

RESEARCH ARTICLE

# Rainfall changes affect the algae dominance in tank bromeliad ecosystems

Aliny Patricia Flauzino Pires<sup>1,2,3\*</sup>, Juliana da Silva Leal<sup>1</sup>, Edwin T. H. M. Peeters<sup>4</sup>

**1** Departamento de Ecologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, **2** Fundação Brasileira para o Desenvolvimento Sustentável, Rio de Janeiro, Brazil, **3** Brazilian Research Network on Climate Change—Rede Clima, Instituto Nacional de Pesquisas Espaciais, São José dos Campos, São Paulo, Brazil, **4** Department of Environmental Sciences, Aquatic Ecology and Water Quality Management Group, Wageningen University, Wageningen, The Netherlands

\* [alinyfp@igmail.com](mailto:alinyfp@igmail.com)



## Abstract

Climate change and biodiversity loss have been reported as major disturbances in the biosphere which can trigger changes in the structure and functioning of natural ecosystems. Nonetheless, empirical studies demonstrating how both factors interact to affect shifts in aquatic ecosystems are still unexplored. Here, we experimentally test how changes in rainfall distribution and litter diversity affect the occurrence of the algae-dominated condition in tank bromeliad ecosystems. Tank bromeliads are miniature aquatic ecosystems shaped by the rainwater and allochthonous detritus accumulated in the bases of their leaves. Here, we demonstrated that changes in the rainfall distribution were able to reduce the chlorophyll-*a* concentration in the water of bromeliad tanks affecting significantly the occurrence of algae-dominated conditions. On the other hand, litter diversity did not affect the algae dominance irrespective to the rainfall scenario. We suggest that rainfall changes may compromise important self-reinforcing mechanisms responsible for maintaining high levels of algae on tank bromeliads ecosystems. We summarized these results into a theoretical model which suggests that tank bromeliads may show two different regimes, determined by the bromeliad ability in taking up nutrients from the water and by the total amount of light entering the tank. We concluded that predicted climate changes might promote regime shifts in tropical aquatic ecosystems by shaping their structure and the relative importance of other regulating factors.

## OPEN ACCESS

**Citation:** Pires APF, Leal JdS, Peeters ETHM (2017) Rainfall changes affect the algae dominance in tank bromeliad ecosystems. PLoS ONE 12(4): e0175436. <https://doi.org/10.1371/journal.pone.0175436>

**Editor:** Ulrike Gertrud Munderloh, University of Minnesota, UNITED STATES

**Received:** November 30, 2016

**Accepted:** March 27, 2017

**Published:** April 19, 2017

**Copyright:** © 2017 Pires et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Data Availability Statement:** All relevant data are within the paper and its Supporting Information files.

**Funding:** Financial support was provided by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Project 476926/2012-2), FAPERJ (Fundação de Apoio à Pesquisa do Estado do Rio de Janeiro, Project E-26/103316/2011) and CENPES/PETROBRAS (Project "Impact of Global Climate Changes on Tropical Continental Aquatic Ecosystems"). APFP and JSL are grateful to CNPq and CAPES for graduate scholarships. This paper is

## Introduction

Ecosystems face many concurrent stressors which may cause great changes in their functioning. It has been proposed that climate change and biodiversity loss could promote large shifts in natural ecosystems compromising important ecological services [1–3]. Also, tropical aquatic ecosystems are affected by these two stressors since most of them are only fed by rain and because these ecosystems are prone to high rates of biodiversity loss [4].

Climate models predict that changes in rainfall distribution will be the prevalent change across different areas of the globe, but especially in tropical and high latitude areas [5]. Extreme

a contribution of the Brazilian Research Network on Global Climate Change, FINEP/ Rede CLIMA Grant Number 01.13.0353-00. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing interests:** The authors have declared that no competing interests exist.

rainfall events may cause unpredicted changes in aquatic ecosystems and may ease a return to a pristine condition in natural environments [6,7]. Changes in rainfall distribution, such as longer periods of drought followed by extreme rainfall events, may lead to a decrease in water level in lakes over a longer period of time. This might favor the development of submerged aquatic plants and reduce algal growth leading to a shift towards a pristine state [8]. Controlling local stressors has been proposed as one of the most effective ways to prevent ecosystems to shift from one condition to another due to climate changes [1,9].

Biodiversity loss is considered one of the major drivers of the current global changes [2]. On the other hand, biologically diverse systems may ensure ecosystems against disturbances and can make them more resistant to the effects of climate changes [10–13]. Species-rich ecosystems seem to be more stable to environmental changes than species-poor systems through various mechanisms, including asynchrony and compensatory population dynamics, different resistance strategies and portfolio effects [14,15]. In this way, biodiversity may favor the maintenance of a pristine condition, especially given the predicted climate change [10]. In aquatic ecosystems, leaf litter is an important food source and substrate and its diversity can provide insurance face to climate changes [16–18]. Empirical approaches could help to identify the underlying mechanisms and to reveal potential interactions between biodiversity and climate change [15,19]. However, experiments unraveling how climate changes and biodiversity jointly drive natural aquatic environments are scarce probably due to the great challenge in manipulating whole ecosystems.

Alternatively, tank bromeliads have been increasingly used as model ecosystems to test many ecological questions [16,20–23]. These natural microcosms are miniature aquatic ecosystems that are shaped by rainwater and allochthonous detritus accumulated in the bases of their leaves. They have great experimental potential due to their handling and replication ability [20]. Empirical studies indicate that these ecosystems can be dominated by two different food sources. Primarily, tank bromeliads ecosystems would be sustained by the allochthonous detritus from the surrounding vegetation (i.e.:“brown” condition); or alternatively, by the autochthonous organic matter, mainly algae growing in the water tank (i.e.:“green” condition) [24,25]. Multiple environmental factors are associated with this green and brown conditions and the importance of algae in these systems has been more and more recognized in the last years [25–28]. Studies suggest that bromeliad size, the amount of organic matter and light inputs are the most important factors to determine algae biomass in these ecosystems [24,26,29]. Increases in bromeliad size can ensure the maintenance of an aquatic condition favoring algae colonization and allowing higher decomposition rates [26] which may be beneficial for the development of an algae-dominated condition. Occasional inputs of organic matter will also regulate algae biomass in tank bromeliads by controlling nutrients concentrations therein [29]. Recently, it has also been demonstrated that light input can regulate algal biomass in tank-bromeliad ecosystems and explain the relative importance of the allochthonous contribution to the system [24]. Ecosystems with greater light inputs had their food webs more closely related to algae than those less exposed to sunlight [24].

