Received: 11 September 2023 | Revised: 3 January 2024 | Accepted: 8 January 2024

DOI: 10.1111/ele.14372

LETTER

ECOLOGY LETTERS cnrs WILEY

Skin microbiome disturbance linked to drought-associated amphibian disease

Shannon Buttimer[1,2] | **Diego Moura-Campos**[3,4] | **Sasha E. Greenspan**[5] | **Wesley J. Neely**[5,6] | **Lucas Ferrante**[7] | **Luís Felipe Toledo**[4] | **C. Guilherme Becker**[1,2]

[1]Department of Biology, The Pennsylvania State University, University Park, Pennsylvania, USA

[2]One Health Microbiome Center, Center for Infectious Disease Dynamics, Ecology Institute, Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, Pennsylvania, USA

[3]Division of Ecology and Evolution, Research School of Biology, The Australian National University, Canberra, Australian Capital Territory, Australia

[4]Departamento de Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil

[5]Department of Biological Sciences, The University of Alabama, Tuscaloosa, Alabama, USA

[6]Department of Biology, Texas State University, San Marcos, Texas, USA

[7]Universidade Federal do Amazonas, Manaus, Amazonas, Brazil

Correspondence
Shannon Buttimer, 606 Mueller Laboratory, The Pennsylvania State University, University Park, PA 16802, USA.
Email: shannon.buttimer@gmail.com

Funding information
National Science Foundation, Grant/Award Number: DEB 2227340 and IOS 2303908

Editor: Barbara A Han

Abstract
The onset of global climate change has led to abnormal rainfall patterns, disrupting associations between wildlife and their symbiotic microorganisms. We monitored a population of pumpkin toadlets and their skin bacteria in the Brazilian Atlantic Forest during a drought. Given the recognized ability of some amphibian skin bacteria to inhibit the widespread fungal pathogen *Batrachochytrium dendrobatidis* (Bd), we investigated links between skin microbiome health, susceptibility to Bd and host mortality during a die-off event. We found that rainfall deficit was an indirect predictor of Bd loads through microbiome disruption, while its direct effect on Bd was weak. The microbiome was characterized by fewer putative Bd-inhibitory bacteria following the drought, which points to a one-month lagged effect of drought on the microbiome that may have increased toadlet susceptibility to Bd. Our study underscores the capacity of rainfall variability to disturb complex host–microbiome interactions and alter wildlife disease dynamics.

KEYWORDS
amphibian, bacteria, chytridiomycosis, climate change, climate variability, disease, drought, microbiome, symbiosis, tropical ecology

INTRODUCTION

Rainfall anomalies are predicted to become more frequent and intense even under conservative climate change scenarios (Pörtner et al., 2022). Increased variability in precipitation and temperature is expected to affect species differently and may lead to disrupted relationships between previously mutualistic organisms (Bénard et al., 2020; Iltis et al., 2022). Behavioural and physiological changes in both hosts and symbionts in

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Shannon Buttimer and Diego Moura-Campos co-first authors.

response to climatic anomalies can lead to cascading effects that result in disrupted community dynamics (Hentschel, 2021; Hom & Penn, 2021; Keeler et al., 2021; Six, 2009). The context dependency of host–symbiont relationships can have equivalently profound effects on disease resistance (Hector et al., 2022). Disease resistance can be impacted by changes in host-associated symbiont communities both directly through competition or production of pathogen-inhibitory compounds (Brucker et al., 2008; Woodhams et al., 2015) and indirectly through mediation of host immune responses (Ford & King, 2016; Fuess et al., 2020). Disruptions to symbiotic associations can be caused by the exclusion or reduction of particular microbial taxa with anti-pathogen properties or measured through changes in compositional variance at the community level, which can indicate that symbiotic communities are in a state of dysbiosis (Ahmed et al., 2019; Zaneveld et al., 2017).

The Anna Karenina principle (AKP) is the idea that increased dispersion, or higher deviation from the average community composition, may indicate a diseased, less-functional state (i.e., dysbiosis). The term stems from Tolstoy's opening line of *Anna Karenina*, "Happy families are alike; each unhappy family is unhappy in its own way." The concept is gaining traction in microbiome research, where it describes how stressors tend to have destabilizing effects on host-associated microbiomes (Arnault et al., 2023; Zaneveld et al., 2017). Ma (2020) showed that about 50% of human microbiome-associated diseases exhibit AKP effects. AKP effects have been detected in many wildlife-bacterial systems and are linked to multiple types of stressors. Posadas et al. (2022) showed that temperature-sensitive sponge species had variable bacterial communities that became even more stochastically dispersed following experimental warming. In frogs, Jiménez et al. (2020) and Neely et al. (2021) detected increased skin microbiome dispersion in hosts living in disturbed habitats when compared to those living in less disturbed ones. Hernández-Gómez et al. (2020) also found higher dispersion of salamander skin microbiomes in forests dominated by invasive vegetation.

Amphibians and their skin bacterial communities (i.e., a major component of their microbiome) are an especially important and well-studied host–microbiome system. This is primarily attributed to the essential role the microbiome plays in protecting amphibians against the globally distributed waterborne fungal pathogen *Batrachochytrium dendrobatidis* (Bd; Bates et al., 2018; Rebollar et al., 2020; Chen et al., 2022). A large number of amphibian-associated bacteria have been identified as Bd-inhibitory in vitro (Woodhams et al., 2015). Bd is a directly transmitted aquatic fungal pathogen that has been one of the main drivers of amphibian population declines worldwide (Fisher & Garner, 2020). Currently, over 40% of amphibians are classified as threatened with extinction, which is the highest proportion for any class of vertebrate (IUCN, 2022). Anuran die-offs and extirpations linked to Bd in North and South America have been correlated with climatic anomalies, including droughts, highlighting the need to investigate how rainfall anomalies may interact with amphibian defences, including the skin microbiome (Adams et al., 2017; Briggs et al., 2010; Jiménez & Sommer, 2017; Kupferberg et al., 2021; Moura-Campos et al., 2021; Rollins-Smith, 2017). Buttimer et al. (2021) found that the composition of skin bacterial communities on sympatric salamanders correlated with mean annual precipitation. Changes in both rainfall and temperature have also been associated with significant shifts in the microbiome (Greenspan et al., 2020; Varela et al., 2018; Woodhams et al., 2020) and have even been linked to survival after Bd infection (Longo & Zamudio, 2017; Neely et al., 2020). It is less clear, however, how local climatic variability, or deviations from historical averages to which endemic taxa are adapted, might influence the interactions between the amphibian skin microbiome function and Bd infection dynamics.

