

## The Teaone River: a snapshot of a tropical river from the coastal region of Ecuador

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Received: 01/02/18

Accepted: 02/10/18

### ABSTRACT

#### The Teaone River: a snapshot of a tropical river from the coastal region of Ecuador

Despite deforestation and population growth during the last 30 years in Esmeraldas (Northern Ecuador), there is no information about the impact of these changes on the coastal rivers. The Teaone is a 5<sup>th</sup> order river located in Northern Ecuador that originates in the Mache-Chindul mountain range and flows into the Esmeraldas River near its mouth. It flows through a rural landscape that includes pastures, forestry plantations, and some remnants of humid forest. In this work, we have studied watershed land use indicators (percentages of agricultural and forest land in addition to road and housing densities), geomorphology (bed substrate composition and woody debris density and origin), water chemistry (pH, turbidity, conductivity, and oxygen, nitrate, nitrite, and ortho-phosphate concentrations) and benthic diatoms and macroinvertebrates along the river and in its main tributaries. The Teaone watershed has been severely modified and agriculture and forestry plantations occupy more than 50 % of the watershed area. The riverbed is dominated by fine sediment, but we could not establish whether an impact from land use changes had occurred because both natural and anthropogenic sources of fine sediment coexist in the watershed. Nitrate concentrations are high on the tributaries, but low concentrations in the main channel suggest the presence of a strong nitrate sink. On the contrary, phosphate concentrations are low in the tributaries and high in the main channel, which suggest that clothes washing and personal bathing in the river might be important sources of phosphate. The diatoms and macroinvertebrate communities respond in a similar way to environmental changes along the river. Both communities show a stronger relationship with watershed land uses than with water chemistry and in-stream habitat.

**Key words:** tropical rivers, land use, channel assessment, water quality, diatoms, macroinvertebrates

### RESUMEN

#### El río Teaone: una instantánea de un río tropical de la región costera de Ecuador

A pesar de la deforestación y el rápido crecimiento de la población en Esmeraldas (norte de Ecuador), no hay información del impacto de estos cambios en los ríos de la costa. El Teaone es un río de orden 5 situado en el norte de Ecuador que nace en las montañas Mache-Chindul y confluye con el río Esmeraldas cerca de su desembocadura. El río atraviesa un paisaje rural que incluye pastos, plantaciones forestales y algunos remanentes de bosques húmedos. En este trabajo, hemos estudiado los indicadores del uso del suelo en la cuenca (porcentaje de usos agrícolas y de bosque y la densidad de vías de comunicación y viviendas), la geomorfología (composición del lecho fluvial y la densidad y origen de las acumulaciones de madera), la química del agua (pH, turbidez, conductividad, oxígeno y las concentraciones de nitrato, nitrito y ortofosfato) y las diatomeas y macroinvertebrados bentónicos a lo largo del río. La cuenca del río Teaone se encuentra muy modificada y los usos agrícolas y las plantaciones forestales ocupan más del 50 % de la superficie de la misma. El lecho del río está dominado por sedimentos finos, pero no se ha podido establecer si se trata de un impacto causado por los cambios en los usos del suelo porque en la

*cuenca coexisten fuentes naturales y antrópicas de sedimento fino. Las concentraciones de nitrato son altas en los tributarios, pero las bajas concentraciones en el cauce principal sugieren la presencia de un fuerte sumidero de nitrato. Por el contrario, las concentraciones de fósforo son bajas en los tributarios y elevadas en el cauce principal, lo que sugiere que el lavado de ropa y la higiene personal en el río pueden ser fuentes importantes de fosfato. Las comunidades de macroinvertebrados y diatomeas responden de una forma similar a los cambios que ocurren en el río. Ambas comunidades mostraron una relación más fuerte con los usos del suelo en la cuenca que con la química del agua y el hábitat fluvial.*

**Palabras clave:** ríos tropicales, usos del suelo, valoración del cauce, calidad de agua, diatomeas, macroinvertebrados

## INTRODUCTION

The effects and impact of human activities on tropical rivers and streams are poorly understood because the pace of the changes occurring in tropical fluvial systems exceeds the pace of research with respect to understanding them. The responses of these systems might be unexpected because our current knowledge of stream ecology is mostly based on streams in temperate areas (Ramírez *et al.*, 2006). Threats to river and stream ecosystems in the province of Esmeraldas include overfishing, deforestation, agriculture, cattle breeding, illegal gold mining, and discharge of untreated domestic sewage. These problems seem to be exacerbated in the northern part of the province because of weak social, economic, and administrative institutions and the lack of environmental controls on most extractive and productive activities (SENAGUA, 2011). Despite the impact of these problems, lack of channelization or flood protection structures results in no alterations of upstream–downstream and channel–floodplain connectivity, and most rivers and streams can be considered free-flowing systems. However, there are several projects for the construction of weirs and dams to bring water from inland catchments to coastal urban areas and beach resorts. Construction of dams presents a major threat to fluvial systems in tropical areas worldwide, and surveys of aquatic biota in rivers prior to hydrological alterations are needed to understand the impacts of flow regulation and optimize future management of these systems (Pringle *et al.*, 2000). Although the major dam construction projects in Esmeraldas have been delayed, a critical point for data collection, which will be highly valuable for the management of these fluvial systems has been achieved.

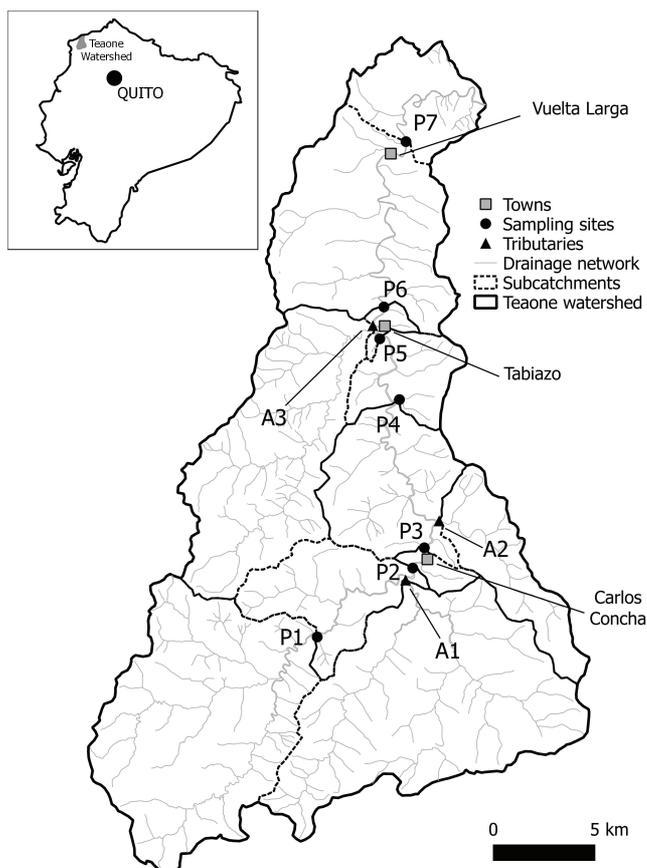
The use of biotic indexes based on diatoms and macroinvertebrates as water quality indicators is well established in developed countries (Bellinger *et al.*, 2006; Bonada *et al.*, 2006; Lavoie *et al.*, 2008; Sánchez-Montoya *et al.*, 2010; Touron-Poncet *et al.*, 2014; Buss *et al.*, 2015; Virtanen & Soininen, 2016), but there is less experience with these bioindicators in developing countries. The application of imported assessment protocols can be useful in order to quickly establish credible monitoring programs in developing countries (Buss *et al.*, 2015), but this is often done without properly testing these indices and lacks important taxonomic and ecological information (Bere, 2015). In Ecuador, most stream ecology studies have focused on the Andean region (Jacobsen *et al.*, 2014; Vimos *et al.*, 2015) and the Guayas watershed (Alvarez-Mieles *et al.*, 2013). Assessment methods have been developed for ecological status monitoring (CERA, ECOSTRIAND, and IMEERA), quality of the riparian vegetation (QRB-And), and water quality based on macroinvertebrate communities (ABI) in the Andean streams (Acosta *et al.*, 2009; Villamarín *et al.*, 2013; Ríos-Touma *et al.*, 2014). However, biotic indexes for water quality assessment are most often imported from other tropical or temperate regions (see a review of monitoring studies in the supplemental materials of Damanik-Ambarita *et al.*, 2016). An additional problem is that many studies are not published in international peer-reviewed journals and remain unknown within the general scientific community (Endara, 2012; Smith, 2015).

