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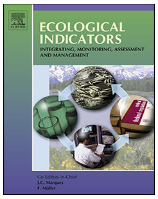

Modelling the effect of directional spatial ecological processes for a river network in Northern Italy

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ABSTRACT

The River Continuum Concept (RCC) and the Riverine Ecosystem Synthesis (RES) are two different theories proposed by river ecologists to describe the response of biotic communities to environmental variability. River network directional patterns are conveniently described by asymmetric eigenvector maps, an eigenfunction-based spatial filtering method specifically proposed for situations where a hypothesized directional spatial process influences the species distribution.

In this work asymmetric eigenvector maps are used in conjunction with canonical redundancy analysis and variation partitioning analysis to describe the distribution of macroinvertebrate communities of a river system in Northern Italy and to test the link between the river theories and the available data. Benthic macrofauna data were collected during the summer of 2009–2013 in 16 rivers, for a total of 283 replicates. We investigate the effects of some measured environmental factors on the benthic macrofauna community, accounting for directional effects intrinsic to the river network structure.

The proposed protocol allows to highlight and discuss some of the features relevant to the two river theories. According to the RCC theory, altitude and temperature were relevant factors affecting the macrozoobenthic community, together with the distance from the spring and water depth. Environmental factors representing local and lateral dimensions were less relevant for explaining the variability of the community composition. Nonetheless a role of the surrounding land use was also found, suggesting the presence of lateral effects due to human activities.

Overall, the results demonstrated that RCC is a reliable model to describe the distribution of macrobenthic communities in river networks. In socio-ecological systems, the local and lateral dimensions postulated by the RES theory could be mainly related to surrounding land use and naturalness degree.

1. Introduction

Historically, the identification of environmental factors which determine the composition of biotic assemblages in lotic ecosystems received a great attention as a challenging topic in aquatic ecology (Minshall et al., 1985). River ecologists proposed different theories to describe the response of biotic communities to environmental variability of lotic ecosystems. In the River Continuum Concept (RCC) theory, Vannote et al. (1980) conceptualized a pristine river as a continuum of chemical, physical and morphological gradients along a longitudinal axis (i.e. from spring to mouth), which rules the distribution of biological communities in order to optimize the use of energetic resources. However, Statzner and Higler (1985) argued that some assumptions of RCC are not always verified and should be excluded from the theory for its larger applicability. Namely, the maximization of yearly energy utilization through species replacement, lack of stream community successions, their time invariance and specific mechanisms leading to higher diversity in midstream communities are not verified or restricted to specific geographic areas. Corkum (1990,1991,1992) highlighted the importance of the surrounding landscape features in determining the distribution of macroinvertebrate communities, demonstrating that similar assemblages occur at different river sites surrounded by the same biome when characteristic vegetation is not disturbed. Although not refusing the longitudinal gradient of physical conditions described by the RCC, the Riverine Ecosystem Synthesis (RES, Thorp et al., 2006) further developed the lateral dimension in

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river theories, describing the river as a mosaic of hydrogeomorphic patches formed by catchment geomorphology and climate, named 'functional process zones'. According to this theory, the distribution of macrobenthic assemblages is mainly affected by the variability of local abiotic conditions and energy sources. With this paper we aim to describe the distribution of macroinvertebrate communities of a river system in Northern Italy and to assess the link of the RCC and RES theories with the available data.

RCC and RES theories have to do with the effects of purely directional patterns and sparse environmental factors on the distribution of the macrobenthic community in a lotic system. Directional patterns are found in river networks, where water flows downstream carrying abiotic particles and biotic organisms. Numerical ecologists have largely contributed to investigate the spatial and environmental effects influencing species distribution models (Legendre and Legendre, 2012). Eigenfunction-based spatial filtering methods allow to adequately represent the gradients influencing species spatial distributions in a river network via latent variables that correspond to directional spatial processes. Asymmetric eigenvector maps (AEM, Blanchet et al., 2008a,2011) were proposed for situations where a hypothesized asymmetric, directional spatial process influences the species distribution at scales ranging from fine to broad (e.g. the directional effects of a river network organized in basins, rivers and monitoring stations). In a community ecology context, AEM eigenfunctions are used to model the directional spatial structure of single species or multi-species communities. Canonical redundancy analysis (RDA) is a multivariate extension of multiple regression, suitable to measure the dependence of numerous sampled taxa on environmental factors and AEMs used as explanatory variables. Their relevance can properly be assessed by a forward selection procedure (Blanchet et al., 2008b), while variation partitioning analysis (VPA, Peres-Neto et al., 2006) can be performed to study the independent and combined importance of purely spatial latent variables and sampled environmental variables on the community.

In this work AEMs describe the geometrical structure of the network of mountain streams and rivers of Northern Italy, RDA allows to detect the relations of the distribution of macrozoobenthic taxa with environmental and structural factors and VPA provides information on which river model better describes the distribution of available data. Eigenvector maps are used for the first time to discuss the connection between the composition of macrozoobenthic communities and RCC and RES theories. The outcomes clarify the role of broad spatial structures and the effects of local conditions and surrounding land use in order to test which river model (RCC or RES) better describes the variability of the macrozoobenthic communities.

2. Materials and methods

2.1. River network data

2.1.1. Biological data

The biological data collection was carried out in 16 rivers located in 5 different basins of Northern Italy (Fig. 1 and Appendix A1). Sixty-one monitoring stations were sampled 3–5 times during the summer of 2009–2013, for a total of 283 replicates. Sampling stations were located within an altitudinal range of 150–1670 m a.s.l., to avoid sampling the lower river sections that are typically highly impacted by intense human activities. Benthic macrofauna was collected sweeping a 40 cm-wide D-frame hand net (mesh size = 500 μm) in an area of 1 m^2. Animals were preserved in 4% formalin solution and later identified in laboratory to the family level, except for Nematoda that were not further classified. Family level is considered a sufficient level for invertebrate community analysis (e.g. Gayraud et al., 2003). Particularly, when applied to multivariate analyses, the results obtained considering higher taxa may more closely reflect gradients or stresses than those based on species data, that are more affected by natural "noise" (Warwick, 1988).

