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Spatial variance of profundal and sublittoral invertebrate benthic communities in response to eutrophication and morphological pressures

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With 3 figures and 3 tables

Abstract: Invertebrate communities inhabiting different lake zones are expected to respond differently to natural environmental variation and anthropogenic stressors. We used multivariate statistical methods in order to quantify the effects of eutrophication and morphological pressures on the spatial structure of the invertebrate benthic communities at two depth zones (profundal and sublittoral) in subalpine lakes in Italy, Germany and Austria . In both lake zones, environmental variables related to eutrophication pressures (mid-lake total phosphorus and chlorophyll-*a*) were significant in structuring the invertebrate community (permutation test: $p < 0.01$). Three variables relating to morphological pressures (diversity of macrophyte growth forms, sum of pressures in the lake shore, and percentage of natural land cover within a 200 m stretch from the lake shore) were significant (permutation test: $p < 0.01$) in the sublittoral zone, while in the profundal zone none of the variables included in the analysis related to morphological pressures were significant in structuring the invertebrate community. Variance partitioning analysis showed that profundal communities were mainly affected by eutrophication (8.6 % of total variance; $p = 0.005$), while in the sublittoral zone eutrophication accounted for only 0.5 % ($p = 0.04$) of total variance. The effects of morphological pressures could be tracked only in the sublittoral zone, where it accounted for 0.8 % of total variance ($p = 0.015$). The spatial component was responsible for a large part of the total variance (58.7 % in the profundal, $p = 0.005$; 44.2 % in the sublittoral zone, $p = 0.005$) and had interactions with stressor variables in both lake zones. Therefore the analysis of spatial patterns should be included in assessment systems relating invertebrate assemblages to pressures.

Key words: lake sublittoral zone, lake profundal zone, invertebrate community, eutrophication, morphological pressures, RDA, variance partitioning.

Introduction

Despite the awareness of the need of a pressure-related assessment tool, a quantitative analysis of the unique effects of different pressures on the structure and composition of lake benthic communities is still lacking. This is due to the difficulty of disentangling the signature of different explanatory variables such as those associated to natural spatial and temporal variability and those associated with different stress-

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ors. Further complications arise from the interaction between stressors which may have synergic or antagonistic effects. Fuelled by the implementation strategy of the Water Framework Directive (WFD, Directive 2000/60/EC), recent applied research focus has been mainly on planktonic indicators and eutrophication pressure (Ptacnik et al. 2009, Noges et al. 2010) owing to their straightforward response to increasing levels of total phosphorus (Carvalho et al. 2009, Phillips et al. 2008). As a consequence, the development of assessment tools based on other organism groups and pressures such as invertebrates and hydromorphological alterations has been slower (O'Toole et al. 2008, McGoff & Sandin 2012 (this issue), Porst et al. 2012 (this issue)). Yet, the WFD requires that the quality of European waters is to be protected and improved in relation to pressure-specific assessment of impact of relevant pressures in a more holistic way using several biological groups (Noges et al. 2009, Solimini et al. 2009).

Solimini et al. (2006) and Solimini & Sandin (2012 (this issue)) reviewed the knowledge of the use of benthic invertebrates as indicators of lake ecological status, focusing on the major anthropogenic pressures affecting European lakes: eutrophication, acidification and hydromorphological alterations. Eutrophication was hypothesised to be more easily detectable in deeper lake zones. The 'signal' from changing communities, with respect to a reference state, should be more visible in the profundal and decrease through the shallower zones from sublittoral to eulittoral (see also Brauns et al. 2007b). In contrast, hydromorphological alterations were expected to have the strongest effect in the littoral zone, followed by the sub-littoral, while the profundal was expected to be hardly affected (Brauns et al. 2007a). Acidification was expected to mostly affect the upper littoral zones of the lake (Table 1).

The variation in the structure and composition of macroinvertebrate benthic communities of lakes is influenced by several different abiotic factors and anthropogenic stressors. Littoral communities were reported to be influenced by the habitat characteristics available to invertebrates, water level fluctuations, morphological alterations of lake shores, wave action and pH (Johnson et al. 2004, Stendera & Johnson 2008, Brauns et al. 2008, Mastrantuono et al. 2008), while profundal communities were influenced by lake nutrient and oxygen levels (Rasmussen & Kalff 1987, Hämäläinen et al. 2003, Stendera & Johnson 2008, Bazzanti et al. 2012 (this issue), Jyväsjärvi et al. 2012 (this issue)). Sublittoral macroinvertebrate communities have been shown to have lower variation among years than profundal communities in density and species number (Hämäläinen et al. 2003) and in some other community metrics (total density, total biomass, taxon richness, Shannon diversity and Benthic Quality index; Johnson 1998) making sublittoral assemblages potentially appropriate in detecting anthropogenic impact (Free et al. 2009a). The amount of variation of different lake zone assemblages that could be explained by natural characteristics is critical to detect alterations due to anthropogenic stressors.

When analysing the processes structuring the macroinvertebrate communities, attention should be paid to spatial factors. The spatial component can be responsible for a large part of the variation of the community due to both direct processes such as dispersal, social organization and species interactions, and indirect processes connected to the spatially structured environmental factors (Borcard et al. 2004, Peres-Neto & Legendre 2010). Therefore, the spatial patterns need to be assessed in order to quantify their contribution to the community variance and consequently to be able to distinguish their effects from those due to environmental factors.

The aim of this paper is to quantify the effects of eutrophication and morphological pressures on the spatial structure of the macroinvertebrate benthic communities at two depth zones (profundal and sublittoral) in subalpine lakes. We used a set of multivariate statistical methods to:

1. extract the relevant spatial axes
2. select the most relevant environmental variables related to each pressure and lake zone
3. partition the community variance among spatial and environmental components and compare the response of the two lake zones

Table 1. Hypothesised impact intensity of different stressors on invertebrate fauna in different lake zones (modified from Solimini et al. 2006). *** = high, ** = medium, * = low, 0 = null. Question marks indicate large uncertainty of the respective hypothesis.

