Revealing the pathways by which agricultural land-use affects stream fish communities in South Brazilian grasslands

RENATO B. DALA-CORTE*, XINGLI GIAM†, JULIAN D. OLDEN†, FERNANDO G. BECKER‡, TAÍS DE F. GUIMARÃES* AND ADRIANO S. MELO§

*Programa de Pós-Graduação em Ecologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
†School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA, U.S.A.
‡Departamento de Ecologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
§Departamento de Ecologia, Universidade Federal de Goiás, Goiânia, GO, Brazil

SUMMARY

1. Understanding mechanisms by which agricultural practices affect freshwater ecosystems helps to inform land-use policies and management strategies aimed at mitigating effects of agriculture on biodiversity. Land-use activities in the catchment, riparian and local scales likely influence stream fish communities via multiple pathways, for instance, by modifying the instream habitat.

2. We investigated the mechanisms driving local stream fish taxonomic richness and functional diversity in South Brazilian grasslands by testing a theoretical path model in which we specified a priori relationships predicting how land use at multiple scales affects instream habitat and fish communities.

3. Agricultural activities adjacent to streams (i.e. local impact on the streambank) and catchment-scale cropland area were positively related to macrophyte cover and negatively associated with coarse particulate organic matter (CPOM, i.e. woody debris and leaf litter). Local impact also increased substrate siltation and homogenisation. Riparian vegetation in the upstream buffer ameliorated instream habitat condition by dampening macrophyte proliferation and providing CPOM.

4. Fish species richness increased with both macrophyte cover and CPOM, revealing multiple pathways of agricultural influence. However, functional diversity decreased only with substrate siltation, revealing a response to agricultural activities adjacent to sample sites. Agricultural streams showed a replacement of benthic and lithophilic species by a larger number of morphologically similar and macrophyte-associated nektonic fish.

5. Our study indicates that land-use adjacent to streams and upstream riparian zones is critical for maintaining taxonomically and functionally diverse fish communities due to their strong effects on instream habitat. Protection and recovery of riparian zones from land-use change can mitigate the effects of agriculture on fish communities in South Brazilian grasslands.

Keywords: cropland, multiscale, functional diversity, riparian, species richness

Introduction

Rapid expansion of agriculture over the past century has left an indelible mark on the world as croplands, and livestock pastures now cover an area larger than many of Earth’s natural biomes (Foley et al., 2005). Ongoing agricultural expansion is expected to have significant consequences for global biodiversity, mainly because it is concentrated in species-rich regions, such as the Neotropics (Gibbs et al., 2010; Ray et al., 2012; Overbeck et al., 2015). However, our understanding of how agriculture affects biodiversity in such region is centred
primarily on forest ecosystems and the terrestrial taxa therein (e.g. Naughton-Treves et al., 2003), whereas the potential effects on freshwater ecosystems of non-forest biomes remain much less understood.

The mechanisms by which agricultural conversion affect freshwater communities are complex; they are often scale dependent and involve numerous interacting processes and pathways (Allan, 2004). Agriculture at the catchment scale increases sediment and nutrient load to streams (Burdon, McIntosh & Harding, 2013), causing reduced habitat quality for benthic and substrate-spawning species (Rabeni & Smale, 1995). The interaction between fine sediment and nutrient inputs can alter primary productivity (e.g. increases macrophyte cover), which in turn influences the trophic structure of communities (Bunn, Davies & Mosisch, 1999). At a finer scale, loss of forest cover in the riparian zone may cause increased input of solar radiation and consequent increase in primary production (Burrell et al., 2014), as well as reduction of woody debris and leaf litter input (Hyatt & Naiman, 2001), and reduction of terrestrial food subsidies for fish (Evangelista et al., 2014).

