Cell Size Distributions of Soil Bacterial and Archaeal Taxa

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Cell size is a key ecological trait of soil microorganisms that determines a wide range of life history attributes, including the efficiency of nutrient acquisition. However, because of the methodological issues associated with determining cell sizes in situ, we have a limited understanding of how cell abundances vary across cell size fractions and whether certain microbial taxa have consistently smaller cells than other taxa. In this study, we extracted cells from three distinct soils and fractionated them into seven size ranges (5 μm to 0.2 μm) by filtration. Cell abundances in each size fraction were determined by direct microscopy, with the taxonomic composition of each size fraction determined by high-throughput sequencing of the 16S rRNA gene. Most of the cells were smaller than cells typically grown in culture, with 59 to 67% of cells <1.2 μm in diameter. Furthermore, each size fraction harbored distinct bacterial and archaeal communities in each of the three soils, and many of the taxa exhibited distinct size distribution patterns, with the smaller size fractions having higher relative abundances of taxa that are rare or poorly characterized (including Acidobacteria, Gemmatimonadetes, Crenarchaeota, Verrucomicrobia, and Elusimicrobia). In general, there was a direct relationship between average cell size and culturability, with those soil taxa that are poorly represented in culture collections tending to be smaller. Size fractionation not only provides important insight into the life history strategies of soil microbial taxa but also is a useful tool to enable more focused investigations into those taxa that remain poorly characterized.

The soil environment harbors an amazing diversity of microorganisms, with thousands of bacterial and archaeal taxa living in individual soil samples (1). These taxa not only have a broad array of physiologies and life history strategies but also can exhibit a wide range of morphologies. In particular, we know that cell size can vary considerably across bacterial and archaeal taxa, from 0.2 to 750 μm in diameter (2). This variation in cell size has important effects on the physiological attributes of cells and their interactions with the soil environment, as cell diameter is inversely related to the surface area-to-volume ratio of cells. The surface area-to-volume ratio is a key determinant of a wide range of cell attributes, including the rate at which cells can take up nutrients from their environment, maintenance energy requirements, growth rates, and rates at which cells can release waste products (3). Despite cell size being a key characteristic of soil microorganisms, we have a limited understanding of microbial size distributions in soil and how cell size may vary across the wide diversity of taxa commonly found in soil.

We know from culture-based studies that individual microbial taxa can exhibit a range of cell sizes depending on environmental conditions and growth stage, with more actively growing cells living in resource-rich environments typically having larger cell diameters (4, 5). Nutrient deprivation has been shown to reduce cell size (3), and not surprisingly, cell sizes in soil are generally considered to be smaller than cell sizes typically measured for the most commonly studied isolates grown under laboratory conditions (4, 6). For example, previous work has shown that the majority of soil bacteria are less than 0.5 μm in diameter (6), with some bacterial cells small enough to pass through 0.2-μm filters (7). These “dwarf” cells appear to be very difficult to cultivate in the laboratory (8) and tend to grow more slowly than larger bacterial taxa (9). There is also some evidence that specific bacterial and archaeal taxa may be relatively more abundant in the smaller cell size fractions (10, 11), with many of these taxa likely retaining their small size after cultivation (6, 10, 12). However, what remains to be determined is if the unique cell size fractions generally represent distinct communities. In other words, we do not know how the intertaxon variability in cell size compares to the cell size variability observed within individual taxa. We hypothesize that cell size has a strong taxonomic signal, with certain taxa having smaller cells than other taxa, a pattern that could result from either consistent taxonomic differences in cell growth rates or genetic constraints on cell size that contribute to differences in cell sizes regardless of growth conditions.

The knowledge gaps in our understanding of cell size distributions in soil persist because it is not easy to quantify variation in cell size in environmental samples. Although cell size can readily be determined for cultured isolates, the vast majority of soil microbial taxa are difficult to cultivate (13), and even for those taxa that can be cultured, cell size distributions in culture may have little to no bearing on the size distributions in soil due to differences in resource availability and environmental conditions (3–5).

