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Low abundant soil bacteria can be metabolically versatile and fast growing

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Abstract. The abundance of species is assumed to depend on their life history traits, such as growth rate and resource specialization. However, this assumption has not been tested for bacteria. Here we investigate how abundance of soil bacteria relates to slow growth and substrate specialization (oligotrophy) vs. fast growth and substrate generalization (copiotrophy). We collected 47 saprotrophic soil bacterial isolates of differing abundances and measured their growth rate and the ability to use a variety of single carbon sources. Opposite to our expectation, there was no relationship between abundance in soil and the measured growth rate or substrate utilization profile (SUP). However, isolates with lower growth rates used fewer substrates than faster growing ones supporting the assumption that growth rate may relate to substrate specialization. Interestingly, growth rate and SUP were correlated with phylogeny, rather than with abundance in soil. Most markedly, Gammaproteobacteria on average grew significantly faster and were able to use more substrates than other bacterial classes, whereas Alphaproteobacteria were growing relatively slowly and used fewer substrates. This finding suggests that growth and substrate utilization are phylogenetically deeply conserved. We conclude that growth rate and substrate utilization of soil bacteria are not general determinants of their abundance. Future studies on explaining bacterial abundance need to determine how other factors, such as competition, predation and abiotic factors may contribute to rarity or abundance in soil bacteria.

Key words: *bacterial abundance; life-history traits; niche breadth; phylogeny.*

Introduction

Many communities contain a few abundant species, whereas most other species are low abundant, or even rare (Magurran and Henderson 2003), so that a general question in ecology is what causes rarity and abundance. However, this question has not yet been extensively addressed with respect to soil bacteria. Recent developments in sequencing technology reveal enormous numbers of belowground bacterial species with approximately 10^4–10^6 different species, based on numbers of operational taxonomic units (OTUs), in one single gram of soil (Bent and Forney 2008). As a consequence of this high diversity, the species abundance distribution of microorganisms in soil is even more skewed than of most other species in their habitat, resulting in a long tail of many low abundant species. Some of these species detected by sequencing might be inactive or relic DNA (Carini et al. 2016), but many were found to be actively contributing to community dynamics in space and time (Shade et al. 2014). This has been named the "rare biosphere" (Sogin et al. 2006). Although there are many studies that provide indications on the functions of rare bacterial species, the causes of their low abundance are poorly investigated.

Species abundance can be driven by a variety of factors. The most fundamental are the local abiotic conditions and the biotic interactions that a species experiences. These act in concert with a species' inherent traits, for example growth rate or substrate utilization, the ability to tolerate abiotic conditions, and habitat range, which can be regarded as measures for niche breadth. Moreover, these factors can act interdependently to shape species abundance (Van der Putten et al. 2010). Here we study which inherent traits can potentially determine the abundance, and especially rarity, of bacterial taxa in soil. Rarity can be defined as either a restriction in habitat and geographic range (i.e., occurrence at few sites) or local abundance (i.e., species density at one site) (Rabinowitz 1981). In our study, we consider rarity as a synonym for a low local abundance.

Studies on macroscopic organisms suggest that rarity of species is often associated with low growth rate and/or low fecundity; this relationship has been found for plants (Murray et al. 2002), as well as for vertebrates such as

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mice and darter fish (Kunin and Gaston 2012). Also for bacteria it has been suggested that rare taxa are slow growing with small cell sizes, which enables escape from predation and viral lysis (Pedrós-Alió 2006). This reflects a life-history strategy of permanent rarity that has indeed been demonstrated for some taxa (Lynch and Neufeld 2015). However, there is also a high number of so-called conditionally rare taxa that can be either rare or abundant at different points in time and space, largely dependent on changes in abiotic conditions (Shade et al. 2014). In addition, bacterial species have been found that are actively growing, but still are rare, possibly due to disproportional competition or predation (Hugoni et al. 2013).

Also niche breadth is suggested to be positively correlated to both range size and local abundance, which we study here (Slatyer et al. 2013). In other words, regionally and locally rare species are supposed to be more likely to be specialists, albeit that highly specialized species can still be locally abundant if suitable conditions are met. Niche breadth for saprotrophic microorganisms has been commonly assessed by measuring the species' substrate utilization profile (SUP), differentiating between substrate specialists with a narrow SUP and generalists that have a wide SUP (Wei et al. 2015). Also for bacteria, generalist species may attain higher abundances, whereas specialists may be more rare (Wilson and Lindow 1994). A narrower range of substrate utilization and high overlap of SUP with other bacterial species are also possible causes of low abundance, since it can lead to increased competition (Wei et al. 2015). On the other hand, studies on decomposition have shown that at least some low abundant bacterial taxa are highly specialized in their substrate use (Pester et al. 2010) and have little niche overlap with more abundant species as their loss leads to a decrease in some aspects of decomposition (Peter et al. 2011, Philippot et al. 2013).

