

TOWARD AN ECOLOGICAL CLASSIFICATION OF SOIL BACTERIA

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Abstract. Although researchers have begun cataloging the incredible diversity of bacteria found in soil, we are largely unable to interpret this information in an ecological context, including which groups of bacteria are most abundant in different soils and why. With this study, we examined how the abundances of major soil bacterial phyla correspond to the biotic and abiotic characteristics of the soil environment to determine if they can be divided into ecologically meaningful categories. To do this, we collected 71 unique soil samples from a wide range of ecosystems across North America and looked for relationships between soil properties and the relative abundances of six dominant bacterial phyla (Acidobacteria, Bacteroidetes, Firmicutes, Actinobacteria, α -Proteobacteria, and the β -Proteobacteria). Of the soil properties measured, net carbon (C) mineralization rate (an index of C availability) was the best predictor of phylum-level abundances. There was a negative correlation between Acidobacteria abundance and C mineralization rates ($r^2 = 0.26$, $P < 0.001$), while the abundances of β -Proteobacteria and Bacteroidetes were positively correlated with C mineralization rates ($r^2 = 0.35$, $P < 0.001$ and $r^2 = 0.34$, $P < 0.001$, respectively). These patterns were explored further using both experimental and meta-analytical approaches. We amended soil cores from a specific site with varying levels of sucrose over a 12-month period to maintain a gradient of elevated C availabilities. This experiment confirmed our survey results: there was a negative relationship between C amendment level and the abundance of Acidobacteria ($r^2 = 0.42$, $P < 0.01$) and a positive relationship for both Bacteroidetes and β -Proteobacteria ($r^2 = 0.38$ and 0.70 , respectively; $P < 0.01$ for each). Further support for a relationship between the relative abundances of these bacterial phyla and C availability was garnered from an analysis of published bacterial clone libraries from bulk and rhizosphere soils. Together our survey, experimental, and meta-analytical results suggest that certain bacterial phyla can be differentiated into copiotrophic and oligotrophic categories that correspond to the r - and K -selected categories used to describe the ecological attributes of plants and animals. By applying the copiotroph–oligotroph concept to soil microorganisms we can make specific predictions about the ecological attributes of various bacterial taxa and better understand the structure and function of soil bacterial communities.

Key words: Acidobacteria; copiotroph; K -selection; microbial diversity; oligotroph; Proteobacteria; quantitative PCR; real-time PCR; r -selection; soil carbon.

INTRODUCTION

The diversity of soil bacterial communities is enormous; a single gram of soil may contain 1×10^3 to 1×10^6 unique “species” of bacteria (Torsvik et al. 2002, Gans et al. 2005, Tringe et al. 2005). While the vast majority of this diversity remains uncharacterized, recent molecular advances (Pace 1997) allow us to survey the full extent of soil bacterial diversity at an ever-increasing pace. Although such molecular surveys provide essential information on the composition of soil bacterial communities, they are only the first step

towards understanding the ecology of such communities. At present, we are largely unable to interpret the taxonomic survey data in an ecologically meaningful manner nor do we know why certain taxa are more abundant in some soils than in others.

A select number of bacterial taxa have been well studied and their ecological characteristics are reasonably well defined. This is the case for those taxa with specific physiological capabilities, such as the ammonia-oxidizing *Nitroso*- genera, the N_2 -fixing *Rhizobium*, and the methane-oxidizing *Methylo*- genera. However, these taxa are the exceptions: the majority of soil bacterial taxa, even those that are numerically dominant, have not been extensively studied and their ecological characteristics remain largely unknown. For example, the phylum Acidobacteria is one of the most abundant taxonomic groups of soil bacteria (Dunbar et al. 1999,

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Liles et al. 2003, Tringe et al. 2005) but we know next to nothing about their physiological capabilities, habitat preferences, and life history attributes (Barns et al. 1999).

Until recently, inadequate methods have been the largest barrier to microbiologists studying the ecology of soil bacterial communities. Traditionally microbiologists have characterized bacteria by studying individual strains that could be cultivated in the laboratory. However this approach, by itself, provides little information on soil bacterial ecology since the vast majority of soil bacteria are unculturable, or at least very difficult to culture (Sait et al. 2002). In the past few decades, molecular methods have become more common and increasingly useful as tools to examine the ecological characteristics of individual soil bacteria and bacterial communities (Liles et al. 2003, Handelsman 2004, Tringe et al. 2005). Although methodological considerations will continue to pose a challenge to soil microbiologists, we now have a toolbox of sufficient size (with new methods being developed regularly) to address some of the more difficult ecological questions.

With many new tools in place, conceptual issues are rapidly replacing methodological issues as the primary barrier to progress in the field of soil bacterial ecology. At present, microbial ecologists lack a set of unifying concepts that can be used to analyze and interpret results from the wide range of studies examining soil bacterial ecology. We propose that such concepts already exist in animal and plant ecology and that, by adapting these concepts to meet the unique requirements of microbial ecology, we can improve our understanding of soil bacterial communities and their functioning in soil.

