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**How to assess the impact of fine sediments on the macroinvertebrate communities of alpine streams? A selection of the best metrics**

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(Article begins on next page)

1 **How to assess the impact of fine sediments on the macroinvertebrate communities of alpine**  
2 **streams? A selection of the best metrics**

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13

14 **Highlights**

- 15 • Siltation results in alterations of the aquatic habitat and biological communities  
16 • To face these sediment-associated impacts specific biomonitoring tools are needed  
17 • Macroinvertebrates are good indicators of physical alterations, including siltation  
18 • We compared the response of several invertebrate community metrics to fine sediment  
19 • We aggregated the most sensitive ones into a stressor-specific multimetric index  
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29 **Abstract**

30 Excessive fine sediment accumulation (i.e., siltation) in streams and rivers originates from several  
31 human activities and globally results in heavy alterations of aquatic habitats and biological  
32 communities. In this study the correlation between fine sediment and several benthic invertebrate  
33 community metrics was tested through a manipulative approach in alpine streams, where siltation  
34 mainly results as a physical alteration (i.e., the clogging of substrate interstices) without the  
35 influence of co-occurring confounding factors. We selected 12 candidate metrics, belonging to three  
36 different categories: compositional, structural and functional. We first carried out a manipulative  
37 experiment where artificial substrates were used to provide standardized conditions of siltation. All  
38 candidate metrics were calculated for each artificial substrate and the selection of the best  
39 combination of metrics was statistically performed with an information-theoretic approach. All  
40 candidate metrics were calculated both at family level and also at a mixed level (family and genus)  
41 in order to account for the systematic resolution. Then, data from a field study on alpine streams  
42 affected by mining activities were used as independent dataset for testing the performance of the  
43 selected metrics. We found that the total taxa richness, the EPT (Ephemeroptera, Plecoptera and  
44 Trichoptera) richness and the abundance of benthic invertebrates associated to rheophilous  
45 conditions and coarse mineral substrates were the most sensitive metrics. When these metrics were  
46 aggregated into a multimetric index in the validation dataset, we observed high and significant  
47 correlations between index values and the quantity of fine sediment for both taxonomic levels,  
48 especially for the mixed level . The findings of this study provide useful tools for biomonitoring the  
49 effects of fine sediment in low order, mountainous streams and contribute to improve our diagnostic  
50 ability on stressor-specific alterations.

51

52 **Key-words:** siltation, benthic invertebrates, multimetric index, ecological assessment, taxonomic  
53 resolution, rivers

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## 61        **1. Introduction**

62        The riverbed colmation by fine sediment is one of the world-wide causes of alteration in streams  
63        and rivers (Owens et al., 2005; Wilkes et al., 2017). Excessive fine sediment inputs can originate  
64        from several anthropogenic sources, including agriculture (Benoy et al., 2012; Burdon et al., 2013),  
65        deforestation and clear-cut practices (Couceiro et al., 2010), road construction (Kaller and Hartman,  
66        2004; Cocchiglia et al., 2012), mining activities (Smolders et al., 2003; Pond et al., 2008), damming  
67        and river flow regulation (Wood and Armitage, 1999; Crosa et al., 2010).

68        Fine sediment in running waters can act as a disturbance not only as suspended solids but also as  
69        settled material and negative consequences of sedimentation on all the components of lotic  
70        ecosystems have been well documented, regardless of the source (Wood and Armitage, 1997;  
71        Henley et al., 2000; Jones et al., 2012). Firstly, the deposition of large amount of fine inorganic  
72        material on the riverbed causes the loss of substratum heterogeneity and micro-habitats (i.e.,  
73        spawning habitat for fish and interstitial spaces for invertebrates). A layer of fine sediment also  
74        hinders the oxygen and chemical exchanges between the bottom and the water column, producing  
75        anoxic or adverse conditions for benthic organisms (i.e., invertebrates and algae). In addition, fine  
76        sediment can cause direct damage to the aquatic organisms, clogging their respiration or feeding  
77        anatomical structures, producing an abrasive stress and dislodging them from the substrate (Bilotta  
78        and Brazier, 2008).

79        In the last decades, benthic invertebrates have been increasingly used in biomonitoring programs  
80        focused on physical alterations in streams, including fine sedimentation (Mebane, 2001; Cover et  
81        al., 2008; Kefford et al., 2010). Indeed, macroinvertebrates have a historical tradition as bio-  
82        indicators: their use to assess the ecological status of lotic ecosystems started at the beginning of the  
83        20<sup>th</sup> century (Rosenberg and Resh, 1993; Bonada et al., 2006), so that they are currently the most  
84        used group in freshwater biomonitoring around the world (Buss et al., 2015).

85        Recently, interesting stressor-specific biotic indices have been developed describing the structure of  
86        macroinvertebrates biological assemblages based on known or hypothesized tolerances of taxa to  
87        fine sedimentation (Table 1). For example, the PSI (Proportion of Sediment-sensitive Index),  
88        developed in the UK, scores each benthic invertebrate taxon according to its sensitivity or tolerance  
89        to fine sediment (Extence et al., 2013). The final index value is then calculated as the proportion of  
90        the most sensitive taxa in the sample (i.e., sampling station), adjusted to their range of abundance.  
91        The index ranges between 0 and 100, and based on its value five different quality classes are set,  
92        varying from completely un-affected by siltation (80-100) to heavy silted (0-20). Similar attempts  
93        have been made by Relyea et al. (2000; 2012) and Hubler et al. (2016) in USA. A different  
94        approach is proposed by Murphy et al. (2015), who assigned the scores to macroinvertebrate taxa  
95        through a multivariate statistical approach, thus overcoming the expert judgment.

96        Despite their strong biological and statistical bases, these indices present some critical issues. First,  
97        they are based on taxonomic identity, thus spatially dependent to the geographical areas where they  
98        have been developed. However, the employment of selected community metrics rather than taxon-  
99        identity scores may be a good solution to overcome the bio-geographical limits. This aspect  
100        introduces a fundamental question: which are the best macroinvertebrate community metrics related  
101        to fine sediment conditions? Literature data show that fine sediment affects several characteristics  
102        of macroinvertebrate communities, such as diversity, total abundance, relative abundance of  
103        functional groups and behavioral patterns (i.e., drift) (Angradi, 1999; Longing et al., 2010;  
104        Descloux et al., 2014). For example, reductions in the taxa richness and abundance of