Previous studies concerning the rivers and streams of the province of Esmeraldas are scarce and fragmentary. There is minimal information about water quality in the Esmeraldas River Watershed. One study describes the concentra-

tions of various emergent pollutants (Voloshenko-Rossin *et al.*, 2015). The impact of agriculture and illegal gold mining on river systems in the north of the province has recently raised concern, and various studies have detected heavy metal concentrations in water, sediments, and fish meat that surpass the current legal quality thresholds (SENAGUA, 2011; Correa *et al.*, 2015). The freshwater fishes of western Ecuador, which includes the province of Esmeraldas, have been described and research is ongoing (Jiménez *et al.*, 2015). There is also some knowledge concerning the taxonomy of benthic macroinvertebrates in the province (Martínez-Sanz, 2013), and Martínez-Sanz *et al.* (2014) studied benthic macroinvertebrate distribution and applied various biotic

indices in pristine and agriculturally impacted coastal streams. Although these studies are important in advancing the knowledge of the water chemistry and biota of rivers and streams, there is still only a small amount of information concerning the interactions between the watershed and channel and between abiotic factors and biological communities along the coastal rivers of Ecuador.

In this work, we studied land use and population distribution within the watershed of the Teaone River and these factors' impacts on channel characteristics and water physicochemistry, calculated mass balances for conductivity, turbidity, nitrate, nitrite, and orthophosphate, and described the benthic diatom and macroinvertebrate communities in the river. The objectives of



**Figure 1.** Map of the study area showing the location of the study sites (P, sites located on the main channel of the Teaone River; A, sites located on the tributaries: A1, Huele river; A2, Moncaume river and A3, Tabiazo river). *Mapa del área de estudio mostrando la localización de los puntos de muestreo (P, puntos localizados en el tramo principal del río Teaone; A, puntos localizados en los tributarios: A1, río Huele; A2, río Moncaume y A3, río Tabiazo).*

this study are: 1) describe the changes in channel habitat, water chemistry, and benthic diatom and macroinvertebrate communities along the river; 2) test the use of two common biotic indices for water quality based on macroinvertebrates; and 3) to search for relationships between water quality and watershed characteristics in addition to between biological communities and environmental variables. We hypothesized that the diatom and macroinvertebrate communities would show changes related to channel, water chemistry, and watershed land uses along the river.

## METHODS

### Study area

The Teaone river is a 5<sup>th</sup> order river of 71 km length that drains a 517.2 km<sup>2</sup> watershed located in the province of Esmeraldas in the Northern Costa region of Ecuador (Fig. 1). The river sources at the Mache-Chindul mountain range within the Mache-Chindul Natural Reserve, travels southward to its confluence with the River Esmeraldas in the city of Esmeraldas, and receives three main tributaries: 1) Huele; 2) Moncaume; and 3) Tabiazo Rivers. The climate in the study area varies from humid tropical in the upper part of the watershed to dry tropical in the watershed outlet. The monthly mean air temperature is constant around 26 °C throughout the year (Fig. S1, available at <http://www.limnetica.net/en/limnetica>). The annual precipitation at Esmeraldas ranges from 296 to 904 mm (2007–2014 period) with a rainy season (winter) between January and May and a dry season (summer) between June and December, which causes two periods of high and low water levels in the Teaone river (Fig. S1) while annual precipitation at the headwaters might reach more than 3000 mm (Fig. S1; MAE, 2005). The mean annual discharge of the Teaone river at Esmeraldas is 5.99 m<sup>3</sup>/s and varied from 4.00 to 9.62 m<sup>3</sup>/s during the 2007 to 2013 period (data from INAHMI).

The watershed's geology is dominated by clay, sandstone, and conglomerates from the Daule group (Middle Miocene to Pliocene) in the middle and lower part of the watershed and clays with calcareous intrusions from the Lower

**Table 2.** Physico-chemical variables at the study sites. *Variables físico-químicas de las estaciones de muestreo.*

Site	Stream order	Length to source (km)	Elevation (m)	Drainage area (km <sup>2</sup> )	Channel width (m)	Channel slope (%)
P1	4 <sup>th</sup>	19.2	59	105.2	23.2 ± 2.8	0.080
P2	5 <sup>th</sup>	27.8	50	247.5	44.3 ± 5.5	0.080
P3	5 <sup>th</sup>	29.4	48	251.9	38.4 ± 8.2	0.080
P4	5 <sup>th</sup>	41.2	31	320.0	35.5 ± 4.6	0.225
P5	5 <sup>th</sup>	46.5	20	338.9	56.3 ± 2.6	0.108
P6	5 <sup>th</sup>	48.7	18	419.7	39.8 ± 7.2	0.108
P7	5 <sup>th</sup>	62.5	7	491.4	30.4 ± 2.8	0.108
A1	4 <sup>th</sup>	27.0 <sup>a</sup>	51	110.9	---	---
A2	3 <sup>rd</sup>	31.0 <sup>a</sup>	47	27.3	---	---
A3	4 <sup>th</sup>	47.4 <sup>a</sup>	19	77.4	---	---

<sup>a</sup>confluence with the Teaone River.

Miocene in the headwaters (DGGM, 1976). Land use in the watershed consists of mainly natural forests (36.3 %) and pastures and low intensity agriculture (61.0 %), while urban areas occupy only 0.6 % of the watershed and are located close to the confluence with the River Esmeraldas (Fig. S2, available at <http://www.limnetica.net/en/limnetica>; MAGAP, 2005). Vegetation varies from tropical evergreen forest in the headwaters to dry forest and low-land semideciduous forest in the lower part of the watershed (MAE, 2005). The density of the road network is low but increases downstream within the city of Esmeraldas (Fig. S2; IGM, 2015a). The Teaone river traverses the rural parishes of Carlos Concha, Tabiazo, and Vuelta Larga with a population of 2354, 2660, and 2997, respectively (INEC, 2010). Although the town centers of Carlos Concha, Tabiazo, and Vuelta Larga are well defined, population is widespread along the river in multiple small communities (Fig. S2; IGM, 2015a). No functional sewage treatment plant exists in Carlos Concha, Tabiazo, and Vuelta Larga, so domestic wastewater is discharged into septic tanks at Carlos Concha and Tabiazo and into the Teaone river through a collector network at Vuelta Larga. The river itself is a focal point for community socialization, and bathing, fishing, washing clothes in the river, and travelling upstream-downstream by foot and mule are common activities. Boat trans-

portation of wood and fruit along the rivers is also common. Tabiazo houses a fluvial beach with several commercial establishments that attracts about a thousand people every weekend.

We selected seven sampling points along the main channel located upstream and downstream of the main populated areas and downstream of the confluences with the three major tributaries (Fig. 1, Table 1). Site P1 was selected as an undisturbed control, sites P2 and P3 were located upstream and downstream of Carlos Concha, site P4 was located downstream of the confluence with the Moncaume river, sites P5 and P6 were located upstream and downstream of Tabiazo, and site P7 was located downstream of Vuelta Larga. Additionally, the three major tributaries (A1 at the Huele River, A2 at the Mouncaune River, and A3 at the Tabiazo River) were also sampled close to the confluence with the Teaone River to estimate the mass balances of major ions in the Teaone River.

