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**CLOGGING IN ALPINE STREAMS: AN INTEGRATED FIELD STUDY**

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**CLOGGING IN ALPINE STREAMS: AN INTEGRATED FIELD STUDY****F. Bona<sup>a\*</sup>, A. Doretto<sup>a</sup>, E. Falasco<sup>a</sup>, V. La Morgia<sup>b</sup>, E. Piano<sup>a</sup>, R. Ajassa<sup>c</sup>, S. Fenoglio<sup>d</sup>**<sup>a</sup> DBIOS, Università degli Studi di Torino, Via Accademia Albertina 13, I-10123 Torino, Italy.<sup>b</sup> ISPRA, Istituto Superiore per la Protezione e la Ricerca Ambientale, via Ca' Fornacetta 9, I-40064 Ozzano Emilia (BO), Italy<sup>c</sup> DST, Università degli Studi di Torino, Via Valperga Caluso, 35, I-10125 Torino<sup>d</sup> DISIT, Università del Piemonte Orientale, Viale Teresa Michel 25, I-15121 Alessandria, Italy.

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**ABSTRACT**

Fine sediment has recently been recognized as one of the major causes of ecological degradation affecting lotic systems. Interestingly, many studies have investigated the impact of clogging on specific compartments of the lotic system, but little or no information is available about the global impact of sedimentation. Aim of this paper is to analyse the influence of fine sedimentation on allochthonous and autochthonous energy inputs and on the structural and functional characteristics of diatom and macroinvertebrate communities. Data were collected in two alpine streams in NW Italy, one interested by the presence of an intense mining area and the other pristine, used as a control. The two rivers greatly differed in terms of suspended solids and bed load characteristics. From ten stations we analysed main physico-chemical characteristics, clogging indicators (using 60 sediment traps), CPOM and photosynthetic pigments amounts, taxonomic and functional characteristics of macroinvertebrate communities. We tested several causal models via path analysis. This approach allowed to identify the weight of different environmental variables on a set of response metrics. Functional traits seem to better reflect the integrated impact originating from quarries in the river basin than traditional community metrics like total abundance and specific richness. This outcome was enforced through the CoInertia Analysis which took in consideration also metrics based on diatom communities. Our study yielded quantitative relations between clogging induced by quarrying activities and the degree of biological impairment and suggests which metrics are more suitable to assess this specific impact.

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3 30 Key words: allochthonous inputs, autochthonous inputs, diatoms, fine sediments,  
4 31 macroinvertebrates, path analysis, rock quarries

## 32 INTRODUCTION

33 In the last decades, anthropogenic pressures have altered morphological and hydrological  
34 features of most lotic systems, so that the transport and deposition of unnatural amounts of fine  
35 sediments has become a key ecological problem (Bilotta and Brazier, 2008). Increased sediment  
36 transport and deposition are common consequences of landscape or channel alterations: agricultural  
37 (Wagenhoff *et al.*, 2011) and logging (Studinski *et al.*, 2012) practices, dams (Baker *et al.*, 2011)  
38 and roads (Angermeier *et al.*, 2004) diffusion, catchment urbanization (Naden, 2010), and mining  
39 (Griffith *et al.* 2012) are among the main causes of this phenomenon. The increase of exposed bank  
40 soils and the removal of riparian vegetation contribute to the massive transport of eroded materials  
41 into lotic systems, where they become suspended or deposited sediments (Hornung and Reynolds,  
42 1995).

43 Increased inputs of fine sediments can result in marked physical modifications of lotic  
44 environments (Owens *et al.*, 2005). Fine sediments adsorb or scatter light within the water column  
45 modifying optical features of water, increasing turbidity and reducing transparency (Davies-Colley  
46 and Smith, 2001). The transport of fine sediments also causes an augmentation of abrasion of  
47 benthic environment (Hedrick *et al.*, 2013). On the other hand, sedimentation alters surface and  
48 hyporheic characteristics of lotic substrates, filling interstitial pore spaces and even smothering the  
49 entire riverbed (Descloux *et al.*, 2010). Increased amounts of fine sediments within the substrate  
50 reduce its permeability to gases, water, nutrients and metabolites (Allan and Castillo 2007).  
51 Moreover, sedimentation diminishes mesohabitat heterogeneity, increasing similarities among pool,  
52 run, and riffle environments (Kemp *et al.*, 2011).

53 These alterations can result in significant changes in many aspects of stream ecosystems  
54 communities. In-stream primary producers seem to be extremely sensitive to sediment increase  
55 (Biggs *et al.*, 1999). With the increase of fine inorganic sediments, chlorophyll-*a* amounts usually  
56 decline and non-living component of periphyton assemblages increases (Yamada and Nakamura,  
57 2002), resulting in a general lowering of photosynthetic efficiency and algal biomass (Quinn *et al.*,  
58 1992; Izagirre *et al.*, 2009). Moreover, sedimentation increase usually produces evident taxonomic  
59 changes in periphytic communities, for example decreasing the proportion of some taxa (Peterson,  
60 1996; Wagenhoff *et al.*, 2011) and enhancing the amount of others (Schofield *et al.*, 2004).

