Supplemental Information – DNA-SIP reveals functional guild diversity and membership for labile and recalcitrant C decomposition in soil

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1 Supplemental Methods

1.1 Soil Collection and Preparation

We collected soils from an organic farm in Penn Yan, New York. Soils are Honoeye/Lima, a silty clay loam on calcareous bedrock. To get a field average, cores (5 cm diameter x 10 cm depth) were collected in duplicate from six different sampling locations around the field using a slide hammer bulk density sampler (coordinates: (1) N 42 40.288 W 77 02.438, (2) N 42 40.296 W 77 02.438, (3) N 42 40.309 W 77 02.445, (4) N 42 40.333 W 77 02.425, (5) N 42 40.340 W 77 02.420, (6) N 42 40.353 W 77 02.417) on November 21, 2011. Soil cores were sieved through (2mm), homogenized by mixing, and stored at 4C until preincubation (within 1-2 week of collection). Carbon and nitrogen content were previously measured for these soils [1]. Reported soil C values for the organic field were 12.15 (\pm s.d. 0.78) mg C g⁻¹ dry soil and 1.16 (\pm s.d. 0.13) mg N g⁻¹ dry soil.

1.2 Cellulose production

Bacterial cellulose was produced by Gluconoacetobacter xylinus grown in Heo and Son [2] liquid minimal media made with 0.1% glucose (one batch with 12 C and another with 13 C-glucose). All cellulose (12 C and 13 C) was grown in 1L Erlenmeyer flasks containing 100 mL Heo and Son minimal media that were inoculated with three colonies of Gluconoacetobacter xylinus from on Heo and Son 0.1% glucose agar plates (using 12 C-glucose) at 30C without inositol. Flasks were incubated statically in the dark at 30C for 2-3 weeks until as thick cellulose pellicule formed. Cellulose pellicules were collected and washed with two parts 1% alconox and autoclaved. Cellulose pellicules were purified by repeated (10x) overnight dialysis in 1 L deionized water. Harvested pellicules were dried overnight (60C) and then cut into pieces and ground using ball grinder until the desired size range (53 μ m - 250 μ m) was achieved (size was checked by dry sieving). Size range was based on particulate organic matter to emulate how microbes may experience cellulose in the environment [3] and for even distribution in microcosms.

Post processing, purity of ground cellulose was checked with *E.coli* cultures, Benedict's reducing sugars assay, Bradford assay, and isotopic analysis. *E.coli* is not able to use cellulose as a C source but is capable of growth on a variety of nutrients available in the Heo and Son medium. Biological

assays consisted of E. coli inoculated into minimal M9 media which lacked a carbon source and was supplemented with either: (1) 0.01% glucose, (2) 2.5 mg purified, ground cellulose, (3) 25 mg purified, ground cellulose, (4) 25 mg purified, ground cellulose and 0.01% glucose. Growth in media was checked by spectrometer (OD450). No measureable growth was observed with either 2 mg or 25 mg cellulose, indicating absence of contaminating nutrients. In addition, the presence of 25 mg cellulose did not inhibit the growth of *E.coli* cultures provided with glucose relative to control, indicating the absence of compounds that may inhibit microbial growth in the purified cellulose.

Purified cellulose was also assayed for residual proteins and sugars using Bradford and Benedict's assays, respectively. Bradford assay was performed as in [4] with a standard curve ranging from 0-2000 μ g ml ⁻¹ BSA. Ground, purified cellulose contained 6.92 μ g protein mg cellulose ⁻¹(*i.e.* 99.31% purity). Reducing sugars were not detected in cellulose using Benedict's reducing sugar assay [5] tested at 10 mg cellulose ml⁻¹. Finally, ¹³C-cellulose had an average 96% \pm 5 (s.d.) degree of ¹³C labeling as determined by isotopic analysis (UCDavis Stable Isotope Facility).

1.3 Soil microcosms

A subset of soil was dried at 105C overnight to determine soil moisture content gravimetrically. Microcosms (35 total) were started by adding the 10 g approximate dry soil weight of the sieved soil to a 250 mL Erlenmeyer flask capped with a butyl rubber stopper to prevent drying. Microcosms were preincubated at 25C for 2 weeks until the soil respiration rate (determined by GCMS measurement of head space CO₂) had stabilized. Sieving causes a transient increase in soil respiration rate presumably due to the liberation of fresh labile soil organic matter [6]. Pre-incubation ensures that this labile organic matter is consumed and/or stabilized prior to the beginning of the experiment. Respiration rate (CO₂) began to plateau around 10 days, with no change in rate after that time. Stoppers were removed for 10 min every 3 days to exchange the headspace with air.

Three parallel treatments were performed with identical amendments of carbon which varied only with respect to ¹³C-labelling as follows: (1) unlabeled control, (2) ¹³C-cellulose (synthesis and purity described above), (3) ¹³C-xylose (98 atom% ¹³C, Sigma Aldrich 666378). 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). Each microcosm received an evenly distributed dry addition of insoluble substrates (2 mg cellulose and $1.2 \text{ mg lignin g dry soil}^{-1}$) and a liquid addition (1.2 mL) of a complex substrate mixture. The complete amendment (dry and liquid additions) was added to each microcosm at 5.3 mg g dry soil⁻¹; representative of natural concentrations [7]. The complex mixture was designed based on switch grass biomass composition [8, 9] to include (by mass) 38% cellulose, 23% lignin, 20% xylose, 3% arabinose, 1\% galactose, 1\% glucose, and 0.5\% mannose, with the remaining 13.5\% mass composed of amino acids (in-house made replica of teknova Cat#C0705) and basal salt mixture (Murashige and Skoog, Sigma M5524) for a final C:N of 10. The volume of the liquid addition was chosen to achieve 50% water holding capacity of the soil. Water holding capacity of 50% was chosen to achieve $\sim 70\%$ water filled pore space in these soils based on soil texture, which is the optimal water content for respiration [10, 10].

Replicate microcosms were harvested (stored at -80°C until nucleic acid processing) at days 1 (control and xylose only), 3, 7, 14, and 30. A subset of microcosm soil for each treatment and time point were isotopically analyzed at Cornell University Stable Isotope Laboratory to determine amount of ¹³C that remained at each time point.