As these aquatic ecosystems are fully fed by rainwater, changes in rainfall could disrupt important mechanisms that regulate algae growth therein. For example, increases in rainfall can raise the water level in tank bromeliad ecosystems [30] and it can favor the light entrance in the tank or speed up litter decomposition which benefits algae dominance. Controversy, rainfall itself can dilute or even overflow the water in the bromeliad tanks which may decrease nutrients concentration and algae in the water. Additionally, bromeliad plants are also able to take up nutrients from the water in their tanks [31,32]. Thus, the effects of rainfall changes on the occurrence of a green condition may be mediated by the ability of tank bromeliads in removing nutrients from the water. However, the interaction between algae and bromeliads

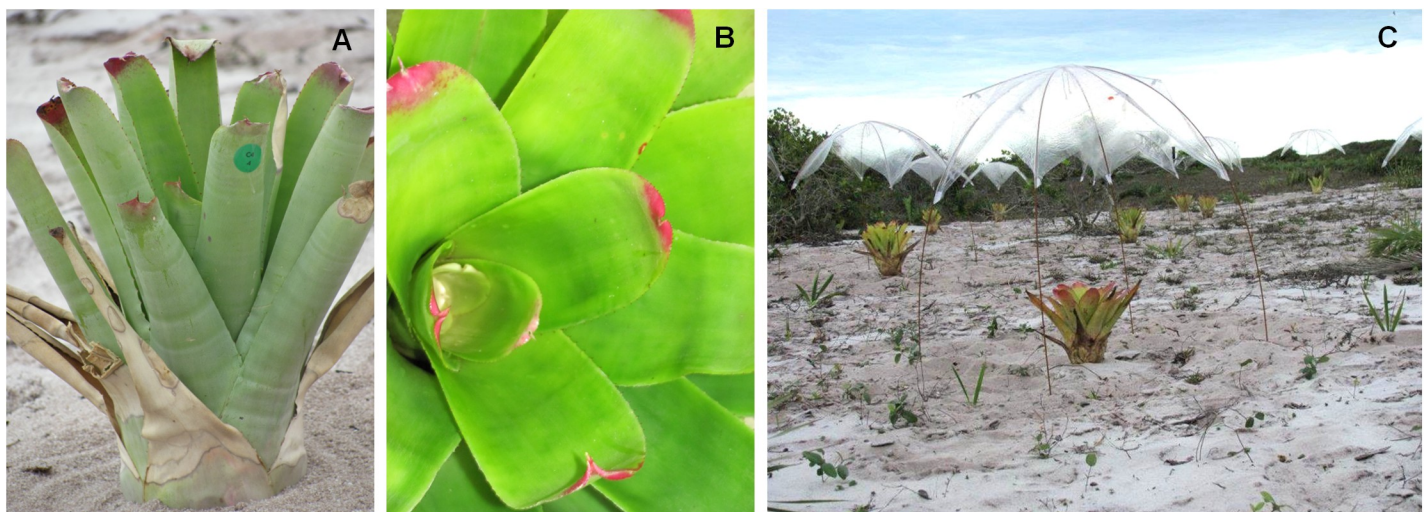
and how they regulate the functioning of phytotelma are considered open questions in the ecology of these ecosystems [33]. Empirical studies using isotopic analysis could solve the main gaps in the relationship between bromeliad nutrition and the water in their tank, including the potential competition by nutrients with algae [33].

In this study, we experimentally test the effects of rainfall changes and biodiversity on the occurrence of algae-dominated conditions in tank bromeliads ecosystems. We controlled biodiversity by manipulating litter diversity in the bromeliads tanks and using all combination of three different species from our field site. Then, we manipulated temporal clustering and amplitude of rainfall by using five different rainfall scenarios predicted to occur in the South-eastern Brazil. We used stable isotopes analysis to verify the potential effect of rainfall changes in regulating the ability of bromeliads in uptaking nutrients from the water in their tank. We hypothesized that (i) changes in rainfall distribution will decrease the occurrence of an algae-dominated condition by suppressing the ability of bromeliads in taking up nutrients from their tanks and (ii) this effect will be minimized by the positive effects of litter diversity on the algae dominance. At last, we discuss whether the occurrence of an algae-dominated condition in tank bromeliads can be regarded as alternative states by providing a conceptual framework based on the actual knowledge about these ecosystems.

## Material and methods

### Experimental design

We experimentally manipulated the amount of water and litter diversity input for the tank bromeliad *Neoregelia cruenta* (Graham) L. B. Sm. (Fig 1), in the Restinga de Jurubatiba National Park, Rio de Janeiro State, Brazil ( $-22^{\circ}14'18''\text{S}$ ,  $41^{\circ}33'35''\text{W}$ ). The Restinga de Jurubatiba National Park is characterized by shrubby vegetation patches in a sandy soil matrix. We used a full-factorial design, composed of five rainfall scenarios crossed with all combinations of three litter species (seven litter combinations, including all 1-, 2- and 3-species combinations), resulting in 35 treatments with five replicates each (175 experimental units). All samplings and procedures were conducted according to the national and international guidelines



**Fig 1. The tank bromeliad *Neoregelia cruenta* used in the current experiment.** (A) Detail of the central tank that hosts the miniature aquatic ecosystem created by the rain water accumulated between the bromeliad leaves (B). Panoramic view of the experiment after shelter installation (C). Each bromeliad was in the field for 84 days and received water as described by their rainfall scenario treatment (300 mm). Experimental details are described in Material and Methods.

<https://doi.org/10.1371/journal.pone.0175436.g001>

and licensed by the Instituto Chico Mendes de Conservação da Biodiversidade, Ministério do Meio Ambiente (research permit 26995-1/2011).

We established the rainfall scenarios based on precipitation projections for Southeastern Brazil for the second half of this century (2071 to 2100). These projections include an increase in temporal clustering of rainfall and in the occurrence of extreme rainfall events, but no changes in the total amount of rain [34]. We established an *Ambient* rainfall scenario based on the daily rainfall pattern over the rainy season (November to February) for our field site during nine years (1997 to 2005; Instituto Brasileiro de Meteorologia, INMET). *Ambient* rainfall scenario was characterized by a 25 mm rainfall event with a six days interval between rainfall events, distributed over 84 days (12 rainfall events) with a total 300 mm of rainfall. For the altered rainfall scenarios, we held the total amount of rainfall added constant (300 mm) but increased the temporal clustering of rainy days (two “clustering” scenarios) and the occurrence of extreme rainfall events (two “amplitude” scenarios). In the clustering scenarios, we decreased the interval between rainfall events; clustering rainfall either as two consecutive rainy days (25 mm per rainy day; 50 mm per two-day event) with a 12-day interval between rain events in a *Medium Clustering* scenario (MC scenario), or as four consecutive rainy days (25mm per rainy day; 100 mm per four-day event) with a 24-day interval between rain events in a *High Clustering* scenario (HC scenario). For the amplitude scenarios, we increased the intensity of each rainfall event and the interval between events. In a *Medium Amplitude* scenario (MA scenario), a one-day 50 mm rainfall event occurred every 14 days, while in a *High Amplitude* scenario (HA scenario), a one-day 100 mm rainfall event occurred every 28 days. As we intentionally constrained our rainfall manipulations to have total equivalent amounts of rain, each altered rainfall scenario necessarily altered several rainfall variables simultaneously. For example, increases in the occurrence of extreme events (*MA* and *HA* rainfall scenarios) resulted in more consecutive days without rain, as it is predicted to occur [35]. For more details about rainfall manipulation, including rainfall scenarios and the watering procedures related to our experimental design, see [16].

We manipulated litter diversity by choosing plants from different metabolic groups ( $C_3$  – *Eugenia uniflora*,  $C_4$  – *Cyperus sp.* and CAM – *Clusia hilariana*) that are potential litter source for tank bromeliads at our field site. We collected  $C_4$  and CAM litter in the field and then washed and dried at 60°C until its mass stabilized (at least 48 hours). *E. uniflora* was cultivated in a greenhouse with controlled irrigation for  $^{15}\text{N}$  enrichment (see next paragraphs). Each bromeliad received 1.2 g dry weighted of its respective litter combination. We choose this amount of litter because it is comparable to the natural amounts found in the main tank of *N. cruenta* bromeliads (unpublished data). We accommodate the untied litter in the bromeliad central tanks because they are more susceptible to the other factors that regulate algal biomass in the tank bromeliads (i.e.: light input, hydrology). In total, all 1-, 2- and 3-species combinations (seven litter combinations) were applied through a substitutive design. It means that all experimental units received the same amount of litter but in different proportions of species. The 1-specie treatments ( $C_3$ ,  $C_4$  and CAM) received 1.2 g of litter from the same litter species; the 2-species treatments ( $C_3$ - $C_4$ ,  $C_3$ -CAM and  $C_4$ -CAM) received 0.6 g of each litter species in the treatment; and in the 3-species treatment ( $C_3$ - $C_4$ -CAM), we added 0.4 g of each litter species.