Growth rate and substrate utilization are two important traits in the oligotrophic-copiotrophic concept (Koch 2001). According to this concept, copiotrophic bacteria exhibit high growth rates under nutrient-rich conditions, whereas oligotrophic bacteria have lower growth rates, but are able to sustain growth under nutrient-poor conditions. Thus, oligotrophs may be at disadvantage under nutrient-rich conditions, but are able to outcompete copiotrophs when the environment is nutrient-poor. In addition, copiotrophs are assumed to be able to adapt to a wide range of habitats and niches marking them as generalists (Koch 2001). Based on this, oligotrophic species are expected to be more specialized. This is further supported by the finding that oligotrophic species often have smaller genomes than copiotrophs (Christie-Oleza et al. 2012). Moreover, fast growing copiotrophic bacteria have been proposed to contain more copies of the 16S rRNA gene, which enables them to more rapidly respond to enhanced resource availability (Roller et al. 2016). The exhibition of oligotrophic and copiotrophic traits could influence the abundance of bacteria in the natural environment and these traits might be conserved in certain classes or phyla (Fierer et al. 2007).

Oligotrophic bacteria are supposed to be slow growing and more specialized in their substrate use (Semenov 1991). Similarly, it is often assumed that low growth rates can lead to bacterial rarity (Pedrós-Alió 2006). However, it has not yet been tested to what extent restricted substrate use and slow growth determine bacterial abundance in soil. Here we investigate the relationship between two intrinsic species traits, growth rate and substrate utilization, and abundance of saprotrophic bacteria in soil to elucidate potential factors shaping microbial communities. We tested three hypotheses: (1) rare bacterial species have on average lower growth rates than abundant species; (2) rare bacterial species utilize fewer carbon-sources than abundant bacterial species; and (3) bacterial species with a lower growth rate are also utilizing fewer carbon-sources than fast-growing species.

In order to test the three hypotheses, we isolated 47 saprotrophic (i.e., organotrophic and heterotrophic) bacterial strains from soil in an old field in The Netherlands and determined relative abundance in soil by blasting the isolate sequences against an OTU reference database generated from a pyrosequencing approach using the same soil. Growth rates were determined experimentally and all strains were exposed to 31 different substrates in order to test relationships between relative abundance, growth rate, the number of carbon sources used, and the substrate utilization profile.

Methods

Isolation of bacteria

Soil samples for bacterial cultivation were taken from a long-term diversity experiment on ex-arable land near Ede (Gelderland, The Netherlands, 52°04′ N 05°45′ E), in which the development of a variety of abiotic and biotic measurements is being followed since abandonment from agriculture in 1995 (Van der Putten et al. 2000). This site was selected because of the high amount of information available on the soil characteristics (Olsen $P = 90 \pm 3$ mg/kg, %orgC = 4.2 ± 0.3, C:N = 16.8 ± 0.1, Min-N = 10.6 ± 0.8 mg/kg, pH = 6.2 ± 0.1) (van de Voorde et al. 2012). Bacteria were isolated by both flow sorting with a flow cytometer (MoFlo Legacy Cell Sorter; Beckman Coulter, Miami, Florida, USA) and dilution plating. Flow sorting was done to increase the chance of isolating rare taxa as those have been found to be overrepresented among the smaller cell size fractions (Portillo et al. 2013). Different approaches and media were chosen to enhance the chances of cultivating a broader range of bacterial species. Several oligotrophic media were used that have been shown to enable the cultivation of many previously uncultured microorganisms (Janssen et al. 2002, Joseph et al. 2003). For the flow sorting approach, the media used were tryptone soy agar at 1/10 and a 1/100 dilution, water yeast agar, dilute nutrient broth solidified with gellan gum, and soil agar with or without addition of nutrients (for composition of media see Appendix S1: Table S1). The media were

used to prepare 96-well agar plates and wetted with phosphate buffer before flow sorting. One day prior to flow sorting 5.4 g of sieved soil were added to 130 mL phosphate buffer (1 g/L KH_2PO_4; pH = 6.5) and shaken for 1.5 h at 120 rpm. Bacterial cells were obtained from soil particles with sonication (2 × 1 min) followed by shaking for 0.5 h and passing the soil solution through a 45 μm sieve. In addition, 130 mL of phosphate buffer without soil added was subjected to the same treatment as a control. The soil solution and the control were stained with SYBR green stain (Sigma-Aldrich, St. Louis, Missouri, USA) at a concentration of 1:100 and stored at 4°C in the dark until flow sorting. Due to the high cell density in the soil samples they were diluted 1:10 with the phosphate buffer to a concentration suitable for counting.

The SYBR green stained particles in the samples were excited with a 488 nm laser beam. Fluorescence was measured at 580 and 530 nm as emitted by the SYBR green stain. Particles with low intensity fluorescence and a size of approximately 0.5 μm were selected and sorted into single wells of the 96-well plates, each containing one of six different media with five plates per medium. The plates were incubated at 20°C and checked regularly for visible bacterial growth.

