Ecography

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## Supplementary material

***Supplementary material***

Ecography

# Biotic interactions hold the key to understanding metacommunity organisation

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## Appendix 1. Study area and environmental features of the study ponds

Several environmental variables (Table A1) were measured *in situ* along transects for each study pond: mean depth (cm), Secchi depth (cm), pH, oxygen (mg $L^{-1}$), conductivity (µS $cm^{-1}$) and turbidity (FTU). To do this, we used calibrated sticks, WTW field probes (Model LF 323) and a portable turbidimeter (Model HACH 2100P), respectively. Water samples were randomly collected at different depths along a shore-centre transect, combined and mixed to form a composite water sample using a cylindrical corer (diameter = 60 mm, length = 1 m). The integrated water samples were then preserved in Pyrex glass bottles at 4 °C. Water chemistry variables were determined in laboratory from the composite water sample and included total nitrogen (mg $L^{-1}$), nitrate (mg $L^{-1}$), ammonium (µg $L^{-1}$), total phosphorus (µg $L^{-1}$), soluble reactive phosphorus (µg $L^{-1}$), total suspended solids (mg $L^{-1}$), volatile suspended solids (mg $L^{-1}$), dissolved organic carbon (mg $L^{-1}$) and chlorophyll *`a´* (mg $L^{-1}$). Nutrient samples were previously fixed with mercuric chloride ($HgCl_2$) and all laboratory analyses followed APHA standards (APHA 1989).

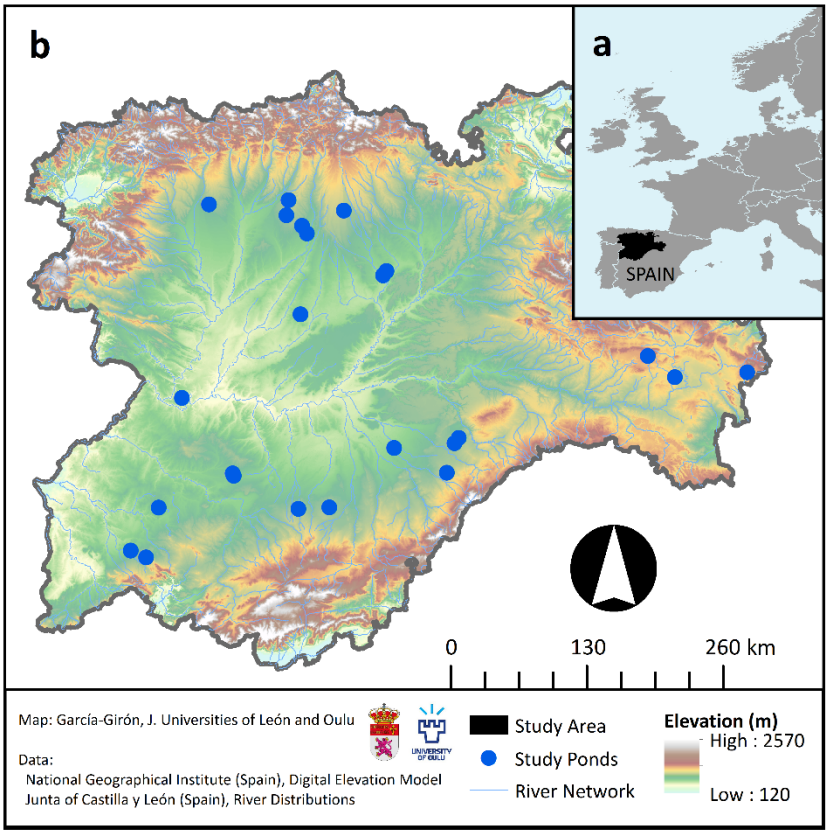


**Fig. A1.** Map showing the location of the study area **(a)** and the ponds (*n*=25) studied **(b)**.

**Table A1.** Summary of the environmental conditions of the 25 study ponds.

| Variables | Units | Abbreviations | Minimum | Maximum | Median | Mean |
|---|---|---|---|---|---|---|
| Pond area | ha | Area | 0.1 | 23.0 | 0.8 | 3.39 |
| Mean Depth | cm | Depth | 31.0 | 168.2 | 77.5 | 85.7 |
| Secchi depth | cm | Secchi | 14.5 | 126.5 | 63.5 | 76.8 |
| pH | | pH | 7.36 | 10.30 | 8.42 | 8.48 |
| Oxygen | mg $L^{-1}$ | Oxygen | 3.24 | 11.98 | 6.13 | 6.08 |
| Conductivity | µS $cm^{-1}$ | Conductivity | 104 | 898 | 265 | 345 |
| Turbidity | FTU | Turbidity | 1.4 | 83.3 | 11.1 | 18.2 |
| Total nitrogen | mg $L^{-1}$ | TN | 0.13 | 5.21 | 1.45 | 1.77 |
| Nitrate | mg $L^{-1}$ | Nitrate | <0.01 | 0.18 | 0.05 | 0.05 |
| Ammonium | µg $L^{-1}$ | Ammonium | <0.01 | 1521.10 | 11.18 | 82.69 |
| Total phosphorus | µg $L^{-1}$ | TP | 41.34 | 2438.60 | 118.63 | 445.06 |
| Soluble reactive phosphorus | µg $L^{-1}$ | SRP | 2.88 | 1928.28 | 15.66 | 161.78 |
| Total suspended solids | mg $L^{-1}$ | TSS | 2.5 | 57.5 | 13.6 | 16.1 |
| Volatile suspended solids | mg $L^{-1}$ | VSS | 1.7 | 24.8 | 7.0 | 9.7 |
| Dissolved organic carbon | mg $L^{-1}$ | DOC | 4.6 | 233.4 | 26.4 | 43.5 |
| Chlorophyll `*a*´ | mg $L^{-1}$ | Chla | 0.67 | 362.71 | 11.87 | 40.27 |
| Woodland | % | Woodland | 0 | 95 | 0 | 6 |
| Water | % | Water | 0 | 5 | 0 | 1 |
| Farming | % | Farming | 0 | 2 | 0 | 1 |
| Urban | % | Urban | 0 | 11 | 2 | 3 |
| Grassland | % | Grassland | 0 | 92 | 18 | 28 |
| Cropland | % | Cropland | 0 | 95 | 75 | 60 |
| Mean annual temperature | °C | Aver.temp | 7.2 | 12.2 | 11.3 | 10.3 |
| Annual temperature range | °C | Temp.range | 28.0 | 29.3 | 28.9 | 28.7 |
| Annual precipitation | mm | Precipitation | 380 | 800 | 484 | 499 |