	Eutrophication	Hydromorphological	Acidification	Combined impacts
Littoral	*	***	***	***?
Sublittoral	**?	*?	**?	**?
Profundal	***	0	?	**?

Our hypothesis was that eutrophication would mostly affect profundal communities, while morphological pressures would mostly affect sublittoral communities. The individual contribution by each of the two pressures to the biotic variance will be examined in the two lake zones.

Material and methods

Study area, and macroinvertebrate sampling

The dataset used for this analysis has been described in previous works (Free et al. 2006, Rossaro et al. 2007, Solimini et al. 2006, Free et al. 2009a) and refers to three sampling campaigns conducted between 2005 and 2008. The first one refers to 12 subalpine Italian lakes sampled in the sublittoral and in the profundal zones in spring 2005, using a Ponar grab (sampled area = 420 cm^2; 3 sites per lake, 2 replicates per site). The second one refers to 46 subalpine lakes sampled in the sublittoral between April and June 2006 in South Germany (15), Italy (16) and Austria (15) using an Ekman grab (sampled area = 225 cm^2; 3 sites per lake, 2 replicates per site; see Free et al. 2006 and Free et al. 2009a for details). Additional profundal data were collected during a sampling campaign in June 2008 in 5 subalpine Austrian lakes using cores (diameter 6 cm), 12 replicates at each site, for a total area of 339 cm^2 per site. Taxa lists were harmonised to species and genus levels and data were expressed as average of densities per site. In total we collected 85 taxa in the 37 profundal sites, and 169 taxa in the 171 sublittoral sites.

Environmental variables

Nine environmental variables were included in the analysis. They were grouped into three sets of variables related to: 1. eutrophication, 2. morphological pressures, and 3. morphometry and alkalinity. The ranges of values for each dataset and their spatial scales are reported in Table 2.

The eutrophication-related variables were: mid-lake chlorophyll-*a* (mid-lake chl-*a*), and mid-lake total phosphorus (mid-lake TP), both averages of water samples collected at different depths (surface, 2.5, 5, 10, 20 m from the surface and 1.5 m above the sediment). Those two variables are known to be important in structuring invertebrate communities (Rasmussen & Kalff 1987). The morphological-pressure-related variables were three indices obtained through the application of lake habitat survey (LHS; Rowan et al. 2004, Rowan et al. 2006) and two land use variables (% urban, and % natural land use) calculated through GIS data within a 200 m band around the lake from the edge of the shore. The LHS variables include: 1. diversity of macrophyte growth form types, derived from the recording of up to ten macrophyte groups during the survey; 2. the sum of pressures, from the presence of 18 potential pressures affecting the riparian zone and the shoreline within a 50 m radius of each site; and 3. the degree of naturalness of the riparian zone, which takes into account riparian vegetation complexity, vegetation longevity and naturalness of land cover (see Free et al. 2009a for more details on the environmental variables). The LHS indices were based on observation of the riparian/littoral zone and of the shoreline close to the lake sites. Therefore those indices have only been calculated for the sublittoral sites as profundal sites could not be matched to any riparian stretch. Moreover, an upscaling of those variables from site to lake level by averaging would not be correct, due to the low number of sites per lake. Morphometry and geology are represented by mid-lake alkalinity and by the index of lake basin shape (ILBS; Nürnberg 1995, Free et al. 2009a), calculated as:

$$\frac{MaximumDepth}{\sqrt{LakeArea}}.$$

The ILBS index synthesises lake area and slope, with high values typical of small deep lakes while low values are typical of large shallow lakes.

Data analysis

Prior to analyses, taxa densities were Hellinger-transformed, as suggested by Legendre & Gallagher (2001). The Hellinger transformation preserves the Euclidean distance among rows and therefore allows the use of Euclidean-based ordination methods such as redundancy analysis (RDA). It also offers the advantage of underweighting rare taxa (Legendre & Gallagher 2001). Environmental variables were standardised (Legendre & Legendre 1998), or the arcsine of the square root was calculated for variables expressed as a percentage (Feld & Hering 2007). This analysis was performed using the R package Vegan (Oksanen et al. 2006; available at http://cran.r-project.org/web/packages/vegan/index.html).

Table 2. Environmental variables: range of the variable values in each dataset and spatial scale of the measurements. The variables are categorised in 3 groups: eutrophication, morphological pressures and morphometry and alkalinity. Variables explanation is provided in the text.

Variable group	Spatial scale	Environmental variable	Profundal	Sublittoral
Eutrophication	lake	Mid-lake Chl-*a* (μg l^{-1})	0.43–28.97	0.24–36.36
	lake	Mid-lake TP (μg l^{-1})	1.70–39.5	1.70–101.13
Morphological pressures	site	Diversity of macrophyte growth form types	–	0.00–5.00
	site	Sum of pressures	–	0.00–6.50
	site	Naturalness of riparian zone	–	0.04–0.96
	within 200 m from the lake shore	Urban land cover (%)	0.00–0.53	0.00–97.80
	within 200 m from the lake shore	Natural land cover (%)	0.08–1.00	0.00–97.06
Morphometry and alkalinity	lake	ILBS	2.38–49.29	2.39–79.42
	lake	Mid-lake alkalinity (meq l^{-1})	0.89–3.00	0.89–5.33

Redundancy analysis (RDA) was used in constrained ordination of taxonomic data. This analysis was performed using the R package Vegan (Oksanen et al. 2006).

