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Article

Effects of low-intensity cattle ranching on amphibians in the Ñeembucú Wetland Complex, Paraguay

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ABSTRACT. Cattle in wetlands impact water quality through waste excretion, which deposits excess nutrients, as well as decreasing the biomass and height of vegetation through trampling and herbivory. Amphibians are sensitive to these changes due to their porous skin and reliance on vegetated microhabitats. Previous studies examining the effect of cattle on amphibians in wetlands report conflicting results, exemplifying the need to avoid overgeneralizations and instead examine specific environments. In the Ñeembucú Wetland Complex in Paraguay, low-intensity cattle ranching is a common practice. This study seeks to understand how the presence of cattle in these operations impacts water chemistry and amphibian species richness, as well as determining which cattle effects (eutrophication, nutrient loading, and decreased vegetation height diversity) have the greatest impact on amphibian communities. We anticipated that increased cattle presence would negatively impact wetland condition, and consequentially lower amphibian species richness. Data was collected over seven weeks at Estancia Santa Ana, a low-intensity cattle ranch in Pilar, Paraguay. Cattle presence was measured through cow footprints and feces densities, while a vegetation survey and water quality testing (nitrate, phosphate, and dissolved oxygen) served as environmental metrics. Pitfall trapping was used to gather information on amphibian species richness and community composition. A Pearson parametric correlation test and Factor Analysis of Mixed Data in R were then used to understand the relationship between variables. Very few statistically relationships were found between variables, and those that existed showed a very weak correlation. This suggests that cow presence does not have as strong of an impact on water quality or amphibian species richness as expected. Terrestrial and aquatic vegetation were found to explain much of the variation among the data, which verifies the importance of amphibians' microhabitats. While future research comparing generalist and specialist species in the area is necessary, these initial results suggest a hopeful future for collaboration on environmental efforts with low-intensity cattle ranchers.

KEYWORDS. Amphibia, cattle ranching, livestock, vegetation structure, eutrophication.

RESUMEN. Efectos de la ganadería de baja intensidad sobre los anfibios en el Complejo de Humedales Ñeembucú, Paraguay. La ganadería en los humedales afecta a la calidad del agua por excreción que añade nutrientes excesivos, además de reducir la altura y biomasa de vegetación a causa de pisar y comerla. Los anfibios son sensibles a estos cambios a causa de su piel porosa y su dependencia de la vegetación como hábitat. Estudios anteriores que investigaban los efectos del ganado en anfibios descubrieron resultados en conflicto, y así es importante evitar generalizar sobre todas las especies y ambientes. En los humedales de Ñeembucú en Paraguay, los ranchos con una densidad baja de vaca y un cambio mínimo al ambiente son muy comunes. Esta investigación trata de entender como la presencia del ganado en los ranchos afecta la calidad del agua y la riqueza de especies de anfibios, además de cuales factores (eutrofización, nutrientes excesivos o menos diversidad de vegetación) tienen el impacto más grande en los anfibios. Esperábamos que una presencia más alta de ganado causaría daño a la calidad del agua, y por eso, habría menos especies de anfibios presentes. Los datos se recogieron por siete semanas en Estancia Santa Ana, una estancia cerca a Pilar, Paraguay. La presencia de la ganadería se midió por la densidad de heces y huellas, y el medioambiente se analizó por un estudio de la vegetación y pruebas del agua (nitrato, fosfato y oxígeno). Se usan trampas pozos para capturar las ranas y coleccionar información sobre la riqueza de especies y la composición de la comunidad. Se usa la prueba de correlación paramétrica de Pearson y un Factor Analysis of Mixed Data en R para entender las relaciones entre las variables. Pocas relaciones de significado estadístico existen, y las que existen muestran una correlación muy débil. Eso sugiere que la ganadería no tenga un impacto muy grande en la calidad del agua o la riqueza de las especies como esperábamos. Vegetación terrestre y acuática explica la mayoría de la variación en los datos, y eso confirma la importancia de los hábitats de los anfibios. Los resultados sugieren un futuro optimista en cuanto a la colaboración entre los esfuerzos de conservación y los rancheros.

PALABRAS-CLAVE. Amphibia, ganadería, ganado, estructura vegetal, eutrofización.

There is estimated to be over 988 million heads of cattle on Earth as of 2020, with a steady increase over at least the previous 4 years (USDA, 2020). Much of this growth can be attributed to increasing global levels of beef and dairy consumption (Subak, 1999). Research on the impacts of large-scale rearing of cattle shows that the industry contributes heavily to climate change, with 14.5% of all greenhouse gases produced by human efforts coming from the livestock sector, and beef and dairy cattle accounting for 65% of these emissions (Subak, 1999; Gerber *et al.*, 2013;

Rojas-Downing, *et al.* 2017). Smaller, low-intensity cattle rearing efforts also have environmental impacts, though it is reasonable to suggest that they exert a greater direct impact on surrounding ecosystems than global climate change (Subak, 1999). These effects are especially pronounced in wetlands within ranching operations, because of the potential for degradation to water quality, vegetation, and shorelines (Chaneton *et al.*, 1996; Dahlin *et al.*, 2005; Schmutzer *et al.*, 2008; Jones *et al.*, 2011).

Degradation of this kind extends beyond just the habitat itself to affect wildlife within the ecosystem. Amphibians are sensitive to environmental changes because of their reliance on both aquatic and terrestrial habitats, as well as their porous skin that absorbs potentially harmful substances from their environment (Brodeur & Candioti, 2017). While amphibians are not as sensitive to chemical changes as originally expected, these species are generally accepted and used as bioindicators, particularly in their egg and larval stages (Bridges *et al.*, 2002; Brodeur & Candioti, 2017; Jansen & Healey, 2003; Kerby *et al.*, 2009; Nowakowski *et al.*, 2017). Amphibians rely on wetlands for reproduction with their early life stages taking place in water, while post-metamorphosis they generally live on land (Bridges *et al.*, 2002; Brodeur & Candioti, 2017). Anuran movements and habitat use vary significantly across species and environments, and average post-breeding movements range from 38 m to 1810 m (Lemckert, 2004). As adults, amphibians regulate the abundance of their prey, including insects, other small invertebrates, and occasionally small vertebrates, as well as serve as food for higher trophic levels, which illustrates their key role in the wetland food web (Brodeur & Candioti, 2017). As a consequence of their position in the ecosystem and their sensitivity to change, amphibians are vulnerable to the impacts of cattle on wetlands (Bridges *et al.*, 2002; Jansen & Healey, 2003; Kerby *et al.*, 2009; Brodeur & Candioti, 2017; Nowakowski *et al.*, 2017).