One such unifying concept, that of an *r*- to *K*-selection continuum (MacArthur and Wilson 1967), is fundamental to ecology and is often used to describe the life history characteristics of plants and animals. In general, *r*-strategists are adapted to maximize their intrinsic rate of growth when resources are abundant while *K*-strategists are adapted to compete and survive when populations are near carrying capacity and resources are limited (Pianka 1970). Although *r*- and *K*-selection theory is recognized as an over-simplification and it has largely been replaced with more elaborate models of life history evolution (Reznick et al. 2002), the general concept is understood by all ecologists and the *r*- and *K*-categories provide a useful framework for comparing the ecological characteristics of different taxa. We expect that the concept of an *r*- to *K*-continuum should apply to both multicellular and unicellular taxa even though the specific ecological attributes used to distinguish *r*- and *K*-strategists may differ.

Microbiologists are more likely to use the terms copiotroph and oligotroph to describe those microorganisms with ecological attributes typical of *r*- and *K*-strategists, respectively, and we use that terminology here. Copiotrophs preferentially consume labile soil

organic C pools, have high nutritional requirements, and can exhibit high growth rates when resource conditions are abundant. In contrast, oligotrophs exhibit slower growth rates and are likely to outcompete copiotrophs in conditions of low nutrient availability due to their higher substrate affinities (Meyer 1994, Tate 2000). It follows then that soils with large amounts of available organic C should favor copiotrophs while oligotrophs should predominate in soils where organic C quality and/or quantity is low. This proposed classification of bacteria into copiotrophs and oligotrophs is not new, a similar idea was promoted in the 1920s (Winogradsky 1924) and variations on the concept have followed (Hirsch et al. 1979, Andrews 1984, Gottschal 1985, Andrews and Harris 1986, Meyer 1994, Padmanabhan et al. 2003). Yet, we do not know if this classification scheme is applicable to soil bacterial communities. More specifically, we do not know if it accurately describes the range of ecological strategies exhibited by those bacterial taxa common in the soil environment and, if so, where specific taxa may reside along the copiotroph-oligotroph spectrum.

We set out to determine if soil bacterial phyla can be divided into ecologically meaningful categories based on the *r*- to *K*-selection continuum. Although each bacterial phylum encompasses an enormous level of phylogenetic and physiological diversity, we hypothesize that some phyla will exhibit either copiotrophic or oligotrophic tendencies and have higher abundances in soils with higher and lower organic C availability, respectively. We test this hypothesis using a newly developed quantitative PCR technique (Fierer et al. 2005a) to measure the abundances of the numerically dominant bacterial phyla in 71 soil samples collected from a wide range of ecosystem types (the “cross-site study”; see Fierer and Jackson 2006). We further test this hypothesis by experimentally manipulating C availability in a single soil type and then measuring the shifts in the relative abundances of those target phyla (the “sucrose amendment experiment”), and by comparing the abundances of those target phyla in bulk and rhizosphere soil bacterial clone libraries (the “meta-analysis”).

METHODS

Cross-site study

A total of 71 unique soils, representing a diverse array of soil and site characteristics, were collected from throughout the United States (Appendix A). We restricted our sampling to soils that are unsaturated for most of the year. Soils were collected near the peak of the plant growing season at each site. The upper 5 cm of mineral soil was collected from 5–10 locations within each site and composited into a single bulk sample. All samples were shipped to the University of California–Santa Barbara within a few days of collection where they were sieved to 4 mm and thoroughly homogenized. A subsample of each soil was stored at -80°C for DNA extraction and molecular analyses.

Climate information for each site was estimated from historical average station data (1971–2000) provided by the National Oceanic and Atmospheric Administration, USA. Average annual soil moisture deficit (in mm H₂O) was estimated as the sum of the differences between mean monthly potential evapotranspiration (PET) and mean monthly precipitation. PET was estimated using Thornthwaite's method with a correction for latitude (Thornthwaite 1948). Ecosystem classification for each site follows Bailey et al. (1994).

Total soil organic C and nitrogen (N) contents were measured on a CE Elantech Model NC2100 elemental analyzer (ThermoQuest Italia, Milan, Italy) with combustion at 625°C and 900°C, respectively. Soil pH was measured after shaking a soil/water (1:1, mass: volume) suspension for 30 min. Particle size analyses were conducted at the Division of Agriculture and Natural Resources Analytical Laboratory, University of California Cooperative Extension (Davis, California, USA) using standard methods. Gravimetric soil moisture contents were determined by drying soils at 120°C for 48 h.

Net C and N mineralization rates were measured on triplicate subsamples (4 g wet mass) incubated for a 50-day period. Before the start of the incubation, all soils were adjusted to 35% of water holding capacity (WHC), by drying at 20°C or wetting with deionized water, and equilibrated at 20°C for 10 days. WHC was measured as the gravimetric water content of soil saturated and allowed to drain over 2 hours in a filter funnel. We adjusted the samples to 35% WHC because the soils varied significantly in terms of their soil moisture contents at the time of collection: soils adjusted to the same WHC should have similar soil water potentials, permitting comparisons of microbial processes between soils across sites without introducing artifacts from differences in ambient moisture (Gulledge and Schimel 1998). A series of six to 10 static incubations per sample were used to measure the average rate of soil CO₂ production over the course of the 50-day incubation period at 20°C, using the method described in Fierer et al. (2005b). Triplicate subsamples were harvested initially and at 50 days for the determination of K₂SO₄-extractable NH₄⁺ and NO₃[−]. Samples were extracted for 1 hour with 0.5 mol/L K₂SO₄ and extractable NH₄⁺ and NO₃[−] concentrations were measured on a Lachat autoanalyzer (Milwaukee, Wisconsin, USA) using Lachat methods 31-107-06-5-A and 12-107-04-1-B, respectively. Net N mineralization was calculated as the change in total extractable N over the course of the 50-day incubation. The measurement of net C and N mineralization rates in this manner provides a coarse assessment of microbially available resource concentrations (Robertson and Paul 2000).