The spatial pattern of the community was quantitatively described using Principal Coordinates of Neighbour Matrices (PCNM, Borcard et al. 2004, Léonard et al. 2008, Brind'Amour et al. 2009). This method produces a set of spatial explanatory variables called PCNM vectors. It involves the building of a matrix of Euclidean distances from the geographical coordinates of the sampling sites. The matrix of Euclidean distances is truncated at a threshold value, corresponding to the largest among the minimum distances to connect pairs of sites. A principal coordinate analysis on the truncated distance matrix is then computed and only the coordinates corresponding to positive eigenvalues are kept. The resulting principal coordinates are the PCNM vectors. This analysis was performed using the R package SpacemakeR (Dray 2006; available at http://r-forge.r-project.org/).

The contribution of the spatial factors and the 3 sets of environmental variables in structuring the macroinvertebrate benthic communities were assessed through the use of variance partitioning with partial RDA (Redundancy Analysis). This method allows the decomposition of the variance of the response matrix (taxa density) among sets of explanatory variables in order to identify their pure and shared contributions to the total variance (Borcard et al. 1992, Legendre & Legendre 1998). Variance partitioning was performed by applying the varpart function of the R library Vegan, (Oksanen et al. 2006) this function computes the RDA-adjusted R^2 values. The adjustment, taking into account the appropriate degrees of freedom, provides a way of comparing models with different numbers of predictors and sample sizes (Peres-Neto et al. 2006). The significance of each fraction was tested with a permutation test for redundancy analysis (Borcard et al. 2011).

Only significant spatial (PCNM vectors) and environmental variables, identified by the forward selection procedure were included in the variance partitioning analysis. The forward selection procedure was implemented in the R package Packfor (Dray 2005; available at http://r-forge.r-project.org/). This procedure, applied to each variable group separately, uses the results of a Monte Carlo permutation test (999 random permutations) to test the significance of the explanatory variables successively entering the model and retains those variables with $p < 0.05$ (Léonard et al. 2008, Brind'Amour et al. 2009).

Results

The RDA constrained ordination of taxonomic composition showed that for the profundal zone the proportion of variance explained by the entire set of environmental variables was 38.57 %, while for the sublittoral zone it was 15.39 %.

In the profundal zone (Fig. 1) the first RDA axis (explained variance: 18.1 %) was positively correlated to mid-lake Chl-*a* and mid-lake TP (scores: 0.59 and 0.80) and negatively correlated to ILBS (score: –0.77). The most important components for the second RDA axis (explained variance: 10.71 %) were alkalinity and mid-lake Chl-*a* (scores: –0.45 and –0.78).

In the sublittoral zone (Fig. 2), the first RDA axis (explained variance: 6.5 %) was positively correlated to Chl-*a* (score: 0.62) and negatively correlated to alkalinity (score: –0.75). The second RDA axis (ex-

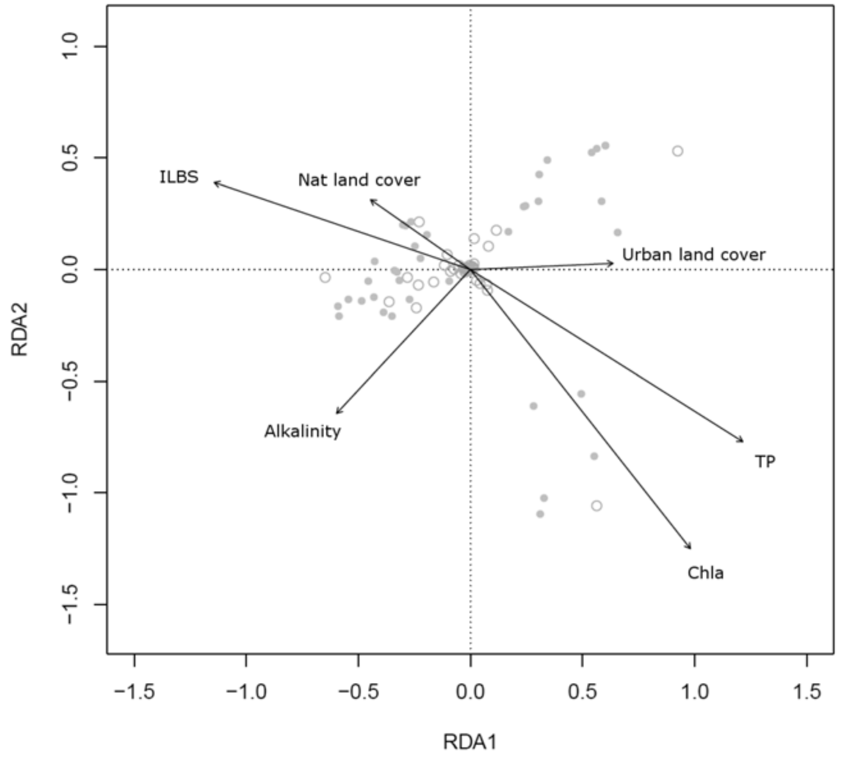

Fig. 1. Profundal zone: RDA biplot scores for constraining variables. Grey dots represent sampling sites, white dots represent species.

plained variance: 3.5 %) represented a gradient of increasing natural land cover and naturalness of riparian zone (scores: 0.39 and 0.32), and decreasing mid-lake TP and mid-lake Chl-*a* (scores: –0.80 and –0.40) from left to right in the ordination.

Spatial component

The PCNM vectors represent a quantification of broad to fine-scale spatial pattern of the study design, the first vectors (broadest/regional scale) depend on the study area surface, while the last vectors (finest/local scale) depend on the truncation distances (Borcard et al. 2004). The truncation distance was 217.5 km for the profundal dataset and 125.6 km for the sublittoral dataset. 9 PCNM vectors were produced for the profundal dataset, 38 PCNM vectors for the sublittoral dataset. The difference in the number of PCNM vectors and truncation distance between the two datasets is due to the different spatial distribution of the sampling sites.

