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Amphibian skin fungal communities vary across host species and do not correlate with infection by a pathogenic fungus

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Summary

Amphibian population declines caused by the fungus *Batrachochytrium dendrobatidis* (Bd) have prompted studies on the bacterial community that resides on amphibian skin. However, studies addressing the fungal portion of these symbiont communities have lagged behind. Using ITS1 amplicon sequencing, we examined the fungal portion of the skin microbiome of temperate and tropical amphibian species currently coexisting with Bd in nature. We assessed cooccurrence patterns between bacterial and fungal OTUs using a subset of samples for which bacterial 16S rRNA gene amplicon data were also available. We determined that fungal communities were dominated by members of the phyla Ascomycota and Basidiomycota, and also by Chytridiomycota in the most aquatic amphibian species. Alpha diversity of the fungal communities differed across host species, and fungal community structure differed across species and regions. However, we did not find a correlation between fungal diversity/community structure and Bd infection, though we did identify significant correlations between Bd and specific OTUs. Moreover, positive bacterial–fungal cooccurrences suggest that positive interactions between these organisms occur in the skin microbiome. Understanding the ecology of amphibian skin fungi, and their interactions with bacteria will complement our knowledge of the factors influencing community assembly and the overall function of these symbiont communities.

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Introduction

The ubiquity, high diversity and function of host-associated microbes is changing our understanding about animal biology (McFall-Ngai *et al*., 2013; Hird, 2017). This is due, in part, to the pivotal role that host-associated microbial communities can play in different aspects of host life history, overall host health, and fitness. For instance, host-associated microbial communities can aid the host in energy uptake and storage (Turnbaugh *et al*., 2006), development (Diaz Heijtz *et al*., 2011), maturation of the immune system (Chung *et al*., 2012), behavior (Ezenwa *et al*., 2012) and protection against pathogens (Flórez *et al*., 2015). Much of this research has focused specifically on the bacteria that inhabit hosts, as they often form the bulk of microbial biomass (Huffnagle and Noverr, 2013).

In the last decade, amphibian skin bacterial communities have been subject to a large amount of research due to their potential protective role against pathogens. This body of research was primarily motivated by outbreaks of the disease chytridiomycosis, associated with population declines and extinctions of several amphibian species across the globe (Berger *et al*., 1998; Bosch *et al*., 2001; Lips *et al*., 2006; Vredenburg *et al*., 2010). Chytridiomycosis is caused by infections of the fungal skin pathogen *Batrachochytrium dendrobatidis* (Bd). Amphibian skin bacteria have been considered a promising area of research to develop a conservation strategy focused on using beneficial bacteria to mitigate Bd infections in endangered amphibians (Bletz *et al*., 2013). This body of research has yielded important insights into the ecology of animal-associated microbes, and into their potential use in controlling emergent fungal diseases due to their capacity to interact with pathogenic agents, such as Bd (Walke and Belden, 2016). For instance, there is evidence that infections by Bd can alter

the structure of amphibian skin bacterial communities (Jani and Briggs, 2014), and that the structure of the skin bacterial communities can be associated with susceptibility of the amphibian host to Bd infection (Lam *et al*., 2010; Becker *et al*., 2015; Rebollar *et al*., 2016). However, studies characterizing and assessing the function of the fungal communities associated with amphibian skin are still scarce and lagging behind those on bacteria (Kueneman *et al*., 2015, 2017; Kearns *et al*., 2017).

In general, in contrast to bacteria, the fungal diversity associated with animals, and their functional roles, remain largely understudied (Huffnagle and Noverr, 2013; Flórez *et al*., 2015; Peay *et al*., 2016). This is an important knowledge gap given that free-living fungi are highly diverse and are known to play important roles in ecological processes (Peay *et al*., 2016). In addition, some have tight mutualistic and antagonistic interactions with plants (Tedersoo *et al*., 2014). Recent research suggests that while the fungal component of animal-associated microbial communities is part of the rare biosphere (<0.1% of microbial cells; Huffnagle and Noverr, 2013), its diversity, which includes novel lineages, is higher than expected (reviewed in Peay *et al*., 2016). In addition, fungi are eukaryotes and are much larger in cell size than bacteria (>100 times larger), and thus, might contribute unique genetic and metabolic traits to their animal hosts (Underhill and Iliev, 2014), and influence the overall attributes of the microbial community.

There is some evidence that fungi can engage in defensive symbiosis with animals. For example, fungus-farming insects (e.g. leaf-cutter ants, termites and bark beetles) can form symbiotic interactions with fungi, in which the symbiotic fungi provide protection to the insects and their fungal gardens against infections by fungal pathogens (reviewed in Flórez *et al*., 2015). Moreover, as fungi can cooccur with bacteria within the host, research assessing the influence of fungi on the structure and function of bacterial communities is an emergent area of interest in host health, including in humans (Huffnagle and Noverr, 2013).

In an initial exploration of the non-bacterial microorganisms associated with amphibian skin, Kueneman *et al*. (2015) identified the microeukaryotes associated with the toad *Anaxyrus boreas.* They found that micro-eukaryotic diversity was substantially higher in subadults and adults in contrast to tadpoles and juveniles. In addition, the authors determined that these communities of microeukaryotes in the subadults and adults were dominated by fungi. There is also evidence that amphibian skin fungal communities can vary across host populations, as was observed in tadpoles of *Alytes obstetricans* (Davis *et al*., 2017). Within the context of Bd infection, Kearns *et al*. (2017) found evidence of a potential defensive role against Bd by the fungal communities associated with the skin of captive amphibians. In addition, Kearns *et al*. (2017) determined that, in contrast to two bacterial probiotics (*Janthinobacterium lividum* and *Flavobacterium johnsoniae*), treating amphibians with a fungal probiotic (*Penicillium expansum*) induced a lower secretion rate of corticosterone (as a measure of stress), and did not suppress the production of antimicrobial peptides by the host. Thus, increasing our understanding of the ecology of amphibian skin fungal communities could provide important insights into the defensive role of symbiotic fungal communities and provide relevant information for current efforts in amphibian conservation.

Using data from field surveys, we assessed the diversity and natural variation of the skin fungal communities across eight species of free-living amphibians (Table 1) and examined whether naturally occurring infections by Bd correlate with changes in the diversity and community structure of the skin fungal communities. In addition, we examined the potential relationship, and cooccurrences, between the composition of bacterial and fungal communities associated with amphibian skin using a subset of samples for which we had both bacterial 16S rRNA and fungal ITS1 amplicon sequencing data. The amphibian species used in this study represent two distinct regions (temperate and tropical), vary in their natural histories (arboreal, terrestrial and aquatic) and reproductive modes, and currently coexist with Bd in localities where the fungus occurs in an endemic infection stage.

Results

Seven phyla comprised the amphibian skin fungal communities (Fig. 1A). However, these communities were dominated by OTUs from the phylum Ascomycota, with a total proportional abundance ranging from 0.43 to 0.64 across the amphibian species (Fig. 1A). The phylum Basidiomycota was the second most abundant on most amphibian species, with the exception of *Notophthalmus viridescens*, with a proportional abundance ranging from 0.12 to 0.38 (Fig. 1A). Interestingly, on *N. viridescens*, the second most abundant phylum was Chytridiomycota, which included 151 OTUs other than Bd (Fig. 1A) and had a proportional abundance of 0.22 in this species. We also determined that the occurrence and relative abundance of fungal families varied substantially across amphibian species, although one family, Davidiellaceae, was present on all the species when accounting for abundant OTUs only, and had a proportional abundance from 0.04 to 0.51 (Fig. 1B). In terms of OTU relative abundance, there was a right-skewed distribution, where a few OTUs were highly abundant relative to the number of rare ones (Fig. 2). The two most abundant OTUs (OTU_1 and OTU_2, both from the phylum Ascomycota), were the only ones present in over half of the samples (OTU_1

Table 1. Amphibian species used in the study, their region of occurrence, locality, sampling dates and sample sizes for the fungal and bacterial datasets.

