

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

Ashley N Campbell and Charles Pepe-Ranney * † ‡, Chantal Koechli, Sean Berthrong, and Daniel H Buckley ‡

*These authors contributed equally to the manuscript and should be considered co-first authors, †Department of Microbiology, Cornell University, New York, USA, and ‡Department of Crop and Soil Sciences, Cornell University, New York, USA

Submitted to Proceedings of the National Academy of Sciences of the United States of America

Abstract

We identified microorganisms participating in xylose and/or cellulose decomposition in soil microcosms using nucleic acid stable isotope probing (SIP) coupled to next generation sequencing. 49 and 63 OTUs assimilated ^{13}C from xylose and cel-
lulose into DNA, respectively. Microorganisms as-
similated xylose-C at days 1, 3, and 7. Cellulose-
C assimilation peaked at day 14 and was main-
tained at day 30. Many SIP-identified cellulose
degraders are members of cosmopolitan but phys-
iologically uncharacterized soil microbial lineages
including *Spartobacteria*, *Chloroflexi* and *Planctomyces*. ^{13}C from Xylose was initially assimilated by *Firmicutes* followed by *Bacteroidetes* and *Actionbacteria*. Trophic interactions may have caused
this temporal pattern of incorporation. Soil C cy-
cling models, however, often disregard bacterial
trophic interactions. Fast growing substrate generalists assimilated xylose-C and slow growing sub-
strate specialists assimilated cellulose-C. Xylose-
C assimilators within time points clustered phylo-
genetically, and cellulose-C assimilators clustered phylogenetically overall. Knowledge of soil C
cycling functional guild diversity, membership and
activity will improve the predictive power of ter-
restrial C flux models.

stable isotope probing | structure-function relationships | soil mi-
crobial ecology | 16S rRNA gene

Abbreviations: C, Carbon; OTU, Operational Taxonomic Unit; SOM,
Soil Organic Matter; BD, Buoyancy Density; SIP, Stable Isotope Prob-
ing

Significance

We have a limited understanding of soil carbon (C) cycling yet soil contains a large fraction of the global C pool. Microorganisms mediate most soil C cycling but have proven difficult to study due to the complexity of soil C biochemistry and the wide range of soil microorganisms participating in C reactions. We demonstrate C use dynam-

ics by soil microbial taxa. Furthermore, we identi-
fied microorganisms involved in cellulose decomposi-
tion that were previously uncharacterized physi-
ologically – cellulose is the most globally abundant
biopolymer. Our results expand knowledge of soil
functional guild diversity and activity which reveal
soil structure-function relationships. This study is
a departure from typical nucleic acid SIP studies
that focus on listing the identities of heavy isotope
labeled organisms. Our approach enables DNA-
SIP to identify ^{13}C labeled microorganisms with
greater resolution producing a better sampling of
functional guilds. This not only allows us to con-
nect function to genetic identity but also allows us
to assess functional guild diversity and uncover
ecological strategies. Further, we demonstrate how
substrate specificity can be assessed from DNA-
SIP data.

Introduction

Excluding plant biomass, there are 2,300 Pg of
carbon (C) in soils worldwide which accounts for
~80% of the global terrestrial C pool [1, 2]. Fungi,
archaea, and bacteria degrade plant biomass that
reaches soil respiring the majority of plant biomass
C producing 10 times more CO_2 annually than an-
thropogenic emissions [3]. Rising atmospheric CO_2
may stimulate plant growth and in turn increase
plant biomass C input to soil [4]. Current climate
change models concur on atmospheric and oceanic,

Reserved for Publication Footnotes

but not terrestrial, global C flux predictions [5]. Contrasting terrestrial C model predictions reflect how little is known about soil C cycling. We need to establish the roles and diversity of soil microbial community members involved with soil C cycling to reconcile inconsistencies in terrestrial C models [6, 7].

Functional guild membership and diversity establish the connections between soil functions and community structure [8]. Microorganisms mediate an estimated 80–90% of soil C cycling [9, 10] but the complexity of soil obfuscates microbial contributions to soil C cycling and the majority of microorganisms resist cultivation in the laboratory. Stable-isotope probing (SIP), however, links genetic identity and activity without cultivation and has expanded our knowledge of microbial contributions to biogeochemical processes [11]. Successful applications of SIP have identified organisms which mediate processes performed by functionally specialized microorganisms of limited diversity such as methanogens [12] but SIP has been less applicable in soil C cycling studies because simultaneous labeling of many different organisms has necessitated prohibitory resolving power. High throughput DNA sequencing technology, however, improves the resolving power of SIP enabling exploration of complex soil C-cycling processes.

This study aimed to observe labile C versus polymeric C assimilation dynamics in the soil microbial community. To soil microcosms we added a mixture of nutrients and C substrates that simulated the composition of plant biomass. All microcosms received the same C substrate mixture where the only difference between treatments was the identity of the isotopically labeled substrate. Specifically, we set up a series of microcosms with three treatments: in one treatment xylose was substituted for its ^{13}C -equivalent, in another cellulose was substituted for its ^{13}C -equivalent, and in the third treatment all substrates in the mixture were unlabeled. We harvested microcosms from each treatment at days 3, 7, 14 and 30 and additionally harvested microcosms receiving ^{13}C -xylose and unlabeled substrates on day 1. We chose to label xylose and cellulose to contrast labile C and polymeric C decomposition, respectively. Post incubation, we sequenced 16S rRNA genes from SIP density fractions with high throughput DNA sequencing technology. Our experimental design allowed us to observe the soil microbial community members that assimilated xylose-C and cellulose-C over time.

