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ECOSYSTEMS

Anuran diversity in ponds associated with soybean plantations

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Abstract: Anurans are considered one of the most threatened animal groups in the world. Agricultural activities are related to water pollution and contamination, which affects biphasic organisms such as amphibians. Brazilian soybean cultivation covers about 36 million hectares and encompasses many remaining ponds used as breeding sites for amphibians. In this study, we evaluated richness, abundance and composition of the anuran communities in ponds with different levels of association with soybean cultivation. A total of 18 anuran species were recorded with an abundance of 421 collected tadpoles and 1230 adult males on average. Ponds presented in soybean plantations were distinct from those adjacent to plantations regarding water properties and tadpole richness and abundance, as well as composition of tadpoles and adults. Ponds inserted in plantations had communities with lower diversity and abundance. One explanation for these results is likely the detrimental effect of soybean management, which suggests that this decrease is a result of community changes. This serves as an alert about the importance of buffer areas around plantations and the use of adequate techniques for pesticide application.

Key words: Amphibians, agriculture, pesticides, soybean plantations.

INTRODUCTION

Anurans are one of the most threatened vertebrate groups in the world (Wake 1991, Beebee & Griffiths 2005, Blaustein et al. 2011, Halliday 2016) due to their close relationship with water and the high rates of human disturbance in the environment in which they are inserted (Cushman 2006, Polo-Cavia et al. 2016). Amphibians are sensitive to small changes in the physical and biological characteristics of their habitat, being generally more sensitive to ecosystem changes than other vertebrates (Haddad & Prado 2005). Like other animals in the aquatic ecosystem, their populations are even impacted by disturbances that are restricted to areas surrounding water resources (Findlay & Houlahan 1997, Toledo et al. 2010, Bridia et al. 2017, Häder et al. 2020). These data are extremely concerning due to the history of conversion, fragmentation and degradation of tropical and subtropical regions, especially due to the advancement of the agricultural frontier (Bencke 2009, Bolzan et al. 2014, Cosset et al. 2018, Liu et al. 2019, Moskowitz et al. 2019).

Brazil is a relevant scenario for this issue since it is one of the largest producers of agricultural commodities and at the same time has the world's highest anuran diversity (Fao 2018, Frost 2019). One of the consequences of this combination is the fact that the country has one of the highest rates of amphibian population decline in the world (SchiesarI et al. 2007, Newbold et al. 2015, Alroy 2017, Ceballos et al. 2017, Powers & Jetz 2019). According to IBGE (2021), the conversion of areas into plantations continued to grow steadily over the last 10 years. And despite the diversity of cultivated crops in Brazil, soybean is one of the commodities with the largest production in the Brazilian territory (EMBRAPA 2019). Due to its tendency of homogenizing environments, soybean cultivation is unsustainable because of its stressors and the direct relationship between the expansion of cultivation areas in the country and the rate of native habitat loss (Domingues & Bermann 2012).

Considering the increasing local extinctions caused by the expansion of agricultural land, a collapse of biodiversity might occur in the near future (Molotoks et al. 2017, González et al. 2020) since the loss and fragmentation of native vegetation reduce food supply (Londero et al. 2017), increase exposure to ultraviolet radiation (Castanãga et al. 2009, Londero et al. 2019) and reduce the number of shelters against predators (Alton et al. 2011). Additionally, amphibians are also affected by water shortage caused by the drainage and irrigation systems in plantations, as well as by the contamination of air and water caused by chemical agents (Vitt et al. 1990, Londres 2011, Carneiro et al. 2015).

Studies of pesticides used in agriculture, such as glyphosate, 2,4-D dimethylamine and N-(phosphonomethyl) glycine, indicate that they change the pH and conductivity parameters and increase the concentration of chloride, nitrate and sulfate anions in water (Schwuger 1973, Cunha et al. 2017, Chamba & Cadena 2019). Moreover, frog communities in locations altered by the conversion and management of cultivation areas tend to be little diversified and have smaller populations. We believe that the structuring of the habitat and even some water parameters, such as conductivity and pH, are significant in determining the distribution and abundance of frogs (Gray et al. 2004, Angulo et al. 2006, Bolzan et al. 2014, Moreira et al. 2015). Thus, this study aimed to assess the richness, abundance and composition of the amphibian community in ponds associated with soybean cultivation. If the presence of plantations modifies the properties of the surrounding habitats (e.g., physical and chemical characteristics of the remaining ponds), decreasing their suitability as breeding sites for frogs, we expect: 1 -communities to be poorer in ponds inserted in plantations than in those adjacent to plantations; 2 - adults and tadpoles to have different patterns of diversity in both types of ponds due to their different levels of water dependence.

