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ORIGINAL ARTICLE

Diversity of protists and bacteria determines predation performance and stability

Muhammad Saleem[1,2], Ingo Fetzer[1,3], Hauke Harms[1] and Antonis Chatzinotas[1]
[1]*Department of Environmental Microbiology, Helmholtz Centre for Environmental Research—UFZ, Leipzig, Germany;* [2]*Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, USA and* [3]*Stockholm Resilience Center, Stockholm University, Kräftriket 2B, Stockholm, Sweden*

Predation influences prey diversity and productivity while it effectuates the flux and reallocation of organic nutrients into biomass at higher trophic levels. However, it is unknown how bacterivorous protists are influenced by the diversity of their bacterial prey. Using 456 microcosms, in which different bacterial mixtures with equal initial cell numbers were exposed to single or multiple predators (*Tetrahymena* sp., *Poterioochromonas* sp. and *Acanthamoeba* sp.), we showed that increasing prey richness enhanced production of single predators. The extent of the response depended, however, on predator identity. Bacterial prey richness had a stabilizing effect on predator performance in that it reduced variability in predator production. Further, prey richness tended to enhance predator evenness in the predation experiment including all three protists predators (multiple predation experiment). However, we also observed a negative relationship between prey richness and predator production in multiple predation experiments. Mathematical analysis of potential ecological mechanisms of positive predator diversity—functioning relationships revealed predator complementarity as a factor responsible for both enhanced predator production and prey reduction. We suggest that the diversity at both trophic levels interactively determines protistan performance and might have implications in microbial ecosystem processes and services.
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Introduction

Human activities pose a serious threat to biological diversity and ecosystem processes on a global scale (Loreau *et al*., 2001). The major concern is that the current loss of top predators will result in an irrevocable disappearance of top-down organismic interactions and alter ecosystem fluxes of matter and energy (Estes *et al.,* 2011). Although the consequences of predator loss have been intensively investigated for various macroscopic ecological systems (for example, Duffy *et al*., 2007; Estes *et al.,* 2011), little is known about microbial food webs and ecological interactions. Bacteria are the main drivers of many biogeochemical cycles, nutrient recycling and trophic transfer of organic carbon and nitrogen (Falkowski *et al.,* 2008). Similar to food web modules composed of larger organisms, top-down control by protistan predators regulates bacterial abundance, community composition and, consequently, has an influence on many microbial ecosystem processes (Simek *et al.,* 1997; Pernthaler, 2005; Corno and Jürgens, 2008; Rosenberg *et al.,* 2009; Bell *et al*, 2010; Glücksman *et al.,* 2010). Although direct influences of top-down control of bacterial communities by protists have been studied earlier (for example, Bell *et al*, 2010; Roberts *et al.,* 2011; Martinez-Garcia *et al.,* 2012), relatively little is known about direct mutual effects of species richness on community structures and performances of the prey or predator communities, respectively. Ecological theory has long predicted that prey richness determines the performance of consumer species and affects the strength of top-down control (DeMott, 1998; Gamfeldt *et al.,* 2005). However, it still remains unclear how the diversity of bacterial prey influences the composition and functional stability of protistan predator communities.

Here we report results of a manipulative laboratory microcosm experiment analyzing diversity effects of prey bacteria on the production of protistan predators and the stability of predator performance. We established a gradient of bacterial species (from mono-cultures to assemblages of five species) in all possible species combinations

Correspondence: A Chatzinotas, Department of Environmental Microbiology, Helmholtz Centre for Environmental Research—UFZ, Permoserstrasse 15, 04318 Leipzig, Germany.
E-mail: antonis.chatzinotas@ufz.de
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($n = 31$). Bacterial communities were then either incubated without predation pressure or exposed to trophic interactions with single- or multiple-protistan predators. After 48 h, we measured the production of protists (cells per ml) and bacteria (colony-forming units) as a proxy for their performance in the system. Using mathematical tools, these results were interpreted in terms of the influence of prey diversity on interaction modes within the next trophic level (that is, the protists; Loreau and Hector, 2001; Fox, 2005), which mainly include complementarity (facilitative interactions or resource partitioning) and selection effects (SEs; dominance by species with particular traits).