Although sequence-based approaches have revolutionized our ability to describe microbial diversity and the physiological attributes of individual taxa, such methods are of limited utility for determining cell sizes. Likewise, although microscopy-based approaches, including fluorescent in situ hybridization, can be used to determine the morphologies of soil microbial taxa that are resistant to culturing (e.g., see reference 14), the logistical difficulties associated with these approaches make it difficult to gain detailed information on how the wide range of taxa found in soil vary with respect to their cell size distributions. To circumvent these limi-
tions, we developed an approach for this study that involves isolating the microbial cells from a soil matrix using a Nycodenz density gradient and then passing the cells through a series of filters with pore sizes ranging from 5 to 0.2 μm. Cell abundances on each of the filters were then determined via microscopy, with the taxonomic identities of the cells in each size fraction determined by high-throughput sequencing of the 16S rRNA gene. Using this combined approach, we were able to determine the size distributions in total cell abundances across the soils and the size distributions of individual bacterial and archaeal taxa to determine if some taxa are consistently larger or smaller than other taxa.

MATERIALS AND METHODS

Soil sample collection and processing. Surface mineral soils (0- to 5-cm depth, no O-horizon material) were collected from a native grassland (tallgrass prairie) in eastern Kansas, a humid deciduous forest in Connecticut, and a cultivated agricultural field located at the Kellogg Biological Station in Michigan. These three soils (which we refer to as prairie, forest, and agricultural soils, respectively) were chosen because they represent a range of soil types and site characteristics (Table 1). Our objective was not to compare how cell size distributions vary across gradients in soil or site characteristics but rather to determine the size distributions of microbial taxa within each of these three distinct soils. All soils were sieved to 2 mm, homogenized, and stored at 4°C until further processing.

Cell separation and pore size fractionation. Four 5-g replicate subsamples of each soil were placed into 50-ml sterile conical tubes, and 5 ml of a cell fixing solution (50 mM tetrasodium pyrophosphate [TSP]; pH 8.0), 4% formaldehyde, and 0.5% Tween 80, as per reference 15) was added to each tube. The resultant slurries were incubated overnight at 4°C to ensure that cells were inactivated to prevent changes in the microbial communities during the cell separation and fractionation procedures. Soil slurries were then vortexed for 15 min, with the larger soil particles allowed to settle to the bottom of the conical tube. Next, 1-ml aliquots of the slurry were aseptically transferred to sterile 2-ml Eppendorf tubes containing 0.5 ml of Nycodenz (Axell, Westbury, NY) solution (80%, wt/vol, prepared in 50 mM sterile TSP buffer), with the Nycodenz density gradient used to isolate bacteria from soil particles by following a procedure described previously (16). Tubes were centrifuged at 10,000 × g for 90 min, after which the upper and middle cell-containing phases were transferred to new sterile 2-ml tubes with 1 ml of the 50 mM TSP buffer. After mixing, the tubes were centrifuged at 20,000 × g for 15 min. The supernatant was discarded and the cell pellet was resuspended in 0.6 ml of the 50 mM TSP buffer containing 4% formaldehyde. The volume of suspended cells from each sample replicate was pooled, and approximately 5 ml of cell suspension was obtained for each of the four replicates per sample. Six negative-control samples (consisting of 50 mM TSP buffer instead of soil slurry) were processed through the Nycodenz density gradient described above to check for any contamination.

Fractionation of microbial cells based on size was accomplished by passing the cell suspension through polycarbonate membrane filters with successively smaller pores (5, 3, 1.2, 0.8, 0.6, 0.4, and 0.2 μm) using a gentle vacuum (<20,000 Pa). The filters as well as 0.5 ml of the flow-through from each filter were retained and stored at −20°C for community analysis. An additional 0.1 ml of the flowthrough was retained and stored at 4°C for cell abundance determination by microscopy (see below).

Determination of cell abundances in the size fractions. The total number of cells in the unseparated soils, in the pellets resulting from the Nycodenz gradient, and on each of the filters obtained from each soil was determined by direct counting procedure on black polycarbonate filters (0.2-μm pore diameter) using epifluorescence microscopy and 4,6-diamidino-2-phenylindole (DAPI). For the unfractinated soil samples, triplicate 1-g subsamples of each soil were suspended in 1 ml of the cell fixing solution described above, incubated overnight, and vortexed for 15 min. Total bacterial abundances were determined in dilutions from the unseparated slurries of the soils, from each filtered fraction, and from the pellets after the cell extraction by incubating aliquots from each diluted suspension with DAPI at a final concentration of 1 μg ml−1 for 10 min in the dark. These solutions were then filtered through a black 0.2-μm polycarbonate membrane filter. Once completely dried, the filters were mounted on paraffin oil and cell numbers were determined from at least 20 fields per sample via epifluorescence microscopy. Negative controls from the cell separation and fractionation processes as well as the solutions used during the dilutions and staining were counted using this approach.