Sucrose amendment experiment

Intact soil monoliths (28.5 cm deep × 15.3 cm diameter) were collected from a mixed hardwood stand

dominated by *Quercus alba* L. within the Duke Forest Teaching and Research Laboratory, North Carolina, USA. The monoliths were collected from the same site as soil DF2 from the cross-site study (see Appendix A). Monoliths were encased within PVC pipe and maintained in a controlled-environment greenhouse at a constant temperature (17.5°C) and moisture throughout the experiment. Monoliths were amended with sucrose, to simulate rhizodeposited carbon, at five different levels: 0, 70, 150, 350, and 800 g C·m^{−2}·yr^{−1}. Following the expectation that soil heterotrophic respiration is dominated by the mineralization of labile carbon pools (Gu et al. 2004, Knorr et al. 2005), we selected these amendment levels to reflect a supply of labile carbon that equates with a broad range of published soil CO₂ efflux rates (Rustad et al. 2001), assuming that 50% of total soil CO₂ efflux is derived from microbial mineralization (Hanson et al. 2000). We used three replicate monoliths per sucrose addition level with eight replicates for the control treatment. Sucrose solution (175 mL of deionized H₂O plus sucrose) was applied twice weekly to the top of each monolith throughout the duration of the 12-month experiment.

At the end of the 12-month period, the top 10 cm of the upper mineral horizon of each monolith was harvested, sieved to 2 mm, and thoroughly homogenized. A subsample of each sieved soil was immediately stored at −80°C for molecular analyses. With the remainder of each sample we measured gravimetric soil moistures, pH, and extractable inorganic N concentrations using the techniques described above. The rates of CO₂ production from the sieved samples were measured over a 60-day period using a series of static incubations (Fierer et al. 2005b); soils were maintained at 20°C and soil moisture levels were held constant during this 60-day period.

Molecular analyses

With the soil samples collected from the cross-site study and sucrose amendment experiment, we wanted to quantify the relative abundances of the major bacterial taxa in soil. We designed primer sets for quantitative PCR (qPCR) assays targeting the most abundant bacterial phyla in soil (Fierer et al. 2005a). Using the suite of qPCR assays we were able to quantify the relative abundances of 16S ribosomal DNA (rDNA) belonging to the following six bacterial groups: Acidobacteria, Actinobacteria, α-Proteobacteria, β-Proteobacteria, Bacteroidetes, and Firmicutes (Appendix B). The α-Proteobacteria qPCR assay may also include 16S rDNA belonging to members of the δ-Proteobacteria group and the Actinobacteria assay may include some Verrucomicrobia (Fierer et al. 2005a). In all cases, taxa are identified using the nomenclature from the most recent version of Bergey's Taxonomic Outline of the Prokaryotes (Garrity et al. 2004). Historically, the Bacteroidetes phylum has also been referred to as the Cytophaga-Flavobacteria-Bacteriodes group (Kirchman

2002) and the Actinobacteria and Firmicutes phyla are sometimes termed the high and low G+C Gram positive bacteria, respectively.

A complete description of the qPCR technique used in this study is available in Fierer et al. (2005a). Briefly, DNA was extracted from each soil sample using the UltraClean Soil DNA kit (MoBio Laboratories, Carlsbad, California, USA), further purified using a Sepharose 4B (Sigma-Aldrich, St. Louis, Missouri, USA) column, and DNA concentrations measured by PicoGreen fluorometry (Molecular Probes, Eugene, Oregon, USA). The qPCR assays were conducted on an ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, California, USA) using SYBRGreen (Molecular Probes) for the fluorescent detection of PCR product accumulation. A dilution series of the appropriate plasmid standard was included with each qPCR run to estimate the copy number of targeted 16S rDNA copies in each soil DNA sample. For each qPCR assay, three replicate runs were conducted per DNA sample and the estimated copy numbers were averaged. The relative abundance of each bacterial group was calculated as the ratio between the number of 16S rDNA copies belonging to the target group (estimated from the individual group-specific qPCR assays) and the total number of bacterial 16S rDNA copies (estimated from a separate "all Bacteria" qPCR assay with a universal bacterial primer set). By using fractional copy numbers, the estimates of relative group abundances should be minimally affected by inter-sample variability in template DNA concentrations (Fierer et al. 2005a).

Meta-analysis

Due to limitations associated with analyzing results from individual studies, we conducted a meta-analysis of published soil clone libraries to determine if the results from the cross-site study and sucrose amendment experiment are in broad agreement with the published literature. We compared the abundances of major bacterial phyla in bulk and rhizosphere soils, with rhizosphere soils expected to have higher overall levels of C availability than bulk soils (Tate 2000). We only included those libraries which used universal bacterial primers for amplification, consisted of more than 60 sequenced clones, and provided adequate taxonomic information (Appendix C). All of the libraries were categorized into those from either bulk soils (16 libraries) or rhizosphere soils (16 libraries). Relative group abundances for the bulk and rhizosphere soils were calculated from the raw abundance data for each library.

