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Unimodal productivity–diversity relationships among bacterial communities in a simple polar soil ecosystem

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Summary

Unlike other macroecological principles, relationships between productivity and diversity have not been effectively tested for microbial communities. Here we describe an experiment in which the availability of resources to soil bacterial communities was manipulated in a model system, the McMurdo Dry Valleys of Antarctica. Mannitol additions were used to simulate a productivity gradient such that a response in bacterial biomass production, taxonomic diversity and functioning (e.g., enzyme activity) were induced. Resource amendment induced a positive linear response in microbial productivity ($P < 0.001$) but a unimodal (hump-shaped) response in microbial diversity at multiple taxonomic scales ($P = 0.035$). Putative oligotrophic (e.g., phyla *Nitrospirae* and *Cyanobacteria*) and copiotrophic (e.g., phylum *Proteobacteria*) taxa were apparent through substantial community turnover along the resource gradient. Soil enzyme activity was inversely related to bacterial biomass but positively related to diversity, suggesting the latter may be a stronger control over enzyme-mediated decomposition. The mechanisms behind this pattern are consistent with macroecological theory of a shift from environmental (e.g., stress tolerance) to biotic (e.g., competition) drivers with increasing resource availability. This evidence is among the first of its kind to document a significant unimodal productivity–diversity relationship for soil bacteria.

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Introduction

Productivity–diversity relationships (PDRs), where species diversity varies with indices of primary productivity (e.g., biomass, resource availability), are among the more common biogeographical patterns observed for organisms. Examples of PDR have been documented and reviewed for both plants (Guo and Berry, 1998; Waide *et al.*, 1999; Mittelbach *et al.*, 2001) and animals (Whiteside and Harmsworth, 1967; Abramsky and Rosenzweig, 1984; Dodson *et al.*, 2000) that show various responses of diversity to productivity. The unimodal (hump-shaped) curve, often the most common pattern, suggests that resource availability can increase species diversity but beyond a certain threshold, the effect becomes negative. This often reflects a switch to biotic mechanisms wherein competition by fast-growing organisms results in the exclusion of other functional groups (Michalet *et al.*, 2006).

The extension of PDR theory to microorganisms is less established. Previous investigations have observed unimodal PDR for *Pseudomonas* genotypes (Kassen *et al.*, 2000) and soil bacteria (Song *et al.*, 2016) in culture-based and aquatic environments (Horner-Devine *et al.*, 2003; Smith, 2007; Logue *et al.*, 2012). Soils, however, present a unique challenge for making these observations. Habitat heterogeneity over multiple nested scales, such as gradients of moisture and oxygen availability across the rhizosphere, aggregates, mineral surfaces and other microsites (Zhou *et al.*, 2002; Horner-Devine *et al.*, 2004), can supersede or otherwise obscure PDR. In spite of this, we are familiar with three published cases of significant microbial PDR in soil: fungi of a long-term biodiversity experiment in a temperate grassland (Waldrop *et al.*, 2006), bacteria along a natural productivity gradient in the McMurdo Dry Valleys of Antarctica (Geyer *et al.*, 2013) and bacteria of an agricultural soil receiving organic (benzoate) amendments (Langenheder and Prosser, 2008). All three investigations report an asymptotic or unimodal PDR but were either correlational in design or integrated a limited range of resource concentrations. Additional evidence is needed to support the claim that soil microorganisms indeed exhibit PDR.

The ability to predict diversity using PDR provides critical opportunities to model ecosystem-scale processes in soil (Wieder *et al.*, 2014). Important among these are functional

groupings of taxa that exhibit predictable traits such as slow, efficient growth of oligotrophs versus rapid, inefficient growth of copiotrophs (Krause *et al.*, 2014; Fierer, 2017). By nature of their growth strategies, these groups are predicted to result in different rates of C utilization and stabilization. For example, oligotrophic organisms may invest in the acquisition of relatively stable soil organic carbon (SOC) resources through enzymatic breakdown and efficiently convert this C into biomass that serves as precursors to SOC. Conversely, copiotrophs flourish when labile C is abundant by rapid growth accomplished at the expense of low efficiency, potentially resulting in less SOC formation (Molenaar *et al.*, 2009; Kallenbach *et al.*, 2016).

Here we describe the results of a laboratory study where the microbially dominated soil of the Antarctic Dry Valleys was used to test PDR theory. Soils of this region exhibit model characteristics for isolating the drivers that influence diversity: a lack of vascular plants and associated rhizosphere (Ball and Virginia, 2014), low faunal diversity and trophic complexity (Adams *et al.*, 2006), steep resource gradients over sub-meter distances (Geyer *et al.*, 2017) and relatively low micro-scale heterogeneity due to coarse textured soils with minimal structure (Bockheim, 1997). Model soils help to establish causative relationships that may be obscured in other systems by confounding variables. Our earlier work from this system documented an asymptote in bacterial diversity along a naturally occurring soil productivity gradient (Geyer *et al.*, 2013; Geyer *et al.*, 2014). The objectives of the present study are to (i) confirm earlier observations with a manipulative experiment and (ii) induce a broader resource gradient than natural field conditions provide in order to capture the full potential response profile of bacterial diversity. We hypothesize that C availability will initially increase the diversity of C-limited bacterial communities but have negative effects at higher concentrations because of competitive exclusion by copiotrophic taxa.