Variance partitioning

The explanatory variables selected by forward selection for each variable-group and for each dataset are reported in Table 3. The variables related to eutrophication (mid-lake TP and Chl-*a*) and those related to lake morphometry and geology (ILBS and mid-lake alkalinity) were significant in both lake zones ($p < 0.05$). The variables related to morphological pressures were not significant in the profundal zone, while in the sublittoral zone three of them (diversity of macrophyte growth form types, sum of pressures and natural land cover) were significant ($p < 0.05$).

The amount of explained variance by the selected set of variables was 52.12 % of total taxa variance for the profundal zone, and 18.90 % for the sublittoral zone.

Eutrophication accounted for 8.55 % of total variance in the profundal zone ($p = 0.005$), while it accounted for 0.49 % in the sublittoral zone (Fig. 3; $p = 0.04$). Morphological pressures were not significant in the profundal zone, while they accounted for 0.82 % of total variance in the sublittoral zone ($p = 0.015$). Lake morphometry and alkalinity explained 3.13 % of total variance in the profundal zone ($p = 0.01$) and 1.57 % in the sublittoral ($p = 0.005$). The spatial component accounted for 30.58 % of total variance in the profundal zone ($p = 0.005$), 8.35 % in the sublittoral ($p = 0.005$). Interactions among the explanatory variable groups explained 9.87 % of total variance in the profundal (0.18 % spatial-eutrophication interactions, 1.83 % spatial-morphometry and alkalinity interactions, 1.68 % eutrophication-morphometry and alkalinity interactions and 6.18 % spatial-eutrophication-morphometry and alkalinity interactions), and 7.66 % in the sublittoral zone, where the main contributions

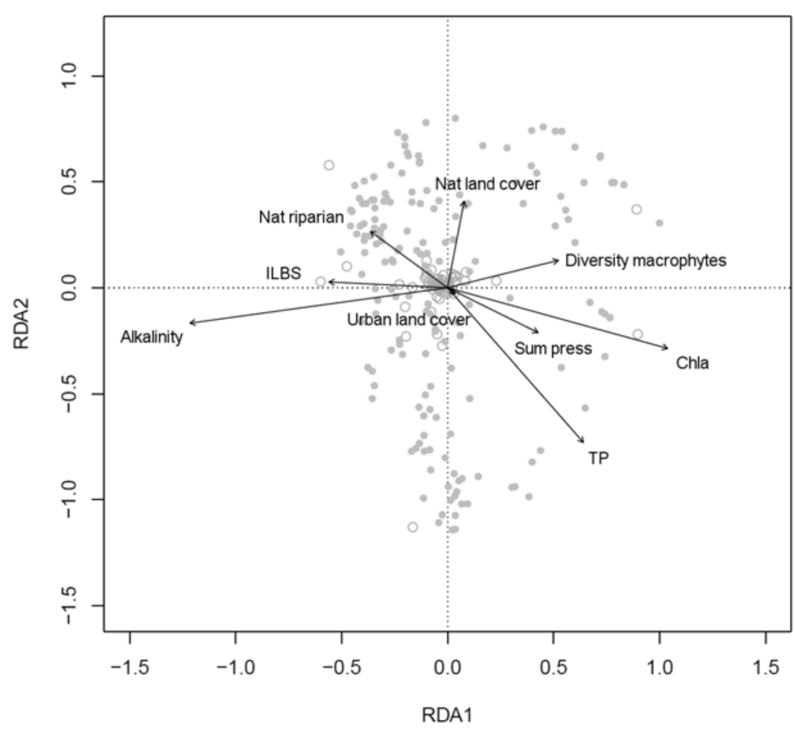

Fig. 2. Sublittoral zone: RDA biplot scores for constraining variables. Grey dots represent sampling sites, white dots represent species.

Table 3. Results of the forward selection procedure applied to each variable group and each dataset separately. The *p* values of the significant variables are reported. For spatial variables, only significant PCNM vectors are reported. Variables explanation is provided in the text.

Variable group	Variable	Profundal zone	Sublittoral zone
Spatial component	PCNM vectors	PCNM 1: $p=0.001$	PCNM 1: $p=0.001$
		PCNM 2: $p=0.001$	PCNM 7: $p=0.001$
		PCNM 4: $p=0.001$	PCNM 23: $p=0.001$
		PCNM 9: $p=0.002$	PCNM 2: $p=0.001$
		PCNM 6: $p=0.038$	PCNM 4: $p=0.004$
			PCNM 33: $p=0.004$
			PCNM 16: $p=0.006$
			PCNM 20: $p=0.007$
			PCNM 5: $p=0.008$
			PCNM 24: $p=0.013$
			PCNM 30: $p=0.049$
Eutrophication	Mid-lake Chl-*a*	$p=0.005$	$p=0.001$
	Mid-lake TP	$p=0.001$	$p=0.001$
Morphological pressures	Diversity of macrophyte growth form types	–	$p=0.001$
	Sum of pressures	–	$p=0.012$
	Naturalness of riparian zone	–	not-selected
	Urban land cover	not-selected	not-selected
	Natural land cover	not-selected	$p=0.001$
Lake morphometry and geology	ILBS	$p=0.001$	$p=0.001$
	Mid-lake alkalinity	$p=0.022$	$p=0.001$

