

## Research

### Structure and vulnerability of the multi-interaction network in macrophyte-dominated lakes

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The network approach is crucial to understand how ecosystems are structured and how they will respond to the disturbances (e.g. the current global change). We have recreated the multi-interaction network of a shallow freshwater lake dominated by submerged macrophytes (Charophytes), a known system very vulnerable to environmental changes, considering both trophic and non-trophic relationships among its elements. To minimize the environmental variability, we established it in an experimental mesocosm, including three habitats: the pelagic, the habitat around the meadow and the periphytic community living on macrophytes. We aimed to study the structure of this network and the roles of its elements, as well as the response of this system to a foreseeable decrease in charophytes due to the global change. Thus, we tested whether there are species in the system that, due to the connections they establish, have central or connecting roles and if the reduction of charophytes affects more the elements that live intimately associated with them. Our results confirm that charophytes are the most central node in the network and that the high-mobility large planktonic herbivores living within the meadow are acting as bridges between the conformant compartments. This suggests a structurally crucial tandem macrophytes-herbivores with the former playing a foundation role (i.e. basal and abundant species centralizing non-trophic interactions) and the latter being connectors in this network. Interestingly, we found that the periphytic elements were those with the highest capacity to affect the other elements of the network when being disturbed. Furthermore, an eventual decrease in the abundance of charophytes will cause a major direct damage to the meadow and periphyton, compartments to which they provide refuge and life support, respectively. Our study highlights the need of approaches encompassing the complex structure of the ecological networks to identify crucial species (such as foundation or connecting species) for their topology and vulnerability geared towards conservation biology.

Keywords: aquatic network, charophyte meadows, foundation species, non-trophic interactions, periphyton, plankton, topology

## Introduction

Aquatic ecosystems comprise numerous habitats or compartments (Tokeshi and Arakaki 2012). These compartments can be defined from pelagic (in the free-water) to benthic environments (over the sediment), including the macrophyte meadows and their planktonic and periphytic associated communities. The connections established intra- and inter-compartments by means of matter and energy flows, contribute to the structural and functional complexity characterizing these systems (Lodge et al. 1988). The role and influence of each compartment in the functioning of aquatic ecosystems is related to their size and shape, e.g. macrophyte meadows are a relatively large part of the habitat in shallow ecosystems and thus an important component (Jeppesen et al. 1998). Moreover, in these ecosystems, where there are two possible alternative states (one dominated by macrophytes and the other dominated by plankton; Scheffer and Jeppesen 2007), the importance of the different compartments, and the shift of one state towards the other, is determinant for the maintenance of the biodiversity and the functioning of the ecosystem (Scheffer and Jeppesen 2007).

The freshwater planktonic (pelagic) food web structure, and its response to disturbances, has been largely studied (Carpenter et al. 1987, Christoffersen et al. 2008). However, the network associated with the macrophyte meadows is less well-known. Charophytes are one of the most widespread macrophyte groups in shallow freshwater ecosystems, which perform a critical ecosystem role (Jeppesen et al. 1997, Hilt and Gross 2008, Rodrigo et al. 2013). By establishing dense meadows, these organisms are capable of modifying not only the abiotic environment (van Donk and van de Bund 2002, Rodrigo et al. 2007), but also the whole community through establishing non-trophic interactions such as competition (direct or indirect) with other primary producers (van Donk and van de Bund 2002, Rojo et al. 2013a, b), providing physical refuge to zooplankton (Blindow et al. 2002), or being inhabited by very specific periphytic assemblages (Rojo et al. 2017).

Regarding non-trophic interactions, in the last few years emphasis has been placed on these types of relationships as an important component of ecosystems (Bascompte et al. 2003, Ings et al. 2009, Kéfi et al. 2012). However, merging non-trophic interactions with the commonly studied trophic ones is not an easy issue to solve and efforts must be done in this direction (Vasas and Jordán 2006, Kéfi et al. 2015). In addition, the role of foundation species is receiving increasing attention (Borst et al. 2018, Ellison 2019). These species are considered crucially important for the ecosystems they inhabit and are distinguished by three features: 1) they are abundant in the system in terms of biomass, 2) they are normally basal species (e.g. primary producers) and 3) they established mainly non-trophic interactions with the other elements of the system (e.g. providing support or refuge for other species or altering ecosystem properties to damage other species; Ellison 2019). Based on these criteria, the submerged macrophytes are a strongly good candidate to exert such a role

in freshwaters. Therefore, a complex aquatic network that includes pelagic, meadow and periphytic habitats emerges with a myriad of imbricated relationships of different natures, both trophic and non-trophic. The construction and analysis of this network is one of the main objectives of this study.

Furthermore, these shallow macrophyte-dominated freshwater systems are particularly vulnerable to global change, and they will see their biodiversity decreased and their biogeochemical cycles altered (Álvarez-Cobelas et al. 2005, Parcerisas et al. 2012). All the habitats in these freshwater systems are expected to be affected, in a direct or indirect way, by environmental changes. In this vein, through experimental approaches at a mesocosm scale (Stewart et al. 2013), the sensitivity of the pelagic communities in these systems has been studied (Carrillo et al. 2017, Deininger et al. 2017, Rojo et al. 2017) as well as the response of macrophytes (Short and Neckles 1999, Barker et al. 2008, Zhang et al. 2019) and benthic communities (Lepori and Robin 2014, Piggott et al. 2015, Cao et al. 2019). Among macrophytes, charophytes have been proved to be very sensitive to changes in environmental factors related to global change such as warming, eutrophication, salinization and ultraviolet radiation (Calero et al. 2017, Rodrigo et al. 2017, Puche et al. 2018, Rojo et al. 2019). These changes are expected to be more acute in shallow ecosystems in Mediterranean semi-arid regions (Jeppesen et al. 2014). However, most of these studies have focused on populations, rather than on higher levels of organization (Woodward et al. 2010). This gap limits our ability to disentangle what elements of these complex networks are more relevant to the system's stability, when faced with the foreseeable changes (IPCC 2014). It is in this context that tackle these systems with a network approach provide a useful tool for recognizing structurally important species, and for establishing the extent of their influence on the response of the whole system to disturbances such as those related with the current global change, thus, allowing a better understanding of the community structure and the ecosystem functioning (Ings et al. 2009, Kéfi et al. 2015, Poisot et al. 2016, Delmas et al. 2017, García-Callejas et al. 2017, Ellison 2019).