Results

Experimental treatment and biogeochemistry

Soil was collected to a depth of 10 cm in December 2012, and returned to Virginia Tech for experimentation. Laboratory treatment established five levels of resource availability: hyperarid field soil that was untreated ('Field'), wetted and warmed laboratory control soil ('Control'), and three treatments similar to lab controls with a single low (0.03 mg C g^{-1}), intermediate (0.10 mg C g^{-1}) or high (0.30 mg C g^{-1}) mannitol amendment. The low rate of addition was chosen to approximate peak dissolved organic carbon (DOC) concentrations of Taylor Valley soil (Geyer *et al.*, 2013), a surrogate for C availability. All treatments were destructively harvested after 12 days of incubation. Amendment of mannitol solutions to soils had no significant effect on specific conductivity (mean = 321.28 μS cm^{-1}) or pH (mean = 9.94) relative to field soil (Table 1). Soil moisture declined consistently among treatments during the experiment from 10% gravimetric (target soil moisture) to an average of 7.02% gravimetric (Table 1) due primarily to evaporative loss that occurred while estimating respiration rates. We consider this amount of drying unlikely to be of biological relevance for arid-adapted communities that generally experience moisture content below 2% in the field. As anticipated, microbial biomass concentrations increased linearly with mannitol addition (slope = 0.040 μg MBC μg^{-1} mannitol-C; P < 0.001), with an overall increase of ~50% between control (mean = 21.52 μg MBC g^{-1}) and high (mean = 33.88 μg MBC g^{-1}) mannitol treatments.

Soil respiration and enzyme activity

Soil respiration rates and cumulative respiration (integrated over the incubation duration) were strongly related to mannitol amendment rates and increased linearly with mannitol addition (slope = 0.12 μg CO_2-C μg^{-1} mannitol-C; P < 0.001). Control and low treatment respiration remained consistently near 1.5 μg CO_2-C g^{-1} day^{-1} throughout the incubation, with the low treatment statistically elevated from control on Day

Table 1. Mean soil factors by experimental treatment.

Factor	Field	Control	MannL	MannI	MannH
pH	9.93	9.97	9.90	9.97	9.96
EC	338.8	322.0	325.0	299.3	335.0
Moisture	0.99 (0.00)[a]	6.96 (0.11)[b]	7.09 (0.09)[b]	7.07 (0.24)[b]	6.99 (0.27)[b]
MBC	12.42 (1.02)[a]	21.52 (1.23)[b]	23.25 (0.93)[b]	29.45 (0.89)[c]	33.88 (2.00)[c]
TN	51.48 (0.74)[a]	44.63 (1.36)[b]	45.32 (1.04)[b]	47.46 (1.54)[bc]	50.69 (0.81)[ac]
CO_2 total	N/A	15.31 (0.29)[a]	19.52 (0.53)[a]	31.82 (0.39)[b]	51.75 (2.01)[c]

Different superscript letters indicate significantly different estimates between treatments by ANOVA (P < 0.05).
Field: field condition of soil collected at 5°C; Control: 25°C and wetted; MannL: control +0.03 mg mannitol-C g^{-1}; MannI: control +0.10 mg mannitol-C g^{-1}; MannH: control +0.30 mg mannitol-C g^{-1}. Standard error in parentheses where sample size was >2 (n = 4 MBC; n = 6 Moisture, TN, CO_2 total). EC = electrical conductivity (μS cm^{-1}); Moisture = gravimetric moisture (g g^{-1} soil); MBC = microbial biomass-C (μg C g^{-1}); TN = total nitrogen (mg N kg^{-1}); CO_2 total = cumulative respiration (μg C g^{-1}).

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4 only ($P < 0.001$). Intermediate treatments were not statistically different from low treatments through the first 4 days, after which respiration peaked at 5.0 μg CO_2-C g^{-1} day^{-1} at Day 8 and remained significantly elevated throughout the incubation. High mannitol treatments were significantly elevated in respiration on all sampling days from all other treatments and peaked at Day 10 at 6.6 μg CO_2-C g^{-1} day^{-1}. Cumulative respiration at the end of the incubation was significantly different ($P < 0.001$) among all +C treatments (Table 1).

