

Supplemental Information - Unearthing the ecology of uncultured bacteria in soil with a high resolution approach to DNA-SIP

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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 3). *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. *Planctomycetes* 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 3).

Finally, a single cellulose responder belonged to the *Melainabacteria* phylum (95% shared SSU rRNA gene sequence identity with *Vamptrovibrio 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 *Vamptrovibrio chlorellavorus* is an obligate predator of green alga [17].

1.2 Supplemental note 2 – Implications for soil-C models

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 [18, 19]. In addition, the diversity of a functionally defined group engaged in a specific C transformation is expected to correlate positively with C lability [19]. 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 3, Figure 4). 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 [21]. 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* [22] and *Paenibacillus* in particular [23] 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 re-

sponders that appeared ^{13}C -labeled at day 7 were members of the *Micrococcales* (Figure 3) and the most abundant ^{13}C -labeled *Micrococcales* OTU at day 7 (OTU.4, Table S1) is annotated as belonging in the *Agromyces*. *Agromyces* are facultative predators that feed on the gram-positive *Micrococcus luteus* in culture [24]. Additionally, certain types of *Bacteroidetes* can assimilate ^{13}C from ^{13}C -labeled *Escherichia coli* added to soil [25]. 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. [26]), 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 [27].

1.4 Supplemental Note 4 – Major C components of plant biomass

We chose specifically to explore the metabolism of xylose and cellulose because these substrates are abundant components of plant biomass which exhibit distinct degradation dynamics. Most plant C is comprised of cellulose (30-50%) followed by hemicellulose (20-40%), and lignin (15-25%) [28]. Hemicellulose, being the most soluble, degrades in the early stages of decomposition. Xylans are often an abundant component of hemicellulose, and xylose is often the most abundant sugar in hemicellulose, comprising as much as 60-90% of xylan in some plants (e.g switchgrass [29]).

1.5 Supplemental Note 5 – Fungal activity

This study focuses on the bacterial response of the soil microbial community to the addition of an amendment that represents organic matter derived from plant biomass. However, the contributions to C-cycling by fungi cannot be disregarded. Until recently, the degradation of low molecular weight substrates, such as the xylose used in this study, was widely assumed to be mediated by bacteria due to their high numbers and rapid growth rate [30] yet fungal as opposed to bacterial load numbers was shown to be more correlated with process rates of easily available C [31]. Most cellulose decomposition is attributed to fungi; their hyphal growth serving as an important strategy for accessing cellulose fibers embedded in the matrix of other plant structural polymers [28], though the relative contributions of bacteria and fungi to the degradation of cellulose in soil is a matter of continuing study [31].

1.6 Supplemental Note 6 – Experimental design

We employed the use of microcosms to observe the soil bacterial community response to the addition of an amendment that represents organic matter derived from plant biomass. Microcosms are not meant to replicate the full extent of spatial and temporal variation observed in the field or across habitats. The purpose of microcosm experiments are to evaluate microbial activity under defined experimental conditions. It is not expected that the microcosm will mimic exactly field conditions, but it is expected that a microorganisms traits can be evaluated by observing how it responds under defined experimental conditions. It is possible that microcosm conditions may enrich for organisms

that are present but inactive in soil under field conditions. Hence, microcosm experiments can demonstrate potential activity, but whether a given activity occurs in field conditions requires additional experimentation. However, the use of microcosm experiments is useful for defining the traits of specific microorganisms. Once microbial traits have been identified it is necessary to make separate tests to determine when these traits are active in the field.

The use of microcosms is meant to control environmental parameters as a means to minimize variability between treatments in an inherently complex system. We sieved and homogenized the soil to minimize unwanted variation associated with spatial heterogeneity so that we could focus on variation resulting from our experimental treatments. Disturbance of soils by sampling, liberates organic matter making it accessible for microbial use (use citation that is the in the methods section for preprocessing). To minimize sampling artifacts we preconditioned all microcosms until soil respiration stabilized (see Methods). Furthermore, we used destructive sampling of replicate microcosms incubated in parallel to eliminate the need for subsampling. All microcosms were treated identically with the sole manipulation being the identity of the ^{13}C -labeled substrate and the time of destructive sampling. Results from amplicon sequencing indicate that bottle to bottle variance was low and far less than variance due to time.