The Atlantic Forest in Brazil is a hotspot of amphibian diversity, home to over 500 species, nearly half of which are endemic (Haddad et al., 2013; Toledo et al., 2021). Bd loads and prevalence vary seasonally, where both tend to rise during the wet winter (Ruggeri et al., 2018). The Atlantic Forest within the state of São Paulo has encountered an escalation in rainfall variability associated with historical deforestation (Webb et al., 2006). This deforestation is linked to an increase in consecutive dry days, as well as an increase in the number of days with rainfall exceeding 100 mm (Marengo et al., 2020; Webb et al., 2005, 2006). The Amazon rainforest also plays a significant role in providing rainfall to the Atlantic Forest through the action of atmospheric rivers and is nearing the threshold of deforestation that could lead to the collapse of this ecosystem service at a continental scale (Lovejoy & Nobre, 2018). Thus, rainfall variability in the Atlantic Forest has been driven by both local and continental-scale climate change, with detrimental impacts on amphibian biodiversity (Ferrante et al., 2023; Rebouças et al., 2021).

A large fraction of neotropical frog biodiversity is composed of direct-developing amphibian species (i.e., species that lack an aquatic larval life stage) which are dependent on high soil moisture levels. Direct-developers can still become infected with Bd through contact with infected frogs or moist surfaces (Becker et al., 2019; Burns et al., 2020; Kolby et al., 2015). One leading hypothesis is that drought might cause amphibians to cluster in wet microhabitats, increasing disease transmission to direct-developing species during periods of low rainfall (Burrowes et al., 2004; Longo et al., 2010), but this mechanism may be confounded by species-specific factors. Martins et al. (2022) found that the Bd-resistant direct-developing species *Haddadus binotatus* has a significantly more diverse and less variable microbiome compared to sympatric susceptible species, indicating that microbiome diversity and stability could be especially important factors for the survival of direct-developers (Greenspan et al., 2022; Piovia-Scott et al., 2017).

The micro-endemic, direct-developing pumpkin toadlet species *Brachycephalus rotenbergae* (Nunes et al., 2021) has been extirpated from parts of its historic Atlantic Forest range without a clear culprit (Toledo et al., 2023). More recently, Moura-Campos et al. (2021) recorded high Bd loads leading to a die-off of these toadlets that was associated with a short-term drought in São Paulo state. However, the mechanism driving the observed disease outbreak remained unclear. In the present study, we tested whether the observed drought event was a significant predictor of disturbances to the amphibian skin microbiome (i.e., AKP effects), and whether microbiome dysbiosis could be a candidate mechanism to explain increased Bd infections and mortality observed in pumpkin toadlets. Using a growing reference sequence dataset of amphibian skin bacteria that have been found to posess Bd-inhibitory properties through challenge assays (Woodhams et al., 2015), we tested for signature differences in the skin bacterial communities of individual *B. rotenbergae* before, during and after the previously documented mortality event. If microbiome dysbiosis is indeed a driving mechanism of disease, we predicted that a decrease in rainfall below historical levels would also lead to a reduction in bacterial taxa within the microbiome that have the capacity to inhibit the growth of Bd.

MATERIALS AND METHODS

Study site

Serra do Japi is a large remnant of continuous rainforest in the Atlantic Forest in the State of São Paulo and is surrounded by vast areas deeply impacted by heavy anthropogenic land use for agriculture and urbanization. The region has two well-defined seasons: a cool/dry season during the months of April—September (median temp range: 16–19°C, median monthly precipitation range: 25–75 mm) and a warm/wet season during October—March (median temp range: 21–22°C, median monthly precipitation range: 125–300 mm). Rainfall during the height of the drought period (December 2018) was about 100 mm, which is less than 50% of the expected average for that month over the past 60 years (Figure S1). The amphibian community at Serra do Japi is comprised of 31 species, including three genera of direct-developers: *Ischnocnema*, *Haddadus* and *Brachycephalus* (Ribeiro et al., 2005). These genera share microhabitats with several aquatic-breeding species which could act as sources of Bd infection (Becker et al., 2019).

Fieldwork

We established 25 sampling areas (720 m^2 each) that were at least 100 m apart. Twelve sampling areas were close to streams, while 13 were at least 70 m distant from streams. We conducted five 20-day field campaigns from June 2018 to February 2019. During each field campaign, we actively searched for toadlets in the forest leaf litter for 1 h in each sampling area. We captured animals using clean plastic bags and then rinsed them with 1 mL of distilled water to remove debris and transient bacteria (Culp et al., 2007). We wore nitrile gloves and swabbed the toadlets following standard procedures (Hyatt et al., 2007). Samples were stored on ice while in the field and then transferred to −20°C until DNA extraction.