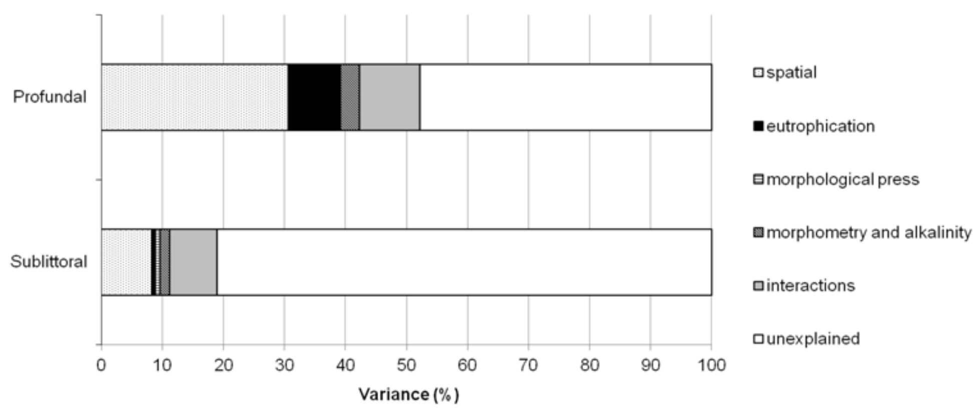

Fig. 3. Variance partitioning using pRDA for each dataset. The pure effect of spatial and environmental variables, the interactions among the 4 groups of variables and the unexplained variance are reported.

were due to spatial – eutrophication (2.36 % of total variance), spatial-morphometry and alkalinity (2.56 % of total variance) and spatial-morphological pressures (1.33 % of the total variance) interactions.

Discussion and conclusions

Since water bodies are subjected to multiple anthropogenic pressures, it is difficult to assess the unique impact of each pressure on the biota (Vinebrooke et al. 2004). A frequent approach to this kind of study is to stratify the sampling design in order to reduce the effects of sources of variation other than the one of interest. For example, Sandin & Hering (2004) studied the impact of organic pollution on stream macroinvertebrates in a large study across Europe and focused the analysis only on water bodies where organic pollution was the unique dominant stressor. In our study, we applied the variance partitioning approach in order to quantify the combined impact of eutrophication and morphological pressures. This method allows a disentangling of the problem of interactions among different groups of explaining factors (Peres-Neto et al. 2006). We also included variables connected to the spatial pattern (PCNM vectors) in the analysis, and variables connected to lake morphometry and geology. Those factors were responsible for a large part of the community variance other than anthropogenic derived disturbances and had synergic effects with the environmental variables related to the two pressures. Furthermore, by including those sets of variables in the variance partitioning analysis we could isolate the pure effects of eutrophication and morphological pressures from the effects due to interactions with the other variables.

Spatial and environmental variables

In the PCNM analysis of spatial pattern, the first vectors are related to the broadest/regional spatial scale while the last vectors are related to the finest scale (Borcard et al. 2004). The first PCNM vectors were highly significant in forward selection ($p = 0.001$) in both datasets (Table 3). This result highlights the importance of the regional scale in both profundal and sublittoral zones. However, in both lake zones, the other significant PCNM vectors represent a mixture of broad, medium and fine scales, indicating the absence of a predominant spatial scale and thus the interdependence between local and regional factors. Similar results were reported also for littoral communities by Johnson & Goedkoop (2002), who stated that regional factors at catchment scale set upper limits and, within these limits, local factors become important in structuring invertebrate community composition.

Eutrophication pressure affects both lake zones, in fact the environmental variables related to this pressure (total phosphorus and Chl-*a*) were significant in structuring the invertebrate community (Table 3), and were important variables in defining the gradient of the RDA ordination (Figs 1 and 2) in both the sublittoral and profundal zone.

RDA ordinations and forward selection results support the hypothesis of the decreasing influence of morphological pressures in structuring invertebrate communities from the sublittoral to the profundal zone (Solimini et al. 2006, Solimini & Sandin 2012 (this issue), Brauns et al. 2007a). Among the variables related to morphological pressure, neither urban land cover nor natural land cover of the 200 m stretch around the lake shore were found to be significant in affecting the profundal communities (Table 3) and had only a marginal role in defining the RDA gradients. Diversity of macrophyte growth form types was highly significant in the sublittoral zone (Table 3). This confirms the well known importance of habitat complexity provided by macrophytes in defining the invertebrate community (Weatherhead & James 2001, Mastrantuono et al. 2008, Free et al. 2009b, McGoff & Irvine 2009), for example Cheruvelil et al. (2002) demonstrated that macrophyte colonization by invertebrates is influenced by plant architecture. Also the natural land cover of lake surroundings and anthropogenic pressures both in the riparian zone and on the shoreline affected the sublittoral community as shown by the forward selection results and the RDA ordinations. The abundance and composition of the sublittoral communities was not affected by the presence and longevity of natural riparian vegetation as synthesized by the index of naturalness of the riparian zone. On the contrary these features are expected to directly affect littoral communities by providing a diverse habitat through the presence of roots and woody debris, known to be important factors in structuring the taxonomic composition of the littoral invertebrate community (Brauns et al. 2007b, Brauns et al. 2008).

Lake morphometry was highly significant in both the profundal and sublittoral zone. Lake area and slope have been demonstrated to be an indicator of anoxia (Nürnberg 1995) and to indirectly affect the invertebrate community through influencing fine sediment distribution and macrophyte growth (Rasmussen & Kalff 1987). In fact, lake area can indicate wind fetch, which determines wave height and thus fine sediments distribution (Smith & Sinclair 1972). Slope influences the ability to retain fine sediments and has been found to be negatively related to the biomass of rooted submerged macrophytes (Duarte & Kalff 1986). Finally, alkalinity is primarily influenced by catchment geology (Lee 1980, Koetsier et al. 1996) and was a significant variable in both the profundal and sublittoral zone (Table 3). Alkalinity, and thus bedrock geology, can be an indicator of the buffering capacity against rapid changes of pH and thus can indirectly affect the invertebrate community through influencing food resources such as primary production and detrital food quantity and quality (Groom & Hildrew 1989, Griffith & Perry 1994, Koetsier et al. 1996).

