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Do Riparian Buffers Protect Stream Invertebrate Communities in South American Atlantic Forest Agricultural Areas?

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Abstract We investigated the influence and relative importance of insecticides and other agricultural stressors in determining variability in invertebrate communities in small streams in intensive soy-production regions of Brazil and Paraguay. In Paraguay we sampled 17 sites on tributaries of the Pirapó River in the state of Itapúa and in Brazil we sampled 18 sites on tributaries of the San Francisco River in the state of Paraná. The riparian buffer zones generally contained native Atlantic forest remnants and/or introduced tree species at various stages of growth. In Brazil the stream buffer width was negatively correlated with sediment insecticide concentrations and buffer width was found to have moderate importance in mitigating effects on some sensitive taxa such as mayflies. However, in both regions insecticides had low relative importance in explaining variability in invertebrate communities, while various habitat parameters were more important. In Brazil, the percent coverage of soft depositional sediment in streams was the most important agriculture-related explanatory variable, and the overall stream-habitat score was the most important variable in Paraguay streams. Paraguay and Brazil both have laws requiring forested riparian buffers. The ample forested riparian buffer zones typical of streams in these regions are likely to have mitigated the effects of pesticides on stream invertebrate communities. This study provides evidence that riparian buffer regulations in the Atlantic Forest region are protecting stream ecosystems from pesticides and other agricultural stressors. Further studies are needed to determine the minimum buffer widths necessary to achieve optimal protection.

Keywords Soy production · Pesticides · Agriculture · Multiple stressors · Stream macroinvertebrates

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Introduction

In recent years, soybean production has become a major export crop for multiple countries in South America, raising concern about environmental impacts. Between 1995 and 2011, soy cultivation area expanded by 126% in Brazil (Castanheira and Freire 2013). In Paraguay, soy cultivation area increased from 1.3 Mha in 2000–2001 to 2 Mha in 2007–2008 (García-Lopez and Arizpe 2010). Land use changes caused by this expansion of soy cultivation are likely to have multiple adverse environmental effects, including reductions in ecosystem complexity, loss of bio-diversity, deforestation, increased erosion, adverse effects

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of agrochemicals, and increased greenhouse gas emissions (Botta et al. 2011; Castanheira and Freire 2013; Lathuilliere et al. 2014).

Conversion of land to intensive agriculture can result in degradation of adjacent streams and stream ecosystems through impacts such as nutrient enrichment, sedimentation, pesticide toxicity, and deforestation (Gücker et al. 2009; Jones et al. 2001; Matthaei et al. 2010). For example, in headwater streams of the Brazilian Cerrado, agricultural streams had higher nutrients, reduced channel morphology, higher velocities, lower microbial biomass, and lower community respiration compared to less disturbed streams (Gücker et al. 2009). Moreover, in a multiple-watershed study that evaluated influence of landscape variables on sediment and nutrient load in the eastern United States, amount of agriculture in the catchment area explained 50% of the variation in total nitrate concentrations (Jones et al. 2001).

Agriculture adjacent to streams can adversely impact benthic macroinvertebrate communities through multiple mechanisms. Agriculture-related stressors can include habitat degradation (e.g., loss of vegetative cover, deposition of fine sediments), hydrological modification (e.g., channelization, less diversity in pool/run/riffle regimes), and impacts to water quality (e.g., pesticide toxicity, nutrient eutrophication, increased turbidity, and conductivity) (Matthaei et al. 2010; Stehle and Schulz 2015; Stone et al. 2005; Whiles et al. 2000). Moreover, pesticides used in agriculture can have severe impacts on stream water quality and ecosystems, and the insecticides used in soy production in South America are known to be especially toxic to aquatic invertebrates (Hunt et al. 2016; Mugni et al. 2011). A recent meta-analysis of 838 studies across 73 countries found that measured insecticide concentrations in water bodies frequently exceeds the regulatory threshold levels for surface waters or sediments (Stehle and Schulz 2015), and another analysis of data from Europe and Australia reported that pesticides reduced both species and family richness of aquatic invertebrate communities (Beketov et al. 2013). The Species at Risk pesticide index ($SPEAR_{pesticides}$) was developed in Europe to evaluate effects of pesticides on benthic macroinvertebrate communities (Liess and Ohe 2005), and has been applied successfully in several continents (Schäfer et al. 2012). We recently applied the $SPEAR_{pesticides}$ index in streams located in soy production regions of Argentina, and found that it performed reasonably well ($r^2 = 0.35$ to 0.42) with only minor modifications consisting of adjusting the sensitivity thresholds for life history traits (Hunt et al. 2017).

As a management strategy, stream buffer width may be one of the most important factors in mitigating transport of pesticides, sediment, and other pollutants to streams in agricultural areas (Bunzel et al. 2014; Jones et al. 2001; Rasmussen et al. 2011b; Stone et al. 2005), and recent regulations in both Brazil and Paraguay require forested riparian buffer zones. For example, in Paraguay, Resolution 485/03 by the Ministry of Agriculture requires a protected zone of 100 m around all water bodies. In Brazil, a new forest code was approved in 2012 (Law No.12.651/12) establishing that riparian buffer zone requirements should vary with the general use of the land adjacent to the water body, the aquatic environment, the stream width, and the size of the rural property. As a general rule for stream widths of 10 m or less, the legislation requires a buffer width of 15 m of native riparian forest in rural areas or 30 m if in areas newly converted for rural activities.

Although there is general consensus that forested riparian buffers are beneficial in protected riparian ecosystems, there is currently very limited quantitative data on the width of protective buffer strips. The objectives of the present study were to evaluate: (1) the effectiveness of forested riparian buffer zones in mitigating adverse effects on streams of this region; and (2) the relative importance of pesticides and other agriculture-related stressors in explaining variation in invertebrate community metrics in Atlantic forest streams. The hypotheses that we tested were: (1) stream buffer width is positively correlated with metrics that describe the community composition; and (2) pesticide levels have higher relative importance than other parameters in explaining variation in the $SPEAR_{pesticides}$ index, while other agricultural stressors have higher relative importance than pesticides in explaining variation in other invertebrate community metrics that are not specific to pesticide pollution.

Materials and methods

Study locations and sampling schedule

The study sites included small streams that flowed through agricultural fields in two intensive soy production regions in the former Atlantic forest habitat of Brazil and Paraguay (Fig. 1). In Paraguay, 17 sites were sampled over two seasons (January and December 2013), and all sampling sites were on tributaries of the Pirapó River in the state of Itapúa. In Brazil, 18 sites were sampled once in November 2013, and all sampling sites were on tributaries of the San Francisco River in the state of Paraná. Both study watersheds were on tributaries of the Paraná River. In general, sampling sites were selected on different tributary streams to minimize potential for spatial pseudoreplication. However, in each watershed we collected replicate samples on one reach to evaluate variability. For these replicates, data were averaged. In Paraguay, the location of some sampling sites along a given reach varied by year, and Fig. 1 shows the sites that were sampled in either or both years.

