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Agriculture impairs stream ecosystem functioning in a tropical catchment

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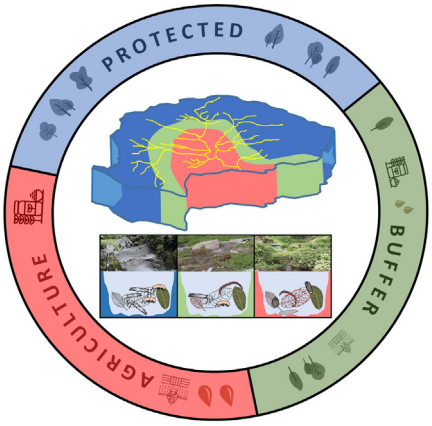
HIGHLIGHTS

- Tropical stream functional integrity was assessed through litter decomposition.
- Decomposition decreased following a rising agricultural influence gradient.
- The reduction was due to impaired detritivore assemblages.
- Microbial decomposition increased but did not compensate for effects on detritivores.

GRAPHICAL ABSTRACT



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ABSTRACT

The expansion of agriculture is particularly worrying in tropical regions of the world, where native forests are being replaced by crops at alarming rates, with severe consequences for biodiversity and ecosystems. However, there is little information about the potential effects of agriculture on the functioning of tropical streams, which is essential if we are to assess the condition and ecological integrity of these ecosystems. We conducted a litter decomposition experiment in streams within a tropical catchment, which were subjected to different degrees of agricultural influence: low (protected area, PA), medium (buffer area, BA) and high (agricultural area, AA). We quantified decomposition rates of litter enclosed within coarse-mesh and fine-mesh bags, which allowed the distinction of microbial and detritivore-mediated decomposition pathways. We used litter of three riparian species representing a gradient in litter quality (*Alnus acuminata* > *Ficus insipida* > *Quercus bumelioides*), and examined

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Leaf litter breakdown
Nutrient concentrations
Pesticide toxicity

detritivore assemblages through the contents of litterbags and benthic samples. We found that the increasing agricultural influence promoted microbial decomposition, probably due to nutrient-mediated stimulation; and inhibited detritivore-mediated and total decomposition because of reduced detritivore numbers, most likely caused by pesticides and sedimentation. Effects were evident for *Alnus* and *Ficus*, but not for *Quercus*, which was barely decomposed across the gradient. Our study provides key evidence about the impact of agriculture on tropical stream ecosystem functioning, which is associated to changes in stream assemblages and may have far-reaching repercussions for global biochemical cycles.

1. Introduction

Human population growth and expansion throughout the world have placed agriculture as a dominant and increasing form of land management globally (Vitousek, 1997; Tilman et al., 2001), which currently occupies ca. 40% of the Earth's land surface (Graeber et al., 2015). The spread of agriculture into native forests is particularly worrying in tropical regions of the world, where forests are being replaced by crops at alarming rates (Gibbs et al., 2010), with severe consequences for biodiversity and ecosystems (Laurance et al., 2014). Within this context, stream ecosystems are of particular concern for two main reasons: firstly, they are substantially impacted by agriculture through inputs of nutrients, sediments and contaminants and the replacement or removal of riparian vegetation (Sala, 2000); and secondly, they experience the largest biodiversity declines, particularly in the tropics, and are among the most endangered ecosystems on Earth (Dudgeon et al., 2006).

It is well known that agricultural practices can negatively impact tropical stream habitats and invertebrate assemblages (Egler et al., 2012; Rizo-Patron et al., 2013; Ruiz-Picos et al., 2016). In general, water quality is reduced, the physical habitat is altered and assemblages are simplified, with a replacement of sensitive by tolerant taxa and loss of biodiversity (Castillo et al., 2006; Rasmussen et al., 2016; Cornejo et al., 2019). However, there is little information about the potential effects of agriculture on the functioning of tropical streams, which is essential if we are to assess the condition and ecological integrity of these ecosystems (Clapcott et al., 2010; von Schiller et al., 2017).

Leaf litter decomposition is a key process in streams, often used as indicator of their ecological integrity (Gessner and Chauvet, 2002; Young et al., 2008). Many streams rely on allochthonous organic matter, mainly in the form of leaf litter (hereafter litter) from riparian plants, as their main energy source (Webster and Benfield, 1986). Once in the water, litter is broken down as a result of physicochemical (i.e., shear stress and leaching of soluble compounds) and biological processes (i.e., decomposition mediated by microorganisms and litter-consuming detritivorous invertebrates; Gessner et al., 1999). Importantly, the rate at which litter is decomposed, and the relative importance of both decomposition pathways (i.e., microbial vs. detritivore-mediated), can inform about the fate of litter carbon and nutrients and the efficiency of the stream food web in capturing and using these elements (Marks, 2019).

Different environmental change drivers can modify the rates and pathways of litter decomposition. For example, the gradual increase in mean temperature promotes microbial decomposition in detriment of detritivore-mediated decomposition (Boyero et al., 2011), while extreme climatic events inhibit microbial decomposition (Correa-Araneda et al., 2020). Agricultural practices involve multiple environmental changes that can act as stressors for stream ecosystems, including nutrient enrichment from fertilizer runoff, increased sedimentation, and the presence of pesticides (Matthaei et al., 2010; Cornejo et al., 2019). Evidence from temperate streams and microcosm experiments indicates that nutrient enrichment can stimulate microbial decomposition (Ferreira et al., 2006; Fernandes et al., 2014; Rossi et al., 2019) while nutrients, pesticides and sedimentation can impair detritivore assemblages and detritivore-mediated decomposition (Woodward et al., 2012; Pérez et al., 2013; Brosed et al., 2016; Chará-Serna and Richardson, 2018).

In many tropical streams, litter decomposition is driven mainly by microorganisms, with a minor contribution of detritivores (Irons et al., 1994; Boyero et al., 2011). Under this scenario, impacts of agriculture on decomposition rates could be expected to be lower than those reported for temperate streams. However, this may not always be the case, because the role of detritivores can be important in some cases, such as at high altitudes (Yule et al., 2009) or in some biogeographic areas (Boyero et al., 2015). Furthermore, decomposition rates and pathways can vary depending on litter type (Martínez et al., 2013), with higher-quality litter generally showing faster detritivore-mediated decomposition rates than more recalcitrant litter (Boyero et al., 2015; Boyero et al., 2016).