Materials and methods

Prey and predator cultures

For easy species differentiation and quantification of final species composition in each assemblage, we used the five bacterial species *Agrobacterium* sp. (B1), *Micrococcus* sp. (B2), *Janthinobacterium* sp. (B3), *Williamsia* sp. (B4) and *Rhodococcus* sp. (B5) each producing colonies of distinct color when grown on agar plates. All bacteria were maintained in Brunner CR-2 medium as described in Saleem *et al.* (2012). All chosen bacteria species are common free-living microorganisms in aquatic and soil ecosystems. We randomly selected bacteria with different growth characteristics (from poorest (B4) to intermediate (B2, B5) to best performers (B1, B3)) to simulate a natural situation where species do not necessarily grow equally (Saleem *et al.,* 2012). Strains B2, B4 and B5 are Gram positive. Although *Janthinobacterium* sp. is known to potentially produce anti-microbial metabolites that could suppress or reduce growth of other species in the mixtures, we did not observe any inhibition zone on agar plates (Saleem *et al.,* 2012). All bacterial strains are deposited at the public Culture Collection of the Helmholtz Centre for Environmental Research—UFZ (http://www.ufz.de/index.php?en=13354) and are available upon request.

The three protist species *Acanthamoeba* sp. (*Acanthamoeba polyphaga*, an amoeba; Huws *et al.*, 2008), *Tetrahymena* sp. (*Tetrahymena pyriformis*, a ciliate) and *Poterioochromonas* sp. (*Poterioochromonas* sp. DS, a flagellate; Tarao *et al.*, 2009) are common bacterivorous predators representing distinct feeding modes (surface feeder, large planktonic feeder and small planktonic feeder, respectively). In addition, they are easy to identify under the microscope due to their distinct morphology (Saleem *et al.*, 2012). *Tetrahymena* sp. and *Poterioochromonas* sp. were maintained in Nutrient broth soytone yeast extract (NSY) ($3\,g\,l^{-1}$) medium (Hahn and Hofle, 1998) at 25 °C without shaking. *Acanthamoeba* sp. was maintained in proteose peptone-yeast-glucose medium (Huws *et al.*, 2008) at 25 °C without shaking. All protist strains were grown axenically (that is, without any bacteria present in the medium) to avoid any transfer of bacteria to the predation experiments. Bacterial precultures were grown in Brunner CR-2 medium and subsequently diluted to the cell numbers desired in the microcosms (as described in Saleem *et al.* (2012)). Grown protist cultures were concentrated by centrifugation and washed with Brunner CR-2 medium; the total initial densities of protists were kept equal (5×10^4 cells) in all predator treatments (Saleem *et al.*, 2012). Controls without microorganisms were used to check for possible contamination.

Experimental design

Both substitutive and additive designs have been widely used in species interaction and biodiversity studies (Loreau and Hector, 2001; Bruno and O'Connor, 2005; Griffin *et al.,* 2009), and their advantages/disadvantages have been broadly discussed (Griffin *et al.,* 2009). In an additive design, the per species densities are equal in single- and multi-species treatments, which results in an increase in total initial density with increasing species richness (Griffin *et al.,* 2009). In a substitutive design, the total initial density is kept constant and thus the density of each species is reduced with increasing species richness (Bruno and O'Connor, 2005; Griffin *et al.,* 2009). Additive designs are used to test the effect of interspecific interactions, whereas substitutive designs serve to examine the balance between intra- and interspecific interactions in ecological communities (Jolliffe, 2000; Griffin *et al.,* 2009).

Using a substitutive design, we assembled all possible combinations of the five bacterial species ($n = 31$) in 24-well micro-titer plates (1.2 ml volume) with an initial total bacterial cell number of 2.11×10^7 cells per microcosm. All community combinations were grown (i) without predators, (ii) in the presence of each individual predator (single predation experiment) and (iii) with all three predators (multiple predation experiments). In most previous biodiversity-ecosystem functioning studies, the authors used random mixtures of species (Loreau and Hector, 2001; Bell *et al.*, 2005; Gravel *et al.*, 2011). However, using randomly mixed communities do carry the risk of obscuring species identity effects, which can potentially be misinterpreted as diversity effects (Schmid *et al.*, 2002; Hector *et al.*, 2011). We used a full factorial design with all possible combinations of microbial species. This experimental approach allows an unbiased statistical analysis, not influenced by species identities and thus not affected by the typical sampling effect that random experimental designs imply. Using a complete factorial combination of species every adjacent diversity level is independent of species identity Thus, it is not necessary to correct statistically for species identity commonly ignored in most random designs (Saleem *et al.*, 2012).