MATERIALS AND METHODS

Study area

The samples were conducted in the city of Cruz Alta, Rio Grande do Sul, southern Brazil. This region is a transition zone between tropical and temperate climates, with four well-defined seasons and uniform precipitation across the year (Overbeck et al. 2007). Originally, the region had a vegetation cover that was transitional between the biomes Pampa (subtropical grassland) and Atlantic Forest (IBGE 2012) but most of it has been converted into agriculture. The sampled area is formed by small unconnected remnants of natural grassland or forests. The landscape is spotted with small (less than 1.5 ha) permanent natural ponds. By the force of the Brazilian law, landowners were obligated to maintain those ponds, and many of them are immersed in agriculture plantations. Usually, ponds offer a significant opportunity for anuran sampling (Madalozzo et al. 2017) and, in our case, their natural distribution in habitat remnants as well as in agriculture plantations give us a good opportunity for sampling design.

Ponds (breeding sites) description

To obtain the lowest possible structural variability between the units, we selected eight permanent ponds similar in shape and size and with similar characteristics of aquatic vegetation. Ponds had an average area of 8000 m², varying a depth from two to five meters, and all had at least two distinct species of macrophytes. Our sampling design was limited to the land owner's permission to access their properties. Once the whole area consists of private properties, it was not possible to conduct a random spatial distribution of samples. Despite this, we were able to distribute sampling ponds to avoid a nested spatial distribution, reducing the spatial correlation among ponds.

Selected ponds were grouped into two treatments: (A) inserted ponds (N=4), located inside and surrounded by soybean plantations, and (B) adjacent ponds, located in natural grassland outside the plantations (N=4). Ponds were located at least 1 km from each other. All ponds have a well-defined margin and have no physical connection with other water bodies or forests. Inserted ponds were surrounded by soybean plantations, which extended for at least 150 m from the pond margin. Adjacent ponds were surrounded by natural grasslands and located 10-15 m from the border of the nearest plantation (Figure 1). Although spatially disconnected from plantations, adjacent ponds are close enough to be affected by them. Our motivation to compare those two groups was based on the hypothesis that the ponds inserted in the plantation should suffer direct actions of agricultural management while in adjacent ponds this influence would be indirect.

