Habitat-host Microbial Associations Across a Gradient in Land Use Intensification in Southern Amazon

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Habitat-host microbial associations across a gradient in land use intensification in Southern Amazon

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ABSTRACT

The conversion of natural habitats to agriculture results in the structural, physical and chemical degradation of ecosystems; and microbial communities respond to change with shifts in diversity and environmental function. Because the structure of amphibian-associated microbiome depends partially on the dynamics of microorganisms in the habitat, we hypothesized that land use would affect the tadpole skin microbiota structure of populations inhabiting water bodies in agricultural lands. To study this, we sampled microbial communities of water bodies and skin of larval amphibian across a gradient of land use intensification represented by cerrado, pastures and soybean fields. We used 16S rRNA high-throughput gene sequencing to characterize the microbial communities. Land use had a strong effect on both host and habitat bacterial communities. Bacterial ASVs richness and diversity in water bodies decreased from pristine habitat to soybean plantations, with the cerrado community differing from pasture and soybean fields. The aquatic microbial community composition and structure were different across the gradient, showing a robust effect of land use on this habitat. The richness and diversity of amphibian-associated bacterial community was lowest in cerrado and highest in pasture populations. The soybean plantation exhibited the most distinct composition and structure of amphibian microbiota while the pasture and cerrado communities were similar. Bacterial ASVs, candidates for biomarkers of the land use effect on both host and water bodies communities, were indicated. Our results highlight the effects of land use intensification as a driver for amphibian microbiome and offer information on the functionality of agro-industrial environments.

INTRODUCTION

Many amphibians have characteristics that make them particularly sensitive to environmental changes, which is why they are often proposed as indicators of habitat quality [1]. These vertebrates are characterized by a humid skin in which they shelter a microbial community in a commensal relationship. Hosted microorganisms seem to benefit from the moisture and nutrition produced by the host's dermal glands and help prevent colonization by foreign microorganisms [2]. A general profile of amphibian skin microbiota is given by the host's taxonomic position, but the environment also contributes to composition and maintenance [3].

Given the environmental transmission of bacteria, the dynamics of microorganism populations in a given habitat is consequential for the amphibian skin microbiota [4], possibly affecting health-related issues. For example, certain physiological disorders are associated with disturbances of the natural structure of the microbiome (Dysbiosis [5]). Changes in the structure of the cutaneous microbiota of the amphibian can lead to greater susceptibility to chytridiomycosis, a major disease in this group [6].

Considerations about environmental influences on skin microbiota must include the impact of ecosystem degradation because this process can modify pathogen-host interactions. This may happen because the habitat degradation causes the loss of regulation of population abundances, which is made by the local biological diversity [7].

For example, the conversion of native habitats into agricultural areas involves transforming areas of diverse vegetation physiognomies, such as savanna and forest, into homogeneous herbaceous counterparts. Land use alters the physical (abiotic) conditions of the environment to which individual amphibians are exposed, expectedly influencing both host ecological performance, the dominant microbial communities, and the interaction among these two components. Relevant variables include, among others, incident solar radiation, temperature, dissolved oxygen, pH, conductivity, water hardness, turbidity, nutrients, and pesticides [8–10]. Soil bacterial communities clearly respond to changes in land use with shifts in composition and abundance [11]. An important consequence of changes in the structure of soil microbial communities is the alteration of their ecological functions, as carbon and nitrogen cycling [12]. However, little is known about the consequences of such shifts in the environmental microbial communities on vertebrates.

Soybean plantations and cattle pastures are widespread agro-industrial activities in Brazil, and involve not only drastic land management but also agrochemical use and soil degradation [9]. Pasture has a lower environmental impact than soybean fields and the conversion of pastures to soybean fields usually involves fire, tilling and liming [9]. Following conversion, yearly land management involves sowing, applying fertilizers and pesticides. Although the response of the environmental microbiome to the impact of forest-pasture and forest-soybean conversions is

poorly understood, it does seem that the implementation of pasture tends to homogenize soil microbial communities [13]. The conversion of Amazon rainforest into large-scale soybean plantation promotes changes in the abundance and composition of soil bacterial community [14]. The responses of amphibians to such changes could be anticipated as drastic; indeed, many species and populations are intolerant of human-dominated landscapes. However, there are also resilient species, able to thrive in harshly modified environments [15]. The Amazonian Arc of Deforestation poses yet one additional question because it is rainfed. In other words, most land management practices coincide with amphibian reproduction so that larval phases occur in severely affected water bodies. Pesticides can affect larval amphibian physiology, behaviour, development and survivorship [16].

The skin microbiota of amphibians is part of the environment-organism interface and a recent review argues that the disruption of microbial diversity associated with vertebrates is a "serious threat to wild populations" and should be recognized as an "essential component" of conservation practices and management practices [17]. A previous study showed that the agriculture implementation may disrupt the gut microbiota of amphibians [18]. This reveals a strong influence of the environment on the amphibian microbiome, given that the intestinal microbiome tends to have greater resilience and stability, due to the selective chemical environment [19].

