

**Studies on diversity and coexistence in an  
experimental microbial community**

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*Forschung ist das simple Vergnügen, etwas zu finden,  
was man früher nicht wusste.*

(Carl Friedrich von Weizäcker)

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# Introduction

*'The diversity of life forms, so numerous that we have yet to identify most of them, is the greatest wonder of this planet. The biosphere is an intricate tapestry of interwoven life forms.'*

(Edward Osborne Wilson 1988)

The term biodiversity was literarily first introduced by E.O. Wilson in 1988 (Wilson 1988) and the concept of biological diversity from which it emerged is progressing since the 19th century and is widely used today. Natural communities may consist of hundreds of species which are all able to coexist. In 2000, Gordon stated that 'the coexistence of similar species in ecological communities is one of the oldest, most studied problems in ecology'. The maintaining of this diversity has long been questioned by ecologists (Gause 1934, Hutchinson 1961, Tilman 1982).

Today, many factors and mechanisms supporting biodiversity and coexistence are in discussion (Tilman 2000). Many researchers point out, that biodiversity is maintained by species interspecific trade-offs between their competitive abilities and their abilities to disperse in space and time, to withstand predation pressure (in a broad sense), to exploit variable resources and to compete for alternative resources in a heterogeneous environment (Tilman 2000, Levine & HilleRisLambers 2009). Other important mechanisms are micro-evolutionary dynamics, which have recently been added to our understanding of species coexistence (Shoresh et al. 2008, Jones et al. 2009). A potentially very important factor had been identified in theoretical considerations - intrinsic non-linear dynamics by means of temporal fluctuations of abundances of organisms in deterministic models (Armstrong & McGehee 1980, Huisman & Weissing 1999, 2001). The question whether biodiversity can persist in uniform environments has puzzled and fascinated biologists since decades (Hutchinson 1961, Wilson 1992). Competition, which theory has a long tradition (Hastings 1980, Tilman 1994, May & Nowak 1994, Lehmann & Tilman 1997, Huisman and Weissing 1999, Huisman & Weissing 2001) is one of the most important factors affecting the coexistence of species. Vito Volterra (1928) was apparently the first who showed mathematically, that the

coexistence of two or more species limited by the same resource is impossible. Experimentally, this was first shown by Gause (1934, 1932), who worked with two species of *Paramecium* and also with two species of *Saccharomyces* competing for the same resource. He found that in an environment in which the environmental parameters are constant, the competition of two species for the same resource leads to the extinction of one interaction partner when one species has even the slightest advantage over another. This phenomenon is called the 'competitive exclusion principle' or 'Gause's law' (Gause 1934). Summing up this principle consequently, one species will always overcome the other. This will either lead to an evolutionary or behavioural shift towards an altered ecological niche or to the extinction of the other. If two species compete for two resources, where one species is limited by resource one and the other by resource two, they may stably coexist, which was theoretically shown by Leon and Tumpson (1975). Overall, these predictions were only supported by a few studies on phytoplankton (Tilman 1977; Holm & Armstrong 1981, Hsu et al. 1981).

In nature one can easily observe, that the competitive exclusion principle often disagrees with natural systems. There are often much more species present than resources available. This was recognized by Hutchinson (1961) who formulated the 'paradox of plankton' which addresses the question why are there so many species in an apparently homogeneous environment? He proposed that an imbalance (seasonal) in the environment could promote a higher diversity of species as would be suggested by theory. Several factors for that are in discussion (Rhode 2012). In fact, Harris (1986) pointed out that, environmental disturbances like weather changes occur very frequently and therefore avoid environmental equilibrium and competitive exclusion within planktonic communities. Another reason why environmental homogeneity seldom occurs is the local perturbation of environments. Scheffer et al. (2003) could show that even in the open ocean, for example swirls are able to generate spatial heterogeneity and therefore prevent environments to reach a stable state of homogeneity. This was supported by Huisman et al. (1999) who found that variances in such disturbances like the mixing of the environment including the species are the determining factor for a great diversity under natural conditions. Therefore, extrinsic factors like weather changes are essential for the maintenance of a high diversity. This is especially the case

for planktonic communities in which diverse species benefit from different wavelengths of light, because they use different photosynthetic pigments which leads to a differentiation into several ecological niches (Stomp et al. 2004) and therefore supports a higher biodiversity. Nevertheless, it has never been tested, if more than three or even more species are able to coexist on one limiting resource under very constant experimental conditions. Additionally, theoretical models predict that internally cyclic behavior may lead to a long term coexistence of species numbers greatly exceeding the number of limiting resources (Armstrong & McGehee 1980, Huisman & Weissing 1999).

Coexisting species are organized in interacting units like food webs. Through trophic linkages, food webs present an energy flow. The behavior of energy in ecosystems can be summarized under the term "energy flow" because energy transformations are directional in contrast to the cyclic behavior of materials (Odum 1968). Every food web contains transfer of energy, top-down effects from producers to consumers, which is the bottom-up control. Some food webs and food chains have opposite top-down effects, which constrict and change bottom-up forces. These can be realized through predators in the food web. These are often very complex systems consist of many species which interact in many ways like mutualism, competition, parasitism and predator-prey relationships. They have been built up over long, evolutionary time scales, and in some cases may contain very old structures which hold information on the nature of the evolutionary changes which occurred in the past. Understanding and also modeling such networks is one of the major challenges in today's natural sciences. The modeling of such networks often goes along with simplifying for the analysis because natural systems are often much more complex regarding all species and links found in a web. Regarding food webs, a main issue is the interpretation of population dynamics and the observed time series data for their biological implications. Population dynamics are triggered by intrinsic and extrinsic parameters. Turchin (2003) pointed out, that studying the dynamic behavior of organisms is crucial to understand their underlying driving forces. Population dynamics underlie different initiations, namely extrinsic- and intrinsic factors. The extrinsic factors can be separated into biotic and abiotic factors. The main important biotic factors are for example competition for the present resources like nutrients and space, and also predator-prey dynamics (Tollrian & Harvell 1999; Persson

et al. 2001). In order to understand the underlying mechanisms of coexistence in the trophic cascades, researchers gather informations like time series data in the field (Turchin 1995; Corno et al. 2008; van der Stap et al. 2008). One major problem in the field is that the investigated populations are faced with irregularity and unpredictable variations in for example food availability or unforeseeable weather changes. This affects the collected time series (population dynamics) data and makes it difficult to investigate intrinsic factors exclusively (Kaitala et al. 1997; Upadhyay & Rai 1997; Bjornstad & Grenfell 2001). To study and to understand population dynamics, laboratory experiments offer a good tool to study intrinsic and extrinsic factors separately and in detail (Cadotte et al. 2005). There are already examples for such investigations on insects (Costantino et al. 1997), protists (Hahn & Höfle 1999), and also for bacteria (van der Stap et al. 2009). Bacteria and protozoans play an important role in the trophic cascade in natural aquatic ecosystems (Weisse et al. 1990; Weitere & Arndt 2003; Weitere et al. 2005). There are many hints, that protozoans like ciliates and heterotrophic flagellates play an important role in the structuring of bacterial community and have an intense impact on it (Boenigk & Arndt 2002; Wey et al. 2008). Laboratory microbial model systems have been widely established to solve ecological questions. Regarding such systems, Jessup et al. (2004) stated: 'The abundance of genetic and physiological information available for commonly used microorganisms, combined with their small size and short generation times, enables the design of replicated experiments across a wide range of spatial and temporal scales.'

Up to now, there are only few laboratory studies dealing with the importance of intrinsic factors regarding the dynamical behavior of microbial populations (e.g. Becks et al. 2005, Becks & Arndt 2008). In 1974, Robert May (May 1974) was one of the first who showed theoretically that in simple food webs population dynamics can show distinct behaviors like stable cycles, stable points and chaos. Moreover, theoretical investigations by Huisman and Weissing (1999, 2001) revealed that oscillations and chaotic behavior are able to support the coexistence of species but the experimental proof is still lacking.

The goal of the present work was to focus on coexistence and its sensitivity to the following parameters: competition, invasion and on the impact of phenotypic plasticity on the coexistence of species. I performed chemostat experiments and used a microbial food web consisting of bacteria as prey organisms and a ciliate as predator. The competition experiments were carried out only with bacteria. Ongoing from that, I analyzed the following hypotheses: 1. Does the replacement of a species by one that is able to form grazing resistant morphotypes enlarge the range of coexistence a different dynamic conditions, 2. How is this food web affected when another bacterium (invader) is added and focusing on the interplay between competition, predation, and invasion, and 3. How does competition influence the coexistence of two up to five bacteria competing for one given resource (with focus on population oscillations)? In the present work, 51 long-term chemostat experiments were performed from which 38 were used for the analyses here.

### **The experimental setup**

In our lab, my colleagues and I established a highly controllable and automated experimental setup for chemostat experiments (Fig. 1). The risk of contaminations with other bacteria or fungi could be minimized due to an automated sampling robot, because no invasive working steps were necessary. The microbial model system consisted of three species, two different bacterial strains, *Pedobacter spec.* and *Acinetobacter johnsonii* ( $\gamma$ -Proteobacteria  $\sim 2.5 \times 1.5 \mu\text{m}$ ), and the bacterivorous ciliate *Tetrahymena pyriformis* as predator. For the competition experiments I used additional bacteria species, which are shown below (Fig. 2) and are characterized in detail in the methods part of Chapter III.

Previous works in our lab done by Lutz Becks (Becks et al. 2005) were carried out with two bacterial preys consisted of *Pedobacter sp.* (cytophaga-flexibacter group,  $1 \times 2 \mu\text{m}$ , morphological stable) and *Brevundimonas sp.* (alpha-proteobacteria,  $1.5 \times 1.5 \mu\text{m}$ , morphological stable). Grazing by the ciliate *Tetrahymena pyriformis* led to different predator-prey population dynamics like stable limit cycles, stable equilibrium and chaotic dynamics by using the dilution rate as bifurcation parameter (the established dilution rates were  $0.45 \text{ d}^{-1}$ ,  $0.5 \text{ d}^{-1}$  and  $0.75 \text{ d}^{-1}$  per day). Coexistence of all three species was



Monsonis 2010) and second experimentally. These results were compared to those of Becks et al. (2005).

This work is subdivided into three chapters. Each chapter is written as a manuscript for a submission to international scientific journals. Each chapter has an appendix which is common in manuscripts to give additional informations and findings supporting the conclusions.

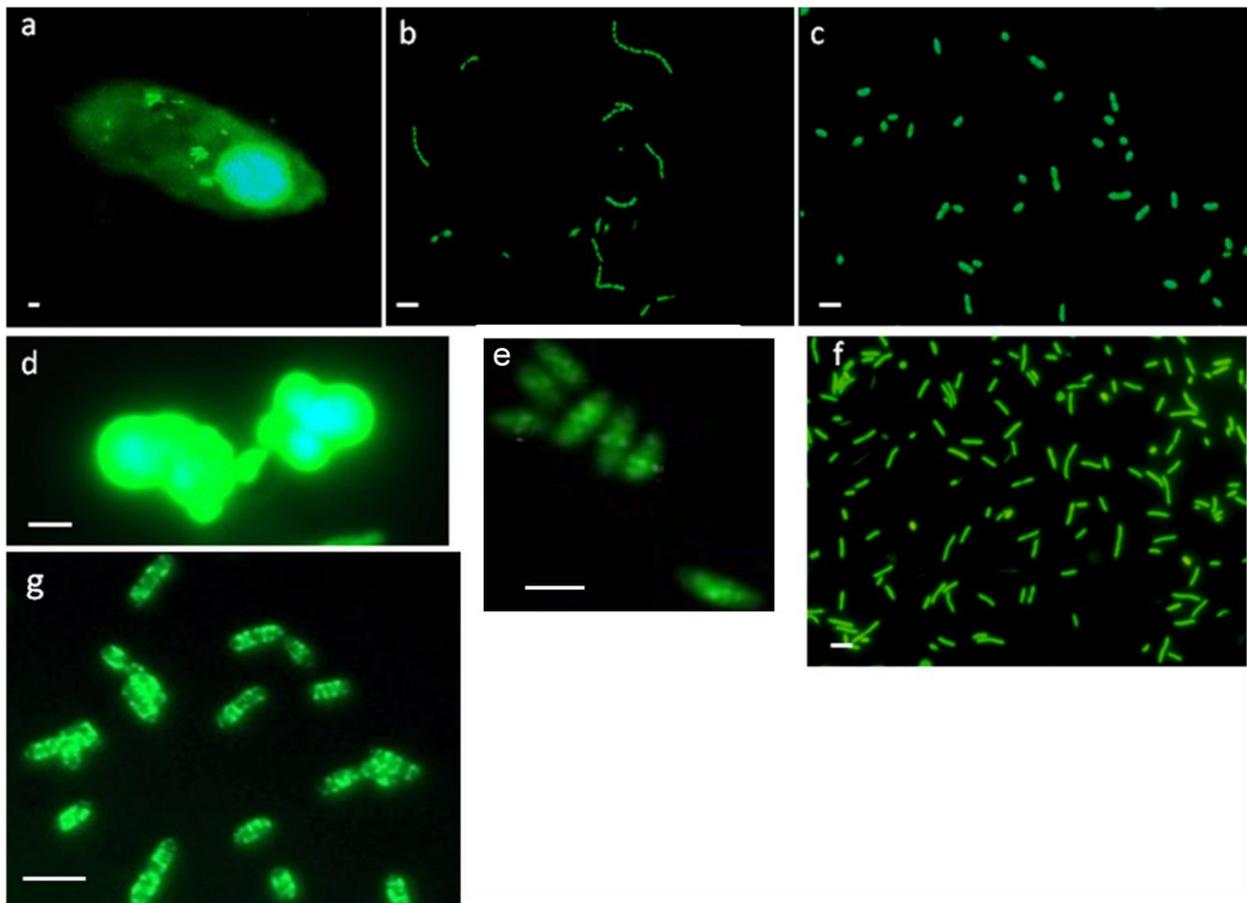


Figure 2. Microscopic photographs of SYBR Green I stained species used for the experimental investigations: a) *Tetrahymena pyriformis*, b) *Acinetobacter johnsonii*, c) *Pedobacter spec.*, d) *Azotobacter vinelandii*, e) *Corynebacterium glutamicum*, f) *Escherichia coli* and g) *Bacillus subtilis* (tagged with GFP). Scale bars indicates 2  $\mu\text{m}$  and refers to all pictures.

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## **Chapter I**

Phenotypic plasticity promotes coexistence of microbes

## Introduction

The coexistence of species may be maintained by species specific trade-offs between competitive abilities and abilities to disperse in space and time, to avoid predation, to use a variety of resources and to compete for alternative resources in a heterogeneous environment (Tilman 2000, Levine & HilleRisLambers 2009). In addition, micro-evolutionary dynamics can also play an important role in the establishment and persistence of biodiversity (Shoresh et al. 2008, Jones et al. 2009). Finally, intrinsic non-linear dynamics had been identified as another important factor allowing coexistence of different species. Theoretical models showed that oscillations of abundances in deterministic models might allow for the coexistence of many species even at limited resources (Armstrong & McGehee 1980, Huisman & Weissing 1999, 2001). The question we address here is whether phenotypic plasticity causing changes in predator avoidance contributes to the coexistence of species at oscillating dynamics and whether the dynamic behavior is influenced. If the latter is true this would have significant influences on our understanding of species interactions. Since experimental proofs are still lacking, non-linear dynamics have mostly been overlooked as important mechanisms supporting coexistence (Huisman & Weissing 1999, Fussmann et al. 2000, Becks et al. 2005, Beninca et al. 2008).

Phenotypic plasticity is wide spread in natural systems including animal and plant species and is involved in structuring of food webs (Agrawal 2001). Inducible defenses often act as predator avoidance mechanisms and has been shown to modify direct interactions between various members of a community (Kerfoot & Sih 1987; Tollrian & Harvell 1999). In the early 70's, John Maynard Smith introduced a mathematical model to study the stability of predator-prey systems (Maynard Smith & Slatkin 1973) and recent theoretical studies on food webs show that changes in the morphotype of any organism within the trophic cascade can drive a system to a broader range of stability (Ramos-Jiliberto et al. 2008).

The following question was analyzed both experimentally and theoretically: Does predator avoidance of grazing resistant organisms' change the dynamic behavior and ultimately the range of coexistence of interacting organisms? We created externally undisturbed conditions to study long-term coexistence of microbial model species in experimental chemostat systems (Becks et al. 2005). Experimental results were compared with that of mathematical models (Levin et al. 1977,

Bohannon & Lenski 1999). Changes in the dilution rates (the volume of the flow-through chemostat system that is replaced by fresh medium per day) were used to allow the system to establish different dynamic behaviors (Becks et al. 2005). Population dynamics were characterized by the estimation of corresponding Lyapunov exponents (Hastings et al. 1993, Turchin 2003).