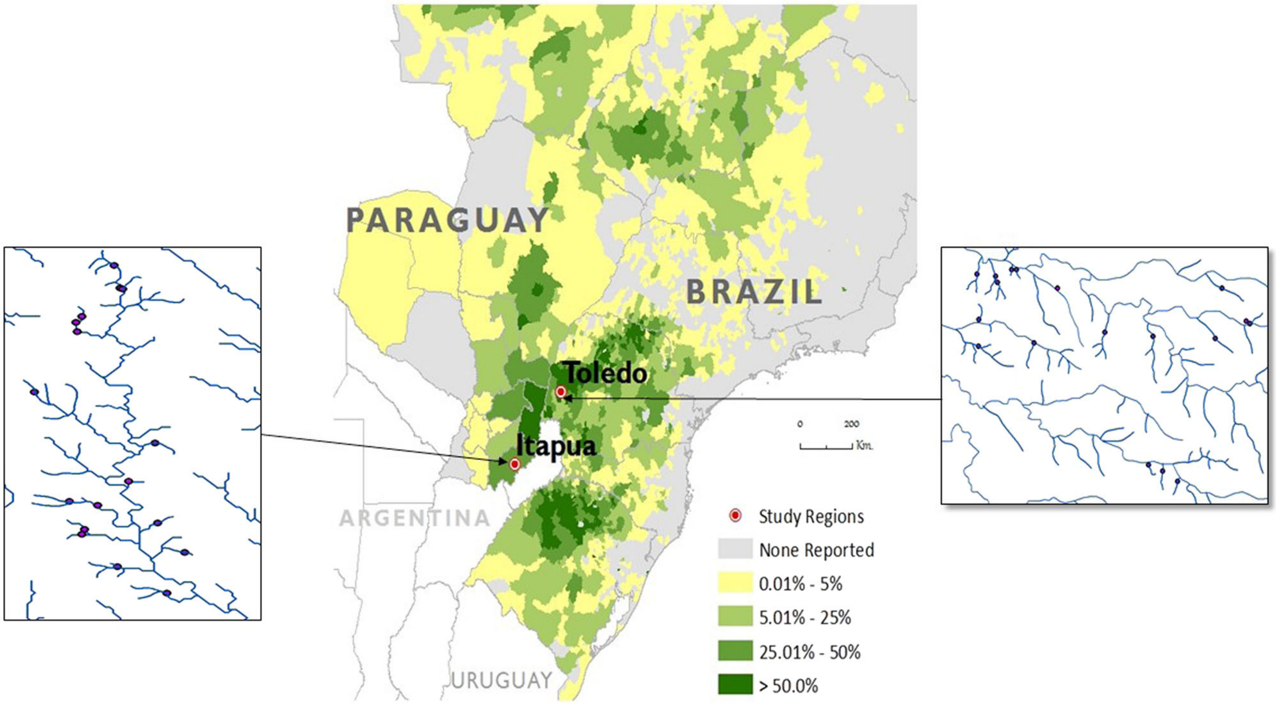

Fig. 1 Overview of study regions and soy production intensity in Brazil and Paraguay, and sampling locations on tributaries of Pirapó River in Itapua, Paraguay, and San Francisco River in Parana, Brazil

Streams selected for the present study were not artificially channelized, and had a minimum buffer strip width of at least 3 m between the stream and the adjacent crop fields. The riparian buffer zones generally contained native Atlantic forest remnants and/or introduced tree species at various stages of growth. Stream depths ranged from 0.12 to 0.81 m, and widths ranged from 2 to 8.5 m (Table 1; Table S2).

Stream sampling was timed to occur during or soon after peak insecticide application periods, which varied depending on planting time. For example, soy can either be planted as an early season crop or a late season crop. The early season crop was generally planted in September or October and harvested in January. The late season crop was typically planted between December and February and harvested several months later. Peak insecticide applications for soy production usually occurred in November and December.

Physico-chemico, habitat, and geographical variables

At each sampling site, pH, conductivity, dissolved oxygen, and temperature were measured during each sampling event with a Yellow Springs Instruments SI 556 multi-parameter probe (Yellow Springs, OH, USA). Turbidity was measured with a portable turbidity meter (Hanna Instruments 93414, Woonsocket, RI, USA). Field test kits were used to measure concentrations of ammonium/ammonia (Sera, Germany), ortho-phosphate (CHEMets K-8510, Midland, VA, USA), and nitrate nitrogen (LaMotte 3354-01, Chestertown, MD, USA). Sediment samples were collected for sediment grain size analysis, and organic carbon analysis by ferrous sulfate titration (USDA 1996).

At each site visit, maximum stream width and depth were measured, and maximum and average water velocities were measured with a current meter (Global Water FP311, College Station, TX, USA). Habitat quality was assessed at each site according to the USEPA Rapid Bioassessment Protocol (RBP) (Barbour et al. 1999) and assigned a score on a scale of 0 to 200. Minimum buffer widths were measured over the reach ~200 m upstream of sampling sites, and these were confirmed with LANDSAT images. Catchments were delineated in GIS using topographical contours to estimate catchment size, and the percent forest and percent agriculture within each catchment were estimated using LANDSAT images. Elevation and stream gradient directly upstream of each site was estimated based on topographical contours.

Sediment sample collection and insecticide analysis

The methods for sediment sample collection and analysis of insecticides have been previously described (Hunt et al. 2016). Briefly, composite sediment samples were prepared from three to five locations at each site, and insecticides were extracted from sediments by sonication (You et al. 2008). Samples were analyzed for pyrethroid insecticides, organochlorinated insecticides, and the organophosphate

Table 1 Summary statistics of site characteristics in each region (average ± standard deviation for all sites)

Parameter	Paraguay[a]	Brazil
Maximum depth (m)	0.50 ± 0.19	0.29 ± 0.16
Maximum width (m)	5.6 ± 1.5	3.8 ± 1.4
Maximum velocity (m/s)	0.45 ± 0.18	0.55 ± 0.15
Gradient (%)	2.4 ± 1.4	4.5 ± 3.5
Elevation (m)	232 ± 81	511 ± 73
Catchment size (Ha)	1604 ± 1226	924 ± 1134
% Cultivated[b]	70.96 ± 11.55	87.66 ± 5.27
Minimum buffer width (m)	89.3 ± 93.4	56.6 ± 87.3
RBP score	155 ± 25	162 ± 12
Water temperature (C)	20.7 ± 1.4	20.6 ± 1.0
Conductivity (uS/cm)	68 ± 15	32.4 ± 12.9
Dissolved oxygen (mg/L)	8.37 ± 1.0	8.79 ± 1.3
Water turbidity	14.8 ± 6.6	17.1 ± 7.6
% sediment fines (silt and clay)	39.72 ± 14.59	65.9 ± 11.6
% sediment TOC	0.78 ± 0.46	2.32 ± 0.68
Total insecticide TU	0.29 ± 0.24	0.21 ± 0.24
Pyrethroid TU	0.23 ± 0.24	0.20 ± 0.23
% soft depositional sediment	28.5 ± 34.8	21.4 ± 18.5
% bedrock	37.1 ± 34.3	16.7 ± 18.1
% large woody debris	5.6 ± 4.2	9.8 ± 6.6
% fine particulate organic matter	2.4 ± 1.1	6.8 ± 4.3
% riffles	29.6 ± 16.5	39.2 ± 25.6
% pool	5.4 ± 7.5	3.6 ± 4.1

[a] For parameters that were measured during both sampling events in Paraguay, statistics are based on values that were averaged over both sampling events

[b] Cultivated area was based on non-forested area, estimated with LANDSAT data

insecticide chloryrifos by either gas chromatography-electron capture detection (GC-ECD) or gas chromatography—mass spectrometry—negative chemical ionization (GC-MS-NCI). Although herbicides, especially glyphosate, are frequently used in soybean production, they were not analyzed because they have been found to have relatively low acute toxicity to invertebrates (Hunt et al. 2016, 2017).

Toxic unit calculation

Insecticide toxic units (TUs) were calculated for all sediment samples:

$$TU = C_i/EC50_i \tag{1}$$

where C_i was the insecticide concentration in sediment normalized for total organic carbon (TOC), and $EC50_i$ was the 10-d median effects concentration (LC50) for each insecticide.

