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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Highlights

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

Abstract

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

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

1. Introduction

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

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

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

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

Despite their strong biological and statistical bases, these indices present some critical issues. First, they are based on taxonomic identity, thus spatially dependent to the geographical areas where they have been developed. However, the employment of selected community metrics rather than taxon-identity scores may be a good solution to overcome the bio-geographical limits. This aspect introduces a fundamental question: which are the best macroinvertebrate community metrics related to fine sediment conditions? Literature data show that fine sediment affects several characteristics of macroinvertebrate communities, such as diversity, total abundance, relative abundance of functional groups and behavioral patterns (i.e., drift) (Angradi, 1999; Longing et al., 2010; Descloux et al., 2014). For example, reductions in the taxa richness and abundance of macroinvertebrates have been typically observed when high levels of siltation occur in the substrate or stream-section, especially among the most sensitive taxa (i.e., EPT – Ephemeroptera, Plecoptera and Trichoptera) (Sutherland et al., 2012; Mathers and Wood, 2016). Conversely, some taxa (i.e., Chiromomidae, Oligochaeta) could benefit from the environmental conditions provided by fine sediment (Ciesielka, and Bailey, 2001; Cover et al., 2008). Also, trait-based classifications of macroinvertebrate taxa have been recently used to assess the response of macroinvertebrate assemblages to fine sediment conditions, with noteworthy results (Pollard and Yuan, 2010; Conroy et al., 2016; Wilkes et al., 2017). Many studies have demonstrated that specific functional groups of invertebrates are particularly affected by siltation (Rabeni et al., 2005; Longing et al., 2010; Doretto et al., 2016). For example, among the functional feeding groups (FFGs) several authors have observed a concomitant decrease in the abundance of scrapers and filterers along a gradient of fine sediment occurrence (Bo et al., 2007; Sutherland et al., 2012). When considering the biological and ecological traits, large body-sized, univoltine and external-gilled organisms appear especially disadvantaged by fine sediment as well as rheophilous and stony-associated taxa (Buendia et al., 2013; Bona et al., 2016).

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

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

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

Index	**Taxonomic resolution**	**Geographical area(s)**	**References**
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
$CoFSI_{sp}$ (Combined Fine Sediment Index)	Genus and species	England and Wales	Murphy et al., 2015

Fourth, to our knowledge, biotic indices measuring the response of macroinvertebrates to fine sedimentation reported in the literature mostly concern the augmentation of fine sediment in streams caused by agriculture (Turley et al., 2014, 2015; Naden et al., 2016). In lowland areas, agriculture-induced sedimentation usually results as a widespread and chronic disturbance, often coupled with organic pollution due to pesticides, fertilizes or urbanization. This may act as a confounding factor on the response of benthic invertebrate assemblages to fine sediment (Turley et al., 2016). By contrast, farming and human settlements are generally scarce in mountainous areas due to their pronounced slope and harsh conditions. Nevertheless, fine sedimentation is today recognized as a primary cause of alteration in alpine streams, originating mainly by acute, localized or episodic sources, such as logging, mining, cross-river constructions or reservoir flushing (Crosa et al., 2010; Milisa et al., 2010; Espa et al., 2015; Bona et al., 2016). These lotic environments are expected to severely suffer the consequences of fine sediment deposition as they are typically dominated by coarse substrata and erosive features (Allan and Castillo, 2007). However, currently few studies have been carried out to investigate the specific effects of fine sediment on benthic macroinvertebrates in alpine streams (but see Espa et al., 2015; Leitner et al., 2015; Doretto et al., 2017). The aims of this study are: i) to investigate what are the best macroinvertebrate community metrics responding to fine sediment deposition in alpine streams and ii) to assess how the taxonomic resolution could affect the relationship between the metrics and fine sediment. In order to investigate the relationship between macroinvertebrates and fine sedimentation at the proper scale, we built up an experimental field study in which standardized conditions of fine sediment were manipulatively determined using artificial substrata (calibration dataset) within one single alpine reach. We then tested the validity of our index on field-collected data obtained from several patches nested into different reaches in two alpine streams (validation dataset).

In particular, we aimed at constructing a multimetric index (MMI) following the algorithm suggested by Schoolmaster et al. (2013). The goal of the algorithm is to produce a maximally sensitive MMI from a given set of candidate metrics and a measure of human disturbance through an information theoretic criterion (Anderson and Burnham, 2002) to inform the process.