61 Likewise, transport and accumulation of fine sediment can have dramatic impacts on the  
62 abundance and composition of benthic invertebrate assemblages (Buendia *et al.*, 2013). High

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3 63 amounts of fine sediments directly affect some taxa through the abrasion and damaging of the most  
4 64 delicate body parts, such as gills and filter-feeding apparatus (Lemly, 1982) or through the burial of  
5 65 less motile organisms (Wood *et al.*, 2005). Moreover, clogging of interstitial spaces reduces habitat  
6 66 availability for many taxa, such as most Plecoptera, Ephemeroptera and Trichoptera (Jones *et al.*,  
7 67 2012). Changes in the functional feeding group structure have been evidenced in clogged streams,  
8 68 probably caused by conspicuous alterations in energy input characteristics (Rabeni *et al.*, 2005; Bo  
9 69 *et al.*, 2007).

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14 70 As evidenced above, ecological and biological impacts of fine sediment deposition have  
15 71 received increasing attention, but unfortunately most studies have so far investigated the response of  
16 72 specific elements or compartments of the lotic biota, while few or no studies were realised with a  
17 73 more integrated and overall approach. Aim of this study is to analyse the influence of unnatural fine  
18 74 sediments deposition on the macroinvertebrate community of a mountain stream at a broad  
19 75 ecological scale, taking also in consideration its influence on autochthonous and allochthonous  
20 76 energy inputs. In particular, we tested: 1) several causal models via path analysis to identify which  
21 77 are the most suitable response metrics to measure the effect of clogging. In this way, we aimed at  
22 78 estimating the direct and indirect weights of each environmental variable on considered metrics; 2)  
23 79 according to an integrated approach, if there are alterations in community composition and  
24 80 functional traits of macroinvertebrates and diatoms in presence of clogging.

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## 34 35 82 **METHODS**

### 36 37 83 *Study area*

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40 84 This study was realized in two third order streams in the Cottian Alps (Piemonte - NW Italy), the  
41 85 Luserna and the Comba Liussa streams. Both are right-bank tributaries of the Pellice river, located  
42 86 about 7 kilometers away from each other, flowing in parallel and sharing the same climatic  
43 87 conditions. The most noticeable difference between the two streams is the evident contrast in fine  
44 88 sediment amounts. Luserna stream drains one of the most important mining areas of Western Alps:  
45 89 approximately 90 quarries lie in this area, dedicated to the extraction of the Luserna Stone, a  
46 90 leucogranitic orthogneiss (Sandrone *et al.*, 2004). The altered land use, and in particular the logging  
47 91 of trees in a large part of the catchment, together with the intense truck and heavy vehicle traffic on  
48 92 unpaved roads, causes an increase of the fine sediment amount in the Luserna. On the contrary, the  
49 93 Comba Liussa stream, selected as control lotic system, is almost unaffected by human activities,  
50 94 with woodland occupying the entire of the catchment. In order to account for the spatial variability

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3 95 in both fine sediment and biological data, seven transects were selected across the Luserna and three  
4 96 across the Comba Liusa stream (Figure 1).

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8 98 *#figure 1 approximately here #*

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#### 11 100 *Data collection*

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13 101 In each sampling transect the following metrics were evaluated: 1) physical- chemical parameters  
14 102 (water dissolved oxygen DO, pH, temperature, conductivity, turbidity) were measured with a  
15 103 multiparametric probe (Hydrolab mod. Quanta), and suspended sediments were determined by  
16 104 gravimetry following the Italian standard methods (APAT-IRSA. CNR, 2003); 2) soluble reactive  
17 105 phosphorous (SRP) nitrate and BOD<sub>5</sub> were determined with a LASA 100 spectrophotometer  
18 106 according to APAT-IRSA CNR standard methods (2003); 3) benthic diatom community, were  
19 107 sampled and treated following the standard procedure (UNI, 2005). Diatoms were identified mainly  
20 108 according to reference floras Krammer and Lange-Bertalot (1986-1991) as well as on recent  
21 109 bibliography including series of Diatoms of Europe Iconographia Diatomologica and relevant  
22 110 scientific papers. Diatom community was evaluated in terms of taxonomic composition, ecological  
23 111 guilds and life forms according to Rimet and Bouchez (2012).

24 112 Within each transect, we selected 6 sampling points where the following metrics were measured:  
25 113 deposited sediments, water velocity, autho- and allochthonous energy inputs and those related to  
26 114 macroinvertebrate assemblage. Deposited sediments were determined by means of sediment traps,  
27 115 using the method proposed by Bond (2002), that is considered particularly advantageous in  
28 116 addressing ecological issues. Each trap consisted in a plastic storage box (165 x 95 x 70 mm), with  
29 117 a piece of wire mesh (20×20 mm openings, 1.5 mm gauge wire), cut to fit just inside the box and  
30 118 placed 30 mm from the top of the trap. In field, the boxes were buried in the streambed such that  
31 119 their tops was flush with the bottom. Once the boxes were in place, the wire mesh was covered by a  
32 120 layer of coarse bed material one clast thick. In this way, fine sediments could enter into the traps,  
33 121 over which local hydraulic conditions were comparable to the whole streambed. All 60 traps were  
34 122 displaced on 28 June 2013 and removed after 17 days. The sediment collected in the traps was  
35 123 returned to the laboratory, where it was dried, sieved into 6 size fractions (thresholds: 0.105, 0.25,  
36 124 0.5, 1, 2 mm) and then weighted. For the statistical analysis only the fraction <0.105 mm was  
37 125 considered (hereinafter fine sediment). Water velocity (0.05 m from the bottom) was measured in  
38 126 each sampling point with a current meter (Mod RHCM Idromar). Inputs coming from  
39 127 photosynthetic activity were measured in triplicate by means of a fluorimetric portable probe (BBE  
40 128 BenthosTorch<sup>®</sup>) expressly designed for benthic algae. The BenthosTorch<sup>®</sup> is a Pulse-Amplitude