1.4 Nucleic acid extraction

Nucleic acids were extracted from 0.25 g soil using a modified Griffiths protocol [11]. Cell were lysed by bead beating for 1 min at 5.5 ms⁻¹ in 2mL lysis tubes containing 0.5 g of 0.1 mm diameter silica/zirconia beads (treated at 300C for 4 hours to remove RNases), 0.5 mL extraction buffer (240 mM Phosphate buffer 0.5% N-lauryl sarcosine), and 0.5 mL phenol-chloroform-isoamyl alcohol (25:24:1) for 1 min at 5.5 ms⁻¹. After lysis, 85 uL 5 M NaCl and 60 uL 10% hexadecyltriammonium bromide (CTAB)/0.7 M NaCl were added to lysis tube, vortexed, chilled for 1 min on ice, and centrifuged at 16,000 x g for 5 min at 4C. The aqueous layer was transferred to a new tube and reserved on ice. To increase DNA recovery, the pellet was back extracted with 85 uL 5 M NaCl and 0.5 mL extraction buffer. The aqueous extract was washed with 0.5 mL chloroform: isoamyl alcohol (24:1). Nucleic acids were precipitated by addition of 2 volumes polyethylene glycol solution (30% PEG 8000, 1.6 M NaCl) on ice for 2 hrs, followed by centrifugation at 16,000 x g, 4C for 30 min. The supernatant was discarded and pellets were washed with 1 mL ice cold 70% EtOH. Pellets were air dried, resuspended in 50 uL TE and stored at -20C. To prepare nucleic acid extracts for isopycnic centrifugation as previously described [12], DNA was size selected (> 4kb) using 1% low melt agarose gel and β -agarase I enzyme extraction per manufacturers protocol (New England Biolab, M0392S). Final resuspension of DNA pellet was in 50 μ L TE.

1.5 Isopycnic centrifugation and fractionation

For each time point in the series isopycnic gradients were setup using a modified protocol [13] for a total of five ¹²C-control, five ¹³C-xylose, and four ¹³C-cellulose microcosms. A density gradient (average density 1.69 g mL⁻¹) solution of 1.762 g cesium chloride (CsCl) ml⁻¹ in gradient buffer solution (pH 8.0 15 mM Tris-HCl, 15 mM EDTA, 15 mM KCl) was used to separate ¹³C-enriched and 12 C-non-enriched DNA. Each gradient was loaded with approximately 5 μ g of DNA and centrifuged on a Beckman Coulter $Optima^{TM}$ MAX-E ultracentrifuge using a TLA-110 fixed-angle rotor for 66 h at 55,000 rpm and room temperature (RT). Fractions of $\sim 100~\mu L$ 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 μ L s⁻¹ [14] into AcroprepTM 96 filter plate (part no. 5035, Pall Life Sciences). The refractive index of each fraction was measured using a Reichart AR200 digital refractometer modified as previously described to measure a volume of 5 μ L [12]. Buoyant density was calculated from the refractive index as previously described [12] using the equation $\rho = a\eta - b$, where ρ is the density of the CsCl (g ml⁻¹), η is the measured refractive index, and a and b are coefficient values of 10.9276 and 13.593, respectively, for CsCl at 20C [15]. The collected DNA fractions were purified by repetitive washing of Acroprep filter wells with TE. Finally, 50 μ L TE was added to each fraction then resuspended DNA was pipetted off the filter into a new microfuge tube.

1.6 DNA Sequencing

For every gradient, 20 fractions between the density range 1.67-1.75 g mL⁻¹ were chosen for sequencing. A total of 14 gradients (280 fractions) and their corresponding bulk DNA extraction (after β -agarase size selection) were amplified for sequencing. Barcoded 454 primers were designed using 454-specific adapter B, 10 bp barcodes [16], 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 μ L 5U μ l⁻¹ AmpliTaq

Gold (Life Technologies, Grand Island, NY; N8080243), 2.5 μ L 10X Buffer II (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 2.5 μ L 25 mM MgCl₂, 4 μ L 5 mM dNTP, 1.25 μ L 10 mg mL⁻¹ BSA, 0.5 μ L 10 μ M BA515F, 1 μ L 5 μ M BA806R, 3 μ L H₂O, 10 μ L 1:30 DNA template) in triplicate and checked by 1% agarose gel. 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. 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)

1.7 Post-Sequencing Analysis

1.7.1 Sequence quality control

Sequences were initially screened by maximum expected errors at a specific read length threshold [17] which has been shown to be as effective as denoising with respect to removing pyrosequencing errors. Specifically, reads were first truncated to 250 nucleotides (nt) (all reads shorter than 250 nt were discarded) and any read that exceeded a maximum expected error threshold of 0.5 was removed. After truncation and max expected error trimming, 87% of original reads remained. The forward primer and barcode was then removed from the high quality, truncated reads. Remaining reads were taxonomically annotated using the "UClust" taxonomic annotation framework in the QIIME software package [18, 19] with cluster seeds from Silva SSU rRNA database [20] 97% sequence identity OTUs as reference (release 111Ref). Reads annotated as "Chloroplast", "Eukaryota", "Archaea", "Unassigned" or "mitochondria" were culled from the dataset. Finally, reads were aligned to the Silva reference alignment provided by the Mothur software package [21] using the Mothur NAST aligner [22]. All reads that did not align to the expected amplicon region of the SSU rRNA gene were discarded. Quality control parameters removed 344,472 of 1,720,480 raw reads.

1.7.2 Sequence clustering

Sequences were distributed into OTUs using the UParse methodology [17]. Specifically, OTU centroids (i.e. seeds) were identified using USearch on non-redundant reads sorted by count. The sequence identity threshold for establishing a new OTU centroid was 97%. With USearch/UParse, potential chimeras are identified during OTU centroid selection and are not allowed to become cluster centroids effectively removing chimeras from the read pool. All quality controlled reads were then mapped to cluster centroids at an identity threshold of 97% again using USearch. 97% of quality controlled reads could be mapped to centroids. Unmapped reads do not count towards sample counts and are removed from downstream analyses. The USearch software version for cluster generation was 7.0.1090.

1.7.3 Phylogenetic analysis

Alignment of OTU centroid SSU rRNA genes was done with SSU-Align which is based on Infernal [23, 24]. Columns in the alignment that were not included in the SSU-Align covariance models or were aligned with poor confidence (less than 95% of characters in a position had posterior probability alignment scores of at least 95%) were masked for phylogenetic reconstruction. Additionally, the

alignment was trimmed to coordinates such that all sequences in the alignment began and ended at the same positions. FastTree [25] was used to reconstruct the phylogeny with default parameters.

