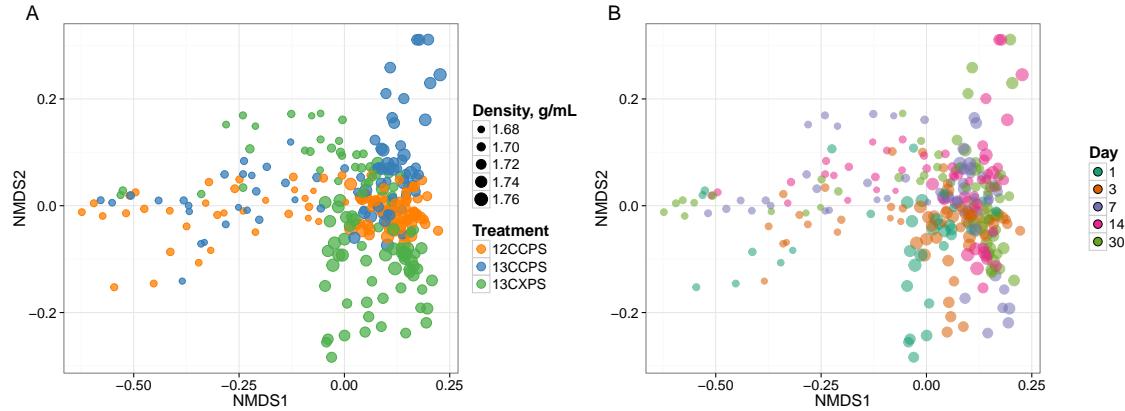
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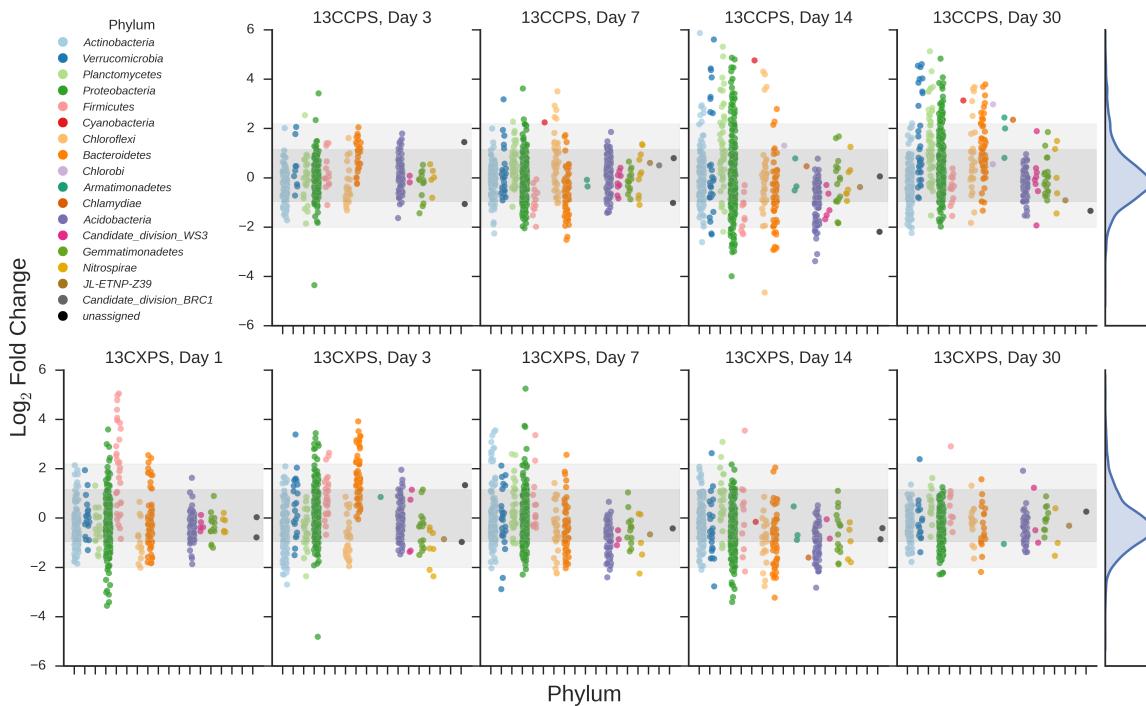
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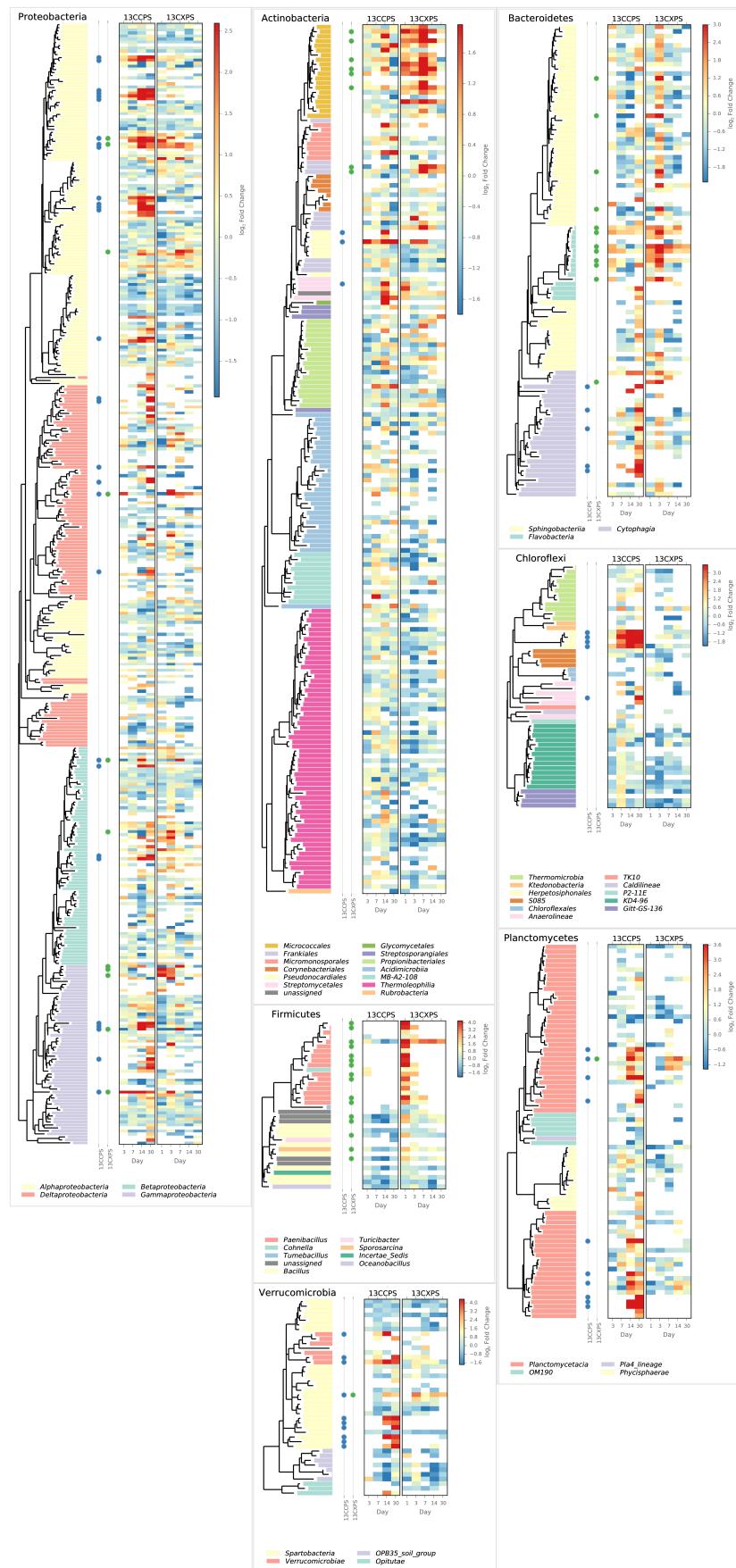
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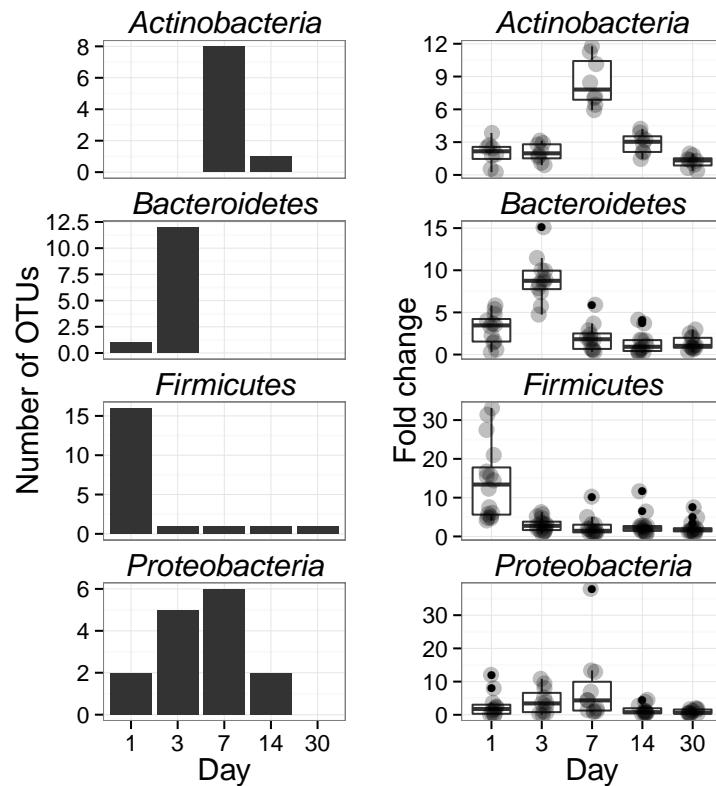
**Fig. 1.** NMDS analysis from weighted unifrac distances of 454 sequence data from SIP fractions of each treatment over time. Twenty fractions from a CsCl gradient fractionation for each treatment at each time point were sequenced (Fig. S1). Each point on the NMDS represents the bacterial composition based on 16S sequencing for a single fraction where the size of the point is representative of the density of that fraction and the colors represent the treatments (A) or days (B).