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3 129 Modulated (PAM) fluorometer. Light pulses of 5 different wavelengths (470, 525, 570, 590 and 610  
4 130 nm) are emitted, and the fluorescence response of the microalgal assemblage is recorded at 690 nm.  
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6 131 The fluorescence signal is proportional to the pigment density of the Cyanobacteria, Chlorophyta  
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8 132 and Bacillariophyta. As confirmed by fluorimetric measures of Chl a, diatoms were the prevailing  
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10 133 primary producers. One benthic sample was collected in each sampling point, using a Surber  
11 134 sampler (250  $\mu\text{m}$  mesh size; 0.062  $\text{m}^2$  area) to evaluate the coarse particulate organic matter  
12 135 (CPOM) and macroinvertebrates. Surber were positioned in the patches of stream bed immediately  
13 136 adjacent (laterally) to sediment traps. Collected substrate was conserved into plastic jars with 75 %  
14 137 ethanol. In the laboratory, CPOM was washed through a 250  $\mu\text{m}$  mesh sieve and subsequently  
15 138 hand-sorted. Then, the material was air dried for 24 h, oven dried (105  $^{\circ}\text{C}$ ) for 24 h, and then  
16 139 weighed with an electronic balance (accuracy 0.001 g). Macroinvertebrates were collected in the  
17 140 benthic samples used for the quantification of CPOM. In the laboratory, organisms were sorted  
18 141 from the substrate, counted and identified to genus level, except for Chironomidae, Simuliidae and  
19 142 early instars of some Trichoptera and Diptera that were identified to the family level. Each taxon  
20 143 was also assigned to a Functional Feeding Group (FFG: scrapers, shredders, collector-gatherers,  
21 144 filterers and predators) according to Merritt *et al.* (2008). Moreover, all taxa were entered into seven  
22 145 biological and five ecological groups, according to the species traits approach of Usseglio-Polatera  
23 146 *et al.* (2000).

#### 24 147 *Statistical analysis*

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26 148 In order to implement the path analysis of our data, we referred to Shipley (2004; 2009). Guidelines  
27 149 provided by this author and used for our paper can be summarized as follows: (a) express the  
28 150 hypothesized causal relationships between the variables in the form of a directed acyclic graph; (b)  
29 151 list each of the  $k$  pairs of variables ( $X_i, X_j$ ) in the graph that do not have an arrow between them; for  
30 152 each pair, list the set of other variables,  $\mathbf{Z}$ , in the graph that are direct causes of either  $X_i$  or  $X_j$ . The  
31 153 pair of variables ( $X_i, X_j$ ) along with its conditioning set  $\mathbf{Z}$  define an independence claim,  $(X_i, X_j) | \mathbf{Z}$ ,  
32 154 and the full set of the  $k$  independence claims defines the basis set  $B_U$ ; (c) for each element in this  
33 155 basis set, obtain the probability,  $p_k$ , that the pair ( $X_i, X_j$ ) is statistically independent conditional on  
34 156 the variables  $\mathbf{Z}$ ; (d) combine the  $k$  probabilities via the  $C$  statistic (Shipley 2009; eqn1 further  
35 157 below) and compare it to a chi-squared distribution with  $2k$  degrees of freedom. Reject the causal  
36 158 model if the  $C$  value is unlikely to have occurred by chance (i.e., below the chosen significance  
37 159 level).

38 160 Four macroinvertebrate-related metrics were selected (total abundance, specific richness, abundance  
39 161 of Shredders and abundance of Ephemeroptera, Plecoptera and Trichoptera ,EPT) and for each of

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3 162 them a causal model was tested based on the same structure (step (a), Figure 2). These graphs  
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5 163 expressed the causal hypothesis that the macroinvertebrate functional metrics are due to three  
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7 164 categories of environmental variables: 1) *quarry pressure*, evaluated through the categorical  
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9 165 variable presence of quarries (QP) in the river basin and the quantitative variable bed load (BL); 2)  
10 166 *hydraulic conditions* measured through water velocity (V); 3) *energetic inputs*, both allogenic  
11 167 (CPOM) and autochthonous (Chl a). Causal relationships between environmental variables were also  
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13 168 considered in the hypothetical model.  
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17 170 #figure 2 approximately here#  
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21 172 The causal structures expressed by the graph were translated into a set of direct separation (d-  
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23 173 separation) statements (step (b), Table I) which predict the conditional probabilistic independences  
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25 174 that must be true if the causal model is correct, as proposed by Shipley (1999, 2000) and applied in  
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27 175 Bona *et al.* (2011). To test each predicted conditional independence (step (c)), we then adopted  
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29 176 generalized mixed modeling approach (as in Shipley 2009), which allowed us to deal with the  
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31 177 different statistical distributions of the variables involved in the models, and to account for the non-  
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33 178 independence of the data collected within each sampling transect. For each element in this basis set,  
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35 179 the probability that the pair  $(X_i, X_j)$  is statistically independent conditional on the variables  $\mathbf{Z}$  is  
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37 180 given by the exact  $p$ -value for the regression coefficient of the variable whose partial slope should  
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39 181 be zero (as reported in Table I).  
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47 185 Starting with this model, to identify a causal graph including only significant path coefficients we  
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49 186 tested all sub-models by gradually reducing the number of paths and variables (Thomas *et al.*,  
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51 187 2007). The overall fit of the final model was tested via the C statistic (step (d)), which follows a chi-  
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53 188 square distribution with degrees of freedom equal to  $2k$  (Shipley, 1999; 2009):  
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$$190 \quad C = -2 \sum_{i=1}^k \ln(p_i) \quad (\text{eqn1})$$