Some main premisses validated by current literature are a) the amphibian skin microbiota is partially dependent on environmental microbiota; and b) the environmental microbial communities are affected by the conversion of native habitats to agriculture. Based on these premisses, this study investigates how a context of land use intensification influences microbial communities associated to amphibians. We hypothesize a link between land use effects and amphibian skin microbiota structure, and assume that such a link would be corroborated by effects on the skin microbiota along a gradient of environmental degradation. We focused on land use in soybean fields and pastures within the Cerrado domain, the original habitat [9] , and compared the skin microbiota of tadpoles in water bodies along a land-use gradient. This is the first study to test the effect of environmental changes on the amphibian host microbiome in a gradient of land use intensification; it also provides information on the functionality of agro-industrial environments linked to microbial communities associated with amphibians.

METHODS

Study region

This study was conducted in the Upper Xingu River Basin in the townships of Canarana, Querência and Água Boa, State of Mato Grosso, Brazil. It is a region of socio-environmental relevance where 20 Indigenous Lands and 10 Protected Areas are in contact with an aggressive

frontier of agricultural expansion known as the ´Amazonian Arc of Deforestation´ [20]. Land use is dominated by extensive cattle ranching and intensive grain production, notably soybean [9, 20]. Apart from protected areas, fragments of native vegetation are found within private land under the protection of the Brazilian Forest Code (Law 4771/1965). Original vegetation cover is a closed-canopy, evergreen seasonal forest that is transitional between the ombrophilous rainforests in the north and the cerrados in the south of the Xingu Basin [21].

In Água Boa, fieldwork was conducted at Campo Alegre Ranch (14˚04'43.52'' S, 53˚01'19.60'' W), an 85,000-ha ranch that comprises ~30,000 ha of pastures and 55,000 ha of cerrado sensu stricto, i.e., an open-canopy shrubby savanna. For local standards, Campo Alegre Ranch is considered a semi-intensive cattle production system, with a stocking density of one head/ha. In Querência and Canarana, fieldwork was conducted at São Luis Ranch (12°41'29.6"S, 52° 23'04.6"W), an intensive soybean producer, and in the INCRA (National Institute of Colonization and Agrarian Reform; 13˚08'19.5" S, 52˚20' 50.4" W) settlement, where cattle are raised in a series of adjacent small properties totalling ~3,500 ha.

Host species

The snouted treefrog *Scinax fuscovarius* (Anura: Hylidae) was selected as host species in the present study. *S. fuscovarius* is widespread in southern, south-eastern and central Brazil as well as in eastern Bolivia, eastern Paraguay, northern Argentina, and northern Uruguay. The species is an open area generalist found in habitats ranging from preserved savannas and shrublands to pastures, crop fields and cities [22]. Its widespread distribution, local abundance and evident tolerance to environmental conditions makes *S. fuscovarius* an ideal model species for assessing how environmental change affects patterns of association among habitat, host and microbial communities. The species presents the typical biphasic pattern of amphibian development with aquatic eggs and larvae and terrestrial adults. We focused on larval phases because water bodies are discrete, clearly defined, and relatively homogeneous habitat patches.

Sampling design

The gradient in land use intensification in our study was represented by a Cerrado *stricto sensu* (i.e., a shrub savanna), two pastures, and one soybean field. Sampling occurred between January and February 2014. Sampling of the larval amphibian skin microbiota was replicated at two levels. First, multiple tadpoles were sampled in each waterbody. The actual number of tadpoles sampled per waterbody depended on availability at the time of collection but was usually > 10 (see below). Second, multiple (>5) waterbodies were sampled in each land use. Waterbodies were ponds and puddles covering a surface area of tens to a few hundred square meters (Figure S1, Supplementary material). Previous studies demonstrated that waterbodies < 120 m^2 in analogous agricultural landscapes are embedded in the terrestrial environment and therefore under

192 the direct effect of land management practices [10]. Waterbodies in each land use were randomly
193 selected among those found to contain *S. fuscovarius* larvae in a pilot survey. We sampled 51
194 tadpoles in 6 waterbodies in the cerrado (8.5 ± 1 individuals per waterbody), 68 tadpoles in 5
195 waterbodies in the pasture at Água Boa (13.6 ± 7.5), 109 tadpoles in 6 waterbodies in the pasture
196 at Canarana (18.2 ± 5.3) and 117 tadpoles in 6 waterbodies in the soybean plantation (19.5 ±
197 10.2). Water was collected from all water bodies the same day.
198 We decided to sample two pastures – at Canarana and Água Boa – because of the
199 biogeographic scenario of our study region, which is at the edge of the Amazon Forest. *S.*
200 *fuscovarius* is an open-area species that does not occur in closed-canopy forests. Therefore, the
201 appropriate reference condition for *S. fuscovarius* and associated microbiota is the cerrado, whose
202 northernmost limit starts ~20km south. Due to extensive land cover change in the region, a
203 preserved cerrado patch was found in Água Boa, which is ~100km south. Pastures are a land use
204 type that is common to both localities and therefore were used as a control for regional effects on
205 bacterial communities.
206
207 **Microbiota sampling**
208 Sterile gloves and one disinfected aquarium net, per water body, were used to capture and
209 handle *S. fuscovarius* tadpoles, which were put in sterile Whirl-Pak® (Nasco) bags for
210 transportation. The skin microbiota was sampled after rinsing the animals individually in 30 ml
211 Milli-Q water to remove the transient microbiota. For each tadpole, a swab was passed five times
212 along the lengths of the dorsum and venter, placed in 1 ml GTE buffer (20% Glucose – 1 M Tris-
213 HCL pH 7.4 – 0.5 M EDTA pH 8.0), and stored immediately in a freezer at − 20°C. The time
214 between amphibian capture and microbiota sampling did not exceed one hour.
215 Sterile Whirl-Pak® bags (Nasco) were used to collect 1L of pond water at a depth of 0-
216 10, immediately after the capture of tadpoles. Pond water was transported to the laboratory and
217 filtered a 0.20 µm pore cellulose acetate filter (Sartorius Stedim Biotech) using a vacuum pump.
218 Membranes were individually preserved in a GTE buffer solution and stored in a freezer at -20 °
219 C. The time between the collection of water and its total filtration was no longer than three hours.
220
221 **DNA extraction e 16s rRNA high-throughput sequencing**
222 The total DNA of each microbial community sample (water or amphibian skin) was
223 extracted using the Power Soil ™ DNA Isolation kit (MoBio Laboratories), following
224 manufacturer's instructions. After extraction, the DNA of all individual skin microbiota of a given
225 waterbody was pooled in a composite sample. This resulted in one pair of microbial samples per
226 waterbody, one composite tadpole microbiota total DNA sample and one water microbiota total
227 DNA sample for each waterbody. The hypervariable region V4 of the 16s rRNA gene (515F,
228 806R) was amplified using the primers: F: GTGYCAGCMGCCGCGGTAA / R:

GGACTACNVGGGTWTCTAAT. The libraries were prepared using the Earth Microbiome Project protocol (www.earthmicrobiome.org) but using 20 μL PCR reactions and dual barcodes instead. The sequencing was done using Illumina MiSeq platform (2 × 300 bp paired-end sequences), 100k reads / sample.

Sequence Analyses

Raw reads were processed using the DADA2 pipeline version 1.16 [23] in the software Microsoft R Open v 4.0.0 [24]. Briefly, the reads were denoised, filtered and clustered as Amplicon Sequence Variants (ASVs) based on the DADA2 algorithm [23]. Reads were truncated at 160 and 150 positions, based on the quality profile observation, using the function plotQualityProfile. After quality filtering, the average read depth was 12,395.6 ± 4,184.6 across samples. Taxonomy was assigned to the ASVs table using the Naïve Bayesian classifier [25] comparing against SILVA reference database v138 [26]. The ASVs sequences were aligned in the platform Qiime 2 v2020.2 [27], using the MAFFT algorithm [28]. The phylogenetic tree was built up with FastTree [29]. ASVs classified as Archaea, chloroplast, mitochondria, or with less than 1000 reads, were discarded, using the Phyloseq v1.32.0 package [30].

Data Analyses

Data analyses were done in the software Microsoft R Open v 4.0.0 [24]. Alpha diversity was assessed with package BAT – Biodiversity Assessment Tools – v 2.1.1 [31]. Differences in microbial alpha diversity according to land use and microbiomes – tadpole skin or water body – were tested using the function anova followed by Tukey HDS pairwise comparisons, with significance level at 0.05: packages vegan and agricolae, respectively [32, 33]. Tests on alpha diversity were conducted after rarefaction by the minimum ASV abundance among all samples.

Analyses of microbial community composition and structure were based on two data transformations. The Compositional Data (CoDa) transformation converts the ASV read count data into compositional data and is based on a centered log-ratio transformation [34]. The codaSeq.clr function was used to run CoDa transformation in the CoDaSeq v0.99.6 package [35]. The Phylogenetic Isometric Log-Ratio (PhILR) transformation consists in an Isometric Log Ratio transform combined with the phylogenetic tree to provide evolutionary information. It is based on the phylogenetic relationship among the ASVs, which is equivalent to the Unifrac metric, but considering the compositional nature of the data [36]. To run PhILR the function philr was used in the Philr v1.14.0 package [36]. Neither of the procedures accept zero values, so a pseudocount of one was previously added. Using CoDa and PhILR transformations, data rarefaction becomes unnecessary [34].

Data transformed by CoDa and PhILR methods were used to build the Euclidean distance matrices and run the Principal Component Analysis (PCA) to visualize the relationships

among samples, using Phyloseq v1.32.0 package [30]. Differences in bacterial community composition and structure among the three land uses and between aquatic and host microbiome were tested by Permutational Multivariate Analysis of Variance (PERMANOVA), using distance matrices, in package vegan with the adonis2 function [32]. Pairwise comparisons between the groups were conducted using PERMANOVA with the adonis.pair function in the EcolUtils v0.1 package [37]. For these analyses, the Euclidean distance matrices obtained by the CoDa and PhILR data transformation were used.

The Random Forest algorithm was used to identify the best predictors, i.e., ASVs, explaining differences observed between groups (i.e., between habitat and host, and among land uses; [38]. This classifier estimates the importance of each predictor based on the Gini criterion, using the best abundance threshold among a subset of predictors randomly selected. Random forest is a machine learning method that consists in creating a set of decision trees based on training data. For that, 100 decision trees were performed by each analysis using the default settings of the randomForest v4.6-14 R package [39]. The importance of each ASV was ranked using the Mean Decrease Gini values and the first highest values were plotted and identified taxonomically.

RESULTS

Land use effects on habitat microbiota

Land use had an effect on the richness ($P = 0.0001$; ANOVA) and diversity ($P = 0.0002$; ANOVA) of microbes inhabiting water bodies occupied by *S. fuscovarius*. ASVs richness and diversity declined from Cerrado to pastures and to soybean fields, although the latter two were not different from each other (Table 1). By contrast, no differences among land uses were observed in the phylogenetic diversity metric ($P = 0.072$; ANOVA).

