

Unearthing the soil carbon food web with high resolution DNA-SIP

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Abstract

We identified microorganisms participating in xylose and/or cellulose decomposition in soil microcosms using nucleic acid stable isotope probing (SIP) coupled to next generation sequencing. 49 and 63 OTUs assimilated ^{13}C from xylose and cellulose into DNA, respectively. Microorganisms assimilated xylose-C at days 1, 3, and 7. Cellulose-C assimilation peaked at day 14 and was maintained at day 30. Many SIP-identified cellulose degraders are members of cosmopolitan but physiologically uncharacterized soil microbial lineages including *Spartobacteria*, *Chloroflexi* and *Planctomycetes*. ^{13}C from Xylose was initially assimilated by *Firmicutes* followed by *Bacteroidetes* and *Actinobacteria*. Trophic interactions may have caused this temporal pattern. Soil C cycling models, however, often disregard bacterial trophic interactions. Fast growing substrate specialists assimilated xylose-C and slow growing substrate specialists assimilated cellulose-C. Cellulose-C assimilators clustered phylogenetically and within time points, phylogenetically coherent groups assimilated xylose-C. Knowledge of soil C-cycling functional guild diversity and activity will improve the predictive power of terrestrial C flux models.

monolayer | structure | x-ray reflectivity | molecular electronics

Abbreviations: SAM, self-assembled monolayer; OTS, octadecyltrichlorosilane

Significance

We have a limited understanding of soil carbon (C) cycling yet soil contains a large fraction of the global C pool. Microorganisms mediate most soil C cycling but have proven difficult to study due to the complexity of soil C biochemistry and the wide range of soil microorganisms participating in C reactions. We demonstrate C use dynamics by soil microbial taxa. Furthermore, we identified microorganisms involved in cellulose, the most globally abundant biopolymer, decomposition that were previously uncharacterized physiologically. Our results expand knowledge of soil

functional guild diversity and activity which reveal soil function-structure relationships.

Introduction

Excluding plant biomass, there are 2,300 Pg of carbon (C) in soils worldwide which accounts for ~80% of the global terrestrial C pool [1, 2]. Fungi, archaea, and bacteria degrade plant biomass that reaches soil. Microorganisms respire the majority of plant biomass producing 10 times more CO_2 annually than anthropogenic emissions [3]. Rising atmospheric CO_2 may stimulate plant growth and in turn increase plant biomass C input to soil [4]. Current climate change models concur on atmospheric and oceanic, but not terrestrial, C predictions [5]. Contrasting terrestrial C model predictions reflect how little is known about soil C cycling. We must establish the roles and diversity of soil microbial community members in soil C cycling to reconcile inconsistencies in terrestrial C models [6, 7].

we must identify the *in situ* activity of soil microbes to establish soil structure function relationships [8]. Microorganisms mediate an estimated 80-90% of soil C cycling [9, 10]. The complexity of soil obscures microbial contributions to soil C cycling and the majority of microorganisms resist cultivation in the laboratory. Stable-isotope probing (SIP) links genetic identity and activity without cultivation and has expanded our knowledge of microbial contributions to biogeochemical processes [11]. Successful applications of SIP have identified organisms which me-

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diate processes performed by functionally specialized microorganisms of limited diversity such as methanogens [12] but SIP has been less applicable in soil C cycling studies because simultaneous labeling of many different organisms required inhibitory resolving power. High throughput DNA sequencing technology, however, improves the resolving power of SIP enabling exploration of complex soil C-cycling patterns.

This study aimed to observe labile C versus polymeric C assimilation dynamics in the soil microbial community. We added mixture of nutrients and C substrates to soil microcosms that simulated the composition of plant biomass. All microcosms received the same C substrate mixture where the only difference between treatments was the identity of the isotopically labeled substrate. Specifically, we set up a series of microcosms with three treatments: in one treatment xylose was substituted for its ^{13}C -equivalent, in another cellulose was substituted for its ^{13}C -equivalent, and in the third treatment all substrates in the mixture were unlabeled. We harvested microcosms at days 1, 3, 7, 14 and 30 except day 1 where only ^{13}C -xylose treated microcosms were harvested. We used labeled xylose and cellulose to contrast labile C and polymeric C decomposition, respectively and we sequenced 16S rRNA genes from SIP density fractions with high throughput DNA sequencing technology. Our experimental design allowed us to observe the soil microbial community members that assimilated xylose-C and cellulose-C over time.

Results

Our experimental design allowed us to track the flow of xylose and cellulose C through the soil microbial community (Figure S1). 5.3 mg C substrate mixture per gram of soil was added to each microcosm representing 18% of the soil C. The mixture included 0.42 mg xylose-C and 0.88 mg cellulose-C per gram soil. Microcosms were harvested at days 1, 3, 7, 14 and 30 during a 30 day incubation. ^{13}C -xylose assimilation peaked immediately and tapered over the 30 day incubation whereas ^{13}C -cellulose assimilation peaked two weeks after amendment additions (Figure 1, Figure S2). See Supplemental Note XX for sequencing and density fractionation statistics. Microcosm treatments (see Methods) are identified in figures by the following code: “13CXPS” refers to the amendment with ^{13}C -xylose (^{13}C Xylose Plant Simulant), “13CCPS” refers to the ^{13}C -cellulose amendment and “12CCPS” refers to the amendment that only contained ^{12}C (i.e. control).

Soil microcosm microbial community changes with time. Changes in the bulk soil microcosm microbial community structure and membership cor-

related significantly with time (Figure S3, p-value 0.23, R^2 0.63, Adonis test [13]). The identity of the ^{13}C -labeled substrate added to the microcosms did not significantly correlate with bulk soil community structure and membership (p-value 0.35). Additionally, microcosm beta diversity was significantly less than gradient fraction beta diversity (Figure S3, p-value 0.003, “betadisper” function R Vegan package [14, 15]). Twenty-nine OTUs significantly changed in relative abundance with time (“BH” adjusted p-value <0.10, [16]) and of these 29 OTUs, 14 were found to incorporate ^{13}C from labeled substrates into biomass (Figure S4). Four taxonomic classes significantly (adjusted P-value ≤ 0.10) changed in abundance: *Bacilli* (decreased), *Flavobacteria* (decreased), *Gammaproteobacteria* (decreased) and *Herpetosiphonales* (increased) (Figure S5). Abundances grouped by phylum for OTUs that incorporated ^{13}C from cellulose increased with time whereas abundances grouped by phylum of OTUs that incorporated ^{13}C from xylose decreased over time although *Proteobacteria* abundance spiked at day 14 (Figure S6).

OTUs that assimilated ^{13}C into DNA. Within the first 7 days of incubation 63% of ^{13}C -xylose was respired and only 6% more was respired from day 7 to 30. At day 30, 30% of the ^{13}C from xylose remained in the soil. An average 16% of the ^{13}C -cellulose added was respired within the first 7 days, 38% by day 14, and 60% by day 30.

We refer to OTUs that putatively incorporated ^{13}C into DNA originally from an isotopically labeled substrate as substrate “responders” (see Supplemental Note XX for operational “response” criteria). There were 19, 19, 15, 6, and 1 ^{13}C -xylose responders at days 1, 3, 7, 14, 30, respectively (Figure S2). The numerically dominant ^{13}C -xylose responder phylum shifted from *Firmicutes* to *Bacteroidetes* and then to *Actinobacteria* across days 1, 3 and 7 (Figure 2, Figure 3). *Proteobacteria* ^{13}C -xylose responders were found at days 1, 3, 7 but peaked at day 7 (Figure 3).

Only 2 and 5 OTUs responded ^{13}C -cellulose at days 3 and 7, respectively. At days 14 and 30, 42 and 39 OTUs responded to ^{13}C -cellulose. (Figure S2). *Proteobacteria*, *Verrucomicrobia*, and *Chloroflexi* had relatively high numbers of responders with strong response across multiple time points (Figure 2). *Verrucomicrobia* ^{13}C -cellulose responders were 70% *Spartobacteria*. *Chloroflexi* responders were annotated belonging to the *Herpetosiphonales* and *Anaerolineae* (Figure ??). *Celvibrio*, a canonical soil cellulose degrader, was found to respond strongly in the microcosms to ^{13}C -cellulose. See Supplemental Note XX for further counts of ^{13}C -responsive OTUs at greater taxonomic resolution.

195 Ecological strategies of ^{13}C responders. ^{13}C -xylose responders are generally more abundant members based on relative abundance in bulk DNA SSU rRNA gene content than ^{13}C -cellulose responders (Figure 4, p-value 0.00028, Wilcoxon Rank Sum test). However, both abundant and rare OTUs responded to ^{13}C -xylose and ^{13}C -cellulose (Figure 4). Two ^{13}C -cellulose responders were not found in any bulk samples (“OTU.862” and “OTU.1312”, Table S1). Of the 10 most abundant 200 responders, 8 are ^{13}C -xylose responders and 6 of these 8 are consistently among the 10 most abundant OTUs in bulk samples.