1.7.4 Identifying OTUs that incorporated ¹³C into their DNA

DNA-SIP is a culture-independent approach that defines identity-function connections in microbial communities [13, 26, 27]. Microbes are identified on the basis of isotope assimilation into DNA. As the buoyant density (BD) of a macromolecule is dependent on many factors in addition to stable isotope incorporation (e.g. GC-content in nucleic acids [28]), labeled nucleic acids from one microbial population may have the same BD as unlabeled nucleic acids from another. Therefore, it is imperative to compare results of isotopic labelling to results obtained with unlabeled controls where everything mimics experimental conditions except that substrates are unlabeled. By contrasting heavy gradient fractions from isotopically labeled samples relative to corresponding fractions from controls, the identities of microbes with labeled nucleic acids can be determined

We used an RNA-Seq differential expression statistical framework [?] to find OTUs enriched in heavy fractions of labeled gradients relative to corresponding density fractions in control gradients (for review of RNA-Seq differential expression statistics applied to microbiome OTU count data see [30]). We use the term differential abundance (coined by [30]) to denote OTUs that have different abundance across sample classes (in this case the only sample class is labeled or control). CsCl gradient fractions were categorized as "heavy" or "light". The heavy category denotes fractions with density values above 1.7125 and below 1.755 g/mL. Since we are only interested in enriched OTUs (labeled versus control), we used a one-sided test to assess the significance of differential abundance (the null hypothesis was that the differential abundance (labeled:control) for an OTU is less than a selected threshold). P-values were corrected with the Benjamini and Hochberg method [31].

We selected a \log_2 fold change null threshold of 0.75 (or a labeled:control differential abundance of 1.68).

DESeq2 was used to calculate moderated \log_2 fold change of labeled:control abundance and corresponding standard errors for the one-sided Wald test. Fold change moderation allows for reliable ranking of fold change for each OTU such that high variance and likely statistically insignificant fold changes are appropriately shrunk and subsequently ranked lower than they would be as raw fold change. OTU DNA that enriches significantly in heavy fractions from 13 C-labeled samples relative to corresponding controls has increased significantly in buoyant density in response to 13 C treatment.

1.7.5 Community and Sequence Analysis

Principal coordinate ordinations depict the relationships between samples. Weighted Unifrac [32] distances were used as the sample distance metric for ordination. The Phyloseq [33] wrapper for Vegan [34] (both R packages) was used to compute sample values along principal coordinate axes. GGplot2 [35] was used to display sample points along the first and second principal axes. Adonis tests [36] were done with 1000 permutations.

Nucleic-acid SIP coupled to microbiome fingerprinting techniques progressed from simple proofof-concept experiments with pure cultures [37] to DGGE, ARISA and/or tRFLP-enabled studies of microcosm microbial assemblages [38]. Recently large experiments employed multiple labeled substrates and high-throughput amplicon and/or shotgun DNA sequencing [39] revealing the relative contributions of sampling location and DNA-SIP gradient density on phylogenetic profile variance from DNA-SIP experiments (CITE Conrad and Neufeld). Although density gradient position can account for approximately XX% of phylogenetic profile variance, soil type appears to represent greater variance than can be established across density gradients from soil DNA. Association of phylogenetic types with "heavy" DNA-SIP density gradient fractions in labeled gradients and not "heavy" fractions in unlabeled control gradients suggests label incorporation into biomass for the associated phylogenetic type. Our study shows that DNA-SIP can also characterize C use in three additional dimensions 1) temporally, isotopic labels can demonstrate C substrate use dynamics on the scale of days both between substrates and for a single substrate, 2) profiling DNA-SIP density gradients along the full gradient length can demonstrate patterns in substrate use specificity, and 3) ¹³C incorporation can be established at high resolution taxonomic groups such as 97% sequence identity OTUs.

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Code to take raw sequencing data through the presented figures (including download and processing of literature environmental datasets) can be found at:

http://nbviewer.ipython.org/github/chuckpr/CSIP_succession_data_analysis

1.8 Buoyant density shift estimates

Upon labeling, DNA from an organism that incorporates exclusively ¹³C 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 ¹³C-cellulose and ¹³C-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 XX).

1.9 Sequencing and density fractionation statistics

Microcosm DNA was density fractionated on CsCl density gradients. 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 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.

2 Phylogenetic affiliation of ¹³C-responsive microorganisms

2.1 Cellulose

2.1.1 Proteobacteria

Proteobacteria represent 46% of all ¹³C-cellulose responding OTUs identified. Cellvibrio accounted for 3% of all proteobacterial ¹³C-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 [40]. All ¹³C-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 cellouronic acid, an oxidized form of cellulose [41].

2.1.2 Verrucomicrobia

Verrucomicrobia, cosmopolitan soil microbes [42], can comprise up to 23% of 16S rRNA gene sequences in high-throughput DNA sequencing surveys of SSU rRNA genes in soil [42] and can account for up to 9.8% of soil 16S rRNA [43]. Many Verrucomicrobia were first isolated in the last decade [44] but only one of the 15 most abundant verrucomicrobial phylotypes in a global soil sample collection shared greater than 93% sequence identity with a cultured isolate [42]. Genomic analyses and physiological profiling of Verrucomicrobia isolates revealed Verrucomicrobia are capable of methanotrophy, diazotrophy, and cellulose degradation [44, 45], yet the function of soil Verrucomicrobia in global C-cycling remains unknown. However, Verrucomicrobia are hypothesized to degrade polysaccharides in many environments [46–48]. Verrucomicrobia comprise 16% of the total ¹³C-cellulose responder OTUs detected, 40% of Verrucomicrobia ¹³C-cellulose responders belong to the uncultured "FukuN18" family originally identified in freshwater lakes [49]. The strongest Verrucomicrobial responder OTU to ¹³C-cellulose shared high sequence identity (97%) with an isolate from Norway tundra soil [50] although growth on cellulose was not assessed for this isolate. Only one other ¹³C-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 [45]. The remaining ¹³C-cellulose Verrucomicrobia responders did not share high sequence identity with any isolates (maximum sequence identity with any isolate 93%). Only two of the ten putative cellulose degrading Verrucomicrobia identified in this experiment share at least 95% sequence identity with an isolate ("OTU.83" and "OTU.627", Table S1). Seven of ten ¹³C-cellulose responding verrucomicrobial OTUs were classified as Spartobacteria which are a numerically dominant family of Verrucomicrobia in SSU rRNA gene surveys of 181 globally distributed soil samples [42]. Given their ubiquity and abundance in soil as well as their demonstrated incorporation of ¹³C from ¹³C-cellulose, Verrucomicrobia lineages, particularly Spartobacteria, may be important contributors to cellulose decomposition on a global scale.