Cattle impact water chemistry through excretion of waste (manure and urine), which is rich in nutrients, especially nitrogen and phosphorus (Dahlin *et al.*, 2005; He *et al.*, 2016). In fact, over 80% of nitrogen and 50% of phosphorus that cattle consume are excreted (Dahlin *et al.*, 2005; Brundage, 2010). Raw excreted manure can leach nutrients into the ground or runoff into nearby surface water--nitrogen, particularly in forms of nitrate and ammonium, is highly soluble, as is phosphorus (Dahlin *et al.*, 2005; He *et al.*, 2016). Both nitrogen and phosphorus are essential for plant growth, and when present in excess in the water, they contribute to eutrophication (Chaneton *et al.*, 1996; He *et al.*, 2016).

Nutrient deposition via cattle excretion is harmful to amphibians in two ways: nitrogen poisoning and eutrophication. Nitrogen in many forms is toxic to amphibians, and nitrate at concentrations higher than 2.5 mg/L has been found to have adverse effects on movement, physical abnormalities, and survival (Schmutzer *et al.*, 2008; Rouse *et al.*, 1999). High nitrogen concentrations, especially in the form of ammonia, also negatively impacts multispecies reproductive success as it can be lethal to embryos, decrease survival of larvae, and reduce postmetamorphic recruitment (Knuston *et al.*, 2004; Burton *et al.*, 2009; Schmutzer *et al.*, 2008). Pollutants in water have a great effect on increased amphibian abnormalities, a small effect on decreased mass and survival, no conclusive effect on hatching time and metamorphosis, and an overall negative impact on amphibians that is more dependent on the type of pollution than the amphibian phylogeny (Egea-Serrano *et al.*, 2012).

Eutrophication also presents an issue because it can lead to the emergence of certain parasites that affect amphibians' early life cycle, cause abnormalities and limit survival (Johnson *et al.*, 2007; Battaglin *et al.*, 2016). While some data suggest that varied oxygen levels between cattle access or non-access wetlands do not strongly correlate with amphibian diversity, a positive relationship was found between dissolved oxygen and species richness (Peltzer & Lajmanovich, 2004, as cited in Schmutzer *et al.*, 2008; Verga *et al.*, 2012). This difference may be due to the fact that dissolved oxygen for amphibian larvae should not fall lower than 2 ml/L in lentic water, and when dissolved oxygen levels are above this critical level, no correlation with amphibian diversity is detected (Schmutzer *et al.*, 2008; Verga *et al.*, 2012).

The presence of cows decreases the biomass of wetland plants due to trampling and herbivory, and there is a negative correlation between the intensity of cattle grazing and the height of plants (Ausden *et al.*, 2005; Jones *et al.*, 2011). Tall plants are important and abundant parts of typical wetland communities, and their loss reduces heterogeneity of wetland plant structures, which is important to the effectiveness of ecosystem regulation and function and provides varied habitat for wildlife (Jones *et al.*, 2011). Amphibians in particular rely on aquatic microhabitats for egg laying, and both aquatic and fringing shoreline vegetation provide protection from predatory fish and inclement weather as adults and tadpoles (Jansen & Healey, 2003; Burton *et al.*, 2009). Degradation or loss of these microhabitats due to grazing negatively impacts amphibians, most notably frog community structure and diversity (Jansen & Healey, 2003; Burton *et al.*, 2009). Increased microhabitats around a wetland may also indirectly influence amphibian community structure and diversity by influencing the structure of macroinvertebrate communities, on which amphibians prey (Brodeur & Candioti, 2017; Gleason *et al.*, 2018)

Despite all this information, conflicting findings exist about the overall impact of cattle on amphibians in wetlands. Mean species richness of overall amphibian larvae is 2.7 times higher and mean larval abundance (for select species) at least 2.9 times higher in non-access wetlands than cattle-access wetlands (SCHMUTZER *et al.*, 2008). Similarly, ponds used by livestock display negatively impacted multispecies reproductive success (KNUTSON *et al.*, 2004). Other data suggest that cattle do not have an overwhelmingly negative effect on amphibians and can even sometimes contribute positively to species that live in the open-canopy (HOWELL *et al.*, 2019). There are no significant differences in species richness between grazed and ungrazed grassland/woodland streams, and species richness increases in ponds grazed by cattle (VERGA *et al.*, 2012). Effects of cattle grazing vary depending on species, and the benefits or drawbacks of cattle grazing cannot be generalized for all amphibians (BURTON *et al.*, 2009). This illustrates the need for further research into the effects of cattle on wetlands and amphibian community composition and species richness, especially taking into account the unique habitat structures and amphibian communities in different areas.

Northeastern Argentina's Chaco ecosystem faces disturbance from cattle grazing, with notable decreases to the biomass of grasses in the Dry Chaco and changes to vegetation structure which affects amphibian species composition in the Humid Chaco (ADAMOLI *et al.*, 1990; CANO & LEYNAUD, 2009). Similarly, in the adjacent Ñeembucú department of Paraguay, much of land is dedicated to low-intensity cattle grazing on estancias, with over 70% of land used for pastures in 2008 (DIRECCIÓN DE CENSOS Y ESTADÍSTICAS AGROPECUARIAS, 2009). As a wetland complex, protecting this ecosystem and its wetland-dependent species is a conservation priority (NEIFF, 2001). To this end, knowing the extent to which cattle ranching disrupts natural ecosystems and the wildlife within them, namely amphibians, will be beneficial in finding balance between the two land uses. This study explored three main research questions: (1) Does increased cattle presence (indicated by cow pie and pugging) correspond with changes to water chemistry of wetlands embedded within cattle ranches? (2) Do varying levels of cattle presence change the species richness of amphibian communities? (3) Which effects (eutrophication, nutrient loading, or decreased vegetation height diversity) are more important in explaining impacts on amphibian communities?

We hypothesized that increased cattle presence (cow pie and pugging density) will negatively impact wetland condition (water chemistry and vegetation), which will change the amphibian species composition and lower species richness. We anticipate that all effects from grazing will play a part in these changes to amphibian communities, but nitrogen levels may be the most prevalent factor.