Cellulose responder buoyant density (BD) shifted further along the density gradient than 205 xylose responder BD in response to ^{13}C incorporation (Figure S7, Figure 4, p-value 1.8610×10^{-6} , Wilcoxon Rank Sum test). ^{13}C -cellulose responder BD shifted on average 0.0163 g mL^{-1} (sd 0.0094) whereas xylose responder BD shifted on average 210 0.0097 g mL^{-1} (sd 0.0094). For reference, 100% ^{13}C DNA BD is 0.04 g mL^{-1} greater than the BD of its ^{12}C counterpart. DNA BD increases as its ratio of ^{13}C to ^{12}C increases. An organism that only assimilates C into DNA from a ^{13}C isotopically labeled source, will have a greater ^{13}C to ^{12}C 215 ratio in its DNA than an organism utilizing a mixture of isotopically labeled and unlabeled C sources (see Supplemental Note XX). We predicted the *rrn* gene copy number for each OTU as described previously [17]. The estimated *rrn* gene copy number for ^{13}C -xylose responders was inversely related to 220 time point of the first response per OTU (p-value 2.02×10^{-15} , Figure S8). OTUs that did not respond at day 1 respond but did respond at day 3 and/or day 7 had fewer estimated *rrn* copy number 225 than OTUs that responded at day 1 (Figure S8).

We assessed phylogenetic clustering of ^{13}C -responsive OTUs with the Nearest Taxon Index (NTI) and the Net Relatedness Index (NRI). 230 Briefly, positive NRI and NTI with corresponding low P-values indicates deep phylogenetic clustering whereas negative NRI with high P-values indicates taxa are overdispersed compared to the null model [18]. NRI and P-values for substrate responder 235 groups suggest ^{13}C -xylose responders are overdispersed (NRI: -1.33, P: 0.90) while ^{13}C -cellulose responders are clustered (NRI: 4.49, P: 0.001). NTI values show that both ^{13}C -cellulose and ^{13}C -xylose 240 responders are clustered near the tips of the tree (NTI: 1.43 (P: 0.072), 2.69 (P: 0.001), respectively).

Discussion

Microbial response to isotopic labels. DNA-SIP can establish functional roles for thousands of phylogenetic types in a single experiment without cultivation. 245

We identified 104 agricultural soil OTUs that incorporated ^{13}C from xylose and/or cellulose into biomass and characterized substrate specificity and C-cycling dynamics for these OTUs. We propose C added to soil microcosms took the following path through the microbial food web (Figure S9): fast-growing *Firmicutes* spore formers first assimilated labile C followed by *Bacteroidetes*, *Actinobacteria* and *Proteobacteria* phylotypes. The *Bacteroidetes*, *Actinobacteria* and *Proteobacteria* phylotypes may have also preyed on the rapidly responding *Firmicutes*. Canonical cellulose degrading bacteria such as *Cellvibrio* and members of cosmopolitan yet functionally uncharacterized soil phylogenetic groups like *Chloroflexi*, *Planctomycetes* and *Verrucomicrobia*, specifically the *Spartobacteria*, decomposed cellulose and cellulose C incorporation into microbial biomass plateaued at day 14 and was maintained through day 30.

Ecological strategies of soil microorganisms participating in the decomposition of organic matter. We assessed ^{13}C -responsive OTU ecological strategy by estimating each OTU's *rrn* gene copy number and the BD shift upon ^{13}C -labeling. *rrn* gene copy number correlates positively with growth rate [19] and BD shift is indicative of substrate specificity (see results). ^{13}C -cellulose responsive OTUs grow slower (Figure 4, Figure S8), have greater substrate specificity (Figure 4), and are generally lower abundance members of the bulk community than ^{13}C -xylose responsive OTUs (Figure 4). The high abundance of xylose responders may also be due in part to high *rrn* gene copy number. ^{13}C -xylose responsive OTUs that incorporate ^{13}C into 250 biomass at day one had greater *rrn* gene copy number than OTUs that responded later to ^{13}C -xylose (Figure 4, Figure S8) suggesting fast-growing microbes assimilated ^{13}C from xylose before slow growers.

NRI values quantify phylogenetic clustering [20] and have been used to assess clustering of soil OTUs that responded similarly to soil wet up [18, 21]. To our knowledge assessing the phylogenetic clustering of OTUs found to incorporate heavy isotopes into biomass during SIP incubations has not been attempted. We found that cellulose and xylose responders are clustered and overdispersed, respectively. This suggests that the ability to degrade cellulose is phylogenetically conserved possibly reflecting the complexity of cellulose degradation biochemistry. The positive relationship between a physiological trait's phylogenetic depth and complexity has been noted previously [22] and the ^{13}C -cellulose response trait depth observed in this study (X.XX 16S rRNA gene sequence divergence) is on the same order as that observed for glycoside hydrolases which are

diagnostic enzymes in cellulose degradation [23]. Overdispersion, as we saw for the ^{13}C -xylose responsive OTUs, may be indicative of a readily horizontally transferred trait and/or a trait that is broadly distributed phylogenetically or in this case may indicate that the group comprises many traits. It's not clear which ^{13}C -xylose responsive organisms were labeled as a result of primary xylose assimilation (see below), and therefore it's not clear if ^{13}C -xylose responsive OTUs in this experiment constitute a single ecologically meaningful group or multiple ecological groups. Temporally defined ^{13}C -xylose responder groups, however, are clearly phylogenetically coherent (Figure XX, Figure XX). For example, most day 1 ^{13}C -xylose responders are members of the *Paenibacillus* (see Supplemental Note XX).

Intuitively we infer C cycling functional guild diversity from the distribution of diagnostic genes in genomes or from screening culture collections for a particular trait CITE. For instance, the wide distribution of the glycolysis operon in microbial genomes is interpreted as evidence that many soil microorganisms participate in glucose turnover CITE. *in situ* functional guild diversity, however, can vary significantly from diversity assessed by functionally screening isolates and/or genomes. Xylose use in soil, for instance, may be less a function of catabolic pathway distribution among genomes and more a function of lifestyle. Soil is characterized by pulse deliveries of nutrients and resources that coincide with environmental phenomena including seasonal change CITE, land management CITE, and rainfall CITE. Therefore, rapid growth rates and/or the rapid resuscitation upon wet up CITE may control labile soil C assimilation. Growth rate and dessication resistance are phylogenetically conserved unlike labile C degradation CITE so labile C assimilation may be constrained by more phylogenetically conserved ecological strategies. DNA-SIP is useful for establishing *in situ* phylogenetic clustering and diversity of functional guilds because DNA-SIP can incorporate life history strategies by targeting active microorganisms. Snapshot estimates of community composition inform soil structure function studies CITE Fierer though labile C decomposition might not be linked to snapshot community structure but rather to community structure dynamics. Fast growing spore formers would not need to maintain high abundance to significantly mediate cycling of pulse delivered resources. This accentuates the usefulness of DNA-SIP for describing soil ecology as DNA-SIP assesses activity which can be decoupled from snapshot abundance.

Need to inforporate Fierer 2007 paper

Paenibacillus Neufeld

365 Thompson "all bands enriched"

Implications for soil C cycling models. Land management, climate, pollution and disturbance can influence soil community composition [24] which in turn influences soil biogeochemical process rates (e.g. [25]). Assessing functional group diversity and establishing identities of functional group members is necessary to predict how biogeochemical process rates will change with community composition [24, 26]. Aggregate biogeochemical processes that are the sum of many subprocesses involve a broad array of taxa and are assumed to be less influenced by community change than narrow processes that involve a single, specific chemical transformation by a smaller suite of microbial participants [24, 26]. Within an aggregate process such as C decomposition, subprocesses can be further classified as broad or narrow [24]. Labile and recalcitrant C decomposition are considered to be carried out by “broad” and “narrow” functional guilds, respectively [24]. However, the diversity of active labile C and insoluble, polymeric C decomposers in soil has not been directly quantified. Notably, we found more OTUs responded to ^{13}C -cellulose, 63, than ^{13}C -xylose, 49. Also, it is possible that many ^{13}C -xylose responders are predatory bacteria as opposed to primary labile C degraders (see below). Cellulose and xylose decomposer functional guilds were non-overlapping in membership – of 104 ^{13}C -responders only 8 responded to both cellulose and xylose – and represented a small fraction of total soil community diversity (Figure 5). While xylose use is undoubtedly more widely distributed among microbial genomes than the ability to degrade cellulose, the number of active xylose utilizers in our microcosms was not greater than the number of cellulose decomposers.

Both ^{13}C -cellulose and ^{13}C -xylose responders largely clustered near the tips of the phylogenetic tree ($\text{NTI} > 0$) at taxonomic levels broader than the OTUs established in this study (Figure S10). Therefore ^{13}C -responders distribute into fewer clades than OTUs (Figure S10). ^{13}C -xylose responders formed clades at depth of X.XX sequence divergence (consenTrait) while our OTUs 410 were established at 0.03 sequence divergence. Active cellulose and xylose responder groups were “narrow” in that few lineages relative to total observed lineages were active participants in cellulose or xylose decomposition but there is no quantitative definition of what constitutes “narrow” versus “broad” in the literature. HGT SENTENCE. TALK ABOUT TRESEDER NUMBERS?