Extracellular enzyme activities measured from a subset of treatments (laboratory control and high mannitol treatments) indicate a significant reduction in the potential activity of most carbon- and nitrogen-acquiring enzymes after amendment of mannitol (Table 2). α-Glucosidase (AG) and β-glucosidase (BG) activities each decreased by 35% ($P = 0.05$, $P = 0.003$ respectively). Protein degrading enzyme activity measured by leucine aminopeptidase (LAP) also declined significantly by 32% ($P = 0.0004$), while activity of POX, a non-specific enzyme known to catalyse the oxidation of more stable, ring-bearing molecules such as phenol-bearing lignins, decreased by 37%. Only the activity of n-acetylglucosaminidase (NAG) was positively affected by mannitol addition, increasing significantly by 22% ($P = 0.001$).

16S rRNA gene sequencing

Bacterial diversity was significantly influenced by mannitol amendments, displaying a clear unimodal PDR (Fig. 1). We chose to measure community diversity with the Shannon index (H') because both the richness and evenness of bacterial taxa were affected by treatment. The diversity pattern was evident when examined across both categorical (e.g., productivity classes of low, intermediate, high) and continuous (e.g., microbial biomass) axes. A second order polynomial relationship ($r^2 = 0.33$, $P = 0.035$) best fit the diversity pattern (at all taxonomic levels) across a gradient of microbial biomass (Fig. 1B) (Waldrop *et al.*, 2006). A pattern was most obvious at the taxonomic scales of genus and order, where maximum diversity was observed for laboratory control and low mannitol (0.03 mg C g^{-1}) treatments ($P < 0.02$). Intermediate (0.10 mg C g^{-1}) and high (0.30 mg C g^{-1}) mannitol additions elicited a decline in diversity to levels found within unmanipulated field soils. A diversity pattern was less apparent at the phylum scale, where diversity was never positively affected by resource availability but declined significantly at intermediate and high mannitol additions.

Multivariate ordination of sequence data using non-metric multidimensional scaling indicates highly significant changes to bacterial community composition along the resource gradient (Fig. 2). All treatments were significantly different (PERMANOVA $P = 0.001$) at genus, order, and phylum scales and shifted consistently along the horizontal axis of all ordinations as resource availability increased. *Post hoc* pairwise comparison of these results suggests that, at all taxonomic scales, treatments are significantly different from one another ($P < 0.05$), except for intermediate versus high treatments ($P > 0.1$). Beta diversity, measured as average within-treatment Bray–Curtis distance, also increased significantly ($P < 0.05$) with mannitol addition at all taxonomic scales (Fig. 3).

Indicator species analysis (ISA) was used to identify the taxa significantly associated with treatments (Table S2). The majority (≥60%) of ISA results at all taxonomic scales were taxa associated with field soil, suggesting that this soil contains the most distinct community. Important field soil taxa include the autotrophic phyla *Cyanobacteria* and *Nitrospirae*, which despite their low overall abundance, both had high indicator values of ~0.61 ($P < 0.02$). Mannitol additions at all concentrations induced a marked shift towards phylum *Proteobacteria.* At finer taxonomic scales, a more nuanced change in community membership was observed. *Nitrospirales* (*Nitrospirae*), an unknown class of *Chloroflexi*, *Lactobacillales* (*Firmicutes*) and *Prochlorales* (*Cyanobacteria)* were the most important indicator taxa in field soil at the order scale (all indicator values >0.54, $P < 0.02$), while *Acidimicrobium* (*Actinobacteria*), *Rhodococcus (Actinobacteria)*, *Prochlorococcus* (*Cyanobacteria*) and an unknown genus of *Chloroflexi* were most important at the genus scale

Table 2. Average activity of extracellular enzymes.

Enzyme	Target	Treatment	Activity (nmole/g OC/h)
α-glucosidase	Starch	Control	1600.64 (24.20)[a]
		MannH	1296.19 (159.71)[a]
β-glucosidase	Cellulose	Control	4204.65 (241.90)[a]
		MannH	2964.29 (194.35)[b]
N-acetylglucosaminidase	Chitin	Control	2247.76 (75.65)[a]
		MannH	2734.90 (85.67)[b]
Leucine aminopeptidase	Protein	Control	421 479.07 (15862.20)[a]
		MannH	288 792.40 (16415.63)[b]
Phenol oxidase	Lignin	Control	1 619 633.00 (30938.79)[a]
		MannH	1 020 740.00 (21379.18)[b]

Control: 25°C and wetted; MannH: control +0.30 mg mannitol-C g^{-1}. Different superscript letters indicate significantly different estimates between treatments by ANOVA ($P < 0.05$). Standard error in parentheses ($n = 6$).

