UNIVERSIDADE FEDERAL DE LAVRAS

**PAULA EVELINE RIBEIRO D'ANUNCIAÇÃO**

# LANDSCAPE AND CLIMATE CHANGES INFLUENCE ON TAXONOMIC AND FUNCTIONAL RICHNESS OF AMPHIBIANS

**LAVRAS – MG**
**2018**

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ecologia Aplicada, área de concentração em Ecologia e Conservação de Recursos Naturais em Paisagens Fragmentadas e Agroecossistemas, para obtenção do título de doutor.

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**LAVRAS – MG**
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APROVADA em 21 de março de 2018,

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**LAVRAS – MG**
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*Dedico esta tese à minha mãe, Jussara, pelo apoio, carinho e incentivo.*

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# RESUMO

A supressão de áreas naturais, chamada perda de hábitat, e a implantação de novos usos da terra leva ao declínio de espécies, extinção em casos extremos e ainda dominância de espécies generalistas e resistentes ao distúrbio. Além de mudanças no uso da terra, o aumento da temperatura atmosférica, por si só ou em sinergia com outras perturbações, causa extinção daquelas espécies incapazes de se adaptar ou se dispersar para locais mais adequados. Anfíbios apresentam características de história de vida que os deixam particularmente sensíveis às perturbações provocadas pelo homem. A sensibilidade dos anfíbios às mudanças antrópicas os tornam modelos ideais para avaliar o impacto, entretanto ainda não é claro a influência dessas mudanças nos seus atributos funcionais. Os objetivos dessa tese foram: i. determinar a suficiência amostral de anfíbios de acordo com a proporção de cobertura florestal utilizando gravadores autônomos; ii. definir os principais preditores ambientais da distribuição de três componentes de diversidade de anfíbios (espécies, grupos funcionais e traços funcionais) e identificar limiares ambientais responsáveis pela substituição da comunidade taxonômica ou funcional; iii. avaliar o efeito das mudanças climáticas na riqueza taxonômica e funcional de anfíbios ao longo da Mata Atlântica de acordo com dois cenários de aumento de temperatura. Utilizando gravadores autônomos, uma nova ferramenta de coleta de dados, e complementando com busca visual a auditiva, determinei a riqueza e composição de anfíbios em cada paisagem amostral. Os principais resultados foram: i. a suficiência amostral de anfíbios não está relacionada com a proporção de cobertura florestal; ii. plantação de eucalipto, corpos d'água e heterogeneidade ambiental são os principais preditores da distribuição dos componentes de diversidade. Os limiares de substituição de comunidades acontecem no início dos gradientes ambientais antrópicos, exceto para heterogeneidade ambiental que apresentou o principal limiar na porção intermediária de seu gradiente. Os diferentes componentes possuem resposta similares; iii. As mudanças climáticas vão ocasionar perda de espécies e funções, porém a perda de funções é mais proeminente. A conversão de áreas naturais em gradientes antropogênicos causa substituição de espécies e funções ainda que represente uma pequena proporção da paisagem. No entanto, a heterogeneidade ambiental mostra que a presença de diferentes usos da terra é benéfica para os anfíbios até a porção intermediária do gradiente, já que a combinação de áreas naturais e antrópicas podem oferecer diferentes recursos. Os cenários de aumento de temperatura são negativos para os anfíbios, a redundância funcional estará comprometida no futuro e, tanto a riqueza taxonômica quanto funcional estará restrita principalmente ao sudeste do Brasil em áreas costeiras. Com relação ao gravadores autônomos, pude indicar um esforço amostral mínimo, porém ainda é necessário mais estudos para determinar o esforço ideal. Ambos os impactos antropogênicos acarretam consequências negativas para a comunidade de anfíbios e os componentes de diversidade apresentam respostas complementares, ainda que similares em muitos casos.

**Palavras-chave:** Gravadores autônomos. Limiares ambientais. Anuros. Mata Atlântica. Aquecimento Global.

# ABSTRACT

Deforestation and suppression of natural areas, called habitat loss, and the implementation of new land uses lead to species decline, extinctions, and dominance of generalist and disturbance-resistant species. In addition to changes in land use, the temperature increase, either alone or in synergy with other disturbances, causes extinction of those species unable to adapt or disperse to suitable places. Amphibians have life history characteristics that make them particularly sensitive to anthropogenic disturbances. The amphibian sensitivity to anthropogenic changes makes them ideal models to evaluate the impact, however the influence of these changes on their functional traits still is not clear. The objectives of this thesis were: i. to determine the amphibians sampling sufficiency according to the forest cover proportion, using automated recorders; ii. to define the main environmental predictors of the three components of amphibian diversity distribution (species, functional groups and functional traits) and to identify environmental thresholds responsible for the turnover of taxonomic or functional community; iii. to evaluate the climate changes effect on the taxonomic and functional richness of amphibians in the Atlantic Forest according to two scenarios of temperature increase. Using automated recording systems, a new data collect tool, I determined the amphibian richness and composition in each sampling landscape. The main results were: i. the amphibians sampling sufficiency is not related to the forest cover proportion; ii. Eucalyptus plantation, water bodies and environmental heterogeneity are the main predictors of the diversity components distribution. Thresholds of community turnover occur at the beginning of anthropogenic environmental gradients, except for environmental heterogeneity that showed the main threshold at the intermediate portion of its gradient. The different components have similar responses; iii. Climate changes will cause species loss and functions, but loss of function is more prominent. The conversion of natural areas into anthropogenic gradients causes species and function substitution, even though the gradient represents a small proportion of the landscape. However, environmental heterogeneity shows that the different land uses are beneficial for amphibians to the intermediate portion of the gradient, since the combination of natural and anthropic areas may offer different resources. The scenarios of temperature increase are negative for amphibians, functional redundancy will be compromised in the future, and both taxonomic and functional richness will be restricted mainly to southeastern Brazil in coastal areas. Regarding to the automated recording systems, I indicated a minimal sampling effort, however it is still necessary more studies to determine the ideal sampling effort. Both anthropogenic impacts have negative consequences for the amphibian community and the components of diversity present complementary responses, although similar in many cases.

**Key-words**: Automated recorder systems – Tipping points – Anurans – Brazilian Atlantic Forest – Global warming.

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# PRIMEIRA PARTE

## 1 GENERAL INTRODUCTION

Anthropogenic activities due to economic growth have severe impact on natural systems, causing biodiversity loss and population declines worldwide (SODHI et al., 2008). The main drivers of biodiversity reduction are habitat loss and fragmentation, over-exploitation of natural resources, invasive species and climate changes (MILLENNIUM ECOSYSTEM ASSESSMENT, 2005). Of these drivers, habitat loss and fragmentation are one primary cause (TILMAN et al., 2001), which is true to amphibian populations as well (STUART et al., 2004). Amphibians are facing worldwide declines, with an estimate of over 160 world species had already become extinct, and at least 43% of describing species are experiencing population declines (STUART et al., 2004). In addition, other causes, such as climate changes, might accelerate the extinction rate (THOMAS et al., 2004). Although the number of studies evaluating the impact of habitat suppression and modification and global warming to amphibian diversity and/or richness is increasing (BEEBEE; GRIFFITHS, 2005; LIPS et al., 2005; BLAUSTEIN et al., 2010; LI et al., 2013), literature about the impact of these threats on other components of diversity, such as functional diversity, is still scarce (RIBEIRO et al., 2017; TRIMBLE; VAN AARDE; 2014 2010; ERNST et al., 2006).

Habitat fragmentation is the process where a large area of natural habitat is converted in smaller patches with a matrix different from the natural habitat, causing isolation between habitat patches (WILCOVE et al., 1986). Habitat loss is the reduction of the habitat amount (FAHRIG, 2003). There are several consequences of those processes, such as increase probability of extinction, decreased species richness and abundance, changes in the species distribution and composition within habitat patches, as well as a general loss of biodiversity (BUTCHART et al. 2010; PIKE et al., 2011b; D’CRUZE; KUMAR, 2011). However, the effects are not uniform among species, whose habitat preferences and the ability to tolerate or explore modified conditions will determine their persistence and survival (PIKE et al., 2011b; PELEGRIN; BUCHER, 2012).

As a consequence of habitat loss, fragmentation and anthropogenic activities, different types of land covers surround the habitat patches, resulting in landscapes formed by a complex mosaic (RICKETTS, 2001). When anthropogenic matrices replace natural habitats, the isolation between these habitats increases. At the same time, the type of matrix can change the predation risk (BIZ et al., 2017). Moreover, depending on matrix structure the permeability of fauna varies, allowing individuals to use these matrices as a complementary source of resources or alternative habitat (PARDINI, 2004, WATLING, 2011). However, the combination of natural and cultivated areas can be positive to the biodiversity since can provide different resources. Recent studies showed this is true to amphibians and the arthropod functional community (GUERRA; ARÁOZ, 2015; GÁMEZ-VIRUÉS et al., 2015). In addition, Fahrig (2017) found that –independent of habitat loss – the majority of responses to habitat fragmentation is positive. However, this finding is related mainly with taxonomic richness, and this may not be the same for functional or phylogenetic diversity. Therefore, we need to evaluate the effects of anthropogenic landscape modification in other levels of diversity – such as functional diversity– in order to fully understand the impact of habitat loss and fragmentation on biodiversity, ecosystem functions and related ecosystem services (TRIMBLE; van AARDE, 2014; RIBEIRO et al., 2017).

Climate change has also been pointed as having high impact on biodiversity. The 20th century was the warmest of the last millennium, with the main change occurring in the last 30 years (JONES et al., 2001). In the current century, the near surface temperatures had already increased 0.5ºC, causing changes in precipitation pattern and increasing the occurrence of extreme weather events (EASTERLING et al., 2000). In a review about the consequences of climate change on biodiversity, Daufresne et al. (2009) suggests three main responses: a) range shifts in geographic distribution; b) changes in phenology and c) reductions of the body size. Then, species can try to find better conditions moving to cooler regions, or anticipate the breeding time to avoid extreme temperatures. If movement or adaptation is not successful, conditions are not suitable anymore leading to the extinction (PETERSON et al., 2005). As an example, because their sensitivity to the weather conditions, tropical coral reefs and amphibians are indicated as the animals that will be more affected by climate changes (PARMESAN, 2006).

The high sensitivity of amphibians is due to a set of characteristics. They present a biphasic life cycle, requiring two different types of habitat to complete the ontogenetic stages (BECKER et al., 2007). The habitat loss and fragmentation cause a disconnection between these suitable habitats, leading to a decrease of reproductive events and individual abundance, lastly species extinction (HARPER et al., 2008; BECKER et al., 2010). The low vagility or movement capacity and the philopatry make them poor dispersers, and within a hostile matrix the connection between the habitats are reduced (SINSCH, 1990; BUCKLEY et al., 2012). Habitat fragmentation and loss in synergism with climate changes can result in worse effects on amphibians, which can cause population declines, even local and regional extinction of the more sensitive species (THOMAS et al., 2004). In addition, because amphibians are ectothermic depending on the external temperatures to regulate their body functions and present a permeable skin to gas exchange (DUELLMAN; TRUEB, 1994), the impact of climate changes can be even higher (CATENAZZI, 2015).

Regardless what type of anthropogenic activity or what component of diversity are being considered in the study to evaluate the impact on biodiversity, it is first necessary to define an effective way to represent the biodiversity. Automated recording systems are a novel sampling tool that allows collecting data from vocalizing animals and provide a faster and better estimation of biodiversity with reduced field effort (DORCAS et al., 2009). Other advantages are the simultaneous sampling in remote and difficult access areas, and permanent record (HUTTO; STUTZMAN, 2009). In addition, they allow the cross-validation of the data by different experts, what increases the accuracy of the information (PEREYRA et al., 2017). However, these recorders can generate a huge amount of data, being a challenge the storage and data processing (ACEVEDO; VILLANUEVA-RIVERA, 2006). One solution to avoid the caveats of this technique is to establish a suitable and efficient minimal time recording schedule.

I divided this thesis in five chapters. The first is the currently chapter, a general introduction. The second one is a methodological study, where I used audio recordings to determine what is the amphibians sampling sufficiency according the forest cover proportion. This second chapter provide the raw data to the second one, where I investigate the influence of different land uses on the distribution of three levels of amphibians' diversity: a) species; b) functional groups; and c) functional traits. After finding the best predictors, I analyzed their anthropogenic environmental gradients to determine where non-linear and rapid changes, called ecological thresholds or tipping points, happened. In addition, I compared the three levels of biodiversity responses. In the fourth chapter, I evaluated the influence of climate changes on amphibians' taxonomic richness and functional richness across the Atlantic Forest hotspot. I used data on amphibian communities for generate habitat suitability models according two different scenarios of climate change. Finally, the last chapter is a general conclusion of the thesis.

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# SEGUNDA PARTE - ARTIGOS

## ARTIGO 1 - Sampling sufficiency using audio recording systems for estimating anuran diversity

*Format based on the guidelines of “Ecological Indicators*”

# Sampling sufficiency using audio recording systems for estimating anuran diversity

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## ABSTRACT

The selection of suitable conservation areas and the reduction of species loss require accurate characterization of biodiversity, which depends on efficient sampling methods. Automated recording systems (ARS) arise as a new tool to improve and to facilitate sampling and monitoring species that generate acoustic signals. However, ARS produce huge datasets, which are time demanding for processing and depend on expert knowledge for efficiently characterizing biodiversity. Moreover, the amount of audio data that is necessary to process is unknown to many taxa, particularly amphibians. In this study, we aim to answer two questions: 1) what is the minimum recording time needed to accurately estimate amphibian species richness? 2) does the total area covered by forest in the surroundings of the recording location influence the minimum recording time? We sampled amphibians using ARS in 10 streams within the Brazilin Atlantic Forest across different amounts of forest cover. From 208,260 recorded minutes, we randomly choose 3,190 to be listen. We constructed richness accumulation curves to define the sampling sufficiency, i.e. richness achieves the asymptote. Regional species richness was 11, and varied from 3 to 9 among landscapes. The needed time was very variable (min= 70, max=770 minutes). We also found no correlation between the minimum time of audio processing and forest cover. To facilitate future studies using automated recorder systems and to ensure a good representation of the community we recommended to record 770 minutes, which it was our maximal time to reach the amphibians sampling sufficiency. In addition, in the end of this work we have also proposed a research agenda to guide next studies about the topic.

**KEY WORDS**

Anura - Survey methods – Acoustic surveys – Soundscape – Bioacoustics

## 2.1 Introduction

Conservation decisions and species management are commonly based on biodiversity patterns, which are accessed by all sorts of monitoring techniques. The sampling method and effort play an important role on conservation programs and ecological studies (Kenkel et al. 1989). Hence, for an effective and accurate sample of a population or community, it is necessary to use a sampling scheme that ensures a good representation of local or regional biodiversity (Gotelli and Colwell, 2001; Koblitz et al., 2017). There is a tendency in ecological studies to enlarge the spatio-temporal range of observations in order to increase our knowledge on biodiversity patterns (Acevedo and Villanueva-Rivera, 2006). However, by increasing the sampling effort, biodiversity surveys might become prohibitively expensive and time consuming. Therefore, studies concerning reasonable sampling efforts are essential to meet a good balance between survey time, cost and the proper characterization of biodiversity patterns.

For vocalizing species such as birds, frogs, primates and insects, Automated Recording Systems (ARS) arise as a less costly alternative when compared to traditional sampling methods, since they provide faster and better estimation of biodiversity with reduced field effort (Dorcas et al., 2009). The approach consists in using audio recorders to detect species that generate acoustic signals, enabling data collection continuously (Blumstein et al., 2011). Although ARS have been already used as a complementary survey technique for a couple of decades (Parris et al,. 1999, Mack and Alonso, 2000; Montambault and Missa, 2002), they arise nowadays as a major sampling technique on biodiversity studies worldwide (Acevedo and Villanueva-Rivera, 2006; Bardeli et al., 2010; Blumstein et al., 2011; Wimmer et al., 2013). ARS offer advantages on biodiversity monitoring, such as a facilitated survey of remote and difficult access areas (Hutto and Stutzman, 2009) and a reduced time spent in field campaigns, due to the possibility of simultaneous sampling. Besides, it guarantees permanent records for long term studies of many organisms that vocalize, providing key information to support conservation and mitigation measures (Acevedo and Villanueva-Rivera, 2006). The collected raw data – *i.e.* the sound records – can be shared with independent experts, allowing cross-validation and reducing species identification errors (Pieretti et al., 2015; Willacy et al., 2015; Pereyra et al., 2017). Moreover, there is less human interference on animal behavior during data collection, reducing the environmental impact of the sampling process (Digby et al., 2013).

ARS also allow researchers to develop studies about soundscape ecology, a new and emergent investigation field (Farina, 2014). In addition to assess biodiversity richness and ecological integrity, the audio recordings are a valuable tool to better understand anthropogenic disturbances, such as land use modifications and climate changes (Liu et al., 2007; Pijanowski et al., 2011). Soundscape analyses allow the investigation about the complexity of signs and codes – the biosemiotic relationships – that vocal animals use to communicate with animals living in the neighborhood (Farina and Pieretti, 2013). However, this monitoring method may produce a huge amount of data, particularly if sampling is conducted continuously and for many sites, which requires large capacity storage systems and a substantial expert time to process the recordings by visually inspecting spectrograms or even listening the audio files (Rempel et al., 2005, Acevedo and Villanueva-Rivera, 2006). Automatic species detection and classification software offer efficient methods to process massive datasets, but using these tools when working with more than a couple of species reduces species classification accuracy (Brandes, 2008, Bardeli et al., 2010). Therefore, the time needed to meet a good balance between audio processing effort and sampling efficiency is still unknown for many taxa worldwide.

Amphibians are among the most diverse vertebrate in tropical ecosystem, however they are facing population declines worldwide (Alford and Richards 1999; Stuart et al., 2004; Skerratt et al. 2007; Blaustein et al., 2010), particularly in the Neotropics (Cortés-Gomes et al., 2015). These animals are sensitive to habitat change, as they may present biphasic life cycle being dependent on aquatic environmental to reproduce and terrestrial habitat in the adult phase (Becker et al., 2007; Becker et al., 2010). Due to this dependency of more than one suitable area, the majority of species shows a positive correlation with forest cover area, using forests as nonbreeding habitat or as areas that allow the movement between wetlands (Houlahan and Findlay, 2003). Then, because of the strong forest-dependency, their importance to ecosystem functions, as well as its high sensitivity to human impact, amphibians are commonly used as bioindicators of habitat disturbance (Storfer, 2003; Buckley et al., 2012). However, to access this important role as bioindicators is necessary to select accurate sampling methods, which allow good representation of community consequently indispensable information to guide mitigation and conservation measures.

In this study, we uses ARS data in order to find a less demanding method to identify amphibian richness derived from large recording datasets. The amphibian vocalizations were recorded at streams that cross native forest remnants. Ten study sites were selected in the Atlantic Forest Biome, within the state of São Paulo, Brazil. We aimed at answering the following questions: a) what is the minimum recording time needed to have an accurate estimate of amphibian species richness at both, landscape and regional levels? b) does the total area covered by forest in the surroundings of the recording location influence the minimum recording time? We hypothesized that each landscape will reach the richness asymptote at different times. Thus, we believe that these times will be positively correlated with the proportion of forest cover –the greater the amount of forest cover, the richer is the landscape and, consequently, the higher the time needed to reach the asymptote (**Fig. 1**).

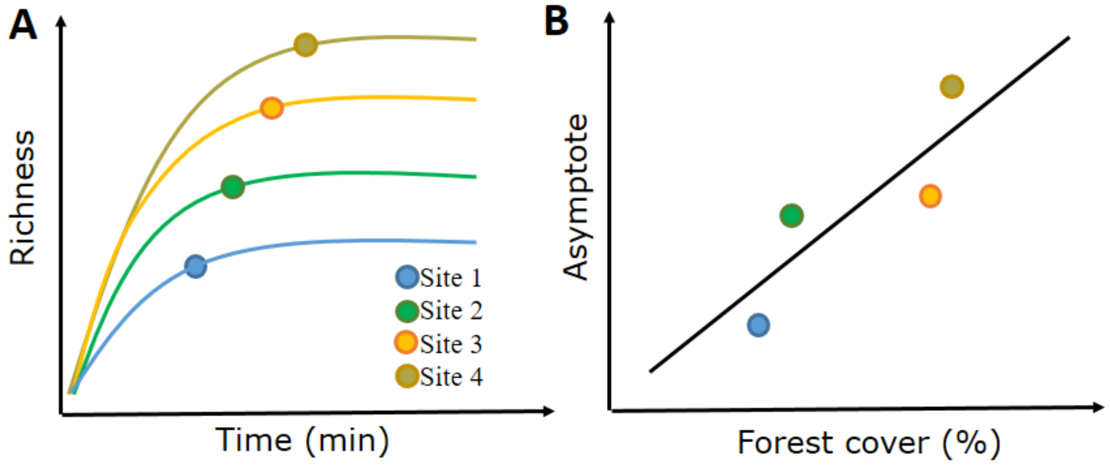

**Figure 1. A.** We expected each site with different forest cover will reach the asymptote or the sampling sufficiency at different times. **B.** The amount of time to reach the asymptote will be positive correlated with forest cover.

## 2.2 Material and Methods

### *2.2.1 Study Area*

The study region comprises the Cantareira-Mantiqueira continuum in the State of São Paulo, Brazil. We selected 10 permanent streams inside forest patches. All selected streams were less then 5 m wide, with low water flux and had the presence of aquatic and riparian vegetation. We defined circular plots with a radius of one kilometer centered at each sampling point for land cover classification. This dimension was defined based on the average displacement for amphibians (Guerry and Hunter, 2002; Wagner et al., 2014). In addition, this has been the scale of response effect of landscape variables on anuran occupancy and diversity on previous studies (Guerry and Hunter, 2002; Van Buskirk, 2005). Forest cover within the plots ranged between 20% and 70%. The distance between streams varied between 2.9 and 56 km (average 27 km), and the elevation varied from 846 to 926 m.a.s.l. Many of the forest remnants are in the intermediate stage of succession (Ribeiro et al., 2009).

We visually interpreted high spatial resolution images available in the Basemap Layer extension of ArcGIS 10 at 1:5,000 geographic scale. Afterwards, we generated land cover maps for each area and calculated percentage forest cover using the LSMetrics software – https://github.com/LEEClab/LS_METRICS (Martello et al. in prep). These maps constitute the landscapes for further analyses.

### *2.2.2 Data Sampling and Species Identification*

We simultaneously installed one audio recorder in each of the 10 landscapes. The Song Meter SM3 (Wildlife Acoustics, Inc.) were placed at 1.5 m high on trees, which were located no more than 3 m away from the streams. We set them up to continuously record data from 6:30 PM until midnight with 48 HZ frequency, being stored as uncompressed ".wav" format, on files of 20 min each. We deployed with four 32 GB SD memory cards plus four D-cell alkaline batteries and two built-in acoustic microphones. At every 15 days, we replaced the memory cards and batteries. We conducted the survey during the breeding season, more specifically between December 2015 and March 2016.

For each landscape, we randomly selected blocks of 10-minutes each as a representative sample of soundscape. Each block was composed by 10 continuous one-minute randomly selected audio recording. Although we used blocks of 10-minutes for extract the audios, we considered every minute as a sampling unit. P.R. Anunciação identified the species of amphibian from their specific calls with the aid of Raven Pro version 1.4 software (Bioacoustics Research Program, 2014).

We used the audio data to construct species accumulation curves and to determine the asymptote point by visual inspection. We defined the optimum interval of processing as the point at which the curve reaches the asymptote (i.e. the plateau). The number of minutes that we processed for each landscape depended on the time of asymptote, which varied between 70 to 770 minutes, in a total of 3,190 processed minutes (Table 1). In addition, we used the total sum of minutes and of species richness to construct a regional accumulation curve.

We used the software R with the iNEXT package (Hsieh et al., 2016) to construct accumulation curves. We managed the audio data with TuneR package (Ligges et al., 2016). To evaluate whether the forest cover affects amphibian detection we performed a linear model wherein the time to reach the asymptote was the response variable and forest cover the predictor, and we considered a significant relation when p value was lower than 0.05.

## 2.3 Results

We recorded 208,260 minutes (c.a. 3,471 hours) using the ARS. From this amount, we processed a total of 3,190 min, which varied among landscapes (min=70; max=770 min.). However only in 2,065 min (64.7%) there was amphibian vocalization (**Table 1**). The amphibian species richness per landscape varied between three and nine species, and 11 species composed the regional pool on our recordings. The species accumulation curve of the total processed minutes or the regional species accumulation curve confirm that 770 min is enough to accurately estimate biodiversity (**Fig. S21** - Supplementary material 1).

By analyzing the accumulation curves for each landscape, we observed that the time needed to reach the asymptote varied between 30 and 650 min (s.d. = 207). In **Figure 1** we present the accumulation curve for six out 10 landscapes, where we indicated both the forest cover and the asymptote.

Regarding the second question, which aimed to evaluate if the amount forest influences the time needed to reach the asymptote, the relationship was not significant ($R^2$= 0.20, p= 0.20, **Figure 2**).

