

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

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## 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  $^{13}\text{C}$  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*.  $^{13}\text{C}$  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  $^{13}\text{C}$  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

Soils worldwide contain 2,300 Pg of carbon (C) which accounts for nearly 80% of the C present in  
the terrestrial biosphere [1, 2]. Microbial soil C respi-  
ration produces annually tenfold more CO<sub>2</sub> than  
fossil fuel emissions [3] yet many global C biogeo-  
chemical flux models ignore microbial physiological  
diversity and its impacts on microbial activity in  
soils despite the significant contribution of micro-  
bial activity to global soil C flux. [4–6]. It re-  
mains difficult to predict the response of soil C to

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changes in climate [7–9] but predictions of climate change feedbacks on soil C flux improve when bio-  
geochemical models explicitly represent microbial physiology [10]. However, we still know little of the ecophysiology of soil microorganisms. Efforts  
that describe soil microorganism ecophysiology relevant to soil C biogeochemical fluxes should assist  
the development and refinement of terrestrial C biogeochemical models [7, 11, 12].

Cellulose comprises most plant C (30–50%) followed by hemicellulose (20–40%), and lignin (15–25%) [13]. Hemicellulose, being the most soluble, degrades most easily as compared to cellulose and lignin, and is targeted in the early stages of decomposition. Hemicellulose composition varies considerably with xylans being the most abundant constituent, themselves composed of differing amounts of xylose, glucose, arabinose, galactose, mannose, and rhamnose [14]. Xylose is often the most abundant sugar in hemicellulose, comprising as much as 60–90% of xylan in some plants (e.g. hardwoods) [?], wheat [16], and switchgrass [17]. Microbes that respire sugars proliferate during the initial stages of decomposition [18, 19], and metabolize as much as 75% of sugar C during the first 5 days of decomposition [20]. In contrast, cellulose degradation rates increase slowly for approximately 15 days and cellulose degradation continues for 30–90 days [20, 21]. It is hypothesized that microbes specialize in the decomposition of specific compounds in fresh, plant-derived organic matter and that these functionally specific guilds of microorganisms proliferate in succession as plant C of decreasing lability is decomposed over time [21, 22, 24?]. For instance, this degradative succession hypothesis posits that rapidly growing plant sugar decomposers respond quickly [25, 26] and are followed by slow growing degraders of plant polymers [25]. Evidence to support the degradative succession hypothesis comes from observing soil respiration dynamics and characterizing microbes cultivated at different stages of decomposition. The degree to which the succession hypothesis presents an accurate model of litter decomposition has been called into question [27? , 28] and it's clear that new approaches are needed to dissect microbial contributions to C transformations in soils.

Functional guild membership and diversity establish the connections between soil functions and community structure [29]. Microorganisms mediate an estimated 80–90% of soil C cycling [30, 31] but the complexity of soil obscures 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 [32]. Successful

applications of SIP have identified organisms which mediate processes performed by functionally specialized microorganisms of limited diversity such as methanogens [33] but SIP has been less applicable in soil C cycling studies because simultaneous labeling of many different organisms requires prohibitive resolving power to successfully employ SIP. High throughput DNA sequencing technology, however, improves the resolving power of SIP enabling exploration of complex soil C-cycling processes.

Though microorganisms mediate 80–90% of the soil C-cycle [30, 31], and the physiological ecology of microorganisms can influence the dynamics of C transformations, terrestrial C-cycle models rarely consider the community composition of soils [34, 35]. Metabolic rates of soil C transformations are measured without knowledge of the organisms that mediate these reactions [31], leaving undefined the importance of community membership towards maintaining ecosystem function [31, 36, 37]. However, microbial community composition can account for significant variation in litter C mineralization [38]. Variation in microbial community composition can be linked most directly to rates of soil processes when discrete functional genes are available to represent those processes (e.g. denitrification [39], nitrification [40–42], methanotrophy [43], and nitrogen fixation [44]). However, the complexity of soil C transformations and the lack of convenient functional genes for describing these transformations has limited progress in characterizing the contributions of individual microbes to the soil C-cycle. Remarkably, we still lack information on the physiology and ecology of the majority of organisms that live in soils. For example, the bacterial phyla Acidobacteria, Chloroflexi, Planctomycetes, and Verrucomicrobia are nearly ubiquitous in soils, they comprise collectively up to 32% of the SSU rRNA genes in soil [45? ], but their contributions to soil processes remain almost completely uncharacterized.