Table 2 Detection frequencies and maximum toxic units (TUs) for each sampling event, for insecticides that had at least one TU value > 0.01

Pesticide	LC50 (ng/g organic carbon)	Statistic	Paraguay Jan 2013	Paraguay Dec 2013	Brazil Nov 2013
Chlorpyrifos	4160[a]	Max TU	0.15	0.05	0.02
		Mean TU	0.04	0.02	0.01
		Frequency[b]	56%	77%	83%
Endosulfan	960[c]	Max TU	0.01	0.04	0.02
		Mean TU	0.00	0.02	0.00
		Frequency[b]	13%	0%	0%
End. sulfate	5220[c]	Max TU	0.05	0.01	0.01
		Mean TU	0.03	0.01	0.00
		Frequency[b]	6%	8%	0%
Cypermethrin	380[a]	Max TU	0.19	0.27	0.83
		Mean TU	0.06	0.10	0.11
		Frequency[b]	31%	8%	44%
L-cyhalothrin	450[a]	Max TU	1.77	0.61	0.16
		Mean TU	0.12	0.11	0.05
		Frequency[b]	6%	8%	39%
Bifenthrin	520[a]	Max TU	0.00	0.14	0.13
		Mean TU	0.00	0.14	0.13
		Frequency[d]	38%	31%	44%
Cyfluthrin	1080[a]	Max TU	<QL	0.05	<QL
		Mean TU	<QL	0.02	<QL
		Frequency[d]	13%	38%	11%
Deltamethrin	790[a]	Max TU	<QL	nd	0.06
		Mean TU	<QL	nd	0.00
		Frequency[d]	13%	0%	6%
Esfenvalerate	1540[a]	Max TU	<QL	nd	0.01
		Mean TU	<QL	nd	0.00
		Frequency[d]	38%	0%	22%
Permethrin	10,830[a]	Max TU	0.02	0.01	0.01
		Mean TU	0.00	0.00	0.00
		Frequency[d]	13%	0%	33%
Total pyrethroid TU[e,g]		Max TU	1.85	0.77	1.03
		Mean TU	0.19	0.28	0.20
Total insecticide TU[f,g]		Max TU	1.89	0.84	1.07
		Mean TU	0.26	0.34	0.21

TUs were calculated as the ratio of the carbon-normalized concentration in sediment over the carbon-normalized LC50. Insecticide concentrations were reported in Hunt et al. (2016a, b)

[a] LC50 for *Hyalella azteca* from Weston and Lydy (2010)

[b] Frequency of detection above the highest quantitation limit of 0.5 ng/g dw in sediment

[c] LC50 for *Chironomus tentans* from You et al. 2004

[d] Frequency of detection above the highest quantitation limit of 0.25 ng/g dw in sediment

[e] Total pyrethroid TU values for each sample were calculated by summing the TU values for each pyrethroid

[f] Total insecticide TU values for each sample were calculated by summing the TU values for each insecticide

[g] A concentration value of half the quantitation limit was assigned for pesticides detected in the sample group but not detected in the sample, or detected < QL in the sample

The sediment LC50 values for freshwater aquatic invertebrates were identified for sensitive species (Table 2; Hunt et al. 2016, 2017). Most of the LC50 values used in the present study were for the amphipod *Hyalella azteca*, which is known to be very sensitive to pyrethroids and chlorpyrifos (Weston and Lydy 2010). Although *H. azteca* does not occur in Brazil or Paraguay, the closely related *H. curvispina* complex occurs throughout South America (Dominguez and Fernandez 2009), and the pesticide sensitivity of *H. curvispina* has been shown to be similar to that of *H. azteca* (Mugni et al. 2013; Hunt, unpublished data). For endosulfan, the LC50 for the more sensitive *Chironomus tentans* was used to calculate TUs, because it is substantially lower than the LC50 for *H. azteca* (You et al. 2004). Toxicity of pesticides in sediment is highly dependent on organic carbon content; therefore, the concentrations were normalized for total organic carbon to calculate TU values.

TU values for all insecticides were summed to calculate total insecticide TUs, and TU values for all pyrethroid insecticides were used to calculate total pyrethroid TUs. When summing TU values, all insecticides that were detected in the data set were included, assigning a concentration of half the quantification limit for pesticides that were not detected in the sample, or detected below the reporting limit. Insecticides that were measured but not detected in the sample group were not included in TU calculation.

Macroinvertebrate collection and identification

Benthic macroinvertebrate samples were collected by kick-sampling with a 30 cm D-frame dip net with 500 µm mesh (Wildco, Yulee, FL, USA). With each net placement, the substrate was disturbed approximately 0.5 m upstream of the net. For the first sampling event in Paraguay, three kick samples, each collected for a period of 30 s, were composited from each site, and all invertebrates from the composite sample were sorted and identified. At four sites, six additional 30 s kick samples were collected several days later because of very low organism counts in the first sampling event. For subsequent sampling events in Paraguay and Brazil, sample size was increased to ensure that a sufficient number of organisms was collected in each sample, and then a subsampling method was used. A larger sample was obtained at each site (30 kick samples, each collected for a period of 20 s), and the sample material was homogenized and divided into 24 quadrats. Organisms from randomly selected quadrats were sorted until a total count of 500 organisms per sample was reached, or until organisms from all quadrats were sorted. This is close to the upper range of counts used in US biomonitoring programs involving fixed-numbers of organisms (Carter and Resh 2013). Once initiating the sorting of a quadrat, it was finished to completion even if the target of 500 organisms was reached before finishing the quadrat.

All samples were preserved in the field in 80% ethanol, later sieved (500 µm) in the laboratory, sorted under 3× magnification, and identified under a stereoscopic microscope. Insects, decapods and amphipods were generally identified to family, genus, or species level, and other taxa were identified by higher taxonomic groups (oligochaetes, nemerteans, turbellarians, leeches, nematodes, gastropods, bivalves) using keys from Dominguez and Fernandez (2009) and Merritt et al. (2008).

$SPEAR_{pesticides}$ index

The $SPEAR_{pesticides}$ index classifies each taxon as either "species at risk" or "species not at risk" based on four biological traits: (1) physiological sensitivity to toxicants; (2) generation time; (3) pesticide exposure potential; and (4) migration ability (Liess and Ohe 2005). In the current version of the $SPEAR_{pesticides}$ index (http://www.systemecology.eu/spearcalc/, Version 0.9.0), binary values are assigned for each trait as follows: (1) physiological sensitivity of 1 for taxa with relative sensitivity > threshold, otherwise 0; (2) generation time sensitivity of 1 for taxa with generation time ≧ threshold, otherwise 0; (3) exposure sensitivity of 1 for epibenthic taxa, or 0 for sediment-dwelling taxa; and (4) migration sensitivity of 0 for organisms with documented ability to migrate rapidly, 1 for all others. A taxon is defined as "species at risk" only if values for all four traits are equal to 1.

The $SPEAR_{pesticides}$ value for each sample is defined as:

$$SPEAR_{pesticides} = \frac{\sum_{i=1}^{n} \log(x_i + 1) \cdot y}{\sum_{i=1}^{n} \log(x_i + 1)} \cdot 100 \quad (2)$$

where n is the number of taxa, x_i is the abundance of the taxon i and y is 1 if taxon i is classified as "species at risk", otherwise 0.

Generation times for each taxon in the established SPEAR database had been previously identified based on European trait databases (http://www.systemecology.eu/spearcalc/, Version 0.9.0). Because generation times of similar multivoltine taxa in the subtropical Atlantic Forest are likely to be shorter than in temperate zones, they likely can reproduce during all seasons; however, sufficient data do not exist to identify generation times of local taxa. In addition, the invertebrate community composition of Atlantic Forest streams is different than communities in the temperate streams where the SPEAR index has been validated. For $SPEAR_{pesticides}$, the default threshold value for physiological sensitivity to pesticides is −0.36 (a taxon must have a relative sensitivity score greater than −0.36 to be considered sensitive). The default threshold value for generation time is 0.5 yr (a taxon must have a generation time of at least 0.5 yr to be considered sensitive). These threshold values can be adjusted based on local invertebrate communities.

In the present study, we applied two versions of the $SPEAR_{pesticides}$ index: the European version with default trait threshold values and described above; and another version with trait threshold values that have been optimized for Argentine Pampas invertebrate communities (Hunt et al., submitted). Although we attempted to use the same approach to optimize the trait threshold values for the Atlantic Forest invertebrate communities, it was not successful because a significant univariate correlation between insecticide TU values and $SPEAR_{pesticides}$ values could not be achieved with the data sets obtained in the present study.

Although in the present study some taxa were identified to genus or species level in some samples, they could not consistently be identified to a level lower than family. Therefore we used family as the lowest taxonomic level for calculation of $SPEAR_{pesticides}$ values. Some families found in the present study were not included in the existing SPEAR database which was based primarily on European taxa; for these missing families we assigned the trait values available for higher taxonomic levels.

