Density and diversity of macroinvertebrates in Colombian Andean streams impacted by mining, agriculture and cattle production

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Background. Mining, agriculture and cattle production are activities that threaten the quality and quantity of water resources in the Colombian Andean region. However, many drainage basins in region have not been subjected to a simultaneous (same climatic period) evaluation of the impact these activities have on the density, diversity and composition of aquatic macroinvertebrates (AMI). The first two of these ecological variables are expected to decrease drastically from zones with no apparent impact towards areas with anthropogenic activity, among which areas with mining activity will present the most impoverished AMI community.

Methods. This study evaluated the AMI density, diversity and composition dissimilarity in streams impacted by gold mining, agriculture and cattle production (sampling zones). Six bimonthly samplings were conducted (February 2014 - February 2015) using a Surber-net. Hydrological, physicochemical and bacteriological parameters (HPCB) were measured in two reference zones and in one zone per impact type. Diversity was evaluated regarding to richness (^{0}D), typical diversity (^{1}D) and effective number of most abundant morphospecies (^{2}D). Compositional dissimilarity was examined through NMDS, ANOSIM tests, and SIMPER.

Results. 7525 individuals of 18 orders, 48 families, 53 genera and 86 morphospecies were collected. The prediction about the density and diversity of AMI was partially fulfilled: the agricultural zone presented an AMI community so more impoverished than the gold mining zone. However, these zones had less diversity than the cattle production and reference zones. AMI density only differed significantly between one reference zone and the agricultural zones, and did not differ significantly from the other sampling zones. The AMI composition in the agricultural zone differed considerably from the other zones. Thus, the increased AMI density coincided with the dominance of pollution tolerant taxa such as *Simulium* in the stream surrounded by agricultural activities

Discussion. The observation of a more impoverished AMI community in areas with agricultural production compared to those of mining or cattle production may reflect the importance of the remaining riparian vegetation, which was scarce in the agricultural zone. Moreover, the reduced AMI richness in the agricultural zone, coincided with the absence of genus, such as *Anacroneuria*, *Marilia,* and *Camelobaetidius*, which are intolerant to deterioration of the biological and physicochemical conditions of the water.

Conclusions. The results suggest that the local impact of agricultural activity may be of equal or greater magnitude than that of mining on AMI density, diversity and composition in a Colombian Andean streams. Future studies should evaluate, over the annual cycle, the effects of the productive activity, the remaining native vegetation cover and the consequent changes in the HPCB parameters of the water on AMI communities in Colombian Andean basins.

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Abstract

Background. Mining, agriculture and cattle production are activities that threaten the quality and quantity of water resources in the Colombian Andes. However, many drainage basins in this region have not been subjected to a simultaneous evaluation of the impact these activities have on the density, diversity and composition of aquatic macroinvertebrates (AMI). The first two of these ecological variables are expected to decrease drastically from zones with no apparent impact towards areas with anthropogenic activity, among which areas with mining activity will present the most impoverished AMI community.

Methods. This study evaluated the density, diversity and composition dissimilarity of AMI in small streams impacted by gold mining, agriculture and cattle production. Parallelly, two reference small streams (where anthropogenic impact was not obvious) were studied. Six bimonthly benthic samplings were conducted (February 2014 - February 2015) in each stream using a Surber-net. Water samples for environmental comparison among afore selected streams, included hydrological, physicochemical and bacteriological parameters (HPCB). Diversity was evaluated as the effective number of RTUs - recognizable taxonomic units (^{q}D) by comparing the richness (^{0}D), typical diversity (^{1}D) and effective number of most abundant RTUs (^{2}D). Compositional dissimilarity was examined through nMDS analysis, ANOSIM tests, and SIMPER.

Results. 7483 organisms were collected, belonging to 14 orders, 42 families and 71 RTUs (57 were at genera and 14 at family levels). Our prediction about density and diversity of AMI (Reference > Cattle production > Agriculture > Mining) was partially fulfilled, where the agriculture-dominated stream presented an AMI community more impoverished (both taxa density and richness) than that of the gold mining stream. However, these streams had less diversity than cattle production and reference streams. Besides, AMI density only differed significantly between one reference stream and the agriculture stream, and did not differ significantly from the others sampling zones. The AMI composition in the agriculture-dominated stream was clearly separated from the other streams. Thus, the increased AMI density coincided with the dominance of pollution tolerant taxa such as *Simulium* in the stream surrounded by agricultural activities.

Discussion. The observation of a more impoverished AMI community in areas with agricultural production compared to those of mining or cattle production may reflect the importance of the remaining riparian vegetation, which was scarce in the stream with agricultural activity. Moreover, the low diversity, and mainly the reduced AMI richness in the agriculture stream, coincided with the absence of insect genera, such as *Anacroneuria*, *Marilia* and *Camelobaetidius*, which are intolerant to deterioration of the biological and physicochemical conditions of the water.

Conclusions. The results suggest that the local impact of agricultural activities may be of equal or greater magnitude than that of mining, in terms of AMI density, diversity and composition in Colombian Andean riverscape. Thus, future studies should systematically evaluate, throughout the annual cycle, the relative effects of the productive land use, the remaining native vegetation cover and the consequent changes in the HPCB parameters of the water on AMI communities in Colombian Andean basins.

Keywords: Aquatic Insects, Hill serie, Biomonitoring, Neotropical region.

Introduction

Over the last four decades, pressure on lotic systems has increased in an accelerated manner at the global level as a consequence of the rapid expansion of areas of anthropogenic exploitation (Haddeland et al., 2014). Among the main threats to global freshwater diversity are overexploitation, water pollution, flow modification, habitat destruction or degradation and invasion of exotic species (Dudgeon et al., 2006; Vörösmarty et al., 2010; Malaj et al., 2014). Reid et al. (2019) explain that there are twelve threats to diversity, including previous ones, more intensified, and some new ones that threaten aquatic ecosystems. However, habitat degradation is one of the main threats to freshwater ecosystems. Continuous overuse increases the deforestation rate of riparian vegetation and runoff, causing changes in the morphology of the water stream. These modified the physicochemical parameters of the water, contributing to the impoverishment of aquatic biodiversity (Etter & Wyngaarden, 2000; Zapata et al., 2007; Larson, Dodds & Veach, 2019).

In particular, different studies have proven how mining, agricultural and cattle production are a threat to the quality of, and access to, hydric resources (Lucia et al., 2017; Grudzinski & Daniels, 2018; Mwangi et al., 2018). In Colombia, agriculture, cattle production and mining over the last decade have put the quality and availability of hydric resources at risk (Chará-Serna et al., 2015; Villada-Bedoya et al., 2017; Villada-Bedoya, Triana-Moreno & Dias, 2017; Ramírez et al., 2018). These activities threaten the lotic systems of the Andes, where the human population of the country presents its highest concentration (Murtinho et al., 2013; Guevara, 2014; Chará-Serna et al., 2015).