We measured temperature using HOBO Pendant™ data loggers (Onset) placed in the centre of each sampling area. The loggers recorded temperature every 6 h across the entire sampling period. We calculated the median daily temperature, maximum and minimum temperature for the previous 3, 7, 30 and 60 days. We obtained rainfall data from a rain gauge located 3 km from the biological reserve and calculated rainfall deviation metrics based on the average accumulated rainfall for each month using a 59-year dataset from the area (Abatzoglou et al., 2018). To calculate the lagged rainfall deviation metric, we subtracted the accumulated rainfall for each month from the expected rainfall for that month. Negative deviation values indicate lower-than-expected rainfall (deficit) and positive values indicate higher-than-expected rainfall (excess) for that month.

Laboratory

We extracted DNA from swabs using Qiagen DNAeasy Blood and Tissue kits following the manufacturer's protocol with an extended overnight incubation period after the addition of proteinase K. Using quantitative polymerase chain reaction (qPCR), we quantified Bd loads following standard protocols (Boyle et al., 2004) with synthetic gBlock™ standards (Integrative DNA Technologies) ranging from 10^2 to 10^6 zoospore genomic equivalents. We ran samples in duplicate and considered them to be positive if both duplicates were positive. We ran mismatched samples in triplicate. We averaged Bd loads between duplicates and log-transformed loads to correct for non-normal distributions of residuals.

To identify prokaryotes present in our samples, we used PCR to amplify the V4 region of the 16S rRNA gene using a dual-index approach (Kozich et al., 2013). We amplified DNA in duplicate, using the following recipe for each sample: 12.2 μL of ultrapure water, 4 μL of 5X Phire Reaction Buffer (Thermo Scientific), 0.4 μL of 2.5 mM dNTPs (Invitrogen), 0.4 μL of Phire Hot Start II DNA Polymerase (Thermo Scientific), 0.5 μL each of 10 μM barcoded forward and reverse primers (Integrated DNA Technologies) and 2 μL of sample DNA. We ran duplicate PCR plates on SimpliAmp thermal cyclers (Thermo Scientific) according to the following protocol: 98°C for 3 min, 38 cycles of 98°C for 5 s, 50°C for 5 s and 72°C for

15 s, then 72°C for 3 min before holding at 12°C. We added negative controls (PCR reagents without template DNA) to the plates to account for any contamination during PCR preparation. We then combined the duplicate plates and visualized the amplicons in 1% agarose gel to estimate DNA concentration in each sample and pooled them in quasi-equimolar amounts (2 μL with strong band intensity, 4 μL with normal band intensity and 6 μL with weak band intensity; Anahtar et al., 2016). We purified the library using the QIAquick Gel Extraction Kit (Qiagen) and sequenced the 16S library using an Illumina MiSeq at Tufts University Core Facility, Boston, MA, USA.

Bioinformatics

We used Quantitative Insights into Microbial Ecology 2 (QIIME2) v2021.2 to join forward and reverse reads and assign reads to sub-OTUs (sOTUs) using the Deblur workflow using default parameters (Amir et al., 2017). We then assigned taxonomy to the sOTUs and exported a phylogenetic tree using the Greengenes database (Bolyen et al., 2019; DeSantis et al., 2006) and removed chloroplast and mitochondrial sequences. To decontaminate sequences, we used the R package *microDecon* which uses proportions of contaminant sOTUs in negative control samples to remove reads identified as contaminants and has been shown to have comparatively high accuracy for identifying contaminant reads (McKnight et al. 2019). We rarefied sequences at a depth of 1300 reads based on the rarefaction curves produced in QIIME 2 (Figure S2). The rarefaction depth is sufficient, yet expectedly low due to the minute physical size of the organism swabbed (Becker et al., 2019). Finally, we calculated sOTU richness and Shannon diversity for each sample in QIIME2. We performed all other downstream analyses in R version 4.2.1 (R Development Core Team, 2022).

Statistical analysis

To identify core microbes (sOTUs present in 80% of samples), we used the R package *microbiome* (Lahti & Shetty, 2023). To determine which sOTUs best-characterized sampling dates with either rainfall deficit or excess (1-month lag), we employed LEfSe (Linear Discriminant Analysis (LDA) Effect Size) analysis using the R package *microbiomeMarker* (Cao, 2023). We narrowed down discriminative sOTUs by using an LDA score (effect size) cutoff of 3 and Kruskal–Wallis test and Wilcoxon test cut-offs of $p < 0.01$.

Using the AmphiBac database containing 16S rRNA sequences of amphibian skin bacteria identified as Bd-inhibitory based on challenge assays that measure the growth of live Bd when grown in conjunction with bacterial metabolites (Woodhams et al., 2015), we checked for matches of our differentially abundant sOTU sequences at 100% similarity. High sequence similarity does not prove that an sOTU is Bd-inhibitory, but suggests it may share similar functional traits as cultured isolates (Muletz-Wolz et al., 2017). We used Fisher's exact test to check for the differences between the proportions of putative Bd-inhibitory sOTUs between excess and deficit rainfall groups.

We ran two generalized linear mixed models (GLMMs) to predict alpha diversity metrics (richness and Shannon diversity) using the R package *glmmTMB*, including an AR1 term to control for temporal autocorrelation and controlling for sampling area by including it as a random effect (Brooks et al., 2022). Our independent variables for both models included: distance to stream, maximum daily temperature, minimum daily temperature, median daily temperature, rainfall deficit (1-month lag), log Bd load (all Bd loads are log-transformed), and an interactive effect between Bd load and rainfall deficit (Moura-Campos et al., 2021). We used AICc for model selection and removed non-significant ($p > 0.05$) variables sequentially, starting with the highest *p*-values, until the ΔAICc dropped by less than 2 points (Burnham & Anderson, 2004; Hocking, 1976). We visually checked residuals for normality.