Additional bioassessment metrics

In addition to the $SPEAR_{pesticides}$ index, we calculated the relative abundance metrics of taxa groups that were selected based on their common occurrence in the region, and/or known high sensitivity or tolerance to pesticides and other pollutants (Table S3) (Chang et al. 2014; Rubach et al. 2010). We also calculated metrics that are used by the local environmental protection agency in Toledo, Brazil including modified Biological Monitoring Working Party (BMWP) scores and average score per taxon (BMWP ASPT) (Daniel Buss, personal communication). Other metrics we calculated included total taxa density per m^2, relative abundance of three most dominant taxa, Shannon–Weaver diversity index, total taxa richness, Coleoptera family richness, Trichoptera family richness, and EPT family richness (Table S3). Samples containing more than 300 organisms were rarefied to a constant size of 300 organisms to reduce the effect of sample size (Barbour and Gerritsen 1996).

Data analysis

We used several statistical methods to evaluate the relationships between environmental variables and invertebrate communities, and to test our hypotheses. We used univariate regression to test the first hypothesis (whether buffer width was correlated with invertebrate community indices), and to evaluate the performance of the indices with regard to various types of stressors. Because riparian buffer zones are expected to have a protective effect on invertebrate communities through multiple mechanisms, we also evaluated the relationship of buffer width with habitat metrics and water and sediment quality parameters for both regions, using ordination methods. Finally, to test the second hypothesis, we applied multiple regression analysis to evaluate the relative importance of each predictor in explaining invertebrate community metrics. All data analyses were carried out in the open-source statistical software R version 3.2.2 (R Development Core Team 2015).

Principle component analysis (PCA) ordination was used to examine patterns in environmental variables for the two study regions and for the high and low buffer width groups. Environmental parameters examined in the PCA included all variables in Table S2, with the exception of water pH, temperature, and dissolved oxygen. pH was not included because of low variation, and DO and temperature were not included because they depend in part on the time of day that sampling was conducted. The R function "prcomp" was used to carry out the PCA, and data for all variables were centered and scaled prior to analysis.

Non-metric multi-dimensional scaling (NMDS) ordination was used to visually examine patterns in community structure and environmental variables for the two study regions, and for high and low buffer width groups (>50 and <50 m). The function "metaMDS" in the "vegan" package in R was used to carry out the NMDS ordination, using Bray–Curtis distance. All taxa counts were square root transformed, and data were standardized using Wisconsin double standardization.

We then used the BIO-ENV procedure (Clarke and Ainsworth 1993) to identify the subset of environmental variables that best explain the variation in community composition. Because the NMDS analysis indicated that community structure was distinct for each of the two regions, we did this analysis separately for each region. BIO-ENV finds the optimum correlation between a community dissimilarity matrix and multiple environmental dissimilarity matrices, with all possible combinations of environmental variables. For the community matrix, we used Bray–Curtis distance, and for the environmental variable matrix we used Euclidean distance. For Paraguay sampling sites, when environmental variable values were measured during both sampling events, average values were used, and invertebrate data collected during two sampling events at the same site were combined. NMDS, correlation analyses, and BIO-ENV were carried out using the "vegan" package in R (Oksanen et al. 2013).

Multiple regression analysis was conducted for each of the 31 invertebrate community metrics (Table S3). Because the NMDS analysis indicated that community structure was distinct for each of the two regions, we performed the analysis separately for the Paraguay and Brazil data sets, and variable values for Paraguay were averaged over the two sampling events. For the full models, we selected the parameters that are both likely to be affected by adjacent agriculture, and to have an effect on invertebrate communities. These parameters included mainly habitat, substrate, and sediment quality predictor variables (RBP score, % riffles, %TOC in sediment, % large woody debris (LWD), % fine particulate organic matter (FPOM), % course particulate organic matter (CPOM), % soft depositional sediment coverage, total insecticide TU (log transformed) and % sediment fines (only in Brazil because sufficient data were not available for all sites in Paraguay). Turbidity was also included, but other water quality parameters (conductivity, pH, temperature) were not included, either because they did not vary much within regions or depended on the time of day sampled. To evaluate the effect of land use and buffer width, we also included % agriculture and buffer width as predictor variables, and to account for the effect of watershed characteristics we included catchment size and elevation.

To avoid using predictor variables with high collinearity, we used a correlation matrix to select variables that were highly correlated with response variables but not with other predictor variables. After initial variable selection, we checked variance inflation factors (VIFs) with the full model to avoid high collinearity ("vif" function in R package "cor"). All predictor variables had VIF values less than three.

We then selected the best predictive models based on Akaike information criterion values corrected for small sample size (AICc) and *p*-values (Barton 2015). The ΔAICc for each model was calculated as the difference between the AICc for the model and the lowest AICc of all models. For each predictor variable in selected models with $\Delta AICc < 4$ and $p\text{-value} < 0.05$, we determined the magnitude and direction of coefficients using multi-model averaging across selected models (Grueber et al. 2011) using the "dredge" and "model.avg" functions in the R package MuMIn version 1.15.1 (Barton 2015). Relative importance of each predictor variable was calculated as the relativized sum of the Akaike weights over all of the selected models containing the variable of interest (Barton 2015). Importance ranged from 0 (i.e., parameter not given any explanatory weight in any of the selected model) to 1 (i.e., parameter included in all selected models).

We then used simple linear regressions to analyze the effect of agriculture and buffer width on each of the habitat, substrate, and sediment, and water quality parameters that were shown to have high importance in invertebrate community response metrics.

Results

Insecticides present

In both regions, the most commonly detected insecticide was chlorpyrifos, followed by the pyrethroids cypermethrin, lambda-cyhalothrin, bifenthrin, cyfluthrin, deltamethrin, esfenvalerate, and permethrin (Table 2). Although chlorpyrifos was detected in most samples, it is less toxic than the pyrethroid insecticides, and the maximum TU values for chlorpyrifos were lower than the total pyrethroid TU values. Endosulfan and its degradate endosulfan sulfate were detected occasionally, but with relatively low TU values. The banned organochlorine insecticides DDT (and its degradates), endrin, chlordane, and aldrin were also detected in some samples, but TU values were always below 0.01. The total insecticide and total pyrethroid TU values were highly correlated, and were similar between the two regions (Hunt et al. 2016, 2017).

Water and sediment quality

The PCA ordination showed that the Paraguay and Brazil sites were clearly distinct on the horizontal axis with respect to certain water quality and sediment quality parameters (organic carbon, % fine sediment, conductivity), but similar with respect to others (insecticide TU values, % soft sediment, and turbidity) (Figs. 2a, d). Generally, percent fine sediments and organic carbon were higher in Brazil than in Paraguay (Table S2), and conductivity was higher in Paraguay than in Brazil (Table S2).

The low and high buffer groups were partially overlapping, but slightly separated with regard to parameters on the vertical axis (insecticide TU values, turbidity, and to a lesser extent, % fines and soft sediment) (Figs. 2b, d). When split into high and low buffer zone groups by region, the low and high groups were still distinct from each other within each region (Fig. 2c), indicating that the differences in pesticide TU values, % soft sediment, and turbidity are more likely to be related to differences in buffer width than to differences between the two regions.

The nutrient results indicated that nutrient concentrations were in the lowest concentration categories (ammonia/ammonium < 0.5 p.p.m., nitrates < 1 p.p.m., ortho-phosphate < 0.1 p.p.m.) for all or most sites in both regions. Four sites in Brazil and five sites in Paraguay had nitrate concentrations of 1–2 p.p.m., and two sites in Brazil had orthophosphate concentrations between 0.1–0.2 p.p.m. Because nutrients concentrations were generally low and did not vary much between sites, nutrients were not included as variables in the statistical analyses.