## Methods

**Chemostat experiments, counting procedure and data analysis.** We established cultures of the ciliate *Tetrahymena pyriformis* (axenic culture from CCAP 1630/1W, average length and width 85 x 22  $\mu\text{m}$ ), the bacteria *Pedobacter spec.* (Cytophaga-Flexibacter-group, 2 x 1  $\mu\text{m}$ ), the bacteria *Brevundimonas sp.* (alpha-proteobacteria, 1.5 x 1.5  $\mu\text{m}$ , morphological stable) and *Acinetobacter johnsonii* ( $\gamma$ -Proteobacteria, 2.5 x 2.5  $\mu\text{m}$ , showing grazing resistant growth forms). Bacteria were always inoculated from deep-frozen stock cultures) in 190 ml glass chemostats at  $20^{\circ}\pm 1^{\circ}\text{C}$  without lightning. The one-stage chemostat systems were fed continuously with sterile medium (0.2 g/l proteose pepton, 0.025 g/l yeast extract) at eleven different dilution rates and mixed by continuous gentle aeration to ensure even distribution of organisms. Chemostats were started with the same inoculum. Samples (0.5 ml) were taken daily at about 13 p.m. from the centre of the chemostats using an automated sampling robot (TecanCavro® RSP 9000). Samplings for the experiments taken out with *Brevundimonas* were done without a sampling robot (see Becks et al. 2005). Bacteria and ciliate samples were fixed with 0.5 ml formaldehyde and stained with SYBR Green I (1:5000 dilution of original stock) for subsequent enumeration with the frame-spotting method (Maruyama et al. 2004) under an epifluorescence microscope (Zeiss Axioskop, Zeiss filterset 01). At least 300 bacteria were counted per sample and for *Tetrahymena* all organisms within 10  $\mu\text{l}$  were counted. Organism abundances represented the average of triplicates taken separately from one chemostat. The total volume of water taken from the chemostats during one sampling was 9.5 ml. The dynamics behaviour of populations was analyzed using calculations of corresponding Lyapunov exponents ( $\lambda$ ) according to the algorithm of Rosenstein (Becks et al. 2005, Becks & Arndt 2008, Hegger et al. 1999, Rosenstein et al. 1993). In general, chaotic dynamics have positive values, systems at steady state have negative Lyapunov exponents and stable limit cycles have value close to zero. For the calculation of the

Lyapunov exponents we used the open–source Software R (Version 2.10.1, available at <http://www.r-project.org/>) including the package RTisean.

## Results

**Experimental results.** Chemostat experiments were designed to compare the range coexistence in a two-prey-one-predator food web with and without grazing resistance of one of the prey species. The experimental food web consisted of the bacterium *Pedobacter* and either the bacterium *Brevundimonas* (no inducible phenotypic plasticity) or the bacterium *Acinetobacter* showing inducible phenotypic plasticity. The bacterivorous ciliate *Tetrahymena pyriformis* served as the model predator and experiments showed that the ciliate was able to induced a phenotypic change in *Acinetobacter* (Appendix Fig. 3). We carried out the experiments at different dilution rates to create different dynamic behaviors (Becks et al. 2005). In experiments with *Acinetobacter* (phenotypic plastic) we found coexistence of all three species for all tested dilution rates (Fig. 1, left panels). In contrast, coexistence was restricted to a smaller range of dilution rates if the experiments were run with the non-inducible bacterium (*Brevundimonas*; Fig. 1, right panels). In addition, in experiments with non-inducible bacteria we observed different population dynamics such as population cycles at a dilution rate of 0.45 per day, (quasi)-chaotic behavior at 0.5 per day, stable equilibrium at 0.75 per day and the extinction of *Brevundimonas* at low dilution rates ( $0.1 \text{ d}^{-1}$ ) and high dilution rates ( $0.9 \text{ d}^{-1}$ ; Fig. 1, right panels, Appendix Tab. 1).

**Model results.** With the aim of testing whether a change in morphology results in a different range of coexistence as a function of changes in the dilution rates, we designed two different two-prey-one-predator food web models based on well established models of Levin et al. (1977) and Bohannan and Lenski (1999). Model 1, without grazing resistance in prey 2 (bacteria) showed the coexistence of the two preys and the predator species between in the range of dilution rates of  $0.64 \text{ d}^{-1}$  and  $1.06 \text{ d}^{-1}$  (Fig. 2 A, upper panel, Appendix Fig. 1 A). At dilution rates lower than  $0.64 \text{ d}^{-1}$ , prey 2 ("*Brevundimonas*") coexisted with the predator ("*Tetrahymena*") while prey 1 ("*Pedobacter*") went extinct. The opposite was found for dilution rates higher than  $1.06 \text{ d}^{-1}$ , where prey 2 went extinct and prey 1 coexisted together with the predator.

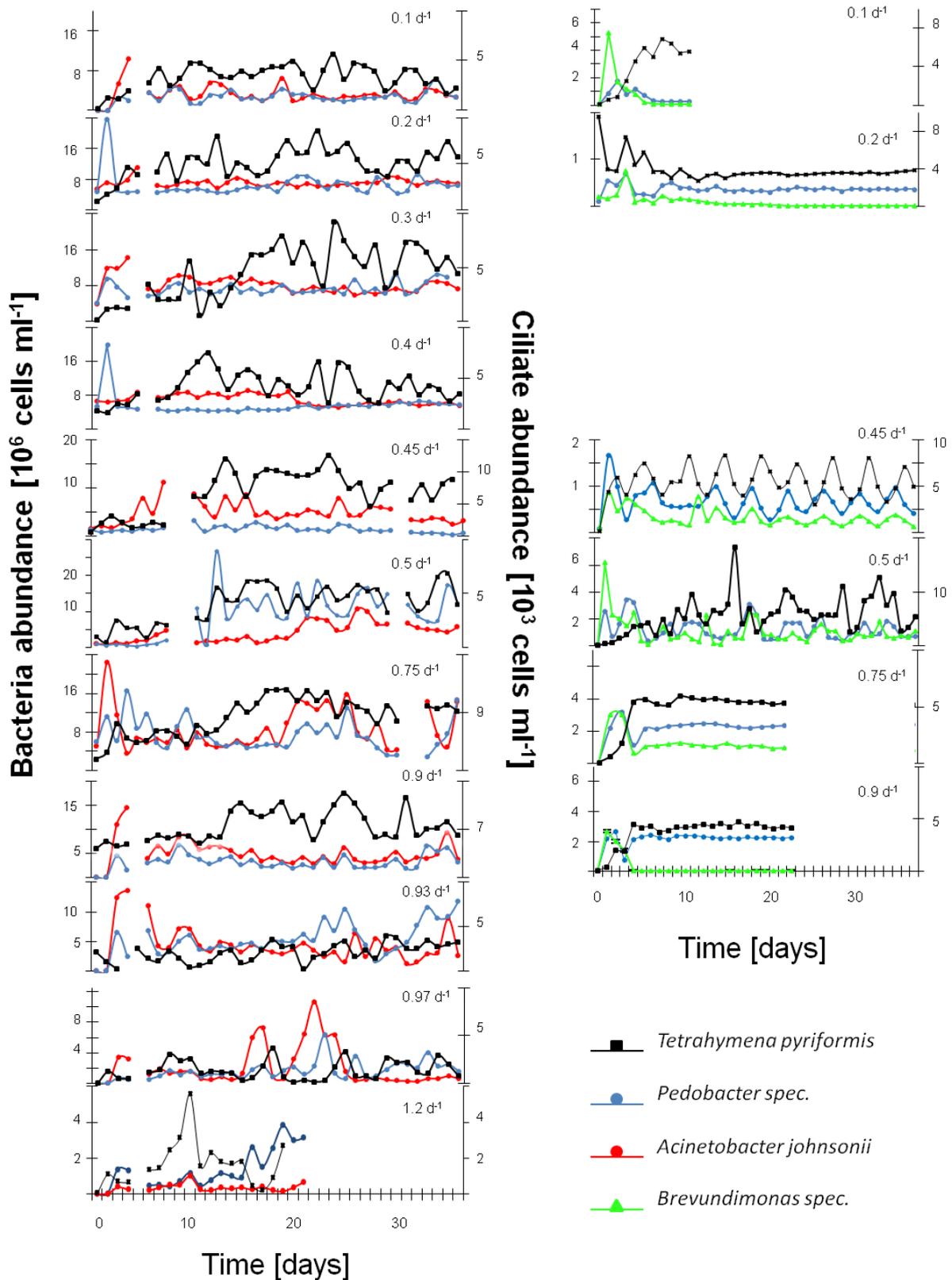


Figure 1. Time series data for all experiments. The left panel shows the experiments with *Acinetobacter* (grazing resistant) and the right panel the results for the experiments with *Brevundimonas* (non grazing resistant) (taken from Becks et al. 2005 and unpublished data). The measurement of variations is given as coefficients of variation (Appendix Fig. 2). Data of the right panel for dilution rates of  $0.45$  to  $0.9 \text{ d}^{-1}$  were taken from Becks et al. (2005).

In model 2, high abundances of the predator ("*Tetrahymena*") induced single cells of prey 2 ("*Acinetobacter*") to aggregate in grazing-resistant colonies and to cause colonies to disintegrate when predator abundances were low. The ability to switch between grazing-resistance at a cost of being less competitive and being single-celled and competitive allows for increased range of coexistence ( $0.14 \text{ d}^{-1}$  to  $1.31 \text{ d}^{-1}$ ; Fig. 2 A, lower panel; Appendix Fig. 1 B) compared to the model 1 without the possibility for changes in grazing resistance.

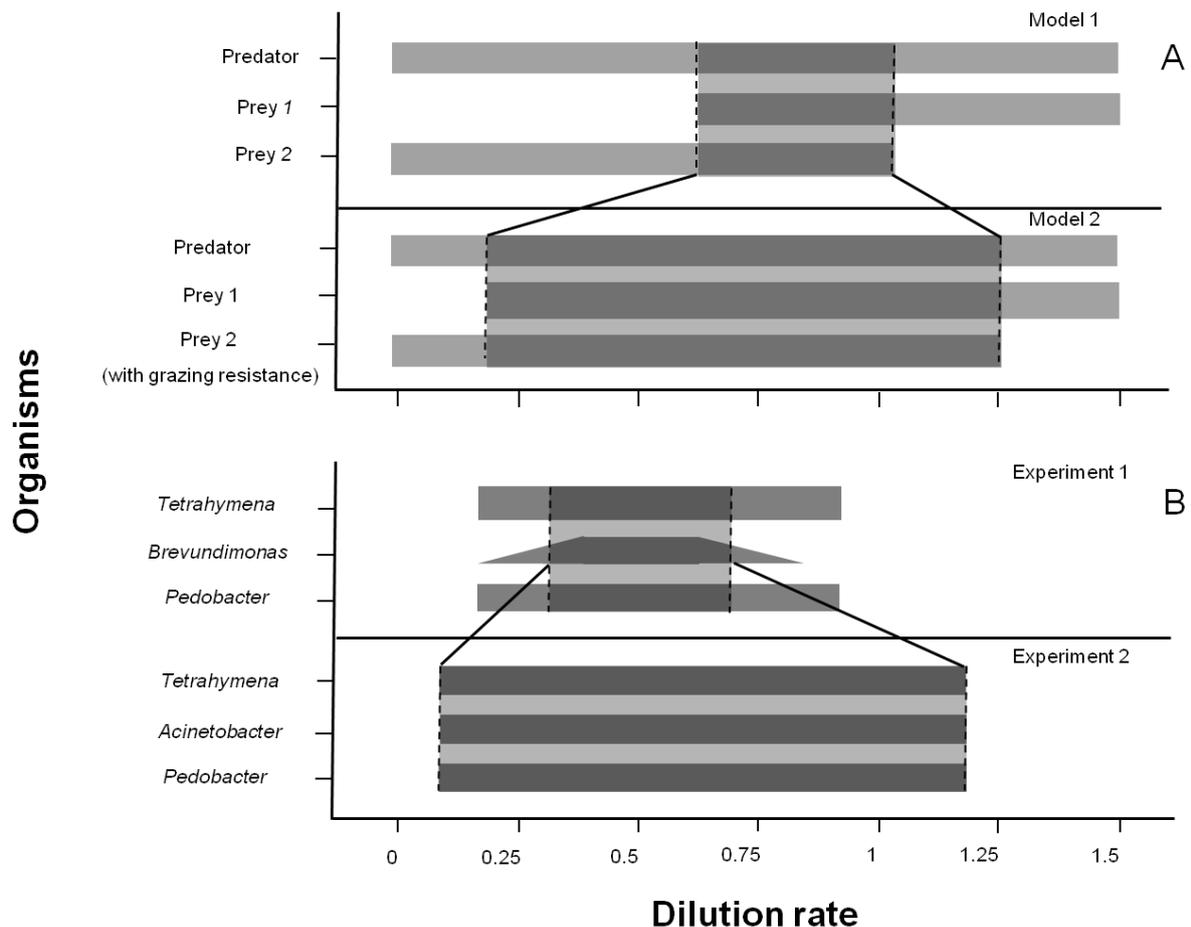


Figure 2. Range of species coexistence in the model and in the experiments. The modeling results (A) are displayed for the model without phenotypic plasticity (upper graph) and the model with inducible phenotypic plasticity by one prey bacterium (lower graph). The experimental results (B) are shown for the experiments where no bacteria showed phenotypic plasticity (upper graph) and the experiments where one bacterium (*Acinetobacter*) showed phenotypic plasticity (lower graph). The areas between the dashed lines indicate the range of possible coexistence of all three species in the food web. Grey bars show the occurrence of each investigated organism. The fading in the bar for *Brevundimonas* indicate the area at which it begins to die out.

## Discussion

Theoretical models showed that inducible predator avoidance behavior can increase the range of coexistence in bi- and tri-trophic food webs (Vos et al. 2004 a, b). This is supported by our own model analysis of a microbial two-prey-one-predator system. In addition, our work shows that not only the range of coexistence but also the dynamical behavior is altered by the presence of inducible defenses.

Previous experiments showed that species' coexistence can be enhanced when induced phenotypic plasticity occurs (Verschoor 2004, van der Stap 2006, 2007, Boeing & Ramcharan 2010, Yoshida et al. 2007). Phenotypic plasticity is wide spread in natural ecosystems in plants and animals (Karban & Baldwin 1997, Tollrian & Harvell 1999b, Vos et al. 2004) ranging from protozoans (Kuhlmann & Heckmann 1985) to terrestrial plants (Karban & Baldwin 1997). Inducible defense has been described for all levels of ecological organization and there is probably no system where it does not play a role (Tollrian & Harvell 1999). Experiments with multicellular plankton organisms (Verschoor 2004, van der Stap 2007) studying effects of inducible defenses gave indications for a phenomenon similar to our experimental results: instable population dynamics and eventually extinction in experiments with organisms without inducible defenses and stable coexistence in experiments with inducible defenses present. Here we could show by working with highly controlled conditions that this phenomenon is due to an intrinsically changed dynamic behavior. The complex effects of nutrient enrichment on the stability and coexistence of species might be interpreted in a similar way. Changes in growth rates due to nutrient enrichment may change the dynamic behavior as it was shown in our experiments without inducible defenses of bacteria (Fig. 1 right panel, Becks et al. 2005). When inducible defenses by one prey bacterium were present the dynamic behavior was dominated by quasi-chaotic oscillations and no extinctions occurred (Fig. 1 left panel, Appendix Tab. 1). Chaos-like patterns have been found in other simple experimental systems (Costantino et al. 1997, Becks et al. 2005, Becks & Arndt 2008) or more complex communities (Graham et al. 2007, Beninca et al. 2008). Whether a system behaves chaotically or stochastically is difficult to judge on a limited data set which experiments can naturally provide. Field experiments could be affected by environmental noise which makes it difficult distinguishing chaotic patterns from noise (Ellner & Turchin 1995, Kaitala et al. 1997, Bjornstad & Grenfell 2001, Vasseur & Yodzis 2004). In contrast to field experiments, our experimental system is well

defined and highly controllable to minimize the occurrence of environmental noise (Jessup et al. 2004, Cadotte et al. 2005) and we suggest that our experimental results derive from intrinsically driven events.

We show both experimentally and theoretically that inducible defenses of prey species may lead to a broad range of coexistence along different growth rates. The corresponding computer model supports the idea that switching in grazing resistance may lead to a fundamentally higher possibility of coexistence in food webs.

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## Appendix

### Mathematical model analyses

To test whether the switch between morphologies results in a wider range of dilution rates at which the coexistence for all species is supported we designed two different two-prey-one-predator food web models. One without grazing resistance and another including a possible switch (inducible defense) between grazing resistant and non-grazing-resistant morphotypes which is triggered by the presence of the predator. We modified a model well established in literature (Levin et al. 1977, Bohannan & Lenski 1999).

#### Model 1: Two-prey-one-predator food web without grazing resistance

In this first model, both prey species are edible but the prey with the better fitness is preferred by the predator. The values of the parameters were chosen according to the experimental food web, which in this model consists of *Tetrahymena pyriformis* as the predatory ciliate, and *Pedobacter spec.* and *Brevundimonas spec.* as prey bacteria.

The system can be described by the following differential equations:

$$\frac{dC}{dt} = (C_0 - C) D - \varepsilon_1 \mu_1(C) N_1 - \varepsilon_2 \mu_2(C) N_2 ,$$

$$\frac{dN_1}{dt} = N_1 \mu_1(C) - P \varphi_1(N_1) - D N_1 ,$$

$$\frac{dN_2}{dt} = N_2 \mu_2(C) - P \varphi_2(N_2) - D N_2 ,$$

$$\frac{dP}{dt} = P \beta_1 \varphi_1(N_1) + P \beta_2 \varphi_2(N_2) - D P ,$$

where  $C$  is the concentration of nutrients in the chemostat,  $N_1$  the abundance of *Acinetobacter*,  $N_2$  the abundance of *Pedobacter* and  $P$  the abundance of *Tetrahymena*. The parameter  $D$  the dilution rate,  $C_0$  represents the concentration of nutrients (resource) and  $\varepsilon_1$  and  $\varepsilon_2$  the reciprocal yield of each prey bacteria  $N_i$  and  $\beta_1$  and  $\beta_2$  the yield of *Tetrahymena* preying upon the bacteria.