Results

Our experimental design allowed us to track the flow of xylose and cellulose C through the soil mi-

crobial community (Figure ??). 5.3 mg C substrate mixture per gram of soil was added to each microcosm representing 18% of the soil C. The mixture included 0.42 mg xylose-C and 0.88 mg cellulose-C per gram soil. Microcosms were harvested at days 1, 3, 7, 14 and 30 during a 30 day incubation. ^{13}C -xylose assimilation peaked immediately and tapered over the 30 day incubation whereas ^{13}C -cellulose assimilation peaked two weeks after amendment additions (Figure 1, Figure ??). See Supplemental Note 1.9 for sequencing and density fractionation statistics. Microcosm treatments (see Methods) are identified in figures by the following code: “13CXPS” refers to the amendment with ^{13}C -xylose (^{13}C Xylose Plant Simulant), “13CCPS” refers to the ^{13}C -cellulose amendment and “12CCPS” refers to the amendment that only contained ^{12}C (i.e. control).

Soil microcosm microbial community changes with time. Changes in the bulk soil microcosm microbial community structure and membership correlated significantly with time (Figure ??, P-value 0.23, R^2 0.63, Adonis test [13]). The identity of the ^{13}C -labeled substrate added to the microcosms did not significantly correlate with bulk soil community structure and membership (P-value 0.35). Additionally, microcosm beta diversity was significantly less than gradient fraction beta diversity (Figure ??, P-value 0.003, “betadisper” function R Vegan package [14, 15]). Twenty-nine OTUs significantly changed in relative abundance with time (“BH” adjusted P-value < 0.10, [16]) and of these 29 OTUs, 14 were found to incorporate ^{13}C from labeled substrates into biomass (Figure ??). Four taxonomic classes significantly (adjusted P-value < 0.10) changed in abundance: *Bacilli* (decreased), *Flavobacteria* (decreased), *Gammaproteobacteria* (decreased) and *Herpetosiphonales* (increased) (Figure ??). Abundances grouped by phylum for OTUs that incorporated ^{13}C from cellulose increased with time whereas abundances grouped by phylum of OTUs that incorporated ^{13}C from xylose decreased over time although *Proteobacteria* abundance spiked at day 14 (Figure ??).

OTUs that assimilated ^{13}C into DNA. Within the first 7 days of incubation 63% of ^{13}C -xylose was respired and only 6% more was respired from day 7 to 30. At day 30, 30% of the ^{13}C from xylose remained in the soil. An average 16% of the ^{13}C -cellulose added was respired within the first 7 days, 38% by day 14, and 60% by day 30.

We refer to OTUs that putatively incorporated ^{13}C into DNA originally from an isotopically labeled substrate as substrate “responders” (see Supplemental Note 1.7.4 for operational “re-

“sponse” criteria). There were 19, 19, 15, 6, and 1 ¹³C-xylose responders at days 1, 3, 7, 14, 30, respectively (Figure ??). The numerically dominant ¹³C-xylose responder phylum shifted from *Firmicutes* to *Bacteroidetes* and then to *Actinobacteria* across days 1, 3 and 7 (Figure 2, Figure 3). *Proteobacteria* ¹³C-xylose responders were found at days 1, 3, 7 but peaked at day 7 (Figure 3).

Only 2 and 5 OTUs responded ¹³C-cellulose at days 3 and 7, respectively. At days 14 and 30, 42 and 39 OTUs responded to ¹³C-cellulose. (Figure ??). *Proteobacteria*, *Verrucomicrobia*, and *Chloroflexi* had relatively high numbers of responders with strong response across multiple time points (Figure 2). *Verrucomicrobia* ¹³C-cellulose responders were 70% *Spartobacteria*. *Chloroflexi* responders were annotated as members of the *Herpetosiphonales* and *Anaerolineae* (Figure ??). *Celvibrio*, a canonical soil cellulose degrader, was found to respond strongly to ¹³C-cellulose in the microcosms. See Supplemental Note 2 for further counts of ¹³C-responsive OTUs at greater taxonomic resolution.

Ecological strategies of ¹³C responders. ¹³C-xylose responders are generally more abundant members based on relative abundance in bulk DNA SSU rRNA gene content than ¹³C-cellulose responders (Figure 4, P-value 0.00028, Wilcoxon Rank Sum test). However, ¹³C-xylose and ¹³C-cellulose responders included both abundant and rare OTUs (Figure 4). Two ¹³C-cellulose responders were not found in any bulk samples (“OTU.862” and “OTU.1312”, Table ??). Of the 10 most abundant responders, 8 are ¹³C-xylose responders and 6 of these 8 are consistently among the 10 most abundant OTUs in bulk samples.

Cellulose-responder-DNA buoyant density (BD) shifted further along the density gradient than xylose-responder-DNA BD in response to ¹³C incorporation (Figure ??, Figure 4, P-value 1.8610x⁻⁰⁶, Wilcoxon Rank Sum test). ¹³C-cellulose-responder-DNA BD shifted on average 0.0163 g mL⁻¹ (sd 0.0094) whereas xylose responder BD shifted on average 0.0097 g mL⁻¹ (sd 0.0094). For reference, 100% ¹³C DNA BD is 0.0435 g mL⁻¹ greater than the BD of its ¹²C counterpart. DNA BD increases as its ratio of ¹³C to ¹²C increases. An organism that only assimilates C into DNA from a ¹³C isotopically labeled source, will have a greater ¹³C to ¹²C ratio in its DNA than an organism utilizing a mixture of isotopically labeled and unlabeled C sources (see Supplemental Note 1.8). We predicted the *rrn* gene copy number for each OTU as described previously [17]. The estimated *rrn* gene copy number for ¹³C-xylose responders was inversely related to the time of first response for each OTU (P-value 2.02x10⁻¹⁵,

Figure ??). OTUs that did not respond at day 1 but did respond at day 3 and/or day 7 had fewer estimated *rrn* copy number than OTUs that responded at day 1 (Figure ??).