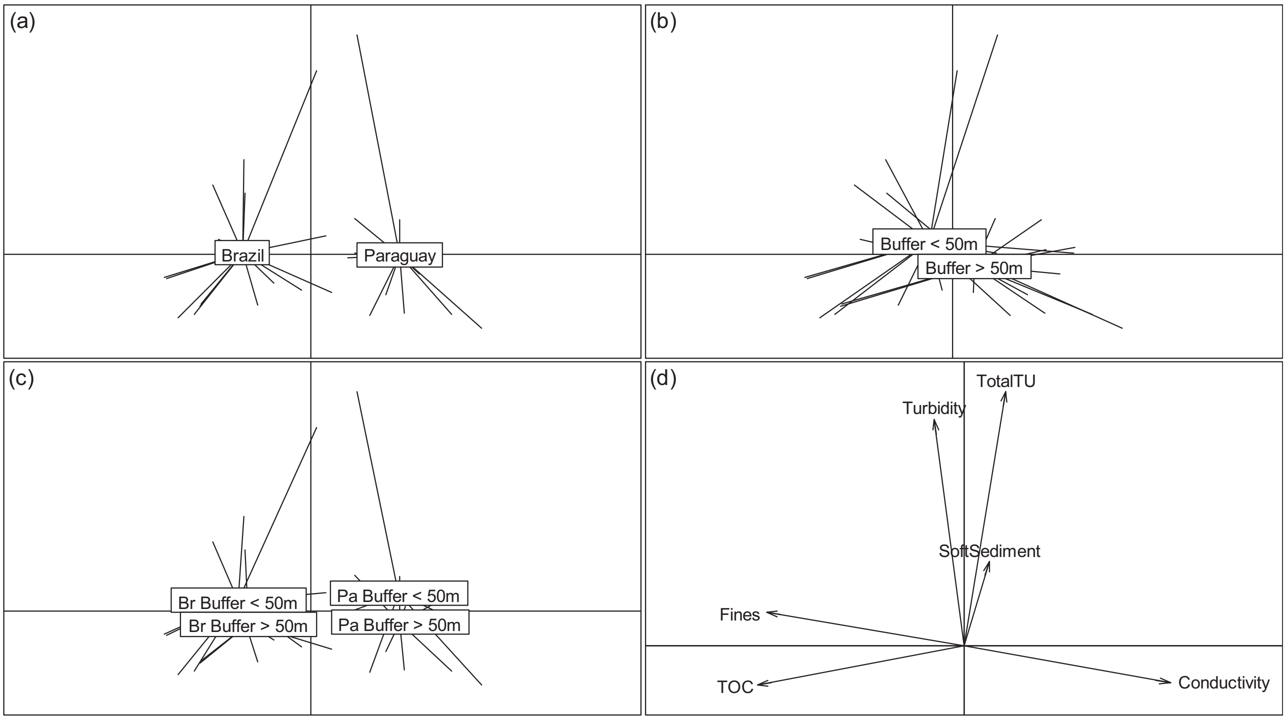

Fig. 2 PCA ordination of water and sediment quality parameters for sampling sites in the two study regions (Brazil and Paraguay) **a**, the high and low buffer width groups **b**, the high and low buffer width groups within each region **c**, and by explanatory variables (**d**). For Paraguay sites that were sampled twice, parameter values were averaged

Habitat quality and landscape characteristics

The PCA ordination showed that the Brazil and Paraguay sampling sites were distinct with respect to certain habitat quality parameters along the vertical axis (Figs. 3a, d). Generally, Brazil sites tended to have higher particulate organic matter and large woody debris, and more riffles, while Paraguay sites tended to have a higher percentage of forest in catchment areas, larger buffer zones, and more bedrock and runs in streams (Fig. 3, Table 1). However, the overall RBP habitat scores were similar for both regions (Table 1).

The low and high buffer groups were also clearly distinct from each other with regard to the same parameters on the vertical axis that separated the regions (Figs. 3b, d). However, when split into low and high buffer zone groups by region, the buffer groups within each region overlapped with each other (Fig. 3c), indicating that the differences in habitat quality were primarily an artifact of the differences in the regional data sets, and not a result of buffer size.

In addition to the differences in habitat parameters, Brazil and Paraguay sampling sites were also distinct with respect to catchment landscape characteristics. Catchment sizes of the Paraguay sites tended to be larger than those in Brazil, hence the stream widths and depths were also generally larger. Elevations of all sites in Brazil were higher than those at Paraguay sites, and stream gradients tended to be slightly higher in Brazil but were not significantly different between regions (Table 1).

Invertebrate community analysis

Invertebrate community composition was similar in the two regions, but there were some differences in relative abundance of certain groups, and in families present (Table S4; Fig. 4). In Paraguay samples, we identified a total of 49 insect families, including 13 families which were not present in samples collected from Brazil sites (Table S4). In Brazil samples, we identified a total of 38 insect families, two of which were not present in samples collected from Paraguay sites. All of the insect families that were found in one region but not the other occurred only rarely in the region where they were detected (<1% relative abundance). In Paraguay, the only decapod found was the family Trichodactylidae, and in Brazil the only decapod found was the family Aeglidae. No ostracods were found in Paraguay samples, but they were found in very small numbers at two sites in Brazil.

In both regions, the dominant family was Chironomidae (Diptera), but relative abundance was substantially higher in Brazil (Table S3). The second most dominant family in both regions was Elmidae (Coleoptera), and in this case relative

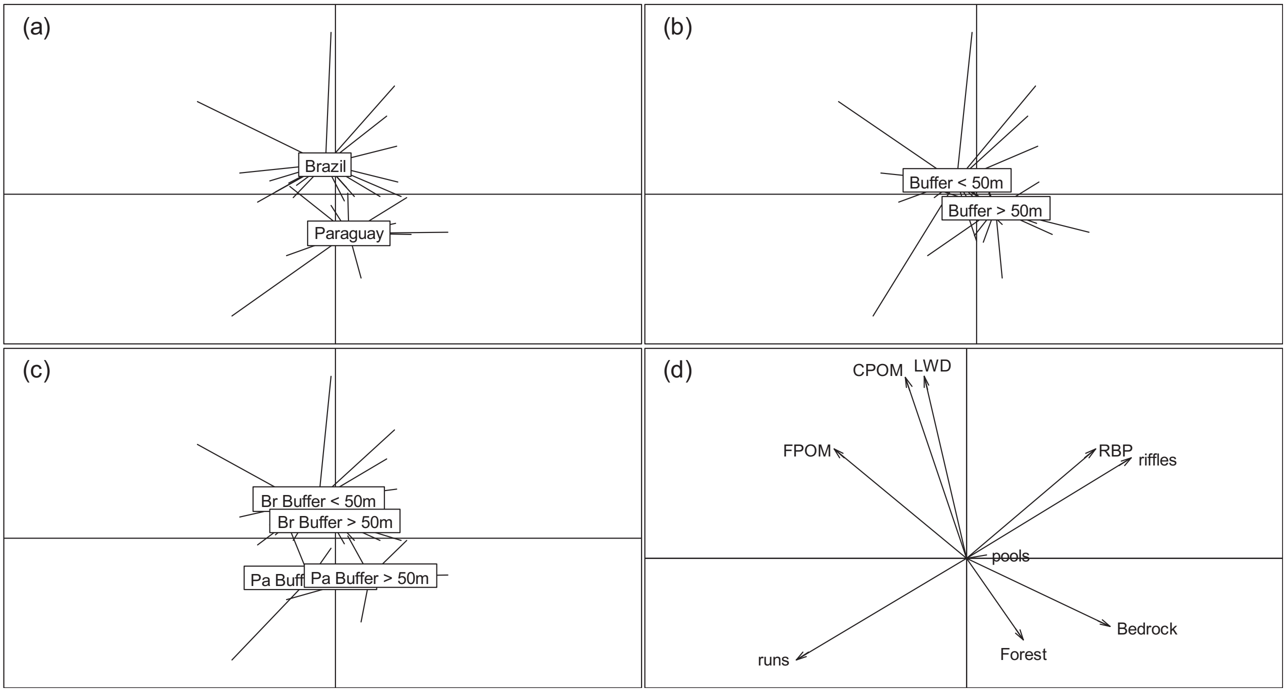

Fig. 3 PCA ordination of habitat quality parameters for sampling sites in the two study regions (Brazil and Paraguay) **a**, the high and low buffer width groups **b**, and the high and low buffer width groups within each region **c**. For Paraguay sites that were sampled twice, parameter values were averaged