Biological samples were organized into a community matrix accounting for 69 taxa observed at 61 monitoring stations. Observed taxa are reported in Appendix A2. Prior to carrying out the statistical analysis, replicates were averaged and the resulting species data were Hellinger transformed (Legendre and Gallagher, 2001): average species abundances were first divided by the site total abundance and the result was square root transformed, with the effect of reducing the importance of very large abundances (Borcard et al., 2011).

2.1.2. Environmental data

With the aim to test the reliability of the RCC and RES river models, a set of quantitative and qualitative environmental factors were measured to detect variability across longitudinal and lateral river dimensions. Latitude, longitude and altitude were recorded using a GPS device (Garmin 72H) and eleven quantitative and qualitative environmental variables were collected (Table 1). Water temperature and dissolved oxygen concentration were measured using a multiparameter probe (YSI Model 85), mean water depth was measured with a metric cord. The distance of the sampling stations from the river spring was measured using Google Earth images. Water samples were taken to the laboratory and filtered (Watman GF/F) before being analyzed. NH_4+ was measured using the Bower and Holm-Hansen protocol (Bower and Holm-Hansen, 1980), while $NO_3–$ was measured with automatic colorimeter method using AutoAnalyser II (Armstrong et al., 1967; APHA et al., 1992). Granulometry was estimated using an analytical sieve shaker (Fritsch Analysensieb DIN 4188). Periphyton and macrophytes, riparian vegetation and surrounding land use were assessed visually.

2.2. Statistical methods

The statistical analysis is focused on the investigation of the effects of environmental factors on the benthic macrofauna community over the study area, accounting for directional spatial effects intrinsic to the river network structure. This formulation allows to highlight and discuss some of the features relevant to the RCC and RES river theories. The analysis proceeds along the workflow depicted in Fig. 2 where available data, statistical tools and their outcomes are highlighted.

2.2.1. Asymmetric eigenvector maps [AEM]

The asymmetric eigenvector map (AEM) framework is an eigenfunction-based spatial filtering method specifically designed to model spatial structures hypothesized to be produced by directional spatial processes. When performing a spatial analysis using AEM, the connections among the sampling sites are expressed by a sites-by-edges matrix that corresponds to a connection diagram. To account for directionality, it is assumed that each most upstream sample of each river is connected to an artificial site that is there solely to give directionality and nothing else (Blanchet et al., 2008a). The sites-by-edges matrix is decomposed into latent spatial variables (eigenvector maps, or eigenfunctions). What results is a set of AEMs that, although constructed together, have independent structure within the different river systems. An illustration of this property of AEMs is given in Blanchet et al. (2011) and a. detailed account of the procedures used to construct AEMs is found in Blanchet et al. (2008a,2011). When constructing AEMs edges can receive weights representing the ease to move along them. In this work, we consider edge weights using two different weight functions based on altitude, originally proposed by Dray et al. (2006) and Blanchet et al. (2008a):

$$f_1(r_{ij}) = 1 - \left(\frac{r_{ij}}{max}(r_{ij})\right)^{\alpha} \ and \ f_2(r_{ij}) = \frac{1}{r_{ij}^{\alpha}} \tag{1}$$

where r_{ij} is the altitude difference between sites i (uppermost site) and j while α is either 1 or 2. AEMs are obtained by the geometric structure of the monitoring station network seen as a set of oriented graphs and can

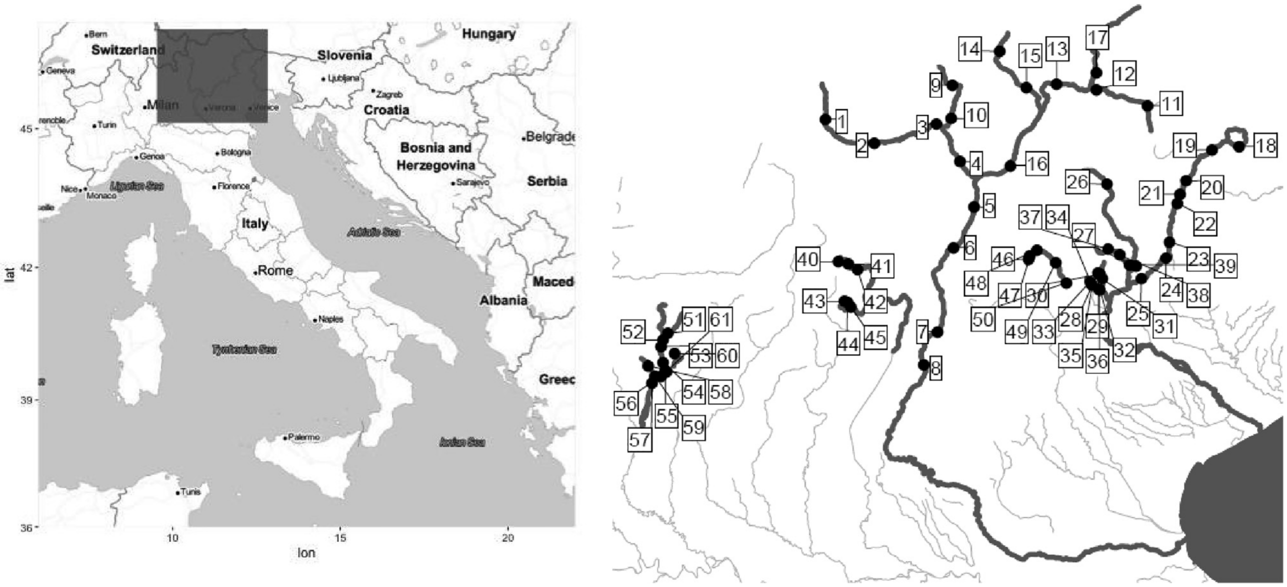

Fig. 1. Left: map of the study area in Northern Italy. Right: map of the river network with 61 monitoring stations. Station ID's correspond to those in Appendix A1.