### Physical characterization

Stream order, river length (km), and drainage area (km<sup>2</sup>) at the study sites were determined on a 1:50 000 map (IGM, 2015a). The percentage of land use in the drainage areas was determined for the four categories used by the Ministerio de Agricultura y Ganadería (MAGAP, 2005): 1) natural forest, which comprises mostly primary forest with varied degrees of intervention; 2) 100 % pasture, which comprises areas of natural and cultivated pasture; and 3) 70 % pasture; and 4) 50 % pasture, both of which consist of areas of pasture mixed with other land uses, including low intensity agriculture (mainly banana and corn), tropical arboriculture (mainly avocado and citrus), and forestry plantations of theca and balsa. The total lengths of roads, paved and unpaved tracks, and the number of houses were estimated on 1:50 000 map (IGM, 2015a) and divided by drainage area to calculate road and house density. Mean elevation (m) and channel slope (%) were estimated from a digital elevation model of 30 m resolution (IGM 2015b). The channel slope was calculated as the height difference divided by the length of the study reach. All the spatial analyses were performed with QGIS (Quantum GIS Development Group, 2012).

The established study reached 200 to 400 m in length as a function of channel width (mean active channel width multiplied by 10). The active channel width was measured with a Nikon Forestry Pro laser telemeter at 10 cross sections. The bed substrate was determined visually at regular 0.5–1.5 m intervals within each cross section. The bed substrate was classified with a caliber following the scale of Wenworth as described in Kondolf *et al.* (2003): 1) bedrock; 2) boulders ( $\text{Ø} > 250 \text{ mm}$ ); 3) cobbles ( $250 \text{ mm} > \text{Ø} > 60 \text{ mm}$ ); 4) gravel ( $60 \text{ mm} > \text{Ø} > 2 \text{ mm}$ ); and 5) sand and clay ( $\text{Ø} < 2 \text{ mm}$ ). Wood density within the active channel was estimated by counting every piece of wood with a diameter  $> 5 \text{ cm}$  in the study reach and dividing the value by the reach area. The origin of each piece of wood was also recorded: 1) artificial, if evidence of cut endings was observed or 2) natural.

### Discharge and water chemistry

Discharge was measured using the mid-section method (Whiting, 2003) with a General Oceanics current meter. Water temperature, dissolved oxygen, conductivity, turbidity, and pH were measured with a Hanna HI9146 oximeter, a Martini EC59 conductivity meter, a Hannah HI93414 turbidity meter, and Macherey-Nagel pH strips. Water samples were collected from three locations in the middle of the channel and mixed to obtain 250 ml composite samples that were stored in polyethylene bottles. Samples were frozen on arrival at the laboratory and thawed and filtered through Whatman GF/F filters before performing the analytical determinations. Samples were analyzed for nitrate content using ultraviolet absorption (Clescerl *et al.*, 1999), nitrite using the diazotization method (Nanocolor test 0-68), and dissolved phosphate using the molybdenum blue method (Nanocolor test 0-76) in a UV/VIS UNICAM spectrophotometer.

Mass balances were calculated for the Teaone River by assuming a complete mixing at the confluences of the tributaries with the main channel:

$$con = \frac{(con_t * Q_t + con_T * Q_T)}{Q_t + Q_T}$$

in which  $con_c$ ,  $con_t$ , and  $con_T$  are the concentrations downstream of the confluence, at the tributary and upstream of the confluence respectively, and  $Q_t$  and  $Q_T$  are the discharges of the tributary and the Teaone River at the confluences, respectively. These estimated concentrations were compared to the observed concentrations in the main channel in order to identify nutrient sources and sinks.

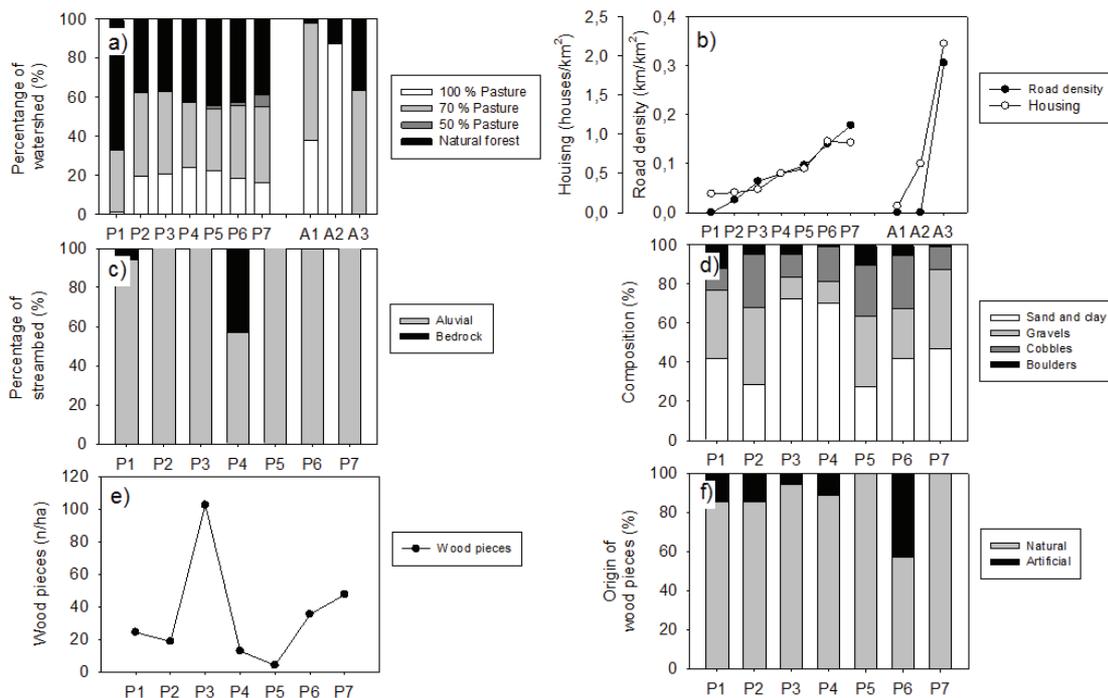
### Benthic diatoms

Three random replicates of periphyton were collected at each site. Each replicate consisted of four stones of 15 to 25 cm diameter collected from the stream. A surface of 25 cm<sup>2</sup> was scraped from each stone (a total of 100 cm<sup>2</sup> of biofilm for a replicate), and the collected biofilms were stored in opaque plastic bottles with 50 ml of

distilled water, fixed with three drops of 3 % Lugol, and kept refrigerated until identification (ACA, 2006). Samples were hand-shaken 30 times for homogenization, and a 1 ml aliquot was taken from each bottle and added to a Sedgewick-Rafter chamber for counting all cells under an OPTIKA inverted microscope at 400x magnification. Diatom identification of genus was done according to Cox (1996). Cell counts were then multiplied by sample volume (50 ml) and divided by the sampled surface (100 cm<sup>2</sup>) in order to calculate cell density in cells/cm<sup>2</sup>.