We explored the above topics by conducting a litter decomposition experiment in streams within a tropical catchment, which were subjected to different degrees of agricultural influence: low (protected area, PA), medium (buffer area, BA) and high influence (agricultural area, AA). We quantified decomposition rates of litter enclosed within fine-mesh and coarse-mesh bags, which allowed the distinction of microbial and detritivore-mediated decomposition pathways; and used litter of three riparian species in order to explore differences due to litter quality. Additionally, we examined detritivore assemblages through the contents of litterbags and benthic samples. We tested the following hypotheses: (1) microbial decomposition increases with agricultural activity (PA < BA < AA) in relation to nutrient enrichment; (2) detritivore-mediated decomposition decreases with agricultural activity (PA > BA > AA) due to a reduction in detritivore abundance and diversity, in relation to increased pesticide concentration and sedimentation; (3) total decomposition decreases with agricultural activity (PA > BA > AA) because the reduction in detritivore-mediated decomposition is higher than the increase in microbial decomposition; and (4) effects of agriculture on decomposition are greater on higher-quality litter types because these are more consumed by detritivores.

2. Material and methods

2.1. Study area and site selection

Our study area was the upper catchment of the Chiriquí Viejo river, located on the Pacific coast of western Panama (8.25–9.00°N, 82.25–83.00°W; Fig. 1). Catchment area is 1376 km^2, the length of the main river is 161 km and the highest altitude is 3474 m a.s.l. at the Barú volcano (ETESA, 2008). The climate is tropical, with minimum, average and maximum temperatures of 17.8, 28.0 and 35.5 °C, respectively (ANAM and CATIE, 2014). Total annual precipitation is 3400 mm on average, with a maximum of 7000 mm at high altitudes and 87.7% occurring in the wet season from May to December (ETESA, 2008).

The catchment is subjected to agricultural practices, but three distinct areas with different degree of alteration can be distinguished: protected, buffer and agricultural areas (PA, BA and AA, respectively). We selected 3 stream sites within each of these areas, with PA sites (S1–S3) located at 2237–2303 m a.s.l. and showing high canopy cover and diverse riparian vegetation (>70%, >40 species); BA sites (S4–S6)

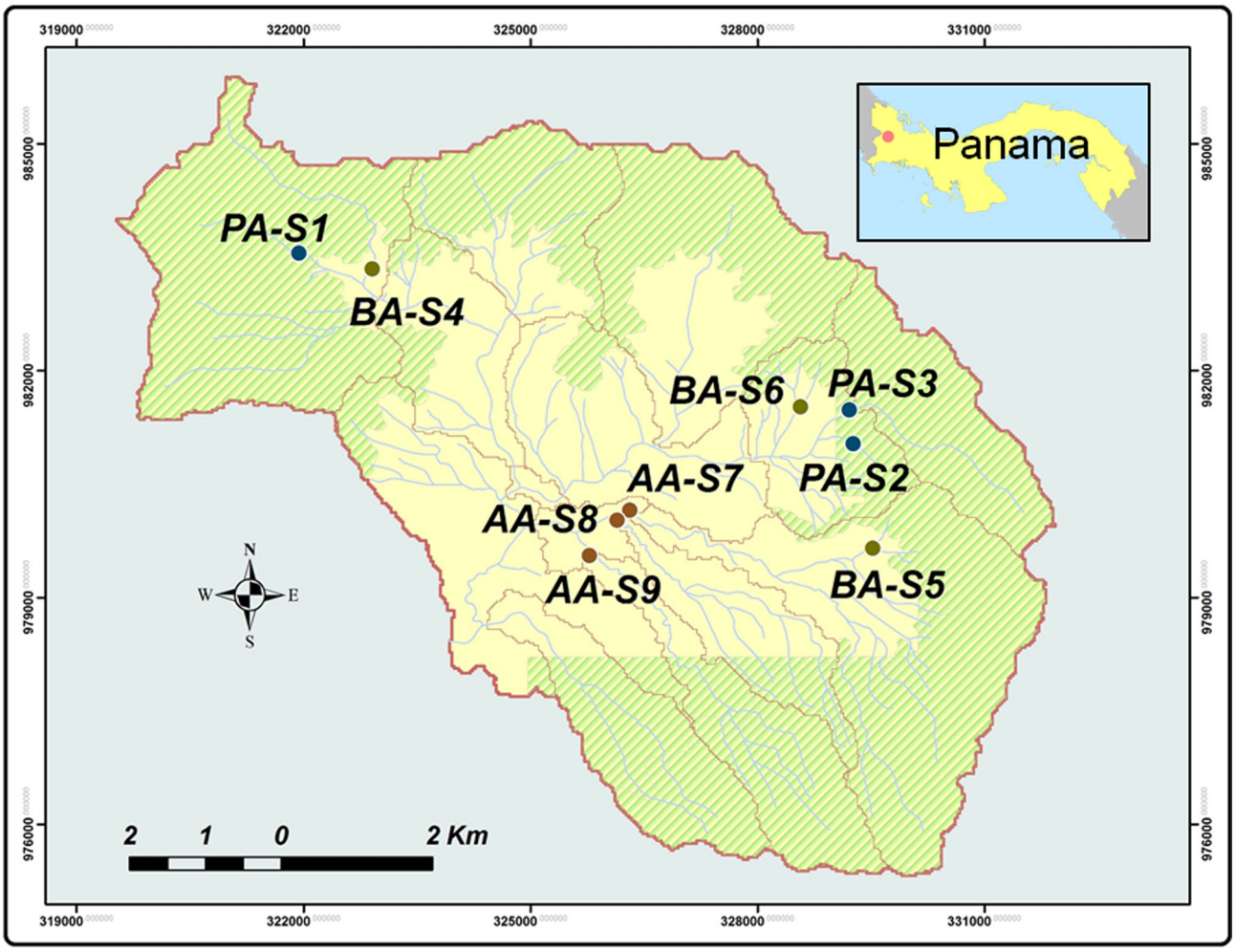

Fig. 1. Location of 9 study sites (S1–S9) in 3 areas with different degree of agricultural influence (protected area, PA; buffer area, BA; and agricultural area, AA) within the Chiriquí Viejo river catchment in western Panama.

at 1675–2143 m a.s.l., with intermediate canopy cover and riparian diversity (40–69%, 21–40 species); and AA sites (S7–S9) at 1708–1888 m a.s.l., with low canopy cover and riparian diversity (<39%, <10 species; Table 1). More altered areas showed higher erosion as a result of vegetation removal (which increases sediment mobilization and deposition or siltation; Izaguirre et al., 2009) and reduced water quality due to pesticide and nutrient release (Cornejo et al., 2019). All sites were 1st or 2nd order independent tributaries of the Chiriquí Viejo river. The study was conducted in April – May 2019.