Total initial predator cell numbers (5×10^4 cells per microcosm) were also kept equal in all incubations. All incubations were run in triplicates resulting in a total of 465 microcosms. After 48 h, cell numbers of all bacterial species were quantified by plating on Brunner CR-2 medium followed by counting the distinctly colored colony-forming units. In addition, 200 µl suspension from each well were fixed with 2% Lugol's iodine solution and stored at 4 °C in the refrigerator until further use for protist cell counting using a Sedgewick-Rafter Cell (Pyser-SGI Limited, Edenbridge, UK) on an inverse microscope (Olympus, CKX-41, Hamburg, Germany).

Statistical analyses

Analysis of variance (ANOVA) with linear fitting on the means was conducted to determine the impact of bacterial species richness on predator production (cells per ml) and evenness (Figures 1a and b, Supplementary Figure 1 and Figure 2). Following the principle of parsimony using linear fitting as simplest model allowed us to detect directly the general trends of the bacterial richness effect on different predators. Results were then investigated for normality of residuals, identification of outliers, homogeneity of variance in the residuals and estimation on no overly influential data points (see Supplementary Information).

(Unaffected) Pielou's evenness i of protists in the multiple predation experiment was calculated (as J = Shannon's diversity index H/Hmax) at the end of the experiments.

Further, a Student's *t*-test was conducted to determine the significance of prey–predator production at the two different predator richness levels (single predation and multiple predations; Figure 3). Moreover, we also tested the combined effect of prey–predator richness on predator production using ANOVA (Supplementary Box 1). In addition, we tested differences of prey production between no-predation, single predation and multiple predation with ANOVA followed by a Tukey's test (Supplementary Figure 2). To determine the relationship between different net biodiversity effect (NBE) components and predator production (Figure 4 and Supplementary Figure 3), linear regression was conducted.

The coefficient of variation (CV; that is, the standard deviation divided by the mean) was used as a stability measure of protist cell production

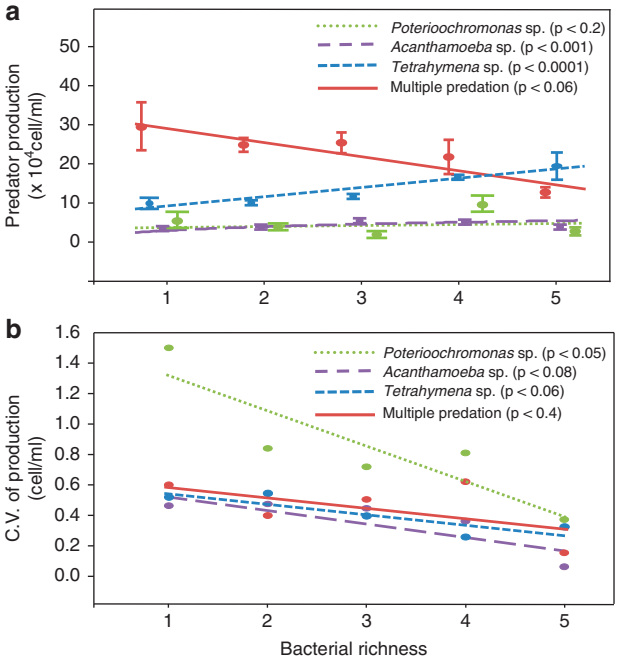

Figure 1 (**a**) Impact of bacterial prey richness on predator production (cell numbers per ml) after 48 h of incubation. Bar represents standard error. Each point corresponds to the average of three replicates of all bacterial species combinations ($n = 31$) at subsequent richness level. The points in **a** are slightly offset horizontally for clarity. (**b**) Impact of bacterial prey richness on variability (coefficient of variability (CV)) in predator production. Each point corresponds to one value of CV (standard deviation divided by the mean, calculated across different bacterial richness levels).

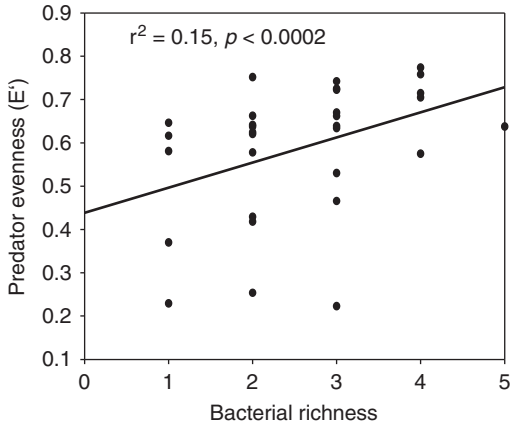

Figure 2 Impact of bacterial prey richness on protistan evenness in multiple predation experiments after 48 h of incubation. Each point corresponds to the average of three replicates.

