ENVIRONMENTAL STRESSOR GRADIENTS HIERARCHICALLY REGULATE MACROZOOBENTHIC COMMUNITY TURNOVER IN LOTIC SYSTEMS OF NORTHERN ITALY

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Abstract

Environmental stressors present a hierarchical influence on freshwater organisms. This study investigates the hierarchy of environmental stressor gradients, which regulate the composition of instream macroinvertebrate communities of northern Italy (Po Valley and the southeastern Alps). Species and environmental data were derived from 585 monitoring sites. Environmental parameters were split into three groups, describing i) ecoregional, ii) hydromorphological and iii) water quality attributes. Partial Redundancy Analysis (partial-RDA) was used to hierarchically rank the group effects, which were expressed as unique (group-specific) and joint effects (of two groups together). Overall, ecoregion explained more variance (30.2%) than hydromorphology (24.8%) and water quality (22.3%). Unique effects were generally low, but ecoregional unique effects were twice as high as those of the other groups. The analysis of single environmental variables highlighted significant effects of anthropogenic impact related to the substrate size composition, riparian vegetation, flow conditions, and *Escherichia coli* (surrogate descriptor of organic-fecal pollution). Such stressor hierarchies can support biodiversity conservation plans, while the high joint effects of stressor groups suggested the need for combined management activities, addressing the respective stressors and stressor groups in concert. Management measures addressing only one stressor group isolated from others are likely to be less effective, or even ineffective.

Keywords gradient analysis · human impact · partial-RDA · CANOCO · Biodiversity conservation plans

Introduction

The intensification of agriculture, mining and industry, the expansion of urban systems, deforestation and climate change during the recent decades have caused a significant alteration of aquatic ecosystems and especially of rivers (Gregory, 2004; Verdonschot et al., 2013). Rivers are usually the first systems affected by anthropogenic impact because: a) they are subjected to pollution from point and non-point sources (Carpenter et al., 1998; Khun et al., 2012), and b) they are usually modified for flood protection, flow regulation, and increased water uses (e.g. domestic use, irrigation, hydroenergy, transportation) (Nilsson et al., 2005; Doledéc & Bernhard, 2008; Elosegi & Sabater, 2013).

The alteration of physical-hydraulic properties and the degradation of the water quality of rivers have an immediate impact on aquatic communities leading to a decline in biodiversity, and alteration of their structural and functional composition (Ward et al., 1999; Ward & Tockner, 2001; Cortelezzi et al., 2013). The communities of benthic macroinvertebrates are considered extremely sensitive to such changes and for this reason they can provide significant information about the biological quality and ecological status of rivers (Armitage et al., 1983; Barbour et al., 1996; Springe et al., 2006; Haslett, 2007). Macroinvertebrates perform a wide range of essential functional roles in the world's freshwater ecosystems (e.g. as herbivores, predators, decomposers, parasites etc) and they also constitute a rich food source for organisms at higher levels of the food web. Because of these biological (functional) roles, they are increasingly being recognized as providers of ecosystem services that have significant measurable economic values, such as dung degradation, pest control and/or nutrition for other wildlife (Losey & Vaughan, 2006; Haslett, 2007). A large number of macroinvertebrate species in Europe are under severe threat of extinction or are already extinct due to ecosystems disturbance by anthropogenic activities (Haslett, 2007; Feld et al., 2011). International conventions, such as the 2010 biodiversity target set by a pan-European initiative to “halt the loss of biodiversity by 2010” (EEA, 2007) have so far not had the desired effect in reversing these conditions, which pose a serious future threat to human society if essential goods and ecosystem services are irreversibly lost (Feld et al., 2011).

Gradient analysis is a suitable method for analyzing the effects of various environmental stressors on macroinvertebrates (Ter Braak, 1986; Ter Braak & Prentice, 1988). This method is commonly used in community ecology to relate the abundance of various species with important environmental gradients or their closely correlated surrogates. Many studies have focused on the analysis of natural environmental and spatial gradients affecting benthic community composition. Of particular interest are those studies, which identify environmental (stressor) gradients partly or fully regulated by anthropogenic interventions related to land-uses (Allan, 2004; Utz et al. 2009), hydromorphological conditions (Carter & Fend, 2001; Merigoux & Doledec, 2004; Bonada et al., 2007) and water quality (Livingstone et al., 2000; Sandin & Hering, 2004). Gradient analysis has to address the problem that joint effects of several or many natural environmental covariates (Feld & Hering, 2007) can not be easily separated. Thus, the studies that incorporate anthropogenic effects have to consider problems associated with: a) higher covariation of anthropogenic and natural gradients in the landscape (difficulty in distinguishing between pure natural and pure anthropogenic gradients), b) the existence of more complex scale-dependent mechanisms, c) nonlinear responses and d) difficulty in separating present-day from past influence (Allan, 2004). These limitations clearly show the difficulties in describing the effects of environmental stressors on macroinvertebrate communities in the watersheds of developed countries since natural gradients are strongly influenced by anthropogenic impact. They additionally lead to significant limitations for planning restoration and management measures where the challenge is to identify and prioritize the main impacts at appropriate scales for implementing effective management practices. Consequently, restoration schemes need to be based on hierarchical analyses. Based on this hierarchy (and possible interaction) of the underlying mechanisms: one stressor may be most important to another, which implies that important stressors have to be mitigated first (Feld et al., 2011). Thus, the development of management practices for the biodiversity conservation of macroinvertebrates in developed countries needs more robust tools that can support the interpretation of their response to natural but also to human driven environmental stressors.

The aim of this study is to develop a hierarchical ranking scheme for environmental gradients, encompassing both anthropogenic impact and natural covariates, and to analyse their effects on the composition of instream macroinvertebrate communities in mountainous streams of the south-eastern Alps and plains of Northern Italy. The two ecoregions lie next to each other and share a dense and extensive hydrographic network consisting of both natural and artificial water pathways. The selection of the specific study area is of great importance because it can provide a general aspect about the driving factors, which regulate the macroinvertebrate communities of the lotic systems in the developed countries. The results of the study can also provide a strong basis for developing management practices for biodiversity conservation.

Materials and Methods

Study area and sampling sites

The study area is situated in Northern Italy and includes the lowland regions of the Po Valley, the foothills and the high altitude areas of the south-eastern Alps (administrative units of Veneto, Trentino-Alto Adige, and Lombardy). The study area spans from 9.51–12.53 decimal degrees West (~240 km) and from 45.45–47.04 decimal degrees North (~180 km) and covers a total area of approximately (56×10^3 km^2) (Fig. 1a).

Altogether, data from 585 river monitoring sites were used in this study, covering a wide range of lotic habitats at different altitudinal zones, different forms of land use and different ecohydrological conditions (Fig. 1a). The extensive hydrographic network consists of natural streams and rivers and artificial water pathways, the latter being mainly in the lowlands (Fig. 1b). Water flow is directed southwards in the uplands and eastwards in the lowlands. Point source pollution at upland sites is limited to organic waste originating from small urban settlements and livestock farms. The lowlands are characterized by a high degree of urbanization and intensive agriculture, with a dense network of artificial ditches regulating the drainage and flow conditions (Castaldelli et al., 2013).

Data collection

Macroinvertebrates were sampled using a 1×1 mm-mesh kick-net within a 50 m reach of each stream covering the whole wetted river cross section between both banks. Sampling was performed during the period 2003–2013 (mid-April to mid-October) at 2–4 sampling events during the same year for each sampling site. The specimens were preserved in 90% alcohol and they were analyzed and classified using a stereo-optical microscope (magnification×50) and an optical microscope (magnification×400). The classification was made up to the level of genus for the taxa belonging to Plecoptera, Ephemeroptera, Odonata, Tricladida and Hirudinea, and up to the family level for the taxa belonging to Bivalvia, Coleoptera, Crustacea, Diptera, Gastropoda, Gordioida Heteroptera, Oligochaeta and Trichoptera. Overall, 98 taxa were identified, with abundances averaged from the 2–4 seasonal samples per site. Rare taxa (frequency <1% of all sites) were excluded from the analysis, resulting in 68 taxa (Table 1). The coarse taxonomic resolution (mixed family and genus level) is not considered problematic in bioassessment studies *per se*, but can significantly influence biodiversity analysis (Waite et al., 2004). For this reason, biodiversity is not included in the analysis and it is only discussed when is necessary from a macroscopic point of view.