2. Materials and Methods

2.1 Calibration dataset

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

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

2.2 Validation dataset

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

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

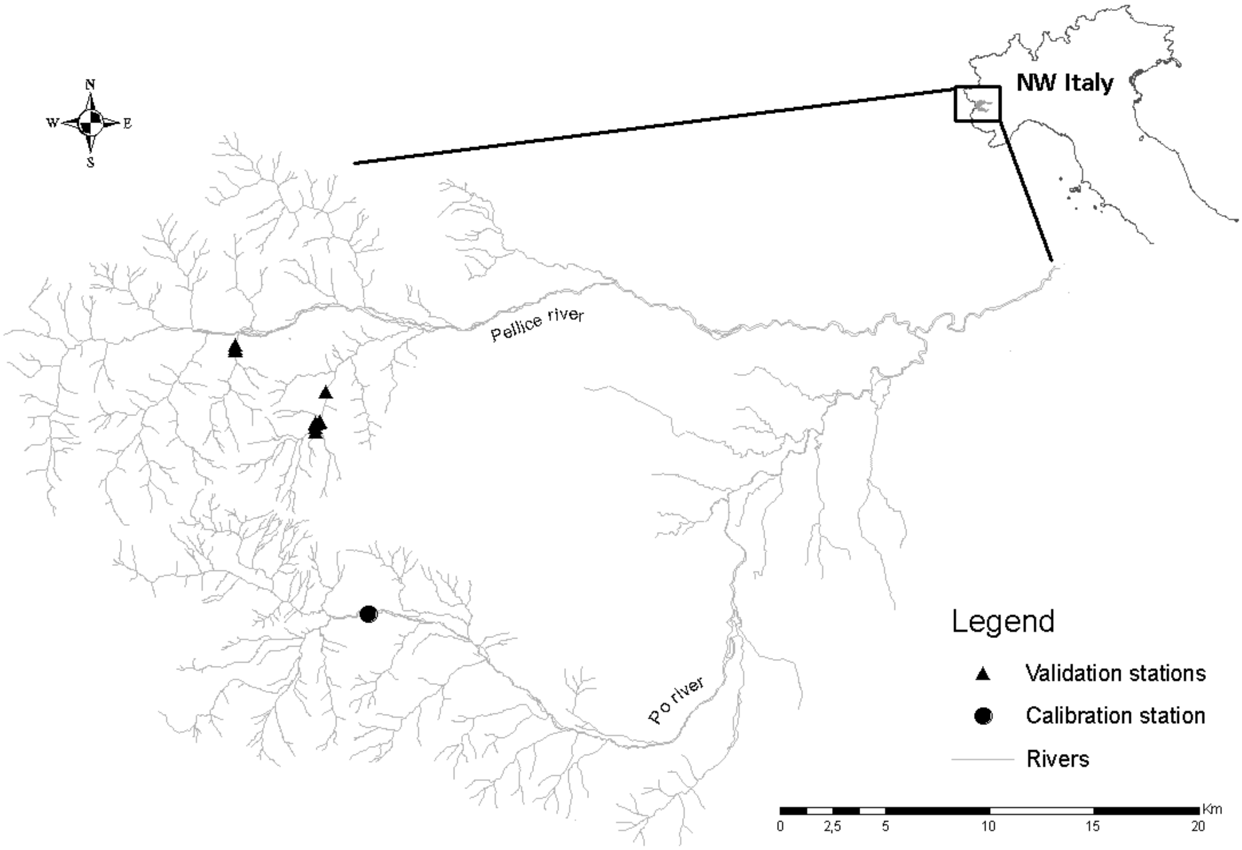

Figure 1. Area of study: circular and triangle dots represent the sampling stations were the calibration and validation experiments were respectively carried out.