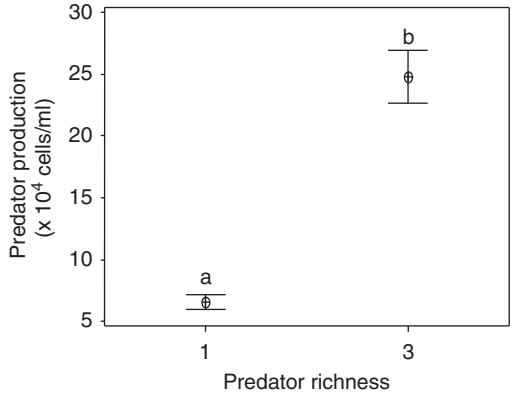

Figure 3 Protist production (that is, cell numbers) in single and multiple predator experiments after 48 h of incubation. Letters above bars indicate statistical difference among different experiments. Bar represents standard error.

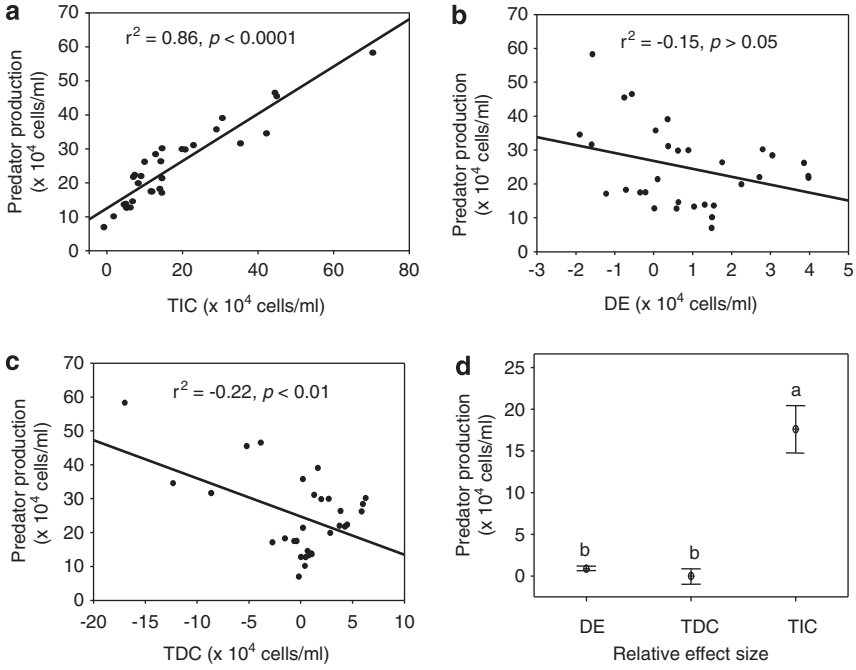

Figure 4 (**a–c**) Relationship of the predator cell numbers in multiple predation experiments with the mathematically partitioned components: trait-independent complementarity (TIC), dominance effect (DE) and trait-dependent complementarity (TDC). (**d**) Average relative effect size of each NBE component (TIC, TDC and DE).

(McCann, 2000; Haddad *et al.*, 2011; Figure 1). Finally, both standard deviation(s) and mean(s) of bacterial cell productions were calculated across different richness levels to calculate CV (Figure 1).

In order to mathematically estimate protist species interactions, we used the tripartite equations by Fox (2005) to partition three types of interspecific interactions whose composite or additive responses determine the NBE, that is, the trait-independent complementarity (TIC), trait-dependent complementarity (TDC) and dominance effect (DE). The NBE is based on the difference between the observed total yield and the expected total yield of a mixture under the null hypothesis (that is, intra- and interspecific interactions are identical). Fox's tripartite equations (Fox, 2005) are derived from Loreau and Hector's (2001) additive bi-partitioning of the NBE into the SE and complementarity effect.

$$\Delta Y = N\overline{\Delta \mathrm{RY}} \times \bar{M} + N\mathrm{cov}\left(M, \mathrm{RY_O} - \frac{\mathrm{RY_O}}{\mathrm{RYT_O}}\right)$$

$$+ N\mathrm{cov}\left(M, \frac{\mathrm{RY_O}}{\mathrm{RYT_O}} - \mathrm{RY_E}\right)$$

ΔY = NBE

N = number of species in mixture

$\bar{M}$ = average mass of all species in monoculture

$\mathrm{RY_O}$ = observed yield of all species in the mixture, that is, cell number of a species in mixture divided by its cell number in monoculture.