We used the *phyloseq* package to calculate Jaccard distances between host bacterial samples (McMurdie & Holmes, 2013). We chose to use a presence/absence method over one based on relative abundance so that we would not dilute the effects of potentially important rare sOTUs (Bletz et al., 2013). Using the *vegan* package, we also calculated Jaccard dispersion (distance from the group centroid) for each sample within each month and compared differences in dispersion between months with the Tukey honest significant difference (Tukey HSD) test (Oksanen et al., 2020). To test for possible drivers of differences in microbiome composition, we ran PERMANOVAs using the adonis2 function in *vegan*, permuted by sampling area. To visualize differences in bacterial community composition between samples, we ordinated using a Principal Components Analysis (PCoA).

To construct structural equation models (SEMs), we used the R package *piecewiseSEM* using generalized least squares equations with corCAR1 temporal autocorrelation structure and blocked by sampling area (Lefcheck et al., 2020). Our starting variables included: distance to stream, maximum daily temperature, minimum daily temperature, median daily temperature, rainfall deficit (1-month lag), rainfall deficit (2-month lag), log Bd load and an interactive effect between Bd load and rainfall deficit (Moura-Campos et al., 2021). We dropped variables from the model to minimize the AICc as we did for the GLMMs (package *MuMIn*; Bartoń, 2020).

To deepen our understanding of regional climate dynamics influenced by continental-scale climatic cycles, we conducted targeted teleconnection analyses as a corroborative method to delineate the origins of rainfall specifically at our research site in Serra do Japi during both the wet and dry seasons (Supporting Methods). Our objective was to highlight and substantiate, through site-specific teleconnection results, the significant moisture inflow from the Amazon to Serra do Japi. These findings, coupled with a growing body of climatological studies (Marengo et al., 2018; Salati et al., 1979; Salati & Vose, 1984; Zemp et al., 2014), strengthen our discussion concerning the potential ramifications of accelerated Amazon deforestation for exacerbating droughts within the Atlantic Forest (Marengo et al., 2018; Webb et al., 2005, 2006) and their cascading adverse effects on amphibians (Ferrante et al., 2023).

RESULTS

A total of nine dead (*n*=4) and dying (*n*=5) *B. rotenbergae* were found during the sampling campaign as previously reported by Moura-Campos et al. (2021). All dead and dying frogs had Bd loads above 200,000 genomic equivalents and seven of the nine showed clinical signs of chytridiomycosis, including skin sloughing, emaciation and lethargy. Five of these skin microbiome swab samples were retained after 16S data normalization through rarefaction.

We detected 6988 unique sOTUs across all 237 skin swab samples. Only two core sOTUs were found in over 80% of samples: *Stenotrophomonas maltophilia* and *Bradyrhizobium* sp. Interestingly, both were detected through LEfSe analysis as differentially abundant between rainfall deficit and excess groups: *Stenotrophomonas maltophilia* was more abundant in samples collected during excess rainfall, while the *Bradyrhizobium* sp. was more abundant following low rainfall (Figure 1a). This indicates that the abundance of these bacteria, which are commonly present on *Brachycephalus rotenbergae*, may depend on rainfall variability. In total, we identified six

