RESEARCH ARTICLE

Freshwater macroinvertebrate traits assessment as complementary to taxonomic information for mining impact detection in the northern Peruvian Andes

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Abstract

Aim: We studied the distribution of freshwater macroinvertebrate taxa and traits to distinguish ecological gradients among the mining-controlled and natural headwaters, and rural and urban economic activity influences.

Location: In 2016's dry season, macroinvertebrate samples were collected at 40 locations in the Mashcon watershed, northern Peruvian Andes. Six locations were in the headwaters directly influenced by mining, eight near-pristine tributary headwaters, 14 agricultural locations at midstream and 12 urban locations downstream.

Methods: Eight traits (five biological and three ecological) were selected according to data availability, and modalities scores were assigned using the weighted and the dominant-trait approaches. The traits relative abundances and abiotic conditions were compared among watershed sections. The ecological interpretability of the ungrouped data was verified with a distance-based redundancy analysis.

Results: The high-altitude mining section had fewer taxa types and abundance, and distinct body forms distributions and prevalent body sizes in macroinvertebrate communities, relatable to the control of the mining headwaters. Physiological and ecological traits (respiration, mobility and attachment, food sources, feeding habits, saprobity and pH preferenda) differed among traits quantification approaches and were less informative at high altitudes. The ecological conditions from the near-pristine tributaries recovered in the vegetated midstream section, to again be affected in the downstream urban section.

Main Conclusions: Our results suggest the presence of ecological impairment despite the excellent physicochemical quality of the water discharged by the mine. The obtainment of autecological information at a higher taxonomic resolution, e.g. for ubiquitous taxa like Acari and Chironomidae, would be needed to advance the freshwater quality assessment of ecologically and hydrogeochemically complex Andean mining ecosystems.

1 | INTRODUCTION

The exploitation of freshwater ecosystems has been largely detrimental (MEA, 2005), as evinced by the increased aquatic biodiversity loss (Harrison et al., 2018). Among the many economic activities threatening freshwater ecosystems, mountaintop mining poses complex environmental pressures that result in a cascade of social-ecological impacts (Palmer et al., 2010; Vela-Almeida et al., 2015). Large-scale mining operations affect key mountainous ecosystem functions such as aquifer recharge and habitat quality maintenance, which in turn affect freshwater flow regimes, biodiversity and ecosystem services (Jeronimo et al., 2015; Mercado-Garcia et al., 2018). The consideration of both direct and indirect influences on ecological interactions is thus crucial to design more sustainable mining approaches (Xu et al., 2018) and mining legacies (Zhao et al., 2018; Zipper et al., 2011). In the exploitation phase, mining watersheds undergo freshwater quality assessments (FWQA) regularly to guarantee the suitability of food production, recreation and biodiversity protection downstream of mining sites. Such a holistic purpose of FWQA requires a mechanistic understanding of the aquatic communities' responses to environmental stressors rather than addressing freshwater quality components separately. Hence the need for ecologically-oriented assessments in the headwaters as well as at downstream reaches of mining watersheds (Mercado-Garcia et al., 2019). Moreover, simple but scientifically sound techniques for FWQA are preferred to induce environmental policy changes (Metze et al., 2017).

A major contribution from the scientific community to FWQA is the simplification of ecological information into one-dimensional values, namely a biological quality or multimetric index, making environmental assessments more accessible to non-expert stakeholders. Taxonomy-based approaches have contributed greatly to such developments (Li et al., 2010; Villamarín et al., 2013). Unfortunately, FWQA in mining contexts lacked progress in bioindicators, multidisciplinary integration and policy relevance aspects (Mercado-Garcia et al., 2019). For instance, the need for calibrating the occurrence of aquatic macroinvertebrates taxa to the site-specific environmental conditions hinders FWQA throughout vast and scarcely studied regions like the Andes (Villamarín et al., 2013). Moreover, although the mining impacts on freshwater macroinvertebrates have been widely reported (Battaglia et al., 2005; Byrne et al., 2012; Dedieu et al., 2015; Ruse & Herrmann, 2000; Zhao et al., 2018), these were mostly demonstrated in the presence of either extreme acidity, peaking metal concentrations or contaminated sediment loads (Gerhardt et al., 2004; Tarras-Wahlberg et al., 2001; Wright & Ryan, 2016). Furthermore, several 'good-quality' macroinvertebrate taxa (e.g. Plecoptera and Coleoptera orders) have been found in streams with low pH and metal pollution (Gerhardt et al., 2004; Mercado-Garcia

et al., 2019; Ruse & Herrmann, 2000; Wright & Ryan, 2016). Studies of mining impacts on macroinvertebrates also argue on whether to aim for higher (Goldschmidt, 2016) or lower taxonomic resolutions (Wright & Ryan, 2016), leaving room for improvement of freshwater bioassessment tools.

The assessment of macroinvertebrates via trait-based approaches (TBA) is promising for advancing FWQA (Menezes et al., 2010). By looking at specific traits in river monitoring sites, the influence of environmental gradients and anthropogenic pressures on biological communities translates into changes in the relative abundance of macroinvertebrate traits. Moreover, TBA offers higher sensitivity because, in the presence of stressors, the distribution of the traits tends to change earlier than the taxonomic composition (Culp et al., 2011). Similar to taxa, traits are influenced by environmental stressors: the traits that impart resistance to stressors prevail, whilst the vulnerable traits fade (Usseglio-Polatera et al., 2000). Since the potential generalization of TBA relies on the use of consensual ecological knowledge, a major limitation remains in the lack of studies in neotropical regions (Van den Brink et al., 2011) and in mining contexts.

1.1 | Aim of this study

The responses of macroinvertebrate traits to mining pressures in the Andes have been still scarcely studied. Generic mining impacts on macroinvertebrates traits include the absence of leaf-like or filamentous gills due to suspended particles (Dedieu et al., 2015), or higher proportions of endobenthic burrowers, collector filterers and operculated gills, which protect against mining debris (Mocq & Hare, 2018), or acid mine drainage causing less functional diversity (He et al., 2015) and smothering by metal flocs. The latter in turn hampers macroinvertebrates that are temporarily attached to substrates, filter-feeders, water column swimmers, free-egg layers or with exposed gills (Reich et al., 2019).

The management of riverine habitats requires substantial ecological characterization and information systematization across multiple geographical regions and stream types (Chapman et al., 2016; Pottgießer & Sommerhäuser, 2008). Since TBA provides mechanistic insights on macroinvertebrate community changes, its application is suited for neotropical ecosystems where freshwater ecological monitoring and knowledge are rather scarce. We conducted a TBA to differentiate gradients in macroinvertebrate communities under different ecosystem types and anthropogenic pressures. The present study complements a previous study that demonstrated a lower biological quality in the mining-impacted headwaters, with insights beyond an excellent physicochemical quality in the headwaters impacted by mining (Mercado-Garcia et al., 2019).