$\mathrm{RY_E}$ = expected relative yield of all species in the mixture, that is, the proportion in which a species was added to the mixture.

$\overline{\Delta \mathrm{RY}}$ = average deviation between $\mathrm{RY_O}$ and $\mathrm{RY_E}$ in the mixture.

$\mathrm{RYT_O}$ = sum of $\mathrm{RY_O}$ of all species in the mixture.

The first term on the right side of the equation ($N\overline{\Delta \mathrm{RY}}\bar{M}$) is the TIC, which is equivalent to complementarity effect according to Loreau and Hector (2001). Similarly, the first covariance term in the above equation ($N\mathrm{cov}\left(M, \mathrm{RY_O} - \frac{\mathrm{RY_O}}{\mathrm{RYT_O}}\right)$) is the TDC, and the last covariance term ($N\mathrm{cov}\left(M, \frac{\mathrm{RY_O}}{\mathrm{RYT_O}} - \mathrm{RY_E}\right)$) is the DE. The sum of TDC and DE is equivalent to SE of Loreau and Hector (2001).

Finally, we estimated the feeding preferences (specialist vs generalist) of the investigated predators (Figure 5). For this, we regressed relative abundance of bacteria in the presence vs absence of different predators in microcosms (Figure 5; Bell *et al.*, 2010). We took a diagonal with slope of 1 and intercept zero as a reference (no-effect model). If the slope of relative abundance deviates from this slope under different predation types, we hypothesized that this will reflect a specialist predation, as specialist predation will change the relative abundance of bacterial species by feeding only on certain species. Nonsignificant deviation of the slope suggests the generalist predation, as generalist predators will more or less impact all species equally. Consequently, generalist predation will not change the relative abundance of the bacteria.

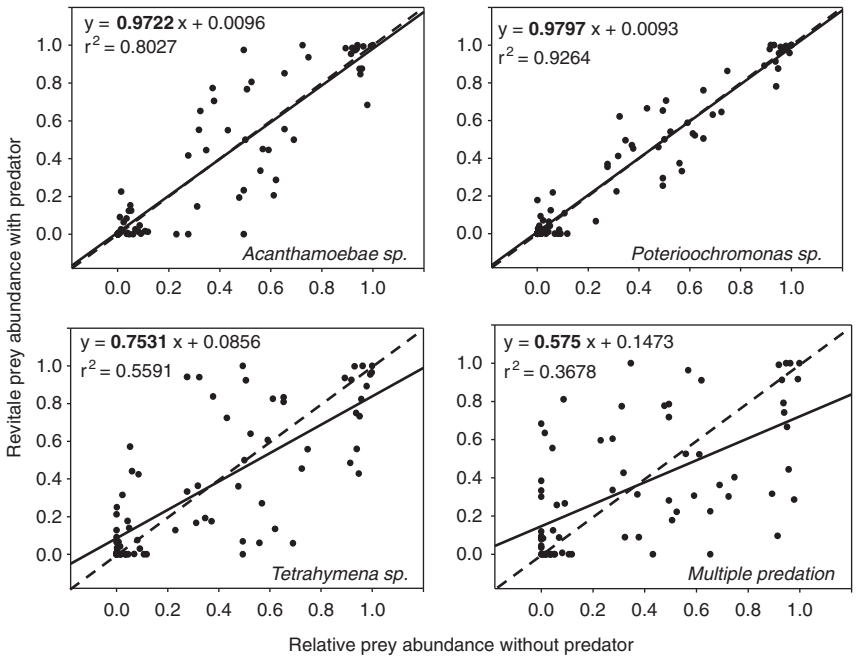

Figure 5 Relative abundance of each bacterial species in the presence of single-protist predators or multiple predators as a function of their relative abundance in the control microcosms without predators.

For estimating the slope, we used standard regression fitting followed by an ANOVA test, testing significance in deviation of the estimated slope against the no-effect model.