Region	Species	Province (Panamá)/ State-County (USA)	Sampling period	Samples infection status (based on ITS1 Illumina reads)		Fungal dataset sample size	Bacterial dataset sample size
				Positive	Negative		
Tropical (Panamá)	*Agalychnis callidryas* (family: Hylidae)	Panamá	July – Sept 2012	7	1	8	8[a]
		Colón	Jul 2013	2	6	8	8[d]
	Dendropsophus ebraccatus (family: Hylidae)	Panamá	July – Sept 2012	2	5	7	7[a]
		Colón	Jul 2013	2	6	8	8[d]
	Silverstoneia flotator (family: Dendrobatidae)	Coclé	Jun 2013	4	2	6	–
		Coclé	Jun 2013	0	6	6	–
	Craugastor fitzingeri (family: Craugastoridae)	Panamá	July – Sept 2012	3	4	7	7[a]
Temperate (United States)	*Rana catesbeiana* (family: Ranidae)	Virginia – Giles	Aug 2010	3	5	8	7[b]
	Pseudacris crucifer (family: Hylidae)	Virginia – Bedford	Mar 2012	7	1	8	7[c]
		Virginia – Giles	Mar 2012	4	2	6	6[c]
	Notophthalmus viridescens (family: Salamandridae)	Virginia – Giles	Apr 2012	4	4	8	–
	Anaxyrus americanus (family: Bufonidae)	Virginia – Montgomery	Mar 2012	1	4	5	5[b]
						85	63

Bacterial 16S rRNA amplicon sequencing data submitted to the NCBI SRA database under the accession numbers: [a]SRP062596 (from Belden *et al*., 2015); [b]SRP062395 (from Walke *et al*., 2015); [c]SRP087497 (from Hughey *et al. in review*); [d]SRP168079 (unpublished).

present in 86% of the samples and OTU_2 in 74%). OTU_1, the most abundant and prevalent OTU, was identified as a fungal species from the genus *Cladosporium* based on sequence alignment of this OTU against the NCBI nucleotide database using BLAST (Basic Local Alignment Search Tool; top hits results: score = 459, query cover 100%, *E*-value = 2e−125 and identity 100%). OTU_2, the second most abundant, was classified as an 'uncultured fungus' from the phylum Ascomycota based on BLAST and the NCBI database (hits results: score = 459, query cover 99%, *E*-value = 2e−125 and identity 100%). These two OTUs were not identified beyond the kingdom or class level using the database UNITE.

We found that richness and Shannon index Hill numbers differed across amphibian species (richness: Chisq = 212.35, $P < 0.001$; Shannon index Hill numbers: Chisq = 90.63, $P < 0.001$). Amphibian species with terrestrial habitats (*Craugastor fitzingeri*, *Silverstoneia flotator* and *Anaxyrus americanus*) seemed to have a higher diversity (Fig. 3A and B; see Supporting Information Table S1 for statistical pairwise comparisons). Despite differences across species in alpha diversity, we found no significant differences between the tropical and temperate species ($N = 4$ species/category) in richness, and possibly weak differences in Shannon index Hill numbers (richness: Chisq = 1.70, $P = 0.19$; Shannon index Hill numbers: Chisq = 3.82, $P = 0.05$). This result suggests that the amphibian host-species plays a more important role in determining the richness and evenness of the skin fungal communities than biogeographic region (Fig. 3A and B). Bd OTU read number (as a proxy for infection load) was not correlated with either richness or Shannon index Hill numbers (Fig. 3C; richness: back-transformed slope = 0.99, SE = 1.95E−4, $R^2 = 0.006$, $P = 0.42$; Shannon index Hill numbers: slope = 4.306E−4, SE = 3.382E−4, $R^2 = 0.007$, $P = 0.21$).

Skin fungal community structure differed across species, regions and Bd infection status (species: pseudo-$F = 2.74$, $R^2 = 0.20$, $P < 0.001$, Fig. 4; region: pseudo-$F = 3.69$, $R^2 = 0.04$, $P < 0.001$; Bd infection status: pseudo-$F = 2.35$, $R2 = 0.03$, $P < 0.001$). However, the factor 'species' explained substantially more variation relative to the factors 'region' and 'Bd infection status', as represented by the R-squared values. The differences in the structure of the skin fungal communities across Bd infection status seemed to be primarily driven by the presence of Bd OTUs at high relative abundances and not to large changes in the composition or relative abundance of other fungal OTUs, as demonstrated by the results from the analysis excluding the Bd OTUs (Fig. 4B, pseudo-$F = 1.13$, $R2 = 0.01$; $P = 0.052$). Finally, there were 55 indicator OTUs (including eight Bd OTUs) that defined the amphibian host species and/or group of species. Of these, 32 were each associated with a single, but not the same, amphibian species (Table 2). Each amphibian species, except *D. ebraccatus*, had unique indicator OTUs.

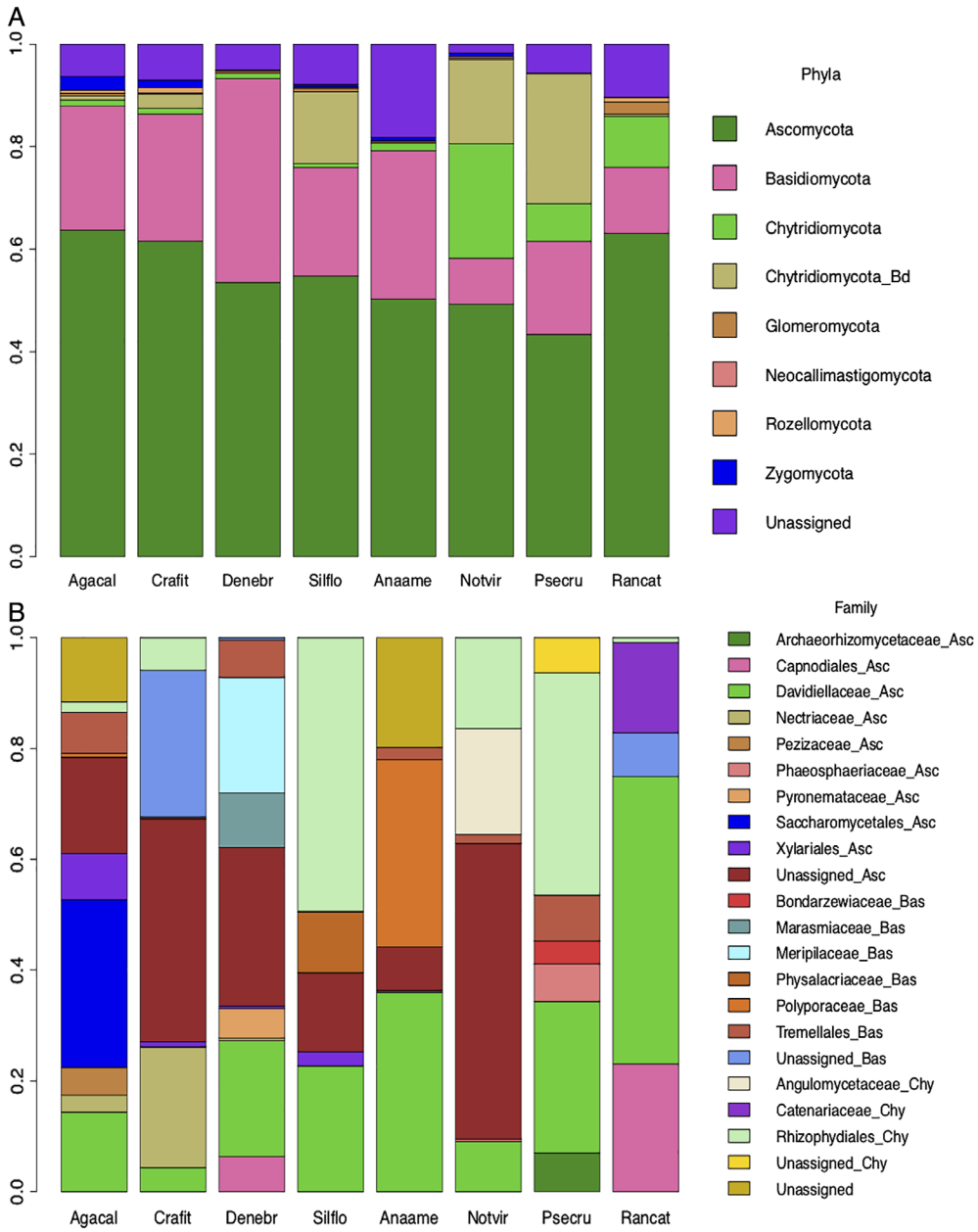

Fig. 1. Stacked barplots showing the relative abundance of the fungal phyla (A) and orders/families (B) across amphibian host species. The taxonomic orders and families denoted in the figure correspond to abundant OTUs with a maximum relative abundance cut-off equal or above 0.20. Taxonomic orders represent OTUs with an undefined classification at the family level (*incertae sedis*) based on the database UNITE. Amphibian species are denoted by the first three letters of the genus and the first three letters of the species name. Agacal: *Agalychnis callidryas*; Crafit: *Craugastor fitzingeri*; Denebr: *Dendropsophus ebraccatus*; Silflo: *Silverstoneia flotator*; Anaame: *Anaxyrus americanus*; Notvir: *Notophthalmus viridescens*; Psecru: *Pseudacris crucifer*; Rancat: *Rana catesbeiana.*