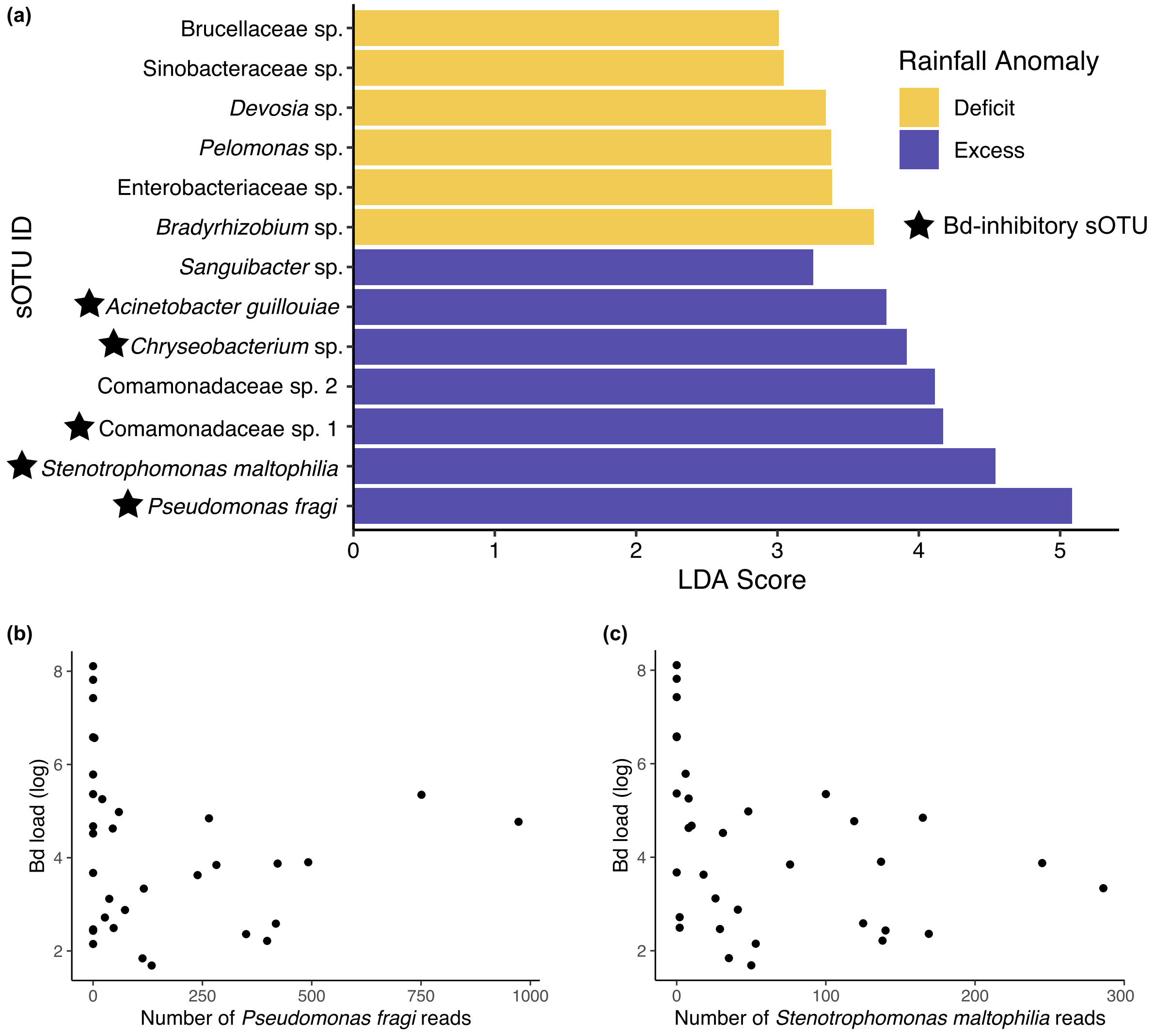

FIGURE 1 (a) Results of LEfSe (LDA Effect Size) analysis. Bacteria with yellow bars are associated with samples taken during rainfall deficit, while bacteria with purple bars are associated with rainfall excess (1-month lag). Stars indicate sOTUs that matched with putative Bd-inhibitory bacterial isolates. Bacteria without stars have unknown Bd-inhibitory function. The relationship between Bd loads of infected frogs and (b) the number of *Pseudomonas fragi* reads and (c) the number of *Stenotrophomonas maltophilia* reads.

sOTUs that were more abundant with rainfall deficit and seven sOTUs that were more abundant with excess rainfall (Figure 1a). Strikingly, none of the six sOTUs associated with rainfall deficit matched isolates found in the Bd-inhibitory database, whereas five of the seven sOTUs associated with excess rainfall matched isolates with putative Bd-inhibitory function. The two sOTUs with the greatest effect sizes showed an inverse relationship between their relative abundance and Bd loads of infected frogs (Figure 1b,c). The excess rainfall group had significantly more known Bd-inhibitory sOTUs than the deficit group (Fisher's exact test, $p=0.021$).

In our first generalized linear mixed model, sOTU richness was predicted by Bd load ($\beta=-17.482$, $p<0.001$). Distance to stream ($\beta=-21.008$, $p=0.218$) and 1-month lagged rainfall deviation ($\beta=-0.397$, $p=0.141$) were both retained in the final model (whole-model conditional $R^2=0.129$, Table S1). In the second model, Shannon diversity was predicted by both Bd loads ($\beta=-0.267$, $p<0.001$) and 1-month lagged rainfall deviation ($\beta=-0.008$, $p=0.038$). Distance to stream was retained in the model but was not a significant predictor ($\beta=-0.397$, $p=0.076$, whole-model conditional $R^2=0.113$, Table S1). In general, microbiome diversity was much lower in individual amphibians carrying the highest Bd infection loads (Figure 2).

Bacterial community composition varied with Bd load and rainfall deviation. According to our PERMANOVAs, Jaccard distances were related to Bd load ($r^2=0.009$, $F_{1,237}=2.244$, $p=0.001$, Figure 3a) and rainfall deviation ($r^2=0.007$, $F_{1,237}=1.655$, $p=0.001$, Figure 3b). Sick or dying individuals detected after the observed drought showed elevated microbiome dispersion (Figure 3c). We also detected Bd-negative individuals with high microbiome dispersion during the post-drought period in February (Figure 3c).

Our structural equation models (SEMs) revealed that rainfall deviation did not have a significant direct effect on the Bd loads of *B. rotenbergae* when accounting for microbial dispersion, a proxy for microbiome dysbiosis (standardized estimate (β)=0.08, $p=0.34$; Figure 4). Bacterial richness was not significantly associated with rainfall ($\beta=0.14$, $p=0.07$), while higher bacterial richness was negatively correlated with Bd loads ($\beta=-0.25$, $p<0.01$; Figure 4). The first PCoA axis of bacterial community composition was negatively associated with higher rainfall ($\beta=-0.25$, $p<0.01$) but composition along Axis 1 was not significantly correlated with Bd loads ($\beta=0.02$, $p=0.36$; Figure 4). However, rainfall deficit (negative rainfall deviation) predicted high microbial dispersion ($\beta=0.28$, $p<0.01$), which was in turn correlated with high Bd loads ($\beta=0.35$, $p<0.01$; Figure 4).

DISCUSSION

Climatic anomalies in biodiversity hotspots can be detrimental to conservation efforts of endemic species, especially in synergy with infectious disease (Merselis et al., 2018; Neely et al., 2020; Rohr et al., 2013). While sampling the pumpkin toadlet *Brachycephalus rotenbergae*, we detected links between skin microbiome dysbiosis, loss of putative Bd-inhibitory bacteria and lethally high Bd infection loads that followed a period of lower-than-average rainfall. Regardless of infection status, skin microbiome dispersion (a potential indicator of dysbiosis) significantly increased one month after the drought. We also detected a decline in a core bacterium with Bd-inhibitory function following the dry period, pointing to the loss of beneficial symbionts as a possible

