

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

Contents

1	Supplemental Methods	2
1.1	Soil Collection and Preparation	2
1.2	Cellulose production	2
1.3	Soil microcosms	3
1.4	Nucleic acid extraction	4
1.5	Isopycnic centrifugation and fractionation	4
1.6	DNA Sequencing	4
1.7	Post-Sequencing Analysis	5
1.7.1	Sequence quality control	5
1.7.2	Sequence clustering	5
1.7.3	Phylogenetic analysis	5
1.7.4	Identifying OTUs that incorporated ^{13}C into their DNA	6
1.7.5	Community and Sequence Analysis	6
1.8	Buoyant density shift estimates	6
1.9	Sequencing and density fractionation statistics	7
2	Phylogenetic affiliation of ^{13}C-responsive microorganisms	7
2.1	Cellulose	7
2.1.1	Proteobacteria	7
2.1.2	Verrucomicrobia	7
2.1.3	Chloroflexi	8
2.1.4	Others	9
2.2	Xylose	9
3	Supplemental Figures and Tables	11

List of Figures

S1	Experimental Set Up	12
S2	Counts of ^{13}C -responders at each day	13
S3	Ordination of bulk samples	13
S4	Temporal fluctuations for OTUs that changed significantly in abundance with time	14

S5	Relative abundance of Classes over time for Classes that changed significantly in abundance with time	15
S6	Sum of bulk abundances with selected phylum for responder OTUs.	16
S7	Density profile for an example "responder"	17
S8	OTU <i>rrn</i> gene copy number with time and treatment	17
S9	Soil food web	18
S10	Phylogenetic trees	19
S11	Density profile for example "non-responder"	20

List of Tables

S1	¹³ C-cellulose responders BLAST against Living Tree Project	21
S2	¹³ C-xylose responders BLAST against Living Tree Project	25

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 ¹²C and another with ¹³C-glucose). All cellulose (¹²C and ¹³C) 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 ¹²C-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 $\mu\text{g ml}^{-1}$ BSA. Ground, purified cellulose contained 6.92 $\mu\text{g protein mg cellulose}^{-1}$ (*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, ^{13}C -cellulose had an average $96\% \pm 5$ (s.d.) degree of ^{13}C 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 CO_2) 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 (CO_2) 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 ^{13}C -labelling as follows: (1) unlabeled control, (2) ^{13}C -cellulose (synthesis and purity described above), (3) ^{13}C -xylose (98 atom% ^{13}C , 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 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 ^{13}C 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]. Cells were lysed by bead beating for 1 min at 5.5 ms^{-1} in 2 mL lysis tubes containing 0.5 g of 0.1 mm diameter silica/zirconia beads (treated at 300°C 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 μL 5 M NaCl and 60 μL 10% hexadecyltrimmonium bromide (CTAB)/0.7 M NaCl were added to lysis tube, vortexed, chilled for 1 min on ice, and centrifuged at $16,000 \times g$ for 5 min at 4°C. The aqueous layer was transferred to a new tube and reserved on ice. To increase DNA recovery, the pellet was back extracted with 85 μL 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 $16,000 \times g$, 4°C 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 μL TE and stored at -20°C. To prepare nucleic acid extracts for isopycnic centrifugation as previously described [12], DNA was size selected ($> 4\text{ kb}$) 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 μL 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 ^{12}C -control, five ^{13}C -xylose, and four ^{13}C -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 ^{13}C -enriched and ^{12}C -non-enriched DNA. Each gradient was loaded with approximately 5 μg of DNA and centrifuged on a Beckman Coulter OptimaTM MAX-E ultracentrifuge using a TLA-110 fixed-angle rotor for 66 h at 55,000 rpm and room temperature (RT). Fractions of $\sim 100 \mu\text{L}$ 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 $3.3 \mu\text{L s}^{-1}$ [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 μL [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 20°C [15]. The collected DNA fractions were purified by repetitive washing of Acroprep filter wells with TE. Finally, 50 μL 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 $1.67\text{--}1.75 \text{ g mL}^{-1}$ 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 μL 5U μL^{-1} AmpliTaq

Gold (Life Technologies, Grand Island, NY; N8080243), 2.5 μL 10X Buffer II (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 2.5 μL 25 mM MgCl_2 , 4 μL 5 mM dNTP, 1.25 μL 10 mg mL^{-1} BSA, 0.5 μL 10 μM BA515F, 1 μL 5 μM BA806R, 3 μL H_2O , 10 μL 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 ^{13}C 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 heavy 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.

DESeq2 was used to calculate moderated \log_2 fold change of labeled:control abundance and corresponding standard errors. We assessed the statistical significance of fold change values using a one-sided Wald test. The threshold for the null model was set at one standard deviation above the mean fold change for all OTUs. 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 [31] distances were used as the sample distance metric for ordination. The Phyloseq [32] wrapper for Vegan [33] (both R packages) was used to compute sample values along principal coordinate axes. GGplot2 [34] was used to display sample points along the first and second principal axes. Adonis tests [35] were done with 1000 permutations. We used the Picante R package to measure NTI and NRI with the default null model [36]. consenTRAIT values were measured using the R code described in the original consenTRAIT publication [37].

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 ^{13}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}C -cellulose and ^{13}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 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 ^{13}C -responsive microorganisms

2.1 Cellulose

2.1.1 Proteobacteria

Proteobacteria represent 46% of all ^{13}C -cellulose responding OTUs identified. *Cellvibrio* accounted for 3% of all proteobacterial ^{13}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 [38]. All ^{13}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 *Chondomyces* 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 [39].

2.1.2 Verrucomicrobia

Verrucomicrobia, cosmopolitan soil microbes [40], can comprise up to 23% of 16S rRNA gene sequences in high-throughput DNA sequencing surveys of SSU rRNA genes in soil [40] and can account for up to 9.8% of soil 16S rRNA [41]. Many *Verrucomicrobia* were first isolated in the last decade

