

The impacts of wild boars (*Sus scrofa*) on fish community and on instream physical habitat of two small streams in a protected area in Brazil

Correspondence:
Marina F. Moreira
marina.moreira10@gmail.com

 Marina F. Moreira,  Larissa B. S. Milhomens,  Pedro L. C. Uzeda,
 Ana Luiza V. Andrade and  Paulo S. Pompeu

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Despite the wild boar's alarming invasive status, little is known about its impacts on aquatic environments. We assessed whether there were changes in the physical habitat of Neotropical streams and their ichthyofaunas after the record of wild boar populations in a conservation unit in Southeastern Brazil. We characterized the physical habitat and the fish community of two streams five times between 2009 and 2022. We found differences after the wild boar invasion. The amount of fine sediment inside the streams and exposed soil increased, while the bank angle decreased. There were also modifications regarding the fish fauna, including the local disappearance of two species and the constant decline of others. Although the sampling design does not allow for the establishment of a cause-effect relationship, these changes appear to be related to alterations in the physical habitats due to the wild boar's behavior of forming large puddles in the stream beds. This is the first long-term study to make indirect inference of a possible effect of wild boar invasion on fish community level on streams. We highlight the urgent need for similar studies in aquatic environments, especially in tropical regions, where the abundance and distribution of wild boars are increasing.

Keywords: Aquatic community, Conservation unit, Environmental impact, Invasive species, Neotropical streams.

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Departamento de Ecologia e Conservação, Universidade Federal de Lavras - UFLA, Trevo Rotatório Professor Edmir Sá Santos, s/n, 37200-000 Lavras, MG, Brazil. (MFM) marina.moreira10@gmail.com (corresponding author), (LBSM) larissamilhomens@outlook.com, (PLCU) pedro.lc.uzeda@gmail.com, (ALVA) analuizaandrade98@gmail.com, (PSP) pompeups@gmail.com.

Apesar do alarmante estado de invasão do javali, pouco é conhecido sobre seus impactos em ambientes aquáticos. Nós avaliamos se houve modificações no habitat físico de riachos Neotropicais e suas ictiofaunas após o registro de populações de javali em uma unidade de conservação no Sudeste do Brasil. Realizamos a caracterização do habitat físico e da comunidade de peixes de dois riachos por cinco vezes, entre 2009 e 2022. Encontramos diferenças após a invasão do javali. Houve um aumento na quantidade de sedimento fino no interior dos riachos e no solo exposto, além da diminuição do ângulo das margens. Encontramos também mudanças na ictiofauna de ambos os riachos, incluindo o desaparecimento local de duas espécies, e o constante declínio de outras. Embora o desenho experimental não permita o estabelecimento de relações de causa-efeito, essas mudanças parecem estar relacionadas com alterações no habitat físico devido ao comportamento do javali em promover a formação de poças de lama nos riachos. Esse é o primeiro estudo de longo prazo a avaliar possíveis impactos do javali sobre comunidades de peixes de riachos. Nós ressaltamos a urgência de estudos similares em ambientes aquáticos, especialmente na região tropical, onde a abundância e distribuição dos javalis está aumentando.

Palavras-chave: Comunidade aquática, Espécies invasoras, Impacto ambiental, Riachos neotropicais, Unidade de conservação.

INTRODUCTION

The integrity of aquatic ecosystems has been threatened by several factors associated with the expansion of anthropogenic action, related to the multiple use of water and soil. Among the main anthropogenic actions that negatively impact aquatic communities, including ichthyofauna, are the introduction of invasive species, the construction of dams and hydroelectric power plants, water pollution, and destruction of riparian forests (Reis *et al.*, 2017; Santos *et al.*, 2017). Invasive species are considered the second greatest threat to biodiversity loss by Invasive Species Specialist Group (ISSG, 2011), being responsible for over USD 1,288 trillion in economic impacts over the last five decades (Diagne *et al.*, 2021). Among the multiple impacts caused by invasive species worldwide, changes in the composition of ecosystems and a decline in biodiversity are predominant (Doherty *et al.*, 2016; Crystal-Ornelas, Lockwood, 2020).

Wild boars (*Sus scrofa* Linnaeus, 1758) are among the world's most problematic invasive species by Global Invasive Species Database (GISD, 2023). This is attributed to the economic impact associated with the species due to the destruction of cultivated areas and its ability to intensely modify components of the ecosystem, which qualifies the species as an ecosystem engineer (Sandom *et al.*, 2013; Doutel-Ribas *et al.*, 2019). Due to the historical introduction of wild boars in Brazil, their invasion process is currently concentrated in the South and Southeast regions (Pedrosa *et al.*, 2015; Rosa *et al.*, 2017), which represent some of the most important areas in terms of freshwater fish diversity worldwide (Abell *et al.*, 2008; Roxo *et al.*, 2012). With increasing occurrences of this species in the wild, studies have registered its presence around 46 national or state-

protected areas in the country (Kmetiuk *et al.*, 2023), an alarming finding considering the importance of these areas for biodiversity conservation.