For dilution plating, VL55 medium was used with the addition of 0.2 mol/L xylan instead of glucose (Appendix S1: Table S1). This composition has been found to be suitable for the isolation of a wide range of different soil bacterial taxa (Sait et al. 2002). In addition, soil agar was prepared according to the protocol for the soil extract with salts (SES) medium described by Smirnov and Brown (2004) (Appendix S1: Table S1). One gram fresh weight of sieved soil was dispersed in 100 mL phosphate buffer and shaken for 1.5 h. Sonication (2 × 1 min) was followed by shaking for 0.5 h. The soil solution was passed through a 45 μm sieve. This 10^{-2} dilution was serially diluted in phosphate buffer up to 10^{-7}. The 10^{-2} and 10^{-7} dilution were used to inoculate two plates each from both the VL55 and SES medium. One plate was inoculated with 100 μL of inoculum, the other by dipping the inoculation loop into the soil solution using the streak plate method. One plate of each medium was inoculated with 200 μL of the 10^{-7} dilution only using the spread plate method. The plates were checked for colony formation every other day. Colonies visible by eye were purified by subcultivation on the respective medium.

All cultures were repeatedly subcultivated on 1/10 diluted tryptone soy agar prior to the subsequent experiments.

Identification of bacterial cultures

Colony-PCR was performed for amplification of the 16S rRNA gene. Colonies of pure isolates were picked from plates using sterile toothpicks and suspended in 25 μL SDS-lysis buffer (1.21 g/L Tris, 0.372 g/L EDTA, 0.5% SDS). The cells were lysed in a PCR thermocycler at 95°C for 5 min and 1 μL of the suspension was used as a template for PCR. From those isolates recalcitrant to this method, DNA was extracted using either the ZR fungal/bacterial DNA MiniPrep kit (Zymo Research, Irvine, California, USA), the Power soil DNA isolation kit (MO BIO laboratories, Carlsbad, California, USA) or the QIAmp DNA Mini kit (Qiagen, Venlo, The Netherlands) and 1 μL DNA was used as a template in the PCR. 16S rDNA fragments were amplified using the primers pA (5′-AGAGTTT GATCCTGGCTCAG-3′) and 1492r (5′-GRTACCTTG TTACGACTT-3′). Reaction mixes of 25 μL were prepared containing 2.5× PCR buffer, 200 μmol/L of each deoxynucleoside triphosphate, 0.6 μmol/L of each primer and 0.04 μmol/L FastStart High Fidelity Polymerase (Roche, Basel, Switzerland). Amplification was carried out in a PCR C100 Touch Thermocycler (Bio-Rad Laboratories, Hercules, California, USA) using the following schedule: initial 2 min of denaturation at 94°C followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 1 min and extension at 72°C for 45 s. PCR products were sent for Sanger sequencing by Macrogen (Amsterdam, The Netherlands) or Baseclear (Leiden, The Netherlands) using the primer 515f (5′-GTGCCAGCMGCCGCGGTAA-3′).

Sequence quality trimming was done on ABI format chromatograms using the program Phred (Green and Ewing 2002) with a phred quality value P of 0.00317 as trim cut-off. The trimmed sequences were blasted against the greengenes and the SILVA database using the SINA alignment service for phylogenetic affiliation (Pruesse et al. 2012). Sequences were deposited in Genbank under the accession numbers KX503324–KX503369 with the exception of VK47, which had an insufficient sequence-length.

Estimation of relative abundance in field soil

In order to estimate the relative abundance of the isolated strains in the field, their partial 16S rRNA gene sequences were compared to a sequence database containing 267,189 sequences from seven soil replicate samples. The replicates originate from the same soil used for bacterial cultivation. Samples were collected in February 2013 at 10 cm depth at a minimum distance of 30 cm. Sequences were obtained by pyrosequencing and grouped into 7,086 reference OTUs at a 97% identity cut-off by *de novo* clustering (Kurm 2016). The *de novo* clustering method has the advantage that it is independent of a reference database and therefore does not discriminate against less well described lineages (Westcott and Schloss 2015). DNA extraction, sequencing and bioinformatics were done as described earlier (Hol et al. 2015). We are aware of the semi-quantitative nature of pyrosequencing data, but it has been shown that proportional abundances can be approximated which allows separation into rare or abundant taxa (Pilloni et al. 2012).

The trimmed Sanger sequences were blasted against the OTU reference table generated from the respective database using NCBI blastn (NCBI, Bethesda, Maryland, USA). The strains were matched to the OTU with percentage identity above a cut-off of 97%. The strain was

subsequently matched to the closest related OTU. The mean relative abundance of sequences grouped into the respective reference OTU was consequentially taken as the relative abundance of the strain in soil. Several OTUs were not detected in the seven pyrosequencing replicates used for comparison in this study, but were detected in other samples from the respective study, in which different disturbance treatments were applied to the soil indicating that they were present but under detection level in the undisturbed soil. Therefore, their relative abundance is designated as 0. Two strains, VK8 and VK14, could not be reliably assigned to an OTU and therefore were excluded from the data analysis on relative abundance.

