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 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. *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 3).

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

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 responders 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.

May 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 (± s.d. 0.78) mg C g^{-1} dry soil and 1.16 (± 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, $^{13}C_6$-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 $^{13}C_5$-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 [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 ^{13}C-xylose treatment and 10 for the ^{13}C-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 ^{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 [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% 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 [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 ^{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} [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 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 [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 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, 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 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 UClust 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 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 [48]). The centroid selection also included robust chimera screening [48]. 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 [54, 55] 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 [56] 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 [57] distances between samples. The Phyloseq [58] wrapper for Vegan [59] (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) [60].

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 [61], 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 [62]). 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 [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 $\Delta\hat{BD}$ 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. 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 $\Delta\hat{BD}$ value is affected by the amount of ^{13}C-assimilation by the community as a whole.

2.7.5 Finding cultured relatives of OTUs

OTU centroids were compared (BLAST [69, 70]) 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 [71]. 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 [61]. 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.

References

[1] 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 Biol Biochem* 43(7): 1450–1455.

[2] 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.

[3] 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.

[4] 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.

[5] 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.

[6] 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.

[7] 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.

[8] 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.

[9] 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.

[10] Zarda B, Hahn D, Chatzinotas A, Schnhuber W, Neef A, Amann R-I, *et al.* (1997) Analysis of bacterial community structure in bulk soil by in situ hybridization. *Arch Microbiol* 168(3): 185–192.

[11] Chatzinotas A, Sandaa R-A, Schnhuber W, Amann R, Daae F-L, Torsvik V, *et al.* (1998) Analysis of broad-scale differences in microbial community composition of two pristine forest soils. *Syst Appl Microbiol* 21(4): 579–587.

[12] Buckley D-H, Schmidt T-M (2003) Diversity and dynamics of microbial communities in soils from agro-ecosystems. *Environ Microbiol* 5(6): 441–452.

[13] 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.

[14] 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. *Front Microbiol* 2: 94. doi: 10.3389/fmicb.2011.00094

[15] 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.

[16] 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.

[17] Gromov B-V, Mamkaeva K-A (1972) Electron microscopic study of parasitism by *Bdellovibrio chlorellavorus* bacteria on cells of the green alga *Chlorella vulgaris*. *Tsitologiia* 14(2): 256–260.

[18] Schimel J (1995) Ecosystem consequences of microbial diversity and community structure. *Arctic and alpine biodiversity: patterns, causes and ecosystem consequences*. eds. Chapin III F-S, Korner C, Ecological Studies (Springer, Berlin Heidelberg), pp 239–254.

[19] McGuire K-L, Treseder K-K (2010) Microbial communities and their relevance for ecosystem models: Decomposition as a case study. *Soil Biol Biochem* 42(4): 529–535.

[20] Berlemont R, Martiny A-C (2013) Phylogenetic distribution of potential cellulases in bacteria. *Appl Environ Microbiol* 79(5): 1545–1554.

[21] Wieder W-R, Grandy A-S, Kallenbach C-M, Bonan G-B (2014) Integrating microbial physiology and physio-chemical principles in soils with the MIcrobial-MIneral Carbon Stabilization (MIMICS) model. *Biogeosciences* 11(14): 3899–3917.

[22] Cleveland C-C, Nemergut D-R, Schmidt S-K, Townsend A-R (2007) Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition. *Biogeochemistry* 82(3): 229–240.

[23] Verastegui Y, Cheng J, Engel K, Kolczynski D, Mortimer S, Lavigne J, *et al.* (2014) Multisubstrate isotope labeling and metagenomic analysis of active soil bacterial communities. *mBio* 5(4): e01157–14.

[24] Casida L-E (1983) Interaction of *Agromyces ramosus* with other bacteria in soil. *Appl Environ Microbiol* 46(4): 881–888.

[25] Lueders T, Kindler R, Miltner A, Friedrich M-W, Kaestner M (2006) Identification of bacterial micropredators distinctively active in a soil microbial food web. *Appl Environ Microbiol* 72(8): 5342–5348.

[26] Moore J-C, Walter D-E, Hunt H-W (1988) Arthropod regulation of micro- and mesobiota in below-ground detrital food webs. *Annu Rev Entomol* 33(1): 419–435.

[27] Kaiser C, Franklin O, Dieckmann U, Richter A (2014) Microbial community dynamics alleviate stoichiometric constraints during litter decay. *Ecol Lett* 17(6): 680–690.

[28] Lynd L-R, Weimer P-J, van Zyl W-H, Pretorius I-S (2002) Microbial cellulose utilization: fundamentals and biotechnology. *Microbiol Mol Biol Rev* 66(3): 506–577.

[29] Bunnell K, Rich A, Luckett C, Wang Y-J, Martin E, Carrier D-J (2013) Plant maturity effects on the physicochemical properties and dilute acid hydrolysis of switchgrass (*panicum virgatum*, l.) hemicelluloses. *ACS Sustain Chem Eng* 1(6): 649–654.

[30] de Boer W, Folman L-B, Summerbell R-C, Boddy L (2005) Living in a fungal world: impact of fungi on soil bacterial niche development. *FEMS microbiol rev* 29(4): 795–811.