Our aims in this study are: 1) to recreate the multi-interaction network organized around the charophyte meadows in a freshwater shallow ecosystem; 2) to characterize the global structure of this network and the topological importance of its elements and 3) to project the effects that a reduction in the abundance of the charophyte meadows would lead to for the constituent species of the network, and the structure of the network as a whole. We hypothesize that: 1) charophytes will exhibit a central role in the network, mainly due to the set of non-trophic interactions in which they participate; 2) among the three compartments considered, the meadow compartment, and specifically the organisms with greater mobility will play an important connecting role in the system and 3) faced with a reduction in the abundance of charophytes, the periphyton compartment and elements of the meadow that benefit from the shelter and support provided by these macrophytes will be adversely affected.

We developed an experimental shallow ecosystem whose elements and interactions we know well (Fig. 1). The experimental control of the abiotic environment in the mesocosm avoid the great variability that this type of shallow ecosystems can exhibit in nature (Stewart et al. 2013), allowing us to address our goals and to test the hypotheses focussed in its multi-interaction network.

## Material and methods

### The ecological community and its multi-interaction network construction

A freshwater ecosystem was recreated in an experimental mesocosm. In order to build its multi-interaction network and assess its structure and vulnerability, it was crucial to

have tight control over the conditions to which the system was submitted and to better delimit the compartments considered. These needs are covered by the use of mesocosms, a useful tool that offers greater tractability than whole-ecosystem manipulations (Stewart et al. 2013). The mesocosm consisted of a 0.5 m<sup>2</sup> enclosure (length 0.8 × width 0.6 × height 0.4 m) containing 165 l of tap water plus an inoculum of 5 l of water from a coastal lagoon. The bottom of the mesocosm was covered with a substrate layer, the width being 10 cm. The substrate was a mixture of organic compost and gravel in the proportion 2:1. On this base, a layer of sediment from a coastal lagoon (sediment inoculum) was scattered. A charophyte meadow was planted in one of the halves of the mesocosm. The meadow was monospecific, formed by the species *Chara hispida*, a green cosmopolitan macroalgae with erect thallus and regular nodes and internodes. Individuals of this species were planted as groupings (packets) in three rows of three packets each one (a total of nine packets). For the plantation, part of the main axis of the individuals was buried in the sediment. This buried part served to form the rhizoidal system that allowed the fixation of the individuals to the sediment. This plantation method has been described in other studies with these macroalgae (Rojo et al. 2015, 2019, Rodrigo et al. 2017, Puche et al. 2018). There were no charophytes on the remaining half of the mesocosm surface, allowing a more pelagic environment (Fig. 1a). From the water and sediment inoculum, as well as from the planted charophytes, a planktonic and periphytic community emerged. Several aquatic gastropods arose from the sediment in the mesocosm, which were also sampled and considered at the time of building the network. In this recreation of a shallow freshwater ecosystem, as happens naturally in most of them due to their temporary nature, predators such as fish were not present. The mesocosm was maintained at 21°C in a light:dark cycle of 14:10 h. In previous studies (Rodrigo et al. 2013, Rojo et al. 2015, 2017, Rubio et al. 2015) it was demonstrated that these conditions are non-limiting to the growth of charophytes. The physical and chemical variables were measured periodically to detect and subsequently rectify possible deviations from the experimental conditions (Supplementary material Appendix 1 Table A1). The community in the mesocosm was allowed to grow for two months before the sampling process. This period of time was determined based on previous studies claiming that charophytes are well fixed to the sediments and grow properly about two weeks after being planted (Rojo et al. 2015, Rodrigo et al. 2017, Puche et al. 2018). In addition, it is known that plankton, in an undisturbed system, can reach a state of equilibrium before two months (Naselli-Flores et al. 2003). Moreover, we did some previous tests in the mesocosm to ensure the feasibility of this recreation.

In this experimental system, three connected compartments were distinguished: 1) periphyton, a compartment formed by charophytes and all the organisms living on them; 2) meadow, the plankton inhabiting free-water within the meadow and 3) pelagic, the planktonic compartment in the pelagic habitat, furthest from the charophytes (Fig. 1a). Each

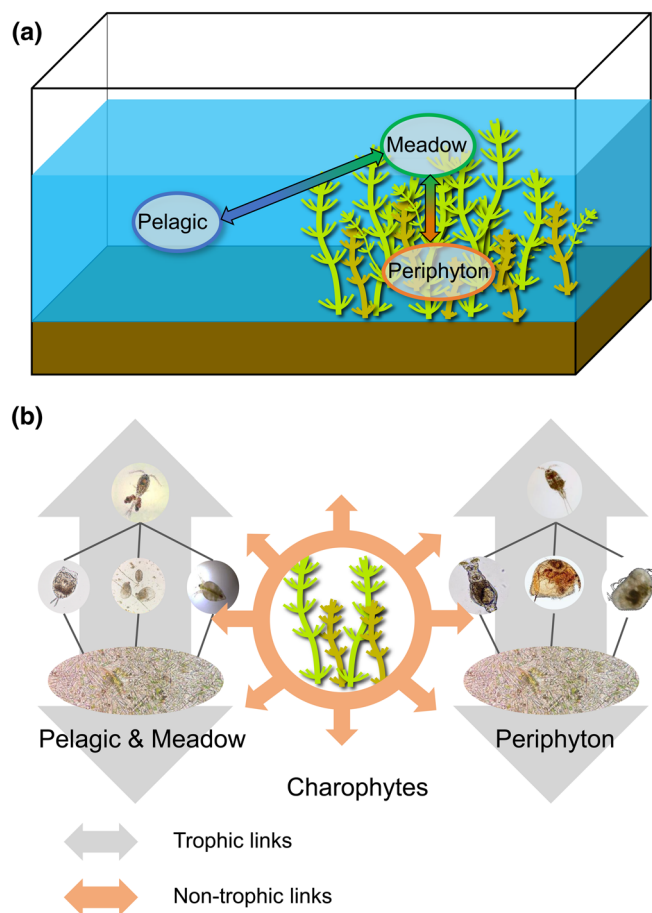


Figure 1. (a) Scheme of the mesocosm where the experimental community was set up with the three compartments represented, (b) the experimental model community with the compartments (pelagic, meadow and periphyton), representing the 'vertical' trophic links and the non-trophic links in all directions. The components in each compartment are organized in rows as autotrophs and heterotrophs (herbivores and carnivores). Charophytes (submerged macrophytes) are presented in the center (although they belong to the periphyton compartment) to highlight their key role in non-trophic interactions in this system.

of these compartments was sampled for autotrophs (phytoplankton/phytobenthos and cyanobacteria) and heterotrophs (bacteria, zooplankton/zoobenthos and gastropods). All the taxa were sampled following the methods described in previous studies (Rodrigo et al. 2003, Villaescusa et al. 2010, Rojo et al. 2012, 2017), and they were identified at the highest possible resolution (Supplementary material Appendix 1 Table A2).

