Mechanisms and consequences of change in aquatic microfauna communities

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CONTENTS

FOREWORD

This dissertation is a cumulative work of the following publications (either published, submitted or in preparation):

Busse AB, Antiqueira PAP, Neutzling AS, Wolf AM, Romero GQ, Petermann JS (2018). Different in the dark: The effect of habitat characteristics on community composition and beta diversity in bromeliad microfauna. *PLoS ONE*, 13 (2): e0191426.

Busse AB, Schoreisz JJ, Petermann JS (*submitted to OIKOS, 29th July 2018*). Predators and priority effects shape microfauna communities in a community transplantation experiment along an elevational gradient.

Busse AB, Schoreisz JJ, Wallner L, Srivastava DS, Petermann JS (*in preparation*). Micro- and macrofauna communities in bromeliad phytotelmata are similar in forest canopy and understory.

Busse AB, Adler Yañez MA, Petermann JS (*in preparation*). Variability in environmental conditions between elevational gradients leads to diverse patterns in microfauna community structure and beta diversity.

GENERAL INTRODUCTION

Community-level research

Biodiversity and ecosystem functions are nowadays catch phrases and their importance for us humans is generally accepted. How they should be maintained is less clear and scientists only agree on the fact that more research is needed to answer this question. Particularly under the aspect of human-induced environmental changes, the future development of biodiversity and ecosystem functions is little predictable (Loreau et al. 2001). It has been demonstrated that environmental change and biodiversity loss affect ecosystem functioning interactively and that interactions can be multifaceted (Pires et al. 2018). The problem is that these interactive effects take place on the community level and that it is normally logistically impossible to include every part of a community in a research study. Community-level research has recently been recognized to be the next step to better predict the effects of environmental changes on biodiversity and ecosystem functions (Suding et al. 2008; Reiss et al. 2009). The use of new model ecosystems such as phytotelmata (i.e. plant-held waters) or ponds make whole-community approaches possible (Srivastava et al. 2004; Meester et al. 2005). In this thesis we used aquatic microfauna communities living in the leaf axils of bromeliad plants to study the processes that structure communities and how the communities are affected by environmental changes.

The bromeliad system

Some species of the Neotropical family *Bromeliaceae* form water-retaining tanks with their leaves (Kitching 2000). Bromeliads can accumulate several litres of rainfall and stemflow water (Cogliatti-Carvalho et al. 2010) divided into small pools between the leaf axils. These water compartments are occupied by a variety of organisms (Picado 1913). Small bacteria, fungi, algae, protozoa, rotifers, nematodes, crustaceans as well as insects and frogs spend part of their life stages or even their entire life span in the bromeliad tank (Kitching 2000). Besides, many taxa from the surrounding environment use the bromeliad water as drinking reservoir or prey on the inhabitants (Bicca-Marques 1992; Romero and Srivastava 2010). This micro-ecosystem in the bromeliads is mainly based on allochthonous leaf litter input as resource base for the inherent food web (Farjalla et al. 2016). The leaf litter is decomposed by detrivores such as bacteria, ciliates, oligochaetes and insect larvae (e.g. chironomids) (Santos et al. 2009; Weisse 2017; Goffredi et al. 2011). The detrivorous organisms are preyed upon by bigger organisms, e.g. mosquito larvae filter free-swimming protozoa such as ciliates from the water (Addicott 1974) and predatory insect larvae (e.g.

damselfly larvae) prey upon smaller insect larvae (Srivastava and Bell 2009). This results in a multi-trophic aquatic food web in a micro-ecosystem with distinct spatial borders making bromeliads the perfect model ecosystem to study whole communities in their natural environment (Srivastava et al. 2004). One of the first detailed studies of the bromeliad-inhabiting fauna was done by Picado (1913). Since then the system has gained attention. However, the investigation of microfauna living in bromeliads has started only recently (Petermann et al. 2015; Kratina et al. 2017; Carrias et al. 2001; Foissner et al. 2003) with the former focus being on macrofauna such as insect larvae (Srivastava 2006; Richardson 1999).

Microfauna

The term microfauna in this study refers to protists such as diatoms, flagellates, ciliates and amoeba as well as rotifers and micro-crustaceans inhabiting the bromeliad tanks. Though they perform basic ecosystem functions in the bromeliad ecosystem, e.g. decomposition and nutrient cycling, very little is known about them and the processes that structure their communities. Many studies from other systems suggest that microfauna communities respond differently to environmental changes than macrofauna communities (Finlay et al. 2004). So, how are we going to predict the effect of environmental changes on natural communities if we are missing information of an important trophic level? This thesis aims to narrow the knowledge gap and suggests further research ideas to provide additional information necessary for the implementation of microfauna communities in the assessment of ecosystem health, because ‘landscapes are healthy when […] the key ecological components are preserved, e.g. […] microfauna […]’ (Rapport et al. 1998).

Study area and sampling

In total, we carried out three field surveys along different types of environmental gradients. Based on the suitability of the field sites, the field survey along the canopy cover gradient was carried out in Brazil and the other two field surveys along gradients of elevation and height were carried out in Costa Rica. The field site in Brazil was Cardoso Island (25°03’S, 48°53’W) situated at the south coast of Sao Paulo State, Brazil, where bromeliads grew abundant on ground level in restinga rainforest (Fig. 1A). In Costa Rica we worked in three different field sites within the Área de Conservación Guanacaste, which were characterized by secondary and primary rainforests, particularly cloud forests in higher elevations (Fig 1B). We took samples along elevational gradients near the field stations ‘Pitilla’ (N 10°59.374’, W 85°25.583’) (Fig 1B+C), ‘Cacao’ (N 10°56.009’, W 85°27.787’) and ‘Santa Maria’ (N 10°48.060’, W 85°19.681’). The survey along the height gradient and a field experiment took place in the vicinity of the field station 'Pitilla'. All field surveys and the field experiment were carried out during the wet seasons (2013-2015). In all approaches we measured a variety of environmental factors depending on the research question in each study (e.g. measurement of oxygen saturation and pH in Costa Rica: Fig 1D). Our experimental setup used bromeliad plants in their natural environment as containers of experimentally transplanted microfauna communities (Fig 1E). Bromeliads high up in the canopy were sampled by use of the single-rope climbing technique (Fig 1F). Microscopic analyses of microfauna communities were all done at 400 x magnification (Fig 1G).

Thesis outline

The central topic of this thesis was to investigate community structuring processes in a bromeliad-inhabiting microfauna community and to determine what happens to these communities if their environment changes. This study was carried out with the fast environmental changes in mind that are momentarily caused by us humans, e.g. climate change, habitat destruction etc., and which will undoubtedly affect and already are affecting biodiversity and ecosystem functioning. This contemporary environmental challenge requires a profound understanding of the processes involved to prevent further biodiversity loss and to develop effective conservation strategies. Here, we studied community structure along several types of environmental gradients to get an overview of the effect of changes in different environmental factors.

In **chapter 1** we investigate microfauna community structure along a canopy cover gradient. This survey focusses on difference in sun-exposure and how this (directly or indirectly) affects microfauna structure. We suggest that bromeliads with higher sun-exposure provide less stable and therefore lower-quality habitats. We hypothesize that community similarity decreases with environmental distance and that beta diversity will change according to shifts in the relative importance of stochastic versus deterministic community structuring processes.

In **chapter 2** we present the results of a community transplantation experiment along an elevational gradient under natural conditions. The aim of the experimental approach was to disentangle effects of environment and trophic interactions on microfauna community structure. We used a full-factorial experimental design to particularly address potential interactions (e.g. between trophic interactions such as resource competition and predation or between environmental change and trophic interactions).

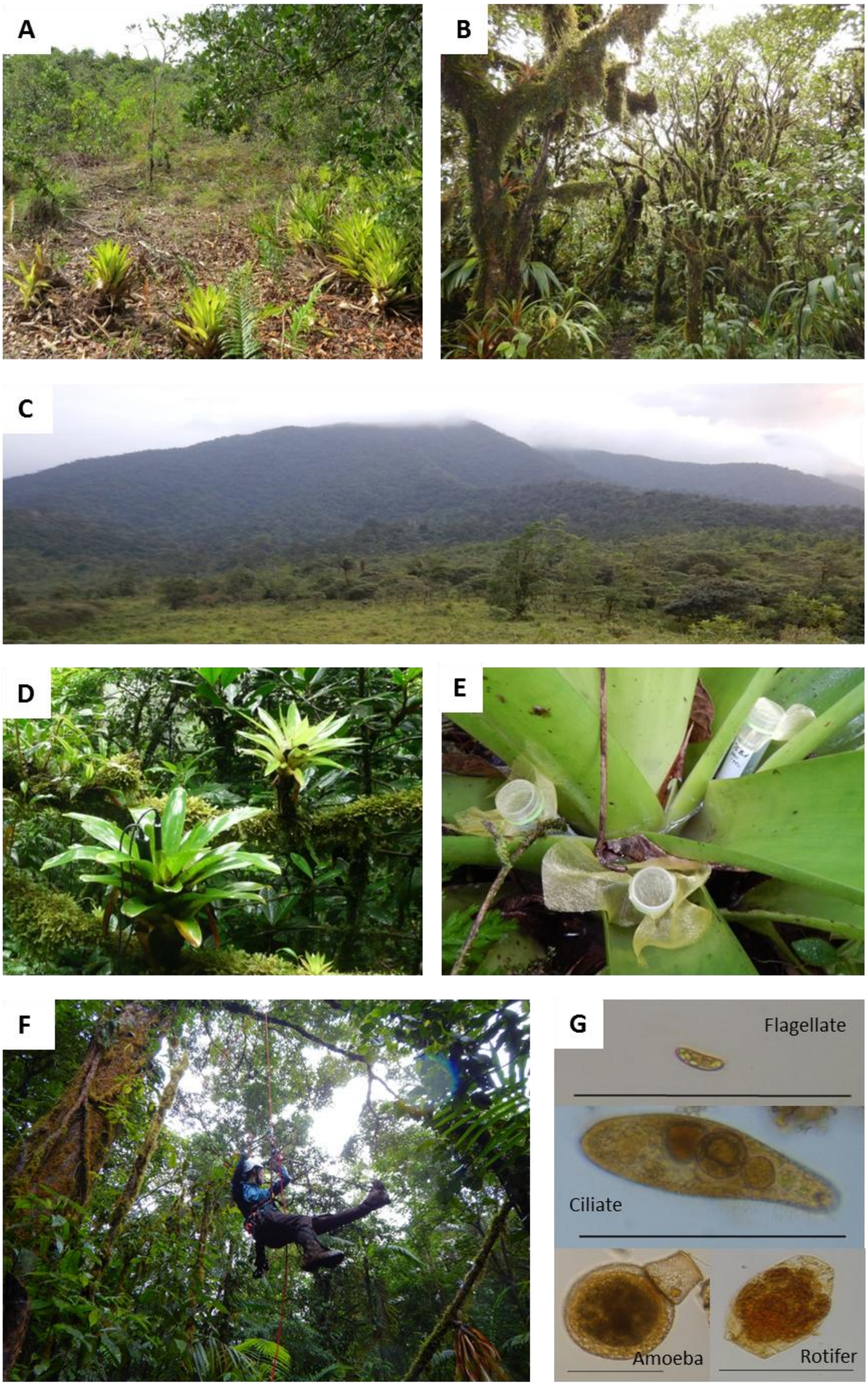

Figure 1: A - Sun-exposed bromeliads in restinga rainforest on Cardoso Island, Brazil. B – Cloud forest at mountain Orosilito (field site 'Pitilla'), Costa Rica. C – Field site Pitilla and surrounding area in Costa Rica. D – Measurement of abiotic environmental parameters (oxygen saturation and pH) in tank bromeliads in Costa Rica. E – Bromeliad as vessel for experimental tubes of a transplantation experiment. F – Single-rope climbing technique to sample canopy bromeliads. G – Selected microfauna organisms inhabiting bromeliads in Costa Rica. Scale bar: 100 µm. Copyright by Pablo A. P. Antiqueira (A) and Annika Busse (B-G).

In **chapter 3** we compare the microfauna communities of canopy and understory bromeliads. Following up on the canopy cover gradient in chapter 1, we assume that environmental conditions change along a height gradient in forest ecosystems based on increasing sun-exposure with increasing height. This comparison was meant to question the exclusive use of understory bromeliads in many bromeliad-related studies.

In **chapter 4** we explore the elevational gradients of three different mountains in terms of naturally occurring patterns in microfauna community structure. This chapter adds information on regional scale to the findings in chapter 2 by providing evidence for the generalizability of community structure patterns found in the field.

In the end follows a general discussion which summarizes the results in a broader context including suggestions for future research questions and applications.

References

Addicott, J. F. 1974. Predation and Prey Community Structure: An Experimental Study of the Effect of Mosquito Larvae on the Protozoan Communities of Pitcher Plants. - Ecology 55: 475–492.

Bicca-Marques, J. C. 1992. Drinking behavior in the black howler monkey (Alouatta caraya). - Folia Primatologica 58: 107–111.

Carrias, J.-F. et al. 2001. A preliminary study of freshwater protozoa in tank bromeliads. - J. Trop. Ecol. 17: 611–617.

Cogliatti-Carvalho, L. et al. 2010. Water volume stored in bromeliad tanks in Brazilian restinga habitats. - Acta Botanica Brasilica 24: 84–95.

Farjalla, V. F. et al. 2016. Terrestrial support of aquatic food webs depends on light inputs: a geographically-replicated test using tank bromeliads. - Ecology 97: 2147–2156.

Finlay, B. J. et al. 2004. Protist diversity is different? - Protist 155: 15–22.

Foissner, W. et al. 2003. Endemic ciliates (Protozoa, Ciliophora) from tank bromeliads (Bromeliaceae): a combined morphological, molecular, and ecological study. - European journal of protistology 39: 365–372.

Goffredi, S. K. et al. 2011. Aquatic microbial habitats within a neotropical rainforest: bromeliads and pH-associated trends in bacterial diversity and composition. - Microbial ecology 61: 529–542.

Kitching, R. L. 2000. Food webs and container habitats. The natural history and ecology of phytotelmata. - Cambridge University Press.

Kratina, P. et al. 2017. Environmental control of the microfaunal community structure in tropical bromeliads. - Ecology and Evolution 7: 1627–1634.

Loreau, M. et al. 2001. Biodiversity and ecosystem functioning: current knowledge and future challenges. - Science 294: 804–808.

Meester, L. de et al. 2005. Ponds and pools as model systems in conservation biology, ecology and evolutionary biology. - Aquatic Conserv: Mar. Freshw. Ecosyst. 15: 715–725.

Petermann, J. S. et al. 2015. Resources alter the structure and increase stochasticity in bromeliad microfauna communities. - PloS one 10: e0118952.

Picado, C. 1913. Les broméliacées épiphytes considérées comme milieu biologique. - Bulletin Scientifique de la France et de la Belgique 47: 215-360.

Pires, A. P. F. et al. 2018. Interactive effects of climate change and biodiversity loss on ecosystem functioning. - Ecology 99: 1203–1213.

Rapport, D. J. et al. 1998. Evaluating landscape health: integrating societal goals and biophysical process. - Journal of Environmental Management 53: 1–15.

Reiss, J. et al. 2009. Emerging horizons in biodiversity and ecosystem functioning research. - Trends in ecology & evolution 24: 505–514.

Richardson, B. A. 1999. The Bromeliad Microcosm and the Assessment of Faunal Diversity in a Neotropical Forest1. - Biotropica 31: 321–336.

Romero, G. Q. and Srivastava, D. S. 2010. Food-web composition affects cross-ecosystem interactions and subsidies. - The Journal of animal ecology 79: 1122–1131.

Santos, R. L. et al. 2009. Survey of Invertebrates Associated with Bromeliads in a Conservation Unit of the Brazilian Atlantic Rain- forest, and its Relevance for Environmental Risk Studies. - Journal of the bromeliad society 59: 241–288.

Srivastava, D. S. et al. 2004. Are natural microcosms useful model systems for ecology? - Trends in ecology & evolution 19: 379–384.

Srivastava, D. S. 2006. Habitat structure, trophic structure and ecosystem function: interactive effects in a bromeliad-insect community. - Oecologia 149: 493–504.

Srivastava, D. S. and Bell, T. 2009. Reducing horizontal and vertical diversity in a foodweb triggers extinctions and impacts functions. - Ecology letters 12: 1016–1028.

Suding, K. N. et al. 2008. Scaling environmental change through the community-level: a trait-based response-and-effect framework for plants. - Global Change Biol 14: 1125–1140.

Weisse, T. 2017. Functional diversity of aquatic ciliates. - European journal of protistology 61: 331–358.

CHAPTER 1

Different in the dark: The effect of habitat characteristics on community composition and beta diversity in bromeliad microfauna

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Abstract

The mechanisms which structure communities have been the focus of a large body of research. Here, we address the question if habitat characteristics describing habitat quality may drive changes in community composition and beta diversity of bromeliad-inhabiting microfauna. In our system, changes in canopy cover along an environmental gradient may affect resource availability, disturbance in form of daily water temperature fluctuations and predation, and thus may lead to changes in community structure of bromeliad microfauna through differences in habitat quality along this gradient. Indeed, we observed distinct changes in microfauna community composition along the environmental gradient explained by changes in the extent of daily water temperature fluctuations. We found beta diversity to be higher under low habitat quality (low canopy cover) than under high habitat quality (high canopy cover), which could potentially be explained by a higher relative importance of stochastic processes under low habitat quality. We also partitioned beta diversity into turnover and nestedness components and we found a nested pattern of beta diversity along the environmental gradient, with communities from the lower-quality habitat being nested subsets of communities from the higher-quality habitat. However, this pattern resulted from an increase in microfauna alpha diversity with an increase in habitat quality. By providing insights into microfauna-environment relationships our results contribute to the mechanistic understanding of community dynamics in small freshwater bodies. Here, we highlight the importance of habitat characteristics representing habitat quality in structuring communities, and suggest that this information may help to improve conservation practices of small freshwater ecosystems.

Introduction

Detailed information about an ecosystem and its structuring processes is crucial for development of effective and sustainable conservation strategies for biodiversity maintenance (Heywood, ed. 1995). Especially freshwater ecosystems, which hold a high proportion of species, are experiencing unprecedented declines in biodiversity (Dudgeon et al. 2006) and are in need of suitable conservation measures (Kalinkat et al. 2017). Earlier studies have shown that parameters like disturbances (Cowell et al. 1987), toxic substances (Hanazato 1998) and spatial connectivity (Jackson et al. 2001) can potentially affect freshwater community composition. Also, several likely drivers of species diversity, e.g. elevation (Richardson et al. 2000) and acidity (Fryer 1980), and their abundances, e.g. resource availability (Naeem 1990) and predation (Sanders and Wickham 1993), have been identified. More recently, it has been noted that for effective conservation the distribution of biodiversity in space has to be taken into account (Socolar et al. 2016). Therefore, the drivers of differences in community composition along spatial or environmental gradients have come into the focus of ecological research (Al-Shami et al. 2013; Brendonck et al. 2015; Shade et al. 2008; Tonkin et al. 2016).

In general, community similarity is assumed to decrease with larger environmental or spatial distances between communities (Anderson et al. 2011). The magnitude of differences in community composition is commonly measured as beta diversity (Anderson et al. 2011; Tuomisto 2010a, 2010b; Whittaker 1960). For example, high beta diversity indicates large differences in composition among local communities within a habitat. Previous research demonstrated that beta diversity can depend on a number of different processes. For example, it can be reduced by strong competitive exclusion (Segre et al. 2014), i.e. conditions where deterministic processes dominate. Beta diversity can also be increased in case of dispersal limitation (Martiny et al. 2011; Wang et al. 2016) or when high rates of random extinction and immigration events led to distinct demographic stochasticity (Arellano and Halffter 2003; Segre et al. 2014). Thus, changes in beta diversity seem to be observed when different types of community-structuring processes change in their relative importance, with a high relative importance of deterministic processes potentially leading to smaller beta diversity and vice versa (Chase et al. 2009; Johnston et al. 2016).

The current knowledge of the conditions under which deterministic versus stochastic processes dominate in their relative importance and affect beta diversity is still ambiguous. For example, harsh environmental conditions, i.e. low habitat quality, may, on the one hand, reduce beta diversity due to strong environmental filtering (Chase 2007) or, on the other hand, increase beta diversity due to dispersal limitation (Jacobsen and Dangles 2012). It has been long known that environmental harshness (or habitat quality) is a key factor in driving community composition by affecting assembly and maintenance processes (Chase 2007; Peckarsky 1983). However, the contradictory evidence described above indicates that the ecological mechanisms governing the natural patterns of beta diversity remain to be explained. Here, we provide further insights into beta diversity disparities by conducting the first study on drivers of microfauna beta diversity in small freshwater bodies along a habitat quality gradient.

We use freshwater microhabitats found in tank bromeliads. These natural microcosms constitute useful model systems for testing various questions in ecology because they are relatively small and easily sampled micro-ecosystems with clear boundaries that can be measured in their entirety (Blaustein and Schwartz 2001; Richardson et al. 2000; Srivastava et al. 2004). Bromeliad-inhabiting communities are per definition metacommunities which “are linked by dispersal of multiple potentially interacting species” (Leibold et al. 2004) and are therefore especially suitable to address research questions related to patterns of community composition along spatial or environmental gradients. Moreover, bromeliads can occur in high densities (Kitching 2000; Williams 1987) which allows for many replicates under comparable environmental conditions. The pools between the leaf axils of water-collecting tank bromeliads are typically colonized by a variety of aquatic organisms. They comprise many different taxa of protists, small metazoans and insect larvae which form a food web based on decomposing leaf litter that falls from the canopy in the tank (Petermann et al. 2015; Srivastava 2006).

We investigate bromeliads along a canopy cover gradient in restinga forest in Brazil. Strong but variable impacts of canopy cover on bromeliad-inhabiting microfauna and invertebrate communities have been observed in former studies (Kratina et al. 2017; Rangel et al. 2016; Serramo Lopez and Iglesias Rios 2001). With our study we aim to identify the canopy-cover related factors that affect bromeliad-inhabiting microfauna communities. The more open sites have lower densities of trees and thus, the bromeliads are exposed to direct sunlight. The more forested sites have a higher density of trees and thus, constitute a more shaded habitat for the bromeliads, thereby potentially providing higher resource amounts for the bromeliad microfauna in terms of greater leaf litter input (Farjalla et al. 2016; de Omena). The addition of these resources has experimentally been shown to favour flagellates and ciliates over algae and amoebae and thus may lead to a shift in community composition (Petermann et al. 2015).

Another likely difference between the bromeliads along the canopy cover gradient, which results from the degree of exposedness to the sun, is the daily variation in water temperature. Daily fluctuations in water temperature are expected to decrease with increasing canopy cover and could potentially affect microfauna richness (Kratina et al. 2017). It has been shown that temperature fluctuations in general and their strength in particular can affect species coexistence and thus diversity (Jiang and Morin 2007; Montagnes and Weisse 2000). As a further difference between communities of different canopy cover, the abundance of protist-feeding mosquito larvae is known to be much higher in sun-exposed bromeliads than in shaded bromeliads (*P.A.P. Antiqueira & G.Q. Romero unpublished data*). This predation by unselective filter-feeders might also influence community composition, e.g. through predator-mediated coexistence (Holt 1984).

In short, bromeliad microcosms may vary along the canopy cover gradient in three major aspects (Fig 1). First, an increase in canopy cover leads to an increase in resource availability. Second, an increase in canopy cover leads to a decrease in solar radiation and thus to less pronounced daily temperature fluctuations. Third, an increase in canopy cover is accompanied by a decrease in predation pressure. Thus, an increase in canopy cover is accompanied by a number of favourable circumstances (e.g. sufficient resource availability, more constant environmental conditions and less predation) which result in less environmental stress and more advantages for the bromeliad-inhabiting microfauna. Hence, the canopy cover gradient likely constitutes a gradient of habitat quality for bromeliad-inhabiting microfauna communities with harsher conditions (lower habitat quality) in more sun-exposed sites and more benign conditions (higher habitat quality) in more forested sites. We use this habitat-quality gradient to study how habitat characteristics may affect community structure, specifically alpha diversity, community composition, beta diversity (Fig 1) and the beta-diversity components nestedness and turnover.

We hypothesize that:

1) Community composition of bromeliad-inhabiting microfauna decreases in similarity with increasing environmental distance along the canopy cover gradient. These differences in community composition are driven by environmental variables that are directly or indirectly related to canopy cover.
2) Beta diversity of bromeliad microfauna changes along the canopy cover gradient due to differences in habitat quality. Whether beta diversity increases or decreases with increasing habitat quality, i.e. with increasing canopy cover, may depend on the relative importance of different types of coexistence processes (i.e. stochastic versus deterministic processes). Thus, we formulate two contrasting expectations (see also Fig 1).
    - *a.* Beta diversity increases with increasing habitat quality, suggesting strong environmental filtering in the harsher environment and higher dispersal limitation in the more benign environment.
    - *b.* Beta diversity decreases with increasing habitat quality, suggesting higher demographic stochasticity and/or dispersal limitation in the harsher environment and stronger competitive exclusion in the more benign environment.

Methods

Study site and system

Samples were taken on Cardoso Island at the south coast of Sao Paulo State, Brazil (25° 03’S, 48°53’W), in September 2013 at the beginning of the wet season. Cardoso island is characterized by mean annual temperatures between 20 and 22°C and mean annual rainfall of 2250 mm (Pessenda et al. 2012). Relative humidity is over 66% in spring (= sampling season of our study) in restinga habitats (Manoel and Mota 2012). Our study was carried out in the northern part of the island within an area of 4.5 km extension. The study site was situated in restinga rainforest, a type of Atlantic rainforest on coastal dunes (Rizzini 1997). On Cardoso Island, restinga rainforest show different vegetation and abiotic conditions along a canopy cover gradient. Less forested restinga (i.e. more sun-exposed habitats for bromeliads and their microfauna communities) and more forested restinga (i.e. more shaded habitats for bromeliads and their microfauna communities). In the less forested habitat, shrub vegetation (maximum 4 m high) is distributed in patches containing lianas with sun-exposed areas between these patches. In the more forested habitat trees range from 6 to 8 m height and may form a relatively continuous canopy cover. Bromeliad density was higher in the more forested restinga (*personal observation*).

Microfauna communities were sampled from water-filled leaf axils of these bromeliad plants. Plant-held waters are commonly referred to as phytotelmata, of which bromeliads constitute only one possible type (Kitching 2000). Tank bromeliads occur almost exclusively in the Neotropics growing on ground level or as epiphytes on branches or trunks. Their funnel-shaped leaf morphology with numerous leaf compartments captures water from above (i.e. rainwater or stem flow) and falling leaf litter from the canopy. Aquatic decomposers such as protozoa and nematodes break down the leaf litter and make the nutrients available for other organisms in the leaf compartment pool and the bromeliad plant. Furthermore, decomposers are prey to various predators within the tank, including larger protozoa, rotifers and insect larvae. This study focuses on microfauna communities including organisms of the size class 5-200 µm such as protozoa (including flagellates, ciliates and amoebae) and rotifers. Bromeliad tanks can occur in high densities in the tropical rainforest holding up to 50,000 L water/hectare (Williams 2006). As such, bromeliad tanks constitute valuable freshwater habitats in the tropics, and may provide important ecological functions, amongst others by being the main breeding ground for semiaquatic insects. Interspecific interactions are not confined to the aquatic bromeliad tank but include the surrounding terrestrial environment because bromeliads provide drinking water reservoirs and preying grounds for many species (Bicca-Marques 1992; Cestari and Pizo 2008; Nadkarni and Matelson 1989). Last but not least, bromeliad microcosms can contain endemic species which are highly adapted to the phytotelm environment thus enhancing species diversity by providing ecological niches (Dunthorn et al. 2012; Foissner et al. 2003). Apart from their ecological importance, bromeliads provide valuable model systems for community research and questions related to the metacommunity concept.

Experimental design

In a stratified random sampling design including four different sites with more sun-exposed or more forested restinga rainforest we selected 78 tank bromeliads of the species *Quesnelia arvensis* Mez. (Bromeliaceae*)* growing on ground level. We collected similar-sized bromeliads (total water volume, mean ± SE: 1386 ± 106 mL) to reduce the effect of habitat size on the studied communities.

Sampling

Portable digital thermometer data loggers (Thermochron® iButton® device - DS1921G) were added to all bromeliads prior to sampling to register the water temperature variation of each bromeliad. From these recorded temperature data three different temperature measurements were calculated: average water temperature, maximum water temperature and coefficient of variation of water temperature (calculated for a time frame of 23 hours). The three variables were strongly correlated (Pearson's correlation: $p \leq 0.001$ for all correlation pairs). To avoid multicollinearity in our analyses, we chose one of the three variables for further analyses: the coefficient of variation, which we considered to be the most representative temperature measurement. The canopy cover was determined for each bromeliad by analysing canopy photos with the program ImageJ (Schneider et al. 2012). Furthermore, during sampling a set of parameters, representing potentially important abiotic and biotic environmental drivers of microfauna community composition, were measured for each bromeliad. First, the number of water-filled bromeliad leaf compartments was counted and bromeliad diameter [cm] and vertical height [cm] were measured. Dissolved oxygen concentration [%] and pH were measured in the field using a multiparameter handheld meter (cyberscan PD 650, Oaklon®). Furthermore, a water sample was collected to analyze turbidity [NTU = nephelometric turbidity unit], chlorophyll a concentration [µg/L], carbon dissolved organic matter (CDOM) [ppb] and ammonium concentration [µM] using a handheld fluorometer (AquaFluor®). Afterwards, to survey the microfauna, a 1 mL water sample per bromeliad was taken from a leaf compartment halfway between the central and outermost leaf compartments and fixed with Lugol's solution. Microfauna were counted as morphotypes for 50 µL of each sample using light microscopy (400 x magnifications). Moreover, the abundance of mosquito larvae per bromeliad was counted in a sample of 17-100mL water (depending on the available volume; mean +/- SE: 80 +/- 2 mL) and the number of mosquitos/100mL was calculated. Total water volume was determined for each bromeliad by extracting all the water.

The study did not involve endangered or protected species. Sampling was carried out under permit 23689-1 issued by Instituto Chico Mendes de Conservação da Biodiversidade.

Statistical analysis

Pairwise dissimilarities and singleton removal

To detect the dissimilarity index best suited to describe our abundance-based data, we performed a preliminary rank index analysis (Faith et al. 1987) using the R package *vegan* (Oksanen et al. 2015). The Bray-Curtis dissimilarity index was identified as the most suitable and was used to obtain an abundance-based dissimilarity matrix for further analyses.

We tested the effect of singleton removal by comparing non-metric multidimensional scaling ordinations (NMDS) with 20 random starts for data sets with and without singletons. Three different definitions of "singletons" were tested according to Poos and Jackson (Poos and Jackson 2012): singletons are defined as species that occur i) in only one site (in our case 3 morphospecies were removed), ii) in less than 5 % of sites (11 morphospecies were removed) and iii) in less than 10 % of sites (17 morphospecies were removed). Procrustes correlation analysis (999 permutations) was used to identify the significance of the congruence between the ordinations with singleton removal and the ordination on the complete data set. None of the three singleton removal strategies showed a significant difference for the community composition (Procrustes correlation coefficient > 0.95, p-value < 0.001 for all three comparisons). Therefore, no singletons were removed prior to statistical analysis.

Community composition and environment

The environmental variables that we measured were tested for multicollinearity. Decisions to remove redundant predictors were based on a combination of correlation coefficients, cluster analysis and biological relevance. After reduction of redundant environmental variables all statistics were done using the following seven of the originally thirteen environmental variables: canopy cover, number of leaf compartments, coefficient of variation of water temperature, pH, turbidity, dissolved oxygen concentration and mosquito larvae abundance.

To determine if community composition changes along the canopy cover gradient we calculated a distance decay plot. It tests for pairwise dissimilarities along an environmental gradient, whereby according to our hypothesis 1) an increase in the difference of canopy cover was expected to result in increasing Bray-Curtis dissimilarity values due to increasing differences in environmental conditions. This relationship was tested with a multiple regression on distance matrices (Legendre et al. 2005; Lichstein 2007) (using the MRM function in the R package *ecodist* (Goslee and Urban 2007)) that is based on permutation tests of significance (999 permutations).

To further investigate which of the canopy cover-related factors drive community composition in particular, we carried out a distance-based redundancy analysis (dbRDA), i.e. a constrained version of principal coordinates analysis (PCoA) (Legendre and Anderson 1999) using measured environmental variables that are related to canopy cover changes (for details on the relationship between environment variables and canopy cover see S1 Figure). The statistical significances of the overall model and single model terms were tested with permutation tests.

Beta diversity

There are many possibilities to measure beta diversity and none of these is perfect (Ricotta 2010). To address the question if beta diversity depended on habitat quality we chose to use multivariate dispersion as a measure of beta diversity (Anderson et al. 2006). Because this analysis can only compare different levels of a categorical variable, we grouped the samples along our continuous canopy cover gradient into two groups (based on the median), the bromeliads in the more sun-exposed habitat, representing the low-quality (harsh) habitat, and the bromeliads in the more shaded habitat, representing the high-quality (benign) habitat. Each group contained 39 bromeliads of the 78 measured in total. For each bromeliad we calculated beta diversity as distance to group centroid based on a Bray-Curtis dissimilarity matrix by using the R function “betadisper” (R package *vegan* (Oksanen et al. 2015)). The calculated distances to group centroid of the two habitats were then compared using a linear model. To correct for a high influence of alpha diversity on patterns in beta diversity, a Raup-Crick null model (Chase et al. 2011) was applied and the resulting matrix was used to calculate differences in beta diversity.

When beta diversity is calculated using pairwise Sørensen dissimilarity, it can be partitioned into two components: turnover (replacement of species by other species in different sites) and nestedness (species loss or gain between sites) (Baselga et al. 2013; Baselga 2010). To identify if differences in community composition were mainly due to species turnover or nestedness, beta diversity was partitioned using the R package *betapart* (Baselga et al. 2013). To assess whether the results for the turnover and nestedness components were greater than expected by chance, we used a null model with 10000 permutations. The null matrix was constrained by the “r1”-method (Patterson and Atmar 1986) which maintains the row frequencies and uses column marginal frequencies as probabilities of selecting species. This method is based on z-scores with positive z values indicating a higher than expected contribution of the turnover or nestedness component.

To quantify the total degree of nestedness along the canopy cover gradient a NODF metric (nestedness measure based on overlap and decreasing fills) was applied (Almeida-Neto et al. 2008; Ulrich 2009). We used the nestedrank-function in the R package *bipartite* (Dormann et al. 2008) to calculate the nestedness rank of communities along the canopy cover gradient. A high rank indicates a more nested community. We used a linear model to test for the effect of canopy cover on nestedness rank. As nestedness is a result of species loss we also tested if alpha diversity changes along the canopy cover gradient using a linear model. Resulting from this, we repeated the first linear model, testing for the effect of canopy cover on nestedness rank with alpha diversity as a co-variable fitted before canopy cover, to differentiate between the effect of alpha diversity and canopy cover on nestedness rank.

All statistical analyses were done in R version 3.0.2 (R Core Team 2013) using the packages *vegan* (Oksanen et al. 2015), *betapart* (Baselga et al. 2013), *bipartite* (Dormann et al. 2008) and *ecodist* (Goslee and Urban 2007).

Results

Abundance and alpha diversity

A total of 35 morphotypes of microfauna were identified from our samples, including flagellates (15 morphotypes), ciliates (9 morphotypes), amoebae (4 morphotypes) and rotifers (7 morphotypes). For a detailed description of morphotypes see S1 Table. On average, flagellates had the highest alpha diversity per bromeliad (2.5 morphotypes ± 0.2 SE/50 µl), followed by ciliates (2.0 morphotypes ± 0.1 SE/50 µl), rotifers (1.5 morphotypes ± 0.1 SE/50 µl) and amoebae (0.6 morphotypes ± 0.1 SE/50 µl). Flagellates also had the highest mean abundance (227 individuals ± 123 SE/50 µL) followed by ciliates (40 individuals ± 8 SE/50 µL), rotifers (6 individuals ± 1 SE/50 µL) and amoebae (5 individuals ± 2 SE/50 µL). Alpha diversity significantly increased with higher canopy cover (Linear model: $F_{1,76}$ = 7.8, p = 0.007, Fig 2). Alpha diversity was not significantly related to any other explanatory variable measured in this study. Log-transformed total microfauna abundance was not related to canopy cover (Linear model: $F_{1,76}$ = 2.1613, p = 0.1455).

Community composition and environment

We investigated if a linear relationship existed between distance in environmental conditions (i.e. canopy cover) and the dissimilarity of communities measured using the Bray-Curtis index. We found that with increasing differences in canopy cover bromeliad microfauna communities became more dissimilar (MRM: $R^2 = 0.026$, $p = 0.001$, Fig 3A). So, community composition changed gradually along the canopy cover gradient. To identify the environmental factors through which changes in canopy cover affected community composition a distance-based RDA was applied (dbRDA model: $F_{70,6} = 1.5$, $p < 0.001$, Fig 3B). The model returned daily fluctuations in water temperature (represented by the coefficient of variation of water temperature), the number of leaf compartments and pH as significant drivers of community composition while the other environmental variables did not show significant effects on community composition (Table 1). We also used raw abundance of mosquito larvae and total water volume per bromeliad as co-variables in the analysis. However, this did not change the results.

Beta diversity

Beta diversity, measured as distance to group centroid, was found to be higher in the sun-exposed (harsh) habitat than in the shaded (benign) habitat (Linear model $F_{1,76} = 10.1$, $p = 0.002$, Fig 4A). This means that communities in the sun-exposed habitat were less similar among each other than the communities in the shaded habitat. However, we repeated the comparison of beta diversity between the two habitats after applying the Raup-Crick null model to the community matrix to correct for differences in alpha diversity (ANOVA, $F_{1,76} = 0.1$, $p = 0.74$, Fig 4B) and found that the significant difference in beta diversity can be explained exclusively by differences in alpha diversity.

To identify the mechanisms that cause potential patterns in beta diversity, we partitioned beta diversity into its two components, turnover and nestedness. A null model analysis showed that the relative importance of nestedness was significantly higher than expected (Fig 5). To further investigate if canopy cover is related to the nestedness component a nestedness rank analysis was applied. Nestedness rank showed a significantly negative relationship with canopy cover (Linear model: $F_{1,76} = 10.737$, $p = 0.0016$). This indicates that communities became less nested with an increase in canopy cover. Using alpha diversity as a co-variable in the model showed that differences in alpha diversity explained the relationship of canopy cover and nestedness rank (Linear model, alpha diversity: $F_{1,75} = 388.5$, $p < 2*10^{-16}$, canopy cover fitted after alpha diversity: $F_{1,75} = 2.8$, $p = 0.098$).

Discussion

The aim of this study was to investigate changes in bromeliad-inhabiting microfauna community composition and their community-structuring processes based on habitat characteristics along a canopy cover gradient. We found that community similarity declines with increasing environmental distance, thus supporting our first hypothesis. The amount of change in community composition, i.e. the beta diversity, differed along the canopy cover gradient, confirming our second hypothesis. The observed differences in beta diversity were linked to differences in alpha diversity. We related this finding to the change in habitat quality along the canopy cover gradient, which seems to lead to a change in the relative importance of different community assembly and maintenance processes.

Community composition and environment

We hypothesized that an increase in environmental distance would lead to an increase in microfauna community dissimilarity along a canopy cover gradient. We found this increase in dissimilarity along the canopy cover gradient and by using null model analyses we showed that these changes were not random. We further hypothesized this change to be driven by environmental variables that change along the canopy cover gradient. Despite the fact that pH, dissolved oxygen concentration, daily fluctuations in water temperature, turbidity, mosquito larvae density and number of bromeliad leaf compartments changed along the canopy cover gradient, only daily fluctuations in water temperature, pH and number of leaves affected microfauna community composition. As the effect of the latter two parameters was only marginal (see Table 1), we focus on explaining the impact of daily fluctuations in water temperature on microfauna communities in the subsequent paragraphs. It may just be mentioned here that plant architectural complexity (in our case number of leaf compartments) can be used as a proxy for habitat heterogeneity (Tews et al. 2004) which is known to have potential effects on arthropod community composition in phytotelmata (Gonçalves-Souza et al. 2011; Naeem 1990) and that pH has been observed to affect only particular functional groups (e.g. amoeba) of bromeliad-inhabiting microfauna (Kratina et al. 2017). We suspect that habitat heterogeneity is not as important for microfauna organisms as for arthropods because microfauna organisms cannot actively rotate among the leaf compartments and we assume that the effect of pH on microfauna community is marginal because only a small part of the community is affected by it.