**Appendix 2. Organismal groups and field surveys of biological communities**

We included a total of 12 organismal groups in our study (Table 1 in the main text*): helophytes, hydrophytes, edible phytoplankton, non-edible phytoplankton, filter-feeding zooplankton, small raptorial zooplankton, big raptorial zooplankton, detritivorous macroinvertebrates, scraping macroinvertebrates, predatory macroinvertebrates, small fish* and *big fish.* Macrophytes and phytoplankton were included because they are primary producers and their diversity and abundance shape the functioning and stability of most lentic ecosystems. We also included different zooplankton groups because they are amongst the most abundant invertebrates in ponds and have a crucial nexus for freshwater trophic webs (Carpenter 2001). Similarly, macroinvertebrates are one of the main animal groups of pond ecosystems, providing linkages between basal food resources (e.g. detritus and algae) and higher trophic levels, thereby playing an important role in material cycling and energy flow. Finally, we included a group of top predators (i.e. fish) because they modify important food web properties via foraging behaviour (Jeppesen et al. 2003).

*Aquatic macrophytes*

Aquatic macrophytes were exhaustively surveyed for each study pond using profiles (i.e. a line from one shore to the opposite shore at a right angle to the shoreline with the longest length). The number of profiles used for each pond varied depending on pond size and shoreline complexity (Jensén 1977). Quadrats (0.5 m × 0.5 m) were placed at varying intervals of 0-5 m depending on the homogeneity of the aquatic flora and percentage coverage of each macrophyte species was estimated in each quadrat as the visual project of each species in the water column onto the pond surface. Mean coverage of each taxon in a pond, including aquatic vascular plants and macroalgae, was determined as the sum of percent coverages of that species in all quadrats divided by the number of quadrats used in the pond.

*Phytoplankton*

At each pond, a subvolume of ca. 250 ml taken from the composite water sample was fixed in Lugol´s iodine and identified to the lowest possible taxonomic level (usually species) following the Utermölh technique

(Utermölh 1958) and using an inverted light microscope. Phytoplankton cells were also counted to determine phytoplankton biomass (µg $mL^{-1}$) from geometric forms of cells.

*Zooplankton*

Zooplankton community composition (incidence data) and community structure (abundance data), including Rotifera, Cladocera and Copepoda, were estimated from 1 to 3 L composite water samples collected on each sampling site and filtered through 50 and 25 µm mesh nets, respectively. Samples were fixed with carbonated water to avoid contraction of the animal teguments, preserved in formalin with a final concentration of 4%, and stored at 4 °C until analysed. Animals were identified using inverted light microscopy and densities were estimated as number of individuals $mL^{-1}$.

*Macroinvertebrates*

To collect macroinvertebrates, we took a three-minute (ponds < 1 ha and/or dominated by one habitat type > 70% of pond surface) to five-minute (three of the ponds with surface area > 1 ha and more than one dominant type of habitat) kick sample (net mesh size of 400 µm) covering most dominant microhabitats in the study ponds (i.e. bare sediments, shores without vegetation, vegetated shores and submerged hydrophytes; Collinson et al. 1995). Macroinvertebrates and associated material were immediately fixed in 96% ethanol and kept in separate jars (one for each pond) at 4 °C until further processing and identification. In the laboratory, a 1/6 subsample of each sample was processed under 10× magnification, but samples containing less than 200 individuals were fully sorted (Trigal et al. 2014). In order to avoid underestimation of richness measures, additional scanning for rare taxa was done following Vinson and Hawkins (1996) and King and Richardson (2002). Animals were identified to genus, except for damaged individuals (usually family), non-insect taxa (e.g. Oligochaeta, family level) and first instar chironomid larvae (subfamily).

*Fish*

Fish were sampled using fyke nets, with one or two nets, depending on the pond area, being set overnight and retrieved the next morning after ca. 18h (Moss et al. 2003). Abundance, as catch per unit effort (CPUE), was estimated for two size classes (i.e. 10 cm < fish < 10 cm).

**Appendix 3. Principal components analysis (PCA) on abiotic environmental features**

We reduced the available environmental variables to a more parsimonious set by performing principal component analysis (PCA) on all transformed abiotic features. To do this, we used the dudi.pca function from the ade4 package (Dray et al. 2018). The first axis was closely associated with water chemistry (including total phosphorus, total suspended solids and chlorophyll `a´), whereas the second axis was strongly related to catchment land use (e.g. cropland and woodland; Fig. A2a and Table A2). The variance captured by the first two axes was 21% and 16%, respectively (Fig. A2b). The third PCA axis (used to assess the sensitivity of the Graphical Lasso to the addition of a new, initially missing environmental predictor, see Supplementary material Appendix 6) was related to a combination of precipitation, nitrate concentration and pond area, and explained 13% of the variance in environmental conditions (Fig. A2b and Table A2).

