Supplemental Information

Contents

1 Supplemental Discussion

1.1 Supplemental note 1 – Phylogenetic affiliation of cellulose responders

Verrucomicrobia represented 16% of the cellulose responders. *Verrucomicrobia* are cosmopolitan soil microorganisms [1] that can make up to 23% of SSU rRNA gene sequences in soils [1] and 9.8% of soil SSU rRNA [2]. Genomic analyses and laboratory experiments show that various isolates within the *Verrucomicrobia* are capable of methanotrophy, diazotrophy, and cellulose degradation [3, 4]. Moreover, *Verrucomicrobia* have been hypothesized to degrade polysaccharides in many environments [5–7]. However, the role of soil *Verrucomicrobia* in global C-cycling remains unknown.

The majority of verrucomicrobial cellulose responders belonged to two clades that fell within the *Spartobacteria* (Figure **??**). *Spartobacteria* outnumbered all other *Verrucomicrobia* phylotypes in SSU rRNA gene surveys of 181 globally distributed soil samples [1]. 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 global cellulose turnover.

Other notable cellulose responders include OTUs in the *Planctomycetes* and *Chloroflexi* both of which have previously been shown to assimilate ^{13}C from ^{13}C-cellulose added to soil [8]. *Planctomycetes* are common in soil [9], comprising 4 to 7% of bacterial cells in many soils [10, 11] and 7% $\pm$ 5% of SSU rRNA [12]. Although soil *Planctomycetes* are widespread, their activities in soil remain uncharacterized. *Plantomycetes* represented 16% of cellulose responders and shared $<$ 92% SSU rRNA gene sequence identity to their most closely related cultured isolates. *Chloroflexi* are known for metabolically diverse lifestyles ranging from anoxygenic phototrophy to organohalide respiration [13] and are among the six most abundant bacterial phyla in soil [9]. Recent studies have focused on *Chloroflexi* roles in C cycling [13–15] and several *Chloroflexi* isolates use cellulose [13–15]. Four of the five *Chloroflexi* cellulose responders belong to a single clade within the *Herpetosiphonales* (Figure **??**).

Finally, a single cellulose responder belonged to the *Melainabacteria* phylum (95% shared SSU rRNA gene sequence identity with *Vampirovibrio chlorellavorus*). The phylogenetic position of *Melainabacteria* is debated but *Melainabacteria* have been proposed to be a non-phototrophic sister phylum to *Cyanobacteria*. An analysis of a *Melainabacteria* genome [16] suggests the genomic capacity to degrade polysaccharides though *Vampirovibrio chlorellavorus* is an obligate predator of green alga [17].

1.2 Supplemental note 2 – Implications for soil-C models

Functional niche characterization for soil microorganisms is necessary to predict whether and how biogeochemical processes vary with microbial community composition. Functional niches are defined by soil microbiologists and have been successfully incorporated into biogeochemical process models (E.g. [18, 19]). In some C models ecological strategies such as growth rate and substrate specificity are parameters for functional niche behavior [19]. The phylogenetic breadth of a functionally defined group is often inferred from the distribution of diagnostic genes across genomes [20] or from the physiology of isolates cultured on laboratory media [21]. For instance, the wide distribution of the glycolysis operon in microbial genomes is interpreted as evidence that many soil microorganisms participate in glucose turnover [22]. However, the functional niche may depend less on the distribution of diagnostic genes across genomes and more on life history traits that allow organisms to compete for a given substrate as it occurs in the soil. For instance, fast growth and rapid resuscitation allow microorganisms to compete for labile C which may often be transient in soil. Hence, life history traits may constrain the diversity of microbes that metabolize a given C source in the soil under a given set of conditions.