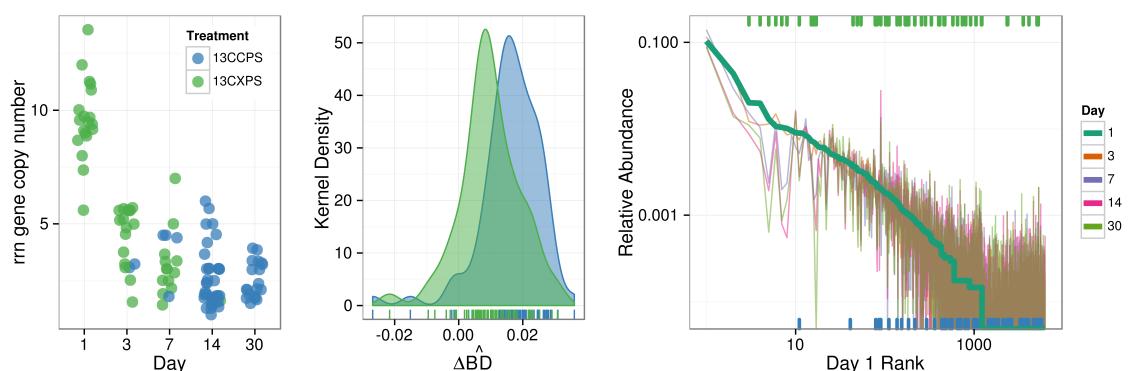
**Fig. 2.** Log<sub>2</sub> fold change of <sup>13</sup>C-responders in cellulose treatment (top) and xylose treatment (bottom). Log<sub>2</sub> fold change is based on the relative abundance in the experimental treatment compared to the control within the density range 1.7125–1.755 g ml<sup>-1</sup>. Taxa are colored by phylum. The last column shows the distribution of all fold change values in each row. The darker gray band represents one standard deviation and the lighter band represents two standard deviations about the mean of all fold change values for both treatments.



**Fig. 3.** Phylum specific trees. Heatmap indicates fold change between heavy fractions of control gradients versus labeled gradients. Dots indicate the position of "responders" to  $^{13}\text{C}$ -xylose (green) or  $^{13}\text{C}$ -cellulose (blue).

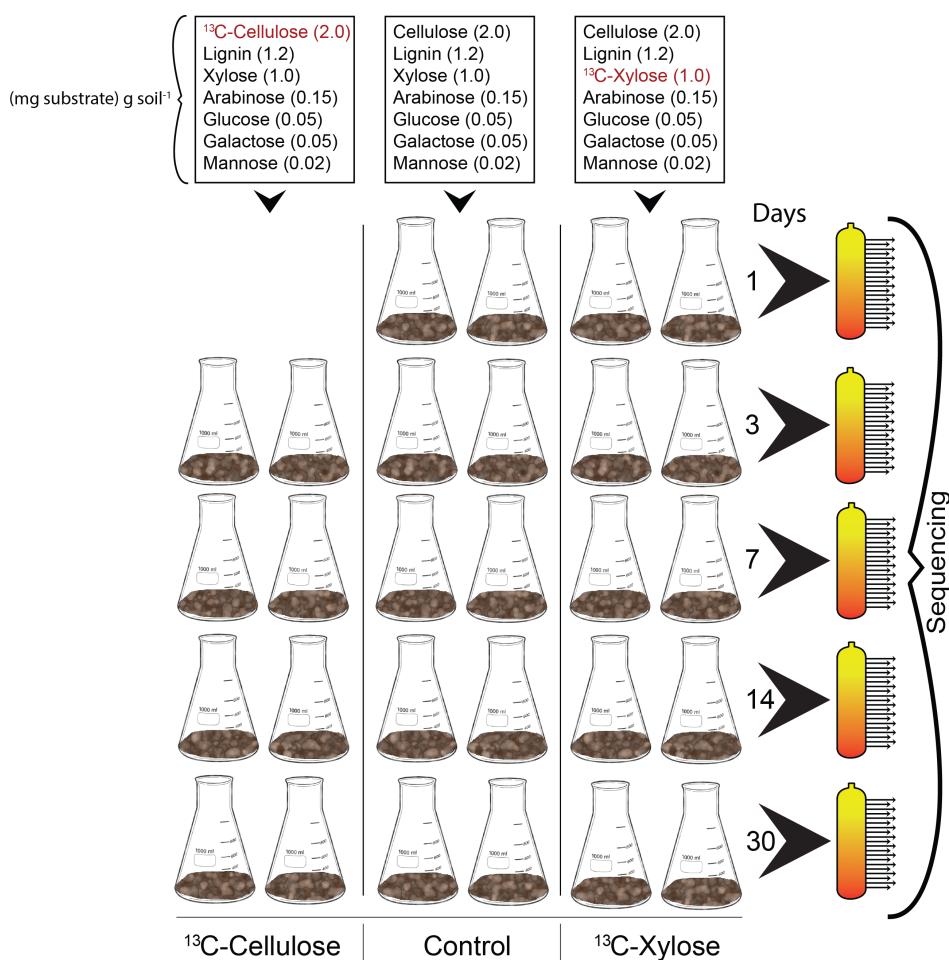


**Fig. 4.** Left column shows counts of  $^{13}\text{C}$ -xylose responders in the *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria* at days 1, 3, 7 and 30. Right panel shows OTU fold enrichment in heavy gradient fractions for  $^{13}\text{C}$ -xylose amendment DNA relative to corresponding control fractions.

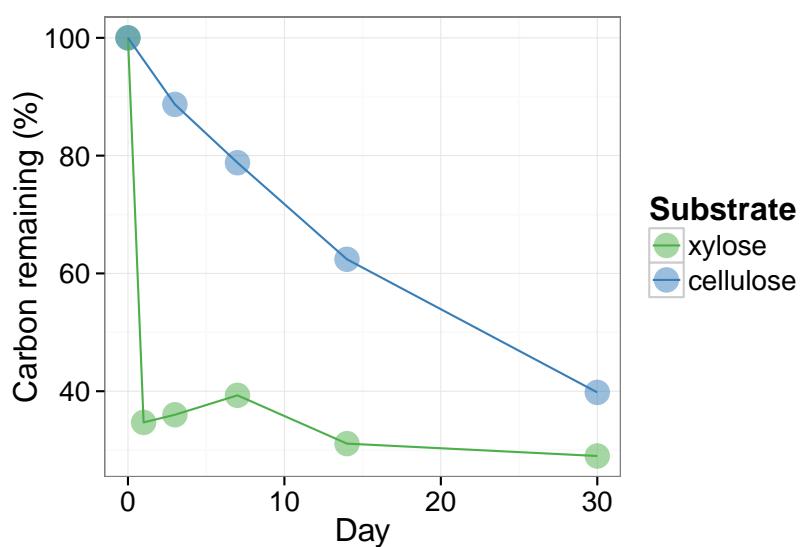


**Fig. 5.**  $^{13}\text{C}$ -responder characteristics based on density shift (A) and rank (B). Kernel density estimation of  $^{13}\text{C}$ -responder's density shift in cellulose treatment (blue) and xylose treatment (green) demonstrates degree of labeling for responders for each respective substrate.  $^{13}\text{C}$ -responders in rank abundance are labeled by substrate (cellulose, blue; xylose, green). Ticks at top indicate location of  $^{13}\text{C}$ -cylose responders in bulk community. Ticks at bottom indicate location of  $^{13}\text{C}$ -cellulose responders in bulk community. OTU rank was assessed from day 1 bulk samples.

