

# Unearthing the soil carbon food web with DNA-SIP

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## Abstract

We describe a high-resolution approach for identifying microbial contributions to soil C-cycling dynamics using nucleic acid stable isotope probing coupled with next generation sequencing (HR-SIP). We amended series of soil microcosms with a complex mixture of model carbon (C) substrates and inorganic nutrients similar to plant biomass. A single C constituent in the C substrate mixture was substituted for its  $^{13}\text{C}$ -labeled equivalent in each microcosm series. Specifically, in separate microcosms we substituted  $^{13}\text{C}$ -xylose or  $^{13}\text{C}$ -cellulose for their unlabeled equivalents. Xylose and cellulose were chosen to represent labile soluble C and polymeric insoluble C, respectively. Microcosm DNA was interrogated for  $^{13}\text{C}$  incorporation at days 1, 3, 7, 14 and 30. Incorporation of  $^{13}\text{C}$  from xylose into microcosm DNA was observed at days 1, 3, and 7, while incorporation of  $^{13}\text{C}$  from cellulose was peaked at day 14 and was maintained through day 30. Of over 6,000 OTUs detected, a total of 49 and 63 unique OTUs assimilated  $^{13}\text{C}$  from xylose and cellulose into DNA, respectively. Xylose assimilating OTUs were more abundant in the microcosm community than cellulose assimilating OTUs, while cellulose OTUs demonstrated a greater substrate specificity than xylose OTUs. Xylose responders exhibited a unique time signature from days one to seven, manifested by a succession from *Firmicutes*, to *Bacteroidetes*, to *Actinobacteria*.  $^{13}\text{C}$ -cellulose incorporating OTUs included members of the *Verrucomicrobia* and *Chloroflexi*.

monolayer | structure | x-ray reflectivity | molecular electronics

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

## Introduction

Excluding plant biomass, there are 2,300 Pg of carbon (C) stored in soils worldwide which accounts for ~80% of the global terrestrial C pool [1, 2]. When organic C from plants reaches soil it is degraded by fungi, archaea, and bacteria. This C is returned to the atmosphere as  $\text{CO}_2$  or remains in the soil as humic substances that can persist up to

2000 years [3]. The majority of plant biomass C in soil is respired and produces 10 times more  $\text{CO}_2$  than anthropogenic emissions on an annual basis [4]. Global changes in atmospheric  $\text{CO}_2$ , temperature, and ecosystem nitrogen inputs are expected to impact primary production and C inputs to soils [5] but it remains difficult to predict the response of soil processes to anthropogenic change [6]. Current climate change models concur on atmospheric and oceanic but not terrestrial C predictions [7]. Contrasting terrestrial C model predictions reflect how little is known about soil C cycling. Inconsistencies in terrestrial modeling could be improved by elucidating the relationship between dissolved organic carbon and microbial communities in soils [8].

An estimated 80-90% of C cycling in soil is mediated by microorganisms [9, 10]. Understanding microbial processing of nutrients in soils presents a special challenge due to the heterogeneous nature of soil ecosystems and methods limitations. Soils are biologically, chemically, and physically complex which affects microbial community composition, diversity, and structure [10]. Confounding factors such as physical protection/aggregation, moisture content, pH, temperature, frequency and type of land disturbance, soil history, mineralogy, N quality and availability, and litter quality all affect the ability of the soil microbial community to access and metabolize C substrates [11, 12]. Further, rates of metabolism are often measured without knowing the identity of the microbial species involved [13] leaving the importance of community membership towards maintaining ecosystem functions unknown [13–15]. Litter bag experiments

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have shown that the community composition of soils can have quantitative and qualitative impacts on the breakdown of plant materials [16]. Reciprocal exchange of litter type and microbial inocula under controlled environmental conditions reveals that differences in community composition can account for 85% of the variation in litter carbon mineralization [17]. In addition, assembled communities of cellulose degraders reveal that the composition of the community has significant impacts on the rate of cellulose degradation [18].

An important step in understanding soil C cycling dynamics is identifying contributions from specific microbial lineages and investigating the relationship between genetic diversity and community structure with function [19]. The vast majority of microorganisms continue to resist cultivation in the laboratory, and even when cultivation is achieved, the traits expressed by a microorganism in culture may not be representative of those expressed when in its natural habitat. Stable-isotope probing (SIP) provides a unique opportunity to link microbial identity to activity and has been utilized to expand our knowledge of biogeochemical processes [20]. The most successful applications of this technique have identified organisms which mediate processes performed by a narrow set of functional guilds such as methanogens [21]. The technique has been less applicable to the study of soil C cycling because of limitations in resolving power as a result of simultaneous labeling of many different organisms in the community. Additionally, molecular applications such as tRFLP, DGGE and cloning that are frequently used in conjunction with SIP provide insufficient resolution of taxon identity and depth of coverage. We have developed an approach called **High Resolution-SIP** (HR-SIP) that employs a complex mixture of substrates added to soil at a low concentration relative to soil organic matter pools along with high throughput DNA sequencing. This greatly expands the ability of nucleic acid SIP to explore complex patterns of C-cycling in microbial communities with increased resolution.

A temporal cascade occurs in natural microbial communities during the plant biomass degradation in which labile C degradation precedes polymeric C degradation [22, 23]. The aim of this study is to track the temporal dynamics of C assimilation through discrete individuals of the soil microbial community to provide greater insight into soil C-cycling. Our experimental approach includes the addition of a soil organic matter (SOM) simulant (a complex mixture of model carbon sources and inorganic nutrients common to plant biomass), where a single C constituent is substituted for its  $^{13}\text{C}$ -labeled equivalent, to soil. Parallel incubations of soils amended with this complex C mixture

allows us to test how different C substrates cascade through discrete taxa within the soil microbial community. In this study we use  $^{13}\text{C}$ -xylose and  $^{13}\text{C}$ -cellulose as a proxy for labile and polymeric C, respectively. We couple nucleic acid stable isotope probing with high throughput DNA sequencing to identify soil microbial community members responsible for specific C transformations. Amplicon sequencing of 16S rRNA gene fragments from many gradient fractions and multiple gradients make it possible to track C assimilation by hundreds of taxa.

## Results

We observed C use dynamics by the soil microbial community by conducting a nucleic acid SIP experiment wherein xylose or cellulose carried the isotopic label. We set up three soil microcosm series. Each microcosm was amended with a C substrate mixture that included cellulose and xylose. The C substrate mixture approximated the chemical composition of freshly degrading plant biomass. The same substrate mixture was added to microcosms in each series, however, for each series except the control, one substrate was substituted for its  $^{13}\text{C}$  counterpart;  $^{13}\text{C}$ -cellulose in one series,  $^{13}\text{C}$ -xylose in another, and no  $^{13}\text{C}$ -labeled substrates in the control. Microcosm amendments are shorthand identified in the following figures by the following code: "13CXPS" refers to the amendment with  $^{13}\text{C}$ -xylose (that is  $^{13}\text{C}$  Xylose Plant Simulant), "13CCPS" refers to the  $^{13}\text{C}$ -cellulose amendment and "12CCPS" refers to the amendment that only contained  $^{12}\text{C}$  substrates (i.e. control). Xylose or cellulose were chosen to carry the isotopic label to contrast C assimilation for labile, soluble C (xylose) versus insoluble, polymeric C (cellulose). 5.3 mg of C substrate mixture per gram soil was added to each microcosm representing 18% of the total soil C. The mixture included 0.42 mg xylose-C and 0.88 mg cellulose-C g soil $^{-1}$ . Microcosms were harvested for DNA extraction at days 1, 3, 7, 14 and 30 during a 30 day incubation.  $^{13}\text{C}$ -xylose assimilation peaked immediately and tapered over the 30 day incubation whereas  $^{13}\text{C}$ -cellulose assimilation peaked two weeks after amendment additions (Figure 1).

Microcosm DNA was density fractionated on CsCl density gradients. We assayed SSU rRNA gene content of CsCl gradient fractions using high-throughput DNA sequencing technology. We sequenced SSU rRNA gene amplicons from a total of 277 CsCl gradient fractions from 14 CsCl gradients and 12 bulk microcosm DNA samples. The SSU rRNA gene data set contained 1,102,685 total sequences. The average number of sequences per sample was 3,816 (sd 3,629) and 265 samples had

over 1,000 sequences. We sequenced SSU rRNA gene amplicons from an average of 19.8 fractions per CsCl gradient ( $sd$  0.57). The average density between fractions was  $0.0040\text{ g mL}^{-1}$ . The sequencing effort recovered a total of 5,940 OTUs. 2,943 of the total 5,940 OTUs were observed in bulk samples. We observed 33 unique phylum and 340 unique genus annotations.

**Soil microcosm microbial community changes with time.** Bulk soil DNA sequencing revealed changes in the soil microcosm microbial community structure and membership correlated significantly with incubation time (Figure S4B, p-value 0.23,  $R^2$  0.63, Adonis test (**author?**) [24]). The identity of the  $^{13}\text{C}$ -labeled substrate added to the microcosms did not significantly correlate with community structure and membership (p-value 0.35). Additionally, microcosm beta diversity was significantly less than gradient fraction beta diversity (p-value 0.003, “betadisper” function R Vegan package (**author?**) [25, 26]). Twenty-nine OTUs significantly changed in relative abundance with time (“BH” adjusted p-value  $<0.10$ , (**author?**) [27]) (Figure S1). OTUs that significantly increased in relative abundance with time included OTUs in the *Verrucomicrobia*, *Proteobacteria*, *Planctomycetes*, *Cyanobacteria*, *Chloroflexi* and *Acidobacteria*. OTUs that significantly decreased in relative abundance with time included OTUs in the *Proteobacteria*, *Firmicutes*, *Bacteroidetes* and *Actinobacteria* (Figure S1). *Proteobacteria* was the only phylum that had OTUs which increased significantly and OTUs that decreased significantly in abundance with time. If sequences were grouped by taxonomic annotations at the class level, only four classes significantly changed in abundance, *Bacilli* (decreased), *Flavobacteria* (decreased), *Gammaproteobacteria* (decreased) and *Herpetosiphonales* (increased) (Figure S2). Of the 29 OTUs that changed significantly in relative abundance with time, 14 are labeled substrate responders (Figure S1).

**OTUs that assimilated  $^{13}\text{C}$  from xylose.** Within the first 7 days of incubation approximately 63% of  $^{13}\text{C}$ -xylose was respired and only an additional 6% more was respired from day 7 to 30, as determined from isotopic analysis of the  $^{13}\text{C}$  remaining in the soil at each time point. At the end of the 30 day incubation 30% of the  $^{13}\text{C}$  from added xylose remained in the soils. The  $^{13}\text{C}$  remaining in the soil from  $^{13}\text{C}$ -xylose addition was likely stabilized by assimilation into microbial biomass and/or microbial conversion into other forms of organic matter. It is also possible that some  $^{13}\text{C}$ -xylose remains unavailable to microbes due to abiotic interactions

in soil [12]. An average 16% of the  $^{13}\text{C}$ -cellulose added was respired within the first 7 days, 38% by day 14, and 60% by day 30.