Table 1
Quantitative and qualitative environmental variables used in the analysis.

Quantitative environmental variables

Variable	Abbr.	Unit	Min	Max
Distance from the spring	DIS	km	1	190
Altitude	ALT	m a.s.l.	150	1670
Mean water depth	DEP	cm	0.5	100
Temperature	TEM	°C	6.8	20.9
Dissolved oxygen	O_2	mg/l	7.26	14.01
NH_4^+	NH_4	mg/l	0.000754	191
NO_3^-	NO_3	mg/l	0.172	2

Qualitative environmental variables

Variable	Abbr.	Categories
Granulometry	GRA	Rocks, Pebbles and gravel, Sand, Silt
Periphyton and macrophytes	PER	Present, Absent
Riparian vegetation	RIP	Trees, Bushes, Grass
Surrounding land use	LAN	Forest, Grassland, Agriculture, Urban

be interpreted as spatial features, possibly influent on species distribution and abundance. From an ecological perspective, AEMs account for the directional spatial forcing among the samples taken in the river network. By construction, AEMs are ordered from the ones capturing very broad spatial structure (e.g. the full range of the river network) to very fine spatial structure (e.g. sites level spatial difference). In other words, AEMs are designed to capture spatial autocorrelation ranging from broad positive scale to fine negative scale in the direction of the spatial forcing. It is important to notice that as AEMs are designed to account for spatial autocorrelation in a specific, user defined, direction, spatial autocorrelation measured transversal to the considered direction may not be accounted for. Thus, AEMs can reveal patterns in taxa composition that can be linked to specific effects occurring along different spatial dimensions and scales.

2.2.2. Canonical redundancy analysis and variable selection [RDA, FORWARD SELECTION and TESTS]

The resulting latent spatial variables are used in canonical redundancy analysis (RDA) to study the directional spatial structure of community composition data. RDA is a constrained ordination method that can be understood as a multivariate extension of multiple regression (Legendre and Legendre, 2012, chapter 11; Rao 1964) and is thus suitable to deal with numerous sampled taxa at once. RDA is obtained by combining a set of multiple linear regressions and applying a principal component analysis to the fitted values. In the RDA framework, an unbiased estimate of the contribution of a set of explanatory variables to the explanation of the multivariate response is given by the adjusted coefficient of multiple determination R_a^2 (Ezekiel, 1930; Legendre et al., 2011). ANOVA-like permutation tests, based on the differences in residual deviance in permutations of nested models (Legendre et al., 2011; Blanchet et al., 2011), are also used to address the significance of the whole model and of each constraining variable. To obtain a parsimonious RDA model a forward selection procedure using two stopping criteria was used (Blanchet et al., 2008b). Specifically, if an explanatory variable included in the forward selected model results in the model having an R_a^2 higher than the R_a^2 calculated using all explanatory variables, this variable is rejected, and the forward selection procedure stops. In addition, if a variable included in the forward selected model is not significant (p-value $>$ 0.05), this variable is rejected, and the forward selection procedure stops. In other words, variables will be included in the model as long as they are significant and that their cumulative R_a^2 is smaller than the R_a^2 calculated using all explanatory variables. This approach has been recommended by Bauman et al. (2018) when the goal is to describe as accurately as possible the system under study. When spatial and/or environmental features are selected, then partial RDA can be used to remove the effects of some conditioning or background variables (Borcard et al., 2011, chapter 6).

2.2.3. Variation partitioning analysis [VPA]

Variation partitioning analysis (VPA, Borcard et al., 1992; Peres-Neto et al., 2006) allows to elaborate on the independent and combined importance of spatial and environmental variables on the river network benthic macrofauna community, thus providing further insights on the role of longitudinal and lateral dimension described by the RCC and RES river models. VPA is calculated by first measuring the amount of variation explained by the full set of explanatory variables (environmental features and AEMs combined) and then by calculating the amount of variation explained solely by environmental features and AEMs independently. Following, we can calculate by subtraction (Borcard et al., 1992) the amount of variation explained solely by environment (Fig. 3, Env) or AEM (Fig. 3, AEM) and by the combination of the two sets of variables (Fig. 3, intersection). Peres-Neto et al. (2006) have shown that it is relevant to use the adjusted coefficient of determination (R_a^2) to measure the amount of variation explained by