We assessed phylogenetic clustering of ¹³C-responsive OTUs with the Nearest Taxon Index (NTI), the Net Relatedness Index (NRI) [18], and the consenTRAIT metric [19]. Briefly, positive NRI and NTI with corresponding low P-values indicates deep phylogenetic clustering whereas negative NRI with high P-values indicates taxa are overdispersed compared to the null model [20]. NRI and P-values for substrate responder groups suggest ¹³C-xylose responders are overdispersed (NRI: -1.33, P: 0.90) while ¹³C-cellulose responders are clustered (NRI: 4.49, P: 0.001). NTI values show that both ¹³C-cellulose and ¹³C-xylose responders are clustered near the tips of the tree (NTI: 1.43 (P: 0.072), 2.69 (P: 0.001), respectively). The consenTRAIT clade depth for ¹³C-xylose and ¹³C-cellulose responders was 0.012 and 0.028 16S rRNA sequence dissimilarity, respectively.

Discussion

Microbial response to isotopic labels. DNA-SIP can establish functional roles for thousands of phylotypes in a single experiment without cultivation. We identified 104 soil OTUs that incorporated ¹³C from xylose and/or cellulose into biomass over time. With this information we can build a conceptual model for the soil food web with respect to xylose and cellulose in our microcosms. We propose xylose and cellulose-C added to soil microcosms took the following path through the microbial food web (Figure ??): fast-growing *Firmicutes* spore formers first assimilated xylose C within 24 hours. Over the next 6 days, biomass from early-responding *Firmicutes* and the remaining xylose-C and was consumed by *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria* phylotypes. Canonical cellulose degrading bacteria like *Celvibrio* and members of cosmopolitan yet functionally uncharacterized soil phylogenetic groups like *Chloroflexi*, *Planctomycetes* and *Verrucomicrobia*, specifically the *Spartobacteria*, decomposed cellulose. Cellulose C incorporation into microbial biomass peaked at day 14 and extended through day 30.

Ecological strategies of soil microorganisms participating in the decomposition of organic matter. Models of soil C cycling rely on functional niches defined by soil microbiologists. In these models ecological strategies such as growth rate and substrate specificity are parameters for functional niche behavior [21]. Functional niches are commonly discovered by observing how community structure changes with changing conditions [22].

In this experiment, DNA-SIP revealed functional niche membership. We also used DNA-SIP data to quantify substrate specificity which is related to the magnitude of DNA BD shift upon ^{13}C labeling (see Results). Moreover, we assessed growth rate of functional niche members by estimating niche member *rrn* gene copy number, a genomic feature reliably extrapolated from phylogeny that is indicative of how fast a microorganism grows [17, 23]. We found that ^{13}C -cellulose responsive OTUs are likely slow growing substrate specialists relative to ^{13}C -xylose responders (Figure 4, Figure ??). We also found that ^{13}C -xylose responsive OTUs that incorporated ^{13}C into biomass at day one grow faster than OTUs that responded later (Figure 4, Figure ??). If OTUs that first responded after day 1 consumed labeled OTUs that responded at day 1, this suggests that predators and/or saprophytes grow slower than microorganisms directly assimilating labile C and has implications for modelling trophic niches. We also note that ^{13}C -cellulose responders are generally lower abundance members of the bulk community than ^{13}C -xylose responsive OTUs (Figure 4), however, high *rrn* gene copy number may inflate ^{13}C -xylose responder abundance.

NRI values quantify phylogenetic clustering [18] and have been used to assess clustering of soil OTUs that responded similarly to soil wet up [20, 24]. To our knowledge, assessing phylogenetic clustering of OTUs found to incorporate heavy isotopes into biomass during SIP incubations has not been attempted. We found that ^{13}C -cellulose and xylose responders are clustered and overdispersed, respectively. This suggests that the ability to degrade cellulose is phylogenetically conserved possibly reflecting the complexity of cellulose degradation biochemistry. The positive relationship between a physiological trait’s phylogenetic depth and its complexity has been noted previously [25] and the clade depth of ^{13}C -cellulose responders, 0.028 16S rRNA gene sequence dissimilarity, is on the same order as that observed for glycoside hydrolases which are diagnostic enzymes for cellulose degradation [26]. Overdispersion, as we saw for the ^{13}C -xylose responsive OTUs, may be indicative of a readily horizontally transferred trait and/or a trait that is broadly distributed phylogenetically, or, in this case may indicate that the overdispersed group includes more than one trait. It’s not clear which ^{13}C -xylose responsive organisms were labeled as a result of primary xylose assimilation (see below), and therefore it’s not clear if ^{13}C -xylose responsive OTUs in this experiment constitute a single ecologically meaningful group or multiple ecological groups. Temporally defined ^{13}C -xylose responder groups, however, were phylogenetically coherent (Figure ??, Figure 3). For example, most

day 1 ^{13}C -xylose responders are members of the *Paenibacillus* (see Supplemental Note 2). Notably, Members of the *Bacillus* [27] and *Paenibacillus* in particular [28] have been previously implicated as labile C decomposers .