2.2. Site characterization

At each site we selected a 100-m long representative stream reach, where we characterized the habitat; measured several physicochemical variables in situ; collected water samples for further physicochemical analyses and determination of pesticides; and sampled benthic invertebrates. We characterized the habitat by measuring mean stream width (m), water depth (cm) and current velocity (m s^{-1}), and visually estimating sediment deposition (%), riparian vegetation cover (%), substrate composition (% of different size classes of mineral substrate: boulder, cobble, gravel, coarse and fine sand, and clay), and organic matter presence [% of streambed covered by CPOM (>1 mm) and FPOM (0.5–1 mm)] (Barbour et al., 1999). We measured pH, temperature (°C), conductivity (μS cm^{-1}), turbidity (NTU) and dissolved oxygen saturation (%) in situ using a multiparametric probe (HACH HQ40d), and current velocity using a flowmeter (Flowatch 12300).

We collected two sets of 2-L water samples from the mid column in the middle of the stream, which were transported to the laboratory on ice and kept at 4 °C for 24 h until their analysis. We analysed the first set of water samples at the Water and Physicochemical Services Laboratory (LASEF-UNACHI, Panama) following standard methods (Rice et al., 2012) for concentrations (mg L^{-1}) of total solids (method SM 2540 B), nitrate (NO_3; SM 4500-NO3 B), phosphate (PO_4; SM 4500 PE), faecal coliforms (SM 9222 D) and biological oxygen demand (BOD_5; SM 5210 B). We analysed the second set of water samples for pesticides at the Plant Health Laboratory from the Agricultural Development Ministry (MIDA, Panama), using two methods: liquid-liquid microextraction (De Romedi et al., 2011) and direct injection (Reemtsma et al., 2013) We used the first method for organophosphates, organochlorines and pyrethroids; we extracted pesticides with ethyl acetate and residuals and quantified them by gas chromatography and mass spectrophotometry (GC-MSMS; limit of quantification: 0.11 μg L^{-1}). We used the second method for triazines, carbamates and other polar pesticides; we injected samples and analysed them with high performance liquid chromatography and triple quadrupole mass spectrophotometer (LC-MSMS; limit of quantification: 0.10 μg L^{-1}) and electrospray ionization with dynamic acquisition (DyMRM mode), which avoids solid phase extraction. The percentage of recovery ranged between 70 and 110% (CV = 11%). We measured linearity by the R^2 coefficient for the individual pesticide calibration curves, which always resulted in $R^2 \geq 0.99$. We analysed each set of samples in duplicate, simultaneously with a laboratory blank; to avoid matrix effects, we used a matrix-matched calibration curve. We

Table 1
Location and physico-chemical characterization of the 9 study sites (S1-S9) located in 3 areas with different degree of agricultural influence (protected area, PA; buffer area, BA; and agricultural area, AA); –, not detected.

Areas	PA Canopy cover >70% No. riparian species >40 Little human influence			BA Canopy cover 40–69% No. riparian species 21–40 Moderate human influence			AA Canopy cover <39% No. riparian species <10 Large human influence		
Sites	S1	S2	S3	S4	S5	S6	S7	S8	S9
Coordinates	322070 984322	329004 981464	328888 981464	322910 983345	328382 979384	327912 980842	326313 980152	325782 979555	325009 979941
Altitude (m asl)	2237	2325	2303	2143	1791	1675	1708	1887	1888
Habitat characterization									
Stream width (m)	10.2	9.8	5.7	10.5	8.7	8.8	9.4	8.2	6.9
Water depth (cm)	52.0	47.0	24.0	57.0	31.0	39.0	35.0	37.0	25.0
Current velocity (m s^{-1})	0.3	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.1
Sediment deposition (%)	35	40	40	75	60	75	90	80	80
Riparian vegetation cover (%)	100	100	100	20	15	10	15	15	5
Inorganic substrate (%)									
Boulder (>256 mm; %)	10	5	0	20	10	30	20	5	0
Cobble (64–256 mm; %)	50	50	20	40	30	55	10	10	0
Gravel (2–64 mm; %)	20	20	20	20	30	5	30	20	10
Coarse sand (0.06-2 mm; %)	10	10	50	10	10	5	20	40	60
Fine sand (0.004–0.006 mm; %)	10	10	10	5	10	5	15	20	20
Clay (<0.004 mm; %)	0	5	10	5	10	0	5	5	10
Organic matter (%)									
Coarse Particulate Organic Matter (CPOM)	70	60	70	60	60	60	40	30	30
Fine Particulate Organic Matter (FPOM)	30	40	30	40	40	40	60	70	70
Physicochemical variables									
pH	7.65	7.05	7.28	7.52	7.33	7.42	7.95	7.63	7.46
Temperature (°C)	13.9	13.6	14.0	13.9	16.6	17.1	17.2	16.7	17.4
Conductivity μS $cm^{-1)}$	25.70	12.08	36.00	15.25	89.30	109.60	115.30	81.80	200.20
Turbidity (NTU)	0.7	0.3	2.9	1.0	5.0	0.5	0.5	11.0	12.8
Oxygen saturation (%)	98.3	96.9	96.0	98.3	93.4	96.0	96.6	96.0	97.2
Biological Oxygen Demand (BOD_5; mgL^{-1})	1.00	1.00	1.00	1.00	1.00	1.00	2.03	1.00	2.14
Total solids (mgL^{-1})	56	80	56	54	113	58	104	105	262
NO_3 (mgL^{-1})	6.6	2.9	12.5	5.9	37.7	15.7	29.9	17.8	58.6
Water-quality-index	77	86	73	75	80	70	68	65	62
Pesticides									
Chlorpyrifos (μgL^{-1})					0.14			10.47	0.32
Cypermethrin (μgL^{-1})							0.38		
Diazinon (μgL^{-1})				1.57					
Pyrazophos (μgL^{-1})						0.58			
TU_{max}	–	–	–	−0.11	−0.33	0.21	−0.23	1.54	0.03

determined pesticide toxicity as the maximum toxic units (TU_{max}) and, when these were below the quantification limit, we considered the values previously reported by Cornejo et al. (2019). Given that toxicity data for tropical stream invertebrates are unavailable, we calculated TU_{max} based on data available for *Daphnia magna* (Liess and Ohe, 2005) calculated according to the following formula:

$$TU_{(D.magna)} = \max_{i=1}^{n}(\log(C_i/LC50_i)) \quad (1)$$

where $TU_{(D.\ magna)}$ is the TU_{max} of *n* pesticides detected in the study site, C_i is the concentration of pesticide *i* ($\mu g\ L^{-1}$), and $LC50_i$ is the 48-h acute median lethal concentration ($\mu g\ L^{-1}$) reported for pesticide *i* in *D. magna*.