2.3 MMI construction

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

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

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

			2013
Ephemeroptera-Plecoptera-Trichoptera richness (EPT S)	Compositional	Richness/diversity	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

In addition to the common and widely used taxa richness, Shannon-Wiener index (H’) and total abundance of benthic invertebrates, some metrics referred to key taxonomic groups were also included. Three of our candidate metrics accounted for the EPT (Ephemeroptera, Plecoptera and Trichoptera) component: EPT richness, EPT% and the ratio between EPT and Diptera (EPT/D). We included these metrics because EPT taxa are among aquatic invertebrates the best adapted to running waters and a key faunal component of the mountain and alpine sections of streams and rivers (Heiber et al., 2005; Fenoglio et al., 2015). Moreover, they are recognized as the most sensitive organisms among freshwater invertebrates so that EPT-based metrics are currently included in biomonitoring indices or programs throughout Europe (Munnè and Pratt, 2009; Gabriels et al., 2010). Similarly, we focused also on Diptera, Oligochaeta and Gastropoda, resulting in three different abundance metrics: the abundance of Chironomidae, Chironomidae/Diptera ratio and 1-GOLD. In general, a strong positive relationship between Diptera, especially Chiromomidae, as well as Oligochaeta and fine sediment is supported by a huge number of literature data (Smolders et al., 2003; Cover et al., 2008; Descloux, et al., 2013). By contrast, 1-GOLD describes the relative proportion of Gastropoda, Oligochaeta and Diptera in the community/sample. This metric was developed in the European WFD (Water Framework Directive) implementation context and it is currently incorporated in the official Italian biomonitoring index (STAR_ICMi; Buffagni et al., 2008). The last three community metrics we selected were based on the functional traits of benthic taxa. The shredders/collector-gatherers describes the ratio between invertebrates feeding directly on coarse particulate organic matter (CPOM) and those feeding on fine particulate organic matter (FPOM). In accordance to the River Continuum Concept (Vannote at al., 1980), the former are mainly located in the upper sections of lotic ecosystems (i.e., low-order streams/reaches) as they strongly depend on the allochthonous input of organic matter (i.e., leaves and vegetal detritus) from the riparian areas. By contrast, the abundance of biological group f and ecological group A (*sensu* Usseglio-Polatera et al., 2000) refer to univoltine, large-sized, rheophilous and stony-associated invertebrates respectively. As all these functional metrics encompass taxa associated to the upper sections of streams, characterized by cold, fast-flowing water and large mineral substratum, we decided to include them among the candidate metrics for evaluating the response of macroinvertebrates to fine sedimentation in alpine streams. To evaluate how taxonomic resolution could affect the response of the metrics to the disturbance, all metrics considered were calculated twice: i) the first time they were derived from taxa identified at family level; ii) the second time they were obtained from inventories in which Plecoptera, Ephemeroptera and Turbellaria were identified to genus level in accordance with Italian pre-WFD official biomonitoring tool (I.B.E. - Ghetti, 1997).

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

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

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

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

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

to obtain an ordinal distribution for the disturbance parameter in the calibration dataset, traps with 0% of fine sediment were assigned to class 0, traps with 50% of fine sediment were assigned to class 1 and traps with 66% of fine sediment were assigned to class 2. We then applied the algorithm proposed by Schoolmaster et al. (2013). First, we selected an initial metric, m_1 and we added m_1 to each of the rest of the metrics, m_j, site-by-site; second, for each m_j, we checked which combination $m_1 + m_j$ had the strongest negative coefficient with D and we selected that one; third, we added the index to each of the remaining metrics m_j site-by-site and we selected the combination of index + m_j that has the strongest negative coefficient with D; finally, we continued this process until the log-likelihood ratio test (see Schoolmaster et al., 2013 for further details) reached the threshold of 3.84, which is the value of the chi-squared distribution that corresponds with $p = 0.05$. We repeated these steps using all metrics as initial metric and this process resulted in a number of potential MMIs equal to the number of metrics considered. We then compared them according to the AICs and selected the one with the lowest AIC value to choose the best one.
Given the categorical distribution of our disturbance parameter, we performed multinomial linear regressions, specifically conceived for categorical dependent variables, with the function *polr* of the package *MASS* (Venables and Ripley, 2002) in R environment (R Core Team, 2015).
The final MMI was then calculated on the validation dataset by averaging the scaled values (ranging from 0 and 1) of the final selected metrics, obtained from the calibration dataset. The MMI in the validation dataset was calculated for each patch and then averaged for each reach. The field observations of fine sediment were converted into an ordinal variable by calculating the relative proportion of fine sediment weight to the total weight of sediments in each patch and then averaged for each reach. Each observation was then assigned to an ordinal class following the same rules used in the calibration dataset (< 50% = class 0; 50%-66% = class 1; > 66% = class 2). Index values in the validation dataset were comprised from 0 (worst condition) to 1 (best condition) and they were therefore correlated to the fine sediment class for each reach through the Pearson correlation test. This process was repeated for metrics obtained at both mixed (i.e, family and genus) and family levels.