The Monod function describes the specific growth rate  $\mu_i(C)$  of the bacteria:

$$\mu_i(C) = \frac{\mu_{\max_i} C}{K_{S_i} + C}, i = 1, 2,$$

where  $K_{S_i}$  the half-saturation constant and  $\mu_{\max_i}$  is the maximum growth rate and of the bacteria.

Similarly, the ciliate feeding response of the Holling II type is given by Monod's model:

$$\phi_i(N_i) = \frac{\phi_{\max_i} N_i}{K_{N_i} + N_i}, i = 1, 2,$$

where  $K_{N_i}$  the half-saturation constant for the predator feeding on bacterium  $N_i$  and  $\phi_{\max_i}$  is the maximum feeding rate and.

### Model 2: Two-prey-one-predator food web with predator induced colony formation

This model investigates a possible switch between the grazing resistant and the grazing vulnerable subpopulations. The switching of morphotypes is theoretically modeled to be triggered by the abundances of *Tetrahymena*. With this target, the flow terms  $\psi_1(N_1, P)$  and  $\psi_2(R, P)$  were introduced into this model:

$$\frac{dC}{dt} = (C_0 - C) D - \varepsilon_1 \mu_1(C) N_1 - \varepsilon_2 \mu_2(C) N_2 - \varepsilon_R \mu_R(C) R,$$

$$\frac{dN_1}{dt} = N_1 \mu_1(C) - P \phi_1(N_1) - \Psi_1(N_1, P) + \Psi_2(R, P) - D N_1,$$

$$\frac{dR}{dt} = R \mu_R(C) + \Psi_1(N_1, P) - \Psi_2(R, P) - D R,$$

$$\frac{dN_2}{dt} = N_2 \mu_2(C) - P \phi_2(N_2) - D N_2,$$

$$\frac{dP}{dt} = P \beta_1 \phi_1(N_1) + P \beta_2 \phi_2(N_2) - D P,$$

With  $\psi_1$  given by:

$$\Psi_1(N_1, P) = \frac{P}{P_{crit}} \alpha N_1,$$

and  $\psi_2$  given by:

$$\Psi_2(R, P) = \frac{P_{crit}}{P} \gamma R.$$

The rate at which cells attach or leave colonies is represented by  $\alpha$  and  $\gamma$ .  $P_{crit}$  [Ind.<sub>Tetra.</sub>/ml] is the critical abundance of *Tetrahymena*. From that, more *Acinetobacter* cells aggregate in colonies than leave them. The value for  $\alpha$  was chosen as 0.01 [h<sup>-1</sup>] and for  $\gamma$  0.002 [h<sup>-1</sup>]. The experimental determination of these values is difficult (Monsonís 2010). The detachment of colonies requires a breakdown of exopolymeric substances, which probably takes more time than the process of the attachment of cells. The value for  $P_{crit}$  was as:  $P_{crit} = 183$  [Ind.<sub>Tetra.</sub>/ml] (Monsonís 2010). The same parameter values and initial conditions as in model 1 were used. The investigated dilution rates ranging from 0 d<sup>-1</sup> to 1.5 d<sup>-1</sup>.

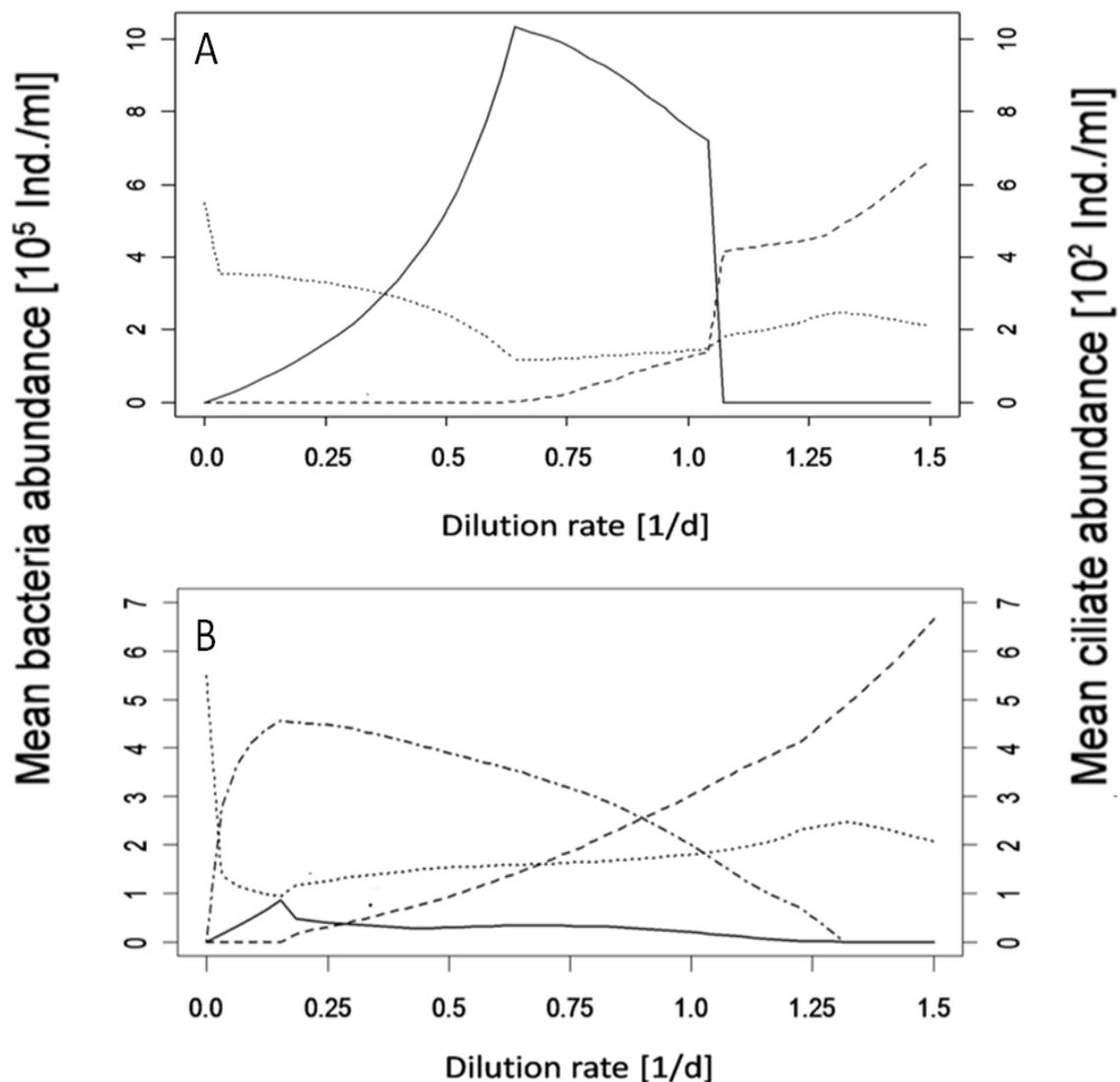
#### Parameter values:

$C_0$	3 $\mu\text{g ml}^{-1}$
$\beta_1$	1/4000 [Ind. <sub>Tetra.</sub> Ind. <sub>i</sub> <sup>-1</sup> ]
$\beta_2$	1/4000 [Ind. <sub>Tetra.</sub> Ind. <sub>i</sub> <sup>-1</sup> ]
$\mu_{max1}$	0.150 [h <sup>-1</sup> ]
$\mu_{max2}$	0.172 [h <sup>-1</sup> ]
$K_{S1}$	0.0274 [ $\mu\text{g/ml}$ ]
$K_{S2}$	0.0020 [ $\mu\text{g/ml}$ ]
$\Phi_{max1}$	150 [Ind. <sub>Acin.</sub> h <sup>-1</sup> Ind. <sub>Tetra.</sub> <sup>-1</sup> ]
$\Phi_{max2}$	450 [Ind. <sub>Pedo.</sub> h <sup>-1</sup> Ind. <sub>Tetra.</sub> <sup>-1</sup> ]
$K_{N1}$	422.000 [Ind. <sub>Acin.</sub> ml <sup>-1</sup> ]
$K_{N2}$	400.000 [Ind. <sub>Pedo.</sub> ml <sup>-1</sup> ]
$\epsilon_1$	2 x 10 <sup>-6</sup> [ $\mu\text{g Ind.}_i^{-1}$ ]
$\epsilon_2$	2 x 10 <sup>-6</sup> [ $\mu\text{g Ind.}_i^{-1}$ ]
Initial bacteria density	10 <sup>5</sup> [Ind. ml <sup>-1</sup> ]
Initial predator density	500 [Ind. ml <sup>-1</sup> ]
Initial nutrient concentration	0 $\mu\text{g ml}^{-1}$

The individual-based units were converted into  $\mu\text{g}$  carbon (*Acinetobacter*: 1.241 x 10<sup>7</sup>  $\mu\text{g}$  carbon Ind.<sup>-1</sup>; *Pedobacter*: 6.65 x 10<sup>-8</sup>  $\mu\text{g}$  carbon Ind.<sup>-1</sup>; *Tetrahymena*: 6.5507 x 10<sup>-3</sup>  $\mu\text{g}$  carbon Ind.<sup>-1</sup>; nutrients: 0.4  $\mu\text{g}$  carbon/ $\mu\text{g}$  glucose) before the model was run

(Heckmann 2009). The parameters used were determined by Heckman (2009) and Monsonis (2010). Both models were developed by David Heckmann (2009) and Mar Monsonis (2010). I used this model to study the range of coexistence of species.

Mean abundances of bacteria and the ciliate at different dilution rates predicted by both models:



Appendix Figure 1. (A) Mean abundances of bacteria and the ciliate at different dilution rates predicted by Model 1 for a time series of 625 days. The stabilisation phase was omitted in the calculation of the mean abundances. Dashed line: prey 1 ("Pedobacter"); solid line: prey 2 ("Brevundimonas"); dotted line: predator ("Tetrahymena"). All of the three species coexist between dilution rates of  $0.64 \text{ d}^{-1}$  and  $1.06 \text{ d}^{-1}$ . (B) Mean abundances of bacteria and ciliates at different dilution rates predicted by model 2 with predator-induced colony formation for a time series of 625 days. The stabilization phase was omitted in the calculation of the mean abundances. Solid line: prey 2 ("Acinetobacter" non-grazing resistant morph); dashed-dotted line: grazing resistant morph of prey 2; dashed line: prey 1 ("Pedobacter"); dotted line: predator ("Tetrahymena"). All species three species coexist between dilution rates of  $0.14 \text{ d}^{-1}$  to  $1.31 \text{ d}^{-1}$ . (Graphs taken from Heckmann 2009)

### Calculated Lyapunov exponents for all experiments:

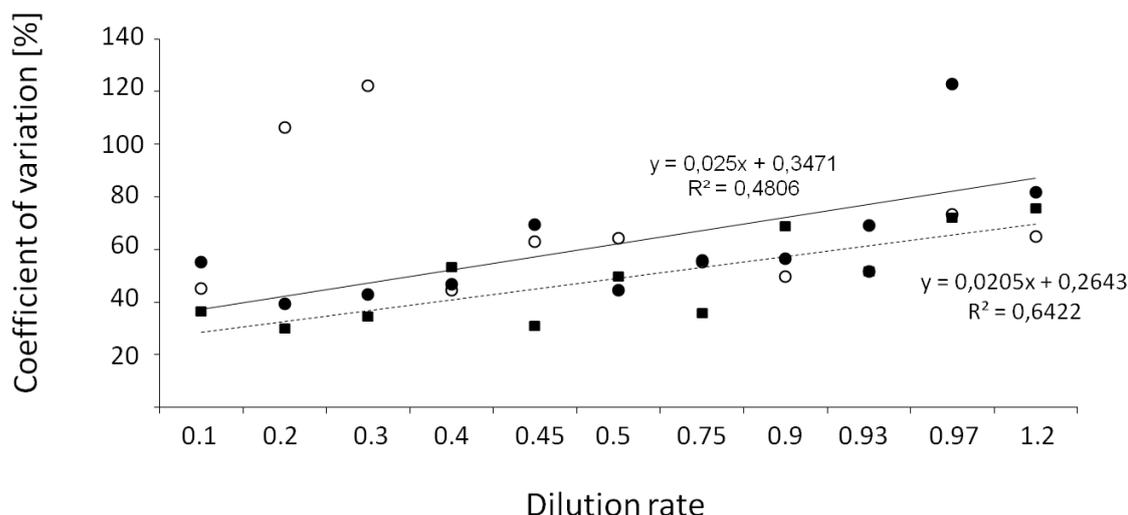
Appendix Table 1: Lyapunov exponents ( $\lambda$ ) for the predator *Tetrahymena* and the prey *Pedobacter* and *Acinetobacter* estimated using the algorithm of Rosenstein et al. (1993) from time series illustrated in Fig. 1. Chemostats a-k display data for the experiments with *Acinetobacter* and l-q the data for the experiments with *Brevundimonas*.

Chemostat	Dilution rate ( $d^{-1}$ )	Dyn. Behav.	<i>Tetrahymena</i>		<i>Acinetobacter</i>		<i>Pedobacter</i>	
			$\lambda$	P	$\lambda$	P	$\lambda$	P
a	0.1	chaos	$0.19 \pm 0.02$	**	$0.19 \pm 0.02$	**	$0.12 \pm 0.01$	**
b	0.2	chaos	$0.25 \pm 0.02$	**	$0.15 \pm 0.03$	*	$0.16 \pm 0.03$	*
c	0.3	chaos	$0.12 \pm 0.01$	**	$0.17 \pm 0.02$	**	$0.29 \pm 0.05$	*
d	0.4	chaos	$0.24 \pm 0.007$	***	$0.19 \pm 0.01$	***	$0.17 \pm 0.007$	***
e	0.45	chaos	$0.32 \pm 0.07$	*	$0.25 \pm 0.09$	*	$0.37 \pm 0.07$	*
f	0.5	chaos	$0.32 \pm 0.08$	*	$0.19 \pm 0.05$	*	$0.21 \pm 0.01$	**
g	0.75	chaos	$0.33 \pm 0.04$	**	$0.39 \pm 0.05$	**	$0.29 \pm 0.08$	*
h	0.9	chaos	$0.24 \pm 0.03$	**	$0.19 \pm 0.009$	***	$0.11 \pm 0.01$	**
l	0.93	chaos	$0.18 \pm 0.01$	**	$0.29 \pm 0.05$	*	$0.18 \pm 0.02$	**
J	0.97	chaos	$0.2 \pm 0.02$	**	$0.24 \pm 0.01$	***	$0.19 \pm 0.02$	**
k	1.2		----- not enough data points available-----					
l	0.1		----- not enough data points available-----					
m	0.2	stable	$-0.12 \pm 0.02$	**	$-0.14 \pm 0.01$	*	$-0.17 \pm 0.01$	***
n	0.45	cycles	$0.02 \pm 0.01$	***	$0.01 \pm 0.02$	**	$0.01 \pm 0.02$	*
o	0.5	chaos	$0.18 \pm 0.01$	**	$0.24 \pm 0.01$	***	$0.22 \pm 0.02$	**
P	0.75	stable	$-0.13 \pm 0.01$	*	$-0.12 \pm 0.01$	*	$0.13 \pm 0.01$	**
q	0.9		----- not enough data points available-----					

Notes: Errors in the  $\lambda$  values correspond to the asymptotic errors of the fit.