At both catchment and riparian scales, the replacement of native vegetation with agriculture affects taxonomic and functional composition of fish communities (Walser & Bart, 1999; Wang et al., 2003; Casatti et al., 2015; Giam et al., 2015). Increased siltation caused by agriculture at catchment scale has been shown to reduce fish species richness (Roth, Allan & Erickson, 1996; Walser & Bart, 1999; Sutherland, Meyer & Gardiner, 2002) and fish biotic integrity (Wang et al., 1997). At the riparian scale, removal of woody vegetation and thus reduction of woody debris and leaf litter may decrease species richness and functional diversity (Stauffer, Goldstein & Newman, 2000; Giam et al., 2015). Removal of riparian vegetation is also related to stream bottom homogenisation via siltation, which modifies fish species composition and abundance of trophic guilds (Casatti, Ferreira & Carvalho, 2009; Zeni & Casatti, 2014). Conversely, riparian deforestation might increase fish species richness by augmenting primary production owing to greater nutrient loading and light input (Lorion & Kennedy, 2009; Burrell et al., 2014; Teixeira-de Mello et al., 2015). Agricultural land use at catchment can also be positively linked to fish species richness (e.g. Harding et al., 1998). Species gains, however, comprise mostly tolerant, water-column species and macrophyte specialists (Harding et al., 1998; Wang, Robertson & Garrison, 2007; Casatti et al., 2012). These different responses highlight the importance of considering simultaneously the multiple pathways by which human land-use affect stream biodiversity, both in terms of taxonomy and functional trait composition (Olden et al., 2010).

A common strategy to mitigate agricultural effects on stream biota is the retention of natural vegetation in the riparian zones (i.e. riparian buffers or reserves; Allan, 2004). Riparian buffers can reduce nutrient and sediment inputs by filtering upland ground water and surface runoff (Lowrance et al., 1997) and limit stream eutrophication via shading (Burrell et al., 2014). Whereas riparian buffers are generally effective in maintaining instream habitat quality, they were not always effective in protecting fish communities (e.g. see Giam et al., 2015 versus Wang, Lyons & Kanieh, 2002, 2006). This suggests that fish may respond to factors other than local habitat structure when catchments are converted to agricultural activities (e.g. see Harding et al., 1998).

Whereas previous research has found contrasting results and indicated that fish communities are influenced by agricultural activities developed at multiple spatial scales, such as catchment, riparian and local (e.g. Roth et al., 1996; Lammert & Allan, 1999; Sály et al., 2011; Feld, 2013), there is limited understanding on how the mechanisms involved vary when multiple spatial scales are evaluated simultaneously. This occurs, in part, because of the correlative nature of multiple scales of agricultural development (King et al., 2005). For instance, one may expect riparian zones to be largely intact in pristine catchments, whereas it is likely that agriculture-dominated catchments also have altered riparian zones. The spatially correlated nature of agricultural development points to the importance of building models that incorporate multiple spatial scales simultaneously. This is particularly relevant if the goal is to better understand the drivers of agricultural effects on fish communities, as agricultural land use can be a proxy for multiple environmental factors (e.g. water quality, pesticides, discharge dynamics and habitat modifications). In our study, we focused on three key instream habitat characteristics to understand how agriculture affects stream fish diversity (substrate, woody debris/leaf litter and macrophyte).

We constructed a global model to test the hypothesis that part of the influence of agricultural land use on fish communities arises from its effect on instream habitat characteristics. We explicitly tested whether agricultural activities at different scales influence fish communities through the same or different pathways of habitat modifications, as well as how these pathways behave when multiple scales are evaluated simultaneously. We aimed to answer the following two specific questions: (i) how does agriculture at catchment, riparian and local spatial...
scales affect taxonomic and functional diversity of stream fish communities? (ii) Can riparian vegetation mitigate effects of agriculture on stream habitat and on fish diversity in agriculture-dominated catchments? Results from this study broadly contribute to essential knowledge regarding how agriculture affects freshwater biodiversity, specifically helping to inform stream conservation in Neotropical grasslands and similar non-forested ecosystems globally.

**Methods**

**Study area**

The South Brazilian grasslands (Campos) comprise the Pampa grasslands and the high-altitude grasslands of the Atlantic forest biome (Fig. 1). This region encompasses freshwater ecoregions with a large number of native and range-restricted fish species (Abell et al., 2008). Mosaics of shrubland and forest patches are spread across natural grassland, especially concentrated along riparian zones. Horses, cattle and sheep were introduced to the Campos in the seventeenth century and since then have become widely used for beef production (Overbeck et al., 2007). In the last three decades, a large part of the native grassland vegetation of the Campos was lost, mostly due to a rapid expansion of soybean, rice, maize and wheat croplands (Overbeck et al., 2007, 2015; IBGE, 2015). This is in spite of South Brazilian grasslands supporting a large number of endemic plant and animal taxa (Pillar et al., 2009).