Intuitively we infer C cycling functional guild diversity from the distribution of diagnostic genes across genomes [26] or from screening culture collections for a particular trait [19]. For instance, the wide distribution of the glycolysis operon in microbial genomes is interpreted as evidence that many soil microorganisms participate in glucose turnover [7]. *In situ* functional guild diversity, however, can vary significantly from diversity assessed by functionally screening isolates and/or genomes. Xylose use in soil, for instance, may be less a function of catabolic pathway distribution across genomes and more a function of lifestyle and few taxonomic groups have participated in labile C decomposition in soil microcosms as inferred by analyzing relative abundance changes in response to labile C input [27, 29]. Phenomena such as seasonal change [30], and rainfall [24] cause nutrient and resource concentrations in soil to fluctuate. Therefore, fast growth and/or rapid resuscitation upon wet up [24] allow microorganisms to favorably compete for labile C resources. Life history ecological strategies tied to phylogeny like growth rate [22] may constrain the diversity of labile C assimilators even though the ability to use labile C is phylogenetically dispersed. DNA-SIP is useful for establishing *in situ* phylogenetic clustering and diversity of functional guilds because DNA-SIP can account for life history strategies by targeting *active* microorganisms. Additionally, snapshot estimates of community composition commonly inform soil structure-function-relationship studies [22] but labile C decomposition might not be linked to snapshot community structure. Alternatively labile C decomposition might be linked specifically to community structure *dynamics*. That is, fast growing spore formers would not need to maintain high abundance to significantly mediate cycling of pulse delivered resources. This accentuates the importance of DNA-SIP for describing soil ecology as DNA-SIP assesses activity which can be decoupled from snapshot abundance.

Implications for soil C cycling models. Land management, climate, pollution and disturbance can influence soil community composition [7] which in turn influences soil biogeochemical process rates (e.g. [31]). Assessing functional group diversity and establishing identities of functional group members is necessary to predict how biogeochemical process rates will change with community composition [7, 32]. Aggregate biogeochemical processes that are the sum of many subprocesses in-

volve a broad array of taxa and are assumed to be less influenced by community change than narrow processes that involve a single, specific chemical transformation by a narrow suite of microbial participants [7, 32]. Within an aggregate process such as C decomposition, subprocesses can be further classified as broad or narrow [7]. In the 420
ory, “broad” and “narrow” functional guilds decompose labile and recalcitrant C, respectively [7]. However, the diversity of active labile C and re- 425
calcitrant C decomposers in soil has not been directly quantified. Notably, we found more OTUs 430 responded to ^{13}C -cellulose, 63, than ^{13}C -xylose, 49. Also, it is possible that many ^{13}C -xylose 435
responders are predatory bacteria or saprophytes as opposed to primary labile C degraders (see below). Cellulose and xylose decomposer functional guilds 440
were non-overlapping in membership – of 104 ^{13}C - 445
responders only 8 responded to both cellulose and xylose – and represented a small fraction of total soil community diversity (Figure 5). While xylose use is undoubtedly more widely distributed 450 among microbial genomes than the ability to de- 455
grade cellulose, the number of unique active cellulose decomposer OTUs outnumbered the number 460
of unique active xylose utilizer OTUs.

Trophic interactions and/or functional groups 445
tuned to different resource concentrations caused 450
the activity succession from *Firmicutes* to *Bac- 455
teroidetes* and finally *Actinobacteria* in response to 460
xylose addition. *Actinobacteria* (e.g. *Agromyces*) 465
and *Bacteroidetes* have been previously implicated 470
as predatory soil bacteria [33, 34], and, in our mi- 475
crocosms *Bacteroidetes* and *Actinobacteria* activ- 480
ity peaked while *Firmicutes* ^{13}C -xylose responder 485
relative abundance plummeted (Figure 2, Figure 3, 490
Figure ??). Considering *Agromyces* and *Bac- 495
teroidetes* phylotypes are likely soil predators, one 500
parsimonious hypothesis for ^{13}C -labelling of *Bac- 505
teroidetes* and *Actinobacteria* with a corresponding 510
decrease in ^{13}C -labeled *Firmicutes* abundance is 515
that *Bacteroidetes* and *Actinobacteria* fed on ^{13}C - 520
labeled *Firmicutes*. Besides predation, mother cell 525
lysis, the last step in sporulation, would release 530
C from spore formers potentially to *Bacteroidetes* 535
and *Actinobacteria*. If the temporal dynamics of 540
 ^{13}C -xylose incorporation are due to trophic inter- 545
actions, predatory bacteria or saprophytes con- 550
sumed many, if not most, fast-growing labile C 555
degraders. Hence, soil C cycling models should 560
include trophic interactions between soil bacteria 565
but rarely do (e.g. [35]). When soil C models do 570
account for predators/saprophytes, trophic inter- 575
actions are predicted to significantly influence C:N 580
ratios of SOM relative to litter C:N, and, recycle 585
significant amounts of microbial biomass [21].