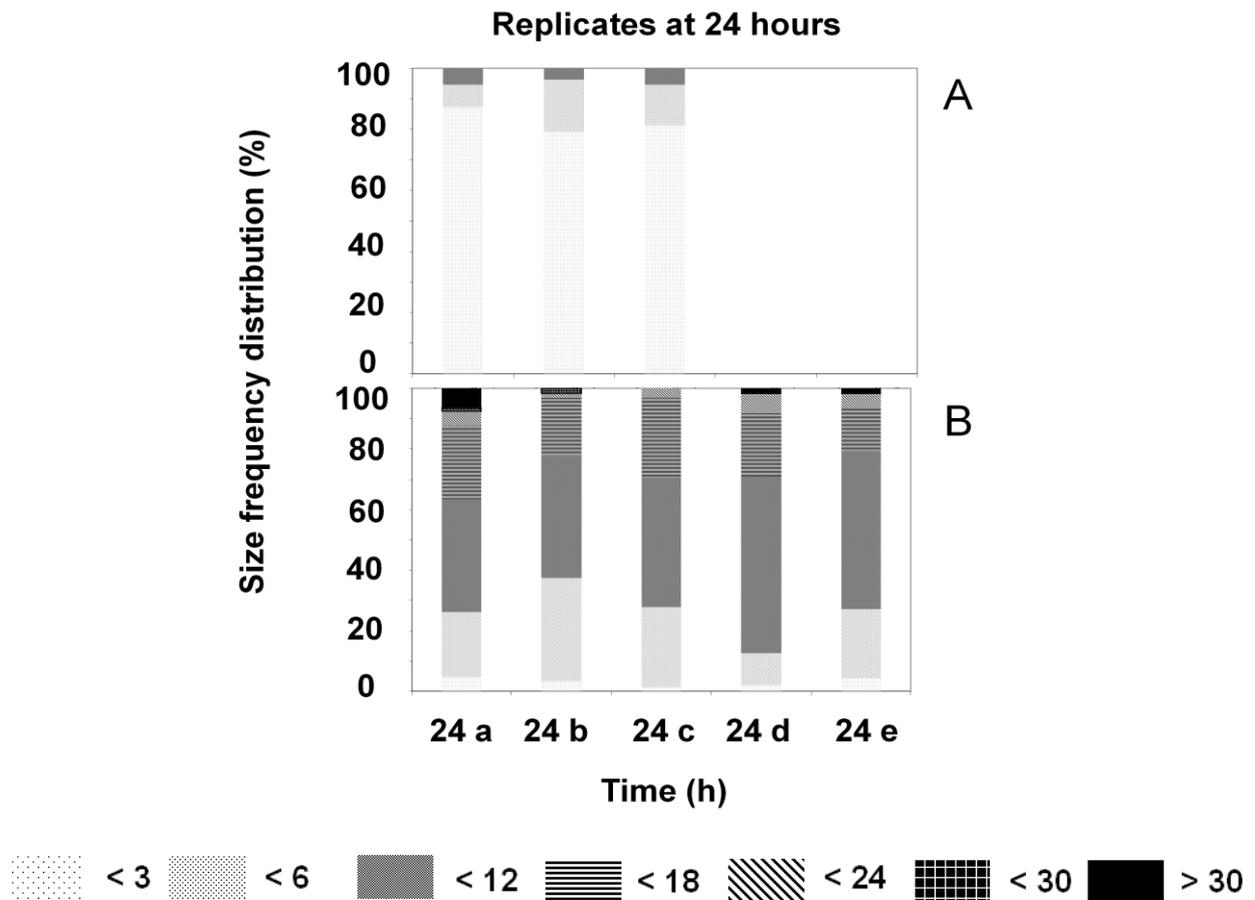
\* P , 0.05; \*\* P , 0.001; \*\*\* P , 0.0001

### Coefficients of variation for all experiments shown in Figure 1:



Appendix Figure 2. Coefficients of variation for all organisms and all experiments (dilution rates). The lines show a regression for *Pedobacter* (dashed line) and *Tetrahymena* (solid line). The line for *Acinetobacter* is not drawn because of the two outliers at the dilution rates 0.2 and 0.3. Open circles display *Acinetobacter*, filled circles *Pedobacter* and boxes *Tetrahymena*. The values for the regression analysis are included.

Investigation of grazing (*Tetrahymena*) induced grazing resistance in *Acinetobacter* in batch experiments:



Appendix Figure 3. Size frequency distribution of *Acinetobacter johnsonii* with and without predation by *Tetrahymena*. A monoxenic culture under batch conditions shows that after 24 hours small fractions of cells are dominating (A, three replicates). Grazing by *Tetrahymena* results in developing large growth forms in batch experiments after 24 hours (B, five replicates). (Graph taken from Willen 2010).

## **Chapter II**

The interplay of competition, predation and invasion in a simple experimental food web with phenotypic plasticity in prey

## Introduction

Biological systems are complex networks, built up by many species that interact in diverse ways, such as competition, mutualism, predator-prey-relationships and parasitism (Drossel & McKane 2003). Predation and competition are important interactions between species (Chase et al. 2002, HilleRisLambers & Dieckmann 2003, Chesson & Kuang 2008). In predation, one species is the resource of the other. Competition can be conceptualized as occurring horizontally on the same resource level, while predation takes place vertically between different resource levels. Competition as well as predation creates environmental stress on the interacting species. There are two ways how competition occurs between species (Amarasekare 2002): 1. Exploitative competition which has an indirect negative effect that results from sharing a common resource (Chase & Gilpin 1974). By reducing resource abundance, each consumer affects the other exclusively (Vance 1984). 2. Territoriality, predation, overgrowth or even chemical competition are involved in interference competition and can be defined as direct negative interactions (Schoener 1983, Amarasekare 2002). This takes place at any abundance level because each competitor changes the ability of the other to exploit the present resource (Vance 1984, Begon et al. 2006).

Predation can affect trophic interactions in diverse ways (Holt & Barfield 2009). The differentiation into generalists and specialist species has to be considered with regard to the coexistence and/or stability of trophic systems. Generalists are able to use a wide variety of different resources and therefore are more successful in a wide range of environmental conditions. Specialists using a narrow range of diets are only able to exist in a narrow range of environmental conditions. The coexistence of populations under competition and predation indicates that these populations have accommodated themselves to each others' presence and have evolved ways to survive with regard to the environmental stressors (Rosenzweig 1971, Tilman 1982, Diehl & Feissel 2000). Predation and competition interact with each other; in simple food webs they are theoretically able to limit or promote diversity (Chesson & Kuang 2008). Another important factor influencing species coexistence and diversity is the invasion of species into long-established biological communities (Wilson 1992). Invasive species can show specific traits or combinations of traits that allow them to outcompete native species. The invasion of alien species can result in extinction of native species which can be mediated by niche displacement, competitive exclusion,

or hybridisation with native species (Elton 1958, Sax et al. 2002, Davis 2003). This is well documented for a wide range of spatially restricted environments (Chown et al. 1998, Sax et al. 2002, Davis 2003, Sax & Gaines 2003, Blackburn et al. 2004).

In nature, all above mentioned aspects of competition cannot be separated from each other and generally act in concert. To disentangle the effect of the different aspects of competition we established a well controlled chemostat system where the species composition was controlled by using axenic cultures only. Three species of bacteria were used as model organisms for competing organisms, a bacterivorous ciliate being able to feed on all three species was taken as a model for a predator. One prey bacterium showed inducible defence (phenotypic plasticity) in the presence of the predator to mimic phenotypic plasticity as an important feature for interacting populations (e.g. Tollrian & Harvell 1999). Up to our knowledge this is the first experimental analysis of the interplay between exploitative competition and apparent competition in a well controlled experimental system.

### **Material and Methods**

The experimental design of the chemostats required sterile conditions. Therefore, all parts of the chemostat systems including medium reservoirs (10 l of PPY 100: 0.2 g l<sup>-1</sup> proteose peptone, Fluka, Munich, Germany, 0.025 g l<sup>-1</sup> yeast extract, Sigma, Steinheim, Germany) were sterilized prior to the experiments. For all experiments, the dilution rate was adjusted to 0.75 per day by automated syringe pumps (Cavro XLP 6000, Modular Syringe Pump, TECAN, Crailsheim, Germany). The experiments were conducted under sterile and constant conditions, for that reason chemostats were kept in a water bath at a temperature of 20 ± 0.3 °C (controlled by JULABO FC600, Seelbach, Germany) and mixed by gentle aeration to warrant a consistent distribution of organisms. The one-stage chemostat systems were inoculated with initial bacterial densities of 10<sup>5</sup> cells/ml from LB-medium overnight cultures. I used cultures of the ciliate *Tetrahymena pyriformis* (axenic culture from CCAP 1630/1W, average length and width 85 x 22 µm), the bacteria *Pedobacter spec.* (Cytophaga-Flexibacter-group, 2 x 1 µm), *Acinetobacter johnsonii*. (γ-Proteobacteria, 2.5\*2.5 µm) and *Azotobacter vinelandii* (~ 4 µm x 3 µm, gram-negative, DSM No. 399, Leibniz Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH). Triplicate samples at 0.5 ml were taken every 24 hours into sterile microcentrifuge tubes via a program-controlled Robot (RSP 9000 Cavro, TECAN, Crailsheim,

Germany). Samples were immediately fixed in 0.5 ml PBS 4 % formaldehyde (8.0 g/l Sodium chloride, Merck, Darmstadt, Germany; 0.2 g/l potassium chloride, Merck, Darmstadt, Germany, 1.42 g/l Na<sub>2</sub>HPO<sub>4</sub> x H<sub>2</sub>O, Merck, Darmstadt, Germany, 0.2 g/l KH<sub>2</sub>PO<sub>4</sub>, AppliChem, Darmstadt, Germany; pH 7.5). For subsequent enumeration with the frame-spotting method (Maruyama et al. 2004) under an epifluorescence microscope (Zeiss Axioskop, Zeiss filterset 01), cells were stained with SYBR Green I (1:5000 dilution of original stock). At least 300 bacteria were counted per sample (10 µl), for enumerating *Tetrahymena* all cells within a volume of 10 µl were counted. Organism abundances represented the average of triplicates taken separately from one chemostat. The total volume of water taken from the chemostats during one sampling was 9.5 ml. Single species systems consisted of *Pedobacter* and *Acinetobacter*. Competition systems contained both *Pedobacter* and *Acinetobacter*. One-predator-two-prey systems were composed of *Pedobacter* and *Acinetobacter* as prey and *Tetrahymena* as the predator. In addition, one experiment was used to study the effect of invasion by *Azotobacter*. All experiments were carried out twofold except for the invasion experiment. The number of chemostats which could be handled at the same time is limited, since chemostats had to be started at the same time to avoid differences in clones due to micro-evolutionary changes. The results for the first experiment are displayed on the left panel and the replicate experiment on the right panel in Figure 1. As *Acinetobacter* shows a great morphological variability (Willen 2010) filament lengths were determined in all experiments (given as the mean value of 300 measured cells per day). These data were collected at day 12, 15, 18 and 21 of the experiments to test whether the organisms occur as single cells or as filaments. The size classes (Appendix Fig. 2) were defined in < 6 µm, ≤10 µm and ≥ 10 µm, whereas *Tetrahymena* is able to feed on particles up to 10 µm and prefers a particle size of about 6 µm (Willen 2010).

The results are displayed in time delay reconstructions (phase space diagrams, see also appendix Fig. 3) to show the dynamic behaviour of the systems and to reveal changes in abundances. The corresponding time series graphs are also shown (Appendix Fig. 1a, b).

## Results

The experimental design of experiments is illustrated in Figure 1. Single species systems consisted of *Pedobacter* and *Acinetobacter* and competition systems

contained both *Pedobacter* and *Acinetobacter*. One-predator-two-prey systems consisted of *Pedobacter* and *Acinetobacter* as prey and *Tetrahymena* as the predator. One experiment was used to study the effect of invasion by *Azotobacter*. Experiments were labelled by the same colour code as in phase space diagrams (Fig. 2).

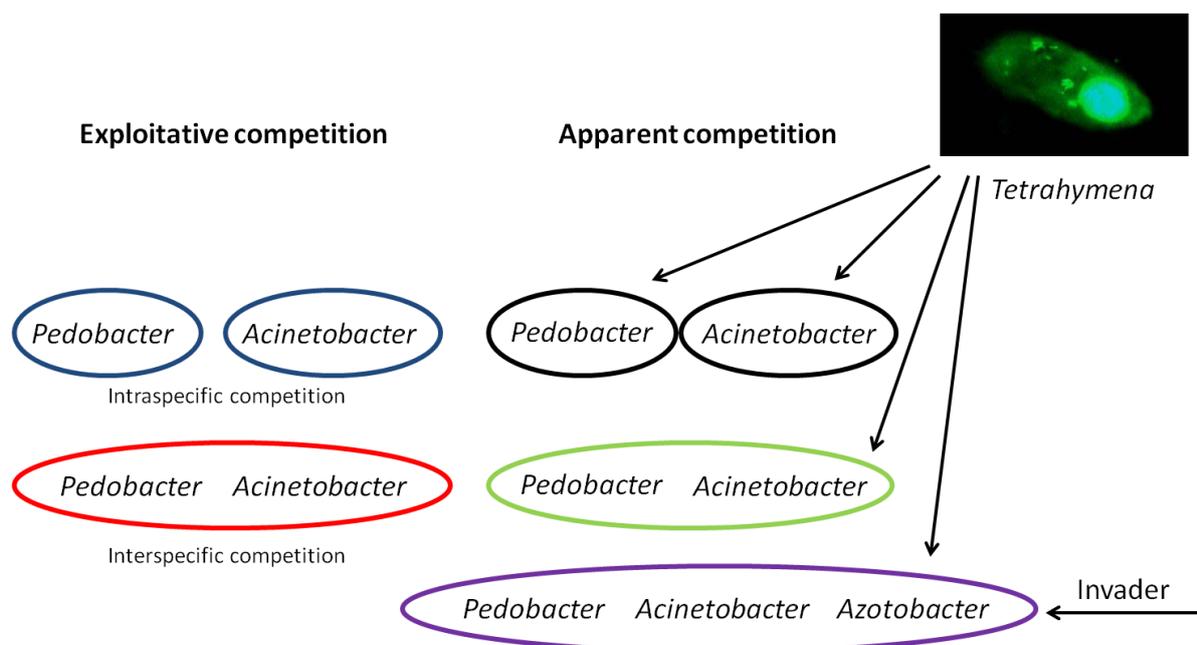


Figure 1. Schematic drawing of the experiments taken out to study the different aspects of competition, predation and invasion in detail. The colour code refers to the one in figure 2. Blue circles: single species systems, red circle: competition systems, black circles: one-predator-one-prey systems, green circle: one-predator-two-prey systems, and purple circle one-predator-two-prey system with invader.

**Single cell experiments.** The single cell experiments with *Pedobacter* and *Acinetobacter* showed a consistent pattern of dynamic behaviour. All populations fluctuated irregularly around a quasi-chaotic attractor. The abundances were nearly constant (Fig. 2 a, b) compared to the replicate except for *Acinetobacter*. Here the abundances differ between the two replicates (Fig. 2 c, d) and decreased from  $4.55 \times 10^7$  cells  $\text{ml}^{-1}$  to  $1.43 \times 10^7$  cells  $\text{ml}^{-1}$ .

**Competition experiments.** *Pedobacter* in the second experimental set seemed to be the better competitor compared to *Acinetobacter* (Fig. 2 b) in comparison to the first set (Fig. 2 a). In the first setup the *Acinetobacter* population shows almost filaments, beginning with a size class of  $10 \mu\text{m}$  (Fig 3), while it showed almost only single cells and short filaments in the second setup (Fig 3). The *Acinetobacter* population in the first setup dominated by large filaments exhibited a lower abundance ( $6.31 \times 10^6$  cells  $\text{ml}^{-1}$ ) (Fig. 2 c) compared to the setup with predominantly

single cells and short filaments ( $3.01 \times 10^7$  cells ml<sup>-1</sup>) (Fig. 2 d, Fig. 3). Also here, the attractors indicate an irregular dynamic behaviour regarding population dynamics.

**One-predator-one-prey systems.** Within the experiments carried out with *Tetrahymena* as predator and either *Pedobacter* or *Acinetobacter* as prey, the bacteria populations showed no difference between the replicates. Also *Tetrahymena* showed no difference in abundance (Fig. 2 d, e). *Acinetobacter* populations were always dominated by large growth forms (Fig. 3).

**One-predator-two-prey system.** The systems consisting of *Tetrahymena* as predator and *Acinetobacter* and *Pedobacter* as prey showed distinct differences between the replicates regarding the abundances of *Acinetobacter*. In the first setup (Fig. 2 c) the population consisted of large filaments (Fig. 3) which served as a predator avoidance. Here the population developed a mean abundance of  $2.38 \times 10^7$  cells ml<sup>-1</sup>. Within the replicates the populations predominantly showed short filaments and single cells (Fig. 3). The abundance reached a mean value of  $5.73 \times 10^5$  cells ml<sup>-1</sup>. Although there is an edible fraction of *Acinetobacter* in the second experimental setup (Fig. 3), the abundances of *Tetrahymena* were lower ( $1.55 \times 10^3$  cells ml<sup>-1</sup>) compared to the setup with grazing protected *Acinetobacter* ( $4.96 \times 10^3$  cells ml<sup>-1</sup>) (Fig. 2 f). The abundances of *Pedobacter* differ only slightly between the two replicate experiments.

**One-predator-two-prey system with invader.** The introduction of *Azotobacter* had a fundamental effect on the other bacteria. *Pedobacter* as well as *Acinetobacter* showed significantly lower abundances. In the case of *Pedobacter*, the population size was much lower compared to the one-predator-two-species system ( $4.56 \times 10^6$  cells ml<sup>-1</sup> compared to  $1.05 \times 10^7$  cells ml<sup>-1</sup>) and *Acinetobacter* showed a change from  $2.38 \times 10^7$  cells ml<sup>-1</sup> to  $1.58 \times 10^6$  cells ml<sup>-1</sup>. Interestingly, the dynamic behaviour was affected with regard to the shape of the attractor. In the one-predator-three-prey system, the population showed a great variability (by occupying more space in the phase space) within its abundances compared to the population behaviour in the one-predator-two-prey system.