Growth rate measurement

Precultures of isolates were prepared in liquid $_{0.1}$TSB (3 g/L tryptone soy broth) on 96-well microplates (Greiner flat-bottom) and incubated for 48 h at 25°C on a flatbed shaker in order to achieve a similar growth stage for all isolates. For the final culture the precultures were inoculated at a concentration of 1:10 into both fresh $_{0.1}$TSB (i.e., low nutrient medium) and full strength TSB (30 g/L tryptone soy broth; i.e., high nutrient medium) on a new microplate. Plates were placed immediately in a Synergy HT microplate reader (Biotek, Winooski, Vermont, USA). The optical density at 600 nm (OD_{600}) was automatically recorded every 30 min over a time period of maximal 60 h with continuous shaking between the measurements and normalized against an uninoculated blank medium measured simultaneously on the same plate. At least five independent measurements were performed for each strain. For isolate VK11, VK13, VK18, VK 20, VK21 and VK 38 1–2 replicates had to be omitted from statistical analysis due to difficulties in obtaining accurate measurements. Some isolates grew poorly in liquid culture, whereas others were prone to form aggregates. Growth models were fitted and growth rates were calculated using the function gcFit from the package grofit in R (Kahm et al. 2010).

Substrate utilization profiling

The substrate utilization profile was assessed by the same principle as in Biolog microplates (Biolog, Hayward, California, USA). Microplates (96-well Greiner flat-bottom) contained a freshly prepared minimal OS medium (Schnider-Keel et al. 2000) with 0.2% Iodonitrotetrazolium-chloride (INT) as a redox dye and 31 different substrates as a sole carbon source at a concentration of 10 mmol/L (6 amino acids, 7 carbohydrates, 11 carboxylic acids, 1 phenolic acid, 4 polymeres and 2 miscellaneous; Appendix S1: Table S2) together with one water control, fitting three assays per plate. The compounds were chosen to resemble substrates present in root exudates (Campbell et al. 1997). The availability of these substrates in the soil at the site of the isolates' origin is, however, unknown.

Precultures in liquid $_{0.1}$TSB were washed three times in phosphate buffer and adjusted to an OD_{600} of 0.5. Subsequently, 20 μL of bacterial suspension were inoculated into 130 μL of medium resulting in a total volume of 150 μL per well. Strains were inoculated in duplicate on two different plates. The experiment was repeated three times for each strain, resulting in an n of 6. Optical density at 590 nm was measured in a microplate reader immediately after inoculation and subsequently once per day over a time period of 7 d. In between, plates were incubated at 25°C on a flatbed shaker.

Data analysis

All statistical calculations were done in R version 3.2.3 (R Core Team 2016).

The mean growth rate in TSB and $_{0.1}$TSB and the difference between the two means was calculated for each strain to account for pseudoreplication. The relationship between relative abundance in soil and mean growth rate was tested with a Spearman rank correlation test. To test for differences in phylogeny at class level between rare and abundant isolates a one-way Anova and Tukey Posthoc test were used. To determine the substrate utilization profile on microplates the slope of color development for each compound was calculated and the color development of the respective control well was subtracted. The compound was considered to be utilized by the inoculated bacterium if the resulting value exceeded a threshold of 0.001. The threshold was based on visual observation of color development compared to the control. A threshold of 0.001 was chosen as a conservative value where the color development clearly exceeded that of the control. In a matrix of compounds and replicates of strains tested, utilization was given the value 1, non-utilization was given a 0. This matrix was used subsequently to calculate the average number of carbon sources used by each strain. Spearman rank correlation tests were performed for number of carbon sources used, relative abundance, and growth rate. To detect potential patterns in the substrate utilization profiles the substrate utilization matrix was analyzed by principal component analysis using the function prcomp(). Effects of relative abundance, growth rate and phylogeny on class level on the matrix were determined by permutational multivariate analysis using the function adonis() from the vegan package (Oksanen et al. 2016). A binary metric of carbon-source utilization was implemented with the function nestednodf(). Two isolates (VK13, VK20) had to be excluded from the substrate utilization analysis due to insufficient growth in precultures and sensitivity to EDTA, respectively.

The number of copies of 16S rRNA genes of the isolated species were estimated by aligning their sequences to a sequence database provided by Větrovský and Baldrian (2013) containing 7,081 16S rRNA gene sequences. The average 16S rRNA gene copy number from all members of the genera they were most closely related with (provided in the same article) was calculated to achieve the best estimate of copy number for the isolated species. Correlations between rRNA gene copy number, genome size, growth rate and number of carbon sources used were performed as described above.

One-way ANOVAs and subsequent Tukey Posthoc tests were used to test for differences in average growth rate, relative abundance, carbon source use and rRNA gene copy numbers between classes of bacterial isolates.