The activity succession from *Firmicutes* to *Bacteroidetes* and finally *Actinobacteria* in response 420 to ^{13}C -xylose addition is a trophic cascade and/or the manifestation functional groups tuned to different resource concentrations. *Actinobacteria* (e.g. *Agromyces*) and *Bacteroidetes* have been previ-

ously implicated as predatory soil bacteria [27, 28],
 425 however, and the activity peak of *Bacteroidetes* and *Actinobacteria* occurred with a corresponding decrease in *Firmicutes* ^{13}C -xylose responder relative abundance. Considering that *Agromyces* and certain *Bacteroidetes* types are likely soil predators
 430 one parsimonious hypothesis for the ^{13}C -labelling of *Bacteroidetes* and *Actinobacteria* with a corresponding decrease in abundance of ^{13}C -labeled *Firmicutes* is that the *Bacteroidetes* and *Actinobacteria* fed on ^{13}C -labeled *Firmicutes*. If the
 435 temporal dynamics of ^{13}C -xylose incorporation are due to trophic interactions, many, if not most, fast-growing labile C degraders were consumed by predatory bacteria. Hence, predatory interactions between soil bacteria may be of importance for
 440 modelling soil C turnover yet intra-bacteria trophic interactions in soil C cycling models are rarely considered (e.g. [29]).

We propose two scenarios in the context of our results wherein C dynamics and fate would be affected by community composition. Genomic evidence shows cellulose degradation is a phylogenetically conserved trait CITE Allison. Our study is the first to evaluate the phylogenetic conservation of soil cellulose degradation *in situ* via DNA-
 450 SIP and our results are consistent with genomic evidence. Decreasing cellulose degraders would diminish cellulose decomposition process rates as few soil microorganisms can fill the phylogenetically conserved cellulose degradation niche. Ecosystem function could be renewed by dispersed cellulose decomposers, however. For labile C decomposition, the absence fast growing spore formers would allow other microbes to assimilate labile C provided dispersal does not enable rapid recolonization CITE. Primary labile C degraders in this study grow fast, and form spores. These distinct ecological strategies might indicate distinct C use dynamics and/or resource allocation. New labile C degraders may metabolize and allocate labile C differently thus changing labile C dynamics and fate. Further, labile C degrader substitution could affect biomass C turnover by predatory bacteria that feed on fast growing, spore forming labile C decomposers. On the other hand, spore formation
 465 enables dispersal which would allow fast growing spore formers to continuously occupy the labile C decomposition niche even when washed out of an environment CITE. One proposed mechanism for similar decomposition rates of labile C across soils
 470 varying in community composition is that labile C can be used widely by microorganisms CITE. An alternative hypothesis for consistent labile C process rates across different soils is that labile C degraders are easily dispersed. Notably, other lineages implicated in rapid labile C turnover include members of the *Actinobacteria* CITE Placella and

many soil *Actinobacteria* form hyphae that facilitate dispersal CITE. The two hypotheses are not mutually exclusive, but our results suggest that environmental conditions unfavorable to fast-growing spore-formers and/or quickly resuscitated, hyphal *Actinobacteria* CITE may impact labile C dynamics and fate.

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 ubiquitous soil lineages participated in cellulose decomposition. Cellulose C degraders 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* order in soil [30]. Labile C in our microcosms was assimilated by fast-growing *Firmicutes* spore formers. *Bacteroidetes* and *Actinobacteria* phylotypes previously implicated as predators may have fed on the fast growing *Firmicutes*. Our results suggest that, cosmopolitan *Spartobacteria* may degrade cellulose on a global scale, tropic interactions within bacteria are important for modeling soil C cycling, and functional guild diversity may be constrained by ecological strategies which can be more phylogenetically conserved than catabolic pathways.

Methods

Additional information on sample collection and analytical methods is provided in SI Materials and Methods.

Twelve soil cores (5 cm diameter x 10 cm depth) were collected from six random sampling locations within an organically managed agricultural field in Penn Yan, New York. Soils were pretreated by sieving (2 mm), homogenizing sieved soil, and pre-incubating 10 g of dry soil weight in flasks for 2 weeks. Soils were amended with a 5.3 mg g soil⁻¹ carbon mixture; representative of natural concentrations [31]. Mixture contained 38% cellulose, 23% lignin, 20% xylose, 3% arabinose, 1% galactose, 1% glucose, and 0.5% mannose by mass, with the remaining 13.5% mass composed of an amino acid (in-house made replica of Teknova C0705) and basal salt mixture (Murashige and Skoog, Sigma Aldrich M5524). Three parallel treatments were performed; (1) unlabeled control, (2) ^{13}C -cellulose, (3) ^{13}C -xylose (98 atom% ^{13}C , Sigma Aldrich). Each treatment had 2 replicates per time point (n = 4) except day 30 which had 4 replicates; total microcosms per treatment n = 12, except ^{13}C -cellulose which was not sampled at day 1, n =

10. Other details relating to substrate addition can be found in SI. Microcosms were sampled de-
540 structively (stored at -80°C until nucleic acid pro-
cessing) at days 1 (control and xylose only), 3, 7,
14, and 30.

Nucleic acids were extracted using a modified
545 Griffiths protocol [32]. To prepare nucleic acid ex-
tracts for isopycnic centrifugation as previously de-
scribed [33], DNA was size selected (>4kb) using
1% low melt agarose gel and β -agarase I enzyme
extraction per manufacturers protocol (New Eng-
land Biolab, M0392S). For each time point in the
550 series isopycnic gradients were setup using a modi-
fied protocol [34] for a total of five ^{12}C -control, five
 ^{13}C -xylose, and four ^{13}C -cellulose microcosms. A
density gradient (average density 1.69 g mL⁻¹) so-
lution of 1.762 g cesium chloride (CsCl) mL⁻¹ in
555 gradient buffer solution (pH 8.0 15 mM Tris-HCl,
15 mM EDTA, 15 mM KCl) was used to separate
 ^{13}C -enriched and ^{12}C -nonenriched DNA. Each
gradient was loaded with approximately 5 μg of
DNA and ultracentrifuged for 66 h at 55,000 rpm
560 and room temperature (RT). Fractions of ~100 μL
were collected from below by displacing the DNA-
CsCl-gradient buffer solution in the centrifugation
tube with water using a syringe pump at a flow
rate of 3.3 $\mu\text{L s}^{-1}$ [35] into AcroprepTM 96 filter
565 plate (Pall Life Sciences 5035). The refractive in-
dex of each fraction was measured using a Reichart
AR200 digital refractometer modified as previously
described [33] to measure a volume of 5 μL . Then
570 buoyant density was calculated from the refractive
index as previously described [33] (see also SI). The
collected DNA fractions were purified by repetitive
washing of Acroprep filter wells with TE. Finally,
575 50 μL TE was added to each fraction then resus-
pended DNA was pipetted off the filter into a new
microfuge tube.
580

For every gradient, 20 fractions were chosen for
sequencing between the density range 1.67-1.75 g
mL⁻¹. Barcoded 454 primers were designed using
454-specific adapter B, 10 bp barcodes [36], a 2
585 bp linker (5'-CA-3'), and 806R primer for reverse
primer (BA806R); and 454-specific adapter A, a
2 bp linker (5'-TC-3'), and 515F primer for for-
ward primer (BA515F). Each fraction was PCR
amplified using 0.25 μL 5 U μl^{-1} AmpliTaq Gold
590 (Life Technologies, Grand Island, NY; N8080243),
2.5 μL 10X Buffer II (100 mM Tris-HCl, pH 8.3,
500 mM KCl), 2.5 μL 25 mM MgCl₂, 4 μL 5
mM dNTP, 1.25 μL 10 mg mL⁻¹ BSA, 0.5 μL
10 μM BA515F, 1 μL 5 μM BA806R, 3 μL H₂O,
595 10 μL 1:30 DNA template) in triplicate. Samples
were normalized either using Pico green quantification
and manual calculation or by SequalPrepTM
normalization plates (Invitrogen, Carlsbad, CA;
A10510), then pooled in equimolar concentrations.
600 Pooled DNA was gel extracted from a 1% agarose
605

gel using Wizard SV gel and PCR clean-up sys-
tem (Promega, Madison, WI; A9281) per man-
ufacturer's protocol. Amplicons were sequenced
610 on Roche 454 FLX system using titanium chem-
istry at Selah Genomics (formerly EnGenCore,
Columbia, SC)

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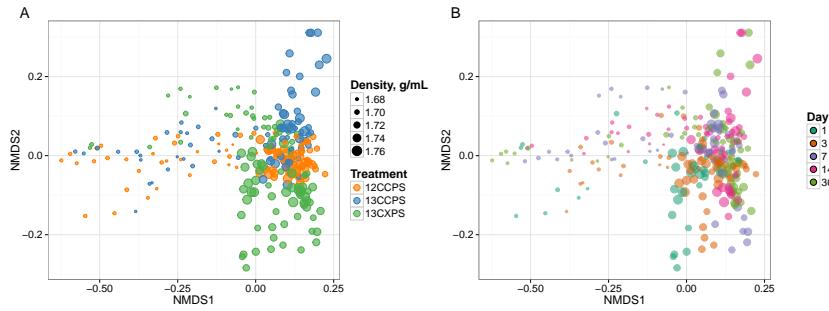


Fig. 1. NMDS analysis from weighted unifrac distances of 454 sequence data from SIP fractions of each treatment over time. Twenty fractions from a CsCl gradient fractionation for each treatment at each time point were sequenced (Fig. S1). Each point on the NMDS represents the bacterial composition based 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).