DNA extraction, PCR amplification, and pyrosequencing. Genomic DNA was extracted directly from soil samples, from the whole-cell fraction separated from the soil particles by Nycodenz gradient, from the pellet remaining after the Nycodenz gradient procedure, and from the different-pore-size filters used for the size fractionation. From each of these 182 individual samples (and the associated negative controls), DNA was extracted using the MoBio Power Soil DNA extraction kit (MoBio, Carlsbad, CA) using the modified protocol described by Lauber et al. (17). A portion of the 16S rRNA gene spanning the V4 hypervariable region was amplified by triplicate PCR amplifications using the bar-coded primer set (515F/806R), PCR mixture, and thermal cycling conditions described by Caporaso et al. (18). This primer set was designed to cover a wide diversity of both Archaea and Bacteria with few biases against individual taxa (19). The replicates were combined for each sample and quantified using a PicoGreen double-stranded-DNA (dsDNA) assay (Invitrogen, Carlsbad, CA). All samples were pooled in equimolar concentrations and sequenced on a HiSeq2000 instrument (Illumina, San Diego, CA) at The University of Colorado Advanced Genomics Facility.

Sequence processing. The raw 100-bp DNA sequences generated by the Illumina HiSeq platform were assigned to their respective samples using associated bar code sequences and filtered based on quality scores using the default parameters in QIIME v.1.5.0-dev (20). Because the sequencing generated a large number (19,422,252) of quality-filtered sequences, traditional de novo picking of operational taxonomic units (OTUs, defined by pairwise sequence similarity) was not practical. Instead, we used an open reference-based OTU picking approach implemented with the QIIME algorithm, “pick_subsampled_reference_otsu_through_otsu_table.py,” which allows OTUs to be picked on a practical timescale while retaining many sequences that are not closely related to those in published databases. Briefly, this algorithm preprocesses sequences using UCLUST (21) and the Greengenes database preclustered at 97%.

TABLE 1 General description of the collection sites, edaphic characteristics, and cell number counts from the three soil types studieda

<table>
<thead>
<tr>
<th>Site</th>
<th>Latitude (°N)</th>
<th>Longitude (°E)</th>
<th>Dominant plant species</th>
<th>pH</th>
<th>% C</th>
<th>% N</th>
<th>% silt + clay</th>
<th>Soil moisture (g H2O/g dry soil)</th>
<th>No. of cells (g dry soil)−1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prairie</td>
<td>39.1</td>
<td>96.6</td>
<td>Andropogon gerardii, Schizachyrium scoparium</td>
<td>6.84</td>
<td>3.74</td>
<td>0.30</td>
<td>78</td>
<td>0.31</td>
<td>1.8 × 1010</td>
</tr>
<tr>
<td>Forest</td>
<td>41.9</td>
<td>72.1</td>
<td>Quercus rubra, Acer rubrum</td>
<td>3.91</td>
<td>4.87</td>
<td>0.32</td>
<td>47</td>
<td>0.62</td>
<td>9.4 × 1010</td>
</tr>
<tr>
<td>Agricultural</td>
<td>42.4</td>
<td>85.4</td>
<td>Solanum tuberosum</td>
<td>5.15</td>
<td>0.60</td>
<td>0.06</td>
<td>26</td>
<td>0.26</td>
<td>2.1 × 1010</td>
</tr>
</tbody>
</table>

a Cell counts were obtained on whole soils without any fractionation of cells by size. Soil texture was determined as described previously (1).
identity (22) to remove sequences with less than 60% similarity (0.2% of
the sequences). Next, sequences were clustered at the 97% sequence si-
milarity level using the same database. Those sequences which failed to
cluster (26% of the prefiltered sequences) were subsampled for de novo
OTU picking, and representative sequences of these OTUs were subse-
quently added to a new reference data set. This process was repeated again
with the new reference data set (reference-based OTU picking followed by
de novo picking). A phylogenetic tree was then constructed using se-
quences aligned by PyNAST (23) in order to calculate phylogenetic dis-
similarity matrices, and as a further quality filtering step, OTUs repre-
sented by less than 2 sequences and those that could not be aligned were
removed. Taxonomy was assigned to OTUs using the RDP classifier (24)
trained on the Greengenes database set at a 50% confidence threshold.
Following OTU picking, samples were rarefied to a constant sequencing
depth (12,000 sequences per sample) for all downstream analyses. Eighty
di samples did not yield a sufficient number of sequences at this rarefaction
depth, so only 102 samples were included in all downstream analyses.