Biogeochemical processes mediated by a broad array of taxa are assumed insensitive to community change relative to processes mediated by a narrow suite of microorganisms [22, 23]. In addition, the diversity of a functionally defined group engaged in a specific C transformation is expected to correlate positively with C lability [22]. However, the diversity of labile C and structural C decomposers in soil has not been quantified directly. We found comparable numbers of OTUs responded to ^{13}C-cellulose and ^{13}C-xylose (63 and 49, respectively). Cellulose responders were phylogenetically clustered suggesting that the ability to degrade cellulose is phylogenetically

conserved. The clade depth of cellulose responders, 0.028 SSU rRNA gene sequence dissimilarity, is on the same order as that observed for glycoside hydrolases which are diagnostic enzymes for cellulose degradation [20]. Xylose responders clustered in terminal branches indicating groups of closely related taxa metabolized xylose but xylose responders also clustered phylogenetically with respect to time of response (Figure **??**, Figure **??**). For example, xylose responders on day 1 are dominated by members of *Paenibacillus*. Thus, microorganisms that degraded labile C and structural C were both limited in diversity. Although the genes for xylose metabolism are likely widespread in the soil community, it's possible only a limited diversity of organisms had the ecological characteristics required to degrade xylose under experimental conditions. Therefore it's possible that only a limited number of taxa actually participate in the metabolism of labile C-sources under a given set of conditions, and hence changes in community composition may alter the dynamics of structural *and* labile C-transformations in soil.

Broadly, we observed labile C use by fast growing generalists and structural C use by slow growing specialists. These results agree with the MIMICS model which simulates leaf litter decomposition by modeling microbial decomposers as two functionally defined groups, copiotrophs or oligotrophs [18]. Including these functional types improved predictions of C storage in response to environmental change. We identified microbial lineages engaged in labile and structural C decomposition that can be defined as copiotrophs or oligotrophs, respectively. We highlight two additional considerations for soil-C process models based on our results. First, soil-C may travel through multiple trophic levels within the bacterial community where each C transfer represents an opportunity for C stabilization in association with soil minerals or C loss by respiration. And second, although labile C consumption is generally considered to be a broad process in terms of microbial participants, we observed that only a small number of related OTUs conclusively consumed xylose-C (see SI for additional discussion) and that fast growth, as opposed the ability to use xylose, may constrain the diversity of microorganisms that process labile-C *in situ* which may often be pulse delivered and transient. The diversity of microbial participants in a biogeochemical process is thought to determine how robust process rates are to changes in community composition. Our understanding of soil C dynamics will likely improve as we develop a more granular understanding of the ecological diversity of microorganisms that mediate C transformations in soil.

1.3 Supplemental note 3 – Evidence for trophic C exchange

Responders did not necessarily assimilate ^{13}C directly from ^{13}C-xylose or ^{13}C-cellulose but, in many ways, knowledge of secondary C degradation and/or microbial biomass turnover may be more interesting with respect to the soil C-cycle than knowledge of primary degradation. The response to xylose suggests xylose-C moved through different trophic levels within the soil bacterial food web. The *Bacilli* degraded xylose first (65% of the xylose-C had been respired by day 1) representing 84% of day 1 xylose responders. *Bacilli* also comprised about 6% of SSU rRNA genes present in non-fractionated DNA on day 1. However, few *Bacilli* remained ^{13}C-labeled by day 3 and their abundance declined reaching about 2% of soil SSU rRNA genes by day 30. Members of the *Bacillus* [24] and *Paenibacillus* in particular [25] have been previously implicated as labile C decomposers. The decline in relative abundance of *Bacilli* could be attributed to mortality and/or sporulation coupled to mother cell lysis. *Bacteroidetes* OTUs appeared ^{13}C-labeled at day 3 concomitant with the decline in relative abundance and loss of ^{13}C-label for *Bacilli*. Finally, *Actinobacteria* appeared ^{13}C-labeled at day 7 as *Bacteroidetes* xylose responders declined in relative abundance and became unlabeled. Hence, it seems reasonable to propose that *Bacteroidetes* and *Actinobacteria* xylose

responders became labeled via the consumption of ^{13}C derived from ^{13}C-labeled microbial biomass as opposed to primary degradation of ^{13}C-xylose.