**Table 1**. Audio recording total sampling effort, minutes processed and accumulation curves' summary of 10 sites with different proportion of forest cover. S= amphibian richness, min= minutes, values with * in the last column means sites that reached the asymptote with estimators.

| Forest cover (%) | S | Sampling effort (min) | Processed (min) | Asymptote (min) |
|---:|:-:|---:|---:|---:|
| 21.7 | 6 | 7410 | 590 | 100 |
| 28 | 5 | 24180 | 770 | 650* |
| 32.5 | 4 | 21840 | 460 | 450* |
| 40 | 4 | 22620 | 70 | 40 |
| 53.9 | 4 | 22230 | 340 | 110* |
| 53.9 | 4 | 22230 | 140 | 100 |
| 57.1 | 8 | 21450 | 340 | 350* |
| 61.9 | 3 | 22230 | 70 | 30 |
| 76.8 | 5 | 21450 | 160 | 160 |
| 77.7 | 9 | 22620 | 250 | 75 |
| *Total* | 11 | 208260 | 3190 | 2065 |

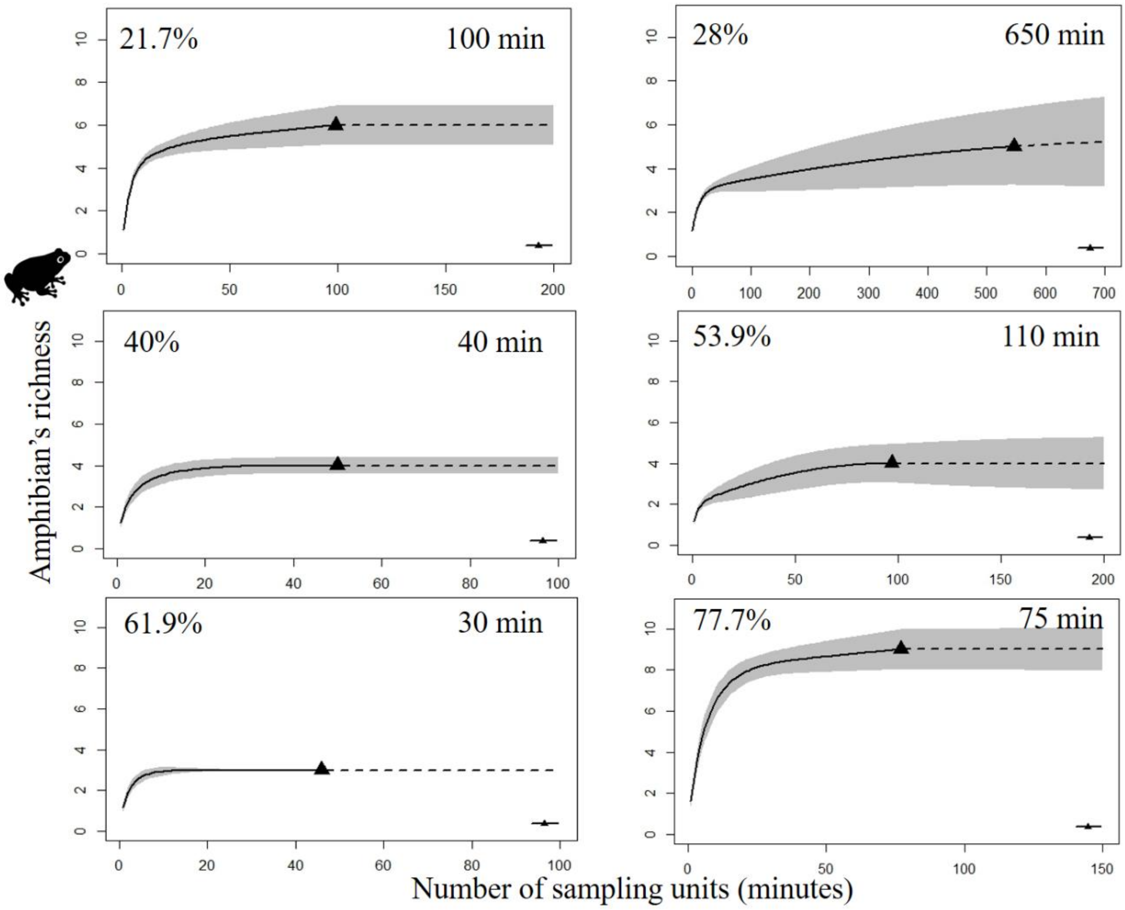

**Figure 1**. Accumulation curves of amphibian richness for six landscapes localized in the state of São Paulo. Numbers in the top left corner are the forest cover (%) and in the top right is the time to reach the richness asymptote. Solid line segments are the rarefaction curves, dotted line segments are the extrapolation curves. The solid triangles represent reference samples.

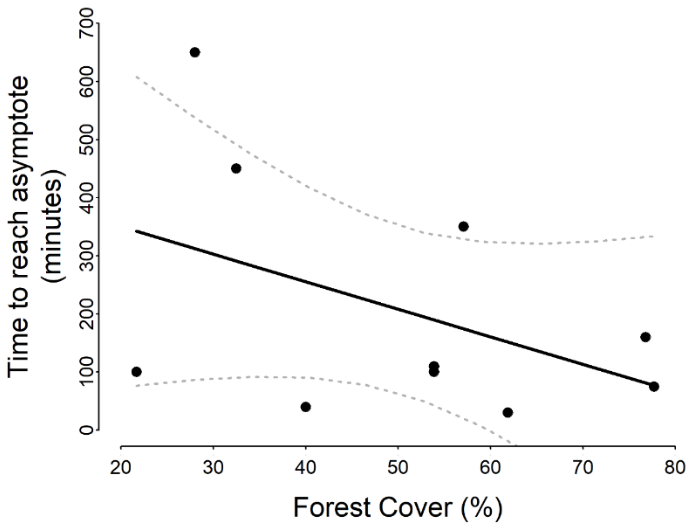

**Figure 2**. Relationship between time to reach the asymptote of amphibian richness and the forest cover for 10 landscapes localized in streams of Atlantic Forest remnants, state of São Paulo, Brazil

## 2.4 Discussion

Improved data collection is important to implement accurate and cost-effective surveying programs (Willacy et al., 2015). ARS represent a sampling method that may facilitate field surveys and provide more accurate data about animals that emit acoustic signals. In this study, we indicate an appropriate sampling duration time to help improving data collection when using ARS. This study represents one of the first guidelines for soundscape in a tropical threatened area. Considering our first research question, we found that each landscape needed different durations of audio listening time to reach the asymptote, which confirm our first hypothesis. However, differently from what we expected in the second research question, we found no correlation between the time that richness reached the asymptote and forest cover.

Our findings, instead of corroborating with the island biogeography theory (MacArthur and Wilson, 1963) and with the habitat diversity hypothesis (Zimmerman and Bierregaard, 1986), seem to agree with Hill et al. (1994). They have found that the sampling effect can mask ecological processes. If the sampling effect is removed, the correlation between species number and area is lower. In our study, we installed one audio recorder in each landscape, independently of the forest cover area, avoiding oversampling larger areas or subsampling smaller ones. Our low correlation is probably the result of the variance explained by the real species-area relationship, as well as other environmental factors that can influence the distribution of amphibian. It is worth to point out that acoustic sampling has its own limitations. Weather conditions like storms and wind produce sounds that might interfere the recorded audio and mask the vocalizations (Towsey et al., 2104; Pieretti et al., 2015). Furthermore, ARS has a lower sensitivity when compared to human listeners (Hutto and Stutzman, 2009), being more efficient to detect loud vocalizations in detriment to the quiet ones (Hsu et al., 2005). In addition, the majority of species vocalize together in the beginning of the night, a phenomenon called chorus that might hampers species identification (Brandes, 2008).

### *2.4.1 Sampling sizes effects on amphibian richness*

Comparing the amphibian richness found in this study with others in the same region, we get similar or even greater species richness. Studies in the Atlantic Forest biome using active search inside forest patches found up to six species (Sabbag and Zina, 2011; Costa et al., 2013; Maffei et al., 2015), while our study results in three to nine species. We have not found a correlation between effort time to reach the asymptote and forest cover, which has advantages and disadvantages. On the one hand, for studies concerning amphibians within forest ecosystem, such as the Atlantic Forest, we have shown that 770 minutes of audio listening would be enough for using ARS sampling. On the other hand, assuming 770 minutes as the minimum means that in some cases we are making 10 times more effort compared to the minimal time found in this study needed to reach the asymptote. One possible explanation to the lack of correlation between the time to reach the asymptote and forest cover is that other environmental variables may be more important to explain the patterns. This includes historical effects of landscape change (Hecnar et al., 1998; Jordan et al., 2009; Zellmer; Knowles, 2009, Silva et al., 2014) and management practices associated with anthropogenic land use (Skole and Tucker, 1993). This spatio-temporal dynamics of landscape structure can have potential effects that will be only detected years later, which is known as the time-lagged response (Ernst et al., 2006; Metzger et al. 2009; Zellmer; Knowles, 2009).

Another possible explanation is that amphibian's distribution and abundance can be attributed to fine spatial scale variables (Parris et al. 2004; Urbina-Cardona et al., 2006; Rojas-Ahumada et al., 2012). We standardized some variables such as stream width, water flux and presence of riparian vegetation; however, other variables like understory density, leaf litter cover and physical structure of the habitat and temperature are examples of important determinants of amphibian diversity (Ernst et al., 2006; Parris et al., 2004; Urbina-Cardona et al., 2006; Rojas-Ahumada et al., 2012). Besides, biotic process such as competition, predation, dispersal, disturbance and disease can also influence the distribution and abundance of amphibians (Parris et al., 2004; Rojas-Ahumada et al., 2012).

### *2.4.2 Sampling effort on audio data: standardize or not?*

To determine a reliable distribution and abundance of amphibians it is common to standardize the sampling effort in auditory surveys of amphibians. USA and Canada researchers defined a standardized survey protocols used by the North American Amphibian Monitoring Program (NAAMP), which are five minutes in duration at each point of roadside next to breeding sites (Weir, 2005). Gibbs (2005) recommends a one-minute survey, Crouch and Paton (2002) and Pierce and Gutzwiller (2004) proposed 10-15 min to a complete survey or to detect at least 90% of amphibian species. In Brazil, it is common to perform auditory surveys starting at sunset, ending at midnight (Benício and Silva, 2017; Maffei et al., 2015; Campos et al., 2013; Zina et al., 2012), which means 6 hours in total. Therefore, there is a lack of standardization of samplings of biodiversity, and we do need to look for new systems to improve amphibian sampling. However, it is always necessary to account for the environmental specificities. The great advantage of ARS in this case is the possibility to increase the survey without fieldwork returns, which can contribute to more accurate biodiversity estimate and a cost-effective monitoring tool as well (Acevedo and Villanueva-Rivera, 2006; Dorcas et al., 2009).

### *2.4.3 A research agenda for ARS and sampling sizes*

- Development of new protocols: more research is necessary in order to determine protocols for data collection in different environments and different species (or communities). A standardized data collection method could improve the quality of the studies, as well as the comparability among them;
- Study about the influence of covariates: to find the right protocol is fundamental to uncover the impact of certain environmental variables on sound recordings, such as storm, thunders, running water. Also, continuous sounds generate by cicadas and crickets for example. Anthropogenic sounds are important to considerate as well, vehicles on roads, engines, bells, sirens can have a huge effect in the quality of the recordings and disturb the species identification;
- Control over the forest cover area variable: we have shown in this study that forest cover has low correlation with the sampling sufficiency of amphibians. However, by fixing this variable it would be possible to access the effects of the environmental heterogeneity within the surroundings;
- Development of improved automatic detection algorithms: continuously develop more efficient algorithms in order to decrease the time spent by experts to filter the recordings, as well as helping to provide more reliable data, without human bias.

## 2.5 Conclusion

By assessing the sampling sufficiency, this paper provides a less time-demanding recording schedule to sampling amphibians in fragmented landscapes. Although we do not find a correlation between the listened minutes to reach richness asymptote and forest cover, we could indicate a minimal sampling effort which can reduce costs with batteries, data storage and expertise time. Because funding is a caveat in ecological researches, sampling techniques that allow more efficient and accurate population monitoring is indispensable to ensure the effectiveness of management and conservation strategies (Willacy et al., 2015).

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## Supplementary Material 1

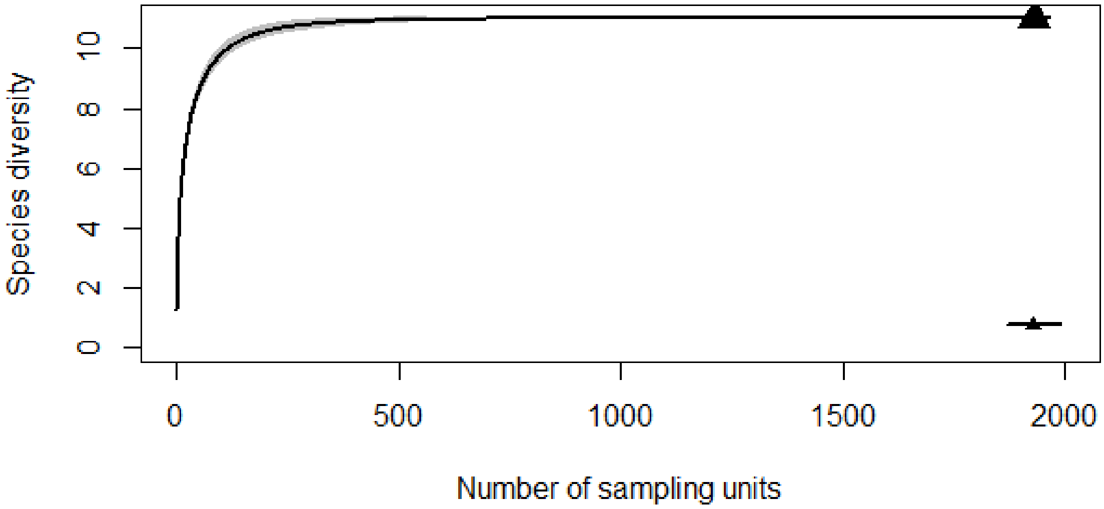

**Fig. S21.** Regional species accumulation curve. We made the sum of time to reach asymptote and respective richness associated of each landscape to generate the regional curve. The regional asymptote is above 600 min and the regional pool of species is 11.

# ARTIGO 2 - Using environmental thresholds to predict taxonomic and functional turnover in anuran communities of a highly fragmented and threatened forest ecosystem

*Format based on the guidelines of "Biological Conservation*"

# Using environmental thresholds to predict taxonomic and functional turnover in anuran communities of a highly fragmented and threatened forest ecosystem

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## ABSTRACT

Anthropogenic environmental gradients can change the dynamic of ecosystems and affect the biodiversity. Ecological thresholds indicate where rapid and non-linear change are happening in these gradients in response to a disturbance, offering the opportunity to avoid species decline and biodiversity loss. Here we first investigate which are the most important environmental predictors of amphibian distribution, accounting for three components of biodiversity: composition, functional groups and functional traits to access the main thresholds in the environmental gradients. In addition, we compare the components of biodiversity responses. To do this we sampling amphibians through automated system recordings and visual and auditory surveys in 15 streams. Using a stream as central point, we defined the sample units surrounding them as a circular area with a 1-km radius and classified the images to determine the proportion of each land cover. Eucalyptus monoculture, water bodies and environmental heterogeneity have power prediction for all components of biodiversity, with the first two showing threshold point right in the beginning of their gradient. Heterogeneity has the main change point in the middle of the gradient. The three components have similar responses, but functional trait approach indicates an amphibian homogenization. The environmental heterogeneity can be positive to amphibians until certain point providing different resources, however above that, the impact of habitat loss can be more evident what is demonstrated by the turnover of species and establishment of generalist species. Ecological thresholds are a valuable tool to guide management and mitigation measures and functional trait approach can offer more accurate responses of anthropogenic environmental gradients.

**KEY WORDS**

Tipping points – Anura – Brazilian Atlantic Forest – Land-use change – Community ecology – Functional trait diversity

## 3.1 Introduction

Fragmentation and habitat loss have been recognized as major threats to biodiversity (Arroyo-Rodríguez et al., 2013; Newbold et al., 2014). Their numerous impacts include an increased probability of extinction, decreased species richness and abundance, and changes in the species distribution and composition within habitat patches, causing biodiversity loss (Ewers and Didham, 2006; Butchart et al., 2010; Pike et al., 2011b). The impacts are fundamental and promote changes in ecological interactions, phenology and geographical distribution (Parmesan, 2006; Lemes and Loyola, 2013). Habitat loss and habitat fragmentation per se (Fahrig, 2003) gradually modify the landscape due to the expansion of urban areas, agriculture, roads and other anthropogenic land cover types. The establishment of human-modified and heterogeneous landscape can have positive, negative or even neutral effects on biodiversity (Fahrig, 2017), which will depend on individual species' response and the ability to tolerate or explore modified environmental conditions (Pike et al., 2011b; Pelegrin and Bucher, 2012; Ernst et al., 2016).

Anthropogenic disturbance creates environmental gradients – such as the amount of silviculture, urbanization, agriculture– which can change the dynamics of entire ecosystems and associated biological communities. Understanding the organismic responses to sudden non-linear changes along anthropogenic environmental gradients (i.e. ecological thresholds *sensu* Toms and Lesperance 2003; Foley et al., 2015) plays therefore pivotal role when designing mitigating measures and management actions (Samhouri et al., 2011; Foley et al., 2015; Magioli et al., 2015; Muylaert et al., 2016). These ecological community thresholds are particularly important because of the evolutionary implications of a synchronous response of species to environmental pressures such as habitat loss and fragmentation (Baker et al., 2010; Muylaert et al. 2016). When the community surpasses the threshold, the decrease in the patch size and increase of isolation intensify the effects on population abundances leading to shifts in community composition (Pardini et al., 2010; Magioli et al. 2015).

Advances in the development of statistical methods that allow identifying/detecting these thresholds provide unique opportunity for the analysis of critical processes that may alter ecosystem dynamics (Kéfi et al., 2014; Roque et al., 2018). Through these newly developed threshold analyses is possible to detect how much suppression of natural habitat and establishment of anthropogenic land uses the biodiversity can support before shows species loss, abundance and biodiversity decline. Threshold identification is relevant mostly in tropical areas, where the species declines have been rigorous due to habitat loss and fragmentation (Watson et al., 2016). However, irrespective of the region, a central question to identify thresholds is what kind of metric can show accurate responses. The responses of different metrics commonly used in ecological studies vary according their sensitivity, what can generate different thresholds. Then, it is advised to previous evaluate the sensitivity of these community metrics or different components of diversity along the environmental gradient and select the ones that have a clear response to the gradient (Roque et al., 2018).

Although taxonomical metrics are commonly used as community descriptors in most studies, they have limited predictive power to estimate the structure and functioning of communities. They can mask essential information about the impacts of anthropogenic land-use (Trimble and van Aarde, 2014), as they designate the same functional weight or ecological importance to different species (Ribeiro et al., 2017; Levrel, 2007). Measures that incorporate species' functional traits (Petchey and Gaston, 2006) can provide more efficient information for understanding the response of species to anthropogenic disturbance (Ernst et al., 2006; Vandewalle et al., 2010; Trimble and van Aarde; 2014; Ribeiro et al., 2017). Besides that, not only the chosen metric but also the response group can offer an anticipate and more accurate response to the impact. Amphibians have a set of life history characteristics, which make them very sensitive to disturbances and frequently show responses to changes before other groups. Their thresholds can act as a signal helping to anticipate the responses of other communities (Roque et al., 2018).

Amphibians are particularly sensitive to habitat change due to their mainly biphasic life cycle, where different ontogenetic stages depend on different environmental conditions (Becker et al., 2007; Harper et al., 2008; Becker et al., 2010). Amphibians also exhibit philopatry, low vagility and ectotherm habits (Buckley et al., 2012; Sinsch, 1990), thus habitat availability and connectivity positively influences survival, population recruitment and successful dispersion. Temporal and spatial changes in these variables can influence the viability and distribution of amphibian populations (Rothermel, 2004; Cushman, 2006; Price et al., 2006; Harper et al., 2008).

Furthermore, due to their role in the flow of matter and energy acting as predator and prey, nutrient transference between terrestrial and aquatic systems and their diversity and abundance, they play important roles in ecosystem functioning (Cortés-Gómez et al., 2015). Loss of these animals can weaken ecosystem resilience and resistance. Due to their sensitivity and ecological importance they are a suitable, sensitive organismal model system to assess the impact of anthropogenic environmental change on community structure, composition, and diversity at both taxonomic and functional levels (Ernst et al., 2006; Ernst and Rödel, 2008; Hölting et al., 2016).

Atlantic Forest is a prominent example of rapidly accelerating fragmentation, mainly to anthropogenic actions, in tropical forests. It is thus considered a highly threatened global biodiversity hotspot (Myers et al., 2000). Currently, the remaining forest ranges from 11.4% to 16% of the original extent (Ribeiro et al., 2009), and almost 72% of the Brazilian population is living in its geographical limits (IBGE, 2010). Recent research shows that heterogeneous environments represented by a variety of land use types, are positive to the composition of the arthropod functional community (Gámez-Virués et al., 2015) and amphibian diversity (Guerra and Aráoz, 2015). The fact that environmental heterogeneity can have positive effects in anthropogenic landscapes (e.g. Guerra and Aráoz, 2015) has recently been highlighted in an elucidating meta-analysis by Fahrig (2017). However, most of the studies incorporated in this analysis exclusively focused on taxonomic richness patterns, but not on functional groups nor functional traits. In addition, no discussion about biodiversity response thresholds are explored across these environmental gradients of spatial heterogeneity.

To assess whether non-linear environmental thresholds can be a crucial element in determining taxonomic and functional community turnover patterns in a highly fragmented Atlantic Forest matrix, we analyzed large-scale anuran amphibian community data sets. The analyses presented are designed to address three main objectives: (1) Quantify the environmental drivers that most contributed for understanding cross-regional community composition patterns along the amount of natural and anthropogenic land-use gradients; (2) Identify critical values along these gradients that correspond to threshold changes in community composition; (3) Assess concordant/non-concordant diversity patterns at the taxonomic and functional levels. We hypothesize that: (1). forest cover and water bodies will be the most important environmental variables to the components of diversity; (2) we will be able to identify environmental thresholds influencing components of diversity, but the changing points vary between them and the environmental variables; (3) the same environmental variables will have different prediction power, according to the components of diversity. The consistency or non-consistency of these patterns will indispensably affect and determine management strategies aimed at safeguarding maximum diversity and function within and across the entire forest landscape. Inconsistent patterns may require indicator-specific strategies whereas consistent patterns may call for a generalized management concept. We hence discuss the potential implications for biodiversity conservation management in highly fragmented Atlantic Forest system.

## 3.2 Methods

### *3.2.1 Study Area and Study Design*

The study area comprises the region between the Serra da Cantareira and Mantiqueira continuum in the São Paulo State, Brazil (**Fig. 1**). It is an important region forming an ecological corridor that connects these mountain chains. It preserves relatively extensive amount of forest with several protected areas, but some important forest remnants are not protected. Due to the humidity and altitude variation, this region presents different types of forest cover (IBGE, 2012). The changes in the landscape (substitution of natural areas by anthropogenic land uses) are related to the slope of terrain. The concentration of changes occurs in flatter sites with altitudinal variation until 1000m, while higher altitudes preserve natural environments (Sartorello, 2014). This region suffers high pressure of the different land uses, with removal of forest remnants in substitution mainly for silviculture in the recent periods. Besides the eucalyptus plantation, the main land uses surrounding the forest patches are urban areas, agriculture and farming. The urban expansion represents a central pressure, dominated by irregular occupation and property speculation (Sartorello, 2014).

Fifteen, first or second order, streams were selected according four criteria: (i) maximum width below 5 m; (ii) low water flux; (iii) presence of aquatic and riparian vegetation; and (iv) the stream had to be localized inside forest patches, which was embedded in landscapes representing a forest cover gradient from 20% to 70% (see **Table S31**). Using each streams as a central point, we defined a circular landscape using a 1-km radius. This radius size represents the average dispersal distance for amphibians (Guerry and Hunter, 2002; Wagner et al., 2014) and with regard to the effect of landscape variables, it has been shown to be a representative response scale for anuran occurrence and diversity patterns (Van Buskirk, 2005; Guerry and Hunter, 2002). The distance between streams varied between 2.9 and 56 km (average 27 km), and the elevation varied from 846 to 926 m.a.s.l

### *3.2.2 Environmental variables*

We mapped the land use, hydrography, and roads of each landscape within ArcGis10 program (ESRI, 2011), using high-resolution satellite image interpretation in a 1:7,000 scale and field verification when necessary. Land use and land cover map include the following classes: 1) forest (from initial to mature forest), 2) silviculture (eucalyptus plantation), 3) bare soil (abandoned pasture and degraded lands), 4) urban areas (cities, small villages, roads and highways), 5) agriculture (permanent and temporary crops), 6) pioneer vegetation (herbaceous stage of forest, and pasture with some trees and shrubs), 7) water bodies (ponds and dams mainly, but also rivers) and 8) wetlands (permanent and temporary flooded areas with or without trees and shrubs) (Fig.1; Table S1). Them we calculated the amount (%) of each cover class for each 1-km landscapes. In addition we calculated environmental heterogeneity or the compositional landscape heterogeneity using the Shannon's diversity index (SHDI, Shannon and Weaver, 1949). We calculated this index based on the number of all cover types within GRASS 7.2.x (GRASS Development Team 2014) throughout LSMetrics package (Niebuhr et al., in prep; https://github.com/LEEClab/LS_METRICS).

### *3.2.3 Amphibian data*

Anuran species occurrence were recorded using automated recording systems, auditory and visual surveys. Audio records (Song Meter SM3 – Wildlife Acoustics, Inc.) were installed on each landscape, placed 1,5 m high on trees no more than 3-m away from the streams. We set them up to simultaneously record data in all landscapes, during the breeding season (December 2015 to March 2016), from 6:30 PM to midnight.

To determine what amount of time was enough to define accurately which specie was present in each landscape, we randomly selected minutes of each recorder, using calls/minute as incidence. We heard the recordings and identified each specie from its specific calls using Raven Pro version 1.4 software (Bioacoustics Research Program, 2014). We used this data to construct species accumulation curves considering the optimum interval of processing as the point at which the curve reaches the asymptote (i.e. the plateau).

Besides the passive acoustic monitoring, we performed auditory and visual surveys at breeding sites to archive a better representation of anuran assemblages within each sampled landscape (Heyer et al., 1994). During the night, two people performed 4-hour samplings for two days on each landscape. We slowly walk around the perimeter of the breeding sites and searching the emergent vegetation and water, further the surrounding terrestrial environment and ground cover, taking notes of amphibian presence. We sampled one stream (the same as for the audio recorder), one pond and one wetland in each sample unit. We used more than one method putting emphasis on passive sampling to reduce observer bias, however keeping standardized effort across the sites. Because of the furtive habits and rarity of many species, our samples represent an assemblage of the entire community, which is a common challenge in tropical herpetofaunal studies (Ribeiro-Júnior et al., 2008).