To construct the multi-interaction network of this experimental system, we grouped the identified taxonomic species according to functional criteria (such as mobility, edibility or toxicity) to define the nodes (Table 1). In the network, (inorganic) nutrients were considered as a node. In this way, exploitation competition between autotrophic organisms is defined by trophic links going from the nodes that represent the autotrophic organisms to the node that represents the nutrients, as suggested by Kéfi et al. (2012). In addition, charophytes, represented also as a node, performed a function that goes beyond the autotrophic role, as they are also the physical support for the entire periphyton compartment considered in the network (Rojo et al. 2017).

The establishment of the links between the nodes of the network was based on the literature and on expert knowledge. These links encompass both trophic and non-trophic relationships (Table 2, Fig. 1b).

### The structure of the network at a global scale

The arrangement of nodes and links of the network was reflected in a  $S \times S$  matrix  $A$  (where  $S$  is the number of nodes in the network). The entries of matrix  $A$ ,  $a_{ij}$ , represent ecological interactions among species (Cohen 1978). Specifically,  $a_{ij}$ , represents the effect (1 positive,  $-1$  negative and zero otherwise) of node  $j$  (in the column) on node  $i$  (in the row). For instance, if charophytes ( $j$ ) provide refuge for zooplankton

( $i$ ), then the effect of charophytes over the zooplankton will be 1. For trophic links, the effect of the predator over the prey was coded as  $-1$ , and the effect of the prey over the predator as 1. For example, it is well known that cyclopoid adult copepods are mainly carnivores. They can prey on, for example, rotifers of the genus *Lecane*. So that, the effect of the copepods over the rotifers will be  $-1$  and the effect of the rotifers over the copepods will be 1. All node dynamics were assumed to be self-damped so the diagonal elements  $a_{ii}$  were assigned a negative value for the construction of the net effect matrix  $N$ . Non-trophic effects were either positive or negative. For network visualization we used the software Gephi.

The topological features of the network were assessed by means of global descriptors. We first recorded the number of nodes ( $S$ ) and links ( $L$ ). From these basic variables, we calculated the directed connectance ( $C$ ; Table 3). This is the proportion of realized interactions relative to the potential number of possible interactions in the network (Martínez 1992). Furthermore, the modularity coefficient (Table 3) was calculated using the algorithm developed by Guimerà and Amaral (2005). This algorithm finds a particular partition of the network that maximizes a function called modularity, bunching closely connected nodes into modules (i.e. sub-systems of non-overlapping strongly interacting species). In our network, four modules emerged by means of this algorithm: module 1, including the charophytes and the entire periphytic community (with primary producers, herbivores and carnivores), module 2 and 3 consisted of pelagic and meadow-related primary producers, respectively, and module 4 which was mainly formed by the planktonic herbivores and carnivores (both pelagic and meadow-related). We also checked the presence of nestedness in the network (Table 3). This metric was defined by Almeida-Neto et al. (2008) and it is based on two features of the matrices: the overlap and the decreasing fill. In a completely nested matrix, overlap means

Table 1. List of the criteria used to define the nodes in the network and the experimental compartment to which they belong. From these compartments, a nutritional classification of the nodes into 'Nutrients', 'Autotrophic' and 'Heterotrophic' is carried out to clarify the different groups of organisms considered. The first column separates the elements that appear in the three compartments from those that are unique to the periphyton compartment.

Compartment	Nutritional criteria	Taxonomic classification	Functional criteria	Nodes in the network
Pelagic, Meadow and Periphyton	nutrients autotrophic	Nutrients	nutrients	nutrients
		Class Chlorophyceae	unicellular, edible colonial, edible	unicellular chlorophytes colonial chlorophytes
		Class Bacillariophyceae	filamentous, non-edible small (<20 $\mu\text{m}$ ), edible large (>20 $\mu\text{m}$ ) edible	filamentous chlorophytes small diatoms big diatoms
		Class Cyanophyceae	colonial, edible filamentous, non-edible	colonial cyanobacteria filamentous cyanobacteria
	heterotrophic	Domain Bacteria	bacteria	bacteria
		Phylum Ciliophora and Nauplii	protists, bacterivore	ciliates
		Class Eurotatoria	small herbivore	rotifers
		Class Branchiopoda	large herbivore	cladocerans
		Class Hexanauplia	large herbivore carnivore	copepodites copepods
		Class Charophyceae	macrophyte	charophytes
Periphyton	autotrophic	Class Charophyceae	macrophyte	charophytes
	heterotrophic	Class Gastropoda	large, benthic herbivore	benthic gastropod

Table 2. List of the non-trophic interactions considered to build the multi-interaction network. For each interaction, the source and the target of the interaction as well as a short description and a reference are shown.