### Benthic macroinvertebrates

Benthic macroinvertebrates were sampled with a kicker net (30 x 20 cm, 300 µm mesh size) from six randomly selected locations within the channel. Only channel areas with coarse bed substrates



**Figure 2.** Indicators of watershed land use and channel characterization at the study sites (P, sites located on the main channel of the Teaone River; A, sites located on the tributaries): (a) land use, (b) housing distribution and road density, (c) percentage of alluvial sediments and bedrock in the channel, (d) streambed composition, (e) wood density at the study sites and (f) origin of wood pieces. *Indicadores de los usos del suelo en la cuenca y caracterización de los puntos de muestreo (P, puntos localizados en el tramo principal del río Teaone; A, puntos localizados en los tributarios): (a) uso del suelo, (b) distribución de viviendas y densidad de vías, (c) porcentajes de sedimentos aluviales y roca madre, (d) composición del lecho fluvial, (e) densidad de madera en los puntos de muestreo y (f) origen de las piezas de madera.*

in riffles were selected as this single habitat sampling technique is most suitable to show differences in water quality between sites (Barbour *et al.*, 1999). Samples were fixed in 70 % ethanol and kept refrigerated until sorting. In the laboratory, benthic macroinvertebrates were sorted and classified to family level in an OPTIKA magnifier at 1.5–4.5x following Domínguez & Fernández (2009) and Martínez-Sanz (2013). Macroinvertebrates were also classified into functional feeding groups following Cummins *et al.* (2005) and Ramírez & Gutierrez-Fonseca (2014). We calculated two biotic indexes for water quality: 1) Biological Monitoring Working Party (BMWP) index adapted by Zamora (2007) for Colombia from Armitage *et al.* (1983) and 2) EPT index calculated as the percentage of individuals that belong to the Ephemeroptera, Plecoptera, and Trichoptera orders (Barbour *et al.*, 1999).

### Sampling schedule

Channel characterization was done during the dry season occurring between May and August of 2015. Water, diatom, and macroinvertebrate samples were collected during a 2-day campaign in August 2015.

### Statistical analysis

We used simple linear correlations to test the relationship among land use indicators and

between water chemistry and land use. Spatial differences in the abundance of diatom genera among the study sites were tested with one-way analysis of variance (site), and multiple comparisons with the Tukey's HSD post-hoc test for log-transformed count data were done.

To search for the relationships between environmental data and biotic communities, we considered three groups of variables: 1) watershed metrics which included watershed area and land uses; 2) channel metrics which included width, slope, bed substrate composition, and wood density; and 3) water chemistry, which included pH, turbidity, oxygen concentration, and saturation, nitrate, nitrite, and phosphate. For each group, a standardized Euclidean distance matrix of the study sites was obtained following Greenacre & Primicerio (2013).

Two dissimilarity matrices were calculated with the macroinvertebrate and diatom data using the Jackard quantitative index following Oksanen *et al.* (2016). Finally, linear correlations between the standardized Euclidean distances and the Jackard indices were used to test the relationships between environmental and the biological data. Because Euclidean distances and Jaccard indices are not independent measurements, the p value of the correlations was calculated with a Mantel analysis following Oksanen *et al.* (2016). Prior to the Mantel analysis, subsets of environmental variables that maximized the correlation with the biological communities were selected with the "bioenv" function. All statistical analyses were

**Table 2.** Physico-chemical variables at the study sites. *Variables físico-químicas de las estaciones de muestreo.*

Site	Discharge (l/s)	Water temperature (°C)	pH	Oxygen (mg/l)	Oxygen saturation (%)	Conductivity (uS/cm)	Turbidity (FAU)	Nitrate (mg NO <sub>3</sub> /l)	Nitrite (µg NO <sub>2</sub> /l)	Phosphate (mg PO <sub>4</sub> /l)
P1	1244	26.3	7.0	7.29	90.7	270	0	< 0.05	< 3	0.58
P2	1835	31.2	7.0	6.27	106.0	293	4	0.18	< 3	0.74
P3	2256	31.4	7.5	7.36	106.7	296	6	0.23	5	0.73
P4	4274	28.7	7.0	8.95	106.0	309	4	0.38	9	0.79
P5	2654	29.8	7.0	6.87	91.6	250	3	< 0.05	< 3	0.87
P6	3947	29.4	7.5	9.08	109.9	412	7	< 0.05	11	0.60
P7	3013	31.9	7.5	6.78	108.0	472	8	0.28	15	0.72
A1	1345	33.5	7.0	6.00	99.5	277	9	0.73	16	0.58
A2	380	29.0	7.5	7.25	91.7	284	6	1.12	22	0.72
A3	352	29.9	8.5	8.46	109.6	900	4	< 0.05	< 3	0.89

performed in R (R Core Team, 2015) with the vegan package (Oksanen *et al.*, 2016).

## RESULTS

### Watershed and channel characterization

Two patches of natural forest, one located in the headwaters of the Teaone river within the Mache-Chindul Natural Reserve and the other located in the central part of the watershed, were present (Fig. S2a, available at <http://www.limnetica.net/en/limnetica>). These patches were surrounded by a mosaic of agricultural and silvicultural uses. Upstream of site P1, natural forest was the dominant land use (Fig. 2a). Between sites P1 and P2, watershed land uses changed sharply but were fairly uniform downstream between sites P2 and P7 with only 37 % – 42 % natural forest cover. On the contrary, pasture and agricultural land dominated in the Huele (A1) and Moncaune River watersheds (A1 and A2, respectively) in which natural forests represented only 1 % and 12 % of the watershed areas, respectively. In the third tributary, Tabiazo river (A3), the percentage of forest cover was similar (36 %) to that observed along the main channel. Houses, roads, and tracks were located in the vicinity of streams and river reaches (Fig. S2b and c, available at <http://www.limnetica.net/en/limnetica>). House and road densities followed a similar pattern and increased steadily along the watershed, but the area with the highest house and road densities was located within the Tabiazo river watershed (Fig. 2b).

All study reaches developed a layer of alluvial sediments that covered 94 % – 100 % of the streambed, except site P4 in which the percentage of alluvial cover in the streambed diminished to 55 % (Fig. 2c). Streambed sediments were mostly composed of fine sediments, gravels, and sand and clay, that represented 68 % – 88 % of the streambed with coarser particles, cobbles, and boulders, representing < 30 % of the streambed (Fig. 2d). The presence of sand and clay was highest at sites P3 and P4, in which fine sediments covered 72 % and 70 % of the streambed, respectively. Coarse substrates in all of the study reaches were also highly embedded with fine sediment.

The amount of wood along the river was low (< 25 pieces/ha) but increased up to 35 to 47 pieces/ha downstream of Tabiazo although the highest density of wood pieces (102 pieces/ha) was observed downstream of Carlos Concha at site P3 (Fig. 2e). Most of the wood pieces located in the channel (88 % of the observed wood pieces) had a natural origin. Small proportions of wood pieces of artificial origin were found between sites P1 and P4 while almost half of the wood pieces observed at site P6 (42 %) had an artificial origin (Fig. 2f).