All bromeliads were dug up and washed to remove all organisms to standardize initial conditions. As bromeliad size is a strong determinant of bromeliad water dynamics [36], we controlled bromeliad size effects in several ways: (i) by choosing bromeliads of approximately 30 cm in diameter; (ii) by ensuring that any residual variation in size was evenly distributed between treatments, and (iii) by measuring the maximum volume of each bromeliad and using this as a covariate in statistical analyses. We measured the maximum volume of each

tank bromeliad by slowly filling them with water using a volumetric beaker until they overflowed [26].

Bromeliads were replanted in the “*restinga*” in a spatially randomized arrangement three months prior to the rainfall manipulation. Each bromeliad received its respective litter treatment and was weekly water-filled during these three months (September to November 2011) to allow community assembly. After this period, each bromeliad was covered with an individual transparent shelter to prevent natural inputs of rain but which allowed light to enter the bromeliads tank as well as organisms’ colonization in these systems (Fig 1C). The rainfall scenarios were simulated experimentally for 3 months during the rainy season (November 2011 to February 2012) by adding mineral water to each bromeliad using a watering can, according to its specific rainfall scenario. We determined the total volume of water to be added in each bromeliad based on its mean surface catchment area [16]. All response variables were sampled at the end of the experiment, five days after the last rainfall event. We sampled at this moment because it allows the system to recover its own dynamic after rainfall disturbance but it still retains water for sampling. Additionally, we decided to sample at the end of the rainfall manipulation to avoid resetting important ecological processes.

We verified the effects of rainfall changes and litter diversity on algae biomass by measuring the chlorophyll-*a* content in the water of the bromeliads tank. We collected the water by turning the bromeliad upside down in a bucket which allowed us to homogenize the water from all tanks, as previously suggested [37]. Then, we filtered 40 ml of water in 0.7  $\mu\text{m}$  pore size cellulose filters that were kept frozen until analysis. Chlorophyll-*a* concentration was determined by spectrophotometry at 665 nm, after extraction with ethanol 90% at 80°C [38]. Recently, it has been reported an unexpected concentration of bacteriochlorophyll-*a* in tank bromeliads ecosystems [39]. Thus, it is possible that part of our measurements does not report only algal but also bacteria biomass. However, as we used 0.7  $\mu\text{m}$  pore size cellulose filters, we consider that most of the bacteriochlorophyll-*a* was not retained in the filter. Also, the relationship between algal biomass and chlorophyll-*a* is widespread and consistent in the ecological literature and chlorophyll-*a* is still considered one of the best proxy measurements of algal biomass in aquatic ecosystems, including tank bromeliads [26,28,40].

Studies in temperate shallow lakes usually assume that algae-dominated systems have chlorophyll-*a* concentration higher than 20  $\mu\text{g L}^{-1}$  [41–43] whereas in tropical shallow lakes this is 40  $\mu\text{g L}^{-1}$  [44]. However, the total amount of water in tank bromeliads may vary greatly which could lead to a misread in the identification of an algae-dominated condition. Thus, we used the distribution of chlorophyll-*a* concentration in the *Ambient* rainfall scenario (S2 Fig) to establish which value identify a different group of ecosystems. We concluded that 80  $\mu\text{g L}^{-1}$  is a reasonable and conservative value to determine the occurrence of an algae-dominated condition. *In situ* visual observations were regularly done during the experiment to verify the temporal consistency of the rainfall scenario and litter diversity effects on determining the occurrence of algae-dominated ecosystems. For methods, data analysis and main results of the qualitative approach see [Supporting Information](#).

We measured nutrients, color and turbidity to verify whether the occurrence of an algae-dominated condition was regulated to changes in these environmental variables. Nutrient concentration and water color were assessed using the filtered water in 0.7  $\mu\text{m}$  pore size cellulose filters (Advantec). Water color was determined spectrophotometrically by light absorption at 430 nm in 1 cm cuvette [45]. The concentrations of dissolved nitrogen and carbon in the water were estimated by using a carbon analyzer (TOC-V CPN, Shimadzu) with a nitrogen analyzer module coupled (TNM-1, Shimadzu). The phosphorus concentration was obtained by spectrophotometry using the colorimetric method with molybdc acid [46]. Turbidity was assessed by measuring the intensity of light scattered using an HI 98703 Hanna turbidimeter.

We used  $^{15}\text{N}$  labeled leaves of *E. uniflora* in all treatments containing  $\text{C}_3$  litter to determine the indirect effects of rainfall on the ability of bromeliad leaves in removing nutrients from their tank water. However, we only analysed these effects in the  $\text{C}_3$ -litter treatments because i) we were mainly interested in the effects of rainfall in regulating the ability of bromeliads in determining the nutrient loading of their tank and ii) we only have  $^{15}\text{N}$  enriched *E. uniflora*, therefore in the mixed treatments several biases could occur associated with nitrogen competition during bromeliad acquisition of nutrients by unbalanced  $^{14}\text{N}/^{15}\text{N}$  ratios among litter species. Thus, our experimental design did not allow us to verify the potential effects of litter diversity on the bromeliad ability to uptake nutrients from their tank because this manipulation is restricted to  $\text{C}_3$  litter treatments.

## Data analysis

We verified the occurrence of different conditions in tank bromeliads by using the bimodality approach [44]. This method is recommended and very useful in studies with limited temporal data [44], such as the present experiment. We tested which type of distribution fitted best to the chlorophyll-*a* data following the recommendations and procedures are given in literature [44]. We used the chlorophyll-*a* concentration values found in the last sampling to perform this analysis. Bimodality was demonstrated using the Bayesian Information Criterion (BIC) values [44]. The lowest BIC value indicates the best distribution for the number of classes used for each variable. For the full description of the bimodality approach, see [44].