Using the 63 samples for which we also had bacterial 16S rRNA gene amplicon data, we found a correlation between the composition of the fungal and bacterial communities (Jaccard, Mantel *r* statistic = 0.23, *P* = 0.001). In addition, we identified 127 significant pairwise correlations between OTUs in the cooccurrence network analysis (Fig. 5; number of nodes: 211; number of edges: 254; average degree: 1.20; connectedness: 0.0057), which included correlations between bacteria (65% of interactions), bacteria–fungi (33%) and fungi (2%). Bacterial OTUs from the phylum Proteobacteria were involved in most of the significant correlations (68%, Fig. 5). The proportion of positive and negative correlations was similar between correlated bacterial OTUs (positive correlations: 48%; negative: 52%); however, positive correlations were predominant between correlated bacterial–fungal OTUs

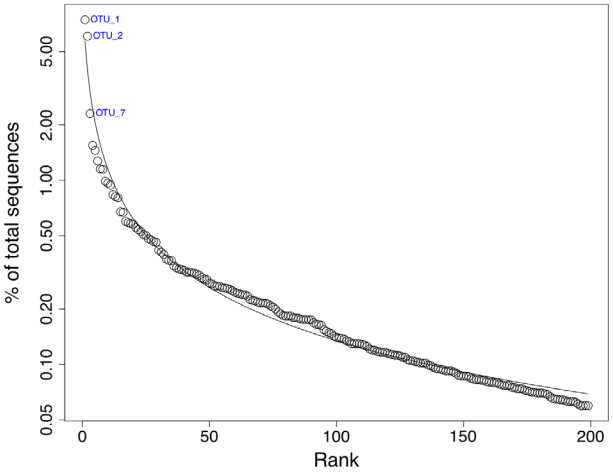

Fig. 2. Diversity/dominance plot showing the rank order of the 200 most abundant OTUs based on the percentage (%) of each one out of the total number of sequences. In this analysis, the abundant OTUs were selected using a maximum relative abundance cut-off equal to or above 0.05. OTU_1 (genus: *Cladosporium*) and OTU_2 represent OTUs from the phylum Ascomycota and OTU_7 represents an OTU identified as Bd. [Color figure can be viewed at wileyonlinelibrary.com]

(positive correlations: 79%; negative: 21%; Fig. 5). While there were few significant correlations between fungal OTUs, all of them were positive.

We found significant correlations, both negative and positive, between Bd OTUs and both bacterial and fungal OTUs (Fig. 5). These correlations included bacterial OTUs from the phylum Proteobacteria (family and direction of correlation: Enterobacteriaceae – negative, Moraxellaceae – negative and Sphingomonadeceae – negative and positive), Firmicutes (Enterococcaceae – positive), Cyanobacteria (Scytonemataceae – positive) and OTUs from the fungal phylum Ascomycota (Nectriaceae – positive).

We initially assigned individuals as Bd+ or Bd– based on a qPCR approach developed by Boyle *et al*. (2004). However, the Bd qPCR results did not align exactly with the Illumina ITS1 amplicon results. Specifically, 72% (N = 26) of the Bd+ individuals based on qPCR were also Bd+ based on Illumina amplicon data, 13 Bd– individuals based on qPCR were Bd+ in the amplicon data, and 10 Bd+ individuals based on qPCR were Bd– in the amplicon data. The 23 individuals that 'switched' infection status had very low infection loads (qPCR: mean = 0.8 zoospore genomic equivalents; Illumina ITS1 amplicon: mean = 6 Bd reads).

The total number of Bd amplicon reads in the Bd+ individuals was correlated with the number of zoospore genomic equivalents estimated with qPCR (second order polynomial regression: $F_{(2,36)} = 30.78$, $P < 0.001$, adjusted-$R^2 = 0.61$; Supporting Information Fig. S1). However, the relationship was not linear, perhaps due in part to some samples with few zoospore genomic equivalents (< 100) based on qPCR having a high number of Bd amplicon reads [above 500 reads] (Supporting Information Table S2). To resolve this for our analyses, we ultimately used the Illumina ITS1 amplicon results to define Bd infection status to stay consistent with the community data produced by amplicon sequencing, including Bd relative abundance.

Bd detection via ITS1 amplicon sequencing resulted in 23 different Bd OTUs in our dataset, which, based on a BLAST search against the NCBI database, seem to be capturing some of the genetic variations within Bd (Supporting Information Table S3). For instance, the Bd OTUs, based on the closest BLAST matches using an 80% query coverage and 95% identity cutoffs, were associated with either a strain of a specific Bd lineage (i.e. Bd-GPL1, Bd-GPL2 and Bd-Brazil) or strains from two lineages. There was variation in the occurrence of these Bd OTUs across amphibian-host species; some were more common. For example, OTU_7 (determined to be most similar to strain CW34 of Bd-GPL2; query coverage 81%, *E*-value = 5e−88, identity 99%) had a higher relative abundance than the other Bd OTUs, and was detected in the majority of the infected amphibians (Supporting Information Fig. S2).

Discussion

We found that the fungal portion of the amphibian skin microbiome was dominated by members of the phyla Ascomycota and Basidiomycota, and, in one instance, by non-Bd taxa in the phylum Chytridiomycota. These are also the major fungal phyla in most natural systems. For instance, the phyla Ascomycota, Basidiomycota and Chytridiomycota were identified, among others, as the taxa with the highest number of OTUs in soil samples from around the world (Tedersoo *et al*., 2014). The phyla Ascomycota and Basidiomycota include a diverse group of species involved in mycorrhizal and endophytic symbiosis (Peay *et al*., 2016). Moreover, animal-associated fungal communities, including human-associated communities, are also dominated by species from the phyla Ascomycota and Basidiomycota (Huffnagle and Noverr, 2013; Peay *et al*., 2016). However, while the fungal diversity we identified on the skin of amphibians might be comprised of fungi involved in commensal and mutualistic interactions, there are also likely transient spores (Peay *et al*., 2016).