It has been previously observed that higher daily fluctuations in water temperature can be found in more sun-exposed bromeliads and it was suspected that this can possibly affect the survival of inhabiting taxa (Laessle 1961). However, fluctuations in temperature are generally considered to be of minor importance to bromeliad-inhabiting species (Weisse et al. 2013a, 2013b). Reasons why the importance of daily temperature fluctuations has been neglected so far could be that many former studies on bromeliad-inhabiting fauna only investigated seasonal temperature changes and not daily temperature fluctuations (Marino et al. 2011; Mestre et al. 2001). Although we found a relatively high change in water temperature during the day for bromeliads exposed to direct sunlight (fluctuations up to 21°C), a recent work from Costa Rica suggests that local microfauna richness (i.e. alpha diversity) peaks at a relatively narrow range of temperatures (23-25°C) (Kratina et al. 2017). This indicates that the bromeliad freshwater habitat is especially challenging for thermally sensitive taxa and could explain the increase in alpha diversity with increasing canopy cover and the related decrease in temperature fluctuations. Besides, we suspect that daily water temperature fluctuations in bromeliad microcosms are even more pronounced in the dry season, when water volume is smaller due to higher evaporation and time of direct exposition to sunlight is longer due to cloudless skies. This means that effects of temperature fluctuations on microfauna communities observed in this study were potentially even more distinct if the samples would have been taken during the dry season.

In general, freshwater ecosystems - especially the smaller ones - are considered particularly vulnerable to changes in climate (Woodward et al. 2010), meaning that a permanent increase in daily temperature fluctuations, e.g. by intensified weather conditions through climate change, could lead to a reduction in species richness in bromeliad micro-ecosystems due to an increase in environmental harshness as known from other studies (Marks et al. 2016; Staddon et al. 1998; Whittaker et al. 2001). A loss in species richness could have cascading effects throughout the food web and might also affect ecosystem functioning by loss of entire functional groups (Petchey et al. 1999).

Beta diversity

We hypothesized that beta diversity, i.e. the magnitude of differences in community composition, of bromeliad-inhabiting microfauna changes along the canopy cover gradient, for example due to changes in the relative importance of different community-structuring processes with habitat quality (Fig 1). Indeed, we found a change from higher beta diversity in the harsher, more sun-exposed habitat to lower beta diversity in the benign, more shaded habitat, along with a contrasting pattern in alpha diversity. The application of the Raup-Crick null model demonstrated that the pattern in beta diversity was caused by changes in alpha diversity along the habitat quality gradient. The increase of microfauna alpha diversity with an increase in habitat quality caused a distinct nestedness pattern in beta diversity. However, we could not determine the environmental variables that were responsible for the change in alpha diversity between habitats; none of our measured variables showed an effect on alpha diversity.

We found that microfauna communities from the harsher habitat were nested subsets of communities from the more benign habitat. Increasing nestedness with declining habitat quality has been previously observed for birds (Fernández-Juricic 2002) and gastropods (Bloch et al. 2007; Hylander et al. 2005); to the best of our knowledge this is the first report of the pattern for microfauna. We found no indication that the nestedness pattern was caused by species loss from a particular microfauna group (flagellates, ciliates, amoebae, rotifers).

While alpha diversity decreased with declining habitat quality, beta diversity simultaneously increased. This increase in beta diversity in harsher environmental conditions can possibly be explained by an increase in the relative importance of stochastic processes such as demographic stochasticity and dispersal limitation (Arellano and Halffter 2003; Chase and Myers 2011; Jacobsen and Dangles 2012).

Based on the decline in alpha diversity with increased environmental harshness we suspect that our microfauna metacommunities were subject to source-sink dynamics (Holt 1984; Pulliam 1988) with the benign habitat providing constant immigrants of microfauna to the harsher habitat. Stochastic immigration events from the benign to the harsh environment and random extinctions, e.g. through short-time droughts or a reduction in habitat size (i.e. water volume) caused by higher temperature fluctuations, would then account for the reduced alpha diversity and higher beta diversity in the harsher habitat. Additionally, higher dispersal limitation within the harsher habitat would add to explaining the less homogeneous distribution of species and thus the higher beta diversity. Higher dispersal limitation in the harsher habitat could be partly due to lower bromeliad density compared with the more benign habitat (*personal observation*).

The higher dispersal limitation in the harsher habitat can possibly also be explained by the mode of dispersal. Microfauna organisms are passive dispersers either transported via wind or animals (Maguire, JR. 1963; Revill et al. 1967; Rogerson and Detwiler 1999; Schlichting, JR. and Sides 1969). Wind dispersal requires the formation of cysts and the exposition of the cysts to wind by, for example, a complete desiccation of the bromeliad tank. However, not all microfauna species are capable of forming cysts and the bromeliads hardly ever dry up completely (*personal observation*). Therefore, the dispersal of microfauna cysts via wind is unlikely, especially over larger spatial scales (Foissner 2006; Horváth et al. 2016). On the other hand, dispersal of aquatic organisms via animal agents has been commonly observed (Serramo Lopez et al. 1999; Vanschoenwinkel et al. 2011). In case of animal dispersal being the predominant dispersal mode, the confinement of animal activity to the more protected forested area could entail increasing dispersal limitation towards the exposed area, which would then explain its higher beta diversity. A possible increasing isolation of bromeliad tanks caused by higher dispersal limitation in the harsher, sun-exposed habitat (caused by a lower density of bromeliads and/or a lower activity of animals acting as dispersal agents) plus the lower alpha diversity in the harsher habitat are coherent with a finding by Chase and Myers (Chase and Myers 2011). They stated that isolation and low alpha diversity are accompanied by an increase in the relative importance of stochastic processes such as ecological drift, random extinctions and chance colonization. Thus, we could confirm prediction b) of our second hypothesis stating that beta diversity decreases with increasing habitat quality (Fig 1) in our system. However, Chase (2007) found the opposite with environmental harshness (in his case drought) favouring the relative importance of deterministic processes (in his case strong environmental filtering) over stochastic processes. We suspect that the identity of the investigated taxa plays a major role in determining which coexistence mechanisms operate because fundamental differences can be observed in the survival strategies (e.g. active or passive dispersal) of smaller versus larger organisms (Astorga et al. 2012; Farjalla et al. 2012; Finlay et al. 2004; Nemergut et al. 2013). Such taxa-dependent difference in the relative importance of coexistence mechanisms add to the complexity of conservation strategies and highlight the importance of clear conservation aims and the awareness of potential side effects for other taxa.

Conclusion

With this study we could show that habitat characteristics describing habitat quality play an important role in structuring bromeliad-inhabiting microfauna communities, presumably through changes in the relative importance of stochastic versus deterministic processes. We observed that the extent of daily fluctuations in water temperature is a driving force of microfauna community composition and that a loss in alpha diversity with decreasing habitat quality leads to a nested pattern in beta diversity. This interlinking of alpha and beta diversity resulting in contrasting patterns in harsh versus benign habitats shows that community structure and community-structuring processes should be studied with attention to detail particularly when communities function as metacommunities. This is especially important when investigating communities with a conservation concern. So far, there is little effort in conserving microfauna (Cotterill et al. 2008; Esteban and Finlay 2010). However, these organisms are definitely understudied, even though they provide fundamental ecosystem functions and are the base of the food web. Our analyses add to the mechanistic understanding of community dynamics in an increasingly used model system and can thus contribute to future theoretical and empirical studies.

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References

Almeida-Neto, M. et al. 2008. A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. - Oikos 117: 1227–1239.

Al-Shami, S. A. et al. 2013. Drivers of beta diversity of macroinvertebrate communities in tropical forest streams. - Freshwater Biology 58: 1126–1137.

Anderson, M. J. et al. 2006. Multivariate dispersion as a measure of beta diversity. - Ecol Letters 9: 683–693.

Anderson, M. J. et al. 2011. Navigating the multiple meanings of β diversity. A roadmap for the practicing ecologist. - Ecology Letters 14: 19–28.

Arellano, L. and Halffter, G. 2003. Gamma diversity: derived from and a determinant of Alpha diversity and Beta diversity. An analysis of three tropical landscapes. - Acta Zoologica Mexicana 90: 27–76.

Astorga, A. et al. 2012. Distance decay of similarity in freshwater communities. Do macro- and microorganisms follow the same rules? - Global Ecology and Biogeography 21: 365–375.

Baselga, A. 2010. Partitioning the turnover and nestedness components of beta diversity. - Global Ecology and Biogeography 19: 134–143.

Baselga, A. et al. 2013. betapart: Partitioning beta diversity into turnover and nestedness components. R package version 1.3. http://CRAN.R-project.org/package=betapart.

Bicca-Marques, J. C. 1992. Drinking Behavior in the Black Howler Monkey (Alouatta caraya). - Folia Primatol 58: 107–111.

Blaustein, L. and Schwartz, S. S. 2001. Why study ecology in temporary pools? - Israel Journal of Zoology 47: 303–312.

Bloch, C. P. et al. 2007. Effects of Large-Scale Disturbance on Metacommunity Structure of Terrestrial Gastropods: Temporal Trends in Nestedness. - Oikos 116: 395–406.

Brendonck, L. et al. 2015. Hydrological stability drives both local and regional diversity patterns in rock pool metacommunities. - Oikos 124: 741–749.

Cestari, C. and Pizo, M. A. 2008. Utilization of epiphytes by birds in a Brazilian atlantic forest. - ORNITOLOGIA NEOTROPICAL 19: 97–107.

Chase, J. M. 2007. Drought mediates the importance of stochastic community assembly. - Proceedings of the National Academy of Sciences 104: 17430–17434.

Chase, J. M. et al. 2009. Predators temper the relative importance of stochastic processes in the assembly of prey metacommunities. - Ecology Letters 12: 1210–1218.

Chase, J. M. et al. 2011. Using null models to disentangle variation in community dissimilarity from variation in α-diversity. - Ecosphere 2: art24.

Chase, J. M. and Myers, J. A. 2011. Disentangling the importance of ecological niches from stochastic processes across scales. - Philosophical Transactions of the Royal Society B: Biological Sciences 366: 2351–2363.

Cotterill, F. P. D. et al. 2008. Conservation of protists. Is it needed at all? - Biodivers Conserv 17: 427–443.

Cowell, B. C. et al. 1987. Recolonization of Small-Scale Disturbances by Benthic Invertebrates in Florida Freshwater Ecosystems. - The Florida Entomologist 70: 1–14.

de Omena, P. M. Effects of predators on bromeliad-aquatic arthropod communities and ecosystem functioning. - Doctoral dissertation, Universidade Estadual de Campinas. 2014.

Dormann, C. F. et al. 2008. Introducing the bipartite Package:Analysing Ecological Networks. - R News 8: 8–11.

Dudgeon, D. et al. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. - Biological reviews of the Cambridge Philosophical Society 81: 163–182.

Dunthorn, M. et al. 2012. Diversity and endemism of ciliates inhabiting Neotropical phytotelmata. - Systematics and Biodiversity 10: 195–205.

Esteban, G. F. and Finlay, B. J. 2010. Conservation work is incomplete without cryptic biodiversity. - Nature 463: 293.

Faith, D. P. et al. 1987. Compositional dissimilarity as a robust measure of ecological distance - In: Prentice, I. C. and van der Maarel, E. (eds.), Theory and models in vegetation science. Springer Netherlands, pp. 57–68.

Farjalla, V. F. et al. 2012. Ecological determinism increases with organism size. - Ecology 93: 1752–1759.

Farjalla, V. F. et al. 2016. Terrestrial support of aquatic food webs depends on light inputs. A geographically-replicated test using tank bromeliads. - Ecology 97: 2147–2156.

Fernández-Juricic, E. 2002. Can human disturbance promote nestedness? A case study with breeding birds in urban habitat fragments. - Oecologia 131: 269–278.

Finlay, B. et al. 2004. Protist Diversity is Different? - Protist 155: 15–22.

Foissner, W. et al. 2003. Endemic ciliates (Protozoa, Ciliophora) from tank bromeliads (Bromeliaceae). A combined morphological, molecular, and ecological study. - European journal of protistology 39: 365–372.

Foissner, W. 2006. Biogeography and Dispersal of Micro-organisms: A Review Emphasizing Protists. - Acta Protozoologica 45: 111–136.

Fryer, G. 1980. Acidity and species diversity in freshwater crustacean faunas. - Freshwater Biol 10: 41–45.

Gonçalves-Souza, T. et al. 2011. Bromeliad architectural complexity and vertical distribution predict spider abundance and richness. - Austral Ecology 36: 476–484.

Goslee, S. C. and Urban, D. L. 2007. The ecodist Package for Dissimilarity-based Analysis of Ecological Data. - J. Stat. Soft. 22.

Hanazato, T. 1998. Response of a zooplankton community to insecticide application in experimental ponds: a review and the implications of the effects of chemicals on the structure and functioning of freshwater communities. - Environmental Pollution 101: 361–373.

Heywood, V. H., ed. 1995. Global biodiversity assessment. - Cambridge University Press.

Holt, R. D. 1984. Spatial heterogeneity, indirect interactions, and the coexistence of prey species. - The American Naturalist 124: 377–406.

Horváth, Z. et al. 2016. Wind dispersal results in a gradient of dispersal limitation and environmental match among discrete aquatic habitats. - Ecography 39: 726–732.

Hylander, K. et al. 2005. Differences in Habitat Quality Explain Nestedness in a Land Snail Meta-Community. - Oikos 108: 351–361.

Jackson, D. A. et al. 2001. What controls who is where in freshwater fish communities – the roles of biotic, abiotic, and spatial factors. - Can. J. Fish. Aquat. Sci. 58: 157–170.

Jacobsen, D. and Dangles, O. 2012. Environmental harshness and global richness patterns in glacier-fed streams. - Global Ecology and Biogeography 21: 647–656.

Jiang, L. and Morin, P. J. 2007. Temperature fluctuation facilitates coexistence of competing species in experimental microbial communities. - The Journal of animal ecology 76: 660–668.

Johnston, N. K. et al. 2016. Predator identity influences metacommunity assembly. - The Journal of animal ecology 85: 1161–1170.

Kalinkat, G. et al. 2017. Flagship umbrella species needed for the conservation of overlooked aquatic biodiversity. - Conservation biology : the journal of the Society for Conservation Biology 31: 481–485.

Kitching, R. L. 2000. Food webs and container habitats. The natural history and ecology of phytotelmata. - Cambridge University Press.

Kratina, P. et al. 2017. Environmental control of the microfaunal community structure in tropical bromeliads. - Ecology and evolution 7: 1627–1634.

Laessle, A. M. 1961. A Micro-Limnological Study of Jamaican Bromeliads. - Ecology 42: 499–517.

Legendre, P. et al. 2005. Analyzing beta diversity: Partitioning the spatial variation of community composition data. - Ecological Monographs 75: 435–450.

Legendre, P. and Anderson, M. J. 1999. Distance-Based Redundancy Analysis: Testing Multispecies Responses in Multifactorial Ecological Experiments. - Ecological Monographs 69: 1–24.

Leibold, M. A. et al. 2004. The metacommunity concept. A framework for multi-scale community ecology. - Ecology Letters 7: 601–613.

Lichstein, J. W. 2007. Multiple regression on distance matrices. A multivariate spatial analysis tool. - Plant Ecol 188: 117–131.

Maguire, B., JR. 1963. The Passive Dispersal of Small Aquatic Organisms and Their Colonization of Isolated Bodies of Water. - Ecological Monographs 33: 161–185.

Manoel, M. C. and Mota, I. Z. 2012. Análise da zona de transição da vegetação da restinga e do mangue a partir de parametros microclimáticos: estudo de caso da Ilha do Cardoso - Cananéia, SP. - Revista Geonorte 3: 734–746.

Marino, N. A. C. et al. 2011. Habitat size determine algae biomass in tank-bromeliads. - Hydrobiologia 678: 191–199.

Marks, C. O. et al. 2016. Tree diversity, tree height and environmental harshness in eastern and western North America. - Ecology Letters 19: 743–751.

Martiny, J. B. H. et al. 2011. Drivers of bacterial beta-diversity depend on spatial scale. - Proceedings of the National Academy of Sciences of the United States of America 108: 7850–7854.

Mestre, L. A. M. et al. 2001. Macroinvertebrate Fauna Associated to the Bromeliad Vriesea inflata of the Atlantic Forest (Paraná State, Southern Brazil). - Brazilian Archives of Biology and Technology 44: 89–94.

Montagnes, D. J. S. and Weisse, T. 2000. Fluctuating temperatures affect growth and production rates of planktonic ciliates. - Aquatic Microbial Ecology 21: 97–102.

Nadkarni, N. M. and Matelson, T. J. 1989. Bird Use of Epiphyte Resources in Neotropical Trees. - The Condor 91: 891–907.

Naeem, S. 1990. Resource Heterogeneity and Community Structure: A Case Study in *Heliconia imbricata* Phytotelmata. - Oecologia 84: 29–38.

Nemergut, D. R. et al. 2013. Patterns and processes of microbial community assembly. - Microbiology and molecular biology reviews : MMBR 77: 342–356.

Oksanen, J. et al. 2015. vegan: Community Ecology Package. R package version 2.2-1. http://CRAN.R-project.org/package=vegan.

Patterson, B. D. and Atmar, W. 1986. Nested subsets and the structure of insular mammalian faunas and archipelagos. - Biological Journal of the Linnean Society 28: 65–82.

Peckarsky, B. L. 1983. Biotic interactions or abiotic limitations? A model of lotic community structure. - in T. D. Fontaine III and S. M. Bartell, editors. Dynamics of lotic ecosystems. Ann Arbor Science, Ann Arbor Michigan, USA.: 303–323.

Pessenda, L. C. R. et al. 2012. Late Quaternary vegetation and coastal environmental changes at Ilha do Cardoso mangrove, southeastern Brazil. - Palaeogeography, Palaeoclimatology, Palaeoecology 363: 57–68.

Petchey, O. L. et al. 1999. Environmental warming alters food-web structure and ecosystem function. - Nature 402: 69–72.

Petermann, J. S. et al. 2015. Resources Alter the Structure and Increase Stochasticity in Bromeliad Microfauna Communities. - PLoS ONE 10: e0118952.

Poos, M. S. and Jackson, D. A. 2012. Addressing the removal of rare species in multivariate bioassessments. The impact of methodological choices. - Ecological Indicators 18: 82–90.

Pulliam, H. R. 1988. Sources, Sinks, and Population Regulation. - The American Naturalist 132: 652–661.

R Core Team 2013. R: A language and environment for statistical computing.

Rangel, J. V. et al. 2016. Assessing the role of canopy cover on the colonization of phytotelmata by aquatic invertebrates. An experiment with the tank-bromeliad Aechmea lingulata. - J Limnol.

Revill, D. L. et al. 1967. Passive Dispersal of Viable Algae and Protozoa By Certain Craneflies and Midges. - Ecology 48: 1023–1027.

Richardson, B. A. et al. 2000. Effects of nutrient availability and other elevational changes on bromeliad populations and their invertebrate communities in a humid tropical forest in Puerto Rico. - Journal of Tropical Ecology 16: 167–188.

Ricotta, C. 2010. On beta diversity decomposition: Trouble shared is not trouble halved. - Ecology 91: 1981–1983.

Rizzini, C. T. 1997. Tratado de Fitogeografia do Brasil. Aspectos ecológicos, sociológicos e florísticos. - Âmbito Cultural Edições.

Rogerson, A. and Detwiler, A. 1999. Abundance of airborne heterotrophic protists in ground level air of South Dakota. - Atmospheric Research 51: 35–44.

Sanders, R. W. and Wickham, S. A. 1993. Planktonic protozoa and metazoa: predation, food quality and population control. - Marine microbial food webs 7: 197–223.

Schlichting, H. E., JR. and Sides, S. L. 1969. The Passive Transport of Aquatic Microorganisms by Selected Hemiptera. - Journal of Ecology 57: 759–764.

Schneider, C. A. et al. 2012. NIH Image to ImageJ. 25 years of image analysis. - Nat Meth 9: 671–675.

Segre, H. et al. 2014. Competitive exclusion, beta diversity, and deterministic vs. stochastic drivers of community assembly. - Ecology Letters 17: 1400–1408.

Serramo Lopez, L. C. et al. 1999. Frogs and Snakes as Phoretic Dispersal Agents of Bromeliad Ostracods (Limnocytheridae: Elpidium) and Annelids (Naididae: Dero). - Biotropica 31: 705–708.

Serramo Lopez, L. C. and Iglesias Rios, R. 2001. Phytotelmata Faunal Communities in Sun-Exposed Versus Shaded Terrestrial Bromeliads from Southeastern Brazil. - Selbyana 22: 219–224.

Shade, A. et al. 2008. The influence of habitat heterogeneity on freshwater bacterial community composition and dynamics. - Environmental microbiology 10: 1057–1067.

Socolar, J. B. et al. 2016. How Should Beta-Diversity Inform Biodiversity Conservation? - Trends in Ecology & Evolution 31: 67–80.

Srivastava, D. S. et al. 2004. Are natural microcosms useful model systems for ecology? - Trends in Ecology & Evolution 19: 379–384.

Srivastava, D. S. 2006. Habitat structure, trophic structure and ecosystem function: interactive effects in a bromeliad-insect community. - Oecologia 149: 493–504.

Staddon, W. J. et al. 1998. Soil microbial diversity and community structure across a climatic gradient in western Canada. - Biodiversity and Conservation 7: 1081–1092.

Tews, J. et al. 2004. Animal species diversity driven by habitat heterogeneity/diversity: the importance of keystone structures. - Journal of Biogeography 31: 79–92.

Tonkin, J. D. et al. 2016. Contrasting metacommunity structure and beta diversity in an aquatic-floodplain system. - Oikos 125: 686–697.

Tuomisto, H. 2010a. A diversity of beta diversities. Straightening up a concept gone awry. Part 1. Defining beta diversity as a function of alpha and gamma diversity. - Ecography 33: 2–22.

Tuomisto, H. 2010b. A diversity of beta diversities. Straightening up a concept gone awry. Part 2. Quantifying beta diversity and related phenomena. - Ecography 33: 23–45.

Ulrich, W. 2009. Nestedness analysis as a tool to identify ecological gradients. - Ecological Questions 11: 27–34.

Vanschoenwinkel, B. et al. 2011. Passive external transport of freshwater invertebrates by elephant and other mud-wallowing mammals in an African savannah habitat. - Freshwater Biology 56: 1606–1619.

Wang, X. et al. 2016. Drivers of bacterial beta diversity in two temperate forests. - Ecol Res 31: 57–64.

Weisse, T. et al. 2013a. Bromeliothrix metopoides, a boom and bust ciliate (Ciliophora, Colpodea) from tank bromeliads. - European journal of protistology 49: 406–419.

Weisse, T. et al. 2013b. Functional ecology of the ciliate Glaucomides bromelicola, and comparison with the sympatric species Bromeliothrix metopoides. - The Journal of eukaryotic microbiology 60: 578–587.

Whittaker, R. H. 1960. Vegetation of the Siskiyou Mountains, Oregon and California. - Ecological Monographs 30: 279–338.

Whittaker, R. J. et al. 2001. Scale and species richness: towards a general, hierarchical theory of species diversity. - Journal of Biogeography 28: 453–470.

Williams, D. D. 1987. The ecology of temporary waters. - Timber Press.

Williams, D. D. 2006. The Biology of Temporary Waters. - Oxford University Press.

Woodward, G. et al. 2010. Climate change and freshwater ecosystems: impacts across multiple levels of organization. - Philosophical transactions of the Royal Society of London. Series B, Biological sciences 365: 2093–2106.

Figures and tables

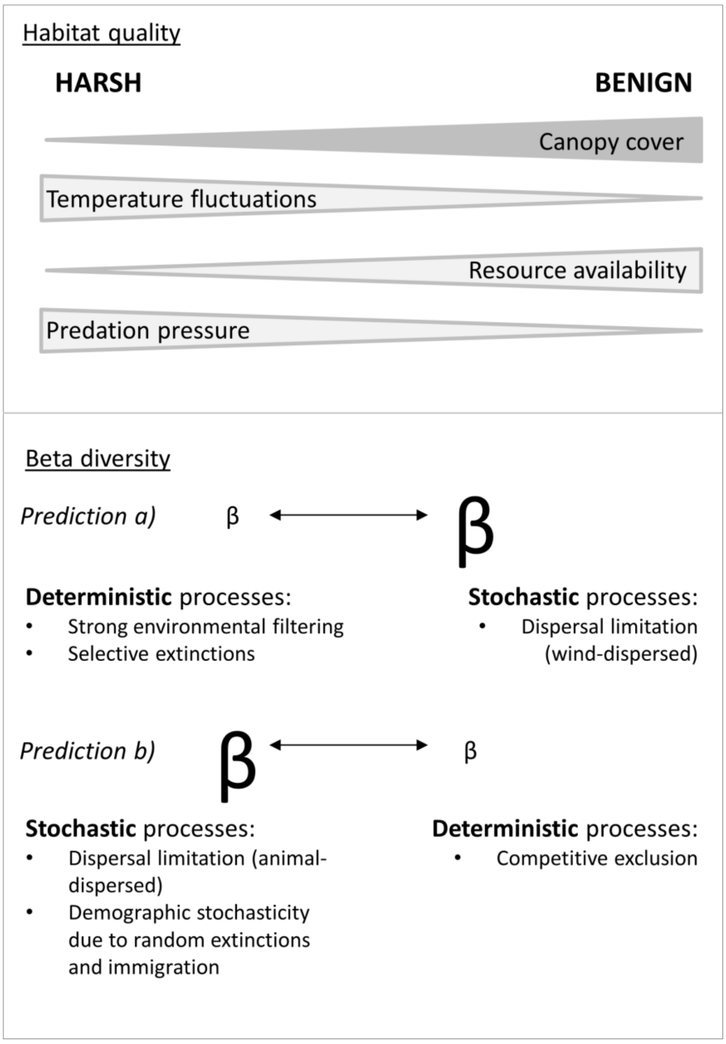


Figure 1: Contrasting predictions concerning the differences in beta diversity of bromeliad-inhabiting microfauna along a habitat quality gradient. At the top, the direction of canopy cover and canopy cover-related factors is given along a habitat-quality gradient. At the bottom, hypothesized beta diversity differences, related processes and mechanisms are shown. According to our first prediction a) of our hypothesis 2), harsh environmental conditions (e.g. higher daily temperature fluctuations and low nutrient availability) result in low beta diversity due to selective extinctions driven by strong environmental filtering. In the more benign but also more forested habitat, trees and dense shrub vegetation may have an effect on microfauna community composition if dispersal of microfauna (or their cysts) is mainly wind-driven. According to the second prediction b) of our hypothesis 2), dispersal limitation is stronger in the harsher habitat assuming that microfauna is primarily dispersed by animals (instead of wind) and that these are less active in the harsher, more exposed area. Furthermore, random extinction (e.g. through higher predation pressure by unselective filter-feeders) and immigration events are probably contributing to higher beta diversity in the harsh habitat while competitive exclusion might lower beta diversity in the benign habitat.

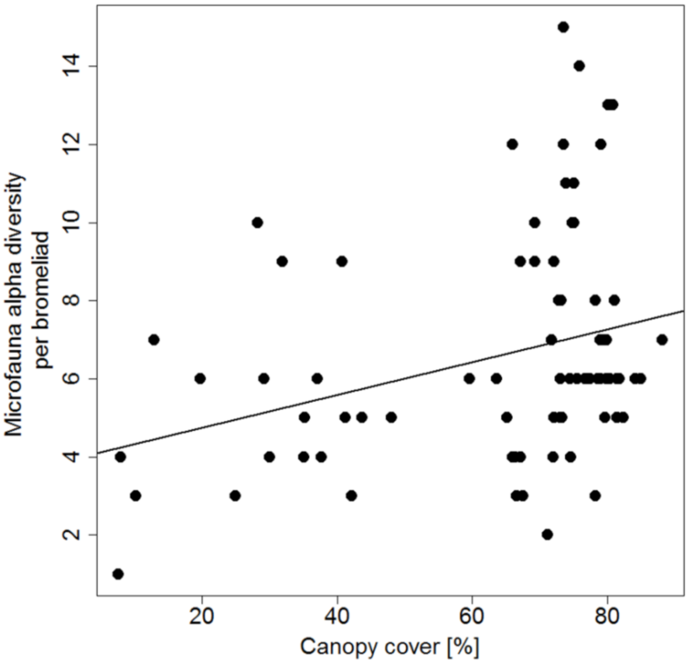


Figure 2: Mircofauna alpha diversity from bromeliads showing a positive linear relationship with canopy cover. Linear model: $F_{1,76}$ = 7.8, p = 0.007. n = 78. Microfauna (including protozoa and small metazoa) were counted as morphotypes in 50 µL of Lugol-fixed water samples.

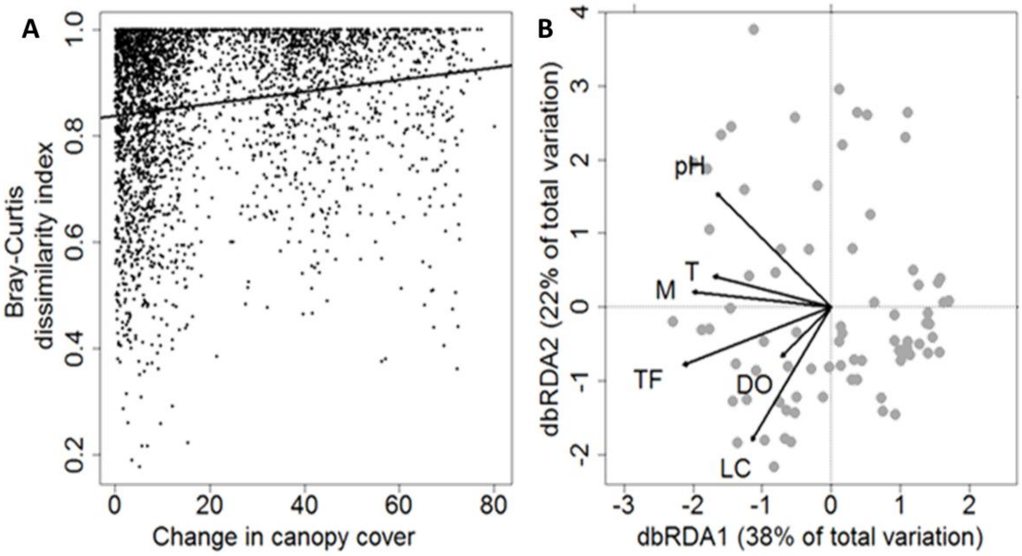


Figure 3: Drivers of bromeliad-inhabiting microfauna community composition. A: Distance-decay plot depicting the effect of change in canopy cover on community dissimilarity of bromeliad-inhabiting microfauna. The continuous line results from multiple regressions on distance matrices (MRM: R^2 = 0.026, p = 0.001) to test for a linear relationship between change in canopy cover and community dissimilarity. B: Distance-based redundancy analysis of bromeliad-inhabiting microfauna communities illustrating the influence of canopy

cover-related factors. pH – pH, T – turbidity [NTU], M – mosquito larvae [per 100 mL], TF – daily water temperature fluctuations measured as coefficient of variation, DO – dissolved oxygen concentration [%], LC – number of leaf compartments per bromeliad. n = 77. Daily fluctuations in water temperature explained the highest proportion of total variation (dbRDA1, Table 1).

Table 1: Results of a permutation test on the distance-based redundancy analysis of the effects of individual environmental variables on bromeliad-inhabiting microfauna communities. Variables with significant effects are highlighted in bold.

	Df	F	P
Number of leaf compartments	1	1.83	**0.023**
Daily fluctuations in water temperature	1	2.23	**0.003**
pH	1	1.85	**0.013**
Turbidity [NTU]	1	1.25	0.187
Dissolved oxygen concentration [%]	1	0.87	0.641
Mosquito larvae [per 100 mL]	1	1.13	0.282
Residuals	70		

Daily fluctuations in water temperature were measured as coefficient of variation. n = 77. Statistically significant effects are printed in bold. Df – degrees of freedom, F – F statistic indicating the variation between the group means, P – p value indicating the significance of the model parameters.

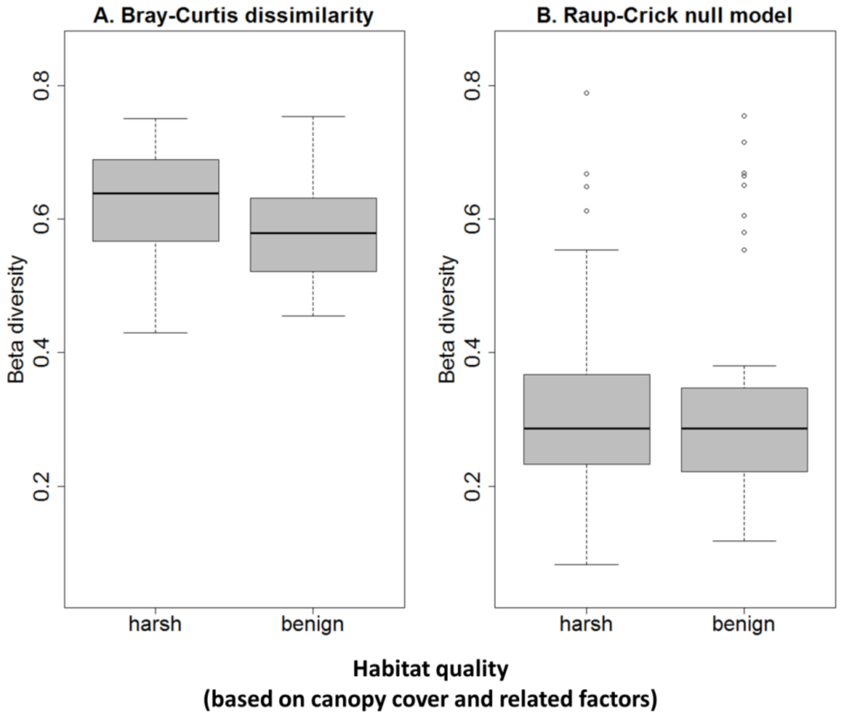
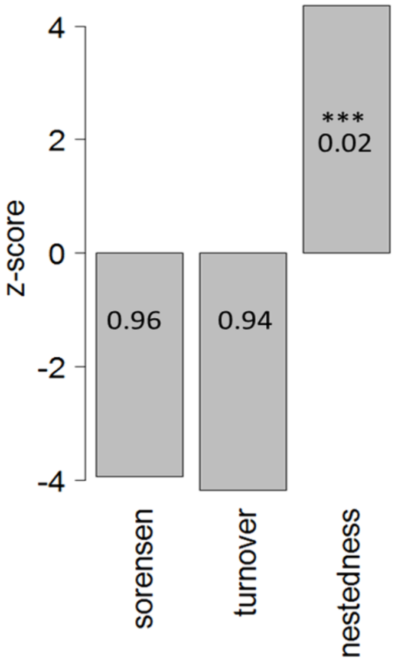


Figure 4: Beta diversity, measured as distance to group centroid, of bromeliad-inhabiting microfauna in two qualitatively distinct habitats. Habitat quality is defined based on canopy cover-related differences in predation pressure, temperature fluctuations and resource availability, which make the sun-exposed side an

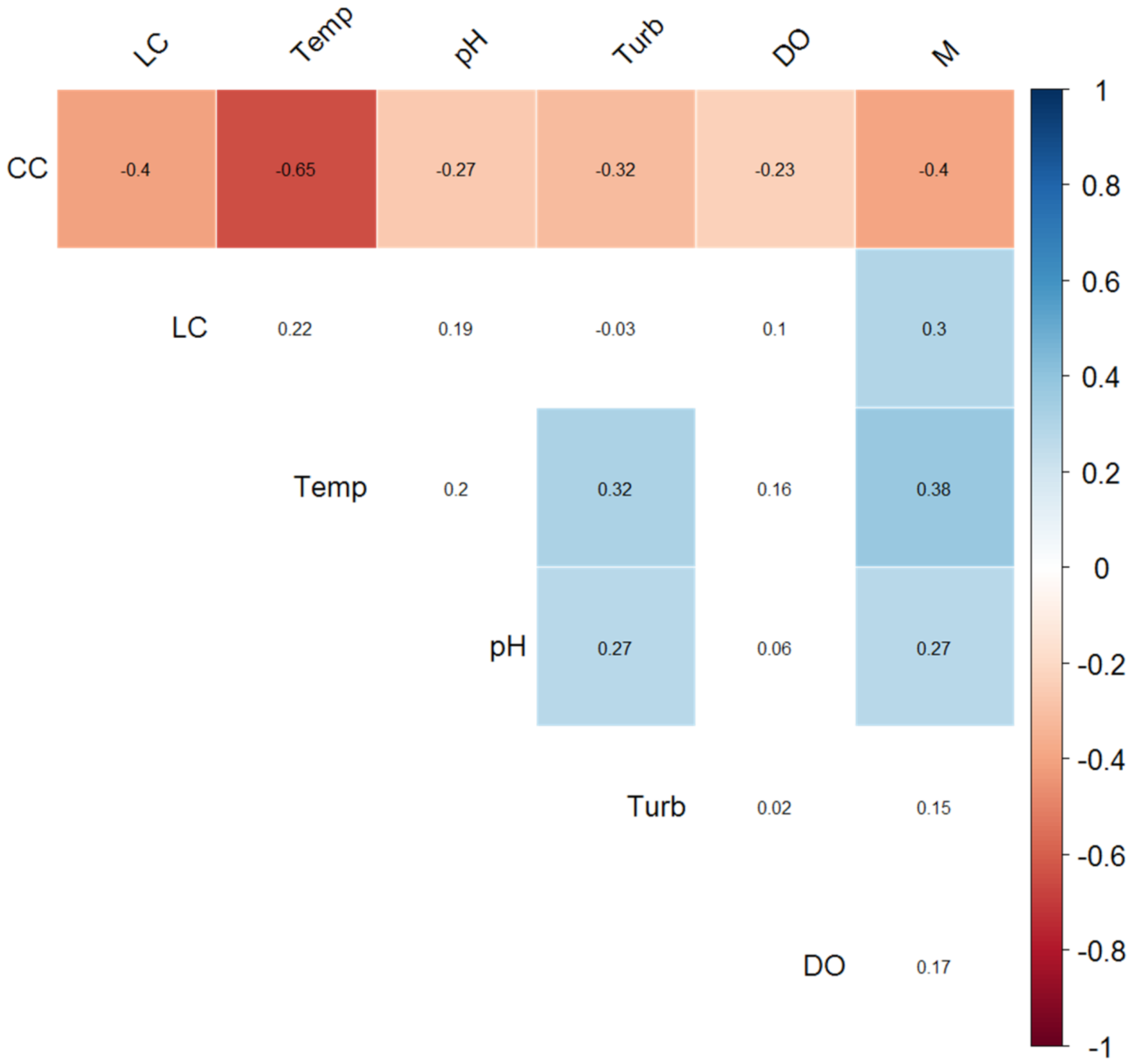
assumingly harsher habitat for microfauna than the shaded side. Beta diversity is significantly different when using a Bray-Curtis dissimilarity matrix (A, linear model, $F_{1,76} = 10.1$, $p = 0.002$). The significant difference is lost when using a Raup-Crick null model to correct for differences in alpha diversity (B, linear model, $F_{1,76} = 0.1$, $p = 0.74$). n = 78.



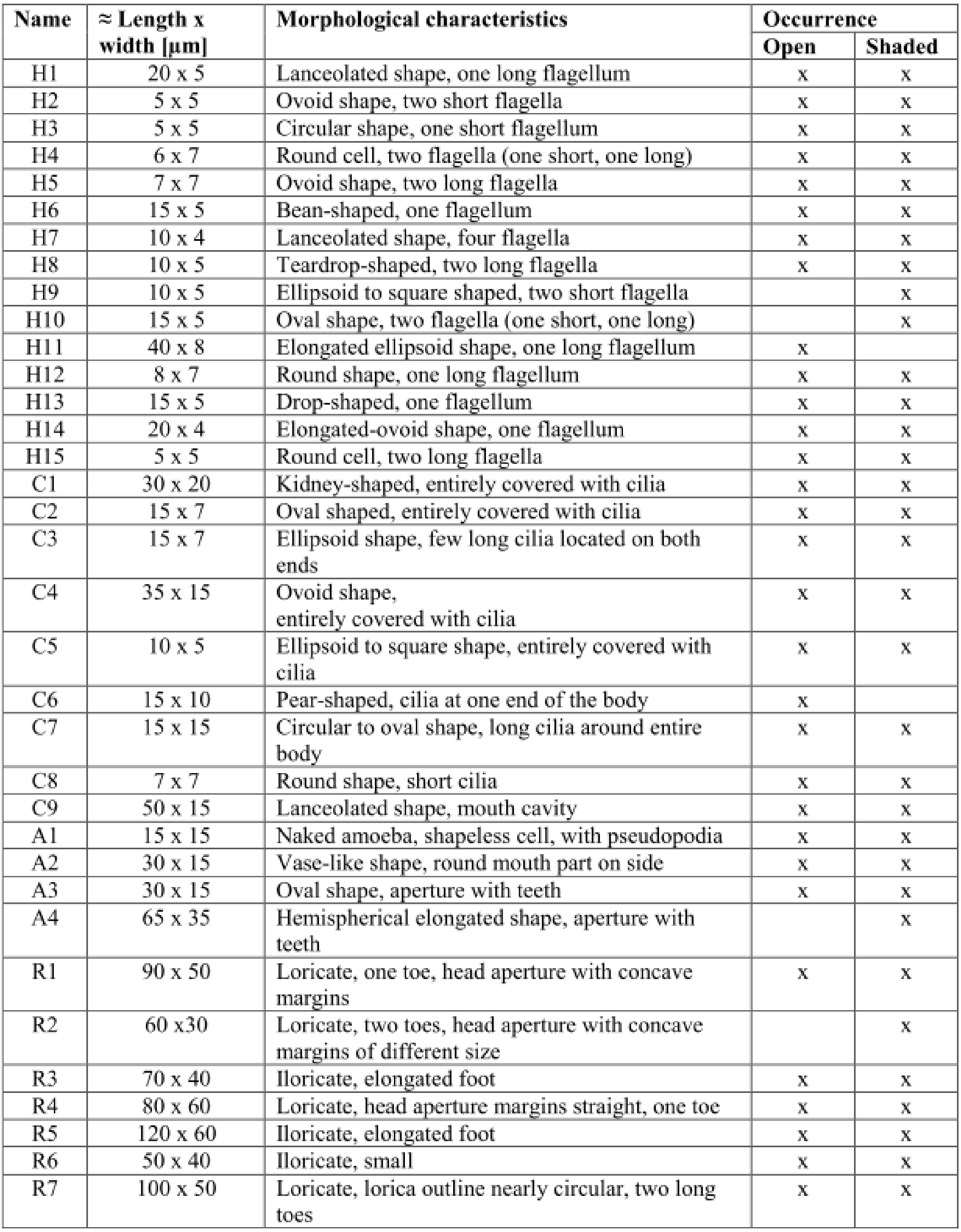
<u>Figure 5:</u> Total beta diversity (Sørensen) and partitioning into turnover and nestedness components for bromeliad microfauna communities. This analysis is based on presence-absence data. Z-scores result from 10000 simulated null model communities using the "r1"-method in the R package *vegan*. A positive z-score indicates that the value is higher than expected by chance. Whether z-scores are significantly different from zero is indicated with asterisks. ***$p < 0.001$. Values given with each bar show raw Sørensen, turnover and nestedness metrics. n = 78. Partitioning of beta diversity revealed that beta diversity in bromeliad microfauna communities is due to nestedness and not turnover.

