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Habitat and food resources use by birds in different landscapes of the State of São Paulo: an historical and isotopic approach

Ana Beatriz Navarro

Dissertation presented to obtain the degree of Master in Science. Area: Applied Ecology

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Bachelor of Science in Biology

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*"Aqueles que não conhecem o passado
estão condenados a repeti-lo"*

George Santayana, 1905

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RESUMO

Uso de habitat e recursos alimentares por aves em diferentes paisagens do Estado de São Paulo: uma abordagem histórica e isotópica

Os impactos antropogênicos causados pela expansão humana sobre áreas originais de florestas exercem fortes ameaças a existência das aves, alterando o uso de habitat e recursos pelas espécies. Análises isotópicas de carbono (medidos por $\delta^{13}C$) e nitrogênio (medidos por $\delta^{15}N$) podem ser úteis no fornecimento de informações sobre modificações espaciais e temporais influenciando no uso de habitat e dieta das aves. O objetivo principal deste estudo foi mensurar modificações isotópicas de carbono e nitrogênio em aves de florestas tropicais enfrentando diversos impactos antropogênicos históricos. O estudo foi desenvolvido no estado de São Paulo devido a intensa expansão humana e devastação das florestas originais, especialmente no século XX. As amostras foram obtidas de penas das asas de diversas espécies que compõem as seguintes guildas: frugívoros, granívoros, insetívoros, nectarívoros e onívoros, provenientes de aves capturadas em campo e de espécimes de museu. Agrupamos os indivíduos segundo os seguintes critérios: 1) indivíduos históricos (coletados até 1970) foram separados dos recentes (coletados de 1985 até 2012), 2) indivíduos recentes (de 2001 a 2019) foram separados segundo a sua presença em paisagens modificadas (menor ou igual a 30% de cobertura florestal) ou em paisagens naturais (igual ou maior a 47% de cobertura florestal). Amostras individuais foram analisadas isotopicamente para obter os valores de $\delta^{13}C$ e $\delta^{15}N$. Calculamos os tamanhos dos nichos isotópicos de cada uma das guildas entre os diferentes grupos através do pacote SIBER no R. Além disso, comparamos os valores médios de $\delta^{13}C$ e $\delta^{15}N$ entre as guildas de cada um dos grupos através dos testes t de Student ou Wilcoxon. Em ambas as comparações, abordagem histórica e de paisagens, os resultados mostraram diferenças significativas nos tamanhos dos nichos isotópicos das guildas de cada um dos grupos. Na abordagem histórica, as guildas tiveram um declínio nos tamanhos dos nichos ao longo dos anos. Na abordagem de paisagens, quase todas as guildas apresentaram nichos menores quando presentes em paisagens modificadas, exceto granívoros. Os valores médios de $\delta^{13}C$ em ambas as abordagens mostraram preferência por forragear nas florestas e pouca mistura com recursos da matriz agrícola. Os valores médios de $\delta^{15}N$ se diferenciaram entre os grupos, mostrando os possíveis efeitos diretos dos impactos antropogênicos sobre a dieta das aves. Apesar das espécies analisadas ainda serem encontradas nas paisagens modificadas de atualmente, a maioria delas mostrou alta dependência dos remanescentes florestais como habitat e fonte de alimento. Considerando que o nicho é formado por variáveis ligadas ao uso de habitat e dieta, a redução no tamanho dos nichos nas guildas pode representar uma ameaça a sobrevivência dessas espécies. Além disso, observamos que os impactos antropogênicos derivados das mudanças no uso do solo, como uso de fertilizantes e homogeneização de habitat, devem ser os fatores relacionados às diferenças nos valores de $\delta^{15}N$. Assim, de modo geral, observa-se que a expansão humana sobre uma região de floresta tropical afetou diretamente a ecologia isotópica das aves.

Palavras-chave: Isótopos estáveis, Ecologia trófica, Antropoceno, Mudanças no uso do solo

ABSTRACT

Habitat and food resources use by birds in different landscapes of the State of São Paulo: an historical and isotopic approach

The anthropogenic impacts caused by human expansion over areas of original forests pose strong threats to birds' existence, altering the habitat and resource use by the species. Isotopic analysis of carbon (measured by $\delta^{13}C$) and nitrogen (measured by $\delta^{15}N$) can be very usefull providing information on spatial and temporal modifications influencing birds' diet and habitat use. The main objective of this study was to measure isotopic modifications of carbon and nitrogen in tropical forest birds facing several historical anthropogenic impacts. This study was carried out in the state of São Paulo due to the intense human expansion and devastation of the original forests, especially in the 20th century. We obtained samples from wing feathers of several species that compose the following guilds: frugivore, granivore, insectivore, nectarivore and omnivore, from alive birds in the field and from specimens in the museum collection. We grouped individuals according to the following criteria: 1) historical individuals (collected until 1970) were separated from the recent ones (collected from 1985 to 2012), 2) recent individuals (from 2001 to 2019) were separated according to their presence in human-modified landscapes (HMLs; less or equal to 30% of forest cover) or in natural landscapes (NLs; equal or over 47% of forest cover). Individual samples were isotopically analyzed to obtain $\delta^{13}C$ and $\delta^{15}N$ values. We calculated the isotopic niche width of each guild within the different groups through the SIBER package in R. In addition, we compared the mean values of $\delta^{13}C$ and $\delta^{15}N$ between the guilds of each group through the Student's t-test or Wilcoxon signed-rank test. In both comparisons, historical and landscape approaches, the results showed significant differences in the isotopic niches widths of the guilds of each group. In the historical approach, all guilds had their niche widths declined over the years. In the landscape approach, almost all guilds had smaller niches when present in HMLs, except the granivores. The mean $\delta^{13}C$ values in both approaches showed that birds prefer to forage in the forests and have a small mixture with resources from the agricultural matrix. The mean $\delta^{15}N$ values differed between groups, showing the direct effects of anthropogenic impacts on birds' diet. Although the analyzed species are still found in nowadays' HMLs, most of them showed a high dependence on forest remnants as habitat and food source. Considering that the niche is formed by variables linked to the habitat use and diet, the reduction in niche width of guilds might pose a threat to the survival of these species in HMLs. Additionally, we observed that anthropogenic impacts derived from land use changes, such as fertilizer use and habitat homogenization, might be influencing the differences in $\delta^{15}N$ values. Thus, in general, it became clear that the human expansion over a region of tropical forest has directly affected the isotopic ecology of birds.

Keywords: Stable isotopes, Trophic ecology, Anthropocene, Land use changes

1. INTRODUCTION

The Anthropocene era is defined by geologists who suggest that planet Earth has entered a new age that is totally dominated by anthropogenic actions (Lewis and Maslin 2015). Although the definition per se is relatively new, the effects of human impact on ecosystems and organisms have been known for a while. Recent studies suggest that the effects of the Anthropocene act directly on the worldwide defaunation process known as a possible sixth mass extinction (Barnosky et al. 2011, Dirzo et al. 2014). Human impacts affect all taxons at different magnitude, in which 17% of terrestrial species are under some degree of extinction risk (Young et al. 2016). In tropical forests highly disturbed by anthropogenic actions there are increases in the proportion of species loss, reaching 30% less bird species when compared to undisturbed habitats (Alroy 2017). In addition, species loss is usually related to the decline of ecosystem functioning and services, such as seed dispersal, pollination and regeneration, carrion removal, and many others that are developed by birds and other organisms (Dirzo et al. 2014).

There are many causes related to the disturbance of habitat and consequently to the species decline. Fragmentation and habitat loss are consequences of human expansion over pristine areas that causes numerous negative impacts on biodiversity (Fahrig 2003). As a result of land use changes, especially through high deforestation rates and advance of agricultural areas, the original habitats have been lost and fragmented by humans (Barraclough and Ghimire 2000). Such impacts are extremely related to the decline of biodiversity in the tropics and all these assemblages reductions are responsible for the trophic cascade effects, considering that species are connected through the food web (Morante-Filho et al. 2018). At a landscape scale, habitat loss can express negative effects on assemblage composition, in which landscapes with more than 50% of forest have more richness and abundance of birds compared to landscapes with until 30% of forest (Morante-Filho et al. 2015). Generally, bird assemblages that inhabit human-modified landscapes are more generalist and tolerate a greater range of habitat degradation (Morante-Filho et al. 2016), often considered as plastic species. Nevertheless, the generalist species are responsible for developing the ecological processes necessary for the ecosystem maintenance in these degraded and fragmented environments (Pizo 2007, Carlo and Morales 2016). Although the greatest birds' conservation efforts are designed to protect endangered and specialists species (Marini and Garcia 2005), which should really be a priority, it is also necessary to assess the effects of anthropogenic threats on generalist birds present in these human-modified landscapes.

Associated with habitat loss and fragmentation, many other impacts can also have negative consequences for biodiversity. Edge effects generate a dichotomy between forest and non-forest, which for many species represents a divergence between suitable habitat and

non-habitat, affecting several vertebrate species (Pfeifer et al. 2017). Some bird species, due to the edge effect, are restricted to the interior of forests, possibly due to a lower availability of food at the edges (Zakaria et al. 2013). Another severe anthropogenic impact on the diversity of native species is the process of savannization of tropical forests with a consequent monogeneric increase of grasses, especially the exotic and economically viable ones (Silvério et al. 2013, Scarano and Ceotto 2015). Future prospects indicate that savannization might further advance over tropical forest areas, increasing the pressure on biodiversity in the tropics (Salazar et al. 2007). Once more, both impacts described above are directly linked to the progressive increase of agricultural areas over tropical forests, consequently affecting the wildlife. Thus, there is an increasingly demand to assess how human expansion and the correlated anthropogenic impacts affects the ecology of species in the tropics.

A tropical region that has been greatly impacted from human expansion over original forest areas is the state of São Paulo in Brazil (Dean 1997). Primitively this state is composed of two biomes that are biodiversity hotspots, the Atlantic Forest and the Cerrado (Mittermeier et al. 2011). In the past, the Atlantic Forest was even compared to the Amazonian biome by virtue of its vast composition of sumptuous trees and magnificent fauna (Cardim 2018). Nevertheless, the anthropogenic impacts on São Paulo were so intense that nowadays only 24% of forest remains covering the state (MapBiomas 2019), wherein both the Atlantic Forest and Cerrado are constrained by human threats (Durigan et al. 2007, Ribeiro et al. 2009). Land use changes to increase areas of agriculture through the suppression of forests were one of the main causes for the current situation of the landscapes of São Paulo. Since the 19th century forests were converted into agriculture, but in the 20th century there was an intensification in human expansion, especially over the western region of the state (Victor et al. 2005). Pastures and sugarcane crops are the main farming in São Paulo, occupying approximately 71% of total land cover (MapBiomas 2019). Especially located in the western and countryside of the state, the agriculture was established there due to the plateaus and fertile soil, contrary to the knife-ridges near the coast which persisted with large forest remnants because of mountain slopes that hampered agriculture expansion (Aguiar et al. 2003, Durigan and Ratter 2006). Although it is the most economically developed state in Brazil, São Paulo had its forests and biodiversity decimated, making it possible to distinguish the composition of communities in the past and recent (e.g. Antunes 2007, Cavarzere et al. 2012).

Atmospheric changes coming from human impacts on Earth has been constantly measured, especially due to the large input of anthropogenic carbon (e.g. CO_2) and nitrogen (e.g. N_2O) (Steffen et al. 2005). The effects on carbon and nitrogen cycles are mainly derived from processes such as land use changes, burning of fossil fuels, industrialization and use of fertilizers in agriculture (Prentice et al. 2001, Schlesinger 2009). The absorption of these elements at the most varied molecular levels occurs in different organisms, from soil

microorganisms, plants, invertebrates to larger vertebrates (Gruber and Galloway 2008). Despite the existing knowledge about anthropogenic carbon and nitrogen inputs directly affecting ecosystems since both are incorporated into natural cycles (Schulze 2000), there are few information on the possible influences over the wildlife, especially through the years of human expansion. Some studies emphasize the impact of agriculture on organisms, primarily by the effect of anthropogenic nitrogen input observed in the species tissues (e.g. Hebert and Wassenaar 2001, Hofmeister et al. 2013), which can also affect the quality of birds' feathers (Møller et al. 2018). Notwithstanding, the consequences that such inputs might have on the patterns of habitat and food resource use by birds are still uncertain.

Stable isotopes are atomic species of the same element that have the same number of protons and different number of neutrons in its nucleus, differing from radioactive isotopes because they do not change their mass over time. In ecological studies the use of isotopic approach is increasing, especially regarding the elements of carbon (^{12}C and ^{13}C) and nitrogen (^{14}N and ^{15}N), due to many information that these isotopes can provide as both are part of the ecosystems' composition (Peterson and Fry 1987, Fry 2008). Plants with distinct photosynthetic pathways (C_3 and C_4) have different proportions of stable carbon isotopes (measured by $\delta^{13}C$) derived from the assimilation process of each pathway (Smith and Epstein 1971). In general, tropical forests are mostly composed of C_3 plants while several agricultural crops are typically C_4 plants (e.g. pasture, sugarcane and corn crops), so that different habitats have different isotopic signatures. Whereas isotopic nitrogen values (measured by $\delta^{15}N$) vary among organisms according to their position in the trophic chain, as apex predators are enriched in ^{15}N following the accumulation of this isotope along the chain levels through organisms (Minagawa and Wada 1984, Post 2002). In addition, anthropogenic impacts on the environment, such as the use of fertilizers in agriculture, influence $\delta^{15}N$ values in the tissues of animals that feed near these sites (Rubenstein and Hobson 2004, Sabat et al. 2013). Considering that animals' composition are consisted of the food eaten 'plus a fill per thousand' derived from tissues isotopic fractionation, isotopic analysis of carbon and nitrogen in animal ecology enables making inferences about the habitat use and the diet of species (Kelly 2000, Boecklen et al. 2011, Ferger et al. 2013).

Regarding the many possibilities of isotopic analysis in ecological matters, there are some subjects rising in attention in ornithological studies, as historical ecology and isotopic landscapes (Hobson 2011). Museum specimens are sample sources for historical studies on a variety of subjects, such as foraging ecology, environmental biogeochemistry and physiology, enabling the reconstruction of historical trends in ecology (Wiley et al. 2017). Long-term studies that aim on how environmental changes influence in foraging patterns are relevant to predict the future conservation of bird species, especially in an anthropogenic world (Norris et al. 2007), issuing crucial information that otherwise would not be possible to collect anymore

with traditional methods and museum specimens. The anthropogenic impacts through the human expansion over the centuries arouse direct consequences to birds and its trophic ecology, which can be measured by isotopic analysis of carbon and nitrogen (Chamberlain et al. 2005). Anthropogenic impacts on species can also be measured through landscape-level isotopic analysis. Species that forage in different habitats usually have distinct isotopic values reflecting the composition of the environment that has been used by them (Pagani-Núñez et al. 2019). In human-modified landscapes, some bird species might differ in their use of available resources between forest remnants and the surrounding agricultural areas (Ferger et al. 2013). Understanding and measuring how organisms use the different landscapes available, such as the human-modified and natural ones, provides better management actions of agricultural areas focusing on the species' conservation in these landscapes (Magioli et al. 2019).

Thus, the main objective of this study was to evaluate and measure isotopic modifications of carbon and nitrogen in tropical forest birds facing anthropogenic impacts, especially derived from land use changes. This document is divided into two independent but complementary chapters. In the first chapter there is an historical approach whereby isotopic analysis was done for birds' individuals ranging from the beginning of the 20th century until recently. The primary objective of chapter one was to assess whether anthropogenic impacts on a region of the tropics have affected the patterns of habitat and food resource use of birds, especially regarding land use changes in which forests were converted into agricultural areas. In chapter two the approach is about landscape-level isotopic analysis, comparing the isotopic values among individuals from landscapes with distinct amounts of forest cover. The main objective of chapter two was to assess whether there are differences in diet and habitat use of birds between human-modified (less or equal to 30% of forest cover) and natural landscapes (equal or more than 47% of forest cover), using the isotopic analysis. Thus, the chapters are linked by trying to identify how human expansion in the landscapes of the study region affected the trophic ecology of birds through isotopic analysis, in relation to the past when deforestation rates were high and in relation to nowadays in which landscapes are composed by distinct amounts of forest.

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2. CHAPTER 1 – CENTENARY ANTHROPOGENIC IMPACTS ON TROPICAL FORESTS REVEALED BY CARBON AND NITROGEN ISOTOPES OF BIRDS

ABSTRACT

Human expansion over the last centuries has posing significant impacts on birds' habitat use and diet. However, it remains a gap of knowledge on how historical anthropogenic impacts have influenced the isotopic ecology of these species, especially in the tropics. So, we aimed in this study to evaluate whether anthropogenic impacts over more than 100 years have influenced in the isotopic niche widths and isotopic carbon and nitrogen signatures of tropical birds. Our study area is a historically and extensively explored tropical region, with high rates of land use change by the shrinkage of forest areas and increase of agricultural fields. We used feathers collected from 26 bird species of five foraging guilds (frugivore, granivore, insectivore, nectarivore and omnivore) to obtain the isotopic signatures. To depict and compare past versus current individuals, we detached our 734 feather samples between the following two groups: historical (from 1897 until 1968) and recent (from 1985 until 2012), for each one of the guilds. Then we measured the width of isotopic niche and mean values of $\delta^{13}C$ and $\delta^{15}N$ for each guild, comparing between the two groups to check whether and how the values changed from past to present. Our results showed that all guilds presented a large reduction in isotopic niche width from the early twentieth century until contemporary. The proportion of reduction from historical to recent ranged from 28% to 70% varying for each guild, wherein frugivores and nectarivores were the guilds that expressed more the reduction, while granivores were the least impacted one. Additionally, our results showed a reduction in the mean values of $\delta^{15}N$ over the years, ranging from 1.7‰ to 3.5‰ depending on the bird guild, whereas the mean values of $\delta^{13}C$ did not changed significantly in almost all guilds. Apparently, reductions in niche widths are totally associated with historical anthropogenic impacts, especially fragmentation and habitat loss, both causes responsible for the decreasing in availability of food resources. Frugivores and nectarivores were the most impacted by the reduction of niche widths, probably because there was a decrease in plant species diversity in these human-modified landscapes. Decline in $\delta^{15}N$ values might also have been influenced by human expansion over the years, mainly due to land use changes, with an increase in agricultural areas and a consequent increase in the use of nitrogen fertilizers. Albeit most of guild species in our study species are considered generalist and common, all of them showed to have been strongly influenced by anthropogenic impacts throughout the twentieth century. Thus, it is extremely relevant to delineate future conservation strategies for these species inhibiting possible local extinctions in a few years, considering that these birds perform important ecological processes in human-modified landscapes.

Keywords: Historical ecology, Trophic ecology, Stable isotopes, Anthropocene, Tropical forest

2.1. Introduction

The detrimental effects of the Anthropocene on wildlife are currently in focus, making studies of historical ecology extremely important, providing relevant information for the conservation of species in anthropogenic scenarios. Several empirical evidences measured over a large timespan endorse the human-induced defaunation in the Anthropocene era (Dirzo et al. 2014), such as land use changes (Newbold et al. 2015), habitat fragmentation (Fahrig 2003), climate change (Şekercioğlu et al. 2012) and pollution (Furness 1993). All these effects of the Anthropocene are linked to trophic cascade effects, generating disturbances in the structure of the trophic chain as a whole (Magioli et al. 2019). Many organisms are directly affected by some of the anthropogenic impacts, from apex predator to basal species, as all of them have an interdependence within the trophic chain (Estes et al. 2011, Garvey and Whiles 2016). In the same way, not only the rarest and specialist species are being threatened by the Anthropocene effects, but also the common and generalist ones (Gaston and Fuller 2007), raising an appeal to built more information on how these species react to human-changes on Earth over the years.

Although the contemporary anthropogenic impacts began at the 19th century in the Northern Hemisphere, the tropics also started to experience such effects some decades after (Lewis and Maslin 2015). In the case of the state of São Paulo, Brazil, a Southern region historically explored and economically developed, anthropogenic impacts intensified during the 20th century, especially the ones derived from land use changes (Cardim 2018). High rates of deforestation for a subsequent agricultural cover were the main causes of drastic land use change impacts in São Paulo (Dean 1997, Victor et al. 2005), which currently has only 24% of forest cover extended mostly at montane knife-ridge near the coast (MapBiomas 2019). This large reduction of forest, especially in the countryside of the state, had an overwhelming influence on bird communities, extinguishing several species previously found there (Antunes 2007, Cavarzere et al. 2012). Besides the decline on forest cover, land use changes usually lead to a homogenization of landscapes due to intensification of monoculture in agriculture, which is also responsible to large declines on biodiversity (Sirami et al. 2019). In addition, the input of anthropogenic nitrogen into the environment due to high use of fertilizers is an indirect effect derived from land use change and increase of agriculture that has been imposing a threat to biodiversity existence (McLaughlin and Mineau 1995). Nevertheless, while we have information on the influence of the direct and indirect impacts of land use changes on species decline, is paramount to strengthen our understanding on the influence of the aforementioned anthropogenic impacts on birds' pattern of food resources and habitat use.

The use of stable isotopes in environmental studies has been increasing recently (Peterson and Fry 1987, Michener and Lajtha 2007, Fry 2008), as well as the application of

isotope analysis in ornithological studies (Inger and Bearhop 2008, Hobson 2011). Using isotopes in dietary studies enables obtaining relevant information to understand species' diet without needing to apply efforts on difficult conventional methods (Inger and Bearhop 2008). As organisms incorporate what they eat, isotopic analysis of a bird tissue provides information on the pattern of using available food resources (DeNiro and Epstein 1978, 1981). Stable carbon isotopes (^{12}C and ^{13}C) are present in different proportions in plants according to their photosynthetic pathways (C_3 or C_4) (Fry 2008). Through the isotopic carbon signature ($\delta^{13}C$), which is a ratio value between both isotopes, it is possible to evaluate which habitat type is used by birds to forage, considering that distinct habitats have different compositions of C_3 and C_4 plants (e.g. forests are mainly C_3; pasture and some agricultural crops are C_4). However, $\delta^{13}C$ is also used in studies on atmospheric changes, mainly caused by the input of anthropogenic CO_2 into ecosystems and its influence on some organisms (Long et al. 2005, Machado and Froehner 2017), although there is still a lack of information about this impact on birds. Stable isotopes of nitrogen (^{14}N and ^{15}N) can be found in organisms in different proportions depending on their position in the trophic chain (Post 2002a, Layman et al. 2012), so that with isotopic signature of nitrogen ($\delta^{15}N$) we can evaluate whether the organism has more plant or animal-based diet and find out its position in the trophic chain. Nevertheless, recent studies also investigated the impacts of anthropogenic nitrogen input on birds, including nitrogen from fertilization in agriculture (Hebert and Wassenaar 2001, Møller et al. 2018), promoting the isotopic analysis as an interesting technology to be used in ecology owing to generates information about the organisms and their relationship with the environment.