Variance partitioning

The most striking results of our analysis were the eutrophication-profundal and hydromorphological-sublittoral pressure-biota relationships (Fig. 3). Although this differing effect of the two pressures on the different zones of lakes was previously hypothesised (Solimini et al. 2006), to date, to our knowledge, no direct quantitative comparison using variance partitioning techniques was available. Our results showed that the profundal communities are mainly affected by eutrophication (Fig. 3). The relationship between lake trophic state and profundal communities is well known, as reported in Solimini et al. (2006), Solimini & Sandin (2012 (this issue)). The input of nutrients enhances littoral and pelagic productivity and leads to an increase of organic matter in the sediments. The degradation of the organic matter causes a decrease of oxygen in the hypolimnion which has direct effects on the profundal macroinvertebrate community (Rasmussen & Kalff 1987, Dinsmore et al. 1999). This process affects the structure of the community, through a decrease of diversity and abundance of hypoxia-sensitive taxa and an increase of hypoxia-tolerant taxa abundances (Bazzanti et al. 1994, Wolfram et al. 2002). In the sublittoral zone the partitioned effect of eutrophication was 17.4 times lower than in the profundal zone. This is consistent with previous studies which demonstrated that the profundal zone is more suitable for detecting early signs of eutrophication than the sublittoral zone (Bazzanti et al. 1994, Hämäläinen et al. 2003).

The variance partitioning results confirm the pattern found with RDA ordination and forward selection, showing a decreasing influence of morphological pressures from the sublittoral to profundal zone. In fact, in the sublittoral, the partitioned effect of morphological pressures was 1.68 times higher than that of eutrophication, while it could not be tracked in the profundal zone. This was owing to morphological pressures being measured at shoreline and riparian zone level only and therefore difficult to spatially associate with profundal sites.

The spatial component was the dominant factor in both the lake zones, representing 58.66 % of the explained variance in the profundal zone and 44.18 % in the sublittoral. This fraction was also responsible for a large part of the interactions among factors. The spatial component accounts for three possible causal factors: spatially-structured environmental or biotic factors not included in the analysis, spatially-structured historical events and spatial autocorrelation in the response matrix (Borcard et al. 2002, Legendre & Legendre 1998). Attempting to account for these factors in more detailed temporal and spatial sampling programmes may improve assessment. Finally, the fraction of unexplained variance (47.88 % in the profundal, 81.1 % in the sublittoral zone) is due to factors not included in the analysis (non spatially-structured factors), random variation and sampling error (Legendre & Legendre 1998). Therefore, in order to reduce the unexplained variance and thus provide a reliable assessment tool based on invertebrate assemblages-pressure relationships, current sampling programs should include a wider array of abiotic and biotic variables at site levels such as fish predation pressure.

When analysing bioassessment methods, Clarke & Hering (2006) identified four possible sources of variation for invertebrate fauna: variation due to the sampling method, that due to the sampling processing, variation due to natural temporal variation and finally that due to stressors and pressures. Our study shows that spatial variation should also be taken into account as a source of variation for invertebrate fauna that need to be assessed in order to isolate the effects of human induced pressures.

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References

Bazzanti, M., Seminara, M. & Tamorri, C., 1994: Depth distribution and relationships to the trophic state of sublittoral and profundal macrobenthos in lake Vico (central Italy). – Limnologica **24** (1): 13–21.

Bazzanti, M., Mastrantuono, L. & Solimini, A. G., 2012: Selecting macroinvertebrate taxa and metrics to assess eutrophication in different depth zones of Mediterranean lakes. – Fundam. Appl. Limnol. **180**: 133–143.

Borcard, D., Gillet, F. & Legendre, P., 2011: Numerical ecology with R. – Springer, New York.

Borcard, D., Legendre, P. & Drapeau, P., 1992: Partialling out the spatial component of ecological variation. – Ecology **73** (3): 1045–1055.

Borcard, D., Legendre, P., Avois-Jacquet, C. & Tuomisto, H., 2004 : Dissecting the spatial structure of ecological data at multiple scales. – Ecology **85** (7): 1826–1832.

Brind'Amour, A., Menot, L., Galéron, J. & Crassous, P., 2009: Spatial organization of a sedimentary macrobenthic community located on the West African Equatorial margin. – Deep-Sea Res. II **56**: 2292–2298.

Brauns, M., Garcia, X. F. & Pusch, M., 2008: Potential effects of water-level fluctuations on littoral invertebrates in lowland lakes. – Hydrobiologia **613**: 5–12.

Brauns, M., Garcia, X. F., Walz, N. & Pusch, M., 2007a: Effects of human shoreline development on littoral macroinvertebrates in lowland lakes. – J. Appl. Ecol. **44**: 1138–1144.

Brauns, M., Garcia, X. F., Pusch, M. & Walz, N., 2007b: Eulittoral macroinvertebrate communities of lowland lakes: discrimination among trophic states. – Freshwat. Biol. **52**: 1022–1032.

Carvalho, L., Solimini, A. G., Phillips, G., Pietilainen, O.-P., Moe, J., Cardoso, A. C., Solheim, A. L., Ott, I., Søndergaard, M., Tartari, G. & Rekolainen, S., 2009: Site-specific chlorophyll reference conditions for lakes in Northern and Western Europe. – Hydrobiologia **633**: 59–66.