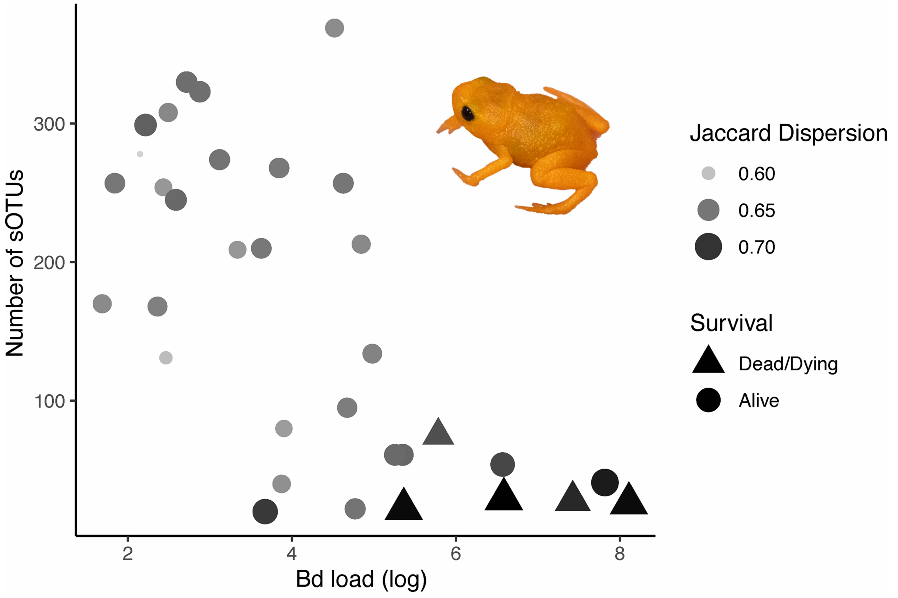

FIGURE 2 The richness of skin bacteria is negatively correlated with Bd loads. Colour and size correspond with Jaccard dispersion values. Shape indicates frog survival. Bd-negative samples have been excluded.

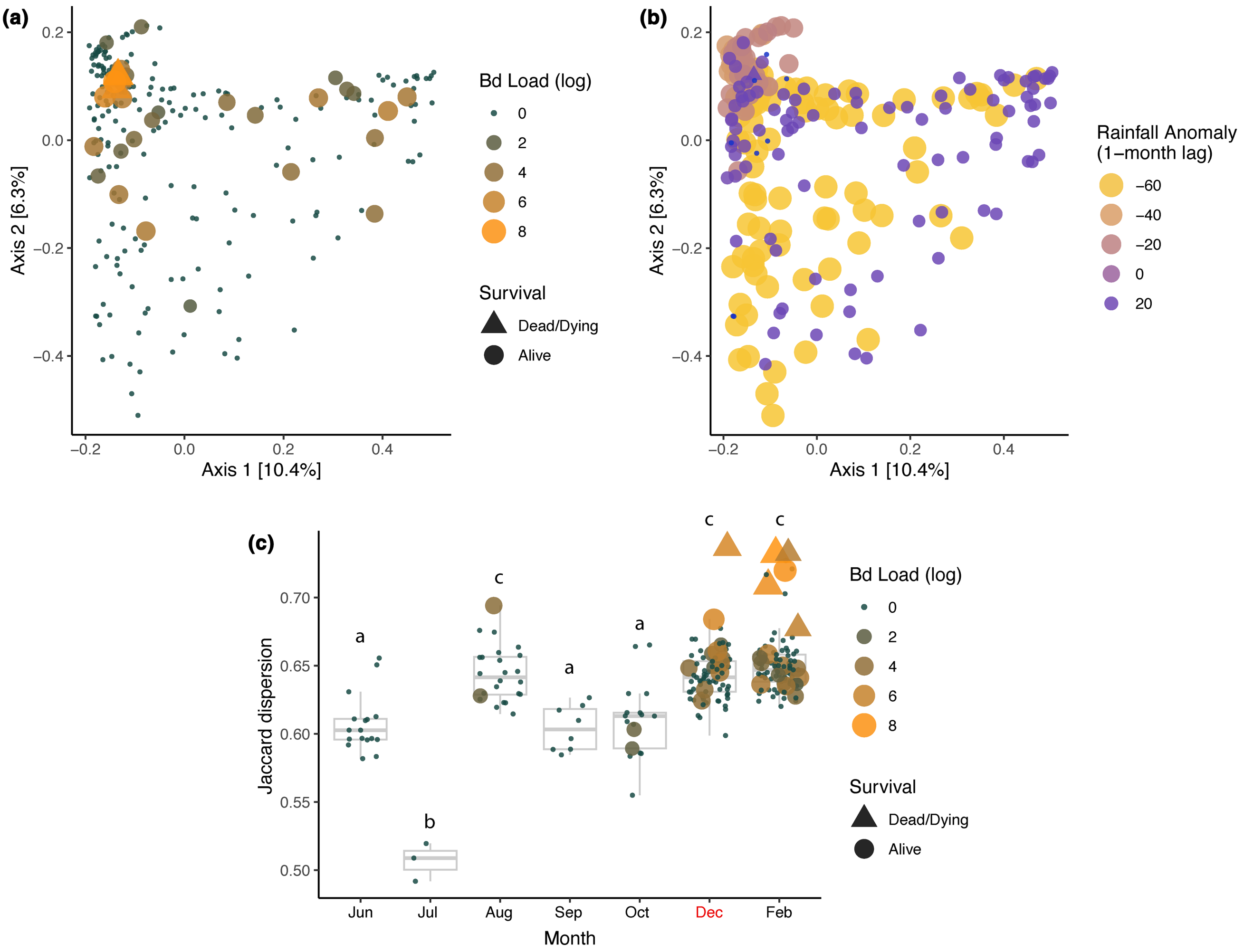

FIGURE 3 Principal component analysis plot of Jaccard distances coloured by (a) Bd load and (b) amount of rainfall deviation (1-month lag). (c) Differences in Jaccard dispersion of *Brachycephalus rotenbergae* microbiomes during each month. Higher dispersion values indicate more variable microbial communities between individuals. Letters indicate significantly different groups according to Tukey's test with a confidence level of 0.95. Unsampled months are not included. The drought was most severe in December, highlighted in red.

explanation for the disease cluster. Our structural equation model showed strong indirect effects of rainfall deficit on Bd loads through microbiome dispersion, surpassing the magnitude of the direct effect of rainfall deficit on Bd loads. This does not necessarily imply that microbiome dysbiosis is the sole mechanism explaining disease; however, the observed robust association between microbiome dispersion and Bd emphasizes the potential role of increased climate variability disrupting host microbiome health, a mechanism driving biodiversity declines that is easily overlooked.