Lately, there has been a greater appeal for the application of isotopic analysis on time scales studies using museum specimens' collections (Wiley et al. 2017). By focusing a large timespan, the isotopic approach produces relevant information on historical ecology, but so far there is a lack of studies with birds on this, especially using isotopic niche analysis. In a bivariate association of $\delta^{13}C$ and $\delta^{15}N$ values it is possible to analyze the width of isotopic niche for a determined species, wherein isotopic niche can be associated with ecological niche as a result of the combination of habitat use information by $\delta^{13}C$ and diet information by $\delta^{15}N$ (Newsome et al. 2007). In addition, knowledge about niche expansion or shrinkage due to landscape changes also enables a better understanding of how human expansion affects the way birds uses the habitat and their diet (Pagani-Núñez et al. 2019). For this reason, the application of isotopic niche analysis can be very useful to conservation strategies of species, prioritizing actions to maintain the habitat and the food resources the organisms use (Newsome et al. 2007). Additionally, through historical ecology it is possible to understand about the past of bird species and how anthropogenic changes on Earth have affected this group (Wiley et al. 2013, Blight et al. 2015). So, with knowledge about the past it is easier to plan future actions for species conservation.

Although the greatest conservation efforts should really focus on endangered birds, as many of them are risking to be lost forever (Marini and Garcia 2005), we should also apply conservation efforts and develop studies that focus on generalist birds (Gaston and Fuller 2007), considering that these species perform an important role at ecological processes in human-modified landscapes (Richmond et al. 2005, Pizo 2007). To lose species that are essential to the fulfillment of various ecological processes might put threats to the ecosystem (Sekercioglu et al. 2004), even if the species are generalist and common. Since species composing the guild present similarity in resource sharing, generally there are fundamental ecological roles associated to each guild. Many of the frugivore birds are responsible for seed-dispersal process consequently perpetuating many plant species, nectarivores pollinate plants ensuring it reproductive success, insectivores regulate the trophic chain through invertebrates' consumption, granivores controls most of weeds and omnivores can perform many of the functions described above depending on main diet (Whelan et al. 2008). The appeal for studies with generalist birds should be increased and also how anthropogenic impacts over timescales has affected and might even affect them further, so that we can be aware of possible future losses of ecological functions, especially at human-modified landscapes.

In this study, we assessed whether anthropogenic impacts over the years, ranging from 20th century until nowadays, affected generalist birds at a formerly forested tropical area in Brazil. Thus, we were able to incorporate the effects of chronic and repetitive habitat change allied to pulses of fertilizers inputs, depicting quantitatively the changes in the isotopic niche of species, and evaluating the niche-constraining and displacement among birds' guilds over years. Achieving an expressive temporal scale, we were able to explore the dimension more rarefied in ecological studies, i.e., the time (McGill 2019), here embracing a timespan of more than one hundred years showing the latent effect of intensive human-induced modifications on the bird fauna. So we measured the isotopic niche width for each of the guilds (frugivore, granivore, insectivore, nectarivore and omnivore), with a hypothesis that over the years there might have had contractions or expansions in guilds' niches widths, probably due to extensive land use changes over the last centuries. We also analyzed and compared $\delta^{13}C$ and $\delta^{15}N$ mean values of all foraging guilds between historical and recent individuals, hypothesizing that there might have had significant changes in $\delta^{13}C$ and $\delta^{15}N$ values over the years possibly caused by anthropogenic impacts, such as agriculture, deforestation and fertilizer use. Finally, we clarified the importance of historical studies for regions that have been highly modified and how these studies might assist in future conservation strategies of species that thrive to survive in these human-modified landscapes (hereafter, HMLs).

2.2. Material and Methods

2.2.1. Study site

The state of São Paulo in Brazil is originally composed by two major biomes of South America, Atlantic Forest and Cerrado; both considered biodiversity hotspots (Mittermeier et al. 2011). Native vegetation cover was predominant in the state until the end of 19th century, wherein anthropogenic impacts started to be more severe at the 20th century (Victor et al. 2005) (Appendix A), specially with agricultural expansion, industrialization and urbanization. Coffee crops were predominant in the state during the 19th century, mainly due to the external market that made this type of cultivation very profitable to landowners. As coffee crops were essentially developed under fertile lands formerly dominated by native forests, after the exacerbated use of the land and few nutrients remained in the soil, the pastures were the main agricultural use, completing the cycle known as forest/agriculture/pasture. The extensive use of pastures for demarcation of vacant land was also a strategy widely used to ensure the legitimation of land holdings in the state, which greatly increased pasture areas in the 20th century. Moreover, with the economic market of coffee fallen in the early 20th century, many land use was changed to another type of cultivation, especially sugarcane crops. So on, it started the predominance of the sugarcane economic market in São Paulo, which goes until present. Nowadays, the state is composed of approximately 24% of forest, 71% of agriculture (mainly pasture and sugarcane crops), 3% of non-vegetated areas (mainly urbanization) and 2% of others land covers (MapBiomas 2019) (Appendix A). The majority of large remaining forest areas are extended in the knife-ridges near the coast, where mountain slopes makes agriculture difficult to advance (Aguiar et al. 2003). Whereas the western portion of the state, commonly named the countryside, possess fertile soils and plateau-based topography, which favored all the previously mentioned agriculture to be developed there (Durigan and Ratter 2006). Therefore, we carefully choose to use samples that were collected in these western anthropogenic areas (Figure 1).

Our historical samples from museum collection, individuals ranging from 1897 until 1968, were collected in 37 different municipalities (Appendix B), wherein eight of them had more birds collected, mainly due to the magnitude of historical expeditions in those areas (municipalities named Anhembi, Avaré, Itatiba, Amparo, Lins, Itapetininga, Matão and Franca). Our recent samples, individuals ranging from 1985 until 2012, were mainly from a field expedition at Corumbataí river basin (Luz, 2013). This region is very representative of the habitat modification situation of the entire state, considering that forest remanants cover only 12% of the area immersed by an agriculture matrix (Valente and Vettorazzi 2005).
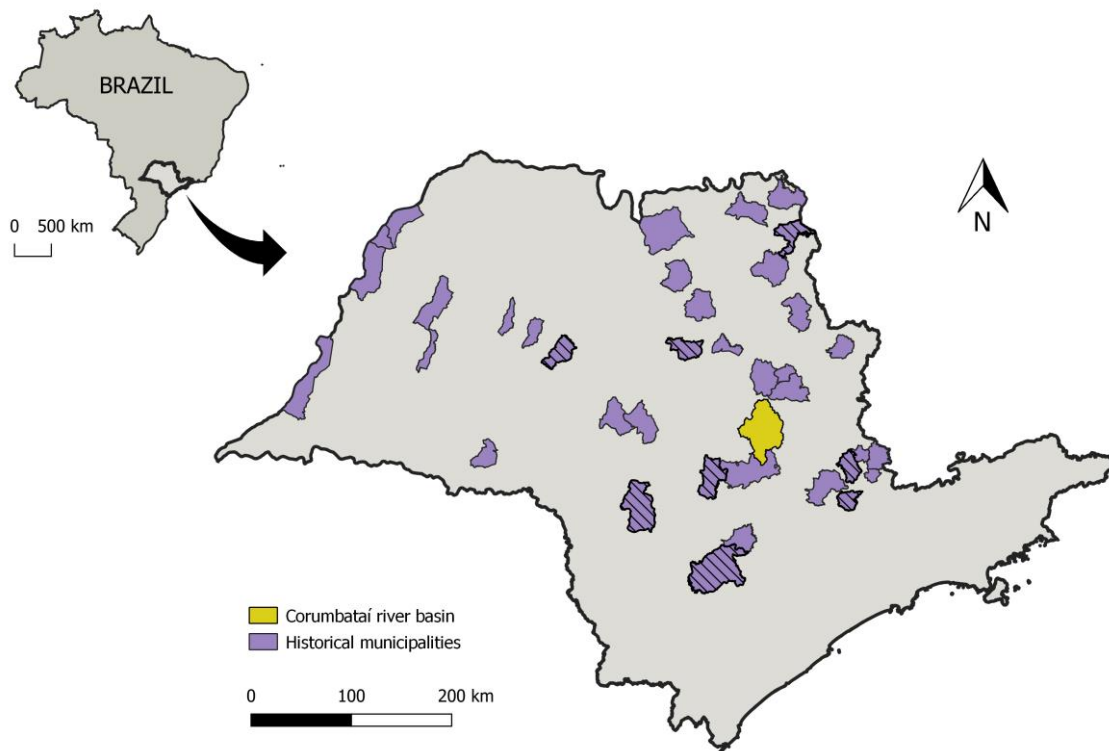
Figure 1.   Localization of municipalities where historical samples were collected in historical expeditions (purple) and localization of Corumbataí river basin, local of recent field expedition (yellow). The eight main locations of historical expeditions are with black dashes (Anhembi, Avaré, Itatiba, Amparo, Lins, Itapetininga, Matão and Franca).

2.2.2. Collection of samples

We used the vane of wing feathers to perform the isotopic analysis, considering that this tissue provides information about diet and habitat use in months, differently of other tissues that provide information about distinct time intervals (Hobson 2011). Besides that, we also considered to use feathers regarding the advantage to obtain this tissue in museums and at field expeditions (Smith et al. 2003).

To compose the historical samples, we collected feathers belonging to birds' collection of the Museum of Zoology of the University of São Paulo (MZUSP) ranging from 1897 until 1968. To compose the recent group, we sampled feathers predominantly collected at a field expedition occurred between 2011 and 2012, but also some few samples from the museum ranging from 1985 until 1994. We considered 1970s threshold as a landmark to segregate these two groups (i.e., historical and recent), based on three literature-based assumptions: 1) the stabilization of land use and land cover by anthropogenic factors in Latin America and São Paulo state (Houghton et al. 1991, Victor et al. 2005); 2) the increased rate

of consumption and production of nitrogenous fertilizers in Brazil (FAO 2019, IFA 2019); and 3) the increased rate of agricultural use, N_2O and CO_2 emissions derived from synthetic nitrogenous fertilizers in Brazil (FAO 2019).

We sampled a total of 734 individuals' feathers (historical = 286 and recent = 448) from a total of 26 species (historical = 26 and recent = 22), belonging to five foraging guilds (frugivores, granivores, insectivores, nectarivores and omnivores) (Appendix B). The 22 species belonging to the recent group are also in the composition of the historical group. Notwithstanding the above, to increase the number of historical individuals, we sampled four species more for this specific group, totalizing 26 species (Frugivores - *Chiroxiphia caudata*, *Euphonia chlorotica* and *Tangara cayana*; Granivore - *Leptotila rufaxilla*), which was possible considering that these guilds and birds uses food resources very similarly (see Willis 1979, Motta Junior 1990, Wilman et al. 2014).

In addition to feather samples, we collected food resources, such as invertebrates and seeds, which were available to birds during the field expedition in the recent period (Appendix C). The invertebrates were collected *ad libitum* (N = 70), while seeds were removed from birds' faeces of the individuals that defecated during sampling (N = 56), being subsequently identified at the lowest taxonomic level. We searched at the "Luiz de Queiroz" Herbarium (ESA) for historical samples of these plants that were identified at species or genus level (Appendix C), collecting the available tissue for isotopic analysis (seed, flower or leaf) (N = 22). Similarly, as group division of historical and recent birds, we used the same landmark of 1970 for the plants.

2.2.3. Isotopic analysis

We used part of the second primary remige (wing feather) of the individuals sampled to proceed with isotopic analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). Firstly, we cleaned the feathers with 70% alcohol to remove possible contaminants. No other chemical procedure was necessary, especially with historical samples, for the reason that preservation of the specimens in the museum is controlled only by temperature regulation. To prepare each feather sample, we removed a small section of the vane, approximately 0.5cm², without inclusion of rachis (Wiley et al. 2010). Finally, these tiny sections were packed in tin capsules (8x5mm or 5x2.5mm). To prepare food resources samples, we ground the invertebrates, seeds and leaves separately, in order to obtain a homogeneous mixture. Subsequently, we included approximately 2μg of plant samples and approximately 1μg of invertebrate samples in tin capsules (5x2.5mm).

All samples were analyzed in a Carlo Erba CNH-1110 elemental analyzer coupled to a Thermo Scientific Delta Plus mass spectrometer, transforming and purifing the sample into gas. This process is responsible for transforming the carbon of the sample into CO_2 and nitrogen into N_2, so the mass spectrometer provides isotopic values expressed in delta (δ) per thousand (‰), following the equation: $\delta^{13}C$ or $\delta^{15}N = [(Rsample/Rstandard) - 1] \times 1000$, where R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. To reference our samples, we compared the values with carbon and nitrogen international standards (Pee Dee Belemnite – V-PDB and atmospheric air, respectively). We analysed all the samples in the Laboratory of Isotopic Ecology of the Center for Nuclear Energy in Agriculture (CENA) and University of São Paulo (USP) at Piracicaba, São Paulo, Brazil.

2.2.4. Statistical analysis

Isotopic fractionation is the difference between the consumer tissue and the food consumed, wherein this value varies for each type of tissue analyzed (e.g., feather, claw, blood) (Hobson and Clark 1992). Each species has a different fractionation value since some characteristics might influence this variation, as physiological and anatomical conditions (Caut et al. 2009, Pecquerie et al. 2010). Thus, we calculated the fractionation values of carbon and nitrogen for each bird species through SIDER package in R ("Stable Isotope Discrimination Estimation in R") (Healy et al. 2017). We used the corrected isotopic values in subsequent analyzes (Appendix D).

The isotopic niche is representative of the ecological niche by using some variables about habitat use ($\delta^{13}C$) and position in the trophic chain and diet ($\delta^{15}N$) (Newsome et al. 2007). We calculated the isotopic niche width through SIBER package in R ("Stable Isotope Bayesian Ellipses in R") (Jackson et al. 2011). We used the niche width measure named Standard Ellipse Area corrected for small samples (SEAc) which is based on the maximum likelihood estimates of the means and covariance matrices of each group (historical and recent) and gives the results per thousand square (‰²). Besides total SEAc that consider almost the full range of individuals values of $\delta^{13}C$ and $\delta^{15}N$ composing the groups (p.interval = 0.95), we also calculated the mean SEAc which consider only the bivariate means to predict the ellipse width. To calculate ellipse area overlap between the two groups we also based on the maximum likelihood estimates of the means and covariance matrices of each group, posteriorly calculating in percentage how much of historical ellipse was overlapped by the recent one and vice-versa.

To ensure that we could group the historical birds collected in different municipalities within São Paulo, considering that the state is composed of distinct biomes and ecoregions

according to WWF (World Wide Fund for Nature), we divide historical individuals according to each of these classifications: 1) biome: Atlantic Forest and Cerrado (Appendix A); and 2) ecoregion: Alto Paraná Atlantic Forest, Cerrado and Serra do Mar Coastal Forest (Appendix A). Subsequently—considering the data distribution— we used Student's t-test or Wilcoxon signed-rank test to compare the means of $\delta^{13}C$ and $\delta^{15}N$ between individuals whitin these two major biomes. We also used one-way analysis of variance (ANOVA) or Kruskal-Wallis test to compare the means of $\delta^{13}C$ and $\delta^{15}N$ within ecoregions. Finally, we used Student's t-test or Wilcoxon signed-rank test to compare the mean values of $\delta^{13}C$ and $\delta^{15}N$ within historical and recent birds for each foraging guild, but also within historical and recent plant food resources. We ran all tests through R software version 3.5.2 (R Core Team 2018).

2.3. Results

All guilds showed a large significant reduction in the width of isotopic niche (SEAc) over the years (Figure 2). We observed the highest rate when comparing historical (<1970s) and recent (>1970s) individuals of nectarivores ($N_{hist.nec}$ = 64, $N_{rec.nec}$ = 57) and frugivores ($N_{hist.frug}$ = 50, $N_{rec.frug}$ = 69), with a rate of approximately 70% of reduction for both ($SEAC_{hist.nec}$ = 19.2‰² to $SEAC_{rec.nec}$ = 5.9‰² and $SEAC_{hist.frug}$ = 16‰² to $SEAC_{rec.frug}$ = 4.9‰²). The insectivores ($N_{hist.inse}$ = 67, $N_{rec.inse}$ = 176) and omnivores ($N_{hist.omni}$ = 77, $N_{rec.omni}$ = 132) had relatively high rates of reduction, 52% and 40% respectively ($SEAC_{hist.inse}$ = 13.3‰² to $SEAC_{rec.inse}$ = 6.4‰² and $SEAC_{hist.omni}$ = 13‰² to $SEAC_{rec.omni}$ = 7.7‰²). While the granivores ($N_{hist.gran}$ = 28, $N_{rec.gran}$ = 14) were the least impacted, with 28% of niche width reduction ($SEAC_{hist.gran}$ = 16.5‰² to $SEAC_{rec.gran}$ = 11.9‰²).

In addition to the crude niche reduction, our results showed a high overlap rate between the niches of recent individuals and the historical ones for all the guilds. Generally, the historic space of niche embodies a large portion of the recent-based niche space (Figure 2). In this case, the nectarivore guild had the maximum overlap (100%), while granivores showed a lower overlap (50.9%), although it was still a high rate. However, the niches of the historical individuals had also overlap with the recent ones, but in a smaller proportion (Figure 2), wherein omnivores and insectivores were the guilds with the greatest overlap, 43% and 41.3%, respectively.

Comparing the means of $\delta^{13}C$ and $\delta^{15}N$ between individuals of different biomes ($N_{atlantic.forest}$ = 135, $N_{cerrado}$ = 151) and ecoregions ($N_{alto.paraná}$ = 82, $N_{cerrado}$ = 151, $N_{serra.mar}$ = 53), we ensured the possibility to compare the historical groups with the recent ones (Appendix E). In both cases, we found a non-significant difference among the means of $\delta^{13}C$ (biome: Atlantic Forest = -23.8 ± 2.8‰ and Cerrado = -23.8 ± 2.3‰, p = 0.57; ecoregion: Alto Paraná Atlantic

Forest = -24.3 ± 2.7‰, Cerrado = -23.8 ± 2.3‰ and Serra do Mar Coastal Forest = -23.2 ± 2.7‰, p = 0.07) and $\delta^{15}N$ (biome: Atlantic Forest = 6.5 ± 2.3‰ and Cerrado = 7.1 ± 2.6‰, p = 0.051; ecoregion: Alto Paraná Atlantic Forest = 6.5 ± 2.2‰, Cerrado = 7.1 ± 2.6‰ and Serra do Mar Coastal Forest = 6.7 ± 2.6‰, p = 0.13), results that support the subsequent comparisons.