Cheruvelil, K. S., Soranno, P. A., Madsen, J. D. & Roberson, M. J., 2002: Plant architecture and epiphytic macroinvertebrate communities: the role of an exotic dissected macrophyte. – J. N. Am. Benthol. Soc. **21**: 261–277.

Clarke, R. T. & Hering, D., 2006: Errors and uncertainty in bioassessment methods – major results and conclusions from the STAR project and their application using STARBUGS. – Hydrobiologia **566**: 433–439.

Dinsmore, W. P., Scrimgeour, G. J. & Prepas, E. E., 1999: Empirical relationships between profundal macroinvertebrate biomass and environmental variables in boreal lakes of Alberta, Canada. – Freshwat. Biol. **41**: 91–100.

Dray, S., 2005: Packfor: R Package for Forward Selection, available at http://r-forge.r-project.org/

Dray, S., Legendre, P. & Peres-Neto, P. R., 2006: SpacemakeR: spatial modelling. http://r-forge.r-project.org/R

Duarte, C. M. & Kalff, J., 1986: Littoral slope as a predictor of the maximum biomass of submerged macrophyte communities. – Limnol. Oceanogr. **31** (5): 1072–1080.

European Community, 2000: Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. – Official Journal of the European Communities **L327**: 1–72.

Feld, C. K. & Hering, D., 2007: Community structure or function: effects of environmental stress on benthic macroinvertebrates at different spatial scales. – Freshwat. Biol. **52**: 1380–1399.

Free, G., Bowman, J., McGarrigle, M. L., Caroni, R., Donnelly, K., Tierney, D., Trodd, W. & Little, R., 2009b: The identification, characterization and conservation value of isoetid lakes in Ireland. – Aquat. Conserv.: Mar. Freshwat. Ecosyst. **19**: 264–273.

Free, G., Solimini, A. G., Cardoso, A. C., Rossaro, B., Marziali, L., Giacchini, R., Paracchini, B., Ghiani, M., Gawlik, B. & Vaccaro, S., 2006: An ecological assessment system for subalpine lakes using macroinvertebrates. Preliminary investigations. – EUR 22558 EN

Free, G., Solimini, A. G., Rossaro, B., Marziali, L., Giacchini, R., Paracchini, B., Ghiani, M., Vaccaro, S., Gawlik, B. M., Fresner, R., Santner, G., Schönhuber, M. & Cardoso, A. C., 2009a: Modelling lake macroinvertebrate species in the shallow sublittoral: relative roles of habitat, lake morphology, aquatic chemistry and sediment composition. – Hydrobiologia **633**: 123–136.

Griffith, M. P. & Perry, S. A., 1994: Secondary production of macroinvertebrate shredders in headwater streams with different baseflow alkalinity. – J. N. Am. Benthol. Soc. **13** (3): 345–356.

Groom, A. P. & Hildrew, A. G., 1989: Food quality for detritivores in streams of contrasting pH. – J. Anim. Ecol. **58**: 863–881.

Hämäläinen, H., Luotonen, H., Koskenniemi, E. & Liljaniemi, P., 2003: Inter-annual variation in macroinvertebrate communities in a shallow forest lake in eastern Finland during 1990–2001. – Hydrobiologia **506–509**: 389–397.

Johnson, R. K., 1998: Spatiotemporal variability of temperate lake macroinvertebrate communities: detection of impact. – Ecol. Appl. **8** (1): 61–70.

Johnson, R. K. & Goedkoop, W., 2002: Littoral macroinvertebrate communities: spatial scale and ecological relationships. – Freshwat. Biol. **47**: 1840–1854.

Johnson, R. K., Goedkoop, W. & Sandin, L., 2004: Spatial scale and ecological relationships between the macroinvertebrate communities of stony habitats of streams and lakes. – Freshwat. Biol. **49**: 1179–1194.

Jyväsjärvi, J., Aroviita, J. & Hämäläinen, H., 2012: Performance of profundal macroinvertebrate assessment in boreal lakes depends on lake depth. – Fundam. Appl. Limnol. **180**: 91–100.

Koetsier, P., Minshall, G. W. & Robinson, C. T., 1996: Benthos and macroinvertebrate drift in six streams differing in alkalinity. – Hydrobiologia **317** (1): 41–49.

Lee, R., 1980: Forest Hydrology. – Columbia University Press, New York.

Legendre, P. & Gallagher, E. D., 2001: Ecologically meaningful transformations for ordination of species data. – Oecologia **129**: 271–280.

Legendre, P. & Legendre, L., 1998: Numerical ecology. Second English edition. – Elsevier Science BV, Amsterdam, The Netherlands.

Léonard, R., Legendre, P., Jean, M. & Bouchard, A., 2008: Using the landscape morphometric context to resolve spatial patterns of submerged macrophyte communities in a fluvial lake. – Landscape Ecol. **23**: 91–105.

Mastrantuono, L., Solimini, A. G., Noges, P. & Bazzanti, M., 2008: Plant-associated invertebrates and hydrological balance in the large volcanic Lake Bracciano (Central Italy) during two years with different water levels. – Hydrobiologia **599**:143–152.

McGoff, E. & Irvine, K., 2009: A test of the association between Lake Habitat Quality Assessment and macroinvertebrate community structure. – Aquat. Conserv.: Mar. Freshwat. Ecosyst. **19**: 520–533.

McGoff, E. & Sandin, L., 2012: Catchment land-use effects on littoral macroinvertebrates in response to local habitat structure and trophic state. – Fundam. Appl. Limnol. **180**: 111–121.

Noges, P., van de Bund, W., Cardoso A. C., Solimini, A. G. & Heiskanen, A.-S., 2009: Assessment of the ecological status of European surface waters: a work in progress. – Hydrobiologia **633**: 197–211.