[42] 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 [40]. Genomic analyses and physiological profiling of *Verrucomicrobia* isolates revealed *Verrucomicrobia* are capable of methanotrophy, diazotrophy, and cellulose degradation [42, 43], yet the function of soil *Verrucomicrobia* in global C-cycling remains unknown. However, *Verrucomicrobia* are hypothesized to degrade polysaccharides in many environments [44–46]. *Verrucomicrobia* comprise 16% of the total ^{13}C -cellulose responder OTUs detected. 40% of *Verrucomicrobia* ^{13}C -cellulose responders belong to the uncultured “FukuN18” family originally identified in freshwater lakes [47]. The strongest *Verrucomicrobial* responder OTU to ^{13}C -cellulose shared high sequence identity (97%) with an isolate from Norway tundra soil [48] although growth on cellulose was not assessed for this isolate. Only one other ^{13}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 [43]. The remaining ^{13}C -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 ^{13}C -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 [40]. Given their ubiquity and abundance in soil as well as their demonstrated incorporation of ^{13}C from ^{13}C -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 [49]. Recent studies have focused on *Chloroflexi* roles in C cycling [49–51] and several *Chloroflexi* utilize cellulose [49–51]. Cellulose degrading soil *Chloroflexi* have previously been identified in DNA-SIP studies [52] and *Chloroflexi* are among the six most abundant soil phyla commonly recovered soil microbial diversity surveys [53]. The cellulose degrading *Chloroflexi* in this study are only distantly related to isolates S1. *Chloroflexi* are typically not as abundant as *Verrucomicrobia* but are roughly as abundant as *Bacteroidetes* and *Planctomycetes* [53]. Four of five ^{13}C -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 [54]. One non-*Herpetosiphon* *Chloroflexi* OTU also from a poorly characterized *Chloroflexi* lineage (closest cultured isolate matched a proteobacterium at 78% sequence identity) responded to ^{13}C -cellulose (Figure S10). In our study, “*Herpetosiphon*” ^{13}C -cellulose responders did not show a delayed response to ^{13}C -cellulose as compared to other responders but nonetheless could have become labeled by feeding on primary ^{13}C -cellulose degraders. The prey specificity of predatory bacteria is not well established especially *in situ*. ^{13}C -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 ^{13}C -cellulose responders include a *Bacteroidetes* OTU that shares high sequence identity (99%) to *Sporocytophaga myxococcoides* a known cellulose degrader [55], and three *Actinobacteria* OTUs that share high sequence identity (100%) with isolates. One of the three *Actinobacteria* ^{13}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 *Allokutzneria albata* [56, 57] and *Lentzea waywayandensis* [58, 59]; neither isolate decomposes cellulose in culture. Nine *Planctomycetes* OTUs responded to ^{13}C -cellulose but none are within described genera (closest cultured isolate match 91% sequence identity, Table S1) (Figure S10). One ^{13}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” [60]; although, the phylogenetic position of “Melainabacteria” is debated [61]. The catalog of metabolic capabilities associated with cyanobacteria (or candidate phyla previously annotated as cyanobacteria) are quickly expanding [60, 61]. 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 [60]. Although we highlight ^{13}C -cellulose responders that share high sequence identity with described genera, most ^{13}C -cellulose responders uncovered in this experiment are not closely related to cultured isolates (Table S1). *Acidobacteria* did not pass or operational criteria for assessing ^{13}C 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 ^{13}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}C -xylose responder is closely related to isolates annotated as members of *Bacillus*, *Paenibacillus* or *Lysinibacillus*. *Bacteroidetes* ^{13}C -xylose responders are predominantly closely related to *Flavobacterium* species (5 of 8 total responders) (Table S2). Only one *Bacteroidetes* ^{13}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, [62]). Other *Bacteroidetes* responders share high sequence identities with canonical soil genera including *Dyadobacter*, *Solibius* and *Terrimonas*. Six of the 8 *Actinobacteria* ^{13}C -xylose responders are in the *Micrococcales* order. One ^{13}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 [63]. It is not possible to determine the specific origin of assimilated ^{13}C in a DNA-SIP experiment. ^{13}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}C labeled *Agromyces* OTU was assimilating ^{13}C primarily by predation if the *Agromyces* OTU was selective enough with respect to its prey that it primarily attacked ^{13}C -xylose assimilating organisms.

3 Supplemental Figures and Tables

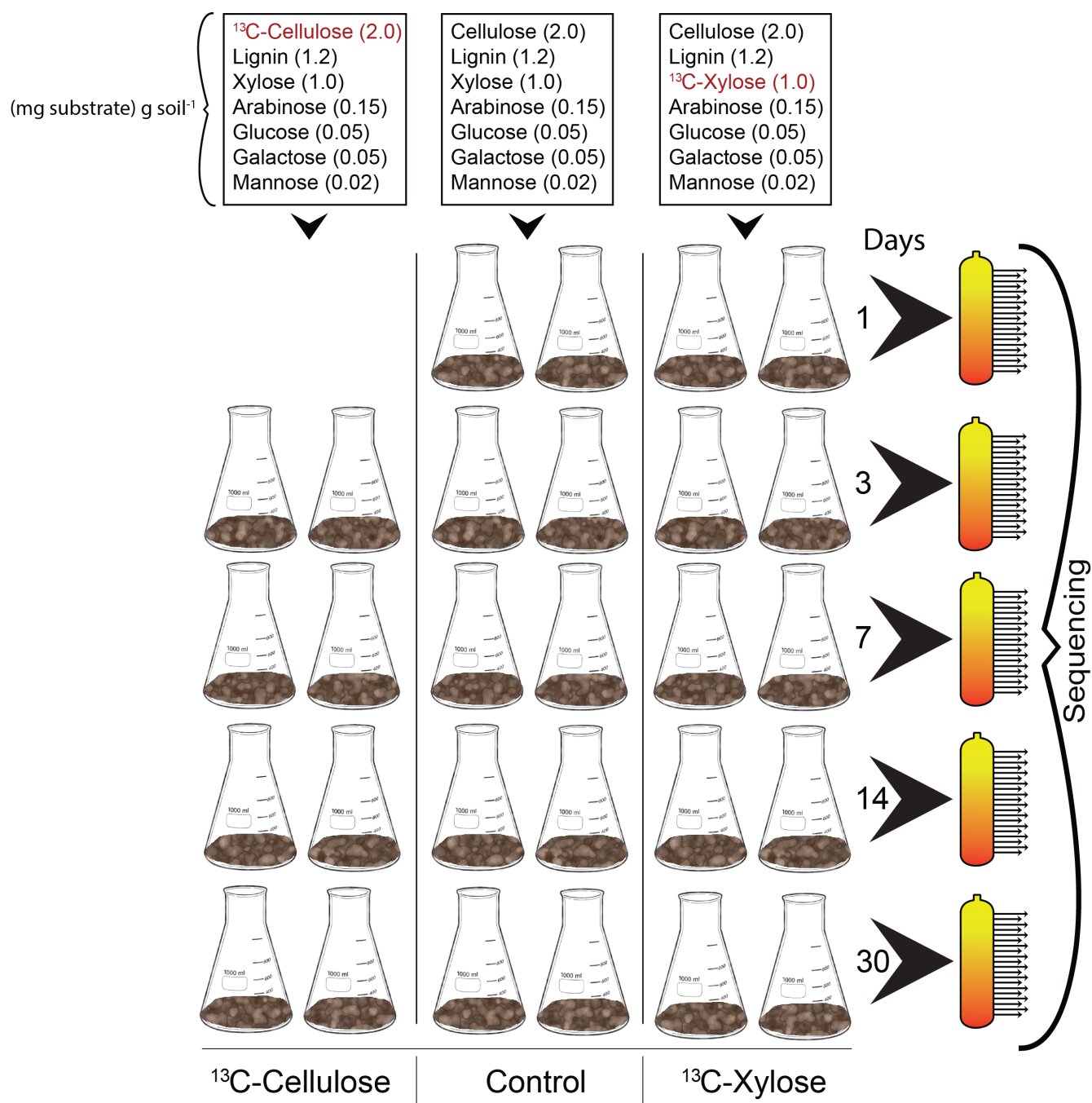


Figure S1: The experimental design. A carbon mixture, in addition to inorganic salts and amino acids (not shown here), was added to each soil microcosm where the only difference between treatments is the ¹³C-labeled isotope (in red). At days 1, 3, 7, 14, and 30 replicate microcosms were destructively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

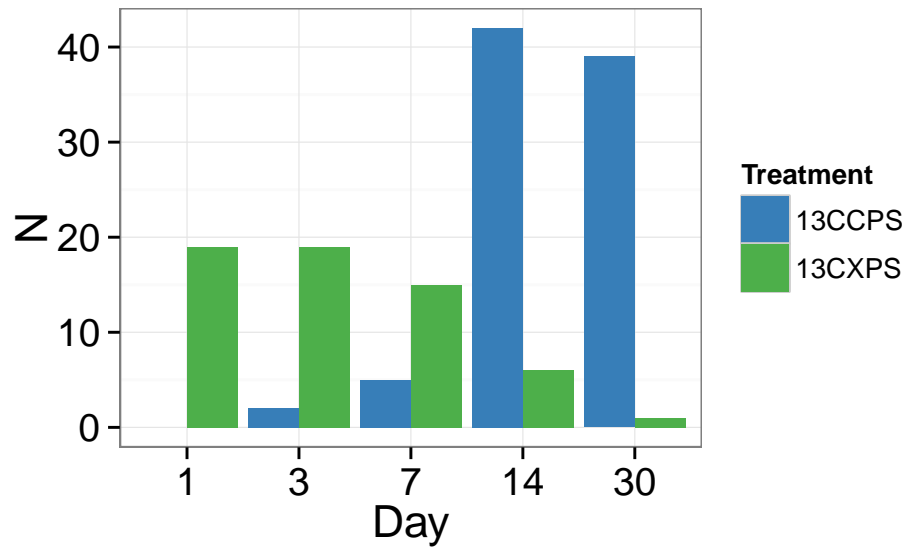


Figure S2: Counts of responders to each isotopically labeled substrate (cellulose and xylose) over time.