The mean values of $\delta^{15}N$ had decreased over the years from historical to recent individuals in all foraging guilds (Figure 3a; Table 1), decreasing in a range of 2.5-3.5‰ for frugivores, granivores, insectivores and omnivores, while for the nectarivores the reduction was 1.7‰. The mean values of $\delta^{13}C$ had not a significative change from historical to recent individuals for almost all guilds (Figure 3b; Table 1), with a maximum reduction of 1.4‰ for granivores and a minimum of 0.04‰ for nectarivores. All these $\delta^{13}C$ means are close to the representative range of forest areas values, composed mainly by C_3 plants (-24‰ to -38‰), except for historical granivores ($\delta^{13}C$ = -21.8‰). We also compared the means of $\delta^{15}N$ and $\delta^{13}C$ among historical and recent individuals of the plants analyzed as possible food resources to birds (Table 1), wherein $\delta^{15}N$ showed a decrease of 3.5‰ and $\delta^{13}C$ a decrease of 2.1‰ over the years (Appendix F).
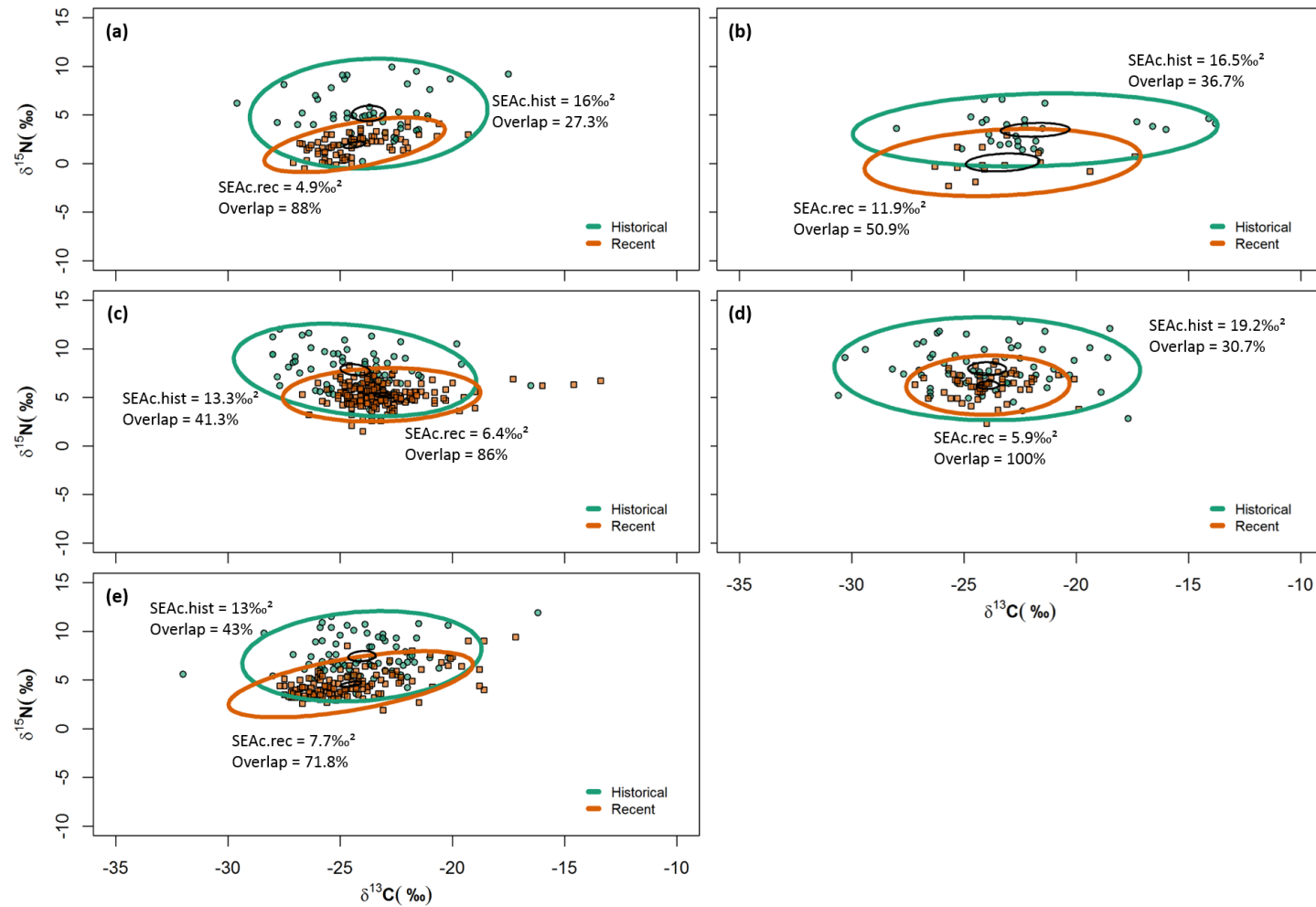
Figure 2. Individual values of $\delta^{13}C$ and $\delta^{15}N$ of the birds that comprise each of the following guilds: (a) frugivore, (b) granivore, (c) insectivore, (d) nectarivore and (e) omnivore, and their respective isotopic niches. Isotopic niches width value is next to the historical ($SEAc_{hist}$) and recent ($SEAc_{rec}$) ellipses. The overlap rate of historical individuals over the recent ones is presented next to $SEAc_{hist}$ value, while overlap of the recent over historical is next to $SEAc_{rec}$ value.
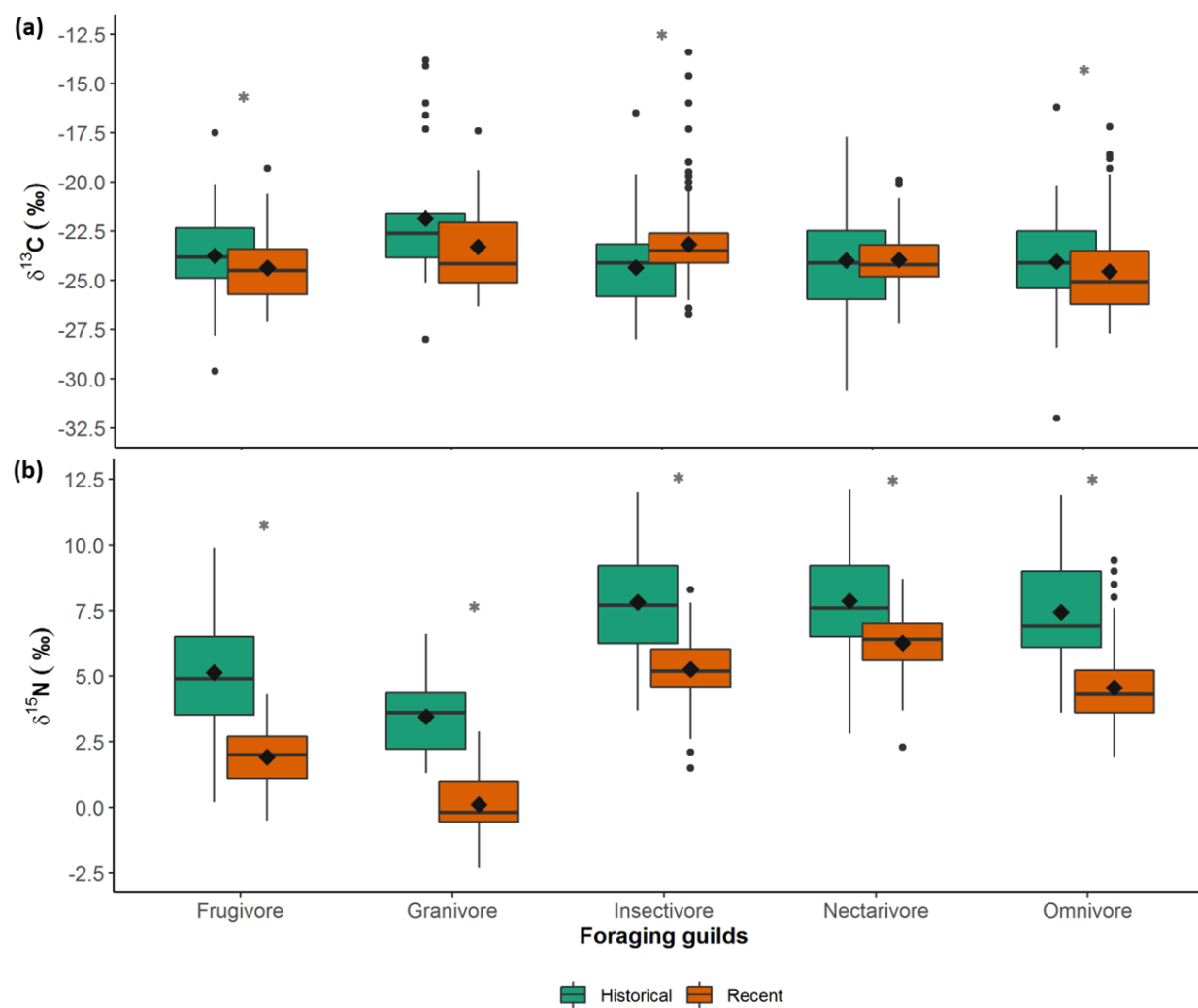
Figure 3. Boxplot of (a) $\delta^{13}C$ and (b) $\delta^{15}N$ for each of the foraging guilds (frugivore, granivore, insectivore, nectarivore and omnivore) comparing historical (green) and recent (orange) individuals. Boxplots show means (diamonds), medians, quartiles and outliers. Asterisks indicate a significant difference between historical and recent individuals' $\delta^{13}C$ and $\delta^{15}N$ means.

Table 1. Means and standard deviations (SD) of $\delta^{13}C$ and $\delta^{15}N$ for each foraging guild (frugivore, granivore, insectivore, nectarivore and omnivore) and food resources (plants and invertebrates) used for comparison (Student's t-test and Wilcoxon signed-rank test) between historical and recent individuals, and P-value (significant when <0.05).

| | | Isotope | Historical | | Recent | | P |
			Mean	SD	Mean	SD	
Foraging guilds	Frugivore	$\delta^{13}C$	-23.7‰	2.2‰	-24.4‰	1.6‰	0.04
		$\delta^{15}N$	5.1‰	2.3‰	1.9‰	1.1‰	<0.0001
	Granivore	$\delta^{13}C$	-21.8‰	3.3‰	-23.3‰	2.5‰	0.06
		$\delta^{15}N$	3.5‰	1.5‰	0.1‰	1.4‰	<0.0001
	Insectivore	$\delta^{13}C$	-24.3‰	2.2‰	-23.2‰	1.8‰	0.0002
		$\delta^{15}N$	7.8‰	1.9‰	5.3‰	1.1‰	<0.0001
	Nectarivore	$\delta^{13}C$	-24.0‰	2.8‰	-23.9‰	1.5‰	0.9
		$\delta^{15}N$	7.9‰	2.2‰	6.2‰	1.2‰	<0.0001
	Omnivore	$\delta^{13}C$	-24.0‰	2.2‰	-24.5‰	2.2‰	0.009
		$\delta^{15}N$	7.4‰	1.9‰	4.5‰	1.4‰	<0.0001
Food resources	Plants	$\delta^{13}C$	-28.0‰	2.0‰	-30.1‰	2.6‰	0.0007
		$\delta^{15}N$	5.6‰	3.6‰	2.1‰	1.6‰	0.0002
	Invertebrates	$\delta^{13}C$	-	-	-26.7‰	2.9‰	-
		$\delta^{15}N$	-	-	4.6‰	3.1‰	-

Our results of $\delta^{15}N$ showed a clear structuration of the trophic chain for both the historical and the recent groups, wherein the mainly plant-based guilds remained below guilds that forage mainly at invertebrates (Figure 4). The nectarivore guild was an exception for both historical and recent group, presenting higher mean values of $\delta^{15}N$ than the other guilds. Nevertheless, this result might be linked to diet complementation with invertebrates and accelerated metabolism of hummingbirds.
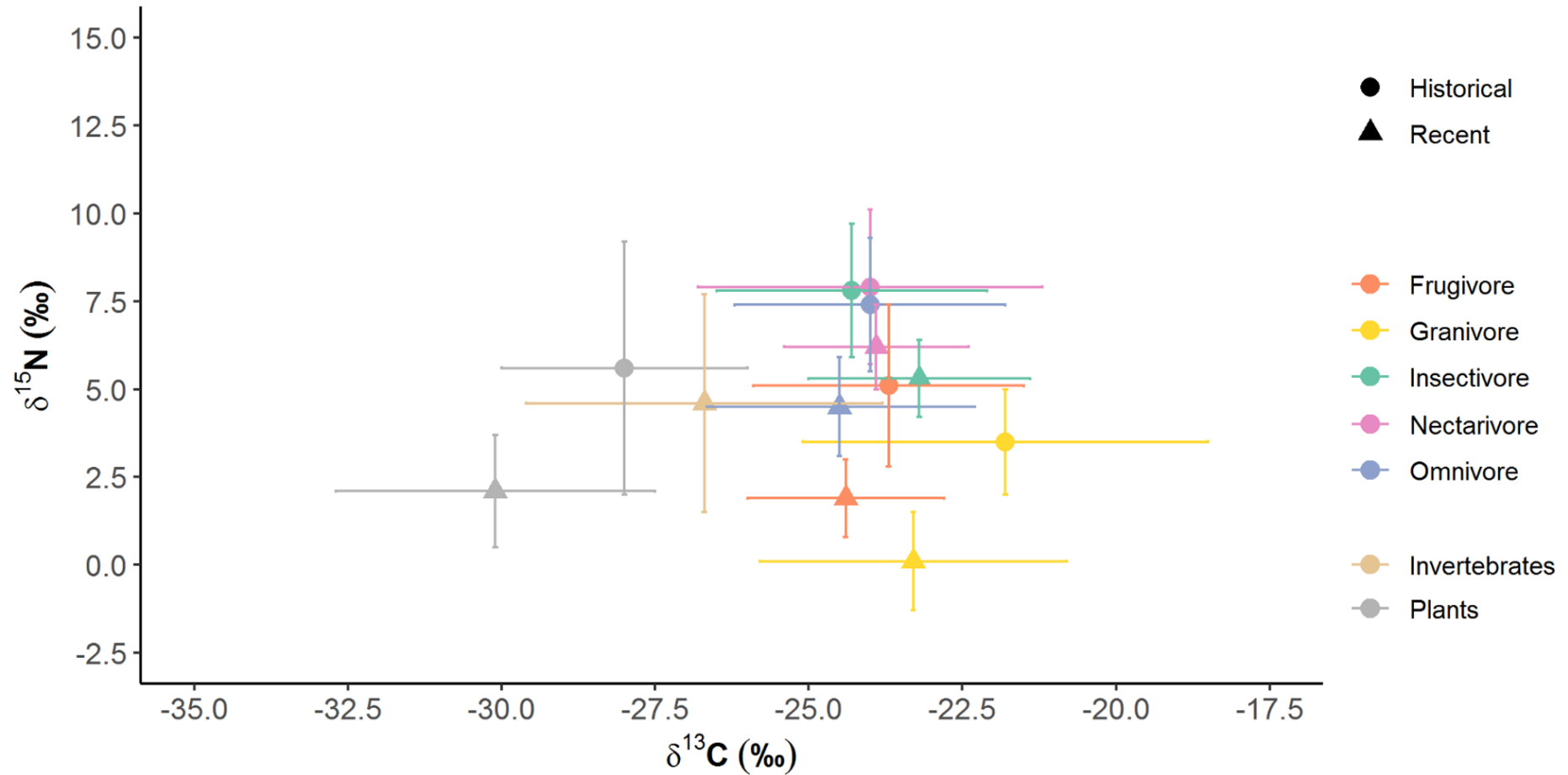
Figure 4. Means and standard deviations of δ13C and δ15N values for the foraging guilds (frugivore, granivore, insectivore, nectarivore and omnivore) and food resources (plants and invertebrates) of the historical (circle) and recent (triangle) groups. Values of δ15N show the structuration of the trophic chain with plant-based guilds at the base and guilds that forage mainly at invertebrates at the top.

2.4. Discussion

2.4.1. Anthropogenic impacts on isotopic niches

All guilds showed a significant high reduction in the width from historical to recent isotopic niches, ranging from 28% to 70% of decline depending on the guild. Apparently, the greatest cause of this niche width reduction was the human expansion in the state of São Paulo during the 20th century, which was responsible by a quantitative and qualitative change throughout the countryside plateaus. This state had its forest cover reduced and replaced mainly by agriculture over the last hundred years of intense devastation (Victor et al. 2005, Cardim 2018). It is estimated that originally there were 82% of forest cover in the state, 58% of forest remained near the coast and at the countryside at the beginning of the 20th century (Victor et al. 2005), remaining only a quarter nowadays, extremely concentrated at coastal mountains (MapBiomas 2019). Following the habitat loss, one of the most important drivers of biodiversity decline, other local threatening processes such as environmental degradation and reduced availability of food resources are triggered (Lindenmayer and Fischer 2006). Beyond the pervasive depletion of many vertebrate population, many species of plants and invertebrates also declined largely due to habitat loss (Dirzo and Raven 2003), leading to a cascade-effect upon ecosystem functioning and bird assembling, since these organisms serve as a basal food resource for several birds and compose the milestone strata of several species diet. We observed that historical niches of all foraging guilds were broader than recent niches. Generally broader isotopic niches represent varied diets mainly composed by many food resources, while more restricted diets are represented by smaller niches (Han et al. 2019). This recent niche bottleneck probably might be associated with the qualitative and quantitative decline in food resources to birds surviving within the high HMLs typically present along the Southeast Brazilian plateaus. As impacts of anthropogenic threats might influence for contractions in the Hutchinsonian realized niche leading some species to decline (Scheele et al. 2017), there is a great concern that this broad reduction might represent a threat of local extinction to the species composing these guilds in a few years.

Nectarivores and frugivores had the highest reduction rates of isotopic niche over the years, approximately 70% for both. These guilds were, probably, directly affected by habitat loss and shrinking native flesh-fruited plant species on forest remnants. Although most studies focus on the impacts of the Anthropocene on vertebrates' decline (Dirzo et al. 2014, Johnson et al. 2017, Bogoni et al. 2018), some studies show the same effects on native plant species (Ellis et al. 2012, Vellend et al. 2017). The low availability of food resources to nectarivores and frugivores nowadays can be observed in this shrinkage of niches, as both guilds had broader niches in the past for the reason they had a high diversity and abundance of food.

Both are currently the narrowest guilds with the smallest niche widths, probably due to the decline of many species that serve as food for birds (e.g. Rigueira et al. 2013) and the increased rate of invasion by exotic plant species (e.g. Zenni and Ziller 2011).

Granivores seemed to be less impacted by anthropogenic activities over the years, although they still had a 28% reduction in the historical niche compared to the recent niche bidimensional space. The greater plasticity of granivores enable many bird species to thrive in open environments or forest edges (Giraudo et al. 2008), which must be one of the causes for this guild to have the lowest reduction rate. However, the invasion of exotic species, especially grasses used for economic purposes, was crucial for the decline of native grasses diversity (D'Antonio and Vitousek 1992, Zenni and Ziller 2011), which may have been significant for a more restricted recent niche despite granivores' plasticity. In the past, the width of frugivores isotopic niche was wider than granivores niche, a pattern that has been totally reversed nowadays in which granivores have the widest niche in relation to all other guilds. There are biological evidences that show the savannization process that the Atlantic Forest has been going through over the years, causing changes in vegetation structure and enabling the establishment of grasses (Scarano and Ceotto 2015), a fact that corroborates greater availability of resources for granivores than for others guilds, despite grass species not being as diverse as in the past.

The significant reduction rates among historical and recent isotopic niches of insectivores (52%) and omnivores (40%) should also be related to the effects of human expansion in the tropics. Despite having less attention than other groups, invertebrates are suffering direct consequences of the Anthropocene as population declining and species extinction from local to regional scales (Dirzo et al. 2014). This decrease in the invertebrate population has brought direct negative consequences for insectivorous birds, through reducing the availability of basal food resources and consequently promoting a trophic cascade effect, since organisms depend on the maintenance of the food web to obtain resources and promote their survival (Lister and Garcia 2018). The same impacts on other guilds should also have an effect on omnivores, as the species that compose this guild consume a variety of available food resources, relying mostly on fruits and invertebrates. Nevertheless, the decline in omnivores niche width was not as expressive as in the other guilds, which might be a result of the capacity of diet adaptation according to food seasonality, so making species' diet more varied despite the general reduction of resources in the environment. The generalized omnivore diet aforementioned is observed by the width of recent isotopic niche, being the second largest after granivores.

Anthropogenic negative effects resulted also in a high overlap of recent over historical niches from 51% to 100% for all foraging guilds, showing that the guilds are highly dependent on the same food resources consumed in the past, despite all landscape changes in São

Paulo. The vast majority of the species that compose the five guilds are considered common and generalist in terms of habitat use, and normally generalist species are less affected by landscape changes (Carrara et al. 2015). Also, these species are responsible for the fulfillment of ecological processes and maintenance of the ecosystem in degraded environments, which mark the importance of their existence in these landscapes (Pizo 2007, Carlo and Morales 2016). Nevertheless, our results showed that even generalist species might be struggling from a reduction in the diversity of resources and do not have so much plasticity to search for new sources, which might compromise species survival. As is the case observed for nectarivores, in which 100% of the recent niche is overlapped by the historical, showing the high dependence of this guild on a small amplitude of the historical resources that persist.

The overlap of historical niches over the recent was lower than the opposite overlap, ranging from 27% to 43%, which further corroborates declining in resource and habitat availability. Landscape homogenization can lead to a reduction on biodiversity and even intensifying the similarity of plant and animal communities within HMLs (Dormann et al. 2007, Sirami et al. 2019), explaining the decrease in diversity of habitat and consequently food resources for birds nowadays. Besides that, landscapes homogenization is one of the negative effects of anthropogenic threats that directly affect bird communities often promoting generalists over specialist species (McKinney and Lockwood 1999, Clavel et al. 2011). Although we have not analyzed any typically specialist bird, we suppose that it is likely to observe great changes with further isotopic niche analysis, considering that such species have declined to the point of no longer being found in the state. Notwithstanding, despite resisting to anthropogenic impacts the generalists analyzed in the study do not show to be in full plenitude when compared with the historical baseline, demonstrating the negative influence of landscape homogenization.

Maintaining the structure of the trophic chain is essential for the conservation of natural ecosystem cycles (Pimm 1982), but the availability of food resources and anthropogenic disturbances might influence this structuring (Post 2002b). It is possible to estimate the position in the trophic chain using $\delta^{15}N$ values, as distinct consumers incorporate different proportions of ^{15}N depending on whether their diet is more plant or animal based (Post 2002a). We observed that in the past and recently the structure of the trophic chain was maintained, wherein plant-based guilds (granivores and frugivores) were closer to the base opposing to guilds that consume more invertebrates (insectivores and omnivores). Exception observed only for nectarivores, which presented $\delta^{15}N$ higher than the other guilds, both in the past and recently. Nevertheless, most of the nectarivores are hummingbirds, this group has accelerated metabolism and often complements the diet with invertebrates influencing the fractionation of nitrogen isotopes providing a higher proportion of ^{15}N in tissues (Hardesty 2009).

Historical and isotopic ecology has received more attention recently, helping not only to identify patterns on the use of habitat and food resources by some species, but also to develop strategies for conservation and management of these organisms (Newsome et al. 2007, Wiley et al. 2017). However, most of these studies were with aquatic species (e.g. Chamberlain et al. 2005, Becker and Beissinger 2006, Blight et al. 2015, Møller et al. 2018) and less attention is given to the terrestrial organisms (e.g. Chamberlain et al. 2005, English et al. 2018). Although the birds analyzed in the study are relatively common in the forest remnants of the countryside of São Paulo, our results showed that anthropogenic impacts were very evident on them. Emphasis in historical ecological studies should become more frequent and also greater conservation awareness should be given to generalist species, since these birds perform essential functions for the maintenance of the ecosystem in these HMLs.