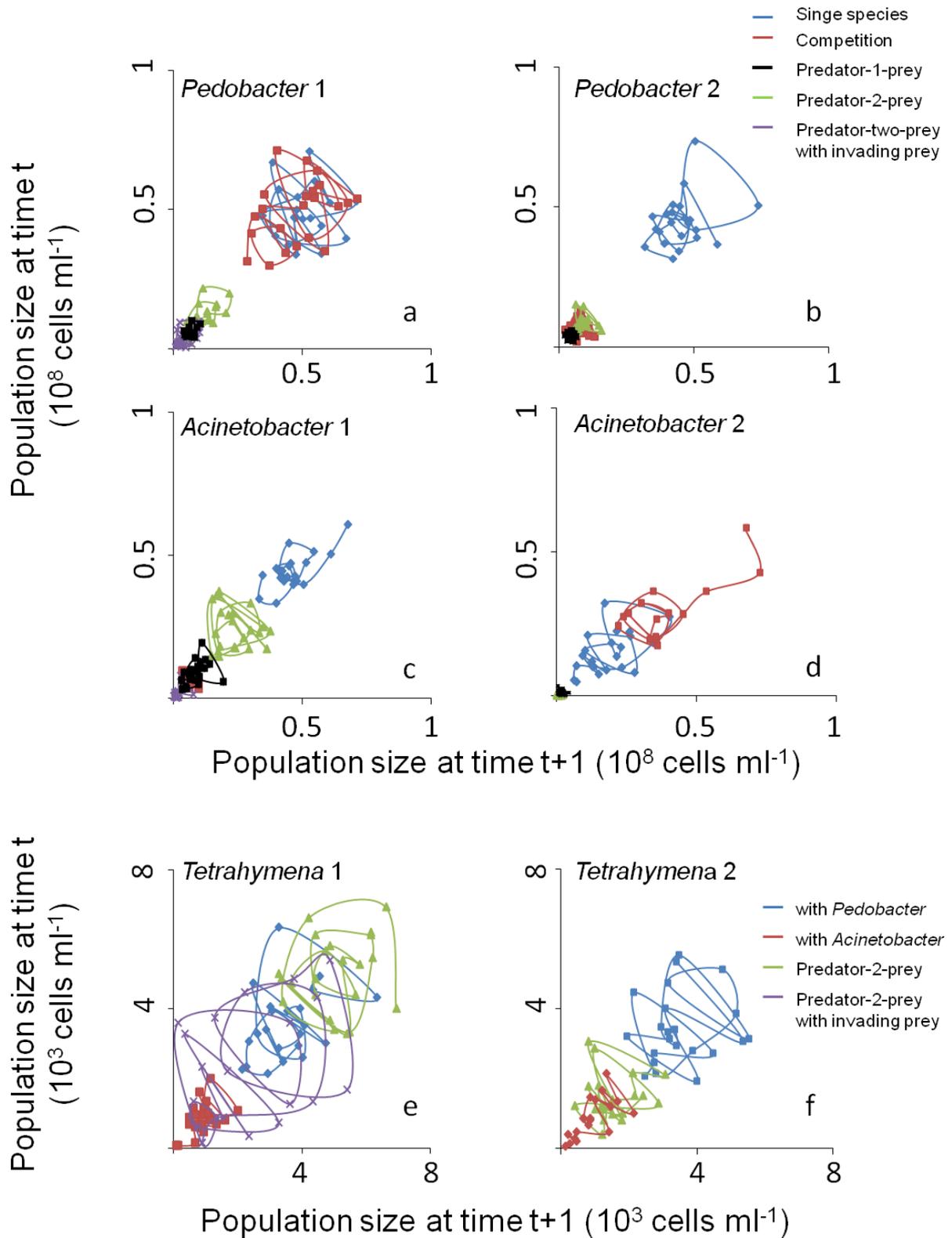


Figure 2. Phase space diagrams of all experiments. From a-d the red lines show the results for the competition experiments (*Pedobacter* and *Acinetobacter*), the blue lines the single species system, the green lines the predator-two-prey system (*Tetrahymena*, *Pedobacter* and *Acinetobacter*), the purple line the predator-three-prey system (*Tetrahymena*, *Pedobacter*, *Acinetobacter* and *Azotobacter*) and the black line the system for single species with predation. From d-e the data for *Tetrahymena* are displayed. Here the blue line shows *Tetrahymena* with *Pedobacter*, the red line with *Acinetobacter*, the green line the predator-two-prey system and the purple line the predator-three-prey system. All data displayed are taken from day 12 to day 35 of the experiments.

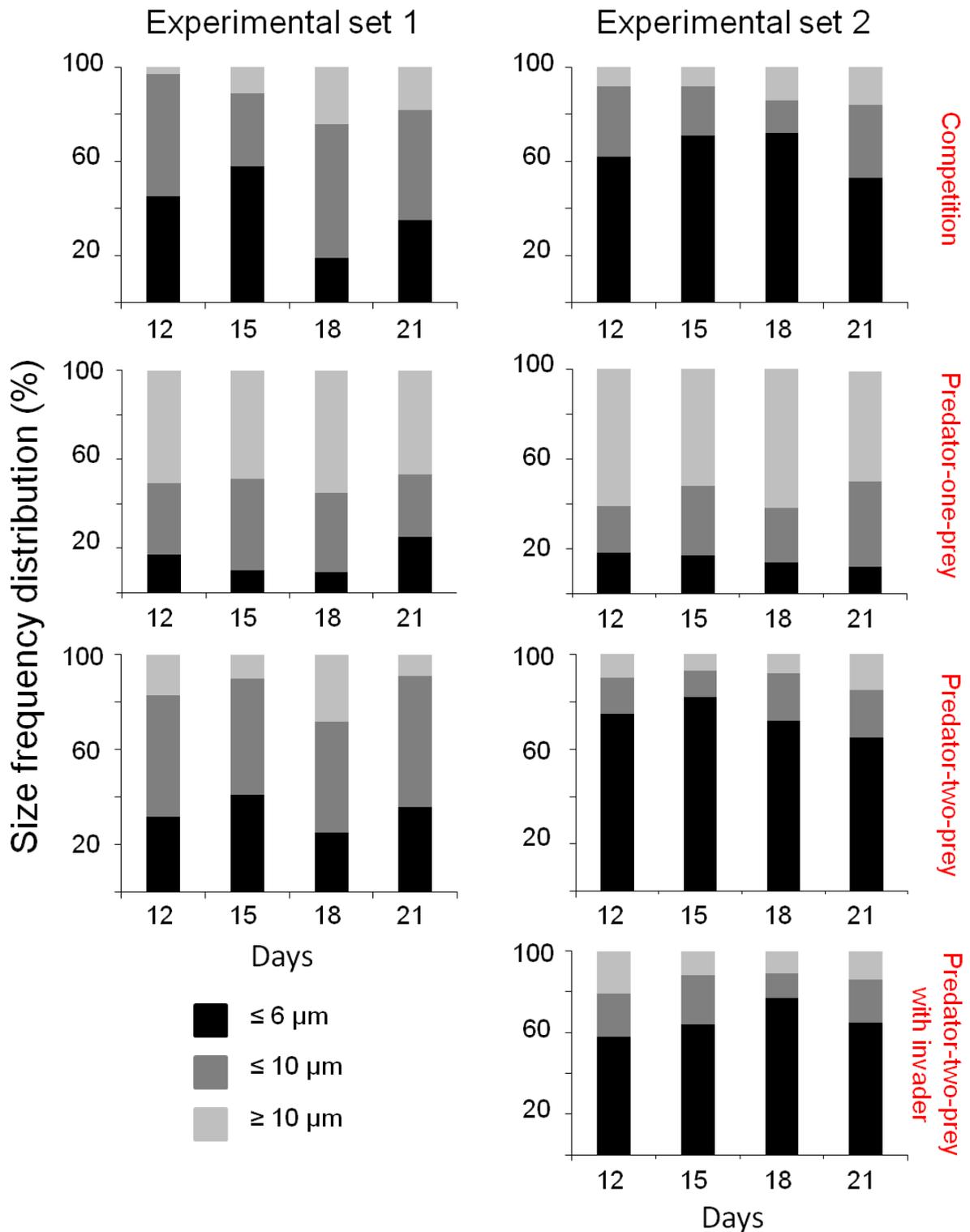


Figure 3. Fraction of the different filament lengths ( $\mu\text{m}$ ) of the *Acinetobacter* populations for the days 12, 15, 18 and 21 in the replicated experiments and different treatments. The competition experiments are displayed at the top, followed by the one-predator-one-prey systems, the one-predator-two-prey systems, and the one-predator-two-prey system with the invader (*Azotobacter*).

## Discussion

The aim of this study was to investigate the interplay of competition, predation and (species) invasion in an experimental microbial food web under highly controlled conditions. The competition experiments with *Acinetobacter* and *Pedobacter* showed that both organisms are able to coexist and that the outcome of competition is dependent on the bacterial morphological appearance. *Acinetobacter* is able to form colonies which serve as a protection from grazing, but a certain morphological variability also randomly occurs in monoxenic cultures (Willen 2010). In the experiments where mostly single cells and short filaments were present (Fig. 2 d and Fig. 3), they were able to compete more successfully (see also Wagner et al. 2006, Young 2008) and exhibit higher abundances than in the replicated experiment where the population showed larger growth forms (Fig. 2 c and Fig. 3). The coexistence *per se* of both bacteria is probably due to the non-selective nutrient source (PPY, proteose peptone extract) which evidently supplies enough energy and essential nutrients for both bacterial strains.

In the one-predator-one-prey experiments where *Tetrahymena* grazed upon *Acinetobacter*, there was a difference regarding the abundances between the two replicates. In the second experiment the abundances were in the same range. The morphotypes of both experiments showed no significant difference in the fractions of morphotypes (Fig. 3). Within both experiments large filaments prevailed, which might be the reason for the low abundances of *Tetrahymena* compared to all other experiments as there was only a small fraction of edible cells.

The one-predator-two-prey systems were also affected by the morphology of *Acinetobacter*. In the experiment (Fig. 2 c and Fig. 3), where *Acinetobacter* cells mainly appear as long filaments, the abundance was much higher than in the other replicate (Fig. 2 d and Fig. 3). The populations of *Pedobacter* behaved like in the competition experiments.

In the last experiment where an additional bacterium was introduced as an invader, there was a fundamental change in the abundances of the other bacteria detectable. The introduction of *Azotobacter* into the system affected the other interacting bacteria by reducing their abundances. *Pedobacter* (Fig. 2 a) as well as *Acinetobacter* (Fig. 2 c) showed a significant decrease in their cell numbers. This effect should be due to the additional competitor in the system. Here, the interplay of predation and additional competition (due to an invader) reinforced the pressure on the interaction

partner. This pressure is probably due to *Azotobacter* exploiting the present resources. Similar effects have been described in literature (Shurin & Allen 2001, Chase et al. 2002, HilleRisLambers & Dieckmann 2003, Chesson & Kuang 2008) showing that invasive species are able to shrink populations of other interacting species. This is a general phenomenon: Invasion of other species into established systems faces species with additional competition (Mooney & Cleland 2001, Sax et al. 2002) and predation (Elton 1958, Sax & Gaines 2008).

Within this study it was shown, that the interaction between species like competition and predation affected each other's appearance by means of changes in abundances. Undisturbed and highly controllable conditions allowed studies on those intrinsically driven aspects in detail and to shed light on the their interplay within interacting communities. Species showed different behaviors regarding their abundances which was indicated by changes in the corresponding attractors in the phase space diagrams. Furthermore, there were differences between replicated experiments that indicate that the morphological appearance (growth form of *Acinetobacter*) may also influence species interactions. Especially bacteria have developed various ways to react on predation pressure and competition like showing phenotypic plasticity (Pernthaler 2005).

The fact that populations showed fluctuations (oscillations) which were indicated by quasi-chaotic attractors in the phase space diagrams gave a hint that non-linear behaviour might also support the coexistence of interacting species which might be overlooked in field experiments. Additionally, diminutive changes in experimental starting conditions may also support chaotic oscillations (bounded population dynamics) (Turchin 2003).

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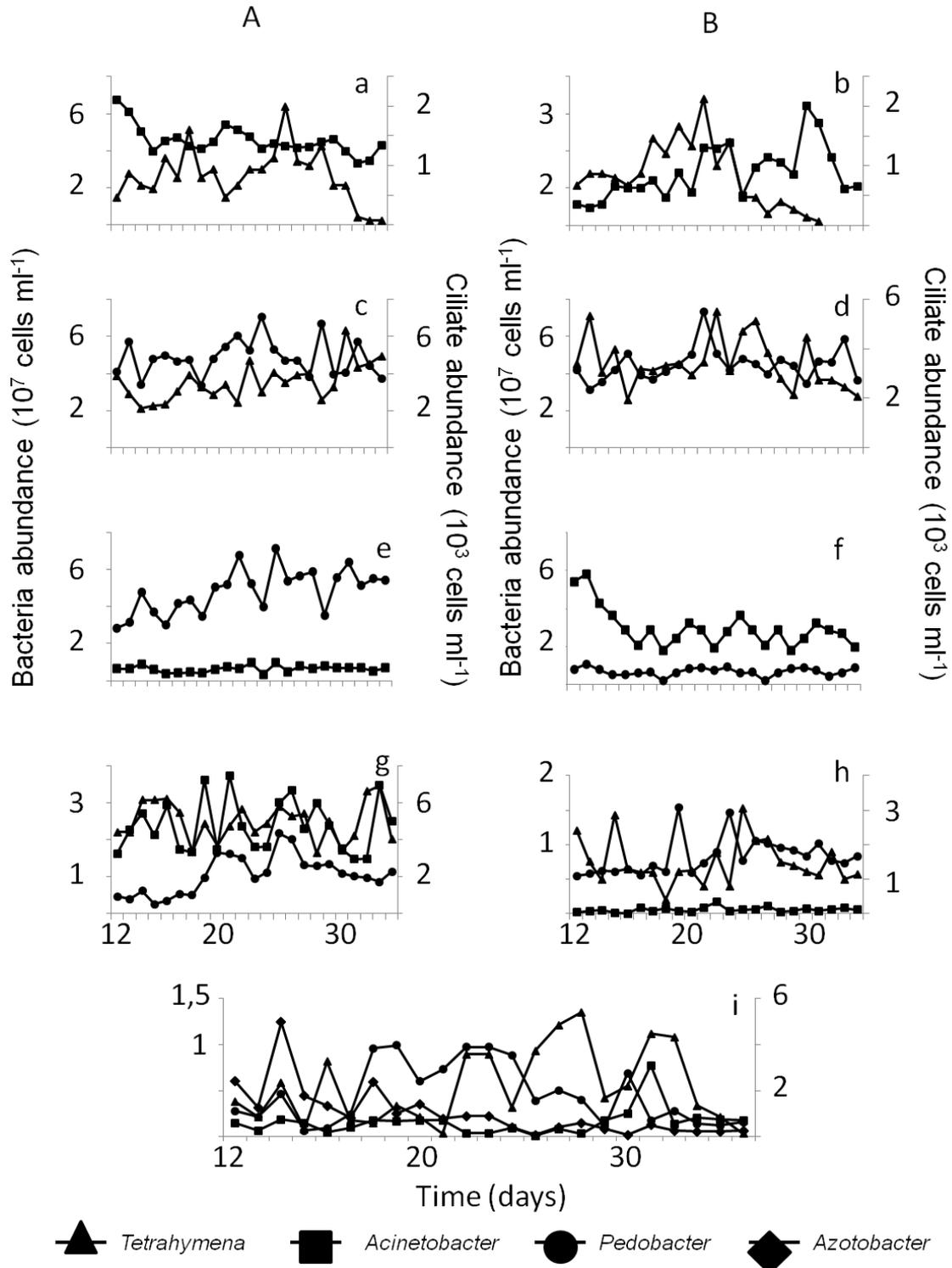
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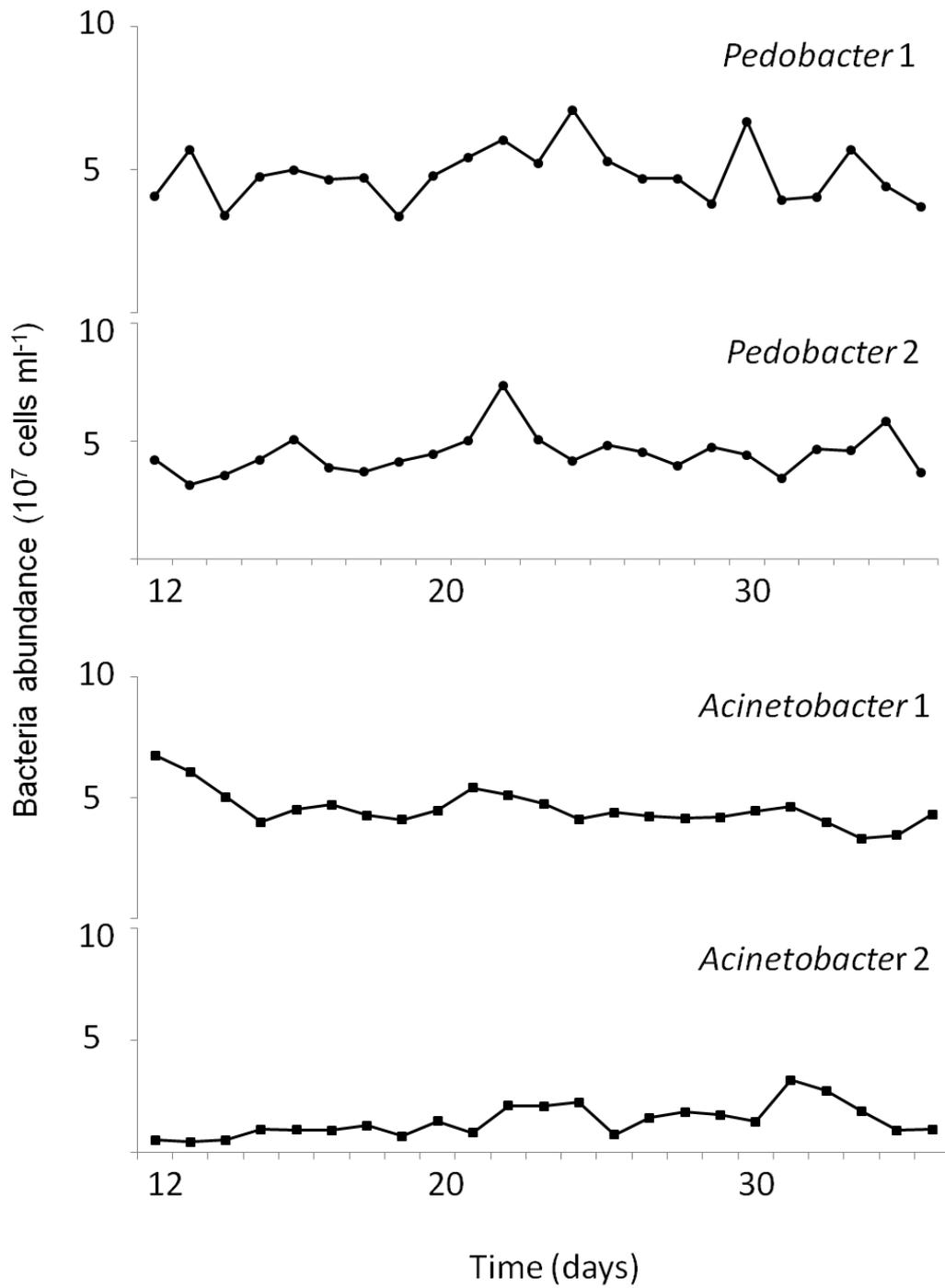
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## Appendix Chapter III