### *3.2.4 Functional traits*

We had a priori a set of eight functional response traits about amphibian characteristics that would be affected by anthropogenic environmental gradients (the chosen trait set after collinearity test is in the **Table 1**). We choose the traits set according the studies of Henle et al. (2004) who reviewed the traits that have been suggested as predictors of species sensitivity. The dimorphism, movement index, and mouth area were continuous traits and we measured in 10 male specimens/species from Célio F.B. Haddad amphibian collection, housed in the Universidade Estadual Paulista (UNESP), Rio Claro, SP (CFBH). The explanation of how we measured each continuous trait is on Table S31. The other five traits –activity, reproductive mode, calling site, ecotype and reproduction habitat – were categorical and the information were taken from literature (Haddad et al., 2013). Moreover, the female SVL (snout venter length), which we used to calculate the dimorphism, was also obtained from the literature (Haddad et al., 2013).

### *3.2.5 Data preparation*

We tested collinearity between environmental variables using Variance Inflation Factors (VIF) with a threshold of 0.4 as exclusion criteria. Only low correlated variables were selected. Thus, we reduced the original set of nine to seven variables (**Table S32**- Supplementary Material 2). Although forest amount had collinearity problem, presenting the VIF value greater than the exclusion threshold, we decided to keep it on our set of variables because of our interest on this landscape metric.

The taxonomic response variable (SP) is a combination of species list from audio recordings and the species list recorded during active search (**Table S33**- Supplementary Material 2). For the functional response traits, we also used VIF to test for collinearity and the same cutoff value (0.4) of environmental variables. We present the seven functional response traits variables into **Table 1**. Then, we generate the functional response variables: functional response traits (FRT) and functional response groups' composition (FRG). The FRT variable was the sites-by-functional response traits matrix. We constructed that crossing information presented in sites-by-species records and species-by-functional response traits matrix (**Table S34**- Supplementary Material 2). For the last response variable, FRG, we used the complete species list and their respective functional response trait values to create a species-by-traits matrix. Using this matrix, we calculated distance matrices and respective dendrogram with Gower distance, which is indicated when working with continuous and categorical traits (Podani and Schmera 2006,). Then, we used the average method and a cutoff value of 0.3 that is relatively strict, generating nine functional response groups (**Fig. S31**- Supplementary Material 2). Crossing the information of what species belongings to each functional group and sites-by-species records, we generate the sites-by-functional groups matrix (in this case, we assume that the functional group was present in a site whether at least one specie belonging to this group was present – **Table S35**- Supplementary Material 2).

### *3.2.6 Data Analysis*

To identify environmental thresholds between taxonomic (SP) or functional community turnover (FRT or FRG) and the environmental gradient (see Environmental variables section above), we applied a novel statistical approach. The gradientForest analysis (GF, Ellis et al., 2012) can show which explanatory variables most influence species presence and/or abundance. In addition, it allow identifying the values along environment gradients that cause important changes in the response variables. GF is a non-parametric statistical approach based on Random Forests. It is a compilation of regression or classification trees. Where each tree is fitted on a bootstrap sample of biological data through recursive partitioning and the best split at each node is selected based on random subset of the total number of predictor variables. The environmental predictor’s importance for model accuracy is assessed permuting randomly each variable. We ran the GF with the option conditional permutation. A cross-validation was carried out and the out-of-bag sample (observations that were not selected in the bootstrap sample for a given tree) is used to estimate the forest prediction error. The importance overall of each abiotic predictor were calculated by weighting the species-level predictor importance by the species $R^2$ and averaging over species. The cumulative compositional changes along each environmental gradient were calculated joining the normalized splits as cumulative distributions standardized by the observations density and plotting importance curves for each community and each specie. These cumulative importance curves were used to transform the different abiotic variables at each site into a scale of common biological response, which reflected the predicted change in the compositional community and the importance of each abiotic variable. Only $R^2 > 0$ species was included in the final GF model and the predictors for $R^2 < 0$ species are considered without predictive power.

We ran the analysis for three different response variables corresponding to the different diversity aspects / levels (taxonomic vs. functional) (1) amphibian species composition, (2) functional response groups and (3) functional response traits. We performed all the analysis in the freely available software R version 2.15.2 (R Development Core Team, 2011). To construct the accumulation curves, we used the iNEXT package (Hsieh et al., 2016). TuneR was used to manage audio data (Ligges et al., 2016). We calculate collinearity through the function *vifstep* from package *usdm* (Naimi et al., 2014). We used the package FD (Laliberté et al., 2014) and vegan (Oksanen et al., 2008) to generate distance matrices and respective dendrogram. We used the gradientForest package to identify the environmental thresholds and where the species composition is changing along the anthropogenic gradient (Ellis et al., 2012). We ran the GF with the option conditional permutation, which is an additional approach to avoid inflated measures of importance. We choose this option due to keep the forest cover variable.

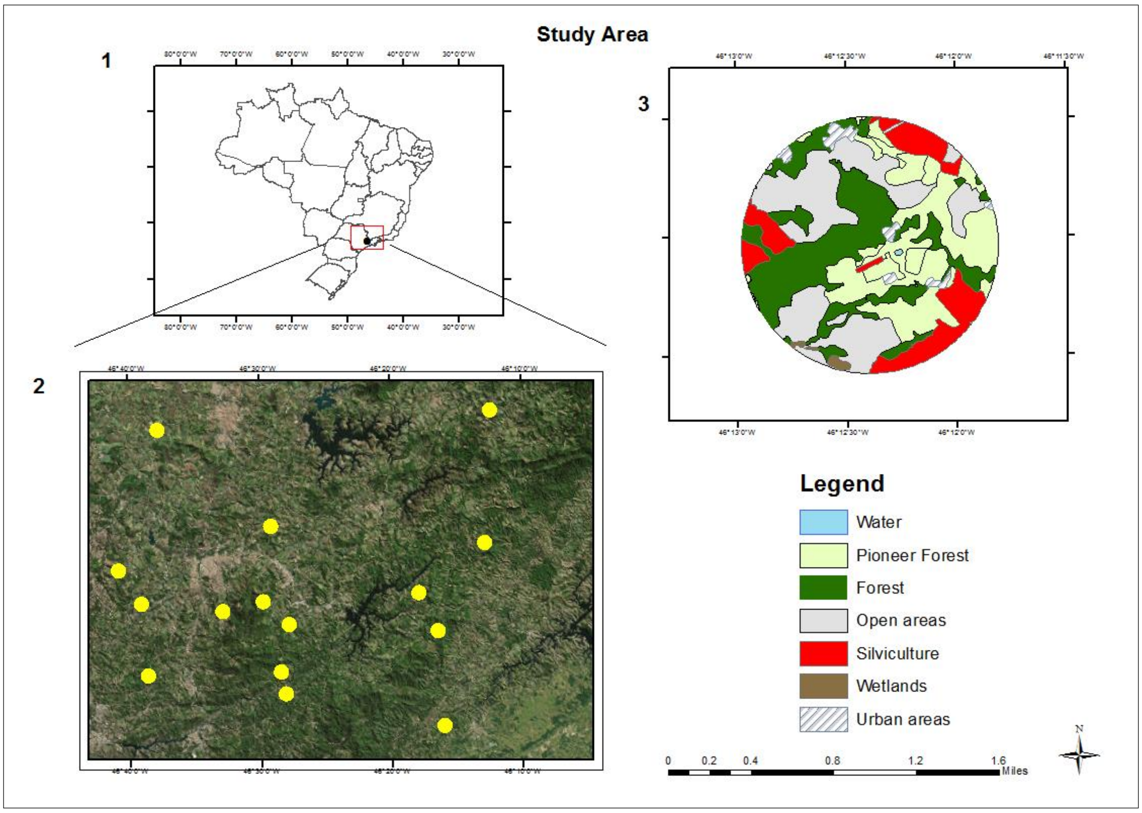

**Figure 1** – Atlantic Forest amphibians' study site. 1. Cantareira-Mantiqueira continuum region in São Paulo State. Brazil. 2. Highlighting the distribution of 1-kilometer radius landscapes. 3. Example of land use classification of one landscape

**Table 1**. Description of response traits measured or compiled from the literature on amphibian species of the Atlantic Forest biome.

| Functional trait | Definition | Specific functions | Ecosystem process relationship |
|---|---|---|---|
| Dimorphism | Male SVL / Female SVL | Sexual selection | Related to the energy flow through trophic chains as predator or prey |
| Movement index | Arm length / leg length | Dispersal ability | Related to the energy flow through trophic chains as predator or prey |
| Mouth width | Depth mouth / width mouth | Foraging tactics, predator-prey relationships | Related to the energy flow through trophic chains as a predator |
| Activity | Diurnal, nocturnal and both | Dispersal ability, predator-prey relationships, sexual selection | Related to the energy flow through trophic chains as predator or prey |
| Reproductive mode | 1 to 39 modes | Dispersal ability, predator-prey relationships, sexual selection | Related to the energy flow through trophic chains as predator or prey |
| Ecotype | Arboreal, leaf-litter, fossorial, terrestrial, arboreal+leaf-litter, semi-aquatic, rheophilic | Dispersal ability, predator-prey relationships | Related to nutrient recycling and energy flow through trophic chains as predator and prey |
| Reproduction habitat | Lentic, lotic or independent | Dispersal ability, predator-prey relationships, sexual selection | Related to the energy flow through trophic chains as predator or prey |

## 3.3 Results

We recorded 39 species belonging to 10 families of amphibian species in the region (**Table S33** - Supplementary Material 2). Of these species, *Aplastodiscus cavicola*, is classified as near threatened and *Lithobates catesbeianus* is an alien species in Brazil. The species richness in the landscapes varied from four to 21 species. The most common family is Hylidae with 23 species, followed by Leptodactylidae and Brachycephalidae families, both presenting four species. Through the recordings from the automated audio recorders we found 11 species, however just one species (*Ischnocnema guentheri*) was exclusively detected using this method.

### *3.3.1 Overall model performance*

The fractions of the variation explained by the selected set of environmental variables oscillated among the taxonomic species richness (SP), functional response group (FRG) and functional response trait (FRT) distribution (**Fig. 2**, **Table 2**). Predictive capacity was restricted to subsets in the respective response variables. For SP, significant predictive power was restricted to 21 out of 39 species (*see* **Table S33**- Supplementary Material 2), for FRG, four out of nine functional groups (FG-5 [*P. boiei*, *R. icterica*, *R. ornata*], FG-6 [*L. fuscus*, *L. latrans*, *P. cuvieri*], FG-7 [*L. catesbeianus*] and FG-8 [*E. cesarii* and *O. americanus*], and for FRG only one out of seven functional traits [mouth width]) (**Table 2**).

**Table 2**. Basic statistics of the gradient Forest models to find the best environmental predictors for the three response variables: SP =species; FRT= functional response traits; FRG= functional response groups. (Predictors pw= number of environmental variables with predictive power; Response variables= total number of species, functional groups and functional traits used in the models; Response pw= number of species, functional groups and functional traits that the environmental variables had predictive power).

| | SP | FRT | FRG |
|---|---|---|---|
| Predictors pw | 7 | 3 | 5 |
| Response variables | 39 | 7 | 9 |
| Response pw | 21 | 1 | 4 |
| Mean $R^2$ (range) | 0.28 (0.28 - 0.58) | 0.06 | 0.13 |

### *3.3.2 Environmental drivers of compositional change*

Overall conditional importance of environmental predictors differed markedly between the three taxonomic and functional response variables (SP, FRG, and FRT) both in terms of the number of most relevant predictors (compare **Fig. 2 a** vs. **b** & **c**), as well as composition (**Fig.**

**2 a-c**). Silviculture, water bodies and spatial heterogeneity had predictive power for all the biodiversity levels. For SP response variable, *L. catesbeianus*, an alien species, is one of the most responsive to the silviculture amount. In addition, forest amount has prediction power only to SP and *A. cavicola*, a near threatened species – the species appears to be stable above 50% of forest (**Fig. S32** - Supplementary Material 2). Urban areas is the most important predictor to species, but not to the other response variables (FRT and FRG). Pioneer forest is the second most important predictor to the functional response groups. The complete list of environmental variables and their predictive power to each response variable is in **Figure 2**. One prominent difference is that more predictors are needed to explain variance in taxonomic composition, when compared to functional response components.

### *3.3.3 Gradient responses and environmental thresholds*

The cumulative importance curves for the three different response variables (SP, FRT, FRG) show strong contrasts in compositional responses to the five most important environmental gradients (three shared and two unique to respective response variables, **Fig. 3**).The shared predictors among the components of diversity (**Fig. 3**) present different rank-order of importance. Silviculture is one of the main predictor, particularly to functional groups response. The cumulative plots show similar patterns of response, the community is stable until 5% of silviculture amount, after that we can see a turnover of species. Water bodies was the most important predictor to functional response traits and the third most important for the other components of diversity. The cumulative plots present very similar patterns of response, with the three components stable between 1.3 and 3% of water bodies amount. Spatial heterogeneity is the last shared predictor and was more important for species and functional traits. The three components of diversity (SP, FRT and FRG) have also similar patterns of response to this environmental variable. Until ~1.3 of the heterogeneity amount, all components show stability and also between 1.7 and 1.8.

Regarding to the threshold points of the shared environmental variables, environmental heterogeneity presents the change points usually around 1.8 of the gradient for each component. For water bodies amount the components of diversity have the principal sections of change between 0.5% and 1%. The environmental variable silviculture, as well as water bodies, has the change point right in the gradient beginning (c.a. 1%). The main threshold points are summarized in the **Table 3**. Density plots showing along the environmental gradient where important compositional changes in the species richness are happening and the cumulative plots showing the SP/FRT/FRG more responsible for these changes on each gradient are in the supplemental material (**Fig. S32**, **Fig. S33** - Supplementary Material 2).

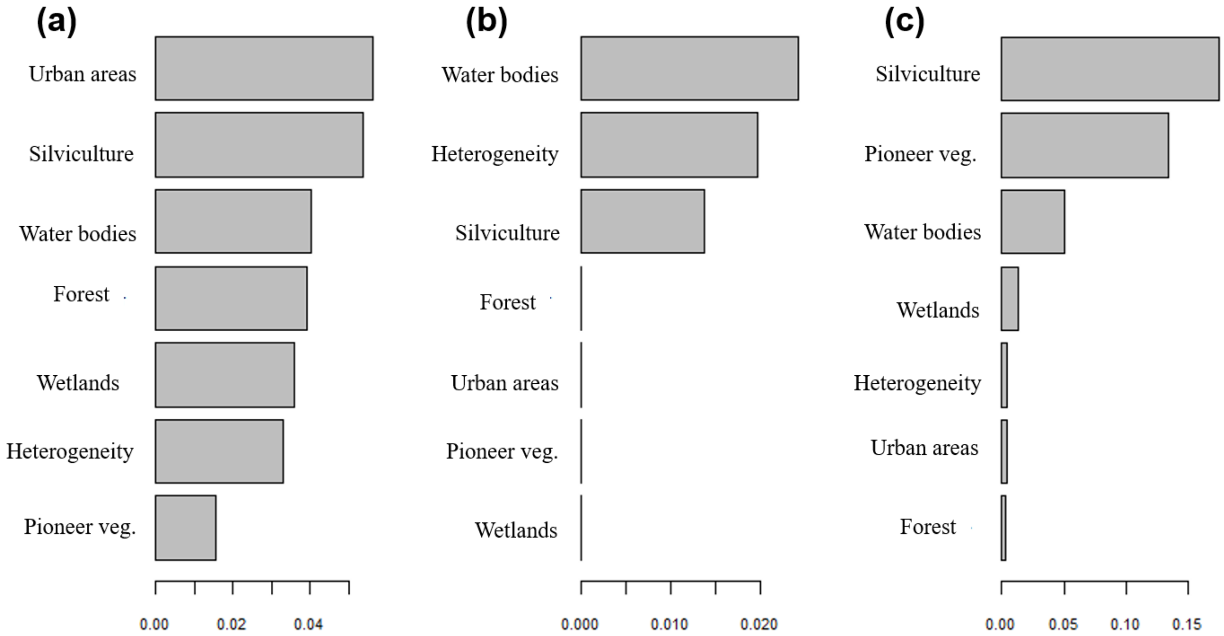

**Fig. 2.** Overall conditional importance of environmental variables for the three components of Atlantic Forest amphibians' diversity. The x axes vary between the predictor groups. (a) Amphibians species composition SP (b) Functional response traits FRT (c) Functional response groups FRG.

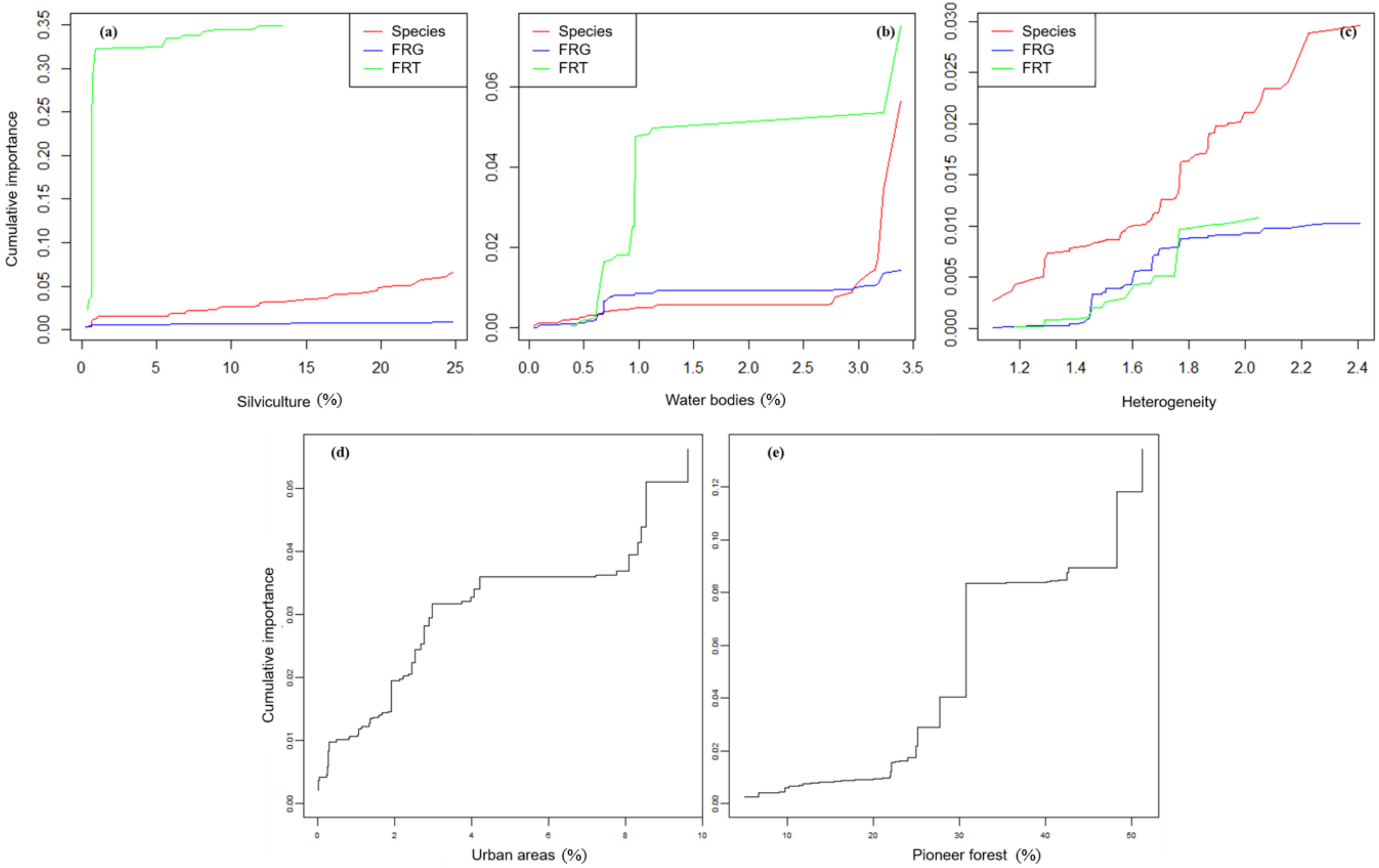

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**Fig. 3** Cumulative importance plots of the environmental variables with predictive power. (a), (b) e (c) are the comparison of the cumulative importance plots to the shared predictors of the three components of Atlantic Forest amphibians' diversity.(d) Cumulative importance plot of urban areas predictor, which is the most important of SP response variable. (e) Cumulative importance plot of pioneer forest, one of the most important predictor to the FRG response

**Table 3.** Summary of the main thresholds in the density plots for the three components of Atlantic Forest amphibians' diversity and their environmental variables with predictive power. The thresholds exhibited are the first and second one most important.

| | Forest | Eucalyptus | Water bodies | Wetlands | Urban areas | Pioneer vegetation | Heterogeneity |
|---|---|---|---|---|---|---|---|
| SP | 70.0 / 60.0 | 1.0 / 25.0 | 0.5 / 3.2 | 0.5 / 3.0 | 0 / 3.0 | 12.0 / 22.0 | 1.3 / 1.8 |
| FRT | - | 1.0 | 0.6 | - | - | - | 1.45 / 1.7 |
| FRG | - | 1.0 | 1.0 | 0.5 | - | 30.0 | 1.8/ 1.6 |

## 3.4 Discussion

Answering our first question, silviculture and environmental heterogeneity are important to amphibian distribution. We have also found that water bodies variable, as we expected, is one of the main predictors of amphibian distribution, being present in all levels of diversity responses. However, forest cover has predictive power only to amphibian species composition, contrary to our expectation. About the second question and the critical values of species turnover, the main threshold points showed a rapid change right in the beginning of the silviculture and water bodies environmental gradients, and for heterogeneity, a big change occurred in the middle of the gradient. The three components of diversity showed concordant diversity patterns answering our last question. However, functional components have lower complexity, presenting less environmental variables with power prediction and these variables are contained in the taxonomic component.

### *3.4.1 Amphibian distribution predictions and thresholds*

The most important environmental predictors were silviculture, water bodies and environmental heterogeneity. These variables have predictive power for all components of diversity, indicating their important influence in the study region. In addition, the thresholds rely on very similar points. Cultivation of silviculture has been encouraged on the justification of improving sequestering atmospheric carbon, an important ecosystem service. It is the main matrix of the study region and it has been expanded in Brazil and in the whole world (FAO, 2001). Even though silviculture presents a similar structure to forest, the majority of studies demonstrated negative impact of this cultivation, with some reporting positive effects (Gardner et al., 2007; Russell and Downs, 2012; Trimble and Van Aarde, 2014). However, it was considered only taxonomical metrics, demonstrating how important is to access other components of diversity as we did here. It is worth to highlight that the American bullfrog (*L.*

*catesbeianus*), introduced in Brazil in the 1930's and with a big invasiveness potential in the whole Atlantic Forest (Giovanelli et al., 2007), is one of the most responsive to the silviculture gradient. This can illustrate how this non-natural habitat can contribute to the establishment and expansion of generalist species. In addition, even though the forest cover had predictive power only to amphibian species response variable, the near threatened species *A. cavicola* was present only in the gradient from the middle. This result also corroborate how the substitution of natural areas by anthropogenic land uses can lead to a prevalence of generalist species.

Species recorded in silviculture sites in general are generalists and with wide distribution (Saccol et al., 2017), similar to species usually living in places that in actual study we called water bodies. Here, this variable is composed mainly by ponds and dams, frequently constructed ones. Natural ponds are, in general in this area, smaller and not always visible in the satellite imagery. Moreover, because the study area has been suffering a big human pressure, constructed water bodies is more common. These dams are common places of generalists and disturbance-tolerate species, because of the lack emergent vegetation cover and the presence of predators, such as fishes (Hazel et al., 2004). Natural and constructed water bodies represent important role in the amphibian conservation, providing habitat for different frog species, with different habitat requirements (Hazel et al., 2004). However, this region seems to be suffering a species homogenization and generalist species being more abundant. Another important variable was environmental heterogeneity, which can be positive due to the combination of natural and cultivated areas for maintenance of amphibian diversity (Guerra and Aráoz, 2015). The positive effect can be visualized in certain points of gradient stabilization between 1.6 and 1.8 (better pattern visualization on FRT).

Silviculture and water bodies show a rapid change right in the beginning of the gradient, indicating these environmental variables modify the composition of amphibians, even though when their proportion in the landscape is not high. The increase of the presence of lentic habitats promotes the establishment of open area species. However, environmental heterogeneity has the more prominent change in the middle of the gradient, close to 1.8%. Higher values indicate loss of natural areas and our results show turnover of species in this sector. This finding corroborates with other studies, which showed until certain point the combination of several land use types contributes to the biodiversity (Gámez-Virués et al., 2015; Guerra and Aráoz, 2015). In addition, the turnover of species on elevated sections of the gradient corroborate with Collins and Fahrig (2016), who found that farmland heterogeneity (structural complexity of cropped areas, considering only cultivated areas) has a negative impact on amphibian diversity. The authors recommend conservation of natural habitats such as forest in agricultural landscapes, as the best option for amphibians. Probably above the threshold, the landscape loses too many natural habitats such as forest, being more suited to generalists and impact-tolerant species.

### *3.4.2 The connection among the biodiversity components*

The similarity of the responses among the three components of diversity for three explanatory variables (silviculture, water bodies and heterogeneity) showed by the predictor cumulative plots indicates that these environmental filters constrain functional traits of organisms, species and communities in a related way (Gutiérrez-Cánovas et al., 2015). The relationship between richness and trait-based approach is complex and context dependent (Naeem and Wright, 2003), here the components of diversity seem to be connected (at least for some explanatory variables). Contrary to recent studies, we find similar responses to environmental gradients among different components of diversity (Palmeirim et al., 2017; Ribeiro et al., 2017, Riemann et al., 2017). Regional environmental filters probably have more pronounced effects on distribution and occurrence of the responsive species and functional groups/traits than species interactions, connectivity, historical events, recruitment variability and local environmental predictors (Pitcher et al., 2012). However, we used presence/absence data and our measures were estimated using mean trait values and within-taxon trait variability have already been demonstrated in such cases to better explain community assembly (Violle et al., 2012).