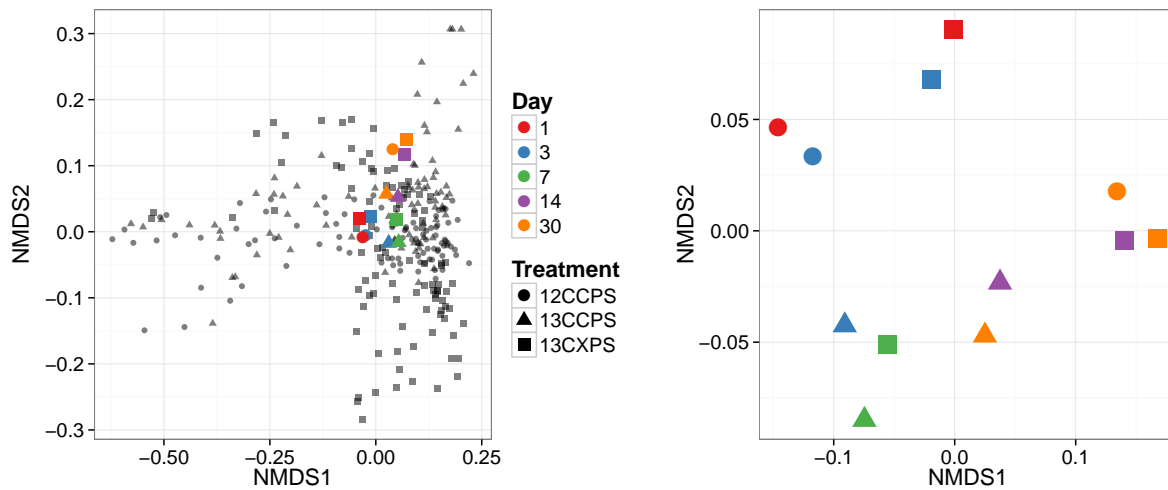


Figure S3: Ordination of bulk gradient fraction phylogenetic profiles.

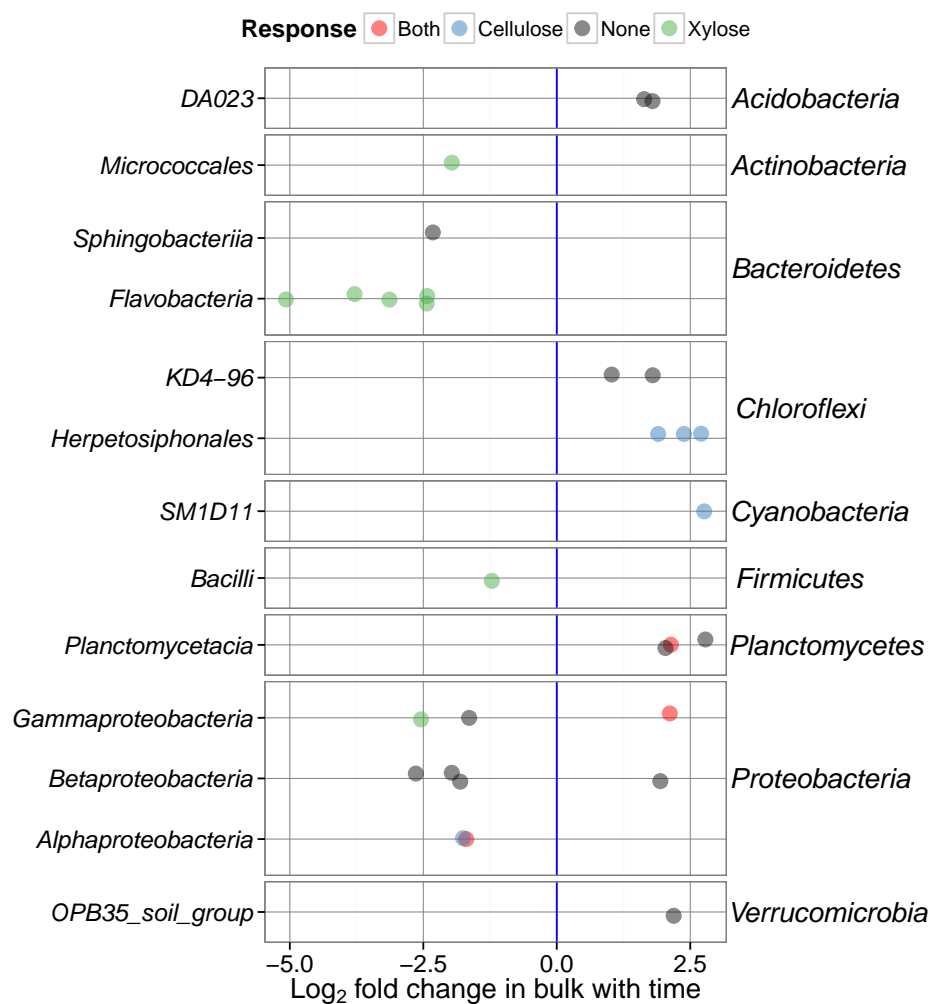


Figure S4: Fold change time^{-1} for OTUs that changed significantly in abundance over time. One panel per phylum (phyla indicated on the right). Taxonomic class indicated on the left.

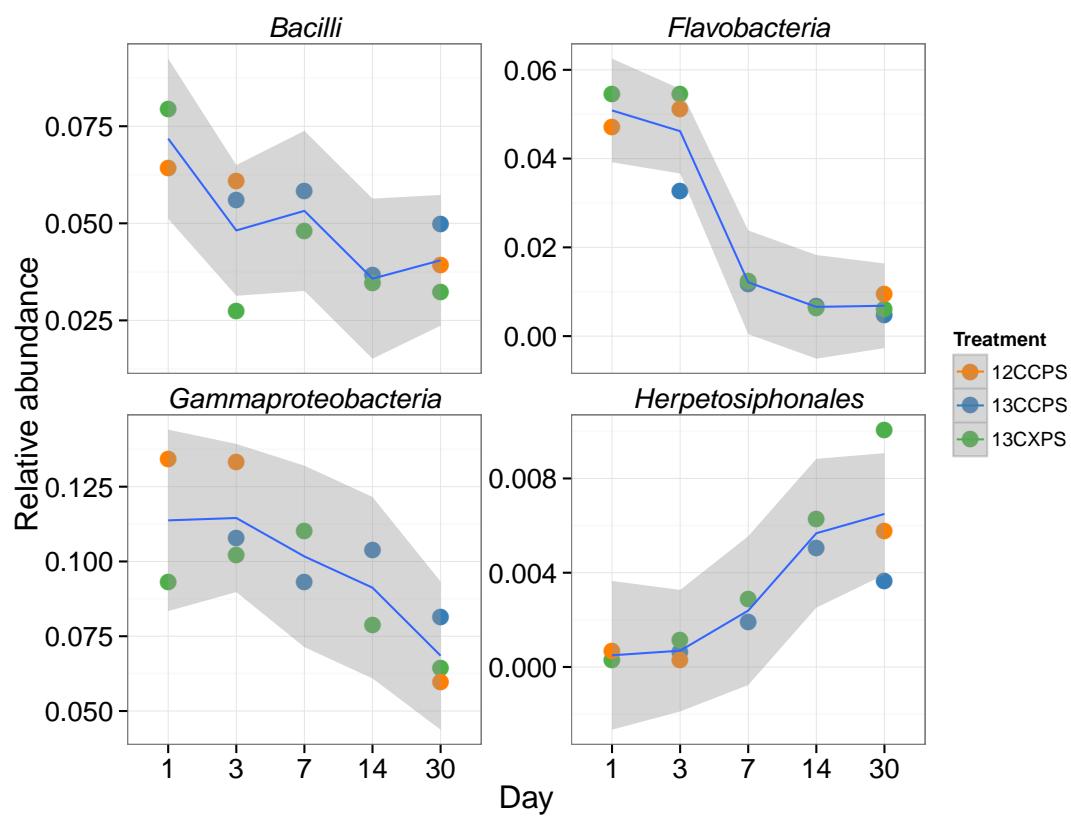


Figure S5: Relative abundance versus day for classes that changed significantly in relative abundance with time.