The inferred physiology of *Actinobacteria* and *Bacteroidetes* xylose responders provides further evidence for C transfer by saprotrophy and/or predation. Most of the *Actinobacteria* xylose responders that appeared ^{13}C-labeled at day 7 were members of the *Micrococcales* (Figure **??**) and the most abundant ^{13}C-labeled *Micrococcales* OTU at day 7 (OTU.4, Table **??**) is annotated as belonging in the *Agromyces*. *Agromyces* are facultative predators that feed on the gram-positive *Micrococcus luteus* in culture [26]. Additionally, certain types of *Bacteroidetes* can assimilate ^{13}C from ^{13}C-labeled *Escherichia coli* added to soil [27]. Alternatively, it is possible that *Bacilli*, *Bacteroidetes*, and *Actinobacteria* are adapted to use xylose at different concentrations and that the observed activity dynamics resulted from changes in xylose concentration over time and/or that *Actinobacteria* and *Bacteroidetes* xylose responders consumed waste products generated by primary xylose metabolism (e.g. organic acids produced during xylose metabolism). These latter two hypotheses cannot explain the sequential loss of ^{13}C-label in combination with the abundance dynamics in non-fractionated DNA, however. If trophic transfer caused the activity dynamics, at least three different ecological groups exchanged C in 7 days. Models of the soil C cycle often exclude trophic interactions between soil bacteria (e.g. [28]), yet when soil C models do account for predators and/or saprophytes, trophic interactions are predicted to have significant effects on the fate of soil C [19].

2 Supplemental Methods

2.1 Soil Collection and Preparation

We collected soils from an organic farm in Penn Yan, New York. Soils were 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 (2mm), homogenized by mixing, and stored at 4 °C until pre-incubation (within 1-2 week of collection). Carbon (C) and nitrogen (N) content were previously measured for these soils [29]. Reported soil C 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 [29].

2.2 Cellulose production

Bacterial cellulose was produced by *Gluconoacetobacter xylinus* grown in Heo and Son [30] minimal media (HS medium) made with 0.1% glucose and without inositol. For the production of ^{13}C-cellulose, $^{13}C_6$-D-glucose, 99 atom % ^{13}C (Isotec) was used. Cellulose was produced in 1L Erlenmeyer flasks containing 100 mL HS medium inoculated with three colonies of *Gluconoacetobacter xylinus* grown on HS agar plates. Flasks were incubated statically in the dark at 30°C for 2-3 weeks. Cellulose pellicules were decanted, rinsed with deionized water, suspended in two volumes of 1% Alconox, and then autoclaved. Cellulose pellicules were purified by dialysis for 12 hr in 1 L deionized water and dialysis was repeated 10 times. Harvested pellicules were dried overnight (60°C), cut into pieces, and ground using a 5100 Mixer/Mill (SPEX SamplePrep, Metuchen, NJ),

and dry sieved to 53 μm-250 μm. The particulate size range was selected to be representative of particulate organic matter in soils (3).

The purity of ground cellulose was checked by biological assay, 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 HS medium. The biological assay consisted of *E. coli* inoculated into minimal M9 media which lacked a C 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 (OD_{450}). No measurable growth was observed with either 2 mg or 25 mg cellulose, indicating absence of contaminating nutrients that can support growth of *E. coli*. 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 in the purified cellulose that could inhibit microbial growth.

Purified cellulose was also assayed for residual proteins and sugars using Bradford and Benedict's assays, respectively. Bradford assay was performed as in [31]. 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 [32] 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).

2.3 Soil microcosms

Microcosms were created by adding 10 g d.w. sieved soil to a 250 mL Erlenmeyer flask capped with a butyl rubber stopper. The headspace was flushed with air every 3 days which was sufficient to prevent anoxia (data not shown). Microcosms were pre-incubated at room temperature 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 [33]. Pre-incubation ensures that this labile organic matter is consumed and/or stabilized prior to the beginning of the experiment. Respiration rate (CO_2) stabilized after 10 days (data not shown).