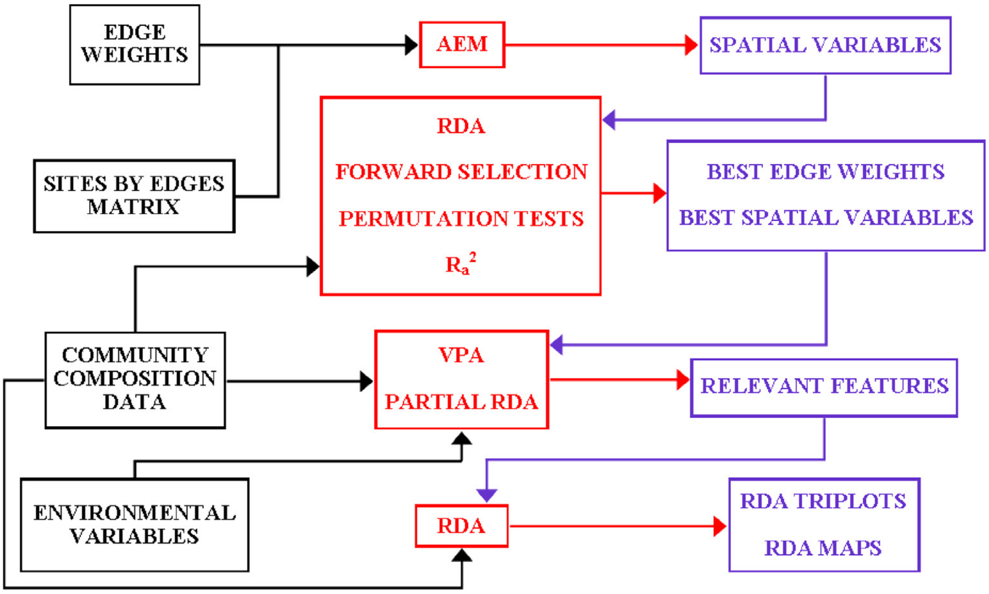

Fig. 2. Workflow of the statistical analysis of the river network data. Black typeface is for the data, red is for methods, blue for output and results.

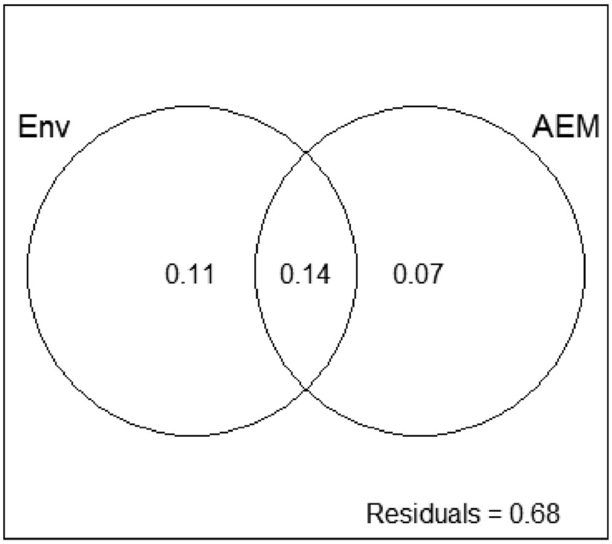

Fig. 3. Venn diagram of the variation partitioning of the community matrix explained by environmental qualitative and quantitative covariates (Env) and spatial variables (AEM).

each fraction of the variation partitioning analysis. Because some of the environmental variables are qualitative, we discretized them into binary dummy variables, allowing us to include them in the analysis.

2.2.4. RDA triplots [RDA]

After variable selection and model comparison, the results of RDA are summarized by the so-called triplots that display sites, taxa and environmental variables in the factorial plane spanned by RDA axes. The choice of one of two distinct scaling schemes leads to *correlation* triplots or *distance* triplots that allow to answer two types of ecological questions: when the interest is to study the relationship among species the correlation triplots should be used, while when the interest is to understand similarities among sites, then the distance triplots should be used. In both cases RDA axes are interpreted according to those variables that approach them away from the origin of the triplot. The methods used in these analyses are available in the “adespatial” (Dray et al., 2018) and “vegan” (Oksanen et al., 2019) R packages.

3. Results

The toolbox briefly described in the previous section was applied to available river network data according to the workflow described in Fig. 2 that will be further developed in this section.

3.1. Definition of directional spatial variables [SPATIAL VARIABLES]

Once the directional river network structure was coded into a 61 sites by 51 edges matrix, each edge was equipped by four alternative weights corresponding to f_1 and f_2 in (1) with $\alpha = 1$ and 2. Consequently, AEM gave rise to four sets of 43 eigenfunctions, corresponding to four alternative specifications of the weights. Within each set, eigenfunctions were divided into those showing significant positive and negative spatial autocorrelation using Moran’s I index (Moran, 1950) as a reference. Positive autocorrelation arises when sites that are close to one another have similar values of the AEM. Negative autocorrelation is the tendency for adjacent locations to show dissimilar values.

3.2. Selection of relevant spatial variables [BEST EDGE WEIGHTS and BEST SPATIAL VARIABLES]

The resulting eight groups of eigenfunctions were used as explanatory variables in RDA to investigate the dependence of the community composition on directional spatial processes. Each group was tested for overall significance using an ANOVA-like permutation test with 999 permutations (based on the differences in residual deviance in permutations of nested models as shown in detail by Legendre et al., 2011; Blanchet et al., 2011). The four sets of negatively autocorrelated spatial variables were all non-significant, while the four positively autocorrelated sets of eigenfunctions were highly significant. Adjusted coefficients of multiple determination R_a^2 were then calculated for each of the four sets of positively autocorrelated spatial variables and the set of AEMs that yielded the largest R_a^2 was used in all subsequent analyses, corresponding to the weight function f_1 with $\alpha = 2$ (values of R_a^2 for the four sets of weights are provided in Appendix A3). Forward selection (Blanchet et al., 2008b) was carried out to determine the relevance of the nineteen AEMs with significant positive autocorrelation resulting in eight latent directional spatial variables to be selected (1, 3, 2, 5, 12, 4, 6, and 10, ordered according to their relevance in the RDA). Selected AEMs explained 21.0% of the total variation (R_a^2) of the community data matrix, accounting for purely spatial features driven by the geometrical structure of the river network. Maps of the eight selected AEMs are displayed in Appendix A4 where some comments and interpretations are also addressed in terms of long-range dependencies and local spatial features.