Linking genetic identity to function in microbial ecosystems has historically relied on microbial cultivation and surveying diagnostic marker genes, but, most microorganisms resist cultivation in the lab by traditional methods (Jannsen) and many processes lack suitable diagnostic genetic markers. Additionally, laboratory cultivation rarely mimics environmental and ecological conditions and diagnostic genes only convey the genomic potential for a function as opposed to *in situ* activity. Stable-isotope probing of nucleic acids (SIP) links the genetic identity of microbes to specific processes bypassing the need for diagnostic genetic markers or microbial cultivation [32]. However, nucleic acid SIP has notable complications including the need to add large amounts of labeled substrate [47], la-

bel dilution resulting in partial labelling of nucleic acids [47–49], the potential for cross-feeding and secondary label incorporation [49–54], and variation in genome G+C content [55–58]. As a result, the most successful applications of SIP have identified functionally specialized microbial functional guilds of limited diversity (e.g. methanotrophs [47]) and the nucleic acid SIP has previously been insufficient to follow isotopic labeling of nucleic acids from many microbes as would be required for analysis of transformations in the soil C-cycle.

High throughput DNA sequencing can be used to enable exploration of major C-cycling processes in soils with nucleic acid SIP. SSU rRNA amplicons can now readily be sequenced from numerous density gradient fractions across multiple samples thereby increasing the resolution of a typical nucleic acid SIP experiment [59]. As a consequence of this increased resolution, it is also possible to use far less isotopically labeled substrate resulting in conditions that are more environmentally realistic [60]. We have employed such a high resolution DNA stable isotope probing approach to explore the incorporation of  $^{13}\text{C}$  labeled xylose and/or glucose into DNA by bacterial taxa over time in an agricultural soil. Specifically, we added a mixture of nutrients and C substrates to soil microcosms that simulated the composition of fresh organic matter from plant biomass. All microcosms received the same C substrate mixture where the only difference between treatments was the identity of the isotopically labeled substrate. We added the C substrate mixture to three series of microcosms: in one series xylose was substituted for its  $^{13}\text{C}$ -equivalent, in another cellulose was substituted for its  $^{13}\text{C}$ -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  $^{13}\text{C}$ -xylose and unlabeled substrates on day 1. The experiment was designed to provide a test of the degradative succession hypothesis as applied to soil bacteria, to identify the bacteria that metabolize xylose and cellulose in soils, and to characterize the temporal dynamics of xylose and cellulose metabolism during the degradation of fresh organic matter.

## Results

Our experimental design allowed us to track the flow of xylose and cellulose-C through the soil microbial community (Figure S1). Substrate mixture, 5.3 mg C 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. Assimilation of  $^{13}\text{C}$ -xylose peaked immediately and tapered over the 30 day incubation whereas  $^{13}\text{C}$ -cellulose assimilation peaked two weeks after amendment additions (Figure 1, Figure S2). 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  $^{13}\text{C}$ -xylose ( $^{13}\text{C}$  Xylose Plant Simulant), “13CCPS” refers to the  $^{13}\text{C}$ -cellulose amendment and “12CCPS” refers to the amendment that only contained  $^{12}\text{C}$  (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 S3, P-value 0.23,  $R^2$  0.63, Adonis test [61]). The identity of the  $^{13}\text{C}$ -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 S3, P-value 0.003, “betadisper” function R Vegan package [62, 63]). Twenty-nine OTUs significantly changed in relative abundance with time (“BH” adjusted P-value < 0.10, [64]) and of these 29 OTUs, 14 were found to incorporate  $^{13}\text{C}$  from labeled substrates into biomass (Figure S4). Four taxonomic classes significantly (adjusted P-value < 0.10) changed in abundance: *Bacilli* (decreased), *Flavobacteria* (decreased), *Gammaproteobacteria* (decreased) and *Herpetosiphonales* (increased) (Figure S5). Abundances grouped by phylum for OTUs that incorporated  $^{13}\text{C}$  from cellulose increased with time whereas abundances grouped by phylum of OTUs that incorporated  $^{13}\text{C}$  from xylose decreased over time although *Proteobacteria* abundance spiked at day 14 (Figure S6).