Among the main environmental impacts caused by invasive wild boars are changes in soil structure and the reduction of vegetation cover, which are mainly related to their resource exploitation of digging and rooting for tubercles, root vegetables and invertebrates (Mitchell *et al.*, 2007; Morais *et al.*, 2019). As an omnivorous generalist, the wild boar presents a wide range of trophic niche, acting both as predator and herbivore, causing local exclusion of native species by predation, competition and habitat modification (Risch *et al.*, 2021). Additionally, their need for body temperature regulation promotes the formation of large puddles in water bodies (Barrett, 1978; Coblenz, Baber, 1987; Cuevas *et al.*, 2013), increasing sedimentation and affecting nutrient cycling, turbidity, pH, and dissolved oxygen concentration of the water (Zengel, Conner, 2008; Helcel *et al.*, 2018). In more severe cases, it may lead to anoxia in this aquatic environment (Bolds *et al.*, 2021). In Southeast Brazil, it is also known that its impacts on streams is more intense than those caused by the native white-lipped peccaries (Rosa *et al.*, 2019).

Changes in instream physical habitats can lead to reduced channel stability and loss of important microhabitats for ichthyofauna (Leal *et al.*, 2014). Stream fish communities are also known to present species-specific microhabitat occupation patterns, inhabiting short river stretches with similar environmental conditions (Roff, 1992; Ahmadi-Nedushan *et al.*, 2006). This high habitat specificity makes fish precise bioindicators for habitat changes in headwater streams, since fish communities respond to environmental changes in different aspects of composition, functional diversity and trophic structure (Sharma *et al.*, 2011; Zeni, Casatti, 2014; Carvalho, Tejerina-Garro, 2015; Peressin *et al.*, 2020). However, studies evaluating the effects of wild boar presence directly on fish fauna are non-existent.

Given this context, our aim was to evaluate whether there were changes in the integrity of two small watercourses and their ichthyofauna which could be associated to the record of wild boar populations in a conservation unit in Southeastern Brazil. We tested the following hypotheses: 1) after the introduction of the wild boar, physical habitat variables will be altered due to the species' behavior of promoting the formation of large puddles in the stream beds, and 2) due to the habitat modification, fish community will be negatively impacted in terms of richness and abundance.

MATERIAL AND METHODS

Study area. Our study was conducted in the Araguari River basin, in a portion located within the Reserva Particular do Patrimônio Natural (RPPN) Galheiros, a protected area in Southeast Brazil (Fig. 1) with an area of 2,694.73 hectares. This RPPN was created in 1995 as a response to the damages and environmental impacts caused by the construction of the Nova Ponte hydroelectric power plant (HPP) by the energy company Companhia Energética de Minas Gerais (CEMIG). Before the construction of Nova Ponte HPP, there were 19 streams in the area, including five headwaters. Some of these streams were flooded by the reservoir, and others are intermittent (BRANDT, 2014). Consequently, we were only able to access and sample two streams.

The surrounding area of RPPN Galheiros faces significant pressure from agriculture and livestock. The Cerrado (Brazilian Savanna) and the Atlantic Forest are the main natural vegetation types within the reserve. With a high biodiversity of fauna and flora, this RPPN is considered to be of great importance for conservation (BRANDT, 2014). The study conducted in a protected area ensures that there has been no change in land use over the years of study, thus preventing any influences of anthropic impacts on the results.

Official records of when the wild boar invasion at RPPN Galheiros occurred are absent. However, prior to 2013, neither the Galheiros staff nor our field team had observed its presence. In the samplings conducted in 2019, 2021, and 2022, the presence of wild boars in the reserve was registered by our team through footprints, sightings, camera recordings, camera traps, and mud marks on tree bases caused by rubbing, including in the area of the streams (Figs. 2A, B). Additionally, since 2019, several sections with turned soil and puddles, clearly formed by the foraging and thermal regulation activities of wild boars, have been found in the sampled streams (Figs. 2C, D).