In contrast to the Anna Karenina effects often seen in microbial systems, stressors like climate change or habitat loss can often lead to a convergence in the species composition of plant and animal communities. In both microbial and non-microbial systems, stressors affect community assembly by mediating niche selection, ecological drift and dispersal (Catano et al., 2017; Vellend et al., 2014). Because microbial communities tend to be more diverse and functionally redundant, they are more likely to contain multiple sOTUs that are able to persist in a niche following a disturbance, allowing for different community compositions with similar functions (Yachi & Loreau, 1999; Zhou & Ning, 2017). In our case, host-associated microbial communities diverged following drought but did not maintain certain members with putative Bd-inhibitory function. These divergent communities were not defined by any known Bd-inhibitory bacteria, whereas communities sampled following excess rainfall were characterized by five different putative Bd-inhibitory bacteria (Figure 1).

Though we cannot determine whether Bd caused increased microbial dispersion or whether Bd loads increased following microbiome destabilization, dispersion of uninfected frogs' microbiomes also increased following the drought, lending support to our hypothesis that the lack of rainfall led to higher microbial dispersion. This could be driven by a combination of factors, including shifts in the environmental pool of bacteria as well as changes in frog immune function, physiology and behaviour during

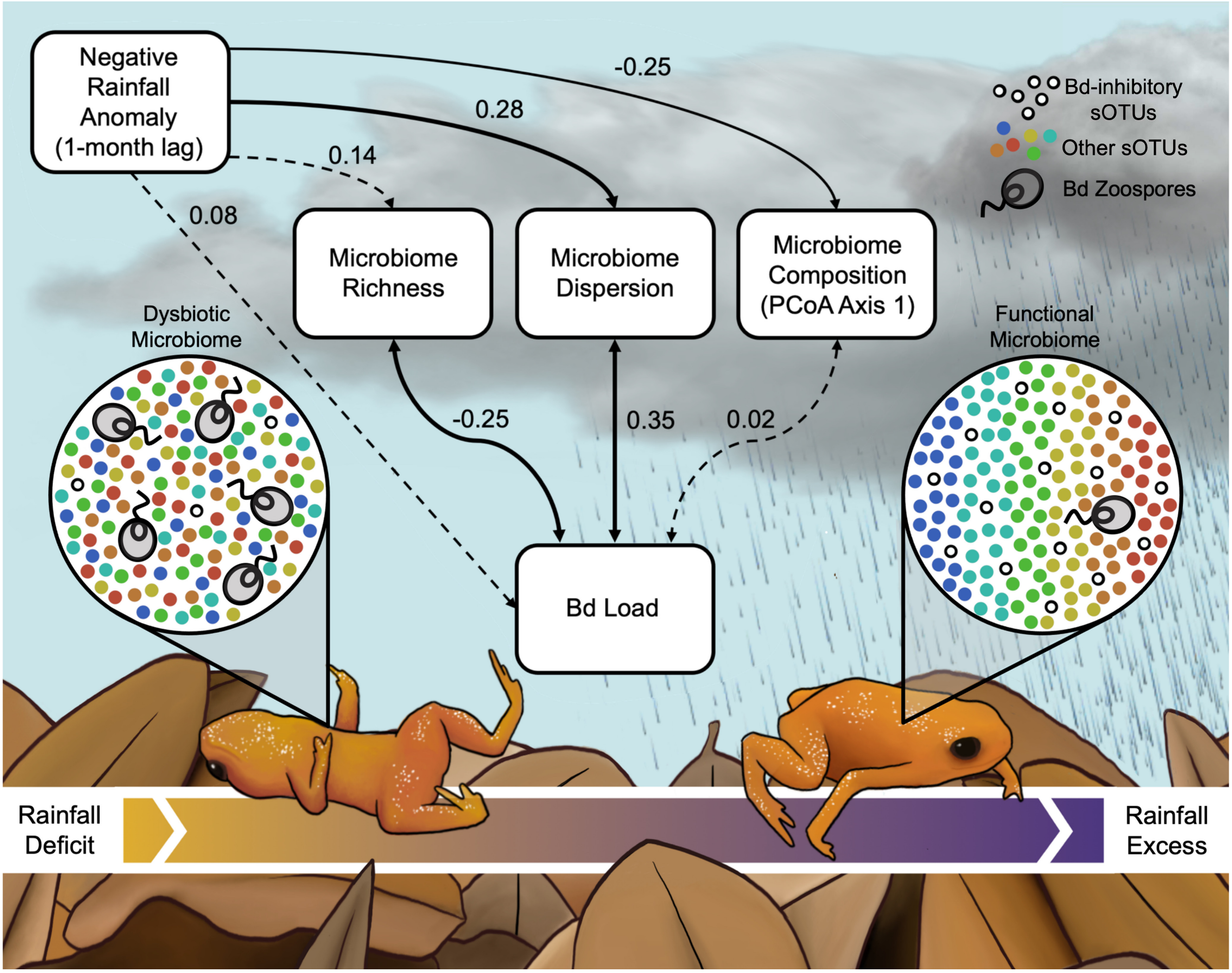

FIGURE 4 SEM linking rainfall deviation (1-month lag, higher values correspond with higher-than-average rainfall), sOTU richness, principal component analysis (PCoA) axis 1 (Jaccard distances), Jaccard dispersion and Bd load. Double-headed arrows indicate bi-directional associations (correlations). Solid lines indicate significant ($p < 0.05$) relationships, while dashed lines indicate non-significant ones. Standardized estimates are listed next to each relationship.