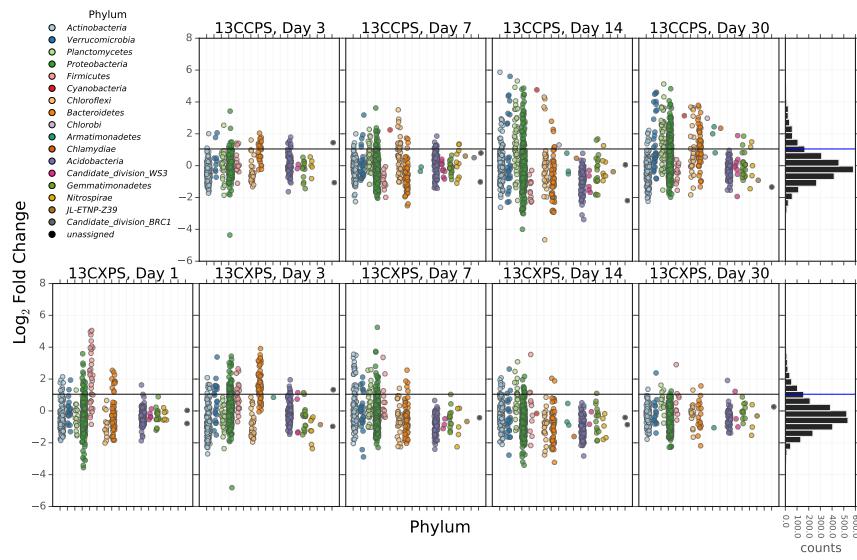


Fig. 2. Log₂ fold change of ¹³C-responders in cellulose treatment (top) and xylose treatment (bottom). Log₂ fold change is based on the relative abundance in the experimental treatment compared to the control within the density range 1.7125–1.755 g ml⁻¹. Taxa are colored by phylum. ‘Counts’ is a histogram of log₂ fold change values.

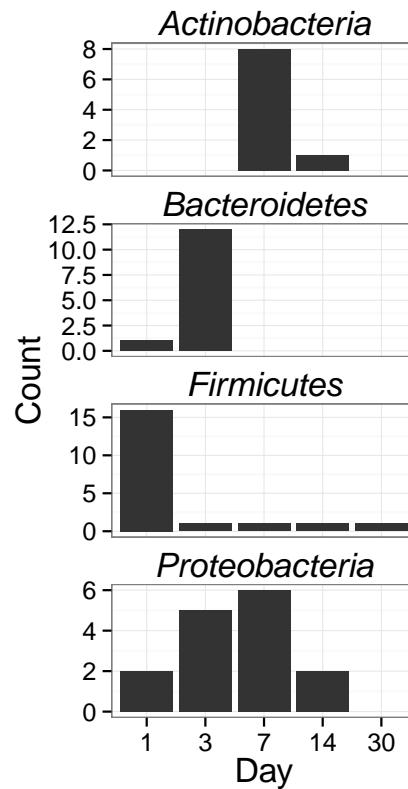


Fig. 3. Counts of ^{13}C -xylose responders in the *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria* at days 1, 3, 7 and 30.

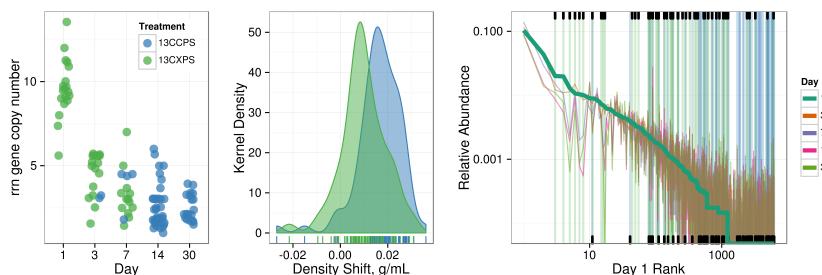


Fig. 4. ^{13}C -responder characteristics based on density shift (A) and rank (B). Kernel density estimation of ^{13}C -responder's density shift in cellulose treatment (blue) and xylose treatment (green) demonstrates degree of labeling for responders for each respective substrate. ^{13}C -responders in rank abundance are labeled by substrate (cellulose, blue; xylose, green). Ticks at top indicate location of ^{13}C -xylose responders in bulk community. Ticks at bottom indicate location of ^{13}C -cellulose responders in bulk community. OTU rank was assessed from day 1 bulk samples.

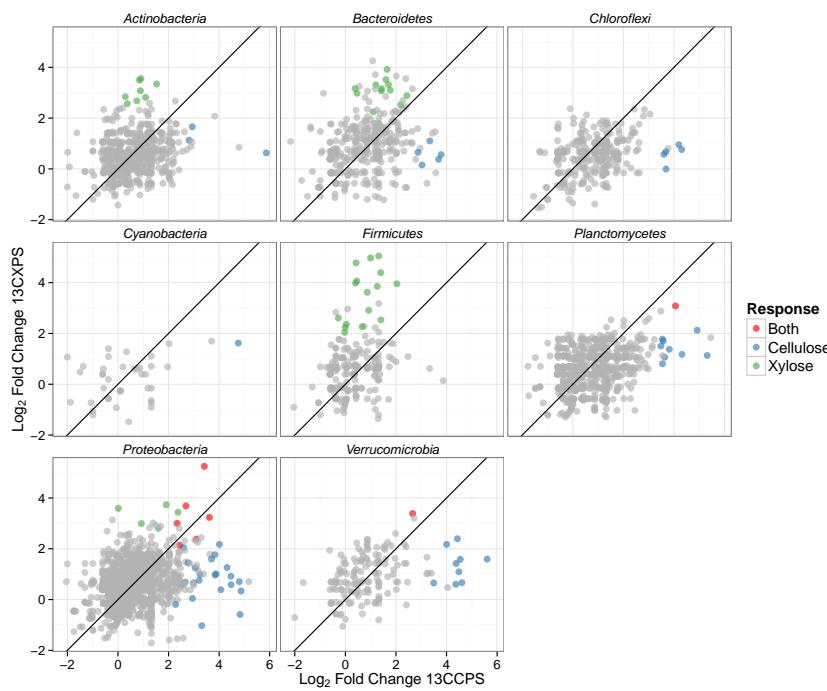
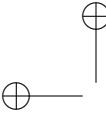


Fig. 5. Maximum log₂ fold change in response to labeled substrate incorporation for each substrate and all OTUs that pass sparsity filtering in both substrate analyses. Points colored by response. Line has slope of 1 with intercept at the origin. OTUs falling in the top-right quadrant responded to both substrates while those in the top-left and bottom-right responded to ¹³C-xylose and ¹³C-cellulose, respectively.



750 **Supplemental Figures and Tables**

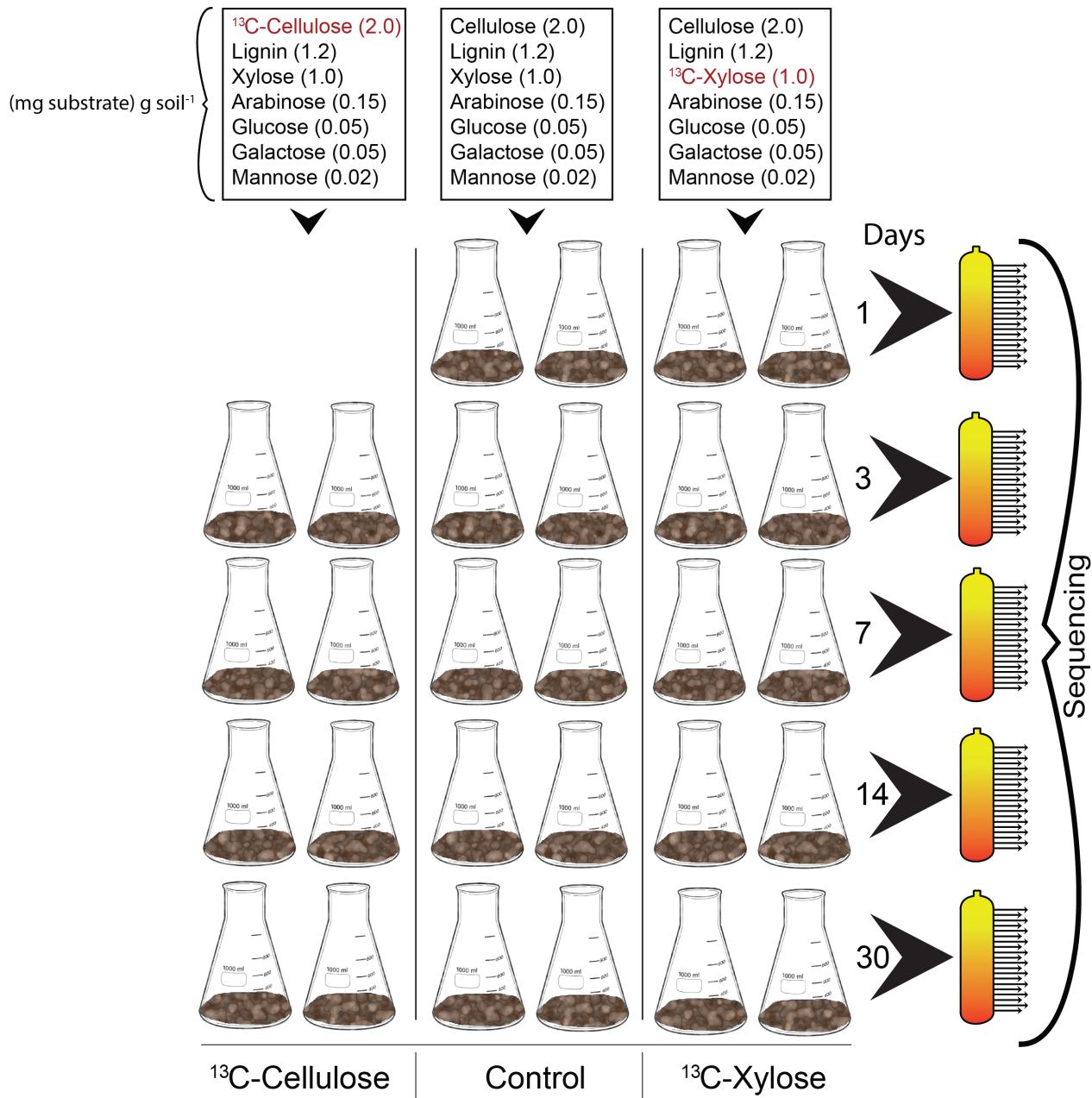


Fig. S1. The experimental design. A carbon mixture, in addition to inorganic salts and amino acids (not shown here), was added to each soil microcosm where the only difference between treatments is the ¹³C-labeled isotope (in red). At days 1, 3, 7, 14, and 30 replicate microcosms were destructively harvested 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.