For the study the impact of human activities on freshwater ecosystems, the aquatic macroinvertebrates (AMI) has been used as bioindicators (e.g., González, Basaguren & Pozo, 2003; Prat et al., 2009; Buss et al., 2017). This is due to the fact that, at both community and population level, these organisms are highly sensitive to changes in the physicochemical properties of the water and to habitat quality (Alonso & Camargo, 2005; Carter, Resh & Hannaford, 2017). Different studies in the Neotropics have evaluated the effect of mining, agricultural and cattle production activities on AMI (e.g., Villamarín-Flores, 2008; Hepp et al., 2010; Mesa, 2010; Miserendino & Masi, 2010; Ordóñez, 2011; Egler et al., 2012; Terneus, Hernández & Racines, 2012; Fierro et al., 2015). In recent years, there has been an increased study in Colombia of the effect on AMI communities of activities of cattle production (e.g. Chará & Murgueitio, 2005; Ramírez et al., 2018), agriculture (e.g. Feijoo, Zúñiga & Camargo, 2005; Galindo-Leva et al., 2012; Villada-Bedoya, Triana-Moreno & Dias, 2017) and mining (Gómez, 2013). These studies have documented changes in the ecological attributes of the AMI as a consequence of anthropogenic alterations to inland water resources.

In the case of species richness, greater values have been recorded in reference streams compared to streams with the influence of mining, agriculture and cattle production (Feijoo, Quintero & Fragoso, 2006; Egler et al., 2012; Terneus, Hernández & Racines, 2012), mainly as a result of the reduction in riparian vegetation and use of polluting substances. In terms of abundance (or density), some studies have recorded greater values in sites with anthropogenic impacts compared to those with higher surrounding vegetation (Chará & Murgueitio, 2005; Miserendino & Masi, 2010). This is due to the dominance of certain taxa, as has been observed in agriculture (Egler et al., 2012) and cattle production-dominated streams (Mesa, 2010; Giraldo et al., 2014). Likewise, AMI composition has also presented important differences between streams with and without evident anthropogenic impact (Hepp et al., 2010).

Among the activities that more degrade the aquatic ecosystem, mining activity has been considered to have strong effects on water quality and quantity due to mining wastes and ecological impairment of the habitats (Cidu, Biddau & Fanfani, 2009; Wright & Ryan, 2016). The diversion of the channel and the removal of organic matter and sediments affect the availability of refuge and food for benthic organisms, making it difficult to colonize and recover long-term communities (Milner & Piorkowsk, 2004). However, few studies have evaluated the effect of mining, agriculture and cattle production in the Andean streams in a simultaneous way (see Villada-Bedoya et al., 2017; Villada-Bedoya, Triana-Moreno & Dias, 2017; Ramírez et al., 2018). It is important to recognize that the Neotropical region presents a wide variety of climatic conditions and habitat heterogeneity, for which reason, patterns of diversity are dynamic and can be influenced by many factors (land use, local geography, availability of riparian vegetation, among others). Hence further knowledge is necessary about patterns of AMI density and diversity (Guevara, 2014; Buss et al., 2017).

This study evaluated the density, diversity and compositional dissimilarity of the AMI in contrasting headwater streams of the Colombian Andes; two near-pristine, and one stream immersed separately in zones with agricultural, cattle production and gold mining activities, in the Chinchiná river basin (Caldas, Colombia). According to the assumed impact of each productive land use, we expected that: 1) AMI density will increase from the two reference streams to those of agriculture, cattle production and mining, 2) this increase in density will reflect an increased dominance of taxa that are tolerant to the water pollution, and 3) there will be a maximum impoverishment of AMI diversity in the zone with gold mining activity.

Materials and Methods

Study area. The selected streams are located on the western slope of the central cordillera of the Colombian Andes, in the municipalities of Villamaría and Manizales (Caldas, Colombia). They are tributaries of the Chinchiná river basin. Five stream length of 100 m per productive activity (agriculture, cattle production and gold mining) and -two- reference conditions, i.e., streams with no evident local anthropogenic impacts, were selected (Fig. 1).

Reference 1 (Ref1): Located in the stream La Elvira, sector Maltería (Manizales: 05°03'10.9"N, 75°24'33.6"W) at 2766 m asl. This area presents riparian vegetation greater than 15 m in width, which is composed mainly of herbaceous plants, shrubs and trees. The most representative plant species include *Aiouea* sp., *Clethra revoluta* Ruiz and Pav., *Dunalia solanacea* Kunth, *Miconia superposita* Wurdack and *Verbesina nudipes* S.F. Blake.

Reference 2 (Ref2): Located in the stream La Floresta (Villamaría: 05°1'42.1"N, 75°31'10.9"W) at 1720 m asl, close to agricultural zones and used as an area of recreation. Its riparian area is more than 15 m in width and presents elements characteristic of a conserved forest (Guariguata & Ostertag, 2002), such as large trees of the families Moraceae (*Ficus* sp., *Coussapoa duquei* Standley), Lauraceae (*Nectandra* sp.) and Boraginaceae (*Cordia panamensis* L. Riley).

Cattle production (CP): Located in the stream Cimitarra, sector Maltería (Manizales:

05°04'32.0"N, 75°24'0.60"W) at 2550 m asl. It is surrounded by grazing pastures, although the cattle have no access to the stream due to the presence of a strip of vegetation of approximately 3 m in width on both banks dominated by species of early succession such as: *Baccharis latifolia* Ruiz and Pavón, *Miconia superposita* Wurdack, *Rubus glaucus* Benth, *Aphelandra acanthus* Nees, *Solanum phaeophyllum* Werderm and *Tibouchina lepidota* Bonpl. In addition, two introduced plant species were recorded: *Pennisetum clandestinum* Hochst. ex Chiov (Poaceae), cultivated as pasture, and *Lachemilla orbiculata* Ruiz & Pav. (Rosaceae), a plant species abundant in grazing pastures of cold climates (Vargas, 2002).

Agriculture (Agr): Corresponding to the stream "Don Alonso" (Villamaría: 05°01'50.79"N, 75°31'39.59"W) at 1849 m asl. The riparian vegetation is practically absent (only small shrubs, grasses, and sparse herbaceous persist). In addition, this area has closer gardens and vegetable cultivars, alternating with the following species: *Brassica oleracea* var. *capitata* Linnaeus and *Brassica oleracea* var. *italica* Linnaeus, *Sechium edule*. (Jacq.) Sw., *Musa velutina* H. Wendl. and Drude, *Guadua angustifolia* Kunth, *Urera baccifera* (L.) Gaudich., *Piper* cf. *crassinervium* Kunth, *Montanoa quadrangularis* Schultz Bipontianus, *Cecropia angustifolia* Trécul.