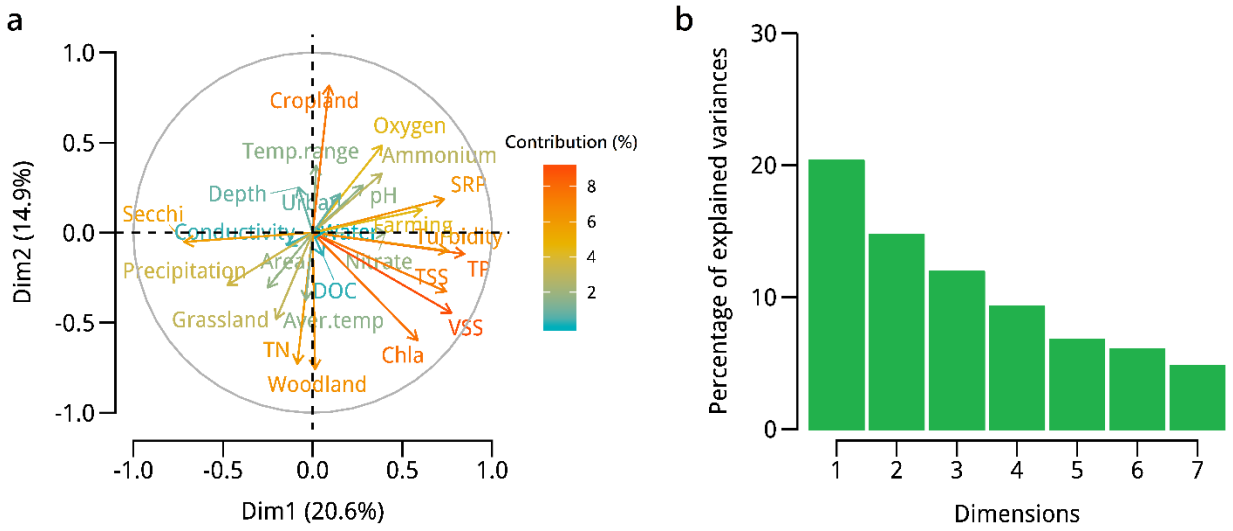


**Fig. A2. (a)** Biplot of the principal component analysis (PCA) performed on 25 (transformed) environmental variables representing local conditions, catchment land use and climate features. See **Table A1** for short names. **(b)** Bar plot of eigenvalues on the principal components of (transformed) environmental features.

**Table A2.** Correlations between the environmental features and the first three axes (PCA1, PCA2 and PCA3) of the principal component analysis. Check **Table A1** for abbreviations.

| | PCA1 | PCA2 | PCA3 |
|---|---|---|---|
| Area | -0,25 | -0,30 | -0,60 |
| Depth | -0,08 | 0,25 | -0,27 |
| Secchi | -0,72 | -0,05 | 0,04 |
| pH | 0,28 | 0,26 | -0,03 |
| Oxygen | 0,39 | 0,48 | -0,53 |
| Conductivity | -0,14 | -0,07 | 0,59 |
| Turbidity | 0,75 | -0,10 | -0,28 |
| TN | -0,09 | -0,73 | -0,11 |
| Nitrate | 0,41 | -0,01 | -0,64 |
| Ammonium | 0,39 | 0,33 | -0,36 |
| TP | 0,85 | -0,12 | 0,25 |
| SRP | 0,73 | 0,19 | 0,14 |
| TSS | 0,74 | -0,33 | -0,04 |
| VSS | 0,77 | -0,45 | -0,07 |
| DOC | 0,06 | -0,13 | -0,01 |
| Chla | 0,58 | -0,60 | 0,19 |
| Woodland | 0,01 | -0,76 | 0,30 |
| Water | 0,06 | 0,01 | -0,25 |
| Farming | 0,61 | 0,13 | -0,01 |
| Urban | 0,15 | 0,21 | 0,21 |
| Grassland | -0,20 | -0,48 | -0,46 |
| Cropland | 0,09 | 0,82 | 0,18 |
| Aver.temp | -0,04 | -0,37 | 0,54 |
| Temp.range | 0,02 | 0,37 | 0,27 |
| Precipitation | -0,48 | -0,29 | -0,65 |

**Appendix 4. Assessing the uncertainty of the empirical partial correlation networks**

We assessed the uncertainty of the empirical partial correlation networks using a random resampling of the sites. To do this, we first built a set of data considering a quartile criterion (here randomly selecting 50% - 50Q- and 75% -75Q- of the sites, respectively) on the same original pool ($N$ =25) of both community composition and community structure. We then compared the weighted degrees of the empirical and simulated networks using paired samples $t$-tests (Ross and Willson 2017). Importantly, patterns of the 50Q and 75Q datasets confirmed the inferred conditional dependencies between pairs of organismal groups detected by the original empirical networks (i.e. no significant differences, Fig. A3), both for variation in community composition (50Q, $t$ = 0.96, $p$ = 0.35; 75Q, $t$ = 0.54, $p$ = 0.59) and community structure (50Q, $t$ = 0.31 $p$ = 0.76; 75Q, $t$ = 0.40, $p$ = 0.69), thereby guaranteeing the confidence of the analyses.