2.4.2. Anthropogenic impacts on $\delta^{15}N$ and $\delta^{13}C$ means

We observed a great (typically larger than 2‰) reduction in $\delta^{15}N$ means from historical to recent individuals for all foraging guilds, which might be directly related to land use changes in São Paulo, especially due to deforestation and increase of agriculture. Albeit most isotopic ornithological studies use $\delta^{15}N$ to infer the position of a particular organism in the trophic chain and about its diet (e.g. Kelly 2000, Norris et al. 2007), some isotopic studies also report the influence of anthropogenic impacts on the species through the $\delta^{15}N$ values (e.g. Hebert and Wassenaar 2001, Møller et al. 2018). Among these impacts we can infer about the influence of agricultural use of fertilizers, as through $\delta^{15}N$ is possible to differentiate the synthetic from the organic, where the former has lower $\delta^{15}N$ (Bateman and Kelly 2007, Choi et al. 2017). The use of nitrogen fertilizers in Brazil has been increasing since 1960 (FAO 2019, IFA 2019), as well as direct N_2O emissions from synthetic fertilizers (FAO 2019), wherein the state of São Paulo was already an important consumer in the past and continues to be until today (Cibantos and Larson 1974, IPNI 2019). Although we still do not have a complete understanding of the effects of anthropogenic nitrogen input on the environment, it is well known that much of the application in agriculture escapes to other locations, including forests (Schlesinger 2009), and consequently to the organisms that live in there. Therefore, these birds might be incorporating nitrogen from an anthropogenic source, mainly from synthetic fertilizers. Anthropogenic nitrogen inputs in the marine environment is already associated with changes in feather quality (Møller et al. 2018), nevertheless we still lack information about possible morphological, physiological and evolutionary consequences in terrestrial birds.

The mean values of $\delta^{13}C$ did not show a significant change over the years in all five guilds, wherein both historical and recent means were representative of forest habitat in

general. Tropical forests are mainly composed of C_3 plants with $\delta^{13}C$ values ranging from -24‰ to -38‰ (Martinelli et al 2009), while the areas of agriculture in the state of São Paulo are mainly pasture and sugarcane (MapBiomas 2019), which are C_4 plants and the values range from -11‰ to -15‰ (Martinelli et al 2009). Considering that all the guilds showed to prefer forest habitat through $\delta^{13}C$ values, these species depend almost entirely on this habitat to search for food resources. However, there is a narrow difference between the $\delta^{13}C$ of birds and C_3 plants (food resource) which might represent a possible mixture in small proportion with C_4 resources from the forest edge or even from agricultural matrix. Such difference is greater with granivores, since this guild has species more adapted to open environments.

Additionally, the difference between $\delta^{13}C$ mean values between historical and recent individuals might also be related to atmospheric changes due to anthropogenic impacts. Increasing rates of land use change, deforestation and pollution were the main causes to raise the input of CO_2 in the atmosphere, especially after the industrial revolution (Leuenberger 2007, Werner et al. 2007). After 1900 the CO_2 input was so high that the atmospheric value of $\delta^{13}C$ was reduced by approximately -1.5‰ in the Southern Hemisphere (Leuenberger 2007). Considering that the incorporation of CO_2 occurs through photosynthesis and consequently the carbon incorporated in plants is transferred to consumers, it is reasonable that the difference observed in the atmosphere also occurs in organisms. Nevertheless, with our results we could not conclude any considerations in this regard, but we strongly suggest that further studies verify whether anthropogenic impacts on the atmosphere reflect on birds' fauna.

2.5. Conclusion

There was a large reduction in the width of isotopic niches of all foraging guilds throughout the 20th and 21st centuries in the countryside of the state of São Paulo which could be related to anthropogenic activities, especially land use changes through deforestation by the increase of agricultural fields. Considering that isotopic niche is representative of ecological niche, since it contains information on the habitat use and diet of organisms, a reduction in niche width might show a local threat to the species of these guilds (frugivores, granivores, insectivores, nectarivores and omnivores). Moreover, all guilds had a high overlap of recent niches with historical ones, showing that these birds depend on a small breadth of food resources that still persist in these HMLs, mainly due to the large decline in plant species and invertebrates' diversity. The increased consumption of nitrogen fertilizers in agriculture is another important anthropogenic impact on the environment, which might be associated with the evident reduction in $\delta^{15}N$ values among historical and recent individuals. Although the species analyzed in this study are considered generalists, these birds are the responsible to

perform the ecological functions in fragmented forest remnants. Therefore, we suggest an increase in conservation actions towards these species, ensuring that birds and the ecological processes developed by them do not be vanished. Once we detected a detrimental decrease in bidimensional dietary niche of birds over a century, we depicted more intimately the effects of habitat loss and changes on ecosystem functioning. Our results emphasize that birds can be used as a proxy to understand these effects, but also guide public policy to environmental enrichment.

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3. CHAPTER 2 – ISOTOPIC NICHE, HABITAT USE AND DIET OF BIRDS IN HUMAN-MODIFIED AND NATURAL LANDSCAPES

ABSTRACT

Habitat loss, especially due to land use changes, represents one of the greatest contemporary anthropogenic impacts on tropical forest birds. Reduction of forests and consequent decline of biodiversity pose threats to the perpetuation of several species in human-modified landscapes (HMLs). The use of stable isotope analysis provides relevant information on the ecological niche, habitat use and diet of the species. We investigated whether birds of five guilds (frugivore, granivore, insectivore, nectarivore and omnivore) differ in niche width, habitat use and food resources use between HMLs and natural landscapes (NL), using stable carbon and nitrogen isotopes. Our study sites comprise 28 landscapes located in the state of São Paulo in Brazil, an extensively explored tropical region. According to the existing literature, we grouped 54 bird species into five guilds (frugivore, granivore, insectivore, nectarivore and omnivore). We divided bird individuals into two landscape groups according to the percentage of forest cover, with a total of 846 individuals in HMLs (less or equal to 30% of forest cover) and 199 individuals in NLs (equal or more than 47% of forest cover). Considering that ecological niche is well represented by isotopic niche, we calculated the width of isotopic niches for each guild comparing among the different landscape groups. In addition, we calculated the mean values of δ^{13}C and δ^{15}N also for each of the guilds in HMLs and NLs. Frugivores, insectivores, nectarivores and omnivores showed narrower isotopic niches in the HMLs and wider niches in the NLs, while granivores showed the opposite pattern. Omnivores and nectarivores showed a decline of 44% in niche width in HMLs, while granivores presented an expansion of 27%. In HMLs the mean values of δ^{13}C and δ^{15}N were significantly higher than in the NLs, likewise the distribution of δ^{15}N values that also differed among landscapes. Reductions in niche width of most guilds might be associated with the effects of habitat loss on HMLs. In these degraded and fragmented environments, there is a reduction not only of habitat proportion, but also in the availability of food resources to almost all species. The granivores were the only guild that seems to be most adapted to anthropogenic changes in landscape, such as savannization of tropical forests. Moreover, in HMLs the guilds have δ^{13}C values representative of a possible mixture of resources from forest and agricultural fields, although the preference is to forage in the forest remnants, as presented by individuals in NLs. The grouped distribution of δ^{15}N values in HMLs might be related to lower resource availability in these environments, contrary to the heterogeneous distribution observed in NLs due to greater food plenitude. Therefore, if we expect to perpetuate the diversity of guilds in HMLs, there should be a major concern to offer birds more biodiversed habitats.

Keywords: Ecological niche, Guilds, Trophic cascade effect, Agriculture, Trophic ecology

3.1. Introduction

Land use changes constitute one of the greatest threats to biodiversity caused by the Anthropocene, especially due to the high rates of deforestation and expansion of agricultural areas, which have increased considerably over the last fifty years (Steffen et al. 2005). These anthropogenic changes in landscapes directly affect the availability of habitat and food resources to fauna, wherein human-modified landscapes (HMLs) differ from natural

landscapes in the provision of both (Magioli et al. 2019). Also, land use changes are usually linked to habitat loss and fragmentation, which are anthropogenic processes responsible for the decline of several organisms, since the lack of adequate habitat hinders the success of some species (Fahrig 2003, Haddad et al. 2015). Still in face of the Anthropocene, there are significant compositional changes and declines of diversity in the communities of animals and plants as consequence of all anthropogenic impacts (Dirzo et al. 2014, Vellend et al. 2017), many of which serve as direct food resources for other organisms. All of these multi-taxon declines implement negative changes in foodwebs since the existence of one species necessarily depends on several others as a food source (Estes et al. 2011, Bogoni et al. 2019).

Birds are one of the many taxa influenced by anthopogenic impacts at landscape scales, having its communities changed significantly in HMLs in comparison with the natural ones (Pardini et al. 2009, Zurita and Bellocq 2010, Martensen et al. 2012). In the neotropical forest gradient, there is a threshold of forest cover that can explains the levels of species diversity, where landscapes composed of until 30% of forest has less bird richness and abundance compared to landscapes with more than 50% of forest (Martensen et al. 2012, Morante-Filho et al. 2015). In HMLs, with a lower percentage of forest cover, the diversity of bird assemblages is dominated by generalist species regarding the use of habitat and diet (Carrara et al. 2015). Usually, generalist birds are considered less sensitive to changes in habitats (Devictor et al. 2008), although even then they depend on forest remnants and secondary forests to persist in the landscape (Pardini et al. 2009). Thus, based on their ecological requirements (e.g., resource use) these species are responsible to develop much of the ecological processes required to maintain the ecosystem along a gradient of degraded environments (Pizo 2007, Barros et al. 2019). Species that rely on the same resources are considered to be part of the same guild, differing from the functional group, which considers the ecological functions performed by species to group them together (Blondel 2003). Nevertheless, many of the species that compose guilds are involved in ecological processes, especially due to their diets, not mattering if they are specialists or generalists. Understanding the pattern of birds' habitat and food resource use provides not only improved knowledge of the ecology of species, but also provides an idea about their interaction within the landscapes under different degrees of anthropogenic influence and their roles in these ecosystems functioning.

To understand the species' habitat and resource use across a spatiotemporal scale, the use of stable isotopes has been increasing in ecological studies of birds. This use was captained mainly due to the efficiency of obtaining precise information of their diet and habitat use, which with other methods would cost too many time and efforts (e.g. Inger and Bearhop 2008, Hobson 2011). Whereas these conventional methods used in foraging studies are difficult to apply in some cases (e.g. Sutherland 2004, Garvey and Whiles 2016), to use stable

isotopes of carbon and nitrogen is an alternative to be considered in order to complement these other methods (Kelly 2000, Boecklen et al. 2011). The chemical composition of organisms' tissues is nothing more than the food consumed by it plus an isotopic fractionation rate due to the inherent multiple factors in the tissue (DeNiro and Epstein 1978, 1981). Previous studies of isotopic ecology of birds were done aiming to evaluate the distinct signatures expressed by multiple tissues, such as feathers, muscle, blood and claws (Boecklen et al. 2011), highlight is made for the use of feathers considering the advantage of easily collect this tissue in museums and field expeditions (Smith et al. 2003, Wiley et al. 2017), but also the fact it is a metabolically inert tissue providing responses in a temporal scale of weeks or months (Hobson and Clark 1992a). Stable isotopes of carbon (^{12}C and ^{13}C) are reliable for predicting resource and habitat use by the species, especially as plants with different photosynthetic pathways (C$_3$ and C$_4$) incorporate each isotope in different proportions, measured using the δ^{13}C notation (Inger and Bearhop 2008). Forests, mostly composed of C$_3$ plants, are distinguished from agricultural areas, mainly composed of C$_4$ plants (e.g. pasture, sugarcane), which is the main component of several tropical landscapes. Through δ^{15}N, stable isotopes of nitrogen (^{14}N and ^{15}N) are often used to predict the species' position in the trophic chain, considering that ascending on chain the organisms incorporate more ^{15}N (Post 2002) and also these isotopes infer about the influence of anthropogenic nitrogen input into the environment on organisms (Hebert and Wassenaar 2001).

The concept of ecological niche has been widely used in several studies focused on understanding the relationship among species and their environment and have recently gained more importance (Chase and Leibold 2003). Niches are composed of a multidimensional space of biotic and abiotic variables, such as the availability of food resources and diversity of habitats, so through the niche breadth, it is possible to comprehend relevant information about species ecology (Sexton et al. 2017). To assess the environmental impacts on niche breadth reduction of declining species characterizes the niche reduction hypothesis, in which such contraction can pose a threat of local extinction to species (Scheele et al. 2017). While niche expansion sometimes highlights evolutionary adaptation of species to various environmental changes caused by anthropogenic threats (Pagani-Núñez et al. 2019). The isotopic niche is considered representative of the ecological niche, as through the combination of δ^{13}C and δ^{15}N values it is possible to provide information on the habitat use and diet of species (Newsome et al. 2007). Some studies have used this new approach and found relevant information about niche width changes in anthropogenic habitats and the possible consequences for species conservation in these environments (e.g. Galetti et al. 2016, Pagani-Núñez et al. 2019, Magioli et al. 2019). Facing a changing world with constant modifications at landscape scales, it is essential to understand how and if species adapt to the available food resources in HMLs, and manage to use it as habitat.

We thus investigate the patterns of resource and habitat use by five guilds of birds (frugivore, granivore, insectivore, nectarivore and omnivore) in landscapes with different percentages of forest cover. To do so, we determined the isotopic values of carbon (δ^{13}C) and nitrogen (δ^{15}N) for each guild and we compared individuals between natural landscapes (NLs; equal or more than 47% of forest cover) and HMLs (less or equal to 30% of forest cover). In addition, considering the importance of isotopic niches to understand the relationship of species with the environment, we calculated the niche width using δ^{13}C and δ^{15}N values for the five guilds and compared between these two types of landscapes to evaluate possible changes in their width. Thus, we hypothesized that given the anthropogenic impacts on tropical forests—especially regarding land use changes with agriculture expansion and deforestation—the bird species that compose the five foraging guilds would show significant negative differences in isotopic niche width in HMLs when compared with the relictual natural habitats. We also hypothesized that in HMLs the bird foraging guilds could be prone to a greater mixture of food resources from both forest and agricultural areas compared to NLs, measured by δ^{13}C values. The same case for the δ^{15}N values, in which we expected to find differences to the detriment of landscape components since anthropogenic changes affect negatively resource availability and trophic chain structure.

3.2. Material and Methods

3.2.1. Study sites

Our study comprised 28 distinct landscapes located in the state of São Paulo, Brazil (Figure 1). São Paulo is a Brazilian state that encompasses portions of two tropical hotspots, the Atlantic Forest and the Cerrado (Mittermeier et al. 2011). Historically, forests used to cover almost entirely this region with an estimated 82% of forest cover (Victor et al. 2005). Nowadays, only 24% of forest cover is remaining, extending near the knife-ridges and coastal regions, known as Serra do Mar corridor and Paranapiacaba ecological continuum (Rezende et al. 2018, MapBiomas 2019). The pattern of high fragmentation and forest loss in the western portion of the state, opposed to the more forested coast region, is due to the history of agriculture expansion in São Paulo, that was not previously established near the coast considering the steep mountain slopes in the Serra do Mar region (Aguiar et al. 2003).

The percentage of forest cover in a landscape might be an extinction threshold for several species, in which fragmented landscapes with less than 30% of forest differ greatly in biodiversity from those with more than 50% (Morante-Filho et al. 2015). Considering that Morante-Filho et al. 2015 analysed birds in different landscapes of the Atlantic Forest, one of the biomes of São Paulo state, we replicated the methodology in our landscapes, delimiting

each one as a buffer of 2km-radial and an area of approximately 13km². For each one of the defined landscapes we obtained the percentage of forest cover from a land use and land cover classification map with 5m resolution satellite images, available at the Brazilian Foundation for Sustainable Development (FBDS, 2019). We obtained the land use (in percentages) according to the following categories: forest formation (native tree vegetation with continuous canopy), non-forest formation (native shrub or herbaceous vegetation), water, built area (e.g. urbanization), anthropogenic area (e.g. agriculture) and planted forest. Thus, we grouped our landscapes into two groups according to the forest cover percentage: 1) less or equal to 30% of forest cover (named as human-modified landscapes, HMLs), and 2) equal or more than 47% of forest cover (named as natural landscapes, NLs). The delimitation of buffers on sites and calculation of percentages of each land use were performed using the QGIS version 3.2.2 software, based on the geographic coordinates of each study site, expressed as a UTM projection (Datum SIRGAS 2000).

The HMLs comprised 12 sites ranging from 6% to 26% of forest cover (Appendix G), while NLs contained 16 sites ranging from 47% to 99% of forest (Appendix H). The landscapes showed a great variation in the percentage of forest cover among themselves, as well as in the percentage of anthropogenic area. This pronounced dichotomy with regard to land use in our landscapes is precisely that observed when comparing the western side of the state (more agriculture) with the region near the coast (more forest).
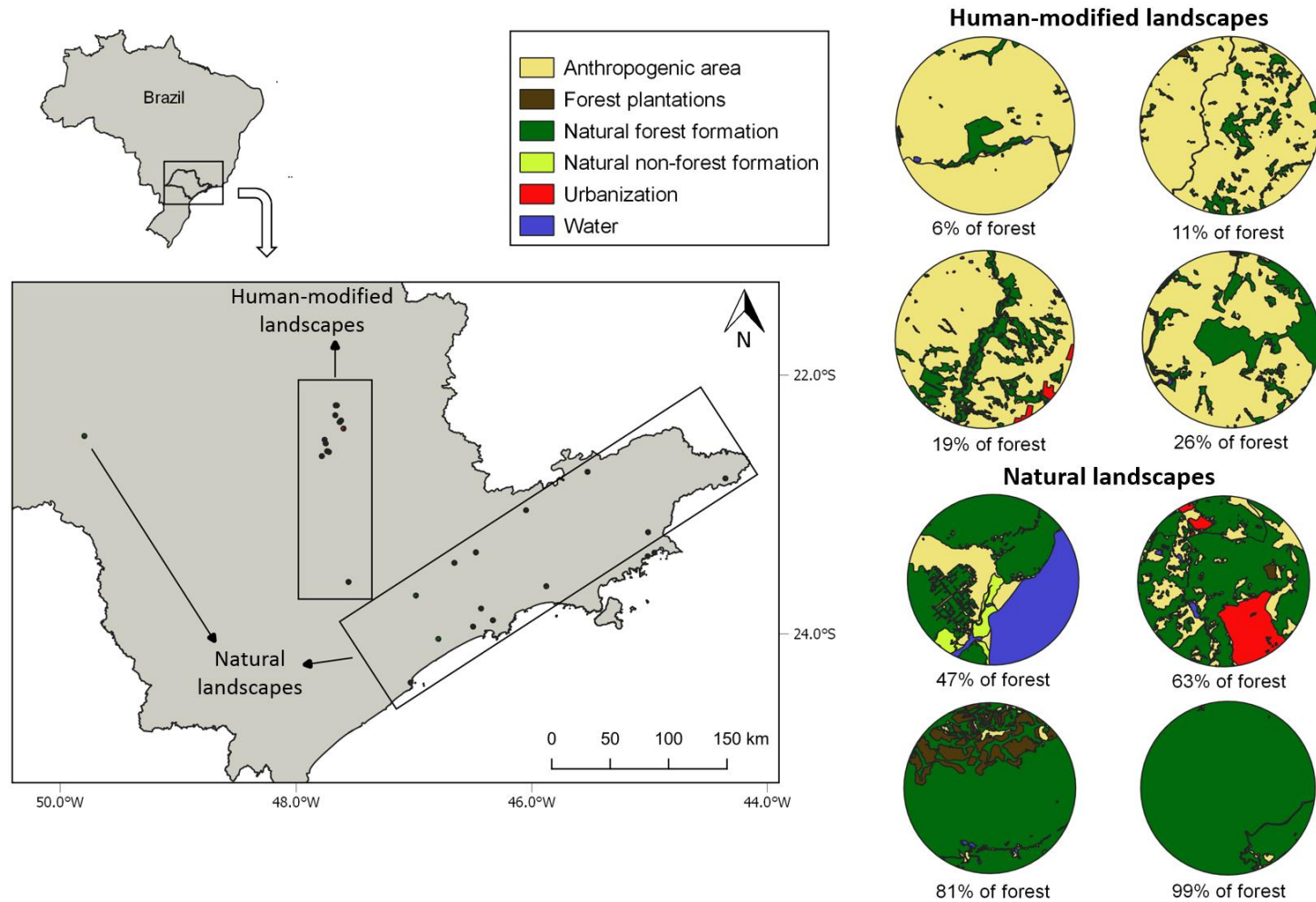
Figure 1. Map of the 28 landscapes in the state of São Paulo, southeastern Brazil. The exact location is represented on the grey map, differentiating between the two established groups: human-modified landscapes (HMLs) and natural landscapes (NLs). The circular buffers in the right are just a sample of the diversity of landscapes with different percentages of forest cover (all landscapes in Appendices E and F), in which the group (a) consists of 12 sites and (b) 16 sites.

3.2.2. Collection of samples

We organized the individual samples of birds into five guilds (frugivore, granivore, insectivore, nectarivore and omnivore) according to the existing literature (see Willis 1979, Motta Junior 1990, Wilman et al. 2014). This condensation allows general evaluations about the diet of the groups, since species in the same guild forage from similar food resources (Blondel 2003, Wilman et al. 2014). Thus, we assembled five species (143 individuals) in frugivores, 10 species (111 individuals) in granivores, 20 species (488 individuals) in insectivores, nine species (107 individuals) in nectarivores and 10 species (196 individuals) in omnivores (Appendix I). Among the 1045 individuals sampled, we divided them into two groups according to the percentage of forest cover of the landscapes. The group of birds captured in HMLs has a total of 846 samples, while the group of birds of NLs has a total of 199 samples.

We used wing feather samples to perform the isotopic analysis, considering that it is a tissue full of information about the diet and easy to be collected in museum collections and field expeditions (Smith et al. 2003). In addition, the comparison between individuals is only possible when it is the same tissue, as different tissues have different isotopic discrimination factors (Caut et al. 2009). To increase the number of individuals belonging to each of the guilds and landscape groups the feather samples were collected by different methods. The individuals belonging to the HMLs were mostly collected during field expeditions using mist nets, but some individuals were collected *ad libitum* when found dead. By contrast, samples of individuals belonging to the NLs were mostly collected from museum specimens and some in field expeditions. Thus, our tissue sampling ranged from individuals collected from 2001 to 2019, considering museum specimen samples and the ones collected in different field expeditions. Nevertheless, distinct years of samples collection should not be an important factor for differences in the values of isotopic signatures, since among these last 20 years there were no significant changes in the landscapes of the state of São Paulo, especially considering forest cover (MapBiomas 2019).