Mining (Mi): Located on the stream La Elvira (Manizales: 05°03'4.4"N, 75°24'33.1"W) at 2725 m asl. Its riparian zone is fragmented by land use change through activities of auriferous mining extraction using mercury. The stream presents vegetation comprising grazing pastures and secondary forest with an approximate width of 1 to 2 m, dominated by grasses (*Pennisetum clandestinum* Hochst. ex Chiov), herbaceous plants (*Coniza bonariensis* (L.) Cronquist), *Hypochaeris radicata* L., *Taraxacum officinale* G. H. Weber ex Wigg, *Lachemilla orbiculata*

Ruiz and Pavón, *Plantago major* L., *Gunnera brephogea* Linden & André) and some juvenile trees (*Baccharis latifolia* Ruiz and Pavón and *Miconia* cf *theaezans* Bonpl.).

Collection of organisms. The AMI density (ind/m^2), diversity and composition of RTUs were evaluated based on the RBP - Rapid Bioassessment Protocols (Barbour et al., 1999). We used a Surber net (30 x 30 cm, 250 µm mesh size) with three replicates per substrate (leaf litter, rock and sediment; Aazami et al., 2015), in six sampling events per stream (between February 2014 and February 2015), totalizing 54 samples per zone. The collected material was fixed in vials containing 96% alcohol and the AMI identified to the lowest taxonomic practical level (usually genus) using the taxonomic keys of Merritt & Cummins (1996), Domínguez et al. (2006) and Domínguez & Fernández (2009). Specimen collection permits were regulated by Resolution 1166 of October 9th, 2014, issued by the National Environmental Licenses Authority (ANLA) of Colombia and by Decree 1376 of June 27th, 2013 from the Colombian Ministry of Environment and Sustainable Development. The material was deposited in the Entomological Collection of the Programa de Biología of the Universidad de Caldas - CEBUC (certified collection under register: No 188 by Instituto de Investigación de Recursos Naturales Alexander von Humboldt).

Hydrological, physicochemical, and bacteriological parameters. The environmental characterization of the sampling streams included five supplementary hydrological parameters, as well as 27 hydrological, physicochemical and bacteriological parameters (hereafter HPCB), plus elevation (m asl). Among the hydrological parameters, the water flow volume (m^3/s) was measured in each sampling event and mean weekly precipitation per month of sampling was recorded (mm/week) (IDEAM, 2015). In February, July and November 2014, the following water and stream parameters were measured (*in situ*, Table 1): velocity (m/s), width (m), depth (cm), temperature (Temp, °C), pH, conductivity (Con, µS/m) and dissolved oxygen (DO, mg/L). Temperature, pH and conductivity were measured with a multiparameter equipment OAKLON brand model PH/CON 300, and dissolved oxygen was measured with Lutron brand dissolved oxygen meter model do-5510. Water samples were taken and transported to the IQ&A certified laboratory (Ingenieros químicos y asociados S.A., Manizales, Colombia) for determination of the following parameters (Table 1): chlorides (Ch, mg/L), sulphates (SO_4, mg/L), nitrites (NO_2, mg/L), phosphates (PO_4, mg/L), fats and oils (FO, mg/L), biochemical oxygen demand (BOD, mg/L), chemical oxygen demand (COD, mg/L), total dissolved solids (TS, mg/L), total suspended solids (TSS, mg/L), ammoniacal nitrogen (NH_3-N, mg/L), aluminum (Al, mg/L), mercury (Hg, mg/L), total iron (Fe, mg/L), lead (Pb, mg/L), cyanide (Cy, mg/L), boron (B, mg/L), *Escherichia coli* (Ecoli, CFU/100 mL) and total coliforms (Tc, CFU/100 mL) (Chará, 2003; Sánchez, 2004).

Data analysis. Values of AMI density among sampling zones were analyzed with a non-parametric repeated measures Friedman test (n = 6 sampling events) and particular differences among streams were identified with a *post-hoc* Nemenyi test (Zar, 2010). Diversity was estimated as the effective number of RTUs or diversity order q (^qD; Jost, 2006):

$$^qD = \left(\sum_{i=1}^{S} p_i^q\right)^{1/(1-q)}$$

Where pi is the relative abundance (proportional abundance) of the i-th RTUs. S is the number of RTUs and q-value is the order of the diversity. When q = 0, richness is obtained. When q ≈ 1, the effective number of equally common genera is obtained. This is equivalent to the exponential of the Shannon index of entropy and does not present bias as a result of the presence of either rare or abundant RTUs in the sampling. Finally, when $q = 2$, the value of diversity indicates the effective number of more abundant RTUs in the sampling and is equivalent to the inverse of the Simpson index of entropy (Moreno et al., 2011).

Since the continuous variable of density was used as an abundance measure, estimation of sample coverage ($\hat{C}n$, see Chao & Jost, 2012) *per* stream was not required prior to making the diversity comparisons. In each case, we obtained completeness of 100% (absence of singletons), and the diversity comparison was therefore made directly on the observed values of ^qD. The CI 95% of each expression of diversity (^0D, ^1D, ^2D) was used as a statistical criterion, in which an absence of overlap between the CI 95% indicated significant differences between the values of diversity (Cumming, Fidler & Vaux, 2007; Chao et al., 2020). Estimation of $^qD \pm$ CI 95% was conducted with the package iNEXT of R (Hsieh, Ma & Chao, 2015).

By expressing diversity as the effective number of RTUs and making comparisons under the same and maximum sample coverage (100%), the replication principle is met, and it is possible to calculate the magnitude of the difference in diversity (MD = Sampling Site 2 / Sampling Site 1) among communities (Jost, 2006; Moreno et al., 2011). It is thus possible to determine how many times one zone is more or less diverse than another. In addition, comparison of $^qD \pm$ CI 95% under the effective numbers of RTUs eliminates estimation bias due to the high density of certain aquatic insect groups, such as the dipterans (e.g., Chironomidae). It would be impossible to avoid this bias using the classic protocol for the use of rarefaction curves, which relies on a comparison based on the minimum sample size or minimum abundance.