We surveyed 54 wadeable stream sites across South Brazilian grasslands (Fig. 1; Table 1). Each site was sampled once at an independent catchment (independent upstream area). Sampled streams were of second (16), third (29) and fourth (9) Strahler order. The sampled streams belong to two large river basins: the Uruguay river (36 streams) and the Patos lagoon (18 streams) basins. Whereas the species pool may present some differences between these two river basins, the occurrence patterns of fish groups are very similar (such as the predominance of Characidae and Loricariidae fish species; Bertaco et al., 2016). Average pairwise linear overland distance between all the sampling sites was 196 km (SD 101 km), ranging from 1.1 to 472 km.

We sampled fish from 150-m stream reaches using single-pass electrofishing (EFKO GmbH model FEG 1500). Single-pass electrofishing has been shown to be adequate to detect trends in fish abundance and species richness given standardised effort (Bertrand, Gido & Guy, 2006; Sály et al., 2009; Teixeira-de Mello et al., 2014), and is cost-effective for large geographic-scale studies (Meador, McIntyre & Pollock, 2003). We sampled standardised 150-m long reaches because previous evaluations indicated that representative samples of fish species for small streams can be obtained from reaches 30–40 times the mean wetted width or at least 150 m (Reynolds et al., 2003; Sály et al., 2009; David et al., 2010). We restricted sampling to wadeable streams (mean wetted width = 4.7 m, SD 1.8 m) to ensure comparability of sampling reaches.
Catchment livestock density (animal/km²) 87.1 (25.1) 0.3 73.4

We visually estimated % cropland area and livestock density along 10-m wide margins of the sampling reaches.

The extent and effect of agricultural activities developed at the local, riparian, and catchment scales. In addition, we estimated the density of livestock (i.e. cattle, horse, and sheep) at the catchment scale (i.e. the entire basin area upstream from a given sampling reach) and the riparian scale (i.e. 50-m wide riparian zones on both sides of the stream extending 1-km upstream from a given sampling reach; Morley & Karr, 2002) (Fig. 2) by performing supervised classification of 5-m resolution RapidEye satellite imagery (GeoCatálogo, 2015). Agricultural effects at the catchment scale were quantified as % cropland cover. Because natural riparian zones of larger streams (second order) in the Campos are usually dominated by shrubland and forest, we calculated % woody vegetation at the riparian scale to examine whether preservation of the riparian zone may ameliorate agricultural effects at the catchment scale. In addition, we estimated the density of livestock (i.e. cattle, horse, and sheep) at the catchment scale using municipal livestock density data (IBGE, 2006).

**Instream habitat variables**

We characterised the instream habitat at the 150-m fish sampling reach by quantifying (i) % macrophyte cover, (ii) % of fine sediment on the stream bottom (silt, grain size <0.06 mm), (iii) substrate homogeneity (i.e. the inverse of the Shannon–Wiener index of substrate grain size composition standardised to 0–1), (iv) % leaf litter cover and (v) % woody debris cover. See Appendix S1 and Fig. S1 in Supporting Information for a detailed description of how we quantified these variables.

**Local, riparian and catchment scale**

We assessed the local streambank condition by evaluating the extent and effect of agricultural activities developed along 10-m wide margins of the sampling reaches. We visually estimated % cropland area and livestock use intensity at both stream sides in 11 cross sections along the sampled reaches. We averaged these scores to obtain values per sampling site (see Appendix S1 and Fig. S1 for details).

We characterised land use and land cover at the catchment scale (i.e. the entire basin area upstream from a given sampling reach) and the riparian scale (i.e. 50-m wide riparian zones on both sides of the stream extending 1-km upstream from a given sampling reach; Morley & Karr, 2002) (Fig. 2) by performing supervised classification of 5-m resolution RapidEye satellite imagery (GeoCatálogo, 2015). Agricultural effects at the catchment scale were quantified as % cropland cover. Because natural riparian zones of larger streams (second order) in the Campos are usually dominated by shrubland and forest, we calculated % woody vegetation at the riparian scale to examine whether preservation of the riparian zone may ameliorate agricultural effects at the catchment scale. In addition, we estimated the density of livestock (i.e. cattle, horse, and sheep) at the catchment scale using municipal livestock density data (IBGE, 2006).