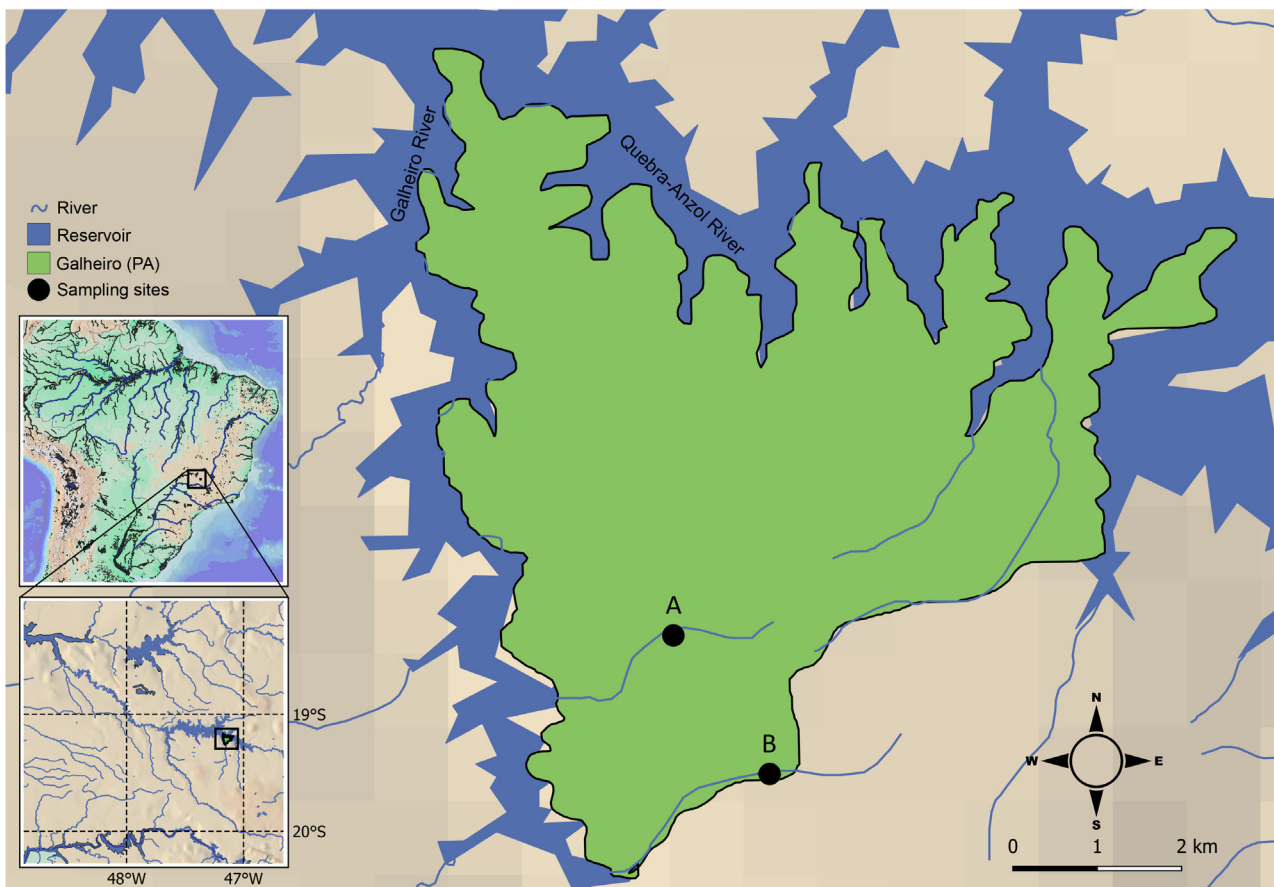


FIGURE 1 | Map of Galheiros protected area (PA), indicating the sampling sites (A and B).