ID	Source	Target	Interaction	Description of interaction	Reference
1	Cyanobacteria (0)	bacteria (+)	stimulation	cyanobacteria release a variety of organic molecules that could stimulate heterotrophic bacteria's growth	Lange 1967, Baines and Pace 1991, Kirkwood et al. 2006
2	Cyanobacteria (0)	microalgae (-)	allelopathy	some groups of cyanobacteria has an antialgal allelopathic activity	Flores and Wolk 1986, Schlegel et al. 1999, Smith and Doan 1999
3	Meadow microalgae	charophyte	shading	phytoplankton development causes a shading effect on macrophytes reducing the amount of light reaching the bottom of the systems	Sand-Jensen and Søndergaard 1981, Ozimek et al. 1991
4	Meadow herbivore zooplankton (+)	charophyte (+)	relaxing competition	grazing by herbivore zooplankton slows microalgal growth benefiting the macrophytes	Zuo et al. 2015
5	Benthic microalgae (+)	charophyte (-)		microalgae living on macrophytes colonizing them and limiting the amount of light that they receive	Sand-Jensen and Søndergaard 1981
6	Benthic cyanobacteria (0)	charophyte (-)	allelopathy	the same effect as in interaction 2	
7	Zoobenthos (+)	charophyte (+)	cleaning	zoobenthos 'clean' macrophytes from epiphytes and provide them with $CO_2$ for photosynthesis	Cheng et al. 2017
8	Charophyte (0)	meadow microalgae and cyanobacteria (-)	allelopathy	macrophytes release allelopathic compounds that inhibit or slow the growth of several groups of microalgae	Gross et al. 2007, Rojo et al. 2013a
9	Charophyte (0)	meadow zooplankton (+)	refuge	charophytes meadows serve as a refuge for zooplankton, protecting them from their predators	van Donk and van de Bund 2002, Rodrigo et al. 2015
10	Charophyte (0)	benthic organisms (+)	vital support	charophytes meadows provide benthic organisms a substrate for living	Rojo et al. 2017

that there is a full overlap of 1s from right to left columns and from down to up rows; while decreasing fill means that there is a decreasing marginal totals (sum of 1s) between all pair of columns and all pairs of rows (Almeida-Neto et al. 2008). The significance of this metric was evaluated after 1000 randomizations of the network using the software ANHIDADO (ver. Bangu 3.0; Guimarães and Guimarães 2006).

### The structure of the network at a node-scale

At a node-scale, we determined the importance of each node in the directed matrices of the network by means of 1) different centrality measures and 2) the alteration of global descriptors that the removal of each node caused in the network.

The centrality measures were: degree centrality ( $C_D$ , the number of interactions established by a node; Freeman 1977, Table 3); closeness centrality ( $C_C$ ) which is a measure of the proximity of a node to all other nodes in the network, and it is based on the shortest path length between pairs of nodes (Freeman 1978, Freeman et al. 1979, Table 3) and betweenness centrality ( $C_B$ ) which gives information about how central a node is, in the sense of being incident to many shortest paths in the network (Freeman 1977, Table 3).

The other approach to the importance of the nodes was the assessment of the response of the global descriptors of the network to the elimination of each node (Solé and Montoya 2001). We performed removals with replacement (one different node each time). After each elimination, we calculated the global descriptors of the network (connectance, modularity and nestedness). In this way, we calculated the alteration in these global parameters by eliminating each node as the difference between their value in the network without the node, and their value in the complete network, normalized by the latter. It should be highlighted that the node 'charophytes' was not eliminated since it is the vital support for all the periphytic community considered and, therefore, its elimination would automatically lead to the elimination of all those nodes in the network. By the same way, the elimination of the node 'nutrients' was not considered for this analysis, since it does not make ecological sense to remove the nutrients from a biological community.

Moreover, based on the modules defined by the modularity algorithm, we assessed the universal roles played by the nodes in the network by means of the within-module degree ( $z$ ) and the participation coefficient ( $P$ ) of each node to determine how important a node is for its module and

Table 3. Global-scale and node-scale structural network attributes measured. The formulae used to calculate their values with a description and the references are provided.

	Equation		Reference
Network global-scale variables			
Directed connectance, C	$C = \frac{L}{S(S-1)}$	Where L is the number of links and S is the number of nodes	Martínez 1992
Modularity, M	$M = \sum_{s=1}^{N_M} \left[ \frac{L_m}{L} - \left( \frac{D_m}{2L} \right)^2 \right]$	Where $N_M$ is the number of modules, L is the number of links in the network, $L_m$ is the number of links between nodes in module m and $D_m$ is the sum of the degrees of the nodes in module m.	Guimerà and Amaral 2005
Nestedness, NODF	$NODF = \frac{\sum D_{\text{paired}}}{\left[ \frac{c(c-1)}{2} \right] + \left[ \frac{r(r-1)}{2} \right]}$	Where $D_{\text{paired}}$ is the averaged paired degrees of nestedness of columns and rows, c is the number of columns and r is the number of rows in the matrix.	Almeida-Neto et al. 2008
Network node-scale variables			
Degree centrality, CD	$CD_i = L_i$	Where $L_i$ is the number of links of node i.	Freeman 1977
Closeness centrality, CC	$CC_i = \frac{S-1}{\sum_{j=1}^S d_{ij}}$	Where S is the number of nodes and $d_{ij}$ is the shortest path length between nodes i and j.	Freeman 1978, Freeman et al. 1979
Betweenness centrality, BC	$CB_i = 2 \times \sum_{j < k; i \neq j} \frac{g_{jk}(i)}{(S-1)(S-2)}$	Where $g_{jk}$ is the number of paths between j and k, while $g_{jk}(i)$ is the number of these paths that include node i and S is the number of nodes.	Freeman 1977
Within module z-score, z	$Z_i = \frac{L_i - \bar{L}_{m_i}}{\sigma_{L_{m_i}}}$	Where $L_i$ is the total number of links of node i to other nodes in its module m, $\bar{L}_{m_i}$ is the average of links over all nodes in $m_i$ and $\sigma_{L_{m_i}}$ is the standard deviation of $L_i$ in m.	Guimerà and Amaral 2005
Participation coefficient, P	$P_i = 1 - \sum_{s=1}^{N_M} \left( \frac{L_{im}}{L_i} \right)^2$	Where $N_M$ is the number of modules, $L_{im}$ is the number of links of node i to nodes in module m and $L_i$ is the total number of links of node i.	Guimerà and Amaral 2005
Effectiveness, E	$E_i = \frac{\sum_{j \neq i}  a_{ij} }{S-1}$	Where $a_{ij}$ is the effect of a perturbation in node j over the node i (taken from the net effects matrix), and S is the number of nodes in the network.	This study
Sensitivity, s	$s = \frac{\sum_{j \neq i}  a_{ij} }{S-1}$	Where $a_{ij}$ is the effect of a perturbation in node j over the node i (taken from the net effects matrix), and S is the number of nodes in the network.	This study

for connecting modules, respectively (Guimerà and Amaral 2005, Olesen et al. 2007, Table 3).

All the calculations for these descriptors (except for nestedness) were performed in MATLAB using the Brain Connectivity Toolbox.

