

# Protists have divergent effects on bacterial diversity along a productivity gradient

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**Productivity and predation are thought to be crucial drivers of bacterial diversity. We tested how the productivity–diversity of a natural bacterial community is modified by the presence of protist predators with different feeding preferences. In the absence of predators, there was a unimodal relationship between bacterial diversity and productivity. We found that three protist species (*Bodo*, *Spumella* and *Cyclidium*) had widely divergent effects on bacterial diversity across the productivity gradient. *Bodo* and *Cyclidium* had little effect on the shape of the productivity–diversity gradient, while *Spumella* flattened the relationship. We explain these results in terms of the feeding preferences of these predators.**

**Keywords:** bacteria; productivity; biodiversity; protist; predation

## 1. INTRODUCTION

Productivity (here defined in terms of resource availability) is believed to be one of the principal drivers of bacterial community composition and diversity. While a large body of theory and data suggest that biodiversity is highest at intermediate levels of productivity, this relationship is neither universal for larger organisms (Mittelbach *et al.* 2001) nor for microbes (Smith 2007). Many mechanisms have been proposed to explain the variety of productivity–diversity relationships. We will not review this extensive literature, but one promising mechanism in bacterial communities is that predators modulate the response of the prey community to variation in productivity.

Predators modify the productivity–diversity relationship by altering the abundance and stability of competing prey species (Rosenzweig 1971; Chase *et al.* 2002). For example, simple models have shown that superior resource exploiters dominate unproductive environments, whereas resistance to predation is increasingly favoured as productivity increases (Leibold 1996), a hypothesis that has been confirmed using simplified microcosm communities containing

a single strain of phage (predator) and bacteria (Bohannan & Lenski 2000).

Protists play a pivotal role in structuring natural bacterial communities (Perthaler 2005). Bacterial communities undergo morphological and physiological changes when subjected to protist predators by forming predation-resistant filaments (Hahn *et al.* 2000; Jurgens & Sala 2000; Hahn & Hofle 2001) or by increasing cell size (Corno & Jurgens 2006), but there has been less experimental effort focused on the ecological effects of protist predators on bacterial community diversity. Studies have tended to either involve intraspecific morphological diversity (Meyer & Kassen 2007; Friman *et al.* 2008) simplified, constructed bacterial communities (Jiang & Morin 2005; Jiang & Krumins 2006; Meyer & Kassen 2007; Friman *et al.* 2008), or have been observational studies of natural environments (Hahn & Hofle 2001).

To bridge the gap between simple microcosm communities and the full complexity of a natural ecosystem, we advocate an experimental microcosm approach containing constructed protist communities together with natural bacterial communities. We tested the effects of three protist predators in microcosms that are laboratory analogues of water-filled tree-holes (Bell *et al.* 2005).

## 2. MATERIAL AND METHODS

### (a) *Microcosms*

Freshly fallen beech leaves were collected and stored at  $-20^{\circ}\text{C}$ . Diverse bacterial communities were obtained by incubating 1 g of the beech leaves in 40 ml Chalkey's medium (CM: 10 g NaCl, 0.4 g KCl, 0.6 g  $\text{CaCl}_2$  per litre of water, autoclaved and adjusted to neutral pH) for 2 days at  $20^{\circ}\text{C}$  in closed microcosms. This mixture was vortexed and passed through a  $1\ \mu\text{m}$  filter to separate bacterial cells from larger organisms, and to obtain a bacterial community that was derived from the surface of the beech leaves. A beech leaf broth was obtained by autoclaving 50 g beech leaves in 500 ml CM at  $120^{\circ}\text{C}$  for 30 min. The resulting broth was passed through a  $0.22\ \mu\text{m}$  filter to remove debris and ensure sterility and serially diluted in CM to create the productivity gradient. The level of productivity is expressed as a proportion of the neat broth. Three 24-well plates containing 2 ml of broth were inoculated with  $10^4$  bacterial cells. The bacteria were incubated for 2 days ( $20^{\circ}\text{C}$ ) after which the appropriate microcosms were inoculated with  $10^3$  protist cells (*Bodo* sp., *Spumella* sp. or *Cyclidium* sp.) and incubated for 7 days. Each treatment combination was replicated three times.