**OTUs that assimilated  $^{13}\text{C}$  into DNA.** Within the first 7 days of incubation 63% of  $^{13}\text{C}$ -xylose was respired and only 6% more was respired from day 7 to 30. At day 30, 30% of the  $^{13}\text{C}$  from xylose remained in the soil. An average 16% of the  $^{13}\text{C}$ -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  $^{13}\text{C}$  into DNA originally from an isotopically labeled substrate as substrate “responders” (see Supplemental Note 1.7.4 for operational “response” criteria). There were 19, 19, 15, 6, and 1  $^{13}\text{C}$ -xylose responders at days 1, 3, 7, 14, 30, respectively (Figure S2). The numerically abundant phylum affiliation of  $^{13}\text{C}$ -xylose responders shifted from *Firmicutes* to *Bacteroidetes* and then to *Actinobacteria* across days 1, 3 and 7 (Figure 2,

Figure 3). *Proteobacteria*  $^{13}\text{C}$ -xylose responders were found at days 1, 3, 7 but peaked at day 7 (Figure 3).

Only 2 and 5 OTUs responded to  $^{13}\text{C}$ -cellulose at days 3 and 7, respectively. The number of  $^{13}\text{C}$ -cellulose responders increased at days 14 and 30 to 42 and 39 OTUs, respectively. (Figure S2). Responders in the *Proteobacteria*, *Verrucomicrobia*, and *Chloroflexi* had strong response across multiple time points (Figure 2). *Verrucomicrobia*  $^{13}\text{C}$ -cellulose responders were 70% *Spartobacteria*. *Chloroflexi* responders were annotated as members of the *Herpetosiphonales* and *Anaerolineae* (Figure S10). *Cellvibrio*, a *gammaproteobacterium* and a canonical soil cellulose degrader, was found to respond strongly to  $^{13}\text{C}$ -cellulose in the microcosms. See Supplemental Note 2 for further counts of  $^{13}\text{C}$ -responsive OTUs at greater taxonomic resolution.

**Ecological strategies of  $^{13}\text{C}$  responders.** Responders to  $^{13}\text{C}$ -xylose were generally more abundant in the bulk soil than  $^{13}\text{C}$ -cellulose responders based on relative abundance in bulk DNA SSU rRNA gene content (Figure 4, P-value 0.00028, Wilcoxon Rank Sum test). However,  $^{13}\text{C}$ -xylose and  $^{13}\text{C}$ -cellulose responders included OTUs at both high and low abundance (Figure 4). Two  $^{13}\text{C}$ -cellulose responders were not found in any bulk samples (“OTU.862” and “OTU.1312”, Table S1). Of the 10 most abundant  $^{13}\text{C}$ -responders, 8 responded to  $^{13}\text{C}$ -xylose, 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  $^{13}\text{C}$  incorporation (Figure S7, Figure 4, P-value  $1.8610 \times 10^{-6}$ , Wilcoxon Rank Sum test).  $^{13}\text{C}$ -cellulose-responder-DNA BD shifted on average  $0.0163 \text{ g mL}^{-1}$  (sd 0.0094) whereas xylose responder BD shifted on average  $0.0097 \text{ g mL}^{-1}$  (sd 0.0094). For reference, 100%  $^{13}\text{C}$  DNA BD is  $0.04 \text{ g mL}^{-1}$  greater than the BD of its  $^{12}\text{C}$  counterpart. DNA BD increases as its ratio of  $^{13}\text{C}$  to  $^{12}\text{C}$  increases. An organism that only assimilates C into DNA from a  $^{13}\text{C}$  isotopically labeled source, will have a greater  $^{13}\text{C}$  to  $^{12}\text{C}$  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 by (author?) [65]. The estimated *rrn* gene copy number for  $^{13}\text{C}$ -xylose responders was inversely related to the time of first response for each OTU (P-value  $2.02 \times 10^{-15}$ , Figure S8).