In addition to feather samples, we also sampled food resources, such as, invertebrates and plant seeds collected in HMLs (Appendix J). The seeds were removed from the faeces of birds' individuals captured in the mist nets, subsequently washed and sorted to be isotopically analyzed, totaling 56 samples. The invertebrates with 70 samples were collected *ad libitum* during the field expeditions. Samples of food resources from NLs were not possible to be collected, especially considering that feather samples were obtained mainly from museum specimens. Notwithstanding, this gap in food resource sampling might not represent a problem in subsequent analysis as we grouped the individuals into foraging guilds, precisely because within the guilds there is a similar consumption of food resources by birds.

3.2.3. Isotopic analysis

We used the stable isotopes of carbon and nitrogen obtained through primary feathers of the left-wing in birds. The feathers were cleaned with 70% alcohol to remove possible contaminants and later approximately $0.5cm^2$ from the vane was cut, without including the rachis in the sample (Wiley et al. 2010). The samples were packed in small tin capsules (8 x 5mm or 5 x 2.5mm) and sent to the Laboratory of Isotopic Ecology of the Center for Nuclear Energy in Agriculture (CENA) and University of São Paulo (USP) at Piracicaba, São Paulo, Brazil. The same procedure was performed with food resource samples, in which we ground the invertebrates and seeds separately, in order to obtain a homogeneous mixture, subsequently including approximately 2μg of seed samples and approximately 1μg of invertebrate samples in tin capsules (5 x 2.5 mm).

All samples were combusted in a CNH-1110 elemental analyzer (Carlo Erba) resulting in gases that were subsequently inserted into a coupled continuous flow isotope ratio mass spectrometer (Thermo Scientific Delta Plus) evaluating samples' isotopic composition. This process is responsible for transforming the carbon of the sample into CO_2 and nitrogen into N_2, so the mass spectrometer provides isotopic values expressed in delta (δ) per thousand (‰), following the equation: $\delta^{13}C$ or $\delta^{15}N = [(Rsample/Rstandard) - 1]$ x 1000, where R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. To reference our samples, we compared the values with carbon and nitrogen international standards (Vienna Pee Dee Belemnite – V-PDB and atmospheric air, respectively).

3.2.4. Statistical analysis

The difference between the consumer tissue and the food resource consumed is known as isotopic fractionation, wherein each type of tissue (e.g., feather, claw, blood) might have a distinct value of isotopic fractionation depending on their metabolism (Hobson and Clark 1992b). Isotopic fractionation value varies not only within tissues but also depending on the species analyzed, since each animal has different physiological and anatomical characteristics that might influence this variation (Caut et al. 2009, Pecquerie et al. 2010). Thus, in order to use the corrected isotopic signature of each individual in posterior statistical analysis, we calculated the fractionation values of carbon and nitrogen for each bird species through SIDER package in R ("Stable Isotope Discrimination Estimation in R") (Healy et al. 2017).

Values of $\delta^{13}C$ are associated with individual's habitat use whereas values of $\delta^{15}N$ are related to individual's position in the trophic chain and diet, in a bivariate association of both it is possible to calculate the isotopic niche, which is representative of ecological niche considering the pieces of information obtained by these two isotopic signatures (Newsome et al. 2007). After correcting $\delta^{13}C$ and $\delta^{15}N$ values with SIDER (Appendix K), we calculated the isotopic niche width through SIBER package in R ("Stable Isotope Bayesian Ellipses in R") (Jackson et al. 2011), for each of the five foraging guilds within both groups of landscapes composition (HMLs and NLs). We used the niche width measure named Standard Ellipse Area corrected for small samples (SEAc) which is based on the maximum likelihood estimates of the means and covariance matrices of each group of landscape composition (HMLs and NLs) and gives the results per thousand square (‰²). Besides total SEAc that consider almost the full range of individuals values of $\delta^{13}C$ and $\delta^{15}N$ composing the groups (p.interval = 0.95), we also calculated the mean SEAc which consider only the bivariate means to predict the ellipse width. To calculate ellipse area overlap between the two groups we also based on the maximum likelihood estimates of the means and covariance matrices of each group, posteriorly calculating in percentage how much of HMLs ellipse was overlapped by NLs and vice-versa.

The comparison of mean values of $\delta^{13}C$ and $\delta^{15}N$ between the two groups of landscapes composition for each foraging guild followed Student's t-test and Wilcoxon signed-rank test depending on data distribution. We performed all aforementioned analysis via R software version 3.5.2 (R Core Team 2018).

3.3. Results

The isotopic niches of all foraging guilds showed some degree of change in the width when compared between landscape groups (HMLs: SEAc.30 and NLs: SEAc.50). In the HMLs, frugivores, insectivores, nectarivores and omnivores showed smaller niches when compared to NLs, opposing to the granivores that showed an expansion of niche width with forest loss (Figure 2). Omnivores and nectarivores were the guilds with the greatest reduction in niche width from NLs to HMLs, showing a decline rate of approximately 44% for both (omnivore: SEAc.50 = 13.3‰² to SEAc.30 = 7.3‰²; and nectarivore: SEAc.50 = 13.9‰² to SEAc.30 = 7.8‰²), while insectivores had a reduction of 28% (SEAc.50 = 8.2‰² to SEAc.30 = 5.9‰²) and frugivores of 16% (SEAc.50 = 8.0‰² to SEAc.30 = 6.7‰²). Whereas the granivores presented an expansion rate of 27% from NLs to HMLs (SEAc.50 = 25.1‰² to SEAc.30 = 31.9‰²). In general, the niche overlap between HMLs over NLs was larger than the overlap between NLs over HMLs for almost all foraging guilds, except for granivores (Figure 2). In this circumstance,

the highest overlap rates were for omnivores (81.2%) and nectarivores (75.6%), opposing to the overlap rate obtained by the granivores niche (59.3%). In contrast, the overlap between NLs over HMLs presented lower and very similar rates among guilds, ranging from 42% to 46.8%, except for granivores again with a rate of 75.4% (Figure 2).

The mean values of $\delta^{13}C$ and $\delta^{15}N$ for all foraging guilds were significantly different between the two landscape groups (HMLs and NLs) (Table 1, Figure 3). Birds in NLs showed lower $\delta^{13}C$ values, representing a strict use of forest habitat resources, while birds in HMLs had higher $\delta^{13}C$, which might be possibly due to a mixture of resources from the forest and anthropogenic habitats. We also observed the same pattern for the $\delta^{15}N$ values, in which individuals present in NLs showed lower values than individuals derived from the HMLs. Further, distributions of individual values of $\delta^{13}C$ and $\delta^{15}N$ also varied according to landscape groups (Figure 4), specially referred to nitrogen. Except from the granivores, all the other guilds showed different probabilities of values distributions around the mean according to each landscape group. In NLs the individuals' values are distributed more homogeneously over the total distribution, whereas in HMLs the values are more concentrated near the central tendencies, varying less over the total distribution.

Table 1. Means and standard deviations of $\delta^{13}C$ and $\delta^{15}N$ for each foraging guild (frugivore, granivore, insectivore, nectarivore and omnivore) and food resources (seeds and invertebrates) used for comparison (Student's t-test and Wilcoxon signed-rank test) between individuals from human-modified and natural landscapes, and P-value (significant when <0.05).

| | | Isotope | Human-modified landscapes | | Natural landscapes | | P |
			Mean	SD	Mean	SD	
Foraging guilds	Frugivore	$\delta^{13}C$	-24.5‰	1.9‰	-26.3‰	1.1‰	<0.0001
		$\delta^{15}N$	1.8‰	1.1‰	-0.3‰	2.4‰	<0.0001
	Granivore	$\delta^{13}C$	-17.3‰	5.3‰	-23.5‰	4.4‰	<0.0001
		$\delta^{15}N$	0.9‰	1.9‰	-0.2‰	1.9‰	0.006
	Insectivore	$\delta^{13}C$	-23.8‰	1.6‰	-25.7‰	1.2‰	<0.0001
		$\delta^{15}N$	5.2‰	1.2‰	4.1‰	2.3‰	0.006
	Nectarivore	$\delta^{13}C$	-24.0‰	1.7‰	-25.8‰	2.0‰	<0.0001
		$\delta^{15}N$	6.6‰	1.5‰	4.1‰	2.2‰	<0.0001
	Omnivore	$\delta^{13}C$	-24.7‰	2.1‰	-27.0‰	2.2‰	<0.0001
		$\delta^{15}N$	4.6‰	1.3‰	3.5‰	1.9‰	0.009
Food resources	Plants	$\delta^{13}C$	-30.1‰	2.6‰	-	-	-
		$\delta^{15}N$	2.1‰	1.6‰	-	-	-
	Invertebrates	$\delta^{13}C$	-26.7‰	2.9‰	-	-	-
		$\delta^{15}N$	4.6‰	3.1‰	-	-	-
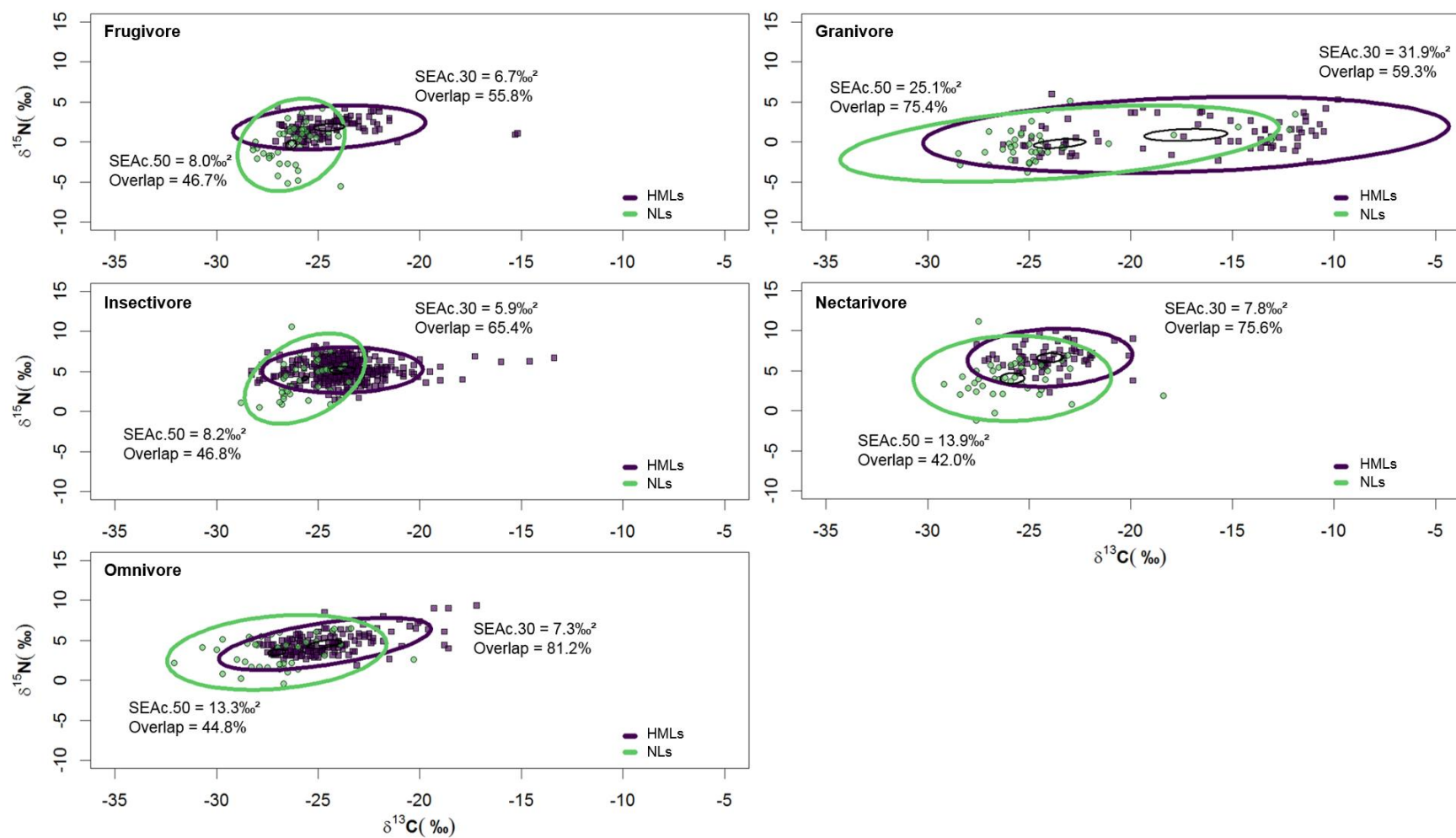
Figure 2. Isotopic niche of the five foraging guilds analyzed: frugivore, granivore, insectivore, nectarivore and omnivore. Dots represent isotopic individual values of birds that compose each guild, also separated by landscape groups (human-modified landscapes; HMLs - purple; and natural landscapes; NLs - green). The value of isotopic niche width (SEAc) for each guild is located next to each landscape group in the graph (HMLs: SEAc.30; and NLs: SEAc.50). The percentage of niche overlap of the HML over the NL is below SEAc.30 and the percentage of niche overlap of the NL over the HML is below SEAc.50.
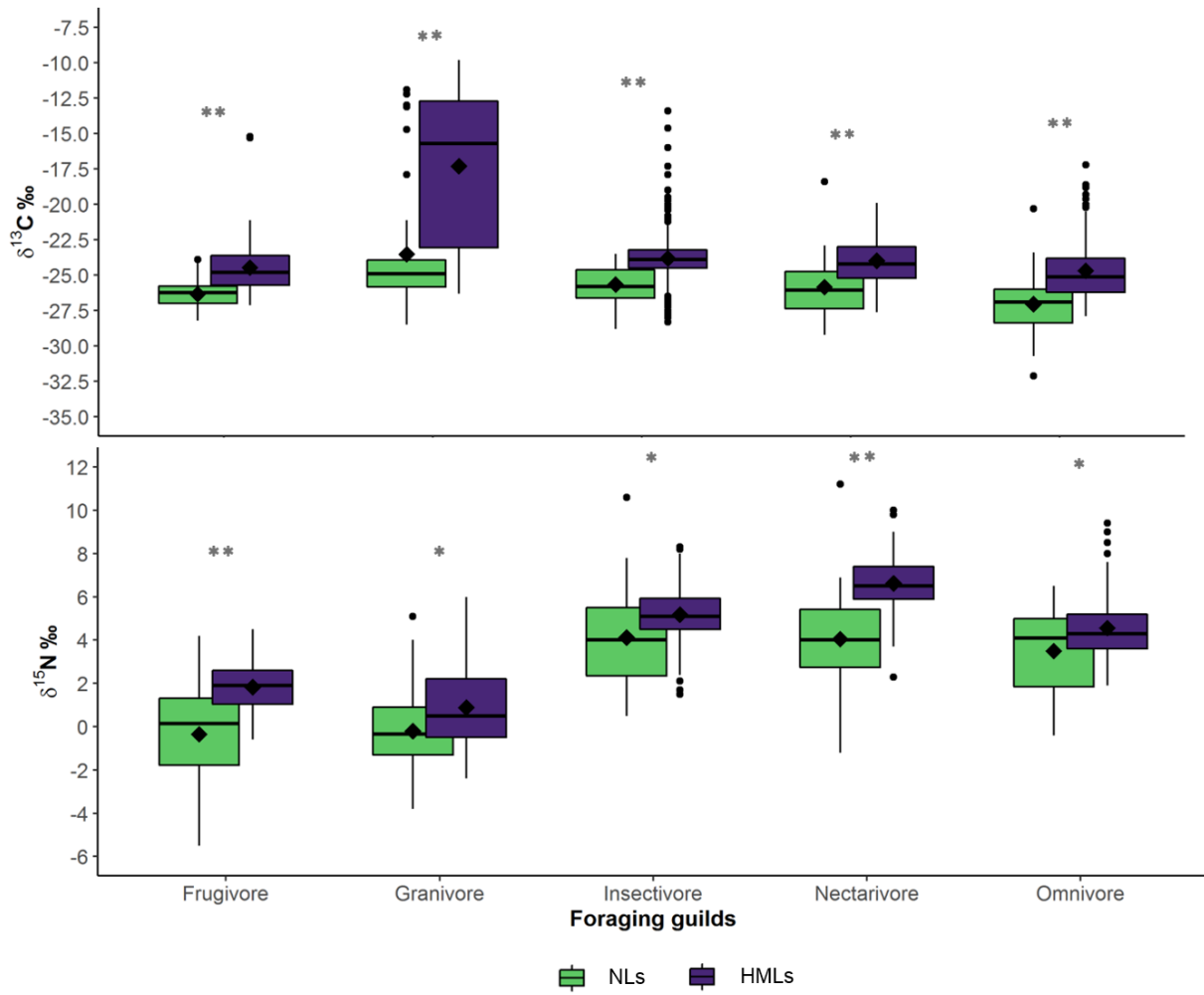
Figure 3. Boxplot of δ¹³C and δ¹⁵N for each of the foraging guilds (frugivore, granivore, insectivore, nectarivore and omnivore) comparing individuals in human-modified landscapes (HMLs – purple) and individuals in natural landscapes (NLs – green). Boxplots show means (diamonds), medians, quartiles and outliers. Asterisks indicate a significant difference between historical and recent individuals' δ¹³C and δ¹⁵N means (* means p<0.01; ** means p<0.0001).
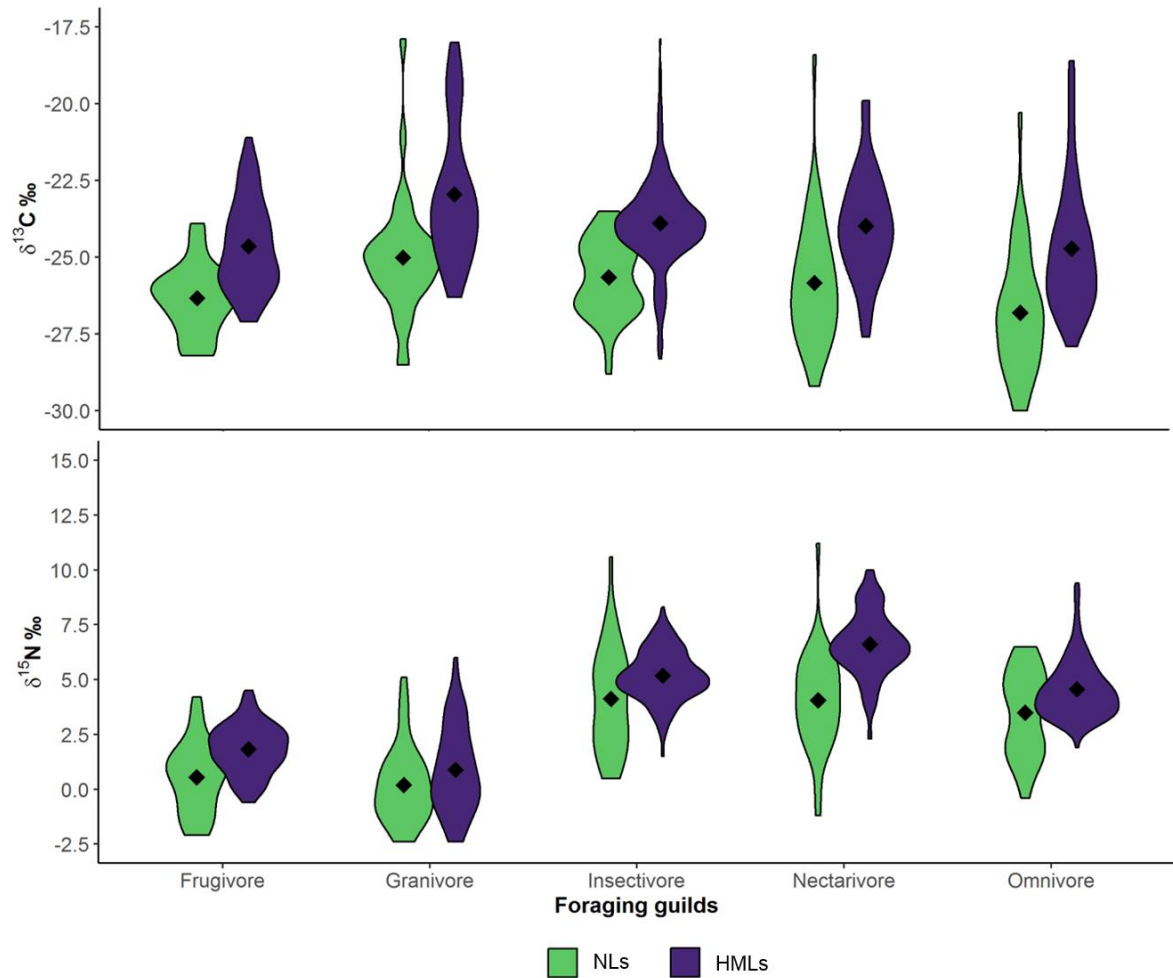
Figure 4. Violinplot of $\delta^{13}C$ and $\delta^{15}N$ values for each of the foraging guilds (frugivore, granivore, insectivore, nectarivore and omnivore) to visualize data distribution and probability density of individuals belonging to each landscape group (Human-modified landscapes; HMLs – purple; and Natural landscapes; NLs – green). Wider sections represent a higher probability of individuals with that range of value, while skinnier sections represent a lower probability.