### (b) *Bacteria*

Bacteria and protists were fixed in 1 per cent paraformaldehyde and stained with the DNA specific stain SYBR Green I (Molecular Probes). Samples were analysed using a Becton Dickinson FACS Calibur flow cytometer using internal standards ( $2.49\ \mu\text{m}$  beads). Protists were counted according to the methodology described by Zubkov *et al.* (2007). Bacterial diversity was assessed using terminal restriction fragment length polymorphism (tRFLP) where amplified bacterial DNA is cut using a site-specific endonuclease, and the sizes of the resulting restriction fragments are measured. Our tRFLP protocol is described by Thomson *et al.* (in press). Each restriction fragment was considered to be a taxonomic unit. The relative abundance of each taxon was calculated as the relative intensity of each tRFLP band within a sample.

## 3. RESULTS

In the absence of predators, increasing resource concentration caused a progressive increase in the total number of bacterial cells (electronic supplementary material, figure S1; table 1). The increase did not correspond to an increase in bacterial diversity. Rather, a different suite of taxa was selected at high

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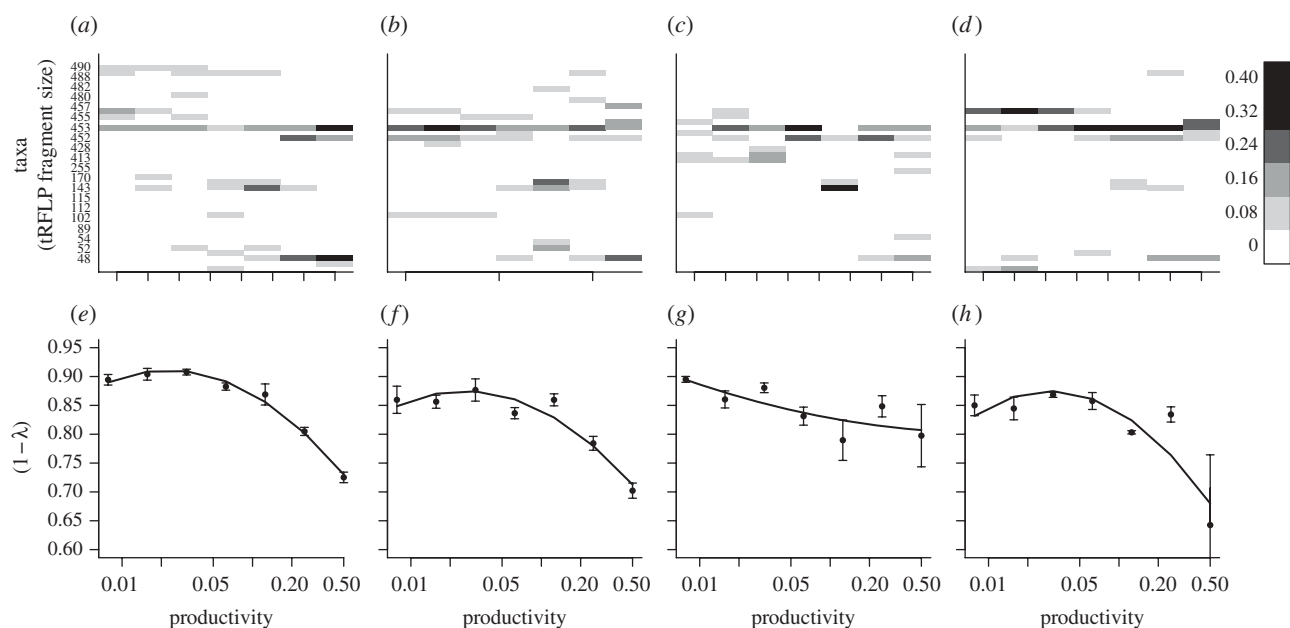


Figure 1. Composition and diversity of the microcosm bacterial communities. The top row of panels shows the mean relative abundance of each bacterial taxon across the productivity gradient for (a) no predators, (b) *Bodo* sp., (c) *Spumella* sp., and (d) *Cyclidium* sp. Colour intensity indicates the relative abundance of each bacterial taxon. The tRFLP fragment size (number of base pairs) associated with each taxon is shown on the y-axis. (e–h) shows the bacterial biodiversity, measured as the complement of the Simpson index ( $1 - \lambda$ ), across the productivity gradient for each predator treatment. Broadly similar results are obtained if the Shannon–Wiener index is used (electronic supplementary material, figure S2). Curves are fitted regression lines.