2.3. Litter decomposition experiment

We selected 3 riparian tree species that were common in the study area and differed in litter quality, which was assessed through specific leaf area [SLA; the ratio of leaf area (mm^2) to leaf dry mass (DM; mg)] and nitrogen (N) concentration (%); SLA was quantified by cutting 20 discs from different air-dried leaves of each species using a 17-mm diameter cork borer, avoiding main leaf nerves, and weighing them to the nearest 0.01 mg; N concentrations were obtained from unpublished data (mean ± SE; L. Boyero, unpubl.). The species were *Alnus acuminata* Kunth. (Betulaceae), with high-quality litter (SLA = 10.8 ± 1.9 $mm^2\ mg^{-1}$; $N = 2.40 \pm 0.08\%$); *Ficus insipida* Willd. (Moraceae), with intermediate-quality litter (SLA = 10.7 ± 1.1 $mm^2\ mg^{-1}$; $N = 1.09 \pm 0.09\%$); and *Quercus bumelioides* Liedm. (Fagaceae), with low-quality litter (SLA = 6.2 ± 1.2 $mm^2\ mg^{-1}$; N concentration unavailable).

We collected recently senesced litter of the 3 species from the riparian forest floor in streams of the study catchment. In the laboratory, litter was air dried and cut in ca. 2 × 2 cm fragments, excluding the basal petiole insertion. We used extra litter to estimate litter mass loss (LML) due to the leaching of soluble compounds: we introduced this litter in glass jars with 400 mL of filtered (100 μm) stream water collected at the experimental site (1 g per species and replicate, n = 3) for 48 h, with water replacement after 24 h; litter from each replicate was oven-dried (70 °C, 72 h) and weighed to estimate the relationship between initial air DM and post-leaching oven DM (López-Rojo et al., 2020).

We prepared 486 sets of fragments (18 per species per site), weighed them individually (1.00 ± 0.05 g), hooked them on safety pins, and introduced them within fine-mesh (0.5 mm) and coarse-mesh (10 mm) bags (20 × 15 cm), with different species in separate bags. On April 23, 2019, we deployed the litterbags at the 9 studied sites, attached with nylon rope to stakes that were hammered into the stream substrate. We collected one third of the bags on day 0 in order to estimate litter mass handling losses (which were observed to be

negligible); and the other two thirds on days 14 and 28, respectively. Bags were collected by placing a net immediately downstream and introducing them into ziplock bags, which were transported to the laboratory on ice. In the laboratory, litter was carefully rinsed using filtered (100-μm) stream water on a 500-μm sieve to remove sediments and invertebrates. Then it was oven dried (70 °C, 72 h), weighed to estimate final DM, incinerated (500 °C, 4 h) and re-weighed to estimate final ash-free dry mass (AFDM). We quantified decomposition through the proportion of LML, calculated as the difference between initial and final AFDM (g) divided by initial AFDM (g), with initial AFDM corrected by the proportion of LML due to leaching. Litter ash content (%) was also used as a proxy for sedimentation to be compared among PA, BA and AA streams.

2.4. Invertebrates

Invertebrates collected from coarse-mesh bags were preserved in 70% ethanol, identified under a stereoscopic microscope to the lowest possible taxonomic level, and separated into litter-consuming detritivores and other invertebrates. Each group was separately oven dried (70 °C for 72 h) and weighed to calculate detritivore and total biomass (mg) per bag. We also recorded detritivore and total invertebrate abundance and taxonomic richness (i.e., number of individuals and taxa, respectively) per bag.

Additionally, we sampled benthic invertebrates twice at each study site (on days 0 and 28 of the decomposition experiment) using a 30-cm wide, 0.5 mm mesh D-net. We used a multihabitat sampling approach, which proportionally covered the main stream habitats (mineral substrate, fine sediment, litter, bank vegetation and submerged macrophytes) present in the study stream reach, with a total of twenty 0.5-m sample units per site (i.e., a total of 3 m^2 per site; Barbour et al., 1999; Cornejo et al., 2019). The net contents were transferred to a 0.5-mm sieve first and then to a white tray, where mineral substrate and organic material were discarded. Samples were introduced in 500-mL bottles, preserved with 96% ethanol and transported to the laboratory, where invertebrates were identified and separated into litter-consuming detritivores and other invertebrates.

Invertebrates from litterbags and benthic samples were sorted and identified to family level at the Freshwater Macroinvertebrate Laboratory at the COZEM-ICGES (Panama) and then identified to genus level and classified based on their feeding type at the Museum of Freshwater Fish and Invertebrates (MUPADI-UNACHI, Panama), using available literature (Hawkes, 1998; Tomanova et al., 2006; Beketov et al., 2009; Gutiérrez-Fonseca, 2010; Menjivar Rosa, 2010; Pacheco-Chaves, 2010; Springer et al., 2010; Ramírez and Gutiérrez-Fonseca, 2014).

2.5. Statistical analyses

We first compared the stream habitat characteristics indicating stream morphology (i.e., stream width, water depth and current velocity) among our 3 study areas to ensure there were no confounding effects, using linear models [lm function in R (R Core Team, 2019)]. We used principal component analysis [PCA; rda function, *vegan* package (Oksanen et al., 2018)] to explore variation among sites in terms of physicochemical and habitat variables that could be indicative of stream impairment due to agriculture (i.e., TU_{max}, water temperature, dissolved oxygen saturation, NO_3 concentration, sediment deposition and riparian vegetation cover; Fig. S1).

To explore how litter decomposition responded to the agricultural influence gradient we used linear mixed-effects models [lme function and restricted maximum likelihood (REML) estimation, *nlme* package (Pinheiro et al., 2018)] with microbial (hypothesis 1), detritivore-mediated (hypothesis 2) and total decomposition (hypothesis 3) as response variables, and different influence degrees (PA, BA and AA) and litter types (*Alnus*, *Ficus* and *Quercus*) as categorical predictors. We included the interaction between agricultural influence and litter type in the models to test whether litter type mediated the agricultural influence on decomposition (hypothesis 4). We included stream sites as a random component (due to our nested sampling design), and tested the improvement of model fit after the inclusion of this component using the Akaike Information Criterion corrected for sample size (AICc; Table S1). We used a variance structure (varIdent function, *nlme* package) to consider different variances across influence degrees and litter types and thus avoid violation of the homogeneity of variances assumption for linear models (Zuur et al., 2009). We defined the optimal variance structure through initial data exploration using multipanel boxplots for each response variable vs. influence degree and litter type, and comparing models with different structures using AICc (Zuur and Ieno, 2015). We inspected residuals from each model to ensure there were no visual patterns or violation of linear model assumptions.

When there was an interaction between influence degree and litter type, we explored and quantified the magnitude of such difference by calculating the average and ordinary nonparametric bootstrapped 95% confidence intervals for microbial, detritivore-mediated and overall decomposition for each litter type. We calculated these confidence intervals using the bias-corrected and accelerated (BCa) method with the boot function in the *boot* package, and based on 1000 bootstrap replicates (Davison and Hinkley, 1997; Canty and Ripley, 2016). Non-overlapping 95% confidence intervals indicated statistically significant differences (Wood, 2005).