Data analyses

All statistical analyses were performed using JMP (SAS Institute 2004). For the cross-site study, a backwards stepwise regression procedure was used to select the model which best predicts the abundance of each bacterial group. We used the following soil and site characteristics in the

statistical analyses: soil pH, moisture, texture, organic C content, C:N ratio, C mineralization rate, N mineralization rate, and select climatic variables (soil moisture deficit, mean annual temperature, mean monthly temperature during the sampling month). The threshold for inclusion in the final model was set at $P < 0.05$. The relationships between group abundances and C mineralization rates were further examined using single factor linear regression models. For the sucrose amendment experiment we tested the effects of sucrose addition rates on the measured abundances of each bacterial group using linear regression models. In all cases, regressions and the corresponding residuals were checked graphically to screen for linearity. If model fit was poor, variables were log-transformed and regression models were recalculated.

RESULTS

Cross-site study

Of the 10 soil and site characteristics examined, C mineralization rate, an index of organic C availability to microorganisms, was the best predictor of group-level abundances (Table 1). The rate of C mineralization was not the only factor related to group abundances, but it explained the greatest amount of variability in group abundances for those target groups that could be adequately predicted with the regression models. In particular, C mineralization rate was a strong predictor of the abundances of three groups: Acidobacteria, β -Proteobacteria, and Bacteroidetes (Table 1, Fig. 1). C mineralization rate alone predicted 26–35% of the variability in the abundances of these three groups (Fig. 1), even though the soils came from a wide range of ecosystem types and there was a high degree of variability in biotic and abiotic soil characteristics (Appendix A). With increasing C mineralization rates, the abundances of Acidobacteria decreased ($P < 0.001$) while the abundances of β -Proteobacteria, and Bacteroidetes increased ($P < 0.001$ in both cases). The abundances of the three other groups: the α -Proteobacteria, Firmicutes, and Actinobacteria could not be adequately predicted by C mineralization rate nor by any of the other soil and site characteristics measured; in all three cases the regression models explained less than 15% of the variability in group abundances (Table 1).

The soil and site characteristics included in the statistical analyses of group abundances are not necessarily independent variables. As a result, there was significant collinearity in a number of cases between measured variables (Appendix D). However, C mineralization rate was significantly correlated with only one other variable (percentage of organic C), and the relationship was relatively weak (Pearson's $r = 0.40$, Appendix D).

Sucrose amendment experiment

To evaluate whether the relationships between C availability and phyla abundances we observed in our cross-site comparison held under controlled conditions, we manip-

TABLE 1. Parameter estimates, proportion of the total model variation explained by factors (in parentheses), and significance levels for the backward stepwise regression models used in the cross-site study.

Phyla	Adjusted r^2	Organic C (%)	Silt + clay (%)	Soil pH	MAT (°C)	SMD (mm)
Acidobacteria†	0.36		0.005* (0.34)			
Actinobacteria	0.14		0.07* (0.49)	−0.9 (0.27)		
α -Proteobacteria	0.06	−0.15 (0.13)				
β -Proteobacteria	0.49				−0.08* (0.15)	
Bacteroidetes	0.44			0.21 (0.10)		
Firmicutes†	0.09					0.001* (1.0)

Notes: Key to abbreviations: MAT, mean annual temperature; SMD, annual soil moisture deficit. The other two characteristics measured (C:N ratio, mean monthly temperature) were not significant predictors of group abundances and hence are not shown.

* $P < 0.01$; ** $P < 0.001$.

† Relative abundances were log-transformed.

ulated C availability in a single soil. The sucrose treatments had strong effects on C and N dynamics; sucrose amendment levels were positively related to C mineralization rates ($r^2 = 0.81$, $P < 0.001$) and negatively related to extractable inorganic N concentrations ($r^2 = 0.53$, $P = 0.02$). Those monoliths receiving the highest level of sucrose addition ($800 \text{ g C} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$) had 183% higher C mineralization rates and 428% lower extractable N concentrations, on average, than those monoliths which received no added sucrose over the 12-month period. Soil pH values ranged from 4.6 to 5.6 and there was a significant, positive, relationship between sucrose amendment levels and soil pH ($r^2 = 0.5$, $P = 0.03$). Even though all monoliths received the same amount of water over the course of the experiment, there was some variability in soil moisture levels between monoliths (data not shown). However, there was no significant relationship between soil moisture and sucrose amendment levels ($r^2 = 0.30$, $P = 0.20$).

Since the abundance of each bacterial group was calculated as the ratio between the number of 16S rDNA copies belonging to the target group and the total number of bacterial 16S rDNA copies, we cannot determine if a specific group is changing its absolute abundance in response to the sucrose additions or if the population size is staying constant and other groups are increasing or decreasing in abundance. Nevertheless, we did find significant relationships between sucrose amendment rates and the relative abundances of three of the bacterial groups, supporting the results of the cross-site study (Fig. 2). With increasing sucrose amendment rates, the relative abundances of Acidobacteria decreased while the relative abundances of β -Proteobacteria and Bacteroidetes increased (Fig. 2). As in the cross-site study, the abundances of the other three groups (α -Proteobacteria, Firmicutes, and Actinobacteria) did not respond in any predictable manner to changes in C availability and there was a high degree of variability between experimental replicates (Fig. 2).