Land use had, in addition, effects on water body bacterial communities both in terms of taxonomic (Adonis - CoDA, F = 3.98, $R^2 = 0.31$, $P = 0.001$; Figure 1a) and phylogenetic composition and structure (Adonis - PhILR, F = 3.44, $R^2 = 0.27$, $P = 0.001$; Figure 1b). In both cases, pairwise comparisons indicated that all land uses were different from each other (Table 2). When analysing Canarana and Água Boa pastures separately (Table S1, Supplementary material), microbial communities in cerrado and pasture water bodies were globally similar to each other but distinct from those in soybean fields; bacterial communities from the two pastures were different from each other in taxonomic (i.e., CoDA) but similar in phylogenetic (i.e., PhILR) community composition and structure.

The random forest analysis indicated 7 bacterial phyla, from 20 ASVs, as the most important predictors to distinguish the water body microbial communities among land uses. Those with highest abundance in cerrado the cerrado were Acidobacteriota, Actinobacteriota,

Bacteroidota and Proteobacteria. The total abundance of these phyla was 4 and 2-fold their abundance in pastures and the soybean field, respectively. Among them, Acidobacteriota was absent in soybean fields, as predictor. By contrast, phyla Bdellovibrionota, Cyanobacteria and Verrucomicrobiota were more abundant in soybean fields, corresponding to a total abundance 10 and 3-fold their abundances in pastures and the cerrado. Cyanobacteria was absent in cerrado and pasture, as predictor (Figure 2a).

Land use effects on host microbiota

Land use had an effect on the richness (p = 0.014; ANOVA) of bacteria colonizing *S. fuscovarius* skin. Larvae from the cerrado and pastures had respectively the lowest and highest ASV richness; richness from larvae from soybean fields was not different from that of other land uses (Table 3). Land use also had effect on the diversity (P = 0.013; ANOVA) of *S. fuscovarius* bacterial community; been more diverse in pastures than in the cerrado or the soybean field, which did not differ from each other. No effect of land use was observed for phylogenetic diversity (P = 0.070; ANOVA).

Land use also had an effect on the taxonomic (Adonis - CoDA, F = 2.71, R^2 = 0.24, P = 0.001; Figure 3a) and phylogenetic (Adonis - PhILR, F = 3.19, R^2 = 0.27, P = 0.001; Figure 3b) composition and structure of bacterial communities hosted by *S. fuscovarius*. Pairwise comparisons demonstrate that, in both cases, soybean communities stand out from pastures and the cerrado, which do not differ from each other (Table 4). When analysed separately, the skin bacterial communities from Canarana and Água Boa pastures could not be differentiated from each other by either the CoDa or PhILR metrics. The general pattern of soybean microbiota differing from those of the cerrado and pastures was maintained (Table S1, Supplementary material).

The 20 most important ASVs explaining the observed effects of land use on the *S. fuscovarius* skin microbiota belonged to 6 bacterial phyla. Among these, Proteobacteria predominated in the host microbiota of the cerrado population, with a 4 and 23-fold increase in abundance relative to pastures and soybean fields, respectively. Phyla Bacteroidota, Desulfobacterota and Cyanobacteria were more abundant in the soybean field. Together, these phyla showed an 8 and 90-fold increase in abundance in soybean fields relative to pastures and cerrado, respectively. Elusimicrobiota and Firmicutes were more abundant in the pasture, with total abundances 9 and 80-fold higher than in the soybean field and the cerrado (Figure 2b).

Comparison between host and habitat microbiota

The microbiota hosted by *S. fuscovarius* differs from the microbiota of their habitat (i.e., the water body). The community analysis revealed that the host and habitat bacterial communities are distinct both in terms of taxonomic (Adonis - CoDA, F = 12.3, R^2 = 0.24, P = 0.001; Figure

4a) and phylogenetic composition and structure (Adonis - PhILR, F = 16.36, $R^2 = 0.29$, $P = 0.001$; Figure 4b).

DISCUSSION

The patterns found in this research indicate that anthropogenic changes of habitat may influence the amphibian-associated skin microbiome. Our reported changes along a gradient of environmental degradation include diversity, structure and composition of microbial communities. The results highlight the effects of land use intensification as a driver for shifts in host microbiomes. We also report drastic results for water bodies, which suggests that impacts may be stronger in aquatic microbial communities than host associated communities.

The literature reports that land transformation into agricultural systems often increases the bacterial alpha diversity and changes the composition of soil microbiome [11]. We show a reduction in water bacterial alpha diversity, in ponds and puddles of agricultural lands. Importantly, the water bodies samples lacked sediments, so that results may be context specific. Independently of this, we assume that changes in the diversity, composition and structure of microbiota in water bodies relate to changes in the physicochemical and biological conditions across environmental gradient. A previous study indicated that biological water bodies properties – as conductivity and turbidity – and the communities of algae, tadpoles, predators and fish are consistently affected by intensity of land use [10]. We observed differences in environmental variables; soybean plantations water bodies had a distinct pattern compared to the water bodies from pastures and cerrado. The latter two were similar for the same variables (Table S2, Supplementary material).