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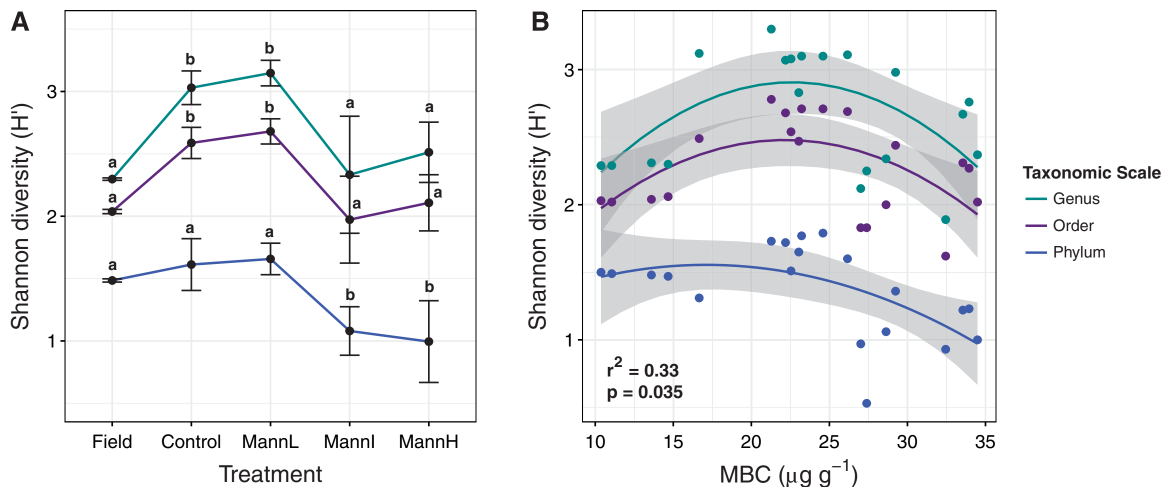

Fig. 1. Bacterial diversity (H') along an *ad hoc* productivity gradient of mannitol amendment (A). Error bars represent one standard deviation. Second order polynomial relationship of bacterial diversity across a gradient of microbial biomass productivity (B). $r^2 = 0.33$, $P = 0.035$ for each regression. Grey shading indicates the 95% confidence interval of regression. Field: field condition of soil collected at 5°C; Control: 25°C and wetted; MannL: control +0.03 mg mannitol-C g^{-1}; MannI: control +0.10 mg mannitol-C g^{-1}; MannH: control +0.30 mg mannitol-C g^{-1}.

(all indicator values >0.75, $P < 0.003$). Laboratory control soils were strongly indicated by multiple *Proteobacteria* such as the genera *Syntrophus* (indicator value = 0.8, $P = 0.002$) and family *Bacteriovoraceae* (indicator value = 0.63, $P = 0.014$). Multiple genera within the order *Rhizobiales* (*Proteobacteria*) and the genus *Arthrobacter* (*Actinobacteria*) significantly characterized the high mannitol treatments at indicator values ~0.40 ($P < 0.04$).

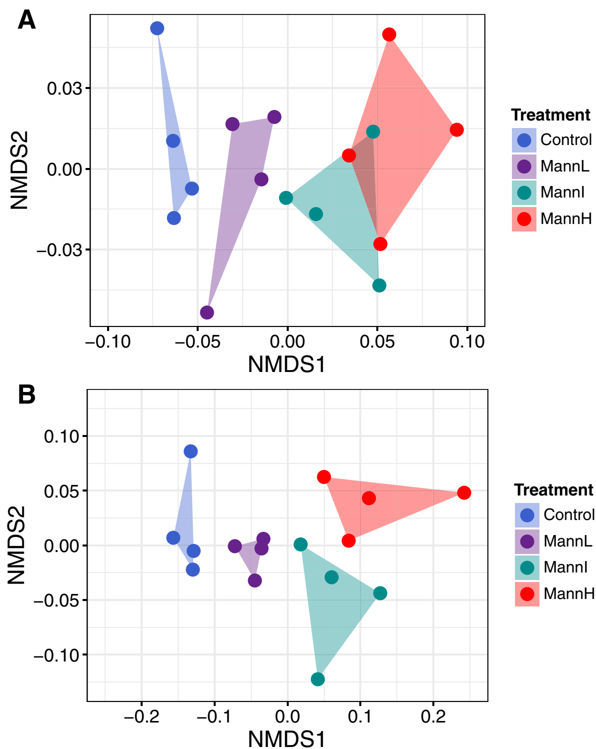

Fig. 2. nMDS of bacterial communities at phylum (A) and genus (B) taxonomic scales using a Bray–Curtis distance metric. Stress = 0.093 (phylum) and 0.076 (genus). Field: field condition of soil collected at 5°C; Control: 25°C and wetted; MannL: control +0.03 mg mannitol-C g^{-1}; MannI: control +0.10 mg mannitol-C g^{-1}; MannH: control +0.30 mg mannitol-C g^{-1}.