A total of 31 environmental parameters were derived for each sampling site (Table 2). Electric conductivity, pH, dissolved oxygen and water temperature were measured *in situ* during invertebrate sampling using a handheld instrument Y.S.I. (Yellow Spring Instruments Inc.). The COD (Dichromate Reflux Method), BOD_5 at 20°C, phosphorus, ammonia and nitrate nitrogen were measured according to APHA (2005). *Escherichia coli (E. coli)* was measured in UFC/100 ml according to MPN method. The remaining environmental parameters represent geographic, hydromorphological and vegetation characteristics (Table 2).

Environmental variables were assigned to three groups representing distinct environmental features (Table 2): Group 1 - "ecoregional gradients" consists of geographic, climatic and vegetation parameters; Group 2 - "hydromorphological gradients" consists of substrate grain size and stream dimensions parameters; Group 3 - "water quality gradients" consists of water quality parameters. Collinear variables with a variance inflation factor VIF>8 were excluded from the analysis (Zuur et al. 2007).

Both taxa and environmental parameters were transformed to reduce normality departures following the methods used by Feld & Hering (2007). Abundance of each taxon (ind. m^{-2}) and environmental parameters, which are not ratios/percentages were transformed using log (x+1). The arcsin $(x/100)^{0.5}$ was used for ratios/percentages while the logit transformation (Warton & Hui, 2011) was also tested but it was not selected for two reasons a) logit transformation does not return results when the ratio is 0 or 1 (100%), b) arcsin transformation showed better performance in general in the procedures which were followed in this study. Ordinal variables and pH were not transformed.

Statistical analysis - Ordination methods and variance partitioning

Detrended Correspondence Analysis (DCA) was used to select the appropriate response model for subsequent direct gradient analysis (ter Braak & Smilauer, 2002; Lepš & Šmilauer, 2003). For the gradient analysis, both Redundancy Analysis (RDA) (linear method) and Canonical Correspondence Analysis (CCA) (unimodal method) were applied on the data, as DCA revealed that the dominant gradient length was between 3 and 4 (Lepš & Šmilauer, 2003). RDA and CCA showed similar results, but RDA explained more variance in the species-environment relationship. Therefore, only RDA results are going to be presented.

Separate RDAs were applied for each group of descriptor variables of Table 2. Each RDA was performed targeting one environmental feature group after partialling out the effects of the parameters of the remaining groups, which were used as co-variables (i.e. partial RDA). Partial RDA was performed for each possible combination of targeted descriptor and co-variables using CANOCO 4.5, based on species correlations and standardized species scores (ter Braak & Smilauer, 2002). Significant descriptors for each group were identified using CANOCO's forward selection procedure and Monte Carlo permutation test (499 permutations) (Feld & Hering, 2007) (Table 2).

A variance partitioning scheme (Borcard et al., 1992; Liu, 1997) was applied for each group of variables based on the overall variance explained by the partial RDAs (sum of all canonical eigenvalues). This procedure allowed the distinction between unique effects (i.e. the variance explained by a single group of variables), joint effects (i.e. the variance jointly explained by variables of two or three groups), and unexplained variance.

Results

Unique effects of ecoregional, hydromorphological and water quality gradients

Overall, the proportion of variance uniquely explained by the three groups of variables was low. Expressed as the sum of all canonical eigenvalues of partial-RDA on taxa, only 5.8%, 2.9% and 2.9% were explained by ecoregional, hydromorphological and water quality variables, respectively. Detailed results of the ordination analysis step by step are given in Tables S.1 and S.2 of the Supplementary Material.

Ecoregional gradients (Group 1)

The first ecoregional gradient is formed by geographic, climatic and vegetation characteristics and explains the majority of variance in the taxa-environment relation (55.8%) (Fig. 2a). Along the first RDA axis, warmer lowland sites with a higher coverage of aquatic vegetation on the right hand side are separated from colder upland sites with forest-dominated riparian vegetation on the left hand side (Fig. 2a and b). The second axis (25.8% variance explained) represents a strong longitudinal gradient (i.e. defined by the longitude and not by the distance from the source).

The corresponding taxa plots confirm the ecoregional transition along the first RDA axis (Fig. 2c-f). The majority of Plecoptera and Ephemeroptera taxa primarily occur at upland sites and are separated from Heteroptera, Odonata, Gastropoda, Bivalvia, Crustacea and Hirudinea taxa, all of which preferably occur at lowland sites. The strong longitudinal gradient along axis 2 separates western from eastern sites, which was found to particularly influence the occurrence of insect taxa (Fig. 2c, d).

Hydromorphological gradients (Group 2)

Two major hydromorphological gradients are observed (Fig. 3a). The first of which (50.4% variance explained) corresponds well with substrate grain size and ordinates sites dominated by finer sediments on the left hand side. Stream size (morphometry) is reflected by the second gradient (18.8% variance explained), thus separating sites along a gradient of stream dimension.

Along the granulometric gradient, many insect taxa (Plecoptera, Ephemeroptera, Trichoptera, Diptera) and Hirudinea show a clear preference for cobbles and gravels, while Gastropoda are particularly related to sites dominated by fine substrata. Overall, 45 taxa out of 68 show a preference to coarser substrata. A more gradual turnover is found along axis 2 showing weak effects of stream dimensions on specific taxa (except some Ephemeroptera and Hirudinea, which seem to prefer smaller upland streams and smaller lowland drainage canals, respectively) (Fig. 3c-f).

Water quality gradients (Group 3)

The parameters of group 3 reveal a pollution gradient along the first RDA axis (33.6% variance explained) mainly described by *E. coli*, which in turn is related to organic-fecal pollution (e.g. urban and livestock wastes) while RDA axis 2 reveals an oxygen depletion gradient explaining 27.3% of the variance (Fig. 4a). Sites, most impacted by organic pollution and oxygen depletion are distributed in the upper left while the least impacted sites can be found at the lower right of the ordination plot (Fig. 4b).

The majority of insects (>80%) are found at less polluted sites (Fig. 4c, d). Some exceptions appear in the case of Ephemeroptera (*Ephemerella*), Coleoptera (Haliplidae), Diptera (Chironomidae, Simuliidae, Blephariceridae), Trichoptera (Limnephilidae) and Odonata (*Orthetrum*). On the other hand, the majority of non-insect taxa (>59%), and especially the Gastropoda, Hirudinea and Gordioida are found at more polluted sites (Fig. 4e, f). The oxygen depletion gradient do not provide general indications about the response of the major taxonomic groups but reveals strong oxygen effects on some taxa such as *Cloeon* and *Helobdella*, which are abundant in less oxygenated environments or *Baetis*, *Calopteryx*, *Platycnemis*, Ceratopogonidae, Gammaridae and *Piscicola*, which are abundant in more oxygenated environments).

The revised water quality standards of EAP Task Force/OECD (2007) approved by UK DEFRA were also used in order to have a better understanding about the overall water quality of the streams in the study area. According to these standards the values of water quality parameters are grouped in five quality classes (I: very high, II: high, III: moderate, IV: low, V: very low quality). Using the standards on the parameters of Group 3, which participated in the gradient analysis, it was found that the 28.2% of sampling sites presents very low water quality (V class) only due to *E. coli* (Table 3). Table 3 verifies the results of gradient analysis, which indicated that *E. coli* was the most important factor of Group 3 in regulating taxa response to pollution.