**Functional diversity**

Functional diversity was based on 14 morphological traits (Table S1; Fig. S2) that reflect fish trophic guilds (e.g. Albouy et al., 2011), spatial occupation in the water column and habitat use (e.g. Leal, Junqueira & Pompeu, 2011). For each trait of each species, an average value was calculated based on measurements of individuals from five size classes whenever possible (Table S1).

We used Rao’s quadratic entropy (Rao’s Q) to quantify fish functional diversity in each sampling site based on species presence–absence data. Rao’s Q measures the mean pairwise distance between species in trait space. Therefore, communities with species that have very different traits will have larger Rao’s Q values than those comprising higher number of species with very similar traits. Rao’s Q does not increase monotonically with species richness (Botta-Dukát, 2005), hence ensuring that a high functional diversity value is not merely an artefact of a high species richness value. We used FD package (Laliberté, Legendre & Shipley, 2014) in R Statistical Environment (R Core Team, 2015) to calculate Rao’s Q values.

**Path models**

Based on the literature, we developed a global theoretical model of how agriculture at different spatial scales may affect fish communities by altering instream habitat (Fig. 3). We performed structural equation modelling...
SEM; Fox, 2010) to examine the support for two submodels that differed in the catchment scale land use (cropland or livestock). We used the lavaan package (Rosseel, 2012) in R (R Core Team, 2015) to perform SEM. We fitted different models for cropland and livestock because whereas these variables were partially correlated \((r = -0.41)\), they may affect fish in different ways. The two path models had the following structure linking terrestrial modifications, instream habitat characteristics and fish diversity (species richness and functional diversity) (Table 1):

1. Terrestrial agricultural effects were specified at the local (streambank), riparian and catchment scales (Figs 2 & 3). We predicted that riparian native vegetation coverage and agricultural activities at these scales would affect fish communities by modifying different aspects of instream habitat.

2. Instream habitat was represented by (i) % macrophyte cover (which was measured directly) and two other latent variables: (ii) substrate siltation and the amount of (iii) coarse particulate organic matter (CPOM). We assumed substrate siltation could be assessed from % silt cover on stream bottom and substrate homogeneity (i.e. high values of both observed variables indicate poor substrate condition). We also assumed that % leaf litter cover and % woody debris cover are representative of both the amount of CPOM available in the stream reach and the structural complexity and microhabitat for fish.

3. Two alpha diversity measures of fish community diversity, which we predicted to be affected differently by instream habitat variables, were used: (i) rarefied species richness and (ii) functional diversity as measured by Rao’s \(Q\). Individual-based rarefaction allowed us to account for the variation in the number of individuals sampled among sites to estimate species richness. It is well known that differences in species number can be an artefact of different individual numbers sampled in communities, rather than actual differences in species richness (Gotelli & Colwell, 2001). Hence, the rarefaction procedure allows for meaningful standardisation and comparison of communities with different numbers of individuals (Gotelli & Colwell, 2001). We performed rarefaction in our study because the number of sampled individuals per stream reach was quite variable (ranging from 105 to 1212 individuals), even though sampled area were similar.

We assessed the goodness-of-fit of each model using the chi-square test \((\chi^2)\): models with low value of \(\chi^2\) and non-significant \(P\)-values \((P > 0.05)\) were considered as good models because they indicate consistency between observed data and the hypothesised model (Grace, 2006). In addition to calculating the direct standardised effect sizes between pairs of variables, we interpreted the results of our path models by calculating the indirect effects (IE) of agricultural land use on fish diversity. Thus, within each model, we calculated the IE of local-, riparian-, and catchment-scale agriculture on fish taxonomic richness/functional diversity via instream habitat variables by tracing (i.e. multiplying) significant \((P \leq 0.05)\) and marginally significant \((0.05 < P < 0.1)\) standardised path coefficients from each of the agricultural effect variables to the community response variable (Legendre & Legendre, 2012).
Spatial autocorrelation

Because spatial autocorrelation could influence our data since some streams were relatively close to each other, we carried out a correction of sample sizes and standard errors of the endogenous variables (response variables) in the path models fitted with SEM. This procedure was performed with the function `spatialCorrect` available in the package `semTools` (Pornprasertmanit et al., 2016) for R (R Core Team, 2015), using geographical coordinates of sampled sites. This function employs Moran’s I statistics to calculate the degree of spatial autocorrelation and the effective sample sizes for all endogeneous variables, as well as returns adjusted standard errors and test statistics for each parameter estimate for each endogeneous variables. Only spatially corrected results are presented.