105 macroinvertebrates have been typically observed when high levels of siltation occur in the substrate  
 106 or stream-section, especially among the most sensitive taxa (i.e., EPT – Ephemeroptera, Plecoptera  
 107 and Trichoptera) (Sutherland et al., 2012; Mathers and Wood, 2016). Conversely, some taxa (i.e.,  
 108 Chironomidae, Oligochaeta) could benefit from the environmental conditions provided by fine  
 109 sediment (Ciesielka, and Bailey, 2001; Cover et al., 2008). Also, trait-based classifications of  
 110 macroinvertebrate taxa have been recently used to assess the response of macroinvertebrate  
 111 assemblages to fine sediment conditions, with noteworthy results (Pollard and Yuan, 2010; Conroy  
 112 et al., 2016; Wilkes et al., 2017). Many studies have demonstrated that specific functional groups of  
 113 invertebrates are particularly affected by siltation (Rabeni et al., 2005; Longing et al., 2010; Doretto  
 114 et al., 2016). For example, among the functional feeding groups (FFGs) several authors have  
 115 observed a concomitant decrease in the abundance of scrapers and filterers along a gradient of fine  
 116 sediment occurrence (Bo et al., 2007; Sutherland et al., 2012). When considering the biological and  
 117 ecological traits, large body-sized, univoltine and external-gilled organisms appear especially  
 118 disadvantaged by fine sediment as well as rheophilous and stony-associated taxa (Buendia et al.,  
 119 2013; Bona et al., 2016).

120 A second problem is represented by the spatial extent. According to Larsen et al. (2009), the best  
 121 spatial extent for directly relating macroinvertebrate communities to fine sedimentation is the patch-  
 122 scale, since the response at the reach-scale is mediated by other factors, such as land use. However,  
 123 in most cases, biotic indices were built on the basis of reach-scaled data, thus hindering the real  
 124 relationship between macroinvertebrate taxa and fine sedimentation (but see Murphy et al., 2015).

125 Third, in the majority of these indices benthic invertebrates are systematically identified at species  
 126 level because these methods rely on species-specific sensitivity/tolerance information (Table 1).  
 127 However, a similar taxonomic resolution is challenging for a routinely biomonitoring and most of  
 128 the Environmental Agencies adopt a different systematic level, mainly family or genus. Moreover,  
 129 species-specific data are not often available for some geographical areas or some invertebrate  
 130 groups.

131

132 Table 1. Fine sediment biotic index recently developed with their systematic and geographical  
 133 applicability details.

<b>Index</b>	<b>Taxonomic resolution</b>	<b>Geographical area(s)</b>	<b>References</b>
PSI (Proportion of Sediment-sensitive Invertebrates)	Family and species	UK	Extence et al., 2013; Glendell et al., 2013; Turley et al., 2014; 2015; 2016
FSBI (Fine Sediment Bioassessment Index)	Genus	USA	Relyea et al., 2000; 2012
BSTI (Biological Sediment Tolerance Index)	OTU (Operational Taxonomic Units: family, genus, species)	Oregon	Hubler et al., 2016
CoFSI <sub>sp</sub> (Combined Fine Sediment Index)	Genus and species	England and Wales	Murphy et al., 2015

134 Fourth, to our knowledge, biotic indices measuring the response of macroinvertebrates to fine  
135 sedimentation reported in the literature mostly concern the augmentation of fine sediment in  
136 streams caused by agriculture (Turley et al., 2014, 2015; Naden et al., 2016). In lowland areas,  
137 agriculture-induced sedimentation usually results as a widespread and chronic disturbance, often  
138 coupled with organic pollution due to pesticides, fertilizers or urbanization. This may act as a  
139 confounding factor on the response of benthic invertebrate assemblages to fine sediment (Turley et  
140 al., 2016). By contrast, farming and human settlements are generally scarce in mountainous areas  
141 due to their pronounced slope and harsh conditions. Nevertheless, fine sedimentation is today  
142 recognized as a primary cause of alteration in alpine streams, originating mainly by acute, localized  
143 or episodic sources, such as logging, mining, cross-river constructions or reservoir flushing (Crosa  
144 et al., 2010; Milisa et al., 2010; Espa et al., 2015; Bona et al., 2016). These lotic environments are  
145 expected to severely suffer the consequences of fine sediment deposition as they are typically  
146 dominated by coarse substrata and erosive features (Allan and Castillo, 2007). However, currently  
147 few studies have been carried out to investigate the specific effects of fine sediment on benthic  
148 macroinvertebrates in alpine streams (but see Espa et al., 2015; Leitner et al., 2015; Doretto et al.,  
149 2017). The aims of this study are: i) to investigate what are the best macroinvertebrate community  
150 metrics responding to fine sediment deposition in alpine streams and ii) to assess how the  
151 taxonomic resolution could affect the relationship between the metrics and fine sediment. In order  
152 to investigate the relationship between macroinvertebrates and fine sedimentation at the proper  
153 scale, we built up an experimental field study in which standardized conditions of fine sediment  
154 were manipulatively determined using artificial substrata (calibration dataset) within one single  
155 alpine reach. We then tested the validity of our index on field-collected data obtained from several  
156 patches nested into different reaches in two alpine streams (validation dataset).  
157 In particular, we aimed at constructing a multimetric index (MMI) following the algorithm  
158 suggested by Schoolmaster et al. (2013). The goal of the algorithm is to produce a maximally  
159 sensitive MMI from a given set of candidate metrics and a measure of human disturbance through  
160 an information theoretic criterion (Anderson and Burnham, 2002) to inform the process.

## 161 **2. Materials and Methods**

### 162 **2.1 Calibration dataset**

164 The study was realized in a homogeneous reach of the upper Po, a typical alpine low-order stream  
165 (Paesana, Monviso Natural Park, NW Italy UTM: 360107E, 4949488N; elevation 730 meters a.s.l.)  
166 (Figure 1). To assess the relationship between fine sediments and benthic macroinvertebrate metrics  
167 at the patch scale in alpine environment, we used artificial substrates to create standardized and  
168 replicable sampling units. We placed artificial substrata in a large and uniform reach of the Po  
169 riverbed, according to a random distribution. Each artificial substratum consisted of a parallelepiped  
170 trap built with a metal net (18 cm long, 6 cm wide and 6 cm high, mesh width 0.8 cm, total volume  
171 = 0.65 dm<sup>3</sup>). We constructed 135 traps, with 3 different levels of clogging. Traps were filled with  
172 different proportions of sand (range size 0.5-1 mm) and pea pebbles (average size 14-20 mm) to  
173 provide three different clogging conditions: 45 traps contained 100% pebbles (without sand, i.e. fine  
174 sediment – WFS), 45 traps contained 50% sand and 50% pebbles (medium level of sedimentation -  
175 MED) and 45 traps contained 66% sand e 33% pebbles (clogging condition – CLO). In the  
176 calibration data, we considered sand proportion as proxy of fine sediment amount.