3. Results

In the calibration dataset, no relevant morpho-hydrological or chemical changes were observed over the entire sampling period. Overall, the sampling reach was characterized by streambed 7.5-10.3 m wide, cold (4.04°C ±0.03 SE), well-oxygenated (99.50 DO% ±4.45 SE) and oligotrophic (conductivity: 172 µS/cm ±0.003 SE; nitrates = 0.70 mg/l; SRP < 0.001 mg/l, BOD_5 = 3.09 mg/l) waters. Mean water depth was 14.4 cm (±0.66 SE), while the average flow velocity was 0.07 m/s (±0.003 SE). We could then exclude an influential effect of environmental parameters on artificial substrata. The average values of the final selected metrics in the three disturbance classes here considered are reported in Supplementary Material.
Before proceeding with the MMI algorithm, we excluded % EPT because in accordance with the protocol provided by Schoolmaster et al. (2013) it can be considered as a duplicate of 1-GOLD. We also excluded the abundance of Chironomidae and the ratio between Chironomidae and Diptera because despite being expected to increase with increasing disturbance they showed an opposite trend. As suggested by Schoolmaster et al. (2013), we excluded them in order to avoid confounding elements. For each of the 9 remnant metrics (Fig. 2), we obtained a potential MMI after the selection process and AIC values are reported in Table 3 and 4.