2.1.3 Chloroflexi

Chloroflexi are known for metabolically dynamic lifestyles ranging from anoxygenic phototrophy to organohalide respiration [51]. Recent studies have focused on *Chloroflexi* roles in C cycling [51–53] and several Chloroflexi utilize cellulose [51–53]. Cellulose degrading soil Chloroflexi have previously been identified in DNA-SIP studies [54] and Chloroflexi are among the six most abundant soil phyla commonly recovered soil microbial diversity surveys [55]. The cellulose degrading Chloroflexi in this study are only distantly related to isolates S1. Chloroflexi are typically not as as abundant as Verrucomicrobia but are roughly as abundant as Bacteroidetes and Planctomycetes [55]. Four of five ¹³C-cellulose responsive Chloroflexi identified in this study are annotated as belonging to the Herpetosiphon although they share less than 95% sequence identity with their closest cultured relative in the Herpetosiphon genus (H. geysericola). H. geysericola is a predatory bacterium shown to prey upon Aerobacter in culture and can also digest cellulose [56]. One non-Herpetosiphon Chloroflexi OTU also from a poorly characterized Chloroflexi lineage (closest cultured isolate matched a proteobacterium at 78% sequence identity) responded to ¹³C-cellulose (Figure S10).In our study, "Herpetosiphon" ¹³C-cellulose responders did not show a delayed response to ¹³C-cellulose as compared to other responders but nonetheless could have become labeled by feeding on primary ¹³C-cellulose degraders. The prey specificity of predatory bacteria is not well established especially in situ. ¹³C-labeling would be positively correlated with prey specificity. If the predator specifically preyed upon one population then it could take on the same labeling percent as that population given enough generations. Preying on multiple types would produce a mixed and dilute labeling signature if some of the prey were not isotopically labeled.

2.1.4 Others

Other notable ¹³C-cellulose responders include a *Bacteroidetes* OTU that shares high sequence identity (99%) to Sporocytophaga myxococcoides a known cellulose degrader [57], and three Actinobacteria OTUs that share high sequence identity (100%) with isolates. One of the three Actinobacteria ¹³C-cellulose responders is in the *Streptomyces*, a genus known to possess cellulose degraders, while the other two share high sequence identity to cultured isolates Allokutzneriz albata [58, 59] and Lentzea waywayandensis [60, 61]; neither isolate decomposes cellulose in culture. Nine Planctomycetes OTUs responded to ¹³C-cellulose but none are within described genera (closest cultured isolate match 91% sequence identity, Table S1) (Figure S10). One ¹³C-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" [62]; although, the phylogenetic position of "Melainabacteria" is debated [63]. The catalog of metabolic capabilities associated with cyanobacteria (or candidate phyla previously annotated as cyanobacteria) are quickly expanding [62, 63]. 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 [62]. Although we highlight ¹³C-cellulose responders that share high sequence identity with described genera, most ¹³C-cellulose responders uncovered in this experiment are not closely related to cultured isolates (Table S1). Acidobacteria did not pass or operational criteria for assessing ¹³C incorporation from cellulose into DNA in our microcosms. Acidobacteria have been found to degrade cellulose in culture CITE and are a numerically significant soil phylum CITE. Acidobacteria have been shown to dominate at low nutrient availability (CITE: cederlund 2014), which may explain why they were not active upon nutrient additions. The Acidobacteria in our microcosms were mainly annotated as belonging to candidate orders in the Silva taxonomic nomenclature. The highest relative abundance for any Acidobacteria order in the bulk samples was 0.20 (order "DA023") and the highest relative abundance of the Acidobacteria phylum was 0.23.

2.2 Xylose

All of the ¹³C-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 ¹³Cxylose responder is closely related to isolates annotated as members of Bacillus, Paenibacillus or Lysinibacillus. Bacteroidetes ¹³C-xylose responders are predominantly closely related to Flavobacterium species (5 of 8 total responders) (Table S2). Only one Bacteroidetes ¹³C-xylose responder is not closely related to a cultured isolate, "OTU.183" (closest LTP BLAST hit, Chitinophaca 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, [64]). Other Bacteroidetes responders share high sequence identities with canonical soil genera including Dyadobacer, Solibius and Terrimonas. Six of the 8 Actinobacteria ¹³C-xylose responders are in the *Micrococcales* order. One ¹³C-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 [65]. It is not possible to determine the specific origin of assimilated ¹³C in a DNA-SIP experiment. ¹³C 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 ¹³C labeled Agromyces OTU was assimilating ¹³C primarily by predation if the Agromyces OTU was selective enough with respect to its prey that it primarily attacked ¹³C-xylose assimilating organisms.

3 Supplemental Figures and Tables

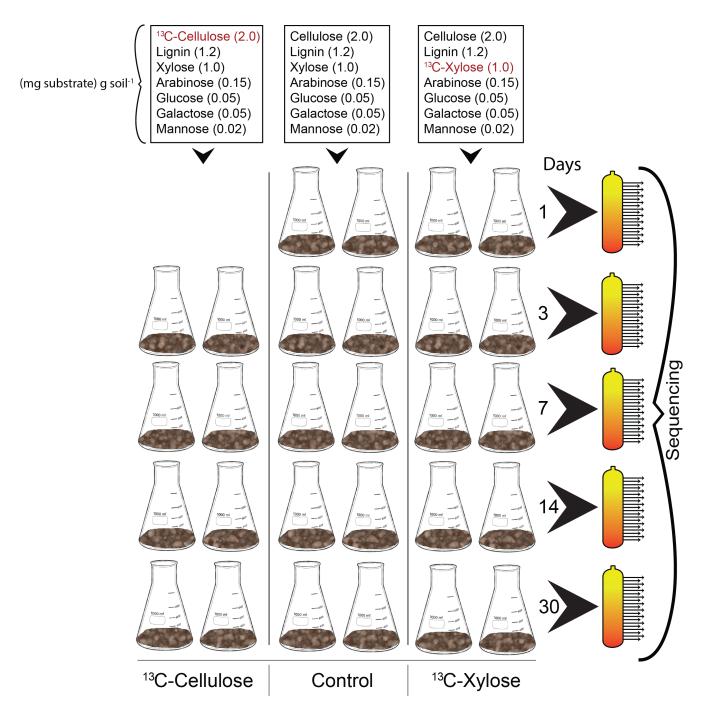


Figure S1: The experimental design. A carbon mixture, in addition to inorganic salts and amino acids (not shown here), was added to each soil microcosm where the only difference between treatments is the 13 C-labeled isotope (in red). At days 1, 3, 7, 14, and 30 replicate microcosms were destructively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

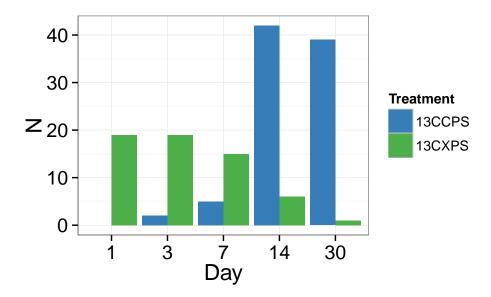


Figure S2: Counts of responders to each isotopically labeled substrate (cellulose and xylose) over time.