We assessed phylogenetic clustering of  $^{13}\text{C}$ -responsive OTUs with the Nearest Taxon Index (NTI), the Net Relatedness Index (NRI) [66], and the consenTRAIT metric [67]. 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 [68]. The consenTRAIT metric is a measure of the average clade depth in a phylogenetic tree for a given collection of tips. NRI and P-values for substrate responder groups suggest  $^{13}\text{C}$ -xylose responders are overdispersed (NRI: -1.33, P: 0.90) while  $^{13}\text{C}$ -cellulose responders are clustered (NRI: 4.49, P: 0.001). NTI values show that both  $^{13}\text{C}$ -cellulose and  $^{13}\text{C}$ -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  $^{13}\text{C}$ -xylose and  $^{13}\text{C}$ -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  $^{13}\text{C}$  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 S9): 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 was consumed by *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria* phylotypes. Canonical cellulose degrading bacteria like *Cellvibrio* 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 [69]. Functional niches are commonly discovered by observing how community structure changes with changing conditions [70]. 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  $^{13}\text{C}$  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 [65, 71]. We found that  $^{13}\text{C}$ -cellulose responsive OTUs are likely slow growing substrate specialists relative to  $^{13}\text{C}$ -xylose responders (Figure 4, Figure S8). We also found that  $^{13}\text{C}$ -xylose responsive OTUs that incorporated  $^{13}\text{C}$  into biomass at day one grow faster than OTUs that responded later (Figure 4, Figure S8). 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  $^{13}\text{C}$ -cellulose responders are generally lower abundance members of the bulk community than  $^{13}\text{C}$ -xylose responsive OTUs (Figure 4), however, high *rrn* gene copy number may inflate  $^{13}\text{C}$ -xylose-responder abundance.

NRI values quantify phylogenetic clustering [66] and have been used to assess clustering of soil OTUs that responded similarly to soil wet up [68, 72]. 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  $^{13}\text{C}$ -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 [73] and the clade depth of  $^{13}\text{C}$ -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 [74]. Overdispersion, as we saw for the  $^{13}\text{C}$ -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  $^{13}\text{C}$ -xylose responsive organisms were labeled as a result of primary xylose assimilation (see below), and therefore it's not clear if  $^{13}\text{C}$ -xylose responsive OTUs in this experiment constitute a single ecologically meaningful group or multiple ecological groups. Temporally defined  $^{13}\text{C}$ -xylose responder groups, however, were phylogenetically coherent (Figure S10, Figure 3). For example, most day 1  $^{13}\text{C}$ -xylose responders are members of the *Paenibacillus* (see Supplemental Note 2). Notably, Members of the *Bacillus* [75] and *Paenibacillus* in particular [59] have been previously implicated as labile C decomposers .

Intuitively we infer C cycling functional guild diversity from the distribution of diagnostic genes across genomes [74] or from screening culture collections for a particular trait [67]. For instance, the wide distribution of the glycolysis operon in microbial genomes is interpreted as evidence that many soil microorganisms participate in glucose turnover [12]. *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 [75, 76]. Phenomena such as seasonal change [77], and rainfall [72] cause nutrient and resource concentrations in soil to fluctuate. Therefore, fast growth and/or rapid resuscitation upon wet up [72] allow microorganisms to favorably compete for labile C resources which are often delivered in pulses. Life history ecological strategies tied to phylogeny like growth rate [70] 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 [70] 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 [12] which in turn influences soil biogeochemical process rates (e.g. [78]). Assessing functional group diversity and establishing identities of functional group members is necessary to predict how biogeochemical process rates will change with community composition [12, 79]. Aggregate biogeochemical processes that are the sum of many subprocesses involve 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 [12, 79]. Within an aggregate process such as C decomposition, subprocesses can be further classified as broad or narrow [12]. In theory, “broad” and “narrow” functional guilds decompose labile and recalcitrant C, respectively [12]. However, the diversity of active labile C and recalcitrant C decomposers in soil has not been directly quantified. Notably, we found more OTUs responded to  $^{13}\text{C}$ -cellulose, 63, than  $^{13}\text{C}$ -xylose, 49. Also, it is possible that many  $^{13}\text{C}$ -xylose responders are predatory bacteria or saprophytes as opposed to primary labile C degraders (see below). Cellulose and xylose decomposer functional guilds were non-overlapping in membership – of 104  $^{13}\text{C}$ -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 among microbial genomes than the ability to degrade cellulose, the number of unique active cellulose decomposer OTUs outnumbered the number of unique active xylose utilizer OTUs.