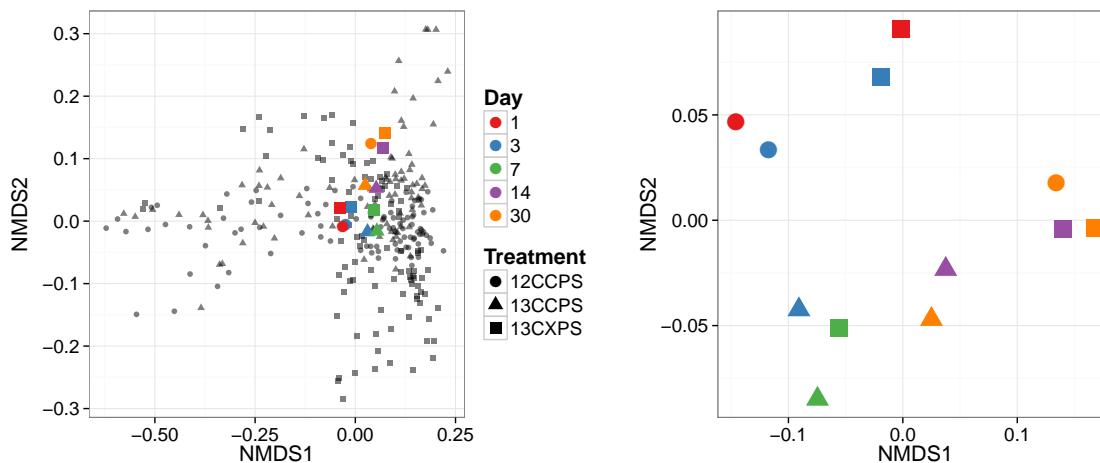
1240 **Supplemental Figures and Tables**



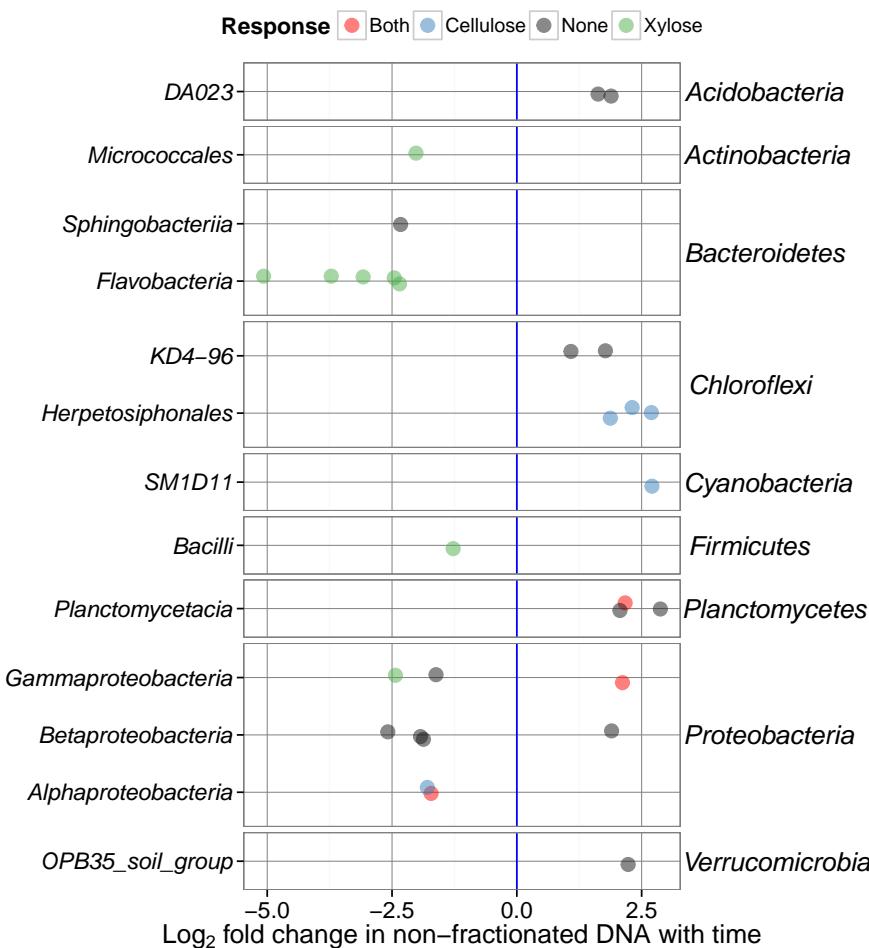
**Fig. S1.** The experimental design. A carbon mixture, in addition to inorganic salts and amino acids (not shown here), was added to each soil microcosm where the only difference between treatments is the <sup>13</sup>C-labeled isotope (in red). At days 1, 3, 7, 14, and 30 replicate microcosms were destructively harvested for downstream molecular applications. Bulk DNA from each treatment and time point ( $n = 14$ ) was CsCl density separated by centrifuged and fractionated (orange tubes wherein each arrow represents a fraction from the density gradient). Fractions were 16S gene sequenced using next generation sequencing technology.



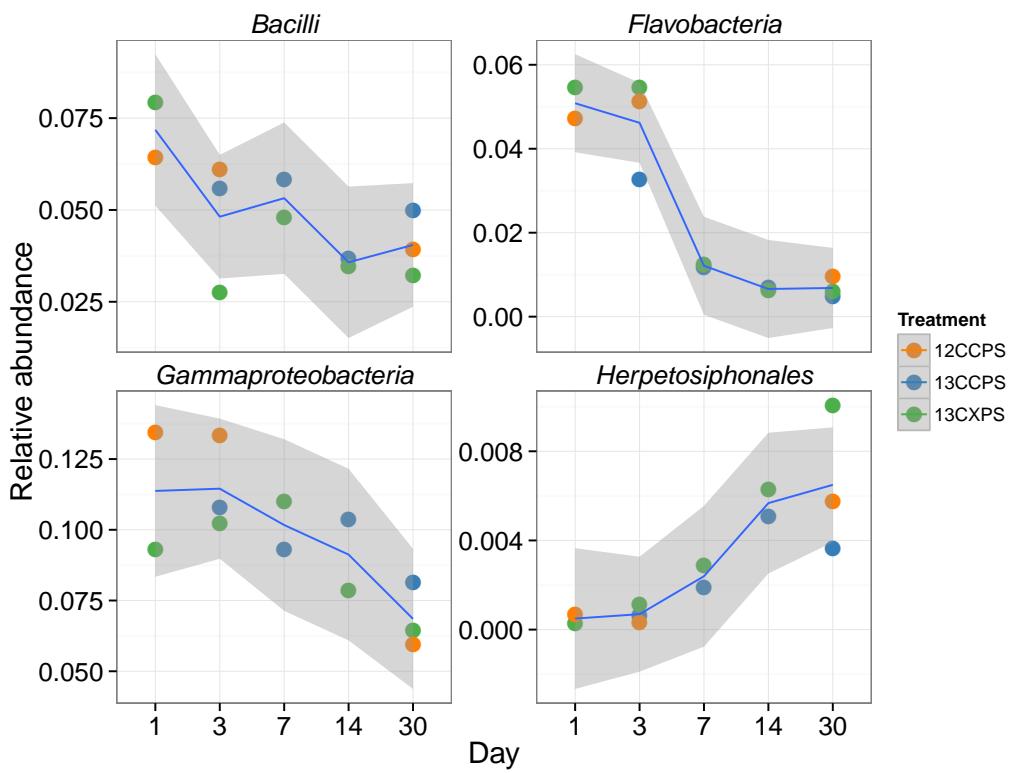
**Fig. S2.** Percentage of added <sup>13</sup>C remaining in soil over time.