Meta-analysis

A robust comparison of bacterial communities in bulk and rhizosphere soils is difficult given the variability between soil types and the relatively small size of the

individual, published clone libraries included in the meta-analysis. Nevertheless, we found that Acidobacteria were less abundant and the β -Proteobacteria (as well as the α - and γ -Proteobacteria) were more abundant in rhizosphere soils than in the bulk soils (Fig. 3), supporting the results from our cross-site and sucrose-amendment studies. In contrast, Bacteroidetes were almost equally abundant in both rhizosphere and bulk soils, but their abundances were quite low and highly variable (Fig. 3). If we compare the relative abundances of the six targeted phyla in soil, we find that the clone library-based estimates (Fig. 3) generally agree with the qPCR-based estimates (Fig. 1, Appendix B) in that the Acidobacteria and α -Proteobacteria are the most abundant bacterial phyla in soil.

DISCUSSION

Copiotrophic and oligotrophic phyla

Together our survey, experimental, and meta-analytical results suggest that some abundant bacterial phyla can be divided into broad ecological categories that correspond to copiotrophic and oligotrophic groups. Bacteria belonging to the Acidobacteria phylum were most abundant in soils with very low resource availability (low C mineralization rates, Fig. 1) and their relative abundances were lowered in an individual soil amended with high concentrations of organic C (Fig. 2). In contrast to the oligotrophic Acidobacteria, the β -Proteobacteria, and Bacteroidetes exhibited copiotrophic attributes; their relative abundances were highest in soils with high C availability either as an intrinsic property of the soil (Fig. 1) or as a result of sucrose amendments (Fig. 2). The meta-analysis provided further support for this ecological categorization, with the Acidobacteria less abundant, and the β -Proteobacteria (as well as the α - and γ -Proteobacteria) more abundant, in rhizosphere soils than in the comparatively C-poor bulk soils (Fig. 3). These results do not suggest that every member of the Acidobacteria, β -Proteobacteria, and Bacteroidetes phyla are distinctly copiotrophic or oligotrophic. For example, the dominant autotrophic ammonia oxidizers are β -Proteobacteria (Bedard and Knowles 1989) yet they are likely to exhibit oligotrophic characteristics. There is an enormous

TABLE 1. Extended.

C mineralization rate ($\mu\text{g C}\cdot\text{g soil}^{-1}\cdot\text{d}^{-1}$)	N mineralization rate ($\mu\text{g N}\cdot\text{g soil}^{-1}\cdot\text{d}^{-1}$)	H ₂ O (%)
−0.04** (0.66)		−0.05 (0.24)
0.33 (1.0)		
0.36** (0.72)		
0.21** (0.76)	0.86** (0.14)	

amount of phylogenetic and physiological diversity within each of the targeted bacterial phyla and it is unlikely that an entire phylum would share common ecological characteristics. However, our results do suggest that the overall abundances of these three soil bacterial taxa respond in a predictable manner to changes in C availability and these taxa, or numerically abundant subgroups within these taxa, can be broadly categorized into copiotrophic or oligotrophic categories.

Although we know of no studies directly comparable to ours, there are independent lines of evidence supporting our division of the taxa, or groups within the taxa, into oligotrophic and copiotrophic categories. In aquatic environments, β -Proteobacteria and Bacteroidetes are commonly associated with substrates rich in organic carbon (Kirchman 2002, Simon et al. 2002, Fazi et al. 2005). In soil, Padmanabhan et al. (2003) found that active members of the β -Proteobacteria and Bacteroidetes phyla were some of the initial metabolizers of labile carbon inputs. Axelrood et al. (2002) and McCaig et al. (1999) have shown that Acidobacteria are generally less abundant and β -Proteobacteria are more abundant in soils with higher concentrations of organic carbon. Similar results were obtained by Héry et al. (2005) in a comparison of unamended soils to soils amended with glucose. Likewise, Marilley and Aragno (1999) found that the relative abundance of Acidobacteria is lower in rhizosphere soil than in bulk soil, which we would expect considering that C availability to microorganisms should be higher in the rhizosphere. However, in each of these studies only a few soils were directly compared and the small size of the clone libraries limits our ability to accurately assess group abundances (Dunbar et al. 2002).

Further support for our categorization of Acidobacteria, β -Proteobacteria, and Bacteroidetes into ecological groups on the basis of soil C availability comes from culture collections. For example, compared to their abundances in clone libraries, Acidobacteria are under-represented in culture collections while members of the Bacteroidetes and β -Proteobacteria groups are commonly isolated from soil and probably over-represented (Dunbar et al. 1999, Smit et al. 2001, Lipson and Schmidt 2004, Floyd et al. 2005). This disparity between clone libraries and culture collections is most striking for the Acidobacteria of which only a handful of cultured

representatives exist despite their high abundance in soil (Fig. 3). Traditional culturing methods are likely to select for microorganisms that can grow rapidly in high resource environments and, hence, the low culturability of Acidobacteria is to be expected. Recent evidence suggests that the culturability of Acidobacteria can be improved considerably if culturing methods are altered (by using long incubation periods and polymeric media) in order to favor the isolation of more oligotrophic bacteria (Janssen et al. 2002, Sait et al. 2002, Joseph et al. 2003, Stevenson et al. 2004).