Trophic interactions and/or functional groups tuned to different resource concentrations caused the activity succession from *Firmicutes* to *Bacteroidetes* and finally *Actinobacteria* in response to xylose addition. *Actinobacteria* (e.g. *Agromyces*) and *Bacteroidetes* have been previously implicated as predatory soil bacteria [80, 81], and, in our microcosms *Bacteroidetes* and *Actinobacteria* activity peaked while *Firmicutes*  $^{13}\text{C}$ -xylose responder relative abundance plummeted (Figure 2, Figure 3, Figure S6). Considering *Agromyces* and *Bacteroidetes* phylotypes are likely soil predators, one parsimonious hypothesis for  $^{13}\text{C}$ -labelling of *Bacteroidetes* and *Actinobacteria* with a corresponding decrease in  $^{13}\text{C}$ -labeled *Firmicutes* abundance is that *Bacteroidetes* and *Actinobacteria* fed on  $^{13}\text{C}$ -labeled *Firmicutes*. Besides predation, mother cell lysis, the last step in sporulation, would release C from spore formers potentially to *Bacteroidetes* and *Actinobacteria*. If the temporal dynamics of  $^{13}\text{C}$ -xylose incorporation are due to trophic interactions, predatory bacteria or saprophytes consumed many, if not most, fast-growing labile C degraders. Hence, soil C cycling models should include trophic interactions between soil bacteria but rarely do (e.g. [82]). When soil C models do account for predators/saprophytes, trophic interactions are predicted to significantly influence C:N ratios of SOM relative to litter C:N, and, recycle significant amounts of microbial biomass [69].

We propose two scenarios in the context of our results whereby community composition could affect C dynamics and fate. Genomic evidence shows cellulose degradation is a phylogenetically conserved trait [74]. 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 [83] 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 [12]. 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* [72] and many soil *Actinobacteria* form hyphae that facilitate dispersal [84]. 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 [85]. Fast-growing *Firmicutes* spore formers assimilated labile C in our microcosms. *Bacteroidetes* 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<sup>-1</sup> carbon mixture; representative of natural concentrations [86]. 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)<sup>13</sup>C-cellulose, (3)<sup>13</sup>C-xylose (98 atom% <sup>13</sup>C, 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 <sup>13</sup>C-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 [87]. To prepare nucleic acid extracts for isopycnic centrifugation as previously described [55], DNA was size selected (>4kb) using 1% low melt agarose gel and  $\beta$ -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 [88] for a total of five <sup>12</sup>C-control, five <sup>13</sup>C-xylose, and four <sup>13</sup>C-cellulose microcosms. A density gradient (average density 1.69 g mL<sup>-1</sup>) solution of 1.762 g cesium chloride (CsCl) ml<sup>-1</sup> in gradient buffer solution (pH 8.0 15 mM Tris-HCl, 15 mM EDTA, 15 mM KCl) was used to separate <sup>13</sup>C-enriched and <sup>12</sup>C-nonenriched DNA. Each gradient was loaded with approximately 5  $\mu$ g of DNA and ultracentrifuged for 66 h at 55,000 rpm and room temperature (RT). Fractions of ~100  $\mu$ L 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  $\mu$ L s<sup>-1</sup> [48] into Acroprep<sup>TM</sup> 96 filter plate (Pall Life Sciences 5035). The refractive index of each fraction was measured using a Reichert AR200 digital refractometer modified as previously described [55] to measure a volume of 5  $\mu$ L. Then buoyant density was calculated from the refractive index as previously described [55] (see also SI). The collected DNA fractions were purified by repetitive washing of Acroprep filter wells with TE. Finally, 50  $\mu$ L 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<sup>-1</sup>. Barcoded 454 primers were designed using 454-specific adapter B, 10 bp barcodes [89], 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  $\mu$ L 5 U  $\mu$ l<sup>-1</sup> AmpliTaq Gold (Life Technologies, Grand Island, NY; N8080243), 2.5  $\mu$ L 10X Buffer II (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 2.5  $\mu$ L 25 mM MgCl<sub>2</sub>, 4  $\mu$ L 5 mM dNTP, 1.25  $\mu$ L 10 mg mL<sup>-1</sup> BSA, 0.5  $\mu$ L 10  $\mu$ M BA515F, 1  $\mu$ L 5  $\mu$ M BA806R, 3  $\mu$ L H<sub>2</sub>O, 10  $\mu$ L 1:30 DNA template) in triplicate. Samples were normalized either using Pico green quantification and manual calculation or by SequalPrep<sup>TM</sup> 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)