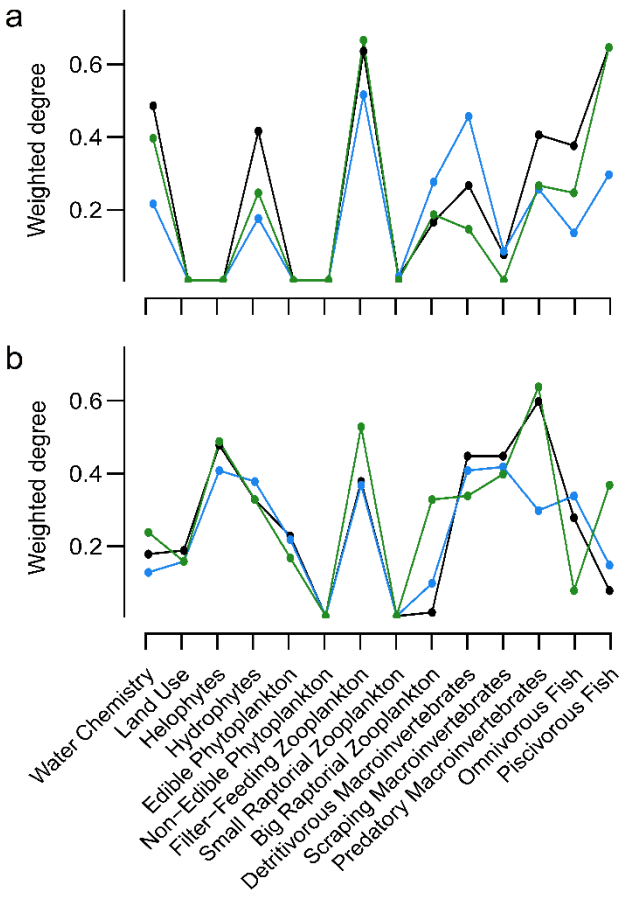


**Fig. A3.** Weighted degrees of the inferred networks (black, original full model; blue, 50Q model; green, 75Q model) for variation in community composition **(a)** and community structure **(b)** of the organismal groups.

**Appendix 5. Marginal and partial correlations for variation in community composition and community structure**

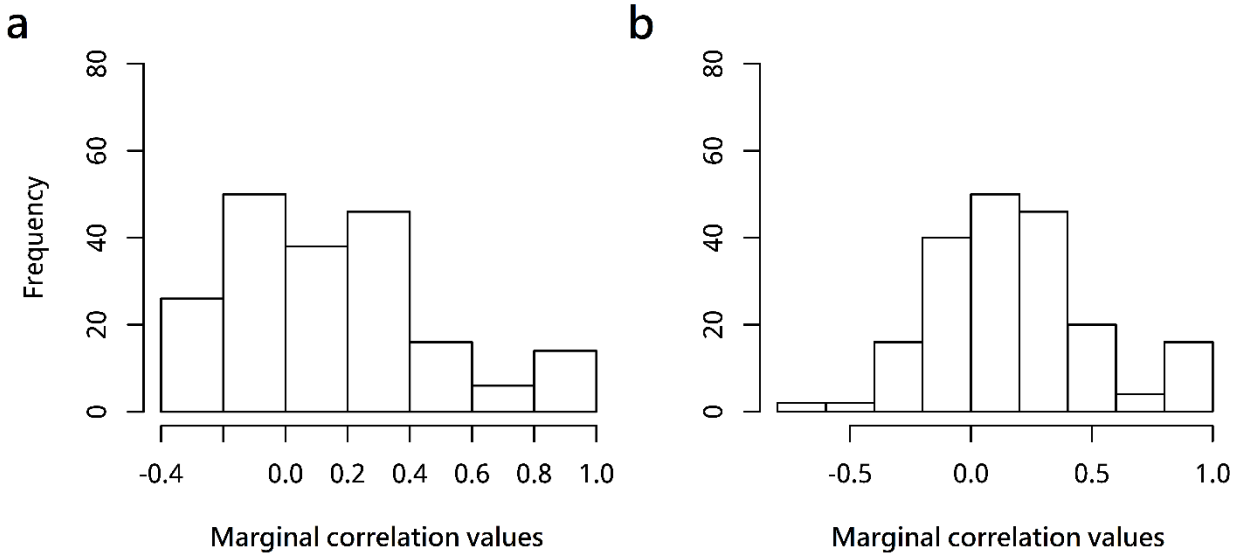


**Fig. A4.** Histograms of the marginal correlation coefficients estimated between variation in community composition **(a)** and community structure **(b)** of major organismal groups and environmental distances.