Results

Isolation of bacteria

Isolation by flow sorting and dilution plating yielded 160 isolates in total, which represented 47 different OTUs. One representative isolate per OTU was used for all further analyses. For complete phylogeny see Supplementary Material (Appendix S1: Table S3). All isolates belonged to the classes Actinobacteria, Bacilli, Gammaproteobacteria, Betaproteobacteria and Alphaproteobacteria. All five classes included isolates of both rare and abundant taxa (Fig. 1). There were no significant differences in mean relative abundance of isolates among classes. It was not possible to compare relative abundances among families statistically, because most families were represented by only one or two isolates.

Relationship between growth rates and relative abundance

Strains grew on average faster in the high nutrient medium than in the low nutrient medium (P-value < 0.01, $F_{1.0} = 65.5$). The growth rates varied considerably among strains, especially in the high nutrient medium, differing with a factor of 30 between the fastest and slowest growing isolate. Variability of measurements was highly strain-specific: several strains were highly variable, whereas others were less so (Appendix S1: Table S4). Overall, there was no significant correlation between growth rate and the relative abundance in soil, neither in low nutrient (P-value = 0.43, $\rho = -0.12$, data not shown), nor in high nutrient media (P-value = 0.64, $\rho = -0.07$; Fig. 2).

Growth rates differed significantly between classes ($P < 0.01$, $F_{4.0} = 10.1$). In nutrient-rich medium Gammaproteobacteria had on average a 1.5× higher growth rate than Actinobacteria and an almost 4× higher growth rate than Alphaproteobacteria, whereas Bacilli had a 3× higher growth rate and Betaproteobacteria a 2.7× higher growth rate than Alphaproteobacteria (Appendix S1: Table S5). In the lower-nutrient medium, however, only Gammaproteobacteria were still faster growing than the other classes except for Betaproteobacteria ($P < 0.01$, $F_{4.0} = 6.7$). Classes with a higher growth rate in TSB also had a higher number of rRNA gene copies ($P < 0.01$, $F_{4.0} = 10.78$); Gammaproteobacteria had a significantly higher number of copies than Alphaproteobacteria and Betaproteobacteria, whereas Bacilli had more rRNA gene copies than members of all other classes except for the Gammaproteobacteria where the difference was only marginally significant. Accordingly, there was a positive correlation between growth rates and the number of rRNA gene copies of the isolates ($P < 0.01$, $\rho = 0.48$ and $P = 0.01$, $\rho = 0.36$ in low and high nutrient medium, respectively; Appendix S1: Table S4).

As the distinct differences in growth rates between classes might have obscured a potential relationship between growth rate and relative abundance we tested for such a relationship within each class. However, also within each single class there was no correlation between relative abundance and growth rate.

Substrate utilization profile

The number of used substrates showed a significant positive association with growth rate in both media

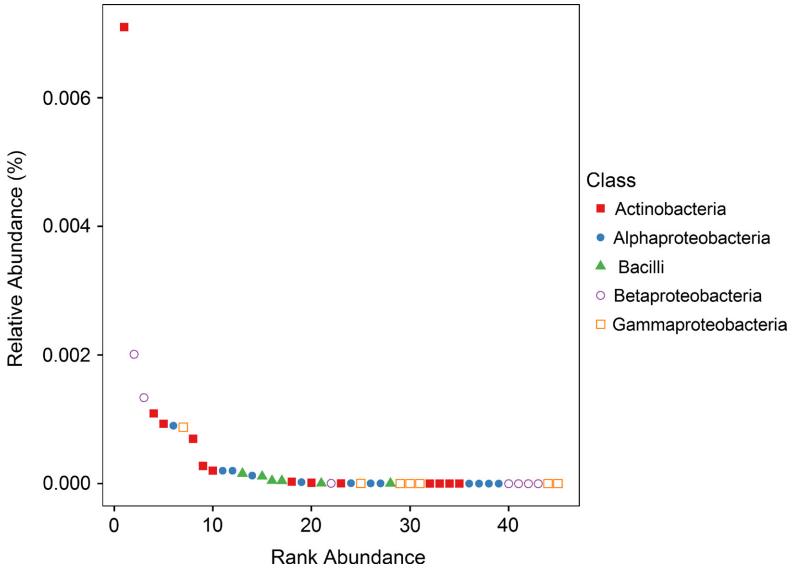

Fig. 1. Relative abundance of isolates plotted against rank abundance; different colors and symbols indicate different phylogenetic classes. [Color figure can be viewed at wileyonlinelibrary.com]

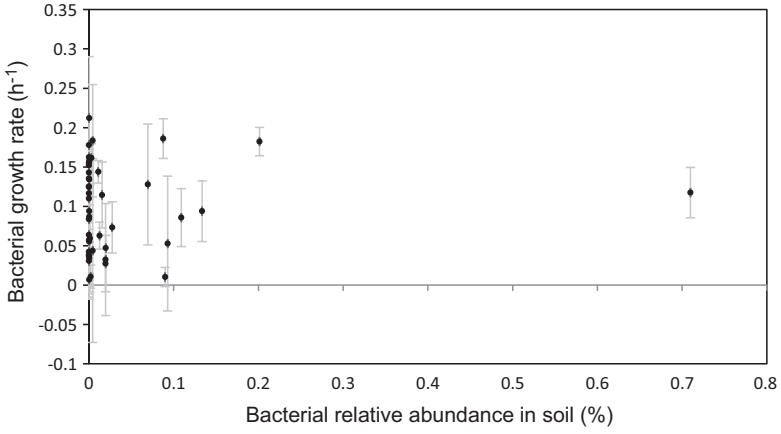

FIG. 2. Average bacterial growth rates in high nutrient medium of single isolates (OD_{600} change h^{-1}) plotted against their relative abundances (%) in soil ($n = 45$); each dot represents a single isolate; the error bars indicate standard deviation.