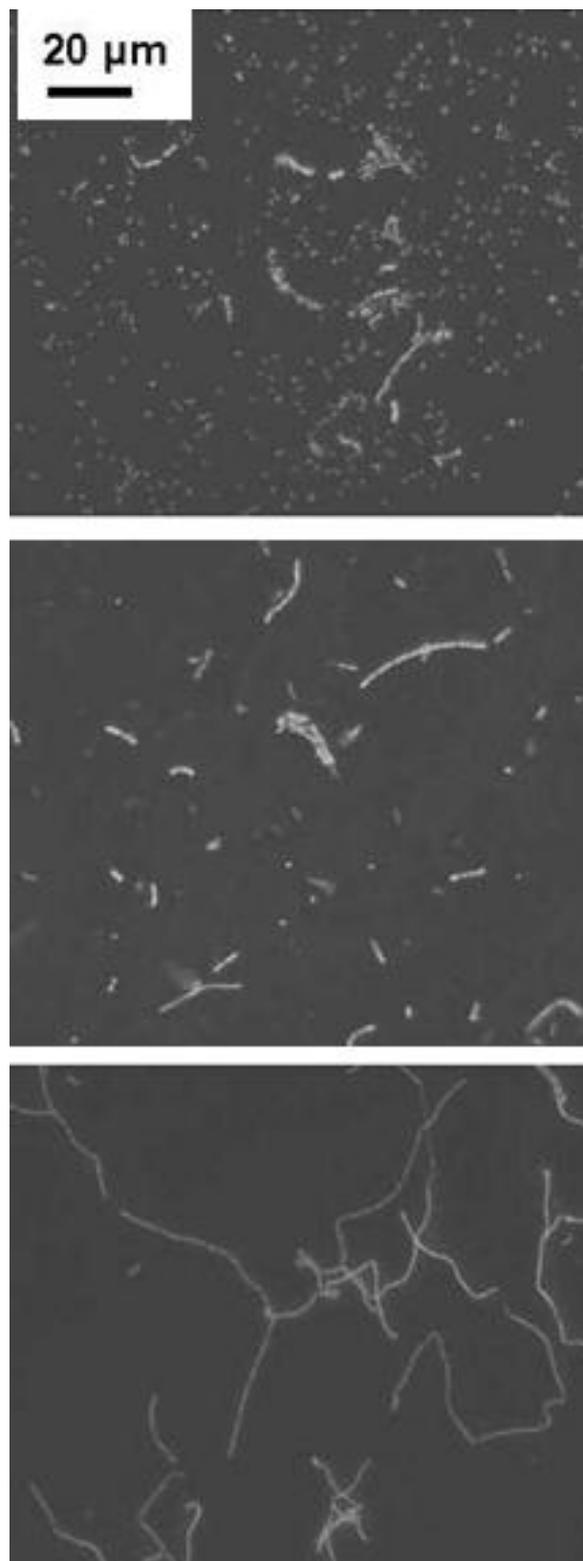
This appendix gives additional supporting material for Chapter II.



Appendix Figure 1a. Time series graphs for all experiments. The predator-one-prey experiments with *Tetrahymena* and *Acinetobacter* are displayed in a and b, the predator-one-prey experiments with *Tetrahymena* and *Pedobacter* in c and d, the competition experiments with *Pedobacter* and *Acinetobacter* in e and f, the predator-two-prey experiments with *Tetrahymena*, *Acinetobacter* and *Pedobacter* in g and h, and the predator-three-prey experiment with *Tetrahymena*, *Acinetobacter*, *Pedobacter* and *Azotobacter* in i. The both experiments (replicates) are labelled with A and B. According to the phase space diagrams, data are displayed from day 12 until day 35.

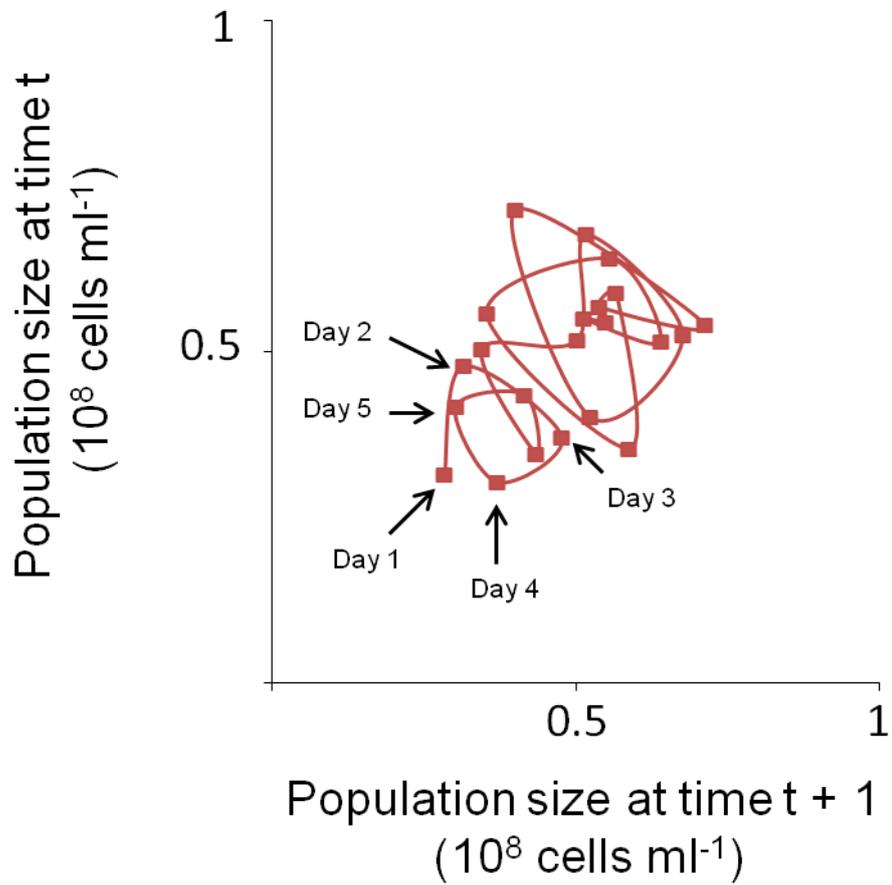


Appendix Figure 1b. Time series graphs for the single cell experiments. The both experiments (replicates) are labelled with 1 and 2. Data are displayed from day 12 until day 35 as they were shown in the phase space diagrams.



Appendix Figure 2. Microscopic pictures of the different morphotypes (filamental growth forms) of *Acinetobacter johnsonii*. The top picture displays the case for single cells. The middle picture shows larger growth forms. The picture at the bottom shows large filamental growth forms, which are inedible for *Tetrahymena*. The scale bar in the top picture refers to all three pictures. (Pictures by Christine Willen)

The following graph explains a phase space diagram:



Appendix Figure 3. Example for a phase space diagram with an attractor showing changes in population size over time. The days 1, 2, 3, 4 and 5 are marked with a black arrow to illustrate the principle of the phase space diagrams.

## **Chapter III**

Biodiversity by species oscillations

## Introduction

When E. O. Wilson introduced the term biodiversity in 1988 in literature (Wilson 1988), the concept of diversity in biological systems from which it originated had been developing since the 19th century and is widely used today. Complexity and diversity are two fascinating features of living beings. The understanding of the origin, structure and functioning of these features is a fundamental challenge in biology. Ecologists have long questioned how this diversity is maintained (Gause 1934, Hutchinson 1961, Tilman 1982). The existence of more species than resources available has been stated to be a paradox (Hutchinson 1961) because when species competing for the same resource one should theoretically outcompeted the other. Obviously in worlds' oceans as the largest habitats where essential resources are scarce the high species richness is perplexing. At present there are several mechanisms discussed, which may contribute to local and global diversity of organisms (Tilman 2000). The coexistence of species may be maintained by interspecific trade-offs between their competitive abilities and their abilities to disperse in space and time, to withstand predation pressure (in a broad sense), to exploit variable resources and to compete for alternative resources in a heterogeneous environment (Tilman 2000, Levine & HilleRisLambers 2009). Micro-evolutionary dynamics have recently been added to our understanding of species coexistence (Shoresh et al. 2008, Jones et al. 2009). An additional, potentially very important factor had been identified in theoretical considerations - intrinsic non-linear dynamics, the temporal fluctuations of abundances of organisms in deterministic models. These may allow the coexistence of many species at limited resources (Armstrong & McGehee 1980, Huisman & Weissing 1999, 2001). Non-linear dynamics as a fundamental driver of species coexistence has mostly been overlooked in the current discussion of mechanisms supporting coexistence (Huisman & Weissing 1999, Fussmann et al. 2000, Becks et al. 2005, Beninca et al. 2008). This is mainly due to the fact that experimental proofs are still lacking.

A fundamental question in environmental sciences is whether or not the diversity in a system is related to its productivity. Theoretical considerations reveal that productivity increases with the number of species in a system (Tilman et al. 1997). Field experiments support theoretical findings (Tilman 1996, Loreau et al. 2001).

Here, we designed experiments to test, whether oscillating populations of competing organisms allow the coexistence under constant environmental conditions. We carried out chemostat experiments supplied with glucose as the sole resource and added up to five different heterotrophic bacteria species (*Bacillus*, *Pedobacter*, *Corynebacterium*, *Azotobacter*, *Escherichia*) as model organisms. Experiments were run under constant substrate supply and flow-through conditions of chemostats (0.75/d) (Becks et al. 2005, Becks & Arndt 2008) at which all experimental organisms can potentially grow. Glucose was chosen as a universally consumable carbon source for the five species. Experimental conditions allowed the establishment of oscillating abundances of the bacteria populations (Becks et al. 2005). Models of competition for abiotic resources may generate fluctuations which lead to a high number of coexisting species (Huisman & Weissing 1999, Tilman 1982).

### Material and Methods

The microbial competition experiments were carried out with five different bacterial strains:

1. *Bacillus subtilis* DB020 (~2  $\mu\text{m}$  x 0.7  $\mu\text{m}$ , gram positive and rod-shaped, kindly provided by Catriona Donovan and Marc Bramkamp, University of Cologne).
2. *Pedobacter* sp. (Cytophaga Flexibacter group, ~1.5  $\mu\text{m}$  x 0.3  $\mu\text{m}$ , isolated by Kristin Beck, kindly provided by Klaus Jürgens, IOW, Warnemünde, Germany).
3. *Corynebacterium glutamicum* CDC010 (~1  $\mu\text{m}$  x 1.2  $\mu\text{m}$ , gram positive and rod-shaped, kindly provided by Catriona Donovan and Marc Bramkamp, University of Cologne).
4. *Escherichia coli* BW30270 (~2  $\mu\text{m}$  x 0.2  $\mu\text{m}$ , gram-negative, rod-shaped, kindly provided by Karin Schnetz, University of Cologne).
5. *Azotobacter vinelandii* (~ 4  $\mu\text{m}$  x 3  $\mu\text{m}$ , gram-negative, DSM No. 399, Leibniz Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH)

All species were cultivated at monoxenic conditions.

The chemostat experiments were carried out in sterile one-stage glass reactors with an average fluid content of 190 ml. All chemostat experiments were performed with the same experimental setup at the same external conditions, temperature (20 °C  $\pm$

0.2), nutrient supply and dilution rate (nutrient inflow of 0.75 per day  $\pm$  0.004). All experiments were started at the same day and with the same inoculums and inoculation density was  $10^6$  cells  $\text{ml}^{-1}$  for all organisms. As an inorganic nutrition source WC minimal medium (Guillard & Lorenzen 1972) was supplied with added glucose at a concentration of 50  $\text{mg l}^{-1}$ . Constant dilution rates were established by automated syringe pumps (Cavro XLP 6000, Modular Syringe Pump, TECAN, Crailsheim, Germany). The computer controlled sampling by a sampling robot (RSP 9000 Cavro, TECAN, Crailsheim, Germany, triplicate samples, 0.5 ml each) took place every 24 hours. All samples were fixed 1:1 in PBS-buffer (phosphate buffered saline: 8.0  $\text{gl}^{-1}$  NaCl; 0.2  $\text{gl}^{-1}$  KCl, Merck, Darmstadt, Germany; 1.42  $\text{gl}^{-1}$   $\text{Na}_2\text{HPO}_4 \times \text{H}_2\text{O}$ , Merck, Darmstadt, Germany; 0.2  $\text{gl}^{-1}$   $\text{KH}_2\text{PO}_4$ , AppliChem, Darmstadt, Germany; pH 7.4) and about 0.01  $\text{gl}^{-1}$  SDS (sodium dodecyl sulphate, Merck, Darmstadt, Germany) containing 4 % formaldehyde (Merck, Darmstadt, Germany).

Bacteria samples were analysed using epifluorescence microscopy. Samples were stained with the fluorescent dye CYBR Green I (Invitrogen, Karlsruhe, Germany, 1:5000 dilution of original stock with sterile distilled water). Staining and sample preparation was carried out using the frame spotting method (Maruyama *et al.* 2004). All bacterial strains (each at least 900 cells in 15  $\mu\text{l}$  total sample volume) were enumerated by a Zeiss epifluorescence microscope (Zeiss Axiophot, Zeiss filter set 43; BP 550/25, FT 570, BP 605/70, 1250x magnification). The chemostat samples were checked for contamination every day, during enumeration. Cell carbon content was calculated using a factor of 0.35  $\text{pgC}/\mu\text{m}^3$  suggested by Bjornsen (1985). The cell volumes were calculated from the mean of the cell lengths and widths. The dynamic behavior of the time series data of the chemostat experiments were analyzed by the largest corresponding Lyapunov exponent ( $\lambda$ ) to quantify the exponential separations of initially close trajectories (Eckmann & Ruelle 1985). Calculations were carried out with the Rtisean package (Rtisean 3.0.14, <http://www.mpipks-dresden.mpg.de/~tisean>) using the algorithm of Rosenstein *et al.* (1993) (embedding dimension:  $m = 1, \dots, 6$ ; number of iterations in time: 15). For evaluation of the delay, the autocorrelation function (ACF) and mutual information revealed different reconstruction delays between 1 – 3. The transient data points at the beginning of the experiments (day 1-10) were always omitted for the analyses. The Lyapunov exponent was estimated from the slope of a straight line fitted to the

linear part of the ln-transformed divergence of the data. The values for the Lyapunov exponents are given for the embedding dimension  $m = 4$ . The corresponding Lyapunov exponents were calculated for the time series data of *Acinetobacter*, *Pedobacter*, *Corynebacterium*, *E. coli*, and *Azotobacter*. Positive estimates of the Lyapunov exponent point to chaotic like irregular dynamics, whereas negative values indicate coexistence at equilibrium.

## Results

The competition experiments with either two, three, four or five species showed a coexistence of all species for the whole experimental period (Fig. 1). Replicated chemostats showed no identical patterns of maxima and minima. Variability of abundances on the basis of calculated coefficients of variation did not significantly increase with the number of coexisting species (Fig. 1, appendix). The analysis of the dynamic behavior of the tested systems showed an overall similar chaotic pattern within all experiments through all populations (see Tab. 1, appendix). There were surprisingly large differences in the Lyapunov exponents regarding the dynamic behavior of single species in the two replicates of one and the same treatment (see Tab. 1, appendix). This becomes evident when one draws the abundance of one species at time  $t$  against the abundance of that species at time  $t+1$  and this in the succession for consecutive values (see phase-space diagrams in Figure 2). Attractor regions are not only changing for one species when it is confronted with competition to different species (as a model species *Bacillus subtilis*, Figure 2a) but also, when individual species are considered in one system (as an example the system with five species is shown for the two replicates (Fig. 2b). According to theory (Turchin 2003), patterns are not repeated. The different attractors occupied by one species (Fig. 2a) or by different species (Fig. 2b) indicate that species interactions are changing from one replicate to the other and between experimental set ups. This illustrates that populations are embedded in a changing world of complex interactions between the species.

A fundamental question in environmental sciences is whether or not the diversity in a system is related to its productivity. In our experimental chemostat systems, the bacteria production in cells per milliliter and day was significantly positively related to the number of species present (Fig. 3a).