Supporting information

<u>S1 Figure:</u> Relationships between the measured environmental variables in bromeliad species *Quesnelia arvensis* Mez. growing on Ilha do Cardoso, Brazil. Significant correlations (significance level = 0.05) are highlighted by colours. The colour legend indicates Pearson correlation coefficients. All measured variables are negatively related to canopy cover. CC – canopy cover [%], LC – number of leaf compartments per bromeliad, Temp – coefficient of variation of water temperature (calculated for a time frame of 23 hours), pH – pH, Turb – turbidity [NTU = nephelometric turbidity unit], DO – dissolved oxygen concentration [%], M – mosquito larva abundance [per 100 mL].



S1 Table: Microfauna found in the bromeliad species *Quesnelia arvensis* Mez. growing in high canopy cover (shaded) environments and low canopy cover (open) environments on Ilha do Cardoso, Brazil. Morphotypes of microfauna with main morphological characteristics and their occurrence in open or shaded bromeliads are presented. H – heterotrophic nanoflagellates, C – ciliates, A – amoebae, R – rotifers. Approximate length and width are noted to give an idea about the size class and proportions.



Name	≈ Length x width [µm]	Morphological characteristics	Occurrence	
			Open	Shaded
H1	20 x 5	Lanceolated shape, one long flagellum	x	x
H2	5 x 5	Ovoid shape, two short flagella	x	x
H3	5 x 5	Circular shape, one short flagellum	x	x
H4	6 x 7	Round cell, two flagella (one short, one long)	x	x
H5	7 x 7	Ovoid shape, two long flagella	x	x
H6	15 x 5	Bean-shaped, one flagellum	x	x
H7	10 x 4	Lanceolated shape, four flagella	x	x
H8	10 x 5	Teardrop-shaped, two long flagella	x	x
H9	10 x 5	Ellipsoid to square shaped, two short flagella		x
H10	15 x 5	Oval shape, two flagella (one short, one long)		x
H11	40 x 8	Elongated ellipsoid shape, one long flagellum	x	
H12	8 x 7	Round shape, one long flagellum	x	x
H13	15 x 5	Drop-shaped, one flagellum	x	x
H14	20 x 4	Elongated-ovoid shape, one flagellum	x	x
H15	5 x 5	Round cell, two long flagella	x	x
C1	30 x 20	Kidney-shaped, entirely covered with cilia	x	x
C2	15 x 7	Oval shaped, entirely covered with cilia	x	x
C3	15 x 7	Ellipsoid shape, few long cilia located on both ends	x	x
C4	35 x 15	Ovoid shape, entirely covered with cilia	x	x
C5	10 x 5	Ellipsoid to square shape, entirely covered with cilia	x	x
C6	15 x 10	Pear-shaped, cilia at one end of the body	x	
C7	15 x 15	Circular to oval shape, long cilia around entire body	x	x
C8	7 x 7	Round shape, short cilia	x	x
C9	50 x 15	Lanceolated shape, mouth cavity	x	x
A1	15 x 15	Naked amoeba, shapeless cell, with pseudopodia	x	x
A2	30 x 15	Vase-like shape, round mouth part on side	x	x
A3	30 x 15	Oval shape, aperture with teeth	x	x
A4	65 x 35	Hemispherical elongated shape, aperture with teeth		x
R1	90 x 50	Loricate, one toe, head aperture with concave margins	x	x
R2	60 x30	Loricate, two toes, head aperture with concave margins of different size		x
R3	70 x 40	Illoricate, elongated foot	x	x
R4	80 x 60	Loricate, head aperture margins straight, one toe	x	x
R5	120 x 60	Illoricate, elongated foot	x	x
R6	50 x 40	Illoricate, small	x	x
R7	100 x 50	Loricate, lorica outline nearly circular, two long toes	x	x

CHAPTER 2

Predators and priority effects shape microfauna communities in a community transplantation experiment along an elevational gradient

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Abstract

Transplantation experiments are a useful method to identify responses of organisms to environmental change. However, they are typically restricted to single or few species. Our experiment was carried out using entire bromeliad-inhabiting microfauna communities which were transplanted along an elevational gradient, simulating environmental change acting on the communities. Additionally, we manipulated trophic interactions, i.e. resource availability and predator presence, thus combining abiotic and biotic effects in a full-factorial experimental design. Using this experiment, we found a strong signal of original elevation in microfauna community structure (abundance, evenness, functional composition) with a shift from amoeba-dominated to flagellate-dominated communities with increasing original elevation. Surprisingly, the transplantation of communities along the elevational gradient did not affect community structure, indicating strong priority effects. Predation decreased microfauna abundance and increased microfauna evenness, specifically in higher original elevation and high resource levels.

In summary, our results show that microfauna communities in bromeliads are primarily shaped by priority effects and predator presence. However, interacting effects (between predator presence and resource availability, as well as between predator presence and original elevation) highlight the usefulness of studies with full-factorial experimental designs to understand community-structuring processes. Bromeliads and other micro-ecosystems provide convenient study systems for community level approaches that could be used in future studies concerning the effects of environmental change (e.g. climate change) on community structure.

Introduction

Human impact on the natural environment is constantly increasing and has reached even the most remote places on earth (Goudie 2013). Landscape structure, water quality and climate are affected by humans in a speed formerly unknown (Steffen et al. 2006). Species differ in their ability to cope with these changes (Khaliq et al. 2014; Garcia-Robledo et al. 2016) and this difference may lead directly or indirectly to alterations in the structure of natural communities through changes in species richness and relative abundances (Brauns 2009; Levitan 1992; Feld and Hering 2007; Littler and Murray 1975; Peres 2000). As changes in community structure can have far-reaching consequences, e.g. through the reduction in ecologically important ecosystem processes (Hillebrand et al. 2007; Tilman et al. 1997), it is important to improve our understanding of the mechanisms that structure natural communities, in order to better predict and counteract the consequences of anthropogenic changes.

Community structure may depend on environmental conditions (e.g. Meehan et al. 2013; McIntyre et al. 2001). Some taxa are very sensitive to environmental change and their presence and abundance are in fact used as indicators of this change (e.g. Bongers and Ferris 1999; Kushlan 1993). Apart from environmental drivers, trophic interactions are considered to be of major importance in shaping communities (Elton 1927). Depending on the system, communities may be more top-down (predator) or bottom-up (resource) controlled (Hillebrand et al. 2007). Environment, competition or predation have often been studied in isolation, but some studies suggest that there are interacting effects. For example, trade-offs between resistance to abiotic stress and competitive ability (Liancourt et al. 2005) have been observed, as well as interactive effects of resource competition and predation (Martin 1988; Ricklefs and O'Rourke 1975; Caswell 1978; Schmidt and Whelan 1998). Similar to abiotic conditions, trophic interactions may change between habitats or along environmental gradients. For example, an increase in CO_2 level has been shown to lead to shifts in plants' competitive ability (Lau et al. 2010) or predation pressure may differ between different elevations (Roslin et al. 2017). In sum, partly interacting effects likely operate and jointly shape community structure, acting upon species richness and relative abundances. However, holistic approaches investigating entire communities under changing conditions are still rare, partly because experimental manipulations are difficult due to the complexity of ecological systems. Phytotelmata, i.e. plant-held waters such as in pitcher plants and bromeliads, constitute relatively simple micro-ecosystems and are therefore ideal model systems for whole-community studies. They also provide convenient units that can be used to study changing environmental conditions by transplanting entire communities and to manipulate trophic interactions in field experiments (Hardwick and Giberson 1996; Srivastava et al. 2004). Here, we use bromeliad-inhabiting microfauna communities, transplant them along a natural environmental gradient and manipulate bottom-up and top-down trophic interactions to study how changes in abiotic and biotic variables and their interacting effects structure natural communities.

Bromeliads are funnel-shaped plants with small rainwater-filled pools between their leaf axils, which are populated by aquatic micro-organisms such as diatoms, flagellates, ciliates, amoeba, rotifers and micro-crustaceans. Detrivorous organisms (e.g. many protozoa), feeding on allochthonous leaf litter, constitute the basic trophic level, on which higher trophic levels prey (e.g. mosquito larvae, thus constituting protozoa predators) (Kitching 2000). Insect communities have long been the main focus of bromeliad research (Frank and Lounibos 2009; Kitching 2000; Petermann et al. 2015a; Srivastava 2006) and only few recent studies have focussed on understanding the role of protozoa and other microfauna in the system (Busse et al. 2018; Carrias et al. 2012; Kratina et al. 2017; Petermann et al. 2015b). Microfauna communities inhabiting bromeliad plants are relatively easy to manipulate, and are expected to respond fast to changes in abiotic and biotic conditions because of relatively short generation times (Srivastava et al. 2004). This makes them well suited for transplantation experiments, in our case along an elevational gradient. Transplantation experiments along elevational gradients have been used to answer questions related to environmental change (Nooten and Hughes 2017) because many factors differ naturally along elevational gradients (Lomolino 2001). Temperature, for example, decreases with increasing elevation, and thus, elevational gradients have been used as natural experiments to study larger-scale effects, for example of climate change (Malhi et al. 2010). A decrease in vegetation density with increasing elevation, as is typically the case on mountain slopes, results in increased light availability for bromeliad-inhabiting communities (Ediriweera et al. 2008) and reduced leaf litter input (i.e. reduced resource availability for bromeliad-inhabiting communities) towards the mountain top (Richardson 1999). Also, it has been observed that abundance of mosquito larvae, which function as a predator on microfauna, decreases with elevation (Eisen et al. 2008). Previous studies have investigated certain effects on the community structure in phytotelmata separately, concluding that different abiotic factors, such as hydrological period (Buosi et al. 2015), exposition to sun (Carrias et al. 2012; Lopez and Rios 2001) and water temperature (Kratina et al. 2017), as well as resource concentration (Petermann et al. 2015b) and predation (Kitching 2000; Addicott 1974) can affect species richness and/or (relative) abundance. We combined the elevational change of environmental conditions, resource availability and predator presence in a field experiment, in which all three factors were crossed in a full-factorial design. We transplanted bromeliad-inhabiting microfauna between bromeliads along an elevational gradient to study the effect of changing environmental conditions. In addition, we manipulated resource availability by altering the amount of available detritus and manipulated predator presence by adding or removing mosquito larvae. This study aims to investigate how changes in abiotic and biotic variables affect community structure and especially, to highlight interacting effects. Specifically, we aim to investigate if a) changes in environmental conditions, simulated by microfauna transplantation along an elevational gradient, affect the community structure of bromeliad-inhabiting microfauna, if b) bottom-up (resource) or top-down (predator) effects or an interaction of these influence the community structure of bromeliad-inhabiting microfauna, or if c) interacting effects between environmental (i.e. elevational) change and trophic interactions (i.e. resource competition and predation) control the community structure of bromeliad-inhabiting microfauna.

Material and Methods

Study site

We carried out a field experiment in the wet season from July to September 2015 along an elevational gradient of 666 m to 1174 m a.s.l. on Orosilito mountain (N 10°59.374', W 85°25.583') in the Área de Conservación Guanacaste (www.acguanacaste.ac.cr), Costa Rica. During the seven weeks of the experiment mean daily precipitation was 15.7 ± 2.3 (mean ± SE) mm and total precipitation amounted to 787.1 mm at 700 m a.s.l. in the study area. Maximum daily temperatures during the experiment ranged from 23°C to 28°C with a mean of 25 ± 0.2 °C at 700 m a.s.l. Minimum daily temperatures during the experiment ranged from 19°C to 22°C with a mean of 21 ± 0.1 °C at 700 m a.s.l. Along the elevational gradient the vegetation changed from secondary rainforest at lower elevations to primary cloud forest at higher elevations. Communities inhabiting bromeliads of the genus *Guzmania* Ruiz & Pav. were studied which occurred along the entire elevational gradient. The elevational minimum in our study was determined by the limit of natural forest and bromeliad occurrence (agricultural land such as orange and cattle ranches cover lower elevations) and the elevational maximum was determined by the mountain’s maximum accessible elevation.

Bromeliad system

Guzmania Ruiz & Pav. bromeliads grow epiphytically or on ground level and form water-collecting containers with their leaves. Additionally to rain and stem-flow water the tanks collect falling leaf litter which is decomposed in the tank, amongst others by protozoa. The bromeliad plant benefits from the unicellular inhabitants which provide nutrients through decomposition which the plant absorbs via the leaves (Leroy et al. 2015). The detritus provides the base for a complex food web consisting of a variety of aquatic organisms, including diatoms, flagellates, ciliates, amoeba, rotifers, micro-crustaceans and insect larvae (Petermann et al. 2015b). Unselective, filter-feeding mosquito larvae are predators of phytotelm microfauna and thus may affect their abundance and diversity (Addicott 1974).

General experimental design

Our community transplantation experiment was set up to test how changes in environmental variables along the elevational gradient, resource availability, predation and their respective interactions affect microfauna community structure using a full-factorial design (Fig 1). The experiment was carried out in 15 mL Falcon test tubes that were inserted into outer bromeliad leaf compartments situated around the centre leaf compartment in similar distances (Fig 2 A & B). Microfauna communities to be added to the test tubes were collected in 30 mL of water as a composite sample from all leaf compartments of each bromeliad. Each test tube contained 3.5 mL of microfauna culture which was filtered (mesh size: 300 µm) beforehand to exclude predatory insect larvae and detritus particles. To allow for different development trajectories of the communities, we filled them up to 7 mL with bottled water, simulating the average natural water volume of single outer leaf compartments in the area (7.4 ± 1.2 mL, n=30). The tubes were covered with a 300 µm mesh-size net to prevent additional detritus input, insect oviposition or other disturbances (e.g. drinking animals) during the experiment, but which still allowed for exchange of air and the input of rain water. Due to input of rainwater the tubes filled up to maximum volume (~17 mL) during the experiment and overflowing was observed occasionally. Overflowing events regularly occur in natural bromeliad leaf compartments and inhabiting organisms are known to adapt their behaviour to avoid being swept out of the bromeliad during rain (Koenraadt and Harrington 2008). Input of water from surrounding bromeliad leaf compartments into the tubes, and thus input of microfauna from neighbouring leaf compartments, was prevented by positioning the tubes with their rim above the maximum water line (Fig 2 B).

Environmental change simulated by microfauna community transplantation along an elevational gradient

The transplantation experiment was carried out along an elevational gradient to simulate changes in environmental conditions, such as temperature and light availability. Transplantation of microfauna communities took place between bromeliad pairs from the lower to the upper half of the elevational gradient and vice versa. A total of 20 bromeliads (*Guzmania sp.*) with a similar morphology and size were chosen along the elevational gradient. Each bromeliad received eight treatment tubes (Fig. 2C). Thereof, four were 'control' treatments, in which microfauna communities were positioned within the same bromeliad they were originally sampled from. The other four were 'transplanted' treatments, in which microfauna communities were moved to another bromeliad up or down the elevational gradient. Hereby, one half of all transplantations took place between bromeliad pairs located at a higher (~450 m) elevational distance from one another and the other half of transplantations took place between bromeliad pairs of lower (~150 m) elevational distance from one another to provide a range of magnitude of environmental change.

Trophic interactions under experimentally manipulated resource availability and predator presence

Resources added to sampling tubes were obtained from a homogenized mixture of detritus sampled along the elevational gradient to exclude the effect of resource quality. Detritus was sterilized by drying at approximately 60°C to constant weight before manually mixing and crushing into approx. 0.5 cm pieces. Previous field survey data (*Busse unpublished data*) showed that resource availability in bromeliads was about 0.02 g detritus/mL at lower elevations and 0.006 g detritus/mL at higher elevations, potentially due to elevational differences in canopy cover. Thus, experimental tubes in bromeliads received a high resource concentration or a low resource concentration as experimental treatment (Fig 1) in addition to the small pieces and dissolved organic compounds that passed through the filter. Additional resource input during experimental duration was prevented by covering the Falcon tubes with a mesh (300 µm mesh size).

Mosquito larvae collected from bromeliads in the field were used as predators. A treatment with 'predators added' contained at total of three mosquito larvae per initial 7 mL. Naturally occurring mosquito densities can vary strongly depending e.g. on habitat (Ngai et al. 2008), bromeliad capacity (Gilbert et al. 2008) or time of year (Addicott 1974). The experimental mosquito density chosen for this experiment lies within the ranges observed for Costa Rican bromeliads (Gilbert et al. 2008) and other phytotelmata (Addicott 1974). The three mosquito larvae used per test tube consisted of one individual of *Anopheles sp.*, one *Culex* sp. and one *Wyeomyia sp.* These three mosquito genera are the most common ones in the experimental area (Ngai et al. 2008). Species of the genera *Culex* and *Anopheles* were observed to filter-feed in the upper part of the water pool, while species of the genus *Wyeomyia* mostly filter-feed on the ground surface of the pool (Gilbert et al. 2008). We observed no differences in feeding mode on species level within the genera (*personal observation*), therefore, identification of mosquito larvae to genus level was considered sufficient to cover different feeding types in the same proportions in the experimental communities. Each test tube initially received two small (early developmental stage, 1st instar, *Wyeomyia* and *Anopheles/Culex*) and one large (late developmental stage, 3rd instar, *Anopheles/Culex*) mosquito larvae. Mosquito presence was visually controlled on a weekly basis. Dead, missing or pupated mosquito larvae were replaced with individuals from the respective genus to keep predator densities constant during the time of experiment. Additional mosquito oviposition during experimental duration was prevented by covering the Falcon tubes with a mesh (300 µm mesh size).

Sampling

On the day of experimental setup we took measurements of oxygen saturation [%], pH and water temperature [°C] in one exemplary, natural leaf compartment per bromeliad. We recorded the location of the bromeliad as height above ground [m]. Additionally, we used a Quantum sensor to measure photosynthetic active radiation (PAR [µmol/m^{2}s] at five points per bromeliad (center of bromeliad, north, east, south and west of center, above the bromeliad). By averaging five light measurements we accounted for small-scale differences in canopy cover. At the end of the experiment 1 mL water samples were taken from all test tubes. All water samples were fixed with Lugol's solution.

Permission for field work and sampling was granted by Sistema Nacional de Áreas de Conservación (SINAC) and Ministerio del Ambiente y Energía (MINAE) in Costa Rica (permit no. ACG-PI-030-2014 and ACG-PI-046-2015).

Identification and counting

Microfauna (i.e. diatoms, flagellates, ciliates, amoeba, rotifers and micro-crustaceans) were counted as morphotypes (see list and pictures, S1) by processing subsamples of 50 µL of each water sample using light microscopy (400x magnification) and using and adding to a photographic key developed by previous projects (Kratina et al. 2017; Petermann et al. 2015b).

Data analysis

We used linear mixed effects models (LMMs) with microfauna treatment (i.e. 'control' versus 'transplanted') nested within bromeliad identity as random effects to analyse the effects of original elevation, experimental elevation, resource availability, predation and the respective interactions on abundance, species richness and evenness of the microfauna community. Original elevation represents information on environmental conditions before the start of the transplantation experiment. Experimental elevation represents information on environmental conditions during the experiment. Comparing effects of original and experimental elevations allows the assessment of the importance of environmental variables versus priority effects.

Abundance data were log-transformed prior to analyses to achieve normality and homoscedasticity. Evenness was calculated as Pielou's J = H'/H'_{max} (Pielou 1966), with $H' = -\sum_i p_i * ln\, p_i$ (Shannon index), with p_i being the proportion of species i, and $H'_{max} = ln\ S$, with S being the total number of species. The species richness and evenness models were additionally run with log-transformed abundance data included as explanatory variable to exclude patterns that were simply due to differences in abundance. We calculated the ratio of the two most frequent taxonomic groups (amoebae/flagellates), using log-transformed abundance data, to identify shifts in dominance patterns between those two groups.

To analyse the influence of environmental parameters (i.e. water temperature [°C], photosynthetic active radiation [µmol/m²s], height above ground [m], pH and oxygen saturation [%], for extreme/average values and effect of elevational gradient see S2) on microfauna abundance and evenness, we used linear mixed effects models with microfauna treatment nested in bromeliad identity as random effects on a subset (i.e. only elevational control treatments without predators but with both resource treatments, see results section) of the available data.

All statistical analyses were done in R version 3.4.2 (R Core Team 2017) using the following packages: *lme4* (Bates et al. 2015), *nlme* (Pinheiro et al. 2017), *shape* (Soetaert 2018) and *vegan* (Oksanen et al. 2017).

Results

We identified a total of 32 morphospecies of microfauna, including one diatom morphospecies, six flagellate morphospecies, four ciliate morphospecies, 18 testate amoeba morphospecies, two rotifer morphospecies and one micro-crustacean morphospecies (see S1). Total abundances varied greatly between samples (S3). This was primarily due to differences in the presence or absence of flagellates, which dominated a community in terms of abundance when they were present. Species richness ranged from 1 to 13 morphospecies per 50 µL sample, with an average of 6 ± 0.2 morphospecies per sample and was mostly driven by a high diversity of amoeba species (S3). While amoebae and flagellates were the most ubiquitous groups, each group occurring in over 85 % of our samples, flagellates dominated the abundance and amoeba the richness of the communities. Crustaceans and rotifers were less common occurring in roughly 30-50% of samples, and ciliates and diatoms were the rarest taxa occurring in less than 30 % of samples (S3).

Microfauna abundance showed an elevational pattern increasing with original elevation (Tab 1, Fig 3A). This pattern remained strong for control communities, i.e. communities that were transplanted in their original elevation (Fig 3B). For communities transplanted to other elevations the elevational pattern was reversed (Fig 3C) indicating that the effect of original elevation was maintained even after many generations of being in the new location.

The patterns of original elevation in abundance, as well as the inverted patterns in evenness, were stronger in communities without predators (Fig 3, S4) revealing an interacting effect between the effect of original elevation and predator presence. While predator presence had a strong negative effect on abundance (Tab 1, Fig 4), resource availability showed a positive effect (Tab 1). The effect of resource availability became more prominent when predators were absent, showing the strong interaction between these two effects (Tab 1, Fig 4). Predator presence decreased microfauna abundance particularly in resource-rich habitats (Fig 4). Microfauna evenness was significantly increased by predator presence (S5). Though resource-predator interactions were shown to significantly affect microfauna community structure, no three-way interaction between environment, resource availability and predation was observed (Tab 1).

Flagellates and amoebae were expected to be particularly important in driving community structure patterns due to their frequent presence in samples (S3). Thus, we investigated the ratio of amoeba/flagellate abundance along the elevational gradient to detect shifts in dominance between these two functional groups. Amoeba/flagellate ratios showed a clear pattern along the original elevational gradient (Fig 5A), demonstrating the shift from amoeba dominated communities at lower elevations to flagellate-dominated communities at higher elevations. This elevational pattern in dominance remained strong for the control communities and was reversed for the communities transplanted to different elevations (Fig 5B+C) with the pattern of original elevation maintained in the communities after transplanting. Of the measured environmental variables that changed along the elevational gradient none had a strong effect on microfauna abundance or evenness (Tab 2).

We found an effect of predator presence on species richness while none of the other parameters had an effect (Tab 1). However, log-transformed abundance used as co-variable in the model explained almost all of the variation in species richness between samples (S6) showing that predation affected microfauna richness via differences in total abundance. When considering only the remaining variation after accounting for abundance, species richness was slightly negatively affected by resource availability (S6).

Discussion

In this study we aimed to identify the interacting effects of environmental change and trophic interactions on microfauna community structure in bromeliads. We found a strong effect of original elevation on community structure (abundance, evenness, functional composition) with a shift from amoeba-dominated to flagellate-dominated communities with increasing original elevation. The transplantation of communities along the elevational gradient did not affect community structure, indicating strong priority effects. Furthermore, predation played a major role in structuring communities by decreasing microfauna abundance and increasing microfauna evenness, specifically in higher original elevation and high resource levels.

Environmental niches versus priority effects

We found that the structure of microfauna communities in bromeliads changed along the elevational (i.e. environmental) gradient. We found an increasing abundance and decreasing evenness of microfauna communities with increasing elevation. No changes in species richness were found along the elevational gradient. For many other taxa patterns in species richness along elevational gradients have been observed; most common are declines in richness with increasing elevation or unimodal patterns (Sanders and Rahbek 2012). Microfauna organisms, however, show fundamental differences from macrofauna such as higher migration rates, lower extinction rates and lower relative number of endemics (Finlay et al. 2004). These differences seem to result in the absence of elevational patterns on the microscopic scale shown previously (e.g. bacteria: Fierer et al. 2011, fungi and protists: Shen et al. 2014). Another reason for the lack of an effect of elevation on species richness in our study could have been a lower taxonomic resolution due to our morphospecies approach, which could be resolved by studies using genetic information in the future.

Community changes in our study were mostly attributed to differences in abundance. Abundance patterns along elevational gradients in previous studies varied greatly between taxa groups, as well as with the length of the elevational gradient and due to other reasons (see for example Hodkinson 2005; Kumar et al. 2009; Samson et al. 1997; Torre and Arrizabalaga 2009). In our study system abundance increased with increasing elevation. This change in total abundance in bromeliad microfauna can mainly be attributed to changes in flagellates abundance. Flagellates were the most abundant group that dominated the bromeliad microfauna communities with individual numbers that were one to two orders of magnitude higher than of the other groups. The second most abundant group and the most species rich group were amoeba. Flagellates and amoebae were also the most frequently occurring groups (i.e. presence-absence). This dominance of the two groups had been observed before in bromeliad microfauna communities (Petermann et al. 2015b), especially in shaded habitats (Carrias et al. 2012). We discovered shifting dominance patterns between amoeba-dominated communities at lower elevations to flagellate-dominated communities at higher elevations. A potential reason for this shift could be habitat stability, for example in terms of water volume. High elevation environments are potentially more stable because they are located in the cloud forest and are constantly wet while lower elevation bromeliads may dry up repeatedly during the dry season (*personal observation*). Some amoeba species are known to outcompete flagellates after rewetting due to faster recovery rates (Geisen et al. 2014) and could thus be the more dominant group in lower elevations at the beginning of the wet season when our experiment was conducted. Distinct effects of hydrological periods in structuring parts of bromeliad inhabiting communities have been observed previously (Buosi et al. 2015).

Unexpectedly, changes in environment by transplanting communities to opposing elevations resulted in a reversal of the elevational effect on abundance, evenness and dominance patterns, thus indicating strong priority effects. Our experiment was designed to be able to compare patterns in community structure related to original elevation with patterns of transplanted experimental elevation to provide evidence of either the effect of environmental niches or priority effects on communities. Thus, if the elevational pattern of community structure that we found had remained the same after transplanting the communities, this would have indicated that environmental niches are important drivers of community structure and similar communities assemble under similar environmental conditions. In contrast, a reversed elevational pattern in community structure indicates that priority effects are at work and that the amount of environmental change was still within the tolerance range of the involved species. Microfauna organisms are able to tolerate a broad range of environmental conditions which enables the species first to arrive to use up most of the available resources, thus preventing other species from establishing a population (= niche-preemption, Fukami 2015). The experimental duration of seven weeks (which equals approximately 20-300 generations of different protozoa taxa (Berninger et al. 1991; Dehority 1998; Schönborn 1986; Weisse et al. 2013)) was considered long enough for the microfauna communities to be able to reassemble as a response to new environmental conditions (Kinnison and Hairston 2007). However, in bacteria and yeast metacommunities priority effects have been observed to persist over multiple generations (Toju et al. 2018). For bromeliad-inhabiting metacommunities that live in seasonally unstable habitats (i.e. changes in water volume) the duration of seven weeks might not be long enough to assess the full impact of environmental change versus priority effects across different seasonal periods. It seems that the communities can cope with shorter disturbances within the natural range of possible changes. In this case, priority effects apparently strongly influence community structure. A longer exposition to a certain set of environmental variables may deterministically drive community assembly and lead to spatial patterns in community structure for example along elevational gradients if environmental conditions remain more or less constant over longer periods (including constant disturbance regimes). Under the aspect of environmental change single weather anomalies will probably not affect bromeliad microfauna, but repeated changes, for example longer dry seasons, could result in lasting changes in the structure of bromeliad microfauna communities.

Trophic interactions

Resource availability and predator presence showed interacting effects on community structure in our field experiment. As expected, high resource levels increased abundance and decreased evenness in accordance with the literature (Petermann et al. 2015b). The presence of predators, here unselective filter-feeding mosquitoes, decreased the abundance of microfauna and thus contributed to more even communities. By increasing evenness predation has a stabilizing effect on community structure, as also shown by other studies (Wilsey and Polley 2002; Wittebolle et al. 2009). In our study, especially predator presence played a major role in structuring the bromeliad-inhabiting microfauna communities, presumably due to the comparatively high predator density. A previous study with bromeliad microfauna found no predator effect, likely also because predator and resource effect were not crossed (Petermann et al. 2015b). Studies in pitcher plants concluded that both processes are important but that bottom-up processes dominate (Hoekman 2007) or that both processes differ in their effect, with resource-limitation being applicable to all parts of a community while predation targets a limited set of groups (Kneitel and Miller 2002). Resource availability and predator presence can vary strongly even within one bromeliad (*personal observation*). For example, a community in an older leaf compartment that has accumulated much debris will not be resource limited and this environment will provide sufficient nutrients for abundant microfauna growth, while a neighbouring leaf compartment may be harbouring a number of mosquito larvae that keep the microfauna less abundant by constant predation. These resource-predator dynamics are potentially not only spatially but also temporally variable, as resource input and insect oviposition are distinct events that can lead to very different conditions in a leaf compartment within few days. This increases the complexity of the interaction between bottom-up and top-down control in bromeliads, as demonstrated for other freshwater systems (Taylor et al. 2015), highlighting the need for further studies on this topic, especially with a high temporal and spatial resolution.

Interacting effects of environment and trophic interactions

We found interacting effects between the effect of elevation and predator presence on microfauna abundance. Generally, few studies investigate interacting effects between the environment and predator presence (but see Chalcraft and Andrews 1999, 1999; Hoekman 2010) because such studies require an extensive and laborious experimental design. Smaller communities such as in bromeliads, however, are particularly suited for investigating this type of question. The relative importance of predation on protists has been observed to decrease with decreasing temperature (Hoekman 2010), i.e. we would have expected a weaker effect of predation at higher elevations. The opposite was true in our experiment. Predation kept microfauna at a generally low abundance with a more even species assemblage along the entire elevational gradient. Thus, predators prevented the few species that are best suited for the environmental conditions from dominating the communities. Our results demonstrate that hidden interacting effects are potentially key to predicting community responses to changing environmental conditions.

Conclusion

Our results show that both, environmental niches and priority effects may be important as drivers of microfauna communities, potentially depending on the temporal scale of the study. Trophic interactions, i.e. resource availability and predation, may jointly and interactively drive community structure, likely with high spatial and temporal dynamics in the bromeliad system. Elevational patterns of microfauna abundance were only visible in the absence of predators, revealing hidden interacting effects between environment and trophic interactions in the bromeliad system and highlighting the usefulness of complex full-factorial experiments to investigate drivers of community structure.

We show that bromeliads, and likely other phytotelm systems, provide convenient model systems for community-level transplantation experiments along environmental gradients, which can be used to help predict effects of environmental change on communities. The use of such model systems will likely become more important in the future to investigate and predict the consequences of anthropogenic environmental change, such as for example climate change, on entire communities. In addition, our results may contribute to the effective protection of bromeliad and other phytotelm systems in the future.

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References

Addicott 1974. Predation and Prey Community Structure: An Experimental Study of the Effect of Mosquito Larvae on the Protozoan Communities of Pitcher Plants. - Ecology 55: 475–492.

Bates, D. et al. 2015. Fitting Linear Mixed-Effects Models Using lme4. - Journal of Statistical Software 67: 1–48, doi:10.18637/jss.v067.i01.

Berninger, U.-G. et al. 1991. Protozoan control of bacterial abundances in freshwater. - Limnol. Oceanogr. 36: 139–147.

Bongers, T. and Ferris, H. 1999. Nematode community structure as a bioindicator in environmental monitoring. - Trends in Ecology & Evolution 14: 224–228.

Brauns, M. 2009. Human impacts on the structure and ecological function of littoral macroinvertebrate communities in lakes.

Buosi, P. R. B. et al. 2015. Effects of Seasonality and Dispersal on the Ciliate Community Inhabiting Bromeliad Phytotelmata in Riparian Vegetation of a Large Tropical River. - The Journal of eukaryotic microbiology 62: 737–749.

Busse, A. et al. 2018. Different in the dark: The effect of habitat characteristics on community composition and beta diversity in bromeliad microfauna. - PloS one 13: e0191426.

Carrias, J.-F. et al. 2012. An ant–plant mutualism induces shifts in the protist community structure of a tank-bromeliad. - Basic and Applied Ecology 13: 698–705.

Caswell, H. 1978. Predator-mediated coexistence: a nonequilibrium model. - The American Naturalist 112: 127–154.

Chalcraft, D. R. and Andrews, R. M. 1999. Predation on lizard eggs by ants: species interactions in a variable physical environment. - Oecologia 119: 285–292.

Dehority, B. A. 1998. Generation times of Epidinium caudatum and Entodinium caudatum, determined in vitro by transferring at various time intervals. - Journal of Animal Science 76: 1189–1196.

Ediriweera, S. et al. 2008. Variation in canopy structure, light and soil nutrition across elevation of a Sri Lankan tropical rain forest. - Forest Ecology and Management 256: 1339–1349.

Eisen, L. et al. 2008. Mosquito Species Richness, Composition, and Abundance along Habitat-Climate-Elevation Gradients in the Northern Colorado Front Range. - Journal of Medical Entomology 45: 800–811.

Elton, C. 1927. Animal ecology. - The Macmillan Company.

Feld, C. K. and Hering, D. 2007. Community structure or function. Effects of environmental stress on benthic macroinvertebrates at different spatial scales. - Freshwater Biol 52: 1380–1399.

Fierer, N. et al. 2011. Microbes do not follow the elevational diversity patterns of plants and animals. - Ecology 92: 797–804.

Finlay, B. J. et al. 2004. Protist Diversity is Different? - Protist 155: 15–22.

Frank, J. H. and Lounibos, L. P. 2009. Insects and allies associated with bromeliads. a review. - Terrestrial arthropod reviews 1: 125–153.

Fukami, T. 2015. Historical Contingency in Community Assembly. Integrating Niches, Species Pools, and Priority Effects. - Annu. Rev. Ecol. Evol. Syst. 46: 1–23.

Garcia-Robledo, C. et al. 2016. Insect species display limited tolerance to high temperatures across tropical elevational gradients. Implications for global warming. - Proceedings of the National Academy of Sciences 113: 680–685.

Geisen, S. et al. 2014. Soil water availability strongly alters the community composition of soil protists. - Pedobiologia 57: 205–213.

Gilbert, B. et al. 2008. Niche partitioning at multiple scales facilitates coexistence among mosquito larvae. - Oikos 117: 944–950.

Goudie, A. S. 2013. The Human Impact on the Natural Environment: Past, Present, and Future.

Hardwick, M. L. and Giberson, D. J. 1996. Aquatic insect populations in transplanted and natural populations of the purple pitcher plant, Sarracenia purpurea, on Prince Edward Island, Canada. - Canadian journal of zoology 74: 1956–1963.

Hillebrand, H. et al. 2007. Consumer versus resource control of producer diversity depends on ecosystem type and producer community structure. - Proceedings of the National Academy of Sciences 104: 10904–10909.

Hodkinson, I. D. 2005. Terrestrial insects along elevation gradients. Species and community responses to altitude. - Biol. Rev. 80: 489–513.

Hoekman, D. 2007. Top-down and bottom-up regulation in a detritus-based aquatic food web: a repeated field experiment using the pitcher plant (Sarracenia purpurea) inquiline community. - The American Midland Naturalist 157: 52–62.

Hoekman, D. 2010. Turning up the heat: Temperature influences the relative importance of top-down and bottom-up effects. - Ecology 91: 2819–2825.

Khaliq, I. et al. 2014. Global variation in thermal tolerances and vulnerability of endotherms to climate change. - Proceedings of the Royal Society of London B: Biological Sciences 281: 20141097.

Kinnison, M. T. and Hairston, N. G. 2007. Eco-evolutionary conservation biology. Contemporary evolution and the dynamics of persistence. - Funct Ecology 21: 444–454.

Kitching, R. L. 2000. Food webs and container habitats: the natural history and ecology of phytotelmata. - Cambridge University Press.

Kneitel, J. M. and Miller, T. E. 2002. Resource and top-predator regulation in the pitcher plant (Sarracenia purpurea) inquiline community. - Ecology 83: 680–688.

Koenraadt, C.J.M. and Harrington, L. C. 2008. Flushing effect of rain on container- inhabiting mosquitoes aedes aegypti and culex pipiens (Diptera: Culicidae). - Journal of Medical Entomology 45: 28–35.

Kratina, P. et al. 2017. Environmental control of the microfaunal community structure in tropical bromeliads. - Ecology and evolution 7: 1627–1634.

Kumar, A. et al. 2009. Elevational Patterns of Diversity and Abundance of Eusocial Paper Wasps (Vespidae) in Costa Rica. - Biotropica 41: 338–346.

Kushlan 1993. Colonial waterbirds as bioindicators of environmental change. - Colonial waterbirds: 223–251.

Lau, J. A. et al. 2010. Species interactions in a changing environment: elevated CO2 alters the ecological and potential evolutionary consequences of competition. - Evolutionary Ecology Research 12: 435–455.

Leroy, C. et al. 2015. The contribution of microorganisms and metazoans to mineral nutrition in bromeliads. - JPECOL 9: 241–255.

Levitan, D. R. 1992. Community structure in times past: influence of human fishing pressure on algal-urchin interactions. - Ecology 73: 1597–1605.

Liancourt, P. et al. 2005. Stress Tolerance and Competitive-Response Ability Determine the Outcome of Biotic Interactions. - Ecology 86: 16-11-1618.

Littler, M. M. and Murray, S. N. 1975. Impact of sewage on the distribution, abundance and community structure of rocky intertidal macro-organisms. - Marine Biology 30: 277–291.

Lomolino, M. V. 2001. Elevation gradients of species-density: historical and prospective views. - Global Ecology and Biogeography 10: 3–13.

Lopez, L. C. S. and Rios, R. I. 2001. Phytotelmata Faunal Communities in Sun-Exposed Versus Shaded Terrestrial Bromeliads from Southeastern Brazil. - Selbyana 22: 219–224.

Malhi, Y. et al. 2010. Introduction. Elevation gradients in the tropics: laboratories for ecosystem ecology and global change research. - Global Change Biology 16: 3171–3175.

Martin, T. E. 1988. On the advantage of being different: nest predation and the coexistence of bird species. - Proceedings of the National Academy of Sciences 85: 2196–2199.

McIntyre, N. E. et al. 2001. Ground arthropod community structure in a heterogeneous urban environment. - Landscape and Urban Planning 52: 257–274.

Meehan, T. D. et al. 2013. Butterfly community structure and landscape composition in agricultural landscapes of the central United States. - Journal of insect conservation 17: 411–419.

Ngai, J. T. et al. 2008. The impact of land-use change on larval insect communities. Testing the role of habitat elements in conservation. - Ecoscience 15: 160–168.