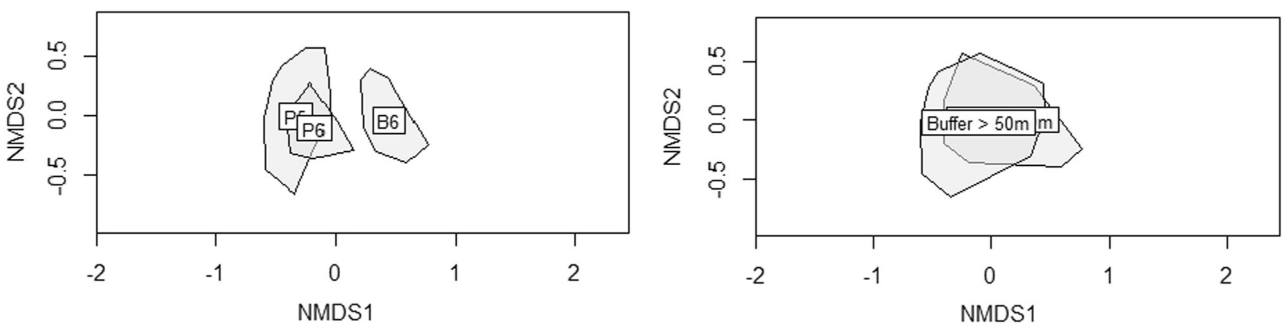

Fig. 4 NMDS results showing sample groups for two events in Paraguay (P5 and P6) and one event in Brazil (B6) and sample groups for sites with low buffer width (<50 m) and high buffer width (>50 m)

abundance was higher in Paraguay. In Paraguay, Leptohyphidae was the most common Ephemeroptera family, while in Brazil, Leptophlebiidae was most common. In Paraguay, trichopterans had higher relative abundance and more families present than in Brazil. Plecopterans were rare in both regions.

The NMDS results indicated that the invertebrate communities were distinct between Brazil and Paraguay (Fig. 4a), but not between low and high buffer groups (Fig. 4b). However, the stress values for the NMDS analysis done in two dimensions was somewhat high for the Brazil data set (0.20), as well as the combined Brazil and Paraguay data set (0.23), indicating that the results should be interpreted only for identifying rough patterns. For the Paraguay data set, stress was lower (0.13).

The BIO-ENV analysis indicated that for the Paraguay data set, only two variables were important in explaining variation in invertebrate communities: stream width and RBP habitat quality score ($r = 0.42$). However, for the Brazil data set, the maximum correlation between community and environmental dissimilarity matrices ($r = 0.39$) was obtained when 10 variables were included in the model: conductivity, turbidity, % soft sediment, % bedrock, % CPOM, buffer width, site elevation, stream gradient, stream width, and stream depth.

Community metrics and relative importance of predictor variables

In both regions, multiple significant models (p-value < 0.05) were selected for four community metrics: %EPT, %EPT-HB, % Ephemeroptera and % Trichoptera. In Brazil, one additional metric (coleoptera richness) had significant models, and in Paraguay nine additional metrics had significant models (Table 3).

In both regions, % agriculture had moderate to high importance (>0.4) and significant effect (average p-value < 0.05) on %EPT, with a negative influence (Table 3). In Brazil, % agriculture also significantly and adversely affected % Ephemeroptera, but in Paraguay % agriculture had no importance in explaining variability in % Ephemeroptera. In Brazil, % agriculture had a negative relationship with %EPT-HB, % Trichoptera, and Coleoptera richness, but with low importance (<0.4) and average p-value > 0.05. In Paraguay, % agriculture had a negative relationship with the ASPT index, with moderate importance (0.46), but average p-value > 0.05.

In Brazil, buffer width appeared to have a mitigating effect, but this relationship was not discernable in Paraguay where most of the sites had minimum buffer width of 100 m or greater. Buffer width had a positive influence and moderate importance on both %EPT-HB and % Ephemeroptera in Brazil, but was significant (average p-value > 0.05) only for % Ephemeroptera. In Paraguay, buffer widths were generally higher than in Brazil and had less variation; in this case buffer width exhibited little or no importance on all response metrics evaluated (Table 3).

Insecticide TU values did not appear to have an explanatory role for the $\mathrm{SPEAR}_{\mathrm{pesticides}}$ index or other invertebrate community metrics in either region. In Brazil, insecticide TU had little or no importance for all response metrics. In Paraguay, insecticide TU did have high importance and a significant relationship for two metrics (% Ephemeroptera and the ASPT index, but the positive direction was opposite to the expected relationship. The $\mathrm{SPEAR}_{\mathrm{pesticides}}$ index values were not significantly correlated with insecticide TU values in either region. In Paraguay, several habitat parameters were the only predictor variables that were important in explaining variability in $\mathrm{SPEAR}_{\mathrm{pesticides}}$ index values. In Brazil, none of the parameters included in the model were significantly correlated with $\mathrm{SPEAR}_{\mathrm{pesticides}}$ index values.

Overall, RBP habitat score was the most important parameter in Paraguay, but was not a good explanatory variable in Brazil. In Paraguay, RBP habitat score had high importance and was significant for eight response metrics, always in the expected direction (positive coefficient for seven sensitive response metrics, and negative for relative abundance of the dominant taxon). However, RBP habitat score had no significant effect (average p-value < 0.05) for any response metric in Brazil, and coefficients for three sensitive response metrics were negative (the opposite direction as expected).

With regard to substrate and water quality parameters, % soft depositional sediment coverage was the best predictor variable in Brazil, but its influence in Paraguay was mixed. In Brazil, this parameter had a significant negative effect with moderate to high importance for three response metrics (%EPT, %EPT-HB, and Coleoptera richness. In Paraguay, while the effect was usually as expected (negative for sensitive response metrics %EPT-HB and SPEAR, and positive for relative abundance of three most dominant taxa) it was not as expected for one metric (positive relationship with rare and sensitive taxa). Several other parameters (% riffles, turbidity, course and fine particulate matter, large woody debris, and sediment TOC) had moderate to high importance and were significant for some response metrics, but effects were not always consistent within or between regions (Table 3).

The effect of % agriculture on % soft sediment coverage and on RBP habitat score was not statistically significant in either region. Similarly, there was no statistically significant relationship between buffer width and % soft sediment coverage or RBP habitat score, although the relationship in Paraguay was close to significant ($p = 0.053$) for soft sediment coverage.

With respect to landscape parameters, catchment area was most important in Brazil, and stream gradient was most important in Paraguay. Generally, catchment area showed a positive relationship with sensitive metrics, while gradient had a negative relationship with sensitive metrics and positive relationship with relative abundance of dominant taxa. In Brazil, catchment size was the most important predictor variable in explaining variability in %EPT-HB and % Ephemeroptera.

Discussion

Relative importance of agricultural stressors

Our study found that stressors related to stream substrate were the most important in determining invertebrate community structure. This result corresponds with those of previous studies (Richards et al. 1993; Wood and Armitage 1997). For example, Richards et al. (1993) found that the most important morphological factors affecting invertebrate communities in streams in an agriculture-dominated region were related to substrate composition and fine sediment distribution. In addition to changing the type of substrate available to invertebrates, deposition of fine sediment can alter stream morphology and decrease structural diversity of

Table 3 Selected model results and relative importance of predictor variables included in averaged models. Includes only response metrics for which significant correlations with predictor variables were found ($p < 0.05$), and models selected for averaging were those with and $\Delta AIC < 4$