We also determined that the newts, *N. viridescens*, harboured a higher occurrence of non-Bd members of the phylum Chytridiomycota relative to the other amphibian species. Chytrids can be abundant in freshwater systems (James *et al*., 2006), so their higher occurrence on *N. viridescens* might be explained, in part, by an increase in exposure to these fungi in the aquatic habitat of adult newts. For instance, an OTU identified as a chytrid from the genus *Angulomyces*, a taxon found in multiple

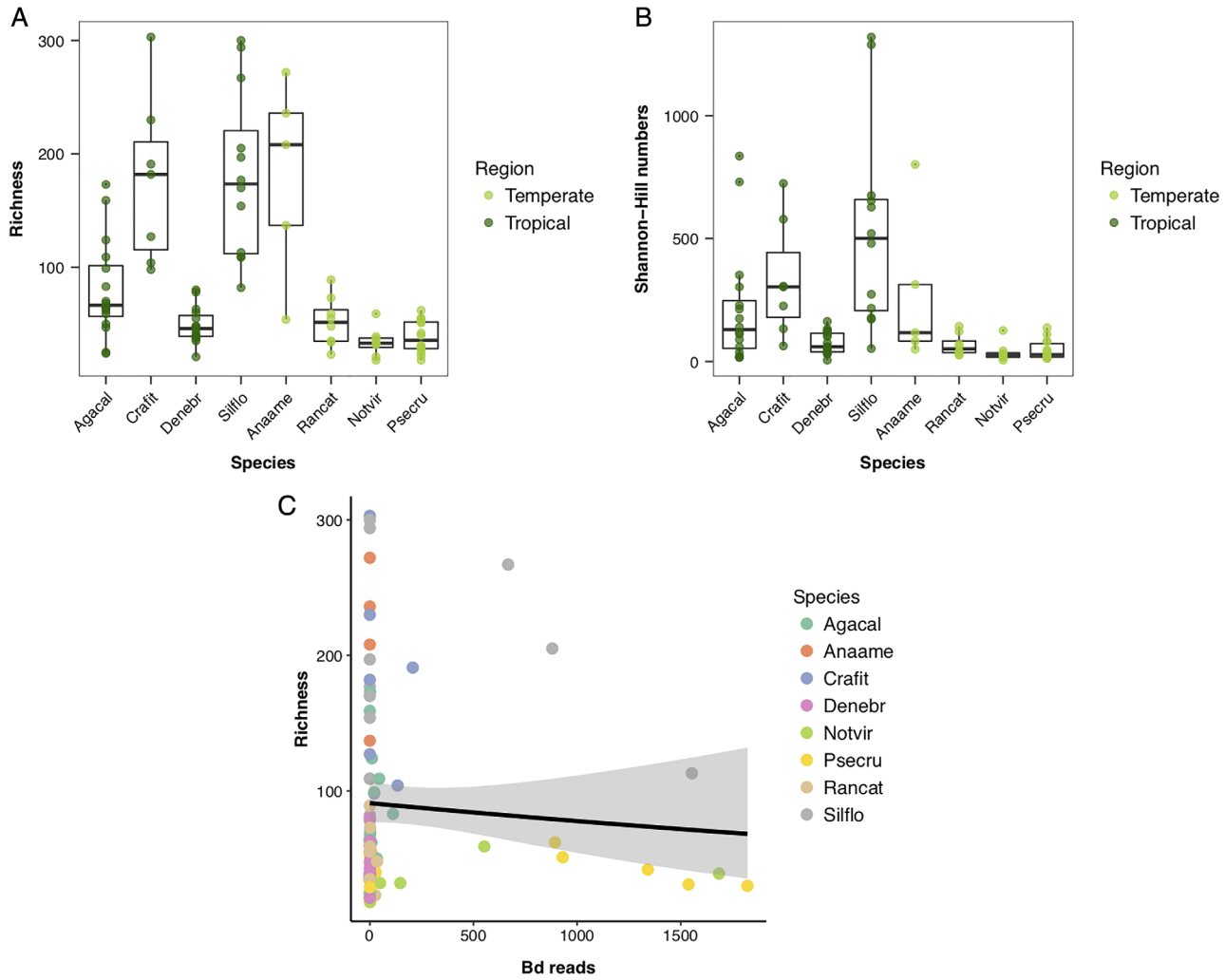

Fig. 3. Alpha diversity values for OTU richness (A) and Shannon Index transformed to Hill numbers (B) across amphibian host species; and the relationship between infection load (number of Bd reads) and richness based on a GLM (R^2 = 0.006, P = 0.42) and 95% CI (C). Points in figures represent the alpha diversity values of each individual amphibian. In C, the amphibian species are denoted by the first three letters of the genus and the first three letters of the species name. Agacal: *Agalychnis callidryas*; Crafit: *Craugastor fitzingeri*; Denebr: *Dendropsophus ebraccatus*; Silflo: *Silverstoneia flotator*; Anaame: *Anaxyrus americanus*; Notvir: *Notophthalmus viridescens*; Psecru: *Pseudacris crucifer*; Rancat: *Rana catesbeiana.* [Color figure can be viewed at wileyonlinelibrary.com]

aquatic habitats from distinct biogeographic regions (Letcher *et al*., 2008; Davis *et al*., 2013), was determined to be an indicator OTU associated with *N. viridescens. N. viridescens* also harboured other chytrids representing more terrestrial taxa, such as the genera *Spizellomyces* (also identified as an indicator OTU) and *Powellomyces*, which are usually found in soil and near the shore of aquatic habitats (Wakefield *et al*., 2010). This result suggests that the composition of the fungal portion of the amphibian skin microbiome can be influenced by the environmental species pool, which has also been observed for amphibian skin bacteria (Muletz *et al*., 2012; Loudon *et al*., 2013; Walke *et al*., 2014). Within this context, we also observed higher fungal community richness in the host species that are more strongly associated with terrestrial habitats, such as the forest floor (*C. fitzingeri*, *S. flotator* and *A. americanus*). This observed diversity pattern might have resulted from the constant exposure of these amphibian species to substrates with very high fungal diversity, such as soil (Peay *et al*., 2016). While we did not systematically test for the effect of host ecology on the amphibian skin fungal communities, our results are consistent with previous work by Bletz *et al*. (2017) on amphibian skin bacterial communities, where terrestrial host species harboured a higher richness of bacterial OTUs compared to the arboreal and aquatic species. Hence, it is possible that host species associations with different habitats lead to exposure to distinct microbial pools, producing some of the observed variations in the fungal diversity of the skin communities.

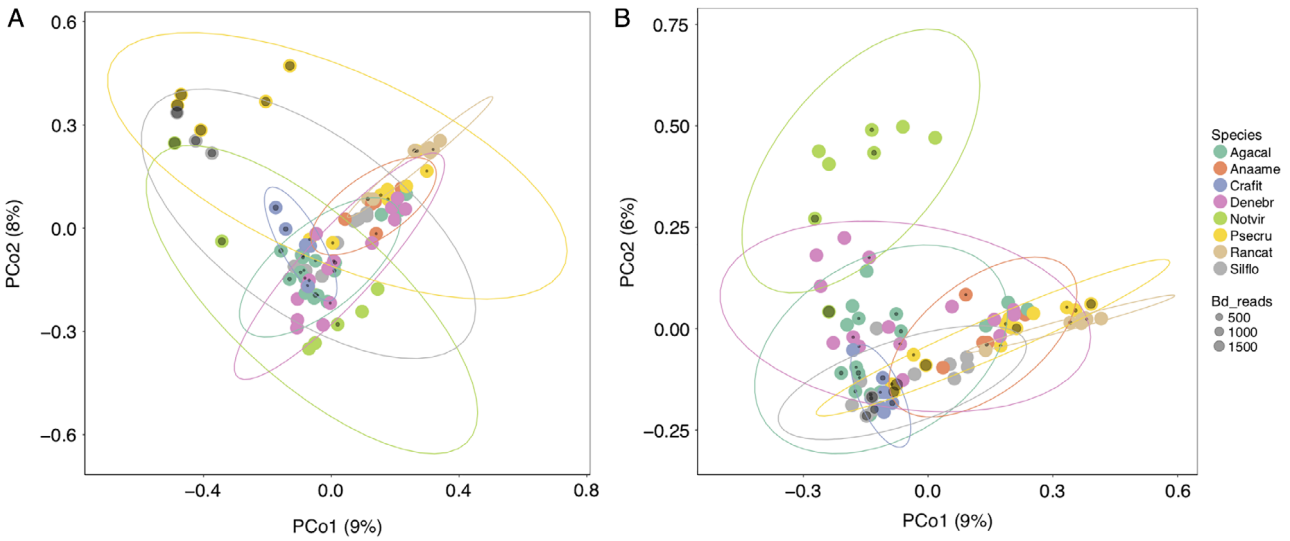

Fig. 4. Beta diversity of fungal communities across amphibian host species using a principal coordinate analysis (PCoA) ordination based on Bray–Curtis dissimilarity distances. The ordinations show the clustering pattern including (A) and excluding Bd OTUs (B) from the analysis. Points represent single individuals from different amphibian species. Dark circles within points represent the number of Bd reads determined for the respective individual. Amphibian species are denoted by the first three letters of the genus and the first three letters of the species name. Agacal: *Agalychnis callidryas*; Crafit: *Craugastor fitzingeri*; Denebr: *Dendropsophus ebraccatus*; Silflo: *Silverstoneia flotator*; Anaame: *Anaxyrus americanus*; Notvir: *Notophthalmus viridescens*; Psecru: *Pseudacris crucifer*; Rancat: *Rana catesbeiana.*