Nooten, S. S. and Hughes, L. 2017. The power of the transplant. Direct assessment of climate change impacts. - Climatic Change 144: 237–255.

Oksanen, J. et al. 2017. vegan: Community Ecology Package. R package version 2.4-4. https://CRAN.R-project.org/package=vegan.

Peres, C. A. 2000. Effects of subsistence hunting on vertebrate community structure in Amazonian forests. - Conservation Biology 14: 240–253.

Petermann, J. S. et al. 2015a. Dominant predators mediate the impact of habitat size on trophic structure in bromeliad invertebrate communities. - Ecology 96: 428–439.

Petermann, J. S. et al. 2015b. Resources alter the structure and increase stochasticity in bromeliad microfauna communities. - PloS one 10: e0118952.

Pielou, E. C. 1966. The measurement of diversity in different types of biological collections. - J. Theor. Biol. 13: 131–144.

Pinheiro, J et al. 2017. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-131, https://CRAN.R-project.org/package=nlme.

R Core Team 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Richardson, B. A. 1999. The Bromeliad Microcosm and the Assessment of Faunal Diversity in a Neotropical Forest1. - Biotropica 31: 321–336.

Ricklefs, R.E. and O'Rourke, K. 1975. Aspect diversity in moths: a temperate-tropical comparison. - Evolution 29: 313–324.

Roslin, T. et al. 2017. Higher predation risk for insect prey at low latitudes and elevations. - Science 356: 742–744.

Samson, D. A. et al. 1997. Ant Diversity and Abundance along an Elevational Gradient in the Philippines. - Biotropica 29: 349–363.

Sanders, N. J. and Rahbek, C. 2012. The patterns and causes of elevational diversity gradients. - Ecography 35: 1–3.

Schmidt, K. A. and Whelan, C. J. 1998. Predator-mediated interactions between and within guilds of nesting songbirds. experimental and observational evidence. - The American Naturalist 152: 393–402.

Schönborn, W. 1986. Population dynamics and production biology of testate amoebae (Rhizopoda, Testacea) in raw humus of two coniferous forest soils. - Archiv für Protistenkunde 132: 325–342.

Shen, C. et al. 2014. Contrasting elevational diversity patterns between eukaryotic soil microbes and plants. - Ecology 95: 3190–3202.

Soetaert, K. 2018. shape: Functions for Plotting Graphical Shapes, Colors R package version 1.4.4., https://CRAN.R-project.org/package=shape.

Srivastava, D. S. et al. 2004. Are natural microcosms useful model systems for ecology? - Trends in Ecology & Evolution 19: 379–384.

Srivastava, D. S. 2006. Habitat structure, trophic structure and ecosystem function. Interactive effects in a bromeliad-insect community. - Oecologia 149: 493–504.

Steffen, W. et al. 2006. Global change and the earth system: a planet under pressure.

Taylor, J. et al. 2015. Top-down and bottom-up interactions in freshwater ecosystems: emerging complexities. In: Trophic ecology: bottom-up and top-down interactions across aquatic and terrestrial systems. - Cambridge University Press., Cambridge, UK: 55–85, doi:10.1017/CBO9781139924856.004.

Tilman, D. et al. 1997. The Influence of Functional Diversity and Composition on Ecosystem Processes. - Science 277: 1300–1302.

Toju, H. et al. 2018. Priority effects can persist across floral generations in nectar microbial metacommunities. - Oikos 127: 345–352.

Torre, I. and Arrizabalaga, A. 2009. Species richness and abundance of small mammals along an elevational gradient of a Mediterranean mountain. - Vie et milieu - Life and environment 59: 203–212.

Weisse, T. et al. 2013. Bromeliothrix metopoides, a boom and bust ciliate (Ciliophora, Colpodea) from tank bromeliads. - European journal of protistology 49: 406–419.

Wilsey, B. J. and Polley, H. W. 2002. Reductions in grassland species evenness increase dicot seedling invasion and spittle bug infestation. - Ecol Letters 5: 676–684.

Wittebolle, L. et al. 2009. Initial community evenness favours functionality under selective stress. - Nature 458: 623–626.

Figures and tables

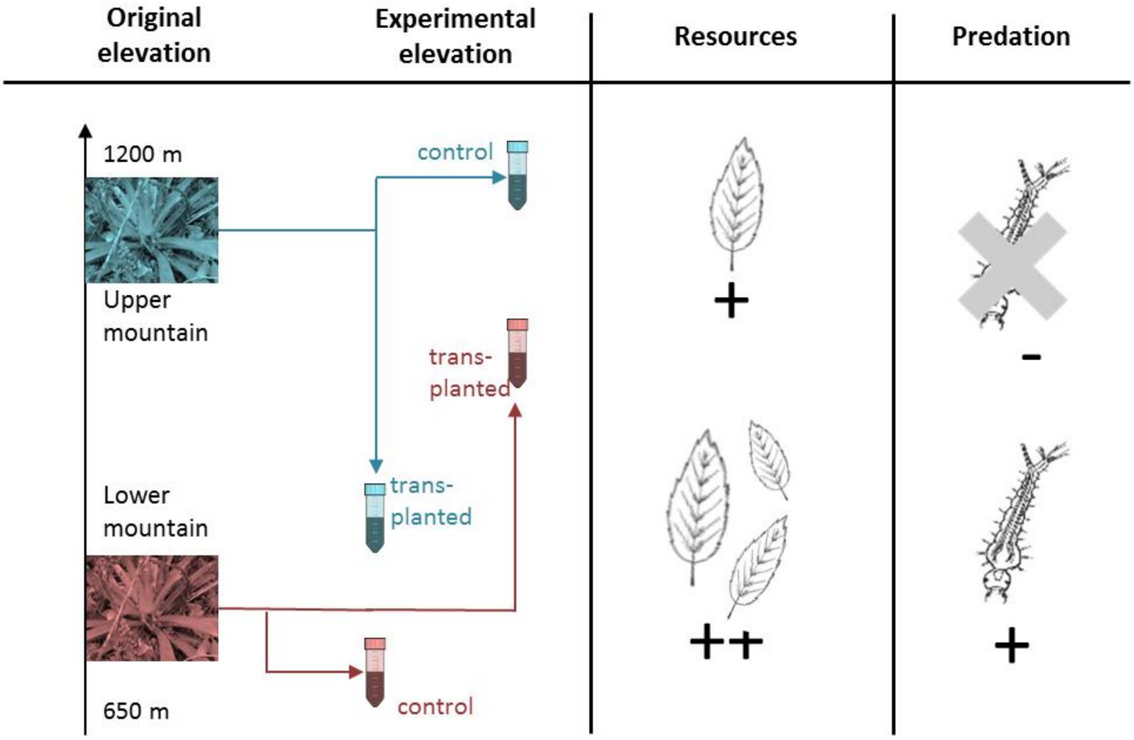


Figure 1: Experimental design of microfauna transplantation experiment along an elevational gradient on Orosilito mountain (Costa Rica) ranging from 666-1174 m a.s.l. Transplantation to experimental elevation, resource availability and predation were crossed experimental treatments in a full-factorial design resulting in eight experimental 15 mL Falcon tubes per bromeliad. Microfauna communities were transplanted between ten bromeliad pairs (upper to lower mountain and vice versa). Detritus was added to experimental tubes as the sole resource for microfauna communities, using two experimental levels: low resource availability and high resource availability. The predator treatment consisted of three filter-feeding mosquito larvae per test tube. Control tubes of the predator treatment did not contain any mosquitoes.

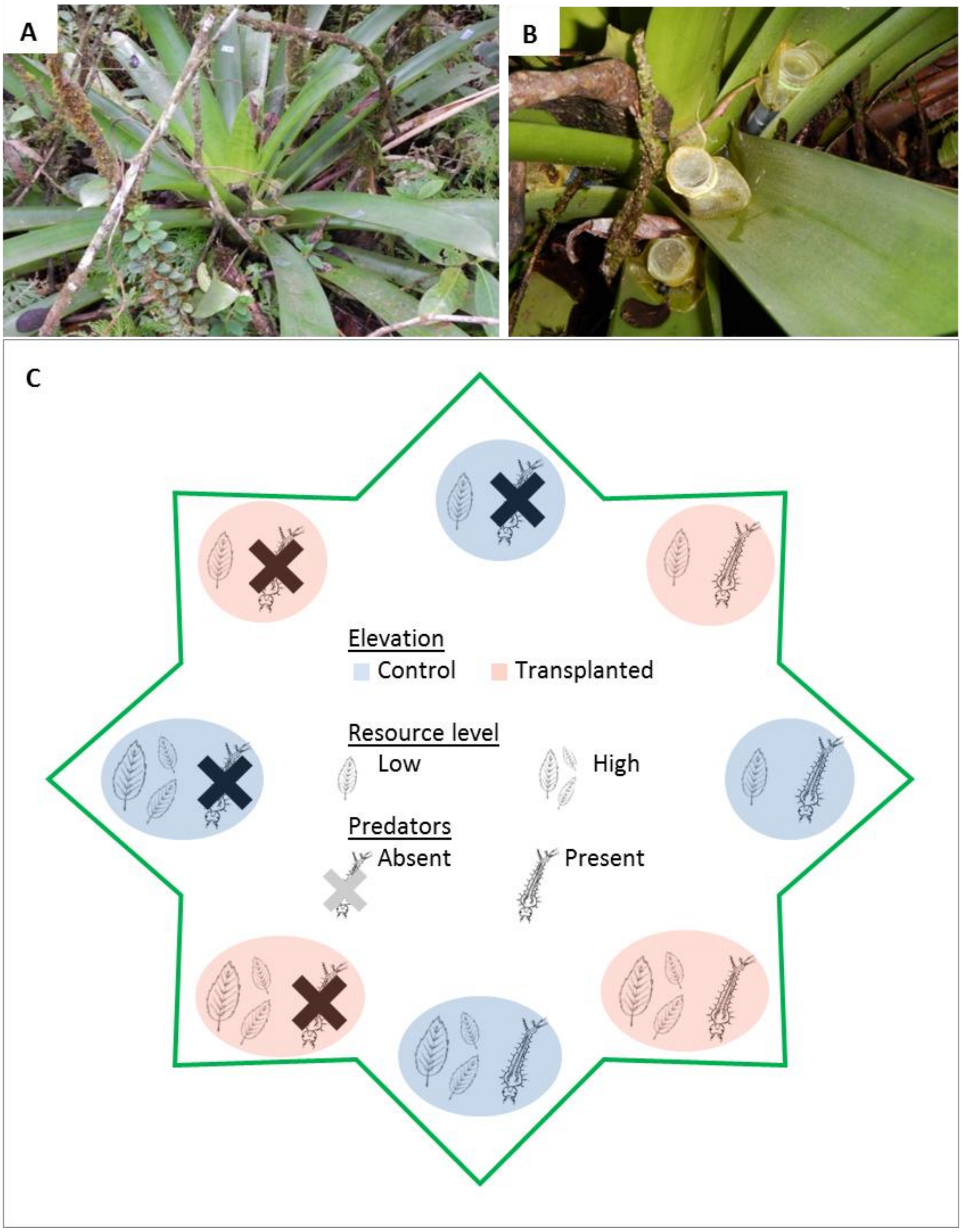


Figure 2: A – Bromeliad in the field and B – Close-up on mesh-covered experimental tubes in which the microfauna communities were kept during the experiment. Mesh size: 300 µm. C – Visualization of the full-factorial experimental design with eight treatments per bromeliad. Control treatments transplanted to same elevation are highlighted in blue and transplanted treatments that were moved to new elevations are highlighted in red. Resource concentration and predator presence are depicted using leaf and mosquito larva symbols, respectively.

Table 1: Results of linear mixed effects model (LMM) analysis for bromeliad-inhabiting microfauna abundance (log-transformed), species richness and evenness with microfauna treatment (i.e. 'control' versus 'transplanted') nested within bromeliad identity as random effects. Data were obtained from a transplantation experiment along an elevational gradient on Orosilito mountain (Costa Rica) ranging from 666-1174 m a.s.l. Transplantation, resource availability and predation were crossed in a full-factorial experimental design resulting in eight treatment tubes per bromeliad. Microfauna communities were transplanted in 15 mL Falcon tubes between ten bromeliad pairs (upper to lower mountain and vice versa). Detritus dry weight was added to experimental tubes as sole resource for microfauna communities, using two experimental levels: 'low' versus 'high'. The predator treatment used three filter-feeding mosquito larvae per test tube. Significant p values ($p \leq 0.05$) are printed in bold. ↑ - Positive relationship. ↓ - Negative relationship. n = 160. For the calculation of evenness one outlier sample with only one individual was excluded.

			Abundance				*Evenness*				*Species richness*		
	numDF		denDF	F-value	p-value		denDF	F-value	p-value		denDF	F-value	p-value
Transplanted elevation	1		18	0.5809	0.4558		18	0.0163	0.8999		18	2.4553	0.1345
Original elevation	1		18	27.1544	**0.0001↑**		18	32.041	**<.0001↓**		18	0.8525	0.3681
Resource	1		108	7.2757	**0.0081↑**		107	6.1362	**0.0148↓**		108	0.4372	0.5099
Predator	1		108	70.9016	**<.0001↓**		107	37.944	**<.0001↑**		108	17.3183	**0.0001↓**
Transplanted elevation:Original elevation	1		18	0.0227	0.882		18	0.05	0.8256		18	0.9591	0.3404
Transplanted elevation:Resource	1		108	1.9836	0.1619		107	0.3014	0.5842		108	0.1694	0.6815
Original elevation:Resource	1		108	1.1492	0.2861		107	0.5484	0.4606		108	1.6238	0.2053
Transplanted elevation:Predator	1		108	2.0942	0.1508		107	0.4323	0.5123		108	0.1168	0.7332
Original elevation:Predator	1		108	11.2711	**0.0011**		107	6.4035	**0.0128**		108	0.3823	0.5377
Resource:Predator	1		108	14.1469	**0.0003**		107	6.5004	**0.0122**		108	2.3007	0.1322
Transplanted elevation:Original elevation:Resource	1		108	0.2882	0.5925		107	0.3425	0.5596		108	0.2122	0.646
Transplanted elevation:Original elevation:Predator	1		108	0.4942	0.4836		107	0.1764	0.6753		108	0.3954	0.5308
Transplanted elevation:Resource:Predator	1		108	0.0422	0.8377		107	0.0671	0.7962		108	1.0621	0.305
Original elevation:Resource:Predator	1		108	0.3366	0.563		107	0.0686	0.7939		108	0.0174	0.8953
Transplanted elevation:Original elevation:Resource:Predator	1		108	1.3088	0.2551		107	2.5683	0.112		108	0.4121	0.5223

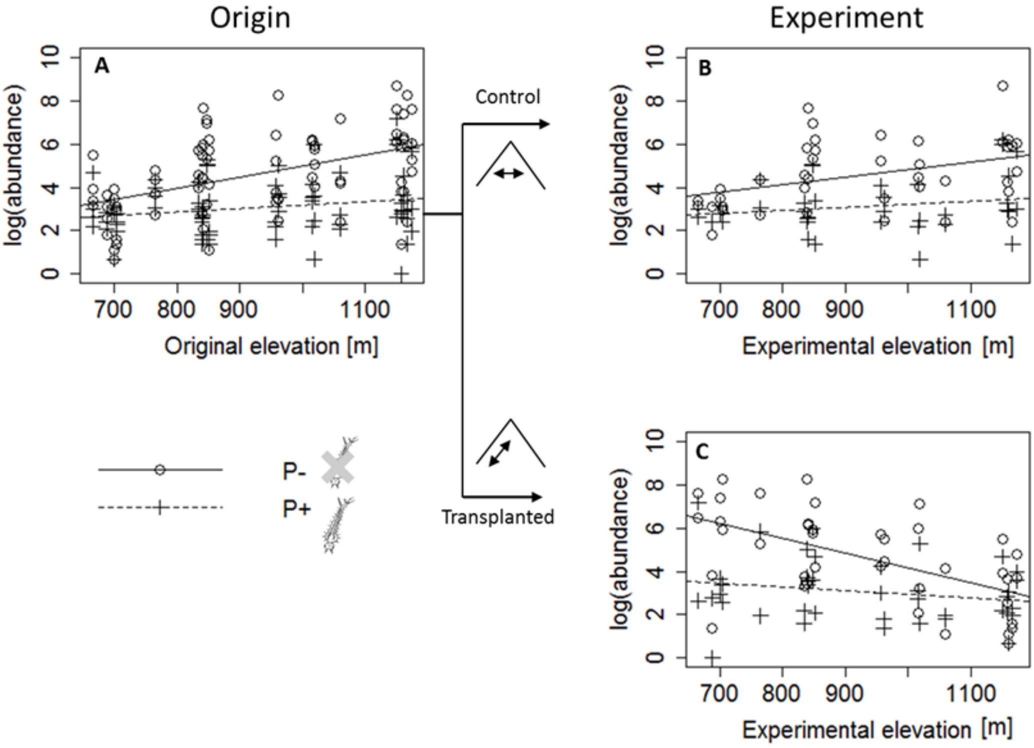


Figure 3: Results of the transplantation experiment demonstrating a strong positive effect of original elevation on total abundance [per 50 µL] of bromeliad-inhabiting microfauna (A). The effect of experimental elevation (B + C) is shown separately for communities transplanted to their original elevation, i.e. controls (B), and communities transplanted to opposing elevations (C). Communities subject to predation by mosquito larvae on microfauna abundance (P+) are shown as dotted line and cross symbols. Communities without predators (P-) are shown as straight line and dots. Duration of experiment: 7 weeks. n = 160.

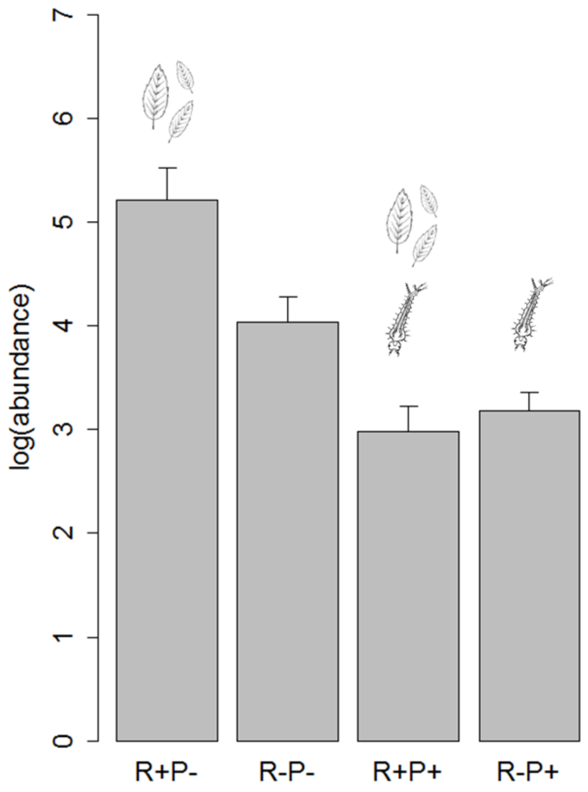


Figure 4: The effect of resource availability and predator presence on the total abundance [per 50 µl] of bromeliad-inhabiting microfauna. Resources increase microfauna abundance, but only in the absence of predators. R+: experimental communities with addition of high amount of detritus, R-: experimental communities with addition of low amount of detritus. P+: predator present P-: predator absent. No main effect of resource availability on microfauna abundance was observed (Tab 1). Predator presence significantly decreased microfauna abundance (Tab 1), especially in resource-rich habitats (Tab 1, sig. interaction between resource addition and predator presence).

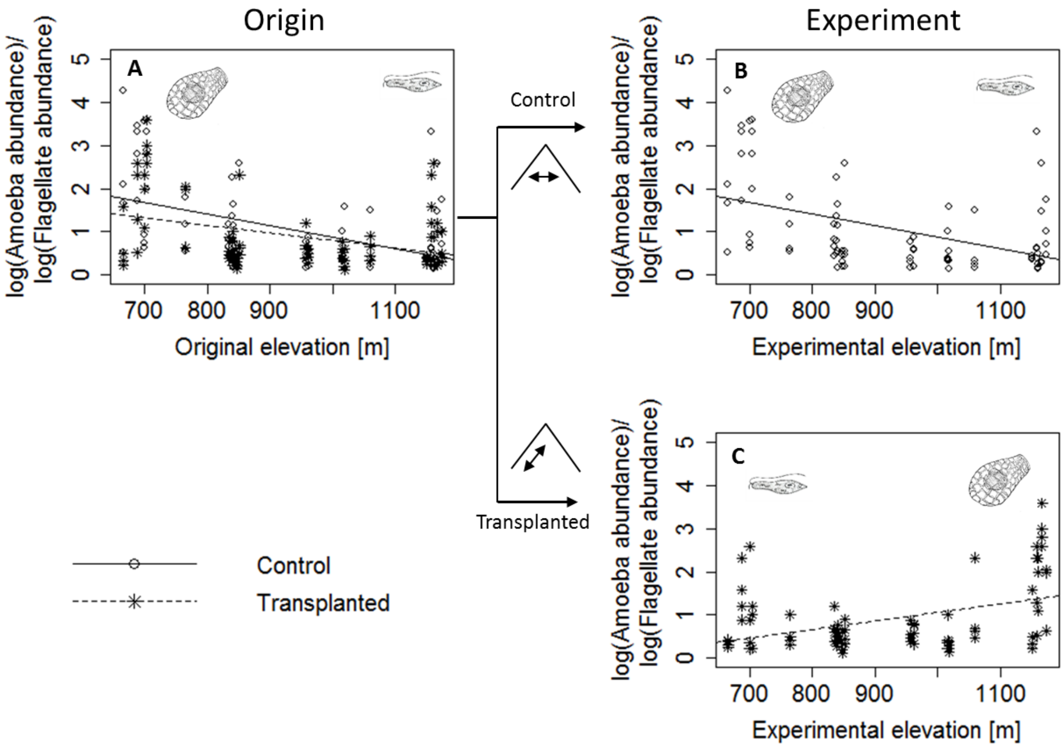


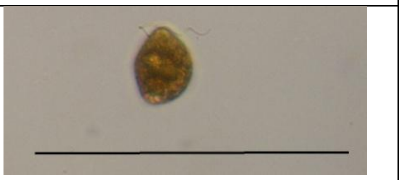
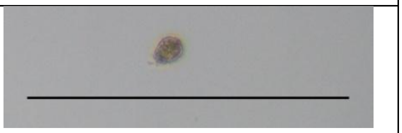
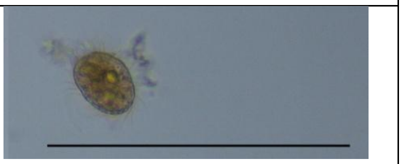
Figure 5: Relation of amoeba abundance (log-transformed) to flagellate abundance (log-transformed) [per 50 µL] in bromeliad-inhabiting microfauna communities along the original elevational gradient (A) and along the elevational gradient during the experiment (B+C), separated by communities transplanted to their original elevation, i.e. controls (B), and communities transplanted to the opposite mountain half (C). Elevational patterns of flagellate and amoeba relative abundance are mirrored after seven weeks of transplantation so that the effect of original elevation is maintained even after 7 weeks of the experiment. n = 160.

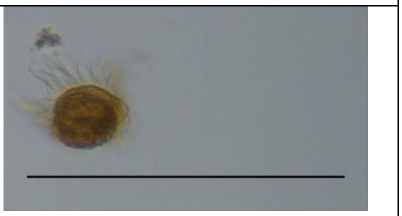
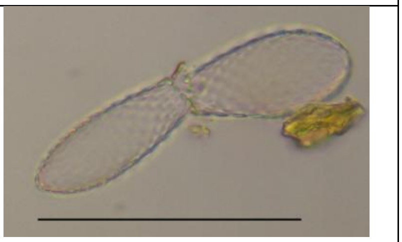
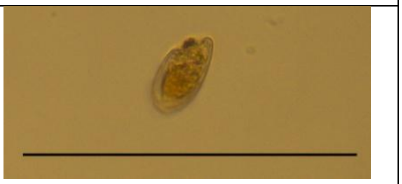
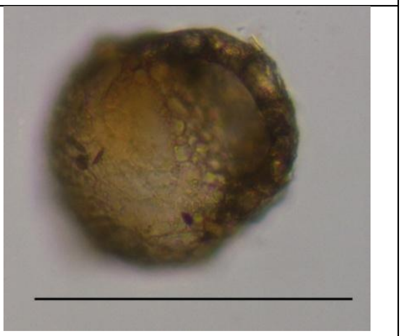
Table 2: Results of linear mixed effects model (LMM) analysis for bromeliad-inhabiting microfauna abundance (log-transformed) and evenness testing the effect of environmental variables, with microfauna treatment (i.e. ‘control’ versus ‘transplanted’) nested within bromeliad identity treated as random effects. This analysis includes only control communities (i.e. samples that were transplanted within their original elevation) and samples without predators to investigate which environmental factors shaped the original community structure. n=40.

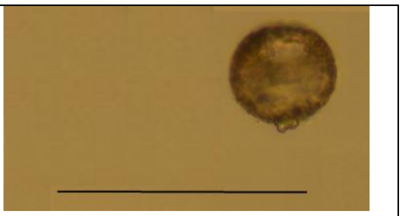
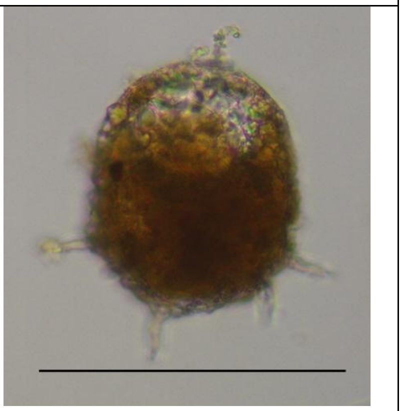
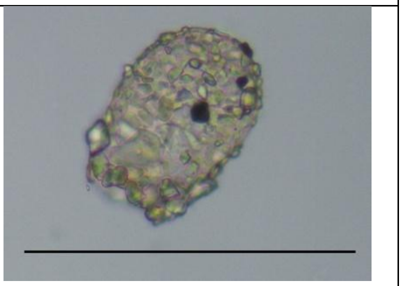
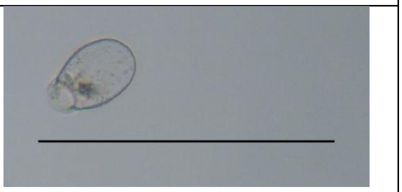
			Abundance		Evenness	
	numDF	denDF	F-value	p-value	F-value	p-value
Water temperature [°C]	1	14	0.32696	0.5765	1.32344	0.2692
PAR [µmol/m²s]	1	14	0.59546	0.4531	0.00012	0.9913
Height above ground [m]	1	14	1.21477	0.289	0.00005	0.9943
pH	1	14	2.52833	0.1341	0.17086	0.6856
Oxygen saturation [%]	1	14	4.8256	**0.0454 ↑**	0.07732	0.785

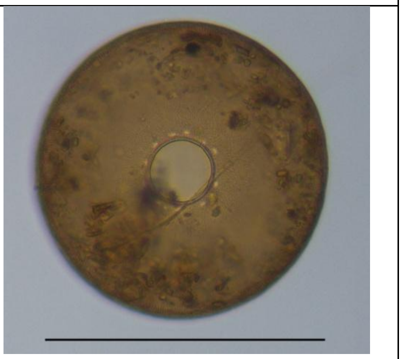
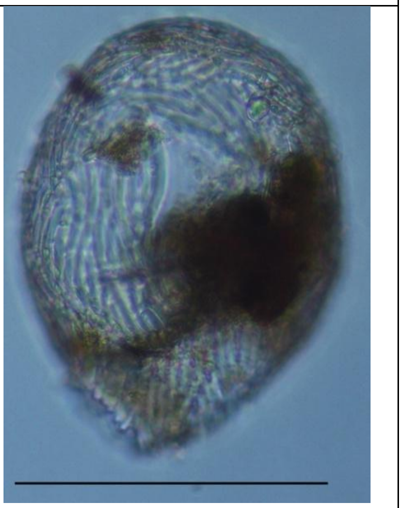
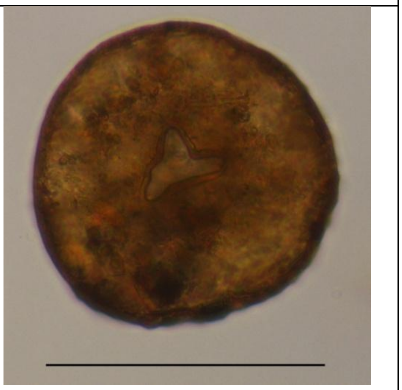
Supplement

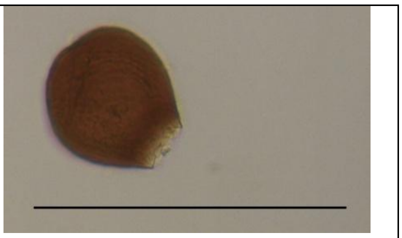
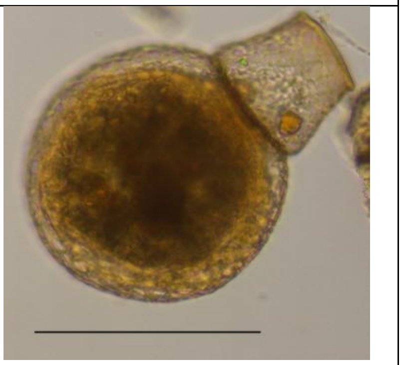
S1: List of all 32 bromeliad-inhabiting morphospecies found in our field experiment in Guanacaste with representative picture, Costa Rica. Scale bar: 100 µm.

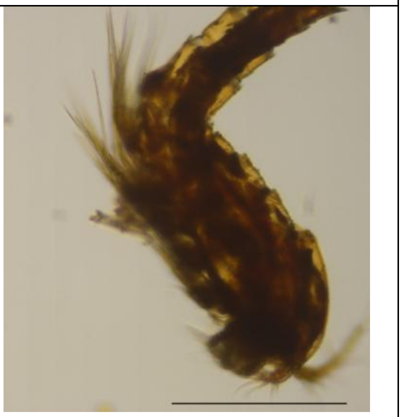
Morphospecies	**Photo**
Diatom 1	
Flagellate 1	
Flagellate 2	
Flagellate 3	
Flagellate 4	
Flagellate 5	
Flagellate 6	
Ciliate 1	

Ciliate 2	
Ciliate 3	
Ciliate 4	
Amoeba 1	
Amoeba 2	
Amoeba 3	
Amoeba 4	
Amoeba 5	

Amoeba 6	
Amoeba 7	
Amoeba 8	
Amoeba 9	
Amoeba 10	

Amoeba 11	
Amoeba 12	
Amoeba 13	
Amoeba 14	

Amoeba 15	
Amoeba 16	
Amoeba 17	
Amoeba 18	
Rotifer 1	

Rotifer 2	
Crustacean 1	 

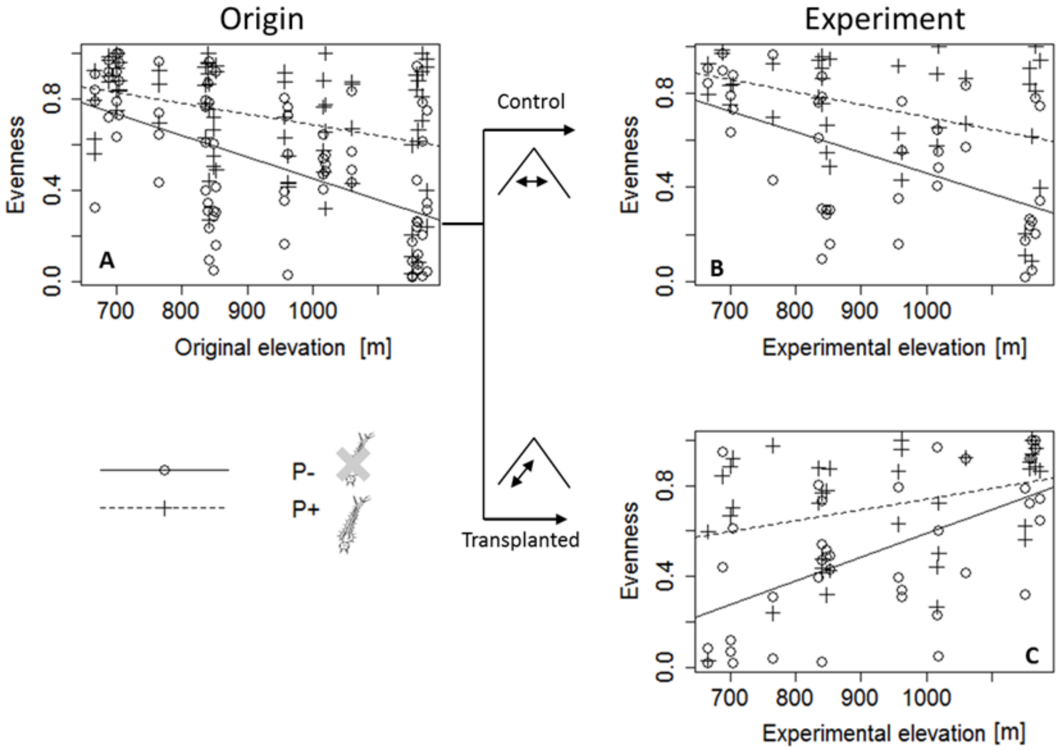
S2: Ranges and mean values of measured environmental variables and effects of elevation on these variables in 20 bromeliads along an elevational gradient in Guanacaste, Costa Rica. Measurements of oxygen saturation [%], pH and water temperature [°C] were taken in one exemplary natural leaf compartment per bromeliad at the start of the experiment. Photosynthetic active radiation (PAR [µmol/m²s]) was measured at five points above each bromeliad. ↑ - positive effect of elevation, ↓ - negative effect of elevation based on a linear regression analysis. ** $p < 0.01$, *** $p < 0.001$.

	Water temperature [°C] ↓ ***	**PAR [µmol/m²s]** ↑ ***	**Height above ground [m]** ↑ **	**pH** ↓ ***	**Oxygen saturation [%]** ↑ ***
Minimum	19.2	0	0	3.1	1.9
Mean	21.7	19.9	1	4.4	43.4
Maximum	23.9	124.6	3.2	5.9	102.3
SE	0.1	2.6	0.1	0.1	2.5

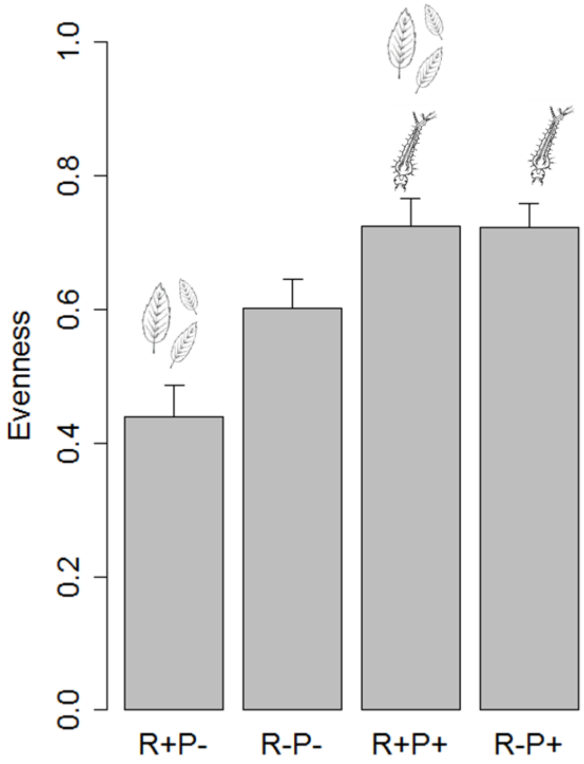
S3: Abundance, richness and frequency of functional groups in bromeliad-inhabiting microfauna per 50 µL sample. Flagellates clearly dominated the communities in terms of abundance and amoeba in terms of richness. No. of samples: 160.

	Abundance			**Species richness**			
	Range per sample	Mean ±SE per sample	Average relative abundance ± SE [%]	Range per sample	Mean ±SE per sample	Average relative species richness ± SE [%]	Frequency [%]
Total	1-6020	260 ± 56		1-13	6 ± 0		
Diatoms	0-11	1 ± 0	1 ± 0	0-1	0 ± 0	3 ± 1	21
Flagellates	0-5989	247 ± 56	60 ± 3	0-5	2 ± 0	29 ± 2	86
Ciliates	0-36	2 ± 0	3 ± 1	0-2	0 ± 0	4 ± 1	23
Amoebae	0-101	9 ± 1	27 ± 2	0-8	3 ± 0	48 ± 2	91
Rotifers	0-17	1 ± 0	5 ± 1	0-2	0 ± 0	7 ± 1	34
Micro-crustaceans	0-15	1 ± 0	4 ± 1	0-1	0 ± 0	9 ± 1	49

S4: Results of the transplantation experiment demonstrating a strong negative effect of original elevation on evenness of bromeliad-inhabiting microfauna (A). The effect of experimental elevation (B + C) is shown separately for communities transplanted to their original elevation, i.e. controls (B), and communities transplanted to opposing elevations (C). Predation by mosquito larvae on microfauna evenness (P+) is shown as dotted line and cross symbols. Absence of predators (P-) is shown as straight line and dots. Duration of experiment: 7 weeks. n = 160.



S5: The effect of resource availability and predator presence on the evenness of bromeliad-inhabiting microfauna. Resources decrease microfauna evenness, but only in the absence of predators. R+: experimental communities with addition of high amount of detritus, R-: experimental communities with addition of low amount of detritus. P+: predator present P-: predator absent. Predator presence significantly increased microfauna evenness.



S6: Results of linear mixed effects model (LMM) analysis for bromeliad-inhabiting microfauna evenness and species richness with microfauna treatment (i.e. ‘control’ versus ‘transplanted’) nested within bromeliad identity treated as random effects. Log-transformed abundance data were included as co-variable in these models (in contrast to the models in Tab. 1 in the main document) to distinguish direct effects of explanatory variables on evenness and species richness from effects via abundance. Data were obtained from a transplantation experiment along an elevational gradient on Orosilito mountain (Costa Rica) ranging from 666-1174 m a.s.l. Transplantation, resource availability and predation were crossed in a full-factorial experimental design resulting in eight treatment tubes per bromeliad. Microfauna communities were transplanted in 15 mL Falcon tubes between ten bromeliad pairs (upper to lower mountain and vice versa). Detritus dry weight was added to experimental tubes as sole resource for microfauna communities, using two experimental levels: ‘low’ versus ‘high’. The predator treatment used three filter-feeding mosquito larvae per test tube. Significant p values ($p \leq 0.05$) are printed in bold. ↑ - Positive relationship. ↓ - Negative relationship. n = 160. For the calculation of evenness one outlier sample with only one individual was excluded.

		Evenness			*Species richness*		
	numDF	denDF	F-value	p-value	denDF	F-value	p-value
log(Abundance)	1	106	468.032	**<.0001↓**	107	75.185	**<.0001↑**
Transplanted elevation	1	18	2.0117	0.1732	18	2.9822	0.1013
Original elevation	1	18	6.9117	**0.017↓**	18	2.9814	0.1013
Resource	1	106	0.1337	0.7154	107	4.6092	**0.0341↓**
Predator	1	106	0.0094	0.9229	107	0.0903	0.7644
Transplanted elevation:Original elevation	1	18	0.0455	0.8335	18	0.8448	0.3702
Transplanted elevation:Resource	1	106	0.247	0.6202	107	1.4423	0.2324
Original elevation:Resource	1	106	0.2352	0.6287	107	0.7766	0.3802
Transplanted elevation:Predator	1	106	0.1381	0.7109	107	0.1365	0.7125
Original elevation:Predator	1	106	0.0634	0.8017	107	1.1868	0.2784
Resource:Predator	1	106	0.1225	0.7271	107	0.112	0.7385
Transplanted elevation:Original elevation:Resource	1	106	0.0001	0.9923	107	0.0515	0.8209
Transplanted elevation:Original elevation:Predator	1	106	0.006	0.9382	107	1.2073	0.2743
Transplanted elevation:Resource:Predator	1	106	0.1767	0.675	107	1.1096	0.2945
Original elevation:Resource:Predator	1	106	0.0033	0.9545	107	0.2206	0.6395
Transplanted elevation:Original elevation:Resource:Predator	1	106	2.0643	0.1537	107	0.0089	0.9248

CHAPTER 3

Micro- and macrofauna communities in bromeliad phytotelmata are similar in forest canopy and understory

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Abstract

Three-dimensional sampling in forest ecosystems has previously revealed vertical stratification patterns in terrestrial community structure. We used single-rope climbing techniques to investigate if vertical patterns exist in community structure of bromeliad-inhabiting macro- and microfauna in a tropical rainforest in Costa Rica. We found that neither macro- nor microfauna communities changed with height above ground. Instead, macrofauna community structure was driven by other environmental factors such as water volume, number of leaves, canopy openness and water temperature. Microfauna community structure was not affected by any of the environmental variables measured, suggesting that seasonal variability, priority effects or trophic interactions (e.g. predation) might be more important in structuring of microfauna communities. Our results justify the use of logistically less challenging samples from bromeliads in the forest understory in future studies using the increasingly important bromeliad model system.