### Net effects matrix: dynamic importance of the nodes and effects of reducing charophytes

As explained above, the community matrix A shows the direct relationships between the elements that comprise it. These relations can have values 1, -1 or 0. From this matrix A, we have calculated the net effect matrix N to assess both direct and indirect influences (i.e. chains of connections) among the elements. To do that, and under the assumption that the system is at an equilibrium state, we simulated 5000 random matrices from matrix A by multiplying each off-diagonal element by a random value sampled from a uniform distribution within the interval (1/2, 2). To the elements within the diagonal ( $a_{ii}$ , self-regulation elements) a value of -3 was

assigned. From each random community matrix A, the net effect matrix N was calculated as  $N = -A^{-1}$  (Novak et al. 2016), thus obtaining 5000 net effects matrices, from which an average net effect matrix was obtained. Its elements  $n_{ij}$  represent the expected long-term change in the equilibrium value of node i due to a constant pressure exerted on node j (Nakajima 1992). With this net-effects matrix, we calculated two metrics of dynamic importance related to the incidence and susceptibility of the nodes in the network. These metrics were effectiveness (i.e. the average capacity of a node to affect the others when being disturbed; Table 3) and sensitivity (i.e. the average susceptibility of a node to be affected by the others when these are disturbed; Table 3). Mathematically, the effectiveness of an element I is calculated as a summation of the net effects of this element over the rest of the elements of the network (sum of rows) and the sensitivity of the element I is the summation of the net effect of the other elements over this element (summations of columns; Table 3). Note that other kinds of 'net effects' have been used in the literature. For example, Ulanowicz and Puccia (1990)

presents their MTI (mixed trophic impact) analysis based on the paths between source and target species in the network. Conversely, our calculations summarize the asymptotic responses of species abundances after parameter disturbances in any species. While Ulanovicz's analysis only considers the paths involved in connecting source and target species, our analysis (based on Levins 1974) also considers the set of species and their interconnections not included in those paths, which Levins (1974) call 'Complementary subsystem' (see also Dambacher et al. 2003). This is a key difference that determines not only differences in the values of net effects but also in their signs, as compared with Ulanovicz's MTI.

Furthermore, a principal component analysis (PCA) was carried out considering these metrics as a multivariate descriptor of the compartments, each node being a variable. In this way, we intended to assess if the compartments considered in the network differ in terms of the values of the nodes for these metrics and which nodes contribute the most to this differentiation.

### Data availability

Data are available from the RODERIC Digital Repository: <<http://roderic.uv.es/handle/10550/70781>> (Puche et al. 2019).

## Results and discussion

### Characterization of the multi-interaction network in a macrophyte-dominated shallow lake

The recreated multi-interaction network of charophyte meadows consisted of a total of 42 nodes (Table 4), distributed into three trophic levels and a nutrients node at a separate level (at the bottom of the network; Fig. 2a). Of these nodes, 52% were primary producers (microalgae, cyanobacteria and charophytes), 31% were herbivores (ciliates, rotifers, cladocerans, cyclopoid copepodites and gastropods) and 7% were carnivores (adult cyclopoid copepods). In addition, the bacteria in each compartment were considered (7% of the nodes), and represented at the row of primary producers, since they are consumers of inorganic nutrients, despite not being photosynthetic organisms. These nodes were interconnected by a total of 240 links. These links represented trophic connections (66%) and non-trophic connections, the latter being positive (21%) and negative (13%). The periphyton and meadow compartments contained the majority of non-trophic interactions (Fig. 2a). In addition, among these, the negative non-trophic relationships occurred mainly among the primary producers (e.g. allelopathy; Gross et al. 2007), while in the positive non-trophic ones the herbivorous organisms were also involved (e.g. the refuge provided by charophytes to zooplankton, or the cleaning of the periphytic microalgae on charophytes carried out by zooplanktonic and zoobenthic herbivores such as the abundant organisms of the genus *Lecane* or the bigger organisms of the genera

*Simocephalus* and *Pleuroxus*; Fig. 2a, van Donk and van de Bund 2002, Cheng et al. 2017). Each node was involved in  $11 \pm 7$  links (mean  $\pm$  SD), the connectance of the network resulted in 0.14 and the modularity coefficient was 0.26 (Table 5). Furthermore, the network showed a significant nested structure (with a NODF of 9.1 and  $p < 0.001$ ; Table 5).

### Roles of the nodes in the multi-interaction network

We found a significant correlation between the centrality measures  $C_D$ ,  $C_C$  and  $C_B$  ( $p < 0.001$ ) (Supplementary material Appendix 1 Fig. A1). That is, a node involved in many links (degree), is both very accessible (closeness) and acts as an intermediary for other nodes in the network (betweenness). Charophytes were the element of the network with the highest values of these metrics (Supplementary material Appendix 1 Table A3), followed by the large herbivores (such as cladocerans of the genera *Simocephalus*, *Pleuroxus* and *Chydorus* and cyclopoid copepodites) living within the meadow. As confirmed by Jordán (2006), these measures of centrality are complementary and end by giving a realistic idea of the importance of the nodes in the network. With this information, decisions related to conservation can be focused on these key nodes.

Analysing the effect of removing each node on the global metrics of the network (connectance, modularity and nestedness) it can be observed that, in absolute value, the nodes of the planktonic compartments (both pelagic and meadow) are those with a greater influence on the global structure of the network (Fig. 3). Going into nodes in more detail, it is remarkable that by eliminating large herbivores in the meadow there is a loss of connectance and nestedness, while the network increases its modularity (Fig. 3). This is because these elements, as mentioned above, have high values of centrality (specifically of degree centrality), that is, they are involved in many interactions and when they are eliminated, the network becomes less connected. The interactions in which large herbivores participate occur in the three compartments considered (pelagic, meadow and periphyton) since they are organisms with high mobility. These organisms living around the macrophytes use them as a refuge, going in and out of the meadow (Blindow et al. 2002, Meyer et al. 2019), they have a broad-spectrum diet (e.g. those of the genus *Simocephalus*) and can feed on virtually all the planktonic primary producers (both in the pelagic, and in the meadow and periphyton compartments; Sterner 1989, Stewart et al. 2017). Therefore, after removing them, the network becomes more modular (the different modules become more isolated by losing those 'bridge' connections between them) and this triggers the loss of the nested structure characterized by the presence of more specialist nodes whose links are 'nested' within the links of more generalist species. On the contrary, the nodes of the periphyton do not seem to have a noticeable influence on the overall structure of the network when they are eliminated (Fig. 3). This reflects that the latter are highly specialist nodes in their relationships (e.g. the periphytic microalgae require

Table 4. List of nodes in the Functional network with their ID and the compartment to which they belong.