In general, the structure of the trophic chain apparently has been maintained in both HMLs and NLs according to our results (Figure 5). The plant-based foraging guilds were located lower in the trophic chain, opposing to guilds with an animal-based diet that was located at the top-level. The only exception was the nectarivore guild that showed the highest values of $\delta^{15}N$ in both landscape groups. Through the comparison of the trophic chains between these different landscapes we observed some dissimilarities. In NLs the frugivores are more distant from the omnivores and insectivores in the trophic chain (distance of 3.8‰ and 4.4‰, respectively), while in HMLs these two guilds are located closer (distance of 2.8‰ and 3.4‰, respectively). The nectarivores also showed a dissimilarity, since in NLs this guild is close to the insectivores (same $\delta^{15}N$ means), while in HMLs nectarivores present even higher values of $\delta^{15}N$ (distance of 2.8‰). The granivores were the only guild that apparently did not show a great change in its position in the trophic chain.
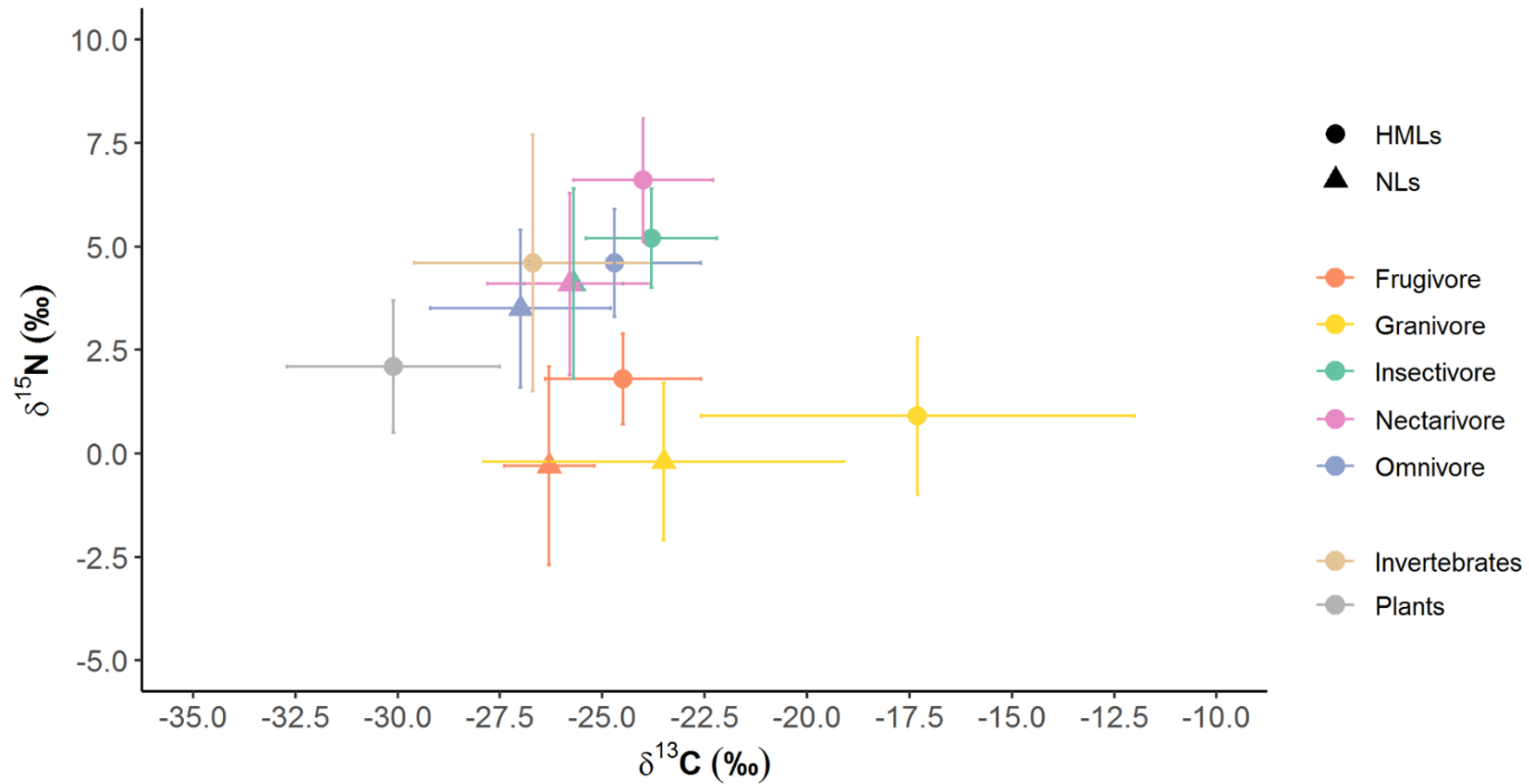
Figure 5.   Means and standard deviations of δ¹³C and δ¹⁵N values for the foraging guilds (frugivore, granivore, insectivore, nectarivore and omnivore) and food resources (seeds and invertebrates) of each landscape group (Human-modified landscapes; HMLs – triangle; and Natural landscapes; NLs – circle). Values of δ¹⁵N show the structuration of the trophic chain with plant-based guilds at the base and guilds that forage mainly at invertebrates at the top.

3.4. Discussion

A non-random reduction in isotopic niche width for most guilds in landscapes less forested was showed by our results, which might be related to a reduction in food availability as a consequence of the process of habitat loss, since it exerts an overwhelming decline of richness and abundance of various organisms (Fahrig 2003), many of them serving as food resource for birds thus generating a cascade-effect upon bird communities. Niche width reductions rates from HMLs to NLs ranged from 16% to 44% depending on the foraging guild, demonstrating that forest loss impact on birds may vary in the intensity according to the guild. This observed pattern of each guild being impacted in a particular magnitude is expected, since each guild experience the implications of fragmentation and habitat loss differently (Bregman et al. 2014). Even though, we found a clear and unanimous pattern reflecting the negative influence on dietary dimension owing to the change and decline in habitat availability plus food resources. Moreover, in NLs the isotopic niches were wider, except for granivores, probably due to greater amplitude of available food resources, as in pristine tropical environments— hardly found nowadays—human impacts are less significant, so there is an increase in the richness and abundance of organisms (Gibson et al. 2011). For some guilds the isotopic niches in NLs are almost two times larger as in HMLs (e.g. omnivore and nectarivore), again demonstrating the importance of natural forest environments for the provision of habitat and resources to birds (Ferger et al. 2013). Contrariwise, granivores had a niche expansion with forest loss being 27% larger in HMLs than in NLs. Usually, species that compose the granivore guild have more plasticity to habitat changes and are adapted to open environments and forest edges (Giraudo et al. 2008), factors that possibly have benefited the expansion of this guild niche. Not only habitat loss, but also savannization of tropical forests due to the increase of grasses, especially exotic ones (e.g. Silvério et al. 2013, Scarano and Ceotto 2015), seem to be propitiating an amplification in the niche width of granivores in HMLs.

In addition to the decline in isotopic niche width, our results of niche overlap also might be related to habitat loss and availability of food resources for birds. Although studies show that habitat loss and forest fragmentation directly affect bird communities and their ecological functions (Morante-Filho et al. 2015, Bovo et al. 2018), there is a knowledge shortfall in understand how these processes interfere at patterns of food resources and habitat use by birds. In our results we observed a contraction in niches of frugivores, insectivores, nectarivores and omnivores and also high overlaps rates ranging from 56% to 81% for these guilds in HMLs over the NLs. In a constantly changing world, facing the pivotal expansion of anthropogenic activities, niche breadth evolution processes might pose a survival condition to species by either expanding, contracting or totally modifying their niches (Sexton et al. 2017).

Thus, it is likely that the species that compose these guilds in HMLs depends mostly on the same food resources available in NLs, which might represent a threat to these species' existence in HMLs, contrary to another study that showed a plasticity of bird species that have amplified their niches in a gradient of urbanization (Pagani-Núnez et al. 2019). Furthermore, the opposite overlap between NLs over HMLs niches ranged from 42% to 47% for these four guilds. Considering these last rates are lower, it might represent the scarcity of resources in HMLs, which are easily found in the NLs, again indicating plenitude of food in more forested landscapes (Morante-Filho et al. 2018). The only exception was the granivore guild showing a greater niche overlap of NLs over HMLs (75%), more than the opposite overlap (59%). Corroborating the savannization process of tropical forests with a substantial increase of grasses, especially in HMLs (Salazar et al. 2007), favoring the establishment of granivores in this environment by increasing habitat and food resources to them.

All guilds showed distinct mean values of $\delta^{13}C$ between the HMLs and NLs, characterizing that birds in less forested landscapes forage more of resources from forest edges, clearings or even from the agricultural matrix. Organisms that consume more than one type of resource (e.g. C_3 and C_4) express in their tissues isotopic signatures representing this mixture and the value correspond to that resource that is mostly consumed (Boecklen et al. 2011). Plants have distinct isotopic values according to photosynthetic pathways, in which C_3 plants, the main composition of tropical forests, have $\delta^{13}C$ ranging from -24‰ to -38‰ (Martinelli et al. 2009, Vitória et al. 2018). The C_4 plants have $\delta^{13}C$ values ranging from -11‰ to -15‰ (Martinelli et al. 2009, Powell et al. 2012), usually these plants are grasses associated with savannas and agricultural areas, wherein landscapes of São Paulo are dominated by pastures and sugarcane crops (MapBiomas 2019). Nevertheless, the guilds' mean values in both landscapes indicate a preference for resources from forests ($\delta^{13}C$ values are near the range of C_3 plants), except for granivores that maximize their foraging outside the forests in HMLs ($\delta^{13}C$ = -17.3‰, closer to the range of C_4 plants). Data distribution within each guild also corroborates that in NLs few individuals have values representative of a mixture of C_3 and C_4 resources, while in HMLs there is a higher probability that individual values of $\delta^{13}C$ to be more than -24‰, meaning there is a greater mixture with resources from open areas. This results corroborates the savannization hypothesis of the tropical forests in South America, responding to various anthropogenic impacts which is expected to pose a threat to plant and animal diversity (Salazar et al. 2007). Thus, we emphasize that despite some individuals tolerate savannization and forage a mixture of food items from different sources (C_3 and C_4 plants), the vast majority of birds prefer food resources from forests (C_3 plants mainly).

The mean $\delta^{15}N$ values of all guilds varied between HMLs and NLs, the values being greater in the first one. Possibly due to the high anthropogenic influence in these landscaspes, considering that agricultural activities cause variations in $\delta^{15}N$ values of organisms

(Rubenstein and Hobson 2004, Magioli et al. 2014, 2019). In NLs the distribution of individual δ^{15}N values was more heterogeneous for frugivore, insectivore, nectarivore and omnivore guilds, while in HMLs the values were more clustered around the mean. Plenitude of food in the environment reduces competition among individuals over the same resources, enabling individuals' foraging specialization (Araújo et al. 2011), as is the case of natural forests that contains more food available to birds. Thus, the distribution result might be related to the existence of a greater plenitude of food resources in the NLs, enabling a foraging specialization among individuals so that there is a larger range of δ^{15}N values within guilds. Contrariwise, in HMLs the grouped values are explained due to a possible simplification of the habitat with consequent reduction in food availability, so individuals are forced to compete basically for the same resources, expressing similar δ^{15}N values. Probably by the reason that individuals relying mostly on the same resources increase competition among themselves, reducing individual specialization (Araújo et al. 2011), which further explains the reductions in isotopic niche widths of most guilds (frugivore, insectivore, nectarivore and omnivore), demonstrating a greater interspecific competition for the same food resources in HMLs. Contrarily, considering that when the breadth of resources is greater there is also an increase in total niche width, in addition to an increase in diet specialization (Costa-Pereira et al. 2019), we observed wider isotopic niches in NLs in our results, seemingly due to a larger plenitude of resources in these landscapes that enable less interspecific competition by providing more specialized food for each species.

Overall, in both landscapes (NLs and HMLs) the trophic chain maintained its structure, wherein guilds with plant-based diets were below the ones with higher consumption of invertebrates. This result conceives an important factor for the perpetuation of ecosystems, since the maintenance and structuring of the trophic chain is essential to sustain all the interchange in foodwebs (Pimm 1982). The only exception was for nectarivores that in both landscapes were above all other guilds with a higher δ^{15}N value. The nectarivore guild, usually composed by hummingbirds, has accelerated metabolism and often complements the diet with invertebrates, influencing the fractionation of nitrogen isotopes and providing a higher proportion of ^{15}N in tissues (Hardesty 2009), which explains the results obtained in our study. Notwithstanding, we observed some significant differences in guilds' position when comparing between NLs and HMLs, as is the case of frugivores, which in HMLs are less distant from omnivores and insectivores in comparison with the same guilds in NLs. In the same way, nectarivores also took different positions between the landscapes, in the NLs showed δ^{15}N means equal to the insectivores, while in the HMLs nectarivores were above the insectivores. This pattern of changes in trophic chain position, especially in guilds mainly dependent on plant resources (e.g. fruits and nectar), might represent a need for further supplementation of diet with invertebrates in HMLs due to reduced availability of priority foods, since habitat loss and

fragmentation were relevant impacts for the decline of many important plant species, which serve as resources for birds in tropical forests (Tabarelli et al. 1999, Silva and Tabarelli 2000). By contrast, granivore was the guild with fewer changes in trophic chain position, again corroborating a probable plasticity in the recurrence of available food resources at forest edges, clearings or even in the agricultural area (Ferger et al. 2013).

A reduction in the availability of food resources might pose a threat to species survival in tropical forests. Impacts such as fragmentation and habitat loss are extremely harmful to birds and their resources (Silva and Tabarelli 2000, Rigueira et al. 2013, Morante-Filho et al. 2015, Pfeifer et al. 2017). Thus, as we observe that most niches are declining in HMLs, unlike wider niches at NLs, we tend to ask: how long will these species tolerate the reduction of their niches (habitat and food resources)? The progressive increase of savannization over tropical forest areas (Salazar et al. 2007, Scarano and Ceotto 2015), in addition to the wide expansion of exotic grass species (Silvério et al. 2013), has generated significant changes in communities' composition in these environments. If the progression of such events persists, is it possible that all guilds will thrive in HMLs or will only granivores remain? If it is desirable to maintain many of the ecological processes in operation, which are intrinsically linked to bird species, we should take more conservation actions in order to perpetuate all these guilds in their habitats.

3.5. Conclusion

We found significant changes in the habitat and resource use by birds depending on the percentage of forest cover in the landscapes, through isotopic analysis of carbon and nitrogen. Our results indicate that habitat loss directly influences the reduction of isotopic niche widths of four guilds (frugivore, insectivore, nectarivore and omnivore) and niche expansion of granivore. In addition, there were also significant differences in $\delta^{13}C$ and $\delta^{15}N$ mean values for all guilds between NLs and HMLs. These changes might be related to the reduction in food resource availability due to habitat loss in HMLs. Thus, birds in HMLs possibly use a mixture of C_3 (mainly forests) and C_4 resources (forest edges, clearings and agricultural areas), despite their preference for foraging in forests. In NLs, all guilds showed values representative of C_3 resources, not presenting mixture with C_4. The difference in $\delta^{15}N$ mean and distribution for all guilds between landscapes might also correspond to the decline of food resources in HMLs. Human-induced impacts on tropical forests, such as land use changes, edge effects and savannization, might have been the main anthropogenic impacts on decline of food availability. The species that compose the five guilds are often linked to the realization of several ecological processes important for the maintenance of the ecosystem. Considering that niche reductions

represent a high risk of species collapse, the observed decline in HMLs might pose a threat of local extinction to almost all guilds and consequently to the ecosystem conservation. Although many species of birds still persist in the HMLs of tropical regions, erstwhile covered mainly by original forests, these highly modified environments are not providing the sufficient dietary and habitat requirements of the species. Thus, it is crucial to maintain the relictual NLs and support further heterogenization actions in the HMLs in order to sustain birds' diversity and the ecological processes developed by them.

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4. CONCLUSION

In both chapters the results showed an isotopic modification in birds' diet as a consequence of past and present anthropogenic impacts in the state of São Paulo, probably related to land cover changes. By comparing historically, all guilds have shrunk their isotopic niches after human expansion increased likewise the deforestation rates. Just as the comparison among landscapes with different percentages of forest cover, in which we also observed a reduction in niche width in the birds present at human-modified landscapes (HMLs; less forested), except for the granivore guild. These results show that birds have had a reduction in their ecological niche, probably due to habitat loss and fragmentation, which might pose a threat to the existence of these species. Considering that the niche is related to the use of habitat and food resources, when verifying shrinkages in guilds' niches it is reasonable to infer that species might be experiencing not only a reduction in availability of habitat, but also of food. Although the species that compose the guilds can still be found in current HMLs with a small percentage of forest cover, these birds are highly dependent on the remaining forests as a habitat and a food source to maintain their existence. Thus, the progression of anthropogenic impacts, especially derived from the advancement of agriculture over natural areas, may influence the birds' patterns of habitat and food resource use in tropical forests.

The historical of human expansion in the state of São Paulo was highly predatory due to the advance of agriculture over original forest areas and occurred intensively just in a few more than 100 years. Nowadays, the state is mostly composed by pasture and sugarcane crops, wherein most of the forest remnants are preserved near the coast region. In chapter one the results showed a high overlap of recent individuals' isotopic niches over historical ones, which might mean that species today depend primarily on the same food resources that was found in the past. Similarly, the results of chapter two presented a high overlap of the isotopic niches of HMLs individuals over the natural landscapes (NLs), also representing a dependence on the resources found in more forested landscapes. Both results showed that, although the species that compose the guilds are able to inhabit anthropogenic environments, the birds have been experiencing a reduction in food availability beyond habitat loss. Since these species need forests to survive, they are not revealing to be able to fully adapt their diets to the reality of HMLs. If we consider that the evolutionary and adaptive processes usually take hundreds of years to happen, we can assume that these approximately 100 years of anthropogenic impacts (accentuated after 1970 in the state of São Paulo) were not sufficient to make species fully adapt to the new landscapes. In order to perpetuate the bird species that are striving to survive in nowadays highly HMLs, we should consider redesigning some landscapes assuring to keep forest habitats adequate and more plentiful of food resources, so these species will not collapse in the future.

The mean $\delta^{13}C$ values of all groups of individuals hereby analyzed revealed that, in general, the species that compose the guilds depend on the forest habitat to obtain their food resources. In both comparisons, historically and among nowadays landscapes with different forest proportions, $\delta^{13}C$ values were representative of a priority to forage in the forests (composed of C_3 plants) and minimal mixing with resources from the agricultural matrix (composed of C_4 plants), mainly for granivore individuals. It is already known that forest remnants are crucial for the maintenance of species, still in this study it became clear that these habitats are essential for the supply of food resources to birds, which are responsible to develop many ecological processes that maintain the ecosystem. There were also changes in the mean values of $\delta^{15}N$ among the guilds in both chapters. In chapter one there was a reduction in $\delta^{15}N$ values over time and the probable cause is the input of anthropogenic nitrogen into the environment, mainly via the application of synthetic nitrogen fertilizer in agriculture. In chapter two it was observed an increase in $\delta^{15}N$ values in individuals from HMLs comparing to those of NLs. Such effect might be related to habitat simplification in HMLs due to the more intense anthropogenic impacts, resulting in a decline in food resources richness and abundance. In this way, the species of all guilds become dependent on the same range of food available in these landscapes. Nevertheless, the nitrogen cycle is complex and has several sources and pathways that can be influenced by anthropogenic inputs and consequently transmitted to organisms. Although it is known that all this anthropogenic nitrogen input is a consequence of human expansion over natural ecosystems, there is still a lack of knowledge on what effects such inputs might cause on the evolutionary and adaptive processes of birds.

Given the above, the results obtained in this study might assist to develop better strategies for the conservation of the bird species of all guilds, especially in HMLs. It is reasonable to consider redesigning these anthropogenic landscapes to provide an increase in forest habitat and therefore enhance the availability of a diverse range of food resources. A greater richness and abundance of food to birds might even promote species specialization into certain items, as observed in NLs. In addition, historical-temporal analysis might assist and improve future studies that aim to predict whether and which birds could collapse with declines in niches widths derived from anthropogenic impacts. In general, we observed that the human expansion over a region of tropical forest has directly affected the isotopic ecology of bird species, even though many of them are considered generalists. Considering that even in these anthropogenic environments the guilds hereby analyzed are responsible for performing various ecological processes, more attention to birds' conservation efforts in HMLs should be assigned in order to keep the ecosystems' function and balance.

APPENDIX

APPENDIX A. Historical (a) and recent (b) land cover in the state of São Paulo/Brazil, showing the amplitude of forest cover at the beginning of 20th century and nowadays is covered mainly by farming fields. The places where historical (purple) and recent (yellow) samples were collected are shown through over the biomes (c) and ecoregions (d) that cover the state of São Paulo/Brazil.
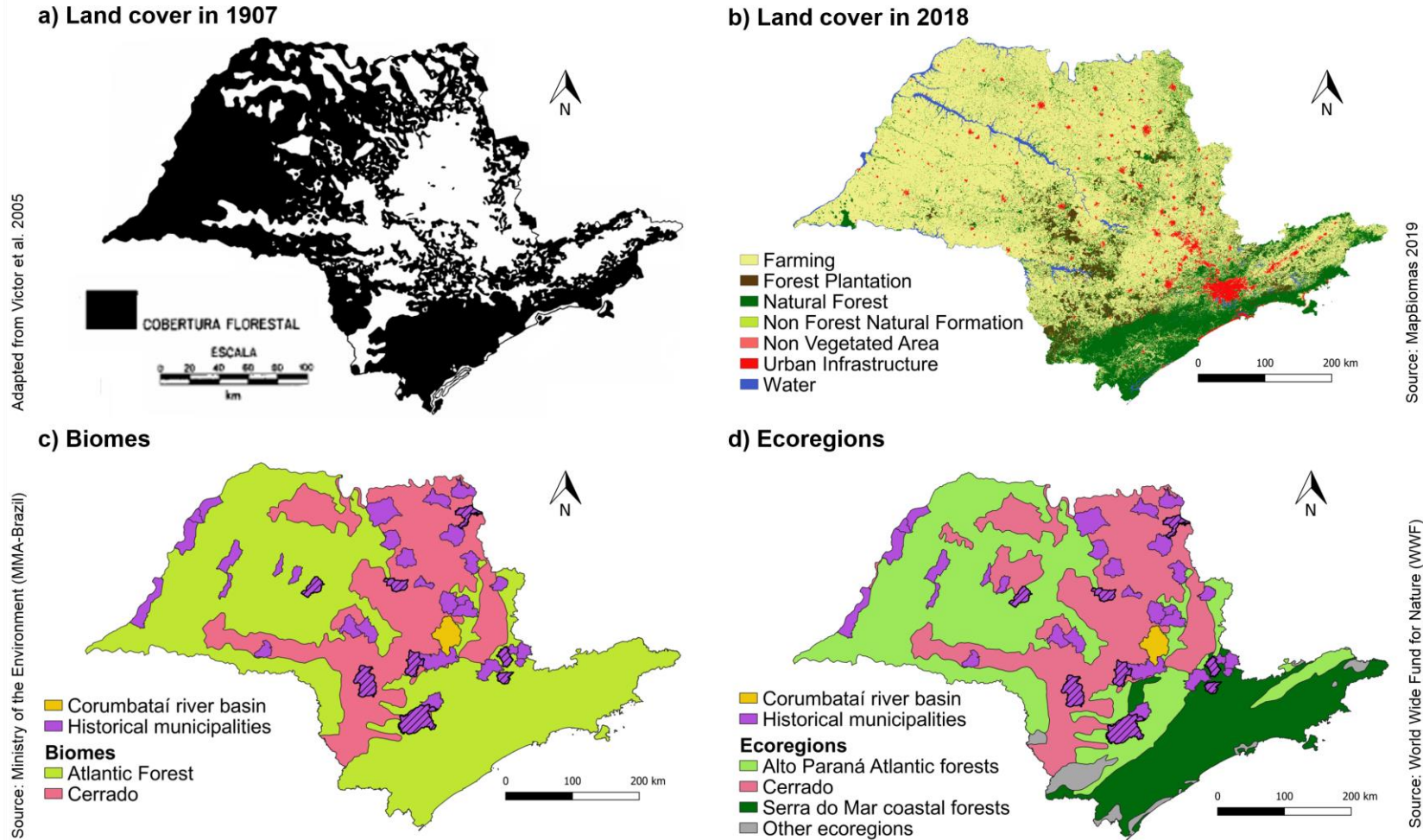
APPENDIX B. Species that compose the five foraging guilds (frugivore, granivore, insectivore, nectarivore and omnivore), their mean values of $\delta^{13}C$ and $\delta^{15}N$, standard deviation (SD), their respective group (historical or recent), how many individuals were sampled, the timespan and localization of field expeditions to collect the individuals.