Introduction

Dimensionality of space is known to be an important factor for trophic interactions and thus a potential driver of species coexistence (Pawar et al. 2012). The assumption that in terrestrial ecosystems species interactions mostly take place on a 2D level while in aquatic ecosystems an additional vertical dimension exists has led to lively discussions on the differences in trophic structures between terrestrial and aquatic ecosystems (Hairston and Hairston 1993; Carr et al. 2003; Shurin et al. 2006). However, the separation of ecosystems into the categories ‘terrestrial’ and ‘aquatic’ has not proven useful (Chase 2000) and the third dimension was soon incorporated into terrestrial ecosystems as well (Drăguţ et al. 2010; Rutten et al. 2015). A pronounced vertical structuring is particularly visible in forest ecosystems (Terborgh and Estes 2013). Forest canopies are still vastly understudied parts of the forest ecosystem due to their difficult accessibility (Lowman 2000), but canopy science has recently gained much interest because first studies have shown that especially the upper forest parts are hotspots of biodiversity (Nakamura et al. 2017).

Bromeliad plants, growing in tropical rain forests of South and Central America, can be found on ground level as well as high up in the canopy (Zotz 2016). The leaves form water-holding tanks and host a diverse community of aquatic micro-organisms such as flagellates, ciliates and amoeba, small crustaceans and rotifers and macro-organisms such as insect larvae. These organisms decompose the accumulating leaf litter or prey on smaller organisms in the tank (Picado 1913). Due to the bromeliad system’s replicability, their tank-inhabiting communities are the ideal study system to investigate questions in community ecology (Srivastava et al. 2004). The numerous studies that have been conducted on bromeliad-inhabiting communities mostly used easily accessible plants on or near ground level. However, the majority of the bromeliads actually grow higher up in the canopy (Isaza et al. 2004). The natural occurrence of bromeliads in canopy and understory makes the third dimension one of the potentially important factors for community structuring processes because a number of environmental variables change along the height gradient (Ulyshen 2011; Petter et al. 2016). In the understory the accumulated leaf area index (i.e. canopy cover) is higher leading to reduced light availability (Castro 2000), lower temperatures (Fowells 1948) and an increased amount of dead organic matter falling to the ground (i.e. available nutrients) (Castro 2000; Rangel et al. 2015). These environmental differences along the height gradient can affect bromeliad morphology, for example tank volume which is a measure of habitat size for the inhabiting organisms, or the number of leaves, a measure of the heterogeneity of their habitat (Cavallero et al. 2011; Benzing 2000), both of which are known to be an important driver of bromeliad community structure (Armbruster et al. 2002). Another possibility is that environmental differences between canopy and understory affect the habitat quality, e.g. more sun-exposed bromeliads in the canopy will likely have stronger daily temperature fluctuations, which has been observed to be an important driver of microfauna community composition and beta diversity (Busse et al. 2018).

Here, we conducted a field study to investigate whether there are differences between bromeliad-inhabiting communities on the ground (in the forest understory) and in different heights from the ground and to identify the environmental variables that might drive these differences in bromeliad community structure. Any differences would then render the exclusive study of understory bromeliads inappropriate to address the full spectrum of bromeliad communities.

Methods

Study Site

Field work was carried out in secondary rainforest around the field station ‘Pitilla’ (N 10°59.374', W 85°25.583', 700 m a.s.l.) in the natural reserve Área de Conservación Guanacaste located in north-western Costa Rica. Samples were taken during the wet season in the years 2000 and 2015. In 2015, detailed measurements of meteorological data were taken: mean daily precipitation was 15.7 ± 2.3 (mean ± SE) mm, total precipitation during the sampling stay amounted to 787.1 mm, maximum daily temperatures ranged from 23°C to 28°C with a mean of 25 ± 0.2 (mean ± SE) °C and minimum daily temperatures ranged from 19°C to 22°C with a mean of 21 ± 0.1 (mean ± SE) °C.

Sampling

We sampled bromeliad pairs, i.e. one bromeliad from the canopy (> 10 m above ground) and one bromeliad from the understory (< 5 m above ground), from around or on twenty-one trees. Understory bromeliads where sampled within a 15 m radius from the canopy bromeliad tree. To assess canopy bromeliads we used single-rope climbing techniques. Bromeliads of the morphologically similar genera *Guzmania* and *Werauhia* with approximately the same diameter (65 ± 4 cm/ mean ± SE) and water volume (114 ± 26 mL/mean ± SE) were chosen. Diameter [cm] (maximum distance of relaxed leaf tips), total number of leaves and height above ground [m] were recorded for each bromeliad. Oxygen saturation [%] (only for 2015 data) and water temperature [°C] (only for 2015 data) were measured using an IntelliCal oxygen electrode (LDO) by HachLange in an exemplary outer water compartment. Light availability (only for 2015 data) was determined via pictures of the canopy above the plant using a camera with a fish-eye lense (Rollei Actioncam S50) to estimate canopy openness [canopy openness or gap fraction in %] using ImageJ PlugIn Hemisperical 2.0.

For microfauna sampling (only for 2015 data) water samples from an outer, water containing leaf compartment were taken per bromeliad and fixed with Lugol's solution. The water volume of the sampled leaf compartment was identified by extracting all water with a pipette after sampling. The remaining water volume of the total bromeliad was identified using the same method. All extracted water was transported to the research station Detritus (only for 2015 data) was extracted from the bromeliad and bagged. With all sampling done, the bromeliad was carefully removed from the tree or ground and transported back to the field station in a plastic bag. There, the macro-organisms remaining in the plant were collected by removing leaf after leaf (2000) or washed out using a high pressure hose (2015) and the bromeliad transportation bag was visually scanned for individuals. Subsequently, macrofauna individuals were collected from the all water and detritus and stored in 70%-ethanol.

Counting

Macrofauna (i.e. insect larvae) were identified and measured using a stereomicroscope and classified into functional groups following and adding to a previously developed key (http://www.zoology.ubc.ca/~srivast/pitilla/, for new morphospecies see S1). Microfauna (i.e. diatoms, flagellates, ciliates, amoeba, rotifers and micro-crustaceans) were counted as morphotypes (see S2) by processing subsamples of 50 µL of each water sample using light microscopy (400x magnification) and using and adding to a photographic key developed in previous projects (Busse et al. 2018).

Permission for field work and sampling was granted by Sistema Nacional de Áreas de Conservación (SINAC) and Ministerio del Ambiente y Energía (MINAE) in Costa Rica (permit no. ACG-PI-046-2015).

Statistics

For the combined macrofauna data set with samples from 2000 and 2015 the effect of total water volume [mL], number of leaves [per bromeliad] and height above ground [m] on community structure (abundance, species richness, community composition) was tested. Differences in abundance and species richness were tested using linear regression models. Abundance data were log-transformed to achieve normality and homoscedasticity. Species richness models include abundance as co-variable fitted before the environmental variables. Differences in community composition were tested using PERMANOVA and visualized using non-metric multidimensional scaling (NMDS). For the analyses of community composition species-by-site matrices were Hellinger-transformed to exclude patterns that were simply due to differences in abundance (Legendre and Gallagher 2001). For macrofauna and microfauna data from 2015, community structure was analyzed the same way but including additional environmental variables which were not available for the data from 2000, i.e. canopy openness [% light pixel], water temperature [°C], oxygen saturation [%] and detritus dry weight [g]. Measures of water volume and detritus dry weight refer to the whole bromeliad for macrofauna and to the leaf compartment for microfauna since this is the relevant habitat size for these organisms. The number of leaf compartments as a variable is only considered for macrofauna analyses because microfauna cannot actively move between the leaf axils.

All statistical analyses were done in R (R Core Team 2017) using the package *vegan* (Oksanen et al. 2017).

Results

A total of 60 macrofauna species were identified, of which 50 were Diptera, 4 Coleoptera, 3 Oligochaeta, 1 Platyhelminthes, 1 Hirudinea and 1 Odonata. The species identified in the two different data sets from 2000 and 2015 showed some overlap with 19 species found in both years. 12 species were exclusively found in the year 2000 and 29 in the year 2015. A total of 28 microfauna morphospecies were differentiated (data only available for 2015), of which 2 were flagellates, 4 ciliates, 19 amoebae, 1 crustacean and 2 rotifers.

Community structure

Total abundance and species richness of neither macro- nor microfauna differed significantly between canopy and understory bromeliads (Fig 1). Macro- and microfauna community composition did not differ significantly between canopy and understory bromeliads (Fig 2). Beta diversity (measured as distance to centroid) did not differ significantly between canopy and understory for neither macrofauna ($F_{1,40} = 0.730$, $p = 0.398$) nor microfauna ($F_{1,20} = 0.731$, $p = 0.403$).

Environment

The environmental variables measured showed no differences between canopy and understory except for canopy openness which was significantly higher in the canopy than in the understory (S3).

To identify the environmental variables which drive community structure in bromeliads we analysed the combined macrofauna data sets from the years 2000 and 2015 using the environmental variables which were measured in both years, i.e. total water volume [mL], number of leaves [1/bromeliad] and height above ground [m]. Neither total water volume nor number of leaves changed with height above ground (S3). Total water volume as a measure of habitat size, was of major importance for community structure, affecting abundance, species richness and community composition (Tab 1), with higher abundance and higher (abundance-controlled) species richness in bromeliads with larger water volumes. Additionally, the number of leaves per bromeliad as a measure of habitat heterogeneity played a minor role for abundance and species richness (Tab 1) with higher numbers of leaves resulting in higher abundance and species richness. Height above ground did not explain any variance in community structure when fitted last (or first) in the models (Tab 1, Tab 2, latter results not shown).

As data on more environmental variables (i.e. canopy openness, water temperature, oxygen saturation and detritus dry weight additionally to total water volume, number of leaves and height above ground) and on microfauna were available for 2015, we conducted an additional analysis for 2015 only, test the effects of the additional variables. Macrofauna abundance was affected by canopy openness (Tab 2) in addition to the effect of water volume which was also found in the combined data set (Tab 1 + 2). The removal of canopy openness from the model due to autocorrelation with height above ground did not result in a significant effect of the latter (*results not shown*). The number of leaves per bromeliad did not have a significant effect on macrofauna abundance in contrast to the result from the analysis of the combined data set (Tab 1 + 2). Macrofauna species richness was affected by water temperature (Tab 2) in addition to effects of water volume and number of leaves which was also found in the combined data set (Tab 1 + 2). No measured environmental variable was found to affect macrofauna community composition here (Tab 2) in contrast to the effect of water volume on community composition found for the combined data set (Tab 1). None of the measured environmental variables affected the structure of the microfauna community (Tab 2). Height above ground was not found to affect either macro- or microfauna community structure (Tab 1 + 2).

Discussion

The aim of our study was to investigate potential differences between canopy and understory in the structure of bromeliad-inhabiting communities. We found no indication that the community structure of either macro- or microfauna changes with height above ground. This result is contrary to our expectations. In literature numerous examples exist showing the forest canopy to harbour different communities than the forest understory, for example in butterflies (DeVries et al. 1997; Schulze et al. 2001), ants (Longino and Nadkarni 1990) and arthropods in general (Ulyshen 2011). However, this pattern did not apply to bromeliad-inhabiting macro- and microfauna.

One reason for the absence of community structure differences could be the lack of pronounced environmental differences between canopy and understory in our study system (see S3). Contrary to former canopy-understory comparisons we investigated discrete habitats, i.e. the bromeliads. Bromeliad water conditions have previously been observed to remain the same regardless of height above ground (Jocque and Kolby 2012) which is supported by our data. While previous findings of differences resulted from changes in habitat structure (e.g. MacArthur and MacArthur 1961), the complexity and structure of the bromeliad units remained unchanged between canopy and understory. It is possible that bromeliads at the highest canopy level which are completely exposed to the sky (and unfortunately, inaccessible to rope-climbing assisted sampling) show more pronounced differences in environmental conditions and do hold different communities. This could be investigated via crane work at suitable sites. In our study, only canopy openness changed along the height gradient (see S3) and could have explained differences in community structure between canopy and understory. Macrofauna abundance increased with canopy openness, but the removal of canopy openness from the model did not result in a significant effect of height above ground *(results not shown)*, showing that canopy openness cannot be a main driver of any height effect on macrofauna abundance. We identified several other environmental variables which did not change with height to play an important part in structuring bromeliad-inhabiting macrofauna communities. First of all, total water volume as a measure of habitat size played a major role in structuring bromeliad macrofauna communities as it was positively related to abundance and species richness. Higher macrofauna abundance and richness in bromeliads with greater water volume were formerly observed by Dézerald et al. (2014), suggesting that some species, e.g. open water species such as culicids, need large water volumes. Not only water volume but also other morphological aspects of the bromeliad, such as the number of leaves per bromeliad as a measure of habitat heterogeneity may shape community structure (Armbruster et al. 2002). We found indeed that the number of leaves per bromeliad affects species richness and marginally also the abundance of bromeliad-inhabiting macrofauna. Bromeliads are compartmentalized and only some of the species such as larger (predatory) taxa, for example damselfly and mosquito larvae (Srivastava and Bell 2009; Frank et al. 1984), can actively move between the leaf compartments. A higher habitat complexity achieved by a greater number of leaf compartments has been shown to reduce predator efficiency (Srivastava 2006) which may result in higher species richness (Freestone et al. 2011). Another factor that influenced species richness in our study was water temperature. Higher water temperature decreased macrofauna richness. The significant effect of water temperature on microfauna richness in bromeliads has been formerly observed (Kratina et al. 2017; Petermann et al. 2015), though it remains unclear if this effect is direct or indirect. Either less species have their tolerance range in higher temperatures or higher water temperatures result in less favourable water conditions (e.g. reduced oxygen saturation, increased acidity (Jocque and Kolby 2012)). The aspect of water temperature is particularly important in the dry season. Bromeliads with higher water temperature are more prone to evaporation, which in the worst case leads to drought during the dry season making survival for species without duration stages or good dispersal abilities impossible (Williams 1987, 1996). Seasonality has been shown various times to play an important role in structuring bromeliad communities (Mondragón-Chaparro and Cruz-Ruiz 2008; Castaño-Meneses 2016; Buosi et al. 2015). Differences in bromeliad community structure between canopy and understory can even be reversed over different seasons of the year (Mestre et al. 2001) demonstrating that (seasonal) changes of environmental conditions need to be taken into account when planning sampling events.

Microfauna community structure was not affected by any of the environmental variables measured in this study. Previous studies showed that microfauna is susceptible to changes in environmental conditions such as daily temperature fluctuations (Busse et al. 2018) and therefore also affected by seasonality (Nolte et al. 2010). Though many microfauna species have duration stages to bridge undesirable (e.g. acidic) water conditions or to survive drought events, they might still be affected via cascading trophic effects due to changes in predation pressure over different seasons. As stated earlier we assume that the exposed canopy bromeliads are more prone to evaporation in the dry season, which means that drought-sensitive predators (such as mosquito larvae which act as predators of microfauna) are more likely to suffer from habitat loss in canopy bromeliads than in understory bromeliads. This would lead to a vertical stratification of predation pressure in the dry season, and this difference in predation pressure could potentially influence microfauna communities as shown previously in bromeliads (Busse et al. submitted, see chapter 2). Therefore, sampling during the dry season, instead of during the wet season as we did, might lead to a different result regarding the similarity of canopy and understory community structure. The lack of difference between communities at different heights may also partly be a result of a potentially lower taxonomic resolution of our morphospecies approach as differences may be subtle, for example a turnover of closely related species as shown previously in ants (Longino and Nadkarni 1990).

Our findings suggest that the sampling of ground bromeliads only may represent an adequate method to assess their community structure. The logistically more laborious canopy sampling does not seem to provide additional information here. It remains to be tested if this finding is consistent at other sampling sites where differences in the environment between canopy and understory may be larger. Primary rain forests, for example, are more strongly structured than secondary rainforests (Barlow et al. 2007; Casas et al. 2016; Ulyshen 2011) and could thus provide a stronger gradient in environmental conditions which might shift community structure along the height gradient.

References

Armbruster, P. et al. 2002. Factors Influencing Community Structure in a South American Tank Bromeliad Fauna. - OIKOS 96: 225–234.

Barlow, J. et al. 2007. Quantifying the biodiversity value of tropical primary, secondary, and plantation forests. - PNAS 104: 18555–18560.

Benzing, D. H. 2000. Bromeliaceae: profile of an adaptive radiation. - Cambridge University Press.

Buosi, P. R. B. et al. 2015. Effects of Seasonality and Dispersal on the Ciliate Community Inhabiting Bromeliad Phytotelmata in Riparian Vegetation of a Large Tropical River. - The Journal of eukaryotic microbiology 62: 737–749.

Busse, A. et al. 2018. Different in the dark: The effect of habitat characteristics on community composition and beta diversity in bromeliad microfauna. - PloS one 13: e0191426.

Carr, M. H. et al. 2003. Comapring marine and terrestrial ecosystems: Implications for the design of coastal marine reserves. - Ecological Applications 13: 90–107.

Casas, G. et al. 2016. Habitat Structure Influences the Diversity, Richness and Composition of Bird Assemblages in Successional Atlantic Rain Forests. - Tropical Conservation Science 9: 503–524.

Castaño-Meneses, G. 2016. Seasonal and altitude effects on the structure of arthropod communities associated with Tillandsia violacea Baker (Bromeliaceae) in a temperate forest of Mexico. - Arthropod-Plant Interactions 10: 403–417.

Castro, F. de 2000. Light spectral composition in a tropical forest. Measurements and model. - Tree Physiology 20: 49–56.

Cavallero, L. et al. 2011. morphological variation of the leaves of Aechmea distichantha Lem. plants from contrasting habitats of a Chaco forest: a trade-off between leaf area and mechanical support. - Revista Brasileira de Biociências 9: 455–464.

Chase, J. M. 2000. Are there real differences among aquatic and terrestrial food webs? - Trends in Ecology & Evolution 15: 408–412.

DeVries, P. J. et al. 1997. Species diversity in vertical, horizontal, and temporal dimensions of a fruitfeeding butterfly community in an Ecuadorian rainforest. - Biological Journal of the Linnean Society 62: 343–364.

Dézerald, O. et al. 2014. Environmental determinants of macroinvertebrate diversity in small water bodies. Insights from tank-bromeliads. - Hydrobiologia 723: 77–86.

Drăguţ, L. et al. 2010. The Third and Fourth Dimensions of Landscape. Towards Conceptual Models of Topographically Complex Landscapes. - Landscape Online 22; 1-10.

Fowells, H. A. 1948. The Temperature Profile In A Forest. - Journal of Forestry 46: 897–899.

Frank, J. H. et al. 1984. On the Bionomics of Bromeliad-Inhabiting Mosquitoes X. Toxorhynchites r. rutilus as a predator of Wyeomyia vanduzeei (Diptera: Culicidae). - Journal of medical entomology 21: 149–158.

Freestone, A. L. et al. 2011. Stronger predation in the tropics shapes species richness patterns in marine communities. - Ecology 92: 983–993.

Hairston, N. G. Jr. and Hairston, N. G. Sr. 1993. Cause-Effect Relationships in Energy Flow, Trophic Structure, and Interspecific Interactions. - The American Naturalist 142: 379–411.

Isaza, C. et al. 2004. Vertical distribution of bromeliads in a montane forest in the Eastern Cordillera of the Colombian Andes. - Selbyana 25: 126–137.

Jocque, M. and Kolby, J. E. 2012. Acidity of tank bromeliad water in a cloud forest, Cusuco National Park, Honduras. - Int. J. Plant Physiol. Biochem. 4: 59–70.

Kratina, P. et al. 2017. Environmental control of the microfaunal community structure in tropical bromeliads. - Ecology and evolution 7: 1627–1634.

Legendre, P. and Gallagher, E. D. 2001. Ecologically meaningful transformations for ordination of species data. - Oecologia 129: 271–280.

Longino, J. T. and Nadkarni, N. M. 1990. A comparison fo ground and canopy leaf litter ants (Hymenoptera: Formicidae) in a neotropical montane forest. - Psyche: A Journal of Entomology 97: 81–93.

Lowman, M. D. 2000. Life in the treetops: adventures of a woman in field biology. - Yale University Press.

MacArthur, R. H. and MacArthur, J. W. 1961. On bird species diversity. - Ecology 42: 594–598.

Mestre, L. A. M. et al. 2001. Macroinvertebrate fauna associated to the bromeliad Vriesea inflata of the Atlantic Forest (Paraná State, southern Brazil). - Braz. arch. biol. technol. 44: 89–94.

Mondragón-Chaparro, D. M. and Cruz-Ruiz, G. I. 2008. Seasonal variation of the macro-arthropod community associated to Tillandsia carlos-hankii (Bromeliaceae) in an oak-pine forest in Oaxaca, Mexico. - Brenesia 70: 11–22.

Nakamura, A. et al. 2017. Forests and Their Canopies. Achievements and Horizons in Canopy Science. - Trends in Ecology & Evolution 32: 438–451.

Nolte, V. et al. 2010. Contrasting seasonal niche separation between rare and abundant taxa conceals the extent of protist diversity. - Molecular ecology 19: 2908–2915.

Oksanen, J. et al. 2017. vegan: Community Ecology Package. R package version 2.4-4, https://CRAN.R-project.org/package=vegan.

Pawar, S. et al. 2012. Dimensionality of consumer search space drives trophic interaction strengths. - Nature 486: 485–489.

Petermann, J. S. et al. 2015. Resources alter the structure and increase stochasticity in bromeliad microfauna communities. - PloS one 10: e0118952.

Petter, G. et al. 2016. Functional leaf traits of vascular epiphytes. Vertical trends within the forest, intra- and interspecific trait variability, and taxonomic signals. - Funct Ecol 30: 188–198.

Picado, C. 1913. Les broméliacées épiphytes considérées comme milieu biologique. Las bromeliáceas epífitas consideradas como medio biológico. - Bulletin Scientifique de la France et de la Belgique 47: 215–360.

R Core Team 2017. R: A Language and Environment for Statistical Computing. - Vienna, Austria, https://www.R-project.org/.

Rangel, J. V. et al. 2015. Assessing the role of canopy cover on the colonization of phytotelmata by aquatic invertebrates. An experiment with the tank-bromeliad Aechmea lingulata. - J Limnol.

Rutten, G. et al. 2015. Vertical and Horizontal Vegetation Structure across Natural and Modified Habitat Types at Mount Kilimanjaro. - PloS one 10: e0138822.

Schulze, C. H. et al. 2001. Understorey versus canopy: patterns of vertical stratification and diversity among Lepidoptera in a Bornean rain forest. - Plant Ecology 153: 133–152.

Shurin, J. B. et al. 2006. All wet or dried up? Real differences between aquatic and terrestrial food webs. - Proceedings. Biological sciences 273: 1–9.

Srivastava, D. S. et al. 2004. Are natural microcosms useful model systems for ecology? - Trends in Ecology & Evolution 19: 379–384.

Srivastava, D. S. 2006. Habitat structure, trophic structure and ecosystem function. Interactive effects in a bromeliad-insect community. - Oecologia 149: 493–504.

Srivastava, D. S. and Bell, T. 2009. Reducing horizontal and vertical diversity in a foodweb triggers extinctions and impacts functions. - Ecology letters 12: 1016–1028.

Terborgh, J. and Estes, J. A. (E.) 2013. Trophic cascades: predators, prey, and the changing dynamics of nature. - Island Press.

Ulyshen, M. D. 2011. Arthropod vertical stratification in temperate deciduous forests. Implications for conservation-oriented management. - Forest Ecology and Management 261: 1479–1489.

Williams, D. D. 1987. The Ecology of Temporary Waters. - Timber Press.

Williams, D. D. 1996. Environmental Constraints in Temporary Fresh Waters and Their Consequences for the Insect Fauna. - Journal of the North American Benthological Society 15: 634–650.

Zotz, G. 2016. Plants on plants-the biology of vascular epiphytes. - Springer.

Figures and tables

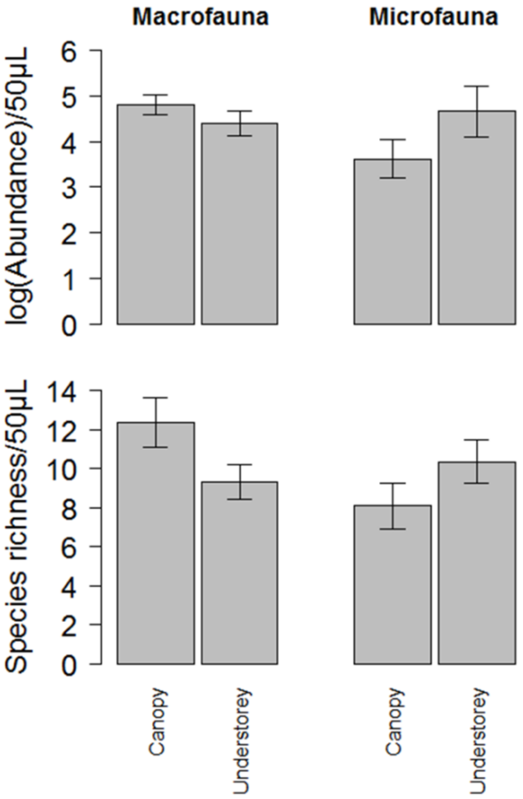


Figure 1: Comparison of total abundance and species richness of macro- and microfauna per 50 µL water sample between canopy and understory bromeliads. The difference between canopy and understory was not significant in any of the cases (i.e. all p>0.05).

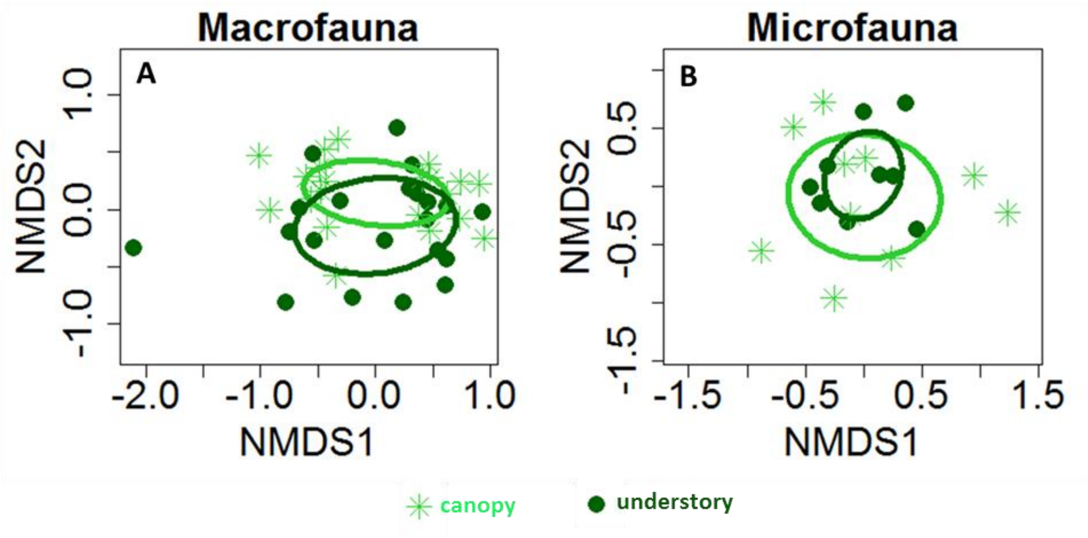


Figure 2: Difference in bromeliad-inhabiting macro- (A) and microfauna (B) communities between canopy and understory. PERMANOVA results: A (macrofauna): n = 42 , $F_{1,40}$ = 1.3, p = 0.230. B (microfauna): n = 22 , $F_{1,20}$ = 1.1, p = 0.278.

Table 1: Results from analyses testing the effect of environmental variables on community structure of bromeliad-inhabiting macrofauna using combined data sets from 2000 and 2015. n = 42. Abundance (log transformed) and species richness were analysed using linear regression analyses. Community composition was analysed using PERMANOVA (number of permutations: 999) with Hellinger-transformed species-by-site matrices. ↑ - positive relationship, ↓ - negative relationship.

Log(Abundance)	Df	F value	P value
Total water volume [mL]	1	9.187	**0.004↑**
Number of leaves [1/bromeliad]	1	8.998	**0.005↑**
Height above ground [m]	1	0.058	0.811
Residuals	38		

Species richness	Df	F value	P value
log(abundance/50 µL)	1	63.065	**<0.001↑**
Total water volume [mL]	1	46.110	**<0.001↑**
Number of leaves [1/bromeliad]	1	6.270	**0.017↑**
Height above ground [m]	1	0.094	0.761
Residuals	37		

Community composition	Df	F.Model	R2	P value
Total water volume [mL]	1	2.837	0.066	**0.016**
Number of leaves [1/bromeliad]	1	1.001	0.023	0.393
Height above ground [m]	1	1.406	0.033	0.163
Residuals	38	0.879		
Total	41			

Table 2: Results from analyses testing the effect of further environmental variables on community structure of bromeliad-inhabiting macro- and microfauna. Macrofauna was analysed on bromeliad level and microfauna on leaf compartment level (one exemplary leaf per bromeliad). Canopy openness was estimated from pictures taken with a fish-eye lense using ImageJ PlugIn Hemisperical 2.0 to determine the percentage of light pixels. Data are from 2015 only because these environmental variables were not measured in 2000. N = 22 (for microfauna 21 due to one missing data point on detritus in one compartment). Abundance (log-transformed) and species richness were analysed using linear regression analyses. Community composition was analysed using PERMANOVA (number of permutations: 999) with Hellinger-transformed species-by-site matrices. ↑ - positive relationship, ↓ - negative relationship.

Log-transformed abundance

	MACROFAUNA			*MICROFAUNA*		
	Df	F value	P value	Df	F value	P value
Canopy openness [%]	1	10.052	**0.007↑**	1	0.585	0.457
Total water volume [mL]	1	4.740	**0.047↑**	1	0.606	0.449
Number of leaves [1/bromeliad]	1	4.439	0.054	-	-	-
Water temperature [°C]	1	0.013	0.912	1	0.008	0.930
Oxygen saturationn [%]	1	0.011	0.918	1	0.029	0.867
Detritus dry weight [g]	1	0.056	0.816	1	0.245	0.629
Height above ground [m]	1	0.425	0.525	1	1.280	0.277
Residuals	14			14		

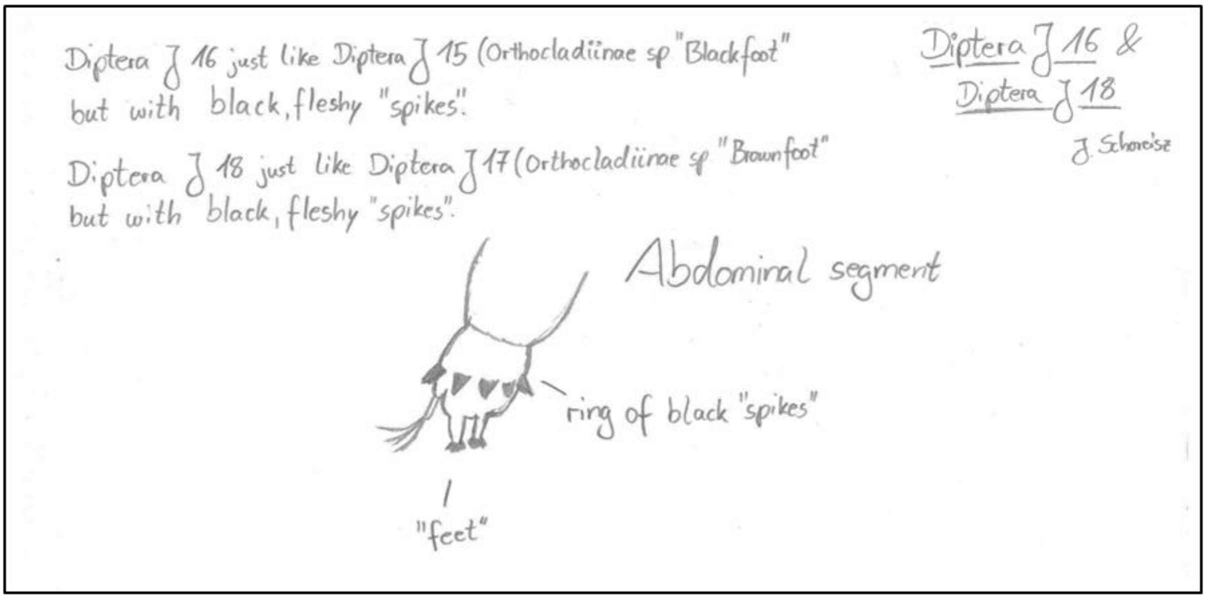
Species richness

	MACROFAUNA			*MICROFAUNA*		
	Df	F value	P value	Df	F value	P value
log(abundance/50 µL)	1	154.079	**<0.001↑**	1	2.281	0.155
Canopy openness [%]	1	0.281	0.605	1	1.155	0.302
Total water volume [mL]	1	59.251	**<0.001↑**	1	0.235	0.636
Number of leaves [1/bromeliad]	1	21.677	**<0.001↑**	-	-	-
Water temperature [°C]	1	21.838	**<0.001↓**	1	0.583	0.459
Oxygen saturationn [%]	1	0.483	0.499	1	1.056	0.323
Detritus dry weight [g]	1	0.089	0.770	1	0.614	0.447
Height above ground [m]	1	0.013	0.912	1	1.831	0.199
Residuals	13			13		

	Community composition							
	MACROFAUNA				*MICROFAUNA*			
	Df	F Model	R²	P value	Df	F Model	R²	P value
Canopy openness [%]	1	1.1778	0.056	0.284	1	0.677	0.038	0.646
Total water volume [mL]	1	1.156	0.056	0.325	1	0.209	0.012	0.977
Number of leaves [1/bromeliad]	1	0.560	0.027	0.880	-	-	-	-
Water temperature [°C]	1	0.944	0.045	0.499	1	0.313	0.018	0.931
Oxygen saturationn [%]	1	0.721	0.035	0.727	1	0.458	0.026	0.847
Detritus dry weight [g]	1	0.514	0.025	0.903	1	1.250	0.070	0.316
Height above ground [m]	1	1.755	0.084	0.063	1	0.965	0.054	0.41
Residuals	14	0.672			14	0.783		
Total	21				20			

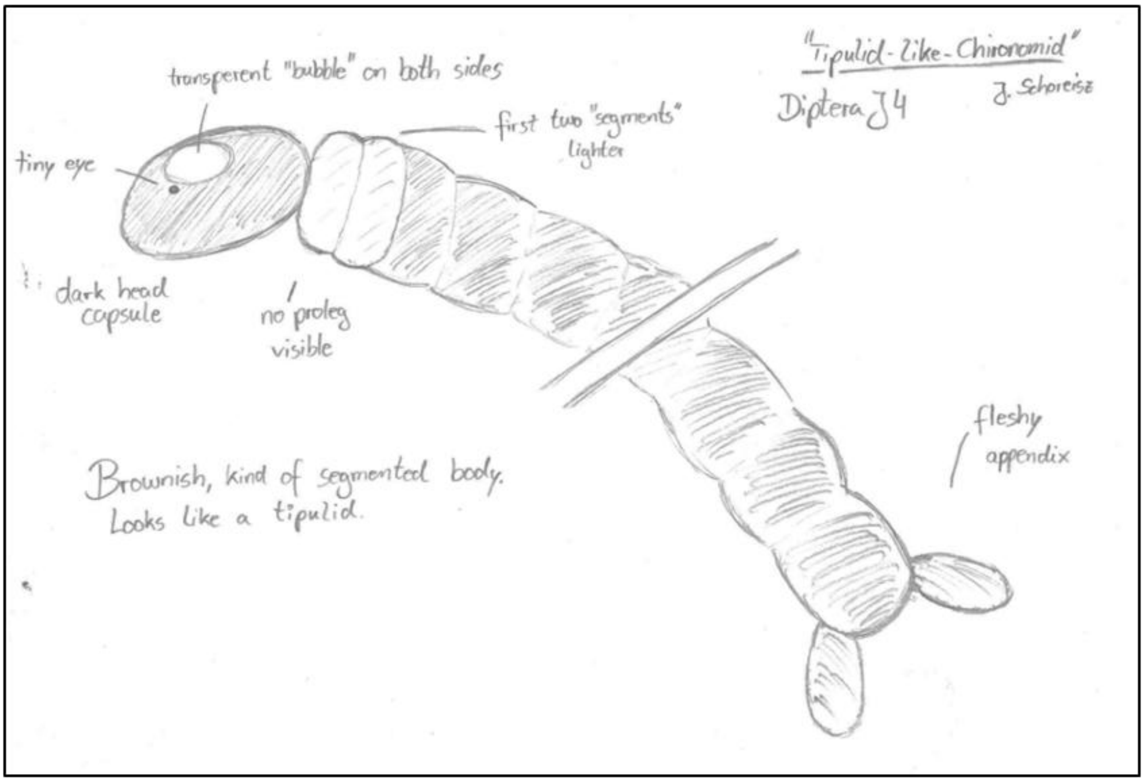
Supplement

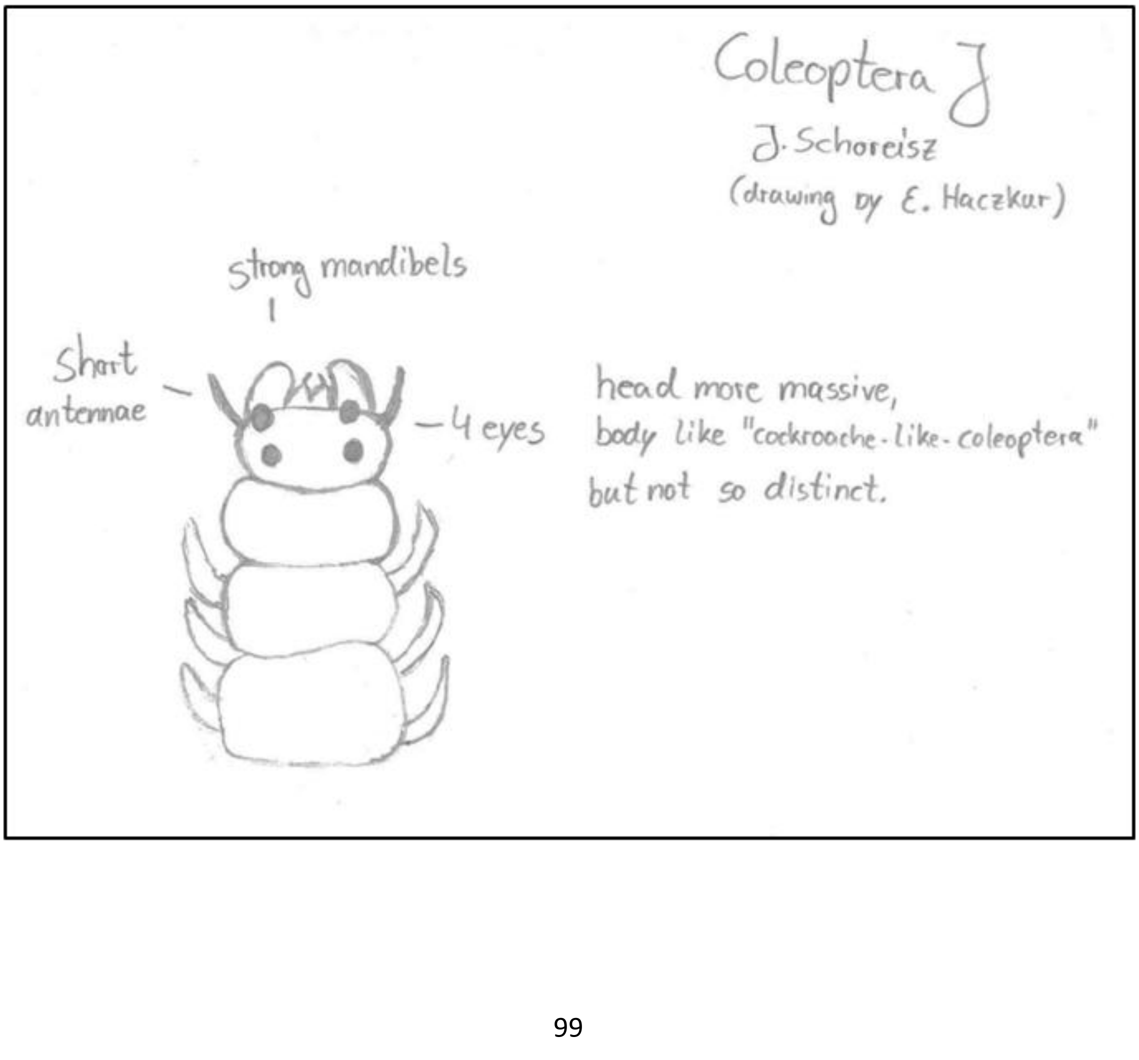
<u>S1:</u> List of three additionally found bromeliad-inhabiting invertebrate morphospecies from a field survey along a height gradient in Guanacaste (Costa Rica) with representative drawings and annotations by Jeremias j. Schoreisz. Previously described morphospecies can be found under: http://www.zoology.ubc.ca/~srivast/pitilla/



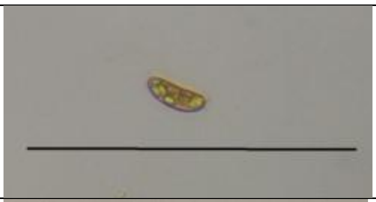
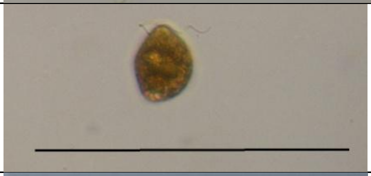
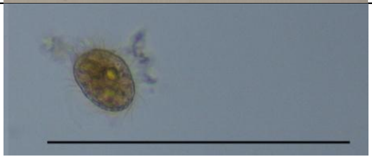
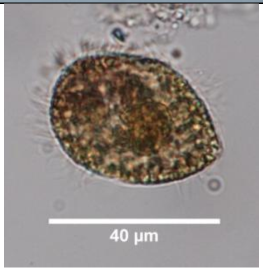
Diptera J 16 just like Diptera J 15 (Orthocladiinae sp "Blackfoot" but with black, fleshy "spikes".