Discussion

The ecosystem consequences of microbial diversity are potentially numerous, particularly for processes that are impacted by the gain or loss of specific taxa, for example, nitrification (Norman and Barrett, 2016). Plant growth, for example, benefits from a diverse soil microbial community because of increased likelihood of beneficial or symbiotic taxa (Tautges *et al.*, 2016; Kolton *et al.*, 2017). Effects on ecosystem processes have been observed even among the broadest taxonomic scales of diversity, such as fungal control over organic matter decomposition (Frey *et al.*, 2014). Understanding the benefits of microbial diversity and its causal drivers may help maintain ecosystem functionality and predict C and nutrient cycling in the face of anthropogenic change.

Our work is among the first non-correlational demonstrations of a significant PDR for soil bacteria. Across an experimental resource gradient of mannitol, microbial biomass scaled positively with resource concentrations while diversity exhibited a unimodal response. This confirms the positive, saturating effect of resource availability on microbial diversity we previously observed along a natural productivity gradient in the McMurdo Valleys (Geyer *et al.*, 2013). DOC concentrations in this region rarely exceed 0.03 mg glucose-C g^{-1}, however, which corresponds to the low mannitol amendment rate used here. Resource additions above this concentration (i.e., MannI, MannH) caused a decline in diversity to levels on par with hyperarid soils, albeit with a substantially different

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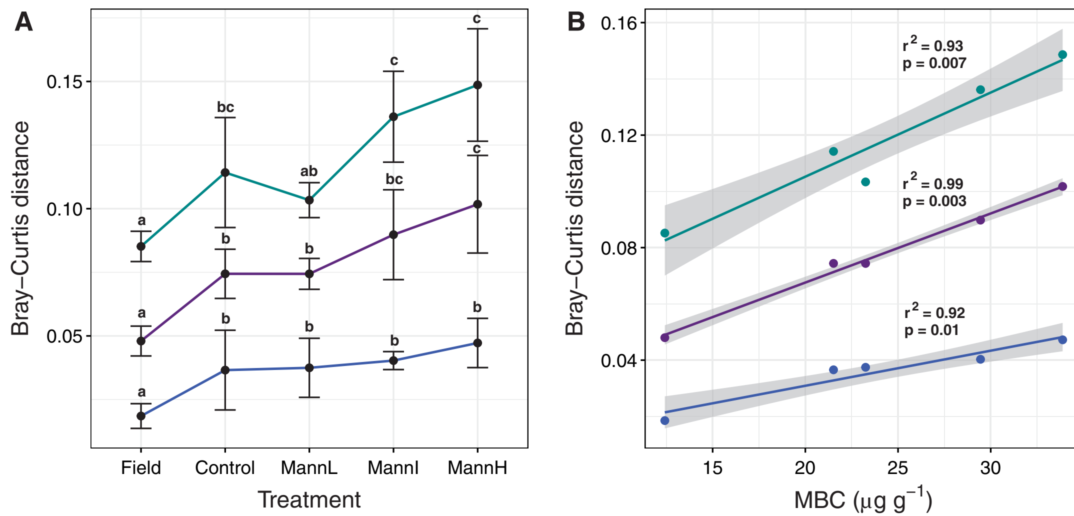

Fig. 3. Average pairwise Bray–Curtis distance (i.e., beta diversity) among experimental replicates (n = 4) across an *ad hoc* productivity gradient of mannitol amendment (A). Error bars represent one standard deviation. Linear regression of average pairwise Bray–Curtis distance among experimental replicates across a gradient of microbial biomass productivity (B). Grey shading indicates 95% confidence interval of regression. Field: field condition of soil collected at 5°C; Control: 25°C and wetted; MannL: control +0.03 mg mannitol-C g^{-1}; MannI: control +0.10 mg mannitol-C g^{-1}; MannH: control +0.30 mg mannitol-C g^{-1}.

composition. A unimodal pattern thus relates microbial diversity to resource availability and (by extension) productivity in this model soil.

Each milligram of mannitol-C added caused linear increases in cumulative respiratory-C and biomass-C by 12% and 4% respectively. These results are consistent with the view that Antarctic soil microorganisms are carbon limited and have access to other nutrients to support growth after carbon limitation is alleviated. It also suggests that these microbes become progressively less efficient in metabolism with mannitol addition, as indicated by the steeper respiratory than biomass response to amendment. The soil used for this experiment (molar C:N ~ 8.20) exhibited total organic carbon concentrations of 0.036%, similar to other estimates from this region and the general assessment of McMurdo Dry Valley soils as among the most carbon depleted on earth (Burkins *et al.*, 2001). In comparison, N is relatively abundant due to dry deposition of nitrogen-laden dusts and salts that accumulate on soil surfaces (Barrett *et al.*, 2005). Whether N additions could have further accelerated microbial activity after alleviation of C limitation we cannot determine, as co-limitation by both C and N is possible (Ball *et al.*, 2018). Our results suggest that C availability is a primary limitation, however. We will discuss the evidence for altered carbon use efficiency below.