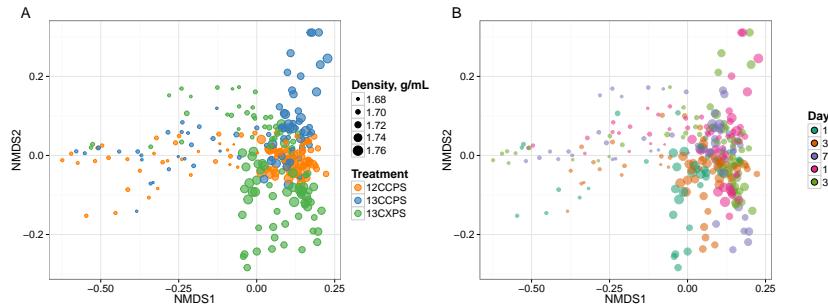
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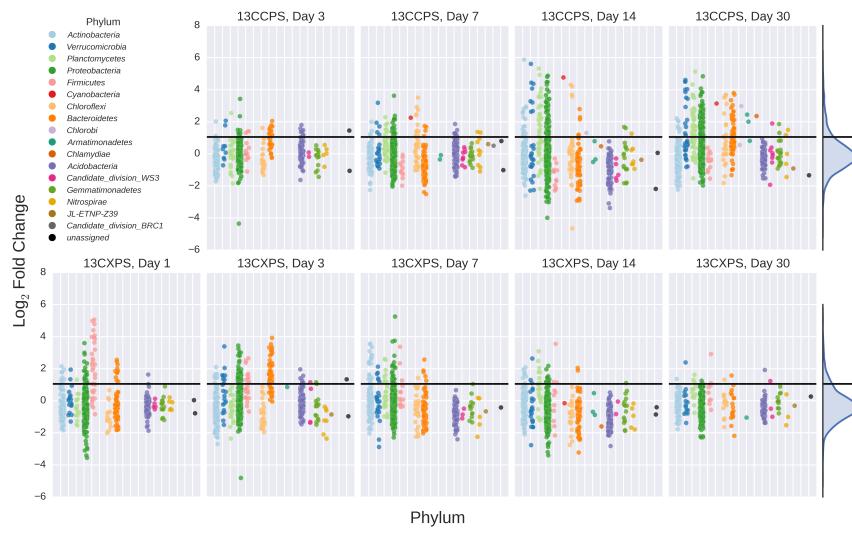
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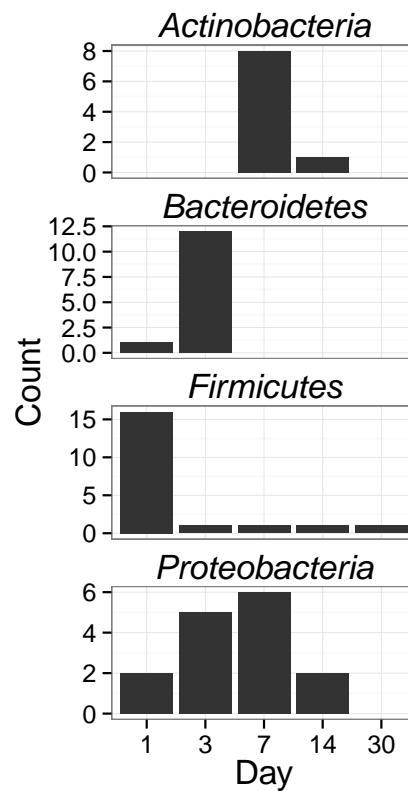
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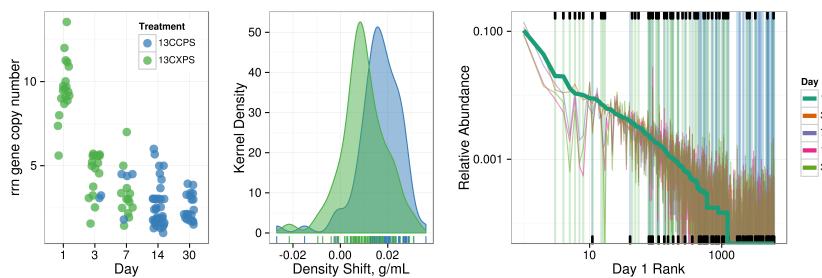
**Fig. 1.** NMDS analysis from weighted unifrac distances of 454 sequence data from SIP fractions of each treatment over time. Twenty fractions from a CsCl gradient fractionation for each treatment at each time point were sequenced (Fig. S1). Each point on the NMDS represents the bacterial composition 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).