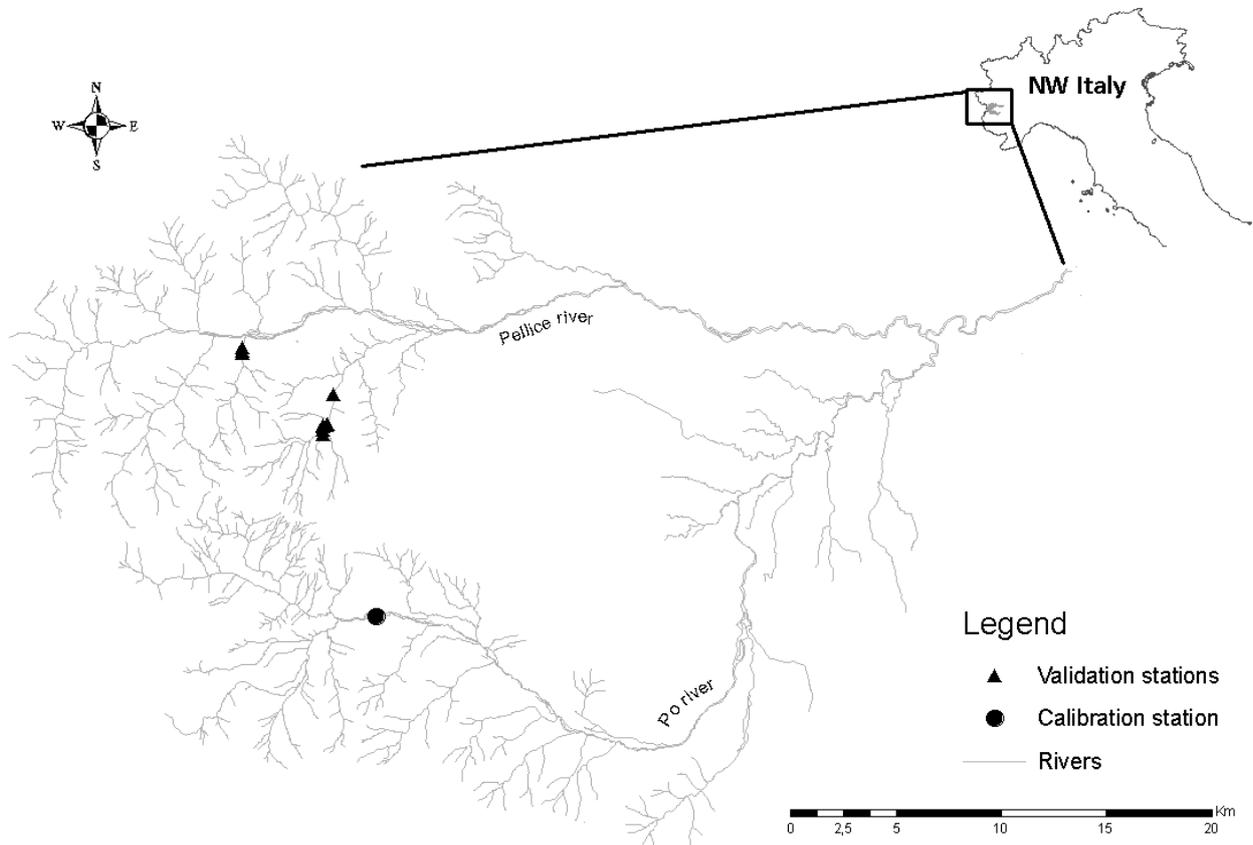
177 All traps were marked with a colored and numbered label and a fine net was applied to their lateral  
178 and basal sides to avoid the loss of fine sediment. Artificial substrata were randomly placed on the  
179 same day, buried in the streambed such that the upper side was flush with the bottom, allowing the  
180 colonization of benthic taxa. We paid attention to guarantee that all artificial substrata were fixed  
181 into the stream bottom with the same orientation and in similar conditions of water depth and  
182 velocity. To evaluate the colonization dynamic of macroinvertebrates on the different clogging  
183 conditions, the artificial substrata were removed on three different sampling dates, namely after 7,  
184 21 and 63 days, for a total of 45 random sampling units (15 for each typology) on each sampling  
185 date. When the cages were removed from the streambed they were suddenly placed into a plastic  
186 bucket and opened. All the content was transferred in separated plastic tins, preserved in 90%  
187 alcohol and returned in laboratory for the sorting and the systematic identification. All benthic  
188 invertebrates were systematically identified until family or genus and counted. Based on their  
189 trophic strategies and their biological and ecological requirements, macroinvertebrates were  
190 classified into the Functional Feeding Groups (FFGs - Merritt et al., 2008) and biological and  
191 ecological traits (Usseglio-Polatera et al., 2000) respectively.

192

## 193 **2.2 Validation dataset**

194 Data for validating the index were collected in a different watershed, comparable to the Po  
195 watershed in terms of physical and chemical variables as well as in terms of human settlement  
196 intensity, to guarantee a wider applicability of the index. For the validation dataset, we thus selected  
197 two third Strahler order streams in the Cottian Alps (Piemonte, NW Italy), the Luserna and the  
198 Comba Liussa streams. They share similar environmental conditions, the only difference being the  
199 presence of quarries in the Luserna which causes augmentation of fine sediments. On the contrary,  
200 the control lotic system is almost unaffected by human activities. Seven reaches were selected  
201 across the Luserna (L1–L7) and three across the Comba Liussa (C1–C3) stream (Figure 1) and in  
202 each of them we selected six roughly equidistant patches. In correspondence of each patch, we  
203 positioned sediment traps, in order to quantitatively characterize each patch in terms of fine  
204 sediment deposition (Bond 2002). Each trap consisted in a plastic storage box (165 × 95 × 70 mm),  
205 with a piece of wire mesh (20 × 20 mm openings; 1.5 mm gauge wire), cut to fit just inside the box  
206 and placed 30 mm from the top of the trap. In the field, the boxes were buried in the streambed such  
207 that their tops were flush with the bottom. Once the boxes were in place, the wire mesh was covered  
208 by a layer of coarse bed material one clast thick. In this way, fine sediments could enter into the  
209 traps, over which local hydraulic conditions were comparable with the whole streambed. All 60  
210 traps were deployed on the same date and removed after 17 days. Samplings were performed when  
211 mining activity in this Alpine area is at its highest level, resulting in an increased load of fine  
212 sediments in the Luserna catchment. This period coincides also with a substantial stability in the  
213 hydrological conditions of the two streams. The fine sediment collected in the traps was returned to  
214 the laboratory, where it was dried and weighted. One benthic sample was collected in each  
215 sampling point, using a Surber sampler (250 μm mesh size; 0.062 m<sup>2</sup> area) to evaluate the  
216 macroinvertebrate community. Surber were positioned in the patches of streambed immediately  
217 after the removal of sediment traps and adjacent (laterally) to where traps were placed. Collected  
218 substrate was conserved into plastic jars with 75% ethanol. In the laboratory, all benthic  
219 invertebrates were systematically identified to family or genus as for the calibration dataset and  
220 counted. We then checked if we had representative communities via accumulation curves

221 (Supplementary Material). Based on their trophic strategies and their biological and ecological  
 222 requirements, macroinvertebrates were classified into the Functional Feeding Groups (FFGs -  
 223 Merritt et al., 2008) and biological and ecological traits (Usseglio-Polatera et al., 2000) respectively.