METHODS

Study site. The Ñeembucú Wetland Complex (ÑWC) is a patchwork of rivers, streams, and flooded grasslands in Southern Paraguay. Estancia Santa Ana is a low-intensity cattle ranch situated in the ÑWC (26°51'07"S, 58°02'31"W) made up of a mosaic of freshwater swamps, grasslands, and forests. Throughout the study period from mid-February to mid-April 2021, the average temperature was 28°C, with a high of 35°C and a low of 16°C. Rainfall was recorded as 62.6 cm over 11 days in February, 361.7 cm over 12 days in March, and 171 cm over 10 days in April.

Data collection. Data was collected over a seven-week period at eight sites located at Estancia Santa Ana (Fig. 1). Four sites were placed at a swamp-forest border and four were placed at a swamp-grassland border. All sites were a minimum of 100 meters apart. This distance is limited due to the size of the property that field work was permitted to be conducted on, as well as a year-long drought which limited the availability of water pools. While not enough data is available to determine confident home range sizes of species in the area, a mean figure of 1,772 m^2 based on 18 studies (generally referring to non-breeding areas) suggests this distance may be enough to reduce data replication (LEMCKERT, 2004). At each site, an 18 meter transect was placed parallel to the edge of the swamp. The distance from the edge was approximately 5 meters, taking into account the potential for future flooding and adjusting for obstacles in the habitat when necessary.

To quantitatively measure the intensity of cattle presence, we measured both the amount of cow pie and the degree of pugging. Surveys were conducted Tuesday through Saturday along each transect, extending 2 meters on either side. Within this transect, we counted the number of cow pies, which were then flagged to avoid recount the following days. Pugging, which is a measure of cow trampling, was classified into ranks from 1-6, with 1 being the least pugged and 6 being completely pugged.

At each site, pitfall traps and plastic drift fences were placed along the 18-meter transect. Each site consisted of five traps, placed three meters apart, with the additional transect length extending past the trap lines. The exact locations for the lines were determined by where they would be least intrusive for the landowner and cattle, accommodating environmental obstacles such as flooding or dense vegetation. These traps were opened on Monday mornings, then checked daily from Tuesday to Saturday, before being closed again Saturday morning. At each site, individuals were collected and brought back to the lab to be identified before either being deposited in the *Colleción Científica de Para La Tierra* in Pilar, Paraguay or being released the next day near the same site but away from the pitfall traps. This allowed us to measure amphibian community composition and species richness, as well as to observe any potential individual morphological abnormalities. It is important to note that some species of frogs, particularly tree frogs, are more likely to escape from pitfall traps, but were recorded as normal when observed. Unfortunately, due to local restrictions, we were unable to conduct frogging trips in the evening to seek out these elusive species.

Fig. 1. The eight sites throughout the study, with all "G" sites situated along grasslands and "F" sites situated along forests at Estancia Santa Ana, Pilar, Paraguay.

A modification of the rapid wetland appraisal index (SPENCER *et al.*, 1998) was used to collect data relevant to wetland condition. This index is able to provide data consistent with long-term monitoring that can be collected rapidly, easily, and consistently among different investigators (SPENCER *et al.*, 1998). For the purposes of this study, we choose the metrics most relevant to our question and added additional metrics to measure, but did not follow the same analysis of these metrics as SPENCER *et al.* (1998). We used three subindices: fringing vegetation, aquatic vegetation, and water quality. Detailed explanations of the significance of sub-indices can be found in SPENCER *et al.* (1998).

The sub index "fringing vegetation" was based on the height diversity of the fringing vegetation strip. Along each transect (used for cow presence and amphibian surveys), extending 2 meters on either side, the presence or absence of vegetation in distinct layers was recorded (shrubs/bushes; grasses; herbs including lichens, mosses, and ground covers), as well as the maximum height of each layer. This data was collected once for each site at the start of data collection.

This sub index "Aquatic vegetation" was measured through cover of aquatic vegetation, attached algae, and spatial heterogeneity. Cover of aquatic vegetation is a visual estimate of the percent coverage of aquatic vegetation from the water surface (none, <25, 26-75, >75, complete coverage). Attached algae is another visual estimate, classifying the amount of macroalgae from little to abundant. Both of these measures were taken in a one-meter-wide portion of the edge of the swamp, parallel to the transects at each site. Spatial heterogeneity is a measure of how many layers of aquatic vegetation are present in each wetland, out of five possible layers (free-floating at surface, free-floating beneath surface, emergent, in substrate with floating leaves, submerged/anchored in substrate). This measure was taken at the same point where water quality tests were conducted. This data was collected once for each site at the start of data collection.

Water quality was measured by levels of nitrate, phosphate, and dissolved oxygen in the wetland. Water was tested at a location perpendicular to the center of the pre-established transects, 1 meter past where the ground became saturated. An ExStik DO600 dissolved oxygen meter was used to record dissolved oxygen levels in mg/L in the field. We aimed to measure dissolved oxygen as early as possible in the morning, to reduce fluctuations caused by photosynthesis. Water samples were also collected from the same location and the levels of nitrate and phosphate were analyzed in the lab using a Hach surface water test kit. These measurements were taken each Monday after opening the pitfall traps, except for the first week.

Data analysis. Data analysis was carried out in R Studio Version 4.0.5. GUI 1.74 Catalina build. For each site, daily numbers for cow pie, cow pugging rank, number of frog species caught, and total number of frogs caught were paired with the weekly nitrogen, phosphate, and dissolved oxygen levels. Using *R*, a Pearson parametric correlation test was used to explore the relationships between all variables.

The packages "FactoMineR" and "factoextra" were used to run a Factor Analysis of Mixed Data on the vegetation survey data and the total species richness found at each site (HUSSON *et al.*, 2020; KASSAMBARA & MUNDT, 2020).

RESULTS

Cattle presence, water quality, and frog surveys. The correlation analysis performed on water quality data, cow presence indicators, and frog survey results indicates 11 statistically significant relationships (α of 0.05) of the 21 relationships described (bold in Tab. I). Of these statistically significant relationships, the highest correlations are found between cow pie-cow pugging, phosphate-number of frogs, nitrate-number of species, phosphate-number of species, and number of frogs-number of species (Tab. I, Fig. 2). Still, excluding cow pie-cow pugging and number of frogs-number of species, both of which have logically medium and large positive correlations, all correlation coefficients fall below |0.3|, making them only small correlations.