2 | METHODS

2.1 | Study area

The Mashcon catchment is in the northern Peruvian Andes (Figure 1), hosting large-scale gold mining at mountaintop besides rural and urban economic activities downstream (Mercado-Garcia et al., 2019). Two high-altitude ecosystems can be differentiated in the catchment, namely jalca and western-slope relict mountainous forest (WSRMF) (MINAM, 2019). The jalca (3500–4000 m.a.s.l.) is an Andean transition between the northern paramo and the humid puna further south, thus having intermediate climatic characteristics (i.e. more humid than the puna but not as heavily rainy nor as cloudy as the paramo) and hosting mostly herbaceous vegetation and evergreen shrubs (Britto, 2017). Besides having floral assemblages like the puna and paramo, the jalca is rich in endemic genera such as Agrostis, Poa, Festuca and Arcytophyllum, among others. Contrary to the paramo, the jalca's mountainous landscapes are continuous (MINAM, 2019). The WSRMF, previously classified within Quechua (Vidal, 2014) or meso-Andean (Britto, 2017) ecosystems, refers to altitudes between 1400 and 3000 m.a.s.l. and it is characterized by epiphytes and 15 metres high canopies (MINAM, 2019). The WSRMF hosts a combination of evergreen shrubs, arboreal and perennial herbaceous vegetation (Vidal, 2014). Due to the temperate climate, human activities proliferate in the WSRMF more than in the jalca. Part of the WSRMF has been converted into agricultural plots and residential areas. The headwaters are formed in the hydrological centre Cajamarca-Hualgayoc, where major gold and copper deposits exist (Bissig et al., 2015). Open-pit gold mining has impacted the freshwater physicochemical quality between 1991 and 1993 (Bury, 2004), besides decreasing the groundwater table in the jalca. Consequently, the Yanacocha mining company installed advanced water treatment facilities (Miller et al., 2008) and an artificial system to recharge the main river headwaters, as required by downstream rural communities and 70% of Cajamarca's municipal water (Vela-Almeida et al., 2016).

2.2 | Biophysical data collection

The freshwater quality was assessed in the dry season of 2016 at 40 locations selected according to potential landuse impacts (e.g. urban, agriculture or mining) and reference conditions (i.e. high tributary headwaters), and their accessibility given the basin's rough topography. The sampling sites belonged to four watershed sections, wherein 6 locations were downstream of the gold mine's artificial recharge system in the jalca, hence named the 'high mining' section (i.e. the Grande River at >3200 m.a.s.l). 8 locations were at a high-altitude pristine-looking section consisting of tributaries of the Grande River, hence named 'high tributaries' section (>3200 m.a.s.l). Another section consisting of 14 locations nearby to patch agriculture and rural settlements was named the 'midstream' section (2800–3000 m.a.s.l.). Lastly, 12 locations in the downstream city of Cajamarca belonged to the 'urban' section (>2600 m.a.s.l.). The hydromorphology was characterized at each location using a standardized protocol (see S2). Physicochemical measurements of temperature, conductivity, pH, turbidity, chlorophyll-a and dissolved oxygen (DO%) were taken using YSI® multiparameter probes. Hach-Lange® laboratory test kits were used for measuring chemical oxygen demand (COD), phosphorus, nitrate, nitrite and ammonium. Sulphates were measured with Hach® SulfaVer® 4 kits. Filtered (0.45 μm) freshwater samples were collected in 50-ml falcon tubes and acidified to 1% HNO3 for dissolved metal analyses. The latter step was repeated without filtering for total metal analyses. A Thermo Fisher Scientific® iCAP

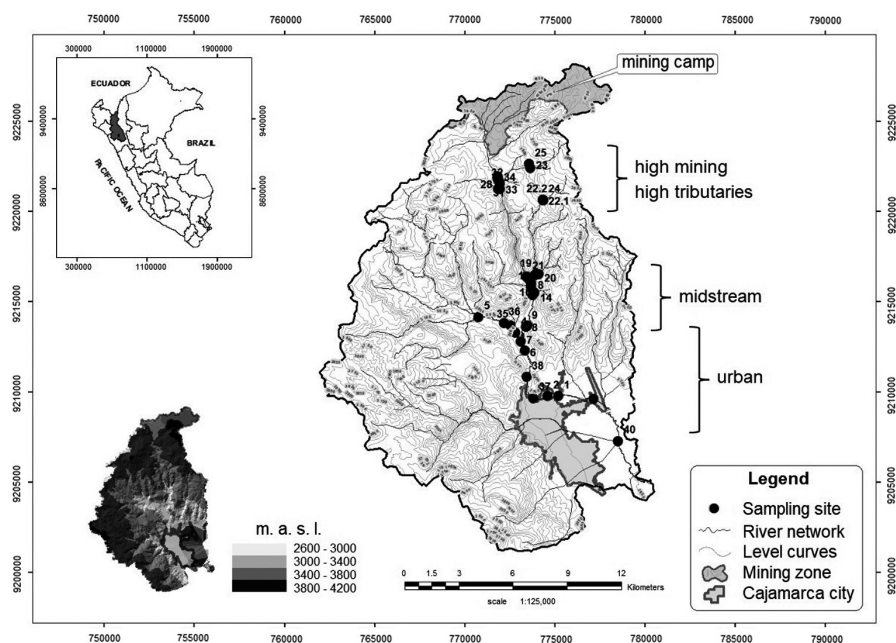**FIGURE 1** The Mashcon watershed in northern Peru, its digital elevation model and the 40 sampling sites divided into 4 watershed sections. The headwaters correspond to the high mining (Grande River) and high tributaries groups. The latter consists of the Grande River's tributaries out of direct mining influence. The peri-urban area includes the Porcon River, which mixes with the Grande River to form the Mashcon River (urban area)

6000 ICP-OES® was used to measure concentrations of cadmium, barium, copper, chromium, iron, nickel, manganese, lead, zinc, cobalt and arsenic. Geocoordinates and altitude were recorded using a Garmin eTrex® HC series GPS.

Freshwater macroinvertebrates were collected with a standardized kick-sampling method, by simultaneously kicking and sweeping the river substrate using a hand-net (frame size 20 × 30 cm; mesh size 500 μm) to sample as many microhabitats as possible, and for 5 minutes at each location (Acosta et al., 2008). Macroinvertebrates were collected from the substrate samples and stored in 75% ethanol. The taxa were counted and identified at the family level by an expert taxonomist, except for the Acari subclass. The level of taxonomic resolution was selected as being sufficient to detect environmental and biodiversity gradients of freshwater macroinvertebrates communities (Mueller et al., 2013).