224  
 225 Figure 1. Area of study: circular and triangle dots represent the sampling stations where the  
 226 calibration and validation experiments were respectively carried out.

227

### 228 2.3 MMI construction

229 We screened the available literature data in order to detect the macroinvertebrate-based metrics  
 230 most sensitive to fine sediment deposition. Selected potential metrics belonged to the three  
 231 categories indicated by Noss (1990)—*compositional*, *structural* and *functional* metrics—and  
 232 provided ecological information in accordance with the four categories indicated by Hering et al.  
 233 (2006)—*composition/abundance*, *richness/diversity*, *sensitivity/tolerance* and *functional traits*  
 234 (Table 2).

235

236 Table 2. Candidate community metrics used in this study and relative categories, ecological  
 237 information and references.

Metric	Category	Ecological information	References
Taxa richness (S)	Compositional	Richness/diversity	Zweig and Rabeni 2001; Buendia et al.

Ephemeroptera-Plecoptera-Trichoptera richness (EPT S)	Compositional	Richness/diversity	2013 Angradi 1999; Zweig and Rabeni 2001; Pollard and Yuan 2010; Buendia et al. 2013;
Inverse relative abundance of Gasteropoda-Oligochaeta-Diptera (1-GOLD)	Structural	Sensitivity/tolerance	Pinto et al., 2004; Buffagni and Erba 2007
Shannon-Wiener index (H')	Compositional	Richness/diversity	Mebane 2001; Zweig and Rabeni 2001; Buendia et al., 2013
Total abundance (N)	Structural	Composition/abundance	Angradi 1999; Zweig and Rabeni 2001; Buendia et al., 2013;
Ratio between Ephemeroptera,-Plecoptera-Trichoptera and Diptera (EPT/D)	Structural	Composition/abundance	Allan et al., 2006; Aura et al., 2010
Ephemeroptera-Plecoptera-Trichoptera percentage (EPT %)	Compositional	Sensitivity/tolerance	Mebane 2001; Buendia et al., 2013; Conroy et al., 2016
Abundance of Chironomidae	Structural	Composition/abundance	Angradi 1999; Zweig and Rabeni 2001;
Chironomidae/Diptera	Structural	Composition/abundance	Helson and Williams 2013
Shredders/Collector-gatherers	Functional	Functional traits	Merritt et al., 2002; Merritt et al., 2016
Abundance of biological group f (univoltine, large-sized taxa)	Functional	Functional traits	Bo et al., 2007; Bona et al., 2016; Doretto et al., 2017
Abundance of ecological group A (rheophilous and stony-associated taxa)	Functional	Functional traits	Bo et al., 2007; Bona et al., 2016; Doretto et al., 2017

238

239 In addition to the common and widely used taxa richness, Shannon-Wiener index (H') and total  
 240 abundance of benthic invertebrates, some metrics referred to key taxonomic groups were also  
 241 included. Three of our candidate metrics accounted for the EPT (Ephemeroptera, Plecoptera and  
 242 Trichoptera) component: EPT richness, EPT% and the ratio between EPT and Diptera (EPT/D). We  
 243 included these metrics because EPT taxa are among aquatic invertebrates the best adapted to  
 244 running waters and a key faunal component of the mountain and alpine sections of streams and  
 245 rivers (Heiber et al., 2005; Fenoglio et al., 2015). Moreover, they are recognized as the most  
 246 sensitive organisms among freshwater invertebrates so that EPT-based metrics are currently  
 247 included in biomonitoring indices or programs throughout Europe (Munnè and Pratt, 2009; Gabriels  
 248 et al., 2010). Similarly, we focused also on Diptera, Oligochaeta and Gastropoda, resulting in three  
 249 different abundance metrics: the abundance of Chironomidae, Chironomidae/Diptera ratio and 1-  
 250 GOLD. In general, a strong positive relationship between Diptera, especially Chiromomidae, as

251 well as Oligochaeta and fine sediment is supported by a huge number of literature data (Smolders et  
252 al., 2003; Cover et al., 2008; Descloux, et al., 2013). By contrast, 1-GOLD describes the relative  
253 proportion of Gastropoda, Oligochaeta and Diptera in the community/sample. This metric was  
254 developed in the European WFD (Water Framework Directive) implementation context and it is  
255 currently incorporated in the official Italian biomonitoring index (STAR\_ICMi; Buffagni et al.,  
256 2008). The last three community metrics we selected were based on the functional traits of benthic  
257 taxa. The shredders/collector-gatherers describes the ratio between invertebrates feeding directly on  
258 coarse particulate organic matter (CPOM) and those feeding on fine particulate organic matter  
259 (FPOM). In accordance to the River Continuum Concept (Vannote et al., 1980), the former are  
260 mainly located in the upper sections of lotic ecosystems (i.e., low-order streams/reaches) as they  
261 strongly depend on the allochthonous input of organic matter (i.e., leaves and vegetal detritus) from  
262 the riparian areas. By contrast, the abundance of biological group f and ecological group A (*sensu*  
263 Usseglio-Polatera et al., 2000) refer to univoltine, large-sized, rheophilous and stony-associated  
264 invertebrates respectively. As all these functional metrics encompass taxa associated to the upper  
265 sections of streams, characterized by cold, fast-flowing water and large mineral substratum, we  
266 decided to include them among the candidate metrics for evaluating the response of  
267 macroinvertebrates to fine sedimentation in alpine streams. To evaluate how taxonomic resolution  
268 could affect the response of the metrics to the disturbance, all metrics considered were calculated  
269 twice: i) the first time they were derived from taxa identified at family level; ii) the second time  
270 they were obtained from inventories in which Plecoptera, Ephemeroptera and Turbellaria were  
271 identified to genus level in accordance with Italian pre-WFD official biomonitoring tool (I.B.E. -  
272 Ghetti, 1997).

273 In accordance with the data preparation protocol provided by Schoolmaster et al. (2013), before  
274 proceeding with the multimetric construction, we removed metrics that contained a large proportion  
275 of zero or duplicated another metric and then rescaled them with the formula:

276

277

$$\frac{m - m_{min}}{m_{max} - m_{min}}$$

278

279 where  $m$  is the observed value of the metric,  $m_{min}$  is the minimum observed value of the metric in  
280 the dataset and  $m_{max}$  is the maximum observed value of the metric in the dataset. In this way, all the  
281 values ranged from 0 to 1, where 0 corresponds to the worst condition and 1 to the best condition.