($P < 0.01$, $\rho = 0.63$ and $P < 0.01$, $\rho = 0.47$ for low and high nutrient media, respectively; Fig. 3B) and with the difference between growth in low and high nutrient medium ($P < 0.01$, $\rho = 0.43$). Gammaproteobacteria, which were growing fastest on average, were able to utilize a higher number of substrates than strains belonging to the other classes ($P < 0.01$, $F_{4.0} = 8.73$). However, we did not detect a relationship between number of substrates utilized and relative abundance in soil (Fig. 3A).

Along the first axis of the principal components analysis the SUPs of the isolates separated out into two groups (Fig. 4). The substrates that contributed to this separation belonged to a variety of different compound classes, i.e., carbohydrates, carboxylic acids, amino acids,

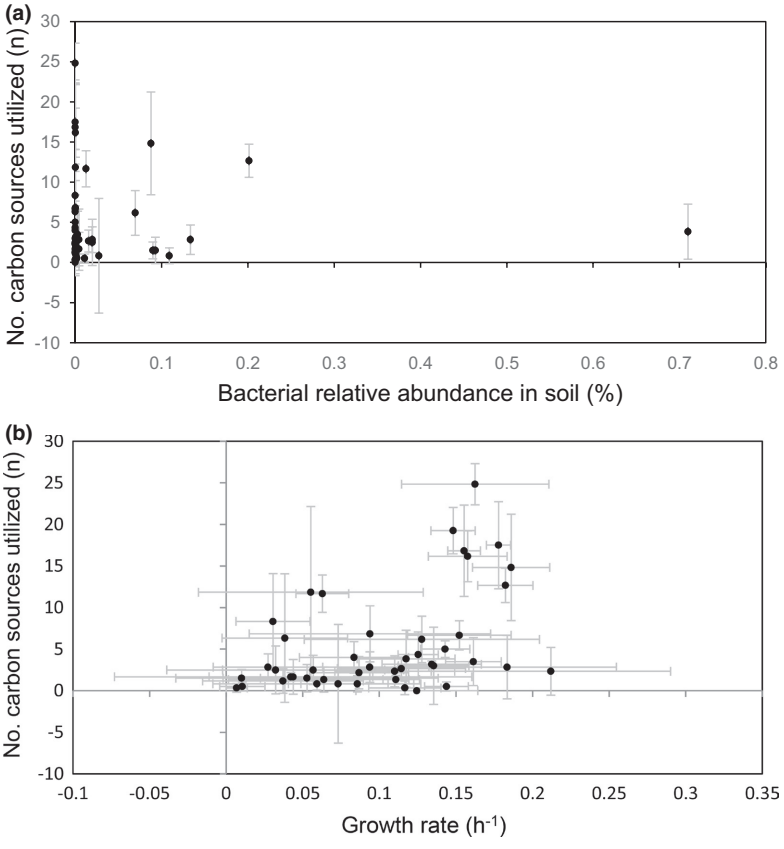

FIG. 3. The average number of carbon sources utilized by bacterial isolates plotted against (A) their relative abundance in soil (%; $n = 43$) and (B) average bacterial growth rate in high nutrient medium (h^{-1}; $n = 45$); dots represent single isolates; the error bars indicate standard deviation.

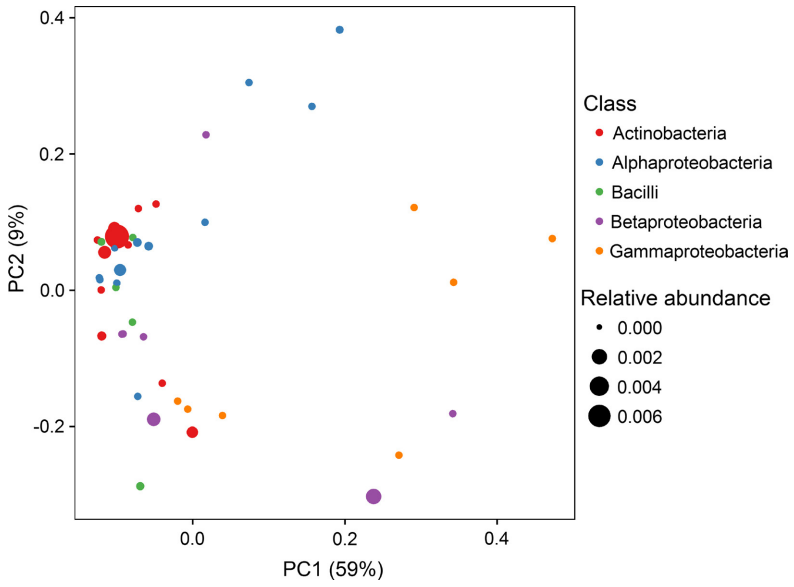

FIG. 4. Principal components analysis of carbon source utilization of all bacterial strains, dots represent single isolates. Colors represent different bacterial classes; the size of the dots represents relative abundance in soil on a continuous scale; proportion of variance explained expressed as percentages on the axes.