We propose two scenarios in the context of our 585
results whereby community composition could af- 590

flect C dynamics and fate. Genomic evidence shows cellulose degradation is a phylogenetically conserved trait [26]. Our study evaluates the phylogenetic conservation of soil cellulose degradation in active microorganisms via DNA-SIP and our results concur with genomic evidence. A decrease in cellulose degrader abundance would diminish cellulose decomposition process rates as few soil microorganisms can fill the phylogenetically conserved cellulose degradation niche. Dispersed cellulose decomposers could renew ecosystem function, however. For labile C decomposition, the absence of fast growing spore formers would allow other microbes to assimilate labile C provided dispersal does not enable rapid recolonization. Primary labile C degraders in this study grow fast, and form spores and these distinct ecological strategies might indicate distinct C use dynamics and/or resource allocation. New labile C degraders may metabolize and allocate labile C differently thus changing labile C dynamics and fate. Further, labile C degrader substitution could affect biomass C turnover by predatory bacteria or saprophytes that feed on fast growing, spore forming labile C decomposers. On the other hand, spore formation enables dispersal [36] which would allow fast growing spore formers to continuously occupy the labile C decomposition niche. One proposed mechanism for similar decomposition rates of labile C across soils varying in community composition is that labile C is decomposed by a diverse suite of soil microorganisms [7]. An alternative hypothesis for consistent labile C process rates across different soils is that labile C degraders disperse readily. Notably, other lineages implicated in rapid labile C turnover include members of the *Actinobacteria* [24] and many soil *Actinobacteria* form hyphae that facilitate dispersal [37]. The two hypotheses are not mutually exclusive, but our results and previous studies suggest that environmental conditions unfavorable to fast-growing spore-formers and/or quickly resuscitated, hyphal *Actinobacteria* may impact labile C dynamics and fate.

Conclusion. Microorganisms sequester atmospheric C and respire SOM influencing climate change on a global scale but we do not know which microorganisms carry out specific soil C transformations. In this experiment microbes from physiologically uncharacterized but ubiquitous soil lineages participated in cellulose decomposition. Cellulose C degraders included members of the *Verrucomicrobia* (*Spartobacteria*), *Chloroflexi*, *Bacteroidetes* and *Planctomycetes*. *Spartobacteria* in particular are globally cosmopolitan soil microorganisms and are often the most abundant *Verrucomicrobia* order in soil [38]. Fast-growing *Firmicutes* spore formers assimilated labile C in our microcosms. *Bac-*

teroidetes and *Actinobacteria* phylotypes, previously implicated as predators, may have fed on the fast growing *Firmicutes*. Our results suggest that, cosmopolitan *Spartobacteria* may degrade cellulose on a global scale, bacterial trophic interactions can significantly impact soil C cycling, and life history ecological strategies such as fast growth constrain functional guild diversity for labile C decomposition.

Methods

Additional information on sample collection and analytical methods is provided in Supplemental Materials and Methods.

Twelve soil cores (5 cm diameter x 10 cm depth) were collected from six random sampling locations within an organically managed agricultural field in Penn Yan, New York. Soils were pretreated by sieving (2 mm), homogenizing sieved soil, and pre-incubating 10 g of dry soil weight in flasks for 2 weeks. Soils were amended with a 5.3 mg g soil⁻¹ carbon mixture; representative of natural concentrations [39]. Mixture contained 38% cellulose, 23% lignin, 20% xylose, 3% arabinose, 1% galactose, 1% glucose, and 0.5% mannose by mass, with the remaining 13.5% mass composed of an amino acid (in-house made replica of Teknova C0705) and basal salt mixture (Murashige and Skoog, Sigma Aldrich M5524). Three parallel treatments were performed; (1) unlabeled control, (2)¹³C-cellulose, (3)¹³C-xylose (98 atom% ¹³C, Sigma Aldrich). Each treatment had 2 replicates per time point (n = 4) except day 30 which had 4 replicates; total microcosms per treatment n = 12, except ¹³C-cellulose which was not sampled at day 1, n = 10. Other details relating to substrate addition can be found in SI. Microcosms were sampled destructively (stored at -80°C until nucleic acid processing) at days 1 (control and xylose only), 3, 7, 14, and 30.

Nucleic acids were extracted using a modified Griffiths protocol [40]. To prepare nucleic acid extracts for isopycnic centrifugation as previously described [41], DNA was size selected (>4kb) using 1% low melt agarose gel and β-agarase I enzyme extraction per manufacturers protocol (New England Biolab, M0392S). For each time point in the series isopycnic gradients were setup using a modified protocol [42] for a total of five ¹²C-control, five ¹³C-xylose, and four ¹³C-cellulose microcosms. A density gradient (average density 1.69 g mL⁻¹) solution of 1.762 g cesium chloride (CsCl) mL⁻¹ in gradient buffer solution (pH 8.0 15 mM Tris-HCl, 15 mM EDTA, 15 mM KCl) was used to separate ¹³C-enriched and ¹²C-nonenriched DNA. Each gradient was loaded with approximately 5 µg of DNA and ultracentrifuged for 66 h at 55,000 rpm

and room temperature (RT). Fractions of ~100 µL were collected from below by displacing the DNA-CsCl-gradient buffer solution in the centrifugation tube with water using a syringe pump at a flow rate of 3.3 µL s⁻¹ [43] into Acroprep™ 96 filter plate (Pall Life Sciences 5035). The refractive index of each fraction was measured using a Reichart AR200 digital refractometer modified as previously described [41] to measure a volume of 5 µL. Then buoyant density was calculated from the refractive index as previously described [41] (see also SI). The collected DNA fractions were purified by repetitive washing of Acroprep filter wells with TE. Finally, 50 µL TE was added to each fraction then resuspended DNA was pipetted off the filter into a new microfuge tube.