Variance partitioning of environmental covariates

The marginal (λ-1) and conditional (λ-A) effects of each covariate in the null-model (RDA with all covariates) show a higher significance for latitude, substrate grain size, riparian vegetation, flow conditions and organic-fecal pollution (i.e. *E. coli*) to control the turnover of invertebrates taxonomic composition (Fig. 5a). The conditional effects (λ-A) suggest that site-specific characteristics are effectively joined to the geographical attributes of latitude and longitude (Fig. 5a).

The unique effects of ecoregional parameters are almost double as high as those found for hydromorphological and water quality parameters (Fig. 5b). Overall, unique effects are generally low (11.6% in total), if contrasted against the partial joint effects of the groups of variables (Fig. 5b). Joint effects ranged 20–25% in individual analyses and averaged roughly 24.9% in the full RDA using all descriptor groups together (i.e. without co-variables) (Fig. 5b, c). The sum of unique and partial joint effects provides the following ranking scheme: ecoregion (30.2%) > hydromorphology (24.8%) > water quality 3 (22.3%) (Fig.5b).

Discussion

Ecoregional gradients

The effect of latitude, which indirectly includes the effects of altitude and consequently climate in our study area, was found to be the most significant descriptor of community composition. Invertebrate communities are controlled both directly and indirectly by climate (Poff et al., 2010). Many macroinvertebrates, mainly insects, in their adulthood live outside the water and their survival and reproduction is strongly associated to climatic conditions while any climate changes would lead to intense local community turnovers, communities relocation or geographical expansion (Nooten et al., 2014; Rasmann et al., 2014; Aluja et al., 2014). Climate, in combination with other factors (e.g. geology) influences the type and production of terrestrial and aquatic vegetation, which in turn influence the sources and types of organic autochthonous and allochthonous materials in the river continuum and their rate of decomposition. These are the main factors, which influence the feeding traits of communities and consequently the taxonomical composition (Sabater et al., 2008; Fernandes et al., 2012; Rugenski & Minshall, 2014).

The effect of riparian vegetation as a driving force to influence community composition was ranked third. The significance of this parameter has also been pointed by Martel et al. (2008) who suggested that larger, longer-lived and possibly more specialized taxa, in particular trichopterans, were more vulnerable to forestry impacts and were replaced by smaller, multivoltine, less specialized invertebrates, such as chironomids. Stone & Wallace (1998) after Noel et al. (1986) also pointed that the reduction of riparian vegetation (through deforestation) may affect the energy flow in the system since lower shading and consequently increase of incident solar radiation may lead to higher water temperatures and aquatic vegetation production. This finding was also evident in our study since riparian vegetation was negatively correlated with aquatic vegetation coverage. Such alterations are responsible to food base changes accompanied by respective changes of community composition, which favor scrappers and filterers when riparian vegetation is reduced (Sabater et al., 1997). Feld et al. (2011) also pointed the positive effects of riparian buffer zones on stream organisms since they reduce fine sediment entry and nutrient-pesticide inflows.

The effect of water temperature, which is also influenced by shading due to riparian vegetation can be associated to a) the tolerance/sensitivity of invertebrates to thermal effects and b) to its interaction with feeding sources and specific feeding traits of species. In the first case, the literature on thermal tolerance is quite restricted and in many cases, clear interpretations cannot be made due to the interference of other factors. A significant contribution to this subject was made by Stewart et al. (2013) who provided the following ranking in terms of upper thermal tolerance Ephemeroptera <

Decapoda < Trichoptera < Mollusca. In the second case, observations from Canadian and Norwegian streams made by Taylor & Andrushchenko (2014) showed that litter decomposition sometimes proceeds faster in small, cool tributaries than in warm and wide rivers because cold-stenothermal, leaf-shredding invertebrates (e.g. *Leuctra* sp.) were more abundant in the cool streams. Similar findings were observed by Bruder et al. (2014) when compared litter decomposition and shredders activity between a tropical and a temperate stream with significantly different water temperatures.

Notably, community composition was also affected by the gradient of longitude. Water flow in the upland regions is directed from north to south, indicating a corresponding habitat connectivity with the downstream watersheds, but not with their adjacent watersheds east- or westwards. Thus, the boundaries of upland watersheds seem to act as habitat barriers for upland communities. Furthermore, upland watersheds of the study region represent different zones of stream ecosystems, which are mainly distinguished into kryal (glacier-melt dominated), krenal (groundwater-fed) and rhithral (seasonal snowmelt dominated). These types create complex mosaics due to the high heterogeneity in the climate, geomorphology and hydrology of alpine and subpolar environments (Gislason et al., 1998; Burgherr & Ward, 2001). Additionally, the largest portion of lowland sites correspond to clusters of sites located in different systems of drainage canals. Drainage networks of different territories act as artificial lowland water basins, which create isolated patches defined by the extent of the drainage system. These systems are extended from west to east and discharge water to large canals and rivers flowing to the same direction defined by the Po river. The spatial extent of each drainage system creates respective barriers along longitude for the lowland communities. Both, the upland and lowland longitudinal changes in community composition can be linked to the general effect called "isolation or accessibility of the sampling site" (Sanchez-Fernandez et al., 2008; Koperski, 2010).

Hydromorphological gradients

Among hydromorphological variables, substrate grain size significantly affected community composition with 45 out of 68 taxa showing a preference to coarser substrata. Rabení et al. (2005) suggested that finer substrate composition can lead to a decline in species richness and diversity, which is supported by our findings. The preference for coarse substrata may also be related to a) the higher taxonomic resolution of most benthic insects compared to other groups such as Oligochaeta and b) the higher mobility and high microhabitat heterogeneity inside coarser substrata which can act as protective mechanism against enemies like predator invertebrates and fishes. The work of Jähnig & Lorenz (2008) showed that artificially driven substrate variability in restored rivers-channels resulted in higher beta diversity.

The flow conditions also had a significant contribution verifying the findings of Bonada et al. (2007) who found that in permanent flow regimes (as in the majority of streams of our study), the habitat stability plays a crucial role for the communities composition. The significant role of habitat stability has also been identified by Castella et al. (2001) for glacier-fed streams from different European territories including the Alps. According to Doisy & Rabeni (2001) flow also played a significant role on benthic food sources.

The secondary effects of stream dimensions which were observed in our study may also be related to the factor of habitat stability since small natural streams are more vulnerable to drought/flood effects (Milner et al., 2001) while small drainage canals may present periodical flow intermissions due to water abstraction (Dewson et al., 2007).

Water quality gradients

The analysis of the water quality parameters indicated, indirectly through *E. coli*, the strong effects of organic-fecal pollution regulated by urban and livestock wastes, and manure-based fertilization practices. *E. coli* is not harmful to invertebrates but it is a surrogate of other harmful parameters while aquatic systems with significantly high *E. coli* concentrations usually present generalized quality degradation. The general observations of taxa response to pollution correspond adequately to the sensitivity/tolerance classification of taxa given by Armitage et al. (1983) and Ghetti (1997) and by the observations of other authors from similar studies (Bottarin & Fano, 1998; Feld & Hering, 2007).

The remaining water quality variables formed a mixed oxygen depletion gradient reflected by respiratory adaptations of several taxa related to 'oxy-regulator' or 'oxy-conformer' behaviours (Nagell, 1977). For example, the tolerance of *Cloeon* and *Helobdella* to oxygen depletion verifies their oxy-regulator behavior observed by Nagell (1977) and Pohle & Hamburger (2005). On the other hand, taxa such as *Baetis*, *Calopteryx*, *Platycnemis*, Ceratopogonidae, Gammaridae and *Piscicola* showed a more oxy-conformer behaviour (their internal oxygen concentrations reflect the external environment) (Olson & Rueger, 1968; Miller, 1993; Connolly et al., 2004).