as well as polymeric compounds. There was a significant association between growth rate and SUP (F-model = 3.83, R^2 = 0.08, P < 0.01; F-model = 2.37, R^2 = 0.05, P = 0.02 in both media respectively). SUPs of strains belonging to different classes were significantly different from each other (F-model: 1.79, R^2 = 0.16, P < 0.01). On the other hand, relative abundance in soil did not correlate with the SUP.

A binary matrix of utilization of every substrate by every isolate showed that niche overlap was relatively high as every compound could be used by at least six isolates. Isolates that utilized only few substrates often used substrates that were also utilized by many other isolates (Appendix S1: Fig. S1).

Discussion

The aim of this study was to elucidate if growth rate and substrate utilization can explain the abundance of saprotrophic bacteria in soil. Growth rate was positively related to the number of substrates a species could use. This indicates that the isolates were distributed along a gradient from oligotrophic to copiotrophic species. Moreover, growth rate and substrate utilization differed with species phylogeny. We did not find relative abundance in soil to be linked to slow growth or restricted substrate use that would have indicated an oligotrophic lifestyle of low abundant species. On the contrary, there was a high variation of these traits among the isolates irrespective of their abundance.

Consequentially, we had to reject our first hypothesis because there was no significant difference in growth rate between rare and abundant strains. Most rare species had potential growth rates as high as or even higher than abundant species. This is supported by the observation that many rare taxa are in fact highly active (Campbell et al. 2011). Some can become abundant upon change of environmental conditions, others are permanently rare in spite of their activity (Hugoni et al. 2013). For a few of the isolated strains, however, their low abundance in soil corresponded with a low growth rate. Pedrós-Alió (2006) suggests that these slow growing strains can persist at low abundances because they are less vulnerable to predation since they have a small cell size and are less likely to be encountered. Interestingly, differences in growth rate between classes diminished with decreased nutrient concentration. This indicates that in even more nutrient-poor environments, such as soil, species identified as slow growers in this study might attain the same as, or even higher growth rates than their counterparts with the higher *in vitro* growth rates.

Secondly, we tested the hypothesis that rare bacterial species are able to utilize fewer carbon sources than more abundant species. This hypothesis was rejected, because the number of substrates used was unrelated to relative abundance. Moreover, substrate utilization showed high overlap between isolates. In other words, there was an overlap in metabolic niche. This was unexpected, because organisms with a narrow niche breadth usually are less abundant than organisms with a wider niche (Tilman 2004, Wamelink et al. 2014). For example, epiphytic bacterial species that use more substrates had higher competitive advantage when co-inoculated (Wilson and Lindow 1994). A similar pattern emerges from invasion experiments; invasion success decreased when the invading strain had large niche overlap in substrate utilization with the resident community (Wei et al. 2015). Therefore, species utilizing few substrates that are also used by many other species are expected to be less abundant in their natural environment

than species with a more general substrate uptake capacity. However, in the present study, niche overlap could have been overestimated, because many substrates used in the SUP assay are easily degradable.

Strains that grew faster were able to utilize a higher number of substrates. Moreover, strains that diverged more in their growth in low and high nutrient medium utilized more substrates than strains showing a less pronounced decrease in growth rate under low nutrient conditions. This is in support of our third hypothesis and the oligotrophy-copiotrophy concept, as copiotrophs respond more strongly to elevated nutrient conditions and are more generalistic in their resource use. In fact, we report for the first time a direct relationship between growth rate in a defined medium and the number of substrates that bacterial isolates utilize in an *in vitro* SUP analysis. Prior studies, on the other hand, have shown the ability of fast growing copiotrophs to quickly respond to labile carbon sources. In an *in situ* study, a relationship between the capability for fast growth and the rapid utilization of easily available carbon sources was shown in highly diverse communities (Goldfarb et al. 2011). Fast growing bacterial taxa respond quickly to addition of labile substrates, whereas slow growing taxa declined in relative abundance upon substrate addition. Although slow growing organisms also might utilize many labile substrates, they are supposed to be less competitive under the employed conditions. Yet, we show that slow growing bacteria are restricted in their substrate use, even when growing in monoculture. Oligotrophic species not only appear to be less competitive with regard to their response to resource availability, as indicated by Goldfarb et al. (2011), but they also lack the ability to utilize as many substrates as copiotrophs in the first place.