2.3 | Estimation of macroinvertebrate trait abundances

The macroinvertebrate taxa counts were complemented with traits information. Eight traits were selected (Table 1) according to the availability of databases of sufficient taxonomic resolution and suited to Andean ecosystems. Traits of macroinvertebrates were obtained from Tomanova et al. (2008), Tomanova and Usseglio-Polatera (2007) and Tachet et al. (2000). These consisted of five biological (maximal body size, food source, body form and respiration) and three ecological (mobility and attachment to substrates, feeding habits, saprobity and pH preferendum) traits (Table 1). A traits matrix was created containing the modalities' affinity scores for 43 taxa (S3). Six families (Blephariceridae, Chordodidae, Curculionidae, Gripopterygidae, Hyalellidae and Hydrobiosidae) were excluded due to lacking traits information.

A fuzzy coding procedure was applied to estimate the weighted traits abundance (Forio et al., 2018), allowing multiple affinities for certain taxa. For example, Baetidae feeds by scraping fine particulate organic matter, microphytes and microinvertebrates from surfaces, but it is also able to feed on deposited debris sometimes. Thus, considering the modalities 'x','y' and 'z' for a given trait, and supposing there are 100 individuals of a taxon, and knowing that this taxon has an affinity of 3 for modality 'x', 1 for modality 'y' and no affinity for modality 'z', then the modality 'x' was given an abundance of 75 counts, modality 'y' 25 counts and modality 'z' 0 counts. In addition, a dominant-trait coding procedure was applied to the initial traits matrix, disregarding the non-dominant-trait modalities. In the above-mentioned Baetidae example, modality 'x' dominates the others, thus the dominant-trait approach assigns 100 counts for modality 'x'. If more than one modality were equally dominant in a taxon, the affinity scores had to be assigned by equal fractions totalling 1, allocating equal counts to the equally dominant modalities. In both quantification methods, the abundance per sampling site was estimated by adding up the modality counts from each taxon.

2.4 | Data processing

The biophysical data of 40 sampling sites were compared among watershed sections using the procedure depicted in Figure S4. The abiotic measurements (S1) were divided in four groups: high mining ($n = 6$), high tributaries ($n = 8$), midstream ($n = 14$) and urban ($n = 12$). The medians and value ranges for each group were identified. The Wilcoxon Rank Sum test and (Welch's) Two-sample t-test were applied to compare the groups by pairs. The Shapiro–Wilk, ANOVA and Kruskal–Wallis tests were used to verify the tests' assumptions. Statistically significant differences were identified at either $p < .05$ or $p < .001$.

The taxa's average abundance per watershed section was assessed as:

$$\frac{\sum_{x_1}^{x_n} a_i}{n} = AA_i \tag{1}$$

where a_i is the sampling site's abundance of taxon 'i'; x_1 is the first sampling site of the watershed section; X_n is the last sampling site of the watershed section; n is the number of sites in the watershed section; and AA_i is the average abundance of taxon 'i' per sample at the watershed section. Taxa resulting in averaged abundances ≤0.2 were excluded from the traits quantification.

Regarding the traits, the relative abundance per watershed section was assessed as:

$$\frac{\sum_{x_1}^{x_n} a_j}{\sum m_1 + \cdots + \sum m_n} x100 = RA_{tm}(\%) \tag{2}$$

where a_j is the sampling site's abundance of a given trait modality 'tm'; x_1 is the first sampling site in the watershed section; X_n is the last sampling site in the watershed section; $\sum m_1$ is the sum of the watershed section sites' abundances for modality 1 of the trait; $\sum m_n$ is the sum of the watershed section sites' abundances for modality n of the trait; and RA_{tm} (%) is the relative abundance of trait modality 'tm' in the watershed section. The latter result was used to generate bar plots, enabling a comparison of traits composition among watershed sections as well as between the weighted and dominant-traits quantification.

The ecological interpretability of the ungrouped data was verified using the R software (R Core Team, 2021) and the 'vegan' package (Oksanen et al., 2020) to conduct a constrained ordination. A detrended correspondence analysis was performed on the ecological data (i.e. environmental variables and biological counts per sampling location, either taxa or traits) to find the length of gradient ('axis length' in R) using the 'decorana' function. Axis lengths below three indicate a redundancy analysis (i.e. linear method) is needed and above three a Canonical Correspondence Analysis (i.e. unimodal method). The distance measures for the ecological data were ranked using 'rankindex' to select the highest index needed for a distance-based Redundancy Analysis (dbRDA). The highest ranked distance indexes (i.e. either Chao or Cao for this study) were specified in the function 'capscale' to produce a dbRDA model. Collinearity of environmental variables was identified by calculating their variance

TABLE 1 Macroinvertebrate traits used in this study

Trait	Modalities	Identifier[a]
1. Maximal body size (Tomanova et al., 2008)	≤0.25 cm	mbs1
	>0.25–0.5 cm	mbs2
	>0.5–1 cm	mbs3
	>1–2 cm	mbs4
	>2–4 cm	mbs5
	>4–8 cm	mbs6
	>8 cm	mbs7
2. Mobility and attachment to substrate (Tomanova et al., 2008)	Flier	mas1
	Surface swimmer	mas2
	Full water swimmer	mas3
	Crawler	mas4
	Epibenthic burrower	mas5
	Endobenthic burrower	mas6
	Temporary attached	mas7
3. Food (Tomanova et al., 2008)	Microorganisms	food1
	Fine detritus ≤1 mm	food2
	Coarse detritus ≥1 mm	food3
	Macrophytes	food4
	Microphytes	food5
	Dead animals >1mm	food6
	Microinvertebrates	food7
	Macroinvertebrates	food8
4. Feeding habits (Tomanova et al., 2008)	Collector gatherer	fhs1
	Shredder	fhs2
	Scraper	fhs3
	Filter feeder	fhs4
	Piercer (plants or animals)	fhs5
	Predator	fhs6
	Parasite	fhs7
5. Body form (Tomanova et al., 2008)	Streamlined	bf1
	Flattened	bf2
	Cylindrical	bf3
	Spherical	bf4
6. Respiration (Tomanova et al., 2008)	Tegument	res1
	Gill	res2
	Plastron	res3
	Stigmata	res4
7. Saprobity (Tachet et al., 2000)	Xenosaprobic	sap1
	Oligosaprobic	sap2

TABLE 1 (Continued)

Trait	Modalities	Identifier[a]
	β-mesosaprobic	sap3
	α-mesosaprobic	sap4
	Polysaprobic	sap5
8. pH preferendum (Tachet et al., 2000)	≤4	pH1
	>4–4.5	pH2
	>4.5–5	pH3
	>5–5.5	pH4
	>5.5–6	pH5
	>6	pH6

[a]Labels for Figure 3a and c.

inflation factors. The latter uses 'vif.cca' on the dbRDA model and removes abiotic variables until all the variance inflation factors are lower than three (Forio et al., 2017). The final three dbRDA models, for the taxa, the weighted and the dominant-traits abundances, were plotted using the 'plot' function.