Isotope incorporation by an OTU is revealed by enrichment of the OTU in heavy CsCl gradient fractions containing  $^{13}\text{C}$  labeled DNA relative to heavy fractions from control gradients containing no  $^{13}\text{C}$  labeled DNA. We refer to OTUs that putatively incorporated  $^{13}\text{C}$  into DNA originally from an isotopically labeled substrate as substrate “responders”. At day 1, 84% of  $^{13}\text{C}$ -xylose responsive OTUs belonged to *Firmicutes*, 11% to *Proteobacteria* and 5% to *Bacteroidetes*. *Firmicutes* responders decreased from 16 OTUs at day 1 to one OTU at day 3 while *Bacteroidetes* responders increased from one OTU at day 1 to 12 OTUs at day 3. The remaining day 3 responders are members of the *Proteobacteria* (26%) and the *Verrucomicrobia* (5%). Day 7 responders were 53% *Actinobacteria*, 40% *Proteobacteria*, and 7% *Firmicutes*. The identities of  $^{13}\text{C}$ -xylose responders change with time. The numerically dominant  $^{13}\text{C}$ -xylose responder phylum shifts from *Firmicutes* to *Bacteroidetes* and then to *Actinobacteria* across days 1, 3 and 7 (Figure 2, Figure 3).

All of the  $^{13}\text{C}$ -xylose responders in the *Firmicutes* phylum are closely related (at least 99% sequence identity) to cultured isolates from genera that are known to form endospores (Table S2). Each  $^{13}\text{C}$ -xylose responder is closely related to isolates annotated as members of *Bacillus*, *Paenibacillus* or *Lysinibacillus*. *Bacteroidetes*  $^{13}\text{C}$ -xylose responders are predominantly closely related to *Flavobacterium* species (5 of 8 total responders) (Table S2). Only one *Bacteroidetes*  $^{13}\text{C}$ -xylose responder is not closely related to a cultured isolate, “OTU.183” (closest LTP BLAST hit, *Chitinophaga sp.*, 89.5% sequence identity, Table S2). OTU.183 shares high sequence identity with environmental clones derived from rhizosphere samples (accession AM158371, unpublished) and the skin microbiome (accession JF219881, (**author?**) [28]). Other *Bacteroidetes* responders share high sequence identities with canonical soil genera including *Dyadobacter*, *Solibius* and *Terrimonas*. Six of the 8 *Actinobacteria*  $^{13}\text{C}$ -xylose responders are in the *Micrococccales* order. One  $^{13}\text{C}$ -xylose responding *Actinobacteria* OTU shares 100% sequence identity with *Agromyces ramosus* (Table S2). *A. ramosus* is a known predatory bacterium but is not dependent on a host for growth in culture [29]. It is not possible to determine the specific origin of assimilated  $^{13}\text{C}$  in a DNA-SIP experiment.  $^{13}\text{C}$  can be passed down through trophic levels although heavy isotope representation in C pools targeted by cross-feeders and predators would be diluted with depth into the trophic cascade. It is possible, however,

that the  $^{13}\text{C}$  labeled *Agromyces* OTU was assimilating  $^{13}\text{C}$  primarily by predation if the *Agromyces* OTU was selective enough with respect to its prey that it primarily attacked  $^{13}\text{C}$ -xylose assimilating organisms.

**Cellulose OTUs.** Only 2 and 5 OTUs had incorporated  $^{13}\text{C}$  from  $^{13}\text{C}$ -cellulose at days 3 and 7, respectively. At days 14 and 30 42 and 39 OTUs incorporated  $^{13}\text{C}$  from  $^{13}\text{C}$ -cellulose into biomass. A *Cellvibrio* and *Sandaracinaceae* OTU assimilated  $^{13}\text{C}$  from  $^{13}\text{C}$ -cellulose at day 3. Day 7  $^{13}\text{C}$ -cellulose responders included the same *Cellvibrio* responder as day 3, a *Verrucomicrobia* OTU and three *Chloroflexi* OTUs. 50% of Day 14 responders belong to *Proteobacteria* (66% Alpha-, 19% Gamma-, and 14% Beta-) followed by 17% *Planctomycetes*, 14% *Verrucomicrobia*, 10% *Chloroflexi*, 7% *Actinobacteria* and 2% cyanobacteria. *Bacteroidetes* OTUs began to incorporate  $^{13}\text{C}$  from cellulose at day 30 (13% of day 30 responders). Other day 30 responding phyla included *Proteobacteria* (30% of day 30 responders; 42% Alpha-, 42% Delta, 8% Gamma-, and 8% Beta-), *Planctomycetes* (20%), *Verrucomicrobia* (20%), *Chloroflexi* (13%) and cyanobacteria (3%). *Proteobacteria*, *Verrucomicrobia*, and *Chloroflexi* had relatively high numbers of responders with strong response across multiple time points (Figure 2).

*Proteobacteria* represent 46% of all  $^{13}\text{C}$ -cellulose responding OTUs identified. *Cellvibrio* accounted for 3% of all proteobacterial  $^{13}\text{C}$ -cellulose responding OTUs detected. *Cellvibrio* was one of the first identified cellulose degrading bacteria and was originally described by Winogradsky in 1929 who named it for its cellulose degrading abilities [30]. All  $^{13}\text{C}$ -cellulose responding *Proteobacteria* share high sequence identity with 16S rRNA genes from sequenced cultured isolates (Table S1) except for “OTU.442” (best cultured isolate match 92% sequence identity in the *Chrondomyces* genus, Table S1) and “OTU.663” (best cultured isolate match outside *Proteobacteria* entirely, *Clostridium* genus, 89% sequence identity, Table S1). Some *Proteobacteria* responders share high sequence identity with isolates in genera known to possess cellulose degraders including *Rhizobium*, *Devosia*, *Stenotrophomonas* and *Cellvibrio*. One *Proteobacteria* OTU shares high sequence identity (100%) with a *Brevundimonas* cultured isolate. *Brevundimonas* has not previously been identified as a cellulose degrader, but has been shown to degrade cellobionic acid, an oxidized form of cellulose [31].

*Verrucomicrobia*, a cosmopolitan soil phylum often found in high abundance [32], are hypothesized to degrade polysaccharides in many environments [32–34]. *Verrucomicrobia* comprise 16% of the to-

tal  $^{13}\text{C}$ -cellulose responder OTUs detected. 40% of *Verrucomicrobia*  $^{13}\text{C}$ -cellulose responders belong to the uncultured “FukuN18” family originally identified in freshwater lakes [35]. The strongest *Verrucomicrobial* responder OTU to  $^{13}\text{C}$ -cellulose shared high sequence identity (97%) with an isolate from Norway tundra soil [36] although growth on cellulose was not assessed for this isolate. Only one other  $^{13}\text{C}$ -cellulose responding *verrucomicrobium* shared high DNA sequence identity with an isolate, “OTU.638” (Table S1) with *Roseimicrobium gellanilyticum* (100% sequence identity) which has been shown to grow on soluble cellulose [37]. The remaining  $^{13}\text{C}$ -cellulose *Verrucomicrobia* responders did not share high sequence identity with any isolates (maximum sequence identity with any isolate 93%).

*Chloroflexi* are known for metabolically dynamic lifestyles ranging from anoxygenic phototrophy to organohalide respiration [38]. Recent studies have focused on *Chloroflexi* roles in C cycling [38–40] and several *Chloroflexi* utilize cellulose [38–40]. Four closely related OTUs in an undescribed *Chloroflexi* lineage (closest matching isolate for all four OTUs: *Herpetosiphon geysericola*, 89% sequence identity, Table S1) responded to  $^{13}\text{C}$ -cellulose (Figure S3). One additional OTU also from a poorly characterized *Chloroflexi* lineage (closest cultured isolate matched a proteobacterium at 78% sequence identity) responded to  $^{13}\text{C}$ -cellulose (Figure S3).

Other notable  $^{13}\text{C}$ -cellulose responders include a *Bacteroidetes* OTU that shares high sequence identity (99%) to *Sporocytophaga myxococcoïdes* a known cellulose degrader [41], and three *Actinobacteria* OTUs that share high sequence identity (100%) with isolates. One of the three *Actinobacteria*  $^{13}\text{C}$ -cellulose responders is in the *Streptomyces*, a genus known to possess cellulose degraders, while the other two share high sequence identity to cultured isolates *Allokutznizeriz albata* [42, 43] and *Lentzea waywayandensis* [44, 45]; neither isolate decomposes cellulose in culture. Nine *Planctomycetes* OTUs responded to  $^{13}\text{C}$ -cellulose but none are within described genera (closest cultured isolate match 91% sequence identity, Table S1) (Figure S3). One  $^{13}\text{C}$ -cellulose responder is annotated as “cyanobacteria”. The cyanobacteria phylum annotation is misleading as the OTU is not closely related to any oxygenic phototrophs (closest cultured isolate match *Vampirovibrio chlorellavorus*, 95% sequence identity, Table S1). A sister clade to the oxygenic phototrophs classically annotated as “cyanobacteria” in SSU rRNA gene reference databases, but does not possess any known phototrophs, has recently been proposed to constitute its own phylum, “Melainabacteria” (author?) [46]; although, the phylogenetic position

of “Melainabacteria” is debated [47]. The catalog of metabolic capabilities associated with cyanobacteria (or candidate phyla previously annotated as cyanobacteria) are quickly expanding [46, 47]. Our findings provide evidence for cellulose degradation within a lineage closely related to but apart from oxygenic phototrophs. Notably, polysaccharide degradation is suggested by an analysis of a “Melainabacteria” genome [46]. Although we highlight  $^{13}\text{C}$ -cellulose responders that share high sequence identity with described genera, most  $^{13}\text{C}$ -cellulose responders uncovered in this experiment are not closely related to cultured isolates (Table S1).

#### **Xylose responders are more abundant members of the soil community than cellulose responders.**

$^{13}\text{C}$ -xylose responders are generally more abundant members based on relative abundance in bulk DNA SSU rRNA gene content than  $^{13}\text{C}$ -cellulose responders (Figure 4, p-value 0.00028). However, both abundant and rare OTUs responded to  $^{13}\text{C}$ -xylose and  $^{13}\text{C}$ -cellulose (Figure 4). For instance, a *Delftia*  $^{13}\text{C}$ -cellulose responder is fairly abundant in the bulk samples (“OTU.5”, Table S1). OTU.5 was on average the 13th most abundant OTU in bulk samples. A  $^{13}\text{C}$ -xylose responder (“OTU.1040”, Table S2) has a mean relative abundance in bulk samples of  $3.57 \times 10^{-5}$ . Two  $^{13}\text{C}$ -cellulose responders were not found in any bulk samples (“OTU.862” and “OTU.1312”, Table S1). Of the 10 most abundant responders, 8 are  $^{13}\text{C}$ -xylose responders and 6 of these 8 are consistently among the 10 most abundant OTUs in bulk samples.