Besides that, in conditions of strong habitat filtering or stressful habitats functional similarity among taxa is bigger due to the pressure on trait evolution by long-standing anthropogenic constant stressors (Ernst et al., 2006; Maire et al. 2012; Gutiérrez-Cánovas et al., 2015). This functional homogenization can have drastic consequences for ecosystem functions with loss of stability and functionally specialized species (Hooper et al, 2005; Pool and Olden, 2012; Ibarra and Martin, 2015).

Mouth width was the only functional trait with power prediction, and can act as an indicator of specie adaptation. Species with lower mouth width and lower displacement were associated with water bodies, conversely bigger mouth width and mobile species were linked with silviculture. Species with bigger mouth can eat small and big preys, having a greater range of feeding resources being generalists and less sensitive to anthropogenic changes (Duellman and Trueb, 1994). Another characteristic of disturbance-tolerate species is the bigger capacity of mobility which allows explore different types of habitats to find necessary resources (Schweiger et al., 2007).

Some studies showed that silviculture can lead to a generalized impoverishment in the taxonomic diversity of amphibians (Russell and Downs, 2012; Gardner et al., 2007) and other taxa (Sousa et al., 1997, 2000). Here we demonstrated the impact can be deeper and reach finest components of diversity. When the response variable is functional response groups, silviculture had prediction power for the group with species that lay eggs in water and have large bodies, which are considered less disturbance-sensitive than small amphibians, which lay eggs in soil (Suazo-Ortuno et al., 2008). In addition, water bodies have predictive power for the same functional group. Moreover, when looking for species response, we have a mix of open area and forest species what reveal that had not clear or incoherent responses opposite to FRT and FRG.

### 3.5 Management recommendations

Finding new tools which are capable to show more accurate responses or which can clarify ecological processes it have been long-standing interest of ecological researches. Ecological thresholds provide opportunity to avoid loss of biodiversity and ecosystem function (King and Richardson, 2003) giving us the reference points of species' capacity to support habitat loss and landscape changing. In addition, to determine which anthropogenic environmental gradient has more influence in biodiversity distribution help to take appropriate mitigation and management measures. Because of that, these approaches give important insights about the impact of anthropogenic environmental gradients on biodiversity and should be considerate by decision makers

To considerate anthropogenic habitat alterations on functional diversity is essential for conservation plans as it let a more complete perception of habitat fragmentation effects on biodiversity than measures based only on species numbers and composition and further allows inferences about possible effects on ecosystem processes (Cadotte et al., 2011; Mouillot et al., 2013). However, the choice what component of biodiversity to use for making decisions depends on the goal of conservation site proposed. If the objective is to protect the whole community, probably it is not necessary go deep in the analysis, only the classic descriptors of biodiversity seem to be enough. Nonetheless, when emblematic, sensitive species are the priorities and when the proposal is to keep the functional integrity, we recommended to explore functional traits and related indexes. Besides that, we have shown that is necessary more environmental predictors to explain variance in taxonomic composition vs. functional response components. This indicates fewer main parameters are required to be controlled to safeguard particular functions/response groups, making this approach easier and faster to be implemented.

While taxonomical indicators cannot be without difficulty extrapolated and applied to different regions, traits and functional groups can generalize across regions (Hodgson et al., 2005; Pont et al., 2006). Moreover, counting species can be easily than measuring traits, but in other situations, identifying every species in a community can be laborious than measuring a small set of traits. Then, this approach can provide a less expensive way for biodiversity monitoring and a way to evaluate the vulnerability of a system considering the complementary and functional redundancy of the species that compose it  and an additional understanding of the spatial and temporal distribution of biodiversity (Petchey and Gaston 2002; Devictor et al., 2010).

Andrén (1994) proposed a threshold of 30% of suitable habitat in the landscape, below this point the impact of habitat fragmentation intensifies, with species being more affected by changes in the landscape. However, there is no information about thresholds of different types of land use and the impact on biodiversity according their increase in landscapes. Our study shows that the important environmental variables or land uses with the predictive power of amphibian distribution have a big impact right at the beginning of the gradient. In other words, the substitution of suitable habitat by anthropogenic land uses can disturb the biodiversity even though their amount in the landscape is not high. The species richness cannot be affected, but there is a turnover of species with the establishment of generalist and impact-tolerant species. Moreover, when it analyzes other components of biodiversity, such as functional response traits, the impact is more evident. However, our understanding of the factors that drive threshold dynamics, and when and how rapidly thresholds will be crossed is currently limited in many systems. Understanding these thresholds and their role in determining biodiversity patterns across anthropogenically altered landscapes represents a major challenge in both basic and applied conservation. We recommended more studies about land uses thresholds in other systems to investigate if the same anthropogenic environmental gradients have common responses, giving right guide to decision makers.

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## Supplementary Material 2

**Table S31**. Explanation of each continuous trait measurements.

| **Continuous Trait** | **Measurement** |
|---|---|
| Dimorphism | Male snout-venter length (SVL) / Female SVL. SVL is the measure from cloaca until the snout. |
| Movement index | Arm length / leg length. As arm we considered the hand measure (from the biggest toe until the carpus) + radio-ulna + humerus. Leg was the combination of feet length (from the biggest toe until the tarsus) + tibiofibula length + femur length (until the cloaca). |
| Mouth area | Depth mouth / width mouth. We measured the mouth length (the distance between the two ends of mouth) and the length of one mouth end until the snout. Then, using the measurement of the length of one mouth end until the snout as a hypotenuse and the measurement of the mouth length/2 as a triangle leg, we calculated the width mouth (other triangle leg) according to Pythagorean theorem. |

**Table S32.** Sites-by-environmental variables matrix showing the localization of the 15 landscapes selected to sample the Atlantic Forest amphibians. In addition, shows the proportion of each land use class in %, except environmental heterogeneity.

| | **Latitude** | **Longitude** | **Forest** | **Silviculture** | **Urban areas** | **Pioneer vegetation** | **Water bodies** | **Wetlands** | **Heterogeneity** |
|---|---|---|---|---|---|---|---|---|---|
| **site 1** | 23° 9'56"S | 46°29'47"W | 77.71 | 0.79 | 0.00 | 14.27 | 0.00 | 0.00 | 0.99 |
| **site 2** | 23°18'46"S | 46°15'59"W | 57.90 | 0.51 | 0.52 | 33.74 | 1.02 | 0.00 | 1.58 |
| **site 3** | 23°16'27"S | 46°28'5"W | 57.08 | 26.38 | 1.61 | 12.28 | 0.14 | 0.26 | 1.63 |
| **site 4** | 23°12'5"S | 46°16'27"W | 53.86 | 15.81 | 0.03 | 11.25 | 0.91 | 0.00 | 1.76 |
| **site 5** | 23° 4'37"S | 46°29'9"W | 32.50 | 0.89 | 2.85 | 27.72 | 0.48 | 0.00 | 1.97 |
| **site 6** | 23°14'54"S | 46°28'26"W | 76.78 | 1.28 | 2.67 | 11.28 | 0.87 | 0.44 | 1.22 |
| **site 7** | 22°56'32"S | 46°12'24"W | 28.05 | 13.07 | 2.24 | 27.74 | 0.08 | 0.62 | 2.12 |
| **site 8** | 23° 7'39"S | 46°40'48"W | 32.79 | 23.16 | 3.14 | 16.10 | 5.43 | 0.00 | 2.33 |
| **site 9** | 23°10'1"S | 46°39'4"W | 24.54 | 10.78 | 13.96 | 16.15 | 0.59 | 0.75 | 2.48 |
| **site 10** | 23°15'5"S | 46°38'36"W | 61.90 | 17.54 | 0.07 | 16.51 | 0.00 | 0.00 | 1.54 |
| **site 11** | 22°57'46"S | 46°37'44"W | 21.65 | 0.00 | 0.48 | 1.91 | 1.33 | 5.68 | 1.38 |
| **site 12** | 23° 5'53"S | 46°12'50"W | 39.98 | 26.38 | 1.61 | 12.28 | 0.14 | 0.26 | 2.02 |
| **site 13** | 23° 9'24"S | 46°17'54"W | 53.87 | 22.12 | 2.23 | 8.08 | 0.44 | 0.00 | 1.77 |
| **site 14** | 23°10'36"S | 46°32'52"W | 24.70 | 0.00 | 5.28 | 68.90 | 0.77 | 0.45 | 1.36 |
| **site 15** | 23°11'33"S | 46°27'48"W | 58.63 | 0.51 | 0.52 | 33.74 | 1.02 | 0.00 | 1.76 |

**Table S33**. Species-by-sites records**:** amphibian species found in each landscape of the study area in the Atlantic Forest biome. Species names in bold are the species which the environmental variables had predictive power.

| **Species/Sites** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Adenomera marmorata* | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 |
| ***Aplastodiscus arildae*** | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***Aplastodiscus cavicola*** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***Aplastodiscus leucopygius*** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 |
| ***Bokermannohyla circumdata*** | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| *Brachycephalus ephippium* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Dendropsophus elegans* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| *Dendropsophus minutus* | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 |
| *Dendropsophus nanus* | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| ***Dendropsophus sanborni*** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| ***Elachistocleis cesarii*** | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| ***Haddadus binotatus*** | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 |
| *Hylodes sazimai* | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| ***Boana albopunctata*** | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 |
| ***Boana bischoffi*** | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***Boana faber*** | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| *Boana polytaenia* | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Boana prasina* | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 |
| *Boana semilineata* | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***Ischnocnema guentheri*** | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| *Ischnocnema juipoca* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| ***Ischnocnema parva*** | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 |
| *Leptodactylus fuscus* | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| *Leptodactylus latrans* | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| ***Lithobates catesbeianus*** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |

**Cont. S33**

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Odontophrynus americanus*** | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 |
| ***Phyllomedusa burmeisteri*** | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Physalaemus cuvieri* | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| ***Proceratophrys boiei*** | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| ***Rhinella icterica*** | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| ***Rhinella ornata*** | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 |
| ***Scinax crospedospilus*** | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 |
| ***Scinax eurydice*** | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| *Scinax fuscomarginatus* | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 |
| *Scinax fuscovarius* | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 |
| *Scinax hayii* | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| *Scinax hiemalis* | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 |
| ***Scinax similis*** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| *Vitreorana uranoscopa* | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Total* | 21 | 12 | 16 | 16 | 14 | 20 | 11 | 16 | 16 | 15 | 12 | 16 | 20 | 7 | 4 |

**Table S34** – Sites-by-functional response traits: complete amphibian functional response traits list to each landscape. Activity: A1 nocturnal; A2 diurnal, A3 both. Reproductive modes: RM1 Egg deposition in still water, tadpole development in pond; RM2 Egg deposition in flowing water, tadpole development in stream; RM3 Egg deposition in subaquatic chamber in stream, tadpole development in stream; RM4 Egg deposition in streamside basin, tadpole development in stream; RM5 Egg deposition in subterranean nest, tadpole development in water after flooding; RM11 Egg deposition in bubble nest on pond, tadpole development in pond; RM23 Non aquatic eggs, direct development; RM24 Eggs on leaves over water, tadpole development in pond, RM32 Egg deposition in foam nest in burrow, tadpole development nonfeeding in burrow. Reproduction habitat: RH1 lentic water bodies, RH2 lotic water bodies, RH3 direct development.

| | Move_index | Mouth widht | Dimorphism | Activity | Reproductive_mode | Ecotype | Reproduction_habitat |
|---|---|---|---|---|---|---|---|
| **site 1** | 0.383 | 11.365 | 0.818 | A1.A2.A3 | RM1.RM1_RM2.RM1_RM4.RM11.RM23.RM3.RM32.RM5 | arboreal.litter.litter.arboreal.rheophilic.terrestrial | RH1.RH2.RH3 |
| **site 2** | 0.369 | 9.798 | 0.794 | A1.A2.A3 | RM1.RM1_RM4.RM11.RM23.RM25.RM3.RM32.RM5 | arboreal.litter.rheophilic.terrestrial | RH1.RH2.RH3 |
| **site 3** | 0.378 | 11.421 | 0.886 | A1.A2.A3 | RM1.RM1_RM2.RM1_RM4.RM2_RM4.RM23.RM32.RM5 | arboreal.litter.terrestrial | RH1.RH3 |
| **site 4** | 0.370 | 11.391 | 0.879 | A1.A3 | RM1.RM1_RM2.RM11.RM23.RM25.RM32.RM5 | arboreal.litter.litter.arboreal.terrestrial | RH1.RH2.RH3 |
| **site 5** | 0.386 | 13.273 | 0.903 | A1.A3 | RM1.RM1_RM2.RM1_RM4.RM11.RM2_RM4.RM23.RM24.RM30.RM5 | arboreal.litter.terrestrial | RH1.RH3 |
| **site 6** | 0.367 | 10.104 | 0.854 | A1.A3 | RM1.RM1_RM2.RM1_RM4.RM11.RM23.RM25.RM30.RM32.RM5 | arboreal.fossorial.litter.litter.arboreal.terrestrial | RH1.RH2.RH3 |
| **site 7** | 0.389 | 14.208 | 0.779 | A1.A2.A3 | RM1_RM2.RM1_RM4.RM11.RM23.RM3.RM32.RM5 | arboreal.litter.rheophilic.terrestrial | RH1.RH2.RH3 |
| **site 8** | 0.383 | 10.753 | 0.909 | A1.A3 | RM1.RM1_RM2.RM1_RM4.RM11.RM23.RM25.RM30.RM32.RM5 | arboreal.fossorial.litter.terrestrial | RH1.RH2.RH3 |
| **site 9** | 0.389 | 12.019 | 0.896 | A1.A3 | RM1.RM1_RM2.RM1_RM4.RM11.RM23.RM25.RM5 | arboreal.litter.terrestrial | RH1.RH2.RH3 |
| **site 10** | 0.369 | 12.704 | 0.871 | A1.A3 | RM1.RM1_RM2.RM1_RM4.RM11.RM2_RM4.RM23.RM30.RM5 | arboreal.litter.litter.arboreal.semi-aquatic.terrestrial | RH1.RH3 |
| **site 11** | 0.367 | 8.075 | 0.887 | A1.A3 | RM1.RM11.RM23.RM32.RM5 | arboreal.litter.terrestrial | RH1.RH3 |
| **site 12** | 0.363 | 11.419 | 0.923 | A1.A3 | RM1.RM1_RM2.RM1_RM4.RM11.RM23.RM30 | arboreal.fossorial.litter.terrestrial | RH1.RH3 |
| **site 13** | 0.387 | 13.351 | 0.837 | A1.A2.A3 | RM1.RM1_RM2.RM1_RM4.RM11.RM2_RM4.RM23.RM3.RM32.RM5 | arboreal.litter.rheophilic.semi-aquatic.terrestrial | RH1.RH2.RH3 |
| **site 14** | 0.365 | 8.932 | 0.873 | A1.A3 | RM1.RM1_RM2.RM23.RM32.RM5 | arboreal.litter | RH1.RH3 |
| **site 15** | 0.339 | 12.680 | 0.880 | A1 | RM1.RM1_RM2.RM23 | arboreal.litter | RH1.RH3 |

**Fig. S31 –** Dendogram constructed using amphibian species found in the study area and their respective functional response traits. It shows the functional response groups (FRG), and to define the nine FRG considered in this study, we used a cut-off of 0.3.

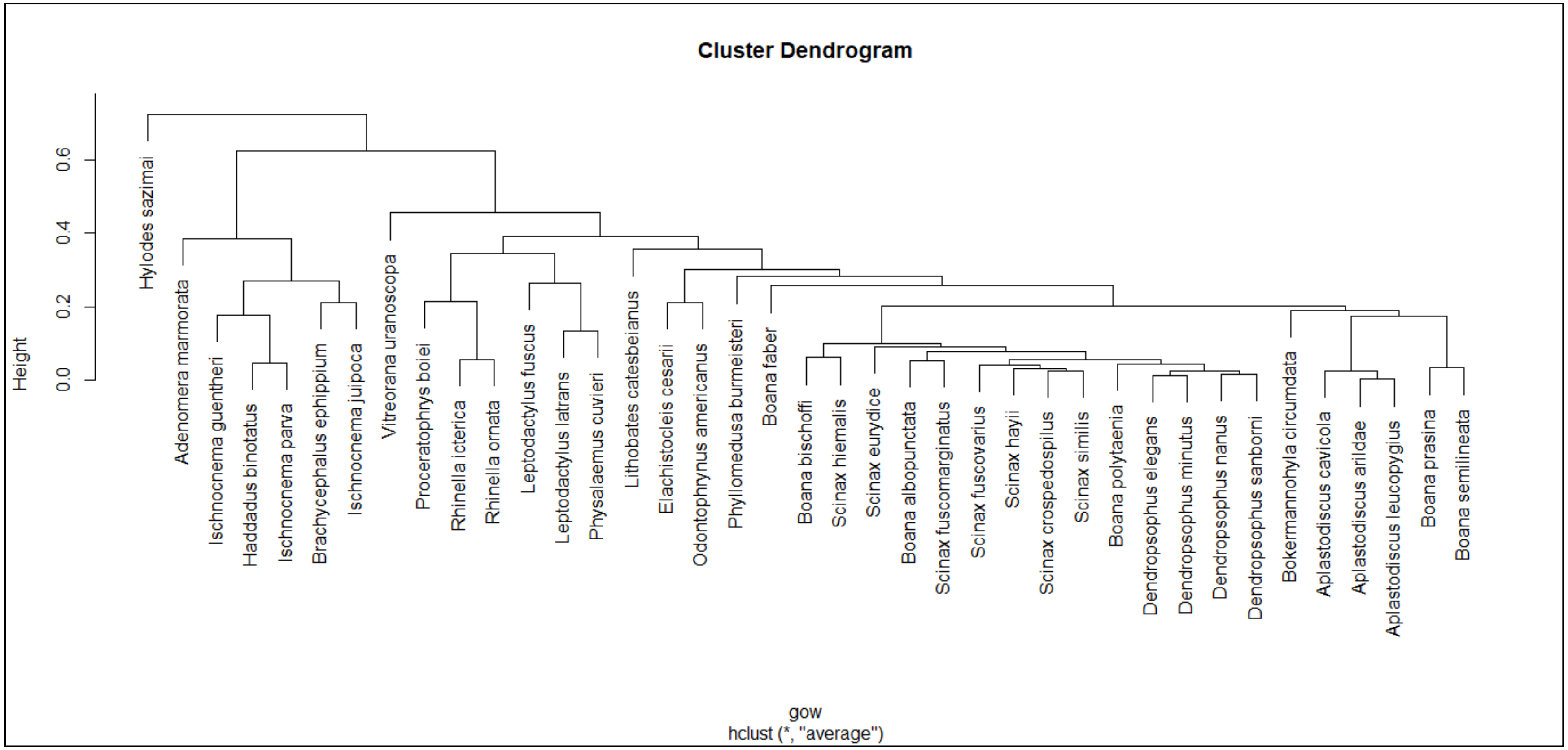
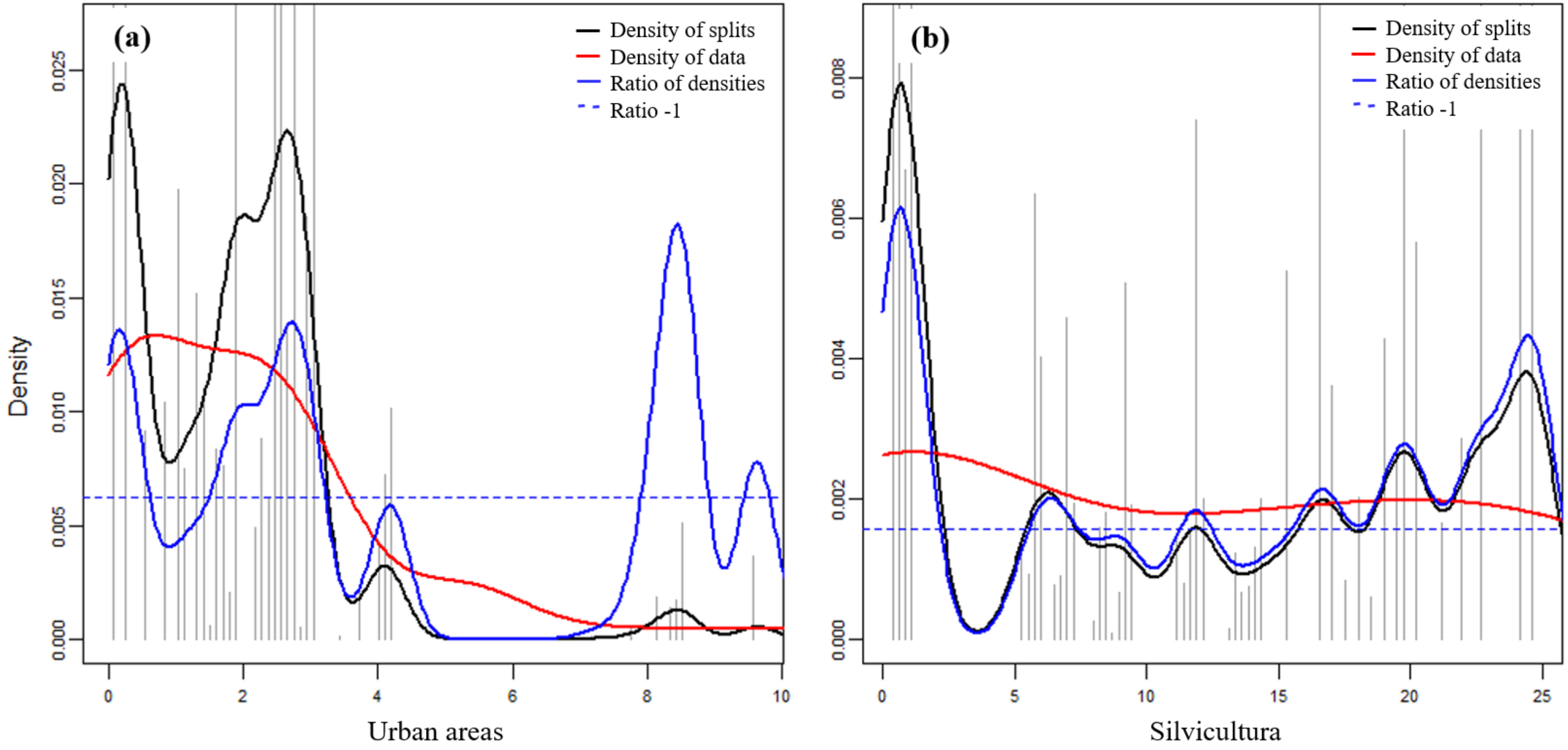
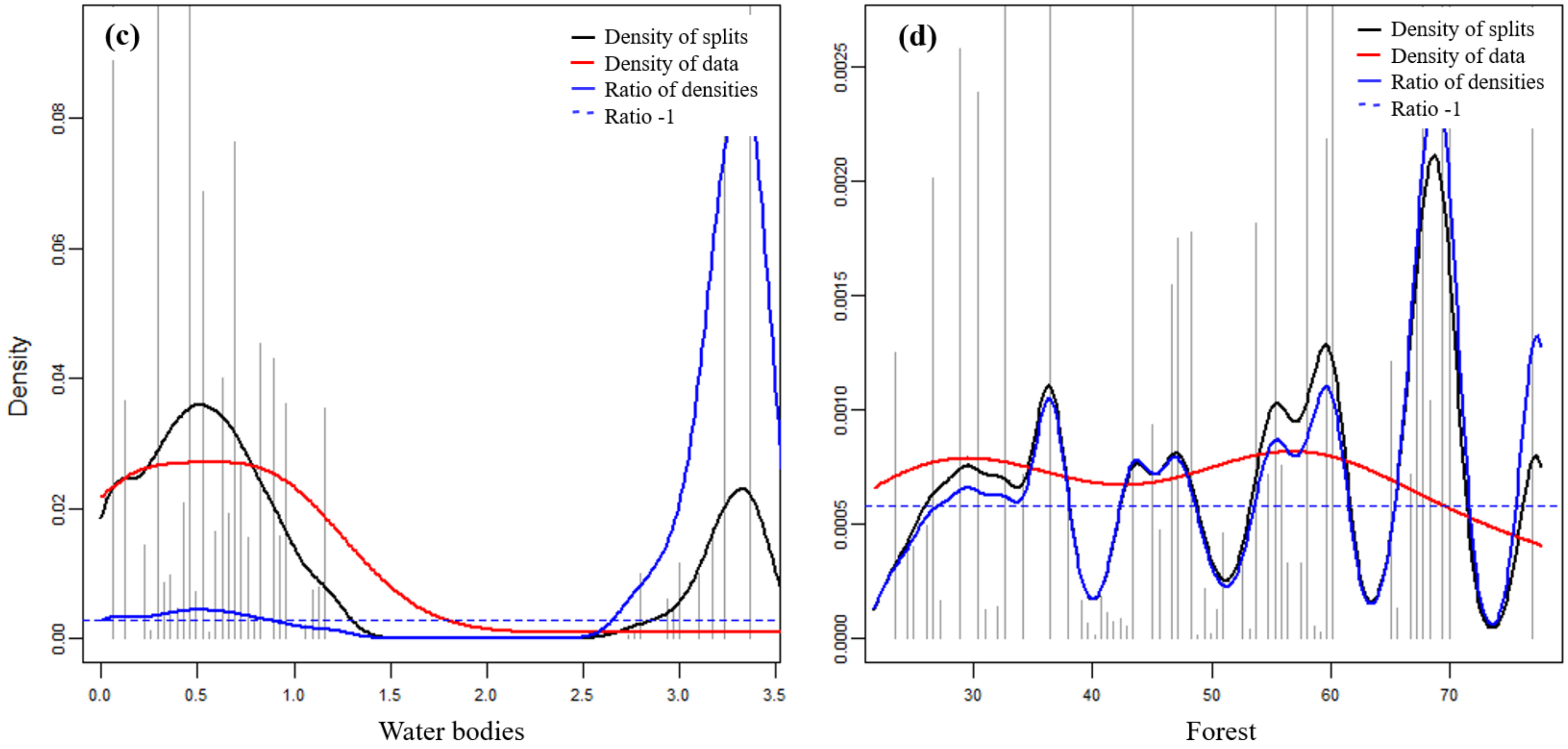


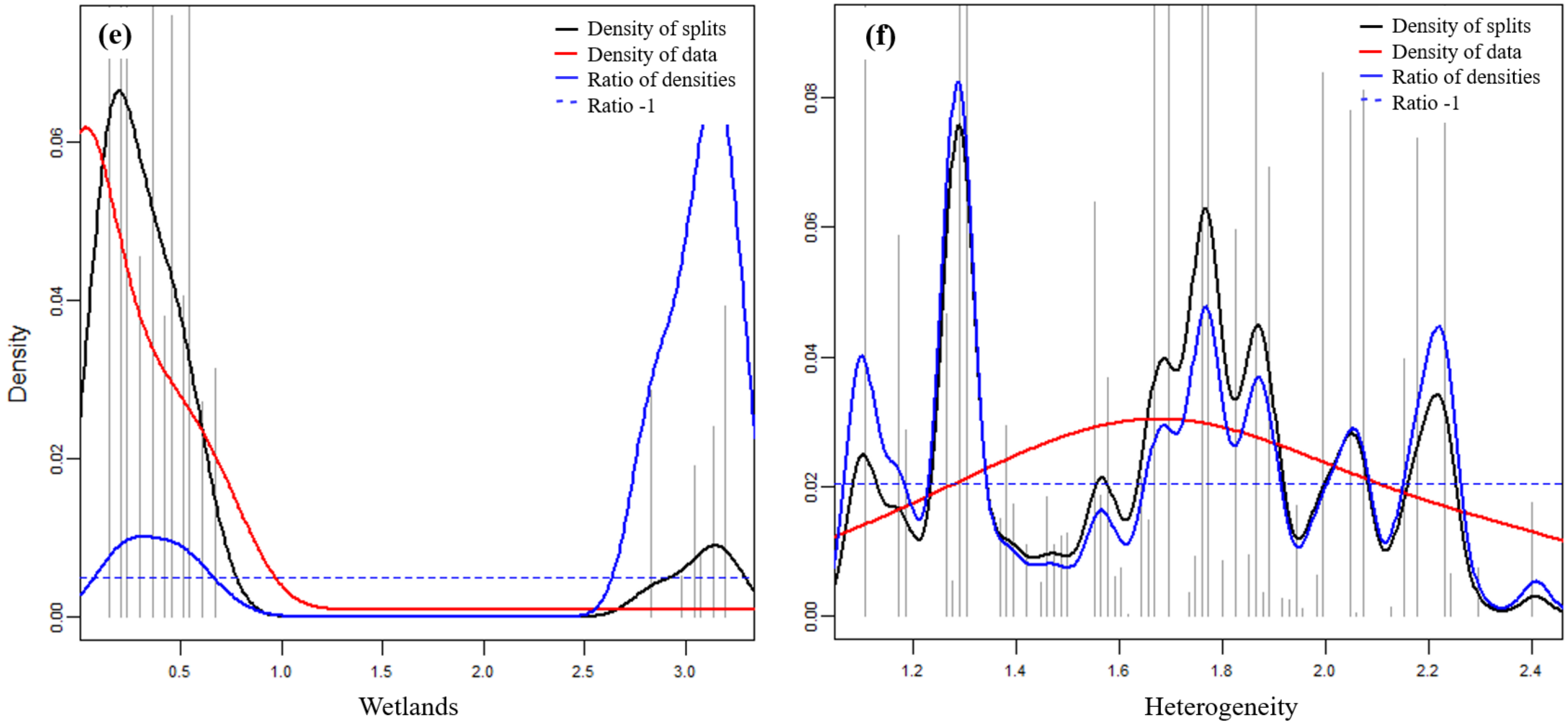
**Table S35** – Sites-by-functional response groups. This matrix was generated crossing the information of what species belongings to each functional group and sites-by-species records.