To compare the microbial communities from the unseparated soil
samples, the cell fractions extracted by Nycodenz gradient, and the cells
retained in each fraction size, we used the phylogenetically based un-
weighted UniFrac metric (25). The resulting distance matrix was used to
determine whether the cell fractions extracted from the different soils
harbored distinct communities by running an analysis of similarity
(ANOSIM) test in PRIMER v6 (26). Likewise, Mantel tests were run in
PRIMER v6 (26) to determine if size fractions more similar in size har-
bored more similar communities. To describe the size distributions of
individual taxa, we focused on those taxonomic groups (phyla, classes, or
orders) represented by more than 1,000 sequences (summed across all size
fractions per soil sample), as we were not confident in our ability to detect
changes in cell size distributions in taxonomic groups represented by
<0.1% of the sequences. The relationships between cell size (determined
by the successive filter pore sizes) and sequence abundances were de-
scribed by fitting a 3rd-order polynomial function in SigmaPlot (Systat
Software, San Jose, CA).

**Nucleotide sequence accession number.** All sequence data from this
study have been deposited in the public EMBL-EBI database (http://www.
ebi.ac.uk/) under the accession number ERP003935.

**RESULTS AND DISCUSSION**

**Cell abundances across size fractions.** The three soils (prairie, forest,
and agricultural) harbored numbers of microbial cells that
were within the same order of magnitude ($10^{10}$ cells g$^{-1}$), with the
forest soil containing the highest cell concentrations, as evident
from the direct cell counts conducted on the unfractionated soil
samples (Table 1). We know from a comparison of cell counts in
the Nycodenz-separated fraction to counts obtained with the un-
fractio nald soils that the majority of the cells were not effectively
separated from the soil matrix using the Nycodenz density gra-
dient approach. We estimate that 13.7%, 2.7%, and 5.2% of the cells
in the prairie, forest, and agricultural untreated soil samples, re-
spectively, were separated from the soil matrix and subsequently
run through the series of size-selecting filters. This efficiency of
cell recovery using the Nycodenz density gradient is low but falls
well within the range of 0.5 to 25% reported in previous studies
that have used a similar approach to separate cells from the soil
matrix (27–30). The variability in cell extraction efficiencies across
the three soils is likely related to differences in soil texture or
organic-matter content (28, 29).

The Nycodenz-separated cells were partitioned into different
size fractions by passage through filters with successively smaller
pore sizes (5, 3, 1.2, 0.8, 0.6, 0.4, and 0.2 μm), and direct cell
counts were performed on each fraction (Fig. 1). Across all soils,
>60% of the cells were smaller than 1.2 μm, confirming results
reported previously (4), with the size distribution in cell abun-
dances varying across different soils. A higher percentage of the
extracted cells from the prairie and forest soil were in the smaller
size classes than in the agricultural soil, where cells were slightly
larger on average (Fig. 1). With only three soils included in this
study, we cannot determine what site or edaphic characteristics
are responsible for the observed differences in size distributions
shown in Fig. 1, but previous work has suggested that such vari-

![Graph showing cell abundances across size fractions](http://www.aem.asm.org/FIG1.png)
ability in cell size distributions could be related to the taxonomic structure of the communities, resource availability, or metabolic state of the microbial cells (10, 14, 27).

Community composition across the studied soils. The microbial communities in the unfractionated samples and the Nycodenz-extracted cells from each of the three soil samples were all dominated by members of the Proteobacteria, Acidobacteria, Firmicutes, Bacteroidetes, Verrucomicrobia, and Actinobacteria phyla (Fig. 2; see also Tables S1 to S3 in the supplemental material), bacterial phyla that are typically the most abundant taxa recovered from molecular surveys of soil microbial communities (17). However, the microbial communities in each of the three soils (prairie, forest, and agricultural) were all significantly different from one another, both before and after the Nycodenz extraction (Fig. 2) (ANOSIM, \( r = 0.87 \) and \( P < 0.001 \)). Because these soils harbored distinct communities, the analysis of the microbial taxa found within each size fraction was conducted separately for each soil.