Responder abundances summed at phylum level generally increased for  $^{13}\text{C}$ -cellulose (Figure S5) whereas  $^{13}\text{C}$ -xylose responder abundances summed at the phylum level decreased over time for *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria* although *Proteobacteria* spiked at day 14 (Figure S5). Bulk abundance trends are roughly consistent with  $^{13}\text{C}$  assimilation.

**Cellulose degraders exhibit higher substrate specificity than xylose utilizers.** Cellulose responders exhibited a greater shift in buoyant density (BD) than xylose responders in response to isotope incorporation (Figure 4, p-value  $1.8610 \times 10^{-6}$ ).  $^{13}\text{C}$ -cellulose responders shifted on average 0.0163 g/mL (sd 0.0094) whereas xylose responders shifted on average 0.0097 (sd 0.0094). For reference, 100%  $^{13}\text{C}$  DNA BD is 0.04 g/mL greater than the BD of its  $^{12}\text{C}$  counterpart. DNA BD increases as its ratio of  $^{13}\text{C}$  to  $^{12}\text{C}$  increases. An organism that only assimilates C into DNA from a  $^{13}\text{C}$  isotopically labeled source, will have a greater  $^{13}\text{C}:\text{ }^{12}\text{C}$  ratio in its DNA than an organism utilizing

a mixture of isotopically labeled and unlabeled C sources. Upon labeling, DNA from an organism that incorporates exclusively  $^{13}\text{C}$  will increase in BD more than DNA from an organism that does not exclusively utilize isotopically labeled C. Therefore the magnitude DNA BD shifts indicate substrate specificity given our experimental design as only one substrate was labeled in each amendment. We measured density shift as the change in an OTU’s density profile center of mass between corresponding control and labeled gradients. BD shifts, however, should not be evaluated on an individual OTU basis as a small number of density shifts are observed for each OTU and the variance of the density shift metric at the level of individual OTUs is unknown. It is therefore more informative to compare density shifts among substrate responder groups. Further, density shifts are based on relative abundance profiles and would be distorted in comparison to density shifts based on absolute abundance profiles and should be interpreted with this transformation in mind. It should also be noted that there was overlap in observed density shifts between  $^{13}\text{C}$ -cellulose and  $^{13}\text{C}$ -xylose responder groups, suggesting that although cellulose degraders are generally more substrate specific than xylose utilizers, each responder group exhibits a range of substrate specificities (Figure 4).

#### **Estimated rrn gene copy number in substrate responder groups.**

$^{13}\text{C}$ -xylose responder estimated *rrn* gene copy number is inversely related time of first response (p-value  $2.02 \times 10^{-15}$ , Figure 5). OTUs that first respond at later time points have fewer estimated *rrn* copy number than OTUs that first respond earlier (Figure 5). *rrn* copy number estimation is a recent advance in microbiome science [48] although the relationship of *rrn* copy number per genome with ecological strategy is well established [49]. Microorganisms with a high *rrn* copy number tend to be fast growers specialized to take advantage of boom-bust environments whereas microorganisms with low *rrn* copy number favor slower growth under lower and more consistent nutrient input [49]. At the beginning of our incubation, OTUs with estimated high *rrn* copy number or “fast-growers” assimilate xylose into biomass and with time slower growers (lower *rrn* copy number) begin to incorporate  $^{13}\text{C}$  from xylose. Further,  $^{13}\text{C}$ -xylose responders have more estimated rRNA operon copy numbers per genome than  $^{13}\text{C}$ -cellulose responders (p-value  $1.878 \times 10^{-9}$ ) suggesting xylose respiring microbes are generally faster growers than cellulose degraders.

## Discussion

Pure culture based studies drove early soil microbial ecology research. Important pure cultures from soil historically included nine genera *Agrobacterium*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Flavobacterium*, *Micromonospora*, *Nocardia*, *Pseudomonas*, and *Streptomyces* ([50] and reviewed by (author?) [51]) but culture-independent surveys of soil microbial diversity revealed soil can harbor 5,000 OTUs per half gram of soil [52]. We recovered almost 6,000 OTUs in this study. Although culturing techniques can produce isolates from diverse soil lineages [53], numerically dominant soil microorganisms are still uncultured and we know little of their ecophysiology [51]. In contrast DNA-SIP can characterize functional roles for thousands of phylotypes in a single experiment. We found 104 OTUs in an agricultural soil that can incorporate C from xylose and/or cellulose into biomass. We also used DNA-SIP to assay substrate specificity and temporal dynamics of C-cycling or soluble and polymeric C degraders. Included in the  $^{13}\text{C}$ -xylose and  $^{13}\text{C}$ -cellulose responsive OTUs were members of numerically dominant yet functionally uncharacterized soil phylogenetic groups such as *Verrucomicrobia*, *Planctomycetes* and *Chloroflexi*.

**Microbial response to isotopic labels.** We propose that C added to soil microcosms in this experiment took the following path through the microbial food web (Figure S9): First, labile C such as xylose was assimilated by fast-growing opportunistic *Firmicutes* spore formers. The remaining labile C and new biomass C was assimilated in succession by slower growing *Bacteroidetes*, *Actinobacteria* and *Proteobacteria* phylotypes that were either tuned to lower C substrate concentrations, were predatory bacteria (e.g. *Agromyces*), and/or were specialized for consuming viral lysate. C from polymeric substrates entered the bacterial community after 14 days. Canonical cellulose degrading bacteria such as *Cellvibrio* degraded cellulose but uncharacterized lineages in the *Chloroflexi*, *Planctomycetes* and *Verrucomicrobia*, specifically the *Spartobacteria*, were also significant contributors to cellulose decomposition.

### Ecological strategies of soil microorganisms participating in the decomposition of organic matter.

We assessed the ecology of  $^{13}\text{C}$ -responsive OTUs by estimating the *rrn* gene copy number and the BD shift upon labeling for each OTU. *rrn* gene copy number correlates positively with growth rate [54] and BD shift is indicative of substrate specificity (see results). We also observed how  $^{13}\text{C}$ -substrate responsive OTUs changed in relative abundance with time in the microcosms and the abundance rank of  $^{13}\text{C}$ -substrate responsive OTUs

in the bulk DNA. Ecological metrics show  $^{13}\text{C}$ -cellulose responsive OTUs grow slower (Figure 5), have greater substrate specificity (Figure 4), and are generally lower abundance than  $^{13}\text{C}$ -xylose responsive OTUs (Figure 4). The higher abundance of xylose responders may also be in part due to of their high *rrn* gene copy number resulting in inflated relative abundance per genome. There are only faint ecological differences within the  $^{13}\text{C}$ -cellulose responsive OTUs but the combination of *rrn* gene copy number, BD shift, abundance rank and relative abundance change over time is consistent with phylum membership (Figure RADVIZ).  $^{13}\text{C}$ -xylose responsive OTU *rrn* gene copy number correlated inversely with the time at which the OTU was first found to incorporate  $^{13}\text{C}$  into DNA (Figure 5) suggesting that fast-growing microbes assimilated  $^{13}\text{C}$  from xylose before slow growers.

Ecological metrics suggest cellulose degraders are substrate specialists that grow slow and are in low bulk abundance. Labile C responder ecological strategies were more varied perhaps because some  $^{13}\text{C}$  labeled microorganisms did not primarily assimilate xylose but became labeled via predatory interactions and/or are saprophytes.  $^{13}\text{C}$ -xylose responsive OTUs are generalists, grow faster and are more abundant when compared to  $^{13}\text{C}$ -cellulose responders.  $^{13}\text{C}$ -xylose responders vary in growth rate and while generally higher abundance than  $^{13}\text{C}$ -cellulose responders can also be low abundance microorganisms. It's not clear whether the observed activity succession from *Firmicutes* to *Bacteroidetes* and finally *Actinobacteria* in response to  $^{13}\text{C}$ -xylose addition marks a trophic cascade or functional groups tuned to different resource concentrations or both. Notably, each temporally defined response group clustered phylogenetically suggesting a uniform ecological strategy (Figure S3). It's also clear that some of the non-*Firmicutes*  $^{13}\text{C}$ -xylose responders are closely related to known predators (*Agromyces*) and many marine predatory bacteria are members of the *Bacteroidetes* ([55]). If the temporal dynamics of  $^{13}\text{C}$ -xylose incorporation are due to trophic interactions, our results suggest that there are many predatory soil bacteria that consume fast-growing, opportunistic, primary labile C assimilating, gram-positive spore-formers. Hence, trophic interactions among soil bacteria may be of importance in soil C turnover models.

**Conclusion.** Microorganisms sequester atmospheric C and respire soil organic matter (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 uncharacterized yet ubiquitous and often abundant soil lineages participated in cel-

lulose decomposition. Cellulose C degraders included members of the *Verrucomicrobia* (*Spartobacteria*), *Chloroflexi*, *Bacteroidetes* and *Planctomycetes*. *Spartobacteria* in particular are abundant microorganisms in many soil biomes and often the most abundant *Verrucomicrobia* order in soil. Our results also suggest that members of the *Bacteroidetes* and *Actinobacteria* act in the cascade of labile, soluble C through soil trophic levels possibly as predators. Both points illustrate the complexity of soil C dynamics.

## 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<sup>-1</sup> carbon mixture; representative of natural concentrations [56]. 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)<sup>13</sup>C-cellulose, (3)<sup>13</sup>C-xylose (98 atom% <sup>13</sup>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 <sup>13</sup>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 destructively (stored at -80°C until nucleic acid processing) at days 1 (control and xylose only), 3, 7, 14, and 30.

Nucleic acids were extracted using a modified Griffiths protocol [57]. To prepare nucleic acid extracts for isopycnic centrifugation as previously described [58], DNA was size selected (>4kb) using 1% low melt agarose gel and  $\beta$ -agarase I enzyme extraction per manufacturers protocol (New England Biolab, M0392S). For each time point in the series isopycnic gradients were setup using a modified protocol [59] for a total of five <sup>12</sup>C-control, five <sup>13</sup>C-xylose, and four <sup>13</sup>C-cellulose microcosms. A density gradient (average density 1.69 g mL<sup>-1</sup>) solution of 1.762 g cesium chloride (CsCl) mL<sup>-1</sup> in gradient buffer solution (pH 8.0 15 mM Tris-HCl, 15 mM EDTA, 15 mM KCl) was used to separate <sup>13</sup>C-enriched and <sup>12</sup>C-nonenriched DNA. Each

gradient was loaded with approximately 5  $\mu$ g of DNA and ultracentrifuged for 66 h at 55,000 rpm and room temperature (RT). Fractions of ~100  $\mu$ L 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  $\mu$ L s<sup>-1</sup> [60] into Acroprep<sup>TM</sup> 96 filter plate (Pall Life Sciences 5035). The refractive index of each fraction was measured using a Reichart AR200 digital refractometer modified as previously described [58] to measure a volume of 5  $\mu$ L. Then buoyant density was calculated from the refractive index as previously described [58] (see also SI). The collected DNA fractions were purified by repetitive washing of Acroprep filter wells with TE. Finally, 50  $\mu$ L TE was added to each fraction then resuspended DNA was pipetted off the filter into a new microfuge tube.