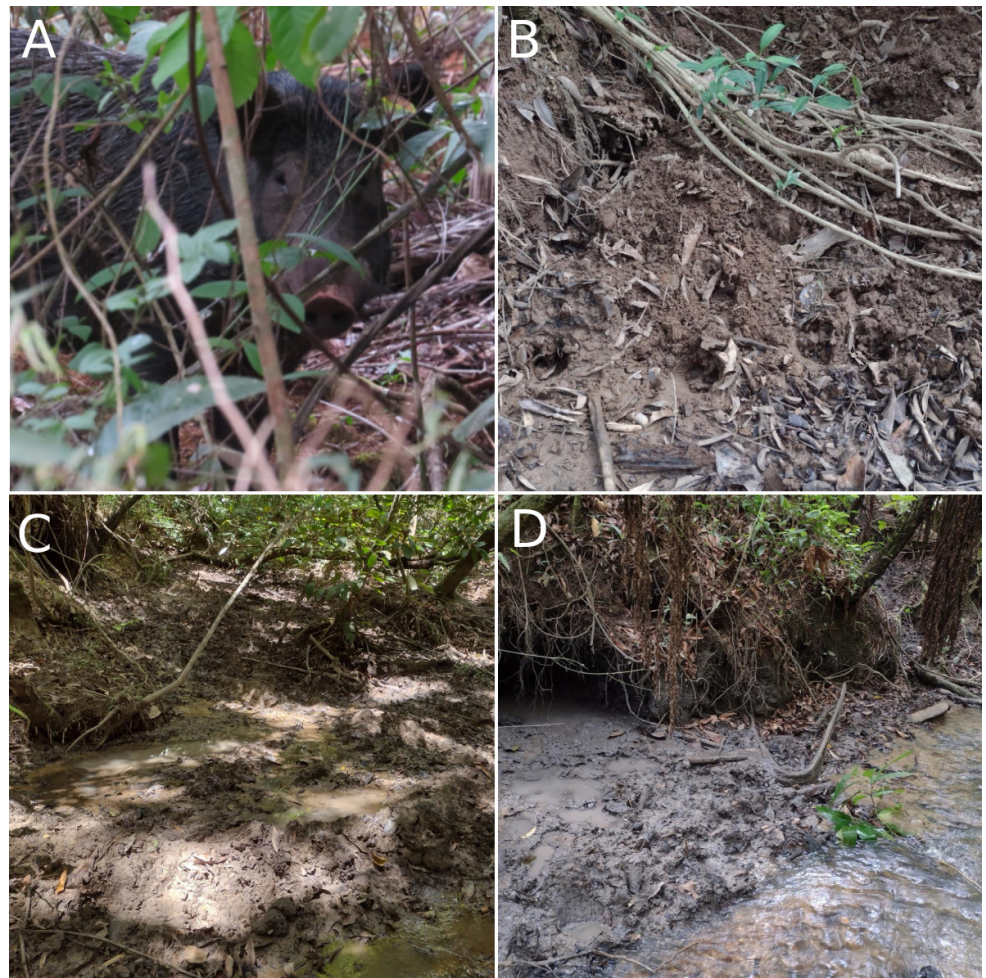


FIGURE 2 | Wild boar records in Galheiros protected area. **A.** Wild boar captured on camera in 2019; **B.** Disturbed soil and wild boar footprints near stream B in 2021; **C.** Disturbed soil and puddles formed by wild boar foraging in stream B area in 2021; and **D.** Disturbed soil and puddles formed by wild boar foraging on the bank of stream B in 2021.

Sampling. Instream physical habitat characterization and ichthyofauna sampling were carried out in two streams of first and second order (A and B, respectively) at RPPN Galheiros. The streams are not connected to each other and flow into one of the branches of the Nova Ponte reservoir. Sampling was conducted on five occasions: September 2009 and 2013, October 2019 and 2022, and November 2021, trying to maintain the same sampled stretch in each stream.

Each stream was divided into ten sections, each with a length of 15 m, by 11 cross-sectional transects, totaling 150 m of sampling. Both physical habitat assessment and fish collection were conducted in each section, moving from downstream to upstream. The method for physical habitat measurements was based on the protocol developed by the U.S. Environmental Protection Agency (Kaufmann *et al.*, 1999; Peck *et al.*, 2001). We selected five instream physical habitat variables to characterize the potential impacts of wild boars, based on previous studies (Rosa *et al.*, 2019). For each of the 11 cross-sectional transects, we measured the wetted width (cm) and the bank angle (0–90 degrees) on both the right and left banks. The bank angle was measured using a

clinometer. Transect characterization also included an assessment of the percentage of bare soil area by visual estimation in a 10 x 10 m plot on each bank. Additionally, we took 10 longitudinal equidistant measurements of thalweg depth (cm) for each section and recorded the presence of fine sediments (silt and sand).

We used sieves with an effort of two people for 12 min for each section. The collected individuals were euthanized in a eugenol solution, fixed in a 10% formaldehyde solution, and later preserved in 70% alcohol. Species were identified in the laboratory and deposited in the Ichthyological Collection of the Universidade Federal de Lavras (CI-UFLA).

Data analysis. All the analyses, with the exception of Discriminant Analysis, were performed using RStudio v. 4.2.2. Regarding the physical habitat, we conducted a Principal Component Analysis (PCA) to identify possible patterns and correlated variables over the years. We used the FactoMineR package (Lê *et al.*, 2008) for the PCA. Additionally, we performed a Discriminant Analysis with Statistica Software v. 14.0.0.15 to assess which variables differed between the periods before (2009; 2013) and after (2019; 2021; 2022) the wild boar presence. To evaluate the sufficiency of the fish community sampling, we performed a species accumulation curve using the vegan package (Oksanen *et al.*, 2022). The difference in species richness between periods with and without the presence of wild boar was tested using a Generalized Linear Mixed Model (GLMM), considering streams as a random effect. The GLMM was performed with lme4 package (Bates *et al.*, 2015). We also compared the fish community abundance across the five sampled years to assess changes following wild boar invasion. For this analysis, we employed Non-metric Multidimensional Scaling (NMDS) and Permutation Multivariate Analysis of Variance (PERMANOVA). The PERMANOVA was used to test the differences between the periods before and after the wild boar presence. Pairwise PERMANOVA was conducted using the pairwiseAdonis package (Martinez Arbizu, 2017), while the other analyses were performed using vegan package (Oksanen *et al.*, 2022).