3 | RESULTS

3.1 | Freshwater abiotic conditions

Table 2 shows the high tributaries and high mining sections have similar altitude distributions, with maximum altitudes of 3299 and 3565 m.a.s.l., respectively. The midstream and urban sections differ between themselves and with the high-altitude sections in terms of altitudinal distribution ($p < .001$), despite the fact that 25% of the urban sites were at midstream altitudes. The lowest, most downstream, location was at 2662 m.a.s.l., whereas the range between 2960 and 3200 m.a.s.l. has no samples due to the rough topography. Except for three heavily polluted urban sites, the DO% was always close to saturation (94–115%). No differences in DO% distributions were found among watershed sections, except between the high tributaries and midstream sections ($p < .05$). The high mining section had the highest maximum velocity, although the midstream section had the highest median and was the only section different from the urban velocity values distribution ($p < .05$). The temperature measurements showed an altitudinal evolution from the colder high tributaries section to warmer water downstream, except the high mining section that showed a higher maximum, minimum and median than the midstream section. Only the high tributaries and midstream sections had similar temperature distributions. The sulphate concentrations fluctuated from below the detection limit in the midstream section to a maximum of 275 mg.l^{-1} in the high tributaries section. The narrowest range of sulphate concentrations corresponded to the high mining section (135–160 mg SO$_4$.l^{-1}), except for a peak of 205 mg SO$_4$.l^{-1} developing after mixing with a high tributary. The pH remained around neutral, except in the high tributaries group, which reached pH values of 4.6 and 5.7. The two high-altitude sections had similar pH value

TABLE 2 Abiotic conditions measured at the sampling locations: medians (variation ranges) and significant differences among watershed sections

Parameter (units)	Watershed sections				Significantly different from
	High mining	**High tributary**	**Midstream**	**Urban**	
Altitude (m.a.s.l.)	3282 (3253–3299)	3525 (3269–3565)	2871 (2800–2925)	2744 (2662–2871)	**u ≠ m & ht **m ≠ hm & ht
Oxygen (% saturation)	102 (101–104)	101 (95–103)	103 (94–105)	106 (22–153)	*m ≠ ht
Velocity (m/s)	0.4 (0.1–1.4)	0.4 (0.1–0.6)	0.5 (0.2–0.8)	0.3 (0.1–0.6)	*u ≠ m
Temperature (°C)	13.4 (13.2–14.2)	12.2 (8.1–14.0)	11.9 (10.4–13.5)	14.9 (12.3–18.5)	**m ≠ u & hm *u ≠ ht &hm
Sulphate (mg/l)	153 (135–205)	103 (12–275)	163 (0–215)	143 (70–230)	
pH	6.9 (6.6–7.0)	6.7 (4.6–7.2)	7.3 (6.9–7.9)	7.3 (7.1–8.2)	**hm ≠ u & m **ht ≠ u & m
Conductivity (µS/cm)	428 (422–439)	318 (78–743)	411 (144–509)	509 (340–933)	*u ≠ m & hm
Turbidity (NTU)	0 (0–0)	0 (0–4)	0 (0–11)	6 (0–366)	*u ≠ m, ht & hm
Chlorophyll-a (µg/l)	0.8 (0.2–1.4)	2.0 (0.2–10.8)	2.1 (0.9–5.5)	5.6 (1.9–22.6)	**u ≠ hm *m ≠ u & hm
Nitrite (mg/l)	0.011 (0.005–0.016)	0.010 (0.005–0.570)	0.005 (0.005–0.018)	0.039 (0.005–0.370)	*u ≠ m
Ammonia (mg/l)	0.29 (0.21–0.29)	0.08 (0.02–1.46)	0.02 (0.02–0.04)	0.02 (0.02–11.90)	**m ≠ hm *u ≠ hm; m ≠ ht
COD (mg/l)	5 (5–5)	5 (5–10)	5 (5–7)	6 (5–47)	*u ≠ m & hm
Particulate Ba (µg/l)	4 (3–5.1)	5 (1–9)	6 (–13.1)	10 (3–333)	*u ≠ m, ht & hm
Dissolved Ba (µg/l)	21 (2–25.2)	52 (24–65.1)	42 (28–147)	51 (45–106)	**hm ≠ u & m *hm ≠ ht
Particulate Mn (µg/l)	5 (3.1–11.2)	6.1 (0–21.8)	7.8 (1.2–21)	22 (11–644.7)	**u ≠ m *u ≠ ht & hm
Dissolved Mn (µg/l)	38.1 (35.6–42)	61.7 (2.2–131.6)	37 (4–302)	167 (64–639)	**u ≠ m & hm *u ≠ ht
Dissolved Zn (µg/l)	28.3 (26.3–108.1)	34 (17.6–75.1)	20 (15.5–429.7)	17.2 (15–32.6)	*u ≠ ht & hm *hm ≠ m

Note: **$p < .001$ *$p < .05$ according to the Wilcoxon rank-sum test for all parameters, except for temperature and sulphate evaluated using the two-sample *t*-test

Abbreviations: u = urban, m = midstream, ht = high tributary, hm = high mining.

distributions, yet were significantly different from the midstream and urban sections ($p < .001$). The electric conductivity values were more stable in the high mining section (422–439 µS/cm) and reached a maximum of 933 µS/cm in the urban section. Regarding metal measurements, the urban section had different distributions for most metals except for dissolved barium and dissolved zinc when compared to, respectively, the high tributaries and midstream sections. The high mining section had different dissolved zinc distributions than the midstream section ($p < .05$), likewise for dissolved barium when comparing it to the high tributaries section ($p < .05$) (Table 2). Moreover, the concentrations of barium and manganese were always higher in dissolved forms than in particulate forms.

3.2 | Bioassessment

Table 3 shows the averaged-per-sample abundance in each watershed section for each taxon, including identifiers serving as labels for Figure 3b. An upstream to a downstream increment of the total

averaged taxa abundances was found, with a contrasting reduction in the high mining section (i.e. one-fourth of the averaged abundance found in the higher-altitude tributaries). Fewer overall taxa types including less Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa corresponded to the high mining section, where the increased relative abundance of Gripopterygidae contrasted with the other watershed sections. Rare taxa, exclusive to a single watershed section, included Planorbidae, Glossiphoniidae, Erpobdellidae (high tributaries section), Pyralidae (high mining section), Blephariceridae and Hydrophilidae (midstream section). From these rare taxa, the scarcest ones (with one specimen per watershed section) were excluded from the traits quantification, thus labelled as NN. Likewise, the NA label identifies the taxa without traits information (Table 3).