The compositional dissimilarity of AMI RTUs was examined with a non-metric multidimensional scaling (nMDS), based on the Bray–Curtis index (Quinn & Keough, 2002). An ANOSIM was used to determine whether the compositional dissimilarity was greater between zones than within them, and the contribution of the RTUs to the dissimilarity was subsequently established using a SIMPER (Quinn & Keough, 2002). In a complementary manner, the patterns of density, diversity and compositional dissimilarity were discussed with respect to HPCB parameters. First, we used a Spearman correlation analysis to examine how changes in AMI density were related to flow and precipitation (Table S1). Secondly, due the HPBC was measured only in three sampling moments (i.e., Feb, Jul, Nov 2014), we carry out a CCA analysis to evaluate the association patterns among AMI's RTUs, sites and HPBC parameters regarding pair-consecutive sampling events of AMIs: Feb14+Apr14; Jul14+Sept14; Nov14+Feb15; this temporal grouping of data was also used for compositional dissimilarity analyzes (see above). To avoid collinearity among HPBC parameters, we applied the Variance Inflation Factor (VIF); thus HPBC parameters with VIF > 10 were not included in CCA analysis (Neter, Wassermn & Kutner, 1990). All statistical analysis was performed using R version 3.2.1 (R Core Team, 2015; See R-code and input data in Data S1).

Results

A total of 7483 organisms were collected, belonging to 14 orders, 42 families and 71 recognizable taxonomic units (RTUs), which 57 were at genera and 14 at family levels (Table S2). Density was significantly higher only in the zone Reference 2 ($Fr = 3.10$, $df = 29$, $p = 0.0163$; Nemenyi *post hoc* test, $p = 0.0163$) (Fig. 2A). The most dominant genus in Reference 1, Reference 2, and Mining streams were *Baetodes*, with 486 (40%), 371 (21%), and 510 (48%)

ind/m^2, respectively. For Cattle production, the highest densities were presented by *Andesiops* (215.5 ind/m^2, 19%) and *Baetodes* (204.9 ind/m^2, 19%). Agriculture stream presented a high representation of *Simulium* (555.7 ind/m^2, 65%). The stream with the greatest AMI density was Reference 2 with 1808.7 ind/m^2, followed by Reference 1 with 1219.8 ind/m^2. These were followed by Cattle production with 1106.5 ind/m^2, Mining with 1074 ind/m^2 and, finally, Agriculture-dominated stream with 852.1 ind/m^2.

According to the 95% CI, the agricultural zone presented the lowest significant values for the three expressions of diversity (qD) (Fig. 2B). In contrast, the other zones sampling were different according to the diversity expression. In the case of the RTUs observed richness (0D), the zones were ordered as follows: Reference 2> Reference 1> Cattle production ≈ Mining) (Fig. 2B). In particular, Reference 2 presented an increment in RTUs richness between 1.3 (Ref2 vs. Ref1) and 4.3 (Ref2 vs Agr) times greater than other sampling zones. Concerning the effective number of equally common RTUs (1D) was obtained the following patterns: (Reference 2 ≈ Cattle production)> Reference 1> Mining. In this case, Reference 2 and Cattle production were between 1.3 and 3.8 times more diverse than other zones. In relation to the effective number of the most abundant RTUs, the zones were ordered in a decreasing pattern (2D): Cattle production> Reference 2> Reference 1> Mining (Fig. 2B), where the magnitude of the difference differed between 1.1 (CP vs Ref2) and 4.3 fold (CP vs. Agr).

No tendency of significant variation was detected in AMI density with respect to water flow (p-value: 0.18 - 0.94) and precipitation (p-value: 0.17 - 0.82). The physicochemical parameters of the water in the studied streams were within the quality thresholds admissible for human and domestic use (articles 38 and 39 of the Colombian Decree 1594 of 1984). The only exceptions were presented in sampling three (July 2014), which, in the Agriculture stream, evidenced values of total coliforms and *E. Coli* that exceeded admissible levels (410,600 CFU/100 mL and 2,417 CFU/100 mL, respectively), and also for the Mining stream, which exceeded the admissible levels for total coliforms (22,470 CFU/100 ml).

Relative to the composition, eight RTUs were shared by the five sampling zones: *Baetodes*, *Simulium*, *Anchytarsus*, *Smicridea*, *Tipula*, *Culoptila* and the subfamilies Chironominae and Orthocladiinae. The nMDS analysis evidenced separation among the different sampling streams (Fig. 3; Stress = 0.13), which is consistent with that found in the ANOSIM. Both tests showed that there were differences among all streams in terms of composition (ANOSIM: R = 0.673, p-value = 0.001). The SIMPER analysis indicated that *Baetodes*, *Simulium* and *Smicridea* were the taxa that contributed most to the differences found among the studied streams. The CCA presented an appreciable association between environmental parameters, sites and macroinvertebrates (Fig. 4: CCA1 + CCA2 = 63.2% of explained variance), where the Agricultural zone has physicochemical profiles and biotic components differentiated and remains separated. As well the Agricultural zone includes the high values of TS (Fig. 4) and lowest values of DO (Table 1), associated with the highest values of density of the taxa *Simulium*, *Chimarra*, *Dugesia, Rhagovelia* and Calopterygidae, while some Ephemeroptera and Coleoptera (*Anchytarsus* and *Heterelmis*) were practically absent in this stream (Table S2). The Cattle production and both Reference streams were associated with high values of DO, in addition with a wide density of the RTUs *Baetodes*, *Mayobaetis*, *Andesiops* and *Anchytarsus* (Fig. 4; Table 1). On the other hand, the Mining stream was strongly associated with the highest phosphate values and high values of TS as in the Agriculture stream (Fig. 4) and presented a decrease in the majority of RTUs previously mentioned.

Discussion

In this study, the Agricultural zone had a greater effect on AMI diversity (lowest values of richness and density) than the Mining zone, which did not follow the expected pattern in our study. These results are probably associated with the traditional horticultural practices (e.g., soil preparation and use of agrochemicals) during the several years in zones of the Chinchiná river basin (Caldas, Colombia: Meza-S. et al., 2012; Chará-Serna et al., 2015; Llano et al., 2016); a land use situation traditionally also occurring along the Andes (Mesa, 2010; Guevara, 2014; Vimos-Lojano et al., 2017). The expansion of agricultural land use strongly reduces the presence of totally pristine headwater ecosystems in many mountainous countries (Vimos-Lojano et al., 2017), where several cultivated areas converge toward mainstream channels (Chará et al., 2007; Chará-Serna et al., 2015).

The higher AMI values of richness and density registered in the references and cattle production zones could be linked to the presence of riparian vegetation and its importance in buffering environmental impacts (e.g., Lenat, 1984; Rivera, 2004; Burdet & Watts, 2009; Egler et al., 2012). However, Reference 2 stream comparatively presented the highest values, which is possibly due to the greater differential contribution of leaf litter from speciose riparian vegetation, producing a greater availability of coarse organic benthic resources in this zone (Gutiérrez-López, Meza-Salazar & Guevara, 2016). It is important to note that the agricultural zone did not have riparian vegetation and this can be the reason for the lowest richness and density values, as found in other studies (e.g., Lenat, 1984; Lenat & Crawford, 1994; Hepp et al., 2010; Egler et al., 2012). Although this study was not aimed at testing the role of the riparian vegetation, this result partially coincides with the idea that the removal of this can have both direct and indirect effects on AMI abundance (Lenat, 1984; Egler et al., 2012) due to degradation of both habitat and water quality (Chará et al., 2007). Indeed, low values of richness in zones of agriculture with similar circumstances has been previously reported by other authors (e.g., Lenat, 1984; Lenat & Crawford, 1994; Hepp et al., 2010; Egler et al., 2012), who argue that deterioration in water quality influences the number of taxa of aquatic invertebrates found.