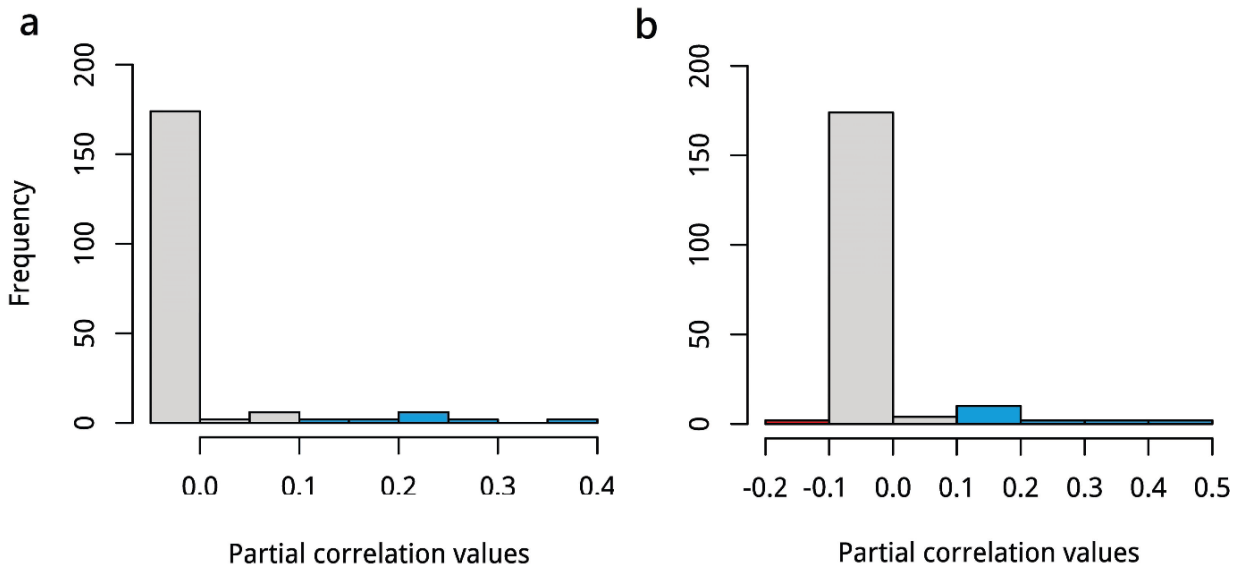


**Fig. A5.** Histograms of the partial correlation coefficients estimated between variation in community composition **(a)** and community structure **(b)** of the organismal groups and environmental distances. Null partial correlation coefficients ($-0.1 \leq partial\ correlation\ value \leq 0.1$) are coloured in grey, partial correlations below the median value of the non-null partial correlation coefficients ($partial\ correlation\ value < -0.1$) are coloured in red, and partial correlations above the median value of the non-null partial correlation coefficients ($partial\ correlation\ value > 0.1$) are coloured in blue.

**Appendix 6. Testing the robustness of the Graphical Lasso to the addition of environmental distances**

We assessed the sensitivity of the Graphical Lasso to the addition of new (initially missing) environmental distances (i.e. PCA axes). To do this, we re-ran the statistical analyses using the 14 previously selected nodes (12 species groups and 2 environmental distances) and added one more environmental distance built from the third axis of the principal component analysis (PCA3; see Supplementary material Appendix 3). For the sake of comparison, we computed both Sørensen and Bray-Curtis dissimilarities for multiple organismal groups. Similarly, we compared the networks inferred with two or three environmental distances using Poisot´s network dissimilarities (Poisot et al. 2012).

Nearly all the trophic networks built using the three environmental distances had positive non-null partial correlation values (Figs. A6 and A7). The overall structure of the networks (i.e. using the Sørensen index and the Bray-Curtis index) was defined by 15 nodes (12 species groups and 3 environmental distances) and 105 possible edges. The highest connectance was found for the partial correlation networks built using community composition (0.12 and 13 undirected edges; Fig. A8a) and the lowest connectance occurred for the network for community structure (0.11 and 12 undirected edges; Fig. A9a).

Adding a third environmental distance to the models did not change the edges between organismal groups for variation in community composition and community structure (see Results in the main text). Briefly, filter-feeding zooplankton (unweighted degree = 4, weighted degree = 0.64) and big fish (unweighted degree = 3, weighted degree = 0.64) remained as the most influential nodes for Sørensen dissimilarities (Fig. A8b), whereas predatory macroinvertebrates (unweighted degree = 3, weighted degree = 0.6) and helophytes (unweighted degree = 3, weighted degree = 0.48) had the strongest effect on variation in community structure of other organismal groups (Fig. A9b). Perhaps more importantly, the additional environmental distance (PCA3) had a small impact on the beta diversity of the organismal groups for both dissimilarity measures (unweighted degree of 1 for community composition, 1 for community structure, whereas the mean unweighted degrees of the partial correlation networks were 1.7 and 1.6, respectively;

weighted degree of 0.23 for community composition data, 0.17 for community structure, whereas the mean values were 0.27 and 0.26, respectively). Similarly, the probabilities of observing a non-null partial correlation between organismal groups´ nodes and the three environmental distances (for the Sørensen dissimilarity index, environmental features = 0.08, organismal groups = 0.13; for the Bray-Curtis dissimilarity index, environmental features = 0.08, organismal groups = 0.12) again emphasised the weaker influence of the abiotic environment on the assembly of pond metacommunities.

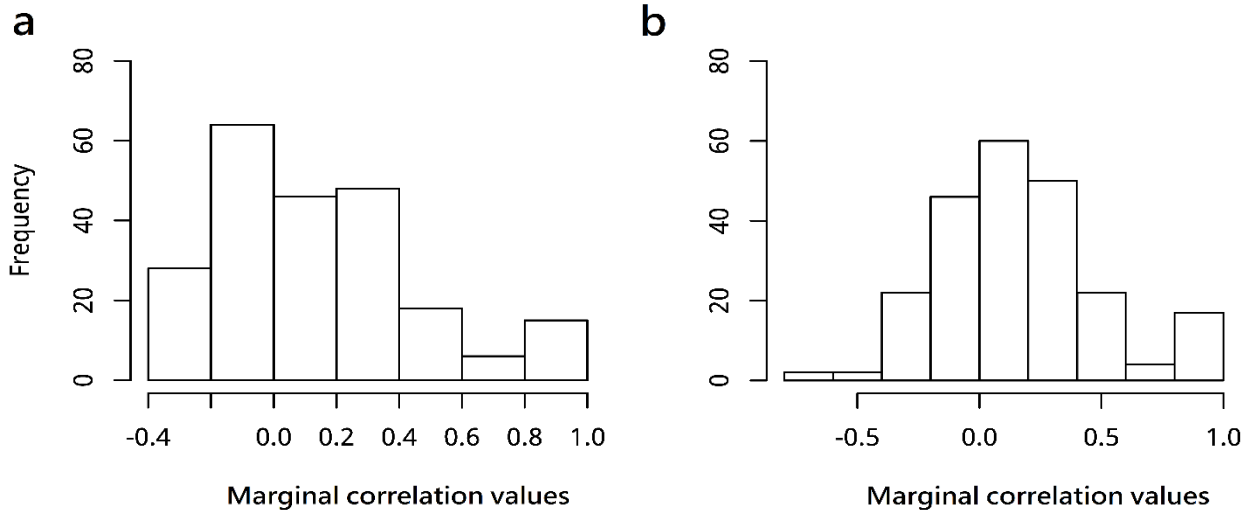


**Fig. A6.** Histograms of the marginal correlation coefficients estimated between variation in community composition **(a)** and community structure **(b)** of major organismal groups and environmental distances.