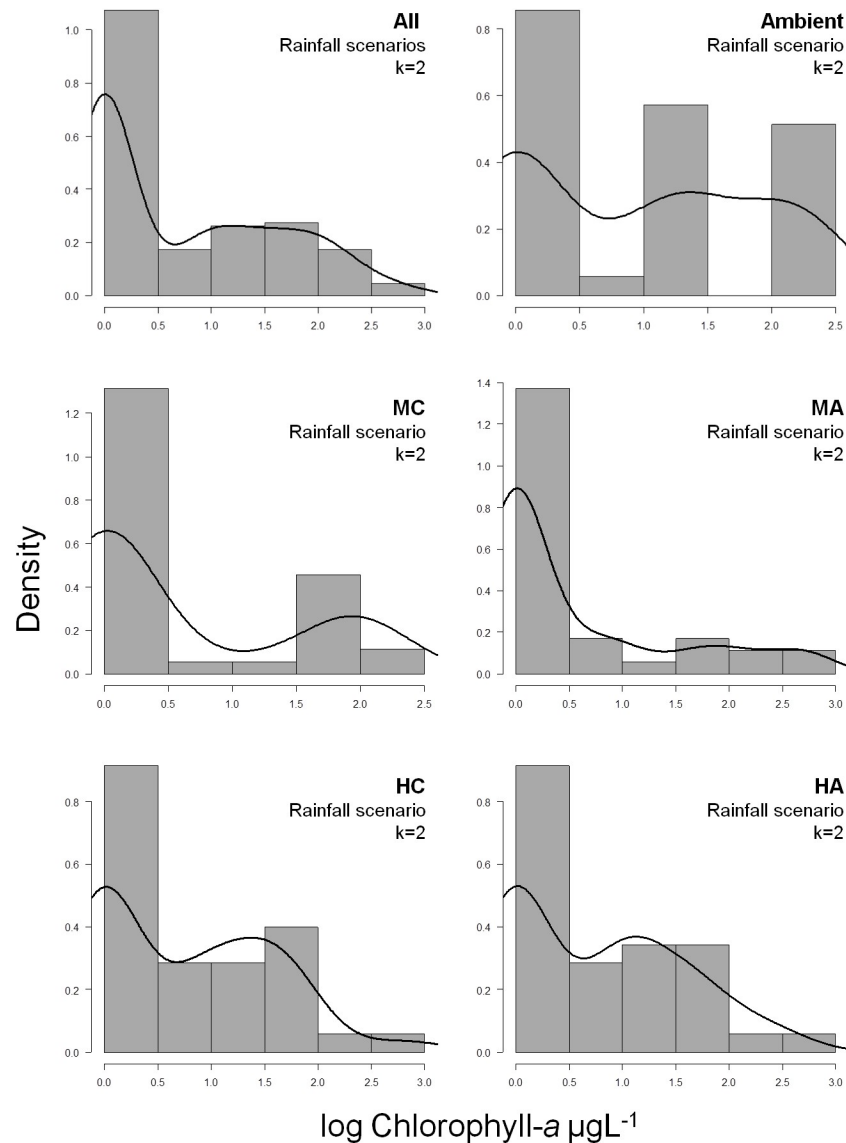
We analyzed the effects of bromeliad maximum volume, rainfall scenario, litter diversity and their interaction on the occurrence of algae dominance by using Generalized Linear Models (GLMs). We also used GLMs to evaluate the effects of water color, turbidity, dissolved nitrogen, dissolved phosphorus and dissolved organic carbon on the occurrence of an algae-dominated condition. For these analyses, we used a binomial distribution and designated the algae-dominated bromeliads by the value “1” and non algae-dominated bromeliads by the value “0”. To check the correlation between chlorophyll-*a* and the  $^{15}\text{N}$  content in the bromeliad leaves, we performed linear models to test the effect of rainfall scenario on the  $^{15}\text{N}$  isotope ratio content in the bromeliad leaves.

All models were performed using R v. 3.2.2 [47]. We carried out the LMs with base functions in R. We obtained the BIC values for the chlorophyll-*a* data in each rainfall scenario using the “*flexmix*” package.

## Results

In all rainfall scenarios and litter diversity treatments, the non algae-dominated condition occurred in at least 70% of all tank bromeliads (Fig 2). Furthermore, the BIC values used in the bimodality approach showed that in all rainfall scenarios two classes best described the distribution of chlorophyll-*a* (Fig 2). We were able to identify one class of bromeliads with very low chlorophyll-*a* concentration and another class with values higher than  $20 \mu\text{g L}^{-1}$  (Fig 2, S1 Fig). The *Ambient* rainfall scenario showed a representative group algae-dominated bromeliads (chlorophyll-*a* higher than  $80 \mu\text{g L}^{-1}$ ; Table A in S1 Appendix; Fig 2), but changes in rainfall distribution decreased the occurrence of them in the HA and HC rainfall scenarios (Table 1; Fig 2).

Rainfall changes affected the algae dominance in tank bromeliads (Table 1, Fig 3) with a significantly lower number algae-dominated conditions in HA ( $z = -1.993$ ,  $P = 0.046$ ) and HC rainfall scenarios ( $z = -2.11$ ,  $P = 0.034$ ). In *Ambient* rainfall scenario, the number of bromeliads dominated by algae was up to 5 folds greater than in HC rainfall scenario (Fig 3). Litter diversity, its interaction with rainfall scenario and bromeliad size did not affect the number of



**Fig 2. Frequency distribution of log-transformed chlorophyll-a ( $\mu\text{g L}^{-1}$ ) in the water of tank bromeliads.** Frequency distribution was determined for all rainfall scenarios ( $n = 175$ ) and *Ambient*, Medium clustering (*MC*), Medium amplitude (*MA*), High clustering (*HC*) and High amplitude (*HA*) rainfall scenarios separately ( $n = 35$ ). The lines depict the probability density described by the best fitting model resulting from the BIC analysis, represented by the  $k$  values in the panels. For statistical details see Table A in [S1 Appendix](#).

<https://doi.org/10.1371/journal.pone.0175436.g002>

algae-dominated ecosystems (Table 1). Additionally, we did not observe any significant effect of water color, turbidity, dissolved nitrogen, dissolved phosphorus and dissolved organic carbon on the occurrence of algae-dominated conditions (Table B in [S1 Appendix](#); For all factors  $P > 0.36$ ).

We found a significant effect of rainfall scenario on the  $\delta^{15}\text{N}$  content in bromeliad leaves ( $F_{4,44} = 5.33$ ,  $P = 0.0013$ ; Fig 4). Tank bromeliads from *HA* rainfall scenario had almost three times higher amount of  $\delta^{15}\text{N}$  in their leaves than observed in *Ambient* rainfall scenario (Fig 4). In  $\text{C}_3$  litter treatments, we also observed a negative correlation between the  $\delta^{15}\text{N}$  in the

**Table 1. Rainfall effects on algae-dominated conditions.** General linear model for the effects of bromeliad volume, rainfall scenario, litter diversity and the interaction between rainfall and litter diversity on the occurrence of algae-dominated conditions. Algae-dominated ecosystems were defined as those which water contained more than 80  $\mu\text{g}$  chlorophyll-*a*  $\text{L}^{-1}$ . Rainfall scenarios *Ambient*, Medium clustering (*MC*), Medium amplitude (*MA*), High clustering (*HC*) and High amplitude (*HA*) are described in the main text. Significant values are presented in bold numbers ( $P < 0.05$ ).

Factors	d.f	$\chi^2$	<i>P</i>
Bromeliad volume	1	0.002	0.963
Rainfall scenario (RS)	4	<b>9.75</b>	<b>0.045</b>
Litter diversity (LD)	1	1.20	0.273
RS x LD	4	6.14	0.189

<https://doi.org/10.1371/journal.pone.0175436.t001>

bromeliad leaves and chlorophyll-*a* concentration in the water sampled at the end of the experiment ( $F_{1,46} = 4.66$ ,  $P = 0.036$ ; best-fit slope =  $-0.7686 \pm 0.3562$ ).