The detailed summary of all statistical tests can be found in the Supplementary Material.

Results

Prey richness affected total predator production (that is, cell numbers) depending on predator identity (Figure 1a). In single-predator incubations, the ciliate *Tetrahymena* sp. responded to higher prey richness with enhanced growth, whereas positive effects were less pronounced for the flagellate *Poterioochromonas* sp. and the amoeba *Acanthamoeba sp*. In contrast, in multiple predation experiments, we observed a marginally significant reduction in predator production ($P<0.06$) with increasing prey richness. The stability of predator production increased significantly at higher prey richness (Figure 1b). The CV decreased with prey diversity regardless of the type or number of predators; however, it was marginally significant in most of the cases (Figure 1b). In the multiple predation experiment, increased prey richness resulted also in higher predator evenness (Figure 2).

Predator richness had a positive effect on predator production (Figure 3), whereas bacterial yields were significantly reduced in the presence of multiple predators indicating stronger exploitation (Supplementary Figure 2). Overall, the combined impact of both prey and predator richness had significant positive impact on the predator production in general (Supplementary Box 1).

Mathematically, partitioning the potential mechanisms responsible for the enhanced protist production in multi-predator systems revealed that TIC was the major determinant of higher predator production and of the positive NBE (Figure 4 and Supplementary Figure 3). The positive correlations of the observed predator cell numbers and the NBE with TIC were highly significant. The three partitioned elements contributed differently to NBE, that is, TIC 80%, the DE 17% and the TDC effect only 3%.

In multi-predator treatments, the three protists responded differently to the bacterial species and species mixtures offered as prey source. *Tetrahymena* sp. generally dominated the predator community and showed the highest production in terms of cell numbers (Supplementary Figure 4). Overall, *Tetrahymena* sp., *Poterioochromonas* sp. and *Acanthamoeba* sp. contributed on average $68 \pm 19\%$, $27 \pm 19\%$ and $4 \pm 2\%$, respectively, to total predator production in the multiple predation experiment. Interestingly, *Poterioochromonas* sp. responded with increased growth whenever exposed to bacterial communities including the *Rhodococcus* sp. strain B5, whereas *Tetrahymena* sp. grew less well on mixtures including the *Janthinobacterium* sp. strain B3 (Supplementary Figure 4). Comparing the relative abundance of prey species growing without and with each predator or a combination of all three predators indicates overall prey community changes as a response to grazing pressure and may reveal preferential removal of distinct prey species when slopes deviate from the diagonal line (Bell *et al*, 2010). *Poterioochromonas* sp. and *Acanthamoeba* sp. generally grazed on all

species present in the respective communities as shown by the slope, which was not different from the 1:1 line (*Poterioochromonas* sp., $P<0.8052$ and *Acanthamoeba* sp., $P<0.8858$; Figure 5). Predation by *Tetrahymena* sp. resulted in a slope, which deviated significantly from the 1:1 line ($P<0.0094$), indicating that this predator preferred the more abundant bacterial species. This effect was even more pronounced when prey communities were exposed to multiple predation ($P<0.000$; Figure 5). However, the relationship between bacterial abundance in the presence vs absence of predators was significantly positive in all predation experiments, the magnitude of specialist predation behavior was in general little pronounced.

Discussion

In predation experiments with single predator species, we observed a positive effect of bacterial prey richness on the extent and stability of predator production, and the evenness of predators (in the multiple predation experiment). However, bacterial prey richness did not result in higher predator cell numbers in microcosms with diverse predator communities.

The bottom-up effect of prey diversity on the production of single predators depended on predator identity (Figure 1a), likely reflecting different feeding modes or adaptation of strains to predation pressure (Matz and Kjelleberg, 2005). The positive response of *Tetrahymena* sp. to increasing prey richness was more pronounced than those of the other two predators. *Tetrahymena* sp. differs from the others by its much larger size and strong filter feeding abilities (Weitere *et al.,* 2005; Dopheide *et al.,* 2011). Final relative abundances of prey species with and without predators indicated that *Poterioochromonas* sp. and *Acanthamoeba* sp. grazed unspecifically, whereas *Tetrahymena* sp. and the three-membered predator community tended to preferentially remove the more dominant bacterial species from the community. We recently showed that the used bacteria differed in their growth in response to predation pressure and that predation on diverse bacterial communities in fact caused a relatively better growth of less productive monocultures (Saleem *et al.,* 2012).