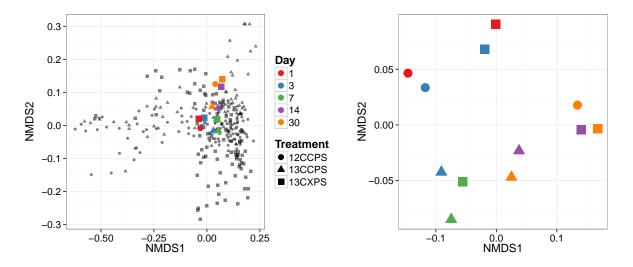


Figure S3: Ordination of bulk gradient fraction phylogenetic profiles.

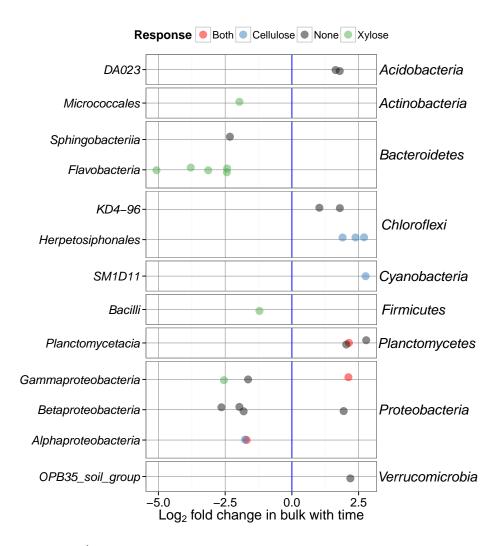


Figure S4: Fold change $time^{-1}$ for OTUs that changed significantly in abundance over time. One panel per phylum (phyla indicated on the right). Taxonomic class indicated on the left.

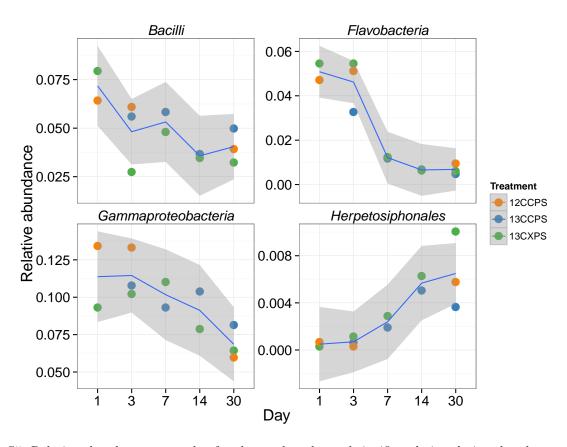


Figure S5: Relative abundance versus day for classes that changed significantly in relative abundance with time.

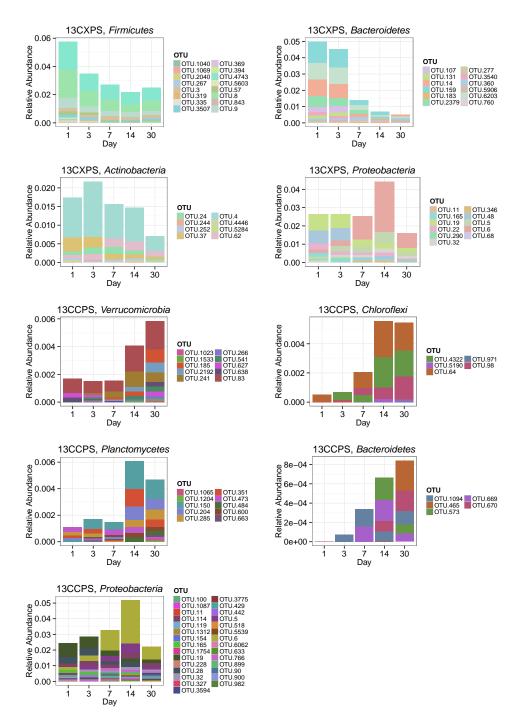


Figure S6: Sum of bulk abundances with selected phylum for responder OTUs.

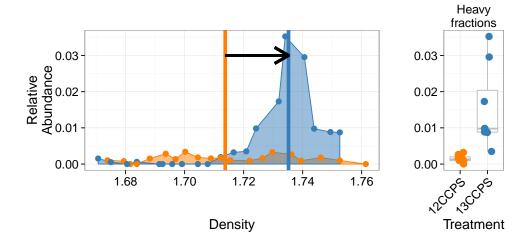


Figure S7: Density profile for a single ¹³C-cellulose "responder" OTU in the labeled gradient, blue, and the control gradient, orange. Vertical lines show center of mass for each density profile and arrow denotes the magnitude and direction of the BD shift upon labeling. Panel at right shows relative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

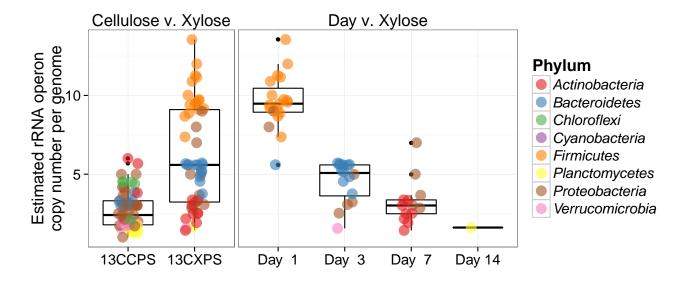


Figure S8: Estimated rRNA operon copy number per genome for 13 C responding OTUS. Panel titles indicate which labeled substrate(s) are depicted.