The Nycodenz separation not only failed to remove all cells from the soil matrix, as described above, but also failed to extract all taxa equally. Although the degrees of taxonomic richness (numbers of OTUs per sample at the rarified sequencing depth) were similar in the Nycodenz-extracted and unextracted samples (\( P > 0.05 \) in all cases), community compositions were significantly different between the Nycodenz-extracted and unextracted
samples for each soil (ANOSIM, r > 0.6 and P < 0.03 for all three soil types). Certain taxa, including members of the Acidobacteria, Bacteroidetes, and Verrucomicrobia phyla, were significantly over-represented in the cell fraction extracted by Nycodenz versus the unextracted soil. In contrast, Firmicutes and Actinobacteria were underrepresented in the Nycodenz extraction from each soil (Fig. 2). This taxonomic bias introduced during the Nycodenz cell extraction process has been noted previously (31) and may be related, in part, to the differential removal of spore-forming bacteria during the Nycodenz extraction process (spores have a higher density than vegetative cells [32]) or due to the tendency of some taxa to be more closely bound to mineral soil particles than other taxa (33).

Although the Nycodenz-separated cells represent a biased subset of the cells in the unextracted soils, the same major taxa were recovered in both the separated and unextracted (whole soil) communities (Fig. 2). More importantly, despite these taxonomic biases, we can still determine whether different cell size fractions harbor distinct bacterial and archaeal communities, and we can assess the cell size distributions of major bacterial and archaeal groups. As many approaches used to determine cell size, including many microscopy-based approaches and the size-fractionation approach used in this study, require that cells be separated from the soil matrix, the bias associated with the Nycodenz approach is somewhat unavoidable until better methods for isolating cells from the soil matrix are developed.

Differences in bacterial communities across size fractions. Microbial community compositions of the different size fractions retained on the filters were significantly different for the three soil samples (ANOSIM, prairie, r = 0.6; forest, r = 0.23; and agricultural, r = 0.76; P < 0.001 at all cases). Moreover, size fractions closer in size harbored more similar communities than we have expected by chance, as determined by Mantel tests (prairie, r = 0.66 and P < 0.001; forest, r = 0.33 and P < 0.01; and agricultural, r = 0.70 and P < 0.001). We know of no comparable studies demonstrating that the communities found in different cell size fractions are distinct in composition; however, our results are qualitatively similar to those reported previously (27) where it was shown the phospholipid fatty acid signatures of cells smaller than 0.4 μm were distinct from those found in bulk soil. Our findings not only highlight that some taxa are often consistently smaller or larger than other taxa, a point discussed in more detail below, but also suggest that the soil microbial community is not a homogeneous entity; that subset of a given community composed of smaller cells will likely interact with the surrounding soil environment in a very different way from the subset with larger cells. For example, microbial taxa of different sizes are likely to differ in their nutrient and energy requirements, their abilities to access smaller-sized soil pores, or their abilities to avoid predation (33–35).

Figures 3 and 4 show the size distributions of major bacterial and archaeal taxa in each of the soils. Since the three soil types harbored such distinct communities (Fig. 2), the relative abun-
dance of individual taxa across the size fractions was dependent on the soil type in question. Likewise, some taxa were only sufficiently abundant in one of the three soil types to accurately determine their size distributions (Fig. 3 and 4). Nevertheless, key patterns emerge highlighting that those bacteria and archaea common in soil often show distinct size distribution patterns, with few taxa being equally abundant in all size classes. For example, at the phylum level we found that Proteobacteria and Actinobacteria were often relatively more abundant in the larger size fractions (>3 μm), while other phyla, including Acidobacteria, AD3, Verrucomicrobia, and Gemmatimondetes, were consistently more abundant in the smaller size fractions (<0.8 μm), with Chloroflexi and Firmicutes being most abundant in the intermediate size fractions. Most of the rare phyla (those representing <2% of the sequences for a given sample) and those phyla represented by few (if any) cultured representatives were most abundant in the smaller size fractions, including Crenarchaeota, Elusimicrobia, TM6, SC3, TM7, ZB2, and WS3 (Fig. 4). However, we reiterate that these phylum-level size distributions were dependent on the soil and cell size cannot always be predicted from the phylum-level designation.