ID	Compartment	Node	Main genus/order	ID	Compartment	Node	Main genus/order	ID	Compartment	Node	Main genus/order
1	Pelagic	nutrients		17	meadow	colonial chlorophytes	<i>Scenedesmus</i>	33	periphyton	big diatoms	<i>Ulnaria</i>
2	Pelagic	bacteria		18	meadow	filamentous chlorophytes	<i>Oedogonium</i>	34	periphyton	colonial cyanobacteria	<i>Chroococcus</i>
3	Pelagic	unicellular chlorophytes	<i>Tetraedron</i>	19	meadow	small diatoms	<i>Cyclotella</i>	35	periphyton	filamentous cyanobacteria	<i>Ulothrix</i>
4	Pelagic	colonial chlorophytes	<i>Scenedesmus</i>	20	meadow	big diatoms	<i>Diploneis</i>	36	periphyton	ciliates	
5	Pelagic	filamentous chlorophytes	<i>Oedogonium</i>	21	meadow	colonial cyanobacteria	<i>Composphaeria</i>	37	periphyton	rotifers	<i>Lecane</i>
6	Pelagic	small diatoms	<i>Cyclotella</i>	22	meadow	filamentous cyanobacteria	<i>Oscillatoria</i>	38	periphyton	cladocerans	<i>Simocephalus</i>
7	Pelagic	big diatoms	<i>Rhopalodia</i>	23	meadow	ciliates		39	periphyton	copepodites	<i>Cyclopoidea</i>
8	Pelagic	colonial cyanobacteria	<i>Chroococcus</i>	24	meadow	rotifers	<i>Lecane</i>	40	periphyton	copepods	<i>Cyclopoidea</i>
9	Pelagic	filamentous cyanobacteria	<i>Oscillatoria</i>	25	meadow	cladocerans	<i>Simocephalus</i>	41	periphyton	Charophyceae	<i>Chara</i>
10	Pelagic	ciliates		26	meadow	copepodites		42	periphyton	Gastropoda	<i>Physella</i>
11	Pelagic	rotifers	<i>Lecane</i>	27	meadow	copepods					
12	Pelagic	cladocerans	<i>Simocephalus</i>	28	periphyton	bacteria					
13	Pelagic	copepodites	<i>Cyclopoidea</i>	29	periphyton	unicellular chlorophytes	<i>Chlorella</i>				
14	Pelagic	copepods		30	periphyton	colonial chlorophytes	<i>Coelastrum</i>				
15	Meadow	bacteria		31	periphyton	filamentous chlorophytes	<i>Oedogonium</i>				
16	Meadow	unicellular chlorophytes	<i>Chlorella</i>	32	periphyton	small diatoms	<i>Cyclotella</i>				

the charophytes' branches as a substrate; Rojo et al. 2017). Changes in the global structure of the network when removing a node have been related to the effects on the stability of the system. In this way, Solé and Montoya (2001) stated that the elimination of central species causes the decrease of the robustness of the network (measured as secondary extinctions generated from the elimination of a node).

Taking into account the modules defined by the algorithm (explained in Material and methods section) and considering the parameter regions proposed by Olesen et al. (2007), it can be observed that, consistently with the importance measures, the charophytes and the large planktonic herbivores living within the meadow play important roles in the network. The charophytes' node was classified as a network hub (Olesen et al. 2007), being very important for their own module and with high participation in the rest of the modules (Fig. 2b). The nodes representing the large meadow-related herbivores (e.g. cladocerans and copepodites) were classified as connector nodes (Olesen et al. 2007), which play an important role connecting the different modules in this system (Fig. 2b). This habitat-coupler role has been similarly described for fish in several freshwater systems (Schindler and Scheuerell 2002). The rest of the nodes played peripheral roles, being nodes immersed in their modules with few connections to the other modules (Fig. 2b). These results are consistent with what was previously mentioned regarding the importance of the nodes in the network, and highlights the crucial role as an influencer that the charophyte-large herbivores tandem plays in the whole system. This role is close to that of the topological keystone species suggested by Jordán et al. (2006). Thus it is highlighted that knowing the 'biological content' of the modules defined in an ecological network is necessary to understand the functioning of these complex systems (Olesen et al. 2007, Jordán et al. 2018).

### Dynamic importance of the nodes in the multi-interaction network

From the net effect matrix N, both the direct and non-direct influences of a node over the others are considered (Nakajima 1992). In this way, the average of the effectiveness of the nodes was greater in the periphyton than in the meadow, and lowest in the pelagic compartment ( $F = 3.8$ ,  $p < 0.05$ ; Fig. 4a). This means that, on average, a sustained and constant disturbance on the nodes of the periphytic community (among which are the charophytes) has the greatest effect on the whole system (Fig. 4b). The non-trophic interactions are key in this effect, since, as we said previously, it is in this compartment where the majority of these types of interactions occur.

Considering the effectiveness as a multivariate descriptor of the compartments (each node being a variable), these can be ordered in a first axis that explains 88% of the total variance (PCA; Supplementary material Appendix 1 Fig. A2). The nodes that, due to their effectiveness, classify to a greater extent the compartments on this axis are the charophytes and the filamentous chlorophytes (Supplementary material Appendix 1 Fig. A2). The charophytes were those with the