For every gradient, 20 fractions were chosen for sequencing between the density range 1.67-1.75 g mL<sup>-1</sup>. Barcoded 454 primers were designed using 454-specific adapter B, 10 bp barcodes [61], a 2 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 forward primer (BA515F). Each fraction was PCR amplified using 0.25  $\mu$ L 5 U  $\mu$ l<sup>-1</sup> AmpliTaq Gold (Life Technologies, Grand Island, NY; N8080243), 2.5  $\mu$ L 10X Buffer II (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 2.5  $\mu$ L 25 mM MgCl<sub>2</sub>, 4  $\mu$ L 5 mM dNTP, 1.25  $\mu$ L 10 mg mL<sup>-1</sup> BSA, 0.5  $\mu$ L 10  $\mu$ M BA515F, 1  $\mu$ L 5  $\mu$ M BA806R, 3  $\mu$ L H<sub>2</sub>O, 10  $\mu$ L 1:30 DNA template) in triplicate. Samples were normalized either using Pico green quantification and manual calculation or by SequalPrep<sup>TM</sup> normalization plates (Invitrogen, Carlsbad, CA; A10510), then pooled in equimolar concentrations. Pooled DNA was gel extracted from a 1% agarose gel using Wizard SV gel and PCR clean-up system (Promega, Madison, WI; A9281) per manufacturer's protocol. Amplicons were sequenced on Roche 454 FLX system using titanium chemistry at Selah Genomics (formerly EnGenCore, Columbia, SC)

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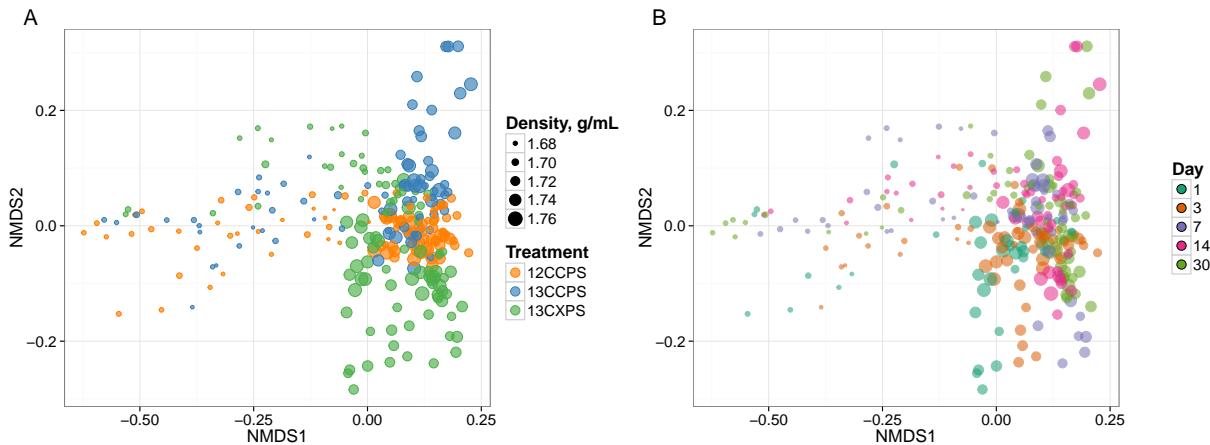
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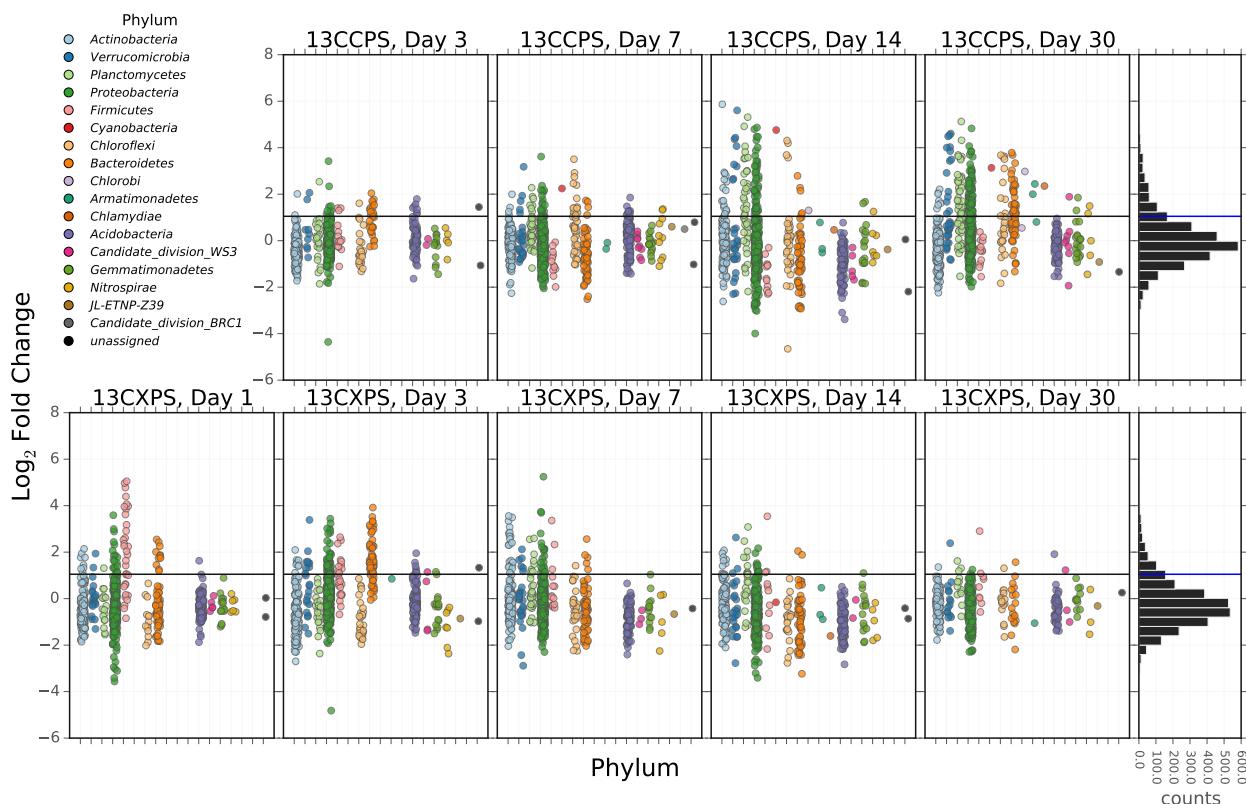
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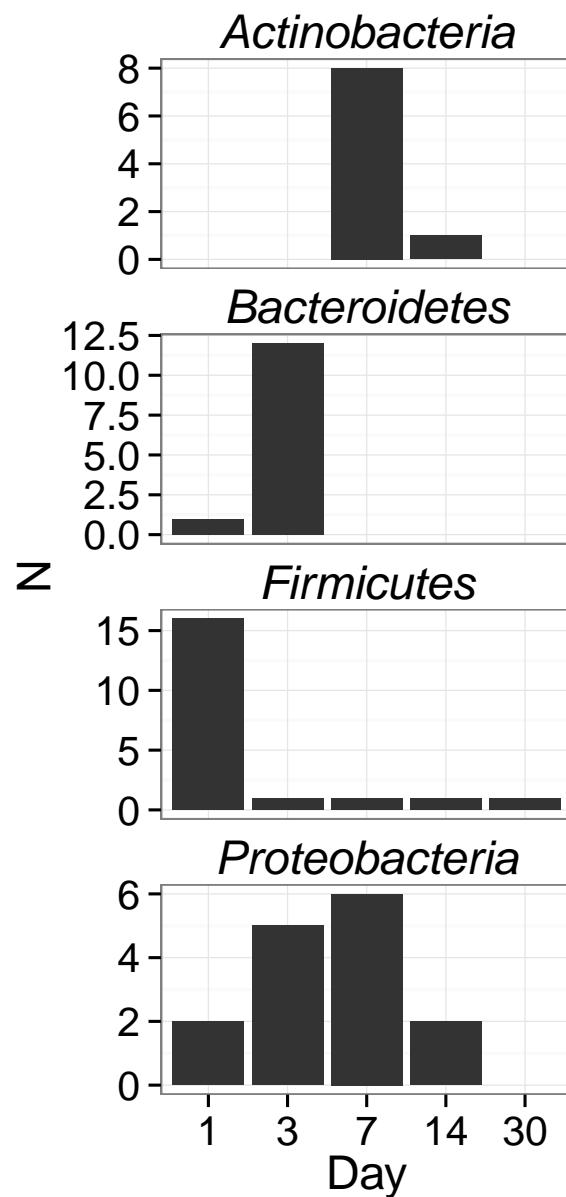
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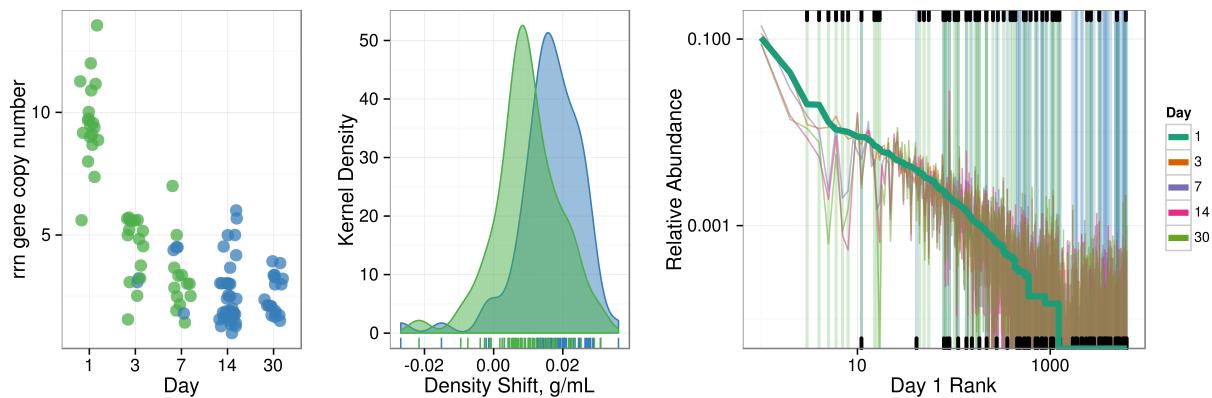
**Fig. 1.** NMDS analysis from weighted unifrac distances of 454 sequence data from SIP fractions of each treatment over time. Twenty fractions from a CsCl gradient fractionation for each treatment at each time point were sequenced (Fig. S1). Each point on the NMDS represents the bacterial composition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



