Supplemental Methods

Soil Collection and Preparation. Soils were collected from an organic farm in Penn Yan, New York. These soils are characterized as 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 randomized 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. Cores were all sieved through a 2mm sieve, homogenized by mixing, and stored at 4C until setup for preincubation (within 1-2 week of collection). Carbon and nitrogen content were previously measured for these soils [1]. Reported values for the organic field were 12.15 (± s.d. 0.78) mg C g^{-1} dry soil and 1.16 (± s.d. 0.13) mg N g^{-1} dry soil.

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) were produced in 1L Erlenmeyer flask containing 100 mL Heo and Son minimal media that were inoculated with three isolated colonies of *Gluconoacetobacter xylinus* grown 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 thick cellulose pellicule had 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 desired size range (53μm - 250μm) was achieved (checked by drying 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 Bradford [4] with a standard curve ranging from 0 - 2000 μg ml $^{-1}$ BSA. Ground, purified cellulose contained 6.92 μg 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% ± 5 (s.d.) degree of ^{13}C labeling as determined by isotopic analysis (UCDavis Stable Isotope Facility).

Soil microcosms. A subset of soil was dried at 105C overnight to determine soil moisture content gravimetrically. Microcosms (35 total) were created by adding the equivalent of 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]. Preincubation 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

Reserved for Publication Footnotes

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 CatC0705) 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 ∼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 -80C 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.

Nucleic acid extraction. Nucleic acids were extracted from 0.25 g soil using a modified Griffiths procotol [11]. Cell lysis was performed 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 presviously 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 μL TE.

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-nonenriched DNA. Each gradient was loaded with approximately 5 μg 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 ∼100 μL were collected from below by displacing the DNA-CsCl-gradient buffer solution in the centrifugation tube with water using a syringe pump at a flow rate of 3.3 μL s^{-1} [14] into Acroprep^{TM} 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 [12] to measure a volume of 5 μL. Then 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 μL TE was added to each fraction then resuspended DNA was pipetted off the filter into a new microfuge tube. The number of 16S rRNA genes of each fraction were quantitated by qPCR (Bio-Rad C1000/CFX96 thermocycler) as described previously [1] using 12.5 μL QuantiFast SYBR green PCR master mix (Qiagen 204056), 1.25 μL 10 μM 515F (5'-GTGCCAGCMGCCGCGGTAA -3'), 1.25 μL 10 μM 806R (5'-GGACTACHVGGGTWTCTAAT-3'), and 1:100 dilution of DNA template. To estimate the abundances of rRNA gene copies, we used standard curves from 10-fold serial dilutions of 16S generated from *Klebsiella pneumonia* using the same primers.

DNA Sequencing. For every gradient, 20 fractions were chosen for sequencing between the density range 1.67-1.75 g mL^{-1}. 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 SequalPrep™ 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)

Post-Sequencing Analysis.

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. 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.

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.

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.

Identifying OTUs that incorporated ^{13}C into their DNA

DNA-SIP is a culture-independent approach towards defining identity-function connections in microbial communities [13, 26, 27]. . Microbes are identified on the basis of isotope assimilation into DNA. As the bouyant 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 the experimental conditions except that unlabeled substrates are used. 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 **(author?)** [30]). We use the term differential abundance (coined by **(author?)** [30]) to denote OTUs that have different proportion means across sample classes (in this case the only sample class is labeled: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 wald-test for differential abundance (the null hypothesis is the labeled:control proportion mean ratio 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 proportion mean ratio of 1.68). DESeq2 was used to calculate the moderated $\log_2$ fold change of labeled:control proportion mean ratios and corresponding standard errors for the Wald test. Mean ratio moderation allows for reliable ratio ranking such that high variance and likely statistically insignificant mean ratios are appropriately shrunk and subsequently ranked lower than they would be as raw ratios. Those OTUs that exhibit a statistically significant increase in proportion in heavy fractions from ^{13}C-labeled samples relative to corresponding controls have increased significantly in bouyant density in response to ^{13}C treatment.

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.

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`

Supplemental Notes

Phylogenetic affiliation of ^{13}C-cellulose and ^{13}C-xylose responsive microorganisms. *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 [37]. All ^{13}C-cellulose responding *Proteobacteria* share high sequence identity with 16S rRNA genes from sequenced cultured isolates (Table **??**) except for "OTU.442" (best cultured isolate match 92% sequence identity in the *Chrondomyces* genus, Table **??**) and "OTU.663" (best cultured isolate match outside *Proteobacteria* entirely, *Clostridium* genus, 89% sequence identity, Table **??**). 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 [38].