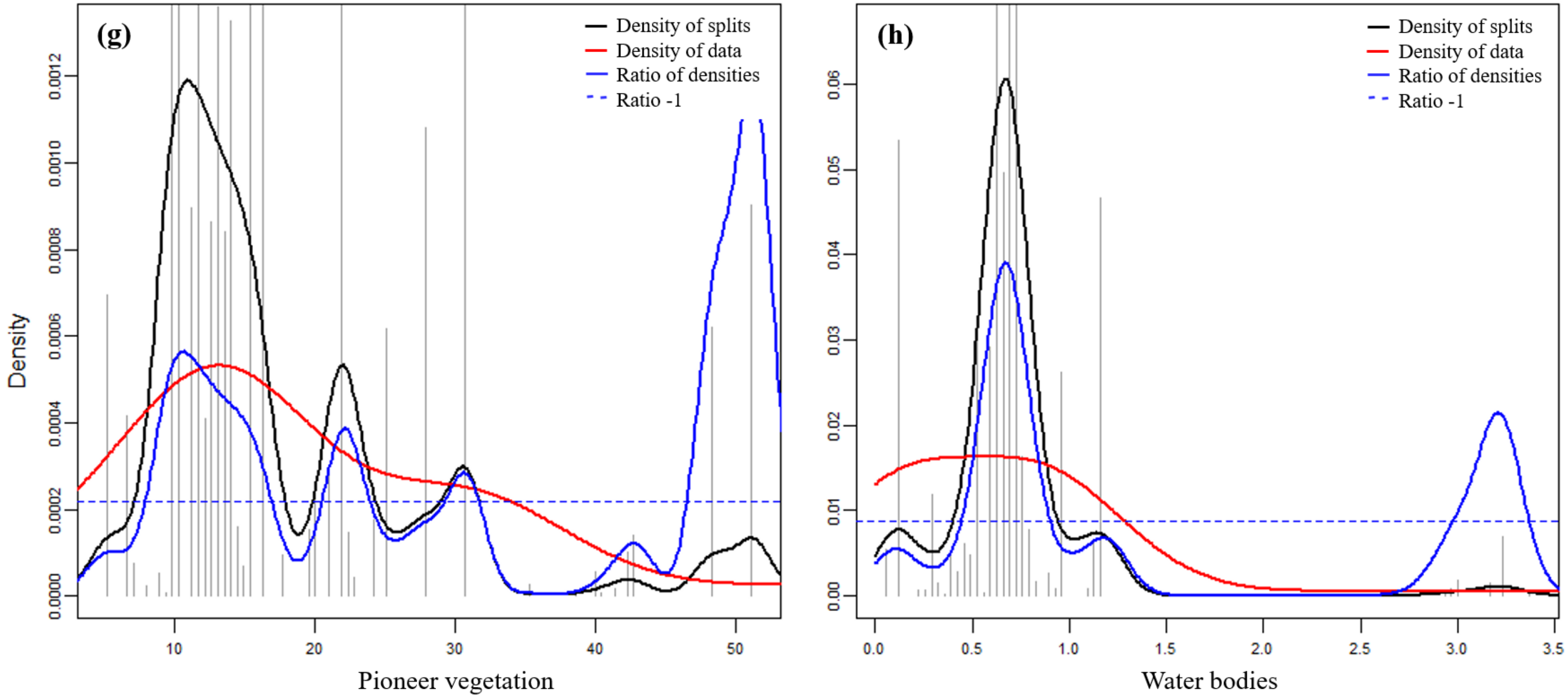
| | **fg_1** | **fg_2** | **fg_3** | **fg_4** | **fg_5** | **fg_6** | **fg_7** | **fg_8** | **fg_9** |
|---|---|---|---|---|---|---|---|---|---|
| **site 1** | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| **site 2** | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 |
| **site 3** | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 |
| **site 4** | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 |
| **site 5** | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| **site 6** | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 |
| **site 7** | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 |
| **site 8** | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 |
| **site 9** | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 |
| **site 10** | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| **site 11** | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 |
| **site 12** | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| **site 13** | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| **site 14** | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| **site 15** | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |

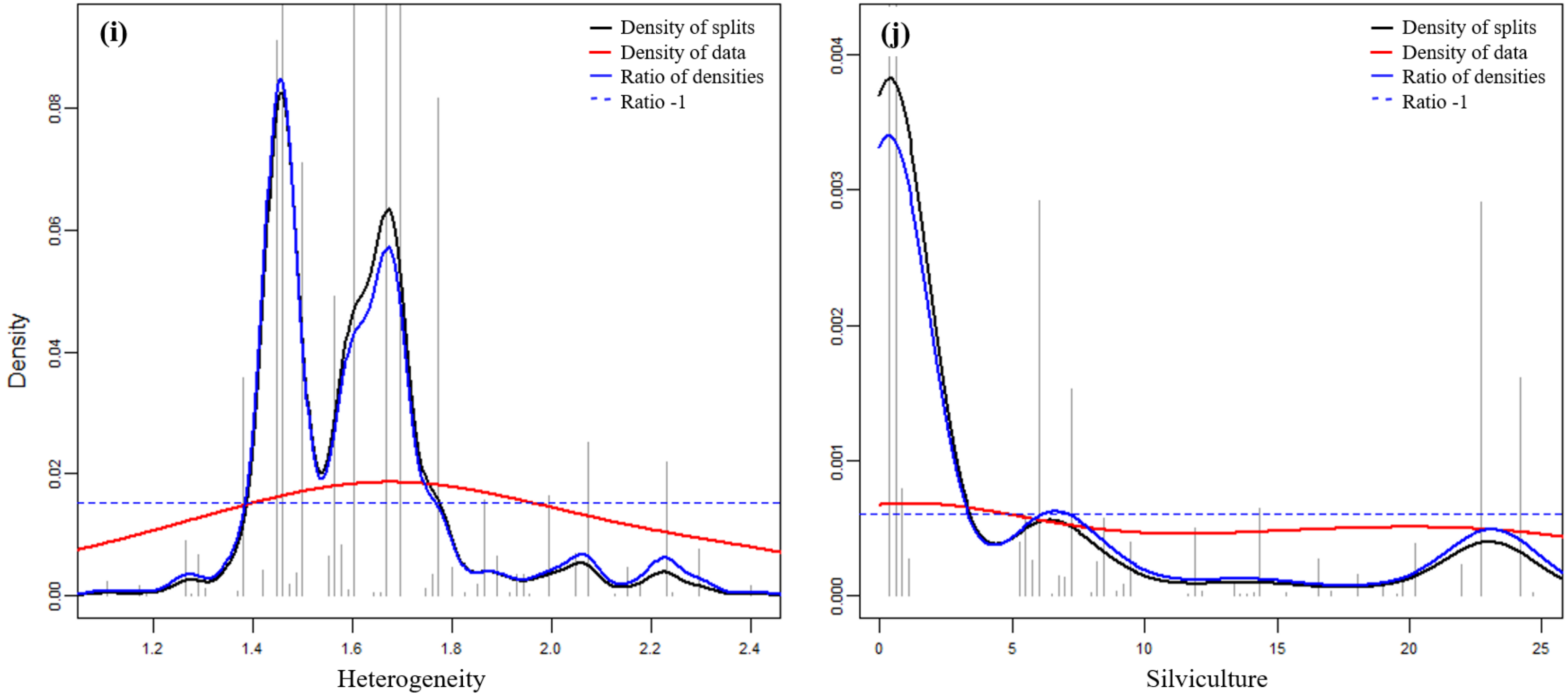
**Fig. S32** – Density plots showing along the environmental gradient where important compositional changes in the species richness are happening. Positions along the gradient where the ratio of split density and density of data is > 1 indicate relatively bigger changes in community composition (horizontal dashed line indicating where the ratio is 1). Abrupt sections of a curve show large change in the occurrence of a taxon. Splits location and importance on gradient (histogram), density of splits (black line) and observations (red line) and ratio of splits standardized by observation density (blue line). (a), (b), (c), (d), (e), (f) e (g) are the environmental gradients to SP. (h), (i) e (j) are the environmental gradients to FRT. (l), (m), (n), (o) e (p) are the environmental gradients to FRG.




(c)
Density of splits
Density of data
Ratio of densities
Ratio -1
Density
0.00
0.02
0.04
0.06
0.08
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Water bodies
(d)
Density of splits
Density of data
Ratio of densities
Ratio -1
0.0000
0.0005
0.0010
0.0015
0.0020
0.0025
30
40
50
60
70
Forest


(e)
Density of splits
Density of data
Ratio of densities
Ratio -1
Density
0.00
0.02
0.04
0.06
0.5
1.0
1.5
2.0
2.5
3.0
Wetlands
(f)
Density of splits
Density of data
Ratio of densities
Ratio -1
0.00
0.02
0.04
0.06
0.08
1.2
1.4
1.6
1.8
2.0
2.2
2.4
Heterogeneity


(g)
Density of splits
Density of data
Ratio of densities
Ratio -1
Density
0.0000
0.0002
0.0004
0.0006
0.0008
0.0010
0.0012
10
20
30
40
50
Pioneer vegetation
(h)
Density of splits
Density of data
Ratio of densities
Ratio -1
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Water bodies


(i)
Density of splits
Density of data
Ratio of densities
Ratio -1
Density
0.00
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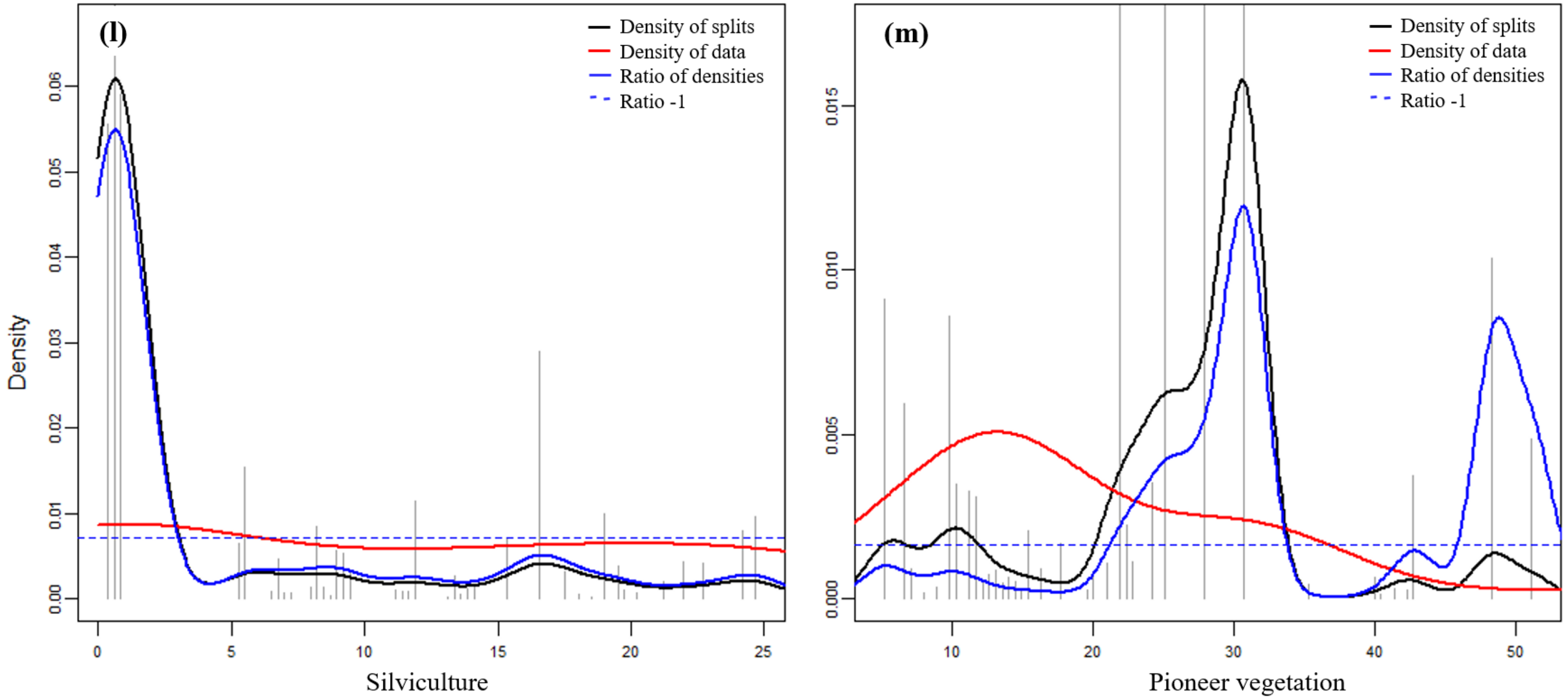
0.04
0.06
0.08
1.2
1.4
1.6
1.8
2.0
2.2
2.4
Heterogeneity
(j)
Density of splits
Density of data
Ratio of densities
Ratio -1
0.000
0.001
0.002
0.003
0.004
0
5
10
15
20
25
Silviculture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5
10
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20
25
Silviculture
(m)
Density of splits
Density of data
Ratio of densities
Ratio -1
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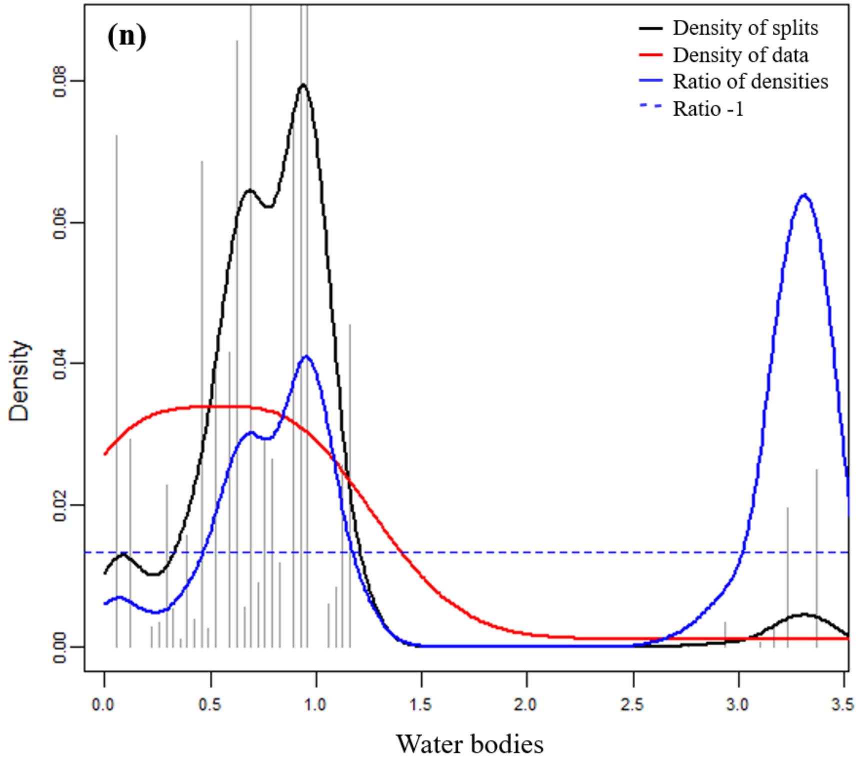
0.005
0.010
0.015
10
20
30
40
50
Pioneer vegetation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Ratio of densities
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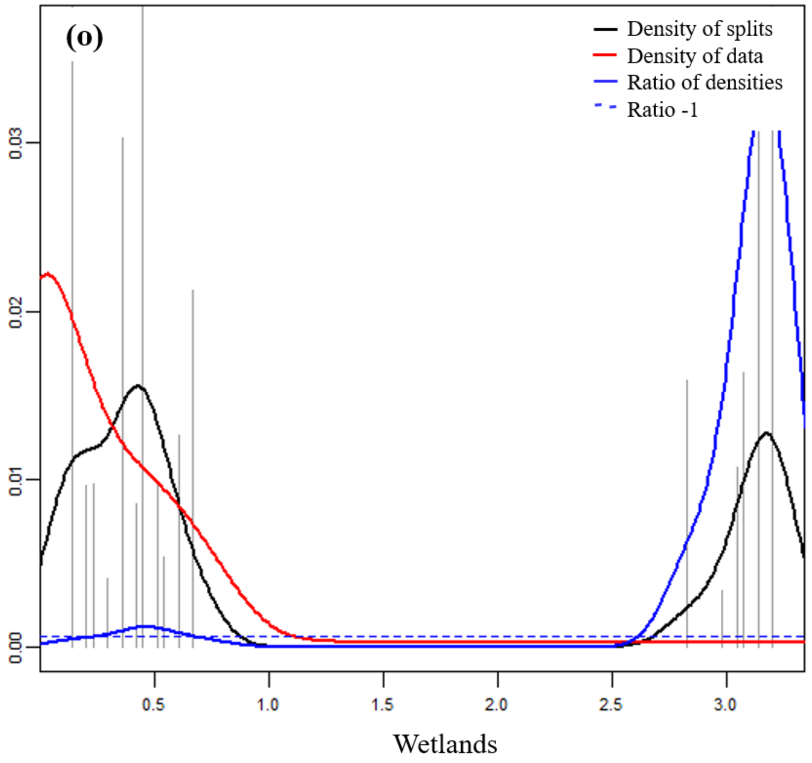
3.0
3.5
Water bodies


(o)
Density of splits
Density of data
Ratio of densities
Ratio -1
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2.5
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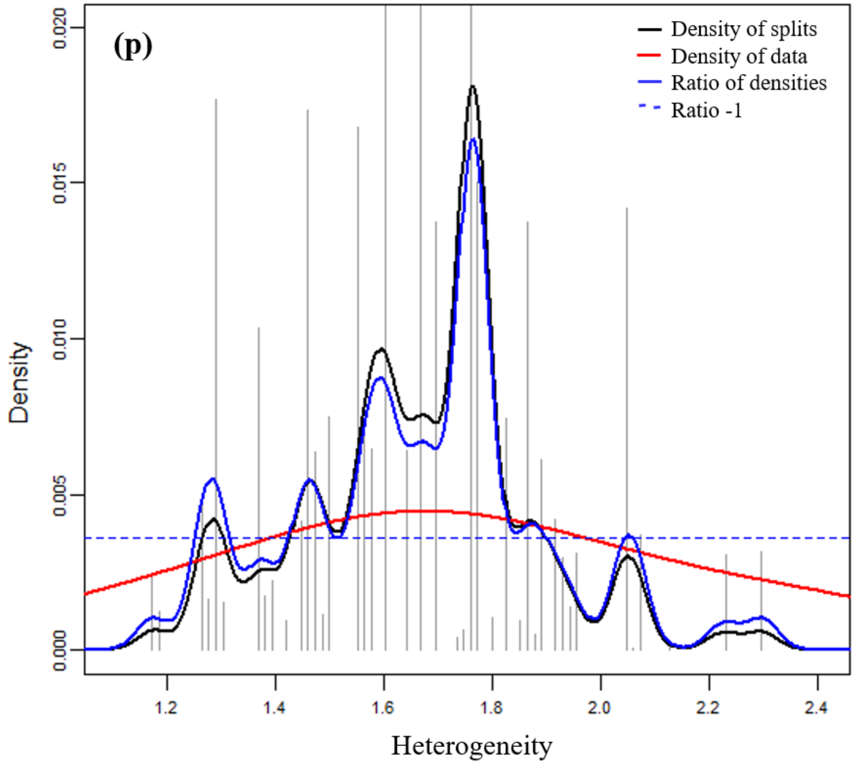
Wetlands


(p)
Density of splits
Density of data
Ratio of densities
Ratio -1
Density
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0.005
0.010
0.015
0.020
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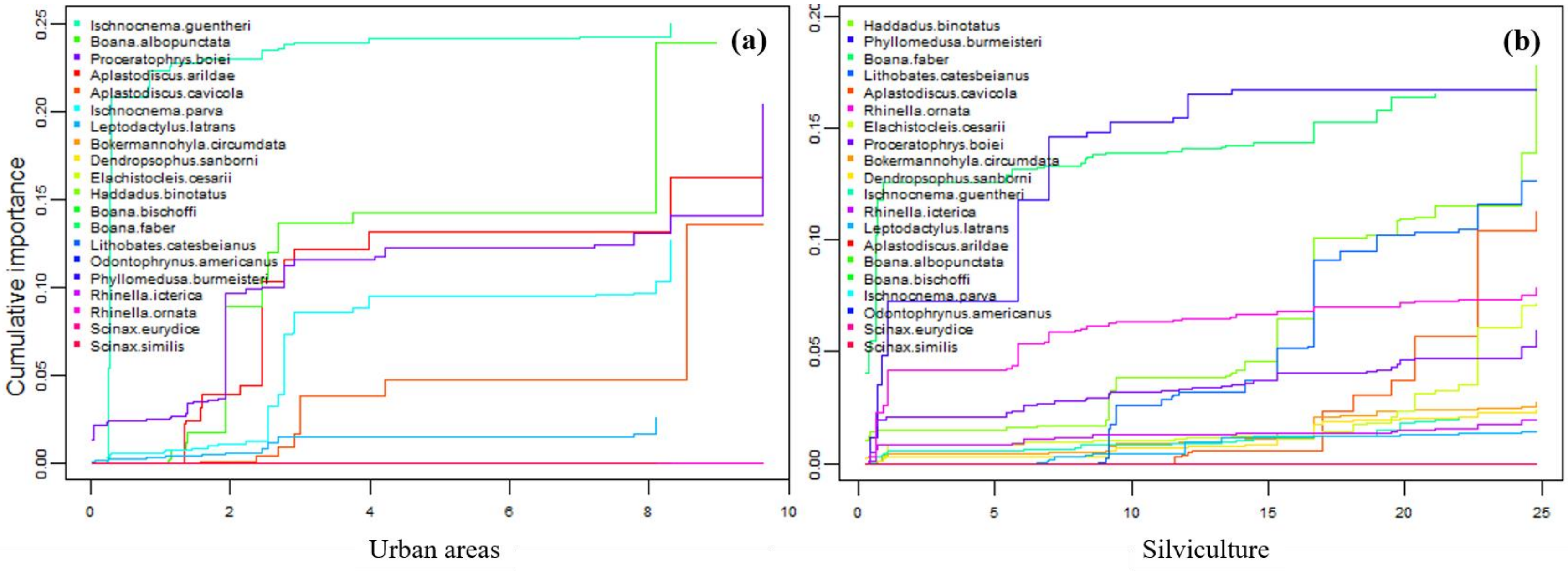
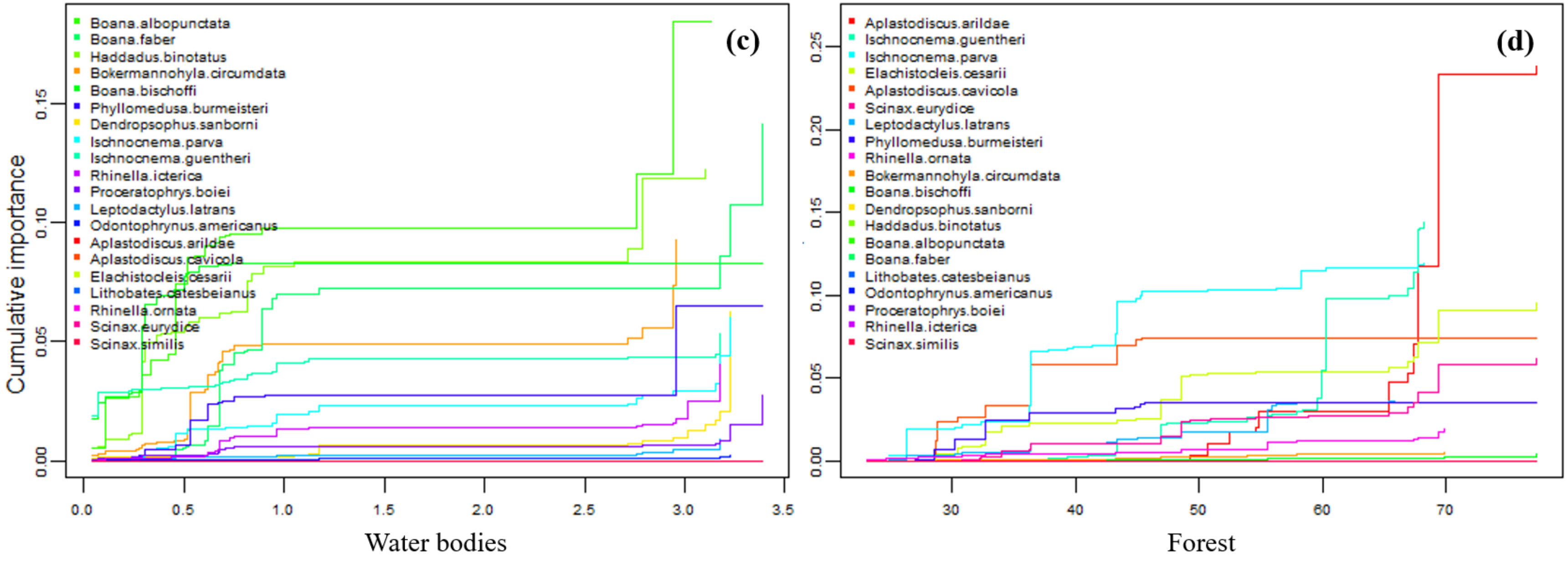
1.4
1.6
1.8
2.0
2.2
2.4
Heterogeneity

**Fig. S33 -** Species cumulative plots showing cumulative change in occurrence of individual species/functional response trait/functional response group, where in the gradient changes occur and which species are most responsible for these changes on each gradient. (a), (b), (c), (d), (e), (f) e (g) are the environmental gradients to SP. (h), (i) e (j) are the environmental gradients to FRT. (l), (m), (n), (o) e (p) are the environmental gradients to FRG.