For every gradient, 20 fractions were chosen for sequencing between the density range 1.67-1.75 g mL⁻¹. Barcoded 454 primers were designed using 454-specific adapter B, 10 bp barcodes [44], a 2 bp linker (5'-CA-3'), and 806R primer for reverse primer (BA806R); and 454-specific adapter A, a 2 bp linker (5'-TC-3'), and 515F primer for forward primer (BA515F). Each fraction was PCR amplified using 0.25 µL 5 U µl⁻¹ AmpliTaq Gold (Life Technologies, Grand Island, NY; N8080243), 2.5 µL 10X Buffer II (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 2.5 µL 25 mM MgCl₂, 4 µL 5 mM dNTP, 1.25 µL 10 mg mL⁻¹ BSA, 0.5 µL 10 µM BA515F, 1 µL 5 µM BA806R, 3 µL H₂O, 10 µL 1:30 DNA template) in triplicate. Samples were normalized either using Pico green quantification and manual calculation or by SequalPrep™ normalization plates (Invitrogen, Carlsbad, CA; A10510), then pooled in equimolar concentrations. Pooled DNA was gel extracted from a 1% agarose gel using Wizard SV gel and PCR clean-up system (Promega, Madison, WI; A9281) per manufacturer's protocol. Amplicons were sequenced on Roche 454 FLX system using titanium chemistry at Selah Genomics (formerly EnGenCore, Columbia, SC)

References

- Amundson R (2001) The carbon budget in soils. *Annu Rev Earth Planet Sci* 29(1): 535–562.
- Batjes N-H (1996) Total carbon and nitrogen in the soils of the world. *European Journal of Soil Science* 47(2): 151–163.
- Chapin F (2002) Principles of terrestrial ecosystem ecology.
- Groenigen K-J, Graaff M-A, Six J, Harris D, Kuikman P, Kessel C (2006) The impact of elevated atmospheric CO₂ on soil C and N dynamics: a meta-analysis. *Managed Ecosystems and CO₂* (Springer Science, Berlin Hei-

- 645 delberg), pp 373–391.
5. Friedlingstein P, Cox P, Betts R, Bopp L, von W-B, Brovkin V, et al. (2006) Climate–carbon cycle feedback analysis: Results from the C4 mip model intercomparison. *J Climate* 19(14): 705 3337–3353.
 6. Neff J-C, Asner G-P (2001) Dissolved organic carbon in terrestrial ecosystems: synthesis and 710 a model. *Ecosystems* 4(1): 29–48.
 7. McGuire K-L, Treseder K-K (2010) Microbial 715 communities and their relevance for ecosystem models: Decomposition as a case study. *Soil Biology and Biochemistry* 42(4): 529–535.
 8. O'Donnell A-G, Seasman M, Macrae A, Waite I, Davies J-T (2002) Plants and fertilisers 720 as drivers of change in microbial community structure and function in soils. *Interactions in the Root Environment: An Integrated Approach* (Springer, Netherlands), pp 135–145.
 9. Coleman D-C, Crossley D-A (1996) Fundamentals of Soil Ecology.
 10. Nannipieri P, Ascher J, Ceccherini M-T, Landi L, Pietramellara G, Renella G (2003) Microbial diversity and soil functions. *European Journal of Soil Science* 54(4): 655–670.
 11. Chen Y, Murrell J-C (2010) When metagenomics meets stable-isotope probing: progress 725 and perspectives. *Trends Microbiol* 18(4): 157–163.
 12. Lu Y (2005) In situ stable isotope probing of methanogenic archaea in the rice rhizosphere. *Science* 309(5737): 1088–1090.
 13. Anderson M-J (2001) A new method for non-parametric multivariate analysis of variance. 730 *Austral Ecology* 26(1): 32–46.
 14. Anderson M-J, Ellingsen K-E, McArdle B-H (2006) Multivariate dispersion as a measure of beta diversity. *Ecology Letters* 9(6): 683–693.
 15. Oksanen J, Kindt R, Legendre P, OHara B, Stevens M-HH, Oksanen M-J, et al. (2007) The 735 vegan package.
 16. 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* 57(1): 289–300.
 17. Kembel S-W, Wu M, Eisen J-A, Green J-L (2012) Incorporating 16S gene copy number 740 information improves estimates of microbial diversity and abundance. *PLoS Comput Biol* 8(10): e1002743.
 18. Webb C-O (2000) Exploring the phylogenetic structure of ecological communities: an example for rain forest trees.. *The American Naturalist* 156(2): 145–155.
 19. Martiny A-C, Treseder K, Pusch G (2013) Phylogenetic conservatism of functional traits 745 in microorganisms. *ISMEJ* 7(4): 830–838.
 20. Evans S-E, Wallenstein M-D (2014) Climate change alters ecological strategies of soil bacteria. *Ecol Lett* 17(2): 155–164.
 21. Kaiser C, Franklin O, Dieckmann U, Richter A (2014) Microbial community dynamics alleviate stoichiometric constraints during litter decay. *Ecol Lett* 17(6): 680–690.
 22. Fierer N, Bradford M-A, Jackson R-B (2007) Toward an ecological classification of soil bacteria. *Ecology* 88(6): 1354–1364.
 23. Klappenbach J, Saxman P, Cole J, Schmidt T (2001) rrndb: the Ribosomal RNA Operon Copy Number Database.. *Nucleic Acids Res* 29(1): 181–184.
 24. Placella S-A, Brodie E-L, Firestone M-K (2012) Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. *PNAS* 109(27): 10931–10936.
 25. Martiny A-C, Treseder K, Pusch G (2013) Phylogenetic conservatism of functional traits in microorganisms. *ISMEJ* 7(4): 830–838.
 26. Berlemont R, Martiny A-C (2013) Phylogenetic distribution of potential cellulases in bacteria. *Appl Environ Microbiol* 79(5): 1545–1554.
 27. 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. ??? 82(3): 229–240.
 28. 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.
 29. Eilers K-G, Lauber C-L, Knight R, Fierer N (2010) Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil. ??? 42(6): 896–903.
 30. Schmidt S-K, Costello E-K, Nemergut D-R, Cleveland C-C, Reed S-C, Weintraub M-N, et al. (2007) Biogeochemical consequences of rapid microbial turnover and seasonal succession in soil. *Ecology* 88(6): 1379–1385.
 31. Berlemont R, Allison S-D, Weihe C, Lu Y, Brodie E-L, Martiny J-BH, et al. (2014) Cellulytic potential under environmental changes in microbial communities from grassland litter. *Front Microbiol* 5: 639.
 32. Schimel J (1995) Ecosystem consequences of microbial diversity and community structure. *Arctic and alpine biodiversity: patterns, causes and ecosystem consequences*, , eds. III P-DFSC, Körner P-DC, Ecological Studies (Springer, Berlin Heidelberg), pp 239–254.