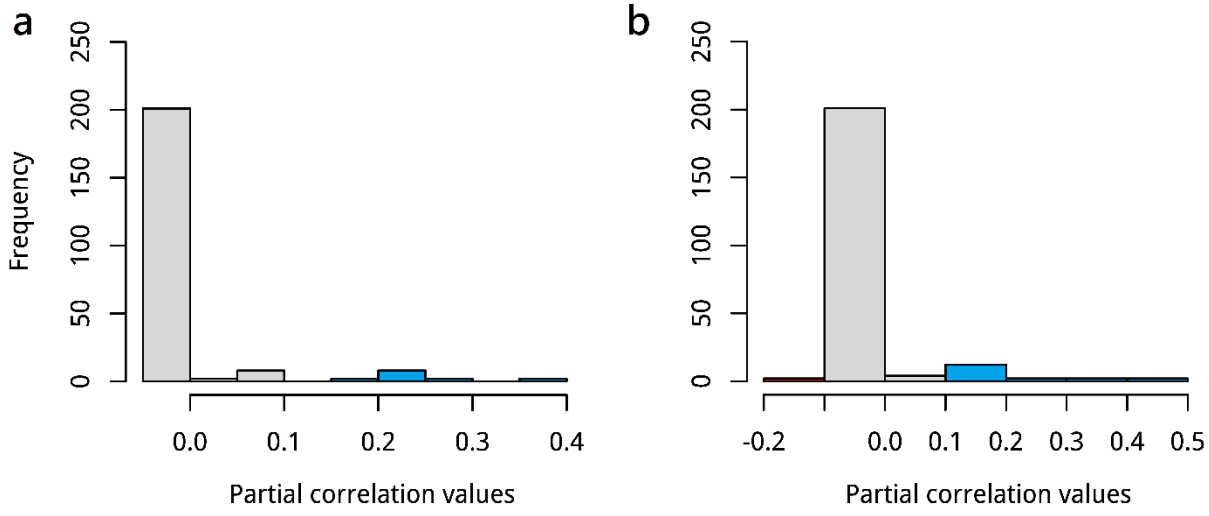


**Fig. A7.** Histograms of the partial correlation coefficients estimated between variation in community composition **(a)** and community structure **(b)** of major organismal groups and environmental distances.

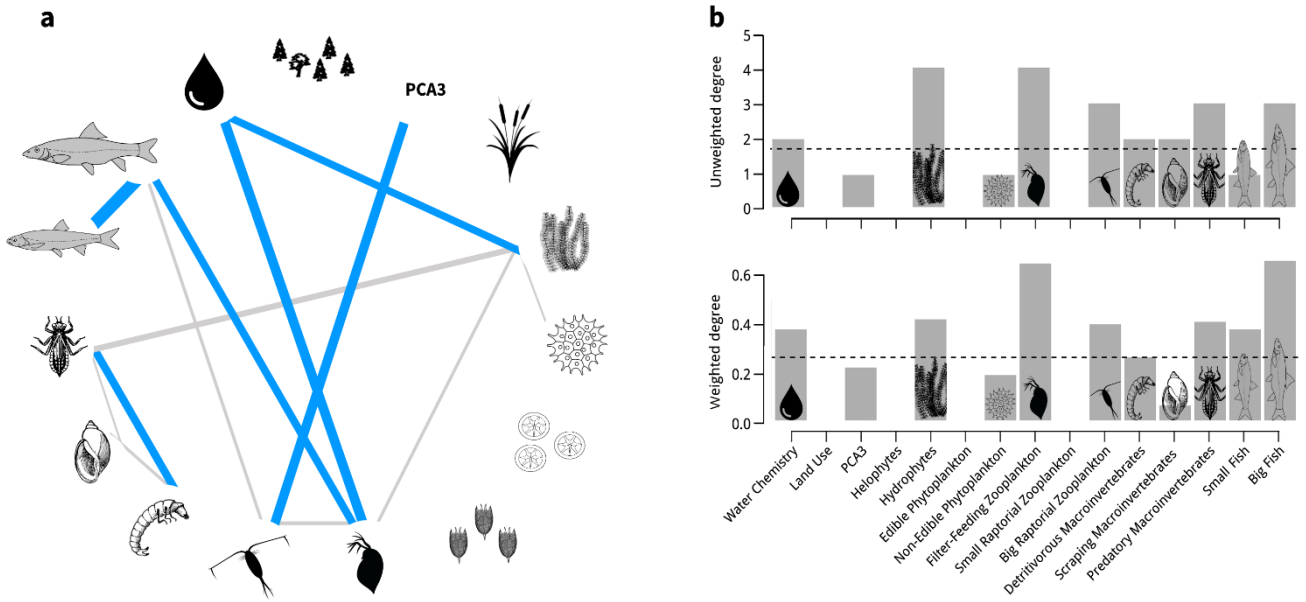


**Fig. A8. (a)** Undirected partial correlation network inferred using the Graphical Lasso between variation in community composition of major organismal groups and the three environmental distances. Each node represents the beta diversity of an organismal group or an environmental distance. Edge thickness is proportional to the value of the partial correlation coefficient (**Fig. A7a**). See the main text for legend. **(b)** Properties of the inferred network. The unweighted degree is the number of neighbours of nodes in a plot (here, the undirected correlation network in **Fig. A8a**). It measures the number of variables that are conditionally dependent on the variable associated with this node. The weighted degree is the sum of the partial correlation coefficients attached to the edges adjacent to this node. Dashed lines represent the mean values of unweighted and weighted degrees.