Vegetation structure and species richness. The Factor Analysis of Mixed Data (FAMD) performed on the data acquired from the terrestrial and aquatic vegetation surveys and species richness from each site reveals that a majority of the variation can be attributed to two dimensions (Tab. II; Fig. 3). Grasses, habitat, and aquatic vegetation cover and shrubs, aquatic vegetation cover, and spatial heterogeneity contribute the most to these two dimensions respectively (Figs 4, 5). The correlations of quantitative and qualitative variables along these two dimensions are varied in their distribution and their level of contribution (Figs 6, 7, 8).

The same FAMD was also performed with a breakdown of the abundance of species found at each site (Fig. 9). While this data does not contribute to answering our study questions, it is interesting to note that species are relatively diverse in the environmental factors that most influence their abundance (Fig. 10). The first dimension (grass height, habitat type, and aquatic vegetation cover) and second dimension (shrub height, aquatic vegetation cover, and spatial heterogeneity) explain a majority of the variation across species. Generally, it appears that higher species diversity and number of individuals were found in areas without grass, with lower aquatic vegetation cover, and higher spatial heterogeneity (Tab. III). Despite this variety in environmental influences, very few of the studied species could be considered specialists (Tab. III).

Tab. I. The correlation coefficients and p-values of all the relationships between water quality factors (nitrate, phosphate, and dissolved oxygen), cow presence indicators (cow pie and cow pugging), and frog survey results (number of total frogs and number of species) at Estancia Santa Ana, Pilar, Paraguay.

row	*column*	*cor*	*p*
Nitrate	**Phosphate**	**-0.175206720**	**1.136568e-02**
Nitrate	**Dissolved Oxygen**	**0.159891346**	**2.105754e-02**
Phosphate	Dissolved Oxygen	-0.029343144	6.402900e-01
Nitrate	Cow Pie	-0.059603340	4.540478e-01
Phosphate	Cow Pie	0.134274599	5.801027e-02
Dissolved Oxygen	Cow Pie	-0.084237195	2.356476e-01
Nitrate	Cow Pugging	-0.112148875	1.579738e-01
Phosphate	**Cow Pugging**	**0.162834479**	**2.123440e-02**
Dissolved Oxygen	Cow Pugging	-0.006552536	9.266289e-01
Cow Pie	**Cow Pugging**	**0.393461597**	**2.608318e-10**
Nitrate	**Number of Frogs**	**-0.180834725**	**1.429532e-02**
Phosphate	**Number of Frogs**	**0.280363220**	**2.150060e-05**
Dissolved Oxygen	**Number of Frogs**	**0.192438911**	**3.919284e-03**
Cow Pie	Number of Frogs	-0.036136606	5.774693e-01
Cow Pugging	Number of Frogs	0.056530661	3.832660e-01
Nitrate	**Number of Species**	**-0.232873559**	**1.511847e-03**
Phosphate	**Number of Species**	**0.224952465**	**7.147633e-04**
Dissolved Oxygen	**Number of Species**	**0.148956282**	**2.612729e-02**
Cow Pie	Number of Species	-0.007299487	9.104315e-01
Cow Pugging	Number of Species	0.059728975	3.568890e-01
Number of Frogs	**Number of Species**	**0.632669179**	**0.000000e+00**

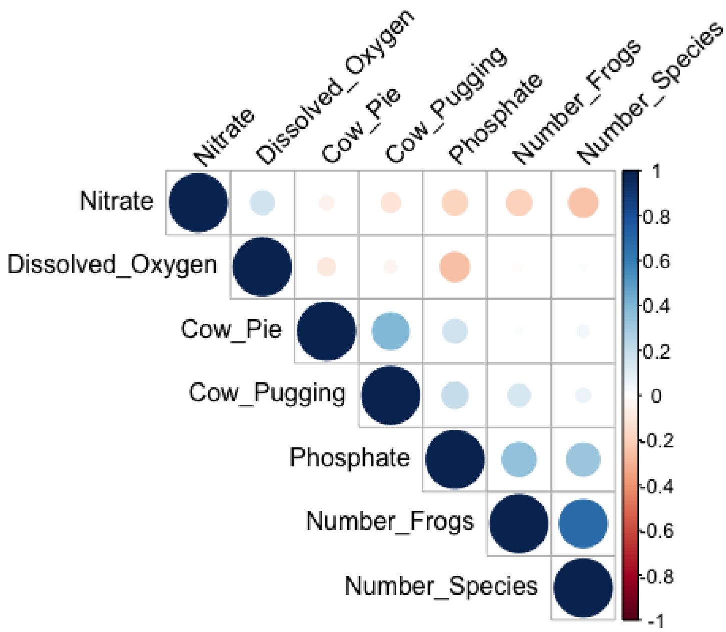


Fig. 2. A correlation graph visualizing the correlation matrix obtained from analysis of water quality factors, cow presence indicators, and frog survey data at Estancia Santa Ana, Pilar, Paraguay. A visual representation of Tab. I.

Tab. II. The Eigenvalues for the first five dimension and how much variance they explain in the data regarding vegetation height structure and species richness at Estancia Santa Ana, Pilar, Paraguay.

	Eigenvalue	Variance Percent	Cumulative Variance Percent
Dim. 1	2.8825369	32.028188	32.02819
Dim. 2	2.7061929	30.068810	62.09700
Dim. 3	2.1254064	23.615627	85.71263
Dim. 4	0.8810299	9.789221	95.50185
Dim. 5	0.2662199	2.957999	98.45984