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3 191 where  $k$  is the number of d-separation statements in the basis set and  $p_i$  is the null probability of the  
4 192 independence test associated with the  $i$ -th independence claim. Mixed models were fitted via the  
5 193 glmmADMB package (Skaug *et al.*, 2013; Fournier *et al.*, 2012) in R 3.0.1 (R Core Team, 2013).  
6  
7 194 To check the consistency of the macroinvertebrate response with that of the diatom community, we  
8 195 related the two community data (diatoms and macroinvertebrates) in a symmetric way i.e. neither  
9 196 set takes the response or predictor role by means of the co-inertia analysis (CoIA). CoIA is a  
10 197 general and flexible way to couple two or more data sets, which are ordered along the axes of co-  
11 198 inertia analysis to maximize covariance (Doledec and Chessel, 1994; Dray *et al.*, 2003). The  
12 199 correlation between the two communities was evaluated with the RV coefficient, which is a  
13 200 multivariate generalization of the Pearson correlation coefficient. This coefficient varies between 0  
14 201 and 1: the closer the coefficient to 1, the stronger the correlation between the datasets. The method  
15 202 finds a common space into which the objects and variables of the data sets can be projected and  
16 203 compared (the distance between sites measures their similarity). For each community we selected  
17 204 the most abundant taxa in the entire dataset (>2% for diatoms and >0.5% for macroinvertebrates).  
18 205 We performed a Monte-Carlo permutation test (9999 permutations in our analysis) to assess the  
19 206 significance of the co-structure of the data tables.  
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## 31 208 RESULTS

### 32 209 33 210 *Water quality, bed load and energy inputs*

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35 212 Data for the water quality, bed load and energy inputs of the two streams are summarised in Table  
36 213 II.

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38 215 *#Table II approximately here#*  
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40 217 Both streams can be classified as oligotrophic and oligosaprobious according to common water  
41 218 quality classifications (e.g. Hofmann, 1994; Van Dam *et al.*, 1994). Nutrient concentrations are  
42 219 similar in the two rivers with the exception of the SRP, which is higher in the Luserna. However,  
43 220 this difference can be attributed to the higher concentration of total suspended sediments (TSS) in  
44 221 the Luserna river which may alter the phosphorous concentration. Indeed, differences in TSS and  
45 222 bed load between the two rivers are highly significant (Mann-Whitney U-test:  $p < 0.001$  for both  
46 223 parameters).  
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3 224 The allochthonous input, mainly represented by terrestrial CPOM, resulted significantly higher in  
4 225 the Comba Liussa than in the Luserna stream (Mann-Whitney U-test:  $p < 0.0001$ ) Also the  
5 226 comparison in terms of primary production highlights significant differences (Mann-Whitney U-  
6 227 test:  $p < 0.0001$  for diatoms and for total chlorophyll *a*).  
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### 12 230 *Path Analysis*

13 231 The path analysis procedure allowed us to define four final models explaining the relationships  
14 232 between environmental variables and macroinvertebrate metrics (Figure 3). In all cases, their fits  
15 233 were satisfactory (according to the values of the C statistic, whose *p* value was  $> 0.05$  for all  
16 234 models) and all the path coefficients reported in Figure 3 were significant. In all models there is a  
17 235 common structure describing the mutual relationships among environmental variables. In particular  
18 236 the QP showed an effect on BL, Chl *a* and CPOM. Any direct effect of the BL on the energetic  
19 237 input was proved, while it negatively influenced the total abundance, the abundance of shredders  
20 238 and the EPT. Also the QP showed a negative effect on richness, abundance of shredders and  
21 239 abundance of EPT. Considering the energetic inputs, the CPOM resulted positively significant for  
22 240 richness and total abundance, while Chl *a* was not included in any final model.

23 241 Water velocity resulted a positive factor for Specific Richness, abundance of shredders and EPT  
24 242 abundance.  
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27 244 *#figure 3 approximately here #*  
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### 30 246 *Co-Inertia*

31 247 Results obtained from CoIA are shown in Figure 4. The RV coefficient is 0.71, confirming a high  
32 248 correlation in the responses of the two biotic communities and the Monte-Carlo permutation test  
33 249 resulted highly significant ( $p < 0.005$ ).  
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36 251 *#figure 4 approximately here#*  
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39 253 The graph reported in Figure 4a shows a clear differentiation of the two rivers. Sampling transects  
40 254 in the control river resulted to be highly differentiated within each other, while in the impacted  
41 255 river, transects are very similar, in particular those closer to the quarries. Only the sampling transect  
42 256 L1, located downstream of the entry of the left tributary, showed a clear differentiation. The  
43 257 impacted river mainly characterized by stress-tolerant taxa with respect of both diatom (Figure 4b)  
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3 258 and macroinvertebrate communities (Figure 4c). Diatom communities in both streams are  
4 259 characterized by sensitive and pioneer taxa with the dominance of *Achnantheidium*, with some  
5 260 noticeable differences within this genus. In the Luserna stream *Achnantheidium minutissimum* is the  
6 261 dominant taxon, belonging to the low profile ecological guild and characterized by the adnate life  
7 262 form, which give it a high tolerance to physical disturbance. In the control stream, *A. minutissimum*  
8 263 is partly substituted by *Achnantheidium pyrenaicum* and *Achnantheidium lineare*, probably less  
9 264 tolerant to physical disturbance. Moreover it is here noticeable the presence of *Gomphonema*  
10 265 *pumilum* var. *elegans* (stalked and typical of mature biofilm) and of *Cocconeis lineata*, which is  
11 266 epiphytic thus strictly related to the presence of aquatic macrophytes.