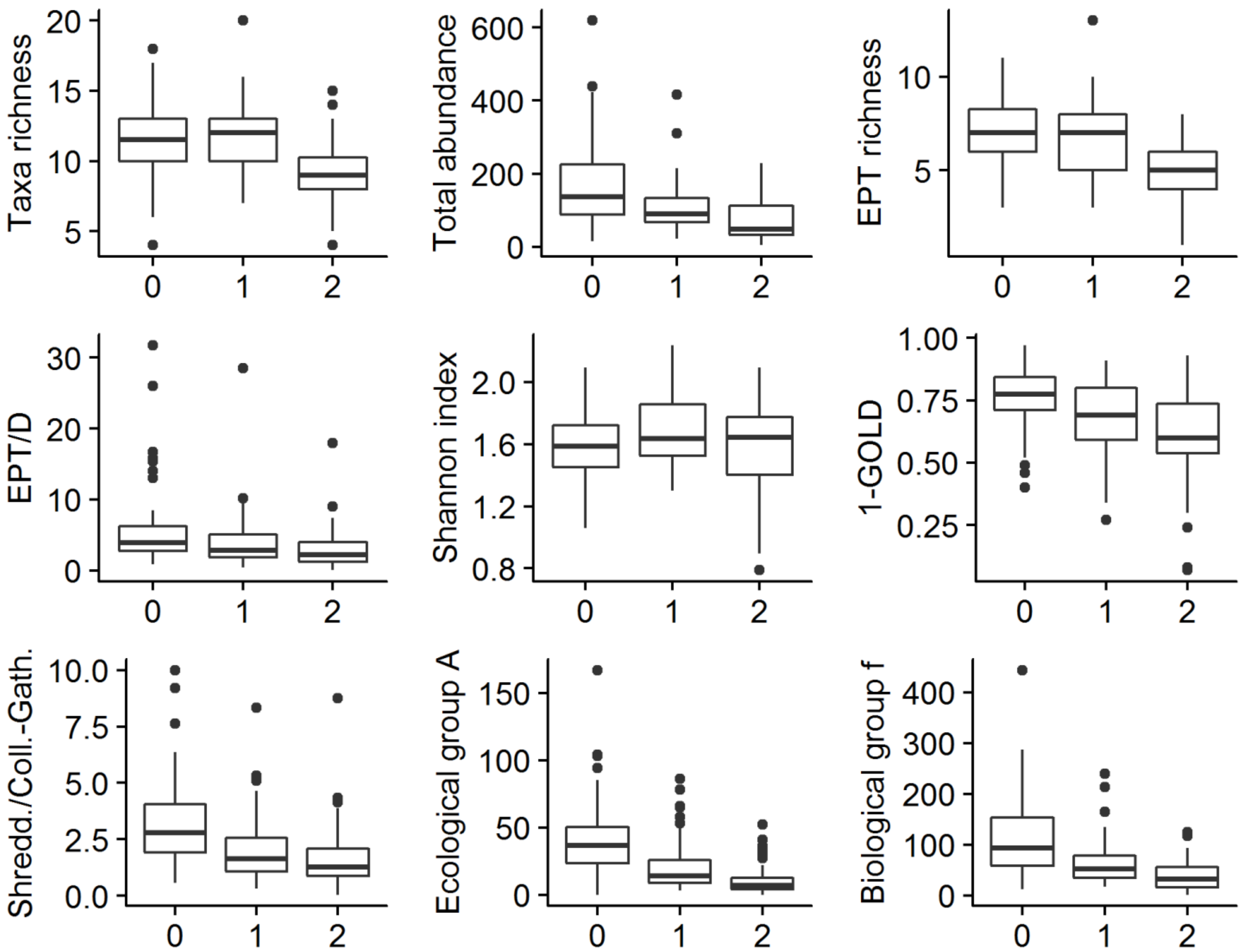

Figure 2. Boxplots represent the response of the candidate metrics to the sediment conditions in the calibration dataset:0 = WFS (0% fine sediment and 100% pebbles), 1 = MED (50% fine sediment and 50% pebbles), 2 = CLO (66% fine sediment and 33% pebbles).

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

Potential models	**AIC**	**ΔAIC**
1) Taxa Richness + EPT Richness + Ecological group A	244.42	**1.39**
2) Total abundance + 1-GOLD + EPT Richness + Taxa Richness	244.70	**1.67**
3) EPT Richness + Taxa Richness + Ecological group A	244.42	**1.39**
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	**1.67**
7) Shredders/Collector-Gatherers + Taxa Richness + EPT Richness + Biological group f	243.03	**0.00**
8) Ecological group A + Taxa Richness + EPT Richness	244.42	**1.39**
9) Biological group f + Taxa Richness + EPT Richness + Shredders/Collector-Gatherers	243.03	**0.00**

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

Potential models	**AIC**	**ΔAIC**
1) Taxa Richness + EPT Richness + Ecological group A	245.88	**1.24**
2) Total abundance + 1-GOLD + EPT Richness + Taxa Richness	247.86	3.22
3) EPT Richness + Taxa Richness + Ecological group A	245.88	**1.24**
4) EPT/D + EPT Richness + Taxa Richness + Biological group f	244.87	**0.23**
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	**0.31**
7) Shredders/Collector-Gatherers + Taxa Richness + EPT Richness + Biological group f	244.64	**0.00**
8) Ecological group A + Taxa Richness + EPT Richness	245.88	**1.24**
9) Biological group f + EPT Richness + Taxa Richness	244.98	**0.34**

For both the family-level and the mixed-level approach, the MMI assembly algorithm identified 7 out of 9 MMIs with ΔAIC < 2. MMIs with values of ΔAIC < 2 are judged to have substantial support, and should be considered as viable alternatives to the model with the lowest AIC. In other words, any MMI can be chosen from the set of models with ΔAIC < 2 without relevant loss of predictive power. Starting from this theoretical background, we preferred to select the most parsimonious solution and we then chose the most recurrent model in both the mixed and family approaches as the final index, instead of creating weighted indices. In fact, a weighted index, including all the metrics composing the models with ΔAIC < 2, would have been more time-consuming, because a higher number of metrics should be calculated, without increasing the predictive power of the index itself. Moreover, it would have required calculating different indices, depending on the taxonomic resolution considered, since the final selected models were different for the two levels. Our final selected index then was that obtained from equation 1 (Table 3 and 4):