Unlike microbial productivity, diversity displayed a clearly unimodal pattern with three apparent phases: low diversity of untreated field soils, elevated diversity of soils experiencing laboratory conditions of wetting/warming and low resource availability and low diversity in soils of high resource availability. Field soil was heavily dominated by the phyla *Actinobacteria* (42%) (Fig. S1) but the autotrophic taxa *Nitrospirae* and *Cyanobacteria* were the strongest indicators. The presence of autotrophic taxa suggests that soil food webs may be more dynamic here than once assumed, sustained by contemporary primary production rather than ancient C alone (Hopkins *et al.*, 2009). Although we cannot confirm it with the present data, many taxa here may be dormant or even environmental DNA preserved by the inherently dry and cold conditions of this region. This may be common for oligotrophic habitats as abiotic severity induces frequent and extended periods of dormancy along with continuous environmental filtering of community members (Chase, 2010; Jones and Lennon, 2010). By this assumption, the realized diversity of organisms at the low end of the productivity gradient may be even lower than estimated and a unimodal pattern of diversity even more striking.

Highest species diversity was observed for incubated soil receiving low mannitol amendment, where the response to treatment was perhaps the activation of previously dormant and underrepresented taxa. Relative abundance of the phyla *Proteobacteria* (43%), *Firmicutes* (19%) and *Bacteroidetes* (17%) displaced the field soil community (Fig. S1). Order *Bdellovibrionales* (*Proteobacteria*), which includes the bacterivorous genera *Peredibacter* and *Bacteriovorax*, was a strong indicator of these soils. Bacterial predation would likely be a successful trophic strategy in these diverse soils. The emergence of genus *Syntrophus* suggests that anaerobic microsites may have formed wherein sulfur cycling might occur syntrophically between this taxon and sulfate reducers

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(Kuever and Schink, 2015). These high diversity soils can also be distinguished from lower diversity field soils by the warming and wetting they received during incubation. A unimodal diversity relationship persists, however, when examined over a continuous gradient of microbial biomass (Fig. 1B). This suggests that productivity remains an important driver of diversity outside of the treatment levels we imposed.

Highest mannitol additions led to dominance by *Proteobacteria* to the near exclusion of other phyla. Almost three-quarters (74%) of the bacterial community in these treatments were comprised of *Proteobacteria*, with *Actinobacteria* (8%), *Bacteroidetes* (6%) and *Firmicutes* (6%) the next most abundant groups. The most common order of *Proteobacteria* was *Rhizobiales*, N fixers whose presence may indicate nitrogen limitation in these treatments although members of this order are metabolically diverse. These results confirm earlier assessments of *Proteobacteria* and, to a lesser degree, *Actinobacteria* (e.g., genus *Arthrobacter*) as copiotrophic taxa (Fierer *et al.*, 2007; Schwartz *et al.*, 2014; Mau *et al.*, 2015), although it is possible that necromass availability may also explain their presence in this study. Interestingly, community composition also became increasingly dissimilar within treatments as resource availability increased (i.e., increasing beta diversity; Fig. 3). This phenomenon has been described in other PDR studies when highly predictable oligotrophic communities formed by environmental filtering are supplanted by faster growing organisms which can attain multiple stable states via relatively stochastic biotic interactions (Chase, 2010; Sokol *et al.*, 2017). To the extent that soil processes are linked to diversity, eutrophic conditions could result in functional stochasiticity.

We observed ecosystem-scale consequences with changes in microbial community composition. Coincident with substrate addition and declining species diversity were reductions in the activities of extracellular enzymes associated with C and N acquisition (Table 2). This decline in activity was particularly striking because it occurred as microbial biomass increased. Community shift towards copiotrophic taxa may have resulted in a shift away from enzyme-mediated carbon acquisition strategies and towards, ostensibly, direct use of amended substrate (*sensu* the copiotrophic hypothesis; Ramirez *et al.*, 2012). An important consequence of reduced diversity may thus be reduced breakdown of structural organic matter, assuming that copiotrophs invest more heavily in resource application (i.e., growth) than acquisition. In contrast to other enzymes, NAG activity responded positively to mannitol addition indicating a possible increase in C and/or N acquisition via chitin degradation. Such a response may have been induced by increased presence and biomass turnover of chitinous fungi, Gram-positive bacteria or microfauna near the end of incubation (Dreesens *et al.*, 2014). As mentioned earlier, our data also suggest that under resource rich conditions microorganisms were transforming C less efficiently. This pattern would be consistent with the understanding of copiotrophic organisms as metabolically inefficient, having higher biomass turnover rates, or perhaps both. The consequences of reduced efficiency could include diversion of C out of soil and into the atmosphere but requires confirmation with other isotopic measures of carbon use efficiency (Manzoni *et al.*, 2012).