The skin microbiota of *S. fuscovarius* larvae may be shaped by variables intrinsic to individuals, including physiological traits affecting the skin milieu [40]. However, these traits may modulate skin microbiota within the major microbiome shifts that seem induced by habitat modification due to agricultural practices. Pastures display higher local diversity and richness of amphibian bacterial community compared to soybean fields, which maintain the richness and diversity typical of preserved Cerrado. However, regarding the composition and structure, this pattern changes dramatically. We interpret these results as evidencing that soybean plantation produces the greatest changes in the microbiota of larval *S. fuscovarius*. It is not yet possible to generalize these findings, not even to compare with previous results, given the nature of our study design. Yet, the study by Lammel et al. [41] shows that the intensity of land use – based on pH, C, litter degradability, pesticides, and nutrient levels – determines the structure of soil bacterial community, in which the soil microbial community of soybean plantation presents the most distinct composition, compared to pasture and cerrado soil communities.

Exposure of the frog *Lithobates pipiens* to a broadspectrum sulfonamide antibiotic, commonly used in livestock, and deposited in pasture soils and aquatic systems, does not alter

alpha diversity, but does change the composition of the skin microbiota [42]. Also, and as already mentioned, the sediment of water bodies may be important. In aquatic systems, sediments act as repositories for materials from anthropogenic activities [43] and are a likely source for tadpole skin microbiota. Which could explain the parallel effects observed in soil [41] and amphibian microbiota in this study.

A collateral finding in this study involves a set of bacterial ASVs that may constitute proper biomarkers of the effect of land use on amphibian and water bodies microbial communities. The phylum Acidobacteriota has already been suggested as a bioindicator of the effects of agricultural management on Amazonian soils [14]. Acidobacteriota, Actinobacteriota and Verrucomicrobiota respond to management practices aimed at soil fertilization in sugarcane plantations [44]. Cyanobacteria is a phylum known as an indicator of freshwater quality, as it clearly responds, in terms of its diversity, to anthropogenic influences and agricultural management [45]. For example, increasing its abundance with phosphorus fertilizer applications [46]. Changes in abundances of members of Bacteroidota and Proteobacteria on amphibian skin were observed after exposition to sulfonamide antibiotic, used in livestock [42]. The prevalence of Firmicutes phylum in pasture soils is a common pattern, probably because of the high carbon availability and resistance to temperature variation and desiccation [47].

This study has demonstrated that the structure of *S. fuscovarius* tadpoles skin microbiota stands out from the microbiota of their habitat. The literature describes that on the skin of amphibians, peptides and microbiota act as complementary systems in their protection against pathogenic microorganisms [48]. These two systems are self-regulated and must act as a filter, preventing colonization by certain environmental microorganisms sensitive to the framework of molecules present on the skin [49, 50]. The findings of this study corroborate a view, in which the structure of the microbiota is given by the physical and physiological characteristics of the organism. Thus, the host species offers a microenvironment that may select microbes that can adapt to it [50].

In summary, the intensification of land use impacts environmental microbial communities, as observed in this and some previous studies, and these findings seem extendable to microbial communities associated with amphibians. In this context, the forest-soybean conversion, relative to forest-pasture counterparts, has greater local impact for amphibian microbial communities. We suggest that changes in habitat may directly affect the biology of amphibian populations; and shifts in host physiology could also modulates its microbiome. We do not address the consequences of observed changes in the amphibian skin microbiota, but we assume functional changes and interactions with host, rooting from shifts in the microbial communities. Therefore, environmental impact may change critical aspects of amphibian biology. Also, the study of microbial communities can lead to valuable indicators of the viability of ecosystems, agricultural productivity and human and animal health.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY

The 16S rRNA gene sequences were deposited at MG-RAST version 4.0.3 under the accession numbers mgm4921536. through mgm4921549.3

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FIGURE LEGENDS

Fig. 1 Principal Components Analysis (PCA) plots of water puddle microbiome by land use. The ordinations are based on Euclidian distance matrices for the taxonomic (a) and phylogenetic (b) bacterial composition; by CoDA and PhILR transformed data, respectively.

Fig. 2 Amplicon sequence variants (ASVs) selected by a Random Forest classification model as predictors best distinguishing water body (a) and *S. fuscovarius* (b) microbiota of cerrado, pasture and soybean fields. The heatmap displays the relative abundances of each predictive bacterial ASV across land uses

Fig. 3 Principal Components Analysis (PCA) plots of *S. fuscovarius* skin microbiome by land use. The ordinations are based on Euclidian distance matrices for the taxonomic (a) and phylogenetic (b) bacterial composition; by CoDA and PhILR transformed data, respectively.

Fig. 4 Plots of the tadpole skin and water microbiomes. Ordinations are Principal Component Analysis of Euclidian distance matrices for the taxonomic (a) and phylogenetic (b) bacterial composition; by CoDA and PhILR transformed data, respectively.

TABLES

TABLE 1 Alpha diversity of water puddle bacterial communities across land uses. Values are given as mean ± standard deviation. Comparisons by means of Tukey at significance level of 0.05.

	Land use		
Diversity metric	**Cerrado**	**Pasture**	**Soy**
ASV richness	224.5 ± 19[a]	149.5 ± 34.5[b]	138.1 ± 21.1[b]
Shannon	4.45 ± 0.2[a]	3.64 ± 0.36[b]	3.35 ± 0.42[b]
Faith's phylogenetic diversity	15.28 ± 1.01[a]	12.24 ± 2.51[a]	13.42± 2.48[a]

For each diversity metric, land uses with the same letter are not significantly different.