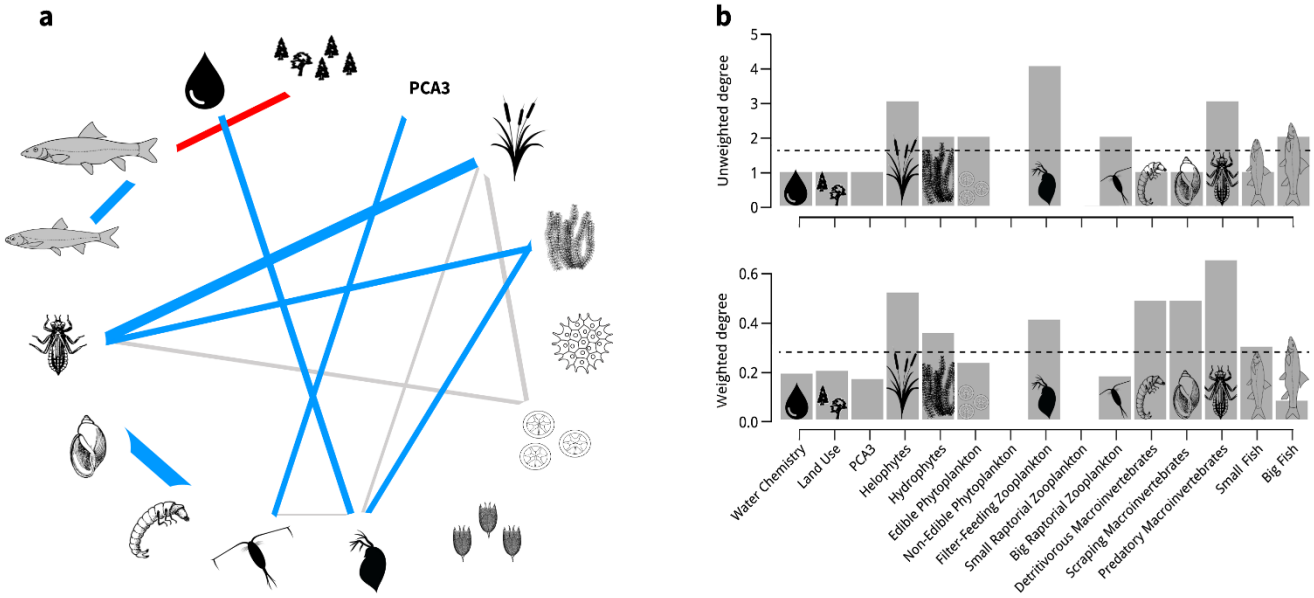


**Fig. A9. (a)** Undirected partial correlation network inferred using the Graphical Lasso between variation in community structure of major organismal groups and the three environmental distances. Each node represents the beta diversity of an organismal group or an environmental distance. Edge thickness is proportional to the value of the partial correlation coefficient (**Fig. A7b**). See the main text for legend. **(b)** Properties of the inferred network. The unweighted degree is the number of neighbours of nodes in a plot (here, the undirected correlation network in **Fig. A9a**). It measures the number of variables that are conditionally dependent on the variable associated with this node. The weighted degree is the sum of the partial correlation coefficients attached to the edges adjacent to this node. Dashed lines represent the mean values of unweighted and weighted degrees.