Region	Response metric	Selected models		Relative variable importance												
		Adj. r^2	AICc	% Agriculture	Buffer width	Insecticide TU	RBP	% Riffles	Turbidity	Soft sediment	CPOM	FPOM	LWD	TOC	Catchment area	Gradient
Brazil	%EPT	0.34 to 0.79	147.8 to 151.7	**0.62** (−)*	0.10 (+)		0.66 (−)	0.12 (−)*	0.17 (+)*	**0.55** (−)*	**0.40** (+)*	0.22 (−)	0.03 (−)	0.29 (−)	0.58 (+)	
	%EPT-HB	0.51 to 0.63	125.3 to 129.0	0.07 (−)	**0.51** (+)			**0.62** (−)*		**0.71** (−)*	0.06 (+)				1.0 (+)*	
	% Ephem.	0.25 to 0.60	141.7 to 145.7	**0.75** (−)*	**0.59** (+)*	0.03 (−)	0.45 (−)	0.04 (−)		0.19 (−)	0.32 (+)	0.04 (−)	0.10 (−)*		**0.93** (+)*	
	% Trich.	0.17 to 0.45	98.1 to 102.0	0.20 (−)	0.07 (−)	0.05 (+)	0.05 (−)	0.14 (−)		0.03 (−)	**0.83** (+)*	**0.83** (−)*	0.04 (−)	0.03 (+)	0.14 (−)	0.03 (−)
	Coleop. richness	0.18 to 0.52	41.1 to 45.0	0.03 (−)	0.14 (+)	0.19 (−)	0.03 (+)		0.38 (−)	**0.80** (−)*		**0.80** (+)*		0.18 (+)	0.39 (−)	
Paraguay	%EPT	0.78 to 0.88	119.5 to 121.8	**0.76** (−)*			1.0 (+)*	1.0 (−)*					1.0 (−)*	1.0 (+)*	1.0 (+)*	
	%EPT-HB	0.27 to 0.50	119.5 to 123.5			0.39 (+)	0.07 (+)*	0.07 (−)*	0.42 (+)	**0.93** (−)*	0.06 (+)		0.07 (−)*	0.43 (−)	0.17 (+)	
	% Ephem.	0.19 to 0.71	116.6 to 120.6			**0.86** (+)*	0.67 (+)	0.34 (−)		0.21 (−)	0.19 (+)		0.09 (−)	0.15 (−)	0.20 (+)	0.30 (−)
	% Trich.	0.19 to 0.49	111.0 to 114.9		0.02 (−)	0.18 (−)	0.14 (+)	0.46 (+)	**0.72** (+)*	0.28 (−)					0.31 (+)	
	EPT richness	0.28 to 0.54	56.1 to 59.7			0.66 (+)	**0.89** (+)*	0.03 (−)		0.16 (−)	0.04 (−)	0.32 (−)	0.30 (−)			
	Trich. Richness			0.02 (+)	0.05 (−)	0.44 (+)	**0.83** (+)*	0.06 (+)*	0.06 (+)	0.16 (−)	0.06 (+)	**0.76** (−)*	0.07 (+)	0.14 (+)		0.02 (−)
	Rare & Sensitive	0.40 to 0.74	47.0 to 51.0				**1.0** (+)*		**0.63** (−)*	**1.0** (+)*			**1.0** (−)*	0.38 (−)*		**1.0** (−)*
	Shannon diversity	0.25 to 0.42	9.1 to 13.1				**0.81** (+)*		0.06 (−)	0.19 (−)*	0.06 (−)		0.10 (−)			**0.64** (−)*
	Dominant taxon	0.27 to 0.47	124.0 to 127.9				1.0 (−)*			0.10 (−)			0.09 (+)	0.08 (+)	0.16 (−)	**0.77** (+)*
	3 dominant taxa	0.28 to 0.43	119.0 to 123.6	0.04 (+)	0.04 (−)	0.05 (−)	0.23 (−)	0.05 (−)	0.04 (+)	**0.86** (+)*	0.04 (+)	0.04 (−)	0.05 (+)	0.04 (+)	0.04 (−)	0.12 (+)
	BMWP	0.34 to 0.69	141.3 to 145.2		0.02 (−)	0.05 (+)	**1.0** (+)*	0.13 (−)	0.51 (−)	0.16 (+)	0.48 (+)	0.22 (+)	0.02 (+)		0.03 (−)	0.07 (−)*
	ASPT	0.21 to 0.73	10.9 to 14.9	0.46 (−)	0.16 (+)	**0.83** (+)*	0.62 (+)	0.12 (−)*		0.05 (−)	0.08 (+)*	0.06 (−)	0.19 (−)	0.13 (−)	0.01 (−)	0.27 (+)
	SPEAR	0.56 to 0.75	92.0 to 96.0	0.14 (+)	0.13 (−)		**0.71** (+)*	0.03 (+)	**0.97** (+)*	**0.41** (−)*		0.05 (+)	0.58 (−)	0.02 (−)	0.07 (+)	0.04 (−)

The coefficient sign for each parameter is indicated after the relative importance value in brackets as (+) for positive correlation and (−) for negative correlation. Parameters that are significantly correlated with response value (average p-value of selected models < 0.05) are indicated with an asterisk. Parameters that are both significantly correlated and have moderated or high importance (>0.4) have values in bold stream habitat by filling pools and covering hard surfaces such as rocks (Richards et al. 1993; Wood and Armitage 1997).

Results, however, did not confirm our hypothesis that pesticide levels have higher relative importance than other parameters in explaining variation in the $SPEAR_{pesticides}$ index. The lack of relationship between insecticide levels and invertebrate community composition was unexpected. Schäfer et al. (2012) found effects to relative abundances of sensitive macroinvertebrate taxa at pesticide concentrations lower than 1/1000 of the median effect concentration (EC50) for *Daphnia magna*. Thus, at the range of total insecticide TU values found in soy production regions in the present study (sampling event means of 0.21 to 0.34, maximums of 0.84 to 1.89) we would expect to find effects on the stream invertebrate communities. As we previously reported, buffer width was significantly correlated with insecticide levels in the streams we studied, and this relationship was especially strong in Brazil where there was substantial variation in buffer width compared to Paraguay (Hunt et al. 2016, 2017). However, none of the analyses conducted in the present study indicated that insecticides in sediments were important in determining invertebrate community structure in either region. Insecticide TU levels were not determined to be important either in the BIO-ENV analysis or in the multiple regression analysis using various bioassessment metrics. The $SPEAR_{pesticides}$ index has been shown to be a sensitive indicator of pesticide effects on invertebrate communities in many different regions of the world (Schäfer et al. 2012; Hunt et al. 2017). However, it was not correlated with insecticide levels in the present study. Unlike other bioassessment indices, the $SPEAR_{pesticides}$ index has been shown to respond selectively to pesticide stressors and to be relatively insensitive to most other stressors when it has been applied in Europe, Australia, and Argentina (Schäfer et al. 2012; Hunt et al. 2017), although its performance can be affected by severe habitat degradation (siltation and channelization) and low dissolved oxygen (Liess and Ohe 2005; Rasmussen et al. 2011a, b; Schäfer et al. 2011, 2007). While the habitat quality and dissolved oxygen of sites in the present study were generally moderate to high, the SPEAR index was significantly correlated with RBP habitat score, turbidity and fine sediments in the Paraguay data set.

There are several factors that may help explain why $SPEAR_{pesticides}$ index values were not significantly correlated with insecticide TU values in either of the Atlantic forest regions we studied. First, the presence of forested riparian buffers along their entire lengths of all streams is likely to increase the resilience and recovery ability of invertebrate communities. Second, the sensitive taxa groups found in the study streams are likely to be sensitive to many habitat and water quality variables in addition to pesticides, potentially confounding the analysis. Third, the $SPEAR_{pesticides}$ index may not work well in tropical and subtropical environments without significant adaptation. Fourth, measurement of the sediment bound insecticides may not be the optimal descriptor of insecticide contamination affecting invertebrates that are predominately in contact with stream water.

There is ample evidence that forested headwaters in Europe provide reservoirs of invertebrate populations that improve the recovery of downstream communities after disturbance from pesticide exposure (Liess and Ohe 2005; Orlinskiy et al. 2015). For example, a study in an agricultural region of Germany found that upstream forested headwaters mitigated the effects of pesticides on downstream invertebrate populations (Orlinskiy et al. 2015). In streams in intensive soy production regions of Argentina, we found similar upper levels of insecticides as in the Brazil and Paraguay study regions, but in Argentina the insecticides were more clearly correlated with impacts on sensitive invertebrate taxa (Hunt et al. 2017). A possible reason for the difference may be that the stream buffers in Argentina were much smaller and were not forested, thus the invertebrate communities were not as resilient.