The diversities ^{1}D and ^{2}D presented a similar pattern, due to the high importance or dominance of the most abundant RTUs in each of the studied streams. The high diversity in the Reference 2 and Cattle production streams, as well as the significantly greater diversity of Reference 1 compared to Mining and Agriculture, could also be related to the presence of riparian vegetation since, although the Cattle production zone presents effects related to this activity, the strips (ca. 3 m in width) of vegetation that exists on both sides of the stream may act to diminish these effects on the AMI community. Niemi & Niemi (1991) indicate that vegetation has a positive effect on streams immersed in cattle production zones since it acts as a barrier to the animals and traps sediments that are transported towards the water bodies by surface runoff. Consequently, Mining and Agriculture streams presented the lowest values of diversity, being significantly minor in the Agriculture stream. These land use changes, in which the riparian vegetation is replaced by human activities such as mining and agriculture, lead to constant alteration of the physical characteristics of the water bodies, which can directly or indirectly influence changes in the spatial and/or temporal diversity of the AMI (Tomanova & Usseglio-Polaterra, 2007; Domínguez

& Fernández, 2009).

To all three diversity expressions (e.i., ^{0}D, ^{1}D, ^{2}D), the lowest values were presented in the stream influenced by agricultural activities. Chará-Serna et al. (2015) reported that one of the most important indirect consequences of agricultural practices for the AMI community is the increase in the values of ammoniacal nitrogen (NH_3-N). In the present study, this parameter did not show values as high as those reported by other authors in Neotropical streams (Mesa, 2010; Vázquez et al., 2011; Chará-Serna et al., 2015) (see Table 1). However, Gücker et al. (2009) explain that, even though the values in streams with agriculture may be low, they still exceed those in zones with no impact. That coincides with our results, in which the values of NH_3-N in the Agriculture stream (0.323 mg/L) were greater than those of both Reference zones (Reference 1: 0.153 mg/L; Reference 2: 0.175 mg/L).

The high representativity and contribution of *Baetodes*, *Andesiops*, *Simulium* and *Smicridea*, as well as the subfamily Orthocladiinae, in the streams evaluated coincide with the results of González et al. (2012) and Meza-S et al. (2012) in the Chinchiná river basin, in which these taxa presented a high abundance. *Baetodes*, *Simulium, Smicridea* and the subfamily Orthocladiinae have a wide distribution in Neotropical basins, covering broad elevational ranges (Sganga & Angrisano, 2005; Sganga & Fontanarrosa, 2006), while *Andesiops* is restricted to zones above 1000 m asl (Gutiérrez & Gomes-Dias, 2015). The nMDS analysis showed a clear separation between Agriculture and the other sampled zones. It is due to the high dominance of *Simulium,* which presents lower values than other streams, and to the absence of taxa that are intolerant of pollution, such as *Anacroneuria, Marilia* and *Camelobaetidius* (Zúñiga & Cardona, 2009). This result demonstrates that the presence of heavy agricultural activity in the sampling zones has a strong effect on the AMI community. Roldán & Ramírez (2008) indicate that a river that has suffered alterations to its natural conditions through contamination processes will reflect these effects through changes in the composition and structure of its aquatic biota. Likewise, García & Rosas (2010) explain that agricultural activities can cause the loss of sensitive taxa, as indeed was the case in our study. The similarity between the Reference 1 and Mining streams is due to both conditions are found on the same stream (i.e., La Elvira stream). The spatial proximity between sampling sites potentially can mask the punctual effect of a disturbance on the AMI community; an effect that is maximized if the sites are located on the same watercourse (Tolonen et al., 2017). Therefore, the density and diversity of AMI in the Mining sampling point may be influenced by the closeness to Reference 1 sampling site. Although our sampling design does not adequately detect the effect of spatial autocorrelation between sampling stations, the results indicated that the spatial proximity does not dampen the impact of Mining on the AMI community and on the water conditions about the HPCB parameters. The compositional dissimilarity between Mining and Reference 1 sampling sites is given by the presence of the genera reported in Reference 1, which are relatively less abundant in Mining (e.g., *Smicridea*, *Andesiops*, and *Nanomis*; Fig. 4). Consequently, the CCA evidenced a clear separation between Mining and Reference 1, where they represent of tolerant groups to conditions of high water contamination by mining activity (e.g., some Chironomidae, Tipulidae, and Empididae) (see Pond et al., 2014). These results coincide with the idea that a point scale, the variation in abundance or the incidence of macroinvertebrate groups can be strongly modulated by the presence and availability of microhabitats (e.g., Burgazzi, Giareschi and Laini, 2019).

The isolation of the Agriculture zone in the CCA and its high values of TS (310.7±209.8) and the lowest values of DO (2.3±0.8) reflect the negative impact of this activity on stream and associated biota. High concentrations of TS on stream were found in both the Agricultural and Mining streams, avoiding the entrance of the light at the ecosystem, affecting the energy flow of the system and, consequently, the productivity levels (Vázquez, Aké-Castillo & Favila, 2011). Furthermore, the increase of the TS is related to the sedimentation rate (Vásquez Zapata, 2009). In turn, the increase in the fine sediment can be a more significant stressor to macroinvertebrates assemblage than increased nutrient concentrations in streams around agricultural areas (Ladrera et al., 2019). Also, this variable can affect to a different group of AMI, for example, taxa adapted to swim, scrapers, shredders, the species that respiration by plastron, gills and also the species of Coleoptera dependent on a bubble or plastron to breath (Hauer & Resh, 1996; Rabeni et al., 2005; Ladrera et al., 2019). On the contrary, the invertebrates living in the mud, burrowers and filter-collector can be favoured because they are feeding on fine sediment.

The low DO promotes the decrease of richness, increasing the density of tolerant organisms as mentioned by Jacobsen & Marín (2008). Both variables (TS and DO) could explain the high abundance of filter-collector organisms relatively tolerant as Simulium and Chimarra. Even though Simulium is generally associated with environments with a high concentration of oxygen (Roldán, 1996; Domínguez & Fernández, 2009; Zúñiga & Cardona, 2009; Villada et al., 2017). However, some species of the Simulium may be more tolerant than others, so it is important to advance in the taxonomic knowledge of the group for an identification at the species level. On the other hand, predators such as Calopterygidae, Dugesia and Rhagovelia can be benefited in these environments due to resource availability, as occur with Rhagovelia that move over the surface layer of water, breathe atmospheric oxygen and feed on dead or dying insects. At the same time, the Calopterygidae are generally associated with substrates at the bottom of streams, where they tolerate low concentrations of dissolved oxygen in water (Domínguez & Fernández, 2009).