TABLE 2 Permutational multivariate analysis of variance (PERMANOVA) for testing the effect of land uses on water bodies microbiota taxonomic (CoDa transformed matrix) and phylogenetic (PhILR transformed matrix) composition.

	Forest <-> Pasture			**Forest <-> Soy**			**Pasture <-> Soy**		
	F	R^2	*P*-value	F	R^2	*P*-value	F	R^2	*P*-value
PhILR	2.903	0.182	0.024	6.122	0.404	0.012	2.619	0.157	0.018
CoDa	3.921	0.231	0.003	6.805	0.430	0.012	2.557	0.154	0.003

Obs.: Bonferroni-adjusted *P*-values for multiple comparisons.

TABLE 3 Alpha diversity of *Scinax* skin bacterial communities across land uses. Values are given as mean ± standard deviation. Comparisons by means of Tukey at significance level of 0.05.

	Land use		
Diversity metric	**Cerrado**	**Pasture**	**Soy**
ASV richness	136.2 ± 34.3[a]	232.8 ± 70.3[b]	186.7 ± 25.4[ab]
Shannon	3.49 ± 0.15[a]	4.17 ± 0.48[b]	3.46 ± 0.44[a]
Faith's phylogenetic diversity	14.42 ± 2.26[a]	18.64 ± 4.11[a]	15.58 ± 2.88[a]

For each diversity metric, land uses with the same letter are not significantly different.

TABLE 4 Permutational multivariate analysis of variance (PERMANOVA) for testing the effect of land uses on *S. fuscovarius* skin microbiota taxonomic (CoDa transformed matrix) and phylogenetic (PhILR transformed matrix) composition.

	Cerrado <-> Pasture			**Cerrado <-> Soy**			**Pasture <-> Soy**		
	F	R^2	*P*-value	F	R^2	*P*-value	F	R^2	*P*-value
PhILR	1.867	0.134	0.117	6.237	0.409	0.009	3.190	0.197	0.003
CoDa	1.958	0.140	0.066	5.086	0.361	0.006	2.503	0.161	0.003

Obs.: Bonferroni-adjusted P-values for multiple comparisons.

Figures

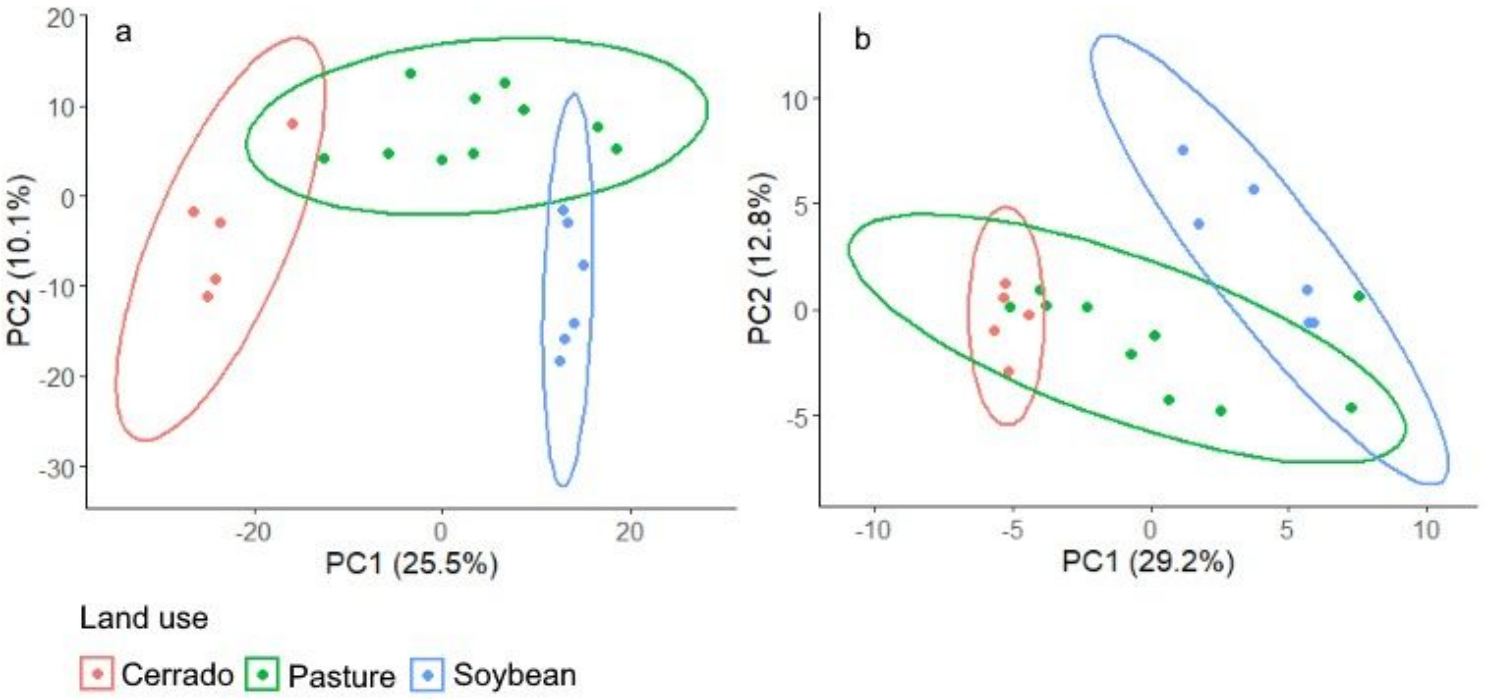

Figure 1

Principal Components Analysis (PCA) plots of water puddle microbiome by land use. The ordinations are based on Euclidian distance matrices for the taxonomic (a) and phylogenetic (b) bacterial composition; by CoDA and PhILR transformed data, respectively.