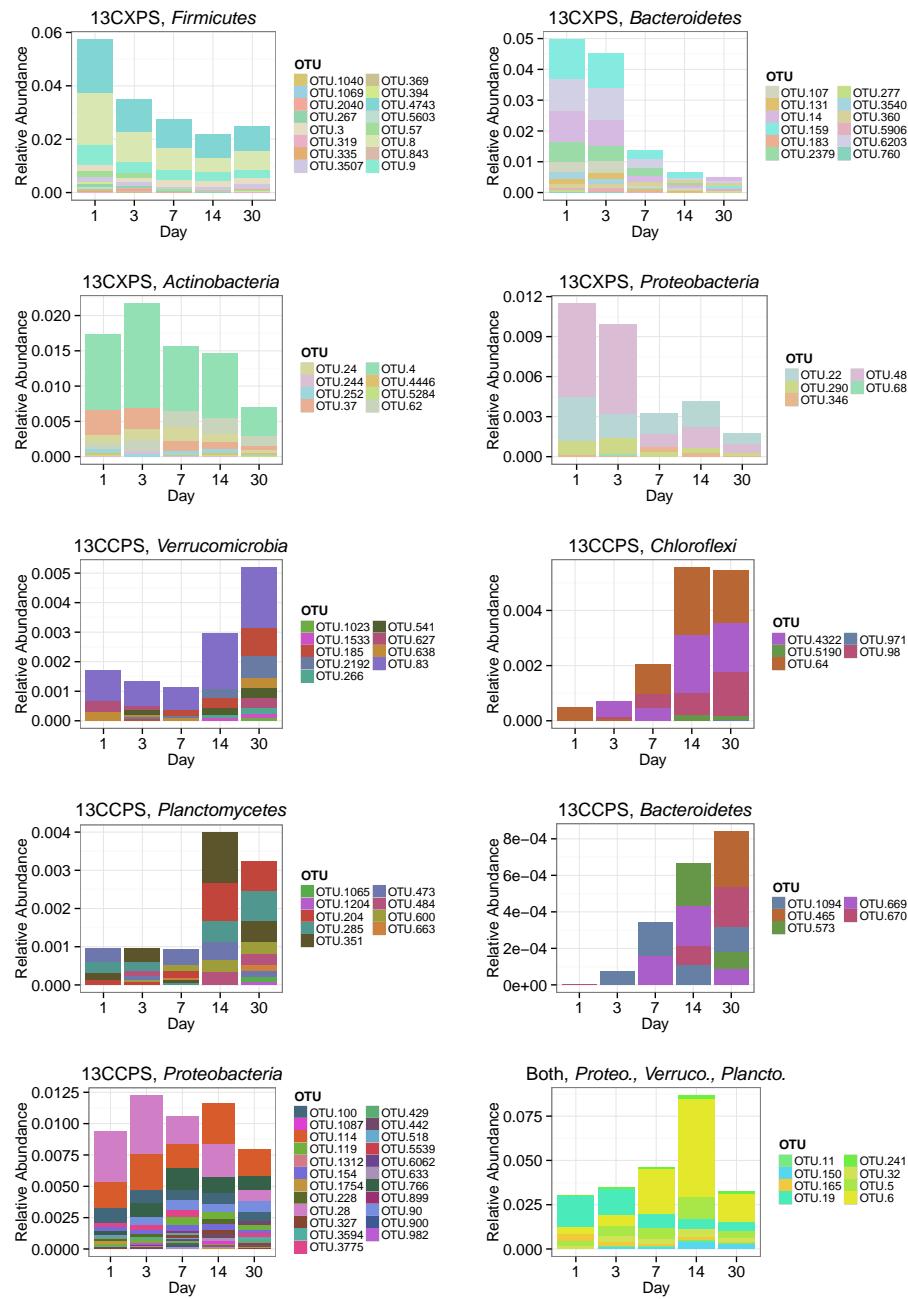
**Fig. S3.** Ordination of bulk gradient fraction phylogenetic profiles.

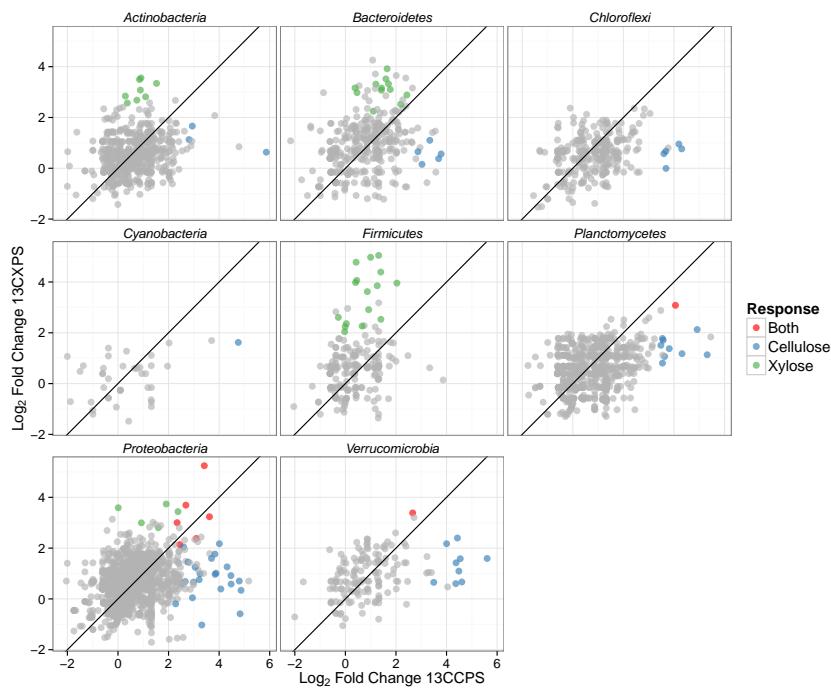


**Fig. S4.** Fold change  $\text{time}^{-1}$  for OTUs that changed significantly in abundance over time. One panel per phylum (phyla indicated on the right). Taxonomic class indicated on the left.

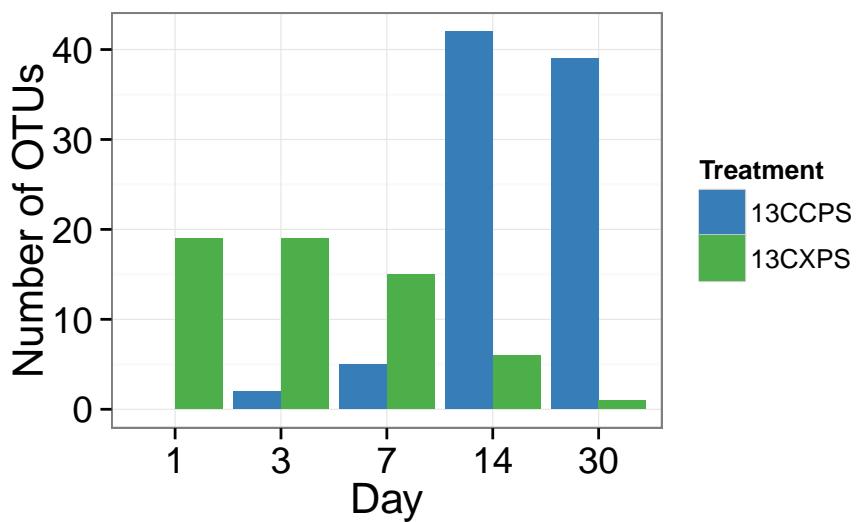


**Fig. S5.** Relative abundance versus day for classes that changed significantly in relative abundance with time.

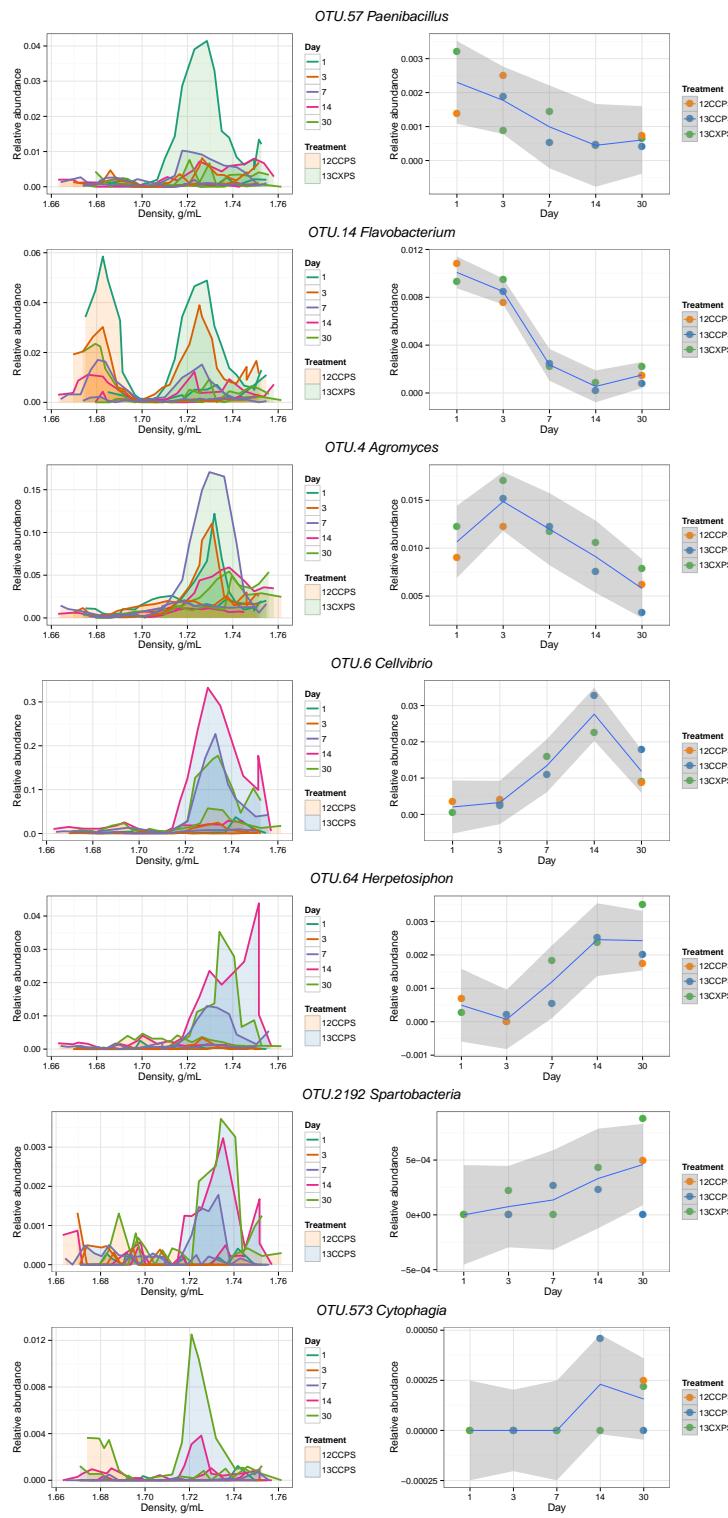
**Fig. S6.** Sum of bulk abundances with selected phylum for responder OTUs.