D ~ Taxa Richness + Ecological group A + EPT richness

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

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

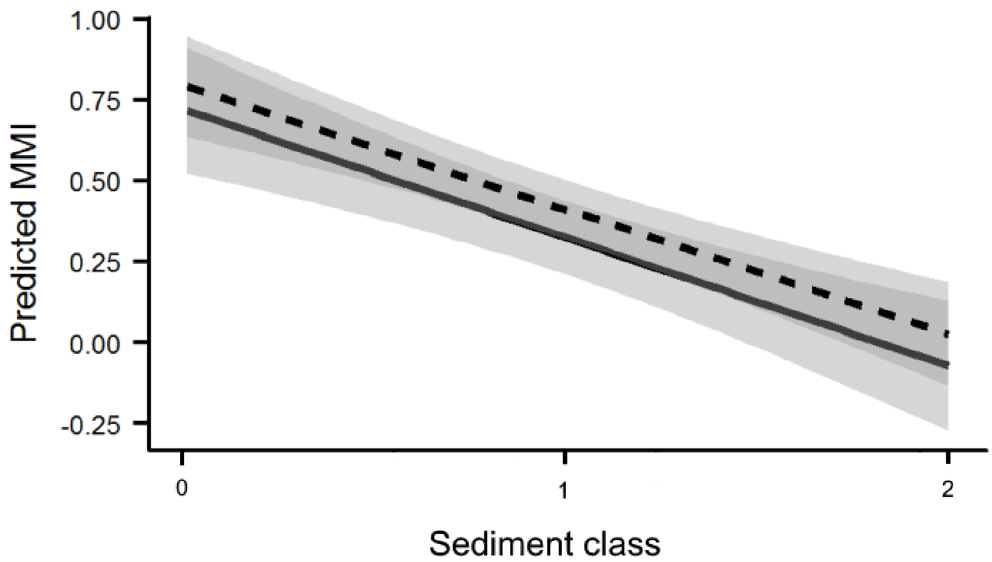

Figure 3. Predicted values and confidence intervals of MMIs (continuous line = family level approach; dashed line = predicted values derived from the mixed level approach) calculated against sediment weight in the validation dataset. Sediment weight is log-transformed for a better graphical representation.

4. Discussion

Despite problems associated to fine sediment being widespread, traditional biomonitoring indices were developed to detect chemical pollution and usually do not correlate with siltation (Angradi, 1999; Zweig and Rabení, 2001; Sutherland al., 2012). For this reason, this topic has recently received an exponential attention so that a new generation of biotic indices has been proposed for measuring the specific effects of fine sediment on macroinvertebrates, mostly in lowland lotic systems (Relyea et al., 2012; Extence et al. 2013; Turley et al., 2014, 2015; Murphy et al., 2015). To our knowledge, our work is the first attempt of building a stressor-specific multimetric index with a manipulative approach aiming at the evaluation of the effect of fine sediment on macroinvertebrates in alpine streams. We are aware of the limitations associated with a manipulative approach, but we are also confident that our experiments resulted highly comparable with real conditions of Alpine streams. In particular, we are confident that the use of sand in the calibration experiment as a proxy of fine sediments could represent a good compromise in Alpine streams. In fact, we had to face some critical issues in the construction, displacement and removal of our traps. It would have been extremely difficult to achieve a similar experimental design using sub-sand fractions, because of high flow velocities and great tractive forces that characterise Alpine running waters. This index may thus represent a promising tool for future biomonitoring assessments for two main reasons: i) using compositional and functional metrics we can overcome the taxonomic constraints intrinsically present in biotic indices (Friberg et al., 2011); ii) this index is highly specific for alpine streams, which represent peculiar ecosystems in which unnatural fine sediment deposition represents one of the main causes of impairment (Wohl, 2006).

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

In this study, three metrics were retained for their integration into a multimetric index evaluating the impacts of siltation in alpine streams. These were diversity metrics, i.e. total taxa richness and richness in Ephemeroptera, Plecoptera and Trichoptera taxa, and a functional metric, i.e. abundance of rheophilous taxa preferring coarse substrata, typical of oligotrophic, alpine habitats (Ecological group A sensu Usseglio-Polatera et al., 2000). Similar results were obtained by Larsen et al. (2011), who observed a negative effect of fine sediment on both diversity and functional metrics. Our results clearly demonstrated how the combination of different categories of metrics, i.e. diversity and functional metrics, reveal the effect of siltation on biotic communities. This is in accordance with literature (Barbour et al., 1996, 1999; Klemm et al., 2003; Bonada et al. 2006; Hering et al. 2006), since, by combining different categories of metrics, the multimetric assessment is regarded as a more reliable tool than assessment methods based on single metrics. Furthermore, the most relevant combination of appropriate metrics thus consisted of three out of the nine metrics tested. In accordance with literature (Menetrey et al. 2011; Schoolmaster et al. 2013), this result shows that the selection of the best combination did not include the maximal number of metrics.

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

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

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

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

5. Conclusions

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

Aknowledgements

We are grateful to Elisa Falasco, Anna Chiara Eandi, Davide Giuliano, Sabrina Mossino and Ilaria Zanin for their help in the field work and in laboratory analysis. This work was realized within the framework of Italian MIUR (Ministry of Education, Universities and Research) - PRIN NOACQUA project.

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