Three parallel treatments were established. Each treatment received the same amendment, where the only difference was the isotopically labeled component in the amendment. The treatments included an unlabeled control treatment and treatments that substituted either ^{13}C-cellulose (synthesized as described above) or $^{13}C_5$-D-xylose (98 atom % ^{13}C (Isotec)) for their unlabeled equivalents. The molecular composition of the amendment was designed to approximate switchgrass biomass with hemicellulose replaced by its constituent monomers [34, 35]. The amendment was added at 5.3 mg g^{-1} d.w. soil which is representative of natural concentrations in soil during early phases of decomposition [36]. The amendment contained by mass: 38% cellulose, 23% lignin, 20% xylose, 3% arabinose, 1% galactose, 1% glucose, and 0.5% mannose, 10.6% amino acids (Teknova C0705), and 2.9% Murashige Skoog basal salt mixture which contains macro- and micronutrients that are associated with plant biomass (Sigma Aldrich M5524). The amendment had a C:N ratio of 10. Cellulose (2 mg cellulose g^{-1} d.w. soil) and lignin (1.2 mg lignin g-1 d.w. soil) were uniformly distributed over the soil surface as a powder and the remaining constituents were added in solution in a volume of 0.12 ml g^{-1} d.w. soil. The volume of liquid was determined in relation to soil moisture to achieve 50% water holding capacity. Water holding capacity of 50% was chosen, in relation to the texture for this soil, to achieve approximately 70% water filled pore space, which

is the optimal water content for respiration [37]. A total of 12 microcosms were established per treatment. Microcosms were sampled destructively on days 1, 3, 7, 14, and 30 and soils were frozen at -80 °C. The cellulose treatment was not sampled on day 1 because it was not expected that significant cellulose metabolism would have occurred within this time. The abbreviation 13CXPS refers to the ^{13}C-xylose treatment (^{13}C Xylose Plant Simulant), 13CCPS refers to the ^{13}C-cellulose treatment and 12CCPS refers to the unlabeled control. A subset of soil from each sample was reserved for isotopic analysis at the Cornell University Stable Isotope Laboratory to determine the mass of ^{13}C remaining in soil.

2.4 Nucleic acid extraction

Nucleic acids were extracted from 0.25 g soil using a modified Griffiths protocol [38]. Cells were lysed by bead beating for 1 min at 5.5 m s^{-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 m s^{-1}. After lysis, 85 μL 5 M NaCl and 60 μL 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 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 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 μL TE and stored at -20 °C. To prepare nucleic acid extracts for isopycnic centrifugation as previously described [39], 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.

2.5 Isopycnic centrifugation and fractionation

We fractionated DNA on density gradients for ^{13}C-xylose treatments (days 1, 3, 7, 14, 30), ^{13}C-cellulose treatments (days 3, 7, 14, 30), and control treatments (days 1, 3 ,7, 14, 30). A total of 5 μg DNA was added to each 4.7 mL CsCl density gradient. Density gradient were composed of 1.69 g mL^{-1} CsCl ml^{-1} in gradient buffer solution (pH 8.0 15 mM Tris-HCl, 15 mM EDTA, 15 mM KCl). Centrifugation was performed at 55,000 rpm 20 °C for 66 hr using a TLA-110 rotor in a Bechman Coulter Optima MAX-E ultracentrifuge. Fractions of $\sim$100 μL were collected from below by displacing the DNA-CsCl-gradient buffer solution in the centrifugation tube with water using a syringe pump at a flow rate of 3.3 μL s^{-1} [40]. Fractions were collected in Acroprep 96 filter plates (part no. 5035, Pall Life Sciences). The refractive index of each fraction was measured using a Reichart AR200 digital refractometer modified as previously described to measure a volume of 5 μL [39]. Buoyant density was calculated from the refractive index as previously described [39] using the equation ρ=$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 [41] and correcting for non-CsCl salts in the gradient buffer. A total of 35 gractions were collected from each gradient and the average density between fractions was 0.0040 g mL^{-1}. The DNA was desalted by washing with TE (5X 200 μL) in the Acroprep filter wells. DNA was resuspeneded in 50 μL TE.