In contrast to the Atlantic Forest streams included in the present study, invertebrate communities in streams of the Argentine Pampas tend to contain large percentages of amphipods, which are very sensitive to pesticides but relatively tolerant of many habitat and water quality parameters (Hunt et al. 2017). When the SPEAR index threshold values were optimized for Argentine Pampas streams, only crustaceans and trichopterans were considered sensitive to pesticides, and amphipods were the most abundant sensitive organisms, making them very important in the performance of the $SPEAR_{pesticides}$ index. In the Atlantic Forest streams, amphipods and other crustaceans were rare, and other sensitive and abundant taxa (such as EPT taxa) tend to be sensitive not only to pesticides but also to many habitat and water quality parameters (Bunzel et al. 2013).

The $SPEAR_{pesticides}$ index has not yet been validated for tropical or subtropical streams. A study in tropical streams of Costa Rica did not find a correlation between pesticide toxic units and a modified version of SPEAR (Rasmussen et al. 2016). However, that study measured only three sites, and all sites suffered from low dissolved oxygen and lacked sensitive taxa. The inconclusive finding with SPEAR in that study may be a result of the study design, including factors such as low number of sampling sites, narrow environmental gradient, and many confounding factors. Although it is possible that the $SPEAR_{pesticides}$ index may not work well in tropical or subtropical environments without significant modification, more study is necessary to determine this.

Although some studies (Schäfer et al. 2011; Hunt et. al 2017) have found strong correlations between pesticide concentrations in sediments and the $SPEAR_{pesticides}$ index, most previous studies applying the $SPEAR_{pesticides}$ index have been based on pesticide concentrations measured in stream water. For the present study, we elected to measure insecticides in sediments rather than surface water because the most commonly used insecticides in the region tend to bind to sediments and not remain dissolved in water (Hunt et al. 2016, 2017). However, there is evidence that some of the newer systemic insecticides such as neonicotinoids are being more widely used in soy production in South America, and these insecticides have high solubility in water (Hunt et al. 2016, 2017). It is possible that insecticide levels in stream sediments did not adequately represent the amount of pesticide exposure that invertebrate communities receive in the present study.

Influence of forested riparian buffers

The present study's findings confirm our hypothesis that stream buffer width is correlated with invertebrate community metrics in Brazil, but reults were inconclusive in Paraguay. The findings in Brazil corroborate findings from other studies in Europe, North America, and South America that have found riparian buffer zones to be important in mitigating transport of pesticides to streams (Rasmussen et al. 2011a, b; Di Marzio 2010; Bunzel et al. 2014; Reichenberger et al. 2007; Aguiar et al. 2015). Another study of Atlantic Forest streams in Brazil found no pesticides except the herbicide atrazine in streams with a forested buffer of ~60 m, while multiple pesticides including chlorpyrifos and lambda-cyhalothrin were detected in streams with 12 and 36 m of forested buffer width (Aguiar et al. 2015). Buffers comprised of grass or shrubs were not as effective in pesticide removal as forested buffers (Aguiar et al. 2015). Global literature reviews have found that many factors could affect the buffer width needed to protect streams from pesticide exposure, including gradient, type of vegetation, soil properties, types of pesticides applied, timing and amount of pesticides applied, and presence of tile drains or drainage ditches that short-circuit the buffer zones (Reichenberger et al. 2007; Bunzel et al. 2014).

The lack of variation in minimum buffer width at the Paraguay sampling sites likely limited a firm conclusion regarding the influence of buffer width in this region. Approximately one half of the Paraguay sites had minimum buffer widths of 100 m, which was the minimum required by law. In contrast, in the Brazil study region where buffer width did not exhibit the same clustered pattern, it was the most important variable in explaining insecticide TU values and also had moderate importance in explaining the variability in several invertebrate community metrics. We previously reported that highest insecticide toxic units in both study regions occurred when buffer zone widths were 20 m or less (Hunt et al. 2016, 2017). Moreover, in that study a multiple regression for the Brazil data set indicated that buffer width was the predictor variable that had the greatest influence on total insecticide TU. The selected model included the following predictor variables: buffer width, percent total organic carbon in sediment, and stream gradient ($r^2 = 0.54$; p-value $= 0.009$). The analysis of relative importance indicated that buffer width contributed 74% of the explained variance in total insecticide TU values, with percent total organic carbon and stream gradient contributing 9 and 17 %, respectively (Hunt et al. 2016, 2017).

Because almost all streams in both regions we studied had relatively large forested stream buffers, it is possible that relative effects of buffer widths in protecting invertebrate communities were less evident in this study than in other similar studies in which streams generally had much smaller buffer width. We measured minimum buffer width observed immediately upstream of each sample site, and confirmed the measurements using LANDSAT images. However, for most streams, the average upstream riparian buffer width was considerably larger than the minimum width measured near the sampling site, and forested buffers typically extended throughout the entire watershed, even around the small headwater streams. Therefore, the streams in the two regions included in the present study can be considered well protected in comparison to streams in many intensive agricultural regions. In contrast, previous studies that have found riparian buffer zones to be effective in mitigating effects on stream invertebrate communities have generally evaluated streams with much smaller protected buffer zones than those in the present study. For example, Whiles et al. (2000) found that land use within 18 m of streams in agricultural areas of Nebraska was correlated with invertebrate bioassessment scores. Another study in Ontario reported that forested area within a 30 m riparian zone was positively correlated with increases in EPT taxa and taxa diversity (Rios and Bailey 2006). In our Brazil study area, where buffer widths were generally lower than those in Paraguay, buffer width did have moderate importance in explaining variability in several invertebrate metrics, while in Paraguay it had little or no importance. It is possible that the higher taxa richness observed in Paraguay streams may be due at least in part to the larger riparian buffer widths compared to the Brazil streams.

Although regulation of pesticide mitigation measures often focuses on application practices, landscape level mitigation measures, such as requiring riparian buffer zones, may be easier to implement and enforce. Bereswill et al. (2014) reviewed global data on the efficacy and practicality of risk mitigation measures for diffuse pesticide entry into aquatic ecosystems, and ranked riparian buffer strips as highly effective for mitigating both spray drift and runoff, with high acceptability and feasibility. However, the implementation and enforcement of new riparian buffer requirements in Brazil has been difficult and controversial, especially in regions with small-scale production where a significant amount of a landowner's productive farmland could be lost with compliance (Alvez et al. 2012). The minimum buffer widths measured at sites included in the present study are not necessarily representative of stream buffer widths typical of the study regions, because we biased our site selection to those with better habitat quality.

Conclusions

Results of this study indicated that non-pesticide agricultural stressors such as habitat quality and deposition of fine sediment were more important than insecticides in affecting invertebrate communities. In particular, the amount of soft depositional sediment had high importance in explaining variability in several invertebrate community metrics in both Paraguay and Brazil, and the RBP habitat score was very important in explaining variability in multiple metrics in Paraguay.

This study did not find a correlation between the $\mathrm{SPEAR}_{\mathrm{pesticides}}$ index or other bioassessment metrics and insecticide TU values in Atlantic forest streams. However, the fact that almost all streams had ample forested riparian buffer zones is likely to have mitigated the effects of pesticides on stream invertebrate communities. In contrast, our previous study in Argentina, where there are no requirements to maintain riparian buffers, found a consistent correlation between the $\mathrm{SPEAR}_{\mathrm{pesticides}}$ index and insecticide levels.

Although results indicated that riparian buffer width was a moderately important predictor variable in Brazil, but had low importance in Paraguay, it is likely that the findings in Paraguay were limited by the lack of variation in minimum buffer width in that region. More study is necessary to determine the optimal relationship between buffer width and the health of stream invertebrate communities in Atlantic forests and other regions.

Although our study results may not apply globally, they are certainly applicable and significant to riparian buffer management in the Atlantic Forest region, and may provide an impetus of how further studies can be designed. For example, future studies should attempt to include a larger proportion of study sites with small buffer widths (less than about 20 m) to better quantify the relationship between buffer size and invertebrate community impacts. Such studies would provide important information to assist in determining scientifically based minimum riparian buffer widths to protect streams and rivers.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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