Although differing feeding strategies and food preferences between protists species are known (Jezbera *et al.,* 2005; Bell *et al.*, 2010), predation may be influenced not only by various prey characteristics but also by the environmental or experimental context (Bruno and O'Connor, 2005). Overall, the positive bottom-up effect of prey richness on predator production is in line with the balanced diet hypothesis, which assumes that predators benefit nutritionally from a diverse resource pool as prey mixtures better satisfy the nutritional requirements of predators than individual prey species (DeMott, 1998; Gamfeldt *et al.,* 2005). A better performance of predator species with mixed prey food might also reflect selective feeding on those prey species that fit the predator's food requirements best (Unsicker *et al.,* 2010). However, contrary to findings of Gamfeldt *et al.* (2005), prey richness negatively affected multiple predator assemblages (Figure 1a). Grazing pressure by multiple predators reduced bacterial abundances probably down to critical levels (Supplementary Figure 2). A possible mechanism might be the synergistic exploitation of bacterial prey by diverse protist predators resulting in enhanced predator production in a mixture (Figure 3). This is further supported by the mathematical partition of bacterial richness effects on protistan predators, which shows that the magnitude of predator complementarity (that is, facilitations or positive interactions) was higher than the SE (that is, competition or trait specific selection). Such potentially 'emergent impacts of multiple predators on prey' (Rosenheim, 1998; Sih *et al.*, 1998) seemed to be more pronounced at lower prey diversity and tended to decrease with increasing prey richness (Figure 1a). All prey bacterial species used in our experiments were edible for the predators. Therefore, we speculate that the decrease in the predator performance with increasing bacterial prey diversity might be potentially due to the expression of positive interactions in diverse prey communities (Saleem *et al.*, 2012) or increasing predator defense strategies (for example, biofilm formation; Matz and Kjelleberg, 2005; Pernthaler, 2005; Douglass *et al.*, 2008; Edwards *et al.*, 2010).

Functional reliability or stability in terms of declining process variability (predator production in our case) across diversity gradient has long been investigated as a fundamental ecosystem parameter (Pimm, 1984; McCann, 2000). Stability relies on interacting species and trophic interactions including the extent of predator feeding specialization and prey–predator connectivity (McCann, 2000; Haddad *et al.*, 2011). We measured stability as the variability in predator production by using the CV, where high values exhibit low ecosystem reliability (Haddad *et al.*, 2011; Leary *et al.*, 2012). Through marginally significant, the variability in predator production decreased with increasing prey species richness in all experiments (Figure 1b). Our finding supported that described by MacArthur (1955), where diversity at the prey level tended to stabilize the consumer population. Our results are also in line with previously published microcosm and field studies on the relationship of diversity, ecosystem functioning and stability in multitrophic systems (McGrady-Steed *et al.*, 1997; Haddad *et al.*, 2011) and suggest that diverse bacterial prey resources are a primary factor to make predator production more predictable.

As predator richness is generally thought to have a positive effect on ecosystem functioning by enhancing re-mineralization processes ('microbial loop') and controlling dominating species within a community (for example, increasing evenness; Duffy *et al.,* 2003, 2007; Saleem *et al*., 2012), alterations in predator richness may lead to cascading impacts on ecosystem functioning depending on the prevailing context (predator–prey traits, habitat types and other environmental factors; Bruno and Cardinale, 2008). Bacterivorous protists notably directly affect bacterial productivity, density and diversity (Pernthaler, 2005). Moreover, there is evidence that changing the diversity of microbial predators may invert the direction of interspecific interactions (Saleem *et al*., 2012). These interactions are responsible for biodiversity effects at both predator and prey level such as complementarity by resource partitioning, facilitative interactions or competitive exclusion of other species by highly productive ones (Loreau and Hector, 2001). The mathematical description of a higher complementarity (TIC) among predator species indicates that there is less competition between predators in terms of resource exploitation and consequently a more efficient utilization of bacterial resources potentially via resource partitioning. This conclusion is further supported by the low magnitude of species DE within the predator community (Figure 4). Our results are consistent with observations made, for example, in some marine ecosystems, where the increase of consumer biomass with higher consumer richness was largely explained by the 'complementarity effect' rather than by 'selection effects' (Duffy *et al.,* 2003; Steiner *et al.,* 2005). Increasing prey diversity allows for a greater niche complementarity for the predators with regard to their feeding modes (Snyder *et al.,* 2006), and hence, may reduce interspecific interference among predators (Byrnes and Stachowicz, 2009). Moreover, resource partitioning may increase the biomass or density of consumers/predators, and consequently decrease the biomass or density of the shared resource (Figure 3; Supplementary Figure 2; Tilman *et al.,* 1997; Bruno and Cardinale, 2008). Such dietary complementarity is considered to be most common in nature and one of the primary mechanisms by which species co-exist (Tilman, 1982; Chesson, 2000; Bruno and Cardinale, 2008). Furthermore, an overall positive impact of bacteria–protist richness on protist production clearly suggests that diversity at both horizontal and vertical levels in the food chain is important to drive the productivity or in other words the 'functioning' of the ecosystem.