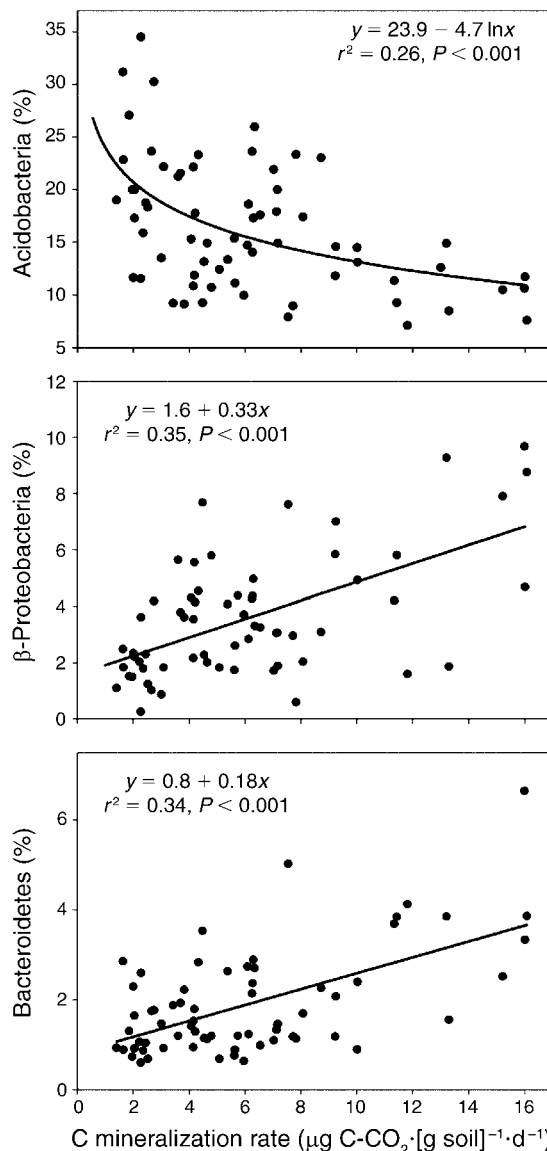


FIG. 1. Relationships between net C mineralization rate (an index of C availability) and the relative abundances of three bacterial groups across the 71 unique soils included in the cross-site study. Regression statistics describe the univariate relationship between the two parameters. Data for individual soils are provided in Appendices A and B.

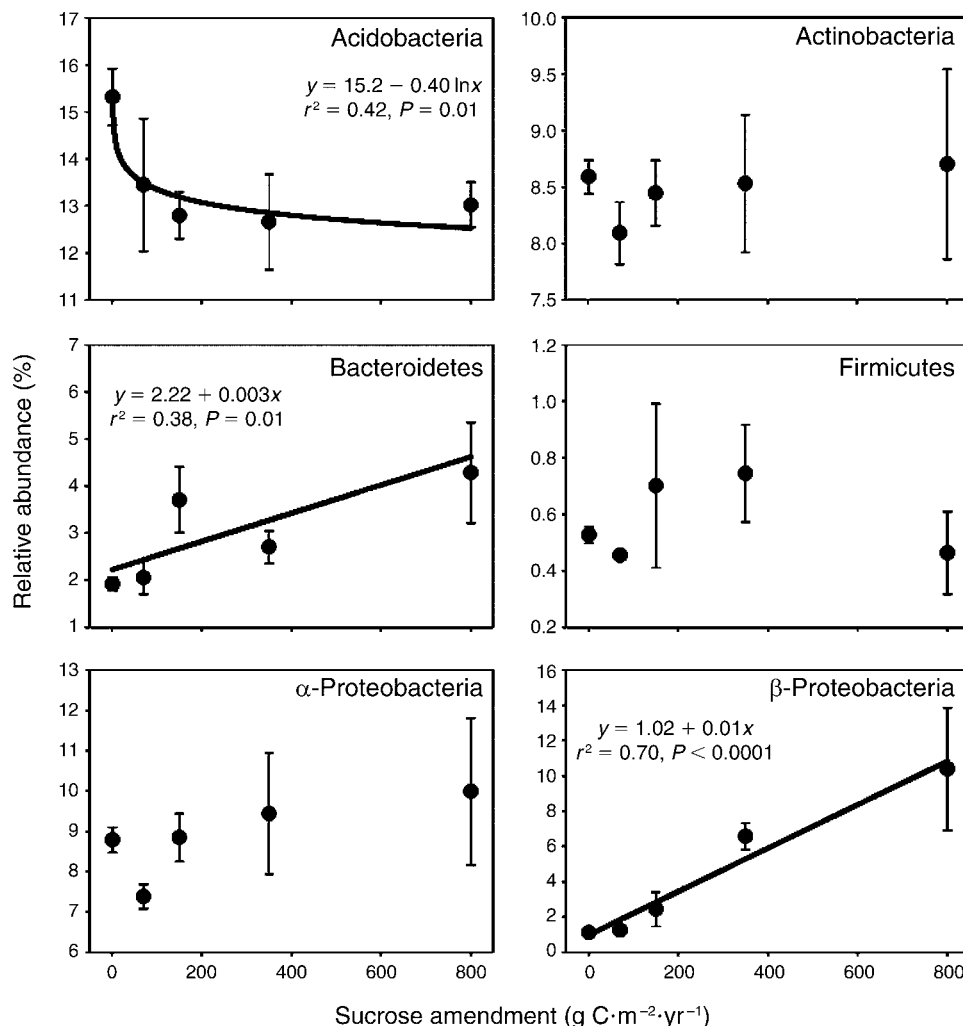


FIG. 2. The effects of sucrose amendment rates on the relative abundances (mean $\pm$ SE) of the six targeted bacterial groups as determined at the end of the 12-month experiment.