We found a trend for a positive relationship between rRNA gene copy number and growth rate, as has also been recently reported by Roller et al. (2016). It is assumed that a high number of rRNA gene copies can enable a fast synthesis of ribosomes and a rapid response of the bacterium to increases in nutrient concentrations (Stevenson and Schmidt 2004). To a certain degree, the number of rRNA gene copies is phylogenetically conserved (Lee et al. 2009). Indeed, our results show that differences in growth rate and substrate utilization correspond to differences in phylogeny. Also the substrate utilization profile differed significantly between phylogenetic classes. For a long time, it has been assumed that even closely related bacterial taxa differ substantially in their traits (Jaspers and Overmann 2004). While it may depend on the specific traits, only recently an *in situ* approach using stable isotope probing showed deeply rooted phylogenetic similarities in microbial activities (Morrissey et al. 2016). In the study by Morrissey et al. (2016) growth and rate of carbon assimilation from glucose were clustered at the phylum level. Alphaproteobacteria were on average more oligotrophic, whereas Beta- and Gammaproteobacteria showed rather copiotrophic traits. This is similar to our results, where Alphaproteobacteria are mostly slow growing and use only few substrates while Gammaproteobacteria grow fast and have a generalistic substrate use. Therefore, we demonstrate that even with *in vitro* substrate utilization tests of single isolates differences between broad taxonomic groups can be measured.

The lack of a relationship between relative abundance and growth rate or substrate use raises the question if the isolates and the general approach used in the present study sufficiently represent conditions in the soil. We were able to cultivate approximately 5% of the bacteria that were collected with the flow sorting approach, which is within the range of frequently reported cultivation efficiencies of bacteria collected from soil (Janssen et al. 2002, Puspita et al. 2012). A large proportion of the isolates are potentially low abundant in the environment. Still, soil hosts many more rare taxa that have not been included in our study. However, we already find considerable variation in growth rate and substrate use within our selection of isolates. The addition of more taxa would most likely have increased the variation. On the other hand, several candidate phyla have been found to contain mostly rare species (Lynch et al. 2012), which indicates that within these phyla shared traits contribute to their low abundance. Other potential limitations must be kept in mind as well, for example species-specific growth rates in other cultivation media or soil might differ from the rates recorded in the present study. Nevertheless, additional experiments with a subset of 10 of our isolates in Luria-Bertani (LB)-medium showed a high correlation between growth rates in the different media (Appendix S1: Fig. S2) indicating that our results are similar across media types. An advantage in the choice of TSB as a growth medium is that it shares no compounds with the SUP assay, so that the results from the two measurements are independent. In addition, we use relatively high nutrient concentrations to assess growth rates. Similar concentrations are likely to occur in the vicinity of plant roots, whereas bulk soil is rather nutrient poor. As mentioned previously, this might change the rank order of species growth rates.

Laboratory conditions are often criticized to poorly resemble the natural environment. However, isolation allows to perform measurements on individual bacterial strains that are to date not possible to perform *in situ*. Especially for low abundant strains isolation-based methods are irreplaceable to study traits of specific species. However, it is notoriously difficult to determine the abundance of a cultured strain in the environment due to the high diversity in soil and phylogenetic identification by short 16S rRNA sequences. It has even been shown that 16S rRNA is often insufficient to distinguish ecologically different strains (Jaspers and Overmann 2004). Although the relative abundance reported in our study might not represent the exact abundance in soil, it is an approximation allowing us to investigate trends in the relationship of abundance with intrinsic bacterial traits.

Overall, our results indicate that the abundance of bacterial species in soil is not solely determined by their growth rate. We also show that a narrow metabolic niche breadth and high overlap in substrate utilization among species do

not necessarily lead to low relative abundance. Metabolic niche breadth is only one component of a species' total niche breadth and other dimensions of a niche, such as habitat range and environmental tolerance might be better correlated with species abundance (Slatyer et al. 2013). We showed a separation of bacterial isolates into more oligotrophic or more copiotrophic species, but these two lifestyles were not related to relative abundance in soil. It is likely that other factors differentially affect bacterial abundance. Apart from abiotic conditions, such as soil pH, temperature or salinity, competitive ability and vulnerability to predation or viral lysis can affect abundance in soil (Neuenschwander et al. 2015, Kirchman 2016). A competitively inferior strain can be low abundant in spite of its ability to grow fast. Similarly, fast growing species could be strongly controlled by protozoan predators or viruses (Simek et al. 1997). As a consequence, there might be considerable temporal and spatial variation in species abundance (Caporaso et al. 2012). However, testing these possibilities requires further studies.

Conclusions

We conclude that bacterial growth rate is positively correlated to the number of carbon sources that a bacterium can use. This indicates that species are distributed along a gradient from oligotrophic to copiotrophic lifestyles. However, we did not find a relationship between relative abundance in soil and growth rate, or substrate use. On the contrary, rare bacterial species showed high variation in these traits. Therefore, we conclude that abundance in soil cannot be explained by growth rate and substrate use alone.

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Supporting Information

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Data Availability

Data associated with this paper are available in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.80724.