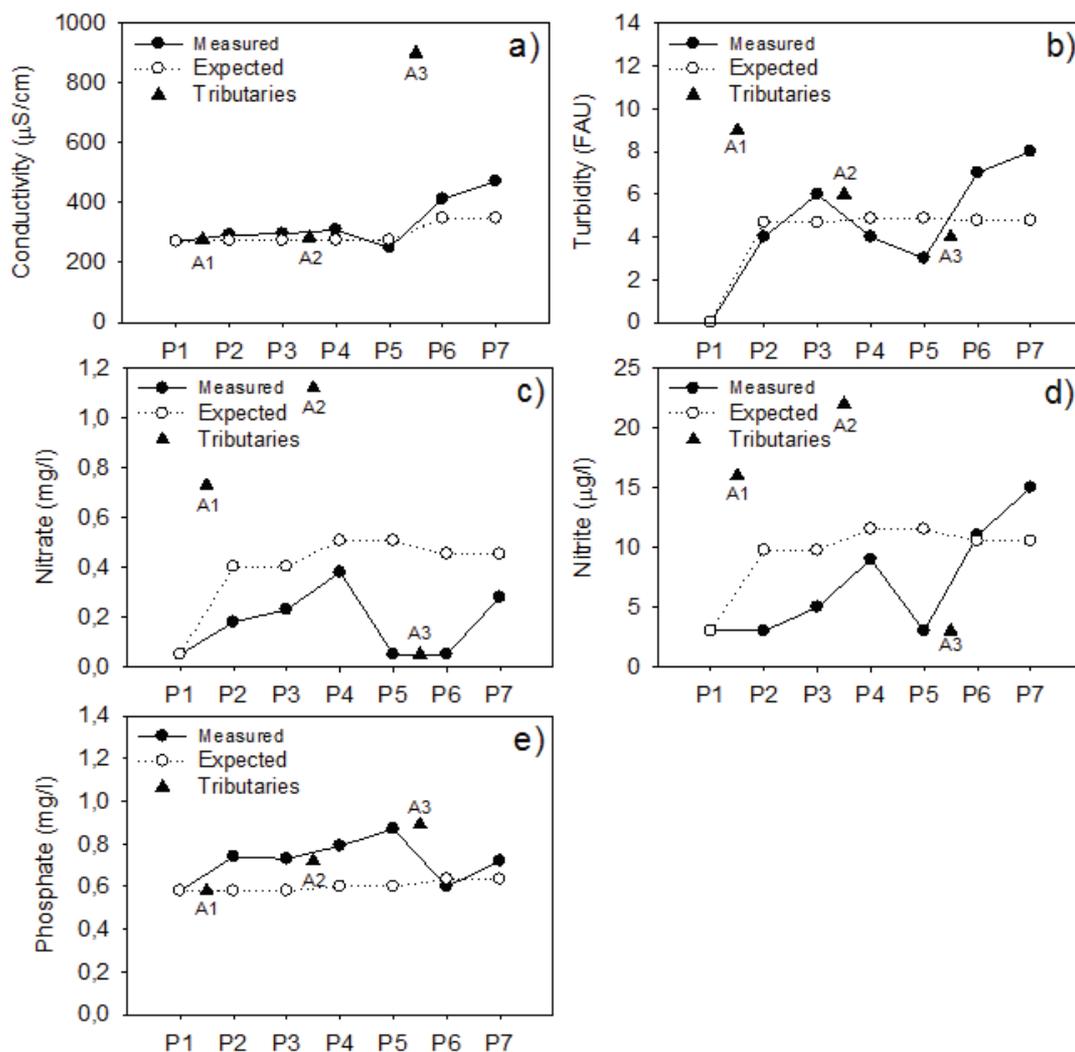
### Discharge and water chemistry

Discharge increased along the Teaone River from 1244 l/s at P1 to 3013 l/s at P7 (Table 2) but peaked at site P4. Water temperature varied between 26.3 and 33.5 °C and pH was circumneutral at all of the study sites, except in the Tabiazo Rver in which it reached 8.5 (Table 2). Water was well-oxygenated at all of the study sites with oxygen concentrations ranging from 6.00 to 9.08 mg/l and oxygen saturation ranging from 90 % to 110 % (Table 2).

Conductivity was uniform between sites P1 and P5 and in the two tributaries located within this part of the river (Huele river, A1 and Moncaune river, A2) and ranged between 250 and 309  $\mu\text{S}/\text{cm}$  (Table 2, Fig. 3a). Downstream of site P5, the Tabiazo river (with a conductivity of 900  $\mu\text{S}/\text{cm}$ ) flows into the Teaone River, and we observed a downstream conductivity increase to 412–472  $\mu\text{S}/\text{cm}$  at sites P6 and P7. Conductivity values calculated using the mass balance were similar to those measured between sites P1 and P5, but they were lower than the measured values at sites P6 and P7, which suggests that an additional source of dissolved solids was present in the lower part of the watershed (Fig. 3a).

Turbidity was < 10 FAU at all of the study sites (Table 2, Fig. 3b). Differences between observed turbidity and values calculated with the mass balance were < 3 FAU, so the mixing model provided good turbidity estimates along the river (Fig. 3b).

Nitrate and nitrite showed similar patterns along the Teaone River and in the tributaries (Table 2, Figs. 3c and d). Nitrate and nitrite

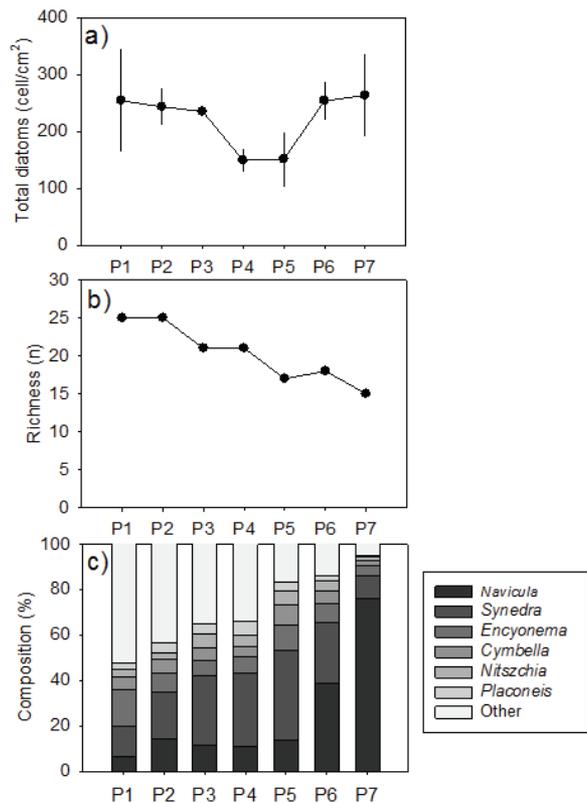


**Figure 3.** Observed physicochemical variables in the Teaone River and estimates based on the mass balances: (a) conductivity, (b) turbidity, (c) nitrate, (d) nitrite, (e) phosphate. *Variables físico-químicas observadas en el río Teaone y valores estimados según el balance de masas: (a) conductividad, (b) turbidez, (c) nitrato, (d) nitrito, (e) fosfato.*

increased from detection levels (0.05 mg/l and 3 µg/l, respectively) at sites P1 and P4 to 0.38 mg/l and 9 µg/l, respectively. Both ions showed higher concentrations (> 0.70 mg/l of nitrate and 15 µg/l of nitrite) in the Huele (A1) and Moncaune (A2) Rivers, respectively, and values below the detection level in the Tabiazo River (A3). The mass balances suggest both a nitrate sink along the river, especially between sites P5 and P6 in which concentrations dropped to detection levels, and a nitrite sink between sites P1 and P5 (Fig. 3c and d).

Phosphate concentrations were very similar at the study sites and ranged between 0.58 and 0.87 mg/l (Table 2, Fig. 3e). The phosphate mass balance predicted lower phosphate concentrations than those observed, which suggests additional phosphate sources between sites P2 and P5.

Conductivity and pH were positively correlated ( $r = 0.91$ ,  $p < 0.001$ , Table S1. available at <http://www.limnetica.net/en/limnetica>), and both correlated with road and house densities (conductivity:  $r = 0.91$ ,  $p < 0.001$  and pH:  $r = 0.81$ ,  $p < 0.01$



**Figure 4.** Benthic diatoms: (a) abundance, (b) richness and (c) relative abundance of the six most common genera. *Diatomeas bentónicas. (a) abundancia, (b) riqueza y (c) abundancia relativa de los seis géneros más abundantes.*

and conductivity:  $r = 0.95, p < 0.001$  and pH:  $r = 0.91, p < 0.001$ , respectively). Nitrate and nitrite concentrations positively correlated ( $r = 0.85, p < 0.01$ ) and were also positively correlated with the percentage of pasture (P100) in the watershed (nitrate:  $r = 0.92, p < 0.001$  and nitrite:  $r = 0.79, p < 0.01$ ) and inversely correlated with the percentage of natural forest (nitrate:  $r = -0.80, p < 0.01$  and nitrite:  $r = 0.70, p < 0.05$ ). Turbidity correlated with nitrite ( $r = 0.70, p < 0.05$ ) and inversely correlated with the percentage of forest in the watershed ( $r = -0.70, p < 0.05$ ).