**Appendix 7. Spatial autocorrelation for variation in community composition and community structure**

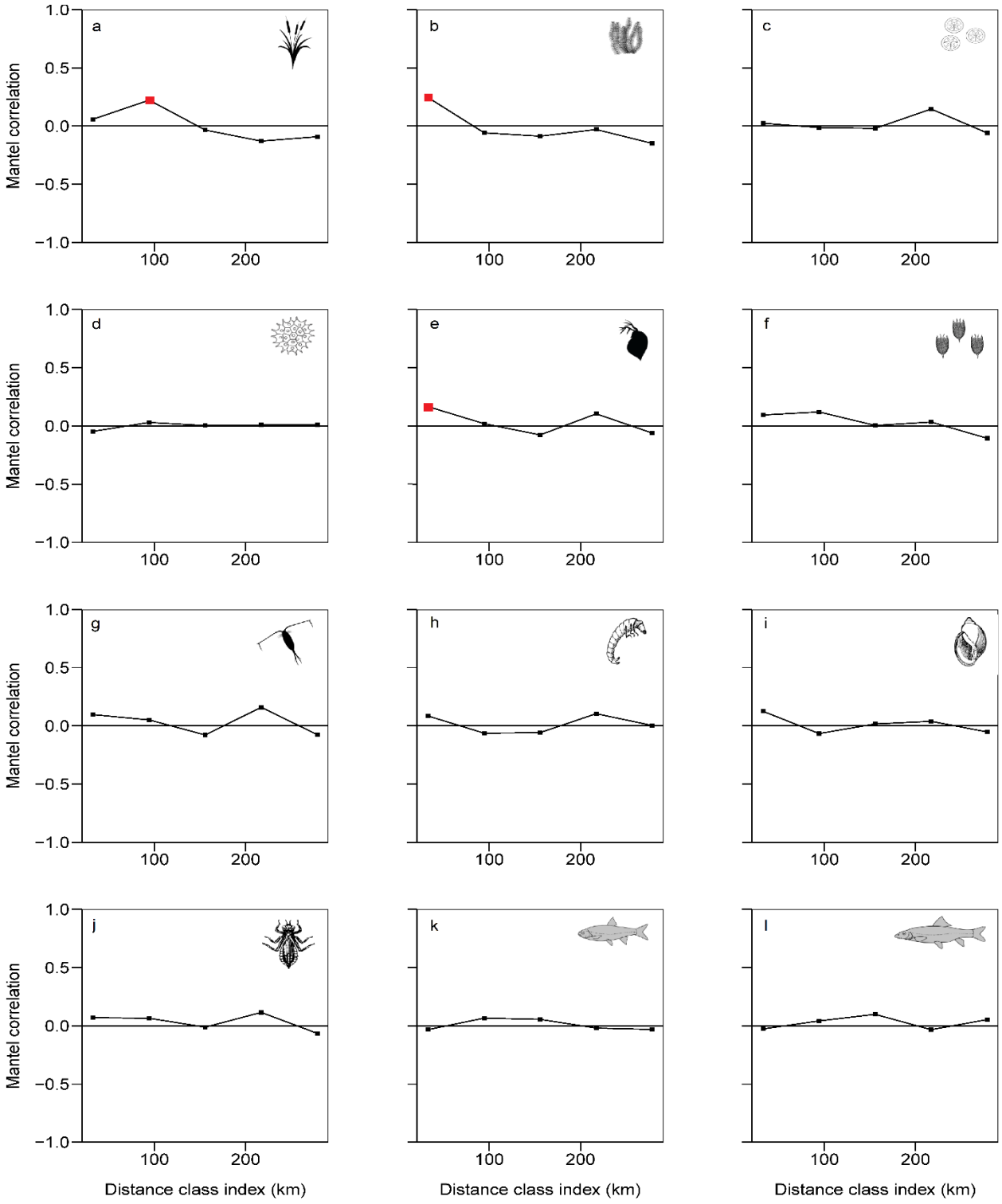


**Fig. A10.** Mantel correlograms showing the spatial structures for variation in community composition. Red squares denote significant spatial autocorrelation (α = 0.05) after Holm correction for multiple testing.

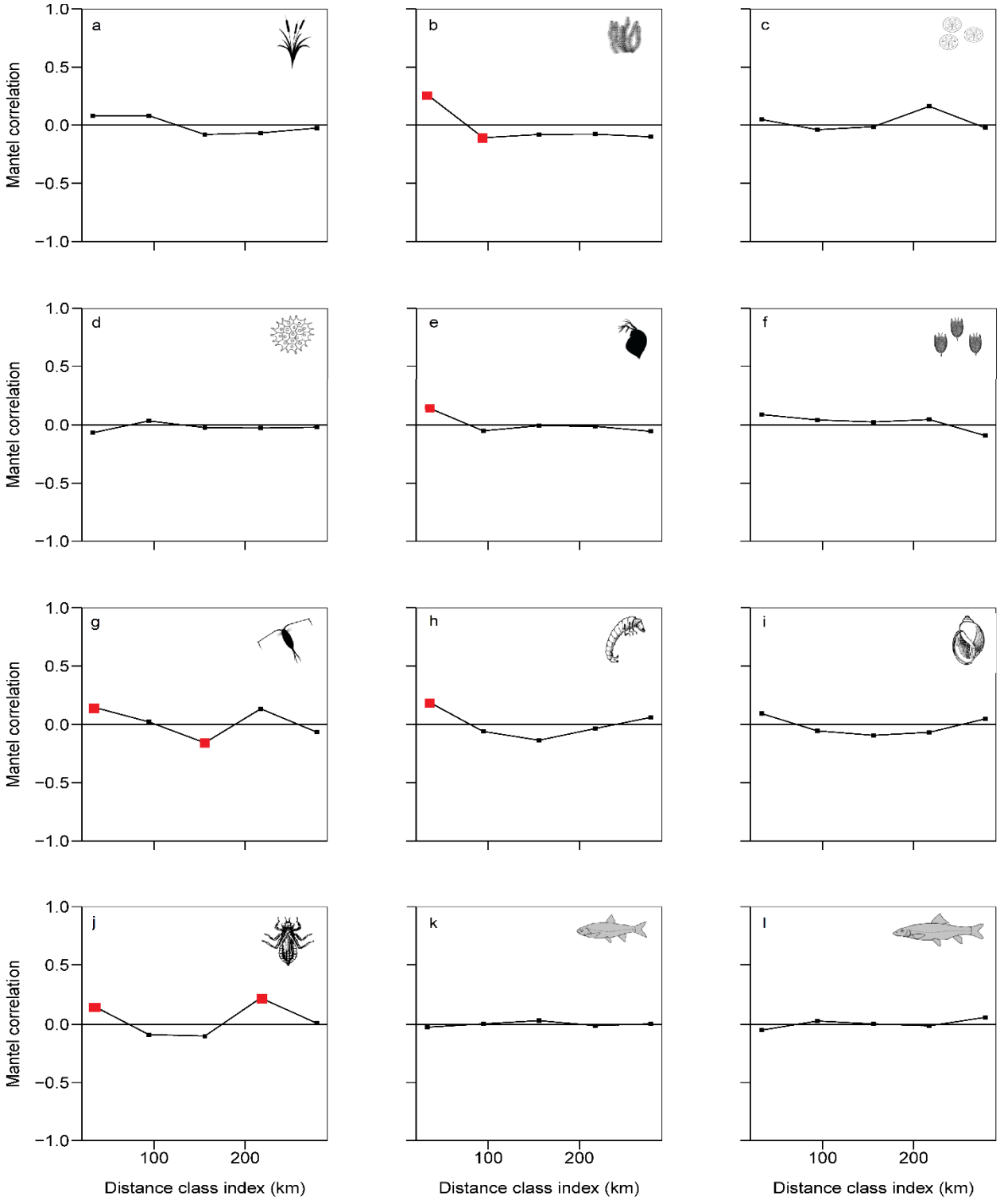


**Fig. A11.** Mantel correlograms showing the spatial structures for variation in community structure. Red squares denote significant spatial autocorrelation (α = 0.05) after Holm correction for multiple testing.

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