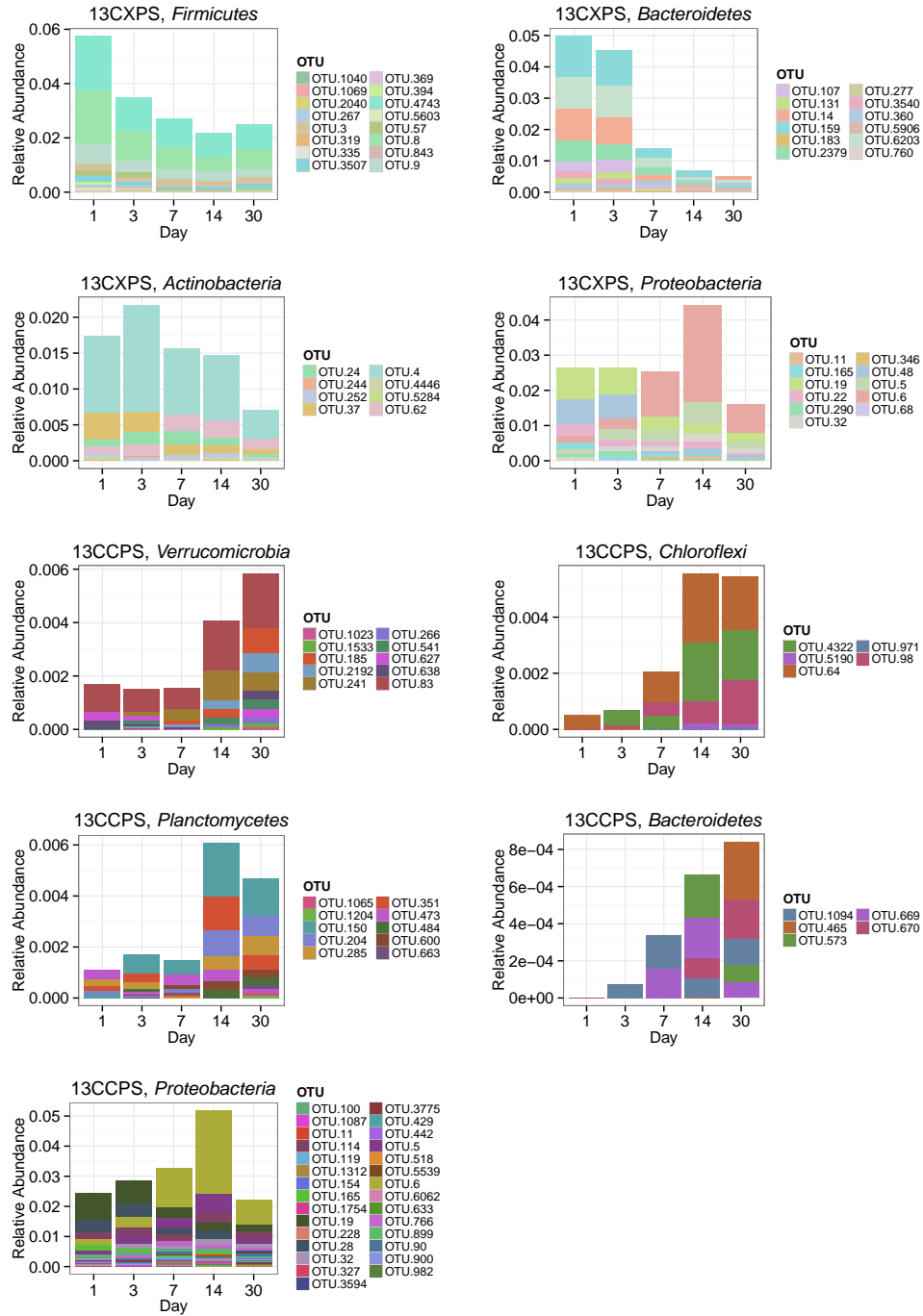


Figure S6: Sum of bulk abundances with selected phylum for responder OTUs.

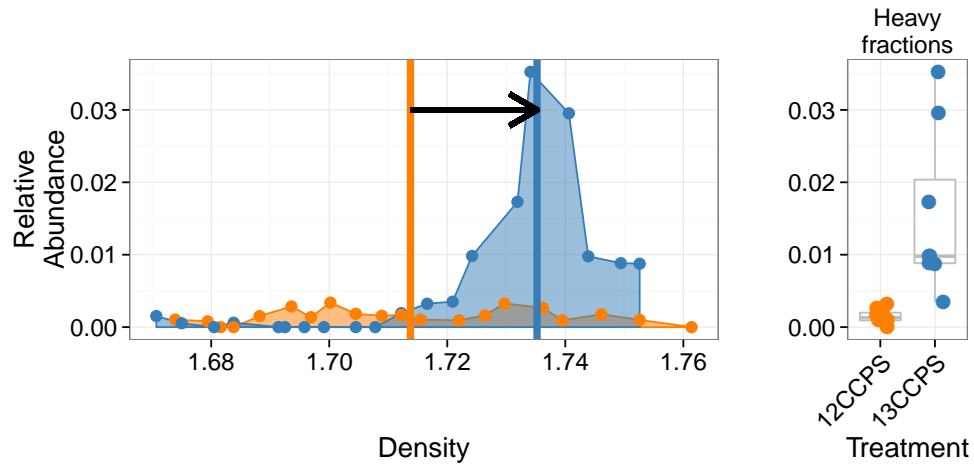


Figure S7: Density profile for a single ^{13}C -cellulose "responder" OTU in the labeled gradient, blue, and the control gradient, orange. Vertical lines show center of mass for each density profile and arrow denotes the magnitude and direction of the BD shift upon labeling. Panel at right shows relative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

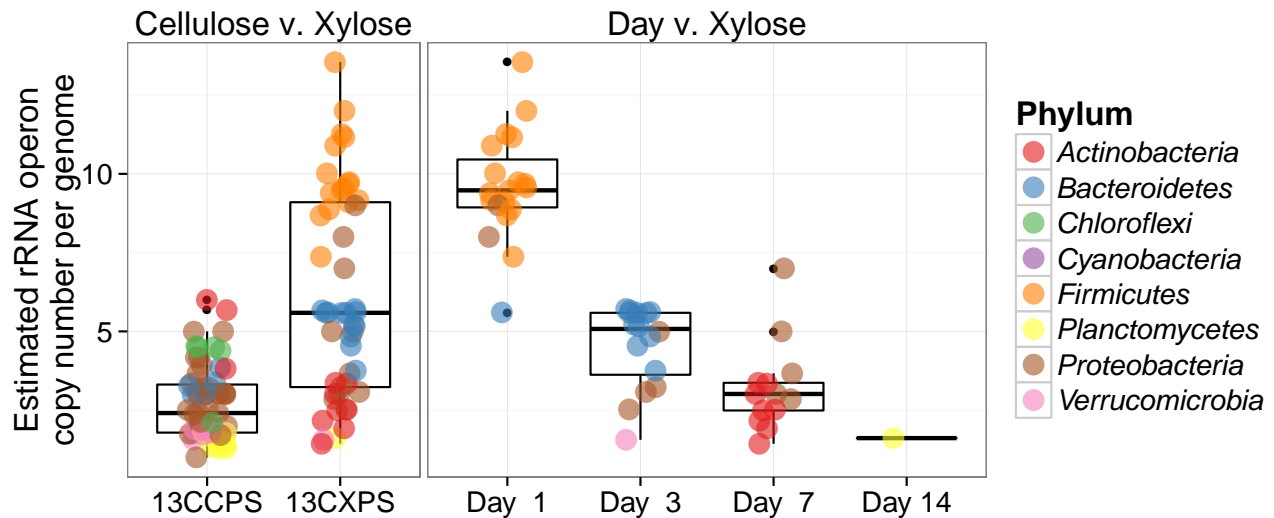


Figure S8: Estimated rRNA operon copy number per genome for ^{13}C responding OTUS. Panel titles indicate which labeled substrate(s) are depicted.