282 Given the manipulative structure of our calibration dataset, we did not adjust metrics for the  
283 covariates effects since traps were placed within the same stream reach, being differentiated only by  
284 the fine sediment quantity. Metrics positively correlated with fine sediment were reflected and those  
285 not correlated were excluded from further analysis.

286 We then applied the algorithm proposed by Schoolmaster et al. (2013) that can be used to generate a  
287 MMI able to discriminate different disturbance conditions from a given set of metrics. This method  
288 produces a MMI with the strongest possible negative correlation with human disturbance through  
289 statistical inference since it assumes that metrics and the final MMI are linear functions of the  
290 measure of disturbance. Potential MMIs are then built as sets of models, where the disturbance  
291 represents the dependent variable and metrics are included as independent variables to be tested  
292 against the disturbance. In accordance with this protocol, the quantity of fine sediment was chosen  
293 as disturbance parameter,  $D$ , which represented the dependent variable in our set of models. In order

294 to obtain an ordinal distribution for the disturbance parameter in the calibration dataset, traps with  
295 0% of fine sediment were assigned to class 0, traps with 50% of fine sediment were assigned to  
296 class 1 and traps with 66% of fine sediment were assigned to class 2. We then applied the algorithm  
297 proposed by Schoolmaster et al. (2013). First, we selected an initial metric,  $m_I$  and we added  $m_I$  to  
298 each of the rest of the metrics,  $m_j$ , site-by-site; second, for each  $m_j$ , we checked which combination  
299  $m_I + m_j$  had the strongest negative coefficient with  $D$  and we selected that one; third, we added the  
300 index to each of the remaining metrics  $m_j$  site-by-site and we selected the combination of index +  $m_j$   
301 that has the strongest negative coefficient with  $D$ ; finally, we continued this process until the log-  
302 likelihood ratio test (see Schoolmaster et al., 2013 for further details) reached the threshold of 3.84,  
303 which is the value of the chi-squared distribution that corresponds with  $p = 0.05$ . We repeated these  
304 steps using all metrics as initial metric and this process resulted in a number of potential MMIs  
305 equal to the number of metrics considered. We then compared them according to the AICs and  
306 selected the one with the lowest AIC value to choose the best one.

307 Given the categorical distribution of our disturbance parameter, we performed multinomial linear  
308 regressions, specifically conceived for categorical dependent variables, with the function *polr* of the  
309 package *MASS* (Venables and Ripley, 2002) in R environment (R Core Team, 2015).

310 The final MMI was then calculated on the validation dataset by averaging the scaled values  
311 (ranging from 0 and 1) of the final selected metrics, obtained from the calibration dataset. The MMI  
312 in the validation dataset was calculated for each patch and then averaged for each reach. The field  
313 observations of fine sediment were converted into an ordinal variable by calculating the relative  
314 proportion of fine sediment weight to the total weight of sediments in each patch and then averaged  
315 for each reach. Each observation was then assigned to an ordinal class following the same rules  
316 used in the calibration dataset (< 50% = class 0; 50%-66% = class 1; > 66% = class 2). Index values  
317 in the validation dataset were comprised from 0 (worst condition) to 1 (best condition) and they  
318 were therefore correlated to the fine sediment class for each reach through the Pearson correlation  
319 test. This process was repeated for metrics obtained at both mixed (i.e, family and genus) and  
320 family levels.