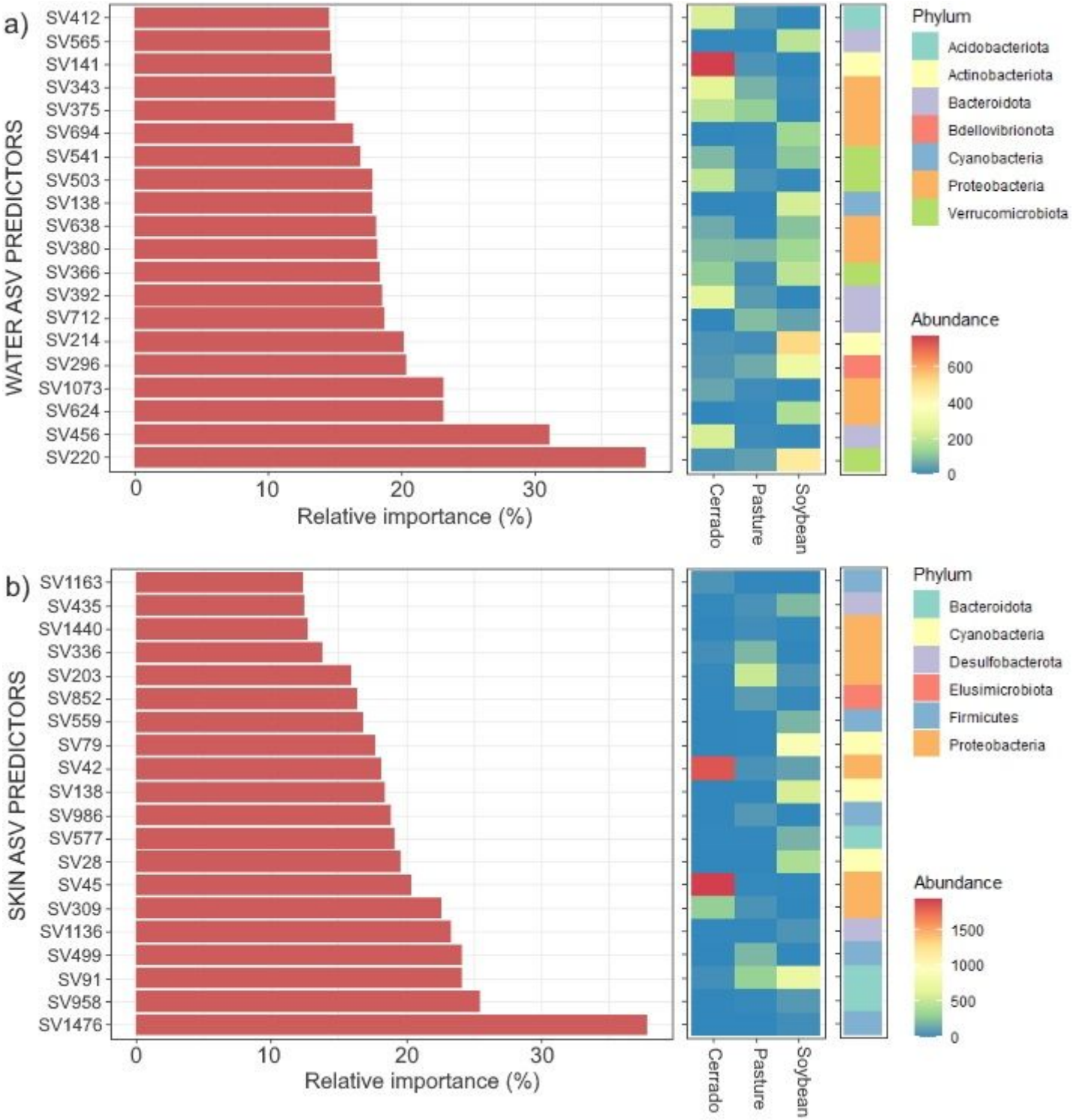

Figure 2

Amplicon sequence variants (ASVs) selected by a Random Forest classification model as predictors best distinguishing water body (a) and S. fuscovarius (b) microbiota of cerrado, pasture and soybean fields. The heatmap displays the relative abundances of each predictive bacterial ASV across land uses