12 267 Considering macroinvertebrates, we collected a total of 15,068 specimens belonging to 53 taxa.  
13 268 Comparing the presence of biological and ecological trait groups in the two rivers we noticed  
14 269 interesting differences. Taxa belonging to the 'f' biological trait group, i.e. medium-sized,  
15 270 monovoltine organisms, mainly crawlers and shredders with aquatic respiration were abundant in  
16 271 the control river while resulted nearby absent in the Luserna. Macroinvertebrates in the impacted  
17 272 river are generally dipterans, belonging to the 'e' biological trait group, i.e. small and medium-sized  
18 273 organisms, short living and plurivoltine, with various feeding habits. Regarding ecological trait  
19 274 classification, in the control river the macrobenthic community was dominated by organisms  
20 275 belonging to the 'C' group, i.e. living in rhithronic environments, oligo to  $\beta$ -mesosaprobic, mainly  
21 276 inhabiting mineral substrates such as cobble and sand with important presence of groups 'A' and 'B'  
22 277 (both generally rheophilous, orophilous and oligosaprobic, inhabiting coarse substrata). These  
23 278 groups are poorly represented in the Luserna river, where in general the most important is the 'F'  
24 279 ecological group, i.e. ecologically adaptable organisms living in semi-lentic habitats, eurythermic,  
25 280 mesosaprobic.

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## 27 282 **DISCUSSION AND CONCLUSIONS**

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29 284 Our study focused on the impacts of quarry activities on some functional and structural parameters  
30 285 of an Alpine stream biota. The occurrence of mining areas in close proximity to streams can impose  
31 286 several distinct stresses at different scales. At reach scale, main alterations refer to water quality  
32 287 (pH, turbidity, fine sediment deposition, concentrations of dissolved metals, deposition of hydrous  
33 288 metal oxides; Niyogi *et al.*, 2002). In this case, the main impact is the increase of fine sediments in  
34 289 the basin. Indeed, 'Luserna gneiss' is a natural stone, and for its extraction only mechanical devices  
35 290 are used. At catchment scale, mining activities imply the removal of riparian vegetation,  
36 291 construction of gravel roads, bank alteration with a significant increase of erosive processes. For

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3 292 such complex impacts, an integrated field-base approach is required. Interestingly, while  
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5 293 experimental work is useful to isolate stressor effects or to analyse selected biological targets (Bo *et*  
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7 294 *al.*, 2007; Connolly and Pearson, 2007), they are almost useless to analyse responses from an  
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9 295 ecosystem point of view (e.g. considering altogether in- and out-stream energetic inputs, benthic  
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11 296 algae, and invertebrate communities).

12 297 With this regard, we expressly adopted two statistical tools which are both able to integrate  
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14 298 responses from multiple factors.

15 299 The path analysis approach highlighted a negative effect of both quarry pressure and bed load  
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17 300 amounts on the traits considered (EPT and Shredders abundance), while the total abundance and the  
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19 301 specific richness are influenced only by one of these factors. Functional traits seem then to better  
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21 302 reflect the integrate impact originating from the presence of quarries in the river basin than  
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23 303 community structure metrics like total abundance and specific richness. From a practical point of  
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25 304 view, this may represent an applied outcome in the environmental assessments of the anthropic  
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27 305 pressure generated by excessive fine sediment concentration. Future management programs should  
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29 306 take into consideration macroinvertebrate functional traits in spite of common community metrics  
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31 307 to detect impacts caused by the bed load.

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33 308 As long as energetic inputs are considered, the quarry pressure in the river basin resulted as the key  
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35 309 factor determining their patterns. We can then hypothesize the landscape morphological alteration  
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37 310 caused by the presence of quarries to be the main responsible of a reduction of energetic inputs. For  
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39 311 instance, the elevated amounts of fine sediments in the streambed combined with the higher  
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41 312 turbidity in the Luserna, resulted in a lower aquatic primary production, due to the reduced survival  
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43 313 possibilities for benthic photoautotrophic organisms, and in a lower CPOM availability, due to the  
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45 314 increased uniformity of the substrate and the consequent reduction of the coarse detritus retention  
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47 315 capacity.

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49 316 The differences between the two rivers also emerged from the Co-Inertia analysis, confirming the  
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51 317 negative effect of the quarries presence in the Luserna catchment. Indeed, the two rivers are clearly  
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53 318 separated, with Luserna transects much more similar between each other than the Comba Liusa  
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55 319 ones. Thus, we can suppose that the anthropic land use and the bed load are responsible of a  
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57 320 homogenization of both the macroinvertebrate and the diatom communities.

58  
59 321 Diatom communities in both streams reflect a good water quality, being composed mainly of  $\beta$ -  
60  
322 mesosaprobous *taxa* typical of mountain streams. In general, the biovolumes of the diatom  
323  
324 communities colonizing the Luserna stretches are lower than those detected in the control stream.  
325  
326 Luserna is characterized by small sized *taxa* with high reproduction rates, generally low profile and  
327  
328 pioneer (Rimet and Bouchez, 2012). Moreover, it is possible to notice a good percentage of motile  
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3 326 taxa (belonging the genera *Nitzschia* and *Navicula*) generally considered siltation tolerant (Bahls,  
4 327 1993). On the contrary, the Comba Liussa hosts a good proportion of stalked taxa belonging to the  
5 328 genus *Gomphonema*, as well as epiphytic taxa such as *Cocconeis*, denoting a certain streambed  
6 329 stability and periphyton maturity. Considering these results, we can conclude that ecological guilds  
7 330 and life forms represent good metrics for the assessment of the unnatural fine sediment impact in  
8 331 mountain rivers, more than commonly used trophic indices.