3.2.1 | Differentiating traits distributions

The traits bar plots (Figure 2) show the relative abundance of the modalities (percentages) in each watershed section for the weighted

TABLE 3 Taxa abundance per watershed section, averaged by the number of samples

Order and (identifier)[a] of family	High mining	High tributaries	Midstream	Urban
Diptera				
(a) Chironomidae	3	11	44.2	460.6
(b) Ceratopogonidae		4.3	6	6
(c) Simuliidae		5.3	6	22.3
(d) Empididae	0.7	0.8	1	11
(e) Limoniinae	8	7	1.6	1.0
(f) Tipulidae		2.8	2.3	1
(g) Culicidae			1	3.2
(h) Dolichopodidae				2
(i) Muscidae	0.2	0.3		1.1
(j) Psychodidae				1.3
(k) Tabanidae		0.1	0.3	
(l) Ephydridae				0.3
NA Blephariceridae			2.1	
Ephemeroptera				
(o) Leptophlebiidae		0.5	2.8	7.7
(m) Baetidae		30.9	237.9	210.6
(n) Leptohyphidae			2	24.8
Plecoptera				
NA Gripopterygidae	45.5	8.4		
Trichoptera				
(p) Hydroptilidae	0.2	6.3	1.8	69.5
(q) Hydropsychidae	0.5	2.1	53.4	2.2
(r) Helicopsychidae	0.2	13.0	30.8	
(s) Leptoceridae	0.2	5.9	22.4	
NA Hydrobiosidae	1	2.4	13.1	1.8
(t) Glossosomatidae		10.9	7.2	1.2
(u) Calamoceratidae	6	7.3	4	0.1
(v) Sericostomatidae		5.9	1.3	
(x) Xiphocentronidae		2		
Coleoptera				
(y) Elmidae	62.3	219	184.5	45.6
(z) Hydraenidae	2.7	21.5	20.6	0.1
(w) Psephenidae		0.6	5.5	
(ia) Dytiscidae		3.8	0.1	1
(ib) Scirtidae	0.2	1.6	1.9	
(ic) Gyrinidae	2.3	1.8	0.2	
(id) Staphylinidae		0.5	0.4	0.2
NN Hydrophilidae			0.2	
NA Curculionidae		1.4		
Basommatophora				
(ig) Physidae	0.2	0.1	18.8	60.5
(ih) Lymnaeidae		28.3		0.1
NN Planorbidae		0.1		

TABLE 3 (Continued)

Order and (identifier)[a] of family	High mining	High tributaries	Midstream	Urban
Amphipoda				
NA Hyalellidae	1.2	42.4	39.9	8.7
Haplotaxida				
(ii) Tubificidae		2.8	15.6	82
Prostigmata				
(ij) Acari (Subclass)	2.2	37.3	0.4	1.2
Seriata				
(ik) Dugesiidae	1.5	22.4	8.5	0.4
Opisthopora				
(ie) Lumbricidae		5.5	7.9	1.3
Odonata				
(im) Aeshnidae		0.3	0.2	0.1
Chordodea				
NA Chordodidae	0.2	0.3	0.1	
Veneroidea				
(io) Sphaeriidae		0.4		
Lepidoptera				
NN Pyralidae	0.2			
Rhynchobdellae				
NN Glossiphoniidae		0.1		
Arhynchobdellida				
NN Erpobdellidae		0.1		
Total sum of average abundances	140.4	621.4	745.6	1090.5
Total number of taxa and EPT orders	22 (7 EPT)	41 (12 EPT)	36 (11 EPT)	31 (8 EPT)

Abbreviations: NA, no traits information; NN, excluded due to its scarce presence.

[a]Labels for Figure 3b.

and dominant-traits quantification methods. An upstream to downstream evolution of the phenotypic traits (Figure 2a and c) is observed, whereas the physiological (Figure 2d and e) and ecological traits (Figure 2b, f, g and h) had comparable distributions among watershed sections. The ecological and physiological traits produced shifted distributions when changing the traits quantification method, whereas the phenotypic traits distributions were more maintained. A few trait modalities were unique to the high-altitude groups, namely the smallest maximum body size (Figure 2a) and the food source microinvertebrates (Figure 2d), although with slightly increased shares in the high tributaries. The mobility and attachment to substrate modalities had similar shares among watershed sections, except for a decrease in crawlers and an increase in epibenthic burrowers in the urban section (Figure 2b). The high mining section had an increased relative abundance of streamlined and flattened body forms (Figure 2c). The shares of the respiration trait were higher for the gill and tegument modalities, while plastron and stigmata made together one-fifth of the total share (Figure 2e). The feeding habits distribution was similar among watershed sections and largely dominated by collector-gatherers. The second-most present feeding habits were shredders and scrapers, with the latter decreasing slightly in the mining group. No share for piercers was found in the midstream section while being minimally present in the other sections (Figure 2f).

All the groups had fine detritus as the main food source followed by macrophytes and coarse detritus, with the latter increasing slightly in the mining group. The weighted quantification yielded no share for microorganisms as a food source in the mining group (Figure 2d). The saprobity trait assigned the highest shares to the beta-mesosaprobic modality, but no clear differences among groups except for an increase of polysaprobic organisms in urban areas (Figure 2g). The pH preferenda distributions were maintained among the watershed sections, except for a slight increment of the modality >4.5–5 in the mining group. The acidophilic modalities (pH ≤ 4.5) made one-fifth of the total share according to the weighted method, whereas the dominant-trait approach yielded their absence (Figure 2h).

3.2.2 | Distance-based redundancy analysis (dbRDA)