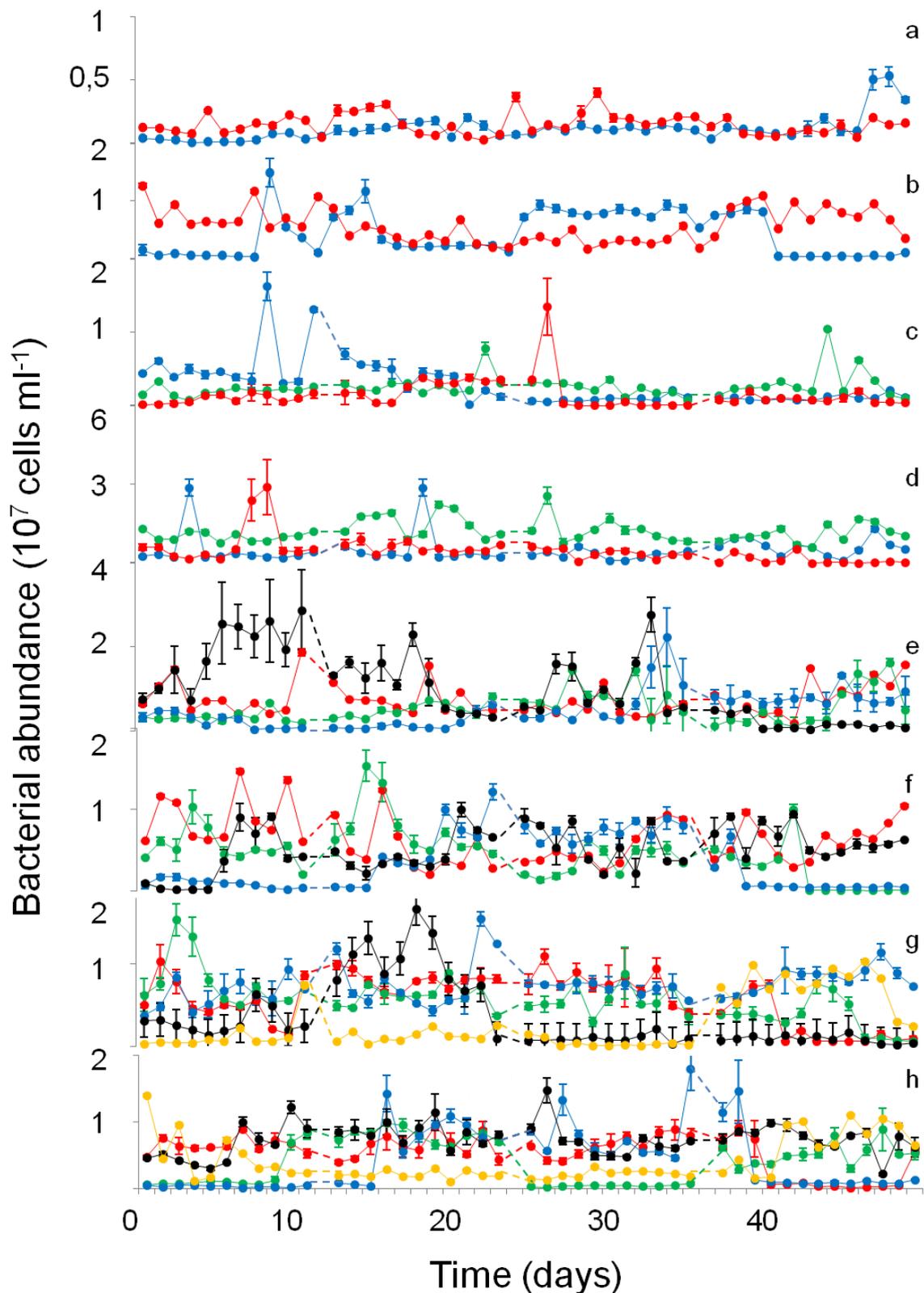


Figure 1. Time series data for the competition experiment with *Pedobacter spec.*, *Bacillus subtilis*, *Corynebacterium glutamicum*, *Escherichia coli* and *Azotobacter vinelandii*. The red lines correspond to *Bacillus*, blue lines to *Pedobacter*, green lines to *Corynebacterium*, black lines to *Escherichia*, and the yellow lines to *Azotobacter*. Vertical error bars indicate  $\pm$  SD of separately taken triplicate samples. In some cases data were missing due to technical problems with the sampling system (days 13, 24, 37) and are data points are joined by dashed lines. Two-species systems: a and b (*Pedobacter* abundance  $\times 5$ ), three-species system: c and d, four-species system: e and f, and five-species system: g and h (*Azotobacter* abundance  $\times 20$ ).

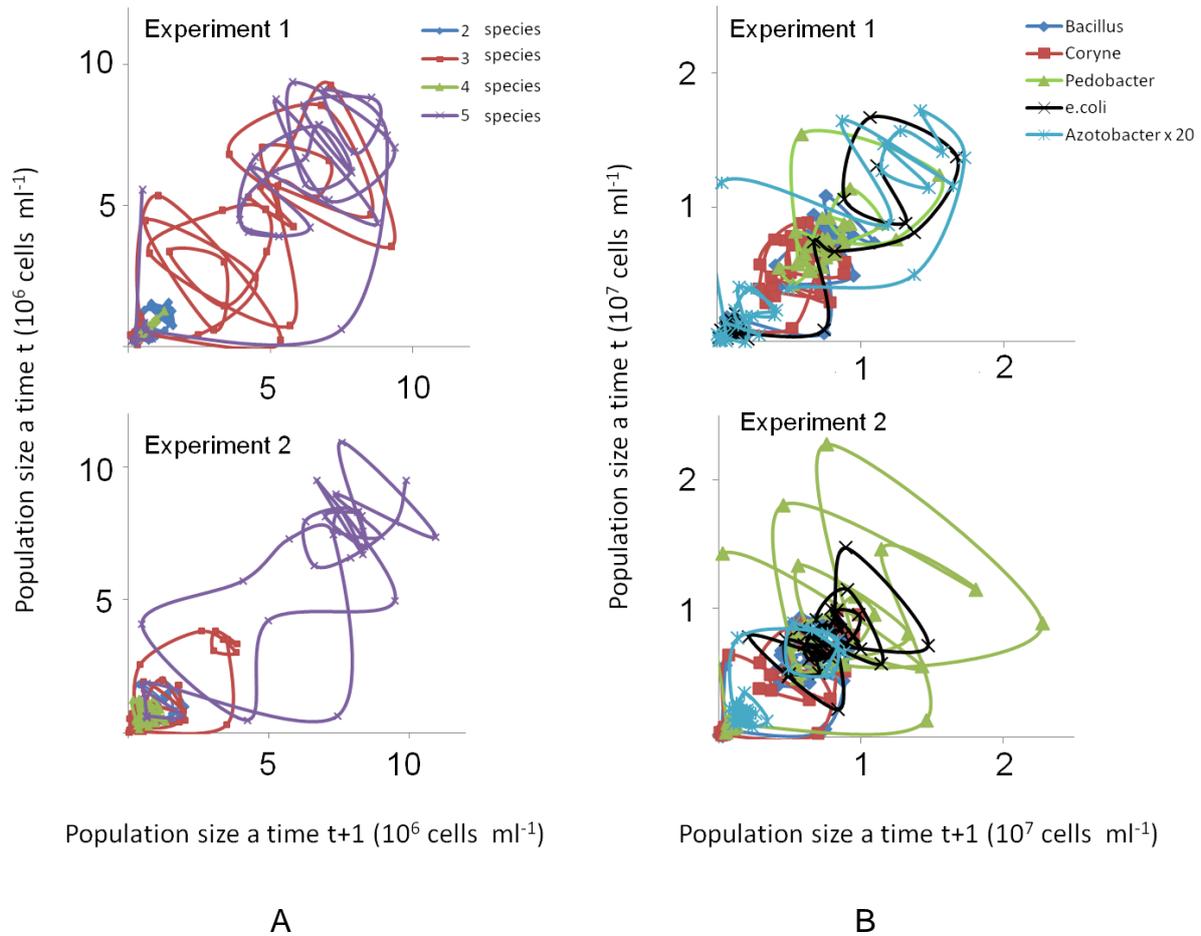


Figure 2. Time delay reconstruction (phase space) for all *Bacillus subtilis* populations within all experiments (A), and for all five species coexisting (B). Data are shown from day 12-49 and for the two replicated experiments (Experiment 1 and 2).

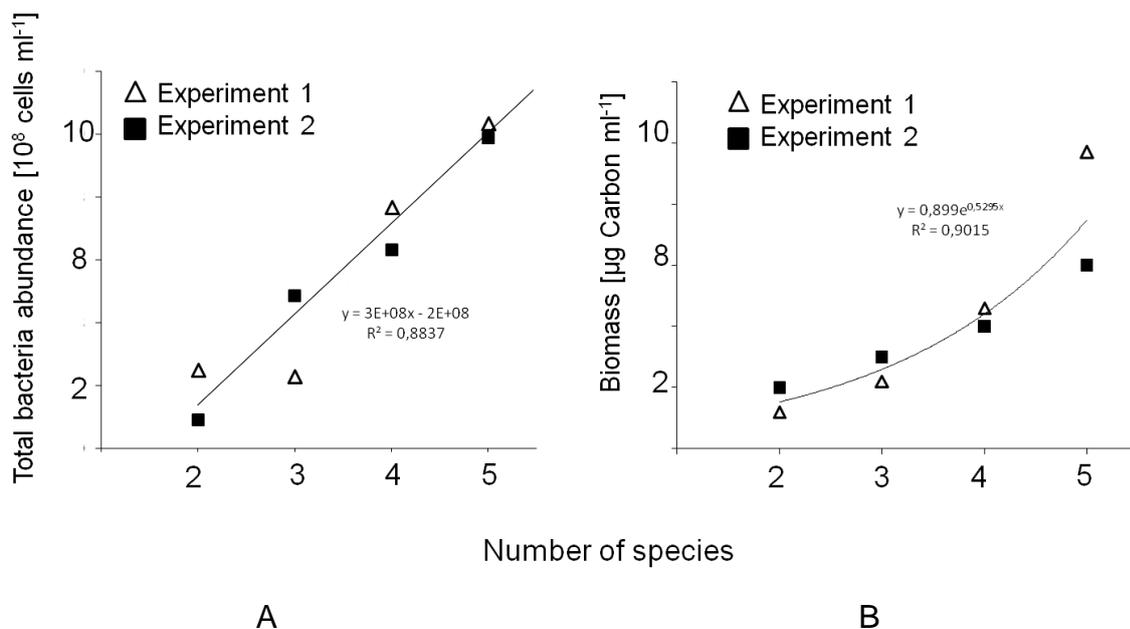


Figure 3. Analyses of total cell numbers and productivity. On the x-axis the experiments with the different species numbers are displayed whereas the y-axis shows total cell numbers (A) and total biomass (B). The triangles and the boxes show the replicated experiments.

And, the same glucose carbon supply revealed a difference in the production of particulate organic matter by a factor of five when either one or five species are present in the system (Fig. 3b).

## **Discussion**

The possibility of non-equilibrium dynamics and chaotic regimes in competition models have already been detected by several theoreticians (Gilpin 1975; May & Leonard 1975; Smale 1976; Armstrong & McGehee 1980), but experimental evidence from controlled systems was still lacking. Theoretical models of Huisman and Weissing (1999) showed that competitive interactions which generate chaos and oscillations may allow the persistence of a great diversity of competitors on only a few resources, thus even in a constant and well-mixed environment. Highly controlled experimental conditions allowed us investigating the effect of competition on the coexistence and population dynamics of up to five species. We showed that all species were able to coexist at competition for one limited resource when abundances were oscillating which could be suggested as an experimental solution of the paradox of plankton (Hutchinson 1961). A novelty in our experimental work with regard to the theoretical considerations by Huisman and Weissing (1999) is that coexistence is also possible when competitors compete for only one limited resource. All our observed microbial dynamics were characterized by a high variability of abundances in the absence of any external stimuli (Fig. 1, appendix), positive Lyapunov exponents of all species dynamics (Tab. 1, appendix), the fact that similar dynamic patterns did never occur in any of the repeated chemostats at slightly different starting conditions (Figs. 1, 2), as well as the agreement of experimental data with theoretical considerations (Huisman and Weissing 1999, 2001). All these characteristics point to deterministic chaos, though a definitive decision is difficult on the basis of the low number of data sets generally available for practical studies (Becks et al 2005, Beninca et al. 2008).

A second conclusion can be drawn from our study. We observed a significant increase of the abundance and biomass of bacteria (at the same dilution rate and with the same substrate supply) with an increasing number of species. This confirms theoretical considerations of plant diversity (Tilman et al. 1997) which have only been complemented by experimental studies on the community level (Tilman et al. 1996,

van Ruijven & Berendse 2005). Here we showed for the first time that under strictly controlled constant carbon supply and other environmental conditions, a known number of species leads to a significant increase in biomass production (Fig. 3). This increase may be explained by the complementarity effect due to slightly differing traits of the different taxa (van Ruijven & Berendse 2005). Theoretically, the productivity should approach a maximum value for a given environment (Tilman et al. 1997). Field observations indicated that productivity stays relatively constant when bacterial diversity has reached a certain level (Reinthal et al. 2005).

Here we disentangled the effect of environmental factors included in field observations from the effect of species properties. We showed that non-linear interactions are a potent mechanism to create coexistence even in a constant environment - an overlooked phenomenon at present. This important mechanism which can only be analyzed in laboratory systems may add to a number of possible other causes of diversity already identified from the field such as competitive abilities, dispersal rates in space and time, predator avoidance, and micro-evolutionary processes (Tilman 2000, Levine & HilleRisLambers 2009). All these latter mechanisms do not explain high species richness observed in constant environments with little spatial segregation (Fierer & Jackson 2005, Bonkowski & Roy 2005) which are common in environments especially for small organisms. Up to now no one was identified as the decisive cause. Finally one can argue that it is a combination of all. It explains the vast species richness observed in many different habitats (Loreau et al. 2001).

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## Appendix to chapter III

Summarized Lyapunov exponents of the time series shown in Fig.1:

Table 1: Lyapunov exponents ( $\lambda$ ) for every species within all competition experiments calculated for the time series data illustrated in Fig. 1.

Chemostat experiment	Dilutionrate ( $d^{-1}$ )	<i>Azotobacter</i>		<i>Bacillus</i>		<i>Pedobacter</i>		<i>E.coli</i>		<i>Corynebacterium</i>	
		$\lambda$	P	$\lambda$	P	$\lambda$	P	$\lambda$	P	$\lambda$	P
1a	0.75	-		$0.22 \pm 0.03$		$0.47 \pm 0.03$ **		-		-	
1b	0.75	-		$0.19 \pm 0.02$ ***		$0.16 \pm 0.02$ **		-		-	
2a	0.75	-		$0.24 \pm 0.03$ **		$0.24 \pm 0.03$ **		-		$0.27 \pm 0.04$ **	
2b	0.75	-		$0.23 \pm 0.01$ ***		$0.15 \pm 0.01$ **		-		$0.18 \pm 0.04$ **	
3a	0.75	-		$0.21 \pm 0.03$ **		$0.36 \pm 0.03$ ***		$0.27 \pm 0.04$ **		$0.27 \pm 0.01$ *	
3b	0.75	-		$0.21 \pm 0.04$ ***		$0.15 \pm 0.03$ ***		$0.30 \pm 0.04$ **		$0.13 \pm 0.03$ *	
4a	0.75	$0.35 \pm 0.009$ **		$0.25 \pm 0.007$ ***		$0.18 \pm 0.02$ **		$0.24 \pm 0.03$ *		$0.15 \pm 0.007$ ***	
4b	0.75	$0.35 \pm 0.005$ ***		$0.21 \pm 0.02$ ***		$0.30 \pm 0.02$ *		$0.18 \pm 0.02$ ***		$0.27 \pm 0.01$ ***	

Notes: Errors in the  $\lambda$  values correspond to the asymptotic errors of the fit.

\* P , 0.05; \*\* P , 0.001; \*\*\* P , 0.0001

This graph shows the analysis of coefficients of variation for all chemostat experiments:

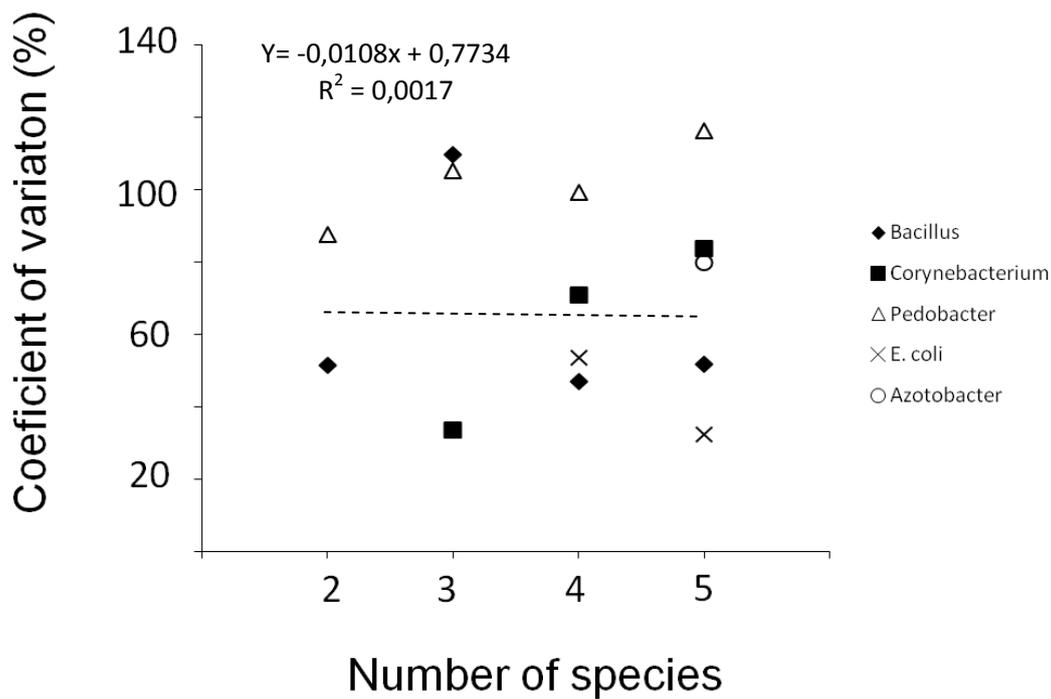


Figure 1. Coefficients of variation for all species within all experiments.