9 332 Regarding the macroinvertebrate communities, benthic coenoses of the control river were composed  
10 333 by taxa typical of mountain environments, characterized by coarse substrate, good intake of  
11 334 particulate organic terrestrial material: here organisms were generally characterized by medium to  
12 335 large size, long life cycles, shredder or scraper feeding habits. On the contrary, the anthropic  
13 336 pressure on the Luserna, and particularly the unnatural presence of high amounts of fine sediment,  
14 337 completely altered the macrobenthic community: here the dominant organisms belonged to  
15 338 ubiquitous and euriecious taxa, with rapid life cycles, small size, which prefer fine or muddy  
16 339 substrates and that are generalist collectors of fine organic particles. The situation improved in the  
17 340 most downstream station, where in the community some stenoecious elements reappeared.

18 341 Indeed, considering that the responses of the two benthic communities to the bed load are highly  
19 342 correlated, it is plausible that the excessive quantity of fine sediment negatively affects not only  
20 343 macroinvertebrates but also diatoms. Further investigation on the effects of the bed load on the  
21 344 diatom community is highly recommended.

22 345 In conclusion, the modelling procedure based on path analysis confirms to be an effective tool in  
23 346 the field of water ecology, particularly when a set of multiple variables must be considered to  
24 347 explain the response of all biological components to an environmental disturbance, as already  
25 348 pointed out by Bona *et al.* (2011).

26 349 Our final suggestion is that resource managers should focus particular attention on reducing erosion  
27 350 in stream catchments and sediment load into streams, because of the multiple and severe effects on  
28 351 the aquatic biota.

29 352

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Table I. The basis set ( $B_U$ ) of d-separation statements implied by our path diagram. As we can see, we have just 3 d-separation statements, since we have no causal parents for the pair ( $V, QT$ ) and their conditioning set  $Z$  is thus empty

d-separation statements	Mixed model	Variable whose partial regression slope should be zero
$(V, Chl\ a) \{QT, BL\}$	$Chl\ a \sim V + QT + BL + (I trsect)$	$V$
$(V, CPOM) \{QT, BL\}$	$CPOM \sim V + QT + BL + (I trsect)$	$V$
$(Chl\ a, CPOM) \{QT, BL\}$	$Chl\ a \sim CPOM + QT + BL + (I trsect)$	$CPOM$

Table II. Summary of water quality data (transects), bed load and energy inputs (sampling points) of the two streams

	Luserna			Comba Liussa		
	N	Mean	SD	N	Mean	SD
Temperature ( $^{\circ}C$ )	7	13.0	0.458	3	14.5	0.072
pH	7	7.1	0.136	3	7.2	0.047
Conductivity ( $mS\ cm^{-1}$ )	7	0.037	0.003	3	0.078	0.000
N-NO <sub>3</sub> ( $mg\ L^{-1}$ )	7	0.819	0.127	3	0.733	0.037
SRP ( $\mu g\ L^{-1}$ )	7	12.8	11.5	3	4.7	2.3
BOD ( $mg\ L^{-1}$ )	7	0.61	0.442	3	1.38	0.366
TSS ( $mg\ L^{-1}$ )	7	147	134	3	0.57	0.120
Turbidity (NTU)	7	37.4	15.0	3	6.70	5.79
Bed Load (g)	42	28.6	19.8	18	3.02	0.565
Chl a total ( $\mu g\ cm^{-2}$ )	42	0.726	0.560	18	3.73	2.08
Chl a diatoms ( $\mu g\ cm^{-2}$ )	42	0.449	0.366	18	3.03	1.95
CPOM (g)	42	0.196	0.286	18	0.416	0.242