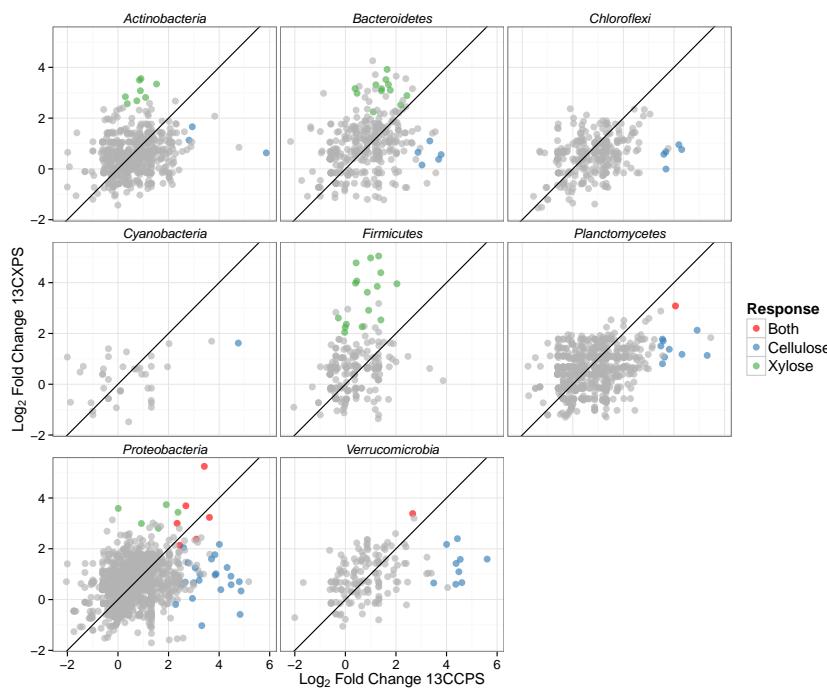
**Fig. 2.** Log<sub>2</sub> fold change of <sup>13</sup>C-responders in cellulose treatment (top) and xylose treatment (bottom). Log<sub>2</sub> fold change is based on the relative abundance in the experimental treatment compared to the control within the density range 1.7125–1.755 g ml<sup>-1</sup>.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



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



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



**Fig. 5.** Maximum log<sub>2</sub> 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 <sup>13</sup>C-xylose and <sup>13</sup>C-cellulose, respectively.