Additionally, trends of oxygen depletion were observed in many sampling sites where the presence of *E. coli* and consequently organic-fecal pollution is suppressed. These observed trends of oxygen depletion may be associated to naturally driven eutrophication trends. The latter suggests that part of the water quality degradation may result from natural causes and not necessarily from human sources. Environments with favorable climatic conditions and available nutrient sources could lead to overproduction of aquatic vegetation and sequestration of dead organic materials justifying such trends. Of course, the probability of human intervention cannot be excluded since nutrient sources may be associated to the use of inorganic fertilizers and/or atmospheric nitrogen deposition (Bergström & Jansson, 2006; Rabalais et al., 2010).

Use of gradients ranking to develop management plans

The development of ranking schemes for gradients or gradients groups are extremely important if anthropogenic interventions are necessary to confront natural threats. For example, if changes in flow and hydraulic conditions of a river have to be performed in order to reduce flood events, additional interventions such as artificial increase of riparian vegetation and additions of artificial coarse substrates could reduce the negative impact of flow changes on biological quality. The ranking scheme can also be used in order to develop management plans for biodiversity conservation/improvement based on the most important environmental parameters taking into account the cost and the effective duration of intervention. For example, if space is available in the riparian area, riparian vegetation enhancement is probably much cheaper and has a longer-duration effect than instream interventions on substrate conditions. Interventions on substrate conditions must be followed by additional interventions in flow conditions in order to be successful with a more permanent effect. For example, it was observed that excessive fine sediment entry from adjacent croplands upstream of a restored system counteracted physical habitat improvements (Larson et al., 2001; Levell & Chang, 2008; Moerke et al., 2004).

The procedure of variance partitioning highlighted the dominance of joint effects of gradients indicating that the interpretation of taxa response to environmental gradients may lead to erroneous conclusions when typological issues remain unconsidered. This was for sure an expected finding since the changes of one group of descriptors usually lead to changes of descriptors in other groups. The fact that the joint effects of environmental feature groups were much higher than their unique effects may turn out to be an advantage for biodiversity conservation planning. This can be justified by the fact that combined interventions of low intensity and lower cost in different types of environmental attributes may lead to more intense changes of community composition due to synergies in comparison to isolated interventions of higher intensity and cost. This finding can justify the observations of Feld et al. (2014) who found small changes of invertebrate communities of lowland rivers due to isolated hydromorphological changes.

Whilst, the water quality group of parameters showed smaller effects than the ecoregional and hydromorphological ones, it is important not to be neglected in restoration interventions. For example, if organic pollution or eutrophication is present in a river stretch that is subjected to restoration, the pollution must be reduced or mitigated before physical habitat and geomorphological processes are being restored. Several restoration studies showed that ongoing water quality problems upstream of a site were the possible causes of restoration failure (Pretty et al., 2003; Roni et al., 2008; Palmer et al., 2010; Feld et al., 2011). In other words, a poor medium “water” flowing in a good matrix is probably an insufficient precondition for recovery. Conversely, if the water quality is sufficient for recovery, it is the chief geomorphological processes or physical structures that may hinder recovery (Shields et al., 2008; Feld et al., 2011). Considering the above, the ranking of parameters in Group 3 (*E. coli*>COD>NO_3>P>BOD_5>NH_4>DO) and the results of Table 3 can set priorities in applying restoration measures to reduce the effects of pollution. Thus, it is easy to select which sites have priority for restoration based on the most important pollution indicators and their degree of severity. For example, there are 165 sites, which belong to V severity class (V-sc) due to *E. coli* (Table 3) (the strongest water pollution gradient) but some of these sites have also another one or more parameters with values belonging to V-sc class. Combining the seven water quality parameters of Table 3, it was found that there are 2 sites with five water quality parameters belonging in V-sc, 4 sites with four water quality parameters belonging in V-sc, 14 sites with three water quality parameters belonging in V-sc, and 41 sites with two water quality parameters belonging in V-sc. The number of water quality parameters belonging in V-sc sets the first base for setting restoration priorities. The second step considers the sites that present the same number of water quality parameters belonging in V-sc, where in this case the priority is regulated by the ranking scheme of the water quality parameters.

The overall analysis provided a representative method for building hierarchical ranking schemes of environmental stressors at large-scale case studies in order to be used for building effective management plans for biodiversity conservation. It has to be mentioned that the analysis was performed based on a large and robust dataset of macroinvertebrates and environmental parameters but lacks a connection with other biological quality attributes such as the response of fish populations in the respective lotic systems. Thus, ranking schemes have to be expanded even to other biological indicators prior to restoration interventions.

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Tables

Table 1 Observed taxa of macroinvertebrate groups and taxonomic level.

Group		Taxonomic level
Bivalvia	F	Pisidiidae
		Sphaeriidae*
		Unionidae
Coleoptera	F	Helodidae*
		Dytiscidae
		Elmidae
		Hydraenidae
		Hydrophilidae*
		Haliplidae
Crustacea	F	Asellidae
		Gammaridae
		Palaemonidae
		Niphargidae
Diptera	F	Dixidae*
		Simuliidae
		Stratiomyidae*
		Chironomidae
		Anthomyiidae
		Athericidae*
		Ceratopogonidae
		Empididae
		Tabanidae*
		Limoniidae
		Blephariceridae
		Psychodidae
		Tipulidae
Gordioida	F	Gordiidae
Heteroptera	F	Corixidae
		Naucoridae
		Nepidae*
Tricladida	G	*Crenobia*
		*Dendrocoelum**
		Dugesia
		*Polycelis**
Ephemeroptera	G	*Caenis**
		*Habrophlebia**
		*Paraleptophlebia**
		Baetis
		Ephemerella
		*Habroleptoides**
		Cloeon
		Epeorus
		Rhithrogena
		Ecdyonurus
Gastropoda	F	Bithyniidae
		Valvatidae
		Ancylidae
		Lymnaeidae
		Neritidae*
		Physidae
		Planorbidae
		Viviparidae*
		Acroloxidae
Trichoptera	F	Brachycentridae*
		Hydropsychidae
		Philopotamidae
		Hydroptilidae
		Odontoceridae*
		Ecnomidae*
		Rhyacophilidae
		Polycentropodidae*
		Beraeidae*
		Glossosomatidae*
		Goeridae*
		Psychomyiidae*
		Leptoceridae*
		Limnephilidae
		Sericostomatidae
Hirudinea	G	*Batracobdella**
		Dina
		Erpobdella
		Glossiphonia
		Helobdella
		Piscicola
Odonata	G	*Calopteryx*
		*Cercion**
		Coenagrion
		Ischnura
		Orthetrum
		Platycnemis
Oligochaeta	F	Enchytraeidae
		Haplotaxidae
		Lumbriculidae
		Tubificidae
		Lumbricidae
		Naididae
Plecoptera	G	*Leuctra*
		*Chloroperla**
		*Dinocras**
		Dyctiogenus
		Isoperla
		Perla
		Perlodes
		Amphinemura
		Brachyptera
		Nemoura
		Protonemura
		*Rhabdiopteryx**

† F corresponds to Family and G corresponds to Genus.

*Rare taxa occurring in <1% of all sampling stations.

Table 2 Groups of environmental parameters, abbreviations, units, type of variable transformation and statistics.