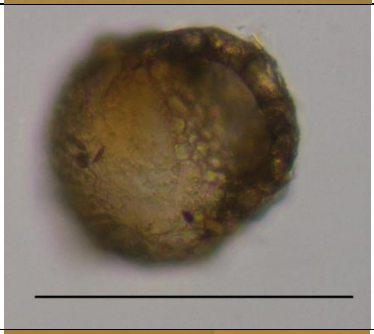
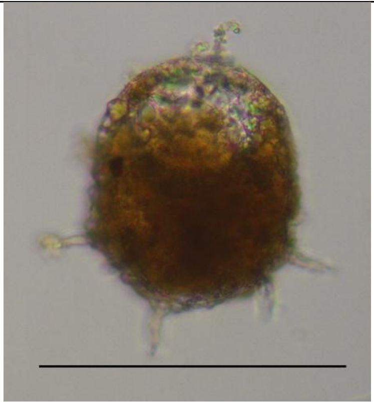
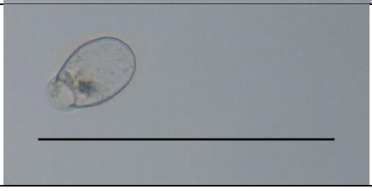
Diptera J 18 just like Diptera J 17 (Orthocladiinae sp "Brownfoot" but with black, fleshy "spikes".

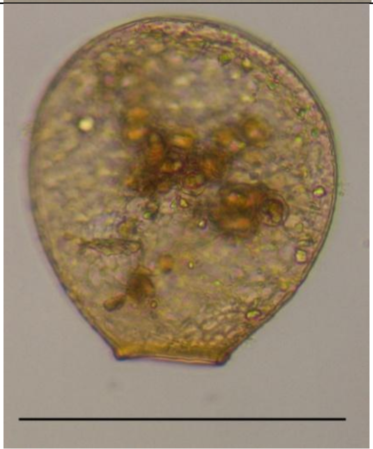
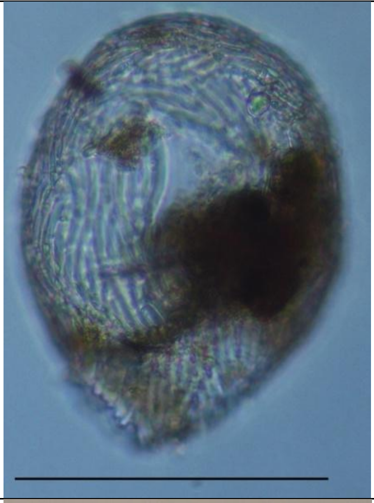

"Tipulid-Like-Chironomid"
J. Schoreisz
Diptera J4
transperent "bubble" on both sides
tiny eye
first two "segments" lighter
dark head capsule
no proleg visible
fleshy appendix
Brownish, kind of segmented body.
Looks like a tipulid.

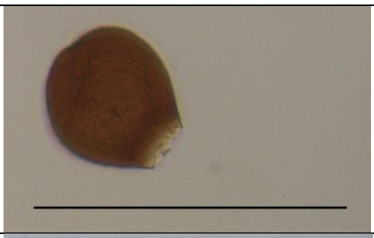

Coleoptera J
J. Schoreisz
(drawing by E. Haczkur)
strong mandibels
Short antennae
4 eyes
head more massive,
body like "cockroache-like-coleoptera"
but not so distinct.

S2: List of all 28 bromeliad-inhabiting microfauna morphospecies found in a field survey along a height gradient in Guanacaste with representative picture, Costa Rica. Scale bar without annotation: 100 µm. Names are based on previous keys.

Morphospecies	Photo
Flagellate 1	
Flagellate 3	
Ciliate 1	
Ciliate 2	
Ciliate 3	
Ciliate 6	
Amoeba 1	
Amoeba 2	

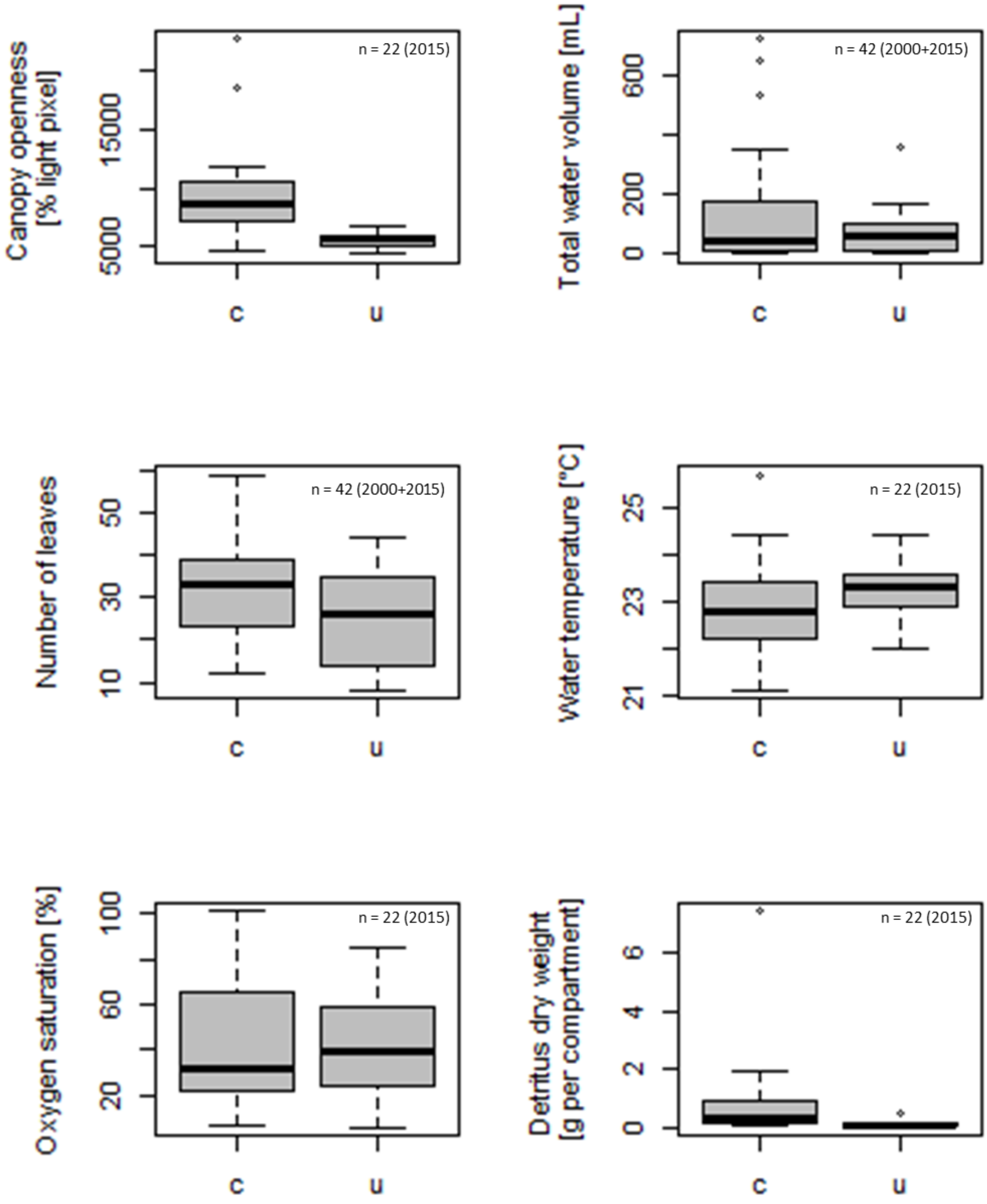
Amoeba 3	
Amoeba 4	
Amoeba 5	
Amoeba 6	
Amoeba 7	
Amoeba 8	
Amoeba 9	

Amoeba 10	
Amoeba 11	
Amoeba 13	
Amoeba 14	

Amoeba 15	
Amoeba 16	
Amoeba 17	
Amoeba 18	
Amoeba 33	
Amoeba 34	

Rotifer 1	
Rotifer 2	
Crustacean 1	

S3: Comparison of environmental parameters in bromeliads between canopy and understory. Only canopy openness differed significantly between canopy and understory ($F_{1,20}$ = 8.169, p = 0.00972). c – canopy, u – understory.



CHAPTER 4

Variability in environmental conditions between elevational gradients leads to diverse patterns in microfauna community structure and beta diversity

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Abstract

Microfauna communities have often been suggested to respond differently to environmental changes than macrofauna communities. To detect patterns in microfauna community structure along environmental gradients, we studied microfauna communities in bromeliads along elevational gradients. We assumed that abiotic variables such as light availability, temperature, pH, oxygen saturation, compartment water volume and resource availability would show consistent patterns along different elevational gradients in the study region (Guanacaste, Costa Rica) which would lead to similar patterns in microfauna community structure. We further assumed beta diversity of microfauna communities to be positively related to elevation based on a decline in resource availability with increasing elevation. We found environmental conditions to differ strongly between the three studied elevational gradients. Linear patterns in microfauna community structure occurred in only one of the three field sites and were related to changes in oxygen saturation. Beta diversity showed contrasting or no patterns along the elevational gradients. In the one case in which beta diversity increased with higher elevation as expected, the pattern resulted from changes in water temperature and not resource availability as we suggested. We conclude that the variability of environmental conditions between elevational gradients is higher than generally assumed making generalizations of community structure and beta diversity patterns difficult.

Introduction

Over the last decades the anthropogenic influence on natural systems has increased considerably (Butchart et al. 2010). Changes in environmental variables due to anthropogenic activities have been observed to lead to changes in community composition (Anderson and Piatt 1999; Schimel and Gulledge 1998). This restructuring of communities can be accompanied by changes in ecological processes and functions (Chin et al. 1999; Schimel and Gulledge 1998). Consequences such as alterations in ecological processes and functions resulting from changes in environmental conditions and community structure are many times little predictable due to the complexity of natural ecosystems. Different scenarios of environmental change have been investigated with the aim to predict the implications of shifting environmental factors, e.g. temperature (Chin et al. 1999), climate (Anderson and Piatt 1999; Schimel and Gulledge 1998), disturbance regime (Schimel and Gulledge 1998) and nutrient availability (Armitage et al. 2005). Particularly well suited for these studies are naturally occurring gradients of environmental factors that allow us to assess the effects of a changing environment under natural conditions. Commonly used gradients are habitat gradients (habitat size (Wellborn et al. 1996), habitat type (Schlosser

1982)), latitudinal gradients (Connolly and Roughgarden 1998), gradients of physical factors such as oxygen (Lüdemann et al. 2000) and temperature (Rodeghiero and Cescatti 2005) or resource availability gradients (Richardson et al. 2000). In this study, we chose an elevational gradient because it likely comprises gradients of a number of ecologically important variables. With increasing elevation both temperature (Rodeghiero and Cescatti 2005) and plant cover (Gutierrez et al. 1998) have been shown to decrease. As plant cover is reduced canopy openness increases with elevation ((Homeier et al. 2010); decreasing leaf area index: (Kitayama and Aiba 2002)). This may result in higher light availability and lower resource input for decomposition processes.

Previous investigations of community composition parameters such as species richness and abundance showed variable patterns along elevational gradients. Earlier theories regarding these parameters assumed a linear decline with increasing elevation which was indeed observed in several studies (crustacean zooplankton species number: Rautio 1998, vascular plant richness: Grytnes 2003, aquatic plant richness: Jones et al. 2003, tree diversity: Homeier et al. 2010, bee abundance: Hoiss et al. 2012). However, species richness and abundance do not always decline monotonically in field studies (Richardson et al. 2000; Chaves-Campos 2004). Unimodal patterns with a peak at mid-elevation were found numerous times (grassland and forest species richness: Wang et al. 2003, vascular plant richness: Grytnes 2003, frog species richness: Fu et al. 2006, relative abundance of ants: Samson et al. 1997). In some studies even the opposite of the initial assumption was found and species richness and abundance increased with increasing elevation (plant species richness: Gutierrez et al. 1998, moth diversity: Brehm et al. 2003, relative abundance of bacteria: Siles and Margesin 2016, abundance of paper wasps: Kumar et al. 2009). These controversial observations might be the result of different driving factors. Many of these investigations focus on animal and plant species. However, the ecologically highly important microorganisms (Arrigo 2005; Finlay et al. 1987; Rosenberg et al. 2007) have been strongly neglected. Some studies indicate that microfauna community structure might exhibit patterns that are different from macrofauna communities (Fierer et al. 2011) which could be based on differences in fundamental characteristics such as rates of migration (Finlay et al. 2004). Nowadays, microfauna communities are increasingly used in ecology to answer questions regarding community-structuring processes (Srivastava et al. 2004). Based on their importance for ecosystems and as model systems, we chose microfauna communities for this study aiming to investigate the mechanisms through which changes in environmental conditions might affect community structure.

We used aquatic microfauna communities from tank-forming bromeliad plants to investigate the effects of changing environmental conditions on community structure. Bromeliads (*Bromeliaceae*) are herbaceous, perennial epiphytes that occur in the warm temperate to tropical regions of the Neotropics (Kitching 2000). Tank bromeliads form phytotelmata, i.e. plant-held waters (Kitching 2001), with their leaf axils (Greeney 2001). In these small water compartments organic matter accumulates in form of leaf litter from the forest canopy and is decomposed by detrivorous organisms living in the bromeliad tank. The decomposed material is used by the bromeliad itself as a nutrient source and also provides the basis for the food web of the aquatic community living in the small water pools. These food webs consist of bacteria, fungi, algae, protozoans, small metazoans such as rotifers and crustaceans, as well as detrivorous, filter-feeding and predatory insects. Bromeliads can contain inhabitants of numerous taxa (Greeney 2001; Maguire 1971) and thus comprise a great part of the aquatic non-fluvial biodiversity in the Neotropics (Carrias et al. 2014; Frank and Lounibos 2009). Therefore, these small water bodies are of great ecological importance, especially because bromeliad plants can occur at very high densities (Kitching 2000). An advantage of the bromeliad micro-ecosystem is that it is less complex and thus more easily comprehensible (Kitching 2001) than whole forest or lake ecosystems, which makes it the perfect model system for the study of community structure (Srivastava 2006).

Previously, mostly invertebrates were investigated in bromeliads (Frank and Lounibos 2009; Lounibos et al. 2003), but recently, the interest has shifted to the microorganisms (Carrias et al. 2012; Dunthorn et al. 2012). Here, we studied single-celled protozoa as well as small metazoa like rotifers and crustaceans. While some invertebrates, e.g. most insect species, spend only their juvenile stage in the bromeliad waters (Montero et al. 2010; Srivastava 2006), microorganisms spend their entire life-time (except for the period of dispersal) in the bromeliad micro-ecosystem. They also have shorter generation times and therefore respond more rapidly to environmental changes (Berger et al. 1997). Elevational changes in the bromeliad macrofauna community were already observed, whereby abundance declined linearly with elevation while species richness showed a mid-elevation peak (Richardson et al. 2000). Literature suggests that changes in microfauna community structure could be similar (Astorga et al. 2012) or completely distinct compared to the patterns found for macrofauna organisms (Finlay et al. 2004; Fierer et al. 2011). A number of environmental factors have been demonstrated to drive microfauna community structure in bromeliads or other systems. For example, bromeliad-inhabiting microfauna communities have been found to be strongly resource controlled (Petermann et al. 2015). An increase in resource input was observed to lead to an increase in species abundance, a decrease in species richness, a shift in community composition and an increase in between-community variation (Petermann et al. 2015). Light availability and related temperature changes were shown to affect community composition of bromeliad microfauna communities (Busse et al. 2018). Habitat size, in this study represented by compartment water volume of the bromeliad, is a known driver of abundance (Marino et al. 2011; Araújo et al. 2007) and we expect compartment volume to increase with elevation due to morphological changes in bromeliad plants that are exposed to more light (Males 2016). Chemical components like oxygen saturation and pH are known to affect microfauna community structure (Louca et al. 2017; Moser and Weisse 2011). Oxygen saturation will likely increase with elevation due to increased light availability and thus higher oxygen-producing algae biomass (Brouard et al. 2011). For pH we assume a decrease with higher elevation based on lower resource availability and thus fewer decomposition processes that would make the water more alkaline (Deano and Robinson 1985; Noble et al. 1996). With this study we investigate how variable these environmental factors are along and between elevational gradients and how they jointly act on microfauna community structure.

With this study we aim to detect if there is an elevational pattern in microfauna community structure and if it can be related to changing environmental factors. We measured temperature, compartment water volume, oxygen saturation, pH, light and resource availability hypothesizing them to change with elevation and therefore to be potential driving forces of community structure. We hypothesize that:

1. Abiotic variables in bromeliads (e.g. light availability, temperature, pH, oxygen saturation, compartment water volume and resource availability) change along elevational gradients (hypothesized direction depicted in Fig 1) leading to changes in community structure.
2. Resource availability is the major driver of changes in microfauna community structure, resulting in lower beta-diversity (between-community dissimilarity) with lower resources, i.e. at higher elevation.

Methods

Study area

The field survey was carried out in the Área de Conservación Guanacaste in northwestern Costa Rica in the beginning of the wet season in 2014. We sampled elevational transects on the volcanoes Orosilito (hereafter referred to as Pitilla based on the name of the field station, N 10°59.374’, W 85°25.583’), Cacao (N 10°56.009’, W 85°27.787’) and Santa Maria (N 10°48.060’, W 85°19.681’). The elevational transects in Cacao (1173-1524 m a.s.l.) and Santa Maria (1536-1906 m a.s.l.) are characterized by primary rainforest while the transect in Pitilla (683-1179 m a.s.l.) is characterized by secondary rainforest in lower elevations and primary rainforest in higher elevations. During the sampling period average minimum air temperature was 20.5 °C and average maximum air temperature was 24.4°C at field site Pitilla. Precipitation amounted to a total of 1044 mm in Pitilla during the sampling period. We suspect average air temperatures in the other two field sites to have been lower according to the higher elevation and total precipitation to have been similar due to constant rain fall during the wet season in all three field sites. The length of each gradient was determined by the natural occurrence of the bromeliad plants in the respective field site.

Sampling

Samples were taken from bromeliads (n_{Pitilla} = 25, n_{Cacao} = 22, $n_{\text{Santa Maria}}$ = 23) of similar size belonging to the morphologically congruent genera *Guzmania* and *Vriesea*. Bromeliad characteristics and abiotic environmental conditions were recorded in form of measurements of photosynthetic active radiation (PAR) [µmol/m²s], compartment water volume [mL], water temperature [°C], oxygen saturation [%], pH and detritus dry weight [g]. PAR was measured with a Quantum sensor at five different points per bromeliad (center of bromeliad, north, east, south and west of center, above the bromeliad). By taking average values of five PAR measurements per bromeliad we accounted for small-scale differences in canopy cover. Measurements of the other five abiotic variables were taken in an exemplary outer leaf compartment that was also used for microfauna sampling. To determine detritus dry weight all leaf litter was extracted from the investigated leaf compartment and dried at approximately 60 °C till constant weight. Microfauna samples were taken by extracting 1 mL of a water-containing outer leaf compartment after mixing with a pipette. Microfauna samples were fixed with Lugol's solution.

Permission for field work and sampling was granted by Sistema Nacional de Áreas de Conservacón (SINAC) and Ministerio del Ambiente y Energía (MINAE) in Costa Rica (permit number: ACG-PI-030-2014).

Identification and counting

Microfauna (i.e. diatoms, flagellates, ciliates, amoebae, rotifers and crustaceans) were counted as morphotypes (see list and pictures S1) by processing subsamples of 50 µL of each water sample using light microscopy (400x magnification) and using and adding to a photographic key developed by previous projects (Busse et al. 2018; Kratina et al. 2017; Petermann et al. 2015).

Diurnal dynamics in abiotic variables

To determine how variable the abiotic characteristics of bromeliad water bodies are, we measured oxygen saturation, pH, water temperature and photosynthetic active radiation from sunrise to sunset (5 am to 6 pm local Costa Rican time). These measurements of daily environmental variability were done three times (3rd June/22th July/11th August 2014). Each time we measured in the same three bromeliads located near the field station Pitilla, of which one was entirely exposed to the sun, one was half-shaded and one was shaded (S2). Measurement procedures were the same as described above and were applied to three different leaf compartments per bromeliad (center/middle/outer leaf compartment). The measurements took place every hour. Weather conditions were noted throughout the day (categories: sunny, cloudy, rainy) to account for precipitation events and changes in cloud cover.

Statistics

We used linear regression analyses to detect environmental changes and changes in abundance and species richness along the elevational gradients. Abundance data were log-transformed prior to analyses to achieve normality and homoscedasticity. We calculated the ratio of the two most frequent taxonomic groups (amoeba/flagellates), using log-transformed abundnce data, to identify shifts in dominance patterns between those two groups. Differences in community composition between the three field sites were analyzed using PERMANOVA (number of permutations = 999). We compared evenness values between the three field sites using linear models. Thereby, evenness was calculated as Pielou's J = H'/H'_{max} (Pielou 1969), with $H' = -\sum_i p_i * ln\, p_i$ (Shannon index), with p_i being the proportion of species *i*, and $H'_{max} = ln\, S$, with S being the total number of species.

To detect the effect of environmental variables on abundance and species richness we carried out stepwise linear regression models. To investigate the effects of environmental variables on community composition we used distance-based redundancy analysis (dbRDA), i.e. a constrained version of principal coordinates analysis (PCoA) (Legendre and Anderson 1999).

Beta diversity can be measured in many different ways, of which none are perfect and their use depends on the objectives of the respective study (Ricotta 2010). Here, we used beta diversity measured as distance to group centroid (Anderson et al. 2006) to make a general comparison of beta diversity between field sites. Based on our hypothesis 1) a change in abiotic variables along the elevational gradient leads to changes in community structure. This means Bray-Curtis dissimilarity values representing differences in community composition should increase with an increase in elevational distance. To determine if beta diversity increases with elevational distance, i.e. if community composition changes along the elevational gradient, we calculated distance decay plots based on Bray-Curtis dissimilarities. We tested this relationship with a multiple regression on distance matrices (Legendre et al. 2005; Lichstein 2007) using the 'MRM' function in the R package 'ecodist' (Goslee and Urban 2007). Based on our hypothesis 2) beta diversity increases with resource availability and decreases with elevation. We assessed the beta diversity along the environmental gradients by calculating pair-wise Bray-Curtis dissimilarities between neighbouring bromeliads along the resource and elevational gradients and tested the relationships with linear regression models.

All statistical analyses were done in R version 3.4.2 (R Core Team 2017) using the following packages: *vegan* (Oksanen J. et al. 2017), *vioplot* (Adler 2005) and *ecodist* (Goslee and Urban 2007).

Results

Variability in abiotic variables along the elevational gradient and in diurnal dynamics

The abiotic variables considered in this study were photosynthetic active radiation [µmol/ m²s], water temperature [°C], pH, oxygen saturation [%], compartment water volume [mL] and resource availability [g detritus dry weight]. We found few changes of environmental variables along the elevational gradients and these effects differed between field sites. Along the elevation gradient near Pitilla station water temperature and pH decreased with increasing elevation (Fig 2D, G). Along the gradient near Cacao water temperature decreased and oxygen saturation increased with increasing elevation (Fig 2E, K). None of the environmental variables showed any elevational pattern in Santa Maria (Fig 2C, F, I, L, O, R).

Diurnal fluctuations in weather conditions were measured from sunrise to sunset on three exemplary days, of which one was on average sunnier, one was characterized by rain and the third was mostly cloudy (S3) at field site Pitilla. Air temperature and PAR fluctuations were highest on the sunniest day (S3 A) and lowest on the most rainy day (S3 B) showing that daily fluctuations in environmental conditions can strongly depend on daily weather conditions.

Oxygen saturation and water temperature fluctuated during the course of the day (Fig 3 + 4) whereas pH stayed mostly constant (*data not shown*). Water temperature fluctuation was higher in the more sun-exposed bromeliads (Fig 3 1-6) and followed a similar daily pattern in all bromeliads and all leaf compartments (Fig 3). Oxygen saturation fluctuated more irregularly during the course of the day and fluctuations varied between leaf compartments within bromeliad plants (Fig 4).

Community structure

In total, 58 microfauna morphospecies were distinguished, including 9 diatoms, 10 flagellates, 7 ciliates, 29 amoeba, 2 rotifers and 1 crustacean (for photographic morphospecies key see S1). The majority of the morphospecies occurred in all three field sites, only few morphospecies were restricted to one field site (Fig 5).

Flagellates and amoeba were the most frequently occuring groups in the samples (Tab 1). Flagellates were the most abundant group and amoeba the most species rich group in all three field sites (Tab 1). Average relative abundances in Pitilla and Cacao were more clearly dominated by flagllates with a mean amoeba/flagellate abundance ratio of 0.58, while in Santa Maria amoebae increase in average relative abundance compared to flagellates, shifting the mean amoeba/flagellate ratio to 0.95 (Tab 1, S4). Elevational patterns of abundance and species richness were only observed in Cacao, were both declined with increasing elevation (Fig 6B + E). Community composition was different between the three field sites (PERMANOVA, number of permutations: 999, $F_{2,67} = 1.768$, $p < 0.05$). Changes in community composition along the elevational gradient were only observed in Cacao (Fig 7B). No differences in beta diversity (measured as distance to group centroid) and evenness of the communities were observed between the different field sites (*data not shown*).

Effect of environment on community structure

Stepwise linear regression analyses revealed significant negative effects of oxygen saturation on abundance in Cacao ($F_{1,20} = 32.4$, $p < 0.001$) and Santa Maria ($F_{1,20} = 4.6$, $p < 0.05$). We further detected significant negative effects of compartment water volume on species richness in Pitilla ($F_{1,23} = 5.3$, $p < 0.05$) and of oxygen saturation on species richness in Cacao ($F_{1,19} = 8.2$, $p < 0.01$). Oxygen saturation completely accounted for the elevational patterns in abundance and species richness in Cacao, and elevation had no additional effect (based on a comparison of two linear models with oxygen saturation and elevation as well as only elevation as predictors, *data not shown*). Distance-based redundancy analysis revealed a significant effect of oxygen saturation on community composition only in Cacao ($F_{1,20} = 4.0$, $p = 0.001$).

Beta diversity along the elevational gradients

Beta diversity did not change along the elevational gradient in Pitilla, decreased with elevation in Cacao and increased with elevation in Santa Maria (Fig 8A-C). In our pairwise approach, data are not independent as each data point (except the first and the last) is used twice (i.e. pairwise calculation between 1-2, 2-3, etc.). Therefore, we ran the analysis with each data point being used only once (i.e. pairwise calculation between 1-2, 3-4, etc.) which produced the same results (*data not shown*). Beta diversity showed no linear changes with changing resources (Fig 8D-F). We tested if the other measured environmental variables had an effect on beta diversity and found that beta diversity in Cacao increased with higher water temperatures ($F_{1,19} = 11.0$, $p < 0.01$).

Discussion

Our results demonstrate that there are differences in how environmental conditions change along elevational gradients. Cacao was the only field site in which linear elevational changes in community structure were observed, mostly caused by the strong elevational pattern of oxygen saturation, which was identified as major driving force. Contrary to our expectations resource availability did not change with elevation and did not affect microfauna community structure or beta diversity. Beta diversity showed variable patterns along the elevational gradients. In the case were beta diversity significantly declined with elevation, showing that communities become more similar towards the mountain top as we expected, the pattern was based on changes in water temperature.

Change in environmental variables along the elevational gradient and their effect on community structure

Only three (pH, water temperature and oxygen saturation) of the six measured environmental variables changed significantly with elevation and not in a consistent manner at all three field sites. The orientation of the sampled elevational transect (i.e. Pitilla: northern, Cacao: western, Santa Maria: southern) accompanied by differences in vegetation could potentially explain differences in environmental patterns. The age of the forests (Pitilla partly secondary forest, Cacao and Santa Maria exclusively primary forest) could also further explain transect differences. Though we sampled the complete transects of bromeliad occurrence in the respective field sites, it is also possible that the length of the environmental gradients was insufficient to render strong enough environmental changes.

The only community structure patterns along elevation were found in Cacao based on differences in oxygen saturation, even though Pitilla exhibited environmental changes (pH and water temperature) along the elevational gradient as well. Water temperature has formerly been observed to affect microfauna communities in bromeliads potentially due to narrow thermal niches of the inhabiting organisms (Kratina et al. 2017). The temperature range measured during our study in Pitilla fits the thermal optimum range described in Kratina et al. (2017) which means the temperature change in our study was probably not large enough to provoke changes in community structure. Also daily fluctuations in temperature, which were formerly observed to affect microfauna community structure in bromeliads (Busse et al. 2018), were probably not large enough in our study to affect community structure due to the fact that all sampling took place in bromeliads with low sun-exposure. pH expectedly decreased with elevation in Pitilla but did not result in community structure changes. The decrease in pH with elevation might be due to differences in leaf litter quality (Tóth et al. 2011), which we did not measure. pH has been observed to show interacting effects with temperature in affecting microfauna (Moser and Weisse 2011). As temperature did not show a sufficient degree of change in Pitilla and as pH did not change during the course of the day like oxygen saturation and water temperature did, this might also explain the lack of a community response to pH. Pitilla did not show changes in oxygen saturation which was the major driving force of community structure changes in Cacao.

Oxygen saturation is an important environmental factor not only for microfauna organisms (Fenchel 2012) but also for microbial communities (Louca et al. 2017) and mosquito larvae (Silberbush et al. 2015), thus affecting several trophic levels in the bromeliad tank. The susceptibility to changes in oxygen saturation of different levels of the bromeliad food web could be an explanation why this environmental factor was the sole predictor of elevational changes in community structure along the elevational gradient in Cacao. The mechanisms (direct/indirect effects) by which changes in oxygen saturation affect microfauna community structure remain to be investigated. Potentially, the irregularity of daily fluctuations in oxygen saturation we observed could play a role in the importance of this environmental factor for community structure. Assuming that oxygen saturation in the leaf compartments is driven by primary production, the existence and abundance of autotroph organisms such as algae as well as exposition to direct sunlight could lead to community differences between the leaf compartments within one bromeliad. Presumably, leaf compartments that are more regularly exposed to sunlight (e.g. center leaf compartments are presumably more sun-exposed than outer leaf compartments) might have a higher abundance of autotroph algae and can thus exhibit faster and stronger changes in oxygen saturation, leading to rapid changes between anaerobic and aerobic conditions in the water. Assuming differences in canopy cover along the elevational gradient, future studies could investigate if daily fluctuations in oxygen saturation are higher in the more sun-exposed higher elevations.

Beta diversity along the elevational gradient

The expected decrease in beta diversity with elevation was only observed in one of three field sites. The driving force behind this change in beta diversity was not a change in resource availability as expected but a change in water temperature. An increase in beta diversity of bromeliad microfauna communities with increasing temperature has been observed before by Busse et al. (2018), with daily temperature fluctuations as possible cause of changes in beta diversity. Though we measured temperatures at only one point in the day along the elevational gradient, we assume that daily temperature fluctuations were stronger at lower elevations than at higher elevations which make our findings consistent with those of Busse et al. (2018). These results do not exclude resource availability as a potential underlying cause because the effect of resource availability might be an interactive one with temperature. Even though detritus dry weight was consistent along the elevational gradient, the availability of the resources could have been different. If higher temperatures at the lower elevations cause higher metabolic rates and thus faster resource decomposition, then fewer resources would be available at lower elevations. The higher competition pressure at lower elevation would favor stochastic competitive assemblies (Tilman 2004) and could thus explain a higher beta diversity at lower elevational sites. The question remains why this pattern in beta diversity was only found in one of the three field sites. The opposing beta diversity trend observed in Santa Maria could not be explained by any of the environmental variables measured leaving us to suggest that further environmental variables such as daily fluctuations in oxygen saturation and leaf litter quality or other community-structuring processes such as dispersal and priority effects should be considered in future studies.

Conclusion

Our results demonstrate that environmental conditions on the regional scale can be so diverse that no general pattern for microfauna community structure along elevational gradients can be described. A change in key environmental factors such as oxygen saturation or water temperature is required for local patterns in microfauna community structure and beta diversity. The elevational patterns we found were particular to one of three studied field sites and stressed the importance of big data sets to identify generalizable patterns in ecology.

References

Adler, D. 2005. vioplot: Violin plot. R package version 0.2, http://wsopuppenkiste.wiso.uni-goettingen.de/~dadler.

Anderson, M. J. et al. 2006. Multivariate dispersion as a measure of beta diversity. - Ecology letters 9: 683–693.

Anderson, P. J. and Piatt, J. F. 1999. Community reorganization in th Gulf of Alaska following ocean climate regime shift. - Marine Ecology Progress Series 189: 117–123.

Araújo, V. et al. 2007. Relationship between invertebrate fauna and bromeliad size. - Braz. J. Biol. 67: 611–617.

Armitage, A. R. et al. 2005. Experimental nutrient enrichment causes complex changes in seagrass, microalgae, and macroalgae community structure in Florida Bay. - Estuaries 28: 422–434.

Arrigo, K. R. 2005. Marine microorganisms and global nutrient cycles. - Nature 437: 349–355, doi:10.1038/nature04158.

Astorga, A. et al. 2012. Distance decay of similarity in freshwater communities: do macro- and microorganisms follow the same rules? - Global Ecology and Biogeography 21: 365–375.

Berger, H. et al. 1997. Bestimmung und Ökologie der Mikrosaprobien nach DIN 38410. 26 Bildbestimmungsschlüssel, 10 Tabellen. - Fischer.

Brehm, G. et al. 2003. Unique elevational diversity patterns of geometrid moths in an Andean montane rainforest. - Ecography 26: 456–466.

Brouard, O. et al. 2011. Are algae relevant to the detritus-based food web in tank-bromeliads? - PloS one 6: e20129.

Busse, A. et al. 2018. Different in the dark: The effect of habitat characteristics on community composition and beta diversity in bromeliad microfauna. - PloS one 13: e0191426.

Butchart, S. H. M. et al. 2010. Global Biodiversity. Indicators of Recent Declines. - Science 328: 1164–1168.

Carrias, J.-F. et al. 2012. An ant–plant mutualism induces shifts in the protist community structure of a tank-bromeliad. - Basic and Applied Ecology 13: 698–705.

Carrias, J.-F. et al. 2014. Two coexisting tank bromeliads host distinct algal communities on a tropical inselberg. - Plant biology 16: 997–1004.

Chaves-Campos, J. 2004. Elevational movements of large frugivorous birds and temporal variation in abundance of fruits along an elevational gradient. - Ornitologia Neotropical 15: 433–445.

Chin, K.-J. et al. 1999. Effect of Temperature on Structure and Function of the Methanogenic Archaeal Community in an AnoxicRice Field Soil. - Applied and Environmental Microbiology 65: 2341–2349.

Connolly, S. R. and Roughgarden, J. 1998. A Latitudinal Gradient in Northeast Pacific Intertidal Community Structure. Evidence for an Oceanographically Based Synthesis of Marine Community Theory. - The American Naturalist 151: 311–326.

Deano, P. M. and Robinson, J. W. 1985. The effect of decaying leaves on the ph and buffer capacity of waters. - Journal of Environmental Science and Health . Part A: Environmental Science and Engineering 20: 903–911.

Dunthorn, M. et al. 2012. Diversity and endemism of ciliates inhabiting Neotropical phytotelmata. - Systematics and Biodiversity 10: 195–205.

Fenchel, T. 2012. Protozoa and Oxygen. - Acta Protozool. 52: 11–20.

Fierer, N. et al. 2011. Microbes do not follow the elevational diversity patterns of plants and animals. - Ecology 92: 797–804.

Finlay, B. J. et al. 2004. Protist Diversity is Different? - Protist 155: 15–22.

Finlay, B. J. et al. 1987. Ciliated Protozoa and other Microorganisms from Two African Soda Lakes (Lake Nakuru and Lake Simbi, Kenya). - Archiv für Protistenkunde 133: 81–91.

Frank, J. H. and Lounibos, L. P. 2009. Insects and allies associated with bromeliads: a review. - Terrestrial arthropod reviews 1: 125–153.

Fu, C. et al. 2006. Elevational patterns of frog species richness and endemic richness in the Hengduan Mountains, China: geometric constraints, area and climate effects. - Ecography 29: 919–927.

Goslee, S. C. and Urban, D. L. 2007. The ecodist package for dissimilarity-based analysis of ecologicaldata. - J. Stat. Soft. 22: 1-19.

Greeney, H. F. 2001. The insects of plant-held waters: a review and bibliography. - Journal of Tropical Ecology 17: 241–260.

Grytnes, J. A. 2003. Species-richness patterns of vascular plants along seven altitudinal transects in Norway. - Ecography 26: 291–300.

Gutierrez et al. 1998. Vegetation in an altitudinal gradient along the Rio Loa in the Atacama Desert of northern Chile. - Journal of Arid Environments 40: 383–399.

Hoiss, B. et al. 2012. Altitude acts as an environmental filter on phylogenetic composition, traits and diversity in bee communities. - Proceedings. Biological sciences 279: 4447–4456.

Homeier, J. et al. 2010. Tree Diversity, Forest Structure and Productivity along Altitudinal and Topographical Gradients in a Species-Rich Ecuadorian Montane Rain Forest. - Biotropica 42: 140–148.

Jones, J. I. et al. 2003. Area, altitude and aquatic plant diversity. - Ecography 26: 411–420.

Kitayama, K. and Aiba, S.-I. 2002. Ecosystem structure and productivity of tropical rain forests along altitudinal gradients with contrasting soil phosphorus pools on Mount Kinabalu, Borneo. - Journal of Ecology 90: 37–51.

Kitching, R. L. 2000. Food webs and container habitats. The natural history and ecology of phytotelmata. - Cambridge University Press.

Kitching, R. L. 2001. Food webs in phytotelmata: "bottom-up" and "top-down" explanations for community structure. - Annual review of entomology 46: 729–760.

Kratina, P. et al. 2017. Environmental control of the microfaunal community structure in tropical bromeliads. - Ecology and Evolution 7: 1627–1634.

Kumar, A. et al. 2009. Elevational Patterns of Diversity and Abundance of Eusocial Paper Wasps (Vespidae) in Costa Rica. - Biotropica 41: 338–346.

Legendre, P. et al. 2005. Analyzing beta diversity: Partitioning the spatial variation of communnity composition data. - Ecological Monographs 75: 435–450.

Legendre, P. and Anderson, M. J. 1999. Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments. - Ecological Monographs 69: 1–24.

Lichstein, J. W. 2007. Multiple regression on distance matrices: a multivariate spatial analysis tool. - Plant Ecology 188: 117–131, https://doi.org/10.1007/s11258-006-9126-3.

Louca, S. et al. 2017. Functional structure of the bromeliad tank microbiome is strongly shaped by local geochemical conditions. - Environmental microbiology 19: 3132–3151.

Lounibos, L. P. et al. 2003. Interactions with native mosquito larvae regulate the production of Aedes albopictus from bromeliads in Florida. - Ecol Entomol 28: 551–558.

Lüdemann, H. et al. 2000. Spatial Changes in the Bacterial Community Structure along a Vertical Oxygen Gradient in Flooded Paddy Soil Cores. - Applied and Environmental Microbiology 66: 754–762.

Maguire, B. 1971. Phytotelmata: Biota and Community Structure Determination in Plant-Held Waters. - Annu. Rev. Ecol. Syst. 2: 439–464.

Males, J. 2016. Think tank: water relations of Bromeliaceae in their evolutionary context. - Bot. J. Linn. Soc. 181: 415–440.

Marino, N. A. C. et al. 2011. Habitat size determine algae biomass in tank-bromeliads. - Hydrobiologia 678: 191–199.

Montero, G. et al. 2010. The phytotelmata and foliage macrofauna assemblages of a bromeliad species in different habitats and seasons. - Insect Conservation and Diversity 3: 92–102.

Moser, M. and Weisse, T. 2011. Combined stress effect of pH and temperature narrows the niche width of flagellates in acid mining lakes. - Journal of plankton research 33: 1023–1032.