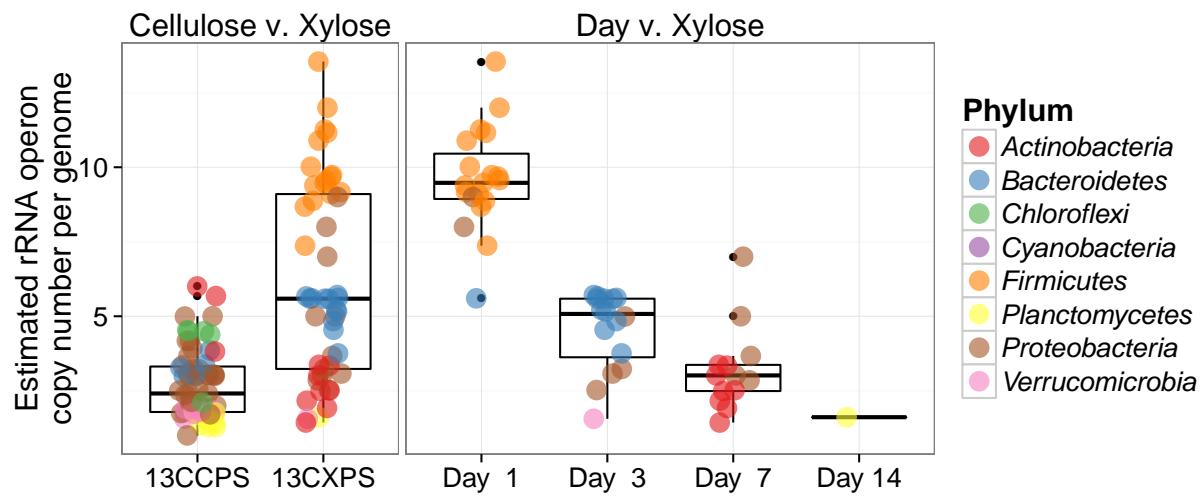
**Fig. 2.** Log<sub>2</sub> fold change of <sup>13</sup>C-responder in cellulose treatment (top) and xylose treatment (bottom). Log<sub>2</sub> fold change is based on the relative abundance in the experimental treatment compared to the control within the density range 1.7125–1.755 g ml<sup>-1</sup>. Taxa are colored by phylum. ‘Counts’ is a histogram of log<sub>2</sub> fold change values.



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

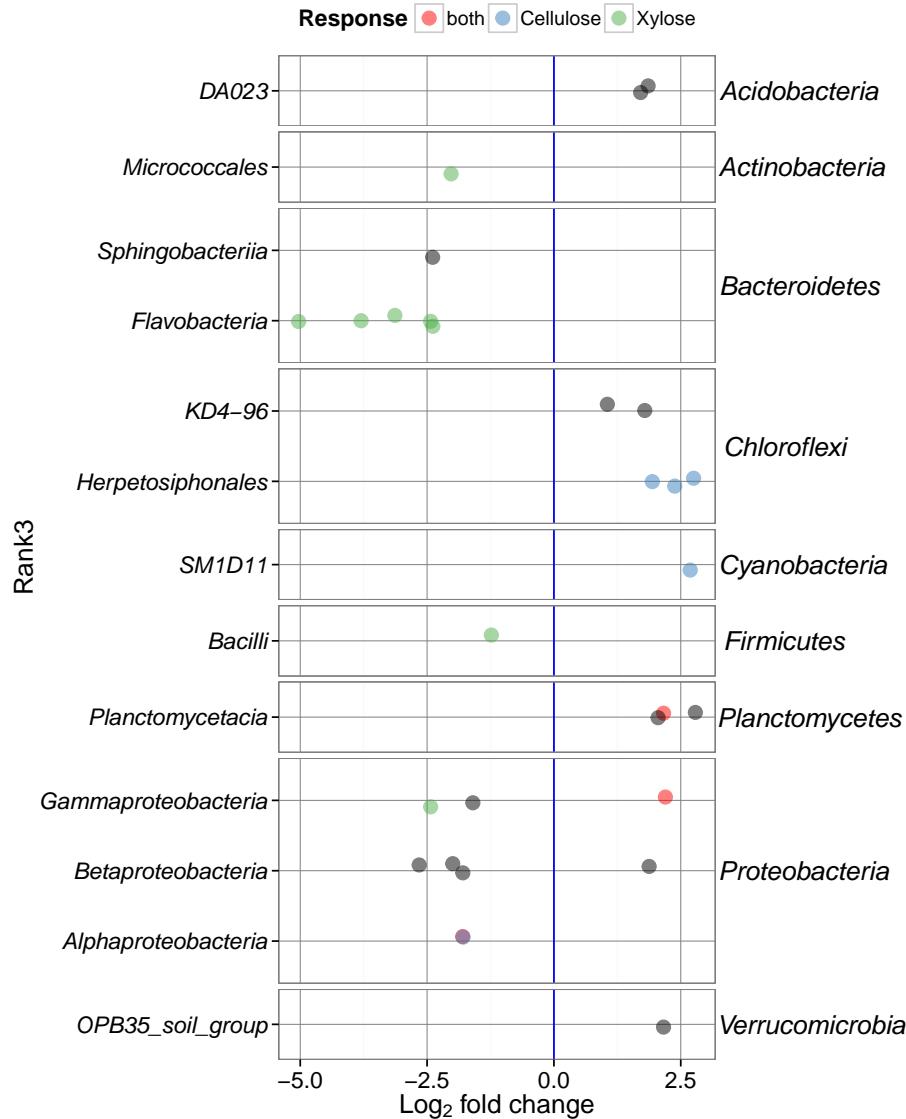


**Fig. 4.**  $^{13}\text{C}$ -responder characteristics based on density shift (A) and rank (B). Kernel density estimation of  $^{13}\text{C}$ -responder's density shift in cellulose treatment (blue) and xylose treatment (green) demonstrates degree of labeling for responders for each respective substrate.  $^{13}\text{C}$ -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.

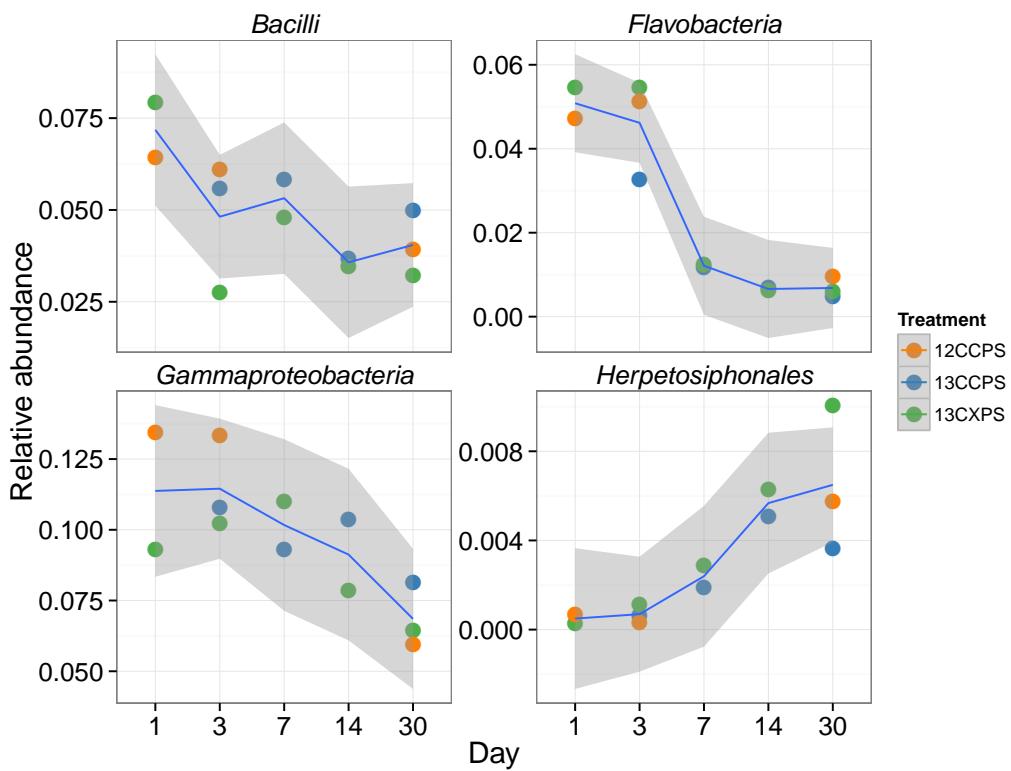


**Fig. 5.** Estimated rRNA operon copy number per genome for  $^{13}\text{C}$  responding OTUS. Panel titles indicate which labeled substrate(s) are depicted.

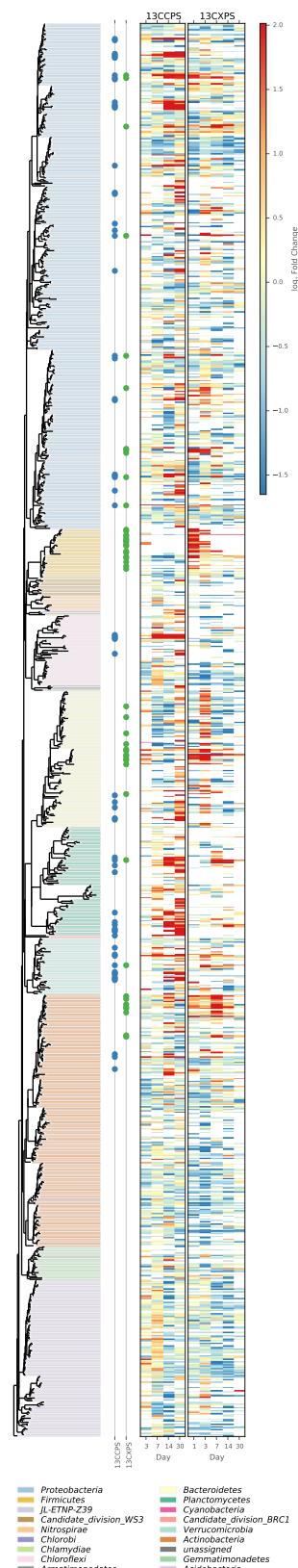
**Supplemental Figures and Tables**



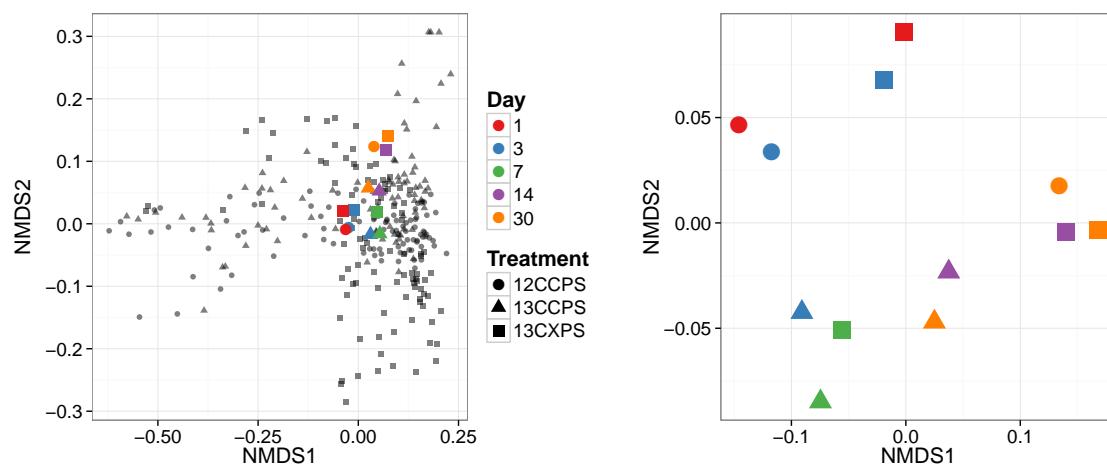
**Fig. S1.** Fold change time<sup>-1</sup> for OTUs that changed significantly in abundance over time. One panel per phylum (phyla indicated on the right). Taxonomic class indicated on the left.