Foraging guild	Species	N individuals	Group	$\delta^{13}C$ mean (‰)	SD	$\delta^{15}N$ mean (‰)	SD	Timespan	Localization
Frugivore	*Antilophia galeata*	7	Historical	-23.0	1.4	3.5	1.2	1900-1963	Municipalities: Avanhandava, Avaré, Batatais, Franca, Porto Ferreira
Frugivore	*Antilophia galeata*	60	Recent	-24.3	1.7	1.9	1.2	1985-2012	Corumbataí river basin / Municipalities: Pederneiras, Pirassununga
Frugivore	*Chiroxiphia caudata*	15	Historical	-24.4	2.0	4.9	2.8	1900-1967	Municipalities: Amparo, Anhembi, Avaré, Franca, Itapetininga, Itatiba, Ituverava, Lins, Matão, Valparaíso
Frugivore	*Euphonia chlorotica*	8	Historical	-24.6	2.4	5.3	1.8	1941-1964	Municipalities: Amparo, Anhembi, Lins
Frugivore	*Manacus manacus*	7	Historical	-24.6	1.7	5.1	2.3	1900-1964	Municipalities: Anhembi, Itatiba, Jaboticabal, Presidente Epitácio, Valparaíso
Frugivore	*Manacus manacus*	9	Recent	-24.8	1.4	1.9	1.1	2012	Corumbataí river basin
Frugivore	*Tangara cayana*	13	Historical	-22.4	2.2	6.3	2.1	1900-1964	Municipalities: Anhembi, Avaré, Batatais, Franca, Itapetininga, Itatiba, Jaboticabal, Matão, Pedregulho, Porto Ferreira
Granivore	*Cyanoloxia brissonii*	9	Historical	-21.3	2.7	3.7	1.4	1901-1964	Municipalities: Anhembi, Avaré, Itatiba, Matão, Rincão
Granivore	*Cyanoloxia brissonii*	5	Recent	-22.5	3.8	-0.9	1.2	2011-2012	Corumbataí river basin
Granivore	*Leptotila rufaxilla*	9	Historical	-23.8	1.9	2.9	1.2	1907-1966	Municipalities: Avaré, Itapetininga, Itatiba, Ituverava, Lins, Matão
Granivore	*Leptotila verreauxi*	10	Historical	-20.6	4.2	3.7	1.9	1926-1946	Municipalities: Amparo, Batatais, Itatiba, Lins, Lucélia, Presidente Epitácio, Serra Negra
Granivore	*Leptotila verreauxi*	9	Recent	-23.8	1.5	0.7	1.2	2011-2012	Corumbataí river basin
Insectivore	*Basileuterus culicivorus*	11	Historical	-26.5	1.4	8.8	2.2	1904-1968	Municipalities: Assis, Avaré, Itapetininga, Itapura, Presidente Epitácio
Insectivore	*Basileuterus culicivorus*	46	Recent	-23.3	1.0	4.9	1.0	2011-2012	Corumbataí river basin
Insectivore	*Conopophaga lineata*	15	Historical	-23.8	1.5	7.3	1.8	1911-1966	Municipalities: Amparo, Anhembi, Avaré, Franca, Itapetininga, Itatiba, Lins, Matão, Pedregulho, Valparaíso

Insectivore	*Conopophaga lineata*	30	Recent	-24.1	0.6	5.6	1.1	2011-2012	Corumbataí river basin
Insectivore	*Dysithamnus mentalis*	15	Historical	-25.1	2.0	8.9	1.7	1904-1964	Municipalities: Amparo, Anhembi, Avaré, Barretos, Bebedour, Ituverava, Lins, Pedregulho, Serra Negra, Valparaíso
Insectivore	*Dysithamnus mentalis*	18	Recent	-24.7	0.6	5.7	1.0	2011-2012	Corumbataí river basin
Insectivore	*Lathrotriccus euleri*	13	Historical	-23.5	2.6	6.9	1.7	1900-1967	Municipalities: Amparo, Anhembi, Avanhandava, Avaré, Bebedouro, Campinas, Glicério, Itapetininga
Insectivore	*Lathrotriccus euleri*	37	Recent	-22.1	1.7	5.4	1.1	2011-2012	Corumbataí river basin
Insectivore	*Myiothlypis flaveola*	6	Historical	-23.0	2.2	7.9	2.3	1901-1963	Municipalities: Matão, Rincão, São José do Rio Pardo
Insectivore	*Myiothlypis flaveola*	34	Recent	-22.7	2.9	5.0	1.4	2011-2012	Corumbataí river basin
Insectivore	*Tolmomyias sulphurescens*	7	Historical	-23.0	1.6	6.7	0.7	1900-1966	Municipalities: Amparo, Bauru, Itapetininga, Itapura, Itatiba, Lins
Insectivore	*Tolmomyias sulphurescens*	11	Recent	-22.5	0.7	5.4	0.9	2011-2012	Corumbataí river basin
Nectarivore	*Amazilia lactea*	10	Historical	-25.1	3.6	8.7	2.5	1904-1964	Municipalities: Amparo, Anhembi, Bebedouro, Itatiba
Nectarivore	*Amazilia lactea*	10	Recent	-24.1	1.7	6.7	1.3	2011-2012	Corumbataí river basin
Nectarivore	*Amazilia versicolor*	13	Historical	-23.0	2.7	8.3	2.2	1904-1965	Municipalities: Anhembi, Avanhandava, Bebedouro, Itapura, Piracicaba
Nectarivore	*Amazilia versicolor*	5	Recent	-25.3	1.8	6.3	1.0	2011-2012	Corumbataí river basin
Nectarivore	*Chlorostilbon lucidus*	13	Historical	-23.3	2.9	8.5	2.0	1902-1964	Municipalities: Anhembi, Avanhandava, Avaré, Bebedouro, Descalvado, Franca, Itatiba
Nectarivore	*Chlorostilbon lucidus*	5	Recent	-23.2	0.7	7.4	0.8	2012	Corumbataí river basin
Nectarivore	*Coereba flaveola*	12	Historical	-24.7	2.7	7.5	2.0	1903-1968	Municipalities: Amparo, Anhembi, Avanhandava, Avaré, Bebedouro, Franca, Itapetininga, Itatiba
Nectarivore	*Coereba flaveola*	13	Recent	-23.6	0.9	5.4	1.4	2012	Corumbataí river basin
Nectarivore	*Phaethornis pretrei*	11	Historical	-23.6	1.9	7.4	2.3	1910-1963	Municipalities: Amparo, Avaré, Franca, Lins, Matão, Socorro, Tatuí
Nectarivore	*Phaethornis pretrei*	14	Recent	-23.1	1.6	6.5	1.0	2011-2012	Corumbataí river basin
Nectarivore	*Thalurania glaucopis*	5	Historical	-25.2	2.3	6.5	1.7	1904-1964	Municipalities: Amparo, Anhembi, Bebedouro, Serra Negra

| Nectarivore	*Thalurania glaucopis*	10	Recent	-25.0	0.5	6.0	0.8	2011-2012	Corumbataí river basin
Omnivore	*Habia rubica*	15	Historical	-25.1	1.4	8.4	2.0	1900-1968	Municipalities: Anhembi, Avaré, Itapetininga, Ituverava, Jaboticabal, Valparaíso
Omnivore	*Habia rubica*	13	Recent	-25.2	0.6	4.0	0.7	2011-2012	Corumbataí river basin
Omnivore	*Myiarchus ferox*	13	Historical	-23.2	1.3	7.6	2.0	1900-1967	Municipalities: Anhembi, Avaré, Bebedouro, Itatiba, Ituverava, Matão, Presidente Epitácio, Rincão
Omnivore	*Myiarchus ferox*	6	Recent	-21.5	2.2	4.8	1.1	2012	Corumbataí river basin
Omnivore	*Ramphocelus carbo*	11	Historical	-24.4	1.7	6.9	2.0	1900-1967	Municipalities: Anhembi, Barretos, Bauru, Ilha Solteira, Ituverava, Jaboticabal, Lins, Matão
Omnivore	*Ramphocelus carbo*	14	Recent	-25.6	0.8	4.0	1.2	2011-2012	Corumbataí river basin
Omnivore	*Tachyphonus coronatus*	13	Historical	-25.1	1.6	6.2	1.3	1897-1967	Municipalities: Amparo, Anhembi, Avaré, Franca, Glicério, Itapetininga, Pinhalzinho, Piracicaba, Valparaíso
Omnivore	*Tachyphonus coronatus*	49	Recent	-26.2	1.1	4.2	1.1	2011-2012	Corumbataí river basin
Omnivore	*Thlypopsis sordida*	10	Historical	-23.0	4.1	7.7	1.9	1927-1965	Municipalities: Amparo, Anhembi, Itatiba, São José do Rio Pardo, Socorro, Tatuí
Omnivore	*Thlypopsis sordida*	9	Recent	-24.4	1.0	4.9	0.9	2012	Corumbataí river basin
Omnivore	*Turdus leucomelas*	15	Historical	-23.2	1.8	7.6	1.6	1900-1968	Municipalities: Amparo, Avaré, Cajuru, Castilho, Itapetininga, Lins, Matão, Rincão
Omnivore	*Turdus leucomelas*	41	Recent	-22.5	2.1	5.2	1.8	2011-2012	Corumbataí river basin

APPENDIX C. Food resources taxons (invertebrates and plants), their mean values of $\delta^{13}C$ and $\delta^{15}N$, standard deviation (SD), the number of individuals collected, the timespan and localization of field expeditions to collect the individuals.

Food resource	Taxon	N individuals	Group	$\delta^{13}C$ mean (‰)	SD	$\delta^{15}N$ mean (‰)	SD	Timespan	Localization
Invertebrate	Blattodea	5	Recent	-25.8	1.5	1.2	1.4	2011-2012	Corumbataí river basin
Invertebrate	Coleoptera	7	Recent	-25.2	2.3	5.4	2.2	2011-2012	Corumbataí river basin
Invertebrate	Diptera	9	Recent	-24.7	3.4	7.1	3.4	2011-2012	Corumbataí river basin
Invertebrate	Hemiptera	8	Recent	-28.0	2.7	2.8	1.9	2011-2012	Corumbataí river basin
Invertebrate	Formicidae	9	Recent	-25.9	1.0	5.1	2.6	2011-2012	Corumbataí river basin
Invertebrate	Vespidae	5	Recent	-25.5	1.3	5.5	1.3	2011-2012	Corumbataí river basin
Invertebrate	Lepidoptera	5	Recent	-28.6	3.7	5.7	5.4	2011-2012	Corumbataí river basin
Invertebrate	Orthoptera	7	Recent	-28.9	2.7	3.5	2.5	2011-2012	Corumbataí river basin
Invertebrate	Arachnida	8	Recent	-26.5	1.4	6.8	1.5	2011-2012	Corumbataí river basin
Invertebrate	Caterpillar	7	Recent	-28.9	3.8	1.6	2.2	2011-2012	Corumbataí river basin
Plant	*Alchornea* sp.	4	Recent	-26.4	0.2	3.7	0.7	2011-2012	Corumbataí river basin
Plant	*Psychotria* sp.	15	Recent	-32.8	2.7	2.8	0.9	2011-2012	Corumbataí river basin
Plant	*Cestrum* sp.	7	Recent	-27.8	1.4	2.1	2.4	2011-2012	Corumbataí river basin
Plant	*Pombalia* sp.	8	Recent	-29.2	1.1	1.4	1.3	2011-2012	Corumbataí river basin
Plant	*Miconia* sp.	7	Recent	-29.4	0.8	2.1	0.3	2011-2012	Corumbataí river basin
Plant	*Rubus* sp.	8	Recent	-29.6	1.1	2.4	2.2	2011-2012	Corumbataí river basin
Plant	Solanaceae or Cucurbitaceae	7	Recent	-31.4	1.3	0.2	1.2	2011-2012	Corumbataí river basin
Plant	*Cestrum* sp.	5	Historical	-28.2	0.9	9.1	3.1	1936-1960	Municipalities: Campinas
Plant	*Miconia* sp.	6	Historical	-28.1	1.3	1.6	2.1	1938-1964	Municipalities: Campinas, Itirapina, Pindorama, São Carlos, São Simão
Plant	*Pombalia* sp.	7	Historical	-26.6	2.5	7.2	2.1	1936-1967	Municipalities: Campinas, Pedreira, Piracicaba
Plant	*Psychotria* sp.	4	Historical	-30.0	1.9	4.6	1.4	1938-1943	Municipalities: Campinas, Piracicaba

APPENDIX D. Fractionation values of carbon and nitrogen for each bird species, calculated to further use them to correct δ¹³C and δ¹⁵N values of each analysed sample.

Guild	Species	$\delta^{13}C$ fractionation value (‰)	$\delta^{15}N$ fractionation value (‰)
Frugivore	*Antilophia galeata*	0.67	5.23
	Chiroxiphia caudata	0.67	5.19
	Euphonia chlorotica	0.68	5.20
	Manacus manacus	0.67	5.20
	Tangara cayana	0.66	5.21
Granivore	*Cyanoloxia brissonii*	0.64	5.21
	Leptotila rufaxilla	0.64	5.20
	Leptotila verreauxi	0.62	5.20
Insectivore	*Basileuterus culicivorus*	1.35	3.16
	Conopophaga lineata	1.38	3.18
	Dysithamnus mentalis	1.35	3.15
	Lathrotriccus euleri	1.35	3.16
	Myiothlypis flaveola	1.33	3.20
	Tolmomyias sulphurescens	1.31	3.16
Nectarivore	*Amazilia lactea*	1.75	3.30
	Amazilia versicolor	1.72	3.33
	Chlorostilbon lucidus	1.75	3.33
	Coereba flaveola	1.79	3.32
	Phaethornis pretrei	1.76	3.31
	Thalurania glaucopis	1.75	3.33
Omnivore	*Habia rubica*	1.80	3.32
	Myiarchus ferox	1.75	3.31
	Ramphocelus carbo	1.77	3.32
	Tachyphonus coronatus	1.78	3.34
	Thlypopsis sordida	1.76	3.32
	Turdus leucomelas	1.80	3.32

APPENDIX E.   Boxplot of $\delta^{13}C$ and $\delta^{15}N$ for A) biomes: Atlantic forest and Cerrado; and B) ecoregions according to WWF: Alto Paraná Atlantic Forest, Cerrado and Serra do Mar Coastal Forest. Boxplots show means (diamonds), medians, quartiles and outliers.
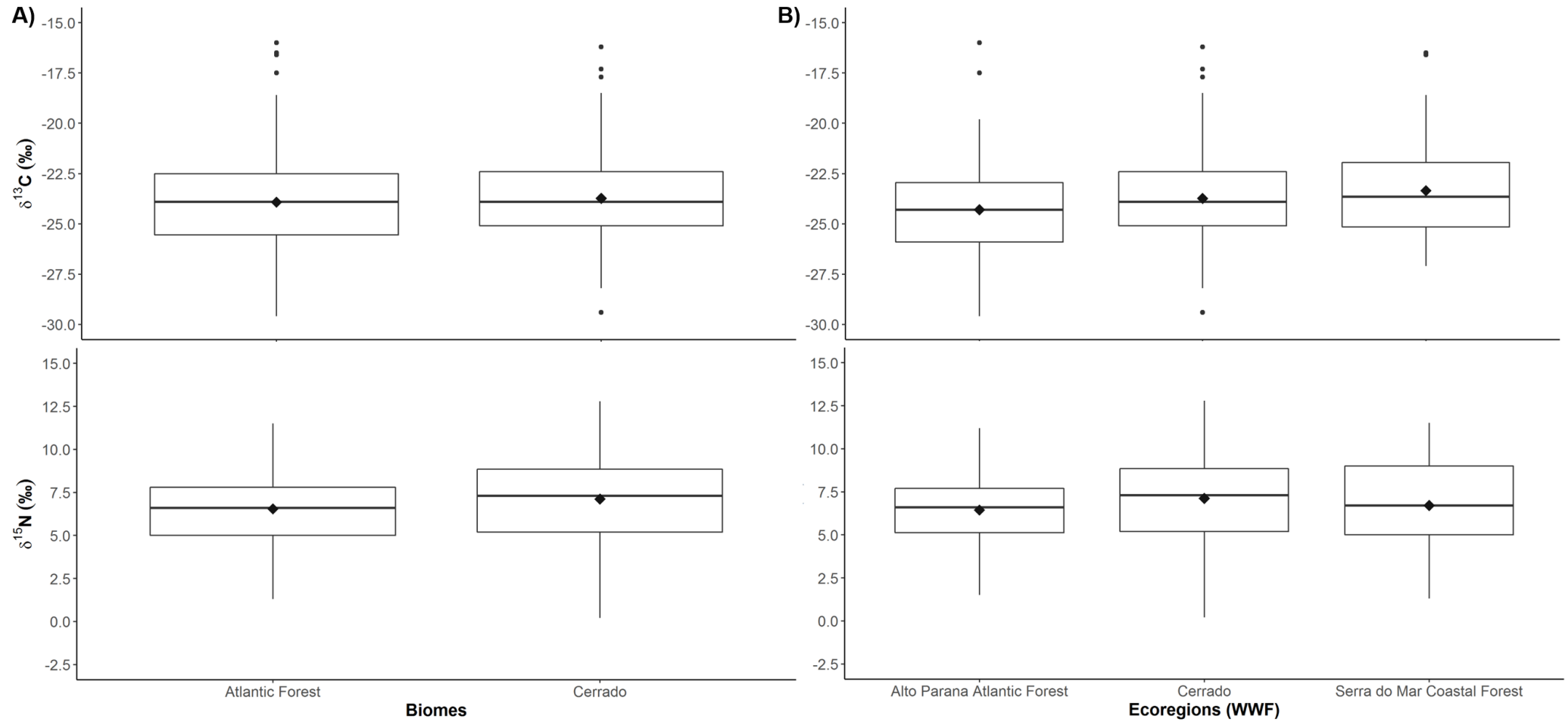
APPENDIX F. Boxplot of $\delta^{13}C$ and $\delta^{15}N$ values of historical and recent plants suitable as food resources for birds. Boxplots show means (diamonds), medians, quartiles and outliers.
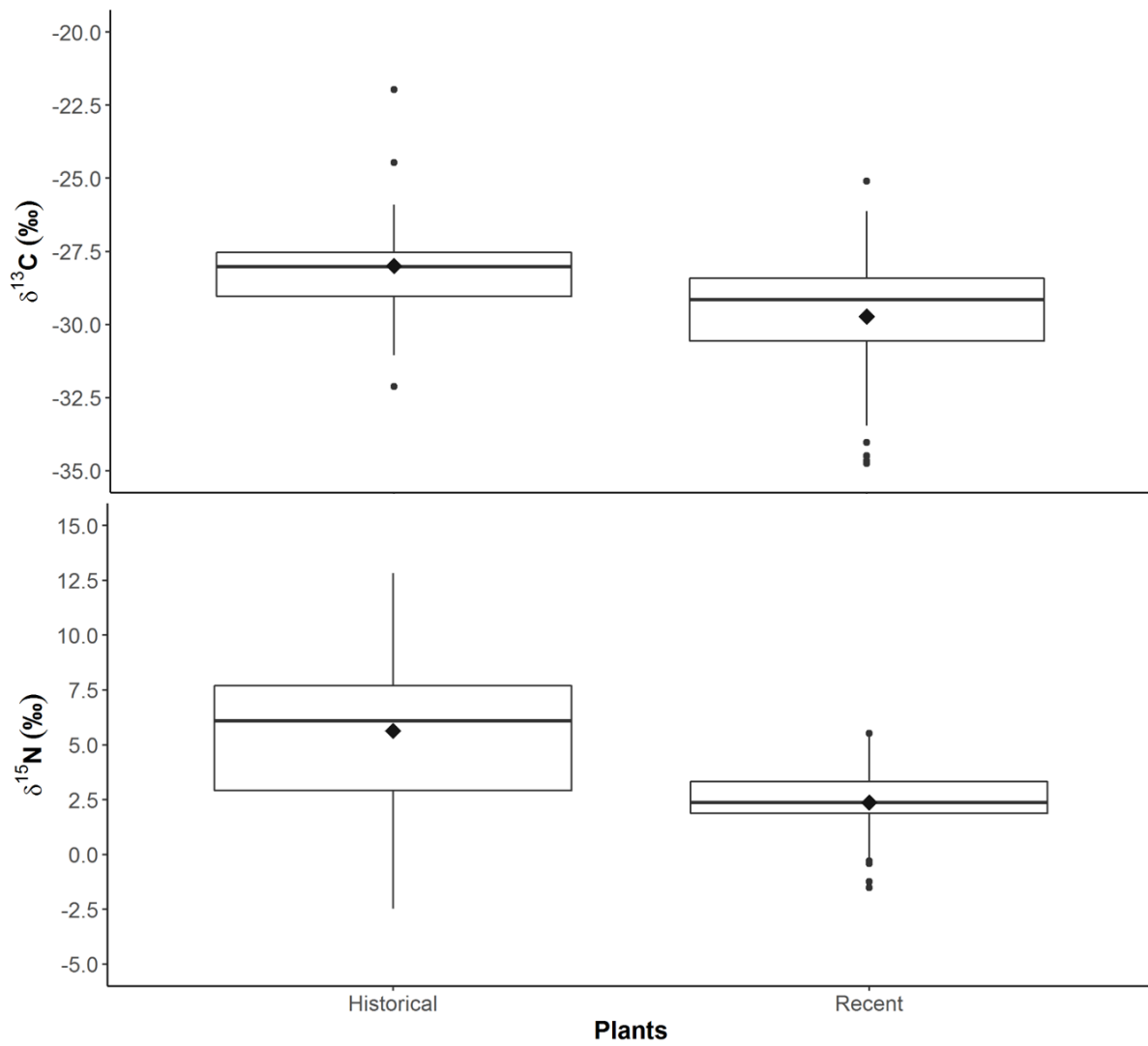
APPENDIX G. All human-modified landscapes (n = 12) composing the group with less or equal amount of 30% of forest cover, ranging from the less forested (6% of forest cover – the first site extremely on the left and on the top) until the most forested site (26% of forest cover – the site on the left of of legend).
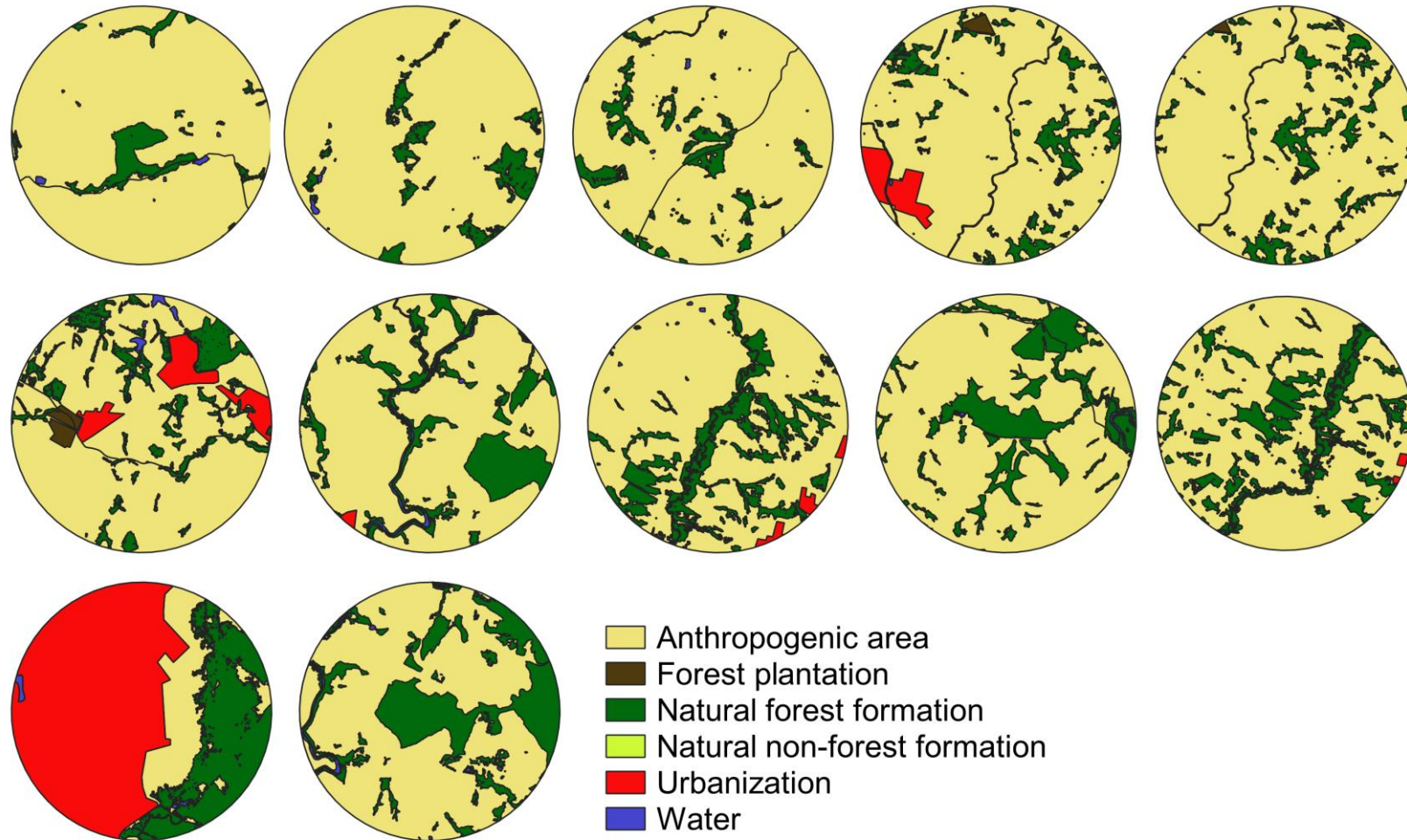
APPENDIX H.   All natural landscapes (n = 16) composing the group with an amount equal or more than 47% of forest cover, ranging from the less forested (47% of forest cover – the first site extremely on the left and on the top) until the most forested site (99% of forest cover – the site on the left of legend).