Lastly, we investigated the variation in invertebrate assemblages separately for litterbags and benthic samples with several methods: we (i) examined assemblage structure with non-metric dimensional scaling (NMDS) based on the Bray Curtis similarity index of abundance data, using the metaMDS function of the *vegan* package; (ii) explored differences among degrees of agricultural influence (PA, BA and AA) and collection times (14 and 28 d) with permutational multivariate analysis of variance (adonis function, *vegan* package); (iii) assessed the contribution of each invertebrate taxon with the similarity percentage procedure SIMPER (simper function, *vegan* package); (iv) examined how detritivore and total invertebrate abundance, richness and biomass in coarse-mesh bags at day 28 responded to the agricultural influence gradient (PA, BA and AA) and litter type (*Alnus*, *Ficus* and *Quercus*) with linear mixed-effects models and confidence intervals (as above); and (v) explored detritivore and total invertebrate abundance and richness variation in benthic samples across the agricultural influence gradient, again with linear mixed-effects models and confidence intervals.

3. Results

3.1. Site characterization

The environmental variables measured at the study sites had the following value ranges: stream width, 5.7–10.5 m; water depth, 25–57 cm; current velocity, 0.1–0.3 m s^{-1}; pH, 7.05–7.95; water temperature, 13.6–17.4 °C, conductivity, 12.18–200.20 μS cm^{-1}; turbidity, 0.33–12.8 mg L^{-1}; dissolved oxygen saturation, 93.4–98.3%; BOD5, 1.00–2.14 mg L^{-1}; total solids, 54.00–262.00 mg L^{-1}; NO_3 concentration, 2.9–58.6 mg L^{-1}; values for each site are given in Table 1. Stream width, water depth and current velocity did not vary among the 3 study areas (width: $F_{2,8} = 0.36$, $p = 0.71$; depth: $F_{2,8} = 0.60$, $p = 0.58$; velocity: $F_{2,8} = 1.33$, $p = 0.33$), which discarded any potential confounding effects of habitat morphology on our results. We detected 4 pesticides in total, including 3 insecticides (chlorpyrifos, cypermethrin and diazinon) and 1 fungicide (pyrazophos); TU_{max} ranged from 0 to 1.54 (Table 1). The first two PCA axes explained 77.6% of the variability among sites; axis 1 (57.4%) separated sites S1–S3 (PA) and S4 (BA) from other sites mainly based on the better status of their riparian vegetation and the lower water temperature and NO_3 concentration; axis 2 (20.2%) further separated sites S1 and S4 based on their higher dissolved oxygen saturation, and sites S6, S7 and S8 based on their higher sediment deposition and TU_{max} (Fig. S1).

3.2. Litter decomposition experiment

After 28 d of instream incubation, the proportion of LML ranged from 0.00 to 0.78 in fine-mesh bags and from 0.02 to 0.93 in coarse-mesh bags (Fig. 2). Linear mixed-effects models revealed a significant interaction between agricultural influence degree and litter type for microbial, detritivore-mediated and total decomposition (Table 2). Microbial decomposition was higher in AA than in PA and BA streams for *Alnus* and *Ficus*, but not for *Quercus*, which showed no differences across the agricultural gradient; detritivore-mediated decomposition was higher in PA than in BA and AA streams for *Alnus*, with *Ficus* showing a similar pattern albeit not significant, and no pattern for *Quercus*; and total decomposition was higher in PA than in BA and AA streams for *Alnus*, and similar across areas for *Ficus* and *Quercus* (Fig. 2). Litter ash contents varied across the agricultural influence gradient after 28 d of incubation; for both mesh types, ash contents were higher in AA than in PA and BA streams for *Alnus* and *Ficus*, but not for *Quercus*, which showed no differences (Table S2, Fig. S2).

Table 2
Results of linear models testing the effects of agricultural influence degree (PA, BA and AA), litter type (*Alnus*, *Ficus* and *Quercus*) and their interaction on microbial, detritivore-mediated and total decomposition (quantified as the proportion of litter mass loss); df = degrees of freedom, F = F statistic; p = p-value.

Response variable	Factor/interaction	df	F	p
Microbial decomposition	Agricultural influence degree (AI)	2	4.86	0.0557
	Litter diversity (LD)	2	104.47	<0.0001
	AI × LD	4	8.32	<0.0001
Detritivore-mediated decomposition	AI	2	0.24	0.7913
	LD	2	13.21	<0.0001
	AI × LD	4	7.23	0.0001
Total decomposition	AI	2	71.25	0.0001
	LD	2	133.34	<0.0001
	AI × LD	4	4.56	0.0026

3.3. Invertebrates

We collected 256 invertebrate individuals from coarse-mesh litterbags, which belonged to 13 genera, 15 families, 8 orders and 4 classes (Table S3); the most common families (representing 86% of all invertebrates collected in litterbags) were the Physidae (Basommatophora; 34.77% of total abundance), Lepidostomatidae (Trichoptera; 19.92%), Tubificidae (Haplotaxida; 14.84%), Simuliidae (Diptera; 9.38%) and Chironomidae (Diptera; 7.42%). In benthic samples there were 2371 individuals belonging to 45 genera, 39 families, 16 orders and 7 classes (Table S4); the most common families (70.72% of all invertebrates found in benthic samples) were the Chironomidae