Phyla for which the copiotroph–oligotroph spectrum may not apply

Three of the taxonomic groups examined in this study: the α -Proteobacteria, Firmicutes, and Actinobacteria, could not be assigned into copiotrophic or oligotrophic categories based on our studies. These results do not suggest that the copiotroph–oligotroph classification scheme is irrelevant for these groups. Rather it suggests that the overall abundances of these groups do not change in a predictable manner to changes in soil C availability. By examining group abundances at finer levels of taxonomic resolution, ecological divisions may become more apparent and subsets of each group could be classified as either copiotrophic or oligotrophic. It is also possible that the copiotroph–oligotroph spectrum may not apply to certain taxa. With additional research, we may be able to discern which factor, or set of factors, better predicts the abundances of Actinobacteria, α -Proteobacteria,

and Firmicutes in soil, improving our ability to classify bacterial taxa into ecological categories. An expanded classification scheme, similar to that described by Grime (1977), may prove to be more useful for assigning bacterial taxa into ecologically meaningful groups.

Ecological attributes of copiotrophs and oligotrophs

We have shown that at least some bacterial taxa (or numerically abundant groups within these taxa) can be classified as either copiotrophic or oligotrophic in relation to resource availability. Although we examined bacterial communities at coarse levels of taxonomic resolution, we expect that the general copiotroph–oligotroph classification scheme will also apply at finer levels of taxonomic resolution. As discussed in *Introduction*, the copiotroph–oligotroph continuum is a direct corollary to the *r*- vs. *K*-selected continuum that is commonly used to describe the ecological attributes of plants and animals. Using the abundant literature on

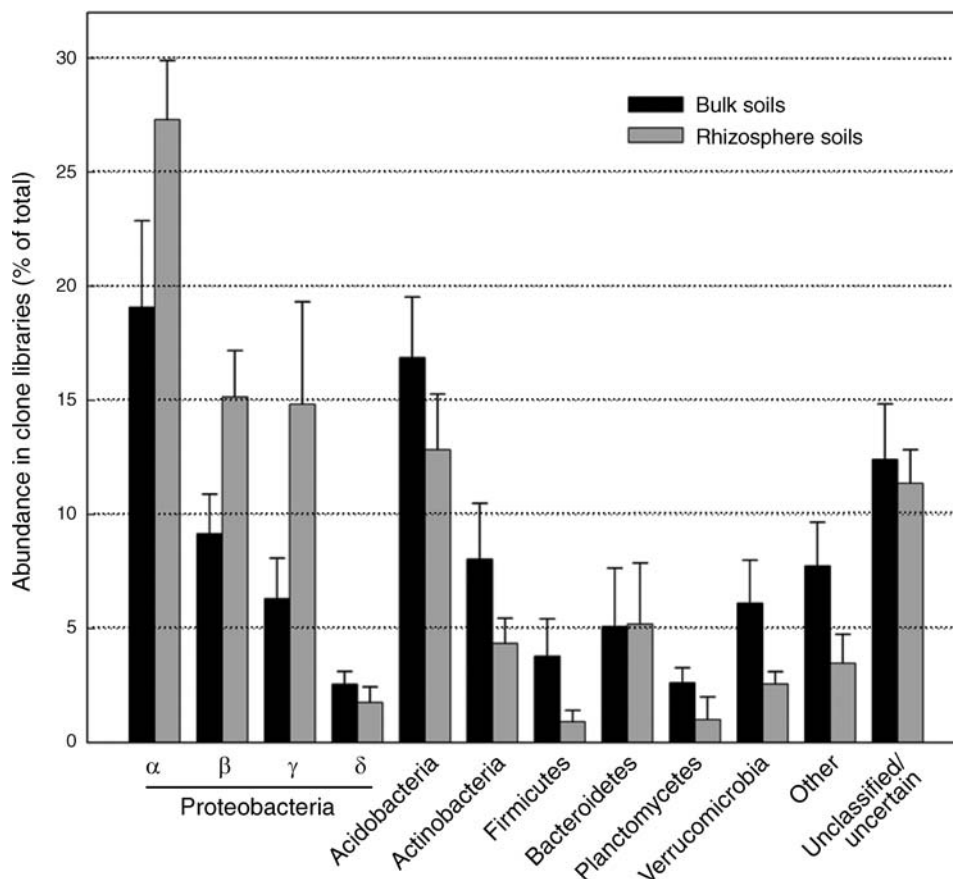


FIG. 3. The relative abundances (mean + SE) of different bacterial phyla in soil as estimated from a meta-analysis of published clone library results reported for rhizosphere and bulk soil samples (see references in Appendix C). A total of 32 libraries were included in the analysis: 16 from bulk soils and 16 from rhizosphere soils.

r- vs. *K*-selection, we can apply the general principles developed by plant and animal ecologists to make some predictions about the ecological attributes and life history patterns of copiotrophic and oligotrophic bacteria (Table 2).

In general, copiotrophic bacteria should have higher growth rates, a greater degree of variability in population size, and lower substrate affinities than oligotrophic bacteria (Table 2). In environments where microorganisms are exposed to sustained environmental stress, particularly where the stress stems from low resource concentrations, oligotrophs are likely to outcompete copiotrophs. We would also expect that microbial succession on substrates rich in organic matter, such as leaf litter or other types of fresh organic detritus, should be dominated by copiotrophs in the earlier stages, with oligotrophs increasing in relative abundance as substrate quality and/or quantity declines over time (Jackson 2003). In contrast, we would expect oligotrophs to be the first to colonize nutrient-poor substrates, such as mineral surfaces, with copiotrophs increasing in abundance as communities mature and develop in size.