Anthropogenic area
Forest plantation
Natural forest formation
Natural non-forest formation
Urbanization
Water
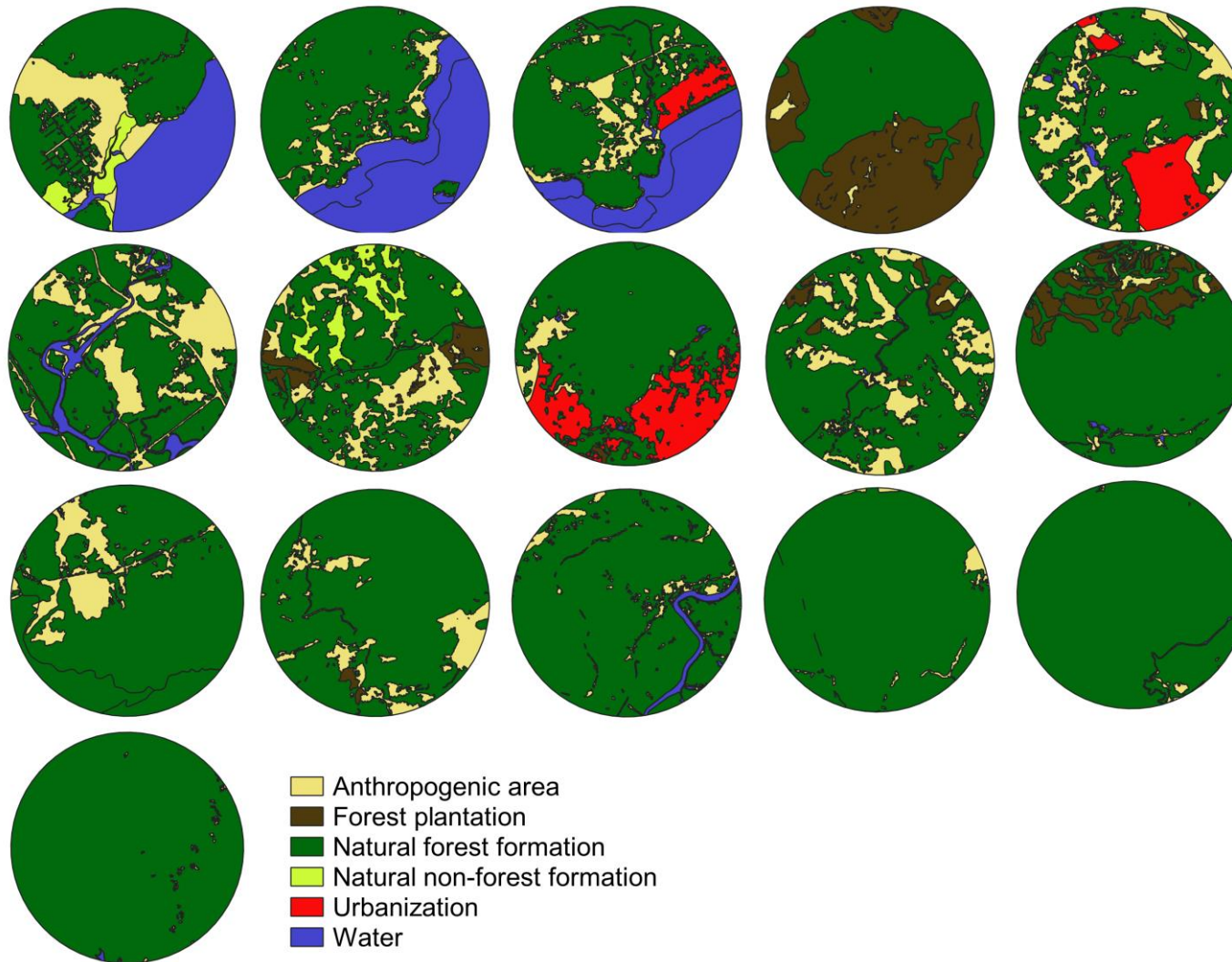
APPENDIX I. Species that compose the five foraging guilds (frugivore, granivore, insectivore, nectarivore and omnivore), the number of sampled individuals, their mean values of δ13C and δ15N, standard deviation (SD) and their respective landscape group (natural or human-modified).

Foraging guild	Species	N individuals	Landscape group	$\delta^{13}C$ mean (‰)	SD	$\delta^{15}N$ mean (‰)	SD
Frugivore	*Antilophia galeata*	72	Human-modified	-24.7	1.5	1.8	1.1
Frugivore	*Manacus manacus*	28	Human-modified	-24.6	1.2	2.1	1.1
Frugivore	*Patagioenas picazuro*	3	Human-modified	-17.2	3.4	0.7	0.6
Frugivore	*Chiroxiphia caudata*	33	Natural	-26.4	1.2	0.0	2.2
Frugivore	*Euphonia chlorotica*	1	Natural	-26.5	-	-5.2	-
Frugivore	*Manacus manacus*	6	Natural	-26.2	0.5	-1.4	2.5
Granivore	*Columbina talpacoti*	8	Human-modified	-15.1	5.0	0.0	1.1
Granivore	*Coryphospingus cucullatus*	1	Human-modified	-13.1	-	0.7	-
Granivore	*Cyanoloxia brissonii*	6	Human-modified	-22.9	3.6	-0.9	1.1
Granivore	*Leptotila verreauxi*	10	Human-modified	-23.8	1.5	1.2	2.0
Granivore	*Tiaris fuliginosus*	33	Human-modified	-15.5	4.9	0.4	1.7
Granivore	*Volatinia jacarina*	3	Human-modified	-11.2	0.7	3.2	1.4
Granivore	*Zenaida auriculata*	3	Human-modified	-18.4	1.6	3.7	0.0
Granivore	*Zonotrichia capensis*	7	Human-modified	-16.8	2.2	3.2	0.7
Granivore	*Haplospiza unicolor*	36	Natural	-23.3	4.6	-0.3	2.0
Granivore	*Leptotila rufaxilla*	2	Natural	-25.2	1.1	1.1	0.4
Granivore	*Leptotila verreauxi*	2	Natural	-25.9	0.3	-0.3	0.1
Insectivore	*Arremon flavirostris*	33	Human-modified	-24.0	1.8	3.8	1.0
Insectivore	*Automolus leucophthalmus*	13	Human-modified	-24.0	1.0	6.1	0.9
Insectivore	*Basileuterus culicivorus*	75	Human-modified	-23.4	0.9	5.0	0.9
Insectivore	*Conopophaga lineata*	39	Human-modified	-24.1	0.5	5.7	1.0
Insectivore	*Dysithamnus mentalis*	20	Human-modified	-24.7	0.6	5.8	1.0
Insectivore	*Lathrotriccus euleri*	44	Human-modified	-22.2	1.7	5.4	1.0
Insectivore	*Leptopogon amaurocephalus*	32	Human-modified	-24.3	0.9	4.7	1.0
Insectivore	*Myiothlypis flaveola*	53	Human-modified	-23.3	2.6	5.0	1.3
Insectivore	*Platyrinchus mystaceus*	59	Human-modified	-23.9	0.6	6.0	0.9
Insectivore	*Pyrrhocoma ruficeps*	15	Human-modified	-26.3	0.7	4.9	1.0
Insectivore	*Synallaxis frontalis*	13	Human-modified	-26.2	1.8	5.3	1.3
Insectivore	*Synallaxis ruficapilla*	16	Human-modified	-24.7	0.8	5.3	1.0
Insectivore	*Thamnophilus caerulescens*	14	Human-modified	-24.2	0.5	4.3	0.9

Insectivore	*Tolmomyias sulphurescens*	16	Human-modified	-22.6	0.7	5.1	0.9
Insectivore	*Veniliornis passerinus*	6	Human-modified	-23.8	1.0	4.7	0.9
Insectivore	*Arremon flavirostris*	3	Natural	-24.9	1.6	4.4	0.8
Insectivore	*Automolus leucophthalmus*	2	Natural	-25.0	0.2	7.1	1.1
Insectivore	*Basileuterus culicivorus*	6	Natural	-25.6	1.2	2.0	0.8
Insectivore	*Conopophaga lineata*	7	Natural	-25.5	0.9	3.6	1.2
Insectivore	*Corythopis delalandi*	1	Natural	-25.0	-	6.5	-
Insectivore	*Lathrotriccus euleri*	5	Natural	-27.0	1.0	4.2	3.7
Insectivore	*Malacoptila striata*	1	Natural	-24.4	-	5.1	-
Insectivore	*Myiothlypis flaveola*	2	Natural	-25.0	0.9	6.5	1.5
Insectivore	*Platyrinchus mystaceus*	1	Natural	-25.9	-	7.3	-
Insectivore	*Pyriglena leucoptera*	3	Natural	-25.2	1.4	6.2	0.9
Insectivore	*Sclerurus scansor*	1	Natural	-24.6	-	5.3	-
Insectivore	*Sittasomus griseicapillus*	2	Natural	-24.2	0.8	5.4	0.5
Insectivore	*Thamnophilus caerulescens*	6	Natural	-26.7	0.9	1.9	1.6
Nectarivore	*Amazilia lactea*	11	Human-modified	-24.4	1.9	8.3	1.3
Nectarivore	*Amazilia versicolor*	6	Human-modified	-24.9	1.9	6.7	1.4
Nectarivore	*Chlorostilbon lucidus*	5	Human-modified	-23.2	0.7	7.4	0.8
Nectarivore	*Coereba flaveola*	17	Human-modified	-23.9	1.2	5.4	1.2
Nectarivore	*Eupetomena macroura*	1	Human-modified	-21.9	-	7.2	-
Nectarivore	*Florisuga fusca*	1	Human-modified	-27.0	-	6.5	-
Nectarivore	*Phaethornis pretrei*	14	Human-modified	-23.1	1.6	6.5	1.0
Nectarivore	*Thalurania glaucopis*	12	Human-modified	-24.5	1.6	6.5	1.4
Nectarivore	*Amazilia versicolor*	10	Natural	-24.8	1.8	5.6	1.0
Nectarivore	*Coereba flaveola*	4	Natural	-26.0	1.4	1.9	1.4
Nectarivore	*Florisuga fusca*	7	Natural	-26.8	0.9	5.7	2.7
Nectarivore	*Phaethornis eurynome*	7	Natural	-26.7	1.4	3.6	1.2
Nectarivore	*Thalurania glaucopis*	12	Natural	-25.6	2.7	2.8	1.9
Omnivore	*Habia rubica*	17	Human-modified	-25.1	0.6	4.1	0.9
Omnivore	*Myiarchus ferox*	6	Human-modified	-21.5	2.2	4.8	1.1
Omnivore	*Ramphocelus carbo*	14	Human-modified	-25.6	0.8	4.0	1.2
Omnivore	*Tachyphonus coronatus*	62	Human-modified	-26.2	1.1	4.3	1.0
Omnivore	*Thlypopsis sordida*	9	Human-modified	-24.4	1.0	4.9	0.9
Omnivore	*Turdus leucomelas*	44	Human-modified	-22.7	2.1	5.1	1.8

Omnivore	*Turdus rufiventris*	5	Human-modified	-23.8	1.4	5.5	0.8
Omnivore	*Dacnis cayana*	1	Natural	-24.2	-	6.4	-
Omnivore	*Habia rubica*	6	Natural	-27.8	1.2	4.2	1.6
Omnivore	*Schiffornis virescens*	16	Natural	-28.6	1.5	2.6	1.7
Omnivore	*Tachyphonus coronatus*	7	Natural	-26.7	1.0	3.0	2.1
Omnivore	*Trichothraupis melanops*	1	Natural	-23.4	-	6.5	-
Omnivore	*Turdus rufiventris*	8	Natural	-24.6	2.0	4.5	1.3

APPENDIX J. Food resources taxons (invertebrates and plants) collected from the human-modified landscapes, the number of individuals, their mean values of δ¹³C and δ¹⁵N, and standard deviation (SD).

Food resource	Taxon	N individuals	δ¹³C mean (‰)	SD	δ¹⁵N mean (‰)	SD
Invertebrate	Blattodea	5	-25.8	1.5	1.2	1.4
Invertebrate	Coleoptera	7	-25.2	2.3	5.4	2.2
Invertebrate	Diptera	9	-24.7	3.4	7.1	3.4
Invertebrate	Hemiptera	8	-28.0	2.7	2.8	1.9
Invertebrate	Formicidae	9	-25.9	1.0	5.1	2.6
Invertebrate	Vespidae	5	-25.5	1.3	5.5	1.3
Invertebrate	Lepidoptera	5	-28.6	3.7	5.7	5.4
Invertebrate	Orthoptera	7	-28.9	2.7	3.5	2.5
Invertebrate	Arachnida	8	-26.5	1.4	6.8	1.5
Invertebrate	Caterpillar	7	-28.9	3.8	1.6	2.2
Plant	*Alchornea* sp.	4	-26.4	0.2	3.7	0.7
Plant	*Psychotria* sp.	15	-32.8	2.7	2.8	0.9
Plant	*Cestrum* sp.	7	-27.8	1.4	2.1	2.4
Plant	*Pombalia* sp.	8	-29.2	1.1	1.4	1.3
Plant	*Miconia* sp.	7	-29.4	0.8	2.1	0.3
Plant	*Rubus* sp.	8	-29.6	1.1	2.4	2.2
Plant	Solanaceae or Cucurbitaceae	7	-31.4	1.3	0.2	1.2

APPENDIX K. Fractionation values of carbon and nitrogen for each bird species, calculated to further use them to correct δ¹³C and δ¹⁵N values of each analysed sample.

Guild	Species	δ¹³C fractionation value (‰)	δ¹⁵N fractionation value (‰)
Frugivore	*Antilophia galeata*	0.67	5.23
	Chiroxiphia caudata	0.67	5.19
	Euphonia chlorotica	0.68	5.20
	Manacus manacus	0.67	5.20
	Patagioenas picazuro	0.61	5.20
Granivore	*Columbina talpacoti*	0.64	5.22
	Coryphospingus cucullatus	0.68	5.21
	Cyanoloxia brissonii	0.64	5.21
	Haplospiza unicolor	0.69	5.20
	Leptotila rufaxilla	0.64	5.20
	Leptotila verreauxi	0.62	5.20
	Tiaris fuliginosus	0.68	5.22
	Volatinia jacarina	0.69	5.23
	Zenaida auriculata	0.66	5.25
	Zonotrichia capensis	0.66	5.21
Insectivore	*Arremon flavirostris*	1.34	3.17
	Automolus leucophthalmus	1.36	3.18
	Basileuterus culicivorus	1.35	3.16
	Conopophaga lineata	1.38	3.18
	Corythopis delalandi	1.34	3.17
	Dysithamnus mentalis	1.35	3.15
	Lathrotriccus euleri	1.35	3.16
	Leptopogon amaurocephalus	1.36	3.18
	Malacoptila striata	1.31	3.16
	Myiothlypis flaveola	1.33	3.20
	Platyrinchus mystaceus	1.31	3.18
	Pyriglena leucoptera	1.36	3.17
	Pyrrhocoma ruficeps	1.34	3.16
	Sclerurus scansor	1.38	3.17
	Sittasomus griseicapillus	1.35	3.17
	Synallaxis frontalis	1.37	3.13
	Synallaxis ruficapilla	1.35	3.16
	Thamnophilus caerulescens	1.35	3.16
	Tolmomyias sulphurescens	1.31	3.16
	Veniliornis passerinus	1.36	3.13
Nectarivores	*Amazilia lactea*	1.75	1.75
	Amazilia versicolor	1.72	3.33
	Chlorostilbon lucidus	1.75	3.33
	Coereba flaveola	1.79	3.32
	Eupetomena macroura	1.75	3.30

	Florisuga fusca	1.75	3.31
	Phaethornis eurynome	1.74	3.31
	Phaethornis pretrei	1.76	3.31
	Thalurania glaucopis	1.75	3.33
Omnivore	*Dacnis cayana*	1.78	3.34
	Habia rubica	1.80	3.32
	Myiarchus ferox	1.75	3.31
	Ramphocelus carbo	1.77	3.32
	Schiffornis virescens	1.77	3.32
	Tachyphonus coronatus	1.78	3.34
	Thlypopsis sordida	1.76	3.32
	Trichothraupis melanops	1.76	3.33
	Turdus leucomelas	1.80	3.32
	Turdus rufiventris	1.80	3.35