[31] Rousk J, Frey S-D (2015) Revisiting the hypothesis that fungal-to-bacterial dominance characterizes turnover of soil organic matter and nutrients. *Ecol Monogr* 85(3): 457–472.

[32] Albertsen M, Karst S-M, Ziegler A-S, Kirkegaard R-H, Nielsen P-H (2015) Back to Basics The Influence of DNA Extraction and Primer Choice on Phylogenetic Analysis of Activated Sludge Communities. *PLoS ONE* 10(7): e0132783.

[33] Parada A-E, Needham D-M, Fuhrman J-A (2015) Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental Microbiology* ???: n/a.

[34] Tremblay J, Singh K, Fern A, Kirton E-S, He S, Woyke T, *et al.* (2015) Primer and platform effects on 16S rRNA tag sequencing. *Evolutionary and Genomic Microbiology* ???: 771.

[35] 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. *Microb Ecol* 66(1): 158–170.

[36] 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. *Biotechnol Appl Biochem* 36(1): 41.

[37] 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.

[38] Benedict S-R (1909) A reagent for the detection of reducing sugars. *Journal of Biological Chemistry* 5(5): 485–487.

[39] 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): 119–124.

[40] 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.

[41] 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.

[42] Schneckenberger K, Demin D, Stahr K, Kuzyakov Y (2008) Microbial utilization and mineralization of ^{14}C glucose added in six orders of concentration to soil. *Soil Biol Biochem* 40(8): 1981–1988.

[43] Linn D-M, Doran J-W (1984) Aerobic and anaerobic microbial populations in no-till and plowed soils. *Soil Sci Soc Am J* 48(4): 794.

[44] Griffiths R-I, Whiteley A-S, O'Donnell A-G, Bailey M-J (2000) Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. *Appl Environ Microbiol* 66(12): 5488–5491.

[45] Buckley D-H, Huangyutitham V, Hsu S-F, Nelson T-A (2007) Stable isotope probing with ^{15}N achieved by disentangling the effects of genome G+C content and isotope enrichment on DNA density. *Appl Environ Microbiol* 73(10): 3189–3195.

[46] 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. *Appl Environ Microbiol* 68(11): 5367–5373.

[47] Birnie G-D (1978) Centrifugal separations in Molecular and cell biology. (Butterworth & Co Publishers Ltd, Boston).

[48] Edgar R-C (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* 10(10): 996–998.

[49] 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 Res* 34(suppl 2): W394–W399.

[50] 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. *Appl Environ Microbiol* 75(23): 7537–7541.

[51] 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. Methods* 7(5): 335–336.

[52] Edgar R-C (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26(19): 2460–2461.

[53] Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, *et al.* (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 41: D590–596.

[54] Nawrocki E-P, Kolbe D-L, Eddy S-R (2009) Infernal 1.0: inference of RNA alignments. *Bioinformatics* 25(10): 1335–1337.

[55] Nawrocki E-P, Eddy S-R (2013) Infernal 1.1: 100-fold faster RNA homology searches. *Bioinformatics* 29(22): 2933–2935.

[56] Price M-N, Dehal P-S, Arkin A-P (2010) FastTree2 - approximately maximum-likelihood trees for large alignments. *PLoS ONE* 5(3): e9490.

[57] Lozupone C, Knight R (2005) UniFrac: a new phylogenetic method for comparing microbial communities. *Appl Environ Microbiol* 71(12): 8228–8235.

[58] 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.

[59] Oksanen J, Blanchet F-G, Kindt R, Legendre P, Minchin P-R, O'Hara R-B, *et al.* (2015) vegan: Community Ecology Package.

[60] Anderson M-J (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecol* 26(1): 32–46.

[61] Love M-I, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15(12): 550.

[62] McMurdie P-J, Holmes S (2014) Waste not, want not: why rarefying microbiome data is inadmissible. *PLoS Comput Biol* 10(4): e1003531.

[63] Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)* 57(1): 289–300.

[64] 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.

[65] Matsen F-A, Kodner R-B, Armbrust E-V (2010) pplacer: linear time maximum-likelihood and Bayesian phylogenetic placement of sequences onto a fixed reference tree. *BMC Bioinformatics* 11: 538.

[66] Kembel S, Cowan P, Helmus M, Cornwell W, Morlon H, Ackerly D, *et al.* (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26: 1463–1464.

[67] Martiny A-C, Treseder K, Pusch G (2013) Phylogenetic conservatism of functional traits in microorganisms. *ISME J* 7(4): 830–838.

[68] Jombart T, Dray S (2010) adephylo: exploratory analyses for the phylogenetic comparative method.. *Bioinformatics* 26: 1907–1909.

[69] Altschul S-F, Gish W, Miller W, Myers E-W, Lipman D-J (1990) Basic local alignment search tool. *J Mol Biol* 215(3): 403–410.

[70] Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, *et al.* (2009) BLAST+: architecture and applications. *BMC Bioinformatics* 10: 421.

[71] Yarza P, Richter M, Peplies J, Euzeby J, Amann R, Schleifer K-H, *et al.* (2008) The All-Species Living Tree project: a 16S rRNA-based phylogenetic tree of all sequenced type strains. *Syst Appl Microbiol* 31(4): 241–250.