This graph shows results obtained by single species chemostat experiments with *Bacillus subtilis* at a dilution rate of 0.75 per day and a glucose concentration of 5 mg per liter where populations reach a stable equilibrium. Both graph show replicated experiments:

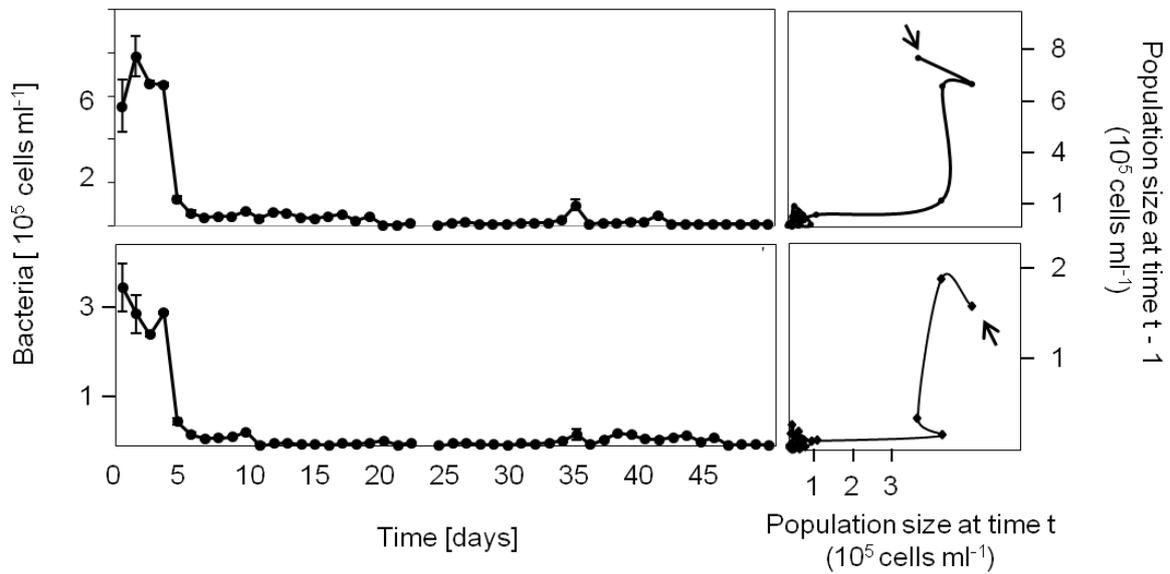


Figure 2. Time series data and time-delay reconstructions given for the corresponding values the single species systems of *Bacillus subtilis* with a glucose concentration of  $5 \text{ mg l}^{-1}$  in WC medium. Vertical error bars indicate  $\pm$  SD of separately taken triplicate samples. The black arrows indicate the starting point of the experiments.

## Discussion

The aim of this work was to shed light on different factors that support and maintain biodiversity and the coexistence of species, respectively. Highly controllable chemostat experiments on microbial systems (predator-prey system and a microbial competition system) were designed for working on the following questions: 1. Is phenotypic plasticity (grazing resistance) of one prey organism within a one-predator-two-prey system able to support and enlarge the range of coexistence of all species, 2. How do competition, predation and invasion interplay in an interacting community, and 3. Are two up to five species competing for the same resource able to coexist in a long term experiment, in accordance to the paradox of plankton (Hutchinson 1961)?

The coexistence of more species than resources available has been stated to be a paradox (Hutchinson 1961) because when species competing for the same resource one should theoretically outcompeted the other. Obviously in worlds' oceans as the largest habitats where essential resources are scarce the high species richness is still perplexing. In nature, competition is an important interaction between species and populations (Chase et al. 2002, HilleRisLambers & Dieckmann 2003, Chesson & Kuang 2008). In the beginning of the last century, it was mathematically shown by Vito Volterra (1928), that the coexistence of two or more species is limited by the same resource is impossible. An early experimental study by Gause (1934, 1932), was able to support that finding. Working with two species of *Paramecium* and with two species of *Saccharomyces* competing for the same resource he was able to show, that in constant environmental parameters, the competition of two species for the same resource leads to the extinction of one interaction partner Gause (1934, 1932). This phenomenon is called the 'Competitive exclusion principle' or 'Gause's law' (Gause 1934). In the present work I addressed the question, whether two up to five bacterial species are able to coexist competing for one limiting resource under highly controlled experimental conditions.

The results of the competition experiments showed that up to five species were able to coexist while competing for 'one' limiting resource. Up to now, this is the first experimental proof as there are no comparable results found in literature. Ayala (1969) showed theoretically and experimentally that two *Drosophila* species competing for limited resources and space were able to coexist. Theoretical analyses by Huisman and Weissing (1999, 2001) revealed that more species than resources

available can persist which was mediated by population oscillations, and regarding population dynamics entering a chaotic regime. In the present study, this was verified by a high variability in species abundances (oscillations) and positive Lyapunov exponents. Additionally, there was evidence that the systems productivity increases significantly in correlation with the number of species present in the experiments. The theoretical proof of the experimental results is still ongoing.

A second experimental setup consisting of a one predator-two-prey system was established to investigate the effect of phenotypic plasticity on the species coexistence at different dynamic conditions (increasing flow rates). Monsonís (2010) and Heckmann (2009) were able to show theoretically that predator induced phenotypic plasticity (grazing resistance) of one prey bacterium enlarged the range of possible coexistence of all species compared to a system where no phenotypic plasticity was present. Corresponding to that model I designed an experiment where

one bacterium (*Acinetobacter johnsonii*) was able to form grazing resistant growth forms which was triggered by the ciliate predator (*Tetrahymena pyriformis*). Compared with the results obtained by Becks et al. (2005, and unpublished data) who worked with a system consisting of the ciliate predator *Tetrahymena pyriformis*, the prey bacteria *Pedobacter sp.* and *Brevundimonas sp.* wherein both were always present as single cells, I was able to show experimentally that the phenotypic response of *Acinetobacter* is probably the cause of the enlarged range of species' coexistence which was supported by our corresponding mathematical analyses. The results obtained by Becks et al. (2005) showed distinct dynamical behaviors, like stable equilibrium, stable limit cycles and chaos. Such behaviors were shown theoretically in the early 70's by Robert May (1974), who focused on intrinsic driven patterns and who showed mathematically that by changing one control parameter (which was the growth rate 'r'), the logistic growth equation exhibits complex dynamic behaviors. For high values of growth rates he found chaos, which was the starting point from which chaos became a research subject for ecologists.

Ongoing from this, the meaning of chaos for population dynamics began to be controversially discussed and still is in debate. The analyses of the dynamic behavior of the experimental systems investigated here give a hint that all populations enter a regime of chaos indicated by 1. Positive Lyapunov exponents, 2. A high variability in abundances, and 3. Unrepeatable patterns with regard to population dynamics.

Furthermore, chaos is characterized by sensitivity to initial conditions (Hastings et al. 1993), which means that diminutive differences in starting values of systems lead to different (chaotic) fluctuations over time (Ellner & Turchin 2005). The occurrence of chaotic population cycles has been shown experimentally for the flour beetle *Tribolium castaneum* (Costantino et al. 1995, Dennis et al. 1997), holarctic microtine rodents (Hansson & Henttonen 1985, Hanski et al. 1993, Falk et al. 1995), voles and lemmings (Stenseth & Ims 1993, Falk et al. 1995), insects (e.g. Dahl & Peckarsky 2002), bacteria populations (Graham et al. 2007) and also in aquatic communities (Beninca et al. 2008). Hanski et al. (1993) pointed out that such results of chaotic fluctuations could be affected by nonlinear ecological interactions. Not only the extrinsically driven forces are affecting the dynamic behavior but also the intrinsically driving forces play an important role. Populations can show different asymptotic or long-term behaviors such as stable equilibria (point attractors), and cyclic or chaotic behavior due to the strength of intrinsic mechanisms (May 1975).

Further, the interplay between extrinsic and intrinsic factors affecting system dynamics and result in spatial and/or temporal population variations (May 1974, Ellner & Turchin 1995, Hastings 2001, Henson et al. 2003). Besides that, the interplay between grazing and competition can be suggested as an additional stabilizing factor in addition to phenotypic plasticity. Stabilizing effects of predation and competition within food webs have often been described (Hofbauer & Sigmund 1989). Moreover, according to the experimental results by Verschoor et al. (2004b), inducible defenses prevent from high amplitude population fluctuation in bi- and tritrophic food chains that may cause an extinction of a population

This work also focused on the interplay of competition, predation and invasion in a simple microbial food web with phenotypic plasticity in one prey. Under highly controllable experimental conditions it was shown that different morphological appearances of one prey can result in different population reactions by means of changing abundances within the replicated experiments. This was detected in the competition experiments wherein small single cell forms act as better competitors than filamental growth forms (Wagner et al. 2006, Young 2008), as well as in the predator-two-prey experiments where grazing protected (inedible) morphotypes were present. The introduction of another bacterium as an invader (*Azotobacter vinelandii*) revealed that there was an apparent reaction of *Acinetobacter* and

*Pedobacter* by means of significantly decreasing abundances. This effect is probably due to additional competition caused by *Azotobacter*. Moreover, it could be suggested that *Azotobacter* is more efficient in exploiting the present resource and therefore is the better competitor. Up to now, effects such as mentioned above have already been described in literature dealing with invasion (Sax et al. 2002, Clavero & Garcia-Berthou 2005).

Recapitulating the present work, I was able to show that the coexistence of species and thus biodiversity is maintained by several (intrinsic) factors and mechanisms. Phenotypic plasticity within the interaction of species was shown to be a fundamental driver of coexistence in the one-predator-two-prey systems, which was detected in the experiments as well as in the corresponding model analyses (Chapter I). Further, within the interplay of competition, predation and invasion there are effects of the composition and complexity of species communities and the morphological appearance of species on species abundances. This was revealed by changing attractors in time delay reconstructions (Chapter II).

Finally, the microbial competition experiments (Chapter III) strikingly showed, that non-linear interactions are a potent mechanism supporting coexistence even in a very constant environment. According to theory, the productivity of the systems increased with the number of present species (Tilman et al. 1997).

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**Abstract**

Biodiversity and the coexistence of species have puzzled and fascinated biologists since decades and is a hotspot in today's natural sciences. Preserving this biodiversity is a great challenge as habitats and environments undergo tremendous changes like climate change and the loss of natural habitats, which are mainly due to anthropogenic influences. The coexistence of numerous species even in homogeneous environments is a stunning feature of natural communities and has been summarized under the term 'paradox of plankton'. Up to now, there are several mechanisms discussed, which may contribute to local and global diversity of organisms. Several interspecific trade offs have been identified maintaining the coexistence of species like their abilities regarding competition and predator avoidance, their capability to disperse in space and time, and their ability to exploit variable resources. Further, micro-evolutionary dynamics supporting the coexistence of species have been added to our knowledge, and deriving from theoretical deterministic models, non-linear dynamics which describe the temporal fluctuation of abundances of organisms. Whereas competition and predation seem to be crucial structural elements within interacting organisms, the intrinsic dynamic behavior – by means of temporal changes in abundance - plays an important role regarding coexistence within a community.

The present work sheds light on different factors affecting the coexistence of species using experimental microbial model systems consisting of a bacterivorous ciliate as the predator and two bacteria strains as prey organism. Additionally, another experimental setup consisting of two up to five bacteria species competing for one limiting resource was investigated. Highly controllable chemostat systems were established to exclude extrinsic disturbances.

According to theoretical analyses I was able to show - experimentally and theoretically - that phenotypic plasticity of one species within a microbial one-predator-two-prey food web enlarges the range of possible coexistence of all species under different dynamic conditions, compared to a food web without phenotypic plasticity. This was accompanied by non-linear (chaotic) population dynamics within all experimental systems showing phenotypic plasticity.

The experiments on the interplay of competition, predation and invasion showed that all aspects have an influence on species coexistence. Under undisturbed controlled conditions all aspects were analyzed in detail and in combination. Populations showed oscillations which were shown by quasi-chaotic attractors in phase space diagrams.

Competition experiments with two up to five bacteria species competing for one limiting resource showed that all organisms were able to coexist which was mediated by species oscillations entering a regime of chaos. Besides that fact it was found, that the productivity (biomass) as well as the total cell numbers – under the same nutrition supply – increased by an increasing number of species in the experimental systems.

Up to now, the occurrence of non-linear dynamics in well controlled experimental studies has been recognized several times and this phenomenon seemed to be more common in natural systems than generally assumed.

## Kurzzusammenfassung

Biodiversität und die Koexistenz von Arten fasziniert und verblüfft Biologen seit Jahrzehnten und stellen einen Schwerpunkt in der heutigen Umweltforschung dar. Der Schutz und die Konservierung dieser Mannigfaltigkeit stellen eine große Herausforderung dar, da die natürlichen Lebensräume sowie die Umwelt enormen Veränderungen unterworfen sind, welche meist in einem anthropogenen Ursprung wurzeln. Die Koexistenz vieler Arten, auch in relativ homogenen Habitaten ist ein faszinierendes Charakteristikum natürlicher Lebensgemeinschaften und wird als ‚Paradox des Planktons‘ bezeichnet. Gegenwärtig werden diverse Ursachen diskutiert, welche vermutlich zur lokalen und globalen Diversität von Organismen beitragen. Einige dieser möglichen Ursachen, die zur Aufrechterhaltung der Koexistenz der Arten beitragen, wurden identifiziert: Das Vermögen der Konkurrenz- und Prädationsvermeidung, die Fähigkeit räumlicher sowie zeitlicher Verteilung, sowie das Vermögen variable Ressourcen zu nutzen. Des Weiteren wurden mikro-evolutionäre Phänomene und Dynamiken identifiziert, sowie, von theoretischen deterministischen Modellen ausgehend, nichtlineare Dynamiken, welche die zeitlichen Schwankungen der Abundanzen von Organismen beschreiben. Diese Aspekte stellen die Schlüsselkomponenten zwischen interagierenden Organismen dar, wobei das intrinsische, nicht lineare dynamische Verhalten in Form von zeitlichen Veränderungen in Abundanzen eine zusätzliche entscheidende Rolle bezüglich der Koexistenz von Arten spielen kann.

Einige dieser Aspekte wurden in der vorliegenden Arbeit untersucht. In Anlehnung an theoretische Analysen konnte experimentell sowie theoretisch gezeigt werden, dass phänotypische Plastizität in einer Bakterienart in einem mikrobiellen Ein-Räuber-zwei-Beute-Nahrungsgewebe den Bereich der möglichen Koexistenz unter sich ändernden experimentellen Bedingungen (Änderungen der Durchflussraten der Chemostate) – im direkten Vergleich zu einem experimentellen Nahrungsgewebe ohne phänotypische Plastizität – erweitern kann. Dies wurde begleitet durch nicht lineare Abundanzschwankungen in den Populationen aller untersuchten Versuchsansätze.

In weiteren Untersuchungen wurde das Zusammenspiel von Konkurrenz, Prädation und Invasion in einer experimentellen mikrobiellen Gemeinschaft untersucht. Unter kontrollierten Bedingungen konnten diese Aspekte detailliert untersucht werden und es konnten Aufschlüsse darüber gewonnen werden, welche Reaktionen (Interaktio-

nen) innerhalb der untersuchten Gemeinschaften stattfinden. Im Versuchsverlauf wurden Veränderungen in den Abundanzen sowie chaotische Schwankungen der Zellzahlen festgestellt.

In Konkurrenzexperimenten von zwei bis zu fünf um eine limitierende Ressource konkurrierende Bakterienarten konnte gezeigt werden, dass alle Arten – vermittelt durch chaotische Abundanzschwankungen – nebeneinander koexistieren konnten. Begleitend dazu wurde herausgefunden, dass die Produktivität (Biomasse) sowie die Gesamtzellzahl bei gleicher Nahrungsverfügbarkeit der experimentellen Systeme mit steigender Artenzahl zunehmen.

Gegenwärtig ist das Auftreten von Chaos in gut kontrollierten experimentellen Studien vereinzelt beobachtet worden, wobei dieses Phänomen jedoch häufiger in der Natur aufzutreten scheint als generell vermutet.

Köln, den 23. April 2012

## **Erklärung**

Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit - einschließlich Abbildungen und Tabellen - die anderen Werken im Wortlaut oder dem Sinn nach entnommen habe, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie noch nicht veröffentlicht worden ist sowie, dass ich solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde.

Die Bestimmungen dieser Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Dr. Hartmut Arndt betreut worden.

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Andre Schieffer