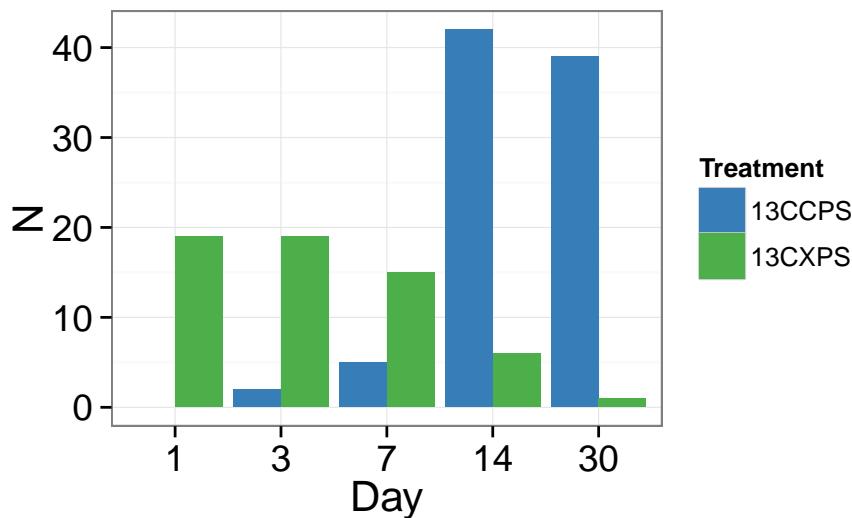


Fig. S2. Counts of responders to each isotopically labeled substrate (cellulose and xylose) over time.

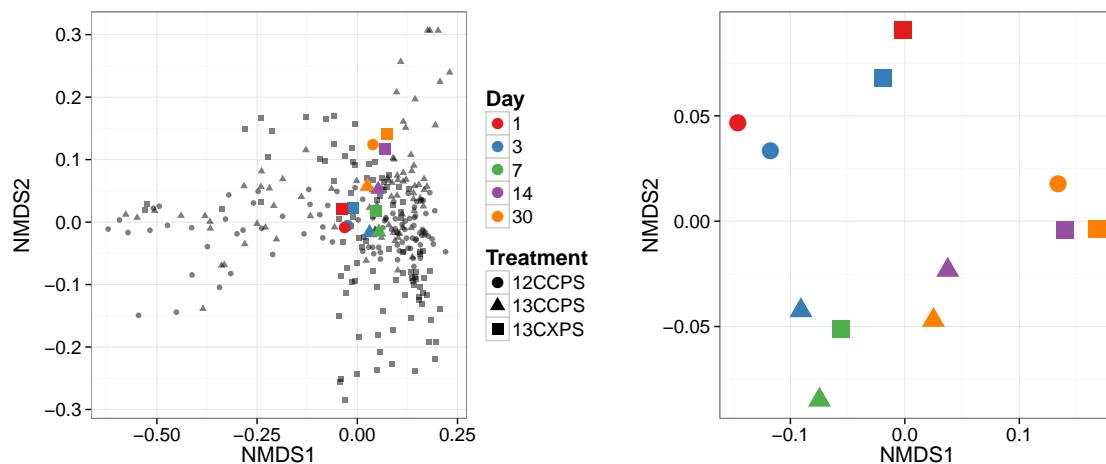


Fig. S3. Ordination of bulk gradient fraction phylogenetic profiles.

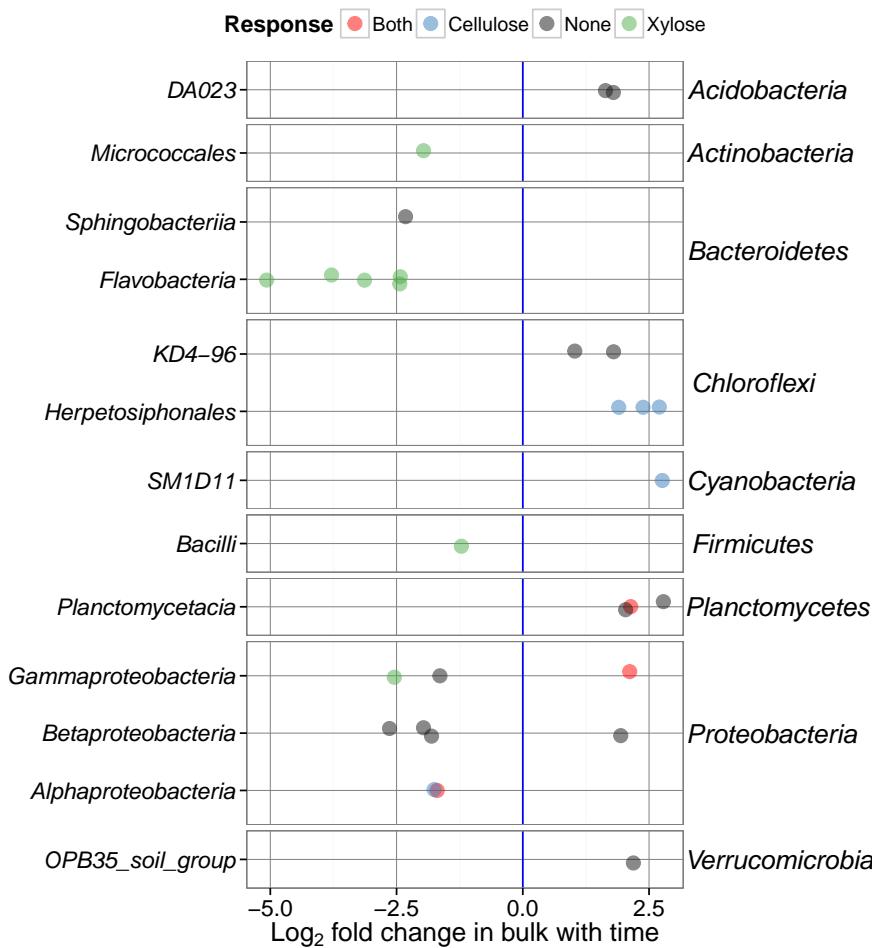


Fig. S4. Fold change time⁻¹ for OTUs that changed significantly in abundance over time. One panel per phylum (phyla indicated on the right). Taxonomic class indicated on the left.