Our results are consistent with prior work on amphibian skin fungal communities. For instance, a study using captive Dendrobatid frogs suggested that all four species harboured a large and diverse group of members of the phylum Ascomycota (Kearns *et al*., 2017). They also determined that the amphibian species thought to be resistant to Bd infection (i.e. *Dendrobates leucomelas*) harboured more Bd-inhibitory fungal taxa from the phylum Ascomycota, as compared to the more susceptible host species. Further research testing Ascomycota isolates from wild amphibians against Bd provides a promising avenue to elucidate the potential for defensive symbiosis between animals and fungi, which remains largely understudied (reviewed in Flórez *et al*., 2015). On the other hand, Kearns *et al*. (2017) also identified several taxa in the phylum Basidiomycota with the ability to facilitate the growth of Bd zoospores. Perhaps the balance of these two major fungal phyla is important in host response to Bd.

In contrast to the findings of Kearns *et al*. (2017), we did not find a strong relationship between naturally occurring Bd infection and fungal community diversity or structure in the free-living amphibians sampled in our study. However, the identification of significant correlations, both negative and positive, between Bd OTUs and both bacterial and fungal OTUs suggest there could still be important interactions between Bd and specific taxa, which might alter disease outcomes, as has been suggested previously (Kueneman *et al*., 2017). Importantly, our results could have been influenced by the small number of amphibian samples we had with high Bd reads. The species used in our study might have had low Bd loads because they are thought to be tolerant or resistant to Bd infection and have extant populations coexisting with Bd at an enzootic stage (Crawford *et al*., 2010; Hughey *et al*., 2014; Rebollar *et al*., 2014). Thus, it may be more difficult with our dataset of wild-sampled amphibians to detect small effects of Bd on community-level metrics.

While our Bd qPCR and Bd ITS1 amplicon data were correlated, there were some differences in the results based on these two methods for identifying Bd positive individuals. This discrepancy suggests that there might be variation in sensitivity between the two methods, in particular for samples with low infection loads, possibly due to some Bd strains not being detected with specific Bd qPCR primers. ITS amplicon sequencing data from skin swabs might allow for the detection of some genetic variation within Bd (Schloegel *et al*., 2012), as potentially inferred by the 23 Bd OTUs identified in our study. However, our ability to further interpret this result is limited by the OTU clustering algorithm used in this study (i.e. UCLUST), which has a low resolution to resolve fine-scale variation in amplicon data (Callahan *et al*., 2017), and can overestimate the number of OTUs in a dataset relative to other algorithms (Callahan *et al*., 2017; Caruso *et al*., 2019). In addition, other studies have found that using multiple molecular markers is necessary to accurately identify Bd genotypes (Byrne *et al*., 2017; O'Hanlon *et al*., 2018). We think this is worthy of further exploration, as it might influence the design and

Table 2. List of indicator OTUs (including eight Bd OTUs) that defined the amphibian host species and/or group of species.

Amphibian-host species/group of species	Indicator OTU ID	Phyla	Order	Genus	Indicator index	*P* value
Agalychnis callidryas	OTU_52	Basidiomycota	Filobasidiales	–	0.30	0.012
	OTU_69	Basidiomycota	Cystofilobasidiales	*Guehomyces*	0.22	0.039
Craugastor fitzingeri	OTU_26	Ascomycota	Hypocreales	*Calonectria*	0.59	0.001
	OTU_254	Ascomycota	Hypocreales	*Calonectria*	0.46	0.001
	OTU_91	Ascomycota	Xylariales	–	0.43	0.002
	OTU_17	Ascomycota	Xylariales	–	0.42	0.004
	OTU_87	Ascomycota	Saccharomycetales	*Yamadazyma*	0.34	0.015
	OTU_128	Zygomycota	Mortierellales	*Mortierella*	0.29	0.011
	OTU_35	Basidiomycota	Agaricales	–	0.29	0.006
Silverstoneia flotator	OTU_45	Ascomycota	Sordariomycetes_ ord_Incertae_sedis	*Phialemoniopsis*	0.32	0.022
	OTU_192	Ascomycota	Hypocreales	*Nectria*	0.25	0.028
Anaxyrus americanus	OTU_44	Unclassified fungi	–	–	0.80	0.001
	OTU_78	Basidiomycota	Cystofilobasidiales	*Mrakiella*	0.79	0.001
	OTU_120	Ascomycota	Capnodiales	*Mycosphaerella*	0.74	0.001
	OTU_500	Ascomycota	Chaetothyriales	*Phaeococcomyces*	0.60	0.001
	OTU_11	Basidiomycota	Polyporales	*Trametes*	0.59	0.002
	OTU_123	Ascomycota	Helotiales	*Oculimacula*	0.40	0.004
	OTU_154	Basidiomycota	Cantharellales	–	0.36	0.007
Notophthalmus viridescens	OTU_19	Chytridiomycota	Rhizophydiales	*Angulomyces*	1.00	0.001
	OTU_34	Chytridiomycota	Spizellomycetales	*Spizellomyces*	0.88	0.001
	OTU_93	Basidiomycota	Sebacinales	–	0.75	0.001
	OTU_86	Ascomycota	Helotiales	–	0.46	0.003
	OTU_53	Chytridiomycota	Rhizophydiales	*Batrachochytrium*	0.41	0.009
Pseudacris crucifer	OTU_169	Unclassified fungi	–	–	0.29	0.01
Rana catesbeiana	OTU_16	Ascomycota	Capnodiales	*Toxicocladosporium*	0.75	0.001
	OTU_60	Ascomycota	Pleosporales	–	0.73	0.001
	OTU_6	Ascomycota	Capnodiales	*Toxicocladosporium*	0.63	0.001
	OTU_43	Chytridiomycota	Blastocladiales	*Catenaria*	0.50	0.001
	OTU_117	Glomeromycota	Diversisporales	*Acaulospora*	0.47	0.007
	OTU_223	Ascomycota	Capnodiales	*Cladosporium*	0.45	0.001
	OTU_59	Ascomycota	Pleosporales	*Leptosphaeria*	0.36	0.011
	OTU_252	Basidiomycota	Sebacinales	*Serendipita*	0.25	0.026
C. fitzingeri and D. ebraccatus	OTU_30	Ascomycota	Saccharomycetales	*Candida*	0.52	0.005
C. fitzingeri and S. flotator	OTU_71	Ascomycota	Dothideales	–	0.42	0.01
	OTU_112	Unclassified fungi	–	–	0.26	0.016
S. flotator and A. americanus	OTU_57	Ascomycota	Hypocreales	*Simplicillium*	0.29	0.031
A. americanus and R. catesbeiana	OTU_21	Ascomycota	–	–	0.61	0.001
N.viridescens and P. crucifer	OTU_4	Chytridiomycota	Rhizophydiales	*Batrachochytrium*	0.38	0.034
A. callidryas, *C. fitzingeri and S. flotator*	OTU_29	Ascomycota	Xylariales	*Monographella*	0.45	0.031
C. fitzingeri, *D. ebraccatus and S. flotator*	OTU_18	Basidiomycota	Sporidiobolales	*Rhodotorula*	0.48	0.006
	OTU_107	Ascomycota	Hyprocreales	*Verticillium*	0.35	0.022
C. fitzingeri, *N. viridescens and P. crucifer*	OTU_2016	Chytridiomycota	Rhizophydiales	*Batrachochytrium*	0.34	0.012
	OTU_2021	Chytridiomycota	Rhizophydiales	*Batrachochytrium*	0.29	0.04
C. fitzingeri, *S. flotator*, *N. viridescens and P. crucifer*	OTU_7	Chytridiomycota	Rhizophydiales	*Batrachochytrium*	0.48	0.035
	OTU_1337	Chytridiomycota	Rhizophydiales	*Batrachochytrium*	0.39	0.01
	OTU_9	Chytridiomycota	Rhizophydiales	*Batrachochytrium*	0.39	0.027
	OTU_92	Chytridiomycota	Rhizophydiales	*Batrachochytrium*	0.34	0.03
A. americanus, *N. viridescens*, *P. crucifer and R. catesbeiana*	OTU_8	Ascomycota	Pleosporales	–	0.48	0.002
A. callidryas, *C. fitzingeri*, *D. ebraccatus*, *S. flotator and R. catesbeiana*	OTU_209	Ascomycota	Pleosporales	–	0.43	0.02
A. callidryas, *D. ebraccatus*, *A. americanus*, *N. virisdescens and P. crucifer*	OTU_41	Basidiomycota	Tremellales	*Cryptococcus*	0.46	0.023
A. callidryas, *C. fitzingeri*, *D. ebraccatus*, *S. flotator*, *A. americanus and N. virisdescens*	OTU_2	Ascomycota	Hyprocreales	–	1.00	0.001