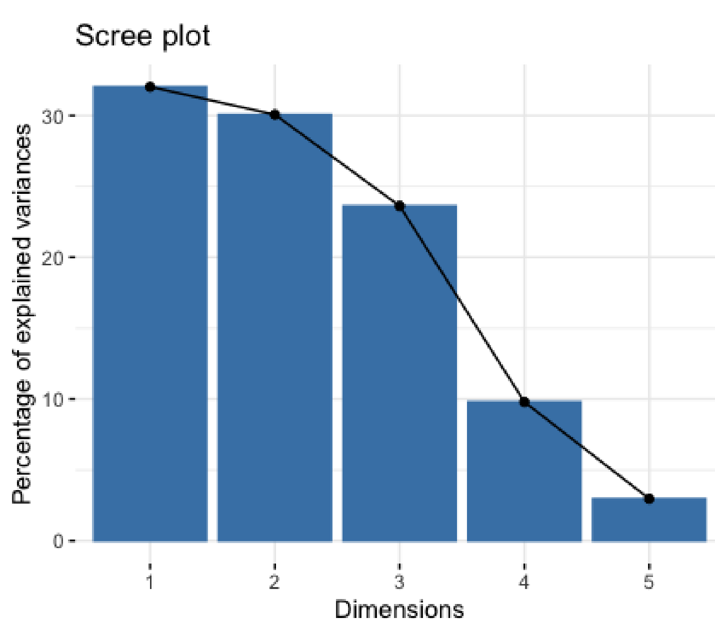
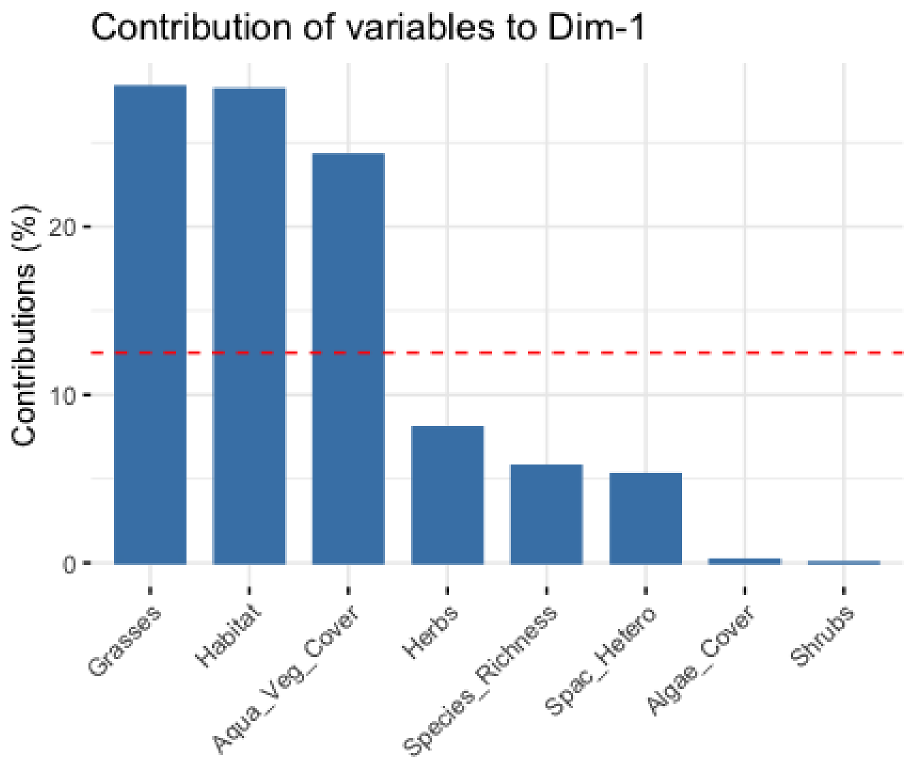
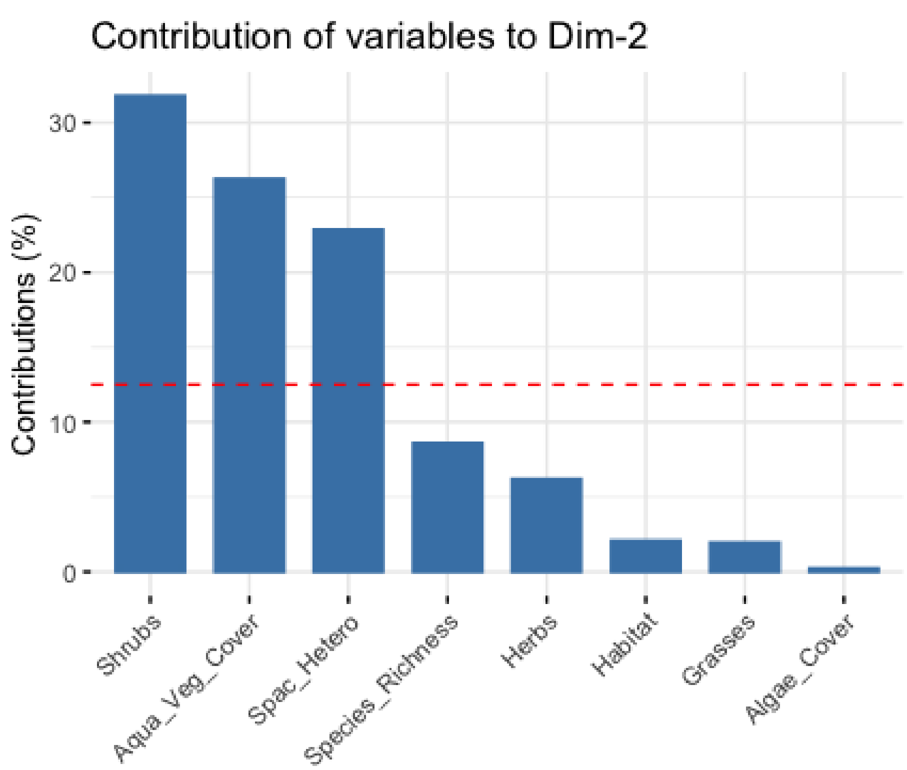


Fig. 3. A Scree plot of eigenvalues, describing how much variance is attributed to each dimension. A visual representation of Tab. II.





Figs 4, 5. The contributions of each variable to dimensions 1 and 2 respectively. The red line marks the expected average contribution.