Verrucomicrobia, a cosmopolitan soil phylum often found in high abundance [39], are hypothesized to degrade polysaccharides in many environments [39–41]. *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 [42]. The strongest *Verrucomicrobial* responder OTU to ^{13}C-cellulose shared high sequence identity (97%) with an isolate from Norway tundra soil [43] 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 **??**) with *Roseimicrobium gellanilyticum* (100% sequence identity) which has been shown to grow on soluble cellulose [44]. The remaining ^{13}C-cellulose *Verrucomicrobia* responders did not share high sequence identity with any isolates (maximum sequence identity with any isolate 93%).

Chloroflexi are known for metabolically dynamic lifestyles ranging from anoxygenic phototrophy to organohalide respiration [45]. Recent studies have focused on *Chloroflexi* roles in C cycling [45–47] and several *Chloroflexi* utilize cellulose [45–47]. Four closely related OTUs in an undescribed *Chloroflexi* lineage (closest matching isolate for all four OTUs: *Herpetosiphon geysericola*, 89% sequence identity, Table **??**) responded to ^{13}C-cellulose (Figure **??**). One additional OTU also from a poorly characterized *Chloroflexi* lineage (closest cultured isolate matched a proteobacterium at 78% sequence identity) responded to ^{13}C-cellulose (Figure **??**).

Other notable ^{13}C-cellulose responders include a *Bacteroidetes* OTU that shares high sequence identity (99%) to *Sporocytophaga myxococcoides* a known cellulose degrader [48], 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 *Allokutzneriz albata* [49, 50] and *Lentzea waywayandensis* [51, 52]; 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 **??**) (Figure **??**). 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 **??**). A sister clade to the oxygenic phototrophs classically annotated as "cyanobacteria" in SSU rRNA gene reference databases, but does not possess any known phototrophs, has recently been proposed to constitute its own phylum, "Melainabacteria" **(author?)** [53]; although, the phylogenetic position of "Melainabacteria" is debated [54]. The catalog of metabolic capabilities associated with cyanobacteria (or candidate phyla previously annotated as cyanobacteria) are quickly expanding [53, 54]. 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 [53]. 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 **??**).

Verrucomicrobia, cosmopolitan soil microbes [55], can comprise up to 23% of 16S rRNA gene sequences in high-throughput DNA sequencing surveys of SSU rRNA genes in soil [55] and can account for up to 9.8% of soil 16S rRNA [56]. Many *Verrucomicrobia* were first isolated in the last decade [57] 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 [55]. Genomic analyses and physiological profiling of *Verrucomicrobia* isolates revealed *Verrucomicrobia* are capable of methanotrophy, diazotrophy, and cellulose degradation [44, 57], yet the function of soil *Verrucomicrobia* in global C-cycling remains unknown. 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 **??**). 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 [55]. 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.

Cellulose degrading soil *Chloroflexi* have previously been identified in DNA-SIP studies [58]. The cellulose degrading *Chloroflexi* in this study are only distantly related to isolates **??**. Chloroflexi are among the six most abundant soil phyla commonly recovered soil microbial diversity surveys [59]. Chloroflexi are typically not as as abundant as *Verrucomicrobia* but are roughly as abundant as *Bacteroidetes* and *Planctomycetes* [59]. 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 [60]. 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.

We also observed ^{13}C-incorporation from cellulose by *Proteobacteria*, *Planctomycetes* and *Bacteroidetes*. Strains in *Proteobacteria*, *Planctomycetes* and *Bacteroidetes* have all been previously implicated in cellulose degradation. *Planctomycetes* is the least studied of the three phyla and only one *Planctomycetes* isolate can grow on cellulose. None of the seven *Planctomycetes* cellulose degraders identified in this experiment are closely related to isolates. *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: ced-

erlund 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.

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 **??**). 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 **??**). Only one *Bacteroidetes* ^{13}C-xylose responder is not closely related to a cultured isolate, "OTU.183" (closest LTP BLAST hit, *Chitinophaca sp.*, 89.5% sequence identity, Table **??**). OTU.183 shares high sequence identity with environmental clones derived from rhizosphere samples (accession AM158371, unpublished) and the skin microbiome (accession JF219881, **(author?)** [61]). Other *Bacteroidetes* responders share high sequence identities with canonical soil genera including *Dyadobacer*, *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 **??**). *A. ramosus* is a known predatory bacterium but is not dependent on a host for growth in culture [62]. 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.