Noble, A. D. et al. 1996. Leaf litter ash alkalinity and neutralisation of soil acidity. - Plant Soil 179: 293–302.

Oksanen J. et al. 2017. vegan: Community Ecology Package. R package version 2.4-5, https://CRAN.R-project.org/package=vegan.

Petermann, J. S. et al. 2015. Resources Alter the Structure and Increase Stochasticity in Bromeliad Microfauna Communities. - PLoS ONE 10: e0118952.

Pielou, E. C. 1969. The measurement of diversity in different types of biological collections. - J. Theor. Biol. 13: 131–144.

R Core Team 2017. R: A language and environment for statistical computing. Foundation for Statistical Computing, Vienna, Austria, https://www.R-project.org/.

Rautio, M. 1998. Community structure of crustacean zooplankton in subarctic ponds - effects of altitude and physical heterogeneity. - Ecography 21: 327–335.

Richardson, B. A. et al. 2000. Effects of nutrient availability and other elevational changes on bromeliad populations and their invertebrate communities in a humid tropical forest in Puerto Rico. - Journal of Tropical Ecology 16: 167–188.

Ricotta, C. 2010. On beta diversity decomposition: Trouble shared is not trouble halved. - Ecology 91: 1981–1983.

Rodeghiero, M. and Cescatti, A. 2005. Main determinants of forest soil respiration along an elevation/temperature gradient in the Italian Alps. - Global Change Biol 11: 1024–1041.

Rosenberg, E. et al. 2007. The role of microorganisms in coral health, disease and evolution. - Nature reviews. Microbiology 5: 355–362.

Samson, D. A. et al. 1997. Ant Diversity and Abundance along an Elevational Gradient in the Philippines1. - Biotropica 29: 349–363.

Schimel, J. P. and Gulledge, J. 1998. Microbial community structure and global trace gases. - Global Change Biol 4: 745–758.

Schlosser, I. J. 1982. Fish Community Structure and Function along Two Habitat Gradients in a Headwater Stream. - Ecological Monographs 52: 395–414.

Silberbush, A. et al. 2015. Dissolved oxygen levels affect the survival and developmental period of the mosquito *Culex pipiens*. - Journal of Vector Ecology 40: 425–427.

Siles, J. A. and Margesin, R. 2016. Abundance and Diversity of Bacterial, Archaeal, and Fungal Communities Along an Altitudinal Gradient in Alpine Forest Soils: What Are the Driving Factors? - Microbial ecology 72: 207–220.

Srivastava, D. S. et al. 2004. Are natural microcosms useful model systems for ecology? - Trends in ecology & evolution 19: 379–384.

Srivastava, D. S. 2006. Habitat structure, trophic structure and ecosystem function: interactive effects in a bromeliad-insect community. - Oecologia 149: 493–504.

Tilman, D. 2004. Niche tradeoffs, neutrality, and community structure: a stochastic theory of resource competition, invasion, and community assembly. - Proceedings of the National Academy of Sciences of the United States of America 101: 10854–10861.

Tóth, J. A. et al. 2011. Effect of Litter Fall on Soil Nutrient Content and pH, and its Consequences in View of Climate Change (Síkfőkút DIRT Project). - Acta Silv. Lign. Hung. 7: 75–86.

Wang, G. et al. 2003. Distribution, species diversity and life-form spectra of plant communities along an altitudinal gradient in the northern slopes of Qilianshan Mountains, Gansu, China. - Plant Ecology 165: 169–181.

Wellborn, G. A. et al. 1996. Mechanisms creating community structure across a freshwater habitat gradient. - Annu. Rev. Ecol. Syst. 27: 337–363.

Figures and tables

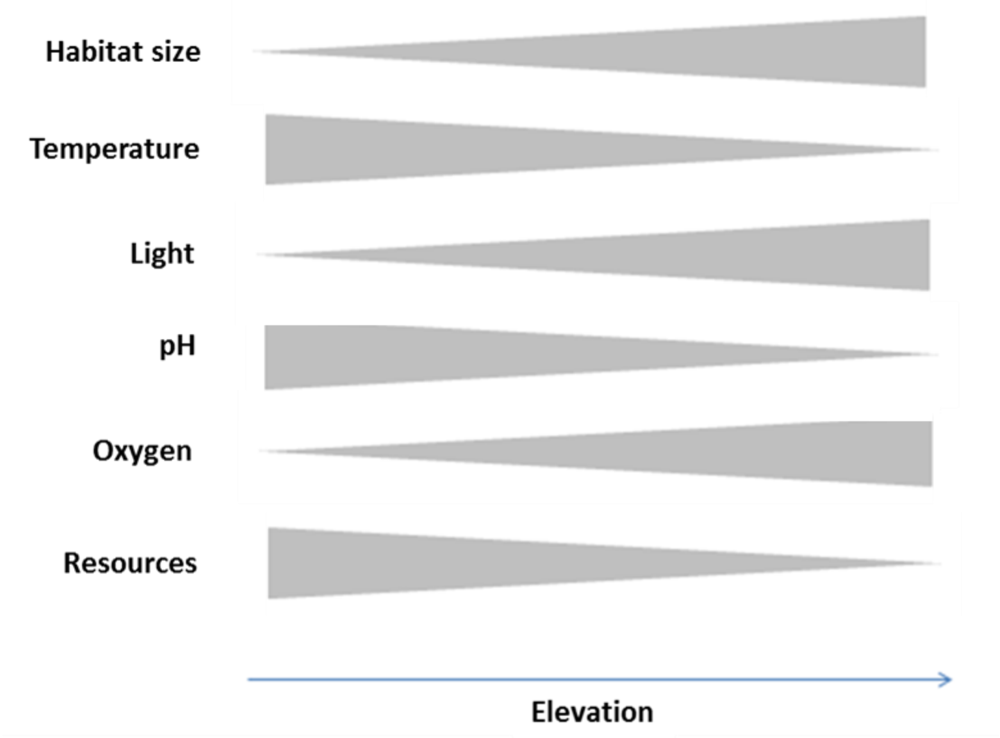


Figure 1: Expected change of selected environmental variables along elevational gradients. Habitat size refers to compartment water volume in the bromeliad plant. Light availability refers to photosynthetic active radiation. Resources are measured as detritus dry weight.

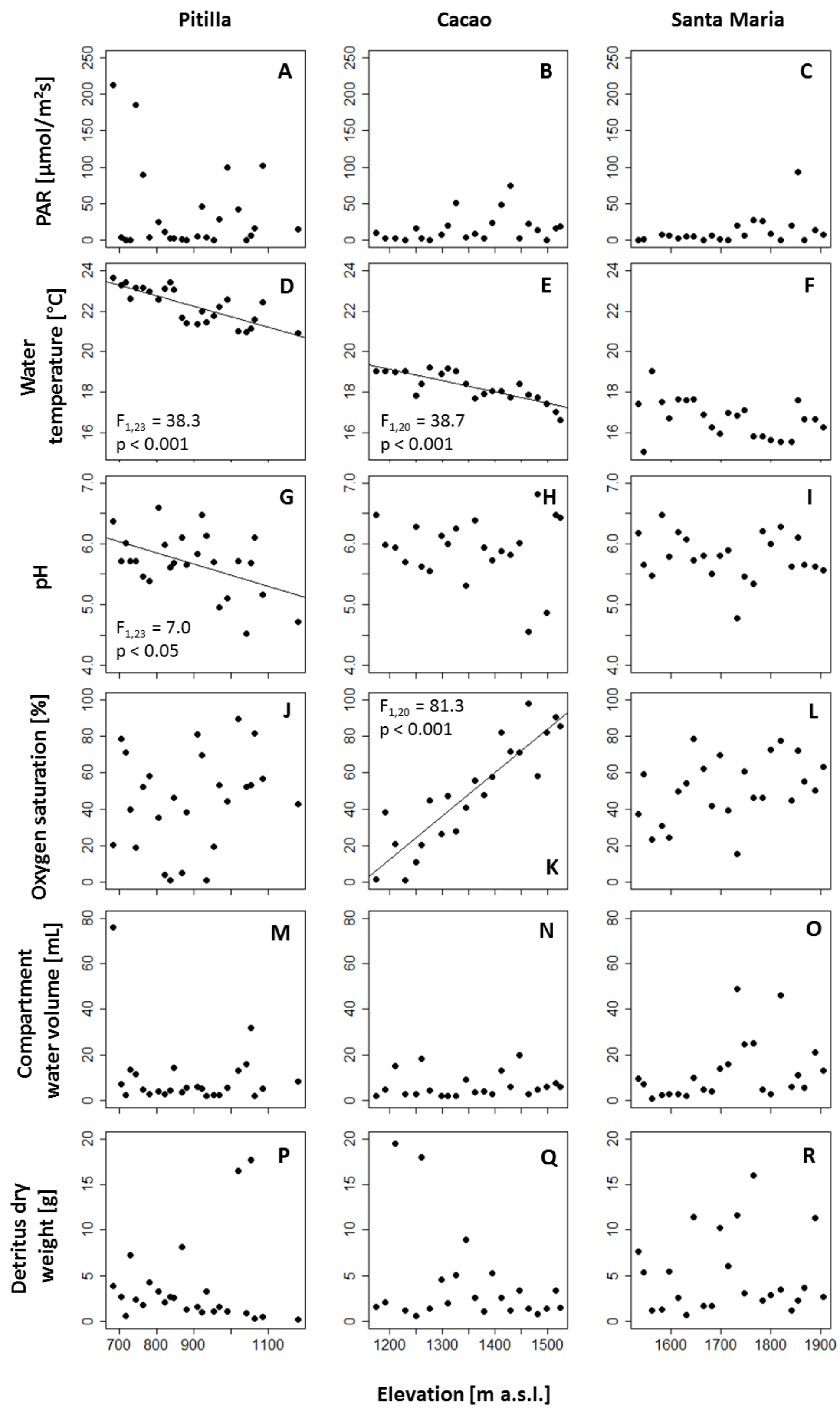


Figure 2: Selected environmental variables in bromeliad tank ecosystems measured along three elevational gradients in Costa Rica. In field site Santa Maria an outlier of photosynthetic active radiation (PAR) at 1562 m with 1019 µmol/m^{2}s was excluded from visualization.

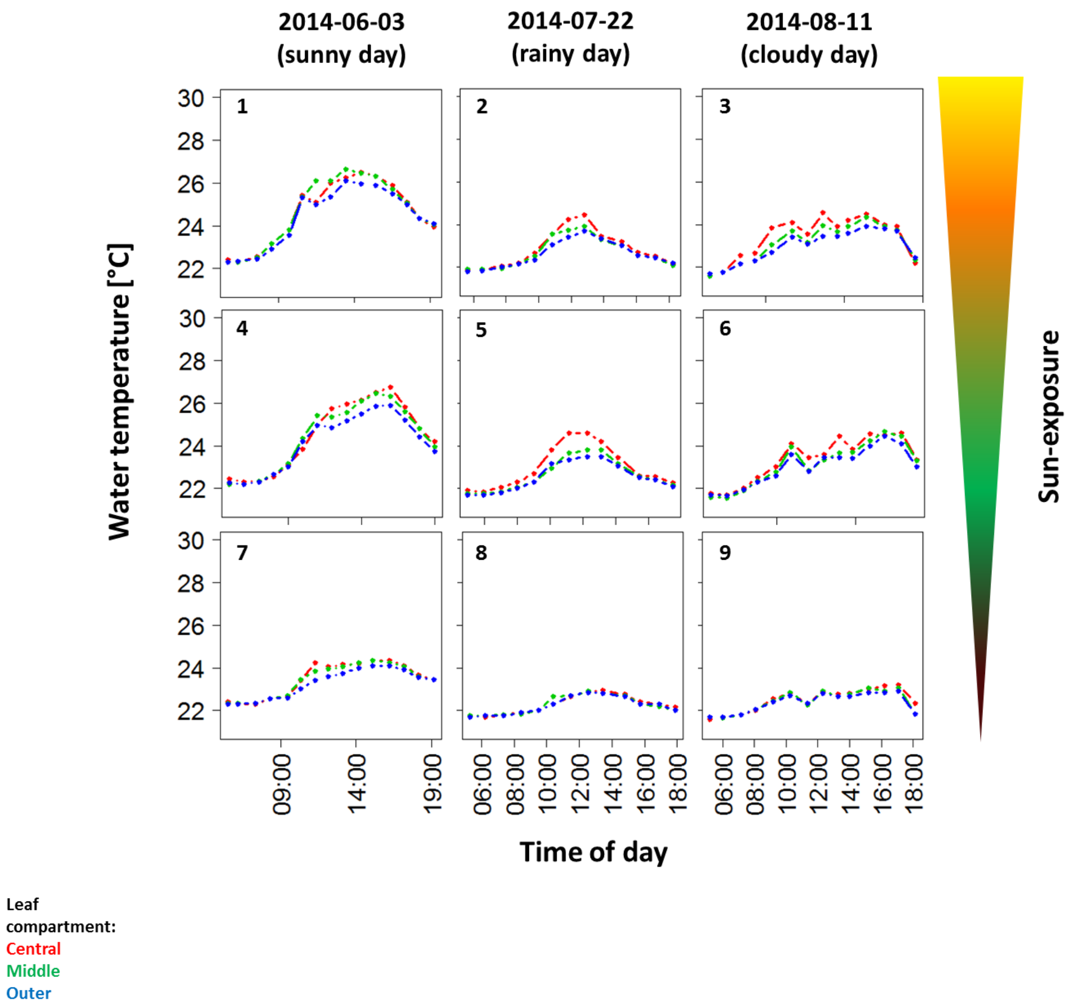
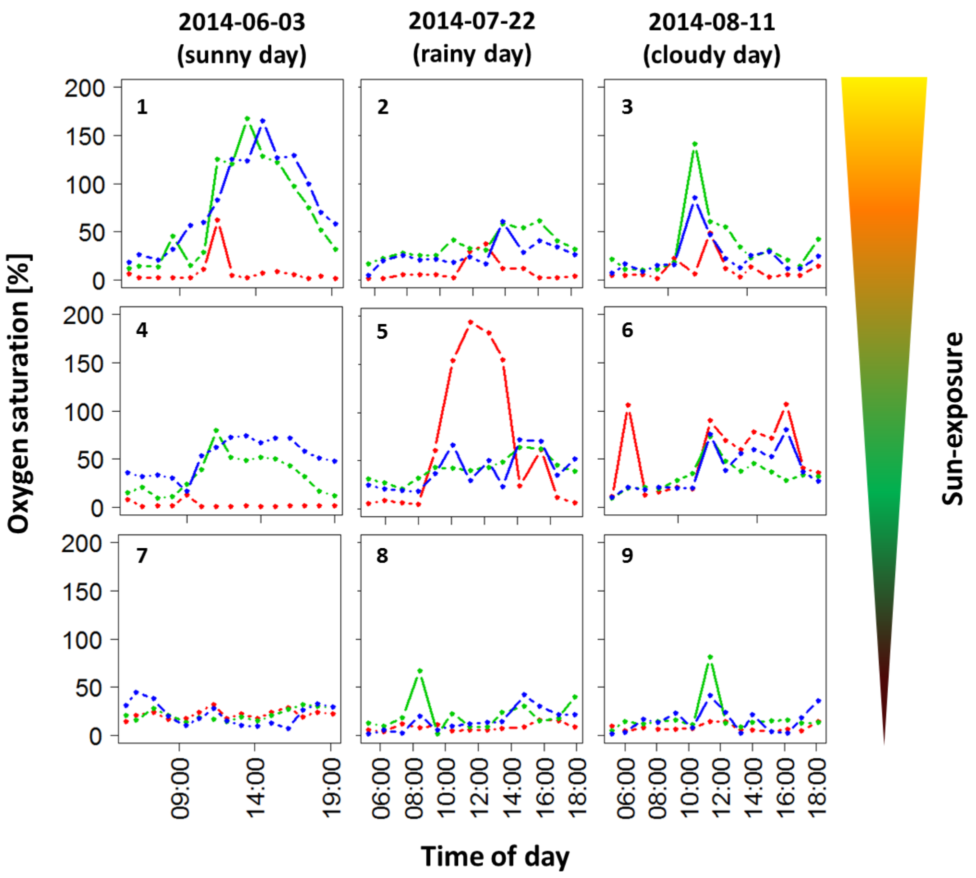


Figure 3: Daily fluctuations in water temperature [°C] in bromeliad leaf compartments. Daily water temperature fluctuations increase with higher sun-exposure and sunnier weather conditions but show a similar pattern independent of bromeliad and leaf compartment.



Leaf compartment:
Central
Middle
Outer

Figure 4: Daily fluctuations in oxygen saturation [%] in bromeliad leaf compartments. Daily fluctuations in oxygen saturation vary inconsistently between leaf compartments. More sun-exposed bromeliads tend to have stronger daily fluctuations in oxygen saturation.

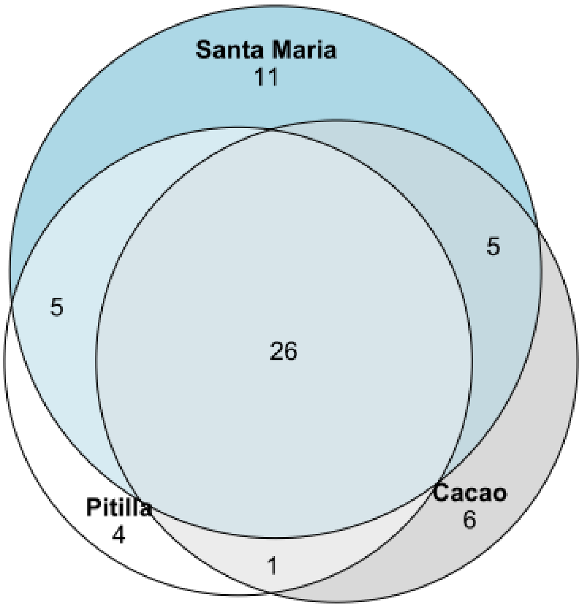


Figure 5: Venn diagram of microfauna morphospecies in Costa Rican bromeliads in three different field sites. Santa Maria had more species that solely occur there, but generally strong overlap in species identities emerged.

Table 1: Abundance, richness and frequency of functional groups in bromeliad-inhabiting microfauna per 50 µL sample in three different field sites in Costa Rica. Flagellates dominated the communities' abundance and amoebae the richness.

ALL

n = 70	**Abundance**			**Species richness**			
	Range per sample	Mean ±SE per sample	Average relative abundance ± SE [%]	Range per sample	Mean ±SE per sample	Average relative species richness ± SE [%]	Frequency [%]
Total	4-4349	284 ± 89		3-20	10 ± 1		
Diatoms	0-207	5 ± 3	3 ± 1	0-8	1 ± 0	6 ± 1	39
Flagellates	0-4328	228 ± 86	44 ± 4	0-6	2 ± 0	18 ± 2	77
Ciliates	0-97	8 ± 2	10 ± 2	0-4	1 ± 0	9 ± 1	56
Amoebae	0-798	37 ± 12	32 ± 3	0-14	5 ± 0	52 ± 2	99
Rotifers	0-13	2 ± 0	4 ± 1	0-2	1 ± 0	9 ± 1	66
Crustaceans	0-25	3 ± 1	6 ± 1	0-1	1 ± 0	7 ± 1	63

PITILLA

n = 25	**Abundance**			**Species richness**			
	Range per sample	Mean ±SE per sample	Average relative abundance ± SE [%]	Range per sample	Mean ±SE per sample	Average relative species richness ± SE [%]	Frequency [%]
Total	7-1633	223 ± 78		3-20	11 ± 1		
Diatoms	0-207	12 ± 8	5 ± 2	0-8	1 ± 0	11 ± 3	60
Flagellates	0-1601	134 ± 65	43 ± 7	0-4	2 ± 0	17 ± 2	84
Ciliates	0-84	8 ± 4	7 ± 3	0-3	1 ± 0	7 ± 2	48
Amoebae	2-798	60 ± 32	31 ± 6	0-12	5 ± 1	48 ± 3	100
Rotifers	0-13	3 ± 1	6 ± 2	0-2	1 ± 0	10 ± 2	72
Crustaceans	0-25	5 ± 1	7 ± 3	0-1	1 ± 0	8 ± 2	68

CACAO

n = 22	**Abundance**			**Species richness**			
	Range per sample	Mean ±SE per sample	Average relative abundance ± SE [%]	Range per sample	Mean ±SE per sample	Average relative species richness ± SE [%]	Frequency [%]
Total	21-4349	505 ± 258		6-17	11 ± 1		
Diatoms	0-5	1 ± 0	1 ± 1	0-2	0 ± 0	3 ± 1	32
Flagellates	0-4328	462 ± 254	50 ± 8	0-6	2 ± 0	21 ± 3	82
Ciliates	0-97	11 ± 5	14 ± 5	0-3	1 ± 0	10 ± 2	64
Amoebae	5-176	29 ± 8	29 ± 5	3-11	6 ± 0	52 ± 2	100
Rotifers	0-5	2 ± 0	3 ± 1	0-2	1 ± 0	8 ± 1	68
Crustaceans	0-7	2 ± 0	3 ± 1	0-1	1 ± 0	6 ± 1	64

SANTA MARIA

n = 23	**Abundance**			**Species richness**			
	Range per sample	Mean ±SE per sample	Average relative abundance ± SE [%]	Range per sample	Mean ±SE per sample	Average relative species richness ± SE [%]	Frequency [%]
Total	4-1314	139 ± 60		3-18	9 ± 1		
Diatoms	0-33	2 ± 1	3 ± 2	0-5	0 ± 0	3 ± 1	22
Flagellates	0-1265	107 ± 58	39 ± 8	0-3	1 ± 0	16 ± 4	65
Ciliates	0-51	6 ± 2	10 ± 4	0-4	1 ± 0	11 ± 3	57
Amoebae	0-167	20 ± 7	37 ± 6	0-14	5 ± 1	55 ± 5	96
Rotifers	0-7	2 ± 0	5 ± 2	0-2	1 ± 0	8 ± 2	57
Crustaceans	0-17	3 ± 1	6 ± 2	0-1	1 ± 0	7 ± 2	57

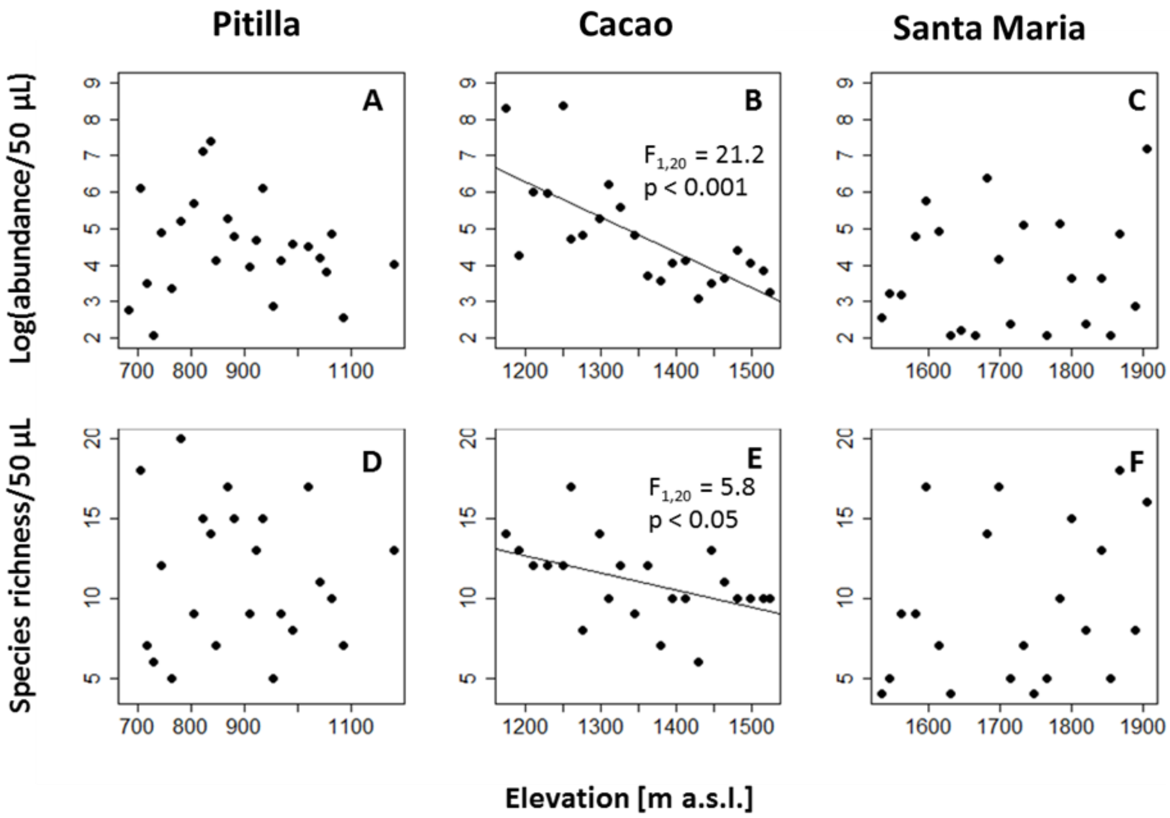


Figure 6: Patterns of log-transformed abundance and species richness of bromeliad-inhabiting microfauna along three elevational gradients in Costa Rica. Significant relationships between log(abundance) and species richness and elevation were observed only in Cacao. Sample volume: 50 µL. Sample sizes: Pitilla = 25, Cacao = 22, Santa Maria = 23.

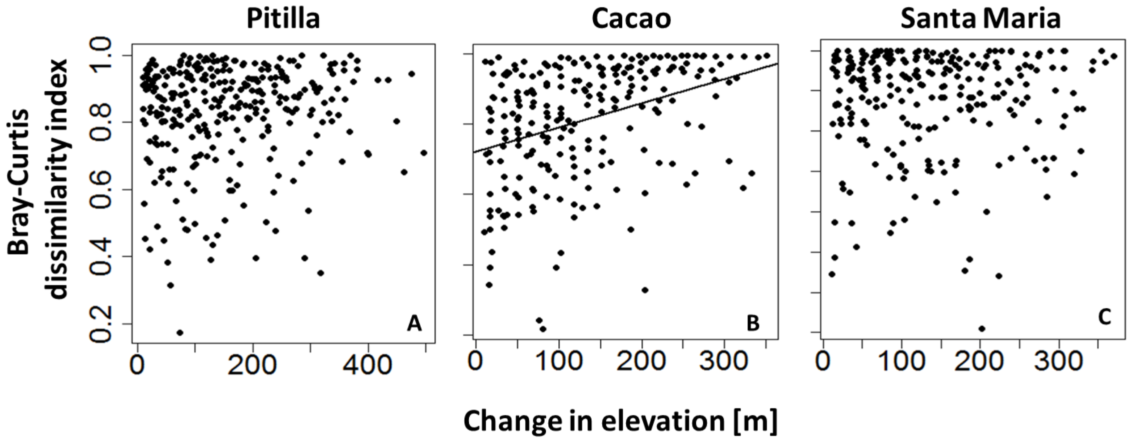


Figure 7: Distance-decay plots representing changes in community dissimilarity of bromeliad-inhabiting microfauna along changes in elevation [m a.s.l.] in three different field sites in Costa Rica. A significnat change in community composition emerges only in the field site Cacao (multiple regression on distance matrices: R^2 = 0.1, $F_{1,229}$ = 33.0, p = 0.001).

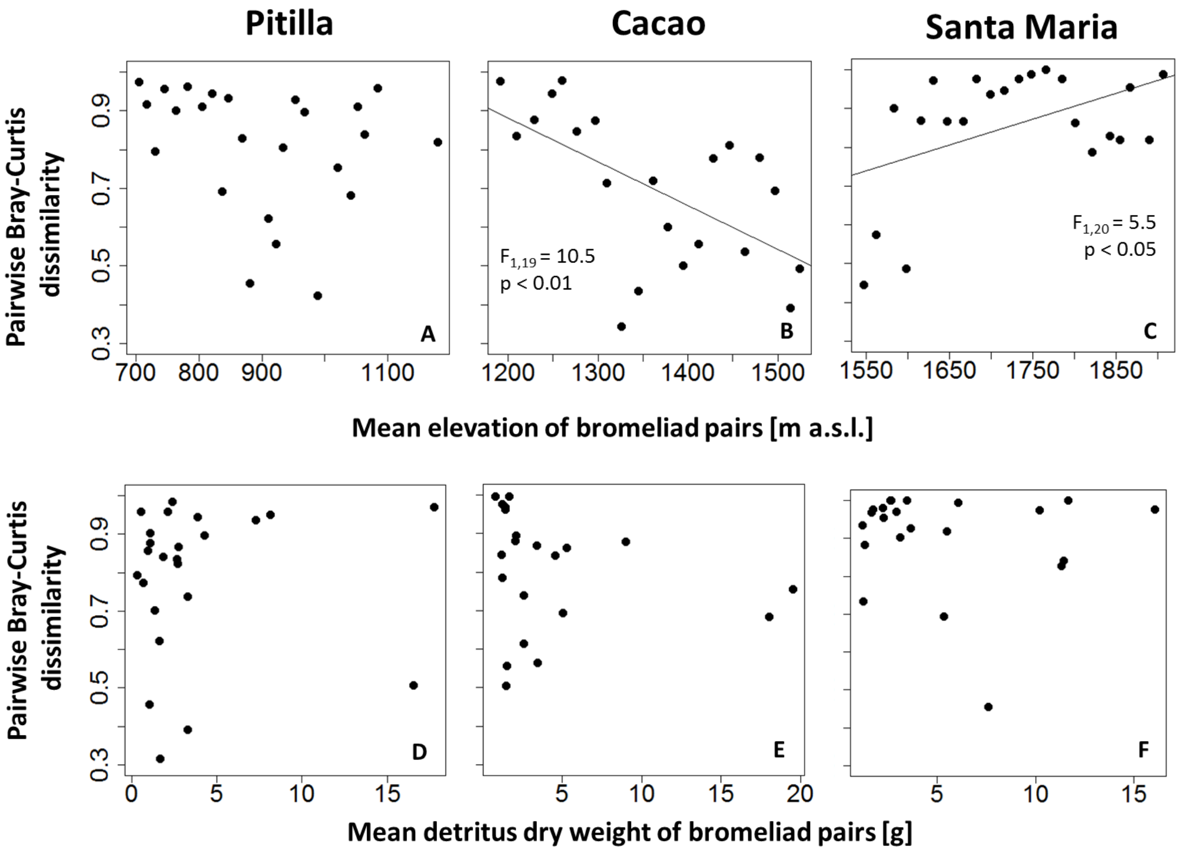
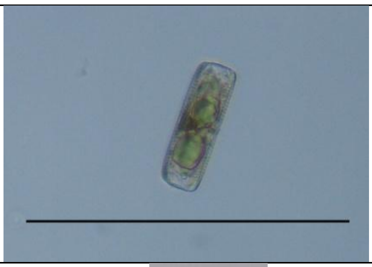
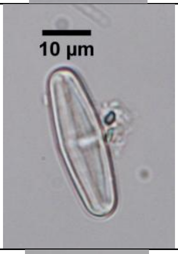
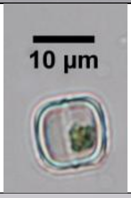
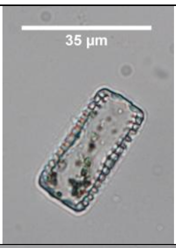
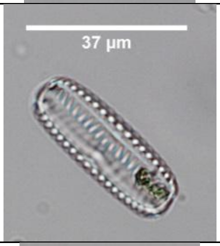
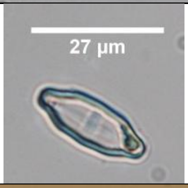
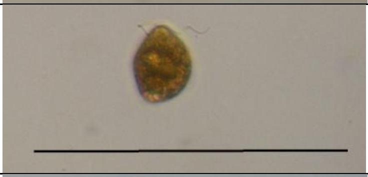
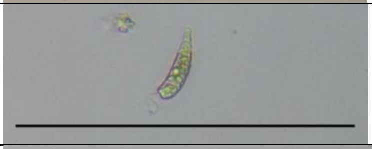
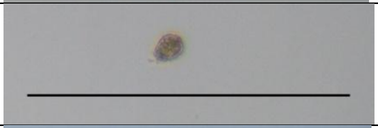


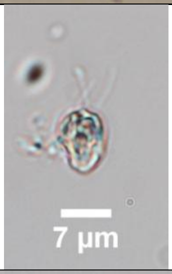
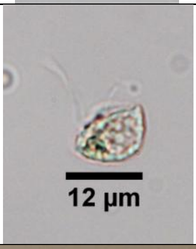
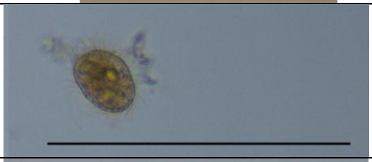
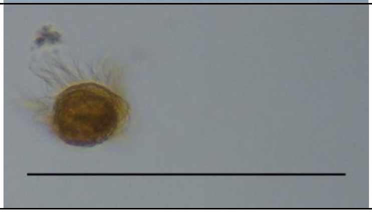
Figure 8: Beta diversity (measured as pairwise Bray-Curtis dissimilarity) of bromeliad-inhabiting microfauna along gradients of resource availability [g detritus dry weight] and elevation [m a.s.l] (mean values between bromeliad pairs next to each other along the respective gradient). Beta diversity did not change along the resource gradient and showed opposing or no patterns along the elevational gradient.

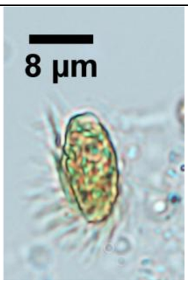
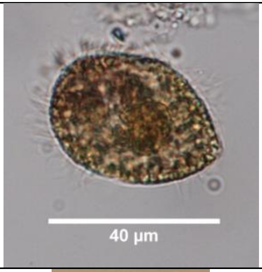
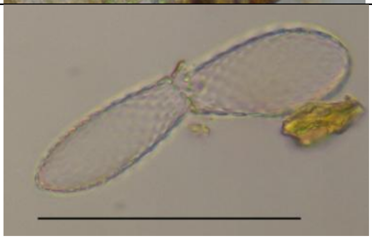
Supplement

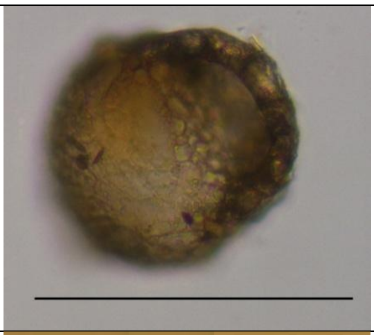
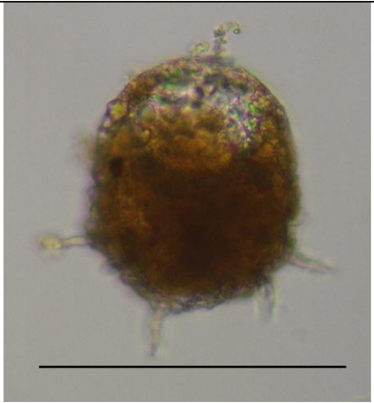
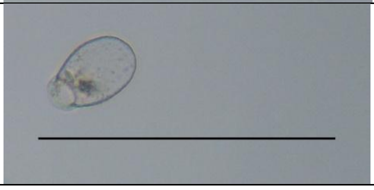
S1: List of all 58 bromeliad-inhabiting microfauna morphospecies found in a field survey along three elevational transects in Guanacaste with representative picture, Costa Rica. Scale bar without annotation: 100 µm. Names are based on previous keys.

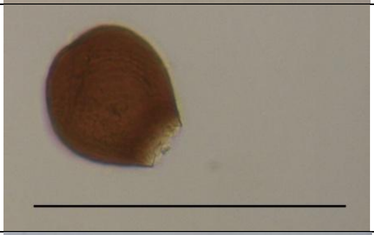
Morphospecies	**Photo**
Diatom 1	
Diatom 2	
Diatom 3	
Diatom 4	
Diatom 5	

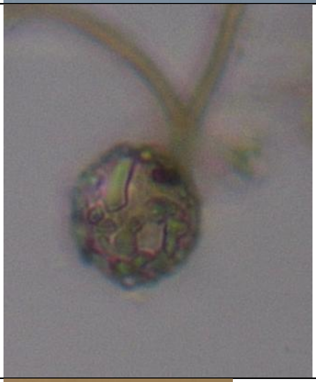
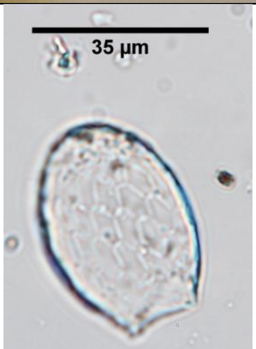
Diatom 6	
Diatom 7	
Diatom 8	
Diatom 9	
Flagellate 1	
Flagellate 2	
Flagellate 3	
Flagellate 4	
Flagellate 5	
Flagellate 6	

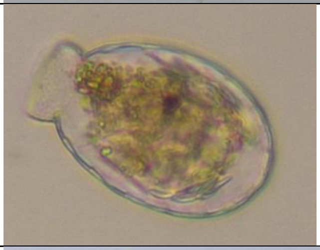
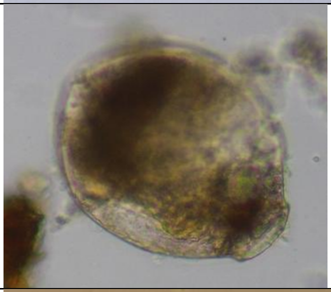
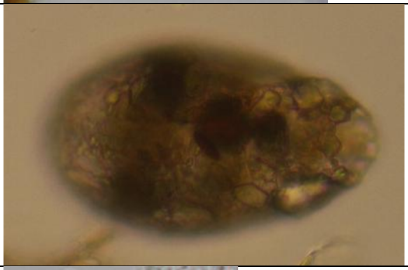
Flagellate 7	
Flagellate 8	
Flagellate 9	
Flagellate 10	
Ciliate 1	
Ciliate 2	
Ciliate 3	
Ciliate 4	

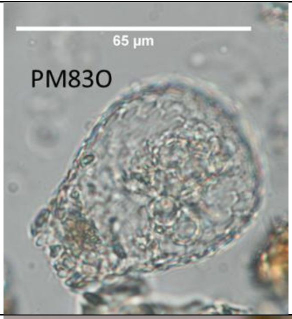
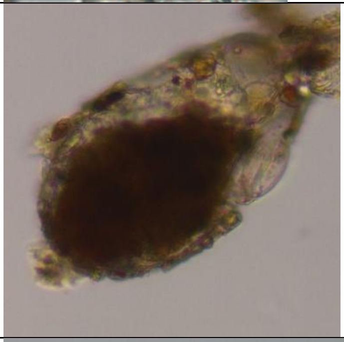
Ciliate 5	
Ciliate 6	
Ciliate 7	
Amoeba 1	
Amoeba 2	
Amoeba 3	
Amoeba 4	

Amoeba 5	
Amoeba 6	
Amoeba 7	
Amoeba 8	
Amoeba 9	

Amoeba 10	
Amoeba 12	
Amoeba 14	
Amoeba 15	
Amoeba 16	

Amoeba 18	
Amoeba 19	
Amoeba 20	
Amoeba 21	
Amoeba 22	
Amoeba 23	

Amoeba 24	
Amoeba 25	
Amoeba 26	
Amoeba 27	
Amoeba 28	
Amoeba 29	

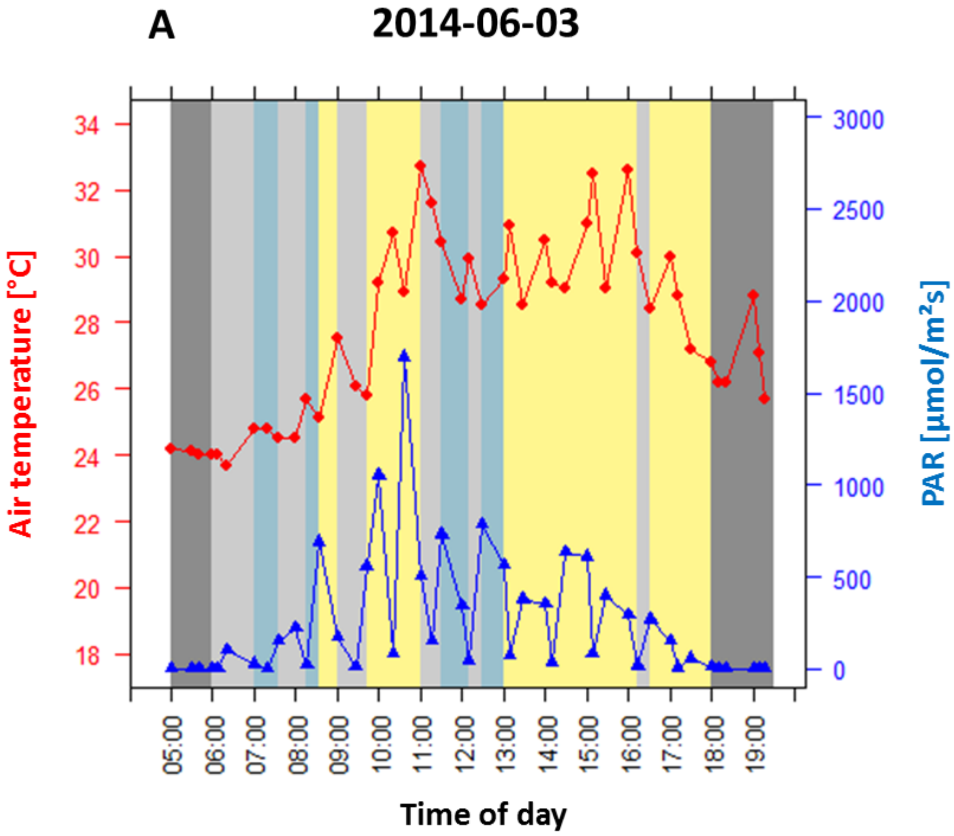
Amoeba 30	
Amoeba 31	
Amoeba 32	
Rotifer 1	
Rotifer 2	

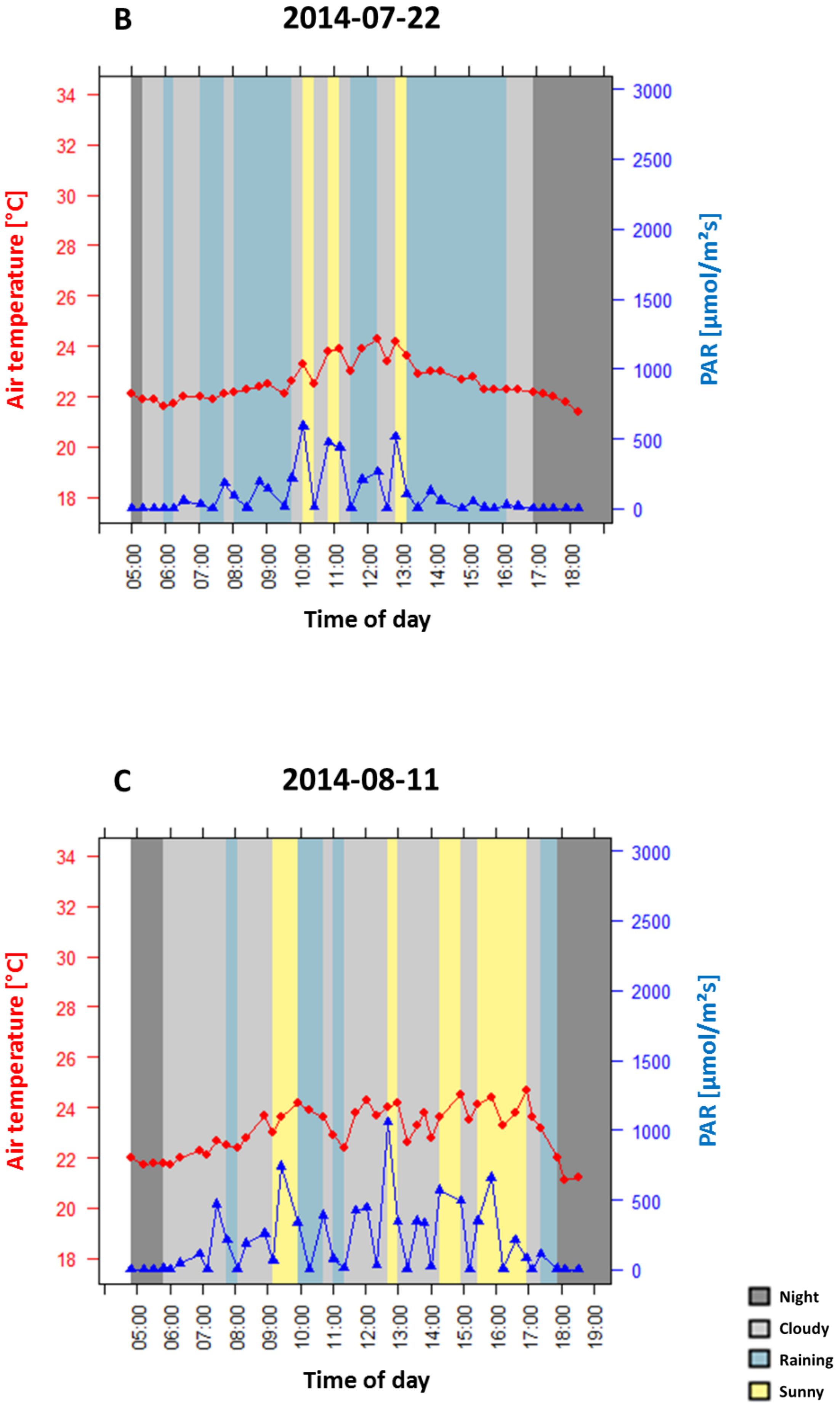
Crustacean 1	

S2: Bromeliads used for daily measurements of fluctuations in environmental variables and their characteristics at field site Pitilla, Costa Rica.