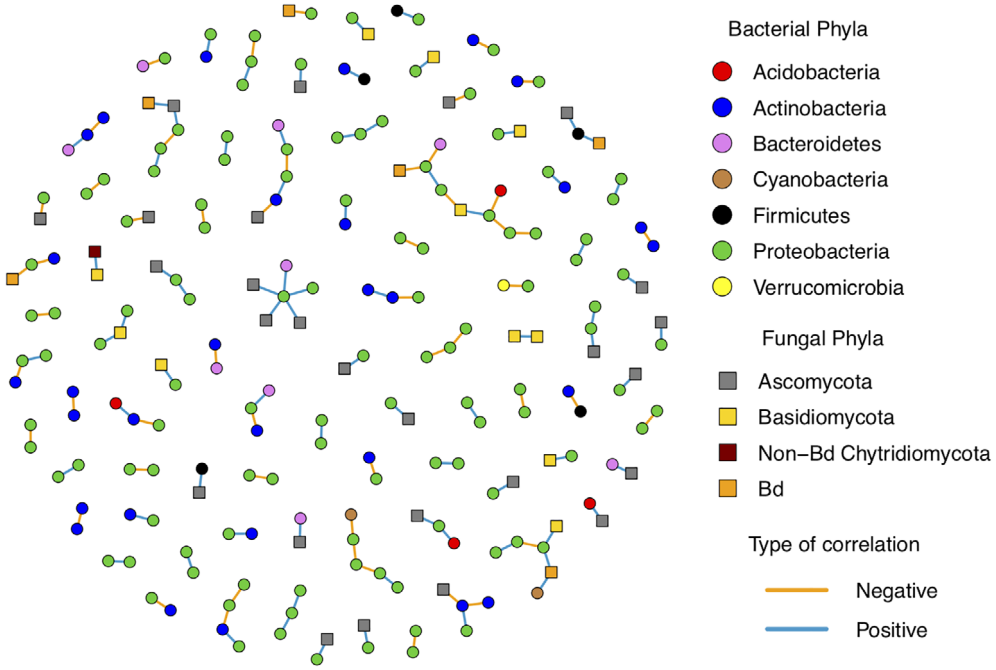

Fig. 5. Significant cooccurrences of fungal and bacterial OTUs from a subset of 63 amphibian skin samples for which both datasets were available. Nodes denote single OTUs, and edges represent significant (pseudo-*P* values <0.05) correlations between OTUs based on SparCC default parameters. The figure includes correlations with coefficients smaller than −0.35 and equal to or larger than 0.35. Node shapes denote taxonomic Domain and colours their respective phylum.

conclusions of studies using these methods. There is some evidence that amplicon sequencing data might sometimes provide a reliable indicator of pathogen occurrence on hosts. For instance, in plant systems, several studies have found correlations between the number of amplicon reads associated with fungal pathogens and disease severity in host tissues (Sapkota *et al*., 2015; Jakuschkin *et al*., 2016).

Relative to diversity patterns of bacterial communities, free-living fungal communities seem to exhibit a higher degree of heterogeneity at local and global scales (Martiny *et al*., 2011; Baldrian *et al*., 2012; Brown and Jumpponen, 2013; Koljalg *et al*., 2013; Peay *et al*., 2016). Within this context, in our study, we observed that the composition of the fungal communities also tended to vary substantially at the family level across amphibian host species. One exception to this was the family Davidiellaceae, which was present on all amphibian species. This family was represented by only one OTU (OTU_1), and it was the most frequent and abundant OTU in our dataset. Interestingly, this OTU was identified as a fungus from the genus *Cladosporium*. Members of this genus have been associated with mycoses in amphibians, such as mycotic dermatitis and mycotic myositis (Taylor, 2001). For instance, the species *Cladosporium cladosporoides* has been associated with the disease chromomycosis, a type of mycotic dermatitis, on invasive *R. catesbeiana* in Korea (Kim *et al*., 2008). In addition, captive amphibians in zoos can also harbour fungi from the genus *Cladosporium* at high relative abundances (Kearns *et al*., 2017), and in some cases, these infections result in cases of chromomycosis (Taylor, 2001). Although we detected a fungal OTU associated with the genus *Cladosporium* across a broad range of amphibians, including *R. catesbeiana*, in which this OTU had the highest relative abundance, we have no evidence that the amphibians sampled in our study had clinical symptoms of mycotic dermatitis. However, our study does provide evidence of how conspicuous and widespread this genus can be across amphibian hosts.

Host-associated microbial communities are subject to strong selective pressure posed by the host via different deterministic processes, which can lead to host species-specific symbiotic communities (Adair and Douglas, 2017). For instance, similar to host-associated bacterial communities, host-specific fungal communities have been observed across a wide range of hosts, including plants, marine invertebrates and terrestrial vertebrates (Huffnagle and Noverr, 2013; Yarden, 2014; Christian *et al*., 2015; Peay *et al*., 2016). Our results provide further support for host-specific fungal communities in amphibians (Kearns *et al*., 2017). Thus, host-associated traits, that either preclude or enhance the colonization of specific microbes (Gallo and Hooper, 2012; Franzenburg *et al*., 2013), are likely to play a pivotal role in shaping these symbiotic fungal communities. In the amphibian skin microbiome system, host specificity of the bacterial

communities has also been observed across a broad range of amphibian species (Kueneman *et al*., 2013; Walke *et al*., 2014; Belden *et al*., 2015; Rebollar *et al*., 2016; Hernández-Gómez *et al*., 2017; Prado-Irwin *et al*., 2017). Interestingly, from a broader scale, relatively little variation in the fungal communities we studied was explained by the biogeographic region of the amphibian species. This suggests that amphibian skin, which contains a combination of mucopolysaccharides, proteins and peptides, might provide a unique habitat for some fungal taxonomic groups in particular (i.e. habitat filtering). This observation is further supported by the pattern observed in amphibian bacterial communities, where the same phyla dominate the bacterial portion of the skin microbiome of amphibians from distinct regions of the world (Kueneman *et al*., 2013; Belden *et al*., 2015; Sabino-Pinto *et al*., 2016; Bletz *et al*., 2017).

Microbial interactions can also influence the subset of microbes that colonize and constitute a host-associated community. In fact, these interactions are generally considered as a deterministic process shaping the diversity and structure of symbiotic communities (Adair and Douglas, 2017), specifically via positive, negative or neutral feedbacks on the microbial species involved in them (Faust and Raes, 2012; Adair and Douglas, 2017). Based on our observed correlation between the composition of the bacterial and fungal communities, and between bacterial and fungal OTUs, our results suggest that cross-domain interactions might be occurring on the skin of amphibians, which is consistent with a previous study (Kueneman *et al*., 2015).