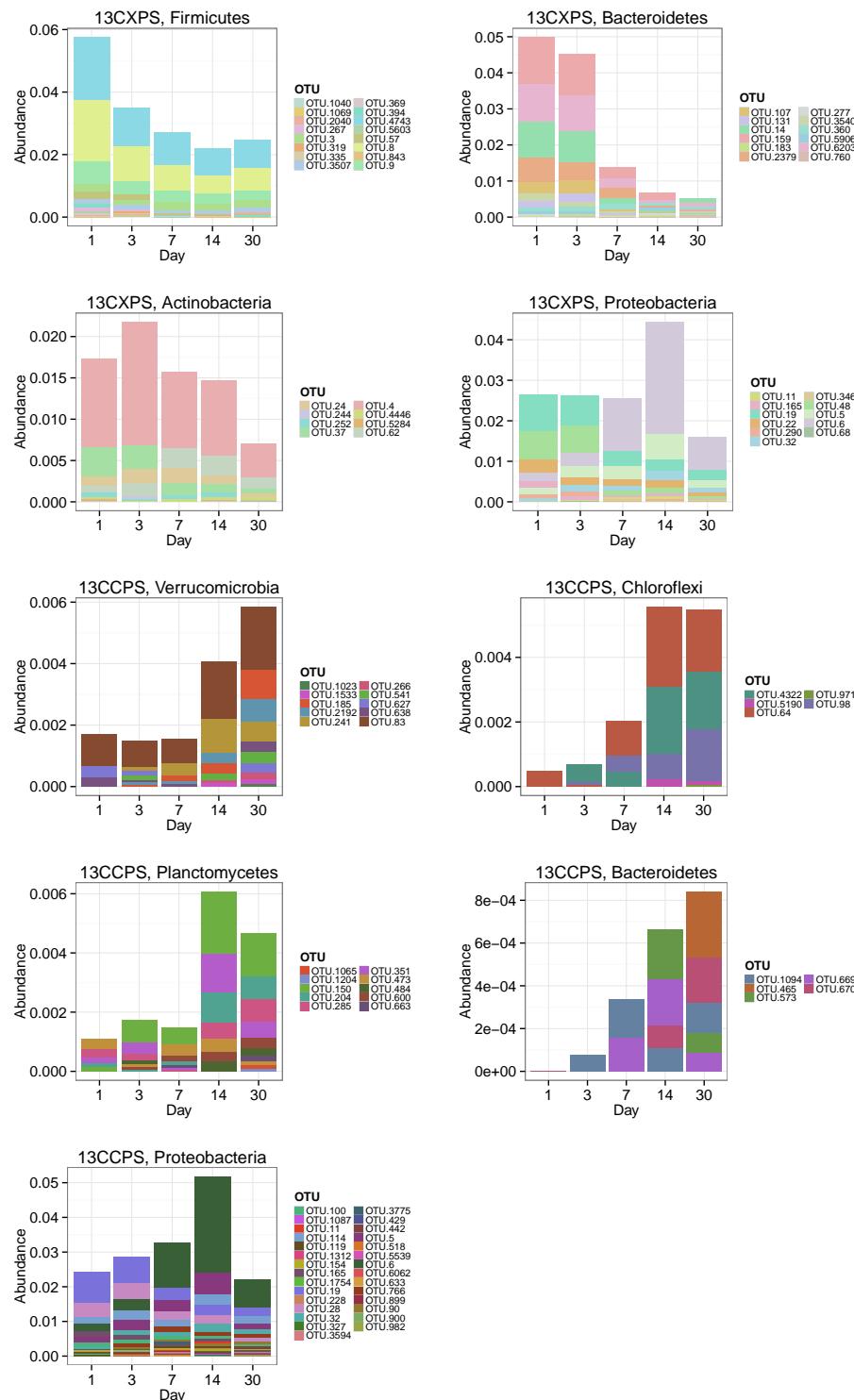
**Fig. S2.** Relative abundance versus day for classes that changed significantly in relative abundance with time.



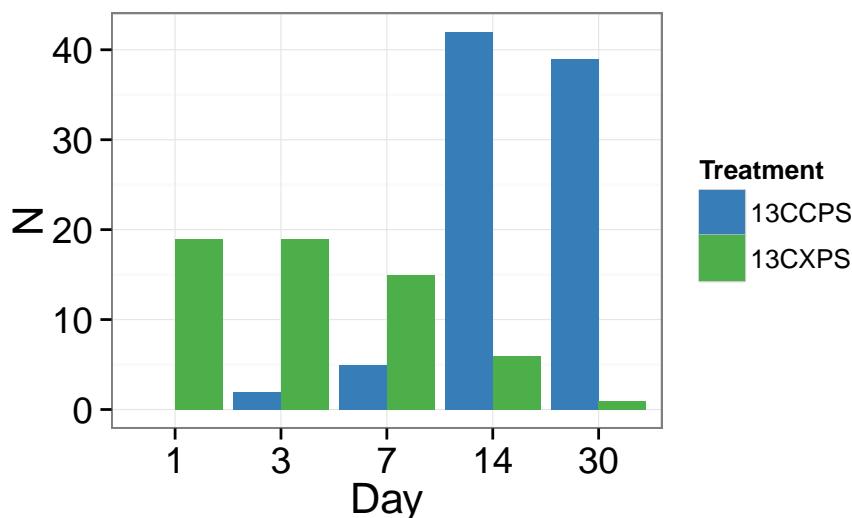
**Fig. S3.** Phylogenetic tree of sequences passing a user defined sparsity threshold (0.6) for at least one day of the time series. Branches are colored by phylum. <sup>13</sup>C-responders for cellulose (blue) and xylose (green) are indicated by a point beside the respective branch. Heatmap demonstrates log<sub>2</sub> fold change of each taxa through the full time series for both treatments (cellulose, left; xylose, right).



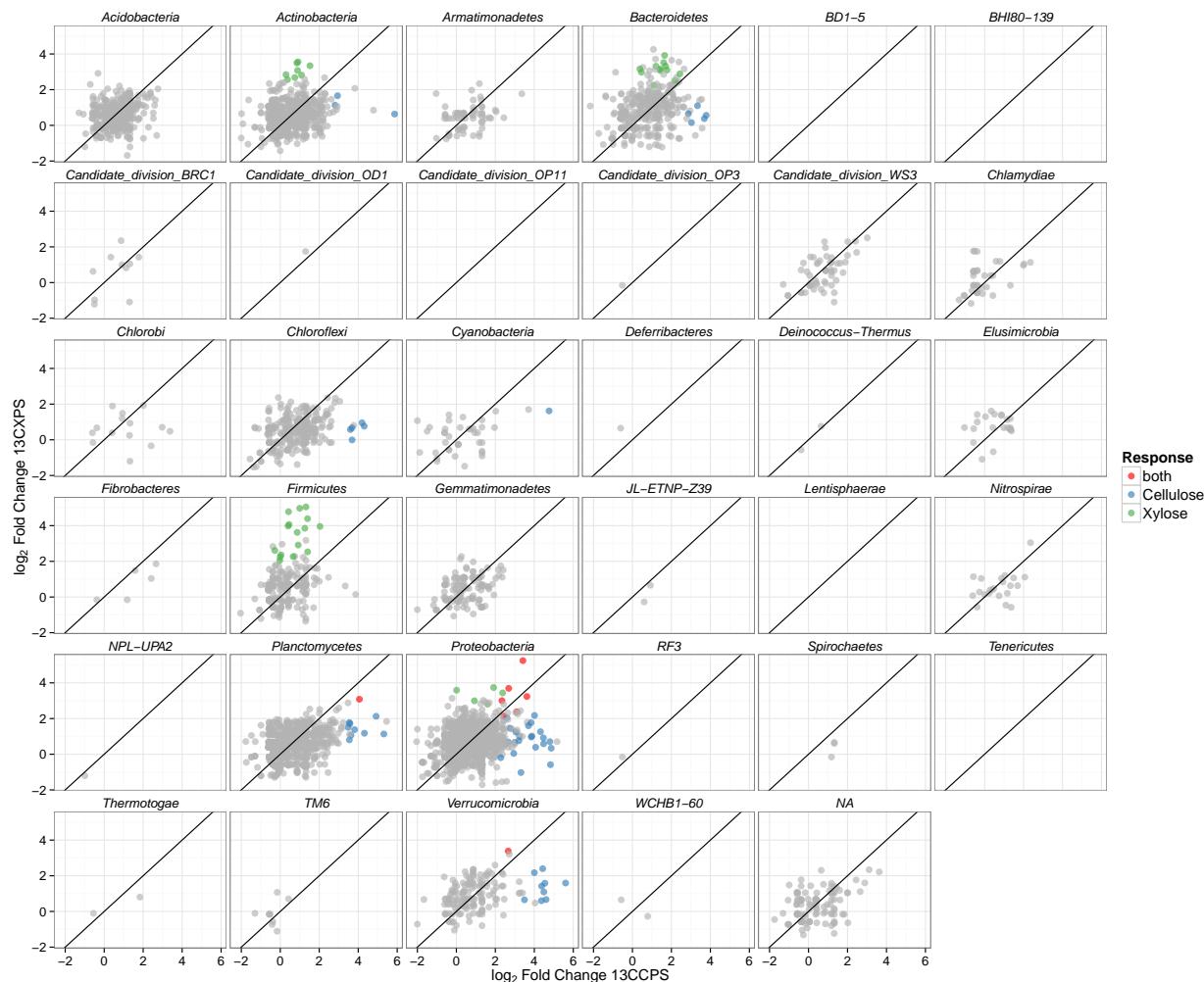
**Fig. S4.** Ordination of bulk gradient fraction phylogenetic profiles.