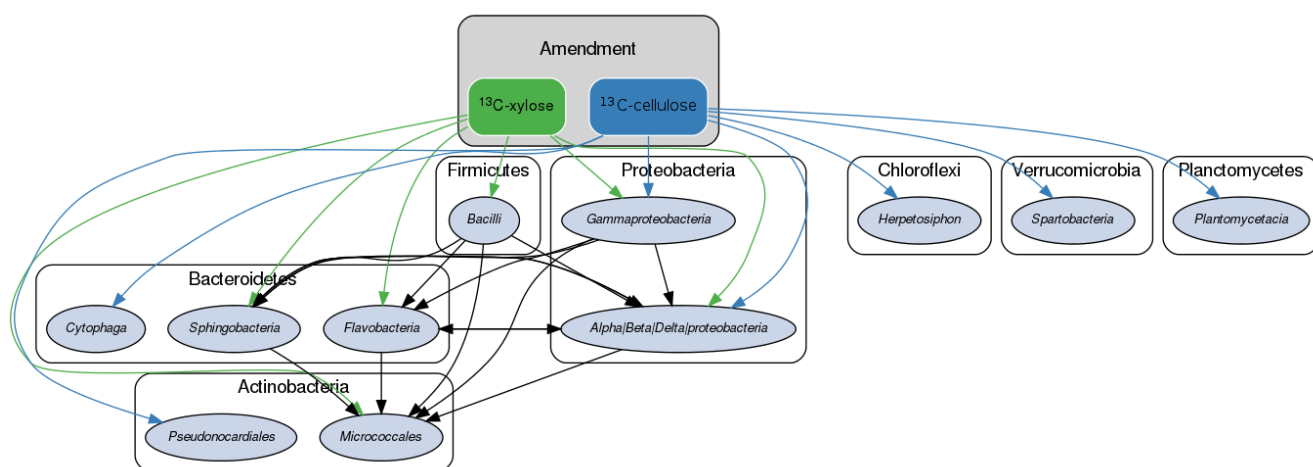


Figure S9: Conceptual model of soil food web in this experiment. Taxa shown possessed at least two ^{13}C responder OTUs for a given C substrate. *Proteobacteria* response was too varied taxonomically to depict at higher taxonomic resolution in this format. Black arrows indicate possible predator/prey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

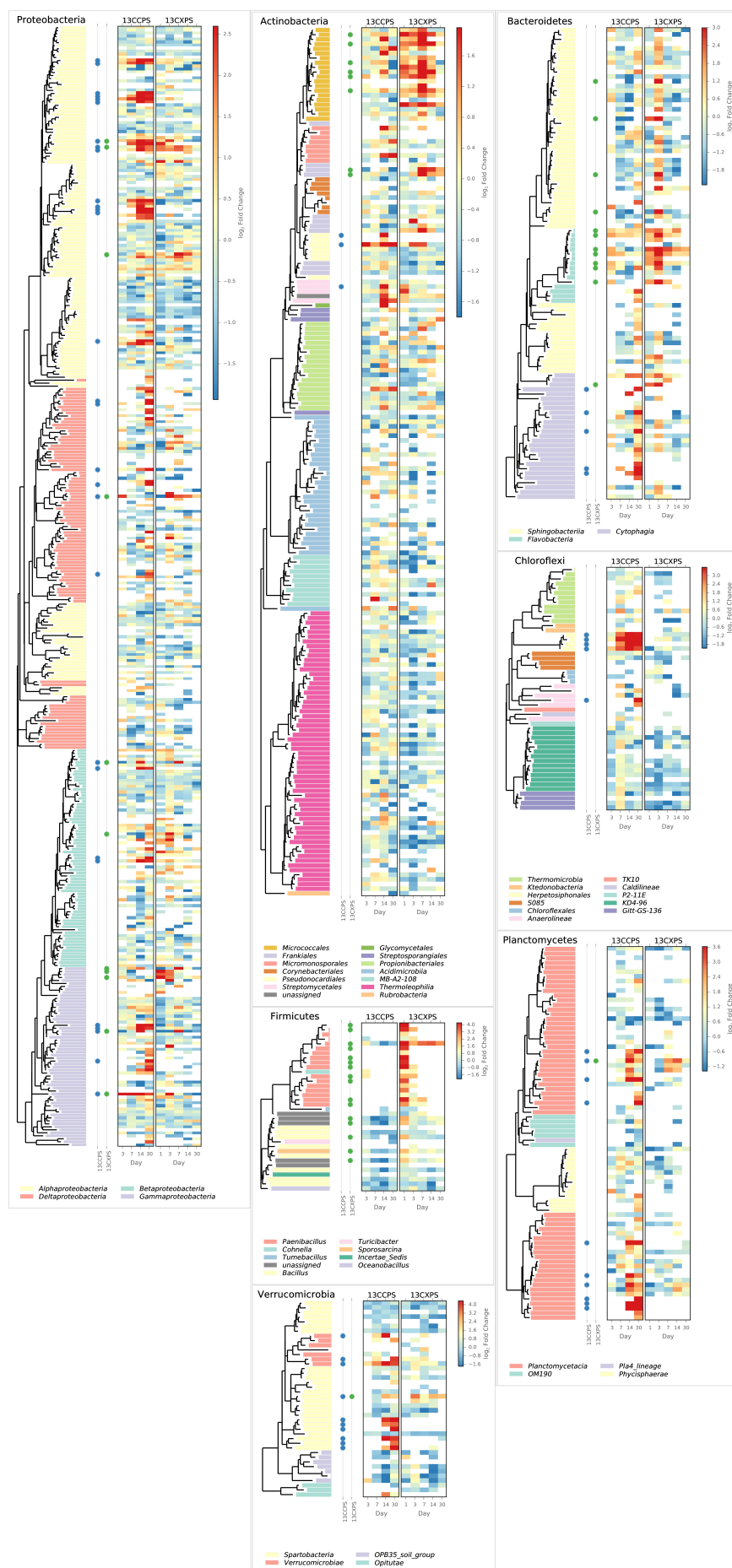


Figure S10: Phylum specific trees. Heatmap indicates ¹⁹log fold change between heavy fractions of control gradients versus labeled gradients. Dots indicate the position of "responders" to ¹³C-xylose (green) or ¹³C-cellulose (blue).

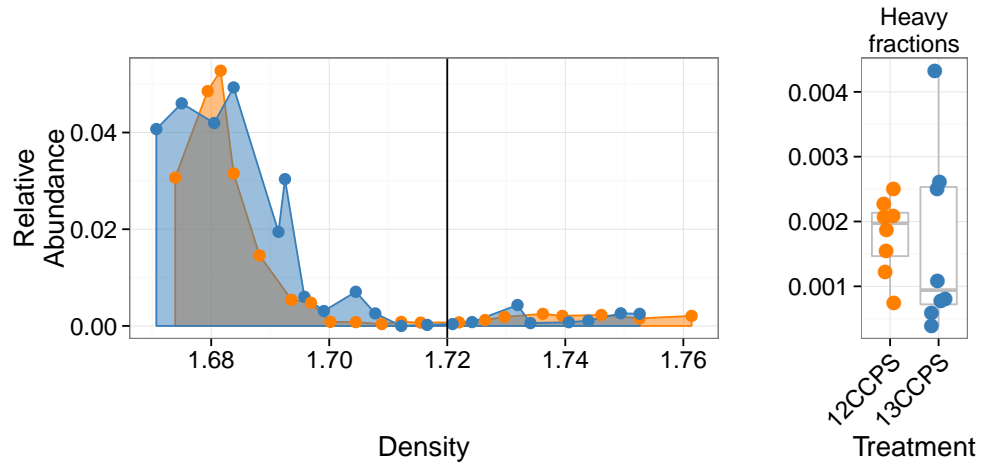


Figure S11: Density profile for a single ^{13}C -cellulose "non-responder" OTU in the labeled gradient, blue, and the control gradient, orange. Vertical line shows where "heavy" fractions begin as defined in our analysis. Panel at right shows relative abundance values in the heavy fractions for each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