Many aspects of field conditions are not reproduced in our microcosms. For instance, (1) Soils are sieved and homogenized and this may disrupt hyphae and spatial arrangements of organisms. (2) Soils are maintained at constant temperature and moisture. (3) The amendment components are combined from separate stocks to allow for isotopic substitution and hence the amendment lacks the structural complexity of plant organic matter. (4) Bacterial cellulose was used to facilitate reproducibility of quality and isotopic labeling. While bacterial cellulose has a crystalline structure similar to plant cellulose and is often used as a substitute for plant cellulose, it is possible that structural differences between bacterial cellulose and plant cellulose may elicit differences in the microbial response.

Our results are subject to biases associated with nucleic acid extraction [32], PCR [33], and sequencing platform [34]. We have employed best practices, to moderate these biases as described in methods and supplemental methods.

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. The agricultural field site has been described previously [35]. 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 [35]. 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 [35].

2.2 Cellulose production

Bacterial cellulose was produced by *Gluconoacetobacter xylinus* grown in Heo and Son [36] minimal media (HS medium) made with 0.1% glucose and without inositol. This cellulose while tractable for use in the lab will not possess the structural complexity of lignocellulosic plant biomass and thus we cannot account for the effects plant biomass structural complexity on which microorganisms utilize cellulose in soil. For the production of ^{13}C -cellulose, $^{13}\text{C}_6\text{-D-glucose}$, 99 atom % ^{13}C (Cambridge Isotope) 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.

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 [37]. 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 [38] 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 headspace CO_2) had stabilized. Sieving causes a transient increase in soil respiration rate presumably due to the liberation of fresh labile soil organic matter [39]. 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 $^{13}\text{C}_5\text{-D-xylose}$ (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 [40, 41]. 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 [42]. 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 micro-nutrients 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 [43]. A total of 12 microcosms were established for the ¹³C-xylose treatment and 10 for the ¹³C-cellulose 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 ¹³C-xylose treatment (¹³C Xylose Plant Simulant), ¹³CCPS refers to the ¹³C-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 ¹³C remaining in soil.

2.4 Nucleic acid extraction

Nucleic acids were extracted from 0.25 g soil using a modified Griffiths protocol [44]. 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% hexadecyltrimmonium 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 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 x 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 [45], 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 ¹³C-xylose treatments (days 1, 3, 7, 14, 30), ¹³C-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 ~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 [46]. 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 [45]. Buoyant density was calculated from the refractive index as previously described [45] 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 [47]. The refractive index (Ri) was corrected to account for the Ri of the gradient buffer using the equation: $Ri_{corrected} = Ri_{observed} - (Ri_{buffer} - 1.3333)$. A total of 35 fractions 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 resuspended 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, 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 *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 µM of each dNTP, 0.5 mg mL⁻¹ BSA, 0.2 µM BA515F, 0.2 µM BA806R, and 10 µL of 1:30 DNA template in 25 µL total volume). The PCR conditions were 95 °C for 5 min 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 manufacturer's protocol. Samples were normalized by SequalPrep normalization plates (Invitrogen, Carlsbad, CA; A10510) or based on PicoGreen DNA quantification 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 [48]. 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 [49, 50]. 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 *AIJUClust* taxonomic annotation framework in [51, 52]. We used 97% cluster seeds from the Silva SSU rRNA database (release 111Ref) [53] as reference for taxonomic annotation (provided on the QIIME website) [53]. Quality control screening filtered out 344,472 of 1,720,480 raw sequencing reads

325 leaving 1,376,008 reads for downstream analyses. Reads annotated as "Chloroplast", "Eukaryota",
326 "Archaea", "Unassigned" or "mitochondria" were culled from the dataset.

327 2.6.3 OTU binning

328 Sequences were distributed into OTUs with a centroid based clustering algorithm (i.e. UPARSE
329 [48]). The centroid selection also included robust chimera screening [48]. OTU centroids were
330 established at a threshold of 97% sequence identity and non-centroid sequences were mapped back
331 to centroids. Reads that could not be mapped to an OTU centroid at greater than or equal to 97%
332 sequence identity were discarded.