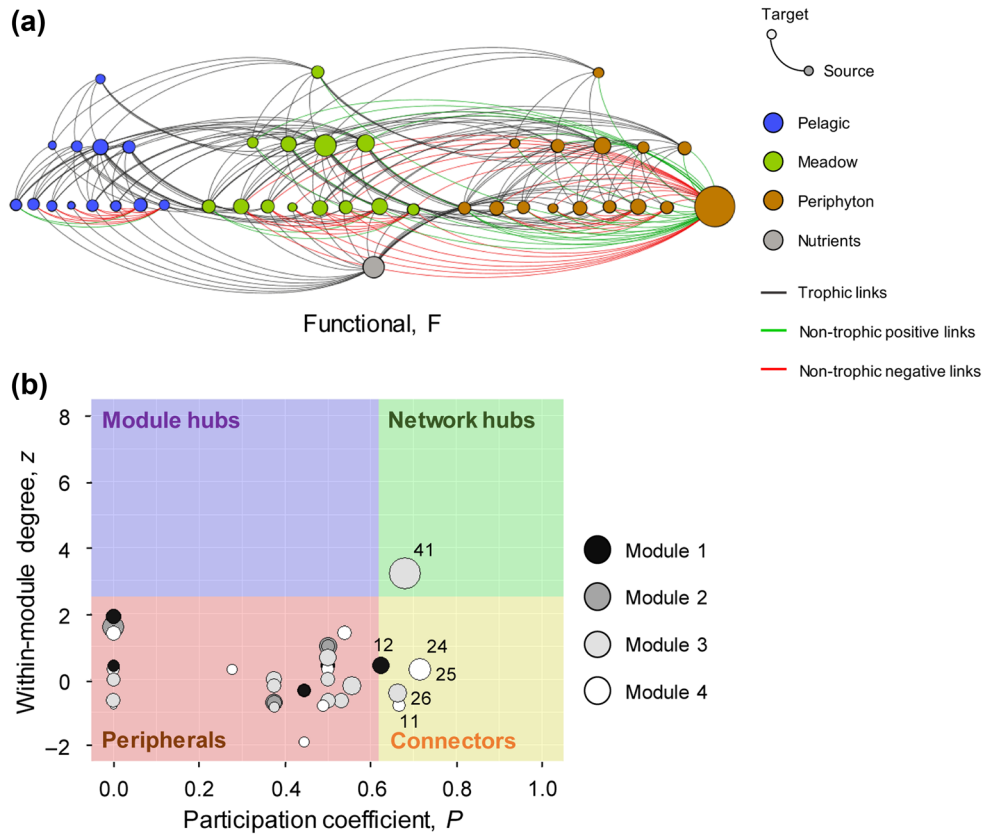


Figure 2. (a) Graphical representation of the multi-interaction functional network. The size of the nodes is proportional to their degree (number of links in which they are involved), and the color represents the experimental compartment to which they belong. Nodes are horizontally distributed in groups according to which compartment they belong to. The vertical distribution corresponds to the trophic position of the nodes, with nutrients at the bottom. The line colors represent the different types of interactions: trophic (black), non-trophic negative (red) and non-trophic positive (green). The curvature of lines connecting the nodes represents the directionality of the interaction, with lines arcing clockwise from the source to the target species. (b) Roles of the nodes of each defined module according to their within-module,  $z$  (y-axis) and their participation coefficient,  $p$  (x-axis). Each circle is a node of the network, their size represents their degree and their color represents the module they belong to. The numbers are the ID of the nodes next to them (Table 4). The parameter regions considered follow those proposed by Olesen et al. (2007).

greatest effectiveness (Fig. 4b), that is, they have the greatest capacity to affect the nodes of the system and do so basically through non-trophic interactions. This feature logically segregates the periphyton compartment (Supplementary material Appendix 1 Fig. A2). In addition, the effectiveness of the filamentous chlorophytes (filaments commonly attached to

the thallus of the charophytes; Rojo et al. 2017) characterizes the meadow compartment compared to the pelagic compartment (Supplementary material Appendix 1 Fig. A2).

Regarding sensitivity, charophytes again demonstrated the highest value, followed by benthic carnivore copepods of the genus *Cyclopoidea* (Fig. 4b). Thus, despite the charophytes having the greatest capacity to affect the different elements that make up the system, they are also the most susceptible to being affected by changes in the other members of the community. However, there were no significant differences between the average sensitivity of the nodes depending on the compartment they belong to.

### Projecting the net effect of a charophyte reduction in the network

Charophytes are very vulnerable to global change factors (Rojo et al. 2015, 2019, Calero et al. 2017, Rodrigo et al. 2017, Puche et al. 2018) and, here, we project the potential

Table 5. Global structural descriptors of the network.  $S$  is the number of nodes,  $L$  is the number of links,  $C$  is the directed connectance,  $M$  is the modularity coefficient and NODF is the descriptor measuring the nestedness of the network with the  $p$ -value associated.

$S$	42
$L$	240
Mean degree (mean $\pm$ SD)	11 $\pm$ 7
$C$	0.1394
$M$	0.2578
Number of modules	4
NODF ( $p$ )	9.1 (0.0)