3.3. Selection of relevant spatial and ecological features [RELEVANT FEATURES]

To select and rank spatial and environmental variables linked with the longitudinal and lateral river dimensions, VPA was carried out with the selected AEMs and qualitative environmental variables as well as quantitative ones (Table 1). VPA showed that both the directional spatial structure and the environmental covariates contribute to the explanation of the species data (Fig. 3). The unique contribution of environmental covariates ($R_a^2 = 0.11$) is larger than that of physiography ($R_a^2 = 0.07$). Partial RDA was also used to calculate and test the amount of variation explained by each of the two sets of variables given (conditional on) the other (Appendix A5 contains some numerical results). The large variation explained jointly by the two sets of variables ($R_a^2 = 0.14$) implies that many of the environmental variables have directional spatial structure (in line with both the RCC and RES river theories). Therefore, we considered only the spatial and non-spatial structures of the environmental explanatory variables as the main cause of variation of the species data and dropped the 7% dependence on AEMs to avoid redundancy.

3.4. Community composition data and environmental factors [RDA TRIPLOTS and RDA MAPS]

According to the results of VPA, our analysis was completed considering RDA constrained by all environmental explanatory variables and no AEMs. Assessing each axis by ANOVA-like permutation tests for the joint effect of all constraints (Legendre et al., 2011) we obtain three significant RDA axes, accounting for about 26% of the total variability in the benthic macrofauna community. Since ecological data are often quite noisy, much higher values of R_a^2 are not to be expected (Borcard et al., 2011). The results of the RDA are here reported by *distance* triplots, while *correlation* triplots are displayed and commented in Appendix A6. To ease readability, each triplot is split into two distance biplots in Fig. 4, highlighting similarities among sites. The two couples of biplots show some interesting connections between the three sets of variables. The first RDA axis is positively influenced by temperature and, as expected, is negatively related to altitude. In the study area, grassland occur mainly at higher altitudes, which is highlighted in Fig. 4 (a, b). The second RDA axis is positively influenced by NH_4 concentrations, with higher concentrations of NH_4 associated to agricultural land where fertilizer pollution and zootechnical discharges are common. Some positive correlations between NH_4 concentration, distance from the spring and mean water depth are also highlighted in Fig. 4 (b), as water depth and nutrient concentration increase going down the river. The unexpected negative relation between NH_4 and NO_3 concentrations is possibly due to NO_3 absorption in the lower stations of the Adige basin, caused by the presence of riparian vegetation. In Fig. 4 (b), monitoring site positions reflect the interpretation of the two axes. In the first quadrant we find stations with deep waters and far from the spring, with large temperatures and NH_4 concentrations (A3-7). On the opposite side, in the third quadrant, stations with higher altitude are found (V1-5, S1-2, Ar1-2, A8). Monitoring stations in the second quadrant of the biplot (Sa1, Bre1-4, Bre6-7, Bra1-2) have higher water temperatures and higher concentrations of NO_3. For some of them (Bre1-4, Bre6-7) anthropic impacts due to surrounding urbanization are reported. Finally, stations in the fourth biplot quadrant have higher altitudes (P1, R1, I1-2, C2, Sa2-3, Se1). In Fig. 4 (c, d) we see that, essentially, the third RDA axis is positively influenced by O_2 concentrations. Silt granulometry is associated with agricultural land use and higher distances from the springs, while sand granulometry is more commonly found in deeper waters. In addition, lower areas are more exposed to agricultural activities and correspond to deeper riverbeds characterized by sandy and silty substrata. According to Fig. 4 (d), stations in the second biplot quadrant (A3-7) are more distant from the springs and have deeper waters with higher concentrations of NH_4. The first quadrant is quite heterogeneous and stations (C1, C4-5, M2, Sa3) are characterized by higher O_2 concentrations. Stations in the third biplot quadrant have higher NO_3 concentrations (Bre1-7, Bra1-2, V2-5, S1-4, Ar1-2, A8).

Fig. 5 shows the maps of the 3 RDA axes that summarize the dependence of the community composition on environmental covariates. As seen with the biplots in Fig. 4, RDA axis 1 is positively correlated with temperature and quite obviously negatively related to altitude. Then, the white dots in Fig. 5 (left panel) highlight areas with lower elevations and higher temperatures. Higher values of RDA axis 2 correspond to higher concentrations of NH_4 and lower concentrations of NO_3 that, as was previously mentioned, seem to mostly characterize the lower Adige basin (Fig. 5 right). Finally, black dots in Fig. 5 (lower panel) represent areas with lower concentrations of O_2, that are expected to increase in areas of higher altitude.

4. Discussion and conclusions

The results we obtained show an example of appropriate analysis in systems where directional spatial information is required to describe the data variation. With river network data, directionality is conceptually fundamental, hence our proposal can be used whenever abundance and environmental measures are to be jointly analysed. There are a few studies (e.g. Blanchet et al., 2011; Bertolo et al., 2012) where directional (AEMs) and non-directional (MEMs) eigenvalue maps have been used side by side to study the relationship between directional and non-directional spatial forcing. These studies focussed on describing the spatial forcing within a stretch of river, in a fluvial lake or in the Atlantic Ocean. In the systems studied in either paper, the authors found that in systems where the directional forcing was unclear, AEMs and MEMs explained independent fractions of the variation in the response variables. These were all situations where there were ecological reasons to believe that directional as well as non-directional pressures could potentially influence the studied system. In this present study, we known that the spatial forces influencing the distribution of benthic macrofauna are mainly influenced by the river flow. As such, not accounting for this information (i.e. spatial directionality in the river network) would lead to a biased model and in turn to misleading results. Note that had our study focussed on organisms less affected by the directionality of the river network, such as aquatic birds or Salmonidae, we would have accounted for space differently.