Parameter	Unit	Transformation	Abbrev.	Min	Max	Mean	St.dev.	[1]Group
Longitude (WGS84 ellipsoid)	Dec. degrees	log(x+1)	long	9.51	12.53	11.66	0.8	1
Latitude (WGS84 ellipsoid)	Dec. degrees	log(x+1)	lat	45.45	47.04	45.96	0.55	1
Altitude	m a.s.l.	log(x+1)	alt	1	2027	411	532	-
Stream width	m	log(x+1)	width	0.5	55	7.6	8.2	2
Mean depth of the riverbed	cm	log(x+1)	meandep	5	150	34	21.1	2
Maximum depth of the riverbed	cm	log(x+1)	maxdep	7	220	56.9	36.6	-
[2]Pool area	%	$\arcsin(x/100)^{0.5}$	pool	0	90	9.4	15.7	-
[2]Riffle area	%	$\arcsin(x/100)^{0.5}$	riffle	0	100	20	28.5	-
[2]Run area	%	$\arcsin(x/100)^{0.5}$	run	0	100	70.6	38.1	-
[3]Rock cover (>350 mm)	%	$\arcsin(x/100)^{0.5}$	rock	0	80	7.7	15.1	-
[3]Boulders cover (350-100 mm)	%	$\arcsin(x/100)^{0.5}$	boulder	0	80	13	16.8	-
[3]Cobbles cover (100-35 mm)	%	$\arcsin(x/100)^{0.5}$	cobble	0	80	15.2	15.9	2
[3]Gravel cover (35-2 mm)	%	$\arcsin(x/100)^{0.5}$	gravel	0	70	9.5	12.5	2
[3]Sand cover (2-1 mm)	%	$\arcsin(x/100)^{0.5}$	sand	0	90	24.7	22.2	-
[3]Silt+clay cover (<1mm)	%	$\arcsin(x/100)^{0.5}$	siltc	0	100	29.8	33.5	2
[4]Water velocity - flow conditions	Ordinal	none	flow	1	7	3.5	1.7	2
[5]Retention of detritus	Ordinal	none	detritus	1	3	1.9	0.6	1
[6]Shading of the riverbed	%	$\arcsin(x/100)^{0.5}$	shade	0	100	12.1	24.4	-
[7]Type of riparian vegetation	Ordinal	none	rip_veg	1	7	3.4	2.04	1
[8]Aquatic vegetation cover	%	$\arcsin(x/100)^{0.5}$	veg_cov	0	100	17.2	27	1
[9]Anthropization	Ordinal	none	anthropi	1	4	2.5	0.9	-
COD	O_2 mg L^{-1}	log(x+1)	COD	0.5	96	10.2	11.5	3
BOD_5	O_2 mg L^{-1}	log(x+1)	BOD	0	22	1.9	2.1	3
Nitrate nitrogen	N mg L^{-1}	log(x+1)	NO3N	0	5	1.1	0.9	3
Ammonia nitrogen	N mg L^{-1}	log(x+1)	NH4N	0	15.3	0.4	1.1	3
Phosphorus	P mg L^{-1}	log(x+1)	PHOSP	0	2.7	0.1	0.2	3
Escherichia coli	UFC/100 mL	log(x+1)	COLI	0	260000	6911	26474	3
Water temperature	°C	log(x+1)	TEMP	0.1	32	13.1	7.4	1
pH	-	none	PH	5.2	10	7.9	0.6	-
Dissolved oxygen	mg L^{-1}	log(x+1)	DO	0.4	20.3	10	2.8	3
Electrical conductivity	μs cm^{-1}	log(x+1)	EC	12	1616	422	232	-

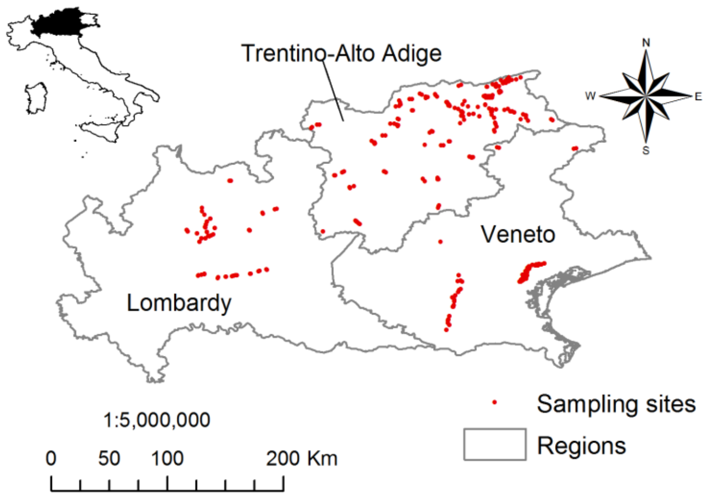
[1]Variables coded "-" not used for final analysis due to collinearity.
[2]Characterization of the watercourse surface (total sum of pool, riffle and run areas percentages equal to 100%).
[3]Substrate grain sizes (total sum of rocks, boulders, cobbles, gravels, sand and silt+clay percentages equal to 100%).
[4]Ordination according to: 1=undetectable/very slow, 2=slow, 3=medium and laminar flow, 4=medium and turbulent flow, 5=high velocity and laminar flow, 6=high velocity and turbulent flow, 7=very high velocity very turbulent flow.
[5]Ordination according to: 1=poor, 2=moderate and 3=high retention of detritus.
[6]The percentage ratio between the distance of trees canopy covering the stream from both sides versus stream width.
[7]Ordination according to: 1=absent, 2=herbaceous, 3=shrub-herbaceous, 4=shrub, 5=forest-herbaceous, 6=forest-shrub, 7=forest.
[8]The percentage coverage of macrophytes in the river bed.
[9]Ordination according to: 1=natural environment with no human presence, 2=natural environment with anthropogenic activities, 3=agricultural land and urbanized areas, 4=fully urbanized areas.

Table 3 Number of sampling sites categorized based on the five water quality classes of EAP Task Force/OECD (2007) for chemical parameters and, *E. coli*.

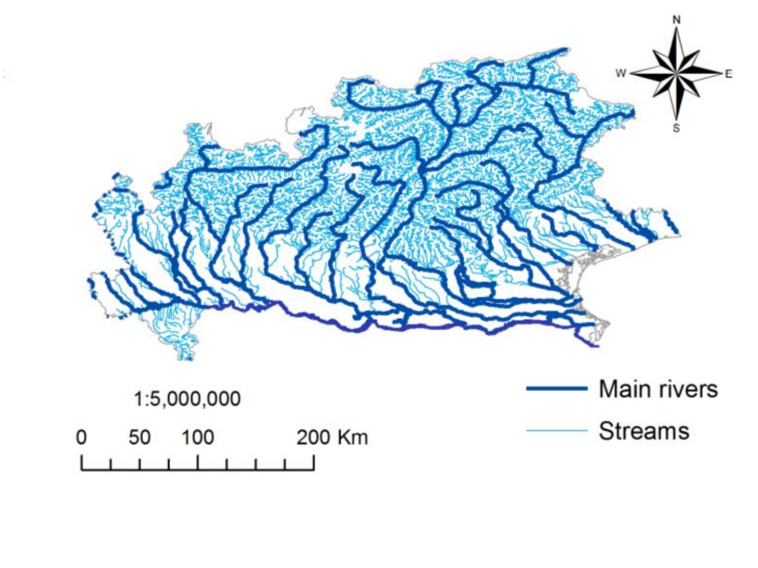
Parameter	Water quality class				
	I	II	III	IV	V
[1]DO	501	38	20	13	13
[2]BOD_5	504	54	6	6	15
[3]COD	248	69	146	41	81
[4]NO_3	344	216	25	0	0
[5]NH_4	426	66	31	52	10
[6]PO_4	292	95	125	62	11
[7]*E.Coli*	271	76	40	33	165

[1](I: ≥7, II: 7-6, III:6-5, IV:5-4, V: <4 mg L^{-1})
[2](I: ≤3, II: 3-5, III: 5-6, IV: 6-7, V: >7 O_2 mg L^{-1})
[3](I: ≤3, II: 3-7, III: 7-15, IV: 15-20, V: >20 O_2 mg L^{-1})
[4](I: ≤1, II: 1-3, III: 3-5.6, IV: 5.6-11.3, V: >11.3 mg N L^{-1})
[5](I: ≤0.2, II: 0.2-0.4, III: 0.4-0.8, IV: 0.8-3.1, V: >3.1 mg N L^{-1})
[6](I: ≤0.05, II: 0.05-0.1, III: 0.1-0.2, IV: 0.2-0.5, V: >0.5 mg P L^{-1})
[7](I: ≤500, II: 500-1000, III:1000-1500, IV:1500-2000, V: >2000 UFC/100 mL)

FIGURES



a)



b)

Fig. 1 a) Sampling sites (locations overlap) and b) hydrographic network in the study area. (source: http://www.eea.europa.eu/data-and-maps/data/european-river-catchments-1).