Cumulative importance
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0.05
0.10
0.15
(c)
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Boana.faber
Haddadus.binotatus
Bokermannohyla.circumdata
Boana.bischoffi
Phyllomedusa.burmeisteri
Dendropsophus.sanborni
Ischnocnema.parva
Ischnocnema.guentheri
Rhinella.icterica
Proceratophrys.boiei
Leptodactylus.latrans
Odontophrynus.americanus
Aplastodiscus.arildae
Aplastodiscus.cavicola
Elachistocleis.cesarii
Lithobates.catesbeianus
Rhinella.ornata
Scinax.eurydice
Scinax.similis
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Water bodies
0.00
0.05
0.10
0.15
0.20
0.25
(d)
Aplastodiscus.arildae
Ischnocnema.guentheri
Ischnocnema.parva
Elachistocleis.cesarii
Aplastodiscus.cavicola
Scinax.eurydice
Leptodactylus.latrans
Phyllomedusa.burmeisteri
Rhinella.ornata
Bokermannohyla.circumdata
Boana.bischoffi
Dendropsophus.sanborni
Haddadus.binotatus
Boana.albopunctata
Boana.faber
Lithobates.catesbeianus
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Rhinella.icterica
Scinax.similis
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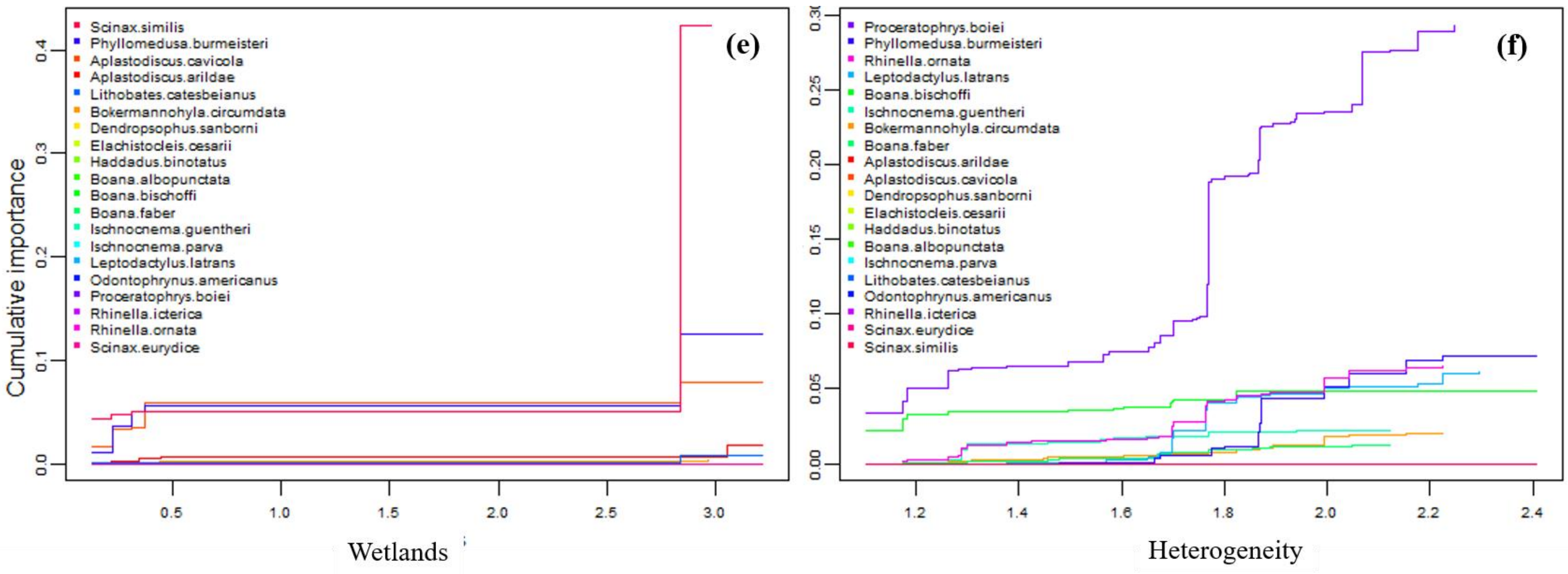
40
50
60
70
Forest


(e)
Cumulative importance
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0.1
0.2
0.3
0.4
Scinax.similis
Phyllomedusa.burmeisteri
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Aplastodiscus.arildae
Lithobates.catesbeianus
Bokermannohyla.circumdata
Dendropsophus.sanborni
Elachistocleis.cesarii
Haddadus.binotatus
Boana.albopunctata
Boana.bischoffi
Boana.faber
Ischnocnema.guentheri
Ischnocnema.parva
Leptodactylus.latrans
Odontophrynus.americanus
Proceratophrys.boiei
Rhinella.icterica
Rhinella.ornata
Scinax.eurydice
0.5
1.0
1.5
2.0
2.5
3.0
Wetlands
(f)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
Proceratophrys.boiei
Phyllomedusa.burmeisteri
Rhinella.ornata
Leptodactylus.latrans
Boana.bischoffi
Ischnocnema.guentheri
Bokermannohyla.circumdata
Boana.faber
Aplastodiscus.arildae
Aplastodiscus.cavicola
Dendropsophus.sanborni
Elachistocleis.cesarii
Haddadus.binotatus
Boana.albopunctata
Ischnocnema.parva
Lithobates.catesbeianus
Odontophrynus.americanus
Rhinella.icterica
Scinax.eurydice
Scinax.similis
1.2
1.4
1.6
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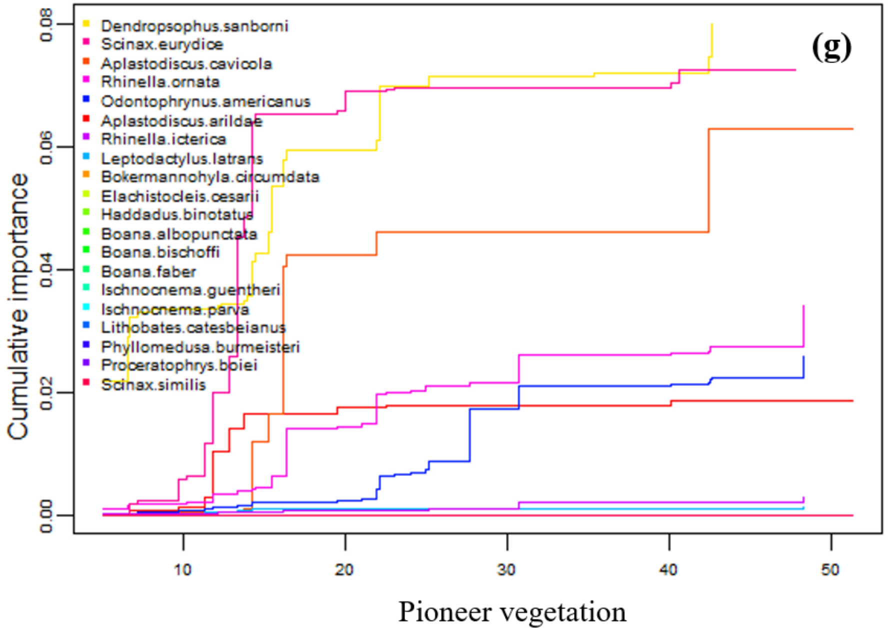
2.4
Heterogeneity


(g)
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Rhinella.ornata
Odontophrynus.americanus
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Rhinella.icterica
Leptodactylus.latrans
Bokermannohyla.circumdata
Elachistocleis.cesarii
Haddadus.binotatus
Boana.albopunctata
Boana.bischoffi
Boana.faber
Ischnocnema.guentheri
Ischnocnema.parva
Lithobates.catesbeianus
Phyllomedusa.burmeisteri
Proceratophrys.boiei
Scinax.similis
Cumulative importance
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10
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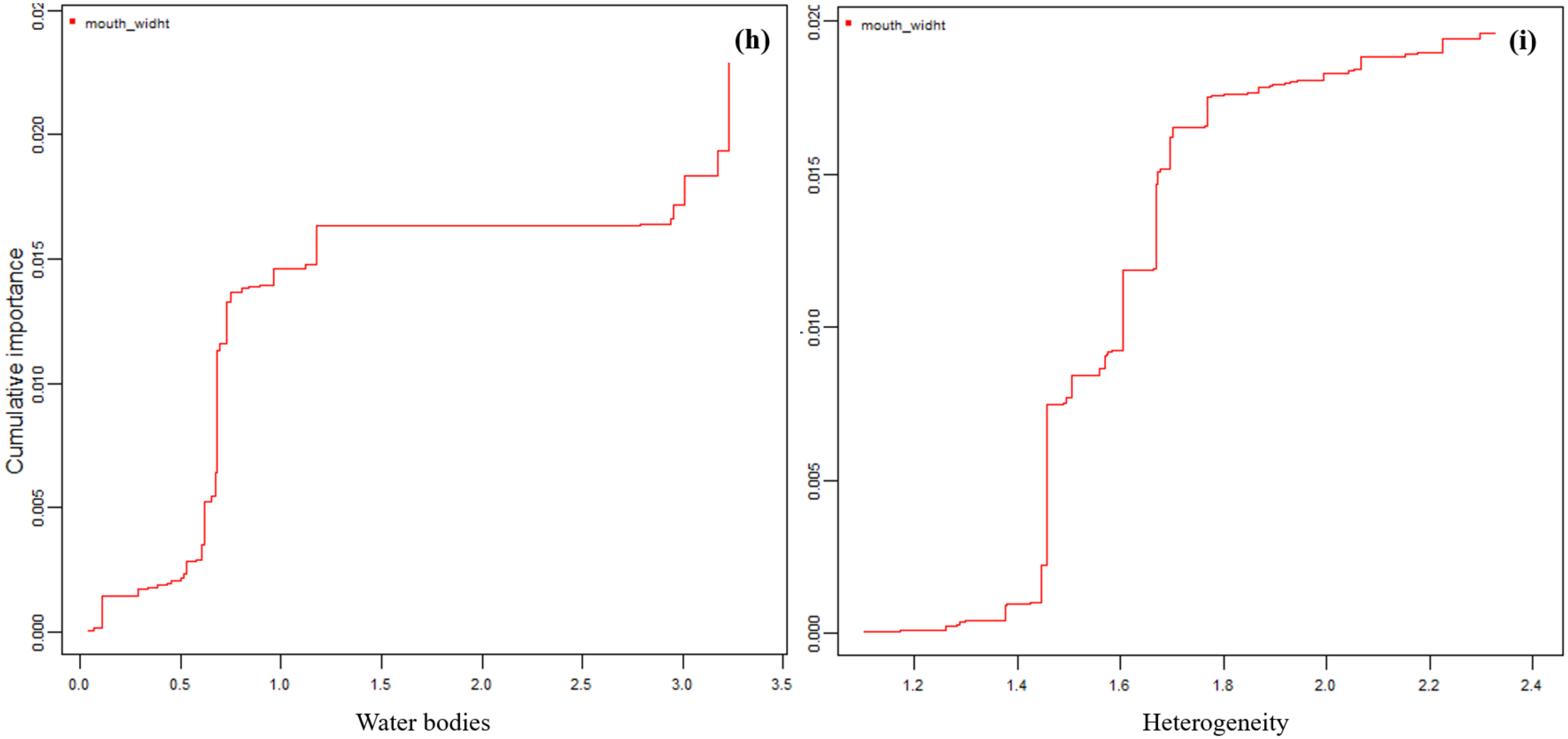
40
50
Pioneer vegetation


mouth_widht
(h)
Cumulative importance
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0.010
0.015
0.020
0.02
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1.0
1.5
2.0
2.5
3.0
3.5
Water bodies
mouth_widht
(i)
0.000
0.005
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1.2
1.4
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2.0
2.2
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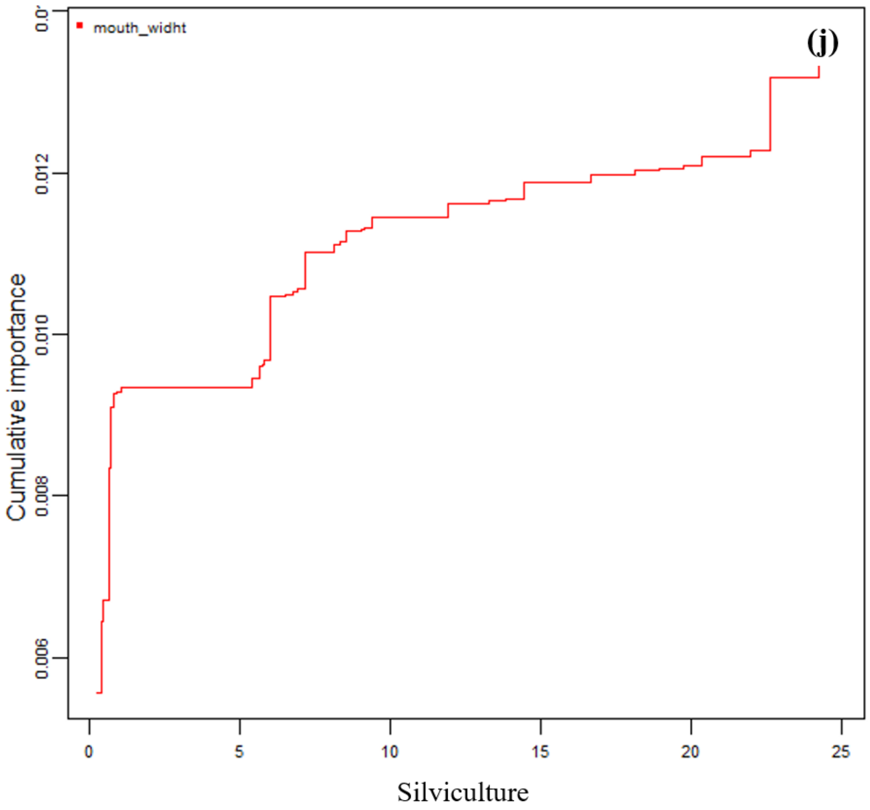
Heterogeneity


mouth_widht
(j)
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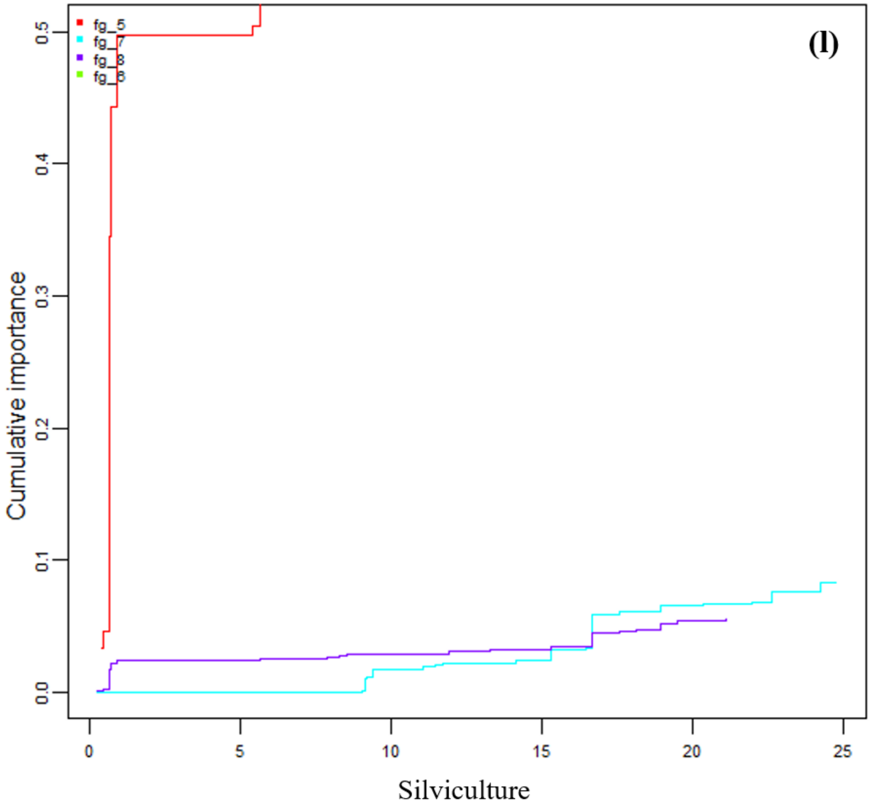
Silviculture


fg_5
fg_7
fg_8
fg_6
(l)
Cumulative importance
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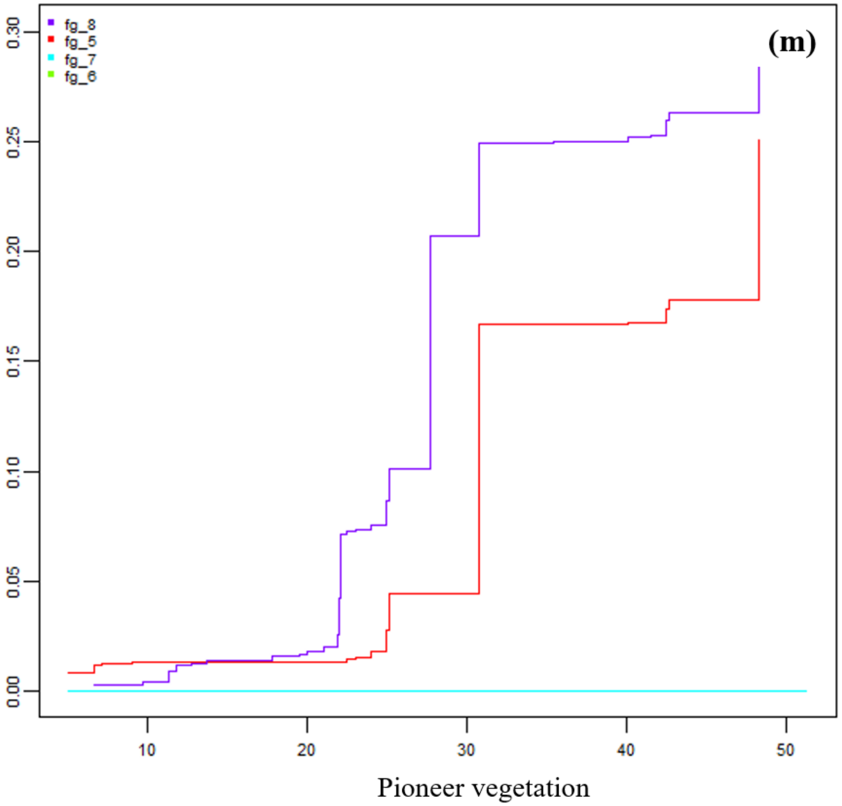
Silviculture


fg_8
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(m)
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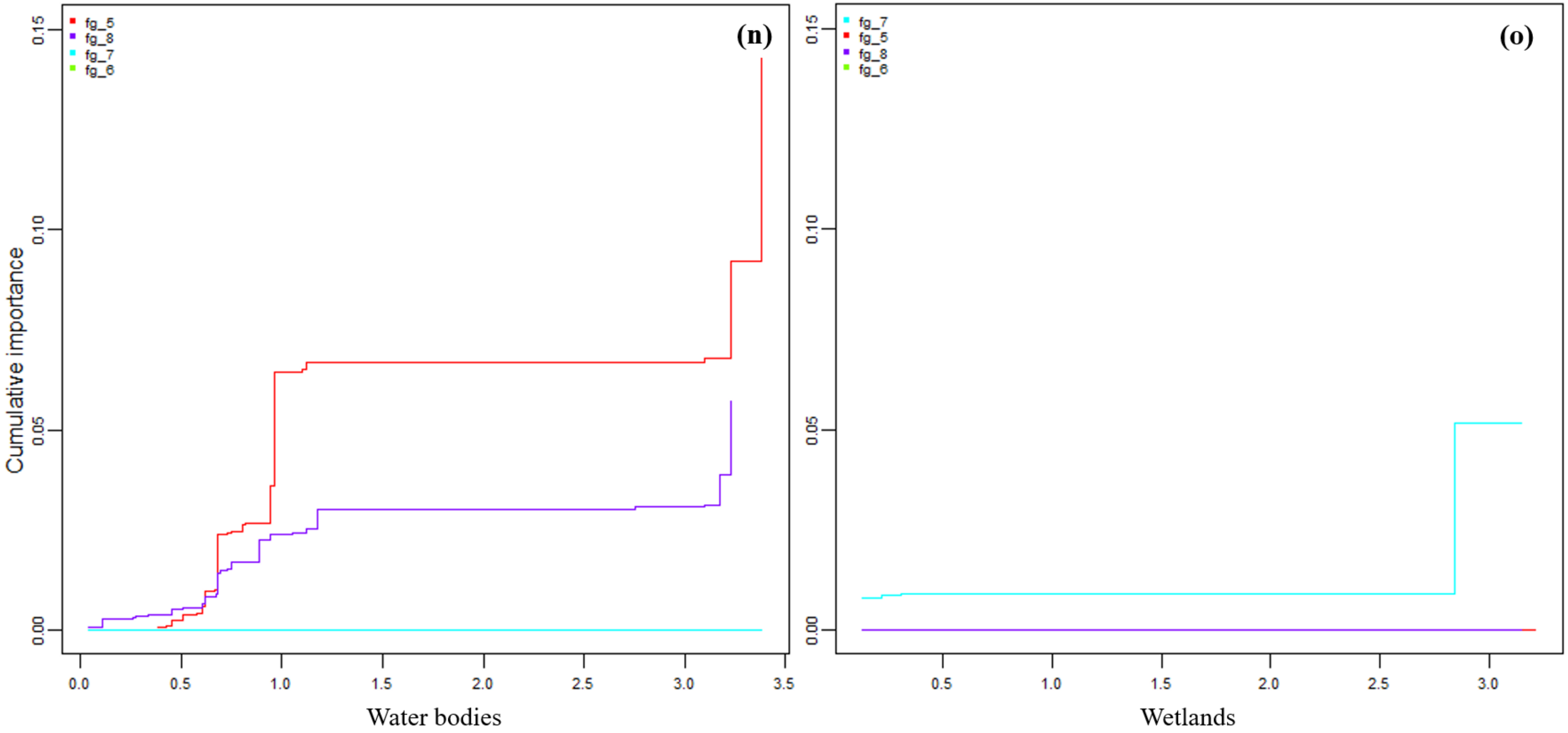
Pioneer vegetation


(n)
🟥 fg_5
🟪 fg_8
🟦 fg_7
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Cumulative importance
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0.15
0.0
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1.0
1.5
2.0
2.5
3.0
3.5
Water bodies
(o)
🟦 fg_7
🟥 fg_5
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🟩 fg_6
0.00
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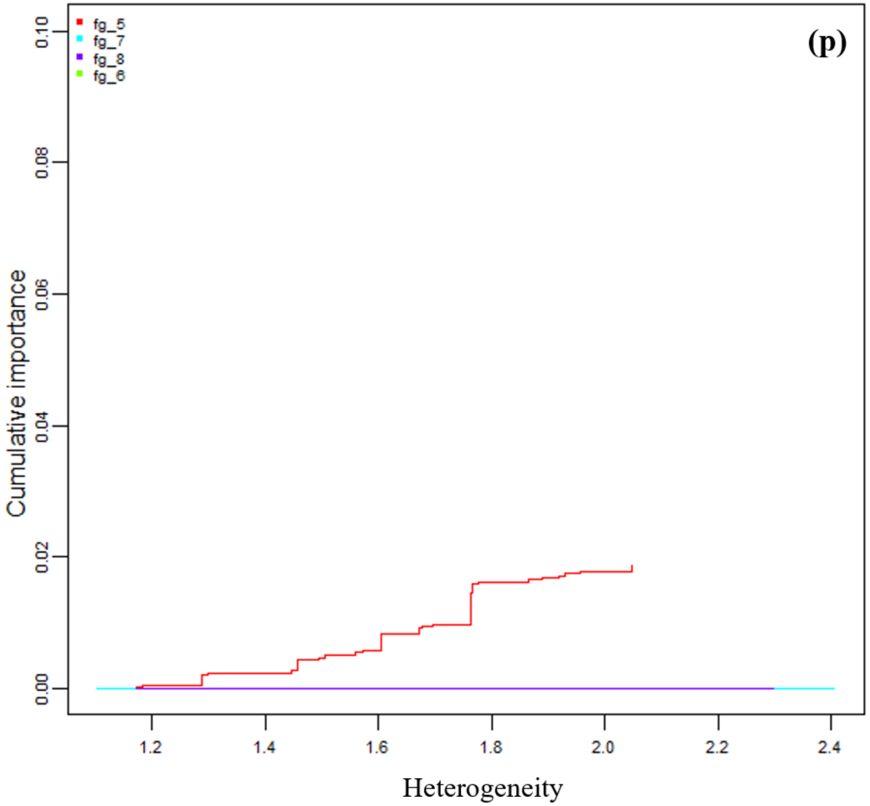
3.0
Wetlands



(p)
fg_5
fg_7
fg_8
fg_6
Cumulative importance
0.00
0.02
0.04
0.06
0.08
0.10
1.2
1.4
1.6
1.8
2.0
2.2
2.4
Heterogeneity

# ARTIGO 3 - Functional and taxonomic amphibian decline due to climate change within Brazilian Atlantic Forest

*Format based on the guidelines of "Diversity and Distributions*"

# Functional and taxonomic amphibian decline due to climate change within Brazilian Atlantic Forest

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[2]São Paulo State University (UNESP), Institute of Biosciences, Department of Ecology, Spatial Ecology and Conservation Lab (LEEC), Rio Claro, SP, Brazil
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## ABSTRACT

Climate changes are one of the key anthropogenic pressures on biodiversity loss. The amphibians are especially sensitive to temperature and precipitation alterations, therefore these species have been negatively affected by climate changes. Herein we applied Ecological Niche Modelling to evaluate the effect of climate changes on taxonomic and functional richness of amphibians within Atlantic Forest biome. We have also evaluated how forest-dependent and open habitat specialists can be affected by climate changes. We expect that (1) both taxonomic and functional amphibian richness will decrease under climate change; (2) functional richness will be more affected than taxonomic richness; (3) forest species will be more affected than open habitat specialists under climate change scenarios. To build taxonomic and functional prediction maps, we used two scenarios of temperature increase (the Representative Concentration Pathway, RCP 4.5 and 8.5) for two different temporal climatic scenarios (2050 and 2070). The temperature increase will cause loss of amphibian's species and functional diversity. However, contrary to our expectation, the negative impact of climate changes is greater in taxonomic richness than functional. Both categories of habitat specialists will lose species, but forest species have greater loss probably due to their high habitat structure requirements. Taxonomic and functional richness give us complementary information about the warming future scenarios, being advised to evaluate both indexes to reach an efficient decision-making about mitigation measures. We also recommended paying special attention on forest species, which seems will be more impacted by the climate changes.