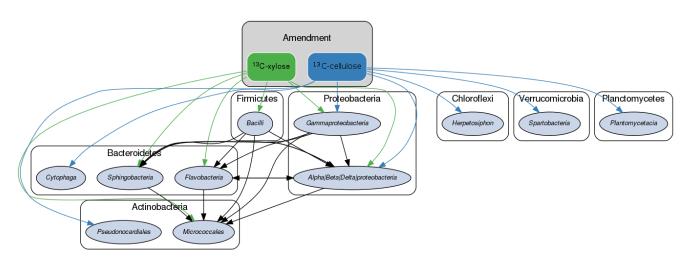


Figure S9: Conceptual model of soil food web in this experiment. Taxa shown possessed at least two 13 C responder OTUs for a given C substrate. Proteobacteria response was too varied taxonomically to depict at higher taxonomic resolution in this format. Black arrows indicate possible predator/prey interactions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

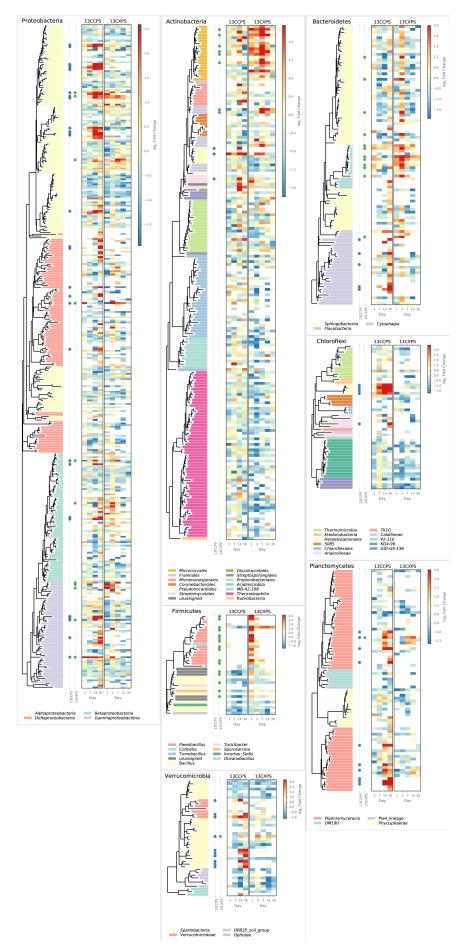


Figure S10: Phylum specific trees. Heatmap indicates fold change between heavy fractions of control gradients versus labeled gradients. Dots indicate the position of "responders" to 13 C-xylose (green) or 13 C-cellulose (blue).

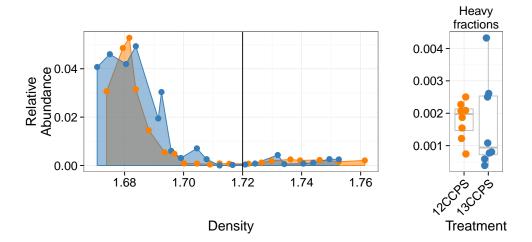


Figure S11: Density profile for a single 13 C-cellulose "non-responder" OTU in the labeled gradient, blue, and the control gradient, orange. Vertical line shows where "heavy" fractions begin as defined in our analysis. Panel at right shows relative abundance values in the heavy fractions for each gradient.

Table S1: 13 C-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	Pseudoxanthomonas sacheonensis, Pseudoxanthomonas dokdonensis	100.0	$Proteobacteria \ Gamma proteobacteria \ Xanthomonadales$
OTU.1023	4.61	30	No hits of at least 90% identity	80.54	$Verru comic robia\ Spartobacteria$ Chthoniobacterales
OTU.1065	5.31	14	No hits of at least 90% identity	84.55	Planctomycetes Planctomycetacia Planctomycetales
OTU.1087	4.32	14	Devosia soli, Devosia crocina, Devosia riboflavina	99.09	Proteobacteria Alphaproteobacteria Rhizobiales
OTU.1094	3.69	30	$Sporocytophaga\ myxococcoides$	99.55	Bacteroidetes Cytophagia Cytophagales
OTU.11	3.41	14	Stenotrophomonas pavanii, Stenotrophomonas maltophilia, Pseudomonas geniculata	99.54	$Proteobacteria \ Gamma proteobacteria \ Xanthomonadales$
OTU.114	2.78	14	Herbaspirillum sp. SUEMI03, Herbaspirillum sp. SUEMI10, Oxalicibacterium solurbis, Herminiimonas fonticola, Oxalicibacterium horti	100.0	Proteobacteria Betaproteobacteria Burkholderiales
OTU.119	3.31	14	Brevundimonas alba	100.0	Proteobacteria Alphaproteobacteria Caulobacterales
OTU.120	4.76	14	Vampirovibrio chlorellavorus	94.52	Cyanobacteria SM1D11 uncultured-bacterium
OTU.1204	4.32	30	Planctomyces limnophilus	91.78	Planctomycetes Planctomycetacia Planctomycetales
OTU.1312	4.07	30	Paucimonas lemoignei	99.54	Proteobacteria Betaproteobacteria Burkholderiales
OTU.132	2.81	14	Streptomyces spp.	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	Pseudoxanthomonas mexicana, Pseudoxanthomonas japonensis	100.0	$Proteobacteria \ Gamma proteobacteria \ Xanthomonadales$
OTU.165	3.1	14	Rhizobium skierniewicense, Rhizobium vignae, Rhizobium larrymoorei, Rhizobium alkalisoli, Rhizobium galegae, Rhizobium huautlense	100.0	Proteobacteria Alphaproteobacteria Rhizobiales