Our results differ from other biodiversity-ecosystem functioning studies, suggesting that predator richness effects were driven by SEs only (that is, DE and TDC; for example, see Bruno and O'Connor (2005) and Douglass *et al.* (2008)). Furthermore, the potential increase in complementarity appears to contradict many previously established paradigms that inter-predator competition, negative interactions and/or intra-guild predation are common and pre-dominant features of predator communities (Polis and Holt, 1992; Arim and Marquet, 2004). If predators interact negatively via intraguild predation or interference, then increasing predator richness may indeed reduce their total impact on prey abundance (Sih *et al.,*1985; Finke and Denno 2004; Schmitz, 2007). Here, we provide contrary evidence that diverse protists mixtures substantially reduced bacterial cell numbers (Supplementary Figure 2). This is further supported by theory (Thebault and Loreau, 2003, 2006), suggesting that increasing generalist predator richness may have a greater effect on prey abundance than increasing specialist predator richness, as well as by field and laboratory studies (Gamfeldt *et al.,* 2005; Byrnes *et al.,* 2006; Griffin *et al.,* 2008) showing that higher consumer or predator biomass because of increasing richness concomitantly resulted in reduced prey biomass.

So far, evenness has been investigated only in the context of predator/herbivore impact on prey/plant community evenness. Predation may, for instance, reduce evenness in plant species by affecting herbivore species (Schmitz, 2008). Similarly, O'Connor and Bruno (2009) and Leroux and Loreau (2009) showed that shrimps significantly reduced prey richness and evenness. However, the impact of prey richness on predator evenness has not received much attention and is basically unknown for microbial systems in the context of trophic interactions (Chen *et al*., 2011). Although our set up included only one type of multiple predation with three protists, the results point to a potentially positive bottom-up effect of bacterial prey richness on protist evenness (Figure 2). This is further in line with some recent evidence on the bottom-up effect of resources on consumer community evenness (Kominoski *et al*., 2011). As any change in biodiversity is first exhibited as shifting relative abundance rather than local extinction (Chapin *et al.,* 2000; Wilsey and Potvin, 2000), evenness is more prone to disturbance than species richness. Species loss from real ecological communities is not a random process, and less productive or competitive species are more susceptible to extinction (Estes *et al*., 2011). Diverse bacterial prey resources seem to maintain the balance of protist predator communities, which are believed to be already threatened by anthropogenic biodiversity alterations (Cotterill *et al.,* 2008). This suggests that the restoration of ecosystem function also requires a reinstatement of species evenness, rather than just the conservation of richness.

An overall enhanced protist production with increasing bacteria–protist diversity does not only follow the current ecology theory of biodiversity-ecosystem functioning (Loreau *et al.,* 2001), but might also offer theory-driven concepts in applied ecological research such as the containment of

invading non-indigenous prey (Harvey *et al.,* 2004), or the biological control of pest species in agroecosystems (Cardinale *et al.,* 2003; Crowder *et al.,* 2010) and wastewater (Kuppardt *et al.,* 2010). Transferring conclusions drawn from miniaturized controlled laboratory experiments to open field situations might be challenging (Douglass *et al.*, 2008; O'Connor and Bruno, 2009). Prey recovery time might be shorter in enclosed, self-contained habitat patches (such as our microcosms) than in open ecosystem, thus leading to an overrating of the importance of protist predators (Ellner *et al.,* 2001; Cardinale *et al.,* 2006). Future studies will require manipulative experimental systems with increasing structural complexities, temporal duration and scale for increasing our knowledge on microbial ecological interactions and testing the relevance of ecological theories for the 'unseen majority' of microbes.

Conflict of Interest

The authors declare no conflict of interest.

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