2.6 DNA Sequencing

2.6.1 PCR amplification of SSU rRNA genes

SSU rRNA genes were amplified from gradient fractions (n = 20 per gradient) and from non-fractionated DNA from soil. Barcoded primers consisted of: 454-specific adapter B, a 10 bp barcode (Reference 90), 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 PCR contained 1.25 U l-1 AmpliTaq Gold (Life Technologies, Grand Island, NY; N8080243), 1X Buffer II (100 mM Tris-HCl, 500 mM KCl, pH 8.3), 2.5 mM MgCl2, 200 M of each dNTP, 0.5 mg ml-1 BSA, 0.2 M BA515F, 0.2 M BA806R, and 10 L of 1:30 DNA template in 25 l total volume). The PCR conditions were 95 °C for 5min followed by 22 cycles of 95°C for 10 s, 53 °C for 30 s, and 72 °C for 30 s, followed by a final elongation at 72 °C for 5 min. Amplification products were checked by 1% agarose gel. Reactions were performed in triplicate and pooled. Amplified DNA was gel purified (1% low melt agarose) using the Wizard SV gel and PCR clean-up system (Promega, Madison, WI; A9281) per manufacturers protocol. Samples were normalized by SequalPrepTM normalization plates (Invitrogen, Carlsbad, CA; A10510) and pooled in equimolar concentration. Amplicons were sequenced on Roche 454 FLX system using titanium chemistry at Selah Genomics (Columbia, SC).

2.6.2 DNA sequence quality control

SSU rRNA gene sequences were initially screened by maximum expected errors at a specific read length threshold [42]. Reads that had more than 0.5 expected errors at a length of 250 nt were discarded. The remaining reads were aligned to the Silva Reference Alignment as provided in the Mothur software package using the Mothur NAST aligner [43, 44]. Reads that did not align to the expected region of the SSU rRNA gene were discarded. After expected error and alignment based quality control. The remaining quality controlled reads were annotated using the UClust taxonomic annotation framework in [45, 46]. We used 97% cluster seeds from the Silva SSU rRNA database (release 111Ref) [47] as reference for taxonomic annotation (provided on the QIIME website) [47]. Quality control screening filtered out 344,472 of 1,720,480 raw sequencing reads leaving 1,376,008 reads for downstream analyses. Reads annotated as "Chlorloplast", "Eukaryota", "Archaea", "Unassigned" or "mitochondria" were culled from the dataset.

2.6.3 OTU binning

Sequences were distributed into OTUs with a centroid based clustering algorithm (i.e. UPARSE [42]). The centroid selection also included robust chimera screening [42]. OTU centroids were established at a threshold of 97% sequence identity and non-centroid sequences were mapped back to centroids. Reads that could not be mapped to an OTU centroid at greater than or equal to 97% sequence identity were discarded.

2.6.4 Phylogenetic reconstruction

We used SSU-Align [48, 49] to align SSU rRNA gene sequences. Columns in the alignment that were aligned with poor confidence ($< 95\%$ of characters had posterior probability $> 95\%$) were not considered when building the phylogenetic tree leaving a multiple sequence alignment of 216 columns. Additionally, the alignment was trimmed to coordinates such that all sequences in the

alignment began and ended at the same positions. FastTree [50] was used with default parameters to build the phylogeny.

2.6.5 Ordination and statistical analysis of differences in SSU rRNA gene composition

NMDS ordination was performed on weighted Unifrac [51] distances between samples. The Phyloseq [52] wrapper for Vegan [53] (both R packages) was used to compute sample values along NMDS axes. The 'adonis' function in Vegan was used to perform Adonis tests (default parameters) [54].

2.7 OTU characteristics

2.7.1 Identifying ^{13}C responders

Figures S11 and S12 demonstrate raw data for responder and non-responder OTUs, respectively. Responders increased in relative abundance in the high density fractions due to ^{13}C-labeling of their DNA. As our data is compositional, often OTUs had consistent *relative* abundance across the density gradients indicating the OTU DNA concentration across the gradient mirrored that of the total DNA concentration. If OTU DNA is centered outside the main distribution of DNA due to G+C content and/or ^{13}C-labeling its relative abundance increases near the center of the OTU DNA concentration profile. Thus, we identified responders by finding OTUs enriched in high density fractions of ^{13}C-treatment gradients relative to control. This technique accounts for the variation in OTU base abundance and the variation in OTU G+C content (and therefore natural buoyant density) because relative abundances in gradient fractions from ^{13}C-treatments are always compared to those in corresponding gradient fractions from control gradients.