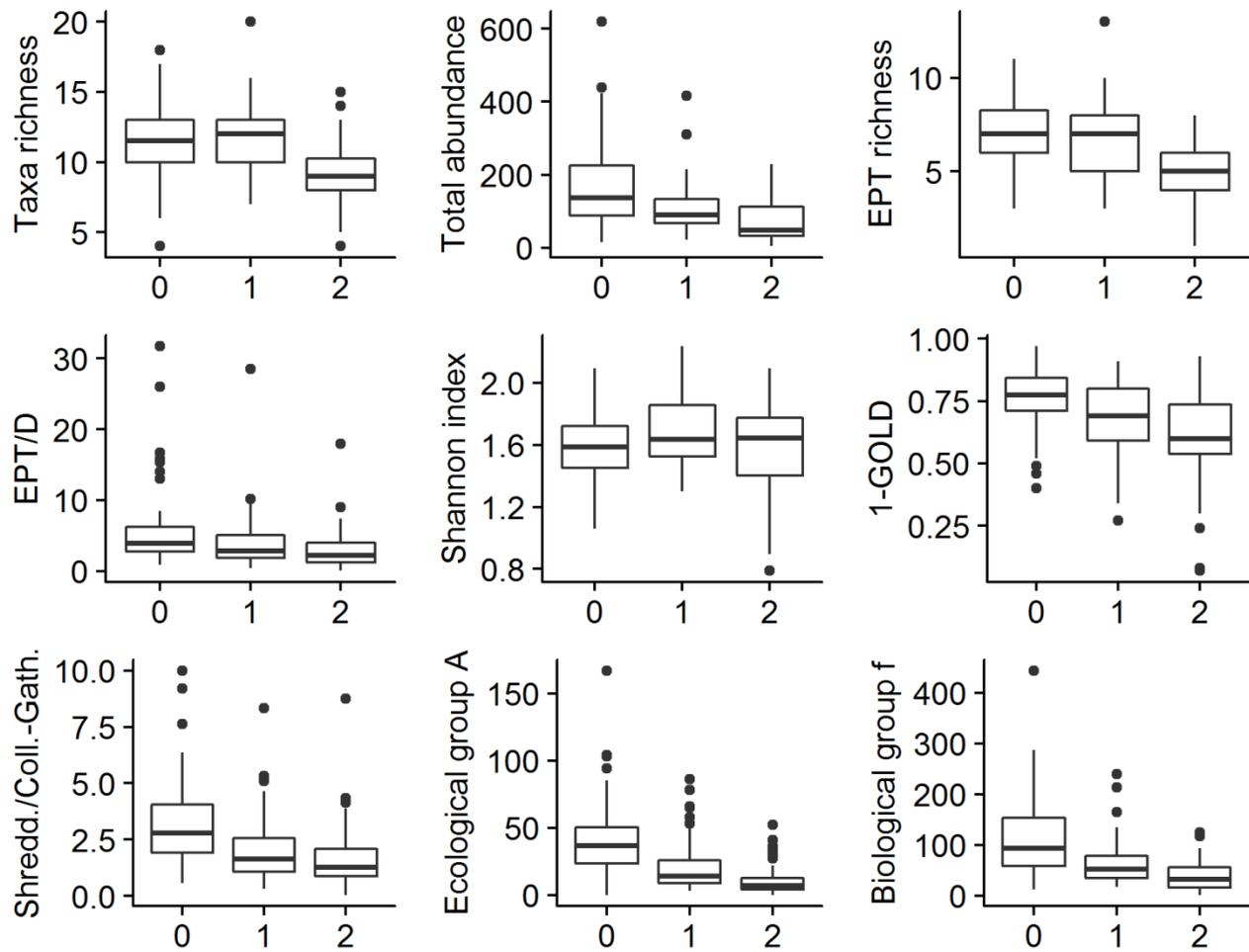
321

### 322 **3. Results**

323 In the calibration dataset, no relevant morpho-hydrological or chemical changes were observed over  
324 the entire sampling period. Overall, the sampling reach was characterized by streambed 7.5-10.3 m  
325 wide, cold ( $4.04^{\circ}\text{C} \pm 0.03$  SE), well-oxygenated ( $99.50$  DO%  $\pm 4.45$  SE) and oligotrophic  
326 (conductivity:  $172 \mu\text{S}/\text{cm} \pm 0.003$  SE; nitrates =  $0.70$  mg/l; SRP <  $0.001$  mg/l, BOD<sub>5</sub> =  $3.09$  mg/l)  
327 waters. Mean water depth was  $14.4$  cm ( $\pm 0.66$  SE), while the average flow velocity was  $0.07$  m/s  
328 ( $\pm 0.003$  SE). We could then exclude an influential effect of environmental parameters on artificial  
329 substrata. The average values of the final selected metrics in the three disturbance classes here  
330 considered are reported in Supplementary Material.

331 Before proceeding with the MMI algorithm, we excluded % EPT because in accordance with the  
332 protocol provided by Schoolmaster et al. (2013) it can be considered as a duplicate of 1-GOLD. We  
333 also excluded the abundance of Chironomidae and the ratio between Chironomidae and Diptera  
334 because despite being expected to increase with increasing disturbance they showed an opposite  
335 trend. As suggested by Schoolmaster et al. (2013), we excluded them in order to avoid confounding  
336 elements. For each of the 9 remnant metrics (Fig. 2), we obtained a potential MMI after the  
337 selection process and AIC values are reported in Table 3 and 4.

338  
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340

341 Figure 2. Boxplots represent the response of the candidate metrics to the sediment conditions in the  
342 calibration dataset: 0 = WFS (0% fine sediment and 100% pebbles), 1 = MED (50% fine sediment  
343 and 50% pebbles), 2 = CLO (66% fine sediment and 33% pebbles).

344

345 Table 3. Final selected models obtained with the family level identification. The AIC column refers  
346 to the AICs values obtained for each model and the  $\Delta$ AIC column refers to the differences between  
347 the AIC of the selected model and the lowest AIC obtained. Values of  $\Delta$ AIC < 2 are reported in  
348 bold.

Potential models	AIC	$\Delta$ AIC
1) Taxa Richness + EPT Richness + Ecological group A	244.42	<b>1.39</b>
2) Total abundance + 1-GOLD + EPT Richness + Taxa Richness	244.70	<b>1.67</b>
3) EPT Richness + Taxa Richness + Ecological group A	244.42	<b>1.39</b>
4) EPT/D + EPT Richness + Taxa Richness + Biological group f	245.38	2.35

5) Shannon + EPT Richness + Taxa Richness + Ecological group A	245.36	2.33
6) 1-GOLD + Total Abundance + EPT Richness + Taxa Richness	244.70	<b>1.67</b>
7) Shredders/Collector-Gatherers + Taxa Richness + EPT Richness + Biological group f	243.03	<b>0.00</b>
8) Ecological group A + Taxa Richness + EPT Richness	244.42	<b>1.39</b>
9) Biological group f + Taxa Richness + EPT Richness + Shredders/Collector-Gatherers	243.03	<b>0.00</b>

349

350 Table 4. Final selected models obtained with the mixed level identification. The AIC column refers  
351 to the AICs values obtained for each model and the  $\Delta$ AIC column refers to the differences between  
352 the AIC of the selected model and the lowest AIC obtained. Values of  $\Delta$ AIC < 2 are reported in  
353 bold.

Potential models	AIC	$\Delta$ AIC
1) Taxa Richness + EPT Richness + Ecological group A	245.88	<b>1.24</b>
2) Total abundance + 1-GOLD + EPT Richness + Taxa Richness	247.86	3.22
3) EPT Richness + Taxa Richness + Ecological group A	245.88	<b>1.24</b>
4) EPT/D + EPT Richness + Taxa Richness + Biological group f	244.87	<b>0.23</b>
5) Shannon + EPT Richness + Taxa Richness + Ecological group A	247.64	3.00
6) 1-GOLD + Total abundance + Taxa Richness + Ecological group A	244.95	<b>0.31</b>
7) Shredders/Collector-Gatherers + Taxa Richness + EPT Richness + Biological group f	244.64	<b>0.00</b>
8) Ecological group A + Taxa Richness + EPT Richness	245.88	<b>1.24</b>
9) Biological group f + EPT Richness + Taxa Richness	244.98	<b>0.34</b>

354

355 For both the family-level and the mixed-level approach, the MMI assembly algorithm identified 7  
356 out of 9 MMIs with  $\Delta$ AIC < 2. MMIs with values of  $\Delta$ AIC < 2 are judged to have substantial  
357 support, and should be considered as viable alternatives to the model with the lowest AIC. In other  
358 words, any MMI can be chosen from the set of models with  $\Delta$ AIC < 2 without relevant loss of  
359 predictive power. Starting from this theoretical background, we preferred to select the most  
360 parsimonious solution and we then chose the most recurrent model in both the mixed and family  
361 approaches as the final index, instead of creating weighted indices. In fact, a weighted index,  
362 including all the metrics composing the models with  $\Delta$ AIC < 2, would have been more time-  
363 consuming, because a higher number of metrics should be calculated, without increasing the

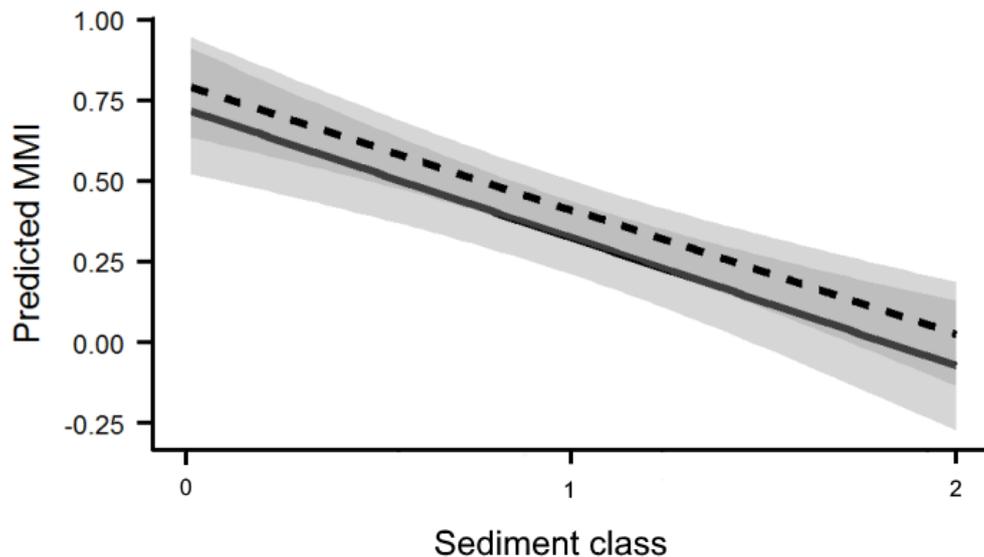
364 predictive power of the index itself. Moreover, it would have required calculating different indices,  
365 depending on the taxonomic resolution considered, since the final selected models were different  
366 for the two levels. Our final selected index then was that obtained from equation 1 (Table 3 and 4):