**Benthic diatoms**

Total diatom density varied between 148 and 263 cells/cm<sup>2</sup> among the study sites (Fig. 4a). The lowest diatom densities were observed at sites P4

**Table 3.** Distribution of the diatom genera among the study sites (relative abundances per site: ●, > 20 %; ●, 10-20 %; ○, 5-10 %; +, < 5 %). *Distribución de los géneros de diatomeas en las estaciones de muestreo (abundancias relativas: ●, > 20 %; ●, 10-20 %; ○, 5-10 %; +, < 5 %).*

	P1	P2	P3	P4	P5	P6	P7
<i>Navicula</i>	○	●	●	●	●	●	●
<i>Synedra</i>	●	●	●	●	●	●	●
<i>Encyonema</i>	●	○	○	○	●	○	+
<i>Cymbella</i>	○	○	○	+	○	○	+
<i>Nitzschia</i>	+	+	○	+	○	+	+
<i>Placoneis</i>	+	+	+	○	+	+	+
<i>Pleurosigma</i>	+	+	+	+	+	+	+
<i>Amphora</i>	+	+	+	+	+	+	+
<i>Caloneis</i>	+	+	+	+	+	+	+
<i>Diatoma</i>	+	+	+	+	+	+	+
<i>Gomphonema</i>	+	+	+	+	+	+	+
<i>Eunotia</i>	+	+	+	+	+	+	+
<i>Pinularia</i>	+	+	+	+	+	+	+
<i>Rhopalodia</i>	○	+	+	+	+	+	
<i>Fragilaria</i>	+	+	+	+	+	+	
<i>Meridion</i>	+	+	+	+	+	+	
<i>Melosira</i>	+	+	+	+	+	+	
<i>Rhoicosphenia</i>	○	+	+	+			
<i>Aulacoseira</i>	+	+	+	+			
<i>Gomphosphenia</i>	+	+	+	+		+	
<i>Gyrosigma</i>	+	+	+	+			
<i>Epithemia</i>	+	+					
<i>Lemnicola</i>	+	+					
<i>Cocconeis</i>	+	+					
<i>Cyclotella</i>	+	+					
<b>Richness</b>	25	25	21	21	17	18	13

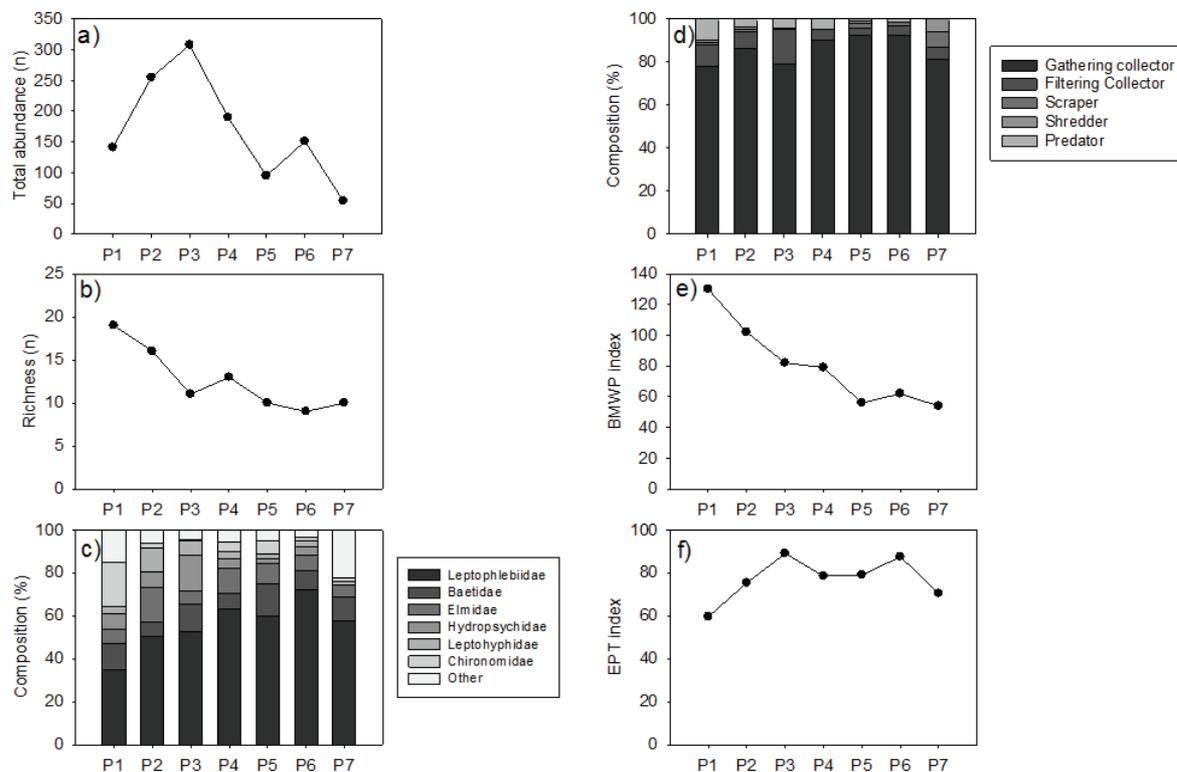
and P5, but differences were significant only between sites P4 and P7 ( $F_{6,14} = 2.8, p < 0.05$ ; Tukey HSD,  $p < 0.05$ , Table S2, available at <http://www.limnetica.net/en/limnetica>).

A total of 25 diatom genera from 19 families were collected from the Teaone River (Table S3, available at <http://www.limnetica.net/en/limnetica>). The most abundant genera were *Navicula*, *Synedra*, *Encyonema*, and *Cymbella*, which accounted for 26.6, 23.0, 8.9, and 5.3 % of the total cell count, respectively. The 25 genera were present at P1, but the richness diminished downstream, and only 15 genera were present at site P7 (Fig. 4b). A group of 13 genera (*Navicula*, *Synedra*, *Encyonema*, *Cymbella*, *Nitzschia*, *Placoneis*, *Pleurosigma*, *Amphora*, *Caloneis*, *Diatoma*, *Gomphonema*, *Eunotia*, and *Pinularia*) was present at all the study sites, and four other genera

(*Rhopalodia*, *Fragilaria*, *Meridion*, and *Melosira*) were only absent from site P7 (Table 3). Among them, the abundance of seven genera showed significant spatial differences (Table S3). *Navicula* ( $F_{6,14} = 32.0$ ,  $p < 0.001$ ; Tukey HSD,  $p < 0.05$ ) showed higher abundance in the lower reaches of the Teaone river (sites P6 and P7), *Synedra* ( $F_{6,14} = 3.0$ ,  $p < 0.05$ ) and *Nitzschia* ( $F_{6,14} = 4.6$ ,  $p < 0.01$ ) showed higher abundance in the middle reaches (sites P3 and P4), while *Pleurosigma* ( $F_{6,14} = 10.6$ ,  $p < 0.001$ ), *Diatoma* ( $F_{6,14} = 14.6$ ,  $p < 0.001$ ), *Eunotia* ( $F_{6,14} = 4.6$ ,  $p < 0.01$ ), *Caloneis* ( $F_{6,14} = 5.4$ ,  $p < 0.01$ ), and *Rhopalodia* ( $F_{5,12} = 5.4$ ,  $p < 0.01$ ) showed higher abundance in the upper reaches (sites P1 and P2). Four genera (*Rhoicosphenia*, *Aulacoseira*, *Gomphosphenia*, and *Gyrosigma*) were absent in the lower reaches of the Teaone River and four others (*Epithemia*, *Lemnicola*, *Cocconeis*, and *Cyclotella*) were only present in the upper reaches (Table 3).