Table S1 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits B	BLAST %ID	Phylum;Class;Order
OTU.1754	4.48	14	Asticcacaulis biprosthecium, Asticcacaulis benevestitus	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	Rhizobium alamii, Rhizobium mesosinicum, Rhizobium mongolense, Arthrobacter viscosus, Rhizobium sullae, Rhizobium yanglingense, Rhizobium loessense	99.54	Proteobacteria Alphaproteobacteria Rhizobiales
OTU.204	3.81	14	No hits of at least 90% identity	nan	Planctomycetes Planctomycetacia Planctomycetales
OTU.2192	3.49	30	No hits of at least 90% identity	83.56	Verrucomicrobia Spartobacteria Chthoniobacterales
OTU.228	2.54	30	Sorangium cellulosum	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	Lentzea waywayandensis, Lentzea flaviverrucosa	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	Rhizobium giardinii, Rhizobium tubonense, Rhizobium tibeticum, Rhizobium mesoamericanum CCGE Rhizobium herbae, Rhizobium endophyticum	99.54 501,	Proteobacteria Alphaproteobacteria Rhizobiales
OTU.285	3.55	30	Blastopirellula marina	90.87	Planctomycetes Planctomycetacia Planctomycetales
OTU.32	2.34	3	Sandaracinus amylolyticus	94.98	Proteobacteria Deltaproteobacteria Myxococcales
OTU.327	2.99	14	Asticcacaulis biprosthecium, Asticcacaulis benevestitus	98.63	Proteobacteria Alphaproteobacteria Caulobacterales
OTU.351	3.54	14	Pirellula staleyi DSM 6068	91.86	Planctomycetes Planctomycetacia Planctomycetales
OTU.3594	3.83	30	Chondromyces robustus	90.41	Proteobacteria Deltaproteobacteria Myxococcales

Table S1 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits B	BLAST %ID	Phylum;Class;Order
OTU.3775	3.88	14	Devosia glacialis, Devosia chinhatensis, Devosia geojensis, Devosia yakushimensis	98.63	Proteobacteria Alphaproteobacteria Rhizobiales
OTU.429	3.7	30	Devosia limi, Devosia psychrophila	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	Chondromyces robustus	92.24	Proteobacteria Deltaproteobacteria Myxococcales
OTU.465	3.79	30	Ohtaekwangia kribbensis	92.73	Bacteroidetes Cytophagia Cytophagales
OTU.473	3.58	14	Pirellula staleyi DSM 6068	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	Delftia tsuruhatensis, Delftia lacustris	100.0	Proteobacteria Betaproteobacteria Burkholderiales
OTU.518	4.8	14	$Hydrogenophaga\ intermedia$	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	Devosia subaequoris	98.17	Proteobacteria Alphaproteobacteria Rhizobiales
OTU.573	3.03	30	$Adhaeribacter\ aerophilus$	92.76	$Bacteroidetes\ Cytophagia$ $Cytophagales$
OTU.6	3.62	7	Cellvibrio fulvus	100.0	Proteobacteria Gammaproteobacteria Pseudomonadales
OTU.600	3.48	30	No hits of at least 90% identity	80.37	Planctomycetes Planctomycetacia Planctomycetales
OTU.6062	4.83	30	Dokdonella sp. DC-3, Luteibacter rhizovicinus	97.26	Proteobacteria Gammaproteobacteria Xanthomonadales
OTU.627	4.43	14	Verrucomicrobiaceae bacterium DC2	a-G7 100.0	Verrucomicrobia Verrucomicrobiae Verrucomicrobiales

Table S1 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.633	3.84	30	No hits of at least 90% identity	89.5	Proteobacteria Deltaproteobacteria Myxococcales
OTU.638	4.0	30	Luteolibacter sp. CCTCC AB 2010 Luteolibacter algae	415, 93.61	Verrucomicrobia Verrucomicrobiae Verrucomicrobiales
OTU.64	4.31	14	No hits of at least 90% identity	89.5	Chloroflexi Herpetosiphonales Herpetosiphonaceae
OTU.663	3.63	30	Pirellula staleyi DSM 6068	90.87	Planctomycetes Planctomycetacia Planctomycetales
OTU.669	3.34	30	Ohtaekwangia koreensis	92.69	Bacteroidetes Cytophagia Cytophagales
OTU.670	2.87	30	Adhaeribacter aerophilus	91.78	Bacteroidetes Cytophagia Cytophagales
OTU.766	3.21	14	Devosia insulae	99.54	Proteobacteria Alphaproteobacteria Rhizobiales
OTU.83	5.61	14	Luteolibacter sp. CCTCC AB 2010	415 97.72	Verrucomicrobia Verrucomicrobiae Verrucomicrobiales
OTU.862	5.87	14	Allokutzneria albata	100.0	Actinobacteria Pseudonocardiales Pseudonocardiaceae
OTU.899	2.28	30	Enhygromyxa salina	97.72	Proteobacteria Deltaproteobacteria Myxococcales
OTU.90	2.94	14	Sphingopyxis panaciterrae, Sphingopyxis chilensis, Sphingopyxis sp. BZ30, Sphingomonas sp.	100.0	Proteobacteria Alphaproteobacteria Sphingomonadales
OTU.900	4.87	14	Brevundimonas vesicularis, Brevundimonas nasdae	100.0	Proteobacteria Alphaproteobacteria Caulobacterales
OTU.971	3.68	30	No hits of at least 90% identity	78.57	Chloroflexi Anaerolineae Anaerolineales
OTU.98	3.68	14	No hits of at least 90% identity	88.18	Chloroflexi Herpetosiphonales Herpetosiphonaceae
OTU.982	4.47	14	Devosia neptuniae	100.0	Proteobacteria Alphaproteobacteria Rhizobiales

^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	Paenibacillus daejeonensis	100.0	Firmicutes Bacilli Bacillales
OTU.1069	3.85	1	Paenibacillus terrigena	100.0	Firmicutes Bacilli Bacillales
OTU.107	2.25	3	Flavobacterium sp. 15C3, Flavobacterium banpakuense	99.54	Bacteroidetes Flavobacteria Flavobacteriales
OTU.11	5.25	7	Stenotrophomonas pavanii, Stenotrophomonas maltophilia, Pseudomonas geniculata	99.54	$Proteobacteria \ Gamma proteobacteria \ Xanthomonadales$
OTU.131	3.07	3	Flavobacterium fluvii, Flavobacteria bacterium HMD1033 Flavobacterium sp. HMD1001	100.0	Bacteroidetes Flavobacteria Flavobacteriales
OTU.14	3.92	3	Flavobacterium oncorhynchi, Flavobacterium glycines, Flavobacterium succinicans	99.09	Bacteroidetes Flavobacteria Flavobacteriales
OTU.150	3.08	14	No hits of at least 90% identity	86.76	Planctomycetes Planctomycetacia Planctomycetales
OTU.159	3.16	3	Flavobacterium hibernum	98.17	Bacteroidetes Flavobacteria Flavobacteriales
OTU.165	2.38	3	Rhizobium skierniewicense, Rhizobium vignae, Rhizobium larrymoorei, Rhizobium alkalisoli, Rhizobium galegae, Rhizobium huautlense	100.0	Proteobacteria Alphaproteobacteria Rhizobiales
OTU.183	3.31	3	No hits of at least 90% identity	89.5	Bacteroidetes Sphingobacteriia Sphingobacteriales
OTU.19	2.14	7	Rhizobium alamii, Rhizobium mesosinicum, Rhizobium mongolense, Arthrobacter viscosus, Rhizobium sullae, Rhizobium yanglingense, Rhizobium loessense	99.54	Proteobacteria Alphaproteobacteria Rhizobiales
OTU.2040	2.91	1	Paenibacillus pectinilyticus	100.0	Firmicutes Bacilli Bacillales
OTU.22	2.8	7	Paracoccus sp. NB88	99.09	Proteobacteria Alphaproteobacteria Rhodobacterales
OTU.2379	3.1	3	Flavobacterium pectinovorum, Flavobacterium sp. CS100	97.72	Bacteroidetes Flavobacteria Flavobacteriales
OTU.24	2.81	7	Cellulomonas aerilata, Cellulomonas humilata, Cellulomonas terrae, Cellulomonas soli, Cellulomonas xylanilytica	100.0	Actinobacteria Micrococcales Cellulomonadaceae
OTU.241	3.38	3	No hits of at least 90% identity	87.73	Verrucomicrobia Spartobacteria Chthoniobacterales