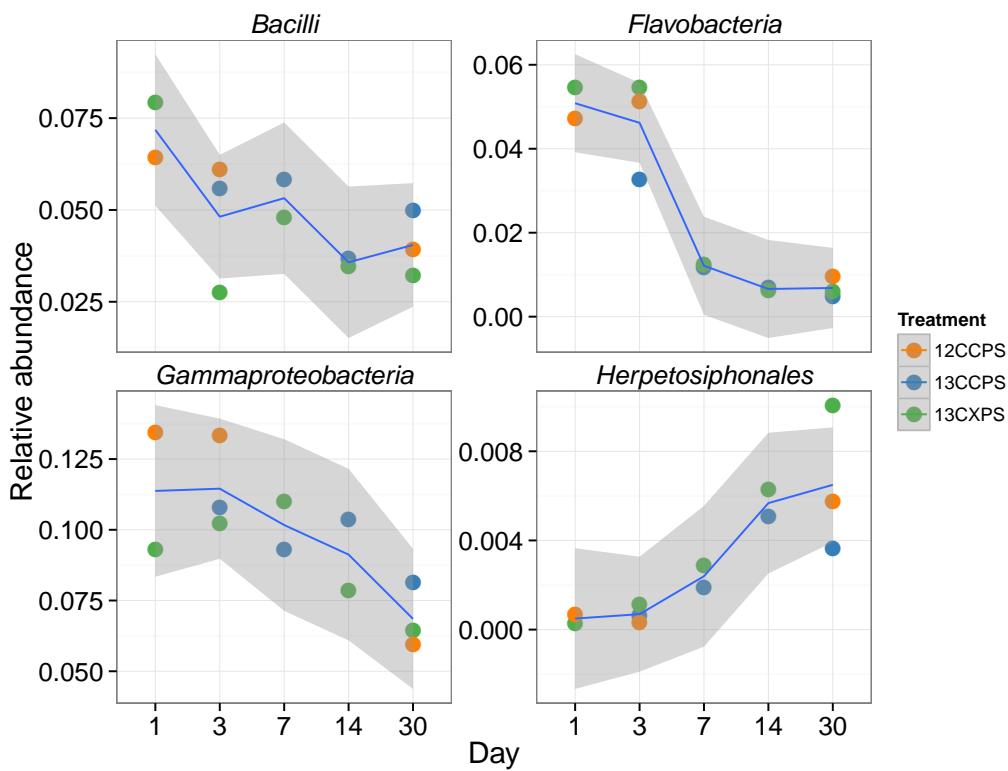
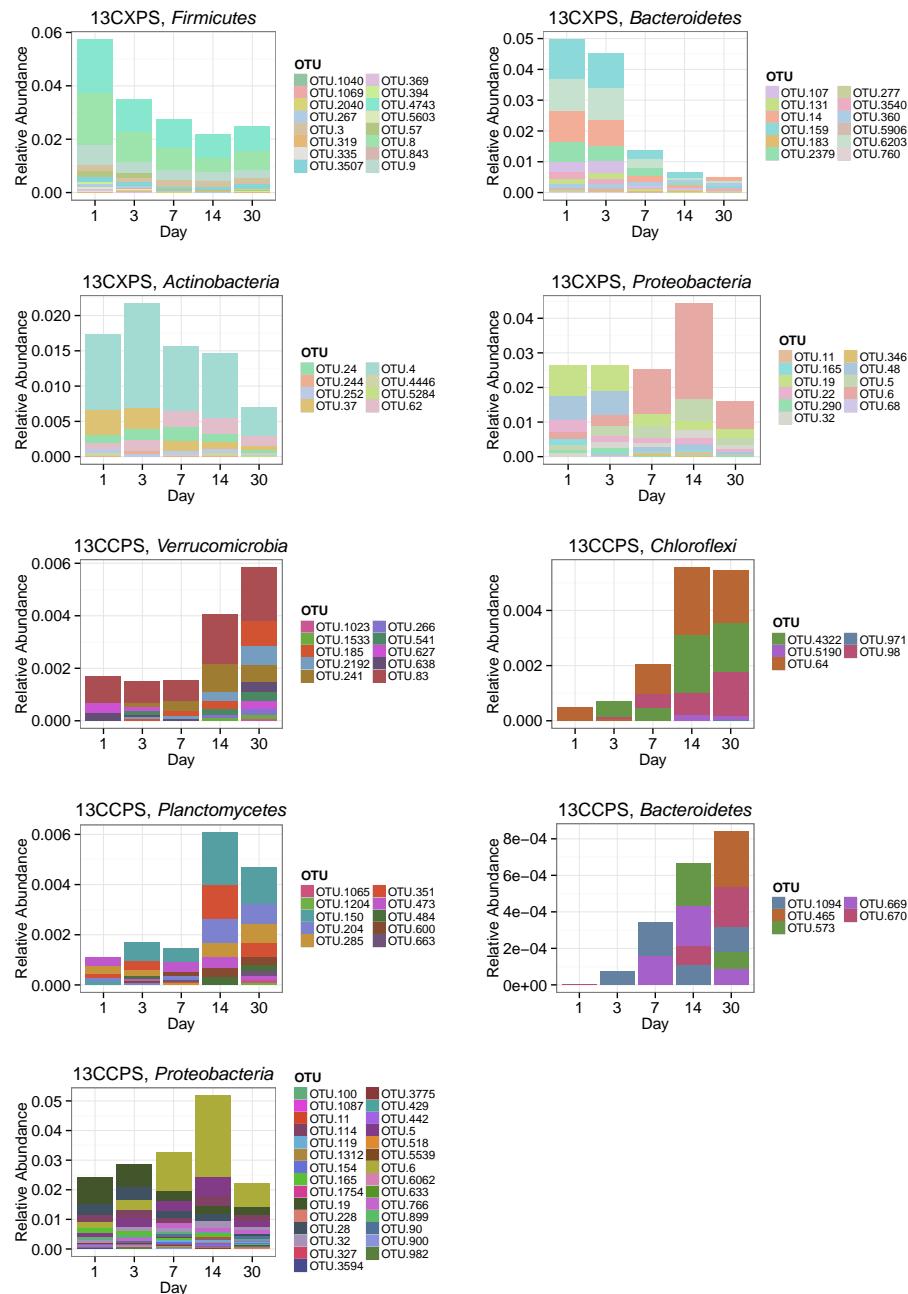


Fig. S5. Relative abundance versus day for classes that changed significantly in relative abundance with time.

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

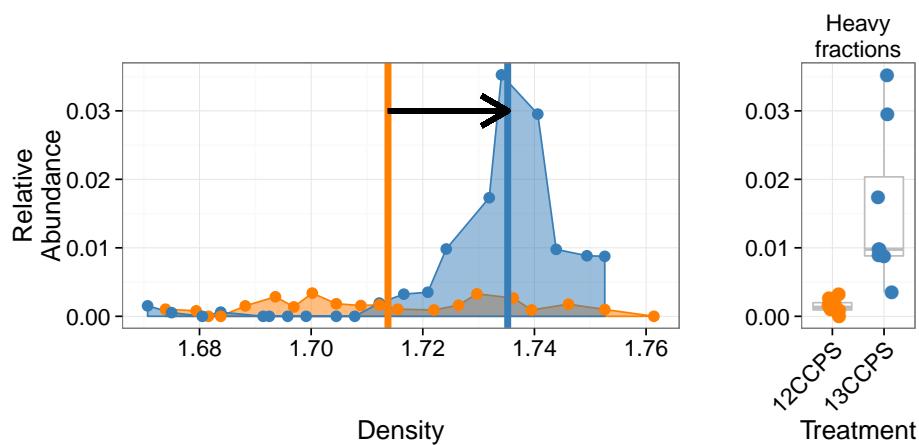


Fig. S7. Density profile for a single ^{13}C -cellulose “responder” OTU in the labeled gradient, blue, and the control gradient, orange. Vertical lines show center of mass for each density profile and arrow denotes the magnitude and direction of the BD shift upon labeling. Panel at right shows relative abundance values in the heavy fractions for each gradient.

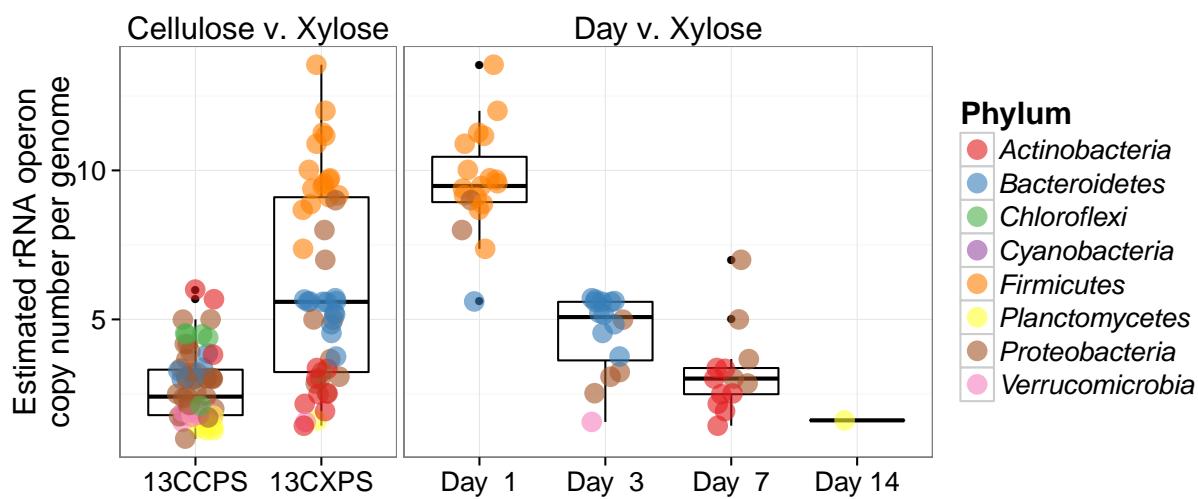


Fig. S8. Estimated rRNA operon copy number per genome for ^{13}C responding OTUs. Panel titles indicate which labeled substrate(s) are depicted.