In summary, we demonstrate that a unimodal PDR can exist for soil bacteria generated by both abiotic (e.g., resource availability) and biotic (e.g., competitive exclusion) drivers. This conclusion strengthens the case that shared ecological forces structure communities of both microorganisms and macro-organisms. Functional consequences of reduced bacterial diversity at high levels of productivity (e.g., elevated microbial biomass) included reduced enzyme activity and perhaps reduced carbon use efficiency. These responses highlight the importance of bacterial diversity, and not biomass alone, for predicting C transformation in soil. Whether our results are generalizable to other systems remains to be demonstrated; the food web complexity and soil heterogeneity of temperate soils may mask PDR. Yet even if resource availability alone cannot predict microbial diversity in non-model systems, our results help to constrain the potential drivers of community structure and associated functionality in more complex soils.

Experimental procedures

Site description

Soil samples for this experiment were collected from lower Taylor Valley in the McMurdo Dry Valleys of Antarctica. This region, and Taylor Valley specifically, has been well characterized by the NSF-supported McMurdo Long Term Ecological Research project (1993-ongoing). Soils are a poorly weathered, dry-permafrost composed of >90% sand-sized particles with little aggregation or horizonation in the top 10 cm (Ugolini and Bockheim, 2008). Salinity and pH are generally high (>9.0 and >500 $\mu S\ cm^{-1}$ respectively) due to accumulation of weathered carbonates and aerially deposited salts (Bockheim, 1997), while moisture and organic matter are extremely limiting (typically <1% gravimetric and <0.03% organic C by weight respectively) (Barrett *et al.*, 2004; Barrett *et al.*, 2006). No vascular plants or vertebrates, and only a limited diversity of microfauna, inhabit these soils (Adams *et al.*, 2006). During the 6–8 week austral summer, 24-h radiation induces ice melt such that localized, dense cryptogamic mats of cyanobacteria and moss form along the margins of streams and lakes. Through this primary production, a surprisingly diverse community of heterotrophs is

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supported (Takacs-Vesbach, 2010). The dominant soil bacteria in this region include many of the more common groups found worldwide including the phyla *Actinobacteria*, *Acidobacteria* and *Bacteroidetes* (Cary *et al.*, 2010).

Earlier work we conducted in this region found bacterial diversity to be positively associated with a natural gradient of soil productivity (i.e., chlorophyll *a* concentrations) (Geyer *et al.*, 2013). Soil was collected for the present experiment in December 2012 from one location outside the influence of any contemporary landscape features (e.g., streams, lakes and nivation hollows). This soil is representative of the region's expansive hyperarid soil habitat and the endemic community of oligotrophic microorganisms. Approximately 5 kg of soil was collected from the surface 10 cm within a 1 m^2 area and stored at −20°C until experimentation.

Experimental incubation

We created an *ad hoc* resource gradient by making organic amendments to commonly sourced soil in replicated (*n* = 6) 75 g glass jar mesocosms. Prior to amendment, all soil was pre-incubated at 25°C for 48 h to rejuvenate the microbial community after frozen storage. Laboratory control soils received sufficient moisture to reach 10% gravimetric content and were incubated throughout the experiment at 25°C. Three experimental treatments received low (0.03 mg C g^{-1}), intermediate (0.10 mg C g^{-1}) and high (0.30 mg C g^{-1}) mannitol addition rates with equivalent wetting and temperature exposure as controls. These concentrations were 3×, 10× and 30× the DOC concentration of field soil at 0.01 mg C g^{-1}. Mannitol is a sugar alcohol previously described as a common photosynthetic derivative of Antarctic cryptogams (Tearle, 1987) that has been used in other C amendment experiments for the region (e.g., Burkins *et al.*, 2001). Five levels of resource availability were observed from this design: hyperarid field soil that did not receive any laboratory treatment ('Field'), wetted and warmed laboratory control soil ('Control'), and treatments similar to the controls with an additional low ('MannL'), intermediate ('MannI') or high ('MannH') mannitol amendment.

All treatments were initiated on the same day and were briefly mixed by spatula to fully incorporate amendment solutions. Incubation progress was monitored through estimation of soil respiration rate every second day. Headspace gas was flushed with CO_2-free air for 10 m, after which mesocosm jars remained sealed overnight before headspace gas was sampled via syringe and CO_2 concentration measured by infrared gas analysis (LI-COR, Lincoln, NE). After 12 days all treatments were harvested and frozen at −20°C when a peak in respiration rate was observed for the MannH treatment. At this time, respiration rate was also significantly elevated in both MannH and MannI treatments relative to the control. MannL respiration rate was significantly elevated from control soil on 1 day only. This approach allowed us to capture a microbial community presumably in or near the stationary phase of growth for intermediate and high C amendment treatments and before the effects of substrate addition was lost.