The Cattle production and Reference 2 zones had associated high values of DO (9.3±3.3 and 5.4±0.63, respectively), suggesting that were the most conserved zones in the study, with the more richness species sensitive to contamination. Zúñiga & Cardona (2009) classified Anchytarsus as sensitive to the pollution confirming those mentioned above since this genus presented high density in these zones. Regarding the genera of Ephemeroptera, several authors indicate that the many genera in the group are sensitive to contamination (e.g., Zedcová et al., 2014; Akamaqwuna et al., 2019). Buss & Salles (2006), mentioned the importance of including the species level for the establishment of the sensibility in water quality monitoring programs. The highest phosphate values (1.2±0.62) and TS (394.7±210) found in the Mining zones, indicates the deterioration that this activity can generate in aquatic ecosystems (Wright & Ryan, 2016), hindering the survival of some genera of macroinvertebrates (Ramírez et al., 2018).

In general, low values of precipitation and water flow volume were associated with high AMI densities in the studied streams. Concomitant results have been found in other Colombian small streams (Rodríguez- Barrios et al., 2007; Longo et al., 2010; Tamaris-Turizo et al., 2013); however, we have no evidence of high variation in density related to either of these environmental variables. Minshall & Robinson (1998) explain that a constant climate pattern, or one of little variation, in the riparian environment translates into lower variability in the AMI dispersion dynamic. Moreover, Smith & Lamp (2008) suggest that the abundance and composition of the AMI community are influenced more by land use than by the seasons of high and low rains, which is consistent with the results of our study.

Despite our attempt to continuously evaluate both physicochemical and biological parameters, the mining and agriculture activities have highly variable management (e.g., frequency and quantity of chemicals used). It is difficult to control this anthropogenic factor, which occurs jointly with natural hydrological patterns (see Friberg, 2014), in the selected small streams. Although these are key elements (i.e., the contribution of natural and anthropogenically-induced changes) for consideration in the patterns of stream macroinvertebrate distribution (e.g., Domisch et al., 2017; Kakouei et al., 2018), this aspect was beyond the scope of the present study due to logistic restrictions. Further studies are necessary to adequately evaluate the variability of AMI due to both anthropogenic and natural pressures. It is recommended that future studies employ a larger number of spatial replicates incorporating the effects of each of the impacts and that a rigorous search of the zones of reference is conducted to ensure the absence of the anthropogenic effects. In addition, evaluation of the heavy metals in the sediment is recommended, since this is where their concentration is likely to be highest (e.g., Dickson et al., 2019).

Conclusions

Contrary to our central hypothesis, the results show that the Agricultural zone had the lowest macroinvertebrate density and diversity. In this sense, beyond the environmental diagnosis based on physicochemical and bacteriological variables, the use of diversity measures (^qD) can be a useful tool to evaluate the impact of human activity on freshwater in-stream biota, since they allow an adequate quantification of changes in the structure of AMI communities, using units with biological sense.

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Table 1 (on next page)

Hydrological, physicochemical, and bacteriological (HPCB) parameters measured in selected streams located on the western slope of the central cordillera of the Colombian Andes, Chinchiná river basin.

The HPCB parameters were measured between one and three times (n) per sampling zone, thus the value of each parameters per sampling showed as mean ± SD.

Parameter	n	Reference 1	Reference 2	Cattle production	Agriculture	Mining
Chemical oxygen demand (mg/L)	3	23.3±14.4	29.67±10.21	25.3±17.9	50.3±58.6	20.3±6.8
Biochemical oxygen demand (mg/L)	3	3.2±0	3.21±5.44	3.21±0	3.2±0	3.2±0
Total dissolved solids (mg/L)	3	110.7±58.6	124±18.33	110.7±32.6	310.7±209.8	394.7±210
Total suspended solids (mg/L)	3	63.2±87.4	15.25±9.73	102.7±167.4	69.7±62	56.4±79.3
Ammoniacal nitrogen (mg/L)	2	0.1±0.08	0.05±0.04	0.1±0.06	0.2±0.2	0.1±0.1
Nitrites (mg/L)	3	0.07±0	0.07±0	0.07±0	0.1±0.03	0.3±0.2
Sulphates (mg/L)	3	21±1	11.33±0.58	6.7±0.6	9.3±3.5	55.7±30.5
Fe (mg/L)	3	0.4±0.4	0.06±0.02	0.2±0.06	0.8±0.9	1.3±0.9
Chlorides (mg/L)	3	2.5±0	2.5±0	2.5±0	3.2±0.8	2.9±0.6
Phosphates (mg/L)	3	0.7±0.62	0.3±0.17	0.4±0.2	0.4±0.3	1.2±0.62
Cyanide (mg/L)	1	*0.001	*0.001	*0.001	*0.001	*0.001
Hg (mg/L)	3	*0.2	*0.2	*0.2	*0.2	*0.2
Al (mg/L)	1	*7.3±0.3	*7.3±0.3	*7.3±0.3	*7.810.3	*7.4±0.3
Pb (mg/L)	2	*0.02±0.01	*0.02±0.01	*0.02±0.01	*0.02±0.01	*0.02±0.01
B (mg/L)	2	*1.1±0.5	*1.1±0.5	*1.1±0.5	*1.1±1.1	*1.1±0.5
Fats and oils (mg/L)	3	0.4±0.2	0.83±0.58	0.8±0.6	0.4±0.2	0.9±0.7
Dissolved oxygen (mg/L)	3	9.3±3.3	4.75±2.64	5.4±0.63	2.3±0.8	5.2±0.6
pH	3	7.6±0.3	8.27±0.08	7.5±0.07	7.7±0.09	7.7±0.2
Temperature (ºC)	3	12.1±0.4	18.9±0.48	13.5±1.15	17.9±0.7	13.1±0.05
Conductivity (µS/m)	3	108±9	99.675±4.35	139±86	33.8±25.5	131.5±55.5
Total coliforms (CFU/100mL)	2	2375	2200	806	209650	2933.5
Escherichia coli (CFU/100mL)	2	12.4±9.7	49	65.9	90.95	6.3
Depth (cm)	3	9.2±3.8	18.2±4.9	10.8±4.02	7.2±2.2	9.5±2.7
Width (m)	3	1.8±0.2	1.82±0.49	1.7±0.6	1.6±0.4	1.9±0.6
Flow (m/s)	3	0.4±0.09		0.4±0.05	0.2±0.05	0.4±0.2

*Below the detection limit.