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

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

Table S1 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.1754	4.48	14	<i>Asticcacaulis biprosthecium</i> , <i>Asticcacaulis benevestitus</i>	96.8	<i>Proteobacteria</i> <i>Alphaproteobacteria</i> <i>Caulobacterales</i>
OTU.185	4.37	14	No hits of at least 90% identity	85.14	<i>Verrucomicrobia</i> <i>Spartobacteria</i> <i>Chthoniobacterales</i>
OTU.19	2.44	14	<i>Rhizobium alarii</i> , <i>Rhizobium mesosinicum</i> , <i>Rhizobium mongolense</i> , <i>Arthrobacter viscosus</i> , <i>Rhizobium sullae</i> , <i>Rhizobium yanglingense</i> , <i>Rhizobium loessense</i>	99.54	<i>Proteobacteria</i> <i>Alphaproteobacteria</i> <i>Rhizobiales</i>
OTU.204	3.81	14	No hits of at least 90% identity	nan	<i>Planctomycetes</i> <i>Planctomycetacia</i> <i>Planctomycetales</i>
OTU.2192	3.49	30	No hits of at least 90% identity	83.56	<i>Verrucomicrobia</i> <i>Spartobacteria</i> <i>Chthoniobacterales</i>
OTU.228	2.54	30	<i>Sorangium cellulosum</i>	98.17	<i>Proteobacteria</i> <i>Deltaproteobacteria</i> <i>Myxococcales</i>
OTU.241	2.66	14	No hits of at least 90% identity	87.73	<i>Verrucomicrobia</i> <i>Spartobacteria</i> <i>Chthoniobacterales</i>
OTU.257	2.94	14	<i>Lentzea waywayandensis</i> , <i>Lentzea flaviverrucosa</i>	100.0	<i>Actinobacteria</i> <i>Pseudonocardiales</i> <i>Pseudonocardiaceae</i>
OTU.266	4.54	30	No hits of at least 90% identity	83.64	<i>Verrucomicrobia</i> <i>Spartobacteria</i> <i>Chthoniobacterales</i>
OTU.28	2.59	14	<i>Rhizobium giardinii</i> , <i>Rhizobium tubonense</i> , <i>Rhizobium tibeticum</i> , <i>Rhizobium mesoamericanum</i> CCGE 501, <i>Rhizobium herbae</i> , <i>Rhizobium endophyticum</i>	99.54	<i>Proteobacteria</i> <i>Alphaproteobacteria</i> <i>Rhizobiales</i>
OTU.285	3.55	30	<i>Blastopirellula marina</i>	90.87	<i>Planctomycetes</i> <i>Planctomycetacia</i> <i>Planctomycetales</i>
OTU.32	2.34	3	<i>Sandaracinus amylolyticus</i>	94.98	<i>Proteobacteria</i> <i>Deltaproteobacteria</i> <i>Myxococcales</i>
OTU.327	2.99	14	<i>Asticcacaulis biprosthecium</i> , <i>Asticcacaulis benevestitus</i>	98.63	<i>Proteobacteria</i> <i>Alphaproteobacteria</i> <i>Caulobacterales</i>
OTU.351	3.54	14	<i>Pirellula staleyi</i> DSM 6068	91.86	<i>Planctomycetes</i> <i>Planctomycetacia</i> <i>Planctomycetales</i>
OTU.3594	3.83	30	<i>Chondromyces robustus</i>	90.41	<i>Proteobacteria</i> <i>Deltaproteobacteria</i> <i>Myxococcales</i>

Table S1 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.3775	3.88	14	<i>Devosia glacialis</i> , <i>Devosia chinhatensis</i> , <i>Devosia geojensis</i> , <i>Devosia yakushimensis</i>	98.63	<i>Proteobacteria</i> <i>Alphaproteobacteria Rhizobiales</i>
OTU.429	3.7	30	<i>Devosia limi</i> , <i>Devosia psychrophila</i>	97.72	<i>Proteobacteria</i> <i>Alphaproteobacteria Rhizobiales</i>
OTU.4322	4.19	14	No hits of at least 90% identity	89.14	<i>Chloroflexi Herpetosiphonales</i> <i>Herpetosiphonaceae</i>
OTU.442	3.05	30	<i>Chondromyces robustus</i>	92.24	<i>Proteobacteria</i> <i>Deltaproteobacteria</i> <i>Myxococcales</i>
OTU.465	3.79	30	<i>Ohtaekwangia kribbensis</i>	92.73	<i>Bacteroidetes Cytophagia</i> <i>Cytophagales</i>
OTU.473	3.58	14	<i>Pirellula staleyi</i> DSM 6068	90.91	<i>Planctomycetes</i> <i>Planctomycetacia</i> <i>Planctomycetales</i>
OTU.484	4.92	14	No hits of at least 90% identity	89.09	<i>Planctomycetes</i> <i>Planctomycetacia</i> <i>Planctomycetales</i>
OTU.5	2.69	14	<i>Delftia tsuruhatensis</i> , <i>Delftia lacustris</i>	100.0	<i>Proteobacteria</i> <i>Betaproteobacteria</i> <i>Burkholderiales</i>
OTU.518	4.8	14	<i>Hydrogenophaga intermedia</i>	100.0	<i>Proteobacteria</i> <i>Betaproteobacteria</i> <i>Burkholderiales</i>
OTU.5190	3.6	30	No hits of at least 90% identity	88.13	<i>Chloroflexi Herpetosiphonales</i> <i>Herpetosiphonaceae</i>
OTU.541	4.49	30	No hits of at least 90% identity	84.23	<i>Verrucomicrobia Spartobacteria</i> <i>Chthoniobacterales</i>
OTU.5539	4.01	14	<i>Devosia subaequoris</i>	98.17	<i>Proteobacteria</i> <i>Alphaproteobacteria Rhizobiales</i>
OTU.573	3.03	30	<i>Adhaeribacter aerophilus</i>	92.76	<i>Bacteroidetes Cytophagia</i> <i>Cytophagales</i>
OTU.6	3.62	7	<i>Cellvibrio fulvus</i>	100.0	<i>Proteobacteria</i> <i>Gammaproteobacteria</i> <i>Pseudomonadales</i>
OTU.600	3.48	30	No hits of at least 90% identity	80.37	<i>Planctomycetes</i> <i>Planctomycetacia</i> <i>Planctomycetales</i>
OTU.6062	4.83	30	<i>Dokdonella</i> sp. DC-3, <i>Luteibacter rhizovicius</i>	97.26	<i>Proteobacteria</i> <i>Gammaproteobacteria</i> <i>Xanthomonadales</i>
OTU.627	4.43	14	<i>Verrucomicrobiaceae</i> bacterium DC2a-G7	100.0	<i>Verrucomicrobia</i> <i>Verrucomicrobiae</i> <i>Verrucomicrobiales</i>