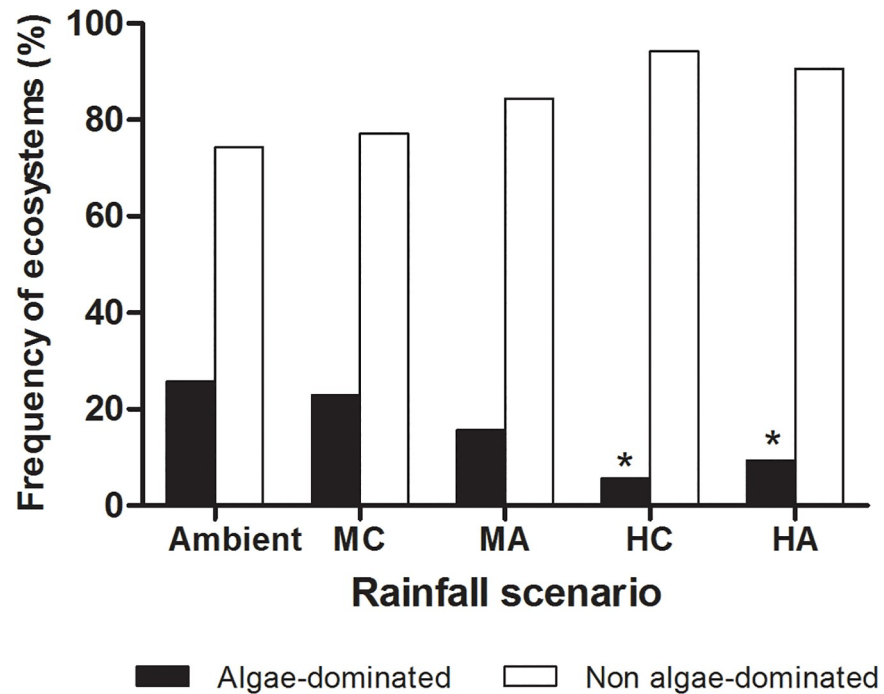
## Discussion

The understanding of how ecosystems shift to critical conditions is a challenging question for ecosystem conservation and management in the face of global changes [1,7]. Our results demonstrated that rainfall changes decreased the occurrence of algae-dominated ecosystems, mainly at *HA* and *HC* rainfall scenario, but litter diversity was not able to mediate this effect. Here, we suggest that predicted changes in rainfall will affect algal biomass in tank bromeliad ecosystems by regulating the ability of bromeliad in uptaking nutrients from the water in their tanks.

We demonstrated that the non algae-dominated condition occurred most in the tank bromeliads *Neoregelia cruenta*. In all rainfall scenarios, we observed a large group of bromeliads where chlorophyll-*a* concentration was below detection level and a smaller group with a detectable source of algae in the water of their tanks. Particularly, in *Ambient* rainfall scenario, we observed a bimodal distribution of chlorophyll-*a* with many observations around zero and a number of observations above 80  $\mu\text{g}$   $\text{L}^{-1}$ . Previous studies using the same tank bromeliad, but at natural conditions and containing different amounts of litter, found a much lower maximum chlorophyll-*a* concentration, around to 27  $\mu\text{g}$   $\text{L}^{-1}$  [26,48]. We suggest that the difference with previous studies is related to the light input in the tanks since light availability seems to be an important determinant of primary productivity of these ecosystems [24]. Our experiment was performed in an open area without surrounding vegetation and canopy cover and thus ensuring that each experimental unit received the same and maximum light input (no dossel cover, Fig 1C).

Our data showed that rainfall changes affected the occurrence of algae-dominated and non-algal conditions in tank bromeliads, which could be indirectly related to the water level in these ecosystems. Interestingly, our isotopic analysis revealed that high changes in rainfall amplitude (*HA* rainfall scenario) increased the content of  $^{15}\text{N}$  in the bromeliad leaves which suggests an enhanced ability in taking up nutrients from the water in these rainfall conditions. This result suggests that, in the Restinga of Jurubatiba, water can be a limiting factor for bromeliads to obtain nutrients. Since bromeliads are able to take up water and nutrients by their foliar trichomes and the availability of water increases the contact of trichomes with nutrients [31,49,50], an increment in the rainfall amplitude may thus increase the contact of trichomes with nutrients, increasing bromeliad nutrition. As consequence, we observed a negative correlation between the total amount of  $^{15}\text{N}$  in bromeliad leaves and the chlorophyll-*a* contents and algal biomass in the water in the tank. Previous studies have reported the interaction between bromeliads nutrition and the behavior of the aquatic ecosystem in their tanks [23,49,51]. Tank

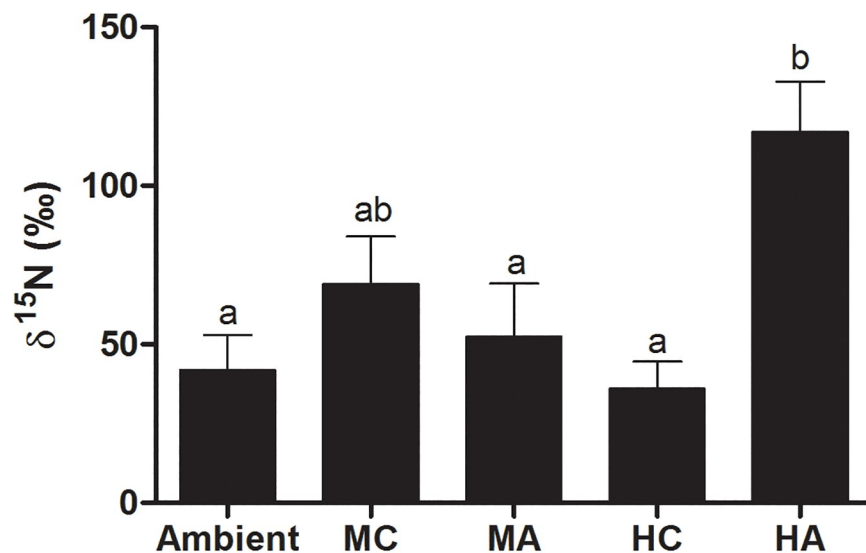




**Fig 3. Effects of rainfall scenarios on the frequency of algae-dominated tank bromeliads ecosystems.** Algae-dominated ecosystems were characterized as those contained more than  $80 \mu\text{g L}^{-1}$ . An asterisk (\*) means significant differences from *Ambient* rainfall scenario ( $P < 0.05$ ). Rainfall scenarios—*Ambient*, Medium clustering (*MC*), Medium amplitude (*MA*), High clustering (*HC*) and High amplitude (*HA*) rainfall scenarios,  $n = 35$ . For statistical details see [Table 1](#).