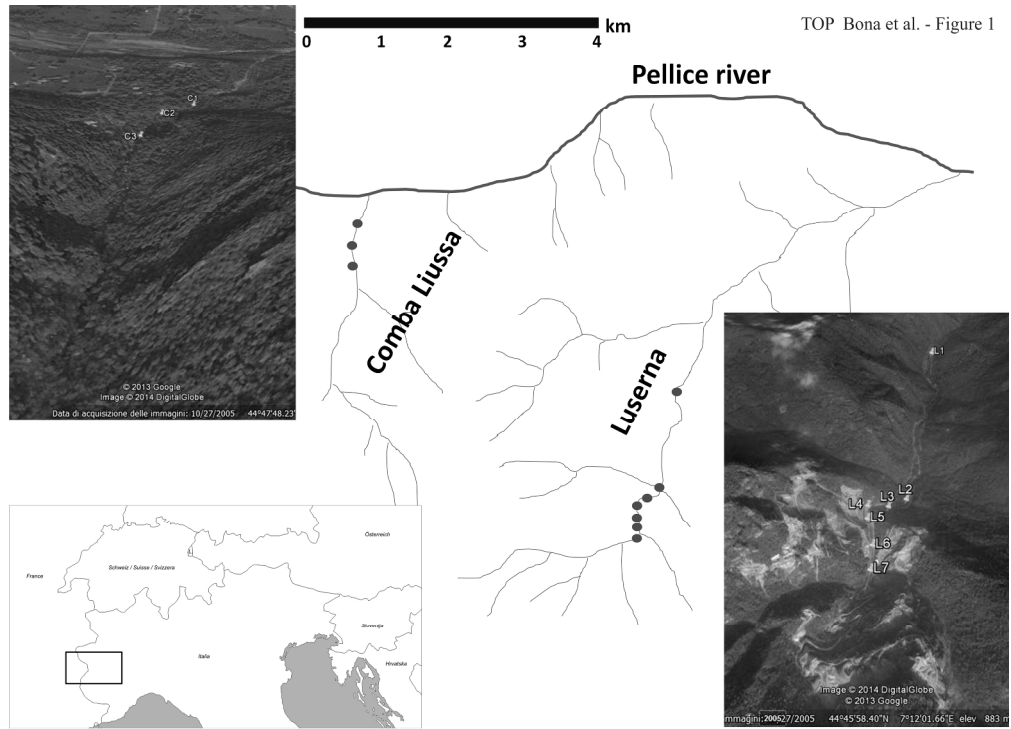


Figure 1. Study area. Google heart images evidence the different anthropic impact in the examined lotic systems: Luserna stream (with important quarry activities) and Comba Liussa stream (utilised as control)  
127x91mm (600 x 600 DPI)

Review

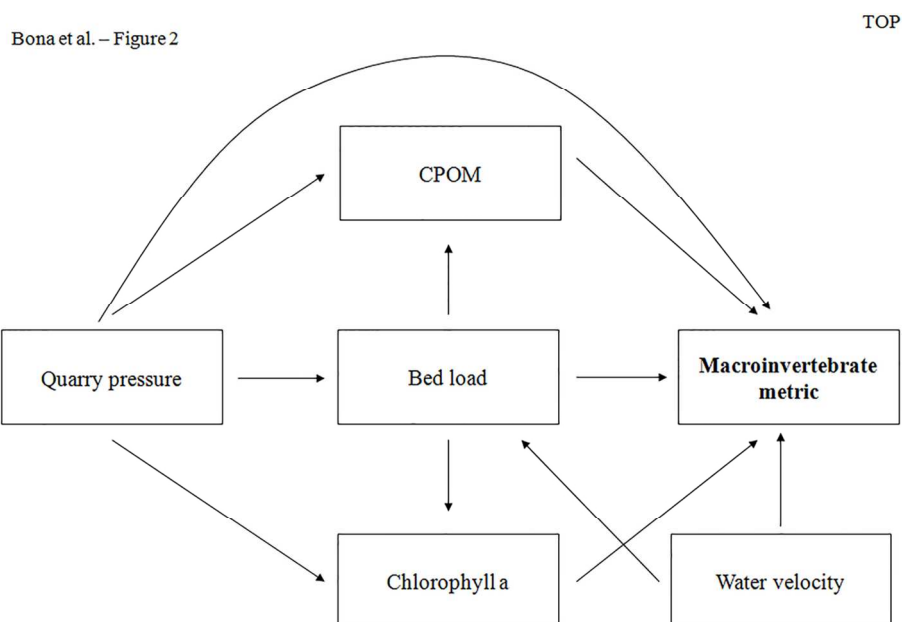


Figure 2. Graph expressing the hypothesized causal model for selected macroinvertebrate metrics. According to this causal model, the water velocity (V) and the quarry pressure (QP) are exogenous variables (their causal parents are not explicitly modeled). Water velocity (V) exerts both a direct and indirect influence, mediated by the bed load (BL), on the macroinvertebrate metrics. Besides affecting macroinvertebrate metrics directly, QP also exerts an indirect influence mediated by the BL, the CPOM and the Chl a. Indeed, the latter variables are endogenous variables (i.e. variables caused by some other variables in the model), affected by V and/or by QP

60x45mm (600 x 600 DPI)