**KEY WORDS**

Anurans – Species declines – Global warming – Functional trait approach – Functional response traits

## 4.1 Introduction

We are facing one of the warmest period on Earth, global average temperature has increased about 0.85 (0.65 to 1.06) °C over the period 1880 to 2012, with this change in the temperature being attributed to the intensification of greenhouse gas emission (IPCC, 2014). The expectation is that global temperature will rise by 0.3 to 4.8 °C by the late-21st century, but different regions will experience different changes, from cooling to warming (Serreze et al., 2000). In the Neotropical region, the predictions are quite similar: increased temperature and length of the dry season, reduced soil moisture and larger interannual variation in rainfall (Hulme; Viner, 1998). Three consequences for the biodiversity are expected worldwide as a response to climate change: (i) changes in phenology, (ii) shift in geographic distributions; and (iii) body-size shrinks (Daufresne et al. 2009).

Amphibians present life-history characteristics such as, permeable and exposed skin, shell-less eggs, complex life cycles, which make them very sensitive to anthropogenic impacts (Cushman, 2006; Wells, 2007; Hof et al., 2011; Catenazzi, 2015). Because they are also ectotherms, the impact of climate change, for instance, can be more severe for them than other animals (*see review* Parmesan, 2006; Li et al., 2013). Moreover, temperature is a key abiotic factor influencing these animals, affecting the aquatic larvae stage, like in the time to hit metamorphosis and growth rates, body size at metamorphosis, mechanisms of gas exchange, and energy metabolism rates (Ultsch et al., 1999). Increases in temperature and dryness can reduce amphibian fecundity (Reading, 2007) and adult recruitment (Lowe, 2012). In a recent meta-analysis the authors showed that amphibians are breeding earlier than other groups, such as butterflies, birds and trees (Parmesan, 2007). However, alterations in body size and morphology of amphibians and poleward shifts in distributions in response to climate change remains with little evidence (Li et al., 2013). Even if climate change has no direct effects on amphibian declines, or it presents a non-clear effect, oscillations in climate will severely affect this taxon. Then, it is necessary to find the best approaches, which can clarify the effects of climate change in the biodiversity. Functional diversity can be key-component to understand the patterns of temperature variations, with some authors suggesting this approach can potentially predict the impacts more accurately (Higgins; Strauss, 2008, Dengler et al., 2014; Pelosi et al., 2014).

Functional traits are efficient to compare broad geographic regions, particularly when we have interest in communities of different taxa (Ernst, 2012; Simberloff; Dayan, 1991). Moreover, with the functional characteristics is possible to investigate changes in species traits along environmental gradients (Vilà-Cabrera et al., 2015; Cornwell; Ackerly, 2009). The interest on functional diversity is rising between ecologist and conservationist (Tsianou; Kallimanis, 2016). The literature points that this approach can be more efficient to comprehend ecosystem resilience and functioning when compared with taxonomic-driven analysis, because functional diversity is linked to these processes more intensely than other components of diversity (Ribeiro et al., 2017; Trimble; Van Aarde, 2014, Flynn et al. 2009, Ernst, 2006). At large extents, the assembly processes can be understood through spatial and temporal patterns of functional diversity (Petchey et al. 2007).

Studies aiming to understand the spatial distribution of functional diversity are still scarce (Thuiller et al., 2015; Tsianou; Kallimanis, 2015). In general, these studies are mainly about whether trait can be predictive of range shift capacity face the climate change, however, spatial distribution of functional diversity rarely has been evaluated (Iverson et al., 2011; Chessmann, 2011; Estrada, 2016). Besides that, there are few studies focusing on the impact of climate changes in amphibians of tropical areas at large scale (Li et al., 2013). Previous studies about the impact of climate changes in the amphibians of the Brazilian Atlantic Forest showed it will be a decreasing number of species under future scenarios and protected areas would become less effective to protect this taxon (Loyola et al., 2013; Lemes et al., 2013). Amphibians had been pointed out as one of the animal groups most negatively affected by climate changes (Parmesan, 2006; Li et al., 2013; Catenazzi, 2015), then we choose this group to develop the first study about predicting models of functional diversity distribution. To this end, we applied ecological niche-based models to assess the effect of climate changes on amphibian communities in Brazilian Atlantic Forest Biome. We addressed three questions: 1) Is the future distribution of amphibian richness according to climate changes different from the current distribution? 2) What is the effect of climate changes on functional and taxonomic richness of amphibians? 3) What is the effect of climate changes on forest and open area species? We expect that (1) due to the amphibians sensitivity to external conditions variation, both taxonomic and functional richness will decrease under climate change; (2) because the functional approach has shown more sensitive to disturbance impact than traditional indexes

(Ernst et al., 2006; Naeem et al., 2012), functional richness will be more affected than taxonomic richness and 3) forest species will be more affected than open habitat specialists under climate change scenarios (Fig. 1).

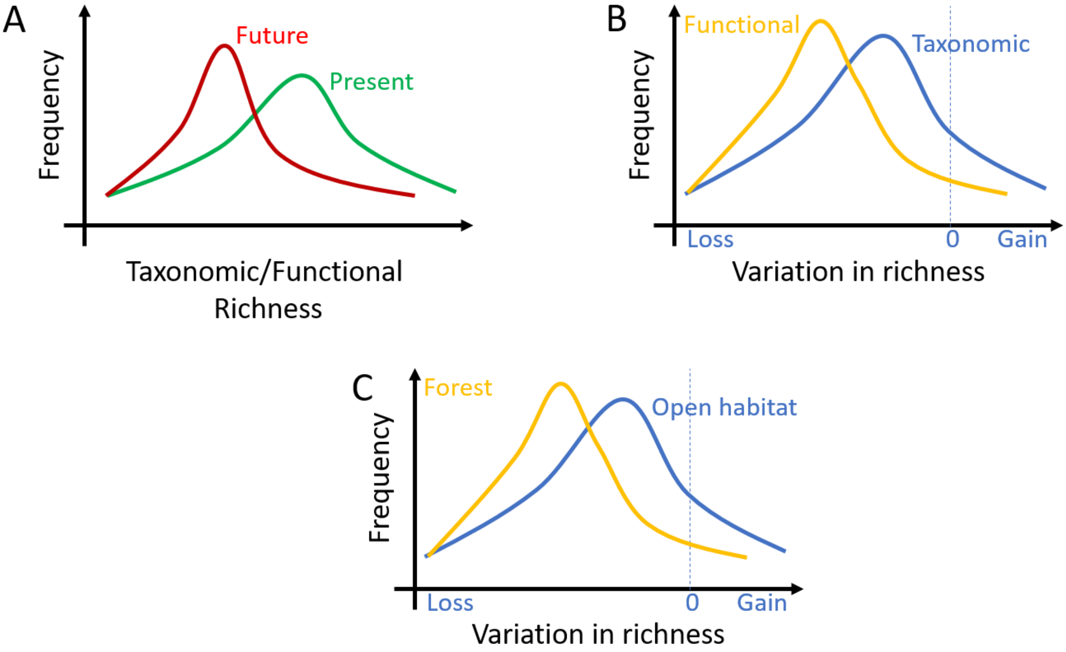

**Figure 1**. Expected patterns for amphibian richness changes under climate chance scenarios. (**A)** Future taxonomic and functional richness will be low when compared to present distribution; (**B)** Functional richness will be more affected than taxonomic richness; (**C**) Forest specialist species will be more affected than open habitat specialists.

## 4.2 Methods

### *4.2.1 Study Area*

The Atlantic Forest originally covered an area of ~150 million ha, distributed between 3º and 31º of latitude south, in a gradient of elevation and ranging from the Atlantic coast to the interior lands of Brazil, Argentina and Paraguay (Ribeiro et al., 2011). Nowadays the biome is highly fragmented, with only 12 to 16% of the original forests remaining, represented by small-sized patches (84% of them are < 50 ha), isolated (average isolation 1,440 meters) and under severe edge effects (half of the remaining forest are less than 100 m from any edge; Ribeiro et al., 2009). Because the biome presents a great number of endemisms, high biodiversity, and due to its highly fragmented condition, the Atlantic Forest is considered a biodiversity hotspot (Mittermeier et al. 1999). However, the fragmentation level is variable, from < 7.1 % at São Francisco and Interior biogeographical sub regions, to 36.5% in the Serra do Mar range.

### *4.2.2 Ecological Niche Modeling*

To predict appropriate current and future areas and thus estimate the change in taxonomic and functional diversity of amphibians in the Atlantic Forest we used Ecological Niche Modeling (ENM). ENMs use algorithms to associate occurrences of species to environmental conditions (generally climatic, such as temperature and precipitation), which allow us to characterize and delimit the niche of the species (Peterson et al. 2011). We used a forecast ensemble approach that combines different types of projections, constructed from various methods and conditions, which results in a more reliable prediction (Araújo and New, 2007, Diniz-Filho et al., 2009).

Presence-only data were obtained from the literature, which used various sources such as undergrad monographs, dissertations, thesis, articles and books (see supplementary material for a complete list of references). These data are a subset of ATLANTIC AMPHIBIANS data paper (Vancine et al., under review) and to our study, this refers more than 240 studies and about 372 sampling points in the database (**Figure 2**). The dataset comprises 172 species and 6,657 records. The number of unique records for the species varied from 10 to 230 (average 39 records). More information about these data can be found at https://github.com/mauriciovancine/ATLANTIC-Amphibians.

We used 19 bioclimatic variables from the WorldClim database at a spatial resolution of 2.5 minutes (Hijmans et al. 2005). We used the present and future projections of two different temporal climatic scenarios (2050 and 2070), for two futures warm climate scenarios, Representative Concentration Pathway (RCP) 4.5 and 8.5. Respectively, these two scenarios yield warming of 2.3 °C and 4.8 °C. RCP 8.5 is the most pessimistic version to date, with continuous greenhouse emissions until 2100 producing the most severe global climate change (Moss et al., 2008). We used the General Circulation Models (GCMs) called ACCESS1-0 developed as collaboration between CSIRO and BoM (Dix et al. 2013).

To avoid collinearity among the variables, we used a factorial analysis with *varimax* rotation (”psych” package, R Development Core Team 2017; Revelle, 2017) to reduce the number of environmental layers. After this step, we selected the five bioclimatic variables that we consider most relevant to the distribution of tropical amphibians, which are dependent of warm and rainy weather conditions to breeding: temperature seasonality (Bio4), mean temperature of the warmest quarter (Bio10), precipitation of wettest month (Bio13), precipitation seasonality (Bio15), and precipitation of driest quarter (Bio17). For the modeling of both present and future scenarios, we defined the geographic extent using the Atlantic Forest

Domain limit available by Ribeiro et al. (2009), which are available at https://github.com/LEEClab/ATLANTIC-limits-shapefiles.

We build the ENMs based in four algorithms, the first two are presence-only methods and the later ones presence-background methods: envelope score – Bioclim (Nix, 1986), Gower distance method – Domain (Gower distance; Carpenter et al. 1993), other two are machine-learning methods – Maximum Entropy – MaxEnt (Phillips and Dudík 2008) and Support Vector Machines (SVM) (Tax and Duin 2004). The ENMs were modeled using “dismo” and “kernlab” R-packages (Hijmans et al., 2015; Karatzoglou et al., 2004). We modeled each species individually using a twofold partition approach with 75% train and 25% test. With this occurrence-partitioned approach, we calculate the evaluation values using True Skill Statistics (TSS) of each model (Allouche et al. 2006). Through bootstrap analysis, for each algorithm we randomized each procedure 5 times, resulting in 20 maps (4 algorithms × 5 times) for each specie. We made the ensemble transforming the 20 continuous maps into binary maps using “maximum sensitivity and specificity” threshold (Liu et al. 2016). This threshold is recommended when presence-only data are available in the niche modeling analysis (Liu et al., 2013, 2016). To predict the final species distribution maps, we overlapped the maps from the same algorithms and the maps between algorithms. Thus, the final maps have cell values varying between 0 and 20, which represents the frequency that the model predicted each cell as suitable of each species. This process was made to present models and used the same thresholds of present models for future models.

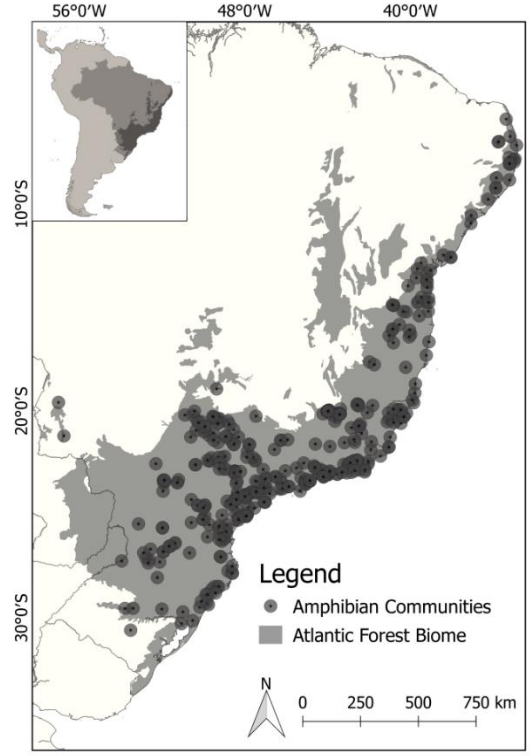

**Figure 2** - Spatial distribution of amphibian surveys (dark gray dots) into Brazilian Atlantic Forest. These data represent a subset of ATLANTIC AMPHIBIANS dataset (Vancine et al., under review). The boundaries are based on Ribeiro et al. (2009).

### *4.2.3 Functional response traits selection*

We selected seven functional traits to calculate functional diversity inferring about amphibian characteristics that would be affected by climate changes (Table 1). The dimorphism, movement index, and mouth area were continuous traits and we measured in male specimens from Célio F. B. Haddad amphibian collection, housed in the Universidade Estadual Paulista (UNESP), Rio Claro, SP (CFBH). We measured the traits of anurans species from the Atlantic Forest which have the minimum number of specimens in biological collections (6 to 20 individuals per species, 129 species) and also have the minimum number of occurrence points equals to 10. The others four traits, activity, reproductive mode, ecotype and reproduction habitat, were categorical and the information were taken from literature. Moreover, the female SVL (snout venter length), which area used to calculate the dimorphism, was also obtained from the literature (Haddad et al., 2013

**Table 1**. Description of the traits measured or compiled from the literature on amphibian species of the Atlantic Forest Biome.

| Functional trait | Definition | Specific functions | Ecosystem process relationship |
|---|---|---|---|
| Dimorphism | Male SVL / Female SVL | Physiology and ecology characters | Related to the energy flow through trophic chains as predator or prey |
| Movement index | Arm length / leg length | Dispersal ability | Related to the energy flow through trophic chains as predator or prey |
| Mouth area | Depth mouth / width mouth | Foraging tactics, predator-prey relationships | Related to the energy flow through trophic chains as a predator |
| Activity | Diurnal, nocturnal and both | Dispersal ability, predator-prey relationships, sexual selection | Related to the energy flow through trophic chains as predator or prey |
| Reproductive mode | 1 to 39 modes | Dispersal ability, predator-prey relationships, sexual selection | Related to the energy flow through trophic chains as predator or prey |
| Ecotype | Arboreal, leaf-litter, fossorial, terrestrial, arboreal+leaf-litter, semi-aquatic, rheophilic | Dispersal ability, predator-prey relationships | Related to nutrient recycling and energy flow through trophic chains as predator and prey |
| Reproduction habitat | Lentic. Lotic or independent | Dispersal ability, predator-prey relationships, sexual selection | Related to the energy flow through trophic chains as predator or prey |

### 4.2.4 Taxonomic and functional diversity

To calculate taxonomic diversity per each pixel of Atlantic Forest biome, we first convert all ENMs in binary maps, using the Lowest Presence Threshold (LPT) to classify each cell, which refers to the lowest frequency that the model predicted the species occurrence (Pearson et al., 2007). Therefore, we classified cell with values equal or higher than LPT as "occurs" (value 1) and cell with lower values than LPT as "does not occur" (value 0). To facilitate the reading of next steps, we consider that "occurs" as presence and "doesn't occur" as absence of the species. By summing these classified maps of all species in a determined scenario, we generated the taxonomic diversity map, wherein the value of each cell represents the potential richness of these cells.

Then, we calculated functional diversity for each cell of the Atlantic Forest biome. To this, we first converted the presence-absence maps into community matrixes, wherein each row represents each cell of the models and columns the species presence or absence (0 or 1 values). Afterwards, we converted categorical variables in fuzzy variables and in sequence in proportion variable using the *prep.fuzzy* variable from the ade4 package (Pavoine 2009). Using all variables, we generate a traits distance matrix with the dist.ktab function and posteriorly we combined this matrix with the presence-absence community matrixes to calculate functional richness (FRic) with the *dbFD* function from FD package. FRic represents the hypervolume in the multivariate trait space that each community occupies. Once our interest was to compare diversity between scenarios, we bind presence-absence community matrixes of all scenarios before calculating FRic. We have also standardized FRic by "global FRic", which represents a community with all species that occurred in all scenarios and have value equal to 1.

After FRic calculation, we separated the cells of each scenario and created a matrix of FRic, and converted it into a map of functional diversity, wherein the value of each cell represents the estimated functional diversity.

### 4.2.5 Analysis

We used Kruskal-Wallis to test the significance of the difference between the present distribution maps and the prediction maps of the four different scenarios of climate change: two different temporal climatic scenarios [2050 and 2070] and two futures warm climate scenarios [RCP4.5 and RCP 8.5]) for each biodiversity level and for the two groups of habitat specialist species. For each biodiversity level (taxonomic and functional richness), we made the subtraction of the future prediction maps and present distribution maps and after that, tested whether the difference is equal to zero with one sample Wilcoxon Signed Rank test. We performed all that tests in the software R, version 3.0.1 (R development core team, 2014). On the above analysis, we used every Atlantic Forest pixel.

## 4.3 Results

### *4.3.1 Influence of climate changes on taxonomic and functional richness*

The comparison of the present and future predictive maps for both taxonomic and functional richness revealed that Brazilian Atlantic forest localized in the interior areas will lose species and functions. The loss of species is greater than functions. This is true for all scenarios; however we highlight the present and the most pessimistic scenarios from both components of biodiversity in the **Figure 3.**

The taxonomic richness shows more species with a narrow distribution than functional richness. In addition, the increasing of temperature will gradually cause a decrease in the distribution range of the amphibian species and functional response traits or in other words, the scenarios of species and functions lost will get worse according the gradual increase of temperature (**Fig. 4**). According the most pessimist scenario, there is a peak of narrow distribution species in both components (**Fig. 4**). The two components of biodiversity show a positive correlation; however it is not a complete overlap, which indicate complementary information (**Fig. S41** – Supplementary material 3). In all future predictions, the range of species distribution will decrease extensively (to both biodiversity levels), as great part of richness difference distributions are negative (**Fig. 5**).

### *4.3.2 Influence of climate changes according to habitat specialization*

With the temperature increase, both categories of habitat specialist species show loss of species with bigger distribution range. However, forest species presents a narrower distribution than open area species. Except for the most pessimistic scenarios that show very similar pattern between the two categories of species. Even open area species keeping the bigger range of distribution, both categories will have a decrease in the number of species with big range of distribution (**Fig. 5**).

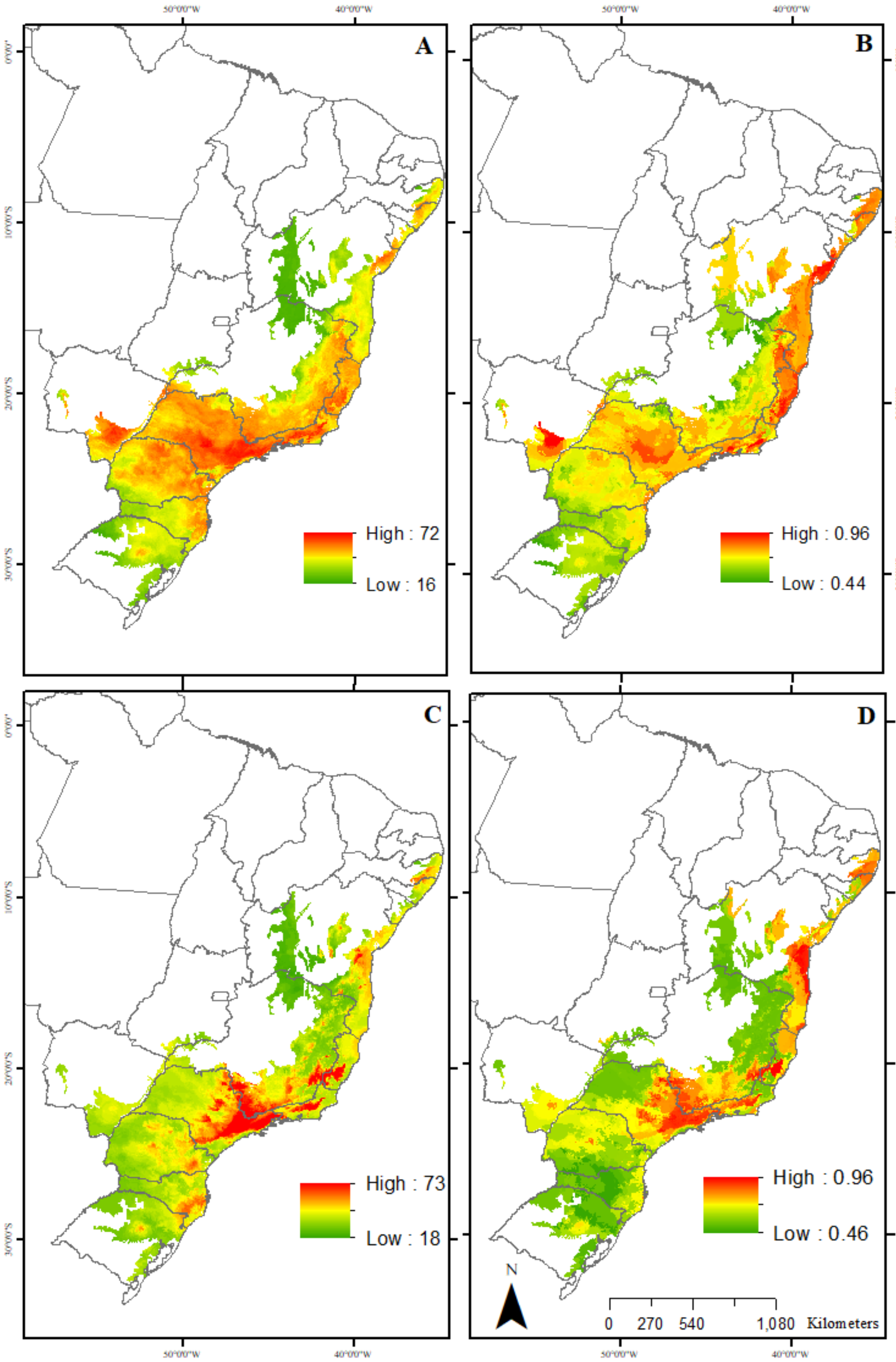

**Figure 3**. Taxonomic richness (left panels) and functional richness (right panels) for Atlantic Forest amphibians for the present (top panels) and for the year 2070 (bottom panels) according the most pessimistic scenarios of temperature increase. **A**. Taxonomic richness of present. **B**. Functional richness of present. **C**. Taxonomic richness of 2070's. **D**. Functional richness of 2070's.