Among them, *Aulacoseira* ( $F_{3,8} = 77.4$ ,  $p < 0.01$ ) and *Gyrosigma* ( $F_{3,8} = 15.2$ ,  $p < 0.01$ ) showed higher abundance in the upper reaches (sites P1 and P2) and *Gomphosphenia* ( $F_{4,10} = 6.9$ ,  $p < 0.01$ ) in the middle reaches (site P3).

In addition to changes in diatom abundance and richness, there were also changes in the composition of the diatom community (Fig. 4c). At site P1, 52 % of the diatom community consisted of a mixture of 19 genera; the most abundant genera were *Encyonema* (16 %), and *Navicula* and *Synedra* only represented 6 % and 13 % of the community, respectively. Between sites P2 and P5, the relative abundance of *Synedra*, the most abundant genera at these sites, increased from 20 % to 39 %, and the abundance of *Navicula* was low (11 %–14 %). At site P6, the abundance of *Navicula* increased to 39 % and became the most abundant genera while the abundance of the other genera diminished. At site P7, *Navicula*



**Figure 5.** Benthic macroinvertebrates: (a) abundance, (b) richness, (c) relative abundance of the five most common families, (d) trophic groups, (e) BMWP index and (f) EPT index. *Macroinvertebrados bentónicos: (a) abundancia, (b) riqueza, (c) abundancia relativa de las cinco familias más abundantes, (d) grupos tróficos, (e) índice BMWP y (f) índice EPT.*

became the dominant genera and represented 75 % of the community.

### Macroinvertebrates

The abundance of macroinvertebrates at the study sites varied between 54 and 308 individuals, peaked in the middle reaches of the Teaone river (site P3) and diminished in the upper and lower reaches (Fig. 5a). A total of 27 families from 11 orders were collected along the river (Table S4, available at <http://www.limnetica.net/en/limnetica>). The most abundant family was Leptophlebiidae that accounted for 55 % of all the collected macroinvertebrates. Other important families were Baetidae, Elmidae, Hydropsychidae, Leptohyphidae, and Chironomidae, which accounted for 10 %, 9 %, 8 %, 6 %, and 4 % of all of the collected macroinvertebrates, respectively.

The highest richness values were observed at site P1 located in the upper reaches of the Teaone River (Fig. 5b). Richness reduction from 19 to 11 families occurred between sites P1 and P3, and richness varied between nine and 13 families in the middle and lower reaches of the Teaone River, respectively (Fig. 5b). Despite changes in richness, the composition of the macroinvertebrate community has remained fairly uniform along the Teaone River (Fig. 5c). A group formed by the six most abundant families dominated the macroinvertebrate community along the river (Hydropsychidae was absent at site P7) and accounted for 78 % to 97 % of the macroinvertebrate community at all the study sites (Fig. 5c, Table 4). Another group of four families (Coenagrionidae, Corydalidae, Corbiculidae, and Naucoridae) was present along the river but in lower abundances (Table 4). A large group of families was absent from the lower reaches of the Teaone. This group included Ceratopogonidae, Psephenidae, Libellulidae, Hydrophilidae, Polycentropodidae, Aeshnidae, Tipulidae and Gomphidae, and five more families consisting of Leptoceridae, Philopotamidae, Ptilodactylidae, Corixidae and Pyralidae, which only were present at site P1. Finally, other families, including Limoniidae, Thiaridae, Psychodidae and Palaemonidae, were absent from site P1 and only appeared in the downstream reaches of the

**Table 4.** Distribution of the macroinvertebrate families among the study sites (relative abundances per site: ●, > 20 %; ●, 10-20 %; ○, 5-10 %; +, < 5 %). *Distribución de las familias de macroinvertebrados en las estaciones de muestreo (abundancias relativas: ●, > 20 %; ●, 10-20 %; ○, 5-10 %; +, < 5 %).*

	P1	P2	P3	P4	P5	P6	P7
Leptophlebiidae	●	●	●	●	●	●	●
Baetidae	●	○	●	○	●	○	●
Leptohyphidae	+	●	○	+	+	+	+
Elmidae	○	●	○	●	○	○	○
Chironomidae	●	+	+	+	○	+	+
Hydropsychidae	○	○	●	+	+	+	
Coenagrionidae	+	+	+	+			
Corydalidae	+	+	+	+	+		
Corbiculidae	+	+		+	+		+
Naucoridae	+		+			+	
Ceratopogonidae	+	+					
Psephenidae	+	+	+				
Libellulidae	+			+			
Hydrophilidae	+			+			
Polycentropodidae		+					
Aeshnidae		+					
Leptoceridae	+						
Philopotamidae	+						
Ptilodactylidae	+						
Corixidae	+						
Pyralidae	+						
Tipulidae				+			+
Limoniidae					+		
Gomphidae		+	+	+			
Thiaridae		+			+	+	+
Psychodidae							+
Palaemonidae							+
<b>Richness</b>	19	16	11	13	10	9	10

Teaone River (sites P5–7). The functional composition of the community was also uniform along the Teaone River (Fig. 5d). The gathering collectors were the most abundant functional group and represented 78 %–93 % of the community. Including gathering and filtering collectors, the collectors represented more than 85 % of the macroinvertebrate community at all the study sites. Among the other functional groups, only predators reached some significance within the community.

We calculated two biotic indexes for water quality: 1) the biological monitoring working party (BMWP) index (Fig. 5e) and 2) the Ephemeroptera, Plecoptera, and Trichoptera (EPT) index (Fig. 5f). The BMWP index showed the highest value (130) at site P1, diminished to 79 between sites P1 and P4, and varied between 54 and 62 in the lower reaches of the Teaone

**Table 5.** Spearman correlations between the Jaccard index of macroinvertebrates (families) and diatom (genera) abundance and the Euclidean distance of chemistry, channel and watershed metrics. A selection of the environmental variables that maximize the correlation and the estimated  $p$  value of the correlations based on the Mantel analysis are also shown. *Correlaciones de Spearman entre los índices de Jaccard de los macroinvertebrados (familias) y de las diatomeas (géneros) y las distancias Euclideas de las variables químicas, del cauce y de la cuenca. También se muestran la selección de variables ambientales que maximiza la correlación y el valor  $p$  estimado de las correlaciones según el análisis de Mantel.*

		Water chemistry	Channel habitat	Watershed metrics
Macroinvertebrate families	Selected variables	Oxygen saturation Nitrite	% Gravel % Cobble Wood density	Drainage area 70 % Pasture 50 % Pasture
	Mantel test	$r = 0.35, n. s.$	$r = 0.01, n. s.$	$r = 0.82, p < 0.001$
Diatom genera	Selected variables	Turbidity Nitrite Phosphate	Channel width Slope % Rock	70 % Pasture Road density
	Mantel test	$r = 0.50, p < 0.05$	$r = -0.03, n. s.$	$r = 0.95, p < 0.001$

river. On the contrary, the EPT index showed the lowest value (60) at site P1, increased downstream, and varied between 75 and 89 in the other study sites.

### Relationships between environmental data and biotic communities

The correlation between the Euclidean distances and the Jaccard indices were low for channel and water chemistry distance matrixes (Table 5). Significant strong correlations appeared between the watershed metrics and the Jaccard indices of both macroinvertebrates and diatoms, which suggests that the biological communities were responding to changes occurring on the watershed scale.