To determine if the differences in the size distributions of individual phyla observed across the soils were related to the predominance of different subphyla in the three soils, we investigated size distributions at finer levels of taxonomic resolution for some of the more abundant phyla (see Fig. S1 in the supplemental material). We found that different groups within a given phylum often exhibited distinct size distribution patterns. For example, some actinobacterial orders (including Actinomycetales) had consistently larger cell sizes than other orders within this phylum (see Fig. S1), patterns that were not obvious at the phylum level of resolution (Fig. 3), with similar patterns observed for individual orders within the Proteobacteria and Bacteroidetes phyla (see Fig. S1). Clearly, different taxonomic groups often have distinct cell sizes, and these patterns are evident at various levels of taxonomic resolution.

What does cell size tell us about microbial life history strategies? There are two possible reasons why many of the soil microbial taxa shown in Fig. 3 and 4 exhibit uneven distributions across the cell size fractions. First, some taxa may be intrinsically larger than other taxa, regardless of growth conditions, resource availability, or growth phase. For example, many actinobacterial taxa are known to have a filamentous growth form that would effectively give them a larger cell size than nonfilamentous bacteria regardless of the specific growth conditions. Alternatively, cell morphology may be a more plastic trait, determined not by the cell genotype but with cell size varying as a function of growth conditions or growth phase. We know that this is true for many cultured taxa that are often smaller when in the stationary phase of growth (36) or when carbon and nutrient resources are limiting (10). In this study, we could not differentiate between these two possible explanations, and in all likelihood, both factors may contribute to the variation in cell size observed across the soil taxa examined. A similar point has been made previously where it has been hypoth-

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**FIG 4** Relative abundances of microbial phyla across the studied size fractions in each soil for those phyla representing less than 2% (on average) of the classified sequences from filter samples. This figure is identical to Fig. 3 except that it shows the less abundant phyla. The symbols representing the sequence abundances are the same as for Fig. 3. The trend line and regression coefficient (r²) are indicated for a specific phylum and soil.
elized that the smaller microorganisms either may represent distinct groups of intrinsically small taxa or may be starved, dormant forms of bigger microbes (10, 37, 38).

Despite the uncertainties in delineating the specific factors driving variation in cell size across taxa, some interesting patterns emerge when we take the data presented in Fig. 3 and 4 and divide the taxa into general size classes (Table 2), as most of the smaller taxa were those with few cultivated representatives. This general pattern suggests that cell size is an important ecological trait, with taxa were those with few cultivated representatives. This general pattern suggests that cell size is an important ecological trait, with smaller taxa having attributes that make them more difficult to culture-based surveys of microbial diversity unless culture conditions, and we would expect smaller cells to be underrepresented in culture conditions, and the ability to occupy microenvironments different from those occupied by larger cells (3). Efforts to characterize soil microbial groups that are underrepresented in culture conditions, using either single-cell isolation or enrichment culturing strategies, could benefit from first size selecting soil microbial cells, as many poorly studied groups appear to have cell sizes that are smaller than average.

Conclusions. Different cell size fractions harbored distinct bacterial and archaeal communities, with many of the taxa exhibiting unique size distribution patterns (few taxa were equally abundant across all size classes). The smallest size fractions contained a higher proportion of rare phyla and candidate divisions (including Acidobacteria, Gemmatimonadetes, Crenarchaeota, Verrucomicrobia, Elusimicrobia, and the candidate divisions AD3, TM6, SC3, TM7, ZB2, and W53) than the larger size fractions. However, soil type was an important factor influencing both the taxa found within different size fractions and the total abundance of cells in each size fraction, highlighting that the size distribution pattern for a given taxonomic group is not constant and can vary depending on soil properties and the members of the taxonomic group. As suspected, cell size is an important ecological trait, and by combining cell size fractionation with other approaches (including genomics-based approaches), we can better understand the ecology and life history strategies of the vast majority of soil microbial taxa that remain poorly characterized.

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REFERENCES