The dbRDA plots (Figure 3) yielded fair representations of the sampling sites, although to a lesser extent when using taxonomic (Figure 3b) and weighted traits information (Figure 3c). The ranking
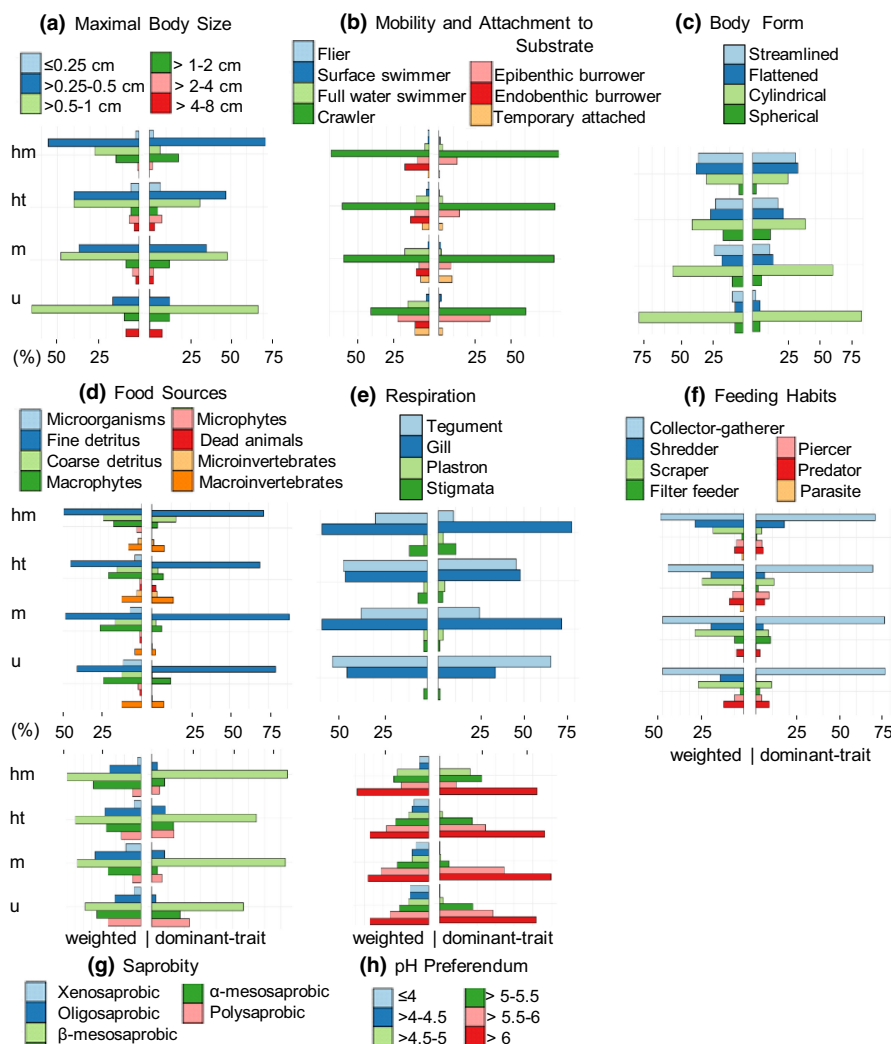
FIGURE 2 Relative traits modalities abundances (%) for the weighted (left) and dominant-trait (right) quantification approaches at the 4 watershed sections (hm = high mining, ht = high tributaries, m = midstream, u = urban). Relative abundances below 1% are excluded

of distance measures yielded the Chao index as the highest for weighted traits, and Cao for dominant-traits and taxonomic information. The watershed sections are indicated in the ordination maps with different symbols representing the sampling sites. Grey labels indicate the biological components. The sampling sites and traits are more separated in the dominant-traits plot. The ordinations were correlated to DO%, altitude, dissolved manganese, dissolved barium, dissolved zinc, particulate manganese, temperature, pH, sulphate, velocity and either nitrate (dominant-traits) or nitrite (taxa-based and weighted traits). In addition, the traits-based ordinations were correlated with chlorophyll-a. The ordination using weighted modalities abundances produced no clear differentiation of the mining-impacted sites, and less scattered modalities than the dominant-traits ordination. The taxa-based ordination yielded most families tightly clustered at the centre of the plot (Figure 3b). An overlap between the high tributaries and midstream groups is visible in the traits-based plots (Figure 3a and c), and to a lesser extent when using taxonomic information (Figure 3b). According to the environmental vectors, the high tributaries and high mining groups followed the direction of increasing altitude and velocity and decreasing pH; the urban section was in the direction of increasing

temperature, dissolved metals except zinc, chlorophyll-a and pH; and the midstream section was in the direction of increasing oxygen saturation and dissolved zinc.

4 | DISCUSSIONS

4.1 | Bioassessment of the gold-mined catchment

From both the naturally and artificially recharged headwaters in the jalca, to the midstream rural settlements and downstream Cajamarca city, the watershed sections were characterized by the differences in abiotic variability (Table 2), macroinvertebrate assemblages (Figure 3b) and averaged abundances (Table 3). By including traits information, we identified an upstream to downstream evolution of phenotypic traits and patterns among watershed sections for the ecological and physiological traits (Table 4). A constrained ordination confirmed that using either traits or taxonomic information helps differentiating the impacted watershed sections, whereas more overlapping between groups and patterns of traits composition corresponded to the more vegetated sections
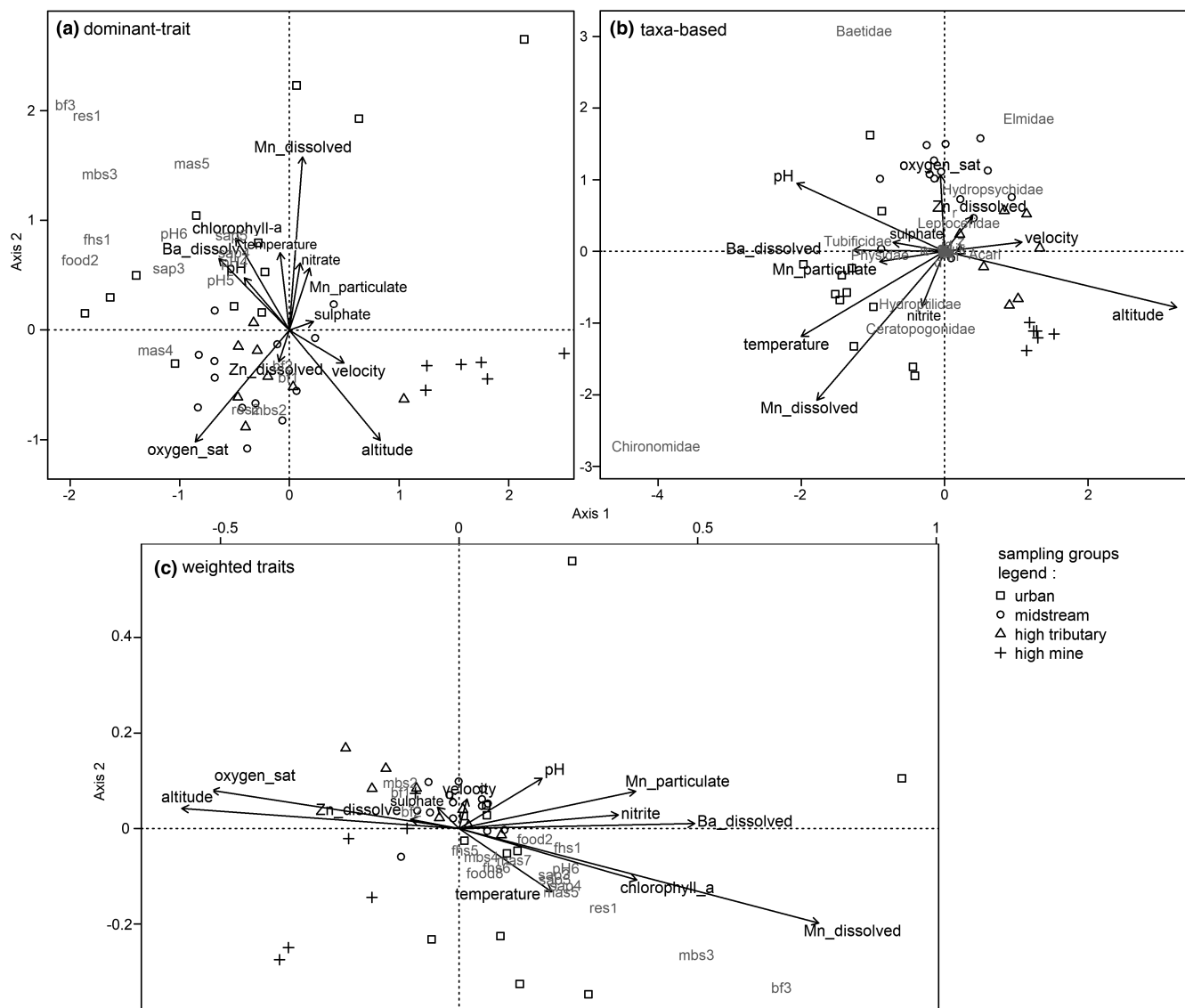
FIGURE 3 Distance-based redundancy analysis (dbRDA) of sampling locations based on (a) dominant-trait abundances, (b) taxa abundances and (c) weighted traits abundances. The length and position of the abiotic vectors indicate, respectively, the strength and direction of the correlation. The trait modalities abbreviations (grey labels, a and c) are those from Table 1 (mbs = maximal body size, mas = mobility and attachment to substrate, food = food sources, fhs = feeding habits, bf = body form, res = mode of respiration, sap = saprobity or sensitivity to organic pollution, pH = pH preferendum). The taxa-type (grey labels, b) abbreviations are those from Table 3 (e.g. r = Helicopsychidae, z = Hydranidae). A few not-shown modalities were overlapping at the plot's centre (i.e. mbs ≤0.25 cm and >2–4 cm; mas full-water swimmer, endobenthic burrower and temporary attached; food coarse detritus, dead animals and microinvertebrates; res plastron and stigmata; bf spherical; fhs shredder and piercer; sap oligosaprobic and pH > 5.5) or remained unreadable near to the plot's centre (i.e. mbs > 1–2 cm and >4–8 cm; food macrophytes and macroinvertebrates; fhs scraper and predator). The scattered trait modalities include mbs ≤ 0.25 and >2–4 cm; mas full-water swimmer, endobenthic burrower and temporary attached; food coarse detritus, dead animals and microinvertebrates; res plastron and stigmata; bf spherical; fhs shredder and piercer; sap oligosaprobic and pH > 5.5–6, >6