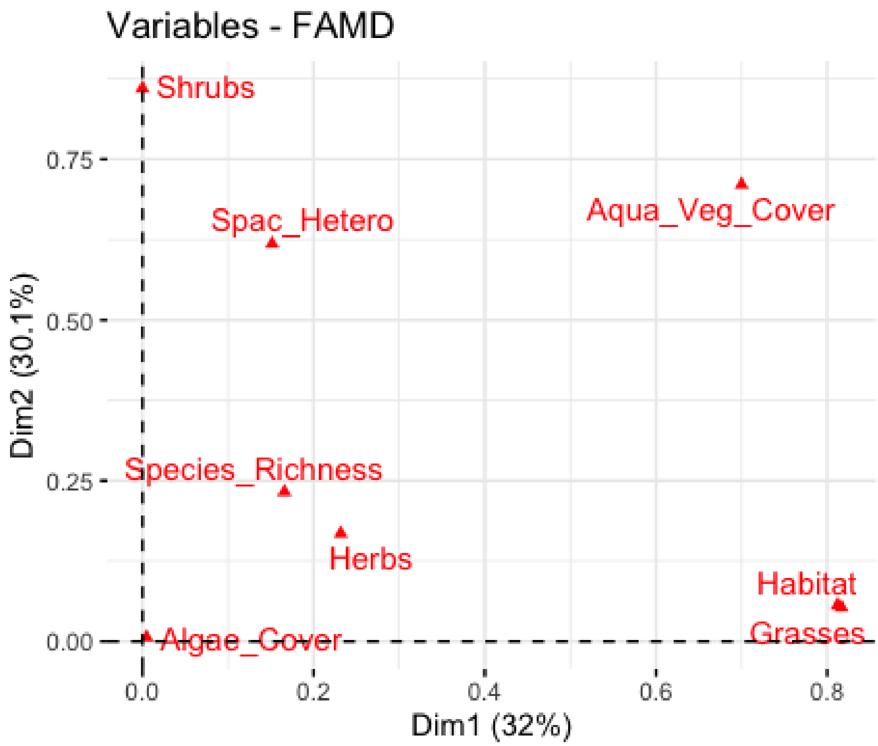


Fig. 6. A plot showing the correlation between both qualitative and quantitative variables along the first two dimensions.

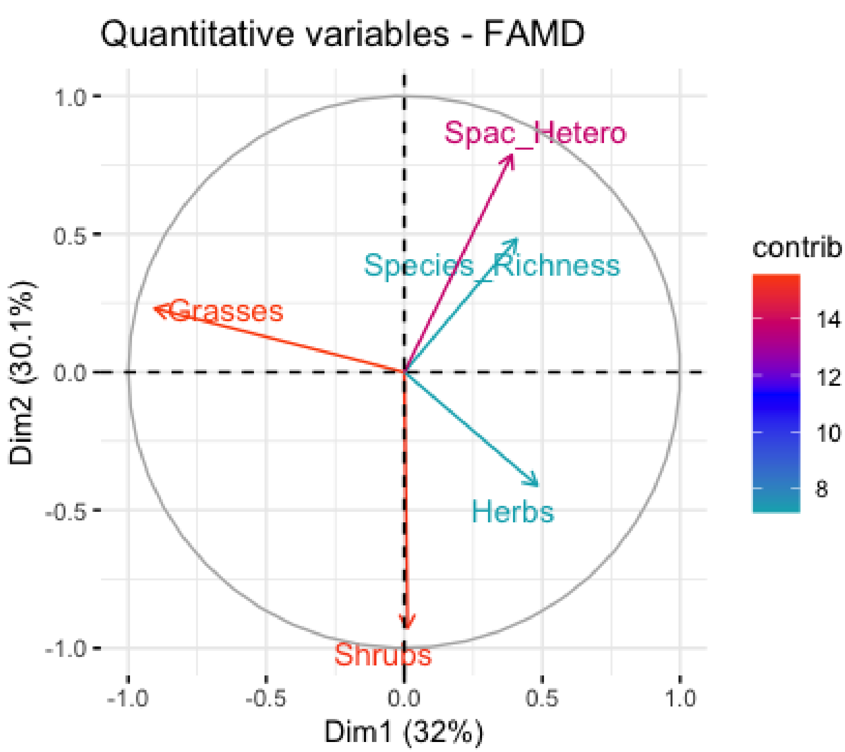


Fig. 7. A correlation circle showing the relationship between quantitative variables, the quality of their representation, and their relationship with the first two dimensions, with colors corresponding to the most contributing variables.

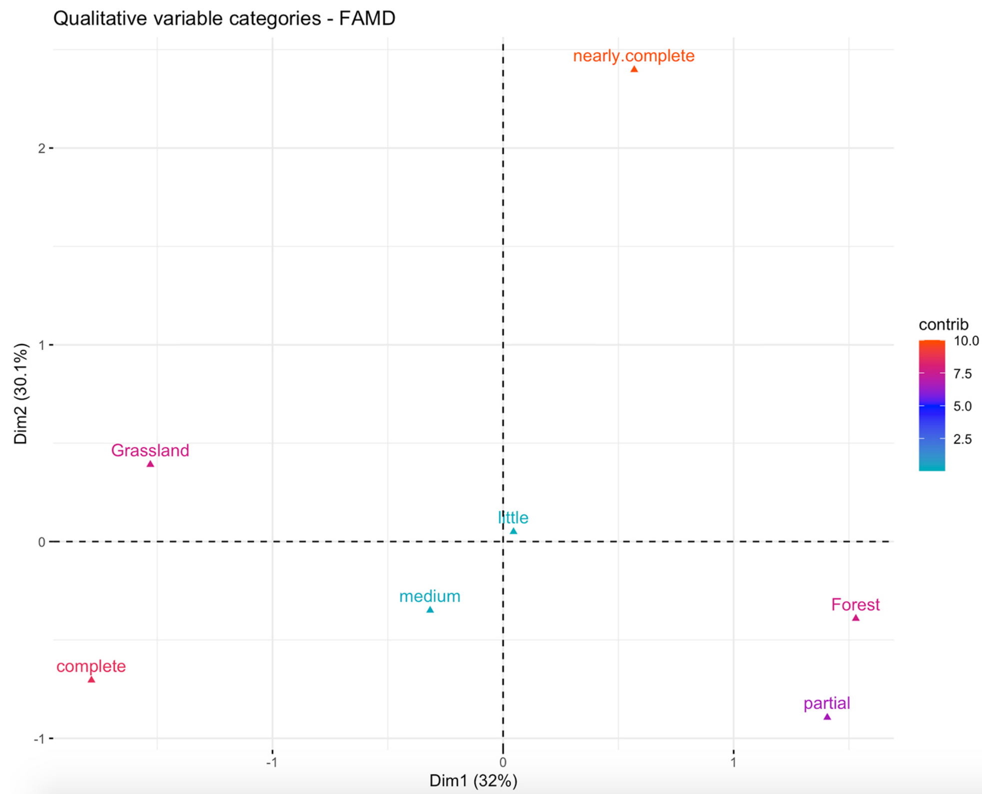


Fig. 8. A plot showing the relationship between qualitative variables, the quality of their representation, and their relationship with the first two dimensions, with colors corresponding to the most contributing variables.