RESULTS

Alterations in the physical habitat of both sampled streams were recorded, aligning with the expected impacts of wild boar activity. Both bare soil and the quantity of fine substrate along the streams increased, while the bank angle decreased (Figs. 3–4; Tabs. 1–2; $p < 0.05$). However, there were no differences in both depth and wetted width before and after the boar presence (Fig. 4).

We recorded eight fish species, four of them in both streams. All species belonged to the Characiformes and Siluriformes orders (Tab. 3). The characid *Psalidodon paranae* (Eigenmann, 1914) was the most abundant species in stream A, while the loricariid *Microlepidogaster arachas* Martins, Calegari & Langeani, 2013 in stream B.

Species richness after the detection of wild boar was significantly lower (GLMM: $t = 3.123$; $p = 0.002$). Stream A showed a progressive decrease, dropping from five species before the boar's invasion (2009–2013) to four in 2019, and further to three (2021–2022) (Fig. 5). Meanwhile, in stream B, after 2009, the richness decreased from seven to six species and has not changed since 2013. Besides the decrease in fish richness

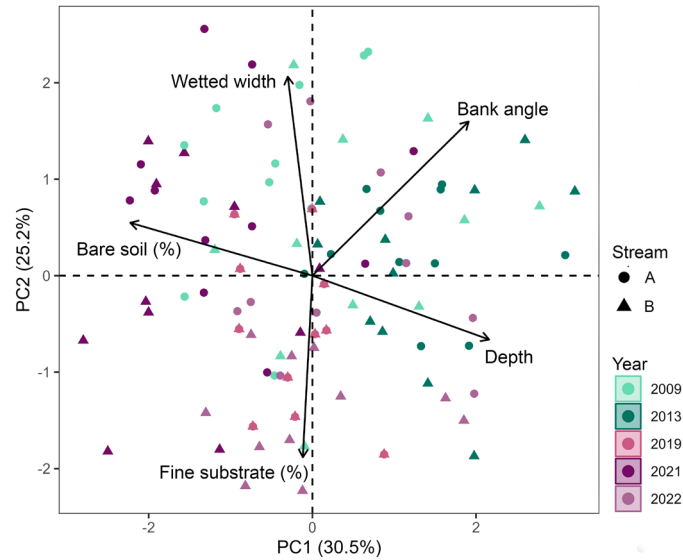


FIGURE 3 | Ordination of the samples (ten sections in both streams) along the first two principal axes of the PCA, based on physical aspects of the two streams between 2009 and 2022. The directions and lengths of the vectors represent eigenvectors of physical habitat variables for each axis.

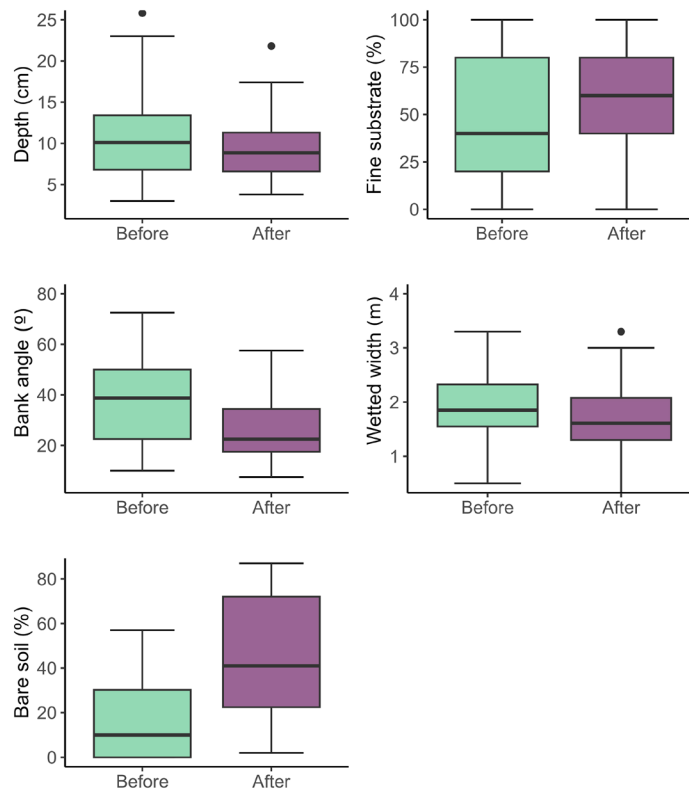


FIGURE 4 | Variation of physical aspects of two streams before (2009; 2013) and after (2019; 2021; 2022) wild boar invasion. The boxplot was created using raw data from the ten sampled sections of both streams combined. Middle line represents the median, and the box the 25% and 75% quartiles. Whiskers represent minimum and maximum values. The points are outliers.