RLQ analysis

To understand potential changes in functional composition caused by agricultural effects mediated by instream habitat modifications, we performed a RLQ analysis using species presence–absence data (Dray et al., 2014). This analysis involves ordinations of the environmental (R), species occurrence (L) and traits (Q) matrices to assess trait associations along environmental gradients. We used the multivariate fourth-corner method permutation test (type I error fixed at 0.05, adjusting the significance level from \(\sqrt{a}\) to \(a\), ter Braak, Cormont & Dray, 2012) to evaluate the relationships between the R, L and Q matrices (Dray et al., 2014). Subsequently, we performed a trait-based clustering of species to identify species clusters associated with changes in instream habitat (Kleyer et al., 2012; Table S2). These analyses were performed using the `ade4` package (Dray, Dufour & Thioulouse, 2015) in R (R Core Team, 2015).

Results

Fish communities

Overall, the sampled stream sites had a total of 116 fish species (for a full list, see Table S3), with a mean of 18.5 (SD 6.7) species per site. All species were native to the studied region. A total of 16 families were represented,
Pathways of agricultural influence on fish diversity

We found support for the two tested path models (Table 2; \( P > 0.05 \)), what indicates that agricultural land-use at different spatial scales shapes patterns in fish species richness and functional diversity via different pathways of instream habitat modifications. Species richness was indirectly affected by land use at three spatial scales (local, riparian and catchment) via macrophyte cover and CPOM. However, functional diversity was only and indirectly related to land use at the local scale (local impact) via substrate siltation (Fig. 4).

Agricultural activity along stream margins, expressed as local impact, had a positive effect on species richness by increasing macrophyte cover \([\text{IE} = 0.09 \text{ (Fig. 4a), and IE} = 0.08 \text{ (Fig. 4b)}]\), but also had a negative effect on species richness by decreasing CPOM \([\text{IE} = -0.10 \text{; Fig. 4b)}\). Also, our models suggest that local impact decreased functional diversity via increase of substrate siltation \([\text{IE} = -0.18 \text{ (when cropland area was the catchment-scale covariate) and IE} = -0.19 \text{ (when livestock density was the catchment-scale covariate)}]\) (Fig. 4a,b).

Compared to agricultural activities at local scale, catchment cropland had a similar relationship with species richness via macrophyte and CPOM (Fig. 4). An increased cropland area in the upland catchment area had a positive indirect effect on species richness by increasing macrophyte cover \([\text{IE} = 0.12 \text{, but also a negative indirect effect on species richness by decreasing CPOM (IE} = -0.13 \text{ (Fig. 4a). Conversely, livestock density at catchment scale was positively associated with CPOM and thus to species richness (IE} = 0.12; \text{Fig. 4b).}\]

The amount of riparian vegetation cover in 1-km long and 50-m wide buffers upstream from sampling sites was also linked to species richness via its influence on macrophyte cover and CPOM, but exerting an opposite effect compared to local impact and catchment cropland (Fig. 4). Riparian vegetation attenuated the influence of local impact and catchment cropland on species richness via its influence on macrophyte and CPOM (Fig. 4). Conversely, livestock density at catchment scale was positively associated with CPOM and thus to species richness (IE = 0.12; Fig. 4b).

The first RLQ axis had the strongest positive correlation with riparian vegetation \((r = 0.60)\) and negative correlation with local impact and macrophyte \((r = -0.71 \text{ and } -0.64)\). The second RLQ axis was more positively correlated with woody debris and leaf litter \((r = 0.77 \text{ and } 0.62)\) and more negatively correlated with cropland at the catchment \((r = -0.78)\) (Table S2). Clustering species according to their traits resulted in four distinct groups, and their locations in the RLQ ordination reflect their occurrences in relation to environmental variables (Fig. 5b). Groups A and B increased in

### Table 2 Structural equation modelling (SEM) results for the two models tested to predict agricultural effects on fish communities via instream habitat modifications (endogenous variables). Low value of \( \chi^2 \) and non-significant P-value \(( P > 0.05)\) indicate low difference between observed data and the hypothesised model. CPOM, coarse particulate organic matter; \( N \), number of sample sites; \( \chi^2 \), chi-square; d.f., degrees of freedom; \( P \), global probability; \( R^2 \), variance explained for each endogenous variable.