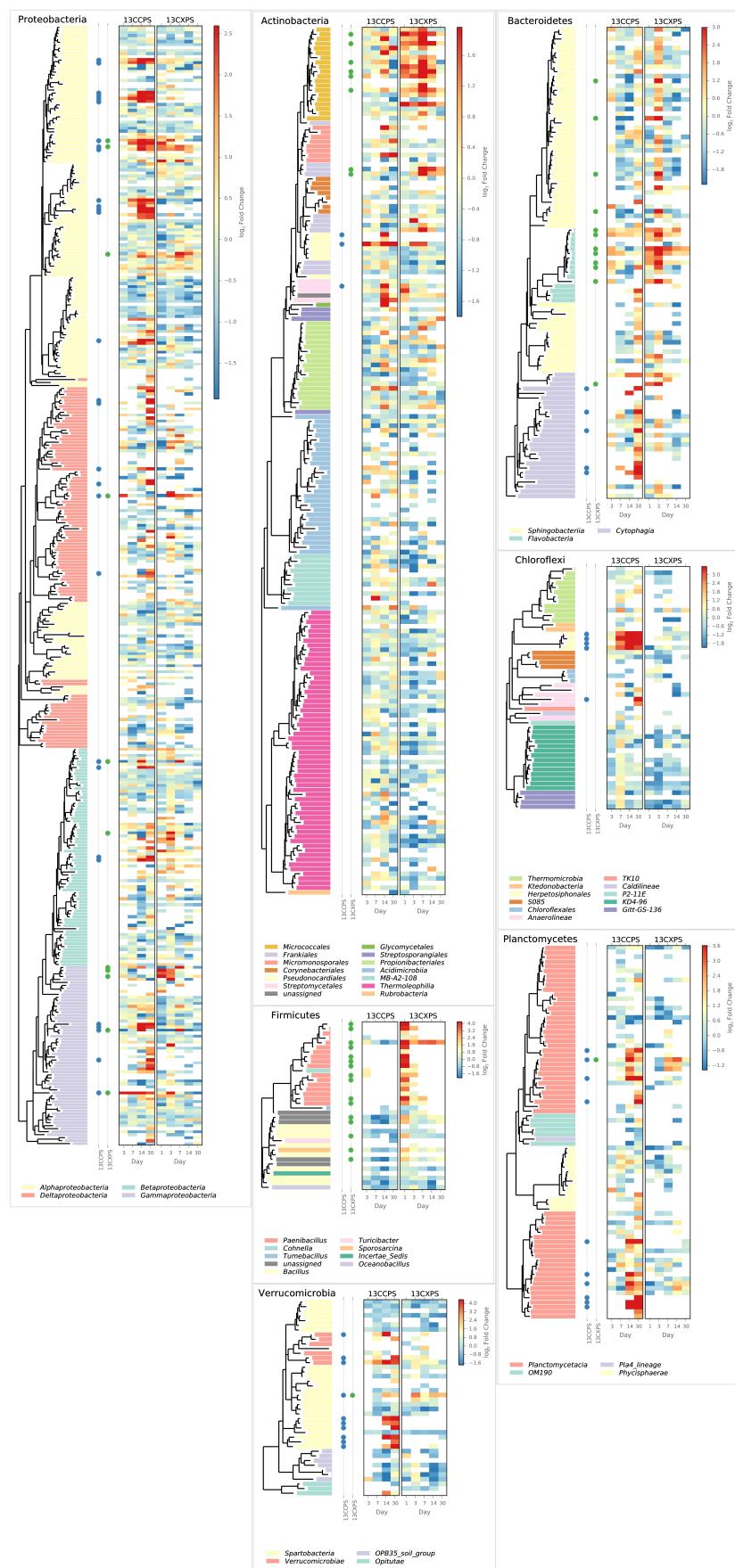
**Fig. S5.** Sum of bulk abundances with selected phylum for responder OTUs.



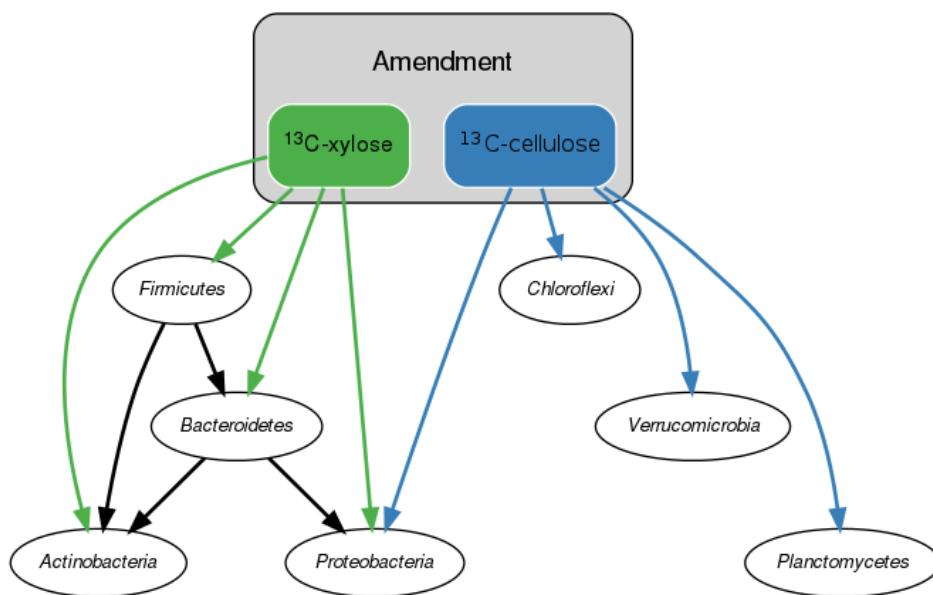
**Fig. S6.** Counts of responders to each isotopically labeled substrate (cellulose and xylose) over time.



**Fig. S7.** Maximum  $\log_2$  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  $^{13}\text{C}$ -xylose and  $^{13}\text{C}$ -cellulose, respectively.



**Fig. S8.** 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. S9.** Conceptual model of soil food web in this experiment.

Table S1: <sup>13</sup>C-cellulose responders BLAST against Living Tree Project

OTU ID	Fold change <sup>a</sup>	Day <sup>b</sup>	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.862	5.87	14	<i>Allokutzneria albata</i>	100.0	<i>Actinobacteria Pseudonocardiales Pseudonocardiaceae</i>
OTU.257	2.94	14	<i>Lentzea waywayandensis, Lentzea flaviverrucosa</i>	100.0	<i>Actinobacteria Pseudonocardiales Pseudonocardiaceae</i>
OTU.132	2.81	14	<i>Streptomyces spp.</i>	100.0	<i>Actinobacteria Streptomycetales Streptomycetaceae</i>
OTU.465	3.79	30	<i>Ohtaekwangia kribbensis</i>	92.73	<i>Bacteroidetes Cytophagia Cytophagales</i>
OTU.1094	3.69	30	<i>Sporocytophaga myxococcoides</i>	99.55	<i>Bacteroidetes Cytophagia Cytophagales</i>
OTU.669	3.34	30	<i>Ohtaekwangia koreensis</i>	92.69	<i>Bacteroidetes Cytophagia Cytophagales</i>
OTU.573	3.03	30	<i>Adhaeribacter aerophilus</i>	92.76	<i>Bacteroidetes Cytophagia Cytophagales</i>
OTU.670	2.87	30	<i>Adhaeribacter aerophilus</i>	91.78	<i>Bacteroidetes Cytophagia Cytophagales</i>
OTU.971	3.68	30	No hits of at least 90% identity	78.57	<i>Chloroflexi Anaerolineae Anaerolineales</i>
OTU.64	4.31	14	No hits of at least 90% identity	89.5	<i>Chloroflexi Herpetosiphonales Herpetosiphonaceae</i>
OTU.4322	4.19	14	No hits of at least 90% identity	89.14	<i>Chloroflexi Herpetosiphonales Herpetosiphonaceae</i>
OTU.98	3.68	14	No hits of at least 90% identity	88.18	<i>Chloroflexi Herpetosiphonales Herpetosiphonaceae</i>
OTU.5190	3.6	30	No hits of at least 90% identity	88.13	<i>Chloroflexi Herpetosiphonales Herpetosiphonaceae</i>
OTU.120	4.76	14	<i>Vampirovibrio chlorellavorus</i>	94.52	<i>Cyanobacteria SM1D11 uncultured-bacterium</i>
OTU.1065	5.31	14	No hits of at least 90% identity	84.55	<i>Planctomycetes Planctomycetacia Planctomycetales</i>
OTU.484	4.92	14	No hits of at least 90% identity	89.09	<i>Planctomycetes Planctomycetacia Planctomycetales</i>
OTU.1204	4.32	30	<i>Planctomyces limnophilus</i>	91.78	<i>Planctomycetes Planctomycetacia Planctomycetales</i>
OTU.150	4.06	14	No hits of at least 90% identity	86.76	<i>Planctomycetes Planctomycetacia Planctomycetales</i>
OTU.663	3.63	30	<i>Pirellula staleyi DSM 6068</i>	90.87	<i>Planctomycetes Planctomycetacia Planctomycetales</i>
OTU.473	3.58	14	<i>Pirellula staleyi DSM 6068</i>	90.91	<i>Planctomycetes Planctomycetacia Planctomycetales</i>
OTU.285	3.55	30	<i>Blastopirellula marina</i>	90.87	<i>Planctomycetes Planctomycetacia Planctomycetales</i>
OTU.351	3.54	14	<i>Pirellula staleyi DSM 6068</i>	91.86	<i>Planctomycetes Planctomycetacia Planctomycetales</i>
OTU.600	3.48	30	No hits of at least 90% identity	80.37	<i>Planctomycetes Planctomycetacia Planctomycetales</i>
OTU.900	4.87	14	<i>Brevundimonas vesicularis, Brevundimonas nasdae</i>	100.0	<i>Proteobacteria Alphaproteobacteria Caulobacterales</i>
OTU.1754	4.48	14	<i>Asticcacaulis biprosthecum, Asticcacaulis benevestitus</i>	96.8	<i>Proteobacteria Alphaproteobacteria Caulobacterales</i>
OTU.119	3.31	14	<i>Brevundimonas alba</i>	100.0	<i>Proteobacteria Alphaproteobacteria Caulobacterales</i>