The performances of AEM, RDA and VPA in identifying factors that influence the distribution of macrobenthic invertebrates were tested with abundance and environmental data for a river system in Northern Italy. The applied methods and observed results provided outcomes that can be extended to river theories and the general ecology of river networks. Given the directionality of the relevant spatial processes, combining AEMs and environmental variables allows to obtain a detailed description of the distribution of the macroinvertebrate community composition. The inclusion of AEMs and non-spatial environmental information in VPA produces a complete and informative model and improves the understanding of the system under study. Environmental variables were shown to be spatially structured and AEMs were not used to explain the species variation in the final RDA model. Nevertheless, AEM eigenfunctions provided some additional insides on the directional forcing of the river system (Appendix A4). The proposed methodology also provided a specific framework to test the link between the RCC and RES theories and the available data. According to the RCC theory (Vannote et al., 1980; Minshall et al., 1985), the altitudinal gradient was found to be a relevant factor affecting the macrozoobenthic community, as altitude and temperature

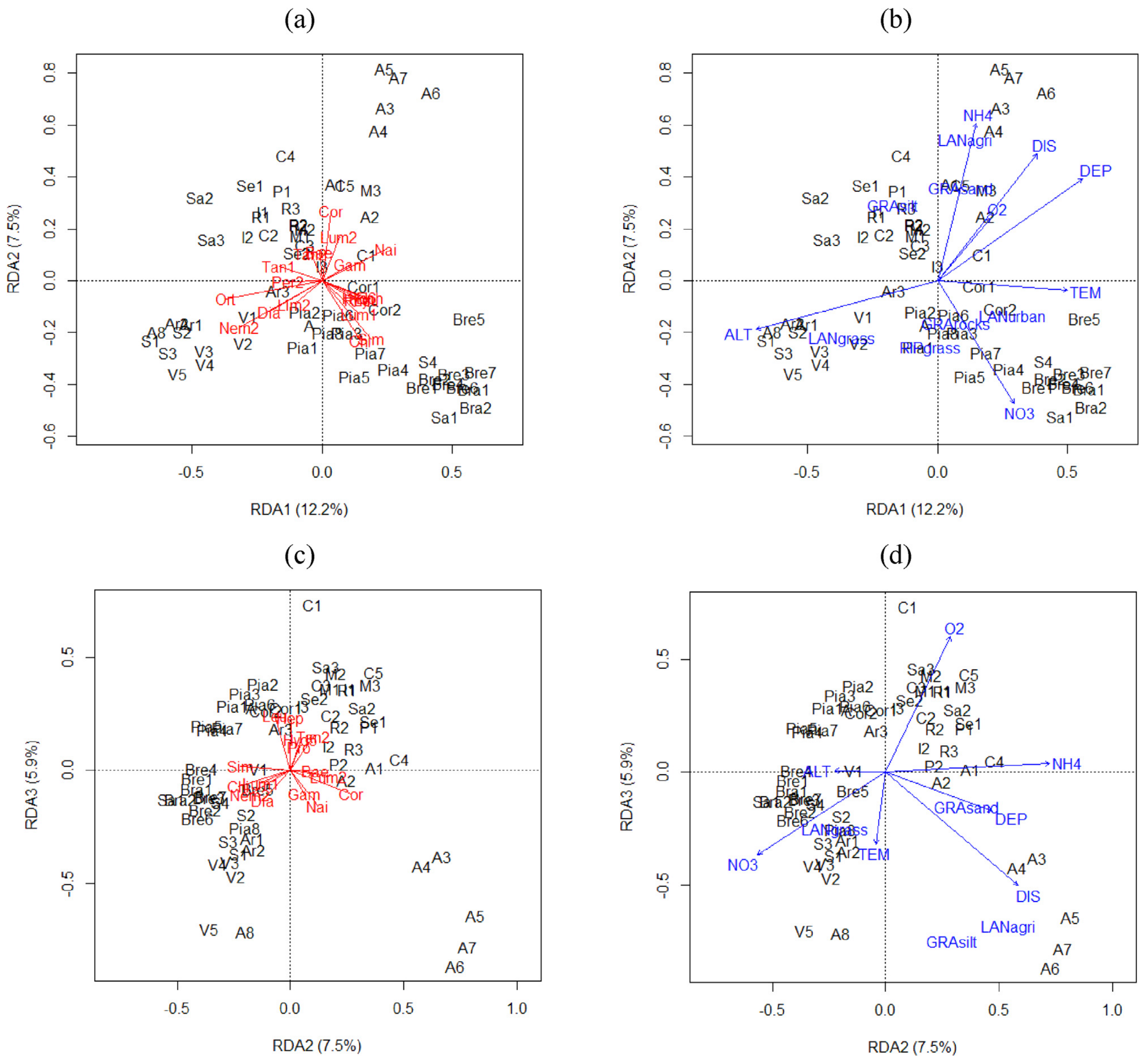

Fig. 4. RDA distance biplots of the Hellinger transformed abundance data constrained by environmental explanatory variables. Sites scores are scaled by eigenvalues. Shorter arrows and relative labels were discarded to make the figure easier to read.

were the most important variables contributing to the main RDA axis. Notably, distance from the spring and water depth were also important factors contributing to the second RDA axis. Nonetheless, the results showed also a role of surrounding land use on determining macrobenthic communities. In fact, NO_3 and NH_4 are the environmental variables that mainly contributed to the second RDA axis, suggesting the lateral effect of human activities on macroinvertebrate communities (in accordance with the RES river model). Such effect is also confirmed by the AEM 1 (Appendix A4), which “isolates” the central Adige stations, dominated by deposit feeder taxa such as Naididae and Tubificidae. These stations are surrounded by cultivated fields and characterized by riparian vegetation with trees, low values of NO_3 and high values of NH_4. Intensive agricultural practices (i.e. the application of fertilizers) and the presence of zootechnical activities are sources of nutrient excesses reaching the water body.