Phylogenetic breadth of ecological strategies. It has been proposed that ecological strategies of soil microorganisms correlate to broad taxonomic groupings CITE (Schimel, Fierer 2007). Our results suggest that while substrate utilization is not defined at the level of OTU, it is also not defined at the phylum or even family level. Ecology captured at broad phylogenetic breadth would indicate that phylogenetic composition would change in response to changing conditions whereas if ecology is defined by high resolution groups, it suggests that community ecology can change without a corresponding change in composition or specifically that extant taxa would rapidly adapt to environmental change CITE. We found that substrate utilization is captured by phylogenetic levels somewhere between the genus and phylum level (Figure **??**). Our measurements did define differences between and within substrate responder groups suggesting there are ecological strategy subgroups within larger groups defined by an affinity for a particular substrate (Figure **??**, Figure **??**). Hence, compositional changes could occur at different phylogenetic scales in response to environmental perturbation.

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 **??**).

***rrn* gene copy number.** *rrn* copy number estimation is a recent advance in microbiome science [63] although the relationship of *rrn* copy number per genome with ecological strategy is well established [64]. Microorganisms with a high *rrn* copy number tend to be fast growers specialized to take advantage of boom-bust environments whereas microorganisms with low *rrn* copy number favor slower growth under lower and more consistent nutrient input [64]. At the beginning of our incubation, OTUs with estimated high *rrn* copy number or "fast-growers" assimilate xylose into biomass and with time slower growers (lower *rrn* copy number) begin to incorporate ^{13}C from xylose. Fur-

ther, ^{13}C-xylose responders have more estimated rRNA operon copy numbers per genome than ^{13}C-cellulose responders (p-value $1.878x10^{-09}$) suggesting xylose respiring microbes are generally faster growers than cellulose degraders.

Sequencing statistics and density fractionation. 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.

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, ODonnell 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 GC 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 15N. *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) Intragenomic 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. Benjamini Y, Hochberg Y (1997) Multiple Hypotheses Testing with Weights. *Scand J Stat* 24(3): 407–418.
32. Lozupone C, Knight R (2005) UniFrac: a New Phylogenetic Method for Comparing Microbial Communities. *Applied and Environmental Microbiology* 71(12): 8228–8235.
33. 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.
34. Dixon P (2003) VEGAN a package of R functions for community ecology. *Journal of Vegetation Science* 14(6): 927–930.
35. Wickham H (2009) ggplot2.
36. Anderson M-J (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26(1): 32–46.
37. Boone D (2001) Bergey's manual of systematic bacteriology.
38. Tavernier M-L, Delattre C, Petit E, Michaud P (2008) β-(1,4)-Polyglucuronic Acids - An Overview. *TOBIOTJ* 2(1): 73–86.
39. 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.
40. 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.
41. 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.
42. 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.
43. 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.
44. 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.
45. 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.
46. 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
47. 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.
48. Vance I, Topham C-M, Blayden S-L, Tampion J (1980) Extracellular Cellulase Production by Sporocytophaga myxococcoides NCIB 8639. *Microbiology* 117(1): 235–241.
49. 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.
50. 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.
51. Labeda D-P, Lyons A-J (1989) Saccharothrix texasensis sp. nov. and Saccharothrix waywayandensis sp. nov.. *Int J Syst Bacteriol* 39(3): 355–358.
52. 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.
53. 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.
54. 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.
55. 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.
56. 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.
57. 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.
58. 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.
59. 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.
60. Lewin R-A (1970) New Herpetosiphon species (Flexibacterales). *Canadian Journal of Microbiology* 16(6): 517–520.
61. 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.
62. Casida L-E (1983) Interaction of Agromyces ramosus with Other Bacteria in Soil.. *Appl Environ Microbiol* 46(4): 881–888.
63. Kembel S-W, Wu M, Eisen J-A, Green J-L (2012) Incorporating 16S gene copy number information improves estimates of microbial diversity and abundance. *PLoS Comput Biol* 8(10): e1002743.
64. Klappenbach J-A, Dunbar J-M, Schmidt T-M (2000) rRNA Operon copy number reflects ecological strategies of bacteria. *Appl Environ Microbiol* 66(4): 1328–1333.