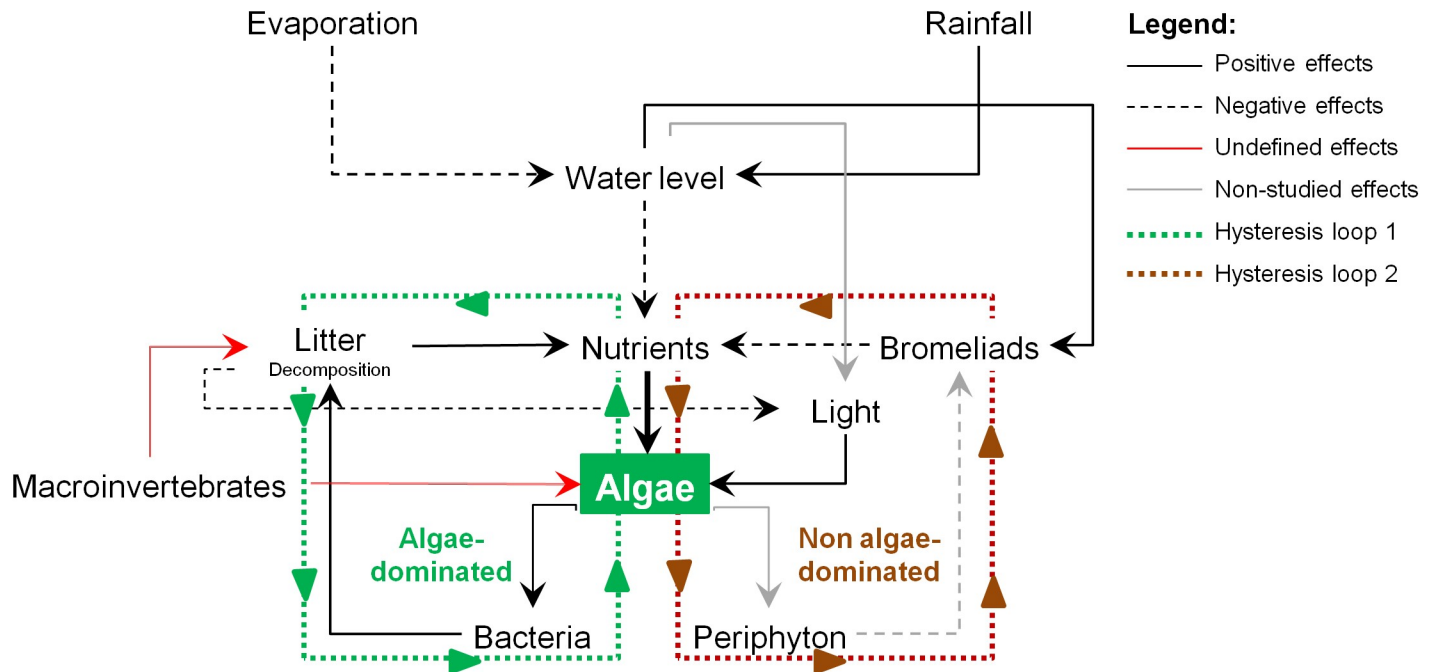
<https://doi.org/10.1371/journal.pone.0175436.g003>

bromeliads shelter numerous microorganisms, algae and metazoan, and have to compete with them for the available nutrients present in the water [50–52]. Since the highest rainfall



**Fig 4.  $\delta^{15}\text{N}$  of *Neoregelia cruenta* leaves after receiving enriched leaves of *Eugenia uniflora* under the following distinct rainfall scenarios: *Ambient*, Medium clustering (*MC*), Medium amplitude (*MA*), High clustering (*HC*) and High amplitude (*HA*).** Bars indicate standard errors of means. Different small letters indicate statistical differences among treatments ( $P < 0.05$ ).

<https://doi.org/10.1371/journal.pone.0175436.g004>



**Fig 5. A conceptual model for alternative stable states in tank bromeliad ecosystems.** Arrows represent direct effects of a variable on the other: continuous line (positive effects); dashed line (negative effects). Red lines represent the undefined effect of macroinvertebrates communities that will depend on several communities' parameters, such as the number of trophic levels and community composition. Gray lines represent potential effects which were still not tested and should be incorporated in future studies. Lines with superimposed arrows demonstrate the hysteresis loops that determine the algae-dominated ecosystems (green loop) and non algae-dominated ecosystems (brown loop).

<https://doi.org/10.1371/journal.pone.0175436.g005>

amplitude favors bromeliad nutrition, bromeliads seem to be the better competitors for nutrients than the algae in this condition. Thus, we suggest that changes in the water level are a crucial regulating factor of the algal biomass in the bromeliads tank, which can be indirectly related to bromeliad size [26] or with rainfall dynamics, as summarized in our proposed conceptual model (Fig 5).

Otherwise, it is important to highlight that each rainfall scenario is described by changes in frequency, intensity and clustering of rain events. For example, in *HA* rainfall scenario, tank-bromeliads have received the greatest rainfall input (100 mm) which implies that at in a short term, these ecosystems presented the wetter conditions and as consequence the most suitable habitat for colonization at this moment. As time goes by the absence of rainfall creates a drought condition which may trigger changes in the whole ecosystem and decrease the occurrence of algae-dominated ecosystems. Thus, *Ambient* rainfall scenario creates a more stable wet condition without great changes in the water level in these ecosystems. In this way, the observed effects of rainfall scenarios reveal the overall balance of all conditions described by them.

Litter diversity did not affect the algae-dominance in tank bromeliads nor minimized the effects of rainfall changes, as we first hypothesized. However, our qualitative approach over time demonstrated that litter diversity may have a positive effect on algae-dominated conditions in the *Ambient* rainfall scenario (see [Supporting Information](#)). In *Ambient* conditions, litter did not prevent light entering the system as the greater amount of water in this scenario raised the water level. On the other hand, in altered rainfall scenarios, bromeliad systems might have a limited amount of water and might be covered by the litter for an extended period. Additionally, litter decomposition could affect the turbidity of the water and

consequently may also control light availability and the concentration of nutrients in the system (Fig 5). As a consequence, the high turbidity or the reduced light input promoted by litter in the tank will limit algal growth despite the availability of nutrients in the tank.

Based on the current experiment and on the mechanisms revealed by previous studies, we built a conceptual model to establish the potential applicability of the alternative stable states theory to tank bromeliads ecosystems. For that, we propose two self-reinforcing mechanisms, based on the current ecological knowledge for tank bromeliads, which could explain the maintenance of green and brown conditions in these systems. We suggest that bromeliads can play a comparable role as submersed macrophytes in shallow lake ecosystems [53] (Fig 5). Submerged macrophytes obtain their nutrients partially from the water, reducing the concentration of nutrients available for algae and microorganisms [23,49,51] and by doing so keeping the lake in a clear state. Bromeliads also take up nutrients from the water in their tank and thus also lower the available nutrients for algae and bacteria (Fig 5). Additionally, the presence of algae in the system may favor microbial activity [40]. This higher microbial activity increases the decomposition rate resulting in a higher release of nutrients which are then available for the algae. These feedbacks seem to reinforce the algae-dominated condition (hysteresis loop 1; Fig 5). Furthermore, periphyton may also have an essential role in regulating the ability of bromeliads taking up nutrients from the water. Periphyton grows on the surface of bromeliad leaves and takes up nutrients by itself, thus reducing the bromeliad ability to take up nutrients from their tank. However, the uptake of nutrients by bromeliads leaves may reduce the provision of labile algal dissolved organic carbon for periphyton. This would maintain the bromeliad leaf surface free of periphyton, allowing for continued removal of nutrients from the water column. These feedbacks may promote another self-reinforcing mechanism for the non algae-dominated condition (hysteresis loop 2; Fig 5). Finally, macroinvertebrates positively contribute to litter decomposition, having a positive effect on nutrient availability and likely in algae growth (Fig 5). However, filter feeding invertebrates may directly remove algae from the water column, but since these animals usually occur in low abundance in bromeliad ecosystems [16,48] it is very unlikely they largely affect algal biomass.

This is the first study that empirically demonstrates how changes in the rainfall and litter diversity may lead to shifts in algal conditions in tank bromeliad ecosystems. We propose some self-reinforcing feedback mechanisms (Fig 5) which make it plausible that those conditions might be alternative stable states. Previous studies attempted to apply the alternative stable state theory in natural microcosms, but they failed in proposing those self-reinforcing mechanisms [19,54]. We strongly suggest to further test the importance of the proposed feedback mechanisms in future studies. Finally, we conclude that changes in rainfall distribution might affect aquatic ecosystems by modifying its structure and the activity of the organisms therein which can trigger regime shifts or push environmental conditions closer to a tipping point.