TABLE 1 | Stream attributes, PCA scores, and contribution to the first three axes for two streams with wild boar presence.

	PCA 1	PCA 2	PCA 3
Depth	0.72918387	-0.22361786	0.29817925
Fine substrate	-0.03934108	-0.6357261	0.68739537
Bank angle	0.64491639	0.53950273	0.07875898
Wetted width	-0.10150226	0.69646478	0.59834833
Bare soil	-0.7516637	0.18517991	0.24005931
Eigenvalue	1.52447501	1.26457061	0.98327543
Variance explained (%)	30.4895001	25.2914121	19.6655085
Cumulative variance (%)	30.4895001	55.7809123	75.4464208

TABLE 2 | Statistics of the Discriminant Correspondence Analysis for the variables measured in two streams, comparing periods before (2009; 2013) and after (2019; 2021; 2022) wild boar presence. Significant values ($p < 0.05$) are indicated in bold.

	F-value	P-value	Tolerance
Depth	0.09	0.76	0.89
Fine substrate	3.99	0.04	0.95
Bank angle	7.38	0.01	0.92
Wetted width	1.78	0.18	0.95
Bare soil	29.76	0.00	0.94

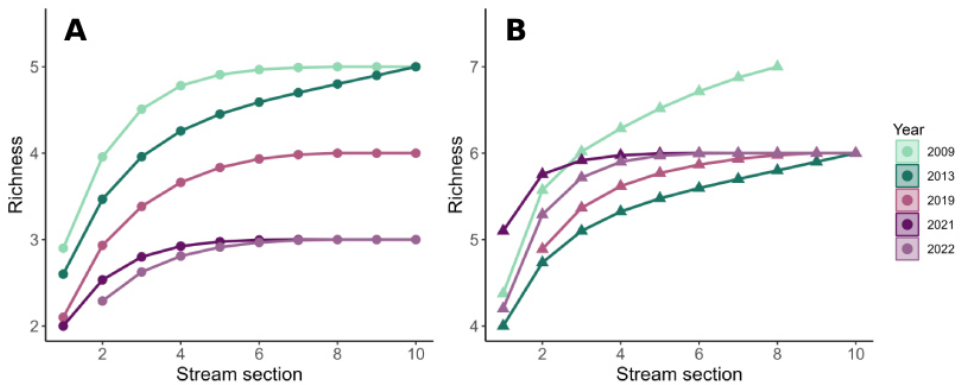


FIGURE 5 | Fish species accumulation curves from streams for each sampled section and year. **A.** Stream A and; **B.** Stream B.

over the years, when considering the abundance data (Fig. 6), we observed a significant differentiation between the communities before and after the boar’s invasion for both streams (PERMANOVA: $p < 0.05$).

Although *P. paranae* was the most abundant species in stream A, by 2019 its abundance decreased to less than half of what was found before the wild boar’s record. In stream B its abundance did not change. Moreover, the most pronounced change in the fish communities was regarding Loricariidae species, which disappeared in sampled stretches

of both streams (Tab. 3). *Hypostomus ancistroides* (Ihering, 1911) was captured only in 2009 and in stream B. While in stream A, this change was marked by the disappearance of *Hypostomus* cf. *nigromaculatus* (Schubart, 1964) after 2013 and *M. arachas* after 2019.