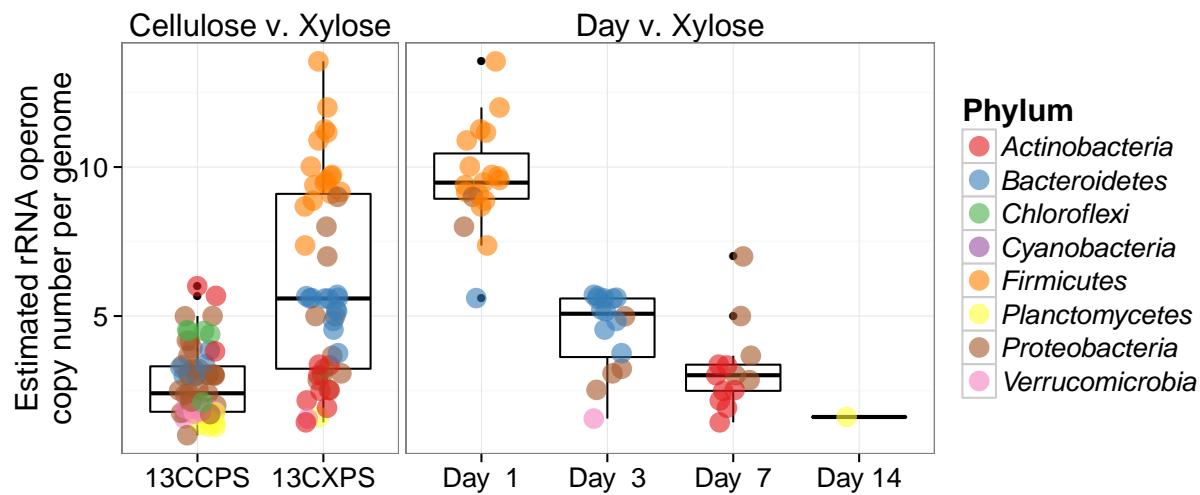
**Fig. S7.** Maximum log<sub>2</sub> fold change in response to labeled substrate incorporation for each substrate and all OTUs that pass sparsity filtering in both substrate analyses. Points colored by response. Line has slope of 1 with intercept at the origin. OTUs falling in the top-right quadrant responded to both substrates while those in the top-left and bottom-right responded to <sup>13</sup>C-xylose and <sup>13</sup>C-cellulose, respectively.



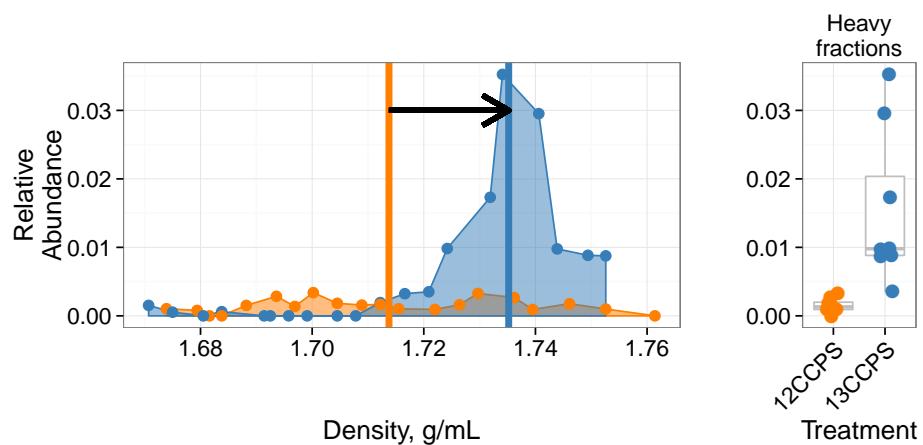
**Fig. S8.** Counts of responders to each isotopically labeled substrate (cellulose and xylose) over time.



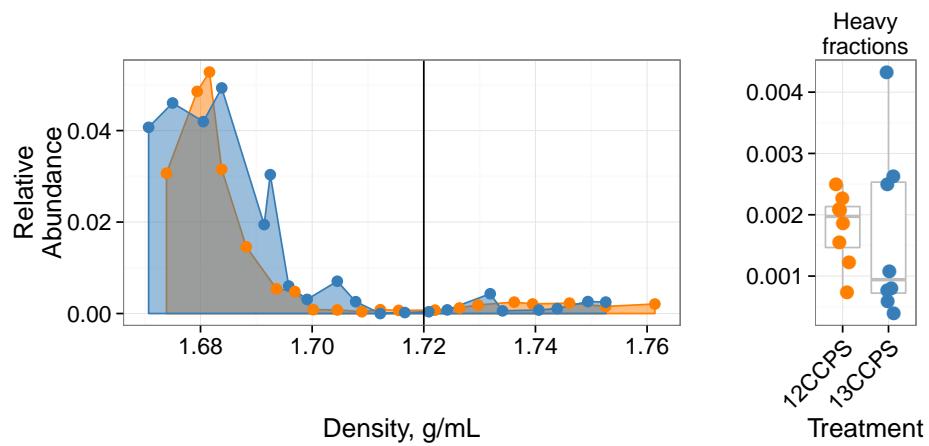
**Fig. S9.** The left column shows DNA-SIP density fraction relative abundances for all gradients for each of the OTUs. Gradient profiles are shaded by treatment where orange represents “control” profiles, blue “<sup>13</sup>C-cellulose”, and green “<sup>13</sup>C-xylose.” The right column shows the abundance of each OTU in non-fractionated DNA (i.e. the DNA that was subsequently fractionated on the density gradient). Enrichment in the heavy end of the gradient in <sup>13</sup>C treatments indicates an OTU has <sup>13</sup>C-labeled DNA that is greater in buoyant density than it would be unlabeled.



**Fig. S10.** Estimated rRNA operon copy number per genome for  $^{13}\text{C}$  responding OTUs. Panel titles indicate which labeled substrate(s) are depicted.



**Fig. S11.** Density profile for a single  $^{13}\text{C}$ -cellulose "responder" OTU in the labeled gradient, blue, and the control gradient, orange. Vertical lines show center of mass for each density profile and arrow denotes the magnitude and direction of the BD shift upon labeling. Panel at right shows relative abundance values in the heavy fractions for each gradient.



**Fig. S12.** Density profile for a single  $^{13}\text{C}$ -cellulose “non-responder” OTU in the labeled gradient, blue, and the control gradient, orange. Vertical line shows where “heavy” fractions begin as defined in our analysis. Panel at right shows relative abundance values in the heavy fractions for each gradient.