Table 1. Analysis of the experimental data. (Degrees of freedom (d.f.) and  $F$ -statistics associated with each of the experimental treatments (predators and productivity). Significant  $p$ -values are indicated with asterisks: \* $p < 0.05$ , \*\* $p < 0.005$ , \*\*\* $p < 0.0005$ .)

source of variance	d.f.	bacterial abundance	bacterial diversity
predators	4	7.6***	4.5**
productivity	1	772.5***	90.3***
(productivity) <sup>2</sup>	1	310.9***	20.2***
predators $\times$ productivity	4	135.7***	1.4
predators $\times$ (productivity) <sup>2</sup>	4	1.5	2.4*
residual	90		

versus low productivity (figure 1a), producing the classic unimodal relationship.

Bacterial abundance also increased across the productivity gradient when predators were present. Predators significantly dampened the increase at intermediate productivities but not at high or low productivities (electronic supplementary material, figure S1). The pattern was mirrored by higher predator abundance at intermediate productivities (electronic supplementary material, figure S1). Unlike bacterial abundance, predator abundance at high productivity was equal to their abundance at low productivity. The pattern of bacterial diversity was notably different for the different protists. The shape of the relationship was similar for *Bodo* and *Cyclidium* compared with the no-protist control (figure 1b,d). *Spumella* created bacterial communities that were markedly different because bacterial diversity

was maintained even at high levels of productivity (quadratic term:  $F_{1,18} = 0.3$ ,  $p = 0.62$ ; figure 1c). The mode of the fitted relationship was within the range of productivity values used in the experiment for the no-protist control and for *Bodo* and *Cyclidium* ( $p < 0.05$ ), but not for *Spumella* ( $p > 0.05$ ) (Mitchell-Olds & Shaw 1987).

If we plot the relative bacterial abundance in the presence and absence of each predator (figure 2), the magnitude to which the slope of the regression deviates from the 1:1 line can be used as a measure of the degree to which the predator is a complete generalist (regression slope equals unity) or an extreme specialist that preferentially removes the most abundant prey species (regression slope is zero). *Bodo* grazed on all taxa (figure 2a), as demonstrated by a significantly positive relationship between bacterial abundance in the presence versus absence of *Bodo* ( $F_{1,38} = 23.8$ ,  $p < 0.0005$ ). The slope of the line was significantly different from unity ( $t_{38} = 2.3$ ,  $p = 0.03$ ) indicating a slight preference for more abundant bacterial taxa. A similar observation was made for microcosms containing *Cyclidium* (figure 2b,  $t_{38} = -1.20$ ,  $p = 0.23$ ). By contrast, *Spumella* removed the more dominant bacteria to a much greater extent than the rarer taxa, apparently allowing some previously rare bacteria to proliferate (figure 2c). The regression slope is consequently not different from zero ( $F_{1,38} = 0.01$ ,  $p = 0.93$ ).

#### 4. DISCUSSION

The results of our productivity–diversity experiments are largely consistent with the experimental and observational results for larger organisms (Mittelbach *et al.* 2001), as well as more simplified microcosm