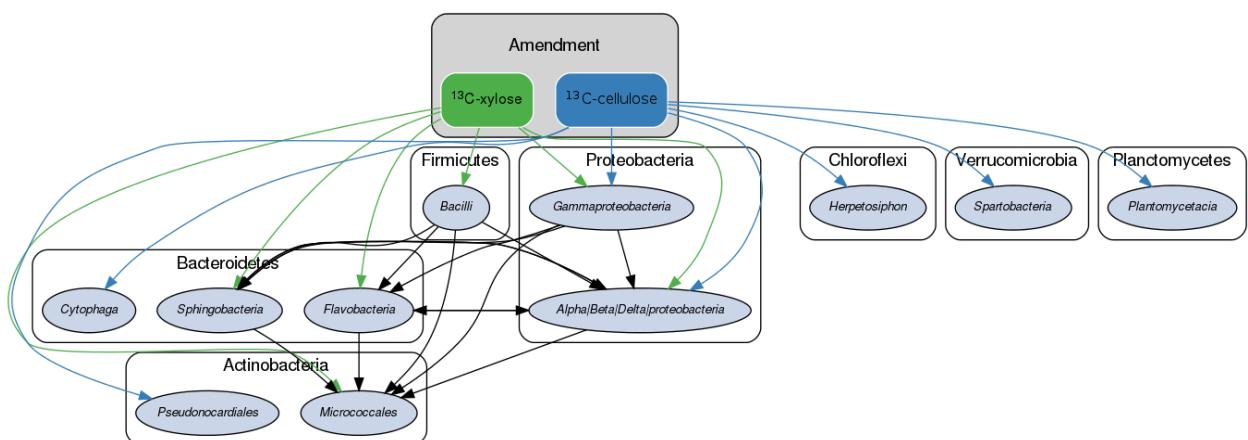


Fig. S9. Conceptual model of soil food web in this experiment. Taxa shown possessed at least two ¹³C responder OTUs for a given C substrate. *Proteobacteria* response was too varied taxonomically to depict at higher taxonomic resolution in this format. Black arrows indicate possible predator/prey interactions whereas colored arrows represent possible routes of primary degradation (green: xylose, blue: cellulose).

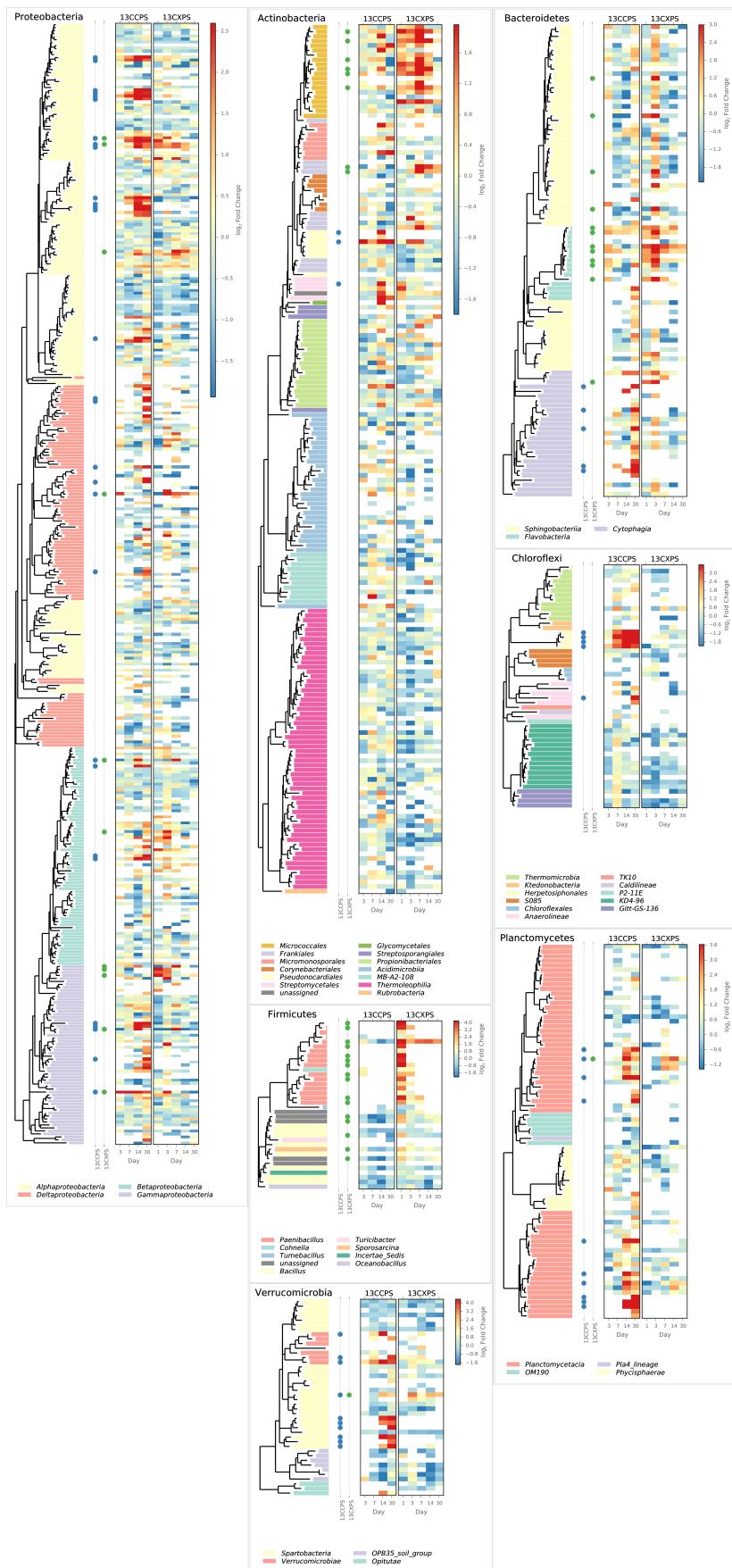


Fig. S10. Phylum specific trees. Heatmap indicates fold change between heavy fractions of control gradients versus labeled gradients. Dots indicate the position of "responders" to ^{13}C -xylose (green) or ^{13}C -cellulose (blue).

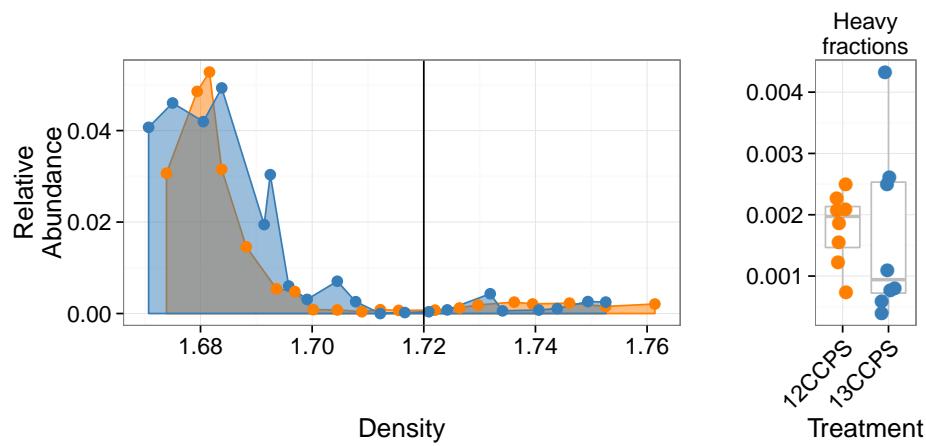


Fig. S11. Density profile for a single ^{13}C -cellulose "non-responder" OTU in the labeled gradient, blue, and the control gradient, orange. Vertical line shows where "heavy" fractions begin as defined in our analysis. Panel at right shows relative abundance values in the heavy fractions for each gradient.

Table S1: ¹³C-cellulose responders BLAST against Living Tree Project

OTU ID	Fold change ^a	Day ^b	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.100	2.66	14	<i>Pseudoxanthomonas sacheonensis</i> , <i>Pseudoxanthomonas dokdonensis</i>	100.0	Proteobacteria Gammaproteobacteria Xanthomonadales
OTU.1023	4.61	30	No hits of at least 90% identity	80.54	Verrucomicrobia Spartobacteria Chthoniobacterales
OTU.1065	5.31	14	No hits of at least 90% identity	84.55	Planctomycetes Planctomycetacia Planctomycetales
OTU.1087	4.32	14	<i>Devsia soli</i> , <i>Devsia crocina</i> , <i>Devsia riboflavina</i>	99.09	Proteobacteria Alphaproteobacteria Rhizobiales
OTU.1094	3.69	30	<i>Sporocytophaga myxococcoides</i>	99.55	Bacteroidetes Cytophagia Cytophagales
OTU.11	3.41	14	<i>Stenotrophomonas pavanii</i> , <i>Stenotrophomonas maltophilia</i> , <i>Pseudomonas geniculata</i>	99.54	Proteobacteria Gammaproteobacteria Xanthomonadales
OTU.114	2.78	14	<i>Herbaspirillum sp. SUEMI03</i> , <i>Herbaspirillum sp. SUEMI10</i> , <i>Oxalicibacterium solurbis</i> , <i>Herminiumonas fonticola</i> , <i>Oxalicibacterium horti</i>	100.0	Proteobacteria Betaproteobacteria Burkholderiales
OTU.119	3.31	14	<i>Brevundimonas alba</i>	100.0	Proteobacteria Alphaproteobacteria Caulobacterales
OTU.120	4.76	14	<i>Vampirovibrio chlorellavorus</i>	94.52	Cyanobacteria SM1D11 uncultured-bacterium
OTU.1204	4.32	30	<i>Planctomyces limnophilus</i>	91.78	Planctomycetes Planctomycetacia Planctomycetales
OTU.1312	4.07	30	<i>Paucimonas lemoignei</i>	99.54	Proteobacteria Betaproteobacteria Burkholderiales
OTU.132	2.81	14	<i>Streptomyces spp.</i>	100.0	Actinobacteria Streptomycetales Streptomycetaceae
OTU.150	4.06	14	No hits of at least 90% identity	86.76	Planctomycetes Planctomycetacia Planctomycetales
OTU.1533	3.43	30	No hits of at least 90% identity	82.27	Verrucomicrobia Spartobacteria Chthoniobacterales
OTU.154	3.24	14	<i>Pseudoxanthomonas mexicana</i> , <i>Pseudoxanthomonas japonensis</i>	100.0	Proteobacteria Gammaproteobacteria Xanthomonadales
OTU.165	3.1	14	<i>Rhizobium skieniewicense</i> , <i>Rhizobium vignae</i> , <i>Rhizobium larrymoorei</i> , <i>Rhizobium alkalisolii</i> , <i>Rhizobium galegae</i> , <i>Rhizobium huautlense</i>	100.0	Proteobacteria Alphaproteobacteria Rhizobiales
OTU.1754	4.48	14	<i>Asticcacaulis biprosthecium</i> , <i>Asticcacaulis benevestitus</i>	96.8	Proteobacteria Alphaproteobacteria Caulobacterales
OTU.185	4.37	14	No hits of at least 90% identity	85.14	Verrucomicrobia Spartobacteria Chthoniobacterales
OTU.19	2.44	14	<i>Rhizobium alamii</i> , <i>Rhizobium mesosinicum</i> , <i>Rhizobium mongolense</i> , <i>Arthrobacter viscosus</i> , <i>Rhizobium sullae</i> , <i>Rhizobium yanglingense</i> , <i>Rhizobium loessense</i>	99.54	Proteobacteria Alphaproteobacteria Rhizobiales
OTU.204	3.81	14	No hits of at least 90% identity	nan	Planctomycetes Planctomycetacia Planctomycetales