While bacteria and fungi can be involved in antagonistic interactions, these organisms also engage in many positive interactions (Artursson *et al*., 2006; Wargo and Hogan, 2006; Frey-Klett *et al*., 2007). For instance, bacterial products can stimulate fungal growth (e.g. auxofuran; Riedlinger *et al*., 2006), can activate genes involved in the production of fungal secondary metabolites (Schroeckh *et al*., 2009), and promote fungal survival (e.g. through protection against antibiotics; Wargo and Hogan, 2006). With this in mind, the high percentage (79%) of positive correlations between bacterial and fungal OTUs in our study suggests that these positive interactions could occur in the amphibian skin microbiome. However, experimental work manipulating the composition of mixed cultures will be required to validate the biological meaning of our observed cooccurrences. Our results suggest that bacteria–fungi interactions might add another layer of complexity to studies focusing on the mechanisms shaping skin community assembly and overall function against Bd, especially considering that Kearns *et al*. (2017) found that a higher proportion of the amphibian skin fungal community inhibited the growth of Bd compared to that of the bacterial community. In addition, future research using a metagenomic or transcriptomic approach could identify genes and metabolic pathways potentially involved in host–fungi interactions (Sharpton, 2014), which would help to elucidate to what degree the amphibian skin fungal communities are symbiotic.

Experimental procedures

Sample collection

We used previously extracted DNA from skin swabs (N = 85) from adults of eight amphibian species – four species from a temperate region and four from a tropical region (Table 1) – and characterized the fungal communities associated with their skin. In addition, to explore potential relationships between the composition of the fungal and bacterial communities, we used a subset of the samples (N = 63, Table 1) for which, with some exceptions, we had previously published bacterial 16S rRNA gene amplicon sequencing data (Belden *et al*., 2015; Walke *et al*., 2015).

The selection of samples aimed to capture a diversity of host species and to examine both Bd− and Bd+ individuals. The infection status used for the selection of samples (total Bd− individuals = 49, and Bd+ = 36) was based on prior results from Taqman real-time PCR [qPCR] (Hughey *et al*., 2014; Rebollar *et al*., 2014). However, there were slight discrepancies in the detection of Bd between prior qPCR results and the amplicon sequencing of the ITS1 region that we performed for this study. Thus, in our final analysis, the infection status and load were based on the rarefied OTU table from the amplicon data in order to stay consistent with the community data produced by amplicon sequencing, and in recognition of the possibility that the detection of Bd sequences might directly influence further statistical analysis. This resulted in small changes in the number of samples of Bd− and Bd+ within each study species (Table 1, total Bd− individuals = 46, and Bd+ = 39).

Individuals were initially sampled with skin swabs for bacterial community analysis and Bd detection between 2010 and 2013, during the spring and summer for the temperate species and during the rainy season for the tropical species. The skin swabs for all species were obtained using the same standardized swabbing technique (Hughey *et al*., 2014; Rebollar *et al*., 2014). Animal use was approved by the Institutional Animal Care and Use Committees of Virginia Tech and the Smithsonian Tropical Research Institute.

The amphibian species used in this study vary in their natural histories and reproductive modes. For instance, the species from the temperate region breed in ponds, which are permanent in the case of *Rana catesbeiana* and *Notophthalmus viridescens* or can also be ephemeral

in the case of *Pseudacris crucifer* and *Anaxyrus americanus*, with the latter species using more terrestrial habitats after metamorphosis. Of the species from the tropical region, *Agalychnis callidryas* and *Dendropsophus ebbraccatus* are arboreal species that breed in ponds, whereas *Silverstoneia flotator* and *Craugastor fitzingeri* are terrestrial species that can be found in the forest along streams and have terrestrial eggs that in the case of *C. fitzingeri* hatch into froglets.

Sample and sequence data processing

DNA from the skin swabs was extracted following the manufacturer's protocol of the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) including an initial lysozyme incubation step at 37 °C for 1 h, as described in previous studies (Hughey *et al*., 2014; Walke *et al*., 2015; Medina *et al*., 2017). The extracted DNA was used as template for the detection and quantification of Bd mentioned earlier, and to conduct amplicon sequencing to determine the skin bacterial (previously done for a subset of the samples) and fungal (in the present study, with samples prepped in 2015) communities.

The assessment of the skin fungal communities was conducted using amplicon sequencing of the ITS1 region with primers ITS1F and ITS2 (Bellemain *et al*., 2010). Primers included adapters and 2 bp linker sequences, and the reverse primer also included a unique 12 bp barcode sequence (Bellemain *et al*., 2010). PCR reactions (25 μl) were prepared with 13 μl of PCR water (Mo Bio Laboratories, Carlsbad, CA), 10 μl of 2.5× 5Prime HotMastermix, 0.5 μl of each primer (10 μM concentration), and 1 μl of template DNA. PCR reactions were conducted using a thermal cycling protocol with an initial temperature of 94 °C for 5 min, 35 cycles of 94 °C for 45 s, 50 °C for 45 s and 72 °C for 90 s, and a final extension at 72 °C for 10 min. Samples were run in triplicate and with a negative control, which lacked template DNA. PCR amplicons from triplicate reactions were pooled and visualized on 2% agarose gels. PCR amplicons were purified using the UltraClean PCR cleanup kit (Mo Bio Laboratories, Carlsbad, CA). Amplicon concentration was quantified using a Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA) following the manufacturer's protocol prior to pooling in equimolar ratios into a composite sample. Fungal amplicons were sequenced on an Illumina MiSeq instrument using a 250 bp paired-end strategy at the Virginia Tech Biocomplexity Institute Sequencing Facility.

Sequencing reads associated with the skin fungal communities were processed as in Sun *et al*. (2017). Sequences were quality filtered and processed using the USEARCH pipeline (Edgar, 2010). Forward and reverse sequences were assembled based on a cut-off of at least 150 bp overlap. Assembled sequences were filtered based on a minimum length of 200 bp and an expected error per read lower than 0.5, which was calculated based on the quality score. Identification and removal of chimera sequences were conducted with UCHIME (Edgar *et al*., 2011), and sequences that matched PhiX (∼500,000), added to increase base diversity in Illumina sequencing, were removed from the dataset using Geneious (Biomatters, Ltd., version 8.1.8). Final sequences were assigned to operational taxonomic units (OTUs) based on a 97% similarity cut-off using the UCLUST method (Edgar, 2010), and aligned against the UNITE database (Koljalg *et al*., 2013). Taxonomy was assigned using the RDP classifier (Wang *et al*., 2007). The resulting OTU table had 2 390 653 sequences, with a sequencing depth per sample that ranged from 1892 to 84 927 (mean sequences/sample: 28 125). The OTU table was rarefied to 1850 reads/sample. This final dataset comprised 4130 fungal OTUs across all samples, and the number of fungal OTUs per sample ranged from 18 to 303 (mean ± SD = 85 ± 74). ITS amplicon sequences were deposited in the NCBI SRA database under the Bioproject PRJNA516413.

In this study, we also aimed to identify relationships between the compositions of the fungal and bacterial communities. For this analysis, we used a subset of the samples included in the fungal dataset (N = 63, Table 1). The bacterial data were compiled from 13 different prior Belden Lab Illumina Mi-Seq 16S rRNA gene amplicon sequencing runs using the QIIME pipeline. The *N. viridescens* samples and one *R. catesbeiana* sample included in the fungal community analysis were never processed in a 16S rRNA gene amplicon sequencing run, and thus they were excluded from this bacterial dataset. All bacterial samples were prepped for sequencing following our standard and published protocol (e.g. Belden *et al*., 2015; Walke *et al*., 2015). The samples were all sequenced at the Dana Farber Cancer Research Institute at Harvard University, Boston, MA, with 10% PhiX added to increase base diversity. Samples were run using a 250 bp or 300 bp single or paired-end strategy. Because of this variation, for the present study, we used only the forward reads of all samples. The bacterial raw Illumina 16S rRNA amplicon data were processed and quality filtered using QIIME (Caporaso *et al*., 2010). We used the default settings for demultiplexing of the forward reads except that we allowed for no errors in the barcode and we set the required phred score at 20. This resulted in a loss of the *S. flotator* samples, as the forward read quality was too low, which resulted in almost all the reads being too short for inclusion in the dataset following this quality filtering. In Geneious (Biomatters, Ltd., version 8.1.8), we trimmed all sequences to 250 bp and extracted those with 250 bp. The remaining sequences were used to create the OTU

table as described in Hughey *et al*. (2017). We rarefied all samples to a sequencing depth of 13 250, which resulted in the loss of one additional sample with low reads from the dataset. The final dataset consisted of 494 bacterial OTUs across 63 samples, with a range of 74–276 bacterial OTUs per sample (mean $\pm$ SD = 179 $\pm$ 45).