### DISCUSSION

The bed of the Teaone River is characterized by fine sediments and gravels. Fine sediment is an important component of rivers and streams, but it has negative effects on stream biota if a sediment excess or deficit occurs (Extence *et al.*, 2013; Turley *et al.*, 2016). Potential sources of fine sediment, as defined by Owens *et al.* (2005) that are present in the Teaone River include agriculture, deforestation, landslides, and bank erosion. However, no clear relationships appeared between

watershed land uses and the composition of the streambed, which varied little along the river. Bed substrate weathering also might be an important source of fine sediment of the channel in the Teaone River because the geology of the watershed is composed of soft consolidated marine sediments. Boulders and cobbles on the margins of the channel are heavily weathered and decompose easily into fragments. Although it is not possible to discern whether natural or anthropogenic sediment sources prevail, the fact that turbidity inversely correlated with the percentage of forest in the watershed suggests some impact of land use changes on the mobility of suspended solids. The amount of fine sediments often correlates inversely with the channel slope or other surrogates of sediment transport capacity (Anlauf & Moffitt, 2010; Naden *et al.*, 2016). In the middle part of the Teaone River (site P4), a geological fault crosses the river and causes an increase on the channel slope and the amount of alluvial sediment in the streambed diminishes. Thus, the large amount of fine sediment in other reaches is also a consequence of its reduced mobility.

Mass balances helped us to understand the processes occurring in the Teaone River. Nitrate sources were located in the tributaries, but nitrate concentrations were low in the main channel and indicate a nitrate sink. Tropical streams show low nitrate and high dissolved organic nitrogen

exports (Perakis & Hedin, 2002), which have been attributed to soil processes that reduce nitrate leaching (Amudson *et al.*, 2003). However, our observations suggest that a significant nitrate load reduction also occurs within the drainage network. Phosphate concentrations were lower in the tributaries than in the main channel because diffuse sources of phosphate such as clothes washing, people bathing with soap, livestock roaming, and heavy horse and mule transportation traffic were present on the banks and in the channel as has been observed in other tropical rivers and streams (Bellinger *et al.*, 2006). The mass balance also suggests that biological processes might be nitrogen limited along the Teaone River.

This work represents the first analysis of the diatom community in a river of Esmeraldas. Diatoms respond to environmental factors and anthropogenic pressures (van Dam *et al.*, 1994; Potapova & Charles, 2007). Along the Teaone River, both genera richness and community compositions changed, and some taxa seemed to reflect the effects of anthropogenic disturbances. The genera *Epithenia*, *Lemnicola*, *Coconeis*, and *Cyclotella* were present at the less impacted sites (P1 and P2), while *Navicula* and *Synedra* were more abundant at impacted sites. Diatom-based water quality indices require high expertise and good taxonomic knowledge for identification at species level (Prygiel *et al.*, 1996; Gómez & Licursi, 2001). Although we observed a clear segregation in the distribution of diatom genera along the river, tolerance ranges for diatom species' environmental conditions within the same genus are ample (van Dam *et al.*, 1994; Potapova & Charles, 2007), which makes it difficult to draw conclusions about the indicator value of higher taxonomic levels. While more detailed taxonomic and ecological information should be gathered, segregation of genera along the river suggests that it may be possible to develop a simple biotic index at the genus level as suggested by Bere (2015).

The benthic macroinvertebrate community is dominated by a few families (Leptophlebiidae, Leptohiphidae, Baetidae, Elmidae, Hydropsychidae, and Chironomidae) and is similar to the communities found in other coastal streams in

Esmeraldas (Martinez-Sanz *et al.*, 2014). The absence of Plecoptera in the region is noticeable as no Plecoptera were found in this study, and Martinez-Sanz *et al.* (2014) only found eight Plecoptera out of 4300 individuals collected. The most abundant families belong to the collector-gatherer functional feeding group; thus, it seems that the macroinvertebrate community is limited by the available food types as leaf litter and periphyton are scarce in the channel (personal observation).

The BMWP Col and the Andean Biotic Index ([ABI]; also derived from the BMWP) indices have been used previously in Ecuador and shown sensitivity to various anthropogenic disturbances (Alvarez-Mieles *et al.*, 2013; Ríos-Touma *et al.*, 2014; Damanik *et al.*, 2016). On the contrary, the validity of the EPT index as an indicator of water quality has been questioned (Martinez-Sanz *et al.*, 2014). Given that the water quality values obtained with the BMWP Col and the EPT indices are contradictory in Esmeraldas, both of them should be used cautiously. The variability of the BMWP Col index among the studied sites is caused by the absence and presence of families that represent < 20 % of macroinvertebrate abundance. Although this index is commonly used in Ecuador, it would be convenient to further test its validity as a water quality monitoring tool in Esmeraldas.

A low correlation between diatom abundances and physicochemical parameters at the time of sampling was observed. Physicochemical variables usually explain a low percentage of the distribution variability of diatoms in aquatic systems (Bere & Tundisi, 2011; Kahlert & Gottschalk, 2014). Diatom communities integrate with the water chemistry over a few weeks, and their response lags behind sudden environmental changes (Lavoie *et al.*, 2008), so community composition is less sensitive to conditions occurring at the moment of sampling. Also, the ecological response of many diatom species to environmental variables is non-linear (Potapova *et al.*, 2004), so a linear correlation is not adequate to model this relationship, but the use of non-linear approaches requires a better understanding of the taxonomy and auto-ecology of the diatoms in the region. Correlations between macroinvertebrate abundances and physicochemical parameters were also low either because these organisms also

integrate water quality over time scales that are not well represented by the conditions at the moment of sampling or because they respond to multiple stressors (Bruns, 2005; Freund & Petty, 2007) and therefore any correlation with a single parameter is low.

In underdeveloped areas, remoteness and lack of infrastructure make it very difficult to establish a recurring water monitoring program for collecting the data required in order to better understand the ecological requirements of these bioindicators. As an alternative, metrics of land use on the watershed scale have also been used to search for relationships between anthropogenic impacts and the composition of the freshwater biological communities (Cuffney *et al.*, 2000; Tanaka *et al.*, 2016). Based on distance metrics, diatoms and macroinvertebrates showed a similar response to watershed metrics, and this response was stronger than in the case of channel and water quality metrics. Thus, the use of watershed metrics to study diatom and macroinvertebrate assemblages is reasonable in areas such as Esmeraldas in which water quality data are scarce and difficult to obtain.

## CONCLUSIONS

The watershed of the Teaone River has been largely modified for cattle breeding and agricultural uses and < 50 % of the watershed is covered with natural forest. The streambed of the Teaone River is mostly composed of gravel, sand, and fine sediments, but an anthropogenic origin for these fine sediments could not be established because different sediment sources coexist within the watersheds. Mass balance studies suggest that nitrate sources are located in the tributaries, while phosphorus sources are related to human activities that occur inside or in the vicinity of the main channel. Nutrient concentrations suggest that river processes are nitrogen-limited. The benthic diatom and macroinvertebrate communities change progressively when moving downstream, and these changes are related to the indicators of watershed land use. The two-water quality biotic indexes used in this study, BMWP Col and EPT, show contradictory values, so they should be used with caution when applied to the rivers and streams of Esmeraldas.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance of N. Goenaga, J. L. Bartolome and J. R. Díez from the University of the Basque Country in designing and implementing this research project. The authors also acknowledge the donation of research equipment and the technical support provided by the University of the Basque Country through projects CUD 2013, CUD 2014 and CUD 2015. M. Barrado was supported by project CUD 2014 for an internship at the Escuela de Gestión Ambiental of the PUCESE (Esmeraldas, Ecuador). M. Guijarro and M. Ortiz were supported by project CUD 2014 for an internship at the Department of Analytical Chemistry of the University of the Basque Country (Leioa, Spain). The Instituto Geográfico Militar of Ecuador (IGM) provided 1:50000 cartography of the study area. The Instituto Nacional de Meteorología e Hidrología (INAMHI) and the Coronel Carlos Concha Torres airport provided meteorological and hydrological data of the study area. The authors also thank meteorologist E. Bastidas from the Instituto Nacional Oceanográfico de la Armada (INOCAR) for providing data from the meteorological station at Esmeraldas harbor. Jon Molinero received a PROMETEO grant from the Secretaría de Educación Superior, Ciencia, Tecnología e Innovación (SENESCYT) of Ecuador and is a member of the IBEPECOR network financed by CYTED (<http://www.ibepecor.csic.es>).

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