osmotic stress in a way that affects skin bacterial recruitment and retention (Estrada et al., 2019; Greenspan et al., 2020; Kueneman et al., 2019; Le Sage et al., 2021). Higher Bd loads were correlated with lower skin bacterial richness and higher dispersion during drought. This particular combination of factors could indicate an overgrowth of sOTUs that are opportunistic pathogens, which may have led to increased stress levels and/or decreased host immune function against Bd (Bénard et al., 2020; Kiesecker, 2011; Rollins-Smith, 2017; Varela et al., 2018; Walke et al., 2015). A better understanding of how skin microbiome dispersion might align with or affect host immunity is sorely needed (Rebollar et al., 2016).

Climatic anomalies should also be expected to affect host behaviour and immunity directly. Drought may affect protective skin mucus or antimicrobial peptides, as their expression is seasonally driven in other amphibian species (Le Sage et al., 2021). Since *Brachycephalus* species lack an aquatic larval stage, they have limited early-life exposure to Bd, leading to a lack of acquired resistance to the pathogen (Mesquita et al., 2017; Ruggeri et al., 2018). Thus, these frogs are at high risk of Bd spillover from aquatic breeding frogs, particularly during times when they move closer to water bodies or congregate (Becker et al., 2019; Ribeiro et al., 2019). Studies focused on host spatial aggregation and interspecific spillover both in the wild and under experimental conditions could allow us to better understand Bd disease dynamics in direct-developers.

Rainfall in the Atlantic Forest has been associated with historical rainfall originating from the Amazon rainforest through the phenomenon known as “Amazonian flying rivers” (Mercier, 2021; Salati et al., 1979; Salati & Vose, 1984; Zemp et al., 2014). Our teleconnection results (refer to Supporting Material) contribute to the existing body of literature by confirming that the state of São Paulo, particularly our specific field site, Serra do Japi, exhibits a dependency on water vapour transported from the Amazon during both the wet and dry seasons (Figures S3 and S4). The Amazon deforestation arc is extending its reach into the untouched central Amazon region via the BR-319 highway, leading to new deforestation cycles (Ferrante et al., 2021). Further deforestation could threaten historical rainfall patterns in southeast Brazil by increasing the number of consecutive dry days (Marengo et al., 2018, 2020; Webb et al., 2005, 2006), potentially leading to additional Bd outbreaks.

Retrospective studies have highlighted a possible link between Bd and historical amphibian population declines in the Atlantic Forest (Carvalho et al., 2017; Rebouças et al., 2021). Despite the long enzootic state of the pathogen in the region, individual and interacting effects of intensifying climate anomalies and disease may disrupt amphibian skin microbiomes and threaten populations. We present drought as a possible stressor that may induce Anna Karenina effects in host-associated bacterial communities, acting in synergy with disease and leading to die-off events. Further, our findings suggest that amphibian disease in the Atlantic Forest could be exacerbated by deforestation in the Amazon through a predicted shift in continental-scale rainfall patterns. Future experiments should aim to test for specific mechanisms of how lack of rainfall affects the microbiome, animal behaviour and pathogen proliferation. Our study highlights the importance of considering broader climatic patterns when assessing the impacts of habitat loss, climate change, disease, symbiosis and their complex interactions on wildlife populations.

AUTHOR CONTRIBUTIONS

SB, DMC, LFT and CGB conceived of the study. DMC conducted fieldwork and DMC, SEG and WJN conducted laboratory work. SB and SEG performed bioinformatics and SB and LF performed data analysis and generated figures. SB wrote the first draft of the manuscript. All authors contributed to subsequent drafts of the manuscript.

ACKNOWLEDGEMENTS

We would like to thank Fundação Serra do Japi and Fazenda Montanhas do Japi for allowing us to use their infrastructure and for logistical support during fieldwork. We would also like to thank everyone who helped with fieldwork and thanks to Daniel Medina and Vanessa Marshall for their help in the laboratory. Finally, we would like to thank Molly Bletz, Ross Whetstone and Doug Woodhams for adding more of our Brazilian isolates to the AmphiBac database.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ele.14372.

DATA AVAILABILITY STATEMENT

Sequence data is available in the NCBI Sequence Read Archive under accession PRJNA1008153. Metadata and code have been uploaded to Figshare (https://doi.org/10.6084/m9.figshare.24119496).

ETHICS STATEMENT

All sampling was approved by the University of Campinas Animal Ethics Committee (CEUA #4744-1/2017, #5440-1/2019), Instituto Chico Mendes de Conservação da Biodiversidade (SISBio #61220-2, #27745-17), Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SISGen #A0B0E76, #AACFE69) and Fundação Serra do Japi.

ORCID

Shannon Buttimer https://orcid.org/0000-0002-4128-5482
Diego Moura-Campos https://orcid.org/0000-0002-9733-1257
Sasha E. Greenspan https://orcid.org/0000-0001-8883-2968
Wesley J. Neely https://orcid.org/0000-0003-4563-5248
Lucas Ferrante https://orcid.org/0000-0003-2636-5713
Luís Felipe Toledo https://orcid.org/0000-0002-4929-9598
C. Guilherme Becker https://orcid.org/0000-0002-5122-8238

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Buttimer, S., Moura-Campos, D., Greenspan, S.E., Neely, W.J., Ferrante, L., Toledo, L.F. et al. (2024) Skin microbiome disturbance linked to drought-associated amphibian disease. *Ecology Letters*, 27, e14372. Available from: https://doi.org/10.1111/ele.14372