## Supporting information

**S1 Appendix. Discussion about the temporal dynamic of the algae dominance in the tank bromeliads ecosystems,  $^{15}\text{N}$  enrichment of *Eugenia uniflora* leaves and tables with the statistic values of the analysis performed.**

(DOCX)

**S1 Fig. Effects of rainfall scenario and litter diversity on the frequency of algae dominance.** We reported the frequency of algae-dominated ecosystems by the percentage of ecosystems that were considered algae-dominated, chlorophyll-*a* values higher than  $80\ \mu\text{g L}^{-1}$ , in each litter diversity level for all rainfall scenarios. *Ambient*, Medium clustering (*MC*), Medium

amplitude (*MA*), High clustering (*HC*) and High amplitude (*HA*) rainfall scenarios are fully described in the main text. \* Non algae-dominated states found in the respective litter diversity and rainfall scenario. For statistical details see [Table 1](#) in the main text.

(TIF)

**S2 Fig. Frequency distribution of chlorophyll-*a* ( $\mu\text{g L}^{-1}$ ) in the water of *Neoregelia cruenta* tanks in *Ambient* rainfall scenario.** The lines depict the probability density described by the best fitting model ( $n = 35$ ).

(TIF)

## Acknowledgments

We are thankful to V. Dib for field assistance and V. F. Farjalla and two anonymous reviewers for comments on the manuscript. Financial support was provided by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Project 476926/2012-2), FAPERJ (Fundação de Apoio à Pesquisa do Estado do Rio de Janeiro, Project E-26/103316/2011) and CENPES/PETROBRAS (Project "Impact of Global Climate Changes on Tropical Continental Aquatic Ecosystems"). APFP and JSL are grateful to CNPq and CAPES for graduate scholarships. This paper is a contribution of the Brazilian Research Network on Global Climate Change, FINEP/Rede CLIMA Grant Number 01.13.0353–00.

## Author Contributions

**Conceptualization:** APFP JSL ETHMP.

**Data curation:** APFP.

**Formal analysis:** APFP ETHMP.

**Funding acquisition:** APFP JSL ETHMP.

**Investigation:** APFP JSL.

**Methodology:** APFP JSL.

**Project administration:** APFP.

**Resources:** APFP JSL.

**Software:** APFP.

**Supervision:** APFP.

**Validation:** APFP JSL ETHMP.

**Visualization:** APFP JSL ETHMP.

**Writing – original draft:** APFP JSL ETHMP.

**Writing – review & editing:** APFP JSL ETHMP.

## References

1. Scheffer M, Barrett S, Carpenter SR, Folke C, Green AJ, Holmgren M, et al. Creating a safe operating space for iconic ecosystems. *Science* (80-). 2015; 347: 1317–1319. <https://doi.org/10.1126/science.aaa3769> PMID: 25792318
2. Rockström J, Steffen W, Noone K, Persson Å, Chapin FS, Lambin E, et al. Planetary boundaries: Exploring the safe operating space for humanity. *Ecol Soc*. 2009; 14: 472–475.

3. Scheffer M, Straile D, Nes EH Van, Hosper H, Nov N. Climatic warming causes regime shifts in lake food webs. *Limnol Oceanogr.* 2008; 46: 1780–1783.
4. Gessner MO, Inchausti P, Persson L, Raffaelli DG, Giller PS. Biodiversity effects on ecosystem functioning: insights from aquatic systems. *Oikos.* 2004; 104: 419–422.
5. IPCC. Climate Change 2013—The Physical Science Basis [Internet]. Intergovernmental Panel on Climate Change, editor. Cambridge: Cambridge University Press; 2014.
6. Holmgren M, Scheffer M. El Niño as a window of opportunity for the restoration of degraded arid ecosystems. *Ecosystems.* 2001; 4: 151–159.
7. Scheffer M, Carpenter SR, Lenton TM, Bascompte J, Brock W, Dakos V, et al. Anticipating critical transitions. *Science (80-).* 2012; 338: 344–348. <https://doi.org/10.1126/science.1225244> PMID: 23087241
8. Scheffer M, Carpenter SR. Catastrophic regime shifts in ecosystems: Linking theory to observation. *Trends Ecol Evol.* 2003; 18: 648–656.
9. Green AJ, Alcorlo P, Peeters ET, Morris EP, Espinar JL, Bravo-Utrera MA, et al. Creating a safe operating space for wetlands in a changing climate. *Front Ecol Environ.* 2017; 1–9.
10. Folke C, Carpenter S, Walker B, Scheffer M, Elmqvist T, Gunderson L, et al. Regime shifts, resilience, and biodiversity in ecosystem management. *Annu Rev Ecol Evol Syst.* 2004; 35: 557–581.
11. Neutel AM, Heesterbeek JAP, van de Koppel J, Hoenderboom G, Vos A, Kaldeway C, et al. Reconciling complexity with stability in naturally assembling food webs. *Nature.* 2007; 449: 599–U11. <https://doi.org/10.1038/nature06154> PMID: 17914396
12. Cardinale BJ, Duffy JE, Gonzalez A, Hooper DU, Perrings C, Venail P, et al. Biodiversity loss and its impact on humanity. *Nature.* 2012; 486: 59–67. <https://doi.org/10.1038/nature11148> PMID: 22678280
13. Loreau M, de Mazancourt C. Biodiversity and ecosystem stability: A synthesis of underlying mechanisms. *Ecol Lett.* 2013; 16: 106–115. <https://doi.org/10.1111/ele.12073> PMID: 23346947
14. Yachi S, Loreau M. Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. *Proc Natl Acad Sci.* 1999; 96: 1463–1468. PMID: 9990046
15. Srivastava DS, Vellend M. Biodiversity-ecosystem functioning research: Is it relevant to conservation? *Annu Rev Ecol Evol Syst.* 2005; 36: 267–294.
16. Pires APF, Marino NAC, Srivastava DS, Farjalla VF. Predicted rainfall changes disrupt trophic interactions in a tropical aquatic ecosystem. *Ecology.* 2016; 97: 2750–2759. <https://doi.org/10.1002/ecy.1501> PMID: 27859129
17. Kominoski JS, Hoellein TJ, Kelly JJ, Pringle CM. Does mixing litter of different qualities alter stream microbial diversity and functioning on individual litter species? *Oikos.* 2009; 118: 457–463.
18. Sanpera-Calbet I, Lecerf A, Chauvet E. Leaf diversity influences in-stream litter decomposition through effects on shredders. *Freshw Biol.* 2009; 54: 1671–1682.
19. Sirota J, Baiser B, Gotelli NJ, Ellison AM. Organic-matter loading determines regime shifts and alternative states in an aquatic ecosystem. *Proc Natl Acad Sci USA.* 2013; 110: 7742–7747. <https://doi.org/10.1073/pnas.1221037110> PMID: 23613583
20. Srivastava DS, Kolasa J, Bengtsson J, Gonzalez A, Lawler SP, Miller TE, et al. Are natural microcosms useful model systems for ecology? *Trends Ecol Evol.* 2004; 19: 379–384. <https://doi.org/10.1016/j.tree.2004.04.010> PMID: 16701289
21. Farjalla VF, Srivastava DS, Marino NAC, Azevedo FD, Dib V, Lopes PM, et al. Ecological determinism increases with organism size. *Ecology.* 2012; 93: 1752–1759. PMID: 22919920
22. Romero GQ, Srivastava DS. Food-web composition affects cross-ecosystem interactions and subsidies. *J Anim Ecol.* 2010; 79: 1122–1131. <https://doi.org/10.1111/j.1365-2656.2010.01716.x> PMID: 20584097
23. Gonçalves AZ, Oliveira RS, Oliveira PS, Romero GQ. Species-Specific Effects of Ant Inhabitants on Bromeliad Nutrition. *PLoS One.* 2016; 11: e0152113. <https://doi.org/10.1371/journal.pone.0152113> PMID: 27002980
24. Farjalla VF, González AL, Céréghino R, Dézerald O, Marino NAC, Piccoli GCO, et al. Terrestrial support of aquatic food webs depends on light inputs: a geographically-replicated test using tank bromeliads. *Ecology.* 2016; 97: 2147–2156. <https://doi.org/10.1002/ecy.1432> PMID: 27859200
25. Srivastava DS, Cardinale BJ, Downing AL, Duffy JE, Jouseau C, Sankaran M, et al. Diversity has stronger top-down than bottom-up effects on decomposition. *Ecology.* 2009; 90: 1073–1083. PMID: 19449701
26. Marino NAC, Guariento RD, Dib V, Azevedo FD, Farjalla VF. Habitat size determine algae biomass in tank-bromeliads. *Hydrobiologia.* 2011; 678: 191–199.
27. Brouard O, Le Jeune AH, Leroy C, Cereghino R, Roux O, Pelozuelo L, et al. Are algae relevant to the detritus-based food web in tank-bromeliads? *PLoS One.* 2011; 6: 1–10.