<table>
<thead>
<tr>
<th>Models</th>
<th>Endogenous variables</th>
<th>N</th>
<th>( \chi^2 )</th>
<th>d.f.</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cropland</td>
<td>Macrophyte</td>
<td>54</td>
<td>30.7</td>
<td>22</td>
<td>0.103</td>
</tr>
<tr>
<td></td>
<td>Substrate</td>
<td></td>
<td></td>
<td></td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>siltation</td>
<td></td>
<td></td>
<td></td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>CPOM</td>
<td></td>
<td></td>
<td></td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Species richness</td>
<td></td>
<td></td>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Functional diversity</td>
<td></td>
<td></td>
<td></td>
<td>0.27</td>
</tr>
<tr>
<td>Livestock</td>
<td>Macrophyte</td>
<td>54</td>
<td>31.5</td>
<td>22</td>
<td>0.087</td>
</tr>
<tr>
<td></td>
<td>Substrate</td>
<td></td>
<td></td>
<td></td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>siltation</td>
<td></td>
<td></td>
<td></td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>CPOM</td>
<td></td>
<td></td>
<td></td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Species richness</td>
<td></td>
<td></td>
<td></td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>Functional diversity</td>
<td></td>
<td></td>
<td></td>
<td>0.24</td>
</tr>
</tbody>
</table>

© 2016 John Wiley & Sons Ltd, *Freshwater Biology*, 61, 1921–1934
Fig. 4 Structural equation models showing the pathways by which agricultural activities developed at multiple scales affect species richness and functional diversity. CPOM = coarse particulate organic matter. Arrow thickness is proportional to the estimated standardised effect size (single headed) or correlation to other variables (double headed), and their values are indicated over the arrows. Squared multiple correlations ($R^2$) indicate the variance of instream (endogenous) factors explained by exogenous factors (catchment, riparian and local).

© 2016 John Wiley & Sons Ltd, *Freshwater Biology*, 61, 1921–1934
agricultural streams, while groups C and D were more common in streams with preserved riparian zones (Fig. 5b).

Traits associated with environmental conditions typical of agriculturally developed streams (i.e. high macrophyte cover and substrate siltation) included compressed and deep body shapes, compressed caudal peduncle, large eyes, terminal to superior mouth position and large head. Species with these traits comprised mainly nektonic omnivorous characins, detritivorous Curimatidae and piscivorous Erythrinidae and Characidae species (Cluster A), as well as several cichlids (Cluster B). Most of these species are water-column dwellers and feeders, and more typical of slow-water habitats. By contrast, fish with long peduncles, dorsal and small eyes, inferior mouth and depressed body, such as the bottom-dwellers periphyton-grazing armoured catfishes (Cluster C), and also nektobenthic elongated-body species of varied groups (Cluster D), were associated with streams with preserved riparian vegetation and with higher CPOM and scarce fine sediment, which typifies streams that experienced low agricultural activity (Table S3, Fig. S3).

Discussion
We found evidence that the effects of agriculture on stream fish communities are scale-dependent; the spatial grain of the agricultural footprint determines the magnitude of the effect on fish communities as well as the pathways through which these effects are likely manifested. Effects of agriculture on fish functional diversity appear to be greater at the local scale due to stream bottom siltation, whereas species richness was linked to terrestrial modifications at multiple scales. The conversion of upland catchment natural grassland vegetation and woody vegetation (shrubland and forests) in riparian zone to agriculture was associated with elevated species richness in adjacent stream reaches. The increased availability of light owing to riparian vegetation removal together with increased nutrient input from upland cropland and from adjacent cropland and farming likely allowed macrophytes to proliferate (Burrell et al., 2014). These modifications on ecosystem conditions may favour the invasion of native species typically found in larger streams, such as detritivorous species (Lorion & Kennedy, 2009), and species tolerant to water quality/