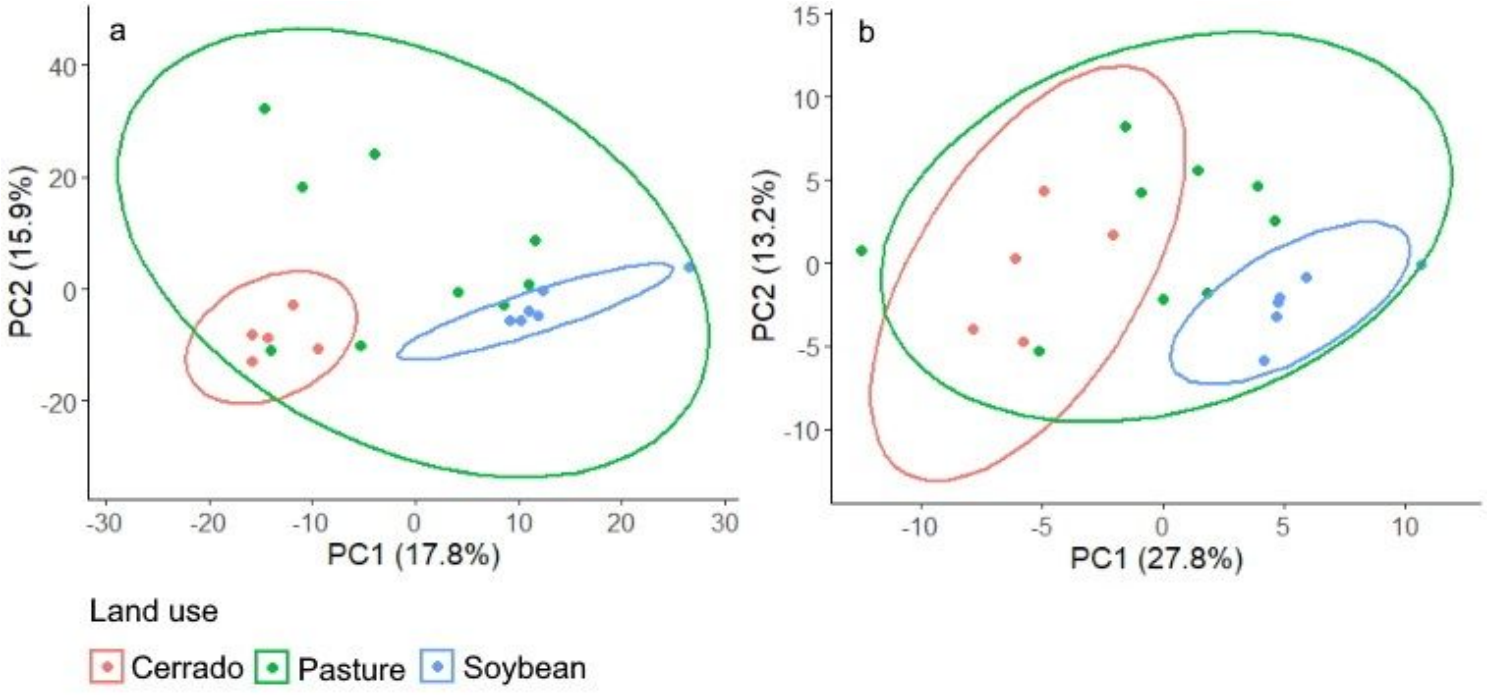

Figure 3

Principal Components Analysis (PCA) plots of S. fuscovarius skin microbiome by land use. The ordinations are based on Euclidian distance matrices for the taxonomic (a) and phylogenetic (b) bacterial composition; by CoDA and PhILR transformed data, respectively.

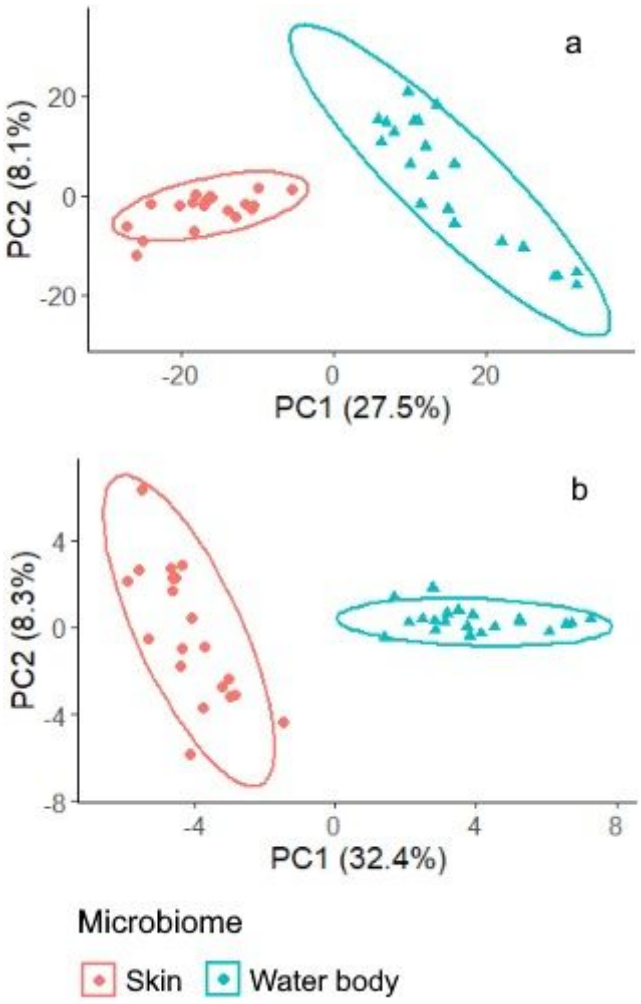

Figure 4

Plots of the tadpole skin and water microbiomes. Ordinations are Principal Component Analysis of Euclidian distance matrices for the taxonomic (a) and phylogenetic (b) bacterial composition; by CoDA and PhILR transformed data, respectively.