(midstream and high tributaries) despite a significant altitudinal gradient. The comparable traits distributions among watershed sections matched the characteristics of a highland watershed according to a study in Bolivian-Andean pristine streams. The feeding habits (Figure 2f) coincided with Tomanova et al. (2007) who found that collectors-gatherers increase in the scarcely vegetated highlands (>3000 m.a.s.l.) while shredders and scrapers decrease due to the limited availability of their food sources (plants and periphyton, respectively). The respiration trait showed no altitudinal

gradient but a general impairment of the aerial breathers (plastron and stigmata) (Figure 2e), contrasting with assessments in the high Ecuadorian Andes demonstrating that the lack of dissolved oxygen affects macroinvertebrate communities as the altitude increase >3000 m.a.s.l. (Burneo & Gunkel, 2003; Jacobsen, 2008). Moreover, the dispersion of benthic organisms by both aquatic and terrestrial directional processes (Dong et al., 2016) hinders the generalization of altitudinal effects on freshwater macroinvertebrates. Dissolved oxygen remained near saturation in the Mashcon

TABLE 4 Summary of findings of this study

Watershed section	Freshwater ecosystem characteristics		Impacts on macroinvertebrates traits and taxa
	Natural	Anthropogenic	
High mining	Jalca's herbaceous and evergreen shrub vegetation More humid than the southern puna, but less rainy than the paramo Bedrock, boulders and cobbles in river substrates.	Concrete-channelled riverbanks Artificially recharged headwaters Decreased phreatic level Stringent water quality Less shading from riverbank vegetation Fastest stream velocity	Mostly streamlined and flattened body forms. Decrease in scrapers and maximal body sizes >0.5–1 cm. Less diversity and abundance of families. Increase in Gripopterygidae.
High tributaries	Jalca ecosystem. Naturally recharged. Substrates like in the high mining section, but more cobbles and fewer boulders. Vegetated riverbanks and pool riffle sequences. Acidic streams.	Scattered agricultural patches and grazing lands Few rural houses	Diverse families. Increase in Acari Increase in small body sizes, mainly >0.25–0.5 cm. Physiological and ecological traits similar to the midstream section
Midstream	Vegetated riverbanks, pool riffle sequences and water temperatures similar to the high tributaries section. Tree groups. Gravel, sand, cobbles and boulders in substrates.	Extensive agricultural patches and grazing lands. Aquaculture. More rural houses and public infrastructures. Water capture plant for Cajamarca city	Diverse and abundant macroinvertebrate communities. Similar body form distributions as the urban section
Urban	Similar climate and vegetation types like the midstream section (WSRMF ecosystems). Broader valley than the upstream sections.	City's concrete infrastructures. Open littering and artisanal stone mining from rivers. Thick sludge layer and eutrophication. Less riverbank vegetation.	Fewer crawlers and more epibenthic burrowers and polysaprobic macroinvertebrates

Abbreviation: WSRMF, Western-slope relict mountainous forest.

watershed thanks to the slope-induced mechanical oxygenation, as reported in high-altitude streams of rough topography (Zhou et al., 2017).

Although the maintenance of physiological and ecological traits makes them less suitable for human impact detection (Menezes et al., 2010), a few slight changes occurred in the anthropized sections. For instance, the removal of riverbank vegetation in urban areas is reflected in fewer shredders as in Villamarín et al. (2013). A decrease in crawlers and increasing polysaprobic shares reflect the deterioration of river bottoms and water quality in the urban section. Conversely, the decrease of tegument respiration in the oxygen-rich high mining section (Figure 2e) is unprecise towards the type of anthropogenic impact since the excluded, yet increased, Gripopterygidae family (Table 2) uses cutaneous respiration in its initial nymphal stages (Gutiérrez-Fonseca, 2010). Andean autecological studies would be needed at different developmental stages of Gripopterygidae. Moreover, the smallest macroinvertebrates were exclusive to both high-altitude sections (>3000 m.a.s.l.), and these have been associated with reference conditions in French Guianese watersheds impacted by artisanal gold mining (Dedieu et al., 2015). The traits shares found in the high tributaries, while different in the high mining section, recovered in the midstream section (Figure 2) coinciding with higher and more fluctuating values of sulphates and electric conductivity nearby rural settlements (Table 2).