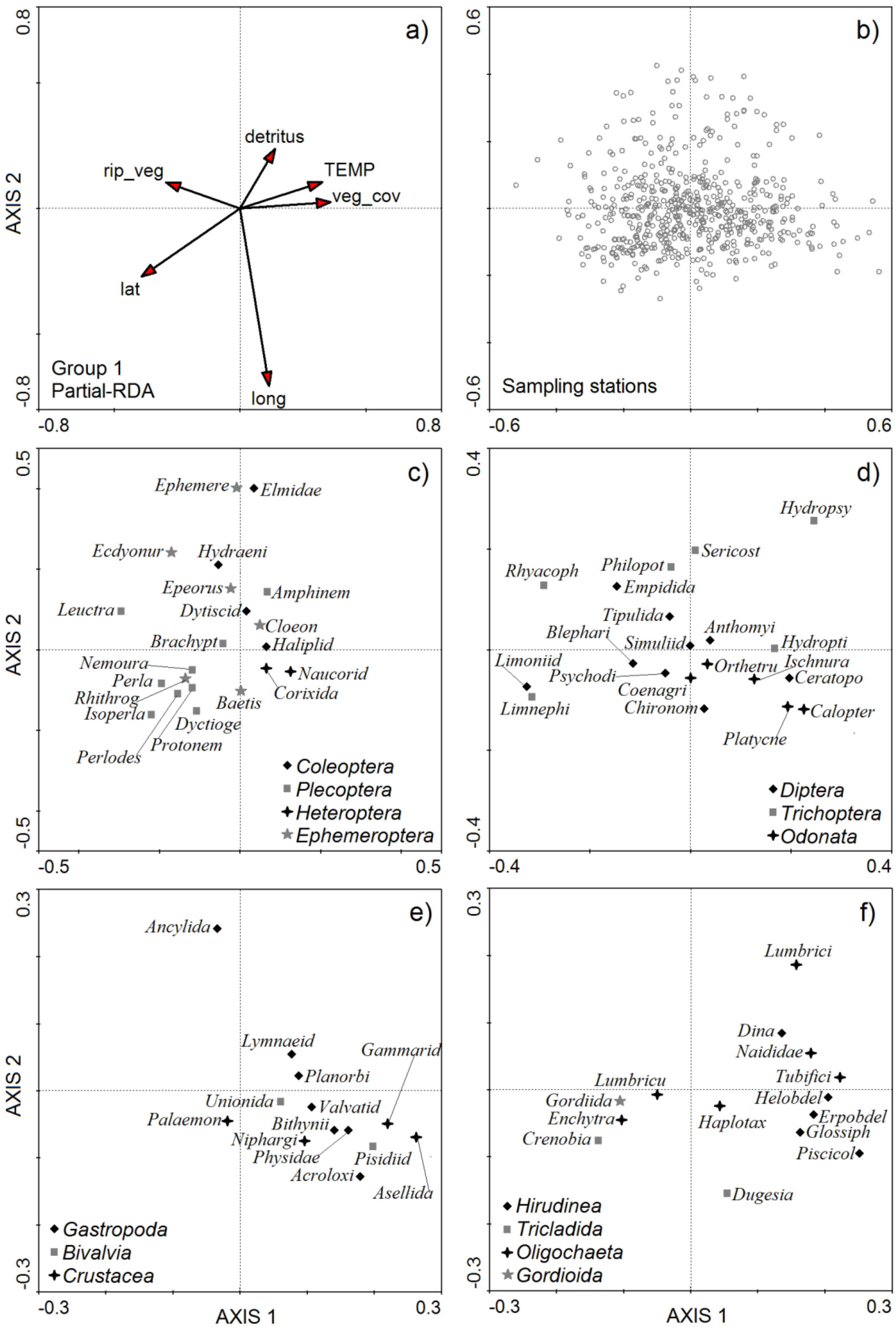


Fig. 2 Partial Redundancy Analysis of 68 taxa using ecoregional (Group 1) parameters as explanatory variables and hydromorphological (Group 2) and water quality (Groups 3) parameters as co-variables.

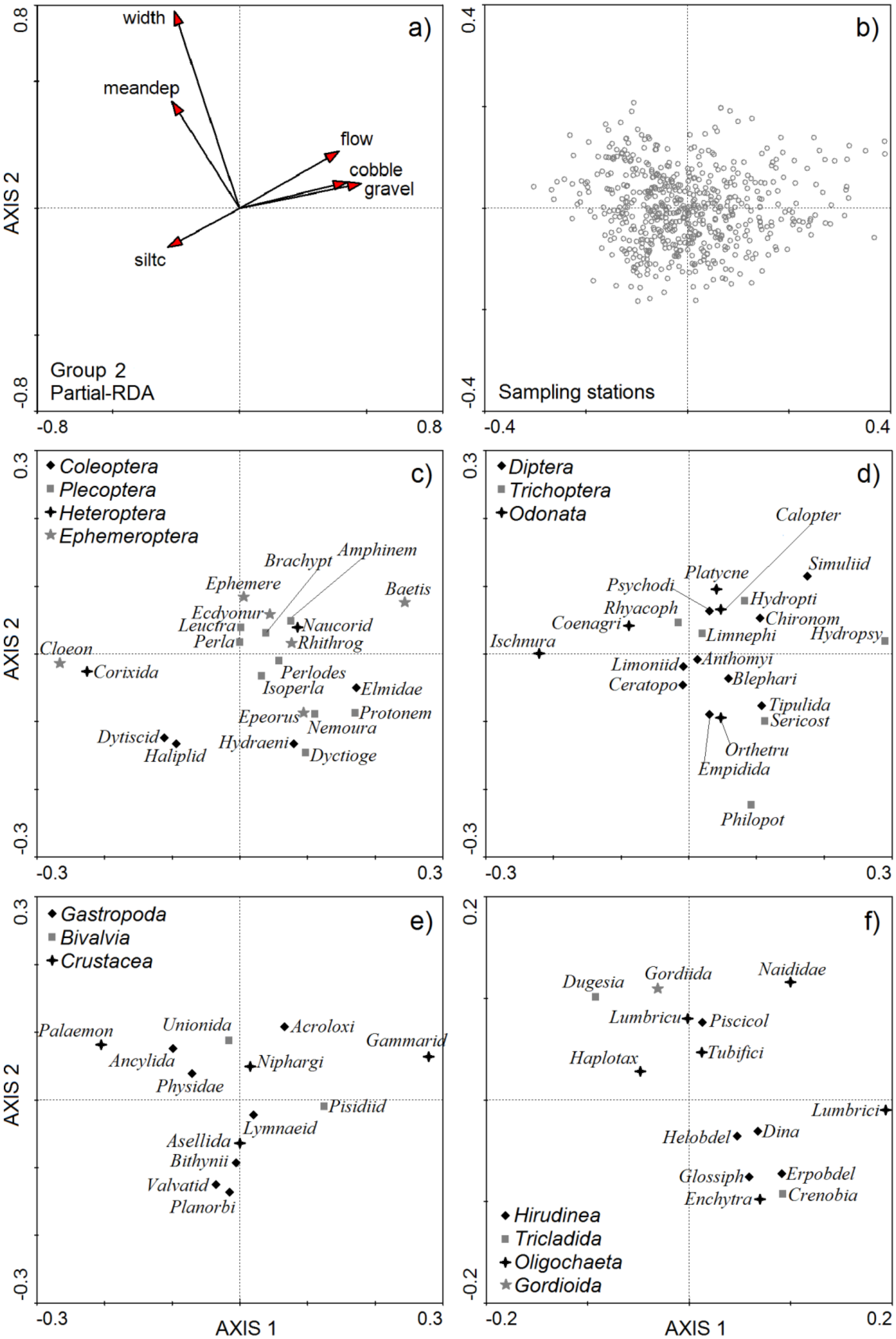


Fig. 3 Partial Redundancy Analysis of 68 taxa using hydromorphological (Group 2) parameters as explanatory variables and ecoregional (Group 1) and water quality (Group 3) parameters as co-variables.