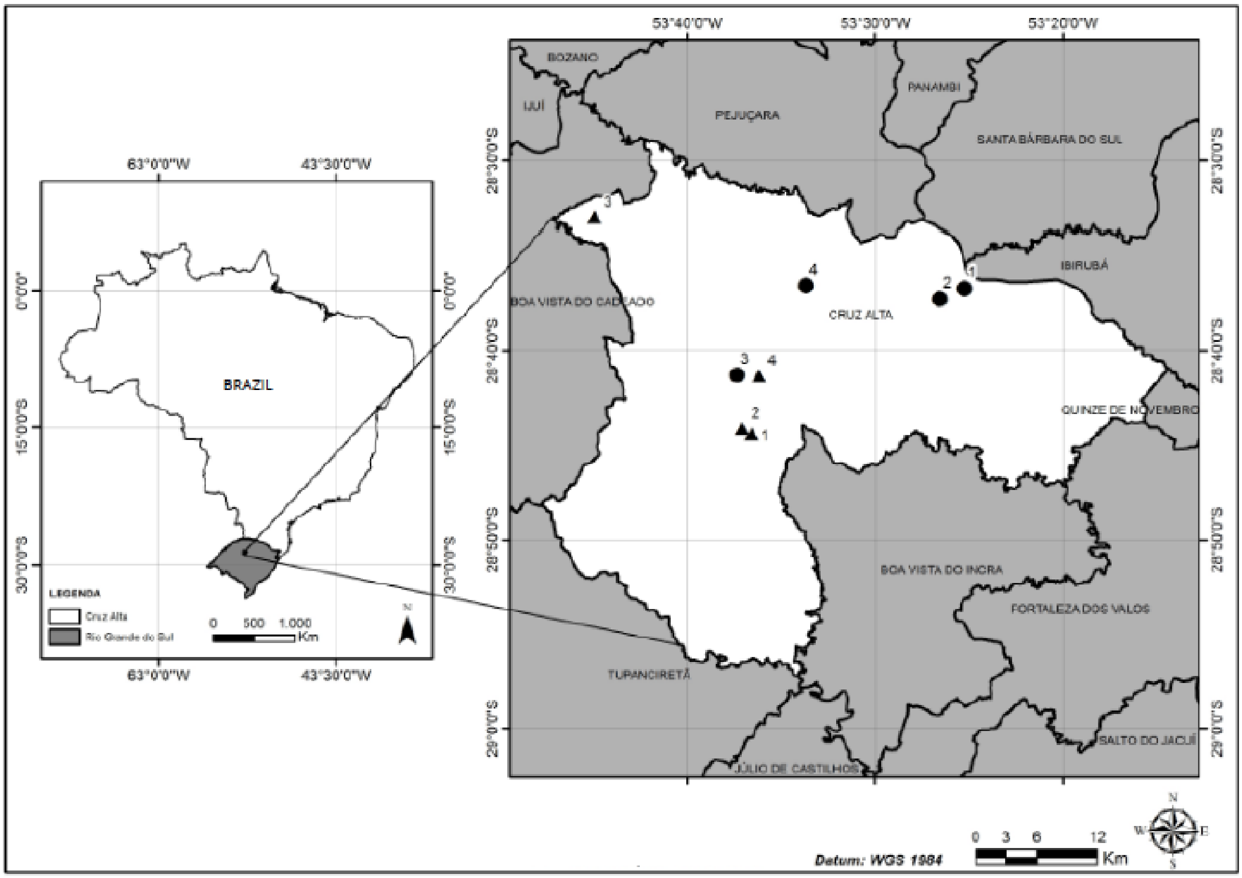


Figure 1. Geographic location of the study area and distribution of sample points. Circles represent inserted ponds and triangles represent adjacent ponds.

Possible impacts over anurans would be greater during land preparation and direct pesticide application.

To test the consistency of our design, we compared the physicochemical properties of the water in the ponds (Supplementary Material - Table SI). We measured temperature, dissolved oxygen, conductivity and pH of the water using a multimeter SensoDirect 150 (Lovibond). Measurements were conducted in all ponds and all amphibian samplings, always in the morning. The PerMANOVA analysis showed that the water of inserted and adjacent ponds had significant differences regarding their physical and chemical parameters (F = 25.81, p = 0.0001). The Mann Whitney test for each measured variable showed that conductivity (U = 196, p = 0.0001) varies significantly between the ponds. See figure 2.

Amphibian sampling

The collections started in 2016 and ended in 2018 and were concentrated between the months of September to May, a period in which anurans are more active due to the warmer climate (Mccain & Sanders 2010, Carvalho-Rocha 2020), and is also the time when soybeans are grown in the sampled region. Each pond was sampled ten times during the whole study. Amphibian samples occurred through techniques of tadpole collection and auditory sampling of adult males. When associated, these techniques are one of the most efficient combinations to record anuran species (Madalozzo et al. 2017). For tadpole collection, we used a 30-cm-diameter dip net, with which we sampled five spots in each pond. Spot selection occurred systematically for the maximum exploration of the pond's perimeter and to limit the samplings to points with a maximum depth of 1 m, where tadpole capture is more likely to occur (Shaffer et al. 1994, Madalozzo et al. 2017). Only the ponds' banks had the conditions described above, which facilitated the sampling. These processes were carried out between 9 am and 7 pm, always by the same collector. At all points, the scan was repeated three times in an area corresponding to a circle with a radius of 1 m (about 3 m²). The collected animals were placed in containers, immersed in xylocaine and later screened to determine their species (Laufer & Barreneche 2008, Machado & Maltchik 2010). All collected specimens are housed in the collection of the laboratory of terrestrial vertebrates at the Universidade do Vale do Rio dos Sinos, RS, Brazil.