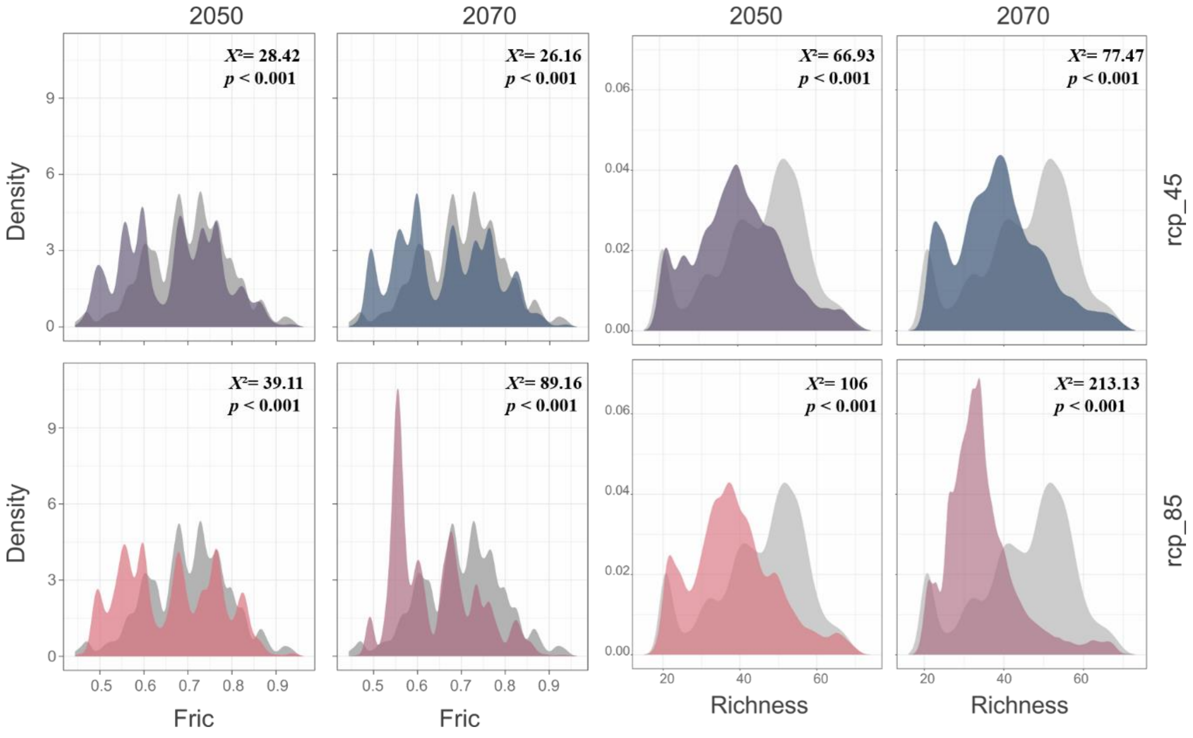

**Figure 4**. Functional and taxonomic richness frequency of Atlantic Forest amphibians according two scenarios of temperature increase (RCP 4.5 and RCP 8.5) and to the years of 2050 and 2070. In gray we represent the present estimates. Fric = fuctional richness; Richness = taxonomic richness.

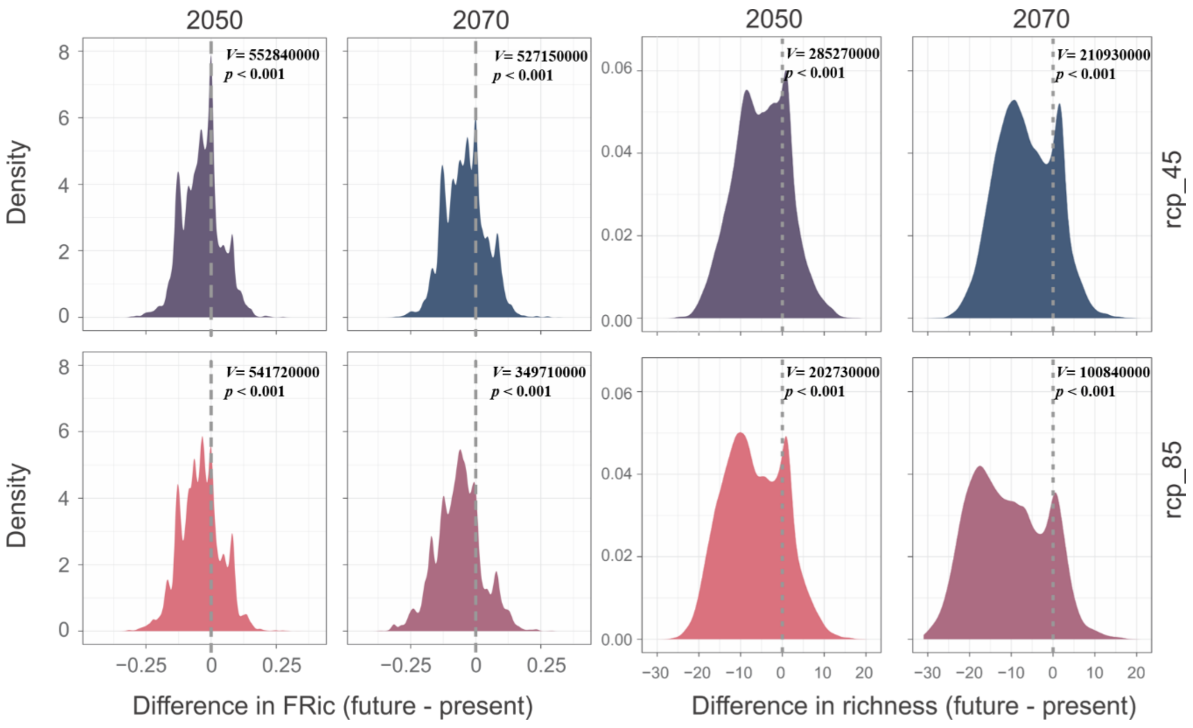

**Figure 5.** Difference between the future and present functional and taxonomic richness of Atlantic Forest amphibians, according two scenarios of temperature increase (RCP 4.5 and RCP 8.5) and two years (2050 and 2070). FRic = functional richness; richness = taxonomic richness. Dashed gray line represents zero of difference.

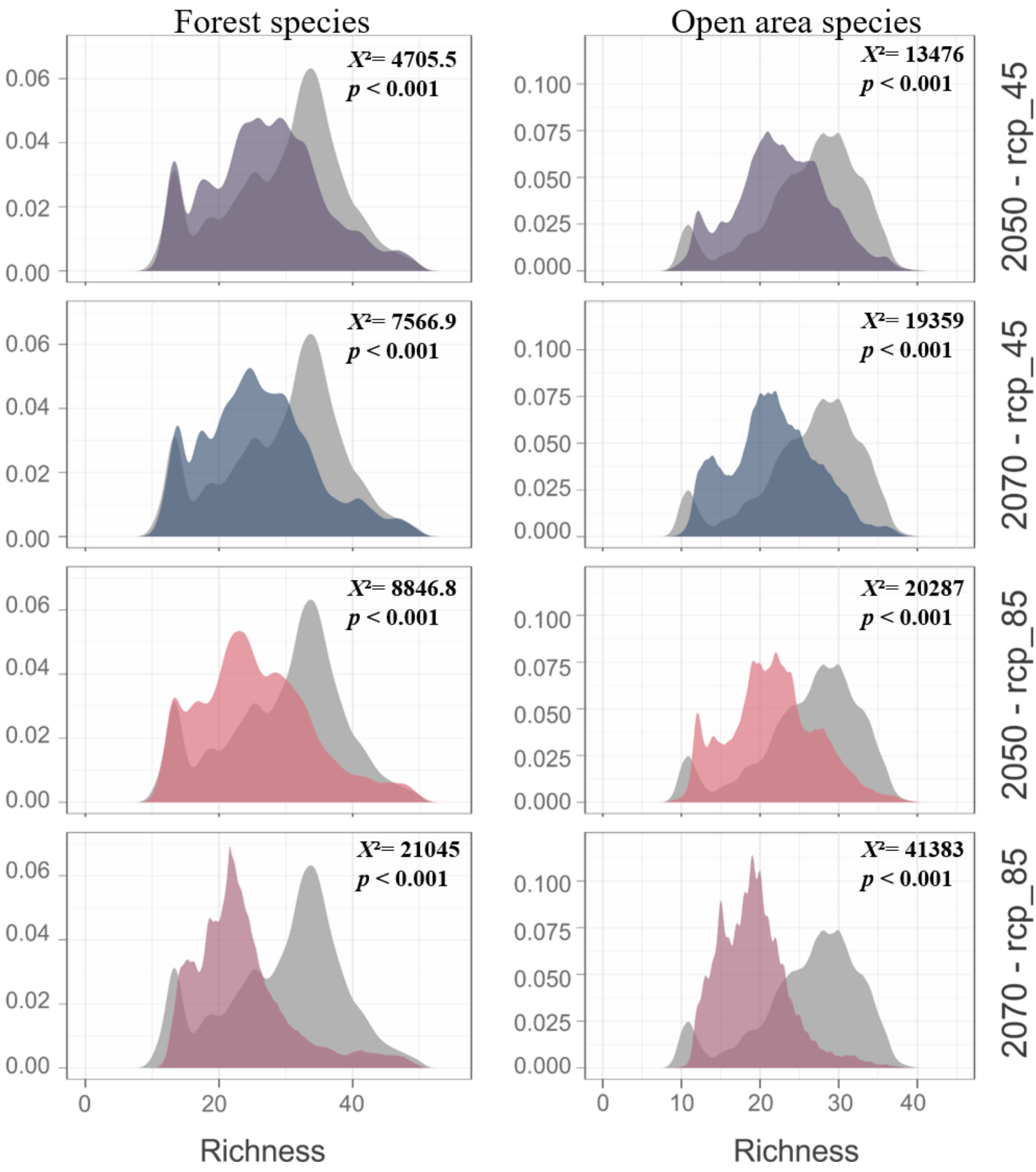

**Figure 5**. Taxonomic richness frequency of Atlantic Forest amphibians according two scenarios of temperature increase (RCP 4.5 and RCP 8.5) and to the years of 2050 and 2070 for the forest species specialists (left panels) and open area specialists (right panels).

## 4.4 Discussion

Climate change will cause severe species loss, compromising species with particular traits, and functional richness will be restricted mainly to regions next to the coast. Moreover, the loss of species seems to be worse, with taxonomic richness being affected more than the functional one. When comparing taxonomic and functional biodiversity components under climate chance we observe that they present complementary information and can offer relevant insights about amphibian conservation. As we expected, species dependent of forest habitats are more sensitive than open-area species and their distribution range will shrink (see also Keinath et al., 2017).

### *4.4.1 Climate changes influence both taxonomic and functional richness*

The Brazilian Atlantic forest is losing species faster than functional response traits. Functional richness will be bigger in the future than taxonomic richness, mainly in the northeast. This result indicates the lost species share similar traits or in other words, we are losing functionally redundant species. The climate changes are filtering out these species with traits that are poorly adapted to the new conditions and permitting colonization by better-adapted species (Mouillot et al., 2013). Even though the two components of biodiversity are not totally correlated, the relationship is positive. Diaz and Cabido (2001) propose this can happen when species converge into relatively discrete functional strategies. However, competition can mask the disturbance impact on the functional structure of communities favoring the coexistence of dissimilar combinations of traits (MacArthur and Levins, 1967; Schamp and Aarssen, 2009). This set of dissimilar traits means lack of functional redundancy, which may disturb the ecosystem process and reduce ecosystem resilience (Davic and Welsh 2004). A future without functionally redundant species can have a huge impact on the ecosystem process, because each species extinction will decrease the functional diversity. Then, the climate changes impact can be even worse than we are seeing in the predictive maps.

Trait-based measures are better predictors of disturbance impact and ecosystem process (Ernst et al., 2006; Naeem et al., 2012; Mouillot et al., 2013), being capable to detect changes in biodiversity due anthropogenic disturbances before extinctions effectively occur (Mouillot et al., 2013). In our study, taxonomic richness shows a pattern of potential lost more evident. This can indicate two responses, or taxonomic richness is more sensitive to climate changes than functional trait approach or the future is not so pessimist how we expected. However, because amphibians are a very sensitive group to temperature and precipitation (Duellman and Trueb, 1994), probably the first option is the correct. Moreover, the correlation between taxonomic and functional richness were found in many studies (Mayfield et al. 2010; Riemann et al., 2017), but the exact relationship between these two components of biodiversity is not clear for the majority of natural systems (Naeem, 2002). In our case, there is not a complete overlapping of the components of biodiversity responses, what makes advised to consider both when takes conservation measures.

A big part of the remaining taxonomic and functional richness of Atlantic Forest Hotspot will be restricted to southeast region close to the coast. This will be a conservation challenge, because this area has a huge property speculation (Dean, 1996), moreover, it is the most developed region of the country. Other studies in the same biome, but evaluating amphibians' richness presented a similar pattern of species loss (Lemes et al., 2013; Loyola et al., 2013). Due to the lack of latitudinal temperature gradient of the lowland regions in the tropics, range shifts probably would happen in higher elevations, such as in southeast of Brazil (Colwell et al. 2008; Carnaval et al., 2009; Lemes et al., 2013). The southeast region, where the main amphibians richness would be concentrated, is a mountainous region and major areas of endemism, acting as important refuges for species under climate changes (Araújo et al., 2011; Klorvuttimontara et al. 2011).

### *4.4.2 Forest dependent are more affected than open habitat species*

Climate changes acting as an environmental filter can often favor generalist species (Ribeiro et al., 2017) such as open area habitat species. The latter species usually have large ranges of distribution and tolerate an extensive diversity of conditions, while small range species to persist require a set of specific conditions (Cooper et al. 2008; Devictor et al. 2010). In our study, we found exactly this pattern, showing the changes in climate will be affect stronger the forest associated species, which are the most sensitive species related to open area species. In addition, we can notice that the response to disturbance is not identical to the species, rejecting the neutral model and giving support for a niche model (Mouillot et al., 2013). The niche processes driving community responses to disturbance can allow prediction of impacts in the structure of functional trait, therefore the functioning of communities (Pakeman, 2011). Then, knowing the increase of temperature will be worse to forest species, we should concentration efforts in this group to avoid lose important functional traits.

The impact of climate changes in species, especially the more sensitive such as forest-dependents, probably will be aggravated by habitat loss and fragmentation. Even though, we have not considered the landscape pattern in our models, the impact of habitat fragmentation and loss is well established, recognized as the major threats to biodiversity (Newbold et al., 2014). The increase of $CO_2$ concentration can lead to an agriculture production intensification, which can generate a larger demand for farming areas (Van Ierland et al., 2001). Besides the agricultural development, landscapes with main anthropogenic land use will continue to change due to increasing urbanization and economic mobility (Opdam and Wascher, 2004). All these factors will contribute to habitat fragmentation and loss, which in synergy with climate changes will decrease the adequate habitat for biodiversity. However, the only study indicating this synergy was developed by Warren et al. (2001), who found that in severely fragmented landscapes there is no range expansion of a British butterfly. Clearly, species associated with forest areas and low dispersal abilities will be the most impacted, also fragmentation seems to increase the climate change impact on biodiversity (Opdam and Wascher, 2004).

### 4.5 Management recommendations

In order to have a more accurate prediction of the climate changes impact, we recommended to investigate what kind of trait is being filtered out by the climate changes. This kind of response will indicate what functional trait is related to this anthropogenic disturbance, in addition reveal which species are more sensitive. Another important approach is to include in the prediction models the landscape pattern to help understand the synergistic effects and reveal what is the role of land cover changes in the biodiversity loss and ecosystem functioning. Moreover, extra studies investigating the role of temperature increase in functional diversity, mainly in tropical areas, are necessary.

This study is the first one to evaluate the effects of climate changes on functional traits of a vertebrate group. We showed that amphibian taxonomic and functional richness are complementary indexes being advised to analyze both responses before taking conclusions. Conservation efforts should be localized in the southeast region of Brazil, which represents an important refuge to amphibians. In addition, special attention on forest associate species is advised. Ecological niche models can offer an important prognosis about species distribution in the future, according anthropogenic disturbances, being a valuable tool to take decisions in conservation and ecosystem management (Wittmann et al., 2016). However, this kind of modeling often only considerate climate variables and species presence data, species functional traits are rarely incorporate in the modeling steps restricting our understanding of the impact and power of decision.

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## Supplementary material 3

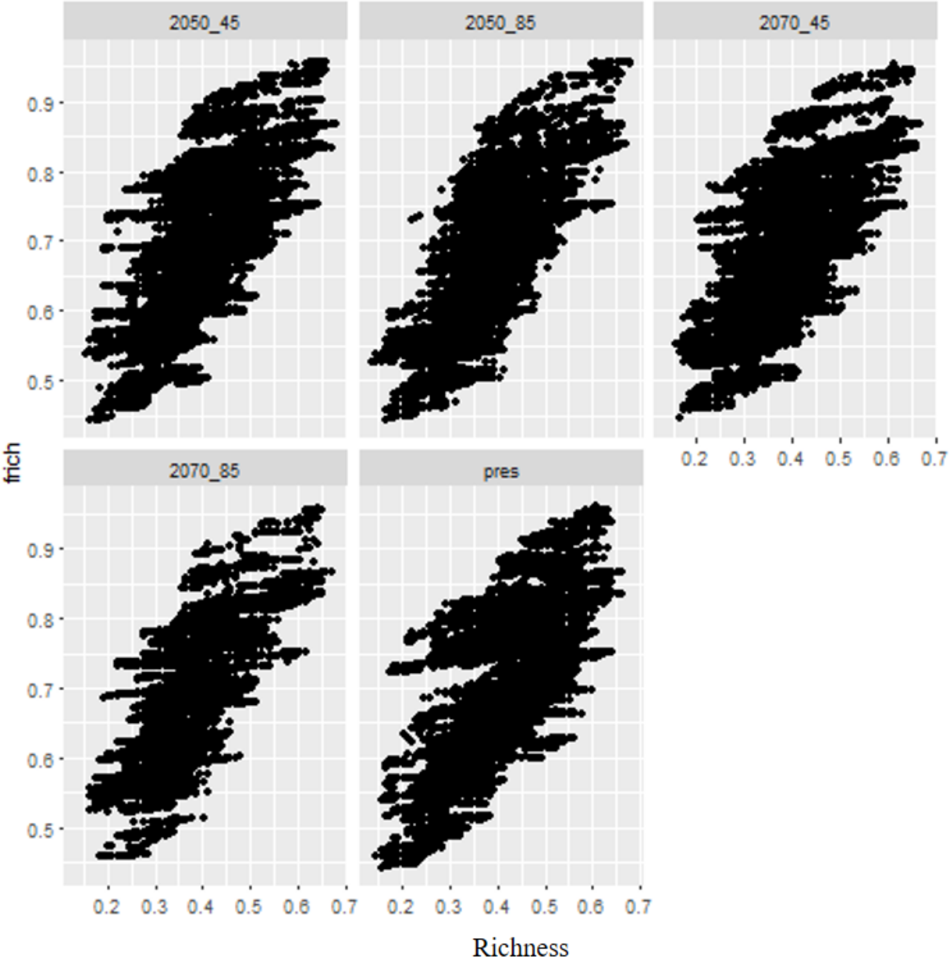

**Fig. S41**. Scatter-plot showing the relationship between functional richness and taxonomic richness of Atlantic Forest amphibians for the present and for the two scenarios of temperature increase, RCP 4.5 (prediction of 2.3ºC temperature increase) and RCP 8.5 (prediction of 4.9ºC temperature increase) for the years of 2050 and 2070. Frich = functional richness; Richness = taxonomic richness.

# CONCLUSION

In this thesis, I aimed to understand the effects of anthropogenic disturbances on amphibians' communities across highly fragmented and threatened forest ecosystem. I used data about amphibians' communities sampled through a new tool called "Automated Recording Systems". This allow simultaneous, long-time and permanent sampling of vocalizing animals, besides cross-validation of the data by experts. In addition, I used auditory and visual surveys of amphibians to supplement the passive acoustic monitoring and avoid locality bias. At landscape level across a habitat loss and fragmentation gradient, I found that anthropogenic environmental gradients, created by suppression of native habitat and establishment of different land uses, cause turnover of amphibians' species, functional response traits and functional response groups. In a large scale level –Atlantic Forest hotspot – I found that temperature increase will causes  taxonomic amphibians' species decline, but this decline is will be smaller for functional diversity amphibians. My findings indicate that trait-approach is complementary to classical measures of diversity, such as taxonomic richness, to elucidate the effects of human-induced changes on biodiversity. Therefore, the combination of these approaches guarantees better understanding of anthropogenic impacts on amphibian communities. The summary of each chapter is presented below.

In the first chapter, I aimed to answer the question "how much sampling time is sufficient to represent amphibian diversity". Automated recording system generate huge amount of the data, and the needed time for characterize the species richness at landscape levels is unknow for many taxa worldwide. In this chapter, I aimed to propose a less time-demanding to identify amphibians, but with an accurate representation of the community. Using accumulation curves, I found that 770 minutes are a suitable amount of time to record and listen to determine the species presence. I have also evaluated if forest amount (%) influences the needed time to reach richness asymptote on a sampling effort-richness curve. I observed no correlation between needed time and forest amount; therefore I suggest that the amount of minutes be a minimum of 770, independent of habitat loss level. Future studies may evaluate the influence of weather and other environmental covariates in the sound recordings. Besides that, the development of improved automatic detection algorithms can decrease the time spent by experts to filter the recordings, as well as helping to provide more reliable data, without human bias.

In the second chapter, my aim was to identify the best environmental predictors of three components of diversity (species, functional response traits and functional response groups) and evaluate where important turnover of species/functional traits/functional groups or ecological thresholds are happening along the anthropogenic environmental gradients. I have also assessed concordant/non-concordant diversity patterns at the taxonomic and functional level. I found that silviculture (eucalyptus monoculture), water bodies and environmental heterogeneity are the main predictors for all components of biodiversity. Moreover, when the interest is explaining taxonomic composition we need more predictors to explain the variance, than we need to explain functional components (functional diversity and traits). Indicating that when the conservation goal is based in the information provide by functional groups or traits, fewer main parameters are required to be controlled, but if the aim is to safeguard overall taxonomic diversity, then additional factors may be important. Regarding to the thresholds, eucalyptus and water bodies amounts showed rapid change in the community already in the beginning of the gradient. Higher the amount of these variables in the landscape, With the changes, occurred establishment of traits associated with generalist species. Consequently, the substitution of suitable habitat by anthropogenic land uses causes disturb on the biodiversity, even though their proportion in the landscape is not high. However, the main threshold of environmental heterogeneity is in the middle of the gradient, indicating that until certain point the combination of natural and anthropogenic areas is beneficial to amphibians, offering complementary resources. The best management measure to amphibians seems to be the conservation of natural habitats such as forest in agricultural landscapes, avoiding predominance of certain type of land uses. Regarding to the responses of the three components of biodiversity, the similarity indicates the environmental filters constrain functional and taxonomic components in a related way. The selection of what component of biodiversity to use for making decisions depends on the conservation goal. If the objective is to protect the whole community, probably it is not necessary the analysis of other levels of biodiversity, only the classic descriptors of biodiversity – such as species diversity, species richness and total abundance - seem to be enough. Nonetheless, when emblematic, sensitive species are the priorities and when the proposal is to keep the functional integrity, we recommended exploring functional traits and related indexes.

In the last chapter, I investigated the influence of climate changes on taxonomic and functional richness of amphibians. Even though recent studies demonstrated functional diversity as a more efficient biodiversity measure for understanding the response of biodiversity to anthropogenic disturbance, with the temperature increase the loss of species is greater than loss of functions. This can happen because the climate changes will exclude species functionally redundant what results in a pattern loss more prominent in taxonomic richness. Despite this difference between the two components of diversity (taxonomic vs functional), a large portion of the remaining taxonomic and functional richness will be concentrate on southeast region of the Atlantic Forest hotspot, particularly close to coastal areas. It will be challenging to the conservation measures, because it is a region that encompass the highest population density in the biome, subject to urban expansion. The two components of diversity showed complementary responses, therefore both taxonomic and functional richness need to be considered when defining conservation strategies for biodiversity conservation and related ecosystem services provision. In addition, the impact of temperature increase will be worse on forest specialist species, probably because this habitat specialist group has higher requirements of habitat structure. Climate changes in synergy with habitat loss and fragmentation probably will be the drivers of the more intense impact on forest species. However, both habitat specialist groups show species loss. We recommend studies evaluating which traits the climate changes are filtering out and to incorporate the landscape pattern. Moreover, more studies about the role of temperature increase in functional diversity are indicated to obtain a clearer response of the impact and guide mitigation measures.

The human-induced changes have negative impact on biodiversity in the present and will continues in the future. Therefore, whether mitigation and conservation measures need to be taken in order to safeguard both species diversity, functional diversity and consequently their ecosystem services and functions. Habitat loss and fragmentation, substitution of native habits by anthropogenic land uses, in synergism with negative climate changes effects, are altering amphibians' community composition leading to predominance of less sensitive and impact-tolerant species. This can have a huge impact on ecosystems functioning because important functions are being lost. Functional component is effective to evaluate and to predict the impact on biodiversity and should be considered as a complementary approach to classical diversity measures.