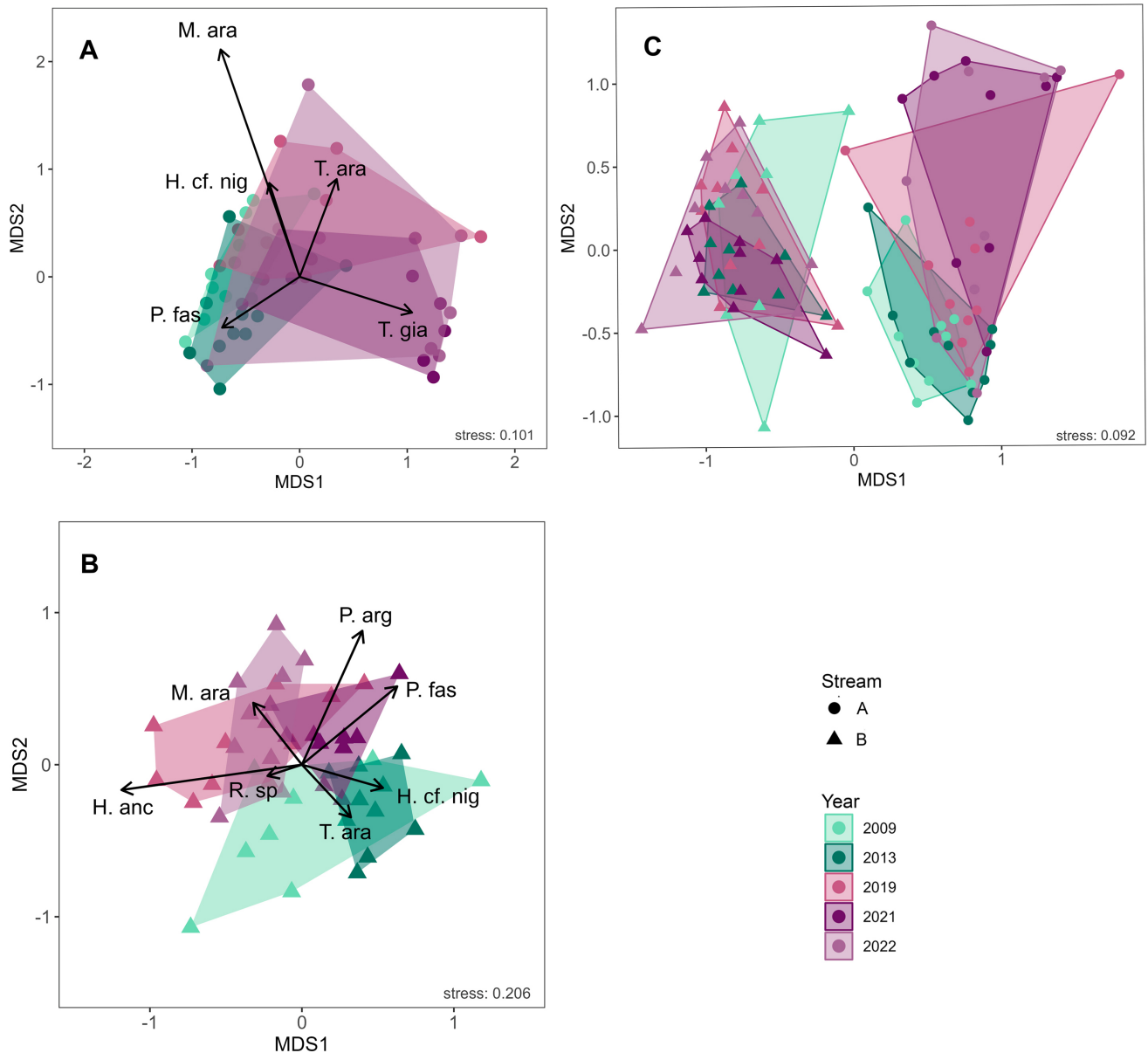


FIGURE 6 | Non-metric multidimensional ordination of species composition of the fish community at stream A (A), B (B), and both streams together (C) in Galheiros PA. Each point represents a sampled section.

TABLE 3 | Voucher numbers and abundance of fish species captured in two streams of Araguari River basin at Galheiros PA. Nomenclatural classification follows Van der Laan, Fricke (2024).

Taxon	Voucher number	Stream A					Stream B				
		2009	2013	2019	2021	2022	2009	2013	2019	2021	2022
Characiformes											
Acestrorhamphidae											
<i>Psalidodon paranae</i> (Eigenmann, 1914)	CI-UFLA 2257	200	340	88	54	92	11	33	26	47	17
Stervadiidae											
<i>Piabina argentea</i> Reinhardt, 1867	CI-UFLA 2258						2	1	4	21	9
Siluriformes											
Heptapteridae											
<i>Rhamdiopsis</i> sp.	CI-UFLA 2259						6	7	11	20	9
Loricariidae											
<i>Microlepidogaster arachas</i> Martins, Calegari & Langeani, 2013	CI-UFLA 2260	13	3	3	0	0	18	39	62	100	95
<i>Hypostomus ancistroides</i> (Ihering, 1911)	CI-UFLA 2261						8	0	0	0	0
<i>Hypostomus</i> cf. <i>nigromaculatus</i> (Schubart, 1964)	CI-UFLA 2262	10	13	0	0	0	53	75	34	49	18
Trichomycteridae											
<i>Trichomycterus araxa</i> Costa, Mattos, Sampaio, Giongo, Almeida & Katz, 2022	CI-UFLA 2263	5	12	4	8	4	22	142	19	91	29
<i>Trichomycterus giarettai</i> Barbosa & Katz, 2016	CI-UFLA 2264	4	18	11	36	23					

DISCUSSION

Our results supported both hypotheses, since we found differences after the wild boar invasion in three of the five instream physical habitat variables, and changes in fish richness and abundance. We cannot completely disregard the influence of natural changes due to random effects or climate influences on the fish community over the thirteen-year duration of our study. However, the recorded changes in the physical habitat are clearly related to modifications caused by the wild boar' instream activities. Therefore, although this inference is indirect, there is evidence that the habitat alteration caused by the wild boar is responsible, at least in part, for the changes in fish communities.