Concurrently with the sampling of tadpoles, adult specimens were registered through an automated registration system (Acevedo & Villanueva-Rivera 2006) consisting of digital recorders (SONY ICD - PX312). The equipment was placed at each pond's shore about 1 m above the soil and set to record the background sounds continuously between 6 pm and 6 am of the following day. The audio files were analyzed, and the first five minutes of each hour were considered a subsample from which we estimated the number of vocalizing individuals of each species (Madalozzo et al. 2017, De Luna-Dias & Carvalho-e-Silva 2019). Later, the data were grouped in abundance classes (Ximenez & Tozetti 2015): (0) no vocalizing individuals of the species; (1) 1–4 vocalizing individuals; (2) 5–9 vocalizing individuals; (3) 10–14 vocalizing individuals; (4) >15 individuals with chorus formation in which individual vocalizations are indistinguishable.

Data analysis

To test the spatial independence of the sampling spots from species composition, we used the Moran's I coefficient. Therefore, we used the occurrence of individual species as variables in a Principal Component Analysis (PCA), in which the first axis was used as the response variable

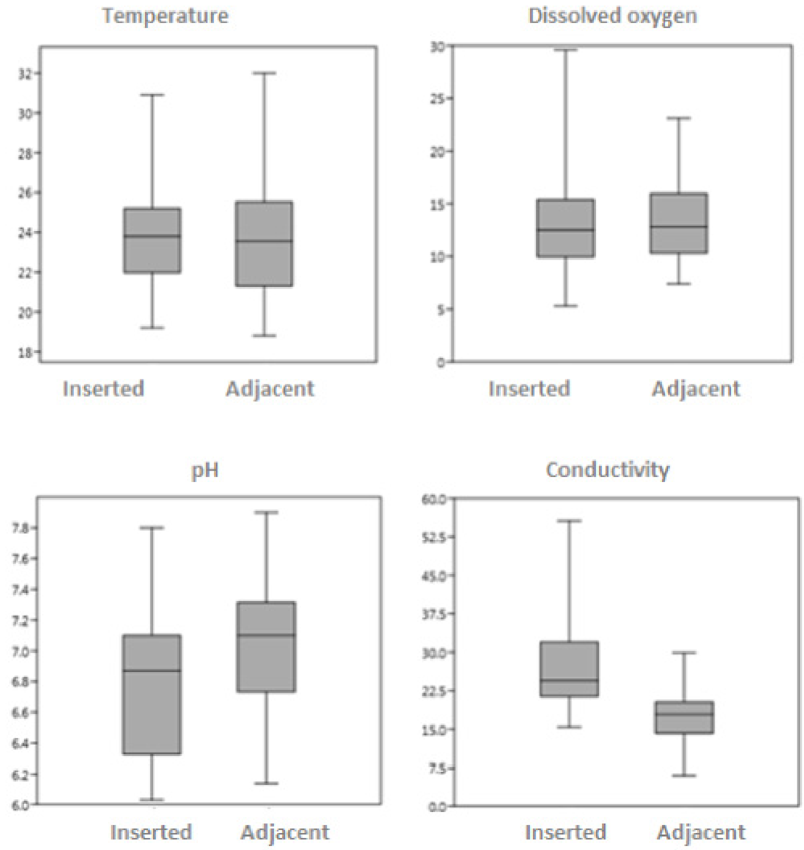


Figure 2. Physical and chemical parameters of water measured in ponds that are inserted or adjacent to soybean plantations.