28. Carrias JF, Céregino R, Brouard O, Pélozuelo L, Dejean A, Couté A, et al. Two coexisting tank bromeliads host distinct algal communities on a tropical inselberg. *Plant Biol*. 2014; 16: 997–1004. <https://doi.org/10.1111/plb.12139> PMID: 24400863
29. Lopez LCS, Da Nóbrega Alves RR, Rios RI. Micro-environmental factors and the endemism of bromeliad aquatic fauna. *Hydrobiologia*. 2009; 625: 151–156.
30. Marino NAC, Srivastava DS, MacDonald AAM, Leal JS, Campos ABA, Farjalla VF. Rainfall and hydrological stability alter the impact of top predators on food web structure and function. *Glob Chang Biol*. 2017; 23: 673–685. <https://doi.org/10.1111/gcb.13399> PMID: 27344007
31. Benzing DH. *Bromeliaceae: profile of an adaptative radiation*. Cambridge: Cambridge University Press; 2000.
32. Goncalves AZ, Mercier H, Mazzafera P, Romero GQ. Spider-fed bromeliads: seasonal and interspecific variation in plant performance. *Ann Bot*. 2011; 107: 1047–1055. <https://doi.org/10.1093/aob/mcr047> PMID: 21385776
33. Leroy C, Carrias JF, Céregino R, Corbara B. The contribution of microorganisms and metazoans to mineral nutrition in bromeliads. *J Plant Ecol*. 2016; 9: 241–255.
34. Marengo JA, Ambrizzi T, da Rocha RP, Alves LM, Cuadra S V., Valverde MC, et al. Future change of climate in South America in the late twenty-first century: intercomparison of scenarios from three regional climate models. *Clim Dyn*. 2010; 35: 1073–1097.
35. Nobre CA, Marengo JA, Seluchi ME, Cuartas LA, Alves LM. Some characteristics and impacts of the drought and water crisis in Southeastern Brazil during 2014 and 2015. *J Water Resour Prot*. 2016; 8: 252–262.
36. Zotz G, Thomas V. How much water is in the tank? Model calculations for two epiphytic bromeliads. *Ann Bot*. 1999; 83: 183–192.
37. Petermann JS, Farjalla V, Jocque M, Kratina P, MacDonald AA, Marino NAC, et al. Dominant predators mediate the impact of habitat size on trophic structure in bromeliad invertebrate communities. *Ecology*. 2015; 96: 428–439. PMID: 26240864
38. Nush EA, Palme G. *Biologische methoden fur die Praxis der Gewässeruntersuchung*. GWF und Wasser/Abwasser. 1975; 116: 562–565.
39. Lehours A-C, Jeune A-H Le, Aguer J-P, Céregino R, Corbara B, Kéralval B, et al. Unexpectedly high bacteriochlorophyll a concentrations in neotropical tank bromeliads. *Environ Microbiol Rep*. 2016; 8: 689–698.
40. Haubrich CS, Pires APF, Esteves F a., Farjalla VF. Bottom-up regulation of bacterial growth in tropical phytotelm bromeliads. *Hydrobiologia*. 2009; 632: 347–353.
41. Jeppesen E, Jensen J, Søndergaard M, Lauridsen T. Trophic dynamics in turbid and clearwater lakes with special emphasis on the role of zooplankton for water clarity. *Hydrobiologia*. 1999; 408/409: 217–231.
42. Koppel J Van De, Herman PMJ, Thoolen P, Heip CHR. Do Alternate Stable States occur in natural ecosystems? Evidence from a tidal flat. *Ecology*. 2001; 82: 3449–3461.
43. Norlin JI, Bayley SE, Ross LCM. Submerged macrophytes, zooplankton and the predominance of low-over high-chlorophyll states in western boreal, shallow-water wetlands. *Freshw Biol*. 2005; 50: 868–881.
44. Kosten S, Vernouij M, Van Nes EH, Sagrario M de LÁG, Clevers JGPW, Scheffer M. Bimodal transparency as an indicator for alternative states in South American lakes. *Freshw Biol*. 2012; 57: 1191–1201.
45. Strome DJ, Miller MC. Photolytic changes in dissolved humic substances. *Verhandlungen Int Vereingung für Theor und Angew Limnol*. 1978; 20: 1248–1254.
46. Mackereth FJH, Heron J, Talling JF. *Water analysis: some revised methods for limnologists*. 36th ed. Cumbria and Dorset; 1978.
47. R Development Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing; 2015.
48. Marino NAC, Srivastava DS, Farjalla VF. Aquatic macroinvertebrate community composition in tank-bromeliads is determined by bromeliad species and its constrained characteristics. *Insect Conserv Divers*. 2013; 6: 372–380.
49. Romero GQ, Nomura F, Gonçalves AZ, Dias NYN, Mercier H, Conforto E de C, et al. Nitrogen fluxes from treefrogs to tank epiphytic bromeliads: An isotopic and physiological approach. *Oecologia*. 2010; 162: 941–949. <https://doi.org/10.1007/s00442-009-1533-4> PMID: 20024585
50. Gonçalves AZ, Hoffmann FL, Mercier H, Mazzafera P, Romero GQ. Phyllosphere bacteria improve animal contribution to plant nutrition. *Biotropica*. 2014; 46: 170–174.

51. Ngai JT, Srivastava DS. Predators accelerate nutrient cycling in a bromeliad ecosystem. *Science* (80-). 2006; 314: 2006–2006.
52. Inselsbacher E, Cambui CA, Richter A, Stange CF, Mercier H, Wanek W. Microbial activities and foliar uptake of nitrogen in the epiphytic bromeliad *Vriesea gigantea*. *New Phytol.* 2007; 175: 311–320. <https://doi.org/10.1111/j.1469-8137.2007.02098.x> PMID: 17587379
53. Scheffer M, Hosper S, Meijer M, Moss B, Jeppesen E. Alternative equilibria in shallow lakes. *Trends Ecol Evol.* 1993; 8: 275–279. [https://doi.org/10.1016/0169-5347\(93\)90254-M](https://doi.org/10.1016/0169-5347(93)90254-M) PMID: 21236168
54. Butler JL, Gotelli NJ, Ellison AM. Linking the brown and green: nutrient transformation and fate in the *Sarracenia* microecosystem. *Ecology.* 2008; 89: 898–904. PMID: 18481512