Table S1 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.327	2.99	14	<i>Asticcacaulis biprosthecum,</i> <i>Asticcacaulis benevestitus</i>	98.63	Proteobacteria Alphaproteobacteria Caulobacterales
OTU.982	4.47	14	<i>Devosia neptuniae</i>	100.0	Proteobacteria Alphaproteobacteria Rhizobiales
OTU.1087	4.32	14	<i>Devosia soli</i> , <i>Devosia crocina</i> , <i>Devosia riboflavina</i>	99.09	Proteobacteria Alphaproteobacteria Rhizobiales
OTU.5539	4.01	14	<i>Devosia subaequoris</i>	98.17	Proteobacteria Alphaproteobacteria Rhizobiales
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.766	3.21	14	<i>Devosia insulae</i>	99.54	Proteobacteria Alphaproteobacteria Rhizobiales
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.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.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.90	2.94	14	<i>Sphingopyxis panaciterrae</i> , <i>Sphingopyxis chilensis</i> , <i>Sphingopyxis sp. BZ30</i> , <i>Sphingomonas sp.</i>	100.0	Proteobacteria Alphaproteobacteria Sphingomonadales
OTU.518	4.8	14	<i>Hydrogenophaga intermedia</i>	100.0	Proteobacteria Betaproteobacteria Burkholderiales
OTU.1312	4.07	30	<i>Paucimonas lemoignei</i>	99.54	Proteobacteria Betaproteobacteria Burkholderiales
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 hortii</i>	100.0	Proteobacteria Betaproteobacteria Burkholderiales
OTU.633	3.84	30	No hits of at least 90% identity	89.5	Proteobacteria Deltaproteobacteria Myxococcales
OTU.3594	3.83	30	<i>Chondromyces robustus</i>	90.41	Proteobacteria Deltaproteobacteria Myxococcales
OTU.442	3.05	30	<i>Chondromyces robustus</i>	92.24	Proteobacteria Deltaproteobacteria Myxococcales
OTU.228	2.54	30	<i>Sorangium cellulosum</i>	98.17	Proteobacteria Deltaproteobacteria Myxococcales

Table S1 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.899	2.28	30	<i>Enhygromyxa salina</i>	97.72	<i>Proteobacteria Deltaproteobacteria Myxococcales</i>
OTU.6	3.62	7	<i>Cellvibrio fulvus</i>	100.0	<i>Proteobacteria Gammaproteobacteria Pseudomonadales</i>
OTU.6062	4.83	30	<i>Dokdonella sp. DC-3, Luteibacter rhizovicinus</i>	97.26	<i>Proteobacteria Gammaproteobacteria Xanthomonadales</i>
OTU.154	3.24	14	<i>Pseudoxanthomonas mexicana, Pseudoxanthomonas japonensis</i>	100.0	<i>Proteobacteria Gammaproteobacteria Xanthomonadales</i>
OTU.100	2.66	14	<i>Pseudoxanthomonas sacheonensis, Pseudoxanthomonas dokdonensis</i>	100.0	<i>Proteobacteria Gammaproteobacteria Xanthomonadales</i>
OTU.1023	4.61	30	No hits of at least 90% identity	80.54	<i>Verrucomicrobia Spartobacteria Chthoniobacterales</i>
OTU.266	4.54	30	No hits of at least 90% identity	83.64	<i>Verrucomicrobia Spartobacteria Chthoniobacterales</i>
OTU.541	4.49	30	No hits of at least 90% identity	84.23	<i>Verrucomicrobia Spartobacteria Chthoniobacterales</i>
OTU.185	4.37	14	No hits of at least 90% identity	85.14	<i>Verrucomicrobia Spartobacteria Chthoniobacterales</i>
OTU.2192	3.49	30	No hits of at least 90% identity	83.56	<i>Verrucomicrobia Spartobacteria Chthoniobacterales</i>
OTU.1533	3.43	30	No hits of at least 90% identity	82.27	<i>Verrucomicrobia Spartobacteria Chthoniobacterales</i>
OTU.83	5.61	14	<i>Luteolibacter sp. CCTCC AB 2010415</i>	97.72	<i>Verrucomicrobia Verrucomicrobiae Verrucomicrobiales</i>
OTU.627	4.43	14	<i>Verrucomicrobiaceae bacterium DC2a-G7</i>	100.0	<i>Verrucomicrobia Verrucomicrobiae Verrucomicrobiales</i>
OTU.638	4.0	30	<i>Luteolibacter sp. CCTCC AB 2010415, Luteolibacter algae</i>	93.61	<i>Verrucomicrobia Verrucomicrobiae Verrucomicrobiales</i>

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

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

OTU ID	Fold change <sup>a</sup>	Day <sup>b</sup>	Top BLAST hits	BLAST %ID	Phylum;Class;Order
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.62	2.57	7	<i>Nakamurella flava</i>	100.0	Actinobacteria Frankiales Nakamurellaceae
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	Actinobacteria Micrococcales Cellulomonadaceae
OTU.4	2.84	7	<i>Agromyces ramosus</i>	100.0	Actinobacteria Micrococcales Microbacteriaceae
OTU.37	2.68	7	<i>Phycicola gilvus</i> , <i>Microterricola viridarii</i> , <i>Frigoribacterium faeni</i> , <i>Frondihabitans sp. RS-15</i> , <i>Frondihabitans australicus</i>	100.0	Actinobacteria Micrococcales Microbacteriaceae
OTU.5284	3.56	7	<i>Isoptericola nanjingensis</i> , <i>Isoptericola hypogaeus</i> , <i>Isoptericola variabilis</i>	98.63	Actinobacteria Micrococcales Promicromonosporaceae
OTU.252	3.34	7	<i>Promicromonospora thailandica</i>	100.0	Actinobacteria Micrococcales Promicromonosporaceae
OTU.244	3.08	7	<i>Cellulosimicrobium funkei</i> , <i>Cellulosimicrobium terreum</i>	100.0	Actinobacteria Micrococcales Promicromonosporaceae
OTU.760	2.89	3	<i>Dyadobacter hamtensis</i>	98.63	Bacteroidetes Cytophagia Cytophagales
OTU.14	3.92	3	<i>Flavobacterium oncorhynchi</i> , <i>Flavobacterium glycines</i> , <i>Flavobacterium succinicans</i>	99.09	Bacteroidetes Flavobacteria Flavobacteriales
OTU.6203	3.32	3	<i>Flavobacterium granuli</i> , <i>Flavobacterium glaciei</i>	100.0	Bacteroidetes Flavobacteria Flavobacteriales
OTU.159	3.16	3	<i>Flavobacterium hibernum</i>	98.17	Bacteroidetes Flavobacteria Flavobacteriales
OTU.2379	3.1	3	<i>Flavobacterium pectinovorum</i> , <i>Flavobacterium sp. CS100</i>	97.72	Bacteroidetes Flavobacteria Flavobacteriales
OTU.131	3.07	3	<i>Flavobacterium fluvii</i> , <i>Flavobacterium bacterium HMD1033</i> , <i>Flavobacterium sp. HMD1001</i>	100.0	Bacteroidetes Flavobacteria Flavobacteriales
OTU.3540	2.52	3	<i>Flavobacterium terrigena</i>	99.54	Bacteroidetes Flavobacteria Flavobacteriales
OTU.107	2.25	3	<i>Flavobacterium sp. 15C3</i> , <i>Flavobacterium banpakuense</i>	99.54	Bacteroidetes Flavobacteria Flavobacteriales
OTU.277	3.52	3	<i>Solibius ginsengiterrae</i>	95.43	Bacteroidetes Sphingobacteriia Sphingobacteriales
OTU.183	3.31	3	No hits of at least 90% identity	89.5	Bacteroidetes Sphingobacteriia Sphingobacteriales
OTU.5906	3.16	3	<i>Terrimonas sp. M-8</i>	96.8	Bacteroidetes Sphingobacteriia Sphingobacteriales
OTU.360	2.98	3	<i>Flavisolibacter ginsengisoli</i>	95.0	Bacteroidetes Sphingobacteriia Sphingobacteriales

Table S2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.369	5.05	1	<i>Paenibacillus sp. D75,</i> <i>Paenibacillus glycanilyticus</i>	100.0	<i>Firmicutes Bacilli Bacillales</i>
OTU.267	4.97	1	<i>Paenibacillus pabuli,</i> <i>Paenibacillus tundrae,</i> <i>Paenibacillus taichungensis,</i> <i>Paenibacillus xylanexedens,</i> <i>Paenibacillus xylanilyticus</i>	100.0	<i>Firmicutes Bacilli Bacillales</i>
OTU.1040	4.78	1	<i>Paenibacillus daejeonensis</i>	100.0	<i>Firmicutes Bacilli Bacillales</i>
OTU.57	4.39	1	<i>Paenibacillus castaneae</i>	98.62	<i>Firmicutes Bacilli Bacillales</i>
OTU.394	4.06	1	<i>Paenibacillus pocheonensis</i>	100.0	<i>Firmicutes Bacilli Bacillales</i>
OTU.319	3.98	1	<i>Paenibacillus xinjiangensis</i>	97.25	<i>Firmicutes Bacilli Bacillales</i>
OTU.5603	3.96	1	<i>Paenibacillus uliginis</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.843	3.62	1	<i>Paenibacillus agarexedens</i>	100.0	<i>Firmicutes Bacilli Bacillales</i>
OTU.2040	2.91	1	<i>Paenibacillus pectinilyticus</i>	100.0	<i>Firmicutes Bacilli Bacillales</i>
OTU.3	2.61	1	[ <i>Brevibacterium</i> ] <i>frigoritolerans</i> , <i>Bacillus sp. LMG 20238</i> , <i>Bacillus coahuilensis m4-4</i> , <i>Bacillus simplex</i>	100.0	<i>Firmicutes Bacilli Bacillales</i>
OTU.335	2.53	1	<i>Paenibacillus thailandensis</i>	98.17	<i>Firmicutes Bacilli Bacillales</i>
OTU.3507	2.36	1	<i>Bacillus spp.</i>	98.63	<i>Firmicutes Bacilli Bacillales</i>
OTU.8	2.26	1	<i>Bacillus niaci</i>	100.0	<i>Firmicutes Bacilli Bacillales</i>
OTU.4743	2.24	1	<i>Lysinibacillus fusiformis</i> , <i>Lysinibacillus sphaericus</i>	99.09	<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>
OTU.22	2.8	7	<i>Paracoccus sp. NB88</i>	99.09	<i>Proteobacteria Alphaproteobacteria Rhodobacterales</i>
OTU.5	3.69	7	<i>Delftia tsuruhatensis</i> , <i>Delftia lacustris</i>	100.0	<i>Proteobacteria Betaproteobacteria Burkholderiales</i>
OTU.346	3.44	3	<i>Pseudoduganella violaceinigra</i>	99.54	<i>Proteobacteria Betaproteobacteria Burkholderiales</i>
OTU.32	3.0	3	<i>Sandaracinus amyloyticus</i>	94.98	<i>Proteobacteria Deltaproteobacteria Myxococcales</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.290	3.59	1	<i>Pantoea spp.</i> , <i>Kluyvera spp.</i> , <i>Klebsiella spp.</i> , <i>Erwinia spp.</i> , <i>Enterobacter spp.</i> , <i>Buttiauxella spp.</i>	100.0	<i>Proteobacteria Gammaproteobacteria Enterobacteriales</i>
OTU.11	5.25	7	<i>Stenotrophomonas pavani</i> i, <i>Stenotrophomonas maltophilia</i> , <i>Pseudomonas geniculata</i>	99.54	<i>Proteobacteria Gammaproteobacteria Xanthomonadales</i>
OTU.48	2.99	1	<i>Aeromonas spp.</i>	100.0	<i>Proteobacteria Gammaproteobacteria aaa34a10</i>
OTU.241	3.38	3	No hits of at least 90% identity	87.73	<i>Verrucomicrobia Spartobacteria Chthoniobacteriales</i>

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