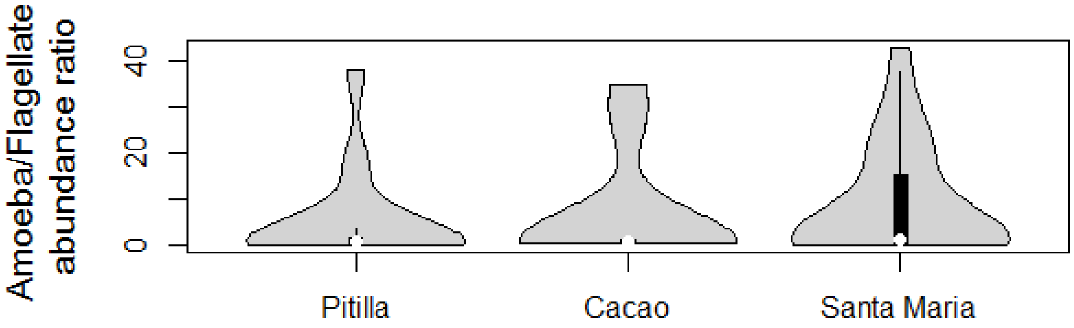
	Location	**Elevation [m]**	**Bromeliad species**	**Diameter (relaxed leaf tips) [cm]**	**Light characteristic**
Bromeliad 1	N 10°59.359' W 85°25.569'	681	*Vriesea sanguinolenta*	65.0	Sun-exposed
Bromeliad 2	N 10°59.360' W 85°25.568'	689	*Vriesea sanguinolenta*	67.3	Half-shaded
Bromeliad 3	N 10°59.338' W 85°25.574'	690	*Vriesea sanguinolenta*	83.2	Shaded

S3: Weather conditions over the course of three measurement days at field site Pitilla, Costa Rica. Measurements of air temperature [°C] and photosynthetic active raditaion (PAR) [µmol/m²s] were taken three times per hour from sunrise to sunset. At each measurement weather condition was generalized as one of four categories (i.e. night, cloudy, raining and sunny) showing that 3rd June 2014 (A) is a relatively sunny day, compared to the more rainy 22th July 2014 (B) and the cloudy 11th August 2014 (C). The differences in the days weather conditions result in differences in the daily fluctuations in air temperature and PAR.




B
2014-07-22
Air temperature [°C]
34
32
30
28
26
24
22
20
18
PAR [µmol/m²s]
3000
2500
2000
1500
1000
500
0
05:00
06:00
07:00
08:00
09:00
10:00
11:00
12:00
13:00
14:00
15:00
16:00
17:00
18:00
Time of day
C
2014-08-11
Air temperature [°C]
34
32
30
28
26
24
22
20
18
PAR [µmol/m²s]
3000
2500
2000
1500
1000
500
0
05:00
06:00
07:00
08:00
09:00
10:00
11:00
12:00
13:00
14:00
15:00
16:00
17:00
18:00
19:00
Time of day
Night
Cloudy
Raining
Sunny

S4: Ratio of log-transformed amoebae abundance and log-transformed flagellate abundance (per 50 µL) for bromeliad-inhabiting microfauna communities in three different field sites. While communities in the field sites Pitilla and Cacao were mostly flagellate-dominated, in Santa Maria amoebae were higher in average relative abundance compared to flagellates shifting the communities slightly towards amoeba-domination. Sample volume: 50 µL. Sample sizes: Pitilla = 25, Cacao = 22, Santa Maria = 23.



GENERAL DISCUSSION

The central topic of this thesis was the investigation of mechanisms and consequences of change in aquatic microfauna communities. We used a combination of observational and experimental approaches to study how habitat characteristics affect community structure by investigating several different types of environmental gradients. Changing environmental conditions along a canopy cover gradient led to changes in community structure (chapter 1) while changes in height above ground (chapter 3) did not result in sufficient environmental differences to affect community structure. Environmental changes along elevational gradients turned out to be variable between field sites, resulting in community structure patterns only when key environmental factors changed sufficiently with elevation (chapter 4). The transplantation of communities along an elevational gradient did not cause an adaptation of communities (chapter 2) supposedly due to insufficient environmental change in the field site of the experiment (chapter 4). If environmental change is not strong enough priority effects and predator presence appear to be the driving forces in structuring communities (chapter 2). The relative importance of stochastic processes like priority effects is likely to change depending on habitat quality (chapter 1) while the importance of environmental change might, apart from being related to the strength of the change, be additionally related to the time scale on which change occurs (chapter 1), e.g. daily fluctuations versus seasonal changes. We also observed interactive effects between environment and species interactions, i.e. predation, (chapter 2) and a strong variability in environmental conditions between field sites (chapter 4) leaving much room for exploration in future studies. This following discussion aims to connect all these findings with one another and to discuss how environmental changes will likely affect bromeliad-inhabiting communities and the surrounding ecosystems and what could be the next steps to improve our understanding of micro-ecosystems and their importance.

Why we should study micro-ecosystems

The micro-ecosystems found in bromeliad tanks have been previously stated to be perfect model systems to address questions in ecology on community level under realistic natural conditions (Srivastava et al. 2004). We could confirm their suitability for the described purpose (all chapters) and found them particularly recommendable for community-level transplantation experiments under natural conditions (chapter 2). Transplantation experiments of whole communities under natural conditions have, to our knowledge, so far only been done with microbial communities (e.g. Bell 2010; Øvreås 2000). We carried out the first transplantation of complete microfauna communities under natural conditions (chapter 2). Contrary to the common assumption that resource availability is a major driver in bromeliad microfauna community (Petermann et al. 2015; Carrias et al. 2012) we found that complex interactions between different species interactions (i.e. resource availability : predator presence) as well as between environment and species interactions (i.e. environment : predator presence) are driving forces of community structure (chapter 2). We suggest that the relative importance of resource availability versus predator presence is variable in time depending on the more limiting factor (i.e. resource concentration versus predator density). The interaction between environment and predator presence highlights the need for full-factorial experimental designs to detect patterns that are otherwise missed (e.g. likely in Petermann et al. 2015). Such intricate procedures as full-factorial experimental designs are only feasible in small ecosystems, which means that we should focus our future endeavours on them. Bromeliads are not the only available micro-ecosystems. Small temporary water habitats can also be found in others plants (e.g. bamboo nodes, tree holes, etc.) or in rock crevices (Williams 1987). These so-called phyto- and lithotelmata occur worldwide (Kitching 2000; Jocque et al. 2010) and have been shown to be of importance for almost all ecosystems as described in the following example for bromeliads. Bromeliads can occur in high densities providing up to 50,000 L/ha of freshwater (Fish 1983). They provide drinking reservoirs (Bicca-Marques 1992), breeding (Frank and Lounibos 2009; Krügel et al. 1995) and feeding habitats (food source can be the community in the bromeliad: Romero and Srivastava 2010, or the bromeliad leaves: Renton 2006; Schmidt and Zotz 2000) and contain a variety of organisms living permanently between the leaf axils (Picado 1913, see morphospecies keys in chapters 2-4). Thus, bromeliads contribute animal and plant biomass that is of relevance to the surrounding food web (Romero and Srivastava 2010; Renton 2006; Schmidt and Zotz 2000) and biodiversity (Foissner et al. 2003). Microfauna living in bromeliads also add important ecological functions such as nutrient cycling to the ecosystem (Leroy et al. 2016). Cocoa pollinators, e.g. biting flies of the genus *Forcipomyia,* breed in bromeliad tanks giving them an additional economic value (Winder 1978). Other crops such as oranges were also observed to benefit from the bromeliads because these provide breeding habitats for predators that reduce herbivory on the orange trees (Hammill et al. 2014). Temporary waters that occur in high densities such as bromeliads can even have climatic effects and contribute to the resistance of the surrounding forest to climate change (Benzing 1998). This demonstrates that bromeliads are not only useful model systems but also important components of functioning ecosystems and that the better understanding of the bromeliad micro-ecosystem is therefore of undeniable importance for conservation and economic reasons.

Why we should study microfauna

Microfauna are one of the oldest living organisms on Earth (Lipps 1993) but they are normally an understudied ‘black box’ because it was always assumed that ‘everything is everywhere’

(Beijerinck 1913) and no further investigation was needed as to the driving environmental variables of their communities. However, recent studies demonstrate that this assumption is not true (La Katz et al. 2005; Foissner 2006; Nolte et al. 2010; Weisse et al. 2011) and that microfauna can even be endemic to special habitats like bromeliads (Foissner et al. 2003). We could show that community structure of bromeliad-inhabiting microfauna does change along environmental gradients if the environmental change of driving factors is large enough, leading to distinct community compositions on small local scales (chapter 1 + 4). Knowing that microfauna respond to environmental changes makes them valuable bioindicators for an early detection of altered environmental conditions (e.g. in biological wastewater treatment: Madoni 2011, or in conservation sites: Radhakrishnan and Jayaprakas 2015) because they respond more rapidly to environmental change than macrofauna due to shorter generation times (Berger et al. 1997). Considering the importance of the ecological functions provided by microfauna, e.g. key roles in fluxes of energy and matter such as nutrient cycling (Leroy et al. 2016) and being prey for higher trophic levels (Gifford 1991), it seems adequate to pay them more attention. Cotterill et al. (2007) go even so far in their argument as to request microfauna to be included in conservation strategies. We showed that habitat quality is very important for microfauna (chapter 1) making them potentially susceptible to habitat quality loss by human-induced changes which stresses the need for microfauna protection.

Consequences of a changing environment

At first glance our results depict microfauna communities as very resistant to environmental changes because they seem to have relatively broad environmental niches (chapter 2-4). Does this mean that climate change will not affect microfauna communities in bromeliads? No, because the temporary scale of environmental fluctuations may play a role (chapter 1) and complex interactive effects are involved in structuring microfauna communities (chapter 2). Bromeliads naturally occur in South and Central America (Kitching 2000), areas for which the climate change prognosis is an increase in temperature (Salazar et al. 2007; Reyer et al. 2017). Higher average temperatures imply higher daily temperature fluctuations which we have identified as important driver of microfauna community composition (chapter 1). Climate change could also indirectly affect microfauna community structure due to changes in species interactions (Tylianakis et al. 2008). Resource competition could be altered if decomposing macrofauna decrease due to higher water temperatures (Rosa 2017) or predation pressure could be increased by higher temperatures due to increased predatory metabolic rates as shown for protozoa-mosquito relationships in pitcher plants (Hoekman 2010). We observed that microfauna communities reached lower abundances if predators were present (chapter 2). Hence, one could jump to the conclusion that decreased microfauna abundances by higher predation rates would result in lower nutrient cycling rates. However, the opposite has been observed in the bromeliad system because the presence of predators accelerates nutrient cycling processes (Ngai and Srivastava 2006) highlighting again the complex interactions that need to be considered to fully understand community-level processes. So, even if microfauna are not necessarily affected directly by small climatic changes, the indirect effects based on species interactions are still not entirely predictable. Indirect effects of climate change could also come in the form of habitat loss. Anjos and Toledo (2018) demonstrate in their study that South American forest ecosystems will be prone to change into savanna or grassland ecosystems under climate change. This loss of canopy cover would result in lower quality habitats leading to an impoverishment of bromeliad communities (chapter 1) with potential loss of ecological functions.

Suggestions for applications and future research

The most obvious application of our results is the confirmation of the validity of taking ground samples as we found no significant differences in canopy and understory community structure (chapter 3). This does not mean that canopy bromeliads should be completely ignored. Probably, many interactions with the surrounding environment only take place between canopy bromeliads and exclusive canopy inhabitants, e.g. obligate canopy-dwelling earthworms (Fragoso and Rojas-Fernández 1996) or herpetofauna (McCracken and Forstner 2014). But with regard to microfauna community structure the sampling of bromeliads in only the understory is justified and thus our results reduce the logistic effort of future studies.

Bromeliads are breeding ground for many organisms (Picado 1913), amongst others for disease-carrying mosquitoes (Pittendrigh 1948). Mosquito larvae filter microfauna prey from the water or from the surface (Wotton et al. 1997; Eisenberg et al. 2000). Testate amoebae are assumedly less adequate prey for mosquito larvae as they are protected by a silica shell (Wheelis 2008). We observed that microfauna communities can shift from flagellate-dominance to amoeba-dominance (chapter 2). It would be interesting to survey if amoeba-dominated communities are less suited for mosquito larvae development and if this could affect mosquito densities in some way. If this is the case, knowledge about community-structuring processes in bromeliads could be applied to manage the dispersal of disease-carrying mosquitoes.

We brought up the issue of time-scale dependence of environmental influences on community structure (chapter 1). Though the range of temperature changes along an elevational gradient is similar to the dimension of change of daily temperature fluctuations, it is particularly the daily fluctuations that seem to influence the community structure (chapter 1 + 2). Further steps should be the experimental analyses of how daily fluctuations affect coexistence of microfauna species. Do stronger daily fluctuations promote coexistence of species if they perform distinct heat stress mechanisms, e.g. resting stages versus inactivity? Literature also gives evidence that long-term fluctuations such as seasonality can affect microfauna community composition (Buosi et al. 2015; Mondragón-Chaparro and Cruz-Ruiz 2008). A very ambitious but nevertheless interesting laboratory experiment could combine the effects of short- and long-term temperature fluctuations in a full-factorial experimental design to disentangle the underlying processes that structure communities and the relative importance of these processes. For example, does the turnover rate of different taxa groups differ along gradients of changing environmental conditions? Do facilitation processes play a role? How important is resource competition for species of the same or different functional groups under different levels of heat stress (e.g. flagellates, ciliates, amoebae)?

Genetic approaches have shown that bromeliad-inhabiting communities are more diverse than typically observed in the morphotype approaches (Rodriguez-Nuñez et al. 2018). We believe that the taxonomic resolution might be a restrictive factor in predicting community changes, because closely related species can show very different behaviour (Longino and Nadkarni 1990). The fact that some ciliates are endemic to bromeliads (Foissner et al. 2003) indicates a high specialisation to the bromeliad environment and supports the need for a taxonomic resolution on species level in future studies. A further step therefore is the taxonomic exploration of the bromeliad-inhabiting community to really assess biodiversity. These identifications should combine genetic approaches with morphological and behavioural studies. Behavioural studies could target questions like cannibalism. The successful implementation of automated counting software like bemovi (Pennekamp et al. 2015) could be used to assess traits like swimming speed and food searching trajectories to observe if these traits change under altered resource competition and/or predation levels.

The issue of microfauna conservation has been considered previously (Cotterill et al. 2008) and might become increasingly important in the future. We have shown that the loss of habitat quality, e.g. through higher daily temperature fluctuations as will locally take place under climate change, impoverishes microfauna communities (chapter 1). Will this affect microfauna in other freshwater and soil ecosystems in the same way? What would this mean for wastewater clearance and soil health, for drinking water availability and productivity of agricultural land? Though not ‘everything is everywhere’ it is undoubtedly true that microfauna perform essential functions in all ecosystems without which those systems would collapse. I therefore recommend to seriously consider microfauna in conservation strategies and that more research should be done as to how this could be realized.

General conclusion

The research papers combined in this thesis reveal the big knowledge gap that still remains with regard to some of the most important organisms within the ecosystems. Microfauna have been here several billions years ago (Arato 2010) and have endured a lot of environmental changes. Though we could show that they seem relatively unsusceptible to small environmental changes, they will not be unaffected by the human-induced alterations taking place at an unprecedented speed that are momentarily occurring on our planet. Many indirect effects will change microfauna community structure and might alter important processes in regard to energy and matter fluxes on the ecosystem level. The results of this thesis are an addition to the already existing calls to recognize the importance of microfauna organisms and to act accordingly in form of further research in this area and to apply the results in conservation strategies.

References

Anjos, L. J. S. and Toledo, P. M. de 2018. Measuring resilience and assessing vulnerability of terrestrial ecosystems to climate change in South America. - PloS one 13: e0194654.

Arato, R. 2010. Protists. Algae, amoebas, plankton, and other protists. - Crabtree Pub. Co.

Beijerinck, M. W. 1913. De infusies en de ontdekking der backteriën. Jaarboek van de Koninklijke Akademie voor Wetenschappen. - Müller, Amsterdam.

Bell, T. 2010. Experimental tests of the bacterial distance-decay relationship. - The ISME journal 4: 1357–1365.

Benzing, D. H. 1998. Vulnerabilities of Tropical Forests to Climate Change: The Significance of Resident Epiphytes. - Climatic Change 39: 519–540.

Berger, H. et al. 1997. Bestimmung und Ökologie der Mikrosaprobien nach DIN 38410. 26 Bildbestimmungsschlüssel, 10 Tabellen. - Fischer.

Bicca-Marques, J. C. 1992. Drinking behavior in the black howler monkey (Alouatta caraya). - Folia primatologica; international journal of primatology 58: 107–111.

Buosi, P. R. B. et al. 2015. Effects of Seasonality and Dispersal on the Ciliate Community Inhabiting Bromeliad Phytotelmata in Riparian Vegetation of a Large Tropical River. - The Journal of eukaryotic microbiology 62: 737–749.

Carrias, J.-F. et al. 2012. An ant–plant mutualism induces shifts in the protist community structure of a tank-bromeliad. - Basic and Applied Ecology 13: 698–705.

Cotterill, F. P. D. et al. 2008. Conservation of protists: is it needed at all? - Biodivers Conserv 17: 427–443.

Eisenberg, J. N. S. et al. 2000. Generalist feeding behaviors of Aedes sierrensis larvae and their effects on protozoan populations. - Ecology 81: 921–935.

Fish, D. 1983. 'Phytotelmata: Flora and Fauna' in Frank, J. H. and Lounibos, L. P. (eds.). - Phytotelmata: terrestrial plants as hosts for aquatic insect communities. Plexus, Medford, NJ: 161–190.

Foissner, W. et al. 2003. Endemic ciliates (Protozoa, Ciliophora) from tank bromeliads (Bromeliaceae): a combined morphological, molecular, and ecological study. - European Journal of Protistology 39: 365–372.

Foissner, W. 2006. Biogeography and Dispersal of Micro-organisms: A Review Emphasizing Protists. - Acta Protozoologica 45: 111–136.

Fragoso, C. and Rojas-Fernández, P. 1996. Earthworms inhabiting bromeliads in Mexican tropical rainforests: ecological and historical determinants. - J. Trop. Ecol. 12: 729–734.

Frank, J. H. and Lounibos, L. P. 2009. Insects and allies associated with bromeliads: a review. - Terrestrial arthropod reviews 1: 125–153.

Gifford, D. J. 1991. The Protozoan-Metazoan Trophic Link In Pelagic Ecosystems. - The Journal of Protozoology 38: 81–86.

Hammill, E. et al. 2014. Bromeliad-associated Reductions in Host Herbivory: Do Epiphytic Bromeliads Act as Commensalists or Mutualists? - Biotropica 46: 78–82.

Hoekman, D. 2010. Turning up the heat: Temperature influences the relative importance of topdown and bottomup effects. - Ecology 91: 2819–2825.

Jocque, M. et al. 2010. Freshwater rock pools: a review of habitat characteristics, faunal diversity and conservation value. - Freshwater Biology 2: e1280.

Kitching, R. L. 2000. Food webs and container habitats. The natural history and ecology of phytotelmata. - Cambridge University Press.

Krügel, P. et al. 1995. Syncope antenori: A Bromeliad Breeding Frog with Free-Swimming, Nonfeeding Tadpoles (Anura, Microhylidae). - Copeia 1995: 955-963.

La Katz et al. 2005. Reframing the 'Everything is everywhere' debate: evidence for high gene flow and diversity in ciliate morphospecies. - Aquat. Microb. Ecol. 41: 55–65.

Lahr, D. J.G. et al. 2015. The Phanerozoic diversification of silica-cycling testate amoebae and its possible links to changes in terrestrial ecosystems. - PeerJ 3: e1234.

Leroy, C. et al. 2016. The contribution of microorganisms and metazoans to mineral nutrition in bromeliads. - JPECOL 9: 241–255.

Lipps, J. H. (ed.) 1993. Fossil prokaryotes and protists. - Blackwell Scientific Publ.

Longino, J. T. and Nadkarni, N. M. 1990. A Comparison of Ground and Canopy Leaf Litter Ants (Hymenoptera: Formicidae) in a Neotropical Montane Forest. - Psyche: A Journal of Entomology 97: 81–93.

McCracken, S. F. and Forstner, M. R. J. 2014. Oil road effects on the anuran community of a high canopy tank bromeliad (Aechmea zebrina) in the upper Amazon basin, Ecuador. - PloS one 9: e85470.

Mondragón-Chaparro, D. M. and Cruz-Ruiz, G. I. 2008. Seasonal variation of the macro-arthropod community associated to Tillandsia carlos-hankii (Bromeliaceae) in an oak-pine forest in Oaxaca, Mexico. - Brenesia 70: 11–22.

Ngai, J. T. and Srivastava, D. S. 2006. Predators accelerate nutrient cycling in a bromeliad ecosystem. - Science 314: 963.

Nolte, V. et al. 2010. Contrasting seasonal niche separation between rare and abundant taxa conceals the extent of protist diversity. - Molecular ecology 19: 2908–2915.

Øvreås, L. 2000. Population and community level approaches for analysing microbial diversity in natural environments. - Ecology Letters 3: 236–251.

Pennekamp, F. et al. 2015. BEMOVI, software for extracting behavior and morphology from videos, illustrated with analyses of microbes. - Ecology and evolution 5: 2584–2595.

Petermann, J. S. et al. 2015. Resources alter the structure and increase stochasticity in bromeliad microfauna communities. - PloS one 10: e0118952.

Picado, M. C. 1913. Les broméliacées épiphytes considerée comme milieu biologique. - Bulletin Scientifique de la France et de la Belgique 47: 215–360.

Pittendrigh, C. S. 1948. The bromeliad-anopheles-malaria complex in Trinidad. I-The bromeliad flora. - Evolution 2: 58–89.

Renton, K. 2006. Diet of Adult and Nestling Scarlet Macaws in Southwest Belize, Central America. - Biotropica 38: 280–283.

Reyer, C. P.O. et al. 2017. Climate change impacts in Latin America and the Caribbean and their implications for development. - Reg Environ Change 17: 1601–1621.

Rodriguez-Nuñez, K. M. et al. 2018. The metagenome of bromeliads phytotelma in Puerto Rico. - Data in brief 16: 19–22.

Romero, G. Q. and Srivastava, D. S. 2010. Food-web composition affects cross-ecosystem interactions and subsidies. - The Journal of animal ecology 79: 1122–1131.

Rosa, E. 2017. Relationship between Climate Factor and Diptera Larvae on Phytotelmata in Dengue Fever’s Endemic Areas in West Sumatera, Indonesia. - American Journal of Zoological Research 5: 1–4.

Salazar, L. F. et al. 2007. Climate change consequences on the biome distribution in tropical South America. - Geophys. Res. Lett. 34.

Schmidt, G. and Zotz, G. 2000. Herbivory in the epiphyte, Vriesea sanguinolenta Cogn. & Marchal (Bromeliaceae). - J. Trop. Ecol. 16: 829–839.

Srivastava, D. S. et al. 2004. Are natural microcosms useful model systems for ecology? - Trends in ecology & evolution 19: 379–384.

Tylianakis, J. M. et al. 2008. Global change and species interactions in terrestrial ecosystems. - Ecology Letters 11: 1351–1363.

Weisse, T. et al. 2011. Significant habitat effects influence protist fitness: evidence for local adaptation from acidic mining lakes. - Ecosphere 2: art134.

Wheelis, M. L. 2008. Principles of modern microbiology. - Jones and Bartlett.

Williams, D. D. 1987. The Ecology of Temporary Waters. - Timber Press.

Winder, J. A. 1978. Cocoa Flower Diptera; Their Identity, Pollinating Activity and Breeding Sites. - PANS 24: 5–18.

Wotton, R. S. et al. 1997. Growth of Anopheles mosquito larvae on dietary microbiota in aquatic surface microlayers. - Med Vet Entomol 11: 65–70.

SUMMARY

Anthropogenic influences on the natural environment are increasingly observed and we only start to comprehend how this will affect biodiversity and ecosystem functioning in the long run. This question is challenging because scientific approaches normally investigate only small parts of a community, focussing on particular taxa or the effect of a restricted number of environmental variables. The predictive power of these studies is questionable because reality is a lot more complex and direct as well as indirect interactions can lead to unexpected outcomes. Whole community approaches under natural environmental conditions are logistically impracticable in most ecosystems due to the sheer impossibility of sampling, for example, an entire forest. Phytotelma, such as bromeliads, provide an ideal solution for this dilemma. These small temporary water bodies contain communities of manageable sizes that can be easily sampled in naturally replicated micro-ecosystems. Most of the previous bromeliad studies have investigated the macrofauna living in bromeliads. Microfauna have been mostly neglected and therefore little is known about their community structure. Microfauna organisms - including flagellates, ciliates, amoeba, rotifers and crustaceans - are the part of the bromeliad-inhabiting communities that this dissertation focusses on. We used a community-level approach to explore community-structuring processes in bromeliad microfauna with the aim to better predict potential effects of environmental changes on biodiversity and ecosystem functioning.

In a field survey along a canopy cover gradient (chapter 1) we investigated the effect of differences in sun-exposure in a restinga rainforest on microfauna community structure. We found strong differences in the environmental conditions which resulted in changes of habitat quality along the canopy cover gradient. This was shown to affect the community structure and beta diversity of bromeliad-inhabiting microfauna via differences in daily temperature fluctuations. With regard to the expected temperature increase through climate change, this result shows that it is not necessarily the direct effect of higher average temperatures that proposes a threat to natural communities but that indirect effects of climate change such as repeated short-time fluctuations in environmental conditions may decrease a habitat’s quality, and thus, lead to a loss of biodiversity and potentially ecological functions.

To disentangle the effects of environmental change and trophic interactions on microfauna community structure we carried out a community-transplantation experiment along an elevational gradient (chapter 2). We used a full-factorial experimental design to particularly address potential interactions between environmental change and trophic interactions. The results showed that bromeliad-inhabiting microfauna communities are also shaped by predator presence and priority effects. Interacting effects played an important role in structuring communities, suggesting that we need to broaden our scientific approaches to fully understand the relationships in natural ecosystems and better predict consequences of human-induced changes.

Though bromeliad plants grow mainly epiphytic, most bromeliad-related studies, including our field survey (chapter 1) and our field experiment (chapter 2), sample exclusively in the understory. Based on the assumption that sun-exposure increased with increasing height and thus leads to changed environmental conditions, we carried out a field survey sampling understory and canopy bromeliads using single-rope climbing techniques (chapter 3). The comparison of microfauna community structure in understory and canopy bromeliads revealed that no change in community structure occurs along the height gradient. This justifies the former bromeliad community approaches with exclusively understory samples.

Finally, we conducted a field survey along three elevational gradients to determine if bromeliad-inhabiting communities change in a generalizable pattern along natural environmental gradients (chapter 4). There was no clear pattern detectable that would allow us to filter out driving environmental factors for community structure in bromeliads on regional scale. The lack of a clear environmental driver of community structure was probably at least partly due to the lack of environmental differences along two of the three gradients.

We conclude from our results that microfauna communities are subject to complex interactions and that it is therefore important to use full-factorial approaches in future studies to disentangle the effects of potential drivers of community structure. So far, we could show that daily temperature fluctuations, predator presence, priority effects and oxygen saturation can play key roles in shaping microfauna communities, but we emphasize that these are strongly dependent on the surrounding environment making general predictions difficult. Human-induced environmental alterations such as climate change are likely to affect bromeliad-inhabiting microfauna communities via indirect effects which might result in alterations of important processes in regard to energy and matter fluxes on the ecosystem level. Based upon these results we recommend the integration of microfauna communities into conservation strategies.

ZUSAMMENFASSUNG

Anthropogene Einflüsse auf unsere Umwelt werden zunehmend beobachtet und wir beginnen gerade erst zu verstehen wie sich diese langfristig auf die Biodiversität und die Ökosystemfunktionen auswirken werden. Die Erforschung dieses Sachverhaltes stellt deshalb eine Herausforderung dar, weil wissenschaftliche Studien normalerweise nur kleine Teile einer Gemeinschaft untersuchen, da sie sich auf bestimmte Taxa oder eine begrenzte Auswahl an Umweltvariablen konzentrieren. Die Vorhersagekraft dieser Studien ist fragwürdig, weil die Realität komplexer ist und sowohl direkte als auch indirekte Interaktionen zu unerwarteten Ergebnissen führen können. Forschungsansätze, die gesamte ökologische Gemeinschaften in ihrem natürlichen Umfeld erforschen, sind aus logistischer Sicht in den meisten Ökosystemen wenig praktikabel, da es z.B. unmöglich ist einen gesamten Wald zu beproben. Mit Phytotelmen wie den Bromelien kann man dieses Problem lösen. Diese temporären Kleinstgewässer beinhalten Gemeinschaften von überschaubarer Größe, welche leicht in den auf natürliche Weise replizierten Mikroökosystemen beprobt werden können. Die meisten der vorangegangenen Bromelienstudien haben die in den Bromelien lebenden Macrofauna-Organismen erforscht. Die Mikrofauna-Organismen wurden meistens vernachlässigt, weshalb wenig über die Zusammensetzung ihrer Gemeinschaften bekannt ist. Mikrofauna-Organismen – inklusive Flagellaten, Ciliaten, Amöben, Rotatorien und Kleinstkrebsen – sind der Teil der in den Bromelien lebenden Gemeinschaft, auf den sich diese Dissertation konzentriert. Wir verwenden einen gemeinschafts-basierten Ansatz, um die für die Mikrofauna-Gemeinschaft relevanten Prozesse zu untersuchen, mit dem Ziel, potentielle Auswirkungen von Umweltveränderungen auf Biodiversität und Ökosystemfunktionen besser hervorsagen zu können.

In einer Felderhebung entlang eines Kronenschluss-Gradienten (Kapitel 1) haben wir den Effekt von unterschiedlichen Sonnenexpositionen in einem „Restinga“-Wald auf die Zusammensetzung der Mikrofauna-Gemeinschaft untersucht. Wir haben starke Unterschiede in den Umweltverhältnissen festgestellt, welche die Habitatqualität entlang des Kronenschluss-Gradienten veränderten. Dies hat sich aufgrund täglicher Temperaturschwankungen auf die Zusammensetzung und die Betadiversität der Mikrofauna-Gemeinschaften in den Bromelien ausgewirkt. Bedenkt man die erwarteten Temperaturerhöhungen im Rahmen des Klimawandels verdeutlichen diese Ergebnisse, dass nicht notwendigerweise die höheren Temperaturmittelwerte eine Gefahr für natürliche Gemeinschaften darstellen, sondern dass indirekte Effekte wie z.B. kurzzeitige Schwankungen der Umweltbedingungen die Habitatqualität herabsetzen können und so zu einem Verlust von Biodiversität und vielleicht sogar von Ökosystemfunktionen führen können.

Um die Einflüsse von Umweltfaktoren und trophischen Interaktionen auf die Zusammensetzung von Mikrofauna-Gemeinschaften zu entflechten, haben wir die Gemeinschaften entlang eines Höhengradienten im Rahmen eines Experimentes verpflanzt (Kapitel 2). Wir haben ein experimentelles Design mit vollständiger Behandlungskombination gewählt, um gezielt potentielle Interaktionen zwischen Umweltparametern und trophischen Interaktionen untersuchen zu können. Die Ergebnisse zeigen, dass die Anwesenheit von Prädatoren und Prioritätseffekten (engl. *priority effects*) für Mikrofauna-Gemeinschaften in Bromelien eine entscheidende Rolle spielen. Interaktionen waren ebenfalls von Bedeutung. Dies suggeriert, dass wissenschaftliche Untersuchungen breiter angelegt werden müssen, um Zusammenhänge in Ökosystemen besser zu verstehen und die Konsequenzen anthropogen verursachter Umweltveränderungen besser hervorsagen zu können.

Obwohl Bromelien meisten epiphytisch wachsen, beproben die meisten Bromelienstudien, inklusive unserer Felderhebung (Kapitel 1) und unseres Feldexperiments (Kapitel 2), nur Bromelien im Unterholz. Ausgehend von der Annahme, dass die Sonnenexposition mit zunehmender Höhe am Baum zunimmt und damit Veränderungen der Umweltbedingungen einhergehen, haben wir mit der Seilklettertechnik Unterholz- und Kronendachbromelien beprobt (Kapitel 3). Der Vergleich der Mikrofauna-Gemeinschaften zwischen den Unterholz- und Kronendachbromelien ergab keine signifikanten Unterschiede in der Gemeinschaftszusammensetzung entlang des Höhengradienten. Dies rechtfertigt die vorangegangenen Studien, welche ausschließlich Unterholzbromelien beprobt haben.

Abschließend haben wir Feldproben entlang von drei Höhengradienten genommen, um zu untersuchen, ob sich Veränderungen in den Gemeinschaftszusammensetzungen der Bromelienbewohner entlang von Umweltgradienten verallgemeinern lassen (Kapitel 4). Wir konnten keine allgemeinen Trends feststellen, anhand welcher wir auf regionaler Ebene allgemein gültige Umweltwirkungsfaktoren für die Zusammensetzung von Bromeliengemeinschaften hätten ableiten können. Das Fehlen eines allgemein gültigen Umweltwirkungsfaktors für die Zusammensetzung der Gemeinschaften kann unter anderem womöglich auf das Fehlen klarer Umweltgradienten auf zwei der drei Beprobungsstandorte zurückgeführt werden.

Basierend auf unseren Ergebnissen schlussfolgern wir, dass in Mikrofauna-Gemeinschaften komplexe Interaktionen wirken und dass daher die Verwendung von experimentellen Designs mit vollständiger Behandlungskombination in zukünftigen Studien wichtig ist, um die potentiellen Effekte auf die Zusammensetzung der Gemeinschaften zu entwirren. Wir konnten bis jetzt zeigen, dass tägliche Temperaturschwankungen, die Anwesenheit von Prädatoren, Prioritätseffekte und die Sauerstoffsättigung eine wichtige Rolle für die Zusammensetzung der Mikrofauna-Gemeinschaften spielen können, aber wir betonen, dass diese stark von der umgebenden Umwelt abhängen und dass allgemeine Vorhersagen daher schwierig sind. Von Menschen verursachte Veränderungen der Umwelt, wie z.B. der Klimawandel, werden über indirekte Effekte vermutlich die Mikrofauna-Gemeinschaften in den Bromelien beeinflussen, was zu einer Veränderung von wichtigen Prozessen im Bereich der Energie- und Materieflüsse auf der Ökosystemebene führen kann. Basierend auf unseren Ergebnissen empfehlen wir Mikrofauna-Gemeinschaften in Schutzstrategien zu integrieren.

CONTRIBUTIONS TO THE PUBLICATIONS

Busse AB, Antiqueira PAP, Neutzling AS, Wolf AM, Romero GQ, Petermann JS (2018). Different in the dark: The effect of habitat characteristics on community composition and beta diversity in bromeliad microfauna. *PLoS ONE*, 13 (2): e0191426.

Own contributions:

GQR and JSP did the conceptualization. PAPA, ASN and AMW did the sampling. AB and JSP did the formal analysis and interpreted the data. AB wrote the first draft; all authors read and edited the manuscript.

Busse AB, Schoreisz JJ, Petermann JS (*submitted to OIKOS, 29th July 2018*). Predators and priority effects shape microfauna communities in a community transplantation experiment along an elevational gradient.

Own contributions:

AB and JSP designed the experiment. AB and JJS performed the experiment. AB and JSP did the formal analysis and interpreted the data. AB wrote the first draft; all authors read and edited the manuscript.

Busse AB, Schoreisz JJ, Wallner L, Srivastava DS, Petermann JS (*in preparation*). Micro- and macrofauna communities in bromeliad phytotelmata are similar in forest canopy and understory.

Own contributions:

AB, JJS and DSS did the sampling. JJS, LW and DSS identified the organisms. AB and JSP did the formal analysis and interpreted the data. AB wrote the first draft; all authors read and edited the manuscript.

Busse AB, Adler Yañez MA, Petermann JS (*in preparation*). Variability in environmental conditions between elevational gradients leads to diverse patterns in microfauna community structure and beta diversity.

Own contributions:

AB and MAAY did the sampling. AB identified the organisms. AB and JSP did the formal analysis and interpreted the data. AB wrote the first draft; all authors read and edited the manuscript.

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