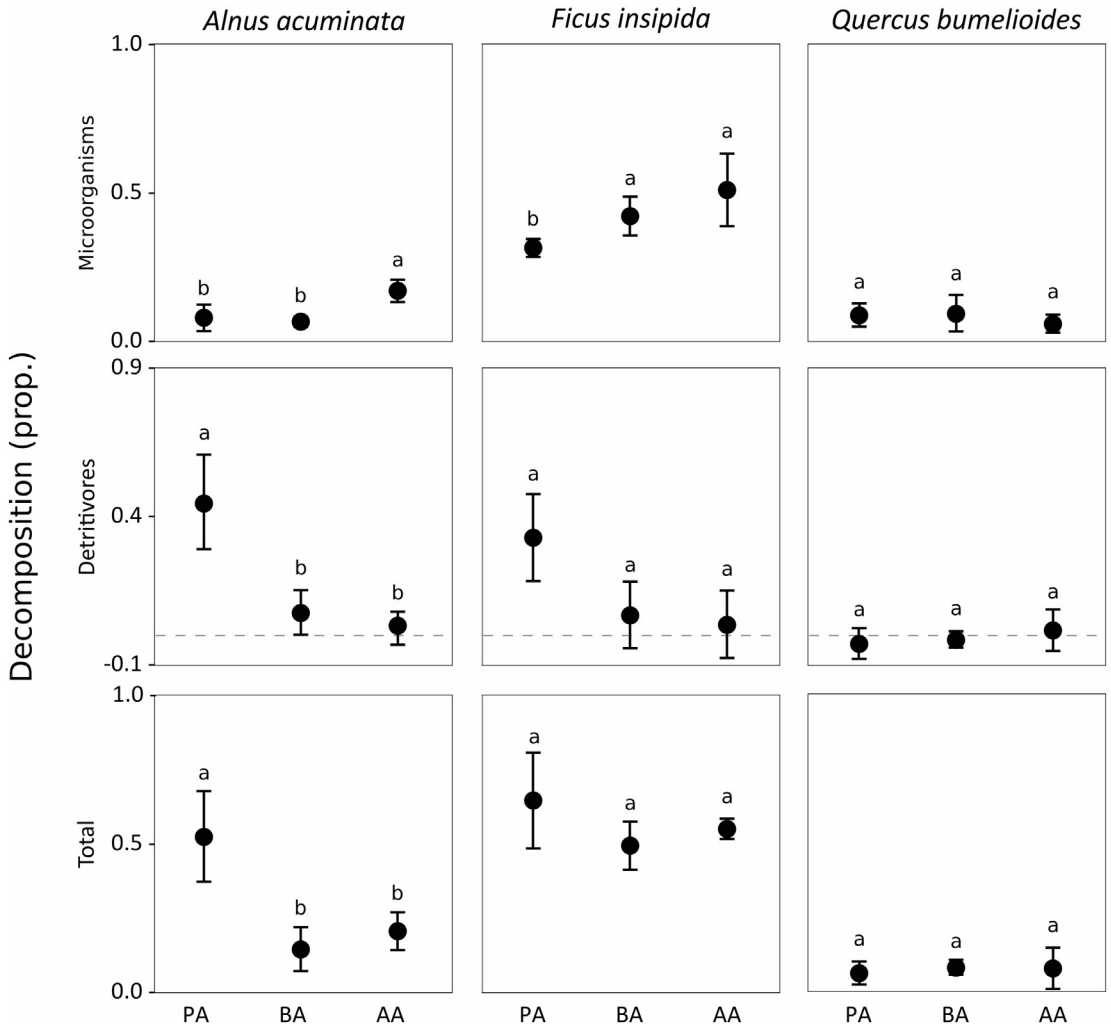

Fig. 2. Microbial, detritivore-mediated and total decomposition (mean ± SE proportion of litter mass loss) of 3 litter types (*Alnus*, *Ficus* and *Quercus*) after 28 d of incubation in streams located at 3 study areas with different degree of agricultural influence (protected area, PA; buffer area, BA; and agricultural area, AA). Different letters indicate significant differences among areas ($p < 0.05$).

(Diptera; 21.31% of total abundance), Simuliidae (Diptera; 13.02%), Physidae (Basommatophora; 12.22%), Tubificidae (Haplotaxida; 7.92%), Baetidae (Ephemeroptera; 5.77%), Hyalellidae (Amphipoda; 5.52%) and Ptilodactylidae (Coleoptera; 4.97%).

The NMDS showed a clear separation of invertebrate assemblages from the 3 areas whit different agricultural influence degree, both for coarse-mesh bags ($p < 0.001$; Fig. 3A) and benthic samples ($p < 0.005$; Fig. 3B). In both cases, PA streams were characterized by the presence of litter-consuming detritivores (mostly Lepidostomatidae), while BA and AA areas had more generalist detrivorous invertebrates (i.e., collector-gatherers and filterers; mostly Chironomidae and Simuliidae in BA and Physidae in AA). We observed a general pattern of greater abundance, richness and biomass of litter-consuming detritivores in litter-bags in PA (345 individuals in total; 3–6 genera per site) compared to BA (45 individuals; 0–4 genera); these invertebrates were absent in AA, where dipterans and gastropods were dominant (Table 3, Fig. S3). These differences were significant for *Alnus* and for richness in *Ficus*, with others being similar albeit not significant. In benthic samples, PA showed greater abundance of litter -consuming detritivores than BA and AA (Table 3).

Table 3
Results of linear models testing the effects of agricultural influence degree (PA, BA and AA), litter type (*Alnus*, *Ficus* and *Quercus*) and their interaction on the abundance, taxonomic richness and biomass of litter-consuming detritivores and total invertebrates found in coarse-mesh bags (Lit.) and benthic samples (Bent.); biomass data unavailable from benthic samples; df = degrees of freedom, F = F statistic; p = p-value.

Response variable	Factor/interaction	df	F	p
Detritivore abundance Bags	Agricultural influence degree (AI)	6	12.57	0.0072
	Litter type (LT)	12	0.89	0.4342
	AI × LT	12	1.48	0.2686
Detritivore richness Bags	AI	6	19.11	<0.0001
	LT	12	1.44	0.2619
	AI × LD	12	2.11	0.1215
Detritivore biomass Bags	AI	6	12.30	0.0075
	LT	12	1.70	0.2244
	AI × LT	12	0.95	0.4700
Invertebrate abundance Bags	AI	6	0.24	0.7931
	LT	12	1.70	0.2245
	AI × LD	12	5.20	0.0115
Invertebrate richness Bags	AI	6	1.00	0.4219
	LT	12	1.30	0.3083
	AI × LD	12	1.30	0.3245
Invertebrate biomass Bags	AI	6	0.12	0.8246
	LT	12	0.18	0.8359
	AI × LT	12	1.55	0.2502
Detritivore abundance Bent.	AI	6	10.36	0.0113
Detritivore richness Bent.	AI	6	2.89	0.1317
Invertebrate abundance Bent.	AI	6	0.04	0.9596
Invertebrate richness Bent.	AI	6	0.18	0.8328

4. Discussion

Studies of litter decomposition in tropical streams flowing through agricultural land are rare, and have provided little evidence about the impacts caused by this human activity. For example, Parnrong et al. (2002) found no differences in litter decomposition between forested and agricultural sites in Thailand; and Torres and Ramírez (2014) found reduced decomposition in agricultural compared to forested streams in Puerto Rico, but did not separate effects mediated by micro-organisms and detritivores. Here we show how an increasing degree of agricultural influence within a tropical catchment in Panama (from protected to buffer to agricultural areas) significantly altered decomposition through effects on microbial activity and detritivore assemblages, and discuss the potential abiotic and biotic drivers of such effects and their consequences for stream ecosystem functioning and global biogeochemical cycles.