Noges, P., Mischke, U., Laugaste, R. & Solimini, A. G., 2010: Analysis of changes over 44 years in the phytoplankton of Lake Vortsjarv (Estonia): the effect of nutrients, climate and the investigator on phytoplankton-based water quality indices. – Hydrobiologia **646**: 33–48.

Nürnberg, G. K., 1995: Quantifying anoxia in lakes. – Limnol. Oceanogr. **406**: 1100–1111.

Oksanen, J., Kindt, R., Legendre, P. & O'Hara, R. B., 2006: Vegan: Community Ecology Package version 1.17–4, http://cran.r-project.org/web/packages/vegan/index.html.

O'Toole, C., Donohue, I., Moe, S. J. & Irvine K., 2008: Nutrient optima and tolerances of benthic invertebrates, the effects of taxonomic resolution and testing of selected metrics in lakes using an extensive European data base. – Aquat. Ecol. **42**: 277–291.

Peres-Neto, P. R. & Legendre, P., 2010: Estimating and controlling for spatial structure in the study of ecological communities. – Global Ecol. Biogeogr. **19**: 174–184.

Peres-Neto, P. R., Legendre, P., Dray, S. & Borcard, D., 2006: Variation partitioning of species data matrices: estimation and comparison of fractions. – Ecology **87** (10): 2614–2625.

Phillips, G., Pietilainen, O.-P., Carvalho, L., Solimini, A. G., Solheim, A. L. & Cardoso, A. C., 2008: Chlorophyll-nutrient relationships of different lake types using a large European dataset. – Aquat. Ecol. **42** (2): 213–226.

Pilotto, F., Free, G., Cardoso, A. C., Wolfram, G. & Solimini, A. G., 2012: Spatial variance of profundal and sublittoral invertebrate benthic communities in response to eutrophication and morphological pressures. – Fundam. Appl. Limnol. **180**: 101–111.

Porst, G., Bader, S., Münch, E. & Pusch, M., 2012: Sampling approaches for the assessment of shoreline development based on littoral macroinvertebrates: the case of Lake Werbellin, Germany. – Fundam. Appl. Limnol. **180**: 123–131.

Ptacnik, R., Solimini, A. G. & Brettum, P., 2009: Performance of a new phytoplankton composition metric along a eutrophication gradient in Nordic lakes. – Hydrobiologia **633**: 75–82.

Rasmussen, J. B. & Kalff, J., 1987: Empirical models for zoobenthic biomass in lakes. – Can. J. Fish. Aquat. Sci. **44**: 990–1001.

Rossaro, B., Marziali, L., Cardoso, A. C., Solimini, A., Free, G. & Giacchini, R., 2007: A biotic index using benthic macroinvertebrates for Italian lakes. – Ecol. Indicators **7**: 412–429.

Rowan, J. S., Duck, R. W., Carwardine, J., Bragg, O. M., Black, A. R. & Cutler, M. E. J., 2004: Development of a Technique for Lake Habitat Survey (LHS): Phase 1. – SNIFFER, Edinburgh.

Rowan, J. S., Carwardine, J., Duck, R. W., Bragg, O. M., Black, A. R., Cutler, M. E. J., Soutar, I. & Boon, P. J., 2006: Development of a technique for lake habitat survey (LHS) with applications for the European Union Water Framework Directive. – Aquat. Conserv.: Mar. Freshwat. Ecosyst. **16**: 637–657.

Sandin, L. & Hering, D., 2004: Comparing macroinvertebrate indices to detect organic pollution across Europe: a contribution to the EC Water Framework Directive intercalibration. – Hydrobiologia **516**: 55–68.

Smith, I. R. & Sinclair, I. J., 1972: Deep water waves in lakes. – Freshwat. Biol. **2**: 387–399.

Solimini, A. G., Free, G., Donohue, I., Irvine, K., Pusch, M., Rossaro, B., Sandin, L. & Cardoso, A. C., 2006: Using benthic macroinvertebrates to assess ecological status of lakes. Current knowledge and way forward to support WFD implementation. Report EUR 22347 EN for the European Commission, Directorate-General Joint Research Centre, Institute for Environment and Sustainability. – Office for Official Publications of the European Communities, Luxembourg, 46 pp.

Solimini, A. G., Ptacnik, R. & Cardoso, A. C., 2009: Towards holistic assessment of the functioning of ecosystems under the Water Framework Directive. – TrAC Trends in Analytical Chemistry **28** (2): 143–149.

Solimini, A. G. & Sandin, L., 2012: The importance of spatial variation of benthic invertebrates for the ecological assessment of European lakes. – Fundam. Appl. Limnol. **180**: 85–89.

Stendera, S. & Johnson, R. K., 2008: Habitat-specific stability and persistence of benthic invertebrate communities in boreal lakes. – Fundam. Appl. Limnol., Arch. Hydrobiol. **171** (4): 311–322.

Vinebrooke, R. D., Cottingham, K. L., Norberg, J., Scheffer, M., Dodson, S. I., Maberly, S. C. & Sommer, U., 2004: Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. – Oikos **104**: 451–457.

Weatherhead, M. A. & James, M. R., 2001: Distribution of macroinvertebrates in relation to physical and biological variables in the littoral zone of nine New Zealand lakes. – Hydrobiologia **462**: 115–129.

Wolfram, G., Kowarc, V. A., Humpesch, U. H. & Siegl, W., 2002: Distribution pattern of benthic invertebrate communities in Traunsee (Austria) in relation to industrial tailings and trophy. – In: Schmidt, R. & Dokulil, M. (eds): Effects of industrial tailings on the ecological integrity of a deep oligotrophic lake (Traunsee, Austria). – Water, Air Soil Pollut. **2**: 63–91.

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