Table S2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.244	3.08	7	Cellulosimicrobium funkei, Cellulosimicrobium terreum	100.0	Actinobacteria Micrococcales Promicromonosporaceae
OTU.252	3.34	7	Promicromonospora thailandica	100.0	Actinobacteria Micrococcales Promicromonosporaceae
OTU.267	4.97	1	Paenibacillus pabuli, Paenibacillus tundrae, Paenibacillus taichungensis, Paenibacillus xylanexedens, Paenibacillus xylanilyticus	100.0	Firmicutes Bacilli Bacillales
OTU.277	3.52	3	Solibius ginsengiterrae	95.43	Bacteroidetes Sphingobacteriia Sphingobacteriales
OTU.290	3.59	1	Pantoea spp., Kluyvera spp., Klebsiella spp., Erwinia spp., Enterobacter spp., Buttiauxella spp.	100.0	Proteobacteria Gammaproteobacteria Enterobacteriales
OTU.3	2.61	1	[Brevibacterium] frigoritolerans, Bacillus sp. LMG 20238, Bacillus coahuilensis m4-4, Bacillus simplex	100.0	Firmicutes Bacilli Bacillales
OTU.319	3.98	1	Paenibacillus xinjiangensis	97.25	Firmicutes Bacilli Bacillales
OTU.32	3.0	3	$Sandaracinus\ amy lolyticus$	94.98	$Proteobacteria \ Delta proteobacteria \ Myxococcales$
OTU.335	2.53	1	Paenibacillus thailandensis	98.17	Firmicutes Bacilli Bacillales
OTU.346	3.44	3	$Pseudoduganella\ violaceinigra$	99.54	Proteobacteria Betaproteobacteria Burkholderiales
OTU.3507	2.36	1	Bacillus spp.	98.63	Firmicutes Bacilli Bacillales
OTU.3540	2.52	3	Flavobacterium terrigena	99.54	Bacteroidetes Flavobacteria Flavobacteriales
OTU.360	2.98	3	$Flavisolibacter\ ginsengisoli$	95.0	Bacteroidetes Sphingobacteriia Sphingobacteriales
OTU.369	5.05	1	Paenibacillus sp. D75, Paenibacillus glycanilyticus	100.0	Firmicutes Bacilli Bacillales
OTU.37	2.68	7	Phycicola gilvus, Microterricola viridarii, Frigoribacterium faeni, Frondihabitans sp. RS-15, Frondihabitans australicus	100.0	Actinobacteria Micrococcales Microbacteriaceae
OTU.394	4.06	1	Paenibacillus pocheonensis	100.0	Firmicutes Bacilli Bacillales
OTU.4	2.84	7	Agromyces ramosus	100.0	Actinobacteria Micrococcales Microbacteriaceae

Table S2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum; Class; Order
OTU.4446	3.49	7	Catenuloplanes niger, Catenuloplanes castaneus, Catenuloplanes atrovinosus, Catenuloplanes crispus, Catenuloplanes nepalensis, Catenuloplanes japonicus	97.72	Actinobacteria Frankiales Nakamurellaceae
OTU.4743	2.24	1	Lysinibacillus fusiformis, Lysinibacillus sphaericus	99.09	Firmicutes Bacilli Bacillales
OTU.48	2.99	1	Aeromonas spp.	100.0	Proteobacteria Gammaproteobacteria aaa34a10
OTU.5	3.69	7	Delftia tsuruhatensis, Delftia lacustris	100.0	Proteobacteria Betaproteobacteria Burkholderiales
OTU.5284	3.56	7	Isoptericola nanjingensis, Isoptericola hypogeus, Isoptericola variabilis	98.63	Actinobacteria Micrococcales Promicromonosporaceae
OTU.5603	3.96	1	Paenibacillus uliginis	100.0	Firmicutes Bacilli Bacillales
OTU.57	4.39	1	Paenibacillus castaneae	98.62	Firmicutes Bacilli Bacillales
OTU.5906	3.16	3	Terrimonas sp. M-8	96.8	Bacteroidetes Sphingobacteriia Sphingobacteriales
OTU.6	3.24	3	Cellvibrio fulvus	100.0	Proteobacteria Gammaproteobacteria Pseudomonadales
OTU.62	2.57	7	Nakamurella flavida	100.0	Actinobacteria Frankiales Nakamurellaceae
OTU.6203	3.32	3	Flavobacterium granuli, Flavobacterium glaciei	100.0	Bacteroidetes Flavobacteria Flavobacteriales
OTU.68	3.74	7	Shigella flexneri, Escherichia fergusonii, Escherichia coli, Shigella sonnei	100.0	Proteobacteria Gammaproteobacteria Enterobacteriales
OTU.760	2.89	3	Dyadobacter hamtensis	98.63	Bacteroidetes Cytophagia Cytophagales
OTU.8	2.26	1	Bacillus niacini	100.0	Firmicutes Bacilli Bacillales
OTU.843	3.62	1	Paenibacillus agarexedens	100.0	Firmicutes Bacilli Bacillales
OTU.9	2.04	1	Bacillus megaterium, Bacillus flexus	100.0	Firmicutes Bacilli Bacillales

 $^{^{\}rm a}$ Maximum observed log_2 of fold change. $^{\rm b}$ Day of maximum fold change.

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