367  $D \sim \text{Taxa Richness} + \text{Ecological group A} + \text{EPT richness}$

368 This model recurred three times for both the mixed and the family level and metrics composing the  
369 final index also represented the most recurrent ones. In particular, in the family-level procedure,  
370 Taxa Richness and EPT Richness were included in all 9 alternative models, while Ecological Group  
371 A was included in 4 out of 9 alternative models. For the mixed-level approach, Taxa Richness was  
372 included in all the alternative models, EPT Richness was included in 8 out of 9 alternative models,  
373 the Ecological group A was included in 5 out of 9 alternative models.

374 In the validation dataset, the index was calculated as the average of the scaled values of the final  
375 metrics. The Pearson correlation test showed high and significant correlations between index values  
376 and the amount of fine sediment at reach level for both the family-level approach ( $r = -0.73$ ,  $P =$   
377  $0.017$ ) and the mixed-level approach ( $r = -0.74$ ,  $P = 0.014$ ). The discrimination capacity of the  
378 index is high for the class 0 of disturbance, while it less powerful in differentiating class 1 and 2,  
379 especially at the family level (Fig. 3).

380



381

382 Figure 3. Predicted values and confidence intervals of MMIs (continuous line = family level  
383 approach; dashed line = predicted values derived from the mixed level approach) calculated against  
384 sediment weight in the validation dataset. Sediment weight is log-transformed for a better graphical  
385 representation.

386

#### 387 4. Discussion

388 Despite problems associated to fine sediment being widespread, traditional biomonitoring indices  
389 were developed to detect chemical pollution and usually do not correlate with siltation (Angradi,  
390 1999; Zweig and Rabení, 2001; Sutherland al., 2012). For this reason, this topic has recently

391 received an exponential attention so that a new generation of biotic indices has been proposed for  
392 measuring the specific effects of fine sediment on macroinvertebrates, mostly in lowland lotic  
393 systems (Relyea et al., 2012; Extence et al. 2013; Turley et al., 2014, 2015; Murphy et al., 2015).  
394 To our knowledge, our work is the first attempt of building a stressor-specific multimetric index  
395 with a manipulative approach aiming at the evaluation of the effect of fine sediment on  
396 macroinvertebrates in alpine streams. We are aware of the limitations associated with a  
397 manipulative approach, but we are also confident that our experiments resulted highly comparable  
398 with real conditions of Alpine streams. In particular, we are confident that the use of sand in the  
399 calibration experiment as a proxy of fine sediments could represent a good compromise in Alpine  
400 streams. In fact, we had to face some critical issues in the construction, displacement and removal  
401 of our traps. It would have been extremely difficult to achieve a similar experimental design using  
402 sub-sand fractions, because of high flow velocities and great tractive forces that characterise Alpine  
403 running waters. This index may thus represent a promising tool for future biomonitoring  
404 assessments for two main reasons: i) using compositional and functional metrics we can overcome  
405 the taxonomic constraints intrinsically present in biotic indices (Friberg et al., 2011); ii) this index is  
406 highly specific for alpine streams, which represent peculiar ecosystems in which unnatural fine  
407 sediment deposition represents one of the main causes of impairment (Wohl, 2006).

408 Compared to other multimetric indices based on macroinvertebrates (Vlek et al., 2004; Couceiro et  
409 al., 2012; Mondy et al., 2012), we here introduced an alternative approach, since the calibration was  
410 conducted at the patch scale while the validation was performed at the reach scale. A number of  
411 other studies have also implied that the ability to detect impacts may be dependent on the choice of  
412 sampling scale (Smiley and Dibble, 2008; Burdon et al., 2013). To keep into account the scale of  
413 response, we built our index through a manipulative experiment at the patch-scale, which is the  
414 most appropriate for measuring the response of macroinvertebrates to fine sediment deposition  
415 (Larsen et al., 2009). While field surveys best represent natural conditions, they may be influenced  
416 by a range of co-varying factors which may alter biological responses (Matthaei et al., 2010;  
417 Robinson et al., 2011; Wagenhoff et al., 2011; Glendell et al., 2014; Turley et al., 2016). Using  
418 manipulative experiments allows for the isolation and control of stressors, minimising confounding  
419 factors (Kochersbergher et al., 2012; O'Callaghan et al., 2015; Piggott et al., 2015; Wang et al.  
420 2016). However, since the stream water quality evaluation and management take place at the reach  
421 scale (Collins and Anthony, 2008; Collins et al., 2011; Murphy et al. 2015), it is at this scale that  
422 investigations must take place. For this reason, the validity of our index was tested through a second  
423 experiment at the reach scale, in order to be applicable for monitoring purposes.

424 In this study, three metrics were retained for their integration into a multimetric index evaluating the  
425 impacts of siltation in alpine streams. These were diversity metrics, i.e. total taxa richness and  
426 richness in Ephemeroptera, Plecoptera and Trichoptera taxa, and a functional metric, i.e. abundance  
427 of rheophilous taxa preferring coarse substrata, typical of oligotrophic, alpine habitats (Ecological  
428 group A sensu Usseglio-Polatera et al., 2000). Similar results were obtained by Larsen et al. (2011),  
429 who observed a negative effect of fine sediment on both diversity and functional metrics. Our  
430 results clearly demonstrated how the combination of different categories of metrics, i.e. diversity  
431 and functional metrics, reveal the effect of siltation on biotic communities. This is in accordance  
432 with literature (Barbour et al., 1996, 1999; Klemm et al., 2003; Bonada et al. 2006; Hering et al.  
433 2006), since, by combining different categories of metrics, the multimetric assessment is regarded  
434 as a more reliable tool than assessment methods based on single metrics. Furthermore, the most

435 relevant combination of appropriate metrics thus consisted of three out of the nine metrics tested. In  
436 accordance with literature (Menetrey et al. 2011; Schoolmaster et al. 2013), this result shows that  
437 the selection of the best combination did not include the maximal number of metrics.