The ecological attributes listed in Table 2 are hypothetical and a paucity of relevant information makes it difficult to ascertain the accuracy of these descriptions. Some of the listed traits would be very difficult to measure in situ and could only be measured by studying bacterial isolates in culture, a difficult task considering that most soil bacteria are not (presently) culturable and isolated strains may switch from copiotrophic to oligotrophic strategies depending on the culture conditions and stage in the life cycle (Hirsch et al. 1979, Gottschal 1985). Despite these limitations, the description of copiotrophic and oligotrophic traits provided in Table 2 may serve as a conceptual framework to help in the design and interpretation of studies attempting to link the structure of soil bacterial communities with their function.

CONCLUSIONS

Based upon the results from our field survey, experimental, and meta-analytical approaches, it seems appropriate to classify specific bacterial phyla, or subsets of these phyla that are numerically abundant in soil, into the ecological categories of copiotroph and oligotroph.

TABLE 2. Ecological attributes that are likely to correspond to copiotrophic and oligotrophic groups of bacteria.

Trait	Copiotrophs	Oligotrophs
Growth rates ($\mu_{\max}$)	High maximum growth rate ($\mu_{\max}$) ¹ when resources are non-limiting, high K_S (substrate concentrations at $\frac{1}{2} \mu_{\max}$). ¹	Low $\mu_{\max}$, outcompeted by copiotrophs in high-resource environments, low K_S .
Growth yield ($Y_{X/S}$) ¹	Low $Y_{X/S}$, inefficient conversion of substrate into cell biomass.	High $Y_{X/S}$: high biomass accumulation per unit substrate, efficient resource utilization.
Maintenance requirements ($s_{\min}$) ¹	High $s_{\min}$, substrate supply rates must be sufficiently high to maintain viability.	Low $s_{\min}$, cells remain viable even when substrates are limited.
Substrate uptake systems	Low specific affinity of cells for substrates (a_A or the $\mu_{\max}:K_S$ ratio), poor competitors when substrates are limited. ²	High specific affinity (a_A), efficient substrate "scavengers," highly capable of simultaneous uptake of mixed substrates. ²
Responsiveness to substrate additions	Brief lag in growth rates after additions of fresh substrate, large proportion of enzymes are produced constitutively.	Long lag time before growth on fresh substrate is maximized, most enzymes are induced, not constitutive.
Temporal variability in population size	High; substrate availability is pulsed producing "feast and famine conditions," fast rates of population turnover, short mean generation times.	Low; supply of substrates is fairly constant (but low), rates of population turnover are slow, long generation times.
Ease of cultivation	High; grow well in nutrient-rich media, visible colonies appear with incubations of short duration.	Low; visible colonies slow to appear, best isolated with nutrient-poor media.
Cell chemistry and morphology	Low C:N and C:P due to high intracellular nucleic acid and protein content, spherical cells with low surface area : volume ratio. ³	Elongated or filamentous cells with high surface area, volume ratio, ³ presence of prosthecae, ⁴ high capacity for intracellular storage of nutrient reserves. ⁵
rRNA operon copy number	High (>5). ⁶	Low (<2). ⁶
Tolerance to environmental stressors (e.g., pH, temperature, drying-rewetting)	Highly sensitive to environmental stress, spore formation common when exposed to suboptimal environmental conditions.	Individual cells can maintain viability under stressful environmental conditions.

Notes: The copiotroph and oligotroph categories are, to some degree, synonymous with the *r*- and *K*-selection categories proposed by Pianka (1970). K_S is the substrate saturation constant, $Y_{X/S}$ is the growth yield, a_A is the specific affinity for substrates, $\mu_{\max}$ is the maximum specific growth rate. Superscript numbers refer to sources: 1, Kovárová-Kovar and Egli (1998), who explain the Monod parameters used to describe growth kinetics ($Y_{X/S}$, $s_{\min}$, $\mu_{\max}$, K_S); 2, Button (1993); 3, Matin (1979); 4, Poindexter (1979); 5, Hirsch et al. (1979); 6, Klappenbach et al. (2000).

We do not suggest that this broad classification scheme is perfect; we have only assessed a coarse level of taxonomic resolution and some of the phyla we examined could not be assigned to positions along the copiotroph–oligotroph continuum. However, we do suggest that the field of soil microbial ecology will benefit by adopting an ecological classification scheme similar to that used by plant and animal ecologists. The copiotroph–oligotroph scheme may serve as a conceptual basis upon which to synthesize the ever-increasing amount of taxonomic data on soil bacterial communities in an ecologically meaningful manner.

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APPENDIX A

Soil and site characteristics for all samples included in the cross-site study (*Ecological Archives* E088-084-A1).

APPENDIX B

The relative abundances of the targeted groups in each soil sample (*Ecological Archives* E088-084-A2).

APPENDIX C

References for the clone library meta-analysis in Fig. 3 (*Ecological Archives* E088-084-A3).

APPENDIX D

Correlation table showing Pearson's r values relating the 10 site and soil characteristics used for statistical analyses of group abundances (*Ecological Archives* E088-084-A4).