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SHORT COMMUNICATION

Phylogenetic organization of bacterial activity

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Phylogeny is an ecologically meaningful way to classify plants and animals, as closely related taxa frequently have similar ecological characteristics, functional traits and effects on ecosystem processes. For bacteria, however, phylogeny has been argued to be an unreliable indicator of an organism's ecology owing to evolutionary processes more common to microbes such as gene loss and lateral gene transfer, as well as convergent evolution. Here we use advanced stable isotope probing with ¹³C and ¹⁸O to show that evolutionary history has ecological significance for *in situ* bacterial activity. Phylogenetic organization in the activity of bacteria sets the stage for characterizing the functional attributes of bacterial taxonomic groups. Connecting identity with function in this way will allow scientists to begin building a mechanistic understanding of how bacterial community composition regulates critical ecosystem functions.

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Microbiologists have hotly debated the relationship between phylogeny and function in microorganisms for decades. Significant genomic variation even within closely related organisms (that is, >97% 16S rRNA gene identity) suggests that even shallow clades may not be ecologically coherent (Doolittle and Zhaxybayeva, 2009). Further, bacterial strains with identical 16S rRNA gene sequences can exhibit distinct growth rates and substrate utilization profiles, suggesting limited niche overlap (Jaspers and Overmann, 2004). However, many functional genes are relatively conserved in prokaryotic phylogeny (Martiny *et al.*, 2013). This conservation of gene content could underlie the accumulating evidence that phylogenetically clustered taxa are similar in their environmental distribution, niche space and functional capabilities, even at high levels of taxonomic organization (for example, order, class, phylum; Philippot *et al.*, 2010; Lennon *et al.*, 2012).

To date, evidence for phylogenetic organization of bacterial traits in intact assemblages has primarily relied on categorizing bacterial responses, such as changes in a taxon's relative abundance (Placella *et al.*, 2012; Evans and Wallenstein, 2014) or the presence in different environments (Morrissey and Franklin, 2015). Such research has provided early evidence that phylogeny can influence bacterial substrate utilization (Goldfarb *et al.*, 2011; Mayali *et al.*, 2014), habitat preference (Morrissey and Franklin, 2015) and life history strategies (Evans and Wallenstein, 2014). Although valuable, these assessments of bacterial traits are incomplete because they fail to capture quantitative variation among taxa. For instance, two taxa may be categorized as 'cellulose utilizing', but one may decompose and assimilate cellulose 10 times faster than the other, exerting more influence on cellulose decomposition rates. Rarely have quantitative measures of *in situ* activity been directly related to bacterial phylogeny (Amend *et al.*, 2016); as a consequence, the significance of phylogeny in determining quantitative variation in microbial function is not well understood. This is likely because of the limited availability of techniques that can quantitatively measure taxon-specific activity. The current work uses a new technique, quantitative stable isotope probing (Hungate *et al.*, 2015), to measure bacterial activity in soil. This technique works by determining

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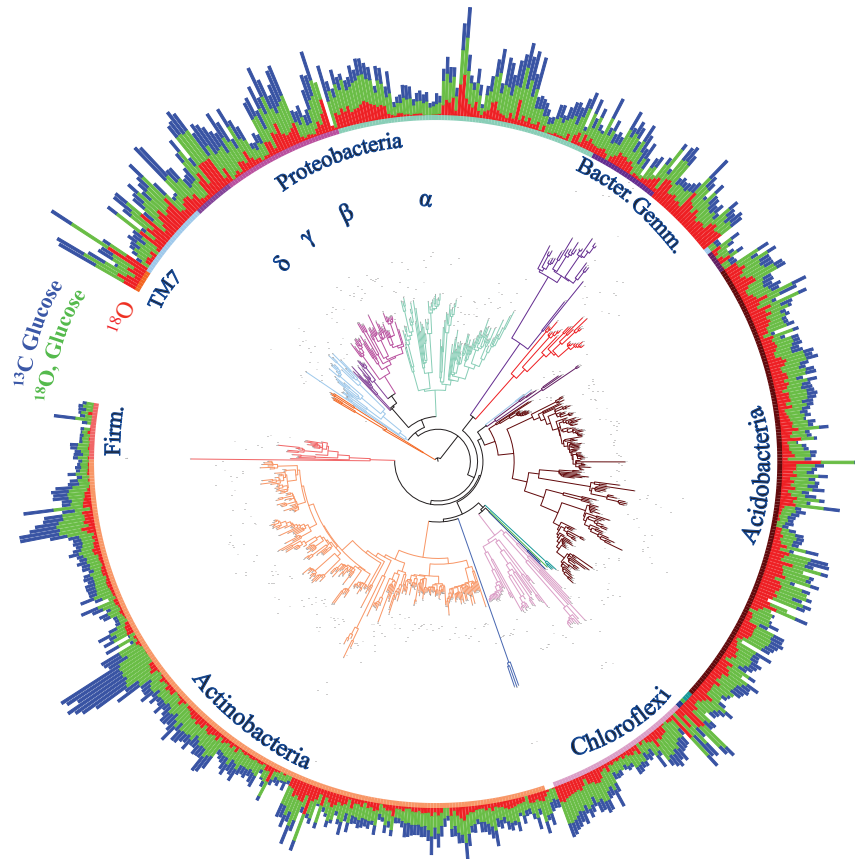