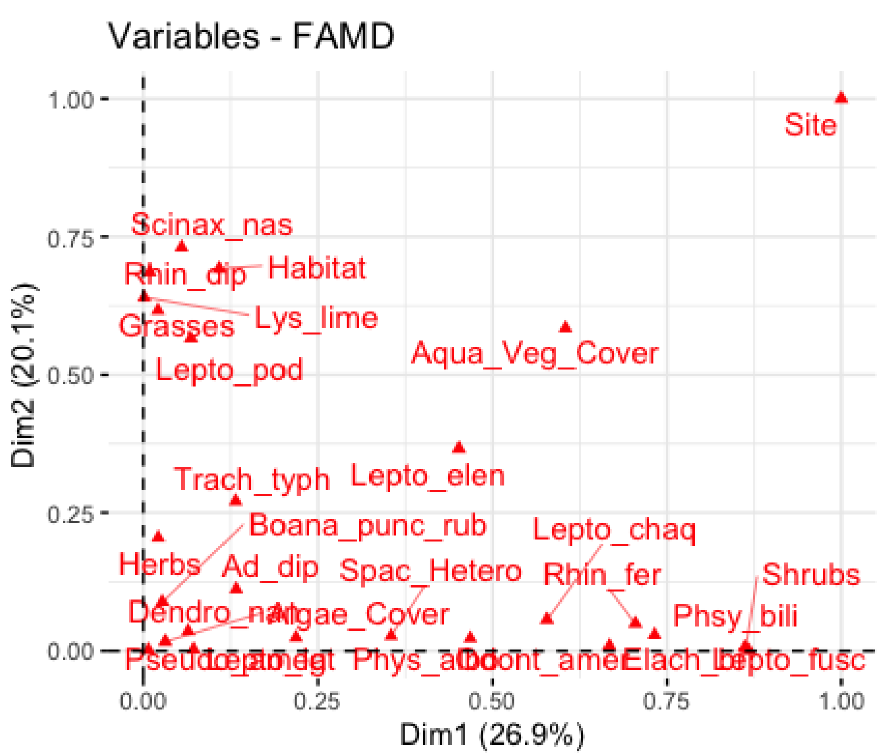


Fig. 9. A plot showing the correlation between frog species and qualitative habitat variables along the first two dimensions at Estancia Santa Ana, Pilar, Paraguay.

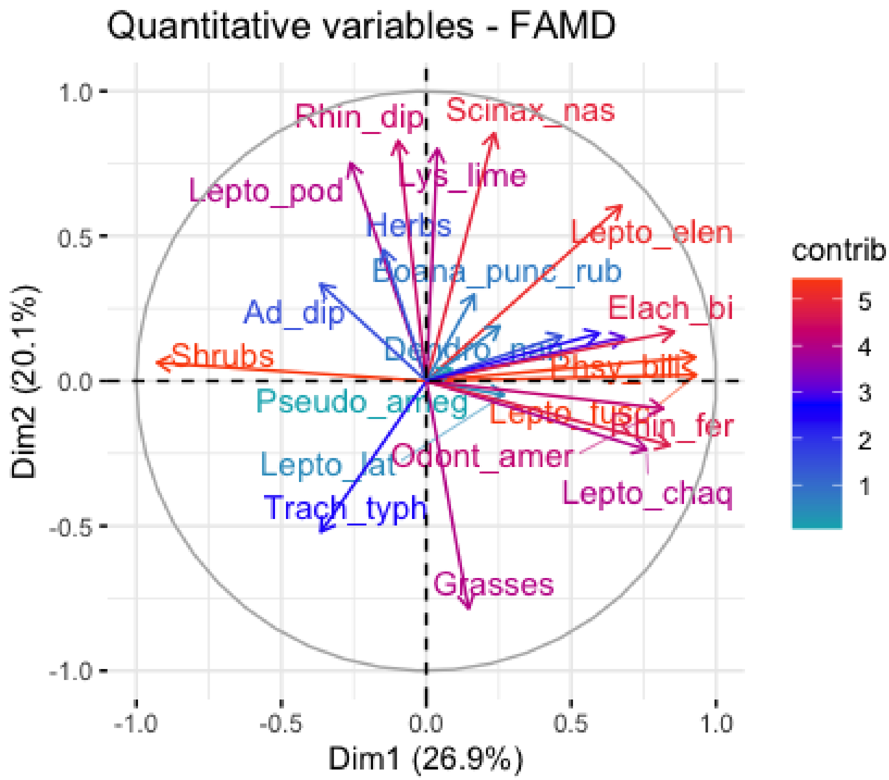


Fig. 10. A correlation circle showing the relationship between frog species, qualitative habitat variables, the quality of their representation, and their relationship with the first two dimensions, with colors corresponding to the most contributing variables.

DISCUSSION

Despite a restricted spatial and temporal scale, this analysis is an important look into the impact of cattle ranching in the currently understudied Humid Chaco of Paraguay. The results indicate that overall, the cattle presence indicators of cow pie and pugging ranking in each transect were lower than initially anticipated. This suggests that the low-intensity ranching practices at Santa Ana limit an overwhelming presence of cattle at any one site, likely also aided by the rotation of cattle to different locations on the estancia over time. Similarly, very low levels of nitrogen were detected, which may be explained by significant nitrate uptake by the plants in the wetland (KIRK & KRONZUCKER, 2005). While this study did not compare water quality data and vegetation structure, there is a possibility that increased spatial heterogeneity contributes to denitrification processes in wetlands, which would explain the low nitrate levels found (WEISNER *et al.*, 1994). In comparison, phosphate levels were higher, which may suggest influencing factors beyond cattle presence, such as fertilizers used for nearby rice farming. Finally, while dissolved oxygen levels fell below the critical 2 mg/L threshold at least once at six sites, these events were relatively uncommon and the average dissolved oxygen levels across the whole study was greater than 2 mg/L for all eight sites (SCHMUTZER *et al.*, 2008). With the understanding of these overall results, we can look at how our analyses answer each of the research questions laid out in this study.

Does increased cattle presence (indicated by cow pie and pugging) correspond with changes to water chemistry of wetlands embedded within cattle ranches? The only statistically significant relationship found between a cow presence indicator and a water quality factor is a very weak positive relationship between cow pugging and phosphate. Both the lack of statistically significant relationships and a very weak correlation in the one statistically significant relationship suggests that cow presence does not have as strong of an influence on water quality as expected. This may be because the intensity of cow presence was low enough to limit cattle effects on the wetland, or it may suggest that cow pie and pugging are not the appropriate factors to explain any changes being seen in water quality.