We used DESeq2 (R package), an RNA-Seq differential expression statistical framework [55], to identify OTUs that were enriched in high density gradient fractions from ^{13}C-treatments relative to corresponding gradient fractions from control treatments (for review of RNA-Seq differential expression statistics applied to microbiome OTU count data see [56]). We define "high density gradient fractions" as gradient fractions whose density falls between 1.7125 and 1.755 g ml^{-1}. Briefly, DESeq2 includes several features that enable robust estimates of standard error in addition to reliable ranking of logarithmic fold change (LFC) (i.e. gamma-Poisson regression coefficients) in OTU relative abundance even with low count OTUs where LFC can often be noisy. Further, statistical evaluation of LFC can be performed with user-selected thresholds, as opposed to the typical null hypothesis that LFC is exactly zero, enabling the most biologically interesting OTUs to be identified for subsequent analyses. For each OTU, we calculated LFC and corresponding standard errors for enrichment in high density gradient fractions of ^{13}C treatments relative to control. Subsequently, a one-sided Wald test was used to statistically assess LFC values. The user-defined null hypothesis was that LFC was less than one standard deviation above the mean of all LFC values. P-values were corrected for multiple comparisons using the Benjamini and Hochberg method [57]. We independently filtered OTUs on the basis of sparsity prior to correcting P-values for multiple comparisons. The sparsity value that yielded the most adjusted P-values less than 0.10 was selected for independent filtering by sparsity. Briefly, OTUs were eliminated if they failed to appear in at least 45% of high density gradient fractions for a given ^{13}C/control treatment pair. These sparse OTUs are unlikely to have sufficient data to allow for the determination of statistical significance. We selected a false discovery rate of 10% to denote statistical significance.

2.7.2 Estimating rrn copy number

We estimated the *rrn* copy number for each OTU as described [58] (i.e. we used the code and reference information provided by the authors [58] directly). In brief, OTU centroid sequences were inserted into a reference SSU rRNA gene phylogeny [59] from organisms of known *rrn* copy number. The *rrn* copy number was then inferred from the phylogenetic placement in the reference phylogeny.

2.7.3 NRI, NTI, and consenTRAIT

NRI and NTI were calculated using the "picante" R package [60]. We used the "independentswap" null model for phylogenetic distribution. The consenTRAIT clade depth for xylose and cellulose responders was calculated using R code from the original publication describing the metric [21] which employs the R "adephylo" package [61].

2.7.4 Buoyant density shift estimates

DNA buoyant density (BD) increases with atom % ^{13}C. Therefore, the magnitude of $\Delta\hat{BD}$ indicates the degreee of isotopic labeling for an OTU. We measured $\Delta\hat{BD}$ as the change in an OTU's density profile center of mass between corresponding control and labeled gradients (Figure S11). Because all gradients did not span the same density range and gradient fractions cannot be taken at specific density positions, we limited our $\Delta\hat{BD}$ analysis to the density range for where all density gradients overlapped. Within this density range we linearly interpolated 20 evenly spaced relative abundance values. The center of mass for an OTU along the density gradient was then the density weighted average where weights were the linearly interpolated relative abundance values. $\Delta\hat{BD}$ values are based on relative abundance profiles and would be distorted in comparison to $\Delta\hat{BD}$ based on absolute DNA concentration profiles and should be interpreted with this transformation in mind.

2.7.5 Finding cultured relatives of OTUs

OTU centroids were compared (BLAST [62, 63]) to sequences in "The All-Species Living Tree" project (LTP). The LTP is a collection of SSU rRNA gene sequences for classified species of Archaea and Bacteria [64]. We used LTP version 115 for analyses in this paper.

2.7.6 OTU changes in relative abundance with time

We identified OTUs that changed in relative abundance over time using DESeq2 [55]. Specifically, we used day treated as an ordered factor as the regressor with LFC of the relative abundance in non-fractionated DNA as the outcome in the general linear model. We used the default DESeq2 base mean independent filtering and disabled the Cook's cutoff outlier detection. The null model was that abundance did not change with time and we assessed significance at a false discovery rate of 10%.

2.8 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.

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