The effects at basin scale involve large scale variables such as those captured by the river continuum, e.g. gradual variations in altitude and distance from the spring, while the river scale reflects regional (i.e. subbasin) variations, such as those related to surrounding land use. Our findings are in line with those of Corkum (1990,1992), who observed significant effects of land use on macroinvertebrate assemblages, and with other studies stressing river theories with various methods. This suggests that anthropic impacts should be carefully considered as disturbing effects when applying river theories to socio-environmental systems. Larsen et al. (2019) tested the RCC theory in the Adige river basin, analysing the variations of functional feeding groups considering stream network topology and spatial autocorrelation. The authors found that the community shift was consistent with the longitudinal gradient, even if variations in water quality and local land use were equally or more important. The general reliability of RCC was demonstrated by Jiang et al. (2011) in a subtropical Asian river system and by Tomanova et al. (2007) in tropical streams, who respectively used a RDA and PCA-based correlation tests to select significant predictors for functional feeding groups.

The AEMs were also successfully used by Mortillaro et al. (2012) to demonstrate the consistence of RCC in predicting the organic matter distribution along the lower Amazon river. Vrebos et al. (2017) used Moran's and Asymmetric Eigenvector Maps to demonstrate that water

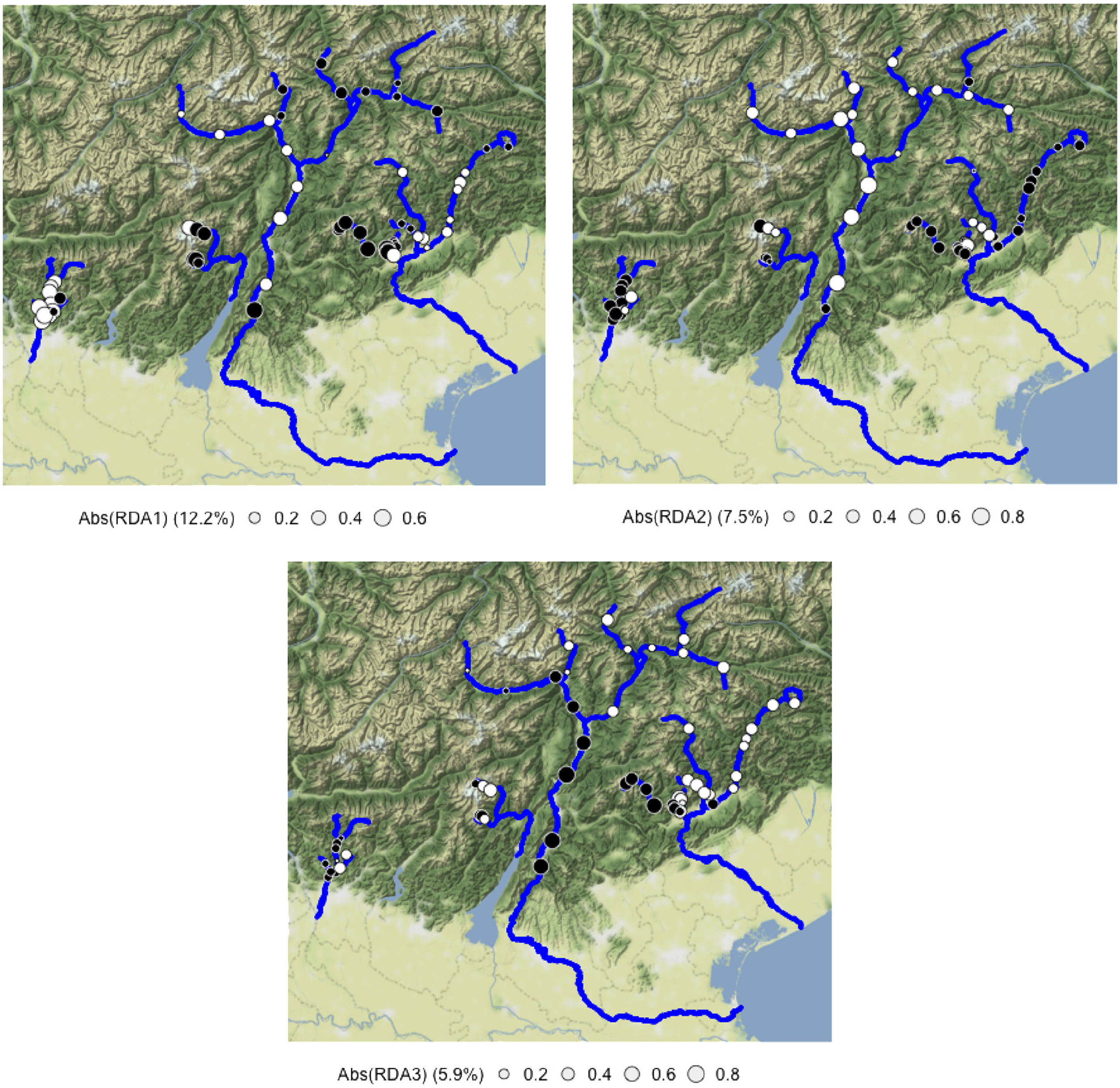

Fig. 5. Spatial distributions of the three significant RDA axes. Dots dimension is proportional to the absolute value of the respective RDA scores (Abs(RDA)). White dots are for positive scores, black for negative ones. In brackets we report the percentage of the of the total variability accounted for by each RDA axis.

quality descriptors are related to land use and other spatial descriptors at different scales of extent rather than a longitudinal gradient. However, these two studies did not consider macroinvertebrate communities.