Figure 1

Study area and sampling zones located on the western slope of the central cordillera of the Colombian Andes, in the Chinchiná river basin (Caldas, Colombia).

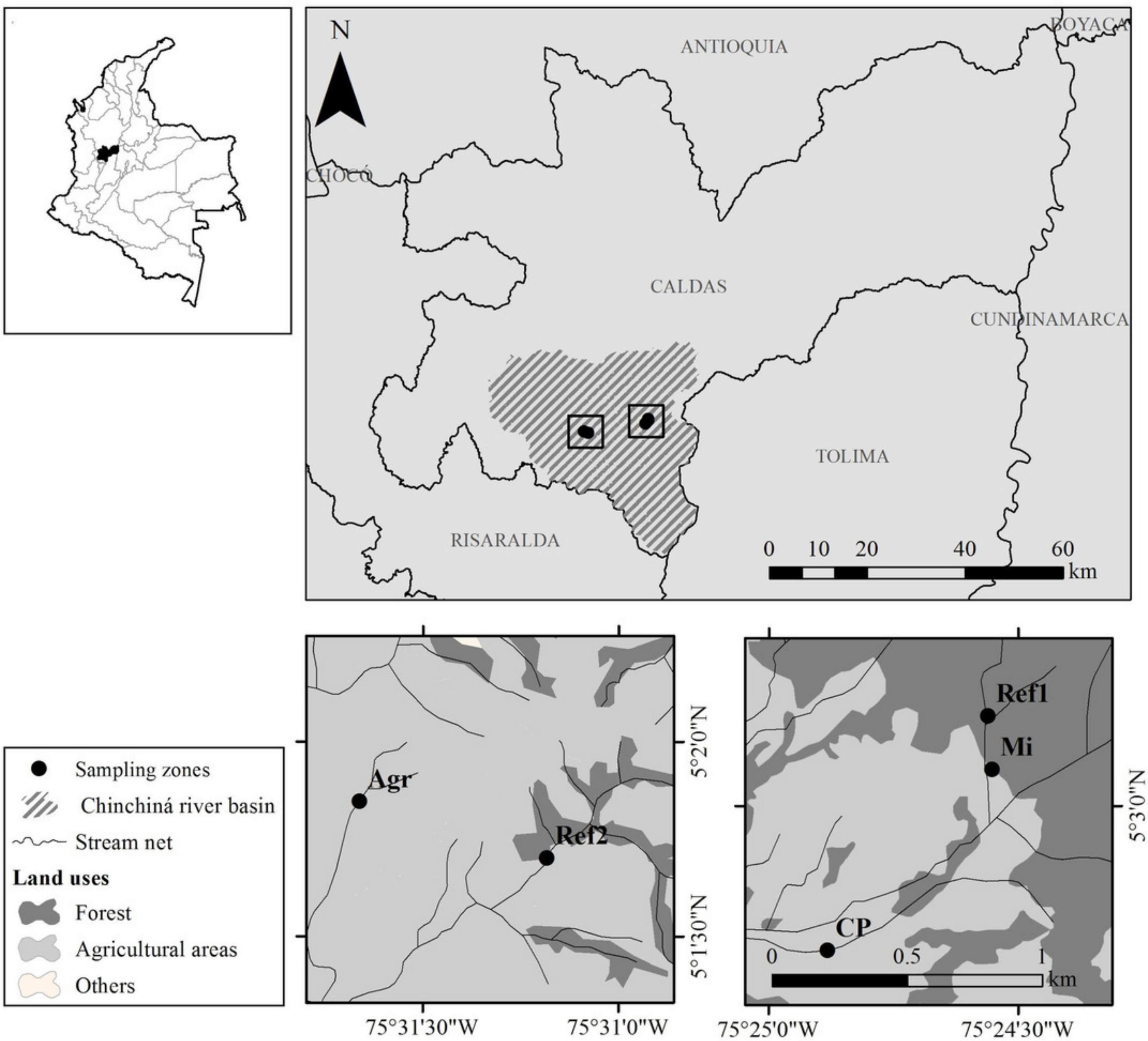

Figure 2

Comparison of the density and diversity of aquatic macroinvertebrates (AMI) in five sampling zones.

(A) Boxplot showing the median AMI density. (B) Patterns of diversity expressions, richness (^{0}D), typical diversity (^{1}D), and effective number of the most abundant morpho-species (^{2}D). The vertical line indicates the CI 95% per ^{q}D. No share letters above boxplot indicate the statistical difference between pairs of the sampling zones. Streams: Ref 1 = Reference 1, Ref 2 = Reference 2, CP = Cattle production, Agr = Agriculture, and Mi = Mining.

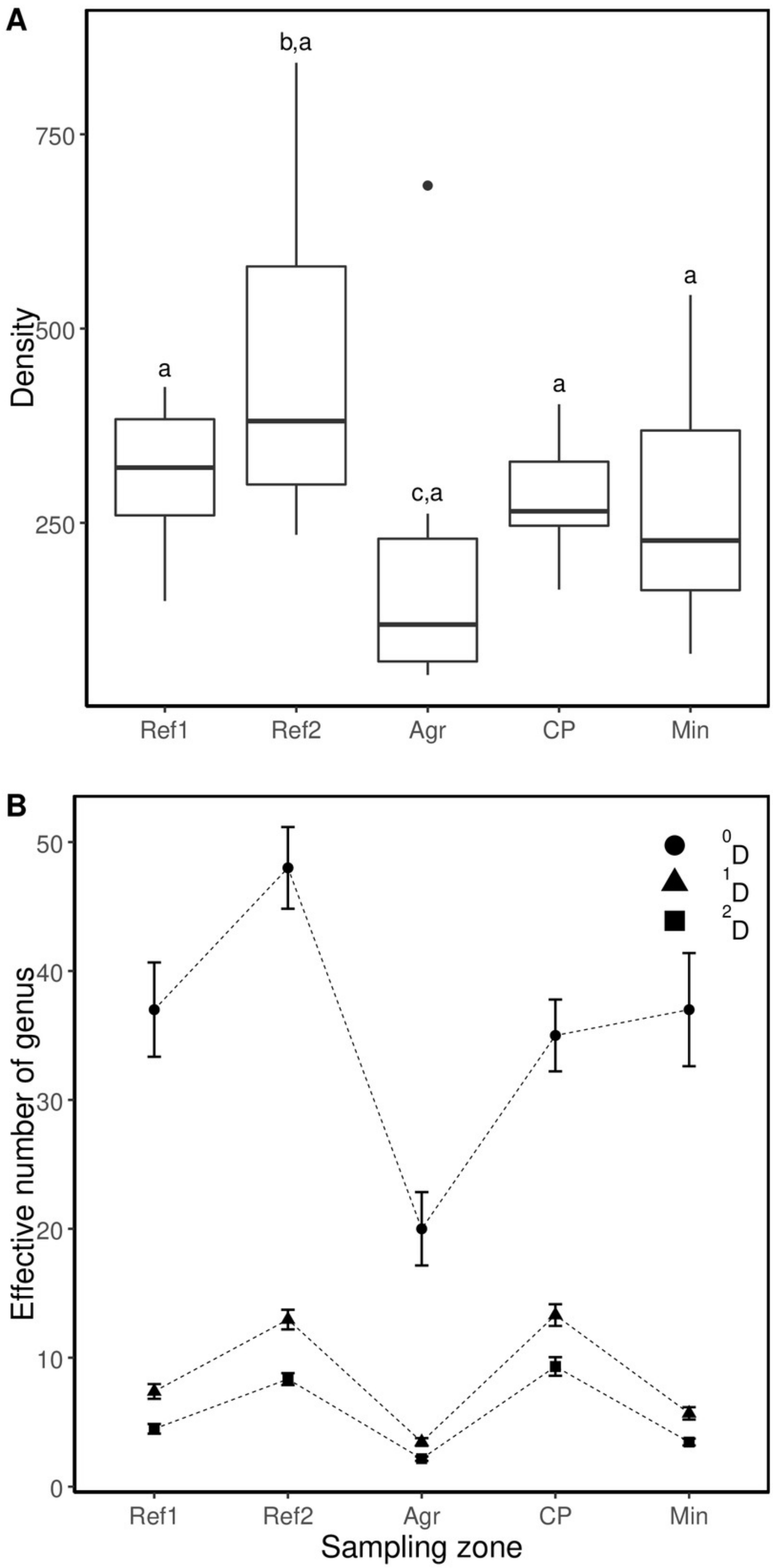
A
b,a
750
a
Density
500
a
a
c,a
250
Ref1
Ref2
Agr
CP
Min
B
50
0D
1D
2D
40
Effective number of genus
30
20
10
0
Ref1
Ref2
Agr
CP
Min
Sampling zone