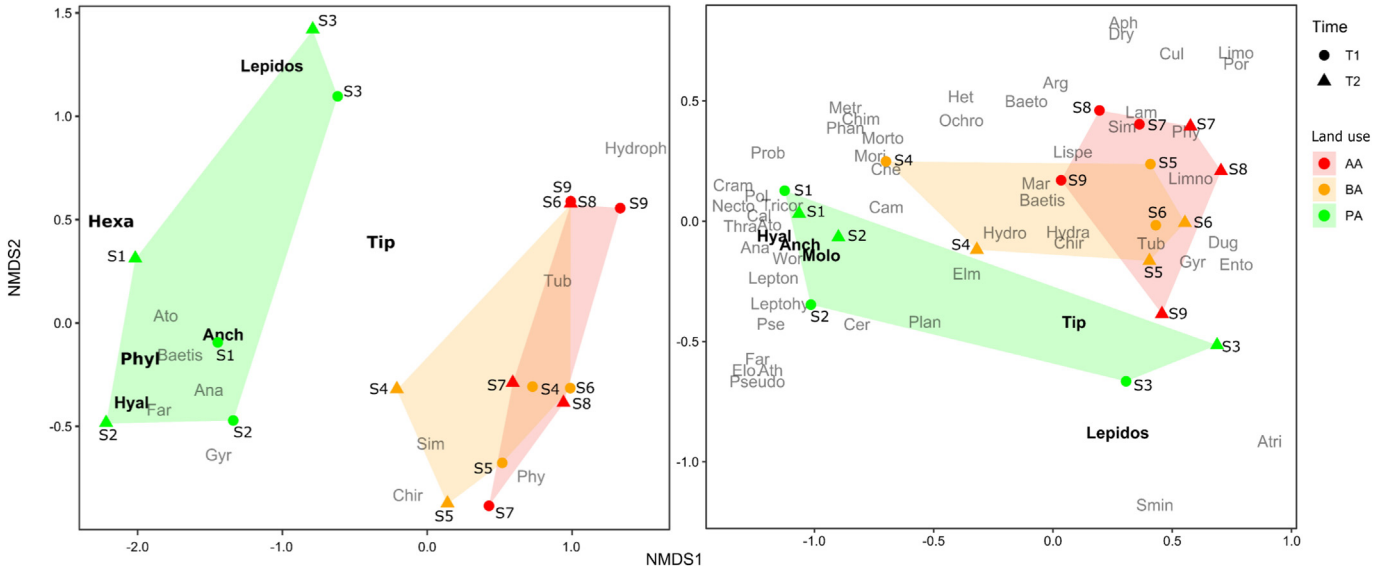

Fig. 3. Non-metric multidimensional scaling (NMDS) ordination of invertebrate assemblages from coarse-mesh bags (A; stress: 0.05953776) and benthic samples (B; stress: 0.1199857) collected from 9 streams located at 3 areas with different degree of agricultural influence (sites S1–S3: protected area, PA; S4–S6: buffer area, BA; and S7–S9: agricultural area, AA). Taxon names are indicated, with litter-consuming detritivores highlighted in bold (Ana, *Anacroneuria*; Anch, *Anchytarsus*; Aph, *Aphrosylus*; Arg, *Argia*; Ath, *Atherix*; Ato, *Atopsyche*; Atri, *Atrichopogon*; Baeti, *Baetis*; Baeto, *Baetodes*; Cal, *Calosopsyche*; Cam, *Camelobaetidius*; Cer, Ceratopogonidae; Che, *Chelifera*; Chim, Chimarra; Chir, Chironomidae; Con, *Contulma*; Cram, Crambidae; Cul, *Culoptila*; Dry, *Dryops*; Dug, *Dugesia*; Elm, Elmidae; Elo, *Elodes*; Ento, Entomobryidae; Far, *Farrodes*; Gyr, *Gyraulus*; Het, *Hetaerina*; Hex, *Hexatoma*; Hyal, *Hyallela*; Hydra, Hydrachnidae; Hydropt; *Hydroptila*; Hydroph, Hydrophilidae; Lam, *Lampyridae*; Lep, Lepidoptera; Lepidos, *Lepidostoma*, Leptohy, *Leptohyphes*; Lepton, *Leptonema*; Limno, *Limnophora*; Limo, *Limonia*; Lispe, *Lispe*, Mar, *Maruina*; Metr, *Metrichia*; Molo, *Molophilus*; Mori, *Moribaetis*; Morto, *Mortoniella*; Necto, *Nectopsyche*; Ochro, *Ochrotrichia*; Phan, *Phanocerus*; Phy, *Physa*; Phyl, *Phylloicus*; Plan, Planariidae; Pol, *Polycentropus*; Por, Porcellionidae; Prob, *Probezzia*; Pse, *Psephenus*; Pseudo, *Pseudothelphusa*; Sim, *Simulium*; Smin, Sminthuridae, Thra, *Thraulodes*; Tip, *Tipula*; Tricor, *Tricorythodes*; Tub, Tubificidae; Wor, *Wormaldia*).

4.1. Agriculture enhanced microbial decomposition

We found that microbial decomposition increased with the degree of agricultural influence, although effects varied with litter type. Decomposition of *Alnus acuminata* increased in the agricultural area compared to the buffer and pristine areas; decomposition of *Ficus insipida* increased in both agricultural and buffer areas compared to the pristine area; and decomposition of *Quercus bumelioides* showed no change across areas and was very low, most likely in relation to its low quality. The faster microbial decomposition of high-quality compared to low-quality litter is well known from temperate areas; for example, *Alnus glutinosa* often decomposes faster than *Quercus robur* in fine-mesh bags (e.g., Lecerf et al., 2005; Monroy et al., 2016), and even intraspecific differences in litter quality of these species induce changes in microbial decomposition (Graça and Poquet, 2014). However, the role of litter quality on microbial sensitivity to environmental impacts is unclear, as different studies have found lower (Gonçalves et al., 2013; Pérez et al., 2014), higher (Chamier, 1987; Pearson and Connolly, 2000) or similar sensitivity (Gulis and Suberkropp, 2003; Bergfur and Friberg, 2012) of high-quality compared to low-quality litter. While dissolved nutrients could be expected to enhance microbial decomposition of recalcitrant litter such as that of *Q. bumelioides* (Esquivel et al., 2019), this was not the case in our study.