Do varying levels of cattle presence change the species richness of amphibian communities? There was no statistically significant relationship found between cow presence indicators and amphibian species richness, nor the number of individuals found at each site, showing that the intensity of cattle presence was not found to greatly impact amphibian species richness. This seems logical if it is accepted that cows did not impact water quality in a

Tab. III. A breakdown of environmental factors and species recorded at each site at Estancia Santa Ana, Pilar, Paraguay. An asterisk indicates a species that may be considered a specialist species due to habitat preferences.

	G1	G2	G3	G4	F1	F2	F3	F4
Fringing vegetation								
Max tree height (m)	–	–	–	–	> 10	> 5	> 10	>10
Max shrub height (cm)	190	172	–	110	132	221	141	137
Max grass height (cm)	231	218	195	–	–	–	–	–
Max herb/ground cover height (cm)	28	24	24	32	25	32	32	30
Aquatic vegetation								
Vegetation Cover (none, <25%, 26-75%, >75%, complete)	complete	complete	> 75%	complete	26-75%	26-75%	> 75%	26-75%
Attached Algae Cover (little, medium, abundant)	little	little	little	medium	little	little	little	little
Spatial Heterogeneity (# of layers)	2	2	5	3	5	2	4	3
Species (# of individuals)								
Adenomera diptyx	1	2	4	1	9	29	6	4
Boana punctata rubrolineata	–	–	–	–	–	–	3	–
Dendrosophus nanus	–	–	–	2	–	–	3	–
Elachistocleis bicolor	2	3	19	1	–	1	13	8
Leptodactylus chaquensis	2	3	23	22	–	4	–	4
Leptodactylus elenae	1	1	8	5	1	1	6	11
Leptodactylus fuscus	–	–	8	4	–	–	1	3
Leptodactylus latrans	16	1	16	25	4	14	4	14
Leptodactylus podicipinus	46	46	90	168	174	382	79	360
**Lysapsus limellum*	–	–	–	–	–	–	1	4
Odontophrynus americanus	–	1	9	4	–	4	4	–
Physalaemus albonotatus	–	32	89	4	21	46	42	30
Physalaemus biligonijerus	–	9	37	32	–	–	21	18
**Pseudopaludicola ameghini*	1	15	3	7	–	–	2	11
**Pseudopaludicola mystacalis*	–	7	4	5	1	1	5	5
Rhinella diptycha	–	–	–	–	1	5	2	20
Rhinella fernandezae	–	–	1	–	–	–	–	–
Scinax nasicus	–	–	2	3	1	1	6	11
Trachycephalus typhonius	1	–	–	–	–	–	–	–

significant way. It is important to note also that this statistical analysis does not consider community composition and the types of frogs (*i.e.*, specialist vs generalist species) recorded at each site. Anecdotally, most of the species recorded are widespread generalist species, which may suggest that wetlands within low-intensity ranching operations are not suitable for specialist species. As future research focuses more on this information, a clearer image may emerge of the impact of cattle on amphibian communities.

Which effects (eutrophication, nutrient loading, or decreased vegetation height diversity) are more important in explaining impacts on amphibian communities? In terms of water quality, statistically significant relationships were found between phosphate and species richness, phosphate and total number of frogs, and nitrate and species richness. The relationship phosphate has with both species richness and total number of frogs is characterized by a weak positive correlation. The fact that it is a positive correlation actually suggests that increased phosphate levels correspond to more frogs and more frog species. This fact is offset by the weak nature of this correlation. In contrast, nitrate and species richness have a negative relationship, meaning that increased nitrate levels lower the number of species found, which can be explained by the negative effects of nitrogen in water (ROUSE *et al.*, 1999; KNUTSON *et al.*, 2004; SCHMUTZER *et al.*, 2008; BURTON *et al.*, 2009). Still, the correlation is weak, which is understood by the low levels of nitrate found at each site, never exceeding the 2.5 mg/L threshold (ROUSE *et al.*, 1999).

All other statistically significant relationships between water quality and amphibian data (N-P, N-dO, N-frog number, dO- frog number, dO-species richness) were found to have only a low correlation. This weak relationship suggests that even though a relationship was found, the influence each variable has on the other is miniscule. As previously mentioned, these results can largely be understood by the low levels of both nitrogen and dissolved oxygen found, typically outside of the range of potentially harmful levels for both nutrients.

The FAMD reveals that a majority of the vegetation and amphibian data can be explained by the first two dimensions, made up of grass height, habitat type, and aquatic vegetation cover, and shrub height, aquatic vegetation cover, and spatial heterogeneity respectively. It is notable that the first dimension is largely made up of terrestrial vegetation, while the second is mainly aquatic vegetation. It makes sense that terrestrial vegetation, particularly grass height and habitat type, would explain a significant amount of variation in this data because of their importance in providing microhabitats for adult frogs within each transect to avoid predators, inclement weather, and the direct sun. The same is true for aquatic vegetation cover, which shows up in both the first two dimensions, because it provides protection for frogs both pre- and post-metamorphosis in the water. In the second dimension, shrub height may explain variation for a similar reason. Spatial heterogeneity likely plays an important role in explaining the data because once again, the various microhabitats in the vertical layers of the water provide different needs for frogs throughout their lifecycle.

In contrast, species richness and herbs (ground cover) height explain very little variation within the data, and algae cover explains essentially none. This reveals that species richness does not strongly influence the terrestrial or aquatic vegetation, which is what would be expected. It makes sense that herb height and algae cover would not explain much variation in the data, because neither are microhabitats that adult frogs, the focus of this study, rely upon strongly.

The implications of these findings actually suggest a hopeful future for the cooperation of conservation and low-intensity cattle ranching. Due to the very low-intensity nature of the ranching, it seems that maintaining natural wetland habitats on estancias may be enough to sufficiently regulate water quality and sustain diverse vegetation structures, and amphibian communities are not at risk of negative impacts from any of these factors. If conservation efforts are clearly presented as simply maintaining natural ecosystems, rather than forcing changes to ranching practices, ranchers may be more willing to work with conservation organizations and feel less threatened by scientific efforts in the area.

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