Table S1:  $^{13}\text{C}$ -xylose responders BLAST against Living Tree Project

| OTU ID   | Fold change <sup>a</sup> | Day <sup>b</sup> | All days <sup>c</sup> | Top BLAST hits   | BLAST %ID | Phylum;Class;Order  |
|----------|--------------------------|------------------|-----------------------|--|-----------|---|
| OTU.1040 | 4.78                     | 1                | 1                     | <i>Paenibacillus daejeonensis</i>  | 100.0     | <i>Firmicutes Bacilli Bacillales</i>                      |
| OTU.1069 | 3.85                     | 1                | 1                     | <i>Paenibacillus terrigena</i>   | 100.0     | <i>Firmicutes Bacilli Bacillales</i>                      |
| OTU.107  | 2.25                     | 3                | 3                     | <i>Flavobacterium sp. 15C3</i> ,<br><i>Flavobacterium banpakuense</i>  | 99.54     | <i>Bacteroidetes Flavobacteria Flavobacteriales</i>       |
| OTU.11   | 5.25                     | 7                | 7                     | <i>Stenotrophomonas pavani</i> i,<br><i>Stenotrophomonas maltophilia</i> ,<br><i>Pseudomonas geniculata</i>  | 99.54     | <i>Proteobacteria Gammaproteobacteria Xanthomonadales</i> |
| OTU.131  | 3.07                     | 3                | 3                     | <i>Flavobacterium fluvii</i> ,<br><i>Flavobacterium bacterium HMD1033</i> ,<br><i>Flavobacterium sp. HMD1001</i>   | 100.0     | <i>Bacteroidetes Flavobacteria Flavobacteriales</i>       |
| OTU.14   | 3.92                     | 3                | 1, 3                  | <i>Flavobacterium oncorhynchi</i> ,<br><i>Flavobacterium glycines</i> ,<br><i>Flavobacterium succinicans</i>   | 99.09     | <i>Bacteroidetes Flavobacteria Flavobacteriales</i>       |
| OTU.150  | 3.08                     | 14               | 14                    | No hits of at least 90% identity   | 86.76     | <i>Planctomycetes Planctomycetacia Planctomycetales</i>   |
| OTU.159  | 3.16                     | 3                | 3                     | <i>Flavobacterium hibernum</i>   | 98.17     | <i>Bacteroidetes Flavobacteria Flavobacteriales</i>       |
| OTU.165  | 2.38                     | 3                | 3                     | <i>Rhizobium skieniewicense</i> ,<br><i>Rhizobium vignae</i> ,<br><i>Rhizobium larrymoorei</i> ,<br><i>Rhizobium alkalisolii</i> ,<br><i>Rhizobium galegae</i> ,<br><i>Rhizobium huautlense</i>                              | 100.0     | <i>Proteobacteria Alphaproteobacteria Rhizobiales</i>     |
| OTU.183  | 3.31                     | 3                | 3                     | No hits of at least 90% identity   | 89.5      | <i>Bacteroidetes Sphingobacteriia Sphingobacteriales</i>  |
| OTU.19   | 2.14                     | 7                | 7                     | <i>Rhizobium alamii</i> ,<br><i>Rhizobium mesosinicum</i> ,<br><i>Rhizobium mongolense</i> ,<br><i>Arthrobacter viscosus</i> ,<br><i>Rhizobium sullae</i> ,<br><i>Rhizobium yanglingense</i> ,<br><i>Rhizobium loessense</i> | 99.54     | <i>Proteobacteria Alphaproteobacteria Rhizobiales</i>     |
| OTU.2040 | 2.91                     | 1                | 1                     | <i>Paenibacillus pectinilyticus</i>  | 100.0     | <i>Firmicutes Bacilli Bacillales</i>                      |
| OTU.22   | 2.8                      | 7                | 7, 14                 | <i>Paracoccus sp. NB88</i>   | 99.09     | <i>Proteobacteria Alphaproteobacteria Rhodobacterales</i> |
| OTU.2379 | 3.1                      | 3                | 3                     | <i>Flavobacterium pectinovorum</i> ,<br><i>Flavobacterium sp. CS100</i>  | 97.72     | <i>Bacteroidetes Flavobacteria Flavobacteriales</i>       |
| OTU.24   | 2.81                     | 7                | 7                     | <i>Cellulomonas aerilata</i> ,<br><i>Cellulomonas humilata</i> ,<br><i>Cellulomonas terrae</i> ,<br><i>Cellulomonas soli</i> ,<br><i>Cellulomonas xylanilytica</i>   | 100.0     | <i>Actinobacteria Micrococcales Cellulomonadaceae</i>     |
| OTU.241  | 3.38                     | 3                | 3, 14                 | No hits of at least 90% identity   | 87.73     | <i>Verrucomicrobia Spartobacteria Chthoniobacteriales</i> |
| OTU.244  | 3.08                     | 7                | 7                     | <i>Cellulosimicrobium funkei</i> ,<br><i>Cellulosimicrobium terreum</i>  | 100.0     | <i>Actinobacteria Micrococcales Promicromonosporaceae</i> |
| OTU.252  | 3.34                     | 7                | 7                     | <i>Promicromonospora thailandica</i>   | 100.0     | <i>Actinobacteria Micrococcales Promicromonosporaceae</i> |
| OTU.267  | 4.97                     | 1                | 1                     | <i>Paenibacillus pabuli</i> ,<br><i>Paenibacillus tundrae</i> ,<br><i>Paenibacillus taichungensis</i> ,<br><i>Paenibacillus xylohexedens</i> ,<br><i>Paenibacillus xylynilyticus</i>   | 100.0     | <i>Firmicutes Bacilli Bacillales</i>                      |
| OTU.277  | 3.52                     | 3                | 3                     | <i>Solibius ginsengiterrae</i>   | 95.43     | <i>Bacteroidetes Sphingobacteriia Sphingobacteriales</i>  |

Table S1 – continued from previous page

| OTU ID   | Fold change | Day | All days           | Top BLAST hits  | BLAST %ID | Phylum;Class;Order  |
|----------|-------------|-----|--------------------|---|-----------|---|
| OTU.290  | 3.59        | 1   | 1                  | <i>Pantoea spp.</i> ,<br><i>Kluyvera spp.</i> ,<br><i>Klebsiella spp.</i> ,<br><i>Erwinia spp.</i> ,<br><i>Enterobacter spp.</i> ,<br><i>Buttiauxella spp.</i>  | 100.0     | <i>Proteobacteria</i><br><i>Gammaproteobacteria</i><br><i>Enterobacteriales</i> |
| OTU.3    | 2.61        | 1   | 1                  | [ <i>Brevibacterium</i> ] <i>frigoritolerans</i> ,<br><i>Bacillus sp. LMG 20238</i> ,<br><i>Bacillus coahuilensis m4-4</i> ,<br><i>Bacillus simplex</i>   | 100.0     | <i>Firmicutes</i> <i>Bacilli</i> <i>Bacillales</i>                              |
| OTU.319  | 3.98        | 1   | 1                  | <i>Paenibacillus xinjiangensis</i>  | 97.25     | <i>Firmicutes</i> <i>Bacilli</i> <i>Bacillales</i>                              |
| OTU.32   | 3.0         | 3   | 3, 7, 14           | <i>Sandaracinus amyloyticus</i>   | 94.98     | <i>Proteobacteria</i> <i>Deltaproteobacteria</i><br><i>Myxococcales</i>         |
| OTU.335  | 2.53        | 1   | 1                  | <i>Paenibacillus thailandensis</i>  | 98.17     | <i>Firmicutes</i> <i>Bacilli</i> <i>Bacillales</i>                              |
| OTU.346  | 3.44        | 3   | 3                  | <i>Pseudoduganella violaceinigra</i>  | 99.54     | <i>Proteobacteria</i> <i>Betaproteobacteria</i><br><i>Burkholderiales</i>       |
| OTU.3507 | 2.36        | 1   | 1                  | <i>Bacillus spp.</i>  | 98.63     | <i>Firmicutes</i> <i>Bacilli</i> <i>Bacillales</i>                              |
| OTU.3540 | 2.52        | 3   | 3                  | <i>Flavobacterium terrigena</i>   | 99.54     | <i>Bacteroidetes</i> <i>Flavobacteria</i><br><i>Flavobacteriales</i>            |
| OTU.360  | 2.98        | 3   | 3                  | <i>Flavisolibacter ginsengisoli</i>   | 95.0      | <i>Bacteroidetes</i> <i>Sphingobacteriia</i><br><i>Sphingobacteriales</i>       |
| OTU.369  | 5.05        | 1   | 1                  | <i>Paenibacillus sp. D75</i> ,<br><i>Paenibacillus glycansilyticus</i>  | 100.0     | <i>Firmicutes</i> <i>Bacilli</i> <i>Bacillales</i>                              |
| OTU.37   | 2.68        | 7   | 7                  | <i>Phycicola gilvus</i> ,<br><i>Microterricola viridarii</i> ,<br><i>Frigoribacterium faeni</i> ,<br><i>Frondihabitans sp. RS-15</i> ,<br><i>Frondihabitans australicus</i>   | 100.0     | <i>Actinobacteria</i> <i>Micrococcales</i><br><i>Microbacteriaceae</i>          |
| OTU.394  | 4.06        | 1   | 1                  | <i>Paenibacillus pocheonensis</i>   | 100.0     | <i>Firmicutes</i> <i>Bacilli</i> <i>Bacillales</i>                              |
| OTU.4    | 2.84        | 7   | 7, 14              | <i>Agromyces ramosus</i>  | 100.0     | <i>Actinobacteria</i> <i>Micrococcales</i><br><i>Microbacteriaceae</i>          |
| OTU.4446 | 3.49        | 7   | 7                  | <i>Catenuloplanes niger</i> ,<br><i>Catenuloplanes castaneus</i> ,<br><i>Catenuloplanes atrovinosus</i> ,<br><i>Catenuloplanes crispus</i> ,<br><i>Catenuloplanes nepalensis</i> ,<br><i>Catenuloplanes japonicus</i> | 97.72     | <i>Actinobacteria</i> <i>Frankiales</i><br><i>Nakamurellaceae</i>               |
| OTU.4743 | 2.24        | 1   | 1                  | <i>Lysinibacillus fusiformis</i> ,<br><i>Lysinibacillus sphaericus</i>  | 99.09     | <i>Firmicutes</i> <i>Bacilli</i> <i>Bacillales</i>                              |
| OTU.48   | 2.99        | 1   | 1, 3               | <i>Aeromonas spp.</i>   | 100.0     | <i>Proteobacteria</i><br><i>Gammaproteobacteria</i> <i>aaa34a10</i>             |
| OTU.5    | 3.69        | 7   | 7                  | <i>Delftia tsuruhatensis</i> ,<br><i>Delftia lacustris</i>  | 100.0     | <i>Proteobacteria</i> <i>Betaproteobacteria</i><br><i>Burkholderiales</i>       |
| OTU.5284 | 3.56        | 7   | 7                  | <i>Isoptericola nanjingensis</i> ,<br><i>Isoptericola hypogaeus</i> ,<br><i>Isoptericola variabilis</i>   | 98.63     | <i>Actinobacteria</i> <i>Micrococcales</i><br><i>Promicromonosporaceae</i>      |
| OTU.5603 | 3.96        | 1   | 1                  | <i>Paenibacillus uliginis</i>   | 100.0     | <i>Firmicutes</i> <i>Bacilli</i> <i>Bacillales</i>                              |
| OTU.57   | 4.39        | 1   | 1, 3, 7, 14,<br>30 | <i>Paenibacillus castaneae</i>  | 98.62     | <i>Firmicutes</i> <i>Bacilli</i> <i>Bacillales</i>                              |
| OTU.5906 | 3.16        | 3   | 3                  | <i>Terrimonas sp. M-8</i>   | 96.8      | <i>Bacteroidetes</i> <i>Sphingobacteriia</i><br><i>Sphingobacteriales</i>       |
| OTU.6    | 3.24        | 3   | 3                  | <i>Cellvibrio fulvus</i>  | 100.0     | <i>Proteobacteria</i><br><i>Gammaproteobacteria</i><br><i>Pseudomonadales</i>   |