Data analysis

In addition to determining the taxonomic composition of the fungal communities associated with the skin of the host species, we assessed variation in alpha and beta diversity of the fungal communities across host species, region (temperate vs. tropical) and Bd infection status (+ or −). As there were differences in the communities among different hosts, we also identified the fungal OTUs driving those differences. Finally, we assessed the relationship between the composition of the fungal and bacterial communities, and whether positive or negative interactions might predominate between these groups. Unless noted, all statistical analyses were conducted using R version 3.3.3 (R Core Team, 2017).

Variation in fungal communities – alpha and beta diversity analysis. Alpha diversity comparisons across species, regions (temperate vs. tropical) and Bd infection status were conducted for richness and for the Shannon index transformed to Hill numbers (the effective number of species). Alpha diversity estimates were calculated for each sample using QIIME. To compare alpha diversity estimates of each metric across species, we fit the estimates to generalized linear models (GLMs). In this analysis, for richness, we used a negative binomial error distribution with the log link function to account for overdispersion, and the model was run using the function glm.nb from the package MASS (Venables and Ripley, 2002). For the Hill numbers calculated from the Shannon index estimates, we used a Gamma error distribution with the inverse link function and the model was run using the function glm from the R stats package. *P* values for the models were estimated by comparing nested models using a likelihood ratio test (Zuur *et al*., 2009; Belden *et al*., 2015). Visual assessments of residual plots with model predictions verified the appropriateness of the error distributions. Pairwise comparisons were conducted by performing Tukey tests using the function glht from the package multcomp (Hothorn *et al*., 2008), which includes multiple comparisons for generalized linear models.

Richness and Shannon index Hill numbers were also compared between regions (tropical vs. temperate). For this analysis, we fit the diversity estimates to generalized linear mixed models (GLMMs), where the random factors in the model consisted of a random effect of 'Site' crossed with a random effect of 'Species'. The error distributions mentioned above for richness and Shannon index Hill numbers were used to construct the models. The GLMMs were run using the functions glmer.nb and glmer for richness and Shannon index Hill numbers, respectively. Both of these functions are from the package lme4 (Bates *et al*., 2014). *P* values for the models were estimated as described above.

An assessment of a potential correlation between alpha diversity and Bd infection load (represented, at the sample level, by the total number of reads summed across the 23 fungal OTUs identified as Bd) was conducted using a generalized linear model (GLM) for richness and a linear model for Shannon index Hill numbers. For richness, the model contained the error distribution and link function described above and used the function glm.nb from the MASS package (Venables and Ripley, 2002). In the case of Shannon index Hill numbers, the diversity estimates were log-transformed to better meet the assumptions of normality and for better model fit, and the model was run using the lm function from the R stats package. The small number of samples containing a high number of Bd reads (> 500 reads) relative to the number of samples with low numbers or no reads limited our ability to account for the random effect of the variables 'Species' and/or 'Site' in these models.

For beta diversity, we assessed the changes in the structure of the fungal communities across host species, regions (temperate vs. tropical) and Bd infection status. We calculated dissimilarity distances across samples based on Bray–Curtis (relative abundance) and Jaccard (presence/absence) dissimilarities. However, given consistent results using either distance metric, we only included the Bray–Curtis results. Statistical comparisons across variables were conducted with permutational multivariate analyses of variance (PERMANOVA; (Anderson, 2001). When comparing the changes in community structure between Bd-infected and non-infected individuals, the argument 'strata' was used to define the group within which to limit the permutations (i.e. amphibian species) in the PERMANOVA, due to the nestedness of the samples within species. In addition, to determine whether the variation in community structure between Bd-infected and non-infected individuals were driven by Bd OTUs, we conducted a second analysis that excluded all Bd OTUs from the dataset. Dissimilarity distances were calculated using the function vegdist and the PERMANOVAs were conducted with the function adonis, both from the vegan package (Okansen *et al*., 2016). Variation in community structure across variables was visualized using principal coordinates analyses (PCoA), with the function cmdscale, also from the vegan package.

Identification of abundant and host-specific fungal OTUs – rank abundance curve and species indicator analysis. To identify the most abundant fungal OTUs

based on their relative abundance, we calculated a rank abundance curve. An OTU rank abundance curve depicts the OTUs in order of absolute or relative abundance, specifically, from the highest rank to the lowest, where the most abundant OTU gets the highest rank and the least abundant gets the lowest rank. Given the high number of OTUs in the dataset with a low relative abundance, we performed a rank abundance curve using only the OTUs with a minimum relative abundance higher than 0.05 in at least one sample (199 OTUs). The rank abundance curve was calculated using the function radfit from the package vegan (Okansen *et al*., 2016).

To identify fungal OTUs associated with specific amphibian species, we conducted a species indicator analysis using the function multipatt from the indicspecies package (De Cáceres and Legendre, 2009). This analysis calculates an indicator value that quantifies the association between OTUs and amphibian species, based on the relative abundance and relative frequency of each OTU, and uses a permutational approach to calculate the significance of the association based on an alpha value of 0.05 (De Cáceres and Legendre, 2009).

Exploring bacteria–fungi relationships. Using the subset of 63 samples for which we had bacterial 16S rRNA gene amplicon data, we assessed the potential relationship between the composition of the fungal and bacterial communities. We used a Mantel test based on Jaccard dissimilarities to test for a correlation between the composition of the fungal and bacterial communities using the function mantel from the vegan package (Okansen *et al*., 2016). Jaccard dissimilarities were used because we could not combine bacterial and fungal relative abundance data in a single dataset, so we converted both datasets to presence/absence metrics for the combined analysis. The Mantel test calculates a Pearson's correlation coefficient (i.e. r_M) that ranges from −1 to +1, and tests for statistical significance of the correlation using a permutational approach.

Potential interactions among bacterial and fungal OTUs were assessed with a cooccurrence network analysis. For this analysis, we combined the non-rarified bacterial and fungal datasets for the subset of 63 samples where both were available, which resulted in an OTU table with a total of 4391 OTUs. Given the decrease in sensitivity to detect correlations as zero counts increase (Weiss *et al*., 2017), we removed the OTUs present in less than 10% (six) of the samples (removed 3685 OTUs) and the OTUs with less than a total sum of 10 reads across all the samples (removed 29 OTUs). The final OTU table had 677 OTUs. The correlations among OTUs were calculated using the default parameters of the method SparCC (Sparse Correlations for Compositional data; Friedman and Alm, 2012) in Python (version 2.7.10). This method uses a permutation-based approach (100 permutations in this case) to calculate bootstrapped pseudo *P* values for each pairwise correlation. Only significant correlations (alpha value = 0.05) with values smaller than −0.35 and larger than 0.35 were used (Kueneman *et al*., 2017). Results were visualized using the igraph package (Csardi and Nepusz, 2006).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Results of *post hoc* comparisons of the skin fungal communities across amphibian host-species based on alpha diversity metrics: richness and Shannon index transformed to Hill numbers.

Table S2. Bd zoospore genomic equivalents based on qPCR, number of Bd sequences based on ITS amplicon sequencing and relative abundances of Bd per sample. This table includes all samples Bd+ based on amplicon sequencing, and that were used in the study. Samples within species are listed in a decreasing order based on Bd relative abundance.

Table S3. List of Bd OTUs and matches from the NCBI nucleotide database using BLAST (based on 80% query coverage and 95% identity cutoffs).

Figure S1. Fitted second order polynomial regression (adjusted-R^2 = 0.61, $P < 0.001$) showing the relationship between the number of zoospore genomic equivalents estimated by qPCR and the number of Bd amplicon reads determined by Illumina sequencing. Each point represents a Bd+ sample.

Figure S2. Heatmap of log-transformed relative abundances of the Bd and other fungal OTUs identified in the study. Rows indicate each OTU, and includes the ID of the Bd OTUs and the taxonomic classification at the family (f) or genus (g) level of the other fungal OTUs. Columns indicate the Bd+ individuals grouped by species.