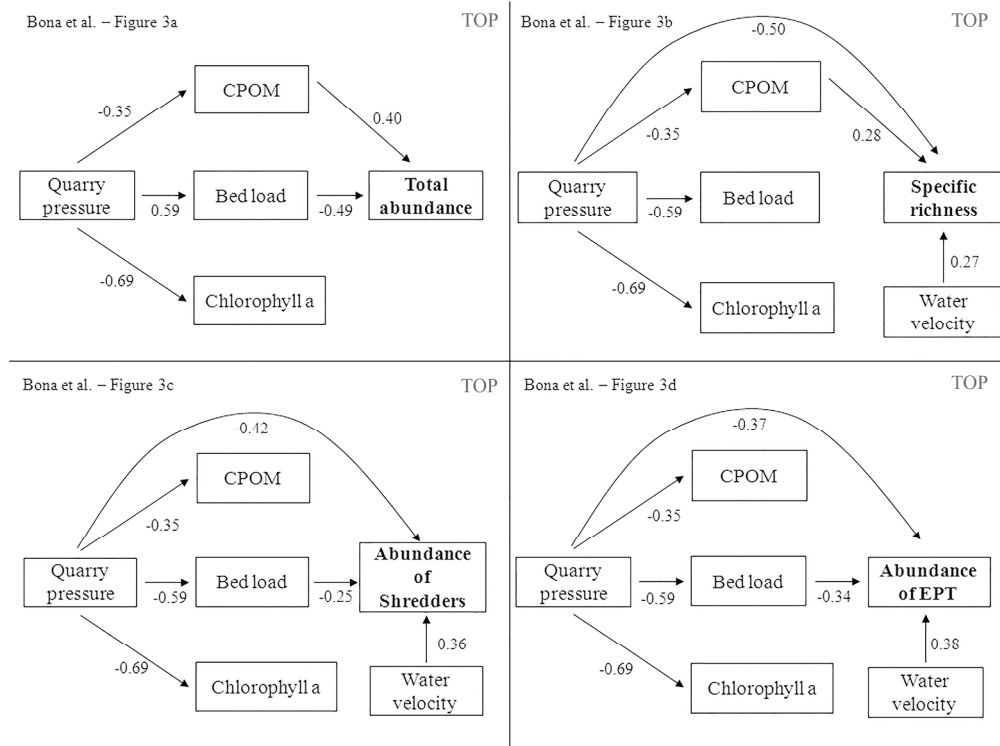


Figure 3. Causal graphs supported by the observed data, with standardized path coefficients (P) for: a) Total abundance: the quarry pressure QP has only an indirect effect on the dependent variable, mediated by the bed load and the CPOM (C = 0.47, df =8); b) Specific richness: QP has both a direct and indirect effect mediated by the CPOM on the dependent variable (C = 0.53, df =16); c) Abundance of Shredders: QP has both a direct and an indirect effect mediated by the bed load on the dependent variable (C = 0.26, df 16); d) Abundance of EPT: QP has both a direct and an indirect effect on the dependent variable mediated by the bed load (C = 0.40, df =16)

120x90mm (600 x 600 DPI)

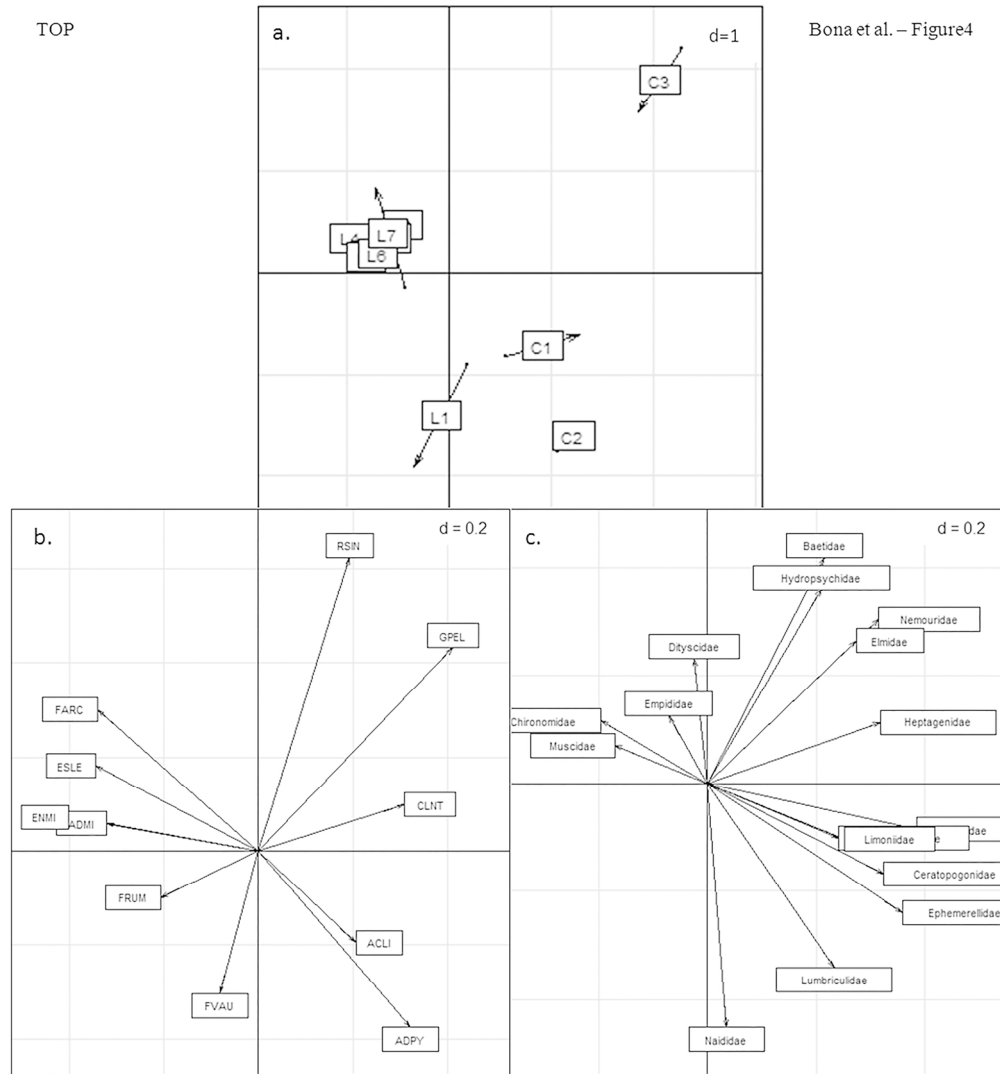


Figure 4. Results of the Co-Inertia analysis with the distribution along of the first two axes of a) sampling transects; b) diatom selected taxa; c) macroinvertebrate selected taxa. ACLI: Achnanthydium lineare, ADMI: Achnanthydium minutissimum, ADPY: Achnanthydium pyrenaicum, CLNT: Cocconeis lineata, ENMI: Encyonema minutum, ESLE: Encyonema silesiacum, FARC: Fragilaria arcus, FRUM: Fragilaria rumpens, FVAU: Fragilaria vaucheriae, GPEL: Gomphonema pumilum var. elegans, RSIN: Reimeria sinuata  
171x184mm (300 x 300 DPI)