Table S1 – continued from previous page

| <b>OTU ID</b> | <b>Fold change</b> | <b>Day</b> | <b>All days</b> | <b>Top BLAST hits</b>  | <b>BLAST %ID</b> | <b>Phylum;Class;Order</b>                                   |
|---------------|--------------------|------------|-----------------|--|------------------|---|
| OTU.62        | 2.57               | 7          | 7               | <i>Nakamurella flava</i>   | 100.0            | <i>Actinobacteria Frankiales Nakamurellaceae</i>            |
| OTU.6203      | 3.32               | 3          | 3               | <i>Flavobacterium granuli</i> ,<br><i>Flavobacterium glaciei</i>   | 100.0            | <i>Bacteroidetes Flavobacteria Flavobacteriales</i>         |
| OTU.68        | 3.74               | 7          | 7               | <i>Shigella flexneri</i> ,<br><i>Escherichia fergusonii</i> ,<br><i>Escherichia coli</i> ,<br><i>Shigella sonnei</i> | 100.0            | <i>Proteobacteria Gammaproteobacteria Enterobacteriales</i> |
| OTU.760       | 2.89               | 3          | 3               | <i>Dyadobacter hamtensis</i>   | 98.63            | <i>Bacteroidetes Cytophagia Cytophagales</i>                |
| OTU.8         | 2.26               | 1          | 1               | <i>Bacillus niaci</i>  | 100.0            | <i>Firmicutes Bacilli Bacillales</i>                        |
| OTU.843       | 3.62               | 1          | 1               | <i>Paenibacillus agarizedens</i>   | 100.0            | <i>Firmicutes Bacilli Bacillales</i>                        |
| OTU.9         | 2.04               | 1          | 1               | <i>Bacillus megaterium</i> ,<br><i>Bacillus flexus</i>   | 100.0            | <i>Firmicutes Bacilli Bacillales</i>                        |

<sup>a</sup> Maximum observed  $\log_2$  of fold change.<sup>b</sup> Day of maximum fold change.<sup>c</sup> All response days.

Table S2: <sup>13</sup>C-cellulose responders BLAST against Living Tree Project

| OTU ID   | Fold change <sup>a</sup> | Day <sup>b</sup> | All days <sup>c</sup> | Top BLAST hits   | BLAST %ID | Phylum;Class;Order  |
|----------|--------------------------|------------------|-----------------------|--|-----------|---|
| OTU.100  | 2.66                     | 14               | 14                    | <i>Pseudoxanthomonas sacheonensis</i> ,<br><i>Pseudoxanthomonas dokdonensis</i>  | 100.0     | <i>Proteobacteria</i><br><i>Gammaproteobacteria</i><br><i>Xanthomonadales</i> |
| OTU.1023 | 4.61                     | 30               | 30                    | No hits of at least 90% identity   | 80.54     | <i>Verrucomicrobia</i> <i>Spartobacteria</i><br><i>Chthoniobacterales</i>     |
| OTU.1065 | 5.31                     | 14               | 14, 30                | No hits of at least 90% identity   | 84.55     | <i>Planctomycetes</i> <i>Planctomycetacia</i><br><i>Planctomycetales</i>      |
| OTU.1087 | 4.32                     | 14               | 14, 30                | <i>Devasia soli</i> ,<br><i>Devasia crocina</i> ,<br><i>Devasia riboflavina</i>  | 99.09     | <i>Proteobacteria</i> <i>Alphaproteobacteria</i><br><i>Rhizobiales</i>        |
| OTU.1094 | 3.69                     | 30               | 30                    | <i>Sporocytophaga myxococcoides</i>  | 99.55     | <i>Bacteroidetes</i> <i>Cytophagia</i><br><i>Cytophagales</i>                 |
| OTU.11   | 3.41                     | 14               | 14                    | <i>Stenotrophomonas pavani</i> i,<br><i>Stenotrophomonas maltophilia</i> ,<br><i>Pseudomonas geniculata</i>  | 99.54     | <i>Proteobacteria</i><br><i>Gammaproteobacteria</i><br><i>Xanthomonadales</i> |
| OTU.114  | 2.78                     | 14               | 14                    | <i>Herbaspirillum sp. SUEMI03</i> ,<br><i>Herbaspirillum sp. SUEMI10</i> ,<br><i>Oxalicibacterium solurbis</i> ,<br><i>Herminiumonas fonticola</i> ,<br><i>Oxalicibacterium horti</i>  | 100.0     | <i>Proteobacteria</i> <i>Betaproteobacteria</i><br><i>Burkholderiales</i>     |
| OTU.119  | 3.31                     | 14               | 14, 30                | <i>Brevundimonas alba</i>  | 100.0     | <i>Proteobacteria</i> <i>Alphaproteobacteria</i><br><i>Caulobacterales</i>    |
| OTU.120  | 4.76                     | 14               | 14, 30                | <i>Vampirovibrio chlorellavorus</i>  | 94.52     | <i>Cyanobacteria</i> <i>SM1D11</i><br>uncultured-bacterium                    |
| OTU.1204 | 4.32                     | 30               | 30                    | <i>Planctomyces limnophilus</i>  | 91.78     | <i>Planctomycetes</i> <i>Planctomycetacia</i><br><i>Planctomycetales</i>      |
| OTU.1312 | 4.07                     | 30               | 30                    | <i>Paucimonas lemoignei</i>  | 99.54     | <i>Proteobacteria</i> <i>Betaproteobacteria</i><br><i>Burkholderiales</i>     |
| OTU.132  | 2.81                     | 14               | 14                    | <i>Streptomyces spp.</i>   | 100.0     | <i>Actinobacteria</i> <i>Streptomycetales</i><br><i>Streptomycetaceae</i>     |
| OTU.150  | 4.06                     | 14               | 14                    | No hits of at least 90% identity   | 86.76     | <i>Planctomycetes</i> <i>Planctomycetacia</i><br><i>Planctomycetales</i>      |
| OTU.1533 | 3.43                     | 30               | 30                    | No hits of at least 90% identity   | 82.27     | <i>Verrucomicrobia</i> <i>Spartobacteria</i><br><i>Chthoniobacterales</i>     |
| OTU.154  | 3.24                     | 14               | 14                    | <i>Pseudoxanthomonas mexicana</i> ,<br><i>Pseudoxanthomonas japonensis</i>   | 100.0     | <i>Proteobacteria</i><br><i>Gammaproteobacteria</i><br><i>Xanthomonadales</i> |
| OTU.165  | 3.1                      | 14               | 14                    | <i>Rhizobium skieniewicense</i> ,<br><i>Rhizobium vignae</i> ,<br><i>Rhizobium larrymoorei</i> ,<br><i>Rhizobium alkalisolii</i> ,<br><i>Rhizobium galegae</i> ,<br><i>Rhizobium huautlense</i>                              | 100.0     | <i>Proteobacteria</i> <i>Alphaproteobacteria</i><br><i>Rhizobiales</i>        |
| OTU.1754 | 4.48                     | 14               | 14                    | <i>Asticcacaulis biprosthecum</i> ,<br><i>Asticcacaulis benevestitus</i>   | 96.8      | <i>Proteobacteria</i> <i>Alphaproteobacteria</i><br><i>Caulobacterales</i>    |
| OTU.185  | 4.37                     | 14               | 14, 30                | No hits of at least 90% identity   | 85.14     | <i>Verrucomicrobia</i> <i>Spartobacteria</i><br><i>Chthoniobacterales</i>     |
| OTU.19   | 2.44                     | 14               | 14                    | <i>Rhizobium alamii</i> ,<br><i>Rhizobium mesosinicum</i> ,<br><i>Rhizobium mongolense</i> ,<br><i>Arthrobacter viscosus</i> ,<br><i>Rhizobium sullae</i> ,<br><i>Rhizobium yanglingense</i> ,<br><i>Rhizobium loessense</i> | 99.54     | <i>Proteobacteria</i> <i>Alphaproteobacteria</i><br><i>Rhizobiales</i>        |