Aspects of stream morphology and its habitats are strongly correlated with land use in the surroundings (Allan, 2004). The transformation of land use in river basins due to anthropic activities at various levels can lead to several changes in streams (Lammert, Allan, 1999; Leal *et al.*, 2016), resulting in degraded habitats (Allan, 2004). For this reason, evaluating the physical aspects of streams located in a conservation area allowed us to assess the effects of wild boars, as no land use changes were observed in the studied watersheds. Our results showed several modifications in the physical habitat of the streams, indicating a decrease in the quality of these environments. Consistent with our findings, a study in streams of Southeast Brazil also observed a decrease in bank angle, as well as an increase in fine sediments due to wild boar activities (Rosa *et al.*, 2019). One of the side effects of invasive species is the modification of conditions and resources in the area where they are established (Spear, Chown, 2009; Keiter, Beasley, 2017). This can be exacerbated in the case of the wild boar, which is considered an ecosystem engineer

(Schaetzen *et al.*, 2018). Through their behavior of rooting, wallowing, and puddle formation they can alter the composition of animal and plant communities, impact water quality and instream physical habitat, and provoke soil changes such as erosion and compaction (Kaller, Kelso, 2006; Dunkell *et al.*, 2011; Barrios-Garcia, Ballari, 2012; Rosa *et al.*, 2019).

The smaller and first-order stream (stream A) showed lower richness and a greater loss of species following the presence of wild boars. Additionally, it exhibited the most pronounced change in overall fish community structure. Smaller areas typically harbor lower species richness (Oberdorff *et al.*, 2019, 2011). Moreover, since wild boars also forage inside streams, smaller rivers may experience a greater portion of alteration due to their activities. Furthermore, although we did not quantify the intensity of the impact in each stream, the more pronounced changes observed visually on the banks of this stream suggest it could be experiencing the effects of wild boar presence for a longer period than stream B. In 2013, our field team recorded the presence of unidentified footprints in the area of stream A. They were possibly wild boar footprints; however, since the boars had not yet been sighted or known to inhabit the reserve before 2013, the possibility of them being wild boar footprints was not considered at that time.

The local extinction of Loricariidae species and decline in the abundance of *P. paranae* in stream A is likely related to the indirect effects of wild boar presence. Although few studies have focused on the understanding of habitat changes caused by wild boars in aquatic biota (Barrios-Garcia, Ballari, 2012), their foraging activities in lagoons have been associated to increased turbidity and changes in the macrophyte community (Doupé *et al.*, 2010), while in streams, they can affect negatively the invertebrates (Kaller, Kelso, 2006). In low-order streams, *P. paranae* consumes mainly aquatic and terrestrial invertebrates and terrestrial plants (Ferreira *et al.*, 2012). Since wild boars can cause modifications in communities of all these organisms mentioned (Kaller, Kelso, 2006; Spear, Chown, 2009; Doupé *et al.*, 2010; Barrios-Garcia, Ballari, 2012), the resources available for *P. paranae* are likely decreasing in stream A.

Regarding the Loricariidae fish, they are bottom-dwelling species, commonly associated with large substrates and rapid waters (Roxo *et al.*, 2017), thus can be sensitive to increases in fine substrate, as we observed after wild boar invasion. The loss of adequate substrate for species of this family could have led to the local disappearance of *M. arachas* and *H. cf. nigromaculatus* in stream A.

Despite the limitations of our study, which included examining only two streams without a control group, we provide valuable insights into the possible impacts of wild boar invasions. Most studies on wild boars have focused on short-term impacts and lasted less than a year (Genov *et al.*, 2017). Our study sheds light on the possible consequences of wild boars on two overlooked aspects: the aquatic environment and long-term effects. The behavior of wild boars could also be leading to impacts on riparian vegetation, and such a relevant aspect deserves future attention. Although wild boar invasion is a worldwide issue, little is known about its impacts on aquatic environments. We emphasize the urgent need for studies in these environments, especially in tropical regions, where the abundance and distribution of wild boars are increasing (Pedrosa *et al.*, 2015; Kmetiuk *et al.*, 2023).

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AUTHORS' CONTRIBUTION

Marina F. Moreira: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing–original draft, Writing–review and editing.

Larissa B. S. Milhomens: Methodology, Writing–original draft, Writing–review and editing.

Pedro L. C. Uzeda: Methodology, Visualization, Writing–original draft, Writing–review and editing.

Ana Luiza V. Andrade: Methodology, Writing–original draft, Writing–review and editing.

Paulo S. Pompeu: Conceptualization, Formal analysis, Funding acquisition, Investigation, Resources, Supervision, Writing–review and editing.

Neotropical Ichthyology



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