Mining pollutants are rather complex in types, sources and ecological effects. The correlation between the traits-based ordinations and the altitude, DO%, stream velocity and dissolved metal measurements points to hydromorphological and geochemical factors steering the community traits. A study of high-altitude water springs in the Cajamarca province found groundwater flows and hydrogeochemical processes, such as pyrite weathering and calcium carbonate dissolution, causing the natural variability of pH and ionic concentrations (Cooper et al., 2010), relatable to the physicochemical quality of the high tributaries and the presence of acidophilic modalities (Figure 2h). Unfortunately, the typification of Andean freshwater ecosystems lacks the characterization of gradients in the physical environment and floral assemblages (Mercado-Garcia et al., 2019; Sylvester et al., 2017; Villamarín et al., 2013). Naturally occurring ranges of salinity and pH in waterbodies enable the in-situ adaptation of microbial assemblages (Waiser & Robarts, 1995), and functional compensations in macroinvertebrate communities despite their changing taxonomic composition (Péru & Dolédec, 2010; Petrin et al., 2008). For instance, the clustering of sampling sites after translating the taxonomic information into weighted traits (Figure 3) suggests functional compensations despite the abiotic and taxa gradients. However, such a coincidence is sometimes deemed as a syndrome yielding redundant ecological

information, making the study of traits trade-offs and compensations (Boersma et al., 2014; Menezes et al., 2010; Schmera et al., 2017) a necessary addition to traits assessments.

The high mining section's physical environment differed from the other rural sections in the sense of having larger boulders in the substrates, less shading, concrete riverbanks and channels, the most fluctuating stream velocities and the narrowest physicochemical quality ranges. Such distinctive abiotic characteristics coincided with a reduced macroinvertebrate community dominated by Elmidae and Gripopterygidae (Table 3), wherein the former contributed largely to the relative abundances of traits in the high mining section. Despite the omission of Gripopterygidae, its phenotypic description in the Colombian Andes coincide with the traits-based differentiation between the high mining and high tributaries sections. This Plecopteran is known to possess strong and articulated thoracic legs and a streamlined body form (Barreto-Vargas et al., 2005), whereas its maximal body size (reported between 0.8 and 1.8 cm), feeding habits, pH preferendum and locomotion are undetermined by the lacking information about its developmental stages and different genera. Yet, the consistent Gripopterygidae traits are relatable to fast-flowing rivers and variable sediment sizes according to Horrigan and Baird (2008).

4.1.1 | Hydromorphological impacts

The increasing modalities for the streamlined and flattened body forms in the mining section (Figure 2c) are presumably induced by hydromorphological changes and an artificial flow regime created by large-scale mining operations. For instance, the impaired Hyalellidae family (Table 3) belongs to the Amphipoda order and looks similar to Gammarus, which is known to have delicate current velocity requirements (Vadher et al., 2018). The hydrological regimes, and the differences in stream size, order and slopes play an important role in the definition of reference conditions for traits compositions (Horrigan & Baird, 2008). Therefore, the bioindicator-based differentiation between mining impacts and Andean environmental pressures has substantial multivariate nature. Villamarín et al. (2013) assessed eight Andean watersheds in Peru and Ecuador and found that the heterogeneity of habitats had more explanatory power than the hydromorphological degradation in the paramo. Likewise, a study of unpolluted streams in the Tibetan Plateau demonstrated that stream power, which is 'the product of the specific weight of water, the discharge and the hydraulic slope' (Zhou et al., 2017), is a good predictor of macroinvertebrate variations in high-altitude ecosystems. Besides a correlation with the benthic biomass, Zhou et al. (2017) suggested that a high stream power reduces the mobility and attachment of macroinvertebrates lacking the necessary morphological and behavioural adaptations to fast flows and large-sized substrates, i.e. dexterously articulated thoracic legs, streamlined or flattened bodies, and strategies to hide from predators and find food (Horrigan & Baird, 2008).

4.1.2 | Physicochemical quality control impacts

The absence of microorganisms as a food source in the high mining group (Figure 2d) might be reflecting a deleterious effect of the controlled physicochemical quality, since chlorination is used at the mining camp to eliminate traces of cyanide before recharging the headwaters (Miller et al., 2008). Microorganisms play an important role in symbiotic (e.g. insect's gut microbiota) and mutualistic (e.g. litter decomposition) interactions with macroinvertebrates (Emilson et al., 2016; Wielkopolan & Obrępalska-Stęplowska, 2016). Likewise, the electric conductivity thresholds for the Hyalellidae's crust formation (Colla & César, 2019) make it a sensitive organism to slight changes in physicochemical water quality. Moreover, the lower share of scrapers (Figure 2) suggests that the mining section lacks periphyton, which is a biologically complex biofilm supporting higher trophic levels. The growth of periphyton in freshwaters of intermediate-to-low acidity and low metals concentrations is governed by thresholds in the water alkalinity and nutrients. For example, calcareous geology reduces algal diversity in periphyton (Smucker et al., 2014). For the Mashcon watershed, although the reactivity, bioavailability and ecotoxicity of metals are expected to increase in dissolved forms (Kravchenko et al., 2014), the physicochemical quality variations constituted healthier freshwater habitats in the high tributaries when compared to the mining section. Presumably, the stringent physicochemical quality in the mining section misrepresents the jalca's natural gradients in physicochemical water quality (Cooper et al., 2010).

4.1.3 | Future research

The lack of macroinvertebrate traits databases for (neo)tropical regions and the growing evidence that bioindicative traits vary among the different genera and species of ubiquitous macroinvertebrates families (Colla & César, 2019; Goldschmidt, 2016; Serra et al., 2017; Wielkopolan & Obrępalska-Stęplowska, 2016) point to a need for autecological studies in Andean mining regions of Peru. The latter is particularly for the scarcely characterized Grypoterigidae and Hyallelidae families, and for ubiquitous taxa like Acari and Chironomidae. Horrigan and Baird (2008) recommended the characterization of freshwater nutrients in flow-sensitive traits assessments, acknowledging the complexity of freshwater ecological interactions. In fact, the sources and requirements of the freshwater nutrients, metals bioavailability and microbial enzymatic activities would be more insightful measures of ecosystem processes (Smucker et al., 2014).

5 | CONCLUSION

We found ecological impairment (less macroinvertebrate diversity and changing phenotypic traits compositions) in the mine-impacted headwaters despite the excellent physicochemical quality of the

water discharged by the mine. Presumably, the environmental norms for large-scale mining operations are unaligned with the ecological requirements of Andean freshwater ecosystems. In addition to the generic mining impacts on freshwater quality, traits-based assessments help understanding the ecological impacts of artificial flow regimes and hydromorphological changes induced by mining operations.

CONFLICT OF INTEREST

The authors declare they have not known competing financial interests nor personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

The raw macroinvertebrate taxa counts per sampling location, the affinity scores for both the weighted and dominant-trait approaches, and the traits counts are in the S3, and the abiotic measurements in the S1 file. The hydromorphological assessment and geocoordinates of the sampling locations are in the S2. Datasets available in https://doi.org/10.5061/dryad.ngf1vhhwj.

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BIOSKETCH

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