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	<i>Proteobacteria</i> <i>Deltaproteobacteria</i> <i>Myxococcales</i>
OTU.638	4.0	30	<i>Luteolibacter</i> sp. CCTCC AB 2010415, <i>Luteolibacter</i> algae	93.61	<i>Verrucomicrobia</i> <i>Verrucomicrobiae</i> <i>Verrucomicrobiales</i>
OTU.64	4.31	14	No hits of at least 90% identity	89.5	<i>Chloroflexi</i> <i>Herpetosiphonales</i> <i>Herpetosiphonaceae</i>
OTU.663	3.63	30	<i>Pirellula staleyi</i> DSM 6068	90.87	<i>Planctomycetes</i> <i>Planctomycetacia</i> <i>Planctomycetales</i>
OTU.669	3.34	30	<i>Ohtaekwangia koreensis</i>	92.69	<i>Bacteroidetes</i> <i>Cytophagia</i> <i>Cytophagales</i>
OTU.670	2.87	30	<i>Adhaeribacter aerophilus</i>	91.78	<i>Bacteroidetes</i> <i>Cytophagia</i> <i>Cytophagales</i>
OTU.766	3.21	14	<i>Devosia insulae</i>	99.54	<i>Proteobacteria</i> <i>Alphaproteobacteria</i> <i>Rhizobiales</i>
OTU.83	5.61	14	<i>Luteolibacter</i> sp. CCTCC AB 2010415	97.72	<i>Verrucomicrobia</i> <i>Verrucomicrobiae</i> <i>Verrucomicrobiales</i>
OTU.862	5.87	14	<i>Allokutzneria albata</i>	100.0	<i>Actinobacteria</i> <i>Pseudonocardiales</i> <i>Pseudonocardiaceae</i>
OTU.899	2.28	30	<i>Enhygromyxa salina</i>	97.72	<i>Proteobacteria</i> <i>Deltaproteobacteria</i> <i>Myxococcales</i>
OTU.90	2.94	14	<i>Sphingopyxis panaciterrae</i> , <i>Sphingopyxis chilensis</i> , <i>Sphingopyxis</i> sp. BZ30, <i>Sphingomonas</i> sp.	100.0	<i>Proteobacteria</i> <i>Alphaproteobacteria</i> <i>Sphingomonadales</i>
OTU.900	4.87	14	<i>Brevundimonas vesicularis</i> , <i>Brevundimonas nasdae</i>	100.0	<i>Proteobacteria</i> <i>Alphaproteobacteria</i> <i>Caulobacteriales</i>
OTU.971	3.68	30	No hits of at least 90% identity	78.57	<i>Chloroflexi</i> <i>Anaerolineae</i> <i>Anaerolineales</i>
OTU.98	3.68	14	No hits of at least 90% identity	88.18	<i>Chloroflexi</i> <i>Herpetosiphonales</i> <i>Herpetosiphonaceae</i>
OTU.982	4.47	14	<i>Devosia neptuniae</i>	100.0	<i>Proteobacteria</i> <i>Alphaproteobacteria</i> <i>Rhizobiales</i>

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

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

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

Table S2 – continued from previous page

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

Table S2 – continued from previous page

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

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

References

- [1] Berthrong S-T, Buckley D-H, Drinkwater L-E (2013) Agricultural Management and Labile Carbon Additions Affect Soil Microbial Community Structure and Interact with Carbon and Nitrogen Cycling. *Microbial Ecology* 66(1): 158–170.
- [2] Heo M-S, Son H-J (2002) Development of an optimized simple chemically defined medium for bacterial cellulose production by *Acetobacter* sp. A9 in shaking cultures. *Biotechnology and Applied Biochemistry* 36(1): 41.
- [3] Cambardella C-A, Elliott E-T (1992) Particulate Soil Organic-Matter Changes across a Grassland Cultivation Sequence. *Soil Science Society of America Journal* 56(3): 777.
- [4] Bradford M-M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72(1-2): 248–254.
- [5] Benedict S-R (1909) A reagent for the detection of reducing sugars. *Journal of Biological Chemistry* 5(5): 485–487.
- [6] Datta R, Vranová V, Pavelka M, Rejšek K, Formánek P (2014) Effect of soil sieving on respiration induced by low-molecular-weight substrates. *International Agrophysics* 28(1): ???.
- [7] Schneckenberger K, Demin D, Stahr K, Kuzyakov Y (2008) Microbial utilization and mineralization of [14C]glucose added in six orders of concentration to soil. *Soil Biology and Biochemistry* 40(8): 1981–1988.
- [8] Yan J, Hu Z, Pu Y, Brummer E-C, Ragauskas A-J (2010) Chemical compositions of four switchgrass populations. *Biomass and Bioenergy* 34(1): 48–53.
- [9] David K, Ragauskas A-J (2010) Switchgrass as an energy crop for biofuel production: A review of its ligno-cellulosic chemical properties. *Energy Environ. Sci.* 3(9): 1182.
- [10] Linn D-M, Doran J-W (1984) Aerobic and Anaerobic Microbial Populations in No-till and Plowed Soils1. *Soil Science Society of America Journal* 48(4): 794.
- [11] Griffiths R-I, Whiteley A-S, O'Donnell A-G, Bailey M-J (2000) Rapid Method for Coextraction of DNA and RNA from Natural Environments for Analysis of Ribosomal DNA- and rRNA-Based Microbial Community Composition. *Applied and Environmental Microbiology* 66(12): 5488–5491.
- [12] Buckley D-H, Huangyutitham V, Hsu S-F, Nelson T-A (2007) Stable Isotope Probing with ¹⁵N Achieved by Disentangling the Effects of Genome G+C Content and Isotope Enrichment on DNA Density. *Applied and Environmental Microbiology* 73(10): 3189–3195.
- [13] Neufeld J-D, Vohra J, Dumont M-G, Lueders T, Manefield M, Friedrich M-W, *et al.* (2007) DNA stable-isotope probing. *Nature Protocols* 2(4): 860–866.
- [14] Manefield M, Whiteley A-S, Griffiths R-I, Bailey M-J (2002) RNA Stable Isotope Probing a Novel Means of Linking Microbial Community Function to Phylogeny. *Applied and Environmental Microbiology* 68(11): 5367–5373.

- [15] Birnie G (1978) Centrifugal Separations: Molecular and Cell Biology.
- [16] Hamady M, Walker J-J, Harris J-K, Gold N-J, Knight R (2008) Error-correcting barcoded primers for pyrosequencing hundreds of samples in multiplex. *Nat Meth* 5(3): 235–237.
- [17] Edgar R-C (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Meth* 10(10): 996–998.
- [18] Edgar R-C (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26(19): 2460–2461.
- [19] Caporaso J-G, Kuczynski J, Stombaugh J, Bittinger K, Bushman F-D, Costello E-K, *et al.* (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Meth* 7(5): 335–336.
- [20] Pruesse E, Quast C, Knittel K, Fuchs B-M, Ludwig W, Peplies J, *et al.* (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research* 35(21): 7188–7196.
- [21] Schloss P-D, Westcott S-L, Ryabin T, Hall J-R, Hartmann M, Hollister E-B, *et al.* (2009) Introducing mothur: Open-Source Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Applied and Environmental Microbiology* 75(23): 7537–7541.
- [22] DeSantis T-Z, Hugenholtz P, Keller K, Brodie E-L, Larsen N, Piceno Y-M, *et al.* (2006) NAST: a multiple sequence alignment server for comparative analysis of 16S rRNA genes. *Nucleic Acids Research* 34(Web Server): W394–W399.
- [23] Nawrocki E-P, Eddy S-R (2013) Infernal 1.1: 100-fold faster RNA homology searches. *Bioinformatics* 29(22): 2933–2935.
- [24] Nawrocki E-P, Kolbe D-L, Eddy S-R (2009) Infernal 1.0: inference of RNA alignments. *Bioinformatics* 25(10): 1335–1337.
- [25] Price M-N, Dehal P-S, Arkin A-P (2009) FastTree: Computing Large Minimum Evolution Trees with Profiles instead of a Distance Matrix. *Molecular Biology and Evolution* 26(7): 1641–1650.
- [26] Buckley D-H (2011) Stable Isotope Probing Techniques Using ^{15}N . *Stable Isotope Probing and Related Technologies* (American Society of Microbiology, ???), pp 129–147.
- [27] Radajewski S, McDonald I-R, Murrell J-C (2003) Stable-isotope probing of nucleic acids: a window to the function of uncultured microorganisms. *Current Opinion in Biotechnology* 14(3): 296–302.
- [28] Youngblut N-D, Buckley D-H (2014) Intra-genomic variation in G + C content and its implications for DNA stable isotope probing. *Environmental Microbiology Reports* ???: n/a.
- [30] McMurdie P-J, Holmes S (2014) Waste Not Want Not: Why Rarefying Microbiome Data Is Inadmissible. *PLoS Computational Biology* 10(4): e1003531.