---

Fig. 5 RLQ analysis of the multiple relationships between species traits and environmental variables related to agricultural activities (a) and the four groups of species clustered according to their set of functional traits (b). First and second axes summarised 60.3 and 32.4% of variation, respectively. The lower-left position of the ordination represents sites with more agricultural effects and upper-right position represents sites with integer riparian vegetation (a). Each point in the ordination plot within clusters (b) represents the species position modelled according to its traits on RLQ axes 1 and 2, and each colour or letter represents a different group. Groups A and B were associated with agricultural streams with increased siltation and macrophyte coverage, while groups C and D were more common in streams with preserved riparian zones and higher density of woody debris and leaf litter. Colour figure can be viewed at wileyonlinelibrary.com.
temperature and habitat structure modifications (Scott & Helfman, 2001). Hence, the increased species richness owing to riparian vegetation removal occurs along with changes in species composition (Teixeira-de Mello et al., 2015). Particularly, we found an increase in those species belonging to richer fish groups and associated with macrophyte habitat. Increasing catchment cropland area and local impact was correlated with reduced species richness via decreased CPOM (i.e. leaf litter and woody debris), whereas riparian vegetation attenuated this effect by maintaining CPOM input to the stream. Higher CPOM may help support a species-rich fish community by spatially concentrating resources such as invertebrates and biofilm (Pringle et al., 1988; Wallace et al., 1997) and acting as favourable microhabitats (Sazima et al., 2006).

Functional diversity was more strongly linked to agricultural activity on adjacent streambanks (local scale). But unlike species richness, the negative association between functional diversity and local agriculture was mediated by a decline in substrate condition by increasing siltation. Increased soil erosion resulting from open cropland and livestock trampling likely contributed to stream bottom siltation and to increase overall substrate homogeneity, which then resulted in the loss of benthic-dwelling and lithophilic fish species. The set of species associated with a better substrate condition was more diffuse in trait space despite having fewer members than the species sets associated with locally impacted, macrophyte-rich stream reaches. Therefore, as streambanks become more impacted by agriculture, our results suggest that species belonging to the functionally diverse benthic and lithophilic species guilds will be replaced by members of the more species rich but functionally redundant nektonic guild typified by traits such as a deep and compressed body, superior mouth position, and large head and eyes. This also explains why functional diversity declined despite an increase in species richness as streams become more affected by agriculture at the local scale.

The retention of woody vegetation in the riparian zone (1-km long 50-m wide buffer) appeared to be important for attenuating the effect of catchment-scale agriculture on species richness by increasing CPOM in streams. Also, by dampening the increase in macrophyte cover, our results suggest that the conservation of riparian vegetation can reduce the effect of agriculture on species richness. Importantly, in addition to the effects of local and riparian scales on CPOM, we found that cropland at the catchment scale reduced CPOM. This suggests catchment cropland may have additional effects on riparian vegetation and that these were not captured at the riparian scale as defined by us. Thus, our results suggest that retaining long continuous stretches (≥1 km) of riparian vegetation is necessary for effective mitigation of catchment-scale agricultural effects on instream habitat and fish species communities. These findings are consistent with previous studies showing that effects at riparian zone can have more pronounced effects on instream habitat, affecting fish communities and ecosystem functioning (Jones et al., 1999; Lorion & Kennedy, 2009; Giam et al., 2015).

In contrast to our expectation, no evidence was found for catchment agriculture effects on substrate siltation. A possible reason is that the study area covered a large region (ca. 110 000 km²), including several soil types with different sensibility to erosion. Moreover, the measure of agricultural land use in the studied catchments did not distinguish different types of cropland, what may have increased the uncertainty regarding how cropland influenced stream siltation. It is plausible, however, that agriculture will have a pronounced effect on stream siltation when it affects streambank vegetation. Also, the footprint of cropland at catchment scale of our sampling sites is low-to-moderate (only seven of the 54 sites with >50% cropland area and one >70%), and there is some previous evidence of stronger effects at higher levels of catchment agricultural cover (Wang et al., 2003, 2006). Thus, we were not able to assess whether riparian vegetation can be effective in preventing agricultural impacts when larger catchment proportions become converted to agricultural use.