to the analysis (Moran's I), with coordinate variables for eight different distance classes. To analyze adult and tadpole richness, we used Generalized Linear Models (GLM) with Poisson distribution. Regarding abundance, the GLMs followed the negative binomial distribution and only the data regarding tadpoles were analyzed because they were real abundance data, in contrast to adults, which had estimated values. These analyses were performed in the statistical program R Project using the package MASS and the function glm.nb. Following, we conducted PerMANOVA tests with quantitative data in the statistical program R Project using the package vegan and the functions adonis and metaMDS to measure variations in the composition of anuran communities. Adults were tested regarding treatment and between steps using a two-way PerMANOVA with Jaccard distance. The graphic representation was done with a non-metric multidimensional scaling (NMDS) with Jaccard distance and two dimensions. For tadpoles, we only tested the differences between treatments using a one-way PerMANOVA with Bray-Curtis distance, which was also graphically represented using NMDS with Bray-Curtis distance and two dimensions. Still trying to measure the difference between the analyzed biological communities and to understand how and why the analyzed biodiversity is distributed between treatments, we performed a beta diversity partitioning analysis. Consequently, we followed the approach of Baselga (2010) that uses the concepts of nestedness and spatial turnover (Harrison et al. 1992, Baselga 2007). Nestedness (βnes) is defined as a process of species loss due to a specific cause that generates community fragmentation, turning sites with lower richness into subsets of richer areas. Species turnover (βsim), on the other hand, indicates the replacement of some species with others due to the habitat's environmental restrictions. Using the program R Project, we generated βsim and βnes values for tadpoles and adults of inserted and adjacent ponds using the package betapart and the function beta.pair (Baselga 2010, 2012).

Later, these values were compared using a t-test to verify which process (nestedness or turnover) prevails among the communities of the studied ponds. We built an abundance-based species ranking, as well as an indicator-species analysis, IndVal, using the package indicspecies and the function multipatt of R Project to determine whether there were tadpole species that could be considered indicator species for any of the environment types. All procedures of this study were previously approved as activities with scientific purposes with a SISBIO license (No. 55351-3) and were also approved by the ethics committee for animal use (CEUA–UNISINOS) (PPECEUA02.2017).

RESULTS

Tadpole richness varied significantly between treatments (GLM = estimate: 1.38; Z value: 1.92; p = 0.054), being higher in adjacent ponds (14 species) than in inserted ponds (8 species). We did not record significant difference in adult richness between inserted and adjacent ponds (GLM = estimate: -0.18; Z value: -0.49; p = 0.62). The number of tadpoles collected in adjacent ponds was larger (331 individuals) than in inserted ponds (90 individuals) (GLM = estimate: 2.05; Z value: 2.32; p = 0.02).

The ranking of species abundance showed different patterns of estimated abundance when life stage was compared (Figures 3 and 4). In all ponds, we observed a steeper curve decline in abundance for tadpoles than for adults. Furthermore, there was a significant variation in the abundance of tadpoles between the inserted and adjacent ponds. Among the

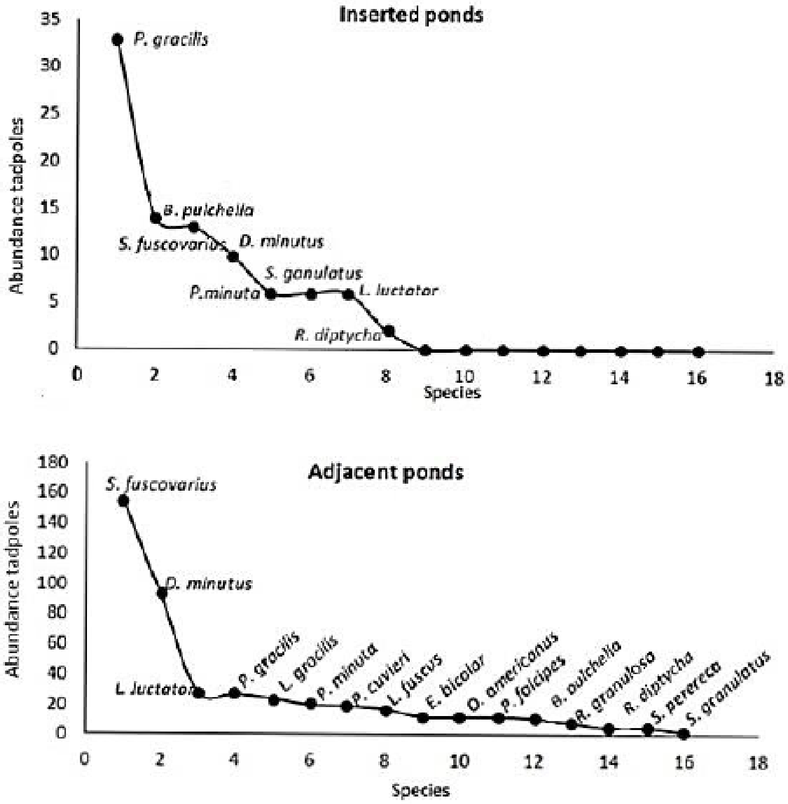


Figure 3. Species ranking of tadpoles collected in ponds inserted in and adjacent to soybean plantations.

tadpole species, *Physalaemus gracilis* was the most abundant in inserted ponds whereas *Scinax fuscovarius* and *Dendropsophus minutus* were more abundant in adjacent ponds. On the other hand, for adults, *Boana pulchella*, *D. minutus* and *Physalaemus cuvieri* were the most abundant species in all ponds.