Data collection and analysis

All biogeochemical and enzyme analyses followed standard protocols developed for the region (Barrett *et al.*, 2004; Geyer *et al.*, 2013). Soil water content was determined gravimetrically after drying at 105°C for 48 h. A 1:2 and 1:5 soil/water slurry was used to measure soil pH and electrical conductivity respectively. Microbial biomass was estimated using the chloroform fumigation extraction technique, wherein DOC was extracted from 20 g of soil using a 0.5 M K_2SO_4 solution. Soil slurries were shaken for 30 min at 150 rpm, centrifuged at 5000 rpm for 10 min to produce particulate-free extracts, and analysed for DOC concentration (OI Model 1010 Total Organic Carbon Analyser, College Station, TX). A paired sample was extracted in the same manner after a 5 days chloroform fumigation and microbial biomass-C estimated as the increase in DOC upon fumigation. Microbial biomass is likely bacterially dominated, as fungal biomass and diversity are limited in these soils (Adams *et al.*, 2006; Rao *et al.*, 2012).

Soil extracellular enzyme activity was estimated for a suite of hydrolytic (AG, BG, NAG and LAP) and oxidative (phenol oxidase, POX) enzymes. Potential activity was measured from 0.5 g soil incubations in the presence of labelled substrates and 50 mM $NaHCO_3$ buffer (pH = 8.2) following the methods of Zeglin *et al.* (2009) (Table S1). Standards used in the POX assay were created by reacting a known mass of L-3,4-dihydroxyphenylalnine with horseradish peroxidase. Triplicate samples were incubated at room temperature on a platform shaker (250 rpm) for a minimum of 2 h and enzyme-induced fluorescence (hydrolytic enzymes) measured by excitation (360 nm) and emission (465 nm) or light absorbance (oxidative enzyme) using a Tecan Infinite M200Pro plate reader (Tecan, Mannedorf, Zurich, Switzerland). In addition to sample incubations, control (buffer only), substrate (substrate + buffer) and standard (standard + buffer) references were analysed to account for other sources of fluorescence. Final activity was normalized to sample SOC content and expressed as activity (nmol) h^{-1} g^{-1} SOC.

Soil DNA was extracted using a cetyltrimethylammonium bromide protocol designed for low biomass samples (Geyer *et al.*, 2013). Four treatment replicates of each extract were submitted to RTL Genomics (Lubbock, TX) for 2 × 300 paired-end MiSeq sequencing of the V3-V6 region of the bacterial 16S rRNA gene. The sequence dataset was denoised using the USEARCH function (Edgar, 2010) and all singletons removed. Chimera detection and removal was performed using UCHIME in de novo mode (Edgar *et al.*, 2011).

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UPARSE was used to create operational taxonomic unit (OTU) clusters at 97% similarity (Edgar, 2013). RDP classifier (April 2014) was used to assign taxonomy to OTUs (Wang *et al.*, 2007). A total of 3088 OTUs were produced, of which 70% could be annotated as bacteria. Rarefaction curves indicate that all soils were well sampled during sequencing (Good's Coverage estimator ≥99%) despite significant differences in total microbial biomass. Sequence data were aggregated to phylum, order and genus levels for comparison across taxonomic scales. All sequence data have been archived as BioProject PRJNA416377 at the National Centre for Biotechnology Information.

OTU data were $\log_e(x + 1)$ transformed prior to multivariate analysis. Community composition was examined by non-metric multidimensional scaling (metaMDS [vegan]) and ISA (indval [labdsv]) was used to determine the taxa significantly responsible for differences in beta diversity among samples. We also report indicator values ranging from 0 to 1, where 1 equals taxa that are always present in a specific treatment (high specificity) but never in others (high fidelity) (Dufrene and Legendre, 1997). Significant differences in community composition and group dispersion were examined by PERMANOVA (adonis[vegan]) and tests for homogeneity of variance (permdisp [vegan]) respectively. Alpha diversity (Shannon $H' = -\Sigma\ p_i \ln p_i$) was estimated from untransformed data in order to accurately represent community evenness. ANOVA and regression were used to test for significant differences in responses across categorical treatments and continuous variables. To meet statistical assumptions, enzyme activities were $\log_{10}$ transformed and alpha diversity estimates were square root transformed. All analyses were performed in R.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Supplementary Fig. 1 Average relative abundance of bacterial phyla by experimental treatment using untransformed data.

Supplementary Table 1 Additional information for enzymatic assays of soils. 4-MUB = 4-methylumbelliferyl.

Supplemental Table 2. Indicator species analysis results at phylum, order, and genus taxonomic scales for all taxa with IndVal ≥0.50 and $p < 0.05$. IndVal: indicator value composed of the specificity and fidelity of taxa's association with Treatment. Frequency is the number of samples (n = 16) where taxa were observed (4 replicate samples per Treatment). Treatments = Field: field condition of soil collected at 5°C; Control: 25°C and wetted; MannL: control +0.03 mg mannitol-C g^{-1}; MannI: control +0.10 mg mannitol-C g^{-1}; MannH: control +0.30 mg mannitol-C g^{-1}.

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