Increased microbial decomposition was most likely due to the higher nutrient concentrations in streams affected by agriculture, which were approximately 3- and 5- fold in the buffer and agricultural areas, respectively, compared to the pristine area. Nutrient enrichment is well known to stimulate microbial activity (Woodward et al., 2012) through increased fungal biomass accrual and sporulation (Gulis et al., 2006) and, possibly, enhanced fungal diversity (Pérez et al., 2018). Such effects have mostly been shown for temperate streams, but there is also evidence of increased microbial decomposition due to eutrophication from the tropics, as shown in studies not related to agriculture (Pearson and Connolly, 2000; Connolly and Pearson, 2013). One tropical study assessing microbial decomposition through an agricultural gradient found an inverse pattern, that is, lower decomposition in agricultural sites (Silva-Junior et al., 2014); however, the number of sites under different degrees of agricultural influence in that study was highly unbalanced and some sites were also affected by urbanization, which makes the interpretation of their results difficult. In our study, the increase in microbial decomposition with agricultural influence suggested that nutrient enrichment effects were greater than any potential negative effects of pesticides (Cornejo et al., 2020) or sedimentation (Martínez et al., 2020) on microorganisms. Such negative effects could be expected to be low, due to the generally high functional redundancy of microbial assemblages (Allison and Martiny, 2008).

4.2. Agriculture impaired detritivore-mediated decomposition

We found that increasing agricultural influence caused a decrease in detritivore-mediated decomposition. Differences were significant only for *A. acuminata* in both the buffer and agricultural areas; *F. insipida* followed a similar trend, but it was not significant; and, again, *Q. bumelioides* showed no differences across the agricultural gradient. The role of litter quality in the sensitivity of detritivore-mediated decomposition to environmental changes is again unclear, due to contrasting results of different studies (Lecerf et al., 2005; Bruder et al., 2011; Woodward et al., 2012; Monroy et al., 2016); in our study, however, only high-quality litter reflected environmental changes, both for microbial and detritivore-mediated decomposition. In contrast, Masese et al. (2014) found reduced detritivore-mediated decomposition in agricultural compared to forested streams in Kenya for both high-quality and low-quality litter (*Croton macrostachyus* and *Syzygium cordatum*, respectively).

Patterns in detritivore-mediated decomposition could be explained by the changes suffered by detritivore assemblages, as reflected in coarse-mesh bags and benthic samples. In both types of samples, we found a clear impact of agriculture on these organisms, which significantly reduced their abundance (30- and 6-fold reduction in bags and benthic samples, respectively) and richness (3-fold reduction in bags and absence in benthic samples from 2 sites) in the buffer area, and almost disappeared in the agricultural area (only 1 individual, found in benthic samples). The litter-consumng detritivores found in the buffer area were *Anchytarsus* (Coleoptera), *Tipula* (Diptera), *Molophilus* (Diptera) and *Hyalella* (Amhipoda), but the Trichoptera present in the pristine area (*Phylloicus* and *Lepidostoma*) were absent. Invertebrate assemblages from buffer and agricultural areas were dominated by other functional groups such as collectors-gatherers and filterers, many of them typical of impacted sites and previously identified in streams affected by agriculture in our study area (Cornejo et al., 2019).

Reduced detritivore abundance and richness were most likely due to the combined effects of sedimentation and pesticide toxicity, as indicated by our measures of litter ash contents and TU_{max} (which were highest in the agricultural area followed by the buffer area) and shown in a previous study that included 13 streams in our study catchment (Cornejo et al., 2019). Sedimentation can promote invertebrate drift downstream (Suren and Jowett, 2001), and pesticides can be toxic and produce lethal and sublethal effects on detritivores (Zubrod et al., 2014; Zubrod et al., 2015; Cornejo et al., 2020); the pesticide chlorpyrifos, in particular, detected at very high concentrations at the agricultural area, was shown to reduce decomposition through sublethal effects on detritivores in an experiment conducted in Canada (Chará-Serna and Richardson, 2018). Experimental evidence of such effects is, however, lacking for tropical detritivores, and reports of comparable patterns from tropical streams are rare. One study conducted in Borneo found reduced detritivore abundance, richness and detritivore-mediated decomposition in streams flowing through areas logged for palm oil plantations and attributed these differences to increased sedimentation, but pesticide concentrations were not examined (Jinggut et al., 2012).

4.3. Decomposition as a key tool for assessing tropical stream integrity

Total decomposition followed a similar pattern to detritivore-mediated decomposition, indicating that enhancement of microbial decomposition did not compensate for the negative effects on detritivores. A similar result was obtained for pesticide effects on total decomposition in a study conducted in France (Magali et al., 2016). Our results thus indicate that the impairment of tropical invertebrate assemblages caused by agriculture (Rasmussen et al., 2016; Cornejo et al., 2019) goes beyond structural changes and has functional repercussions in the ecosystem. Litter decomposition has been long used to assess stream functional integrity in temperate areas (Gessner and Chauvet, 2002), and our study suggests that its use should be extended to the tropics as a complement to structural measures such as biotic indices (Cornejo et al., 2019).

Despite the greater abundance and richness of invertebrates in the buffer area compared to the agricultural area, we found little differences in litter decomposition between both areas: they both showed reduced total and detritivore-mediated decomposition of high-quality litter. This suggests that buffer areas were not efficient in moderating the impacts of agriculture on stream ecosystem functioning, a result that should be considered in management and conservation plans of agricultural catchments. Finally, the consequences of reduced litter decomposition go beyond the altered functioning of the stream ecosystem, because changes in the relative importance of microbial and detritivore-mediated decomposition rates determine how much of the organic carbon contained in litter is outgassed to the atmosphere as carbon dioxide and how much is retained and incorporated into the stream food web (Boyero et al., 2011; Marks, 2019). Given that the extent of agricultural land is rapidly growing in tropical regions (Gibbs et al., 2010), the consequences of these changes for the global carbon cycle could be expected to be large.

CRediT authorship contribution statement

Aydeé Cornejo: Conceptualization, Investigation, Funding acquisition, Project administration, Supervision, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Javier Pérez:** Data curation, Methodology, Validation, Writing - review & editing. **Naiara López-Rojo:** Formal analysis, Methodology, Writing - review & editing. **Alan M. Tonin:** Formal analysis, Writing - review & editing. **Dalys Rovira:** Methodology, Funding acquisition. **Brenda Checa:** Methodology, Funding acquisition. **Nicomedes Jaramillo:** Methodology, Writing - review & editing. **Karina Correa:** Investigation, Data curation, Writing - review & editing. **Allison Villarreal:** Investigation, Data curation, Writing - review & editing. **Víctor Villarreal:** Investigation, Writing - review & editing. **Gabriela García:** Investigation, Writing - review & editing. **Edgar Pérez:** Investigation, Writing - review & editing. **Tomás A. Ríos González:** Investigation, Data curation, Writing - review & editing. **Yusseff Aguirre:** Investigation, Data curation, Writing - review & editing. **Francisco Correa-Araneda:** Writing - review & editing. **Luz Boyero:** Conceptualization, Methodology, Supervision, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.140950.

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