The indicator species analysis pointed out *Dendropsophus minutus* (p = 0.035) and *Scinax fuscovarius* (p = 0.005) as indicators of adjacent ponds. No species was pointed out as an indicator of ponds inserted in soybean plantations. The Moran's I analysis did not detect a significant spatial correlation of the species composition for both adults (mean of the minimum distance class: 0.01 degree; Moran's I = 0.17; p = 0.93) and tadpoles (mean of the minimum distance class: 0.01 degree; Moran's I = 1.10; p = 0.39), suggesting spatial independence between ponds. Community composition varied significantly for both adults (F1:39: 3.64; p = 0.002) and tadpoles (F1:33: 3.36; p = 0.002) (Figure 5). In both, NMDS presented good data consistency, showing a separation highlighted by PerMANOVA.

The beta diversity analysis suggests that there was no significant difference between the processes of nestedness and spatial turnover between inserted ponds (tadpoles t: 1.1979, p: 0.44282; adults t: 3.2006, p: 0.19279) and adjacent ponds (tadpoles t: 1.3142, p: 0.41408; adults t: 3.0288, p: 0.20301). However, we could notice that beta diversity is driven by spatial turnover, which

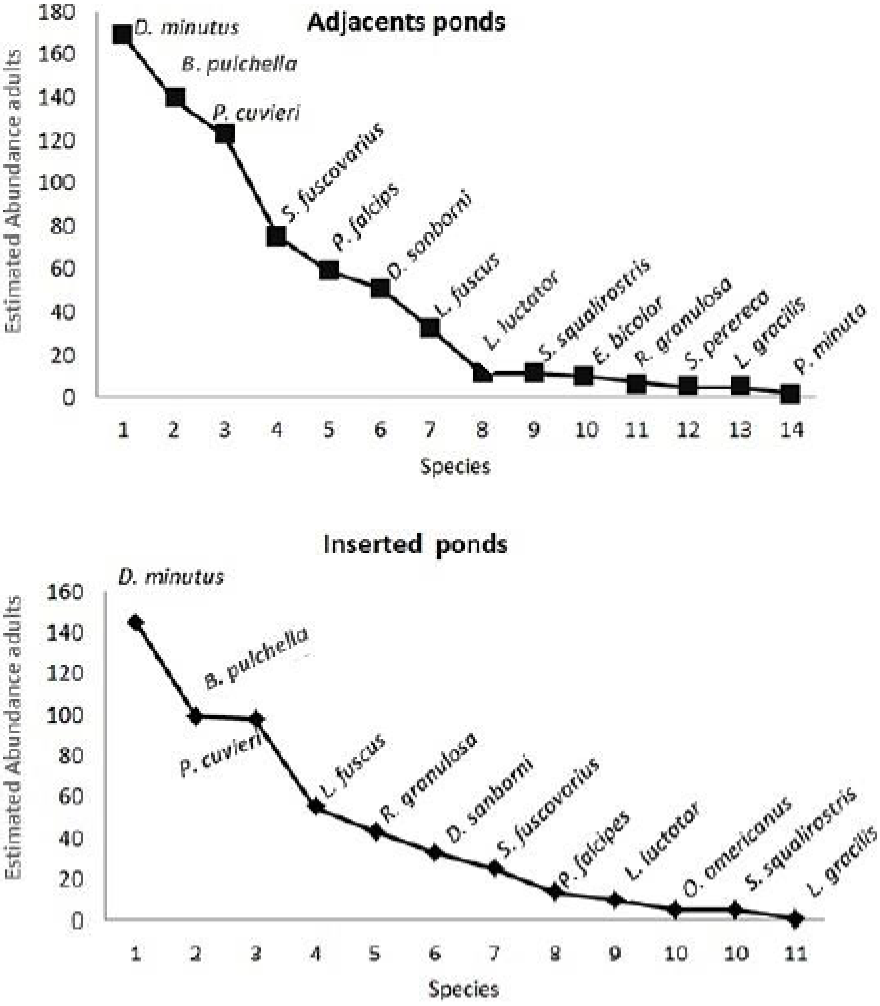
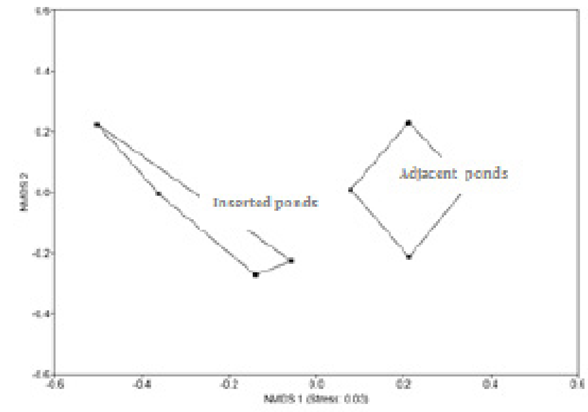
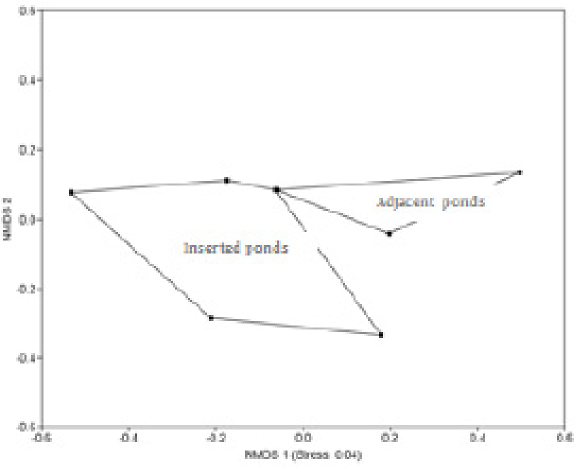