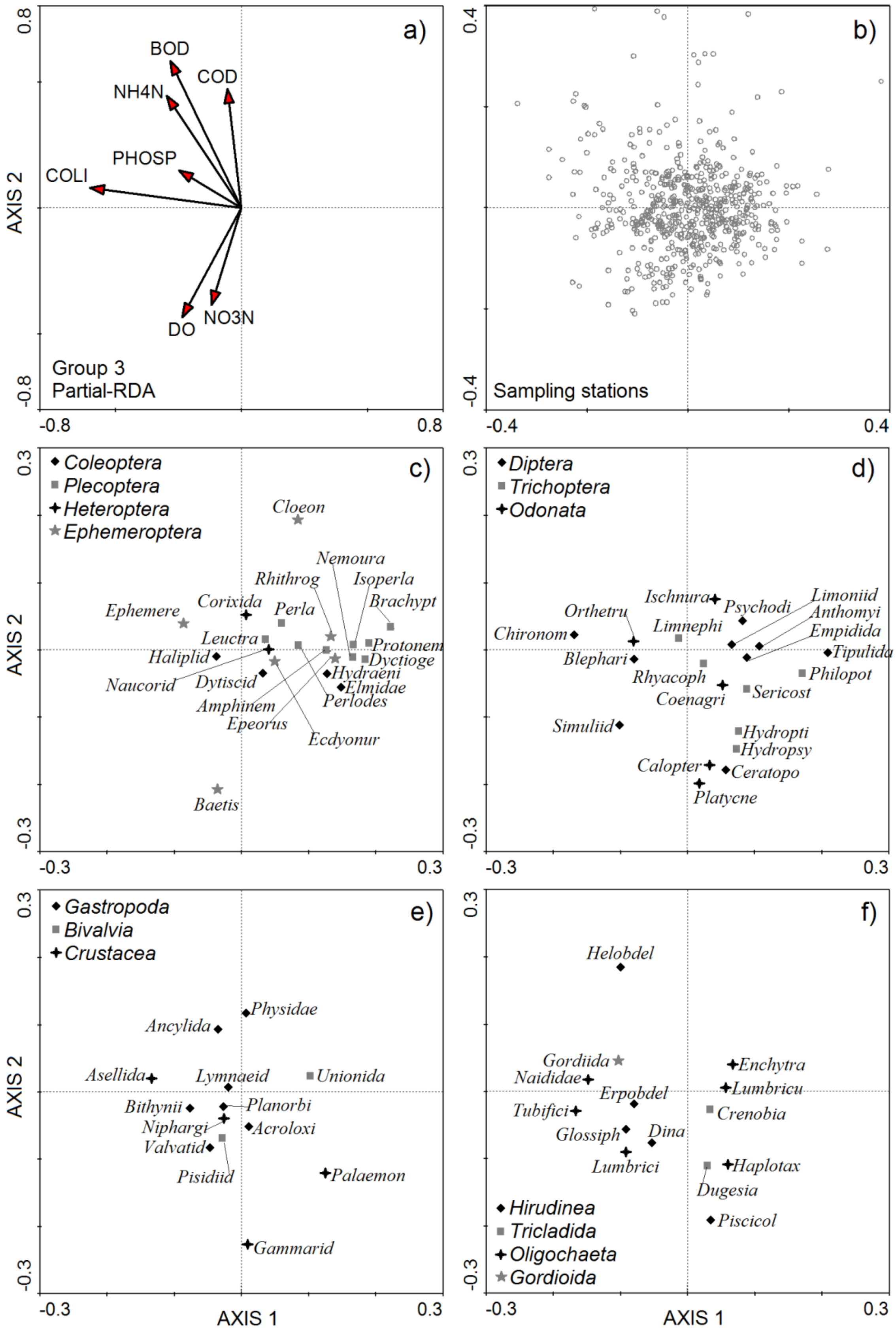


Fig. 4 Partial Redundancy Analysis of 68 taxa using water quality (Group 3) parameters as explanatory variables and ecoregional (Group 1) and hydromorphological (Group 2) parameters as co-variables.