333 2.6.4 Phylogenetic reconstruction

334 We used SSU-Align [54, 55] to align SSU rRNA gene sequences. Columns in the alignment that
335 were aligned with poor confidence ($< 95\%$ of characters had posterior probability $> 95\%$) were
336 not considered when building the phylogenetic tree leaving a multiple sequence alignment of 216
337 columns. Additionally, the alignment was trimmed to coordinates such that all sequences in the
338 alignment began and ended at the same positions. FastTree [56] was used with default parameters
339 to build the phylogeny.

340 2.6.5 Ordination and statistical analysis of differences in SSU rRNA gene composition

341 NMDS ordination was performed on weighted Unifrac [57] distances between samples. The Phyloseq
342 [58] wrapper for Vegan [59] (both R packages) was used to compute sample values along NMDS
343 axes. The 'adonis' function in Vegan was used to perform Adonis tests (default parameters) [60].

344 2.7 OTU characteristics

345 2.7.1 Identifying ^{13}C responders

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

357 We used DESeq2 (R package), an RNA-Seq differential expression statistical framework [61], to
358 identify OTUs that were enriched in high density gradient fractions from ^{13}C -treatments relative
359 to corresponding gradient fractions from control treatments (for review of RNA-Seq differential
360 expression statistics applied to microbiome OTU count data see [62]). We define "high density
361 gradient fractions" as gradient fractions whose density falls between 1.7125 and 1.755 g ml $^{-1}$.
362 Briefly, DESeq2 includes several features that enable robust estimates of standard error in addition
363 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 [63]. 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 [64] (i.e. we used the code and reference information provided by the authors [64] directly). In brief, OTU centroid sequences were inserted into a reference SSU rRNA gene phylogeny [65] 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 [66]. 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 [67] which employs the R “adephylo” package [68].

2.7.4 Buoyant density shift estimates

DNA buoyant density (BD) increases with atom % ^{13}C . Therefore, the magnitude of $\Delta\hat{B}\hat{D}$ indicates the degree of isotopic labeling for an OTU. We measured $\Delta\hat{B}\hat{D}$ 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 $\Delta\hat{B}\hat{D}$ 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. $\Delta\hat{B}\hat{D}$ values are based on relative abundance profiles and would be distorted in comparison to $\Delta\hat{B}\hat{D}$ based on absolute DNA concentration profiles and should be interpreted with this transformation in mind. Additionally, as more DNA is labeled, the relative abundance increase for an OTU in labeled gradient high density fractions relative to control decreases. Therefore, the $\Delta\hat{B}\hat{D}$ value is affected by the amount of ^{13}C -assimilation by the community as a whole.

402 2.7.5 Finding cultured relatives of OTUs

403 OTU centroids were compared (BLAST [69, 70]) to sequences in “The All-Species Living Tree”
404 project (LTP). The LTP is a collection of SSU rRNA gene sequences for classified species of Archaea
405 and Bacteria [71]. We used LTP version 115 for analyses in this paper.

406 2.7.6 OTU changes in relative abundance with time

407 We identified OTUs that changed in relative abundance over time using DESeq2 [61]. Specifically,
408 we used day treated as an ordered factor as the regressor with LFC of the relative abundance in
409 non-fractionated DNA as the outcome in the general linear model. We used the default DESeq2
410 base mean independent filtering and disabled the Cook’s cutoff outlier detection. The null model
411 was that abundance did not change with time and we assessed significance at a false discovery rate
412 of 10%.

413 2.8 Sequencing and density fractionation statistics

414 Microcosm DNA was density fractionated on CsCl density gradients. We sequenced SSU rRNA
415 gene amplicons from a total of 277 CsCl gradient fractions from 14 CsCl gradients and 12 bulk
416 microcosm DNA samples. The SSU rRNA gene data set contained 1,102,685 total sequences. The
417 average number of sequences per sample was 3,816 (sd 3,629) and 265 samples had over 1,000
418 sequences. We sequenced SSU rRNA gene amplicons from an average of 19.8 fractions per CsCl
419 gradient (sd 0.57). The average density between fractions was 0.0040 g mL^{-1} . The sequencing effort
420 recovered a total of 5,940 OTUs. 2,943 of the total 5,940 OTUs were observed in bulk samples. We
421 observed 33 unique phylum and 340 unique genus annotations.

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