Figure 4. Species ranking of adult anurans collected in ponds inserted in and adjacent to soybean plantations.

indicates that species replacement tends to be higher in both adjacent ponds and inserted ponds (Figure 6), in detriment of species loss or gain as suggested by nestedness. Nevertheless, in the adult community, we could notice that turnover decreases and, consequently, nestedness increases.



Tadpoles



Adults

Figure 5. Graphic representation of the difference in the composition of tadpoles and adult anurans between treatments using NMDS.

DISCUSSION

Our data indicate that the species composition of both larval and adult anurans was differentially affected by soybean plantations. However, the most evident differences between the two groups of ponds (inside and adjacent to plantations) were observed in tadpoles. Our results revealed poorer communities and smaller populations of tadpoles in inserted ponds, which could be a result of a higher susceptibility of tadpoles than adults to the effects of the plantations. We believe that this result is driven by changes in the water quality of ponds promoted by agricultural practices. In addition to this, pesticides often used in soybean crops have lethal, sublethal or behavioral effects over native fauna (Agostini et al. 2020, Bolis et al. 2020, Herek et al. 2021). As the larvae of all observed frog species are aquatic, they are directly affected by these environmental changes. This hypothesis is reinforced by differences registered in water conductivity and pH between the two groups of ponds. Such parameters are known to be affected by the presence of glyphosate and other pesticides used in the plantations in the study area. Most of these pesticides increase the concentration of chloride, nitrate and sulfate anions causing such changes in the water (Mekonen et al. 2016, Chamba & Cadena 2019).

At the same time, our results indicate a higher resilience of adults since their community suffered smaller changes between the two groups of ponds. This makes sense since they are less dependent on aquatic habitat than tadpoles, and their greater mobility could minimize agricultural impacts (Petranka et al. 1994, Rudolf & Rödel 2005, Marques & Nomura 2015). We also reinforce that all sampled ponds are unconnected to other ponds or natural habitats, reducing the interference of individual