Figure 3

Non-Metric Multidimensional Scaling (NMDS) analysis based on the Bray - Curtis Index considering each sampling event per zone (Stress=0.13).

The names of AMI RTUs are shown (see Table S2). Streams: Ref 1 = Reference 1, Ref 2 = Reference 2, CP = Cattle production, Agr = Agriculture, and Mi = Mining.

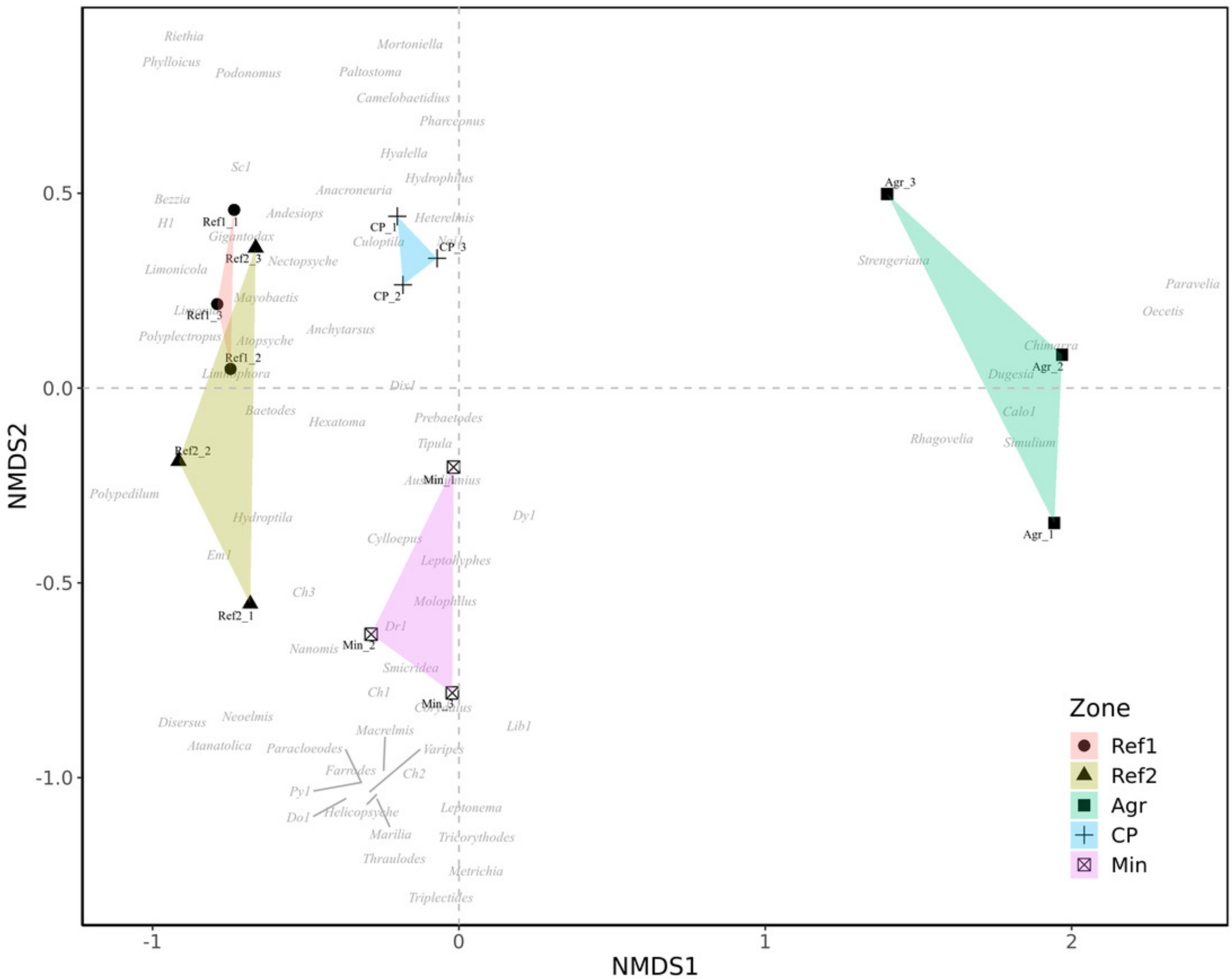

Figure 4

Correspondence Canonical Analysis (CCA) among AMI RTUs composition and eighth hydrological, physicochemical, bacteriological (HPCB) parameters regarding measure events per sampling zone.

The selected HPCB parameters present a VIF < 10. Streams: Ref 1 = Reference 1, Ref 2 = Reference 2, CP = Cattle production, Agr = Agriculture, and Mi = Mining. Mesarure events: 1 = Feb14 + Apr14; 2 = Jul14 + Sept14; 3 = Nov14 + Nov15.