- 760 33. 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.
- 765 34. Casida L-E (1983) Interaction of *Agromyces ramosus* with other bacteria in soil. *Appl Environ Microbiol* 46(4): 881–888.
- 770 35. 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.
- 775 36. Nicholson W-L, Munakata N, Horneck G, Melosh H-J, Setlow P (2000) Resistance of bacillus endospores to extreme terrestrial and extraterrestrial environments. *Microbiol Mol Biol Rev* 64(3): 548–572.
- 780 37. Killham K, Prosser J-I (2007) The prokaryotes. *Soil Microbiology, Ecology and Biochemistry (Third Edition)*, , ed. PAUL E-A (Academic Press, San Diego), pp 119–144.
- 785 38. Bergmann G-T, Bates S-T, Eilers K-G, Lauber C-L, Caporaso J-G, Walters W-A, et al. (2011) The under-recognized dominance of Verrucomicrobia in soil bacterial communities. *Soil Biology and Biochemistry* 43(7): 1450–1455.
39. Schneckenberger K, Demin D, Stahr K, Kuzyakov Y (2008) Microbial utilization and mineralization of ^{14}C glucose added in six or-
- ders of concentration to soil. *Soil Biology and Biochemistry* 40(8): 1981–1988.
- 790 40. 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.
- 795 41. 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.
- 800 42. Neufeld J-D, Vohra J, Dumont M-G, Lueders T, Manefield M, Friedrich M-W, et al. (2007) DNA stable-isotope probing. *Nature Protocols* 2(4): 860–866.
- 805 43. 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.
- 810 44. Hamady M, Walker J-J, Harris J-K, Gold N-J, Knight R (2008) Error-correcting barcoded primers for pyrosequencing hundreds of samples in multiplex. *Nat Meth* 5(3): 235–237.

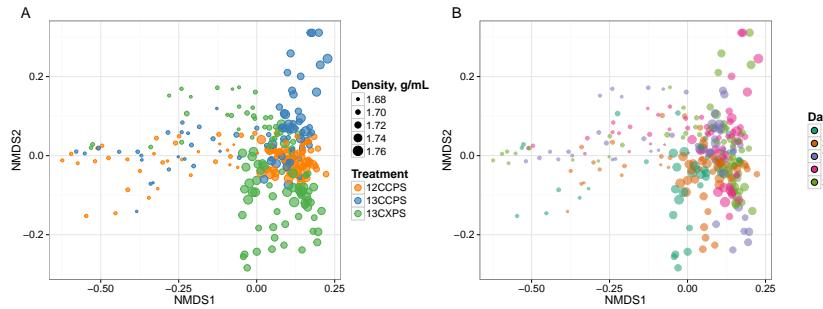


Fig. 1. NMDS analysis from weighted unifrac distances of 454 sequence data from SIP fractions of each treatment over time. Twenty fractions from a CsCl gradient fractionation for each treatment at each time point were sequenced (Fig. S1). Each point on the NMDS represents the bacterial composition based on 16S sequencing for a single fraction where the size of the point is representative of the density of that fraction and the colors represent the treatments (A) or days (B).

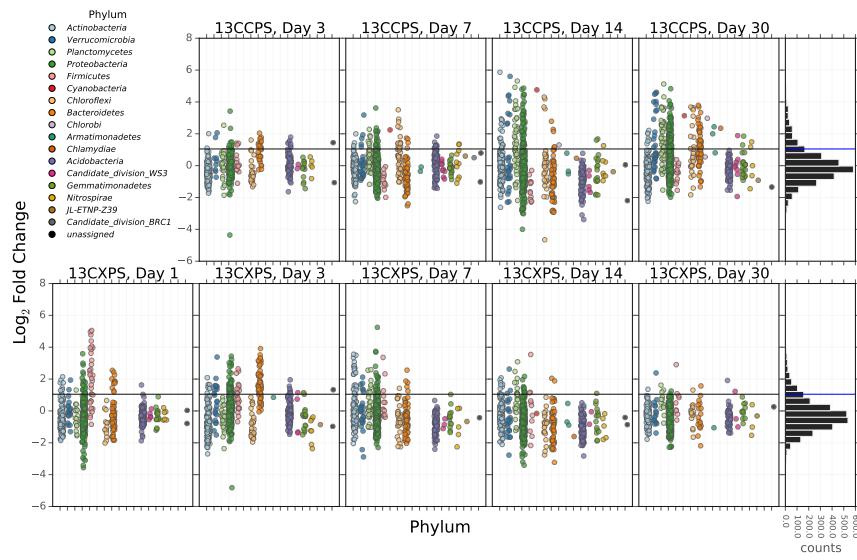


Fig. 2. Log₂ fold change of ¹³C-responders in cellulose treatment (top) and xylose treatment (bottom). Log₂ fold change is based on the relative abundance in the experimental treatment compared to the control within the density range 1.7125–1.755 g ml⁻¹. Taxa are colored by phylum. ‘Counts’ is a histogram of log₂ fold change values.