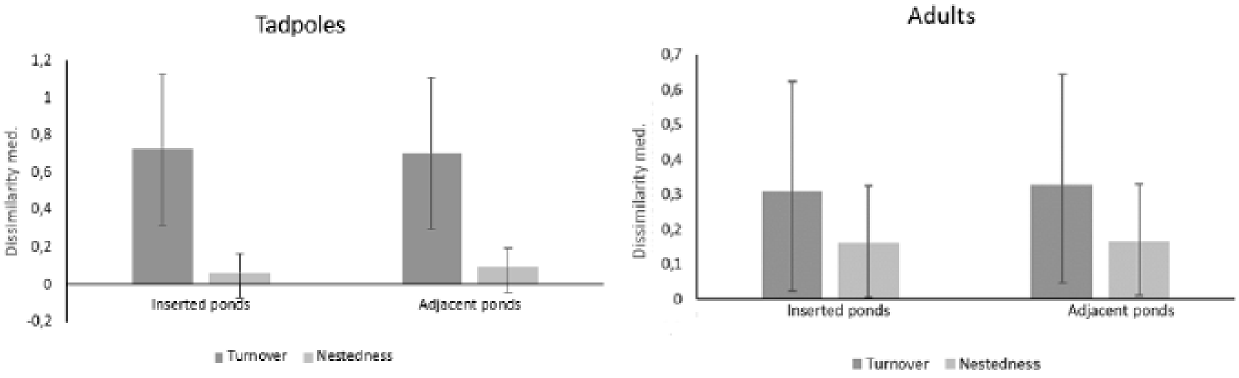


Figure 6. Mean dissimilarity values of species turnover (βsim) and nestedness (βnes) for tadpoles and adult anurans in ponds that are inserted in or adjacent to soybean plantations.

displacement (immigration or emigration) over the results.

Studies report that changes in the physicochemical parameters of the water could lead to a lethal or sublethal effect on frogs (Freda 1986, Muñoz & Bernal 2011, Chamba & Cadena 2019, Herek et al. 2020). Thus, ponds would act as a sinkhole for anurans, since even the most abundant tadpole species in inserted (*P. gracilis*) and adjacent (*S. fuscovarius* and *D. minutus*) ponds had small populations. This indicates that one or two tadpole species tend to stand out numerically in all ponds, but this pattern is not repeated in the adults. The most abundant tadpole species varied between inserted (*P. gracilis*) and adjacent ponds (*S. fuscovarius*, *D. minutus*). For adults, the most abundant species were the same in both inserted and adjacent ponds (*B. pulchella*, *D. minutus* and *P. cuvieri*). This suggests that the location of the ponds affects tadpoles and adults differently.

We identified the tadpoles of *D. minutus* and *S. fuscovarius* as indicators of adjacent ponds. These species have already been described in the literature as susceptible to ecological, morphological and/or physiological changes due to environmental factors (Bokermann 1963, Marques & Nomura 2015, Marques et al. 2018). Therefore, the significant association of *S. fuscovarius* and *D. minutus* only with adjacent ponds reinforces our hypothesis that the two pond groups are different and that the communities and their distributions are more impacted in ponds inserted in soybean culture.

Regarding beta diversity, the difference between ponds indicated a predominant turnover process. However, the adult communities showed smaller differences between nestedness and turnover processes. We have no strong evidence to explain this pattern but we believe that differences in the mobility of tadpoles and adults are a key factor. Adults can access different ponds whereas tadpoles have their whole home range restricted to a single pond.

Given the above, our data show that, even at a short distance (about 10-15m) from agricultural sites, there was a significant increase in population size and species richness in the studied anuran communities. Leading us to believe that there is a real impact of agriculture on frog diversity in lakes located in and around plantations. Therefore, we propose the establishment of buffer zones around plantations in order to avoid significant changes in the habitats surrounding areas used for agriculture. In addition to establishing permanent preservation areas for all water resources. Finally, we believe that these data obtained in the field are in agreement with numerous experiments that have already demonstrated the anura community as sensitive to environmental changes resulting from agricultural activities. In this way, it can help future larger and more in-depth field studies that seek strategies for the conservation of frogs combined with the maintenance of agricultural production.

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SUPPLEMENTARY MATERIAL

Table SI

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Author contributions

GGC and AMT were responsible for the delineation of the study, as well as the collection and identification of the specimens. MSD contributed with statistical analysis, interpretation of the results graph creation. The discussion of the manuscript and its final review were carried out by all authors.