Figure 1 Phylogenetic tree (based on 16S rRNA gene sequences) and isotope incorporation of bacterial taxa in soil. Bars are proportional to the excess atom fraction of ^{18}O or ^{13}C of each taxon's DNA after incubation with ^{13}C -glucose (blue) or H_2^{18}O in the presence (green) or absence (red) of natural abundance glucose. Tree is colored by the phylogenetic group.

the shift in the buoyant density of each taxon's DNA caused by incorporation of a stable isotope tracer during incubation. Because the amount of isotope incorporation is directly proportional to the change in a given taxon's buoyant density, this method enables quantitative measurement of isotope incorporation and thus bacterial activity.

This study aimed to test whether the activity of bacterial taxa in soil exhibits phylogenetic organization. We measured growth and glucose assimilation by exposing soil from a ponderosa pine forest in northern Arizona to ^{18}O -labeled or unlabeled water for 7 days, with or without ^{13}C or unlabeled glucose ($n = 3$ per treatment, see Supplementary Materials for additional methodological details). Using quantitative stable isotope probing, the excess atom fraction ^{18}O in the absence and presence of unlabeled glucose as well as the excess atom fraction ^{13}C from glucose of each taxon's DNA was quantified (Hungate *et al.*, 2015). In brief, this technique uses density ultracentrifugation and high-throughput sequencing to measure changes in the density of microbial DNA following exposure to a heavy isotope. By modeling the effects of genomic GC content and isotope incorporation on the DNA molecular weight and density, this technique can quantitatively estimate

isotope incorporation into DNA irrespective of nucleic acid composition. Because water is a universal substrate for DNA synthesis (Schwartz, 2007), a taxon's genomic ^{18}O content reflects its population growth during the incubation period. Similarly, genomic ^{13}C content provides a quantitative measure of each taxon's carbon assimilation from glucose. To determine whether these activities exhibited phylogenetic organization, we tested for a phylogenetic signal, which is the tendency of related organisms to resemble each other more than would be expected by chance (Pagel, 1999; Blomberg *et al.*, 2003).

All three measures of bacterial activity, growth with and without added glucose as well as carbon assimilation from glucose, were clustered across the 16S rRNA gene-based phylogenetic tree (Figure 1, Table 1). These results provide robust evidence that bacterial evolutionary history has a measurable influence on the activity of bacteria in intact soil communities. Our estimates of λ approached 1, signifying that the variation in bacterial activity was approximately proportional to what would be expected under a neutral drift model of evolution (Freckleton *et al.*, 2002). However, the more conservative Blomberg's K statistics were < 1 , suggesting

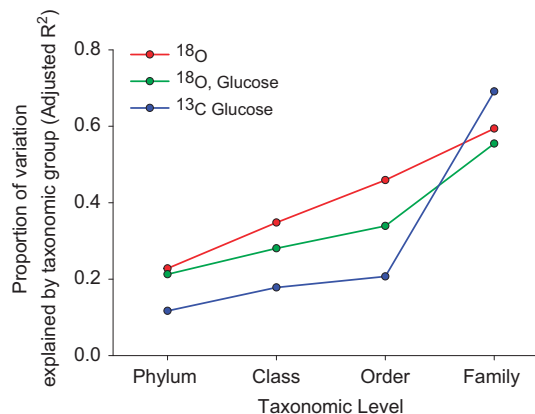
Table 1 Phylogenetic signals associated with bacterial growth in the absence (^{18}O) and presence (^{18}O , Glucose) of added glucose as well as bacterial carbon assimilation from glucose (^{13}C Glucose)

	Blomberg's		Pagel's	
	K	P	λ	P
^{18}O	0.38	0.001	0.97	<0.001
^{18}O , Glucose	0.50	0.001	0.96	<0.001
^{13}C Glucose	0.54	0.001	0.98	<0.001

that relatives were less similar than would be expected under neutral drift (Blomberg *et al.*, 2003). Overall, we detected phylogenetic signals that were similar in strength to those reported for a variety of ecological traits in plants and animals, such as habitat breadth and fecundity (Freckleton *et al.*, 2002; Blomberg *et al.*, 2003).