- [31] Lozupone C, Knight R (2005) UniFrac: a New Phylogenetic Method for Comparing Microbial Communities. *Applied and Environmental Microbiology* 71(12): 8228–8235.
- [32] McMurdie P-J, Holmes S (2013) phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE* 8(4): e61217.
- [33] Dixon P (2003) VEGAN a package of R functions for community ecology. *Journal of Vegetation Science* 14(6): 927–930.
- [34] Wickham H (2009) ggplot2.
- [35] Anderson M-J (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26(1): 32–46.
- [36] Kembel S, Cowan P, Helmus M, Cornwell W, Morlon H, Ackerly D, *et al.* (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26: 1463–1464.
- [37] Martiny A-C, Treseder K, Pusch G (2013) Phylogenetic conservatism of functional traits in microorganisms. *ISMEJ* 7(4): 830–838.
- [38] Boone D (2001) Bergey’s manual of systematic bacteriology.
- [39] Tavernier M-L, Delattre C, Petit E, Michaud P (2008) β -(1,4)-Polyglucuronic Acids - An Overview. *TOBIOTJ* 2(1): 73–86.
- [40] Bergmann G-T, Bates S-T, Eilers K-G, Lauber C-L, Caporaso J-G, Walters W-A, *et al.* (2011) The under-recognized dominance of Verrucomicrobia in soil bacterial communities. *Soil Biology and Biochemistry* 43(7): 1450–1455.
- [41] Buckley D-H, Schmidt T-M (2001) Environmental factors influencing the distribution of rRNA from Verrucomicrobia in soil. *FEMS Microbiol Ecol* 35(1): 105–112.
- [42] Wertz J-T, Kim E, Breznak J-A, Schmidt T-M, Rodrigues J-LM (2011) Genomic and physiological characterization of the verrucomicrobia isolate diplosphaera colitermitum gen. nov. sp. nov., reveals microaerophily and nitrogen fixation genes. *Appl Environ Microbiol* 78(5): 1544–1555.
- [43] Otsuka S, Ueda H, Suenaga T, Uchino Y, Hamada M, Yokota A, *et al.* (2012) Roseimicrobium gellanilyticum gen. nov. sp. nov., a new member of the class Verrucomicrobiae. *Int J Syst Evol Microbiol* 63(Pt 6): 1982–1986.
- [44] Fierer N, Ladau J, Clemente J-C, Leff J-W, Owens S-M, Pollard K-S, *et al.* (2013) Reconstructing the microbial diversity and function of pre-agricultural tallgrass prairie soils in the united states. *Science* 342(6158): 621–624.
- [45] Herlemann D-PR, Lundin D, Labrenz M, Jurgens K, Zheng Z, Aspeborg H, *et al.* (2013) Metagenomic de novo assembly of an aquatic representative of the verrucomicrobial class spartobacteria. *mBio* 4(3): e0056912.
- [46] Chin K-J, Hahn D, Hengstmann U, Liesack W, Janssen P-H (1999) Characterization and identification of numerically abundant culturable bacteria from the anoxic bulk soil of rice paddy microcosms. *Appl Environ Microbiol* 65(11): 5042–5049.

- [47] Parveen B, Mary I, Vellet A, Ravet V, Debroas D (2013) Temporal dynamics and phylogenetic diversity of free-living and particle-associated Verrucomicrobia communities in relation to environmental variables in a mesotrophic lake. *FEMS Microbiol Ecol* 83(1): 189–201.
- [48] Jiang F, Li W, Xiao M, Dai J, Kan W, Chen L, *et al.* (2011) *Luteolibacter luojiensis* sp. nov. isolated from Arctic tundra soil, and emended description of the genus *Luteolibacter*. *Int J Syst Evol Microbiol* 62(Pt 9): 2259–2263.
- [49] Hug L-A, Castelle C-J, Wrighton K-C, Thomas B-C, Sharon I, Frischkorn K-R, *et al.* (2013) Community genomic analyses constrain the distribution of metabolic traits across the Chloroflexi phylum and indicate roles in sediment carbon cycling. *Microbiome* 1(1): 22.
- [50] Goldfarb K-C, Karaoz U, Hanson C-A, Santee C-A, Bradford M-A, Treseder K-K, *et al.* (2011) Differential Growth Responses of Soil Bacterial Taxa to Carbon Substrates of Varying Chemical Recalcitrance. *Frontiers in Microbiology* 2: 94. doi: 10.3389/fmicb.2011.00094
- [51] Cole J-K, Gieler B-A, Heisler D-L, Palisoc M-M, Williams A-J, Dohnalkova A-C, *et al.* (2013) *Kallotenue papyrolyticum* gen. nov. sp. nov., a cellulolytic and filamentous thermophile that represents a novel lineage (Kallotenuales ord. nov., Kallotenuaceae fam. nov.) within the class Chloroflexia. *Int J Syst Evol Microbiol* 63(Pt 12): 4675–4682.
- [52] Schellenberger S, Kolb S, Drake H-L (2010) Metabolic responses of novel cellulolytic and saccharolytic agricultural soil Bacteria to oxygen. *Environ Microbiol* 12(4): 845–861.
- [53] Janssen P-H (2006) Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Appl Environ Microbiol* 72(3): 1719–1728.
- [54] Lewin R-A (1970) New Herpetosiphon species (Flexibacterales). *Canadian Journal of Microbiology* 16(6): 517–520.
- [55] Vance I, Topham C-M, Blayden S-L, Tampion J (1980) Extracellular Cellulase Production by *Sporocytophaga myxococcoides* NCIB 8639. *Microbiology* 117(1): 235–241.
- [56] Labeda D-P, Kroppenstedt R-M (2008) Proposal for the new genus *Allokutzneria* gen. nov. within the suborder Pseudonocardineae and transfer of *Kibdelosporangium albatum* Tomita *et al.* 1993 as *Allokutzneria albata* comb. nov.. *Int J Syst Evol Microbiol* 58(6): 1472–1475.
- [57] Tomita K, Hoshino Y, Miyaki T (1993) *Kibdelosporangium albatum* sp. nov. Producer of the Antiviral Antibiotics Cycloviracins. *Int J Syst Bacteriol* 43(2): 297–301.
- [58] Labeda D-P, Lyons A-J (1989) *Saccharothrix texasensis* sp. nov. and *Saccharothrix waywayandensis* sp. nov.. *Int J Syst Bacteriol* 39(3): 355–358.
- [59] Labeda D-P, Hatano K, Kroppenstedt R-M, Tamura T (2001) Revival of the genus *Lentzea* and proposal for *Lechevalieria* gen. nov. *Int J Syst Evol Microbiol* 51(3): 1045–1050.
- [60] Rienzi S-CD, Sharon I, Wrighton K-C, Koren O, Hug L-A, Thomas B-C, *et al.* (2013) The human gut and groundwater harbor non-photosynthetic bacteria belonging to a new candidate phylum sibling to Cyanobacteria. *eLIFE* 2: e01102.

- [61] Soo R-M, Skennerton C-T, Sekiguchi Y, Imelfort M, Paech S-J, Dennis P-G, *et al.* (2014) An expanded genomic representation of the phylum cyanobacteria. *Genome Biology and Evolution* 6(5): 1031–1045.
- [62] Kong H-H, Oh J, Deming C, Conlan S, Grice E-A, Beatson M-A, *et al.* (2012) Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res* 22(5): 850–859.
- [63] Casida L-E (1983) Interaction of *Agromyces ramosus* with Other Bacteria in Soil.. *Appl Environ Microbiol* 46(4): 881–888.