Table S2 – continued from previous page

| OTU ID   | Fold change | Day | All days  | Top BLAST hits   | BLAST %ID | Phylum;Class;Order                                  |
|----------|-------------|-----|-----------|--|-----------|---|
| OTU.2192 | 3.49        | 30  | 14, 30    | No hits of at least 90% identity   | 83.56     | Verrucomicrobia Spartobacteria Chthoniobacterales   |
| OTU.228  | 2.54        | 30  | 30        | <i>Sorangium cellulosum</i>  | 98.17     | Proteobacteria Deltaproteobacteria Myxococcales     |
| OTU.241  | 2.66        | 14  | 14        | No hits of at least 90% identity   | 87.73     | Verrucomicrobia Spartobacteria Chthoniobacterales   |
| OTU.257  | 2.94        | 14  | 14        | <i>Lentzea waywayandensis</i> ,<br><i>Lentzea flaviverrucosa</i>   | 100.0     | Actinobacteria Pseudonocardiales Pseudonocardiaceae |
| OTU.266  | 4.54        | 30  | 14, 30    | No hits of at least 90% identity   | 83.64     | Verrucomicrobia Spartobacteria Chthoniobacterales   |
| OTU.28   | 2.59        | 14  | 14        | <i>Rhizobium giardinii</i> ,<br><i>Rhizobium tubonense</i> ,<br><i>Rhizobium tibeticum</i> ,<br><i>Rhizobium mesoamericanum CCGE 501</i> ,<br><i>Rhizobium herbae</i> ,<br><i>Rhizobium endophyticum</i> | 99.54     | Proteobacteria Alphaproteobacteria Rhizobiales      |
| OTU.285  | 3.55        | 30  | 14, 30    | <i>Blastopirellula marina</i>  | 90.87     | Planctomycetes Planctomycetacia Planctomycetales    |
| OTU.32   | 2.34        | 3   | 3         | <i>Sandaracinus amyloyticus</i>  | 94.98     | Proteobacteria Deltaproteobacteria Myxococcales     |
| OTU.327  | 2.99        | 14  | 14        | <i>Asticcacaulis biprostheciun</i> ,<br><i>Asticcacaulis benevestitus</i>  | 98.63     | Proteobacteria Alphaproteobacteria Caulobacterales  |
| OTU.351  | 3.54        | 14  | 14, 30    | <i>Pirellula staleyi DSM 6068</i>  | 91.86     | Planctomycetes Planctomycetacia Planctomycetales    |
| OTU.3594 | 3.83        | 30  | 30        | <i>Chondromyces robustus</i>   | 90.41     | Proteobacteria Deltaproteobacteria Myxococcales     |
| OTU.3775 | 3.88        | 14  | 14        | <i>Devasia glacialis</i> ,<br><i>Devasia chinhatensis</i> ,<br><i>Devasia geoensis</i> ,<br><i>Devasia yakushimensis</i>   | 98.63     | Proteobacteria Alphaproteobacteria Rhizobiales      |
| OTU.429  | 3.7         | 30  | 14, 30    | <i>Devasia limi</i> ,<br><i>Devasia psychrophila</i>   | 97.72     | Proteobacteria Alphaproteobacteria Rhizobiales      |
| OTU.4322 | 4.19        | 14  | 7, 14, 30 | No hits of at least 90% identity   | 89.14     | Chloroflexi Herpetosiphonales Herpetosiphonaceae    |
| OTU.442  | 3.05        | 30  | 30        | <i>Chondromyces robustus</i>   | 92.24     | Proteobacteria Deltaproteobacteria Myxococcales     |
| OTU.465  | 3.79        | 30  | 30        | <i>Ohtaekwangia kribbensis</i>   | 92.73     | Bacteroidetes Cytophagia Cytophagales               |
| OTU.473  | 3.58        | 14  | 14        | <i>Pirellula staleyi DSM 6068</i>  | 90.91     | Planctomycetes Planctomycetacia Planctomycetales    |
| OTU.484  | 4.92        | 14  | 14, 30    | No hits of at least 90% identity   | 89.09     | Planctomycetes Planctomycetacia Planctomycetales    |
| OTU.5    | 2.69        | 14  | 14        | <i>Delftia tsuruhatensis</i> ,<br><i>Delftia lacustris</i>   | 100.0     | Proteobacteria Betaproteobacteria Burkholderiales   |
| OTU.518  | 4.8         | 14  | 14        | <i>Hydrogenophaga intermedia</i>   | 100.0     | Proteobacteria Betaproteobacteria Burkholderiales   |
| OTU.5190 | 3.6         | 30  | 14, 30    | No hits of at least 90% identity   | 88.13     | Chloroflexi Herpetosiphonales Herpetosiphonaceae    |
| OTU.541  | 4.49        | 30  | 30        | No hits of at least 90% identity   | 84.23     | Verrucomicrobia Spartobacteria Chthoniobacterales   |
| OTU.5539 | 4.01        | 14  | 14        | <i>Devasia subaequoris</i>   | 98.17     | Proteobacteria Alphaproteobacteria Rhizobiales      |
| OTU.573  | 3.03        | 30  | 30        | <i>Adhaeribacter aerophilus</i>  | 92.76     | Bacteroidetes Cytophagia Cytophagales               |

Table S2 – continued from previous page

| OTU ID   | Fold change | Day | All days  | Top BLAST hits  | BLAST %ID | Phylum;Class;Order                                       |
|----------|-------------|-----|-----------|---|-----------|--|
| OTU.6    | 3.62        | 7   | 3, 7, 14  | <i>Cellvibrio fulvus</i>  | 100.0     | Proteobacteria<br>Gammaproteobacteria<br>Pseudomonadales |
| OTU.600  | 3.48        | 30  | 30        | No hits of at least 90% identity  | 80.37     | Planctomycetes Planctomycetacia<br>Planctomycetales      |
| OTU.6062 | 4.83        | 30  | 30        | <i>Dokdonella sp. DC-3,</i><br><i>Luteibacter rhizovicinus</i>  | 97.26     | Proteobacteria<br>Gammaproteobacteria<br>Xanthomonadales |
| OTU.627  | 4.43        | 14  | 14        | <i>Verrucomicrobiaceae bacterium DC2a-G7</i>  | 100.0     | Verrucomicrobia Verrucomicrobiae<br>Verrucomicrobiales   |
| OTU.633  | 3.84        | 30  | 30        | No hits of at least 90% identity  | 89.5      | Proteobacteria Deltaproteobacteria<br>Myxococcales       |
| OTU.638  | 4.0         | 30  | 30        | <i>Luteolibacter sp. CCTCC AB 2010415,</i><br><i>Luteolibacter algae</i>  | 93.61     | Verrucomicrobia Verrucomicrobiae<br>Verrucomicrobiales   |
| OTU.64   | 4.31        | 14  | 7, 14, 30 | No hits of at least 90% identity  | 89.5      | Chloroflexi Herpetosiphonales<br>Herpetosiphonaceae      |
| OTU.663  | 3.63        | 30  | 30        | <i>Pirellula staleyi DSM 6068</i>   | 90.87     | Planctomycetes Planctomycetacia<br>Planctomycetales      |
| OTU.669  | 3.34        | 30  | 30        | <i>Ohtaekwangia koreensis</i>   | 92.69     | Bacteroidetes Cytophagia<br>Cytophagales                 |
| OTU.670  | 2.87        | 30  | 30        | <i>Adhaeribacter aerophilus</i>   | 91.78     | Bacteroidetes Cytophagia<br>Cytophagales                 |
| OTU.766  | 3.21        | 14  | 14, 30    | <i>Devosia insulae</i>  | 99.54     | Proteobacteria Alphaproteobacteria<br>Rhizobiales        |
| OTU.83   | 5.61        | 14  | 7, 14, 30 | <i>Luteolibacter sp. CCTCC AB 2010415</i>   | 97.72     | Verrucomicrobia Verrucomicrobiae<br>Verrucomicrobiales   |
| OTU.862  | 5.87        | 14  | 14        | <i>Allotkutznheria albata</i>   | 100.0     | Actinobacteria Pseudonocardiales<br>Pseudonocardiaceae   |
| OTU.899  | 2.28        | 30  | 30        | <i>Enhygromyxa salina</i>   | 97.72     | Proteobacteria Deltaproteobacteria<br>Myxococcales       |
| OTU.90   | 2.94        | 14  | 14, 30    | <i>Sphingopyxis panaciterrae,</i><br><i>Sphingopyxis chilensis,</i><br><i>Sphingopyxis sp. BZ30,</i><br><i>Sphingomonas sp.</i> | 100.0     | Proteobacteria Alphaproteobacteria<br>Sphingomonadales   |
| OTU.900  | 4.87        | 14  | 14        | <i>Brevundimonas vesicularis,</i><br><i>Brevundimonas nasdae</i>  | 100.0     | Proteobacteria Alphaproteobacteria<br>Caulobacterales    |
| OTU.971  | 3.68        | 30  | 30        | No hits of at least 90% identity  | 78.57     | Chloroflexi Anaerolineae<br>Anaerolineales               |
| OTU.98   | 3.68        | 14  | 7, 14, 30 | No hits of at least 90% identity  | 88.18     | Chloroflexi Herpetosiphonales<br>Herpetosiphonaceae      |
| OTU.982  | 4.47        | 14  | 14        | <i>Devosia neptuniae</i>  | 100.0     | Proteobacteria Alphaproteobacteria<br>Rhizobiales        |

<sup>a</sup> Maximum observed  $\log_2$  of fold change.<sup>b</sup> Day of maximum fold change.<sup>c</sup> All response days.