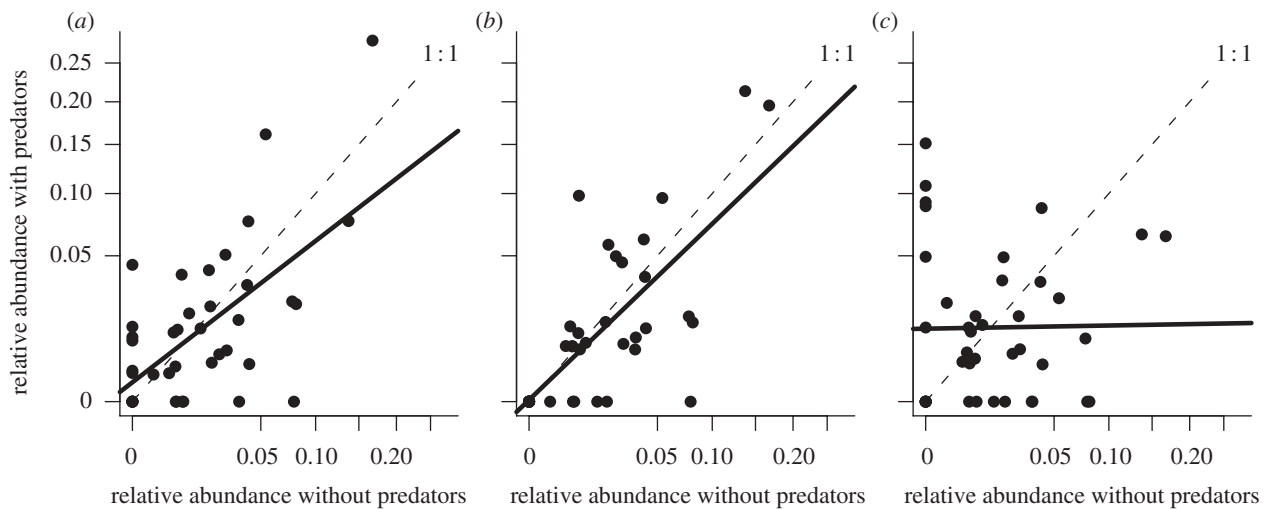


Figure 2. Relative abundance of each bacterial species in the presence of (a) *Bodo* sp., (b) *Cyclidium* sp., and (c) *Spumella* sp. as a function of their relative abundance in microcosms without predators. The axes are on an arcsine transformed square-root scale.

communities (Kassen *et al.* 2000; Hall & Colegrave 2007; Benmayor *et al.* 2008). Even though bacterial abundance increased across the productivity gradient, bacterial diversity increased slightly and then decreased with increasing productivity. That bacterial assemblages at high and low productivity are largely independent suggests a fitness trade-off between high and low productivity environments. At intermediate productivities, diversity is maximized because these assemblages start to cross over.

We have also demonstrated that predators of bacteria can have a wide variety of effects on bacterial diversity and community composition (figure 1). Such a result is unsurprising because previous studies have shown differential effects of protist predators (Simek *et al.* 1997), but the result does emphasize the need to think critically about how different kinds of predator affect bacterial communities. Some of the principal ideas of how predators influence competing prey species revolve around whether particular prey taxa are removed preferentially. Assessing the preferences of protists feeding on diverse bacterial communities containing hundreds or thousands of taxa is problematic, especially as many of the bacteria cannot be isolated and cultured. Feeding trials with individual bacterial strains are therefore not possible in practice, so we determined which bacterial taxa were removed compared with a predator-free control.

The consequence of a uniform *per capita* attack rate is that the addition of the predator will not alter the relative abundance of the prey species in the short term because each species is equally affected by the predator. The data therefore suggest that *Bodo* and *Cyclidium* are acting as generalist predators, at least in our experimental communities (figure 2*a,b*). By contrast, *Spumella* removed the more dominant bacterial species to a much greater extent than the rarer taxa (figure 2*c*), apparently allowing some previously rare bacterial species to proliferate. Generalist predators would decrease the abundance of every prey species resulting in overall lower diversity but a similar pattern of diversity across a productivity gradient is

unaltered. Specialist predators simply allow predator-resistant taxa to proliferate resulting in a relatively flat relationship. This suggests a trade-off between predator resistance and competitive ability.

The experiment is similar to a recent study that constructed microcosm communities with three trophic levels containing bacteria, bacterivores and either a specialist or a generalist predator (Jiang & Morin 2005). In apparent contrast to the current work, generalists reduced the variance in diversity across productivity levels, but the specialist had no effect. It is unclear how far these results can be generalized because the specialist predator had no apparent effect on community diversity, structure or density, whereas the generalist also consumed the prey's resource (bacteria). However, contrasting the two studies further emphasizes the importance of biological details in determining the impact of predators on community diversity.

The observations that predators had little effect on bacterial abundance at high productivity, and that predator abundance declined at high productivity despite higher bacterial abundance (electronic supplementary material, figure S1), strongly imply that the bacterial communities were able to withstand predation pressure. The results lend support to the hypothesis that the cost of resistance is lower at high productivity, resulting in bacterial communities that are able to resist protist predation. Such a conclusion is consistent with observations of the development of grazing-resistant forms across productivity gradients (Corno & Jürgens 2008). A recent chemostat experiment also investigated the effect of nutrient and predator limitation (Corno & Jürgens 2008). Although their experiment documented different patterns of predator and prey abundance across the productivity gradient (both increased linearly), they also concluded that the observed patterns of composition and diversity hinged on a trade-off between predator resistance and growth rate.

Overall, our findings are consistent with relatively simple underlying mechanisms: that there exist life-history

trade-offs in natural bacterial communities, which allows predator-resistant prey to increase with resources availability. That relatively few assumptions are required suggest that these findings are likely to be relevant to a wide range of ecological communities and not only for bacterial communities. Moreover, we have shown that the interactive effects of key ecological variables can be studied in a meaningful way in natural microbial communities, containing largely 'unculturable' bacteria.

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