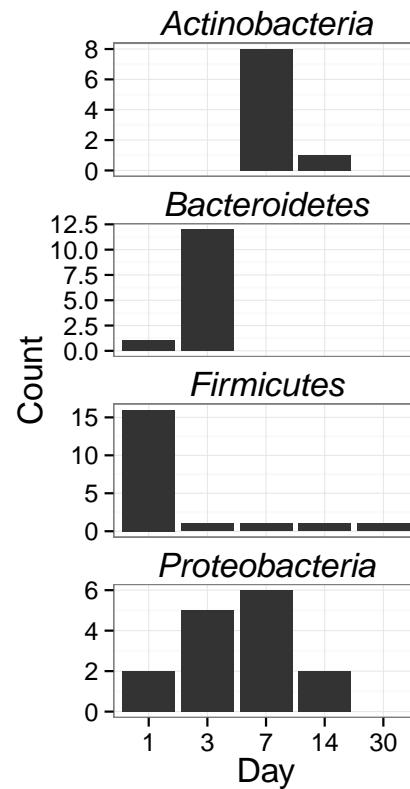


Fig. 3. Counts of ^{13}C -xylose responders in the *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria* at days 1, 3, 7 and 30.

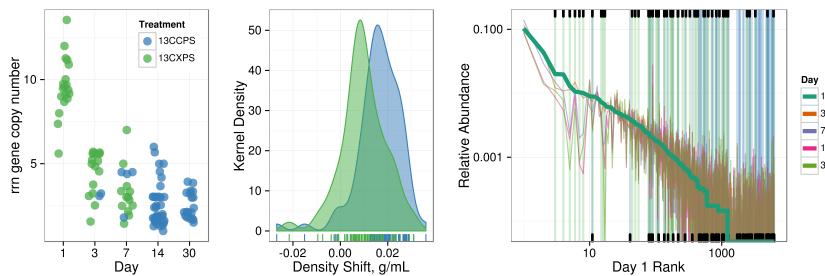


Fig. 4. ^{13}C -responder characteristics based on density shift (A) and rank (B). Kernel density estimation of ^{13}C -responder's density shift in cellulose treatment (blue) and xylose treatment (green) demonstrates degree of labeling for responders for each respective substrate. ^{13}C -responders in rank abundance are labeled by substrate (cellulose, blue; xylose, green). Ticks at top indicate location of ^{13}C -xylose responders in bulk community. Ticks at bottom indicate location of ^{13}C -cellulose responders in bulk community. OTU rank was assessed from day 1 bulk samples.

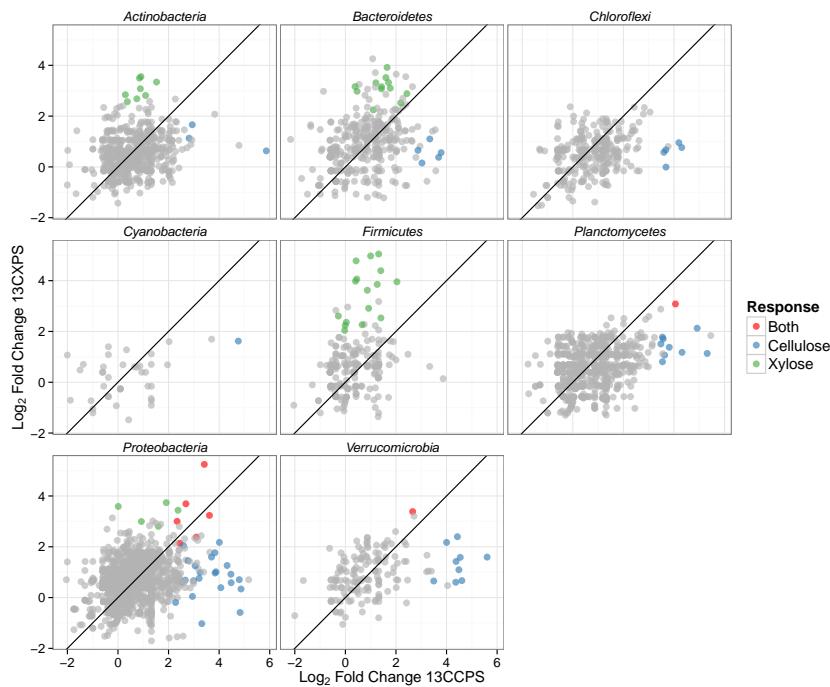


Fig. 5. Maximum log₂ fold change in response to labeled substrate incorporation for each substrate and all OTUs that pass sparsity filtering in both substrate analyses. Points colored by response. Line has slope of 1 with intercept at the origin. OTUs falling in the top-right quadrant responded to both substrates while those in the top-left and bottom-right responded to ¹³C-xylose and ¹³C-cellulose, respectively.