Whereas our findings are consistent with previous studies demonstrating the effects of agricultural activities measured at multiple spatial scales on instream habitat and fish communities (e.g. Wang et al., 1997; Iwata, Nakano & Inoue, 2003; Lorion & Kennedy, 2009; Giam et al., 2015), we provide additional evidence on the different pathways by which agricultural activities at each scale likely affect fish communities. Our results suggest the mechanisms affecting aquatic communities may depend on the spatial scale used to evaluate human impacts. Also, inconsistency in the literature regarding positive or negative responses of species richness to agricultural land use may in part be a consequence of underlying mechanisms associated with different spatial scales (such as macrophyte production versus CPOM input). This result highlights the importance of taking into account the effects of multiple spatial scales (and the different mechanisms operating at each scale) in assessing anthropic impacts on catchments and their stream networks. Furthermore, our results draw

© 2016 John Wiley & Sons Ltd, Freshwater Biology, 61, 1921–1934
attention to the advantages of mechanistic model (e.g. path model) over traditional regression/correlational approaches to address questions of causality in land-use effect on biota.

As unique non-forest ecosystems, such as the South Brazilian grasslands, are increasingly being converted to cropland or used for cattle ranching (Overbeck et al., 2015), an efficient conservation policy is required to protect aquatic biodiversity from the effects of agricultural activities. In 2012, Brazilian legislation on native vegetation protection was modified (Federal Law 12651/2012 or Native Vegetation Law, see Appendix S1 in Overbeck et al., 2015), allowing landowners to use larger portions of riparian areas (Sparovek et al., 2012; Garcia et al., 2013). Ecologists have warned that the reduction in riparian buffer width could result in irreversible environmental degradation including the loss of valuable biodiversity (Metzger et al., 2010; Garcia et al., 2013). Our results provide strong empirical support to these concerns. In the absence of any changes to the current legislation and of policies encouraging the protection riparian zones, we suggest that landowners should voluntarily preserve continuous riparian buffers to effectively reduce negative effects of catchment- and local-scale agriculture. Particularly important is the notion that upstream riparian reserves should be considered as targets for conservation actions.

Acknowledgments

This research was funded by the Programa de Pesquisa em Biodiversidade (PPBio) - Bioma Campos Sulinos (proc. 457503/2012-2) of CNPq. We thank V. P. Pillar, E. Velez and L.R. Malabarba for operational support. We are very grateful to C. Hartmann, M. Camana, M. Dalmolin, L. de Fries, B.A. Meneses, L.R. Podgaiski, V. Basta Zini, V. Lampert, M. Santos, K.O. Bonato and R.A. Silveira for field assistance. We thank the Laboratório de Ictiologia of the UFRGS, especially J. Ferrer and L.R. Malabarba, for their assistance with species identification. Fish sampling was authorised by the Brazilian agency for biodiversity conservation (Instituto Chico Mendes de Conservação da Biodiversidade, ICMBio; SIS-BIO #39672-1) and accomplished to ethical guidelines of the Brazilian National Committee to Control for Animal Experiments (CONCEA) and UFRGS (Comissão de Ética no Uso de Animais of the Universidade Federal do Rio Grande do Sul, CEUA-UFRGS; #24433). R.B.D.C. received a doctorate scholarship from CAPES and A.S.M. received a research fellowship from CNPq (proc. 309412/2014-5). J.D.O. and X.G. were supported by the H. Mason Keeeler Endowed Professorship (School of Aquatic and Fishery Sciences, University of Washington) to J.D.O.

References


Lorion C.M. & Kennedy B.P. (2009) Riparian forest buffers mitigate the effects of deforestation on fish assemblages


Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** Description of the method employed to measure instream habitat variables and to evaluate streambank conditions as an adaptation of the protocol detailed in Kaufmann *et al.* (1999).

**Figure S1.** Illustration of how instream habitat variables and streambank conditions were measured in each sampling site, as a modification of the protocol detailed in Kaufmann *et al.* (1999). Fish communities were sampled in the same stream reaches.

**Figure S2.** Illustration of the 20 morphometric measures used to calculate functional traits related to habitat use and feeding behaviour.

**Figure S3.** Decrease in the relative frequency of occurrence of benthic species (group C) in relation to substrate homogeneity (inverse of Shannon–Wiener diversity index of substrate grain size composition).

**Table S1.** Thirteen functional traits indicative of habitat use/occupation and feeding behaviour used to calculate Rao’s Q functional diversity for stream fish communities. See Fig. S2 for abbreviations.

**Table S2.** Correlations between functional traits, environmental variables and the two first RLQ axes.

**Table S3.** List of fish species surveyed in 54 wadeable streams across South Brazilian grasslands, with indication of their family and respective group assigned according to functional traits.

(Manuscript accepted 1 August 2016)