Table S1 – continued from previous page

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

Table S1 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.573	3.03	30	<i>Adhaeribacter aerophilus</i>	92.76	<i>Bacteroidetes Cytophagia Cytophagales</i>
OTU.6	3.62	7	<i>Cellvibrio fulvus</i>	100.0	<i>Proteobacteria Gammaproteobacteria Pseudomonadales</i>
OTU.600	3.48	30	No hits of at least 90% identity	80.37	<i>Planctomycetes Planctomycetacia Planctomycetales</i>
OTU.6062	4.83	30	<i>Dokdonella sp. DC-3, Luteibacter rhizovicinus</i>	97.26	<i>Proteobacteria Gammaproteobacteria Xanthomonadales</i>
OTU.627	4.43	14	<i>Verrucomicrobiaceae bacterium DC2a-G7</i>	100.0	<i>Verrucomicrobia Verrucomicrobiae Verrucomicrobiales</i>
OTU.633	3.84	30	No hits of at least 90% identity	89.5	<i>Proteobacteria Deltaproteobacteria Myxococcales</i>
OTU.638	4.0	30	<i>Luteolibacter sp. CCTCC AB 2010415, Luteolibacter algae</i>	93.61	<i>Verrucomicrobia Verrucomicrobiae Verrucomicrobiales</i>
OTU.64	4.31	14	No hits of at least 90% identity	89.5	<i>Chloroflexi Herpetosiphonales Herpetosiphonaceae</i>
OTU.663	3.63	30	<i>Pirellula staleyi DSM 6068</i>	90.87	<i>Planctomycetes Planctomycetacia Planctomycetales</i>
OTU.669	3.34	30	<i>Ohtaekwangia koreensis</i>	92.69	<i>Bacteroidetes Cytophagia Cytophagales</i>
OTU.670	2.87	30	<i>Adhaeribacter aerophilus</i>	91.78	<i>Bacteroidetes Cytophagia Cytophagales</i>
OTU.766	3.21	14	<i>Devosia insulae</i>	99.54	<i>Proteobacteria Alphaproteobacteria Rhizobiales</i>
OTU.83	5.61	14	<i>Luteolibacter sp. CCTCC AB 2010415</i>	97.72	<i>Verrucomicrobia Verrucomicrobiae Verrucomicrobiales</i>
OTU.862	5.87	14	<i>Allokutzneria albata</i>	100.0	<i>Actinobacteria Pseudonocardiales Pseudonocardiaceae</i>
OTU.899	2.28	30	<i>Enhygromyxa salina</i>	97.72	<i>Proteobacteria Deltaproteobacteria Myxococcales</i>
OTU.90	2.94	14	<i>Sphingopyxis panaciterrae, Sphingopyxis chilensis, Sphingopyxis sp. BZ30, Sphingomonas sp.</i>	100.0	<i>Proteobacteria Alphaproteobacteria Sphingomonadales</i>
OTU.900	4.87	14	<i>Brevundimonas vesicularis, Brevundimonas nasdae</i>	100.0	<i>Proteobacteria Alphaproteobacteria Caulobacterales</i>
OTU.971	3.68	30	No hits of at least 90% identity	78.57	<i>Chloroflexi Anaerolineae Anaerolineales</i>
OTU.98	3.68	14	No hits of at least 90% identity	88.18	<i>Chloroflexi Herpetosiphonales Herpetosiphonaceae</i>
OTU.982	4.47	14	<i>Devosia neptuniae</i>	100.0	<i>Proteobacteria Alphaproteobacteria Rhizobiales</i>

^a Maximum observed \log_2 of fold change.^b Day of maximum fold change.

Table S2: ^{13}C -xylose responders BLAST against Living Tree Project

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

Table S2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.290	3.59	1	<i>Pantoea spp.</i> , <i>Klugvera spp.</i> , <i>Klebsiella spp.</i> , <i>Erwinia spp.</i> , <i>Enterobacter spp.</i> , <i>Buttiauxella spp.</i>	100.0	Proteobacteria Gammaproteobacteria Enterobacterales
OTU.3	2.61	1	[<i>Brevibacterium</i>] <i>frigoritolerans</i> , <i>Bacillus sp.</i> LMG 20238, <i>Bacillus coahuilensis</i> m4-4, <i>Bacillus simplex</i>	100.0	Firmicutes Bacilli Bacillales
OTU.319	3.98	1	<i>Paenibacillus xinjiangensis</i>	97.25	Firmicutes Bacilli Bacillales
OTU.32	3.0	3	<i>Sandaracinus amyloyticus</i>	94.98	Proteobacteria Deltaproteobacteria Myxococcales
OTU.335	2.53	1	<i>Paenibacillus thailandensis</i>	98.17	Firmicutes Bacilli Bacillales
OTU.346	3.44	3	<i>Pseudoduganella violaceinigra</i>	99.54	Proteobacteria Betaproteobacteria Burkholderiales
OTU.3507	2.36	1	<i>Bacillus spp.</i>	98.63	Firmicutes Bacilli Bacillales
OTU.3540	2.52	3	<i>Flavobacterium terrigena</i>	99.54	Bacteroidetes Flavobacteria Flavobacterales
OTU.360	2.98	3	<i>Flavisolibacter ginsengisoli</i>	95.0	Bacteroidetes Sphingobacteriia Sphingobacterales
OTU.369	5.05	1	<i>Paenibacillus sp.</i> D75, <i>Paenibacillus glycansilyticus</i>	100.0	Firmicutes Bacilli Bacillales
OTU.37	2.68	7	<i>Phycicola gilvus</i> , <i>Microterricola viridarii</i> , <i>Frigeribacterium faeni</i> , <i>Frondihabitans sp.</i> RS-15, <i>Frondihabitans australicus</i>	100.0	Actinobacteria Micrococcales Microbacteriaceae
OTU.394	4.06	1	<i>Paenibacillus pocheonensis</i>	100.0	Firmicutes Bacilli Bacillales
OTU.4	2.84	7	<i>Agromyces ramosus</i>	100.0	Actinobacteria Micrococcales Microbacteriaceae
OTU.4446	3.49	7	<i>Catenuloplanes niger</i> , <i>Catenuloplanes castaneus</i> , <i>Catenuloplanes atrovinosus</i> , <i>Catenuloplanes crispus</i> , <i>Catenuloplanes nepalensis</i> , <i>Catenuloplanes japonicus</i>	97.72	Actinobacteria Frankiales Nakamurellaceae
OTU.4743	2.24	1	<i>Lysinibacillus fusiformis</i> , <i>Lysinibacillus sphaericus</i>	99.09	Firmicutes Bacilli Bacillales
OTU.48	2.99	1	<i>Aeromonas spp.</i>	100.0	Proteobacteria Gammaproteobacteria aaa34a10
OTU.5	3.69	7	<i>Delftia tsuruhatensis</i> , <i>Delftia lacustris</i>	100.0	Proteobacteria Betaproteobacteria Burkholderiales
OTU.5284	3.56	7	<i>Isopotericola nanjingensis</i> , <i>Isopotericola hypogaeus</i> , <i>Isopotericola variabilis</i>	98.63	Actinobacteria Micrococcales Promicromonosporaceae
OTU.5603	3.96	1	<i>Paenibacillus uliginis</i>	100.0	Firmicutes Bacilli Bacillales
OTU.57	4.39	1	<i>Paenibacillus castaneae</i>	98.62	Firmicutes Bacilli Bacillales
OTU.5906	3.16	3	<i>Terrimonas sp.</i> M-8	96.8	Bacteroidetes Sphingobacteriia Sphingobacterales
OTU.6	3.24	3	<i>Cellvibrio fulvus</i>	100.0	Proteobacteria Gammaproteobacteria Pseudomonadales
OTU.62	2.57	7	<i>Nakamurella flava</i>	100.0	Actinobacteria Frankiales Nakamurellaceae

Table S2 – continued from previous page

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

^a Maximum observed \log_2 of fold change.^b Day of maximum fold change.