Examining within- versus among-group variation across bacterial taxonomy also shows evidence of this phylogenetic organization. Taxonomic group, from family to phylum, explained a significant amount of the variation in ^{18}O or ^{13}C excess atom fraction across all taxonomic levels (Figure 2, $P < 0.001$ in all cases). The finding that microbial activities are clustered at the phylum level suggests that variation in bacterial activities arise from physiological and ecological differences deeply rooted in bacterial phylogeny. As would be expected, group identity at finer levels of taxonomic resolution is able to explain increasing amounts of variation in bacterial activity. Family membership explained the majority of variation in growth (that is, ^{18}O incorporation) and carbon incorporation, reflecting the significant differences in the activities of bacterial families (Supplementary Figure S1). For example, all members of the Micrococcaceae family exhibited high ^{13}C assimilation from glucose (ranging from 0.41 to 0.52 ^{13}C excess atom fraction), while assimilation by members of the Solibacteraceae was much lower (ranging from 0.04 to 0.06 ^{13}C excess atom fraction). These findings are consistent with patterns documented in plants, where phylogenetic groups exhibit distinct functional traits, such as leaf size and seed mass (Cornwell *et al.*, 2008).

The ability to use simple carbon substrates is common in bacteria and has been suggested to display only shallow phylogenetic clustering (Martiny *et al.*, 2013), an indication that microbial taxa are functionally redundant with regard to the processing of these compounds (McGuire and Treseder, 2010; Martiny *et al.*, 2013). If the hypothesis of functional redundancy were true, one would expect little-to-no difference in carbon metabolism or assimilation across the bacterial phylogeny, which would produce a weak phylogenetic signal. Our results challenge this notion of functional redundancy: although nearly all organisms incorporated ^{13}C , the degree of enrichment differed dramatically

**Figure 2** Variation in excess atom fraction of ^{18}O or ^{13}C of each taxon's DNA after incubation with ^{13}C -glucose (blue) or H_2^{18}O in the presence (green) or absence (red) of natural abundance glucose explained by group membership at different taxonomic levels. Values reflect linear model results for groups with a minimum of five member taxa.

across taxa and families. Thus, even though the ability to assimilate simple carbon substrates in pure culture may be relatively ubiquitous and display only shallow clustering, the rates of carbon assimilation *in situ* may vary significantly across bacterial phylogeny. In an intact community, carbon assimilation rates likely depend on a variety of ecological traits and physiological systems, such as those governing the transport of substrates into the cell and the organism's growth rate. Our findings highlight potential pitfalls with using qualitative measures, such as genetic potential or substrate utilization tests (Martiny *et al.*, 2013), to infer functional redundancy, which is defined as a quantitative equivalency in the functioning of taxa (Allison and Martiny, 2008).

The variable growth (that is, ^{18}O assimilation) observed here could reflect differing trophic strategies across bacterial taxa. Growth rate, especially in a nutrient-rich environment, is a defining characteristic of trophic strategy, where relatively rapid growth suggests copiotrophy and slow growth indicates oligotrophy (Lauro *et al.*, 2009). Trophic strategy has been linked to many genomic features in bacteria, such as genome size, rRNA operon number and abundance of genes encoding for periplasmic membrane proteins (Lauro *et al.*, 2009). It is likely that a combination of such genomic features underlie the variation in growth observed here. Growth is a process that involves, and is regulated by, many gene systems in bacteria, resulting in phenotypes that reflect an integration of many individual processes. This complexity could be one reason why the phylogenetic signal was so robust, as many gains, losses and changes to individual genes would be required to transition a slow-growing bacterium into a fast-growing bacterium.

Evaluating phylogenetic organization, as carried out in the current work, will allow more accurate

characterization of phylogenetic groups. For instance, prior studies have described Actinobacteria as both oligotrophic (Bastian *et al.*, 2009) and copiotrophic (Leff *et al.*, 2015). Such conflicting results highlight the imprudence of describing high-level taxonomic groups without assessing whether the responses of member taxa are coherent. Our results indicate that Actinobacteria contains both copiotrophic and oligotrophic families (for example, Micrococcaceae and Rubrobacteraceae, respectively). Thus Actinobacteria could be dominated by copiotrophic taxa in one environment and oligotrophic taxa in another. As a consequence, generalizations about trophic strategy at the phylum level may not be possible or appropriate. Although our results are in agreement with some proposed classifications in the literature, such as β - and γ -Proteobacteria as being relatively copiotrophic (Eilers *et al.*, 2010), we suggest researchers evaluate and describe the phylogenetic organization associated with an ecological trait or activity before making generalizations to an entire phylogenetic group.

Microbial community composition affects ecosystem functioning from litter decomposition (Allison *et al.*, 2013) to plant fitness (Lau and Lennon, 2012). However, the mechanisms of these effects are unknown because very little information is available on the distribution of *in situ* activities across prokaryotes. This lack of information has prevented microbial ecologists from testing important questions about the functional redundancy of microorganisms in their natural communities. If bacterial taxa perform a process at the same rate under the same environmental conditions, thereby making them functionally redundant, then a change in the diversity of that community is unlikely to affect the community-level process rate (Allison and Martiny, 2008). However, we found individual taxa and phylogenetic groups to be functionally dissimilar with respect to growth and carbon assimilation, providing a basis for functional differences among phylogenetically distinct communities. Consequently, this research sets the stage for characterizing the functions of microbial taxa and phylogenetic groups in order to build a mechanistic understanding of how phylogenetic community composition influences ecosystem processes.

Conflict of Interest

The authors declare no conflict of interest.

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