438 Concerning diversity metrics, the effect of fine sediment can be significantly measured in terms of  
439 taxa richness and richness of the most stenoeconomic taxa, such as Ephemeroptera, Plecoptera and  
440 Trichoptera, in agreement with recent studies (Couceiro et al., 2011; Leitner et al., 2015; Conroy et  
441 al. 2016; Doretto et al., 2017). Although richness metrics are generally sensitive to natural  
442 variability and seasonality, and thus are influenced by the period of sampling (Bilton al., 2006),  
443 their combination diminishes this effect and thus increases the robustness of the multimetric index  
444 (Dahl and Johnson, 2004; Maloney and Feminella, 2006). Given that many biological impacts  
445 caused by sedimentation are due to sediment deposition (Jones et al., 2012; Glendell et al. 2014),  
446 we here focused only on deposited fine sediment, excluding suspended sediment.

447 Besides diversity metrics, our findings evidenced that functional metrics may also be effective for  
448 measuring the impact of fine sediment deposition. Other studies have evidenced changes in trait-  
449 based metrics to elevated sediment deposition (Rabeni et al., 2005; Archambault et al., 2010; Bona  
450 et al. 2016, Turley et al., 2016), generally focusing on functional feeding and habitat groups. In our  
451 study, the application of ecological and biological groups proposed by Usseglio-Polatera et al.  
452 (2000), which integrate different aspects, like habitat preferences as well as locomotion, may better  
453 integrate the filtering effect of siltation on benthic communities (Doretto et al., 2017). The use of  
454 trait-based indices is a promising approach to help establish the causal relationships between  
455 specific stressors and macroinvertebrate community response (Dolédec and Statzner, 2008; Statzner  
456 and Beche, 2010, Merritt et al., 2016). In this context, indices and functional trait-based metrics are  
457 generally considered more sensitive and showed stronger responses to pressures than taxonomy-  
458 based metrics. Indeed, Dolédec et al. (2006) have demonstrated that functional traits are able to  
459 integrate more general phenomena than taxonomy-based metrics. Our results clearly support the use  
460 of functional metrics to build multi-metric indices to assess river biotic integrity.

461 Contrary to our expectations, total abundance of Chironomidae and the Chironomidae/Diptera ratio  
462 were excluded from the MMI assembly procedure as these metrics showed an inverse relation with  
463 the disturbance (i.e., amount of fine sediment). In the calibration dataset the highest abundance of  
464 Chironomidae was detected in the sediment free substrata (WFS), while the lowest in the clogged  
465 ones (CLO). This finding was unexpected because a huge number of studies have documented high  
466 densities of Chironomidae midges associated to fine sediment conditions (Ciesielka, and Bailey,  
467 2001; Kochersberger et al., 2012; Descloux, et al., 2013). However, literature data depict an unclear  
468 and contrasting situation. Indeed, some authors reported an increment in the Chironomidae  
469 abundance along a gradient of fine sediment amount, while other authors observed significant and  
470 opposite responses according to the sub-families of this taxon (Angradi, 1999; Zweig and Rabeni,  
471 2001). For example, Extence et al. (2013) did not score this family and excluded it for the  
472 calculation of the PSI because of the wide variability in the sensitivity or tolerance to fine sediment.  
473 In this study we systematically identified Chironomidae just at family level, losing information on  
474 the response of each sub-family. This may account for the unexpected response here detected for  
475 this insect group and it surely represents an important aspect that future studies should consider.

476 Finally, we found that both our family-level and mixed-level (family and genus) MMIs significantly  
477 correlated with the amount of fine sediment in the validation dataset. However, the discriminant  
478 capacity was higher for the mixed-level identification than the family level, especially for

479 disturbance classes 1 and 2. Many authors have demonstrated that a mixed-level systematic  
480 identification could improve the performance biotic indices (Schmidt-Kloiber and Nijboer, 2004;  
481 Monk et al., 2012). Our findings are in agreement with their results and highlight the importance of  
482 the systematic resolution in the freshwater biomonitoring. The choice of the adequate systematic  
483 level often reflects a trade-off between the costs associated to the samples processing and the  
484 benefits due to species-specific ecological information. Based on our results, we suggest that a  
485 mixed-level identification of benthic invertebrates may represent a good solution, with the family as  
486 the basic level and the genus for those taxa requiring a higher taxonomic detail, such as EPT or  
487 families that encompass a wide range of species. This option may be very advantageous especially  
488 for those biomonitoring tools aimed to assess the ecological impairment due to specific stressors.

## 489 **5. Conclusions**

490 We are confident that this study could represent an interesting element in the biomonitoring of  
491 siltation impacts. In particular, the index we propose could be effectively employed in alpine  
492 environments, considering reach scale and family/genus taxonomic resolution. The fine sediment  
493 colmation of riverbed is currently recognized as one of the most widespread forms of alteration by  
494 river managers, local agencies and other stakeholders. As a consequence, in the last few years  
495 several biotic indices have been developed to specifically quantify the degree of impairment due to  
496 anthropogenic fine sediment inputs (Relyea et al., 2012; Extence et al., 2013; Turley et al. 2014,  
497 2015; Murphy et al., 2015; Hubler et al., 2016). All these indices focus on the proportion between  
498 sensitive and tolerant benthic invertebrate taxa and rely on valid biological and statistical bases.  
499 However, their routine and large-scale applicability appear limited by some aspects, including the  
500 systematic resolution and the availability of species-specific data on the sensitivity/tolerance to fine  
501 sediment. Moreover, most of them have been developed in an agricultural context and this could  
502 represent a confounding factor due to its chemical changes of the water quality. Unlike the above  
503 mentioned studies, we tested the correlation between several macroinvertebrate community metrics  
504 and fine sediment in alpine streams and we indicated the total richness, the EPT richness and the  
505 abundance of rheophilous, stony-associated invertebrates as the best candidate metrics. Other  
506 studies have examined the relation between benthic invertebrate community metrics and siltation  
507 (Angradi, 1999; Mebane, 2001; Zweig and Rabeni, 2001; Sutherland et al., 2012), but to our  
508 knowledge this is the first study aimed to review the candidate metrics and to combine the selected  
509 ones into a multimetric index. We are aware that our results need to be validated by further  
510 investigations, especially by means of a large-scale survey encompassing a gradient of fine  
511 sediment conditions. However, multimetric indices are today widely recommended for  
512 biomonitoring purposes as they allow the selection of stressor-specific metrics and the applicability  
513 over large geographical areas (Bonada et al, 2006; Nõges et al, 2009; Birk et al., 2012). For  
514 example, a multimetric approach was a common consequence of the European Directive  
515 2000/60/EC (Water Framework Directive) and its implementation in many Member States. For  
516 these reasons, the findings of this study not only may provide practical tools for biomonitoring the  
517 effects of fine sediment but also they may fit with the actual normative scenario.

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