The inverse relation found between NH_4 and NO_3 concentrations could be due to the presence of different pollution sources (i.e. fertilizers application for NO_3 and animal manure for NH_4) and the oxidation processes occurring in riparian soils. Riparian vegetation may significantly affect macrozoobenthic communities of rivers and streams, being a source of allochthonous detritus that supplies additional energy inputs to a broad range of macrobenthic taxa (Cummins et al., 1973; Petersen and Cummins, 1974; Dudgeon, 1988) and increases soil functionality (Mander et al., 2005; Gaglio et al., 2019). Thus, the negative correlation between NH_4 and NO_3 concentrations might depend on the surrounding land use combined with the presence of arboreal or shrubby riparian vegetation. Previous studies have shown that riparian vegetation promotes conditions for denitrification processes (Martin et al., 1999; Schade et al., 2001; Soana et al., 2017). The role of naturalness seems to emerge in AEM 2 (Appendix A4), where, the Brembo river in the Adda basin (Bre3-7) is opposed to the upper Piave basin (Pia4-8). The Brembo river has little or no aquatic vegetation, an urbanized surrounding area and is dominated by deposit feeders' and filter feeders' taxa. Conversely, the Piave basin had the highest abundance of Leuctridae (Plecoptera) in our study area, mainly belonging to the shredders' functional group and is surrounded by woodlands (i.e. high naturalness degree of local and lateral conditions). Although not clearly emerging from the RDA axes, the possible role of the riparian vegetation and naturalness degree as expressions of local and lateral dimensions are the only factors calling for considering the RES river model (Thorp et al., 2006).

The distribution of sampling points in RDA triplots also suggests effects at the river and basin scales on macroinvertebrate communities. From a more general point of view, this highlights that aquatic biodiversity of river networks should be managed from a wide scale perspective. The clustering of the Adige and Piave river sampling stations in the RDA triplots (Fig. 4) can be explained by the river continuum characteristics. The Brembo and Brambilla river stations are clearly isolated in a specific group, suggesting common features related to the Adda basin system.

As far as we know, the idea to use altitude as a way to account for the influence of the samples within the river network is novel. That being said, what is important to note is that we used this approach because it is ecologically relevant for the river system we are studying. This may not be as relevant for all river systems.

The two weight functions used to quantify the importance of edges are two weights functions commonly used in with AEMs (and also Moran's eigenvectors maps, as proposed by Dray et al., 2006) because they weight the importance edges using a concave-down (f_1) and a concave-up (f_2) weight distribution. That is, when f_1 is used, a small altitude difference between two samples will be given much less weight (and thus much less importance) than if f_2 is used. From an ecological perspective, this is interesting because it helps us better understand the river system we are studying. As for the alpha values, there are many different values of alpha that can be used in either of these functions (actually there are an infinity of them). As such, it would have been possible to compare a large range of alpha values. However, comparing a range of alpha values for f_1 and f_2 makes it ecologically challenging to fully grasp the meaning of the one that yields the highest amount of explained variance. For this reason, we focussed on a subset of alpha values that made ecological sense for the river system understudy. An alpha value of 1 for f_1 results in the altitude being accounted for linearly in the system, while an alpha of 2 for f_1 is concave-up. An alpha value of 1 for f_2 is concave-down while an alpha value of 2 for f_2 is even more concave-down.

Overall, the findings show the prevailing effects of river continuum along a spring-mouth gradient, thus supporting the reliability of the RCC river model. Environmental factors representing local and lateral dimensions were less relevant for explaining the variability of the community composition. Land use effects were observed to predominate only in human impacted landscapes (e.g. cultivated systems), confirming the observation of Corkum (1990). No clear evidences were found to support the RES theory (Thorp et al., 2010). However, in socio-ecological systems where human activities and natural components coexist, the local and later dimensions postulated by the RES theory could be affected by anthropic impacts rather than an expression of natural variables acting at lower scale.

Notice that a considerable level of noise, quite common to ecological data, causes only part of the total variability in the benthic macrofauna community is explained by RDA axes. Such noise could be also due to spatial effects that are not related to environmental variables, e.g. dispersal capacity and mass effects. Anyway, macrobenthic communities are used as indicators of ecological conditions because of their limited mobility. In facts, only a limited number of taxa are subject to drift in running waters. Moreover, samplings were carried out during the summer season, when macrobenthic communities reached a stable stage and source-sink dynamics should be less important.

The exclusion of lower river sections and the classification of qualitative environmental variables in few representative classes are possible causes of a further reduction of the capacity of the RDA to capture specific effects on macroinvertebrate communities. Further improvements on the classification of environmental factors, for example including percentage coverage and different leaf decay rates of riparian species, could improve the capturing of local variability. Nevertheless, the AEM analysis suggests some effects of local environmental naturalness degree on macroinvertebrate communities.

CRediT authorship contribution statement

A. Pollice: Methodology, Software, Formal analysis, Writing - original draft, Writing - review & editing. **G. Jona-Lasinio:** Visualization, Supervision, Writing - review & editing. **M. Gaglio:** Data curation, Writing - original draft, Writing - review & editing. **F.G. Blanchet:** Software, Writing - review & editing. **E.A. Fano:** Resources, Conceptualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data and additional results

Supplementary data and additional results to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.106144.

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