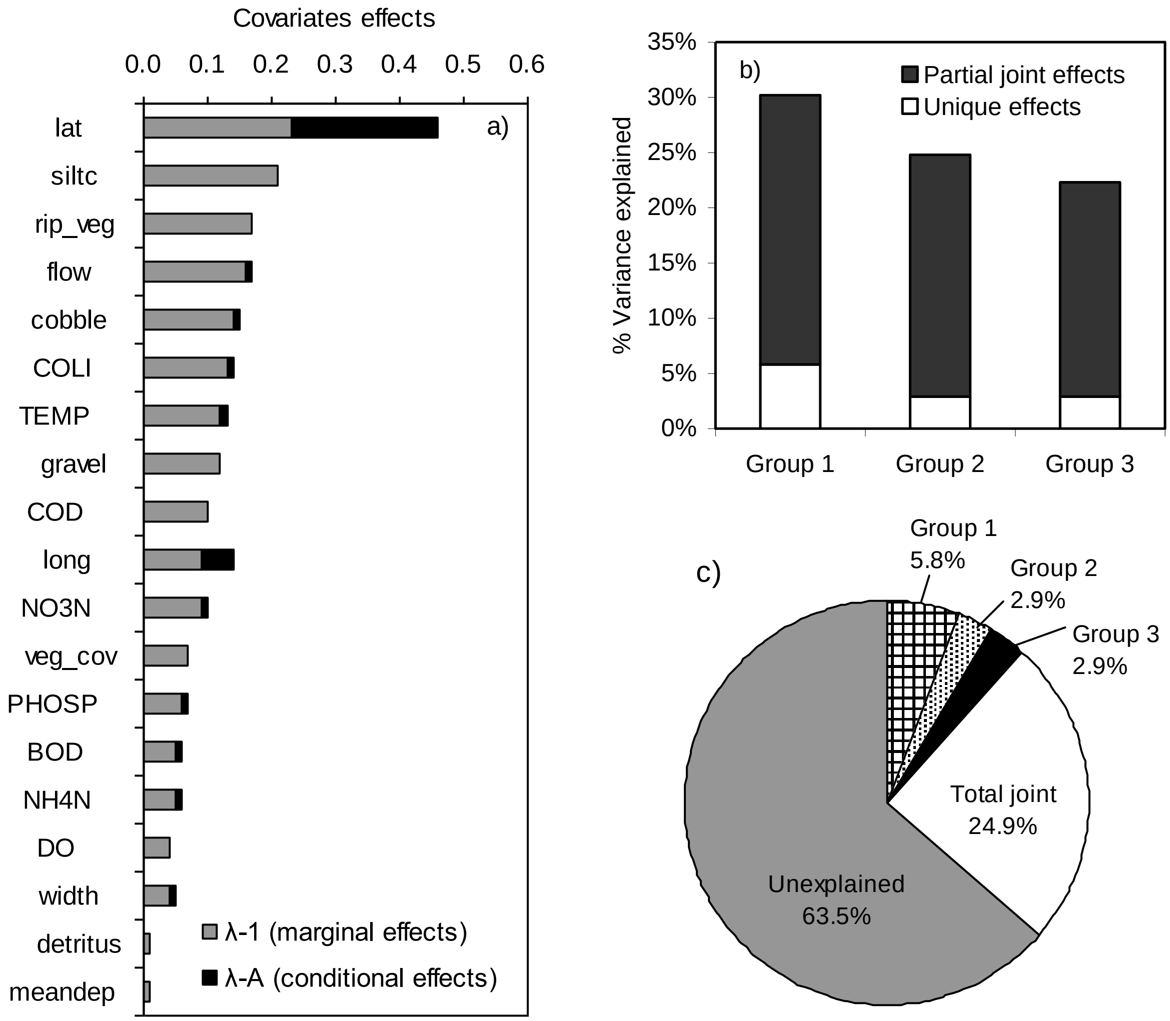


Fig. 5 a) Marginal (λ-1) and conditional (λ-A) effects of each covariate (top-down ranking using λ-1) from the full RDA, b) Unique and partial joint effects for each one of the three groups of variables after partitioning of taxa variance, c) Unique and total joint effects based on partitioning of taxa variance.

Supplementary material

Table S.1 Partial RDA for each group of parameters using a) co-variables (for unique effects), b) RDA for each group without co-variables (for unique+partial joint effects) and c) RDA using all groups (for unique + total joint effects).

a) Partial RDA with co-variables for unique effects	Group 1 (Ecoregion)				Group 2 (Hydromorphology)				Group 3 (Water quality)			
No. taxa	68				68				68			
No. environmental variables	6				6				7			
No. of co-variables	13				13				12			
No. sampling stations	585				585				585			
Eigenvalues (four major axes)	0.032	0.015	0.004	0.003	0.015	0.005	0.003	0.002	0.01	0.008	0.004	0.003
taxa-environment correlations	0.736	0.67	0.485	0.425	0.585	0.456	0.393	0.34	0.534	0.434	0.383	0.393
Cumulative % variance of taxa data	4.7	6.8	7.4	7.8	2.2	3.0	3.5	3.9	1.5	2.7	3.2	3.6
Cumulative % variance of taxa-environment relation	55.8	81.6	88	93.6	50.4	69.2	81.2	89.4	33.6	60.9	74	83.7
Total variance	1.000				1.000				1.000			
Sum of all eigenvalues	0.693				0.663				0.663			
Sum of all canonical eigenvalues	0.058				0.029				0.029			

b) RDA without co-variables (inclusion of partial joint effects)	Group 1 (Ecoregion)				Group 2 (Hydromorphology)				Group 3 (Water quality)			
No. taxa	68				68				68			
No. environmental variables	6				6				7			
No. of co-variables	0				0				0			
No. sampling stations	585				585				585			
Eigenvalues (four major axes)	0.261	0.026	0.006	0.004	0.221	0.011	0.007	0.004	0.187	0.019	0.006	0.005
taxa-environment correlations	0.949	0.693	0.491	0.417	0.879	0.547	0.36	0.393	0.806	0.539	0.477	0.487
Cumulative % variance of taxa data	26.1	28.7	29.3	29.7	22.1	23.2	23.9	24.3	18.7	20.6	21.2	21.7
Cumulative % variance of taxa-environment relation	86.5	95.2	97	98.4	89.2	93.6	96.4	98.2	83.7	92.1	95	97.2
Total variance	1.000				1.000				1.000			
Sum of all eigenvalues	1.000				1.000				1.000			
Sum of all canonical eigenvalues	0.302				0.248				0.223			

c) RDA with all variables (without co-variables)	All groups			
No. taxa	68			
No. environmental variables	19			
No. of co-variables	0			
No. sampling stations	585			
Eigenvalues (four major axes)	0.266	0.035	0.014	0.011
taxa-environment correlations	0.958	0.723	0.604	0.611
Cumulative % variance of taxa data	26.6	30.0	31.5	32.6
Cumulative % variance of taxa-environment relation	72.7	82.2	86.1	89.2
Total variance	1.000			
Sum of all eigenvalues	1.000			
Sum of all canonical eigenvalues	0.365			

Table S.2 Marginal effects (Lambda-1), conditional effects (Lambda-A), statistical significance (*P*,*F*) and variance inflation factors for the selected parameters which are used in the three cases of RDA analysis of Table S.1.

Case of analysis		a) Partial RDA with co-variables for unique effects					b) RDA without co-variables (inclusion of partial joint effects)					c) RDA with all variables (without co-variables)				
	Variable	λ-1	λ-A	*P*	*F*	VIF	λ-1	λ-A	*P*	*F*	VIF	λ-1	λ-A	*P*	*F*	VIF
Group 1	lat	0.03	0.03	1.00	22.42	7.19	0.23	0.23	0.002	173.17	2.93	0.23	0.23	0.002	173.17	7.19
	long	0.01	0.02	1.00	16.65	2.40	0.09	0.05	0.002	44.61	1.30	0.09	0.05	0.002	44.61	2.40
	rip_veg	0.01	0.01	1.00	2.56	2.37	0.17	0.00	0.002	3.28	2.28	0.17	0.00	0.002	2.62	2.37
	veg_cov	0.01	0.00	1.00	3.52	1.57	0.07	0.01	0.002	4.15	1.48	0.07	0.00	0.002	4.40	1.57
	detritus	0.00	0.00	0.51	1.77	1.30	0.01	0.00	0.002	2.21	1.17	0.01	0.00	0.008	1.74	1.30
	TEMP	0.01	0.00	1.00	3.54	2.32	0.12	0.01	0.002	5.12	1.77	0.12	0.01	0.002	4.90	2.32
Group 2	meandep	0.00	0.01	1.00	1.61	1.83	0.01	0.00	0.002	3.81	1.52	0.01	0.00	0.018	1.67	1.83
	width	0.01	0.00	1.00	5.42	2.13	0.04	0.01	0.002	8.01	1.68	0.04	0.01	0.002	7.90	2.13
	cobble	0.01	0.01	1.00	6.66	2.82	0.14	0.01	0.002	3.49	2.63	0.14	0.01	0.002	8.51	2.82
	gravel	0.01	0.00	1.00	3.79	2.20	0.12	0.01	0.002	4.95	2.08	0.12	0.00	0.002	4.01	2.20
	siltc	0.01	0.00	1.00	2.00	5.76	0.21	0.21	0.002	154.89	3.85	0.21	0.00	0.004	2.02	5.76
	flow	0.01	0.01	1.00	5.93	2.66	0.16	0.01	0.002	8.38	2.29	0.16	0.01	0.002	6.39	2.66
Group 3	COLI	0.01	0.01	1.00	6.78	2.44	0.13	0.13	0.002	86.12	1.21	0.13	0.01	0.002	6.77	2.44
	COD	0.00	0.00	1.00	2.58	2.58	0.10	0.04	0.002	31.11	0.37	0.10	0.00	0.002	2.50	2.58
	BOD	0.01	0.00	1.00	4.24	1.73	0.05	0.00	0.002	4.04	0.18	0.05	0.01	0.002	4.41	1.73
	NO3N	0.00	0.00	1.00	3.44	1.96	0.09	0.03	0.002	16.54	0.17	0.09	0.01	0.002	3.38	1.96
	NH4N	0.00	0.01	1.00	2.44	2.21	0.05	0.00	0.002	4.12	0.16	0.05	0.01	0.002	2.71	2.21
	PHOSP	0.00	0.00	1.00	1.85	1.59	0.06	0.01	0.002	4.20	0.05	0.06	0.01	0.004	1.85	1.59
	DO	0.00	0.01	1.00	4.08	1.45	0.04	0.01	0.002	7.71	0.13	0.04	0.00	0.002	4.13	1.45