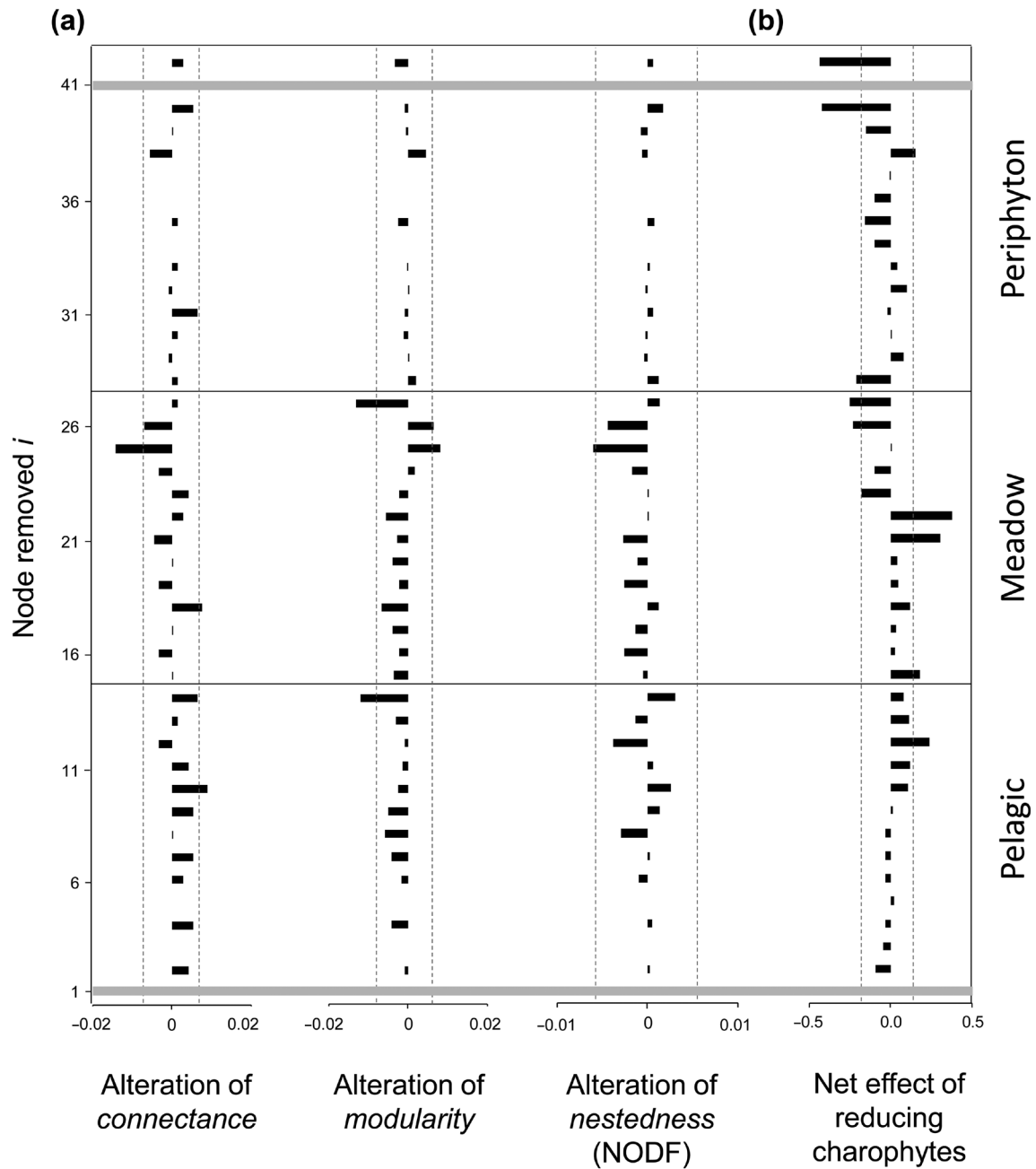


Figure 3. Alteration of connectance, modularity and nestedness of the network after the removal of node  $i$  (calculated as the difference between the values of these descriptors in the network without node  $i$ , and in the network with all the nodes). Dashed lines represent  $\pm 95$ th percentile of the absolute value of deviations from the whole network. Gray bands indicate the nodes not considered for these analyses. The correspondence between the number and the name of the nodes is shown in Table 4.

chain effects of their depletion. Our analyses revealed that the reduction of the equilibrium abundance of this group of macrophytes negatively affects 69% and 47% of the nodes of the meadow and periphyton compartments, respectively (Fig. 5a). In the pelagic compartment there is a percentage of nodes harmed by the decrease in charophytes (31%), while in this compartment a higher percentage of nodes are favored (54%; Fig. 5a). A detailed analysis of the nodes in each compartment shows that in the meadow

compartment the main beneficiaries were the colonial and filamentous cyanobacteria, since they are competing with the charophytes establishing negative non-trophic interactions, such as allelopathy (Rojo et al. 2013a, b, Fig. 5b), and they are, indirectly, strong competitors of the periphytic microalgae that inhabit on the charophytes (Rojo et al. 2017). On the other hand, large herbivores in this compartment, such as cyclopoid copepodites, and carnivores, such as cyclopoid adult copepods, are harmed (Fig. 5b). Again the non-trophic

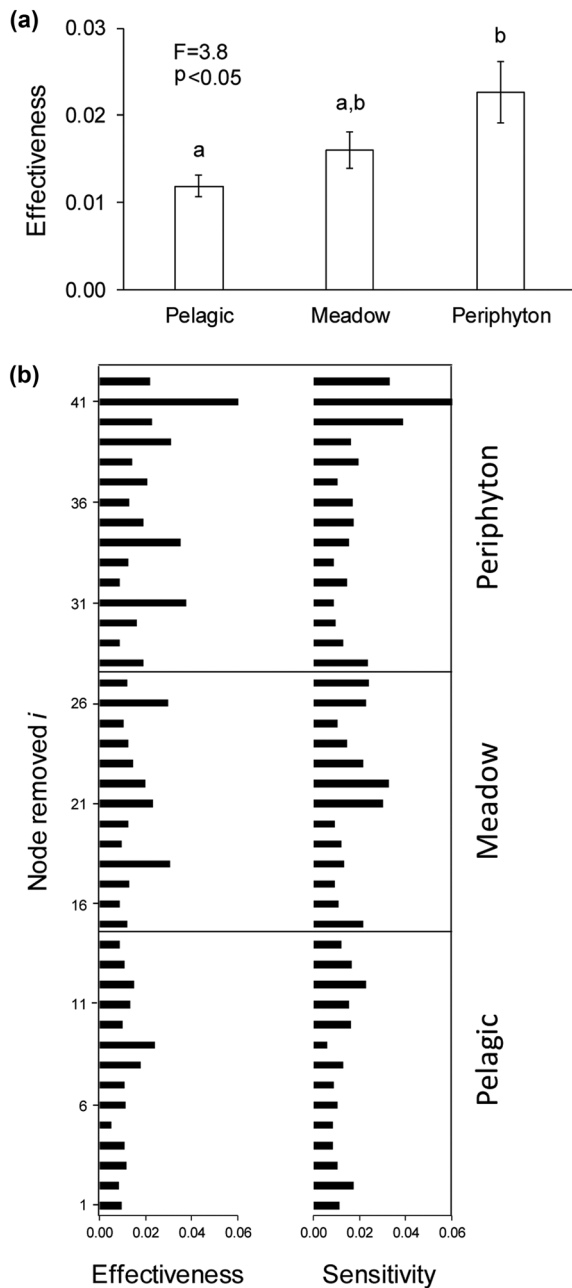


Figure 4. (a) Average values of the node effectiveness in the three compartments. We conducted an ANOVA test to assess the significant differences. Lower-case letters indicate significant differences ( $p < 0.05$ ) within conditions after the Tukey post hoc test. Bars show standard error. (b) Values of effectiveness and sensitivity of each node in the network. The compartments are indicated to the right.

interactions that the charophytes establish with these zooplanktonic organisms play an important role in this effect; by reducing the density in the equilibrium of charophytes, the refuge that these macrophytes provide is lost, and the edible microalgae disappear in favor of non-edible cyanobacteria (both filamentous and colonies; van Donk and van de Bund 2002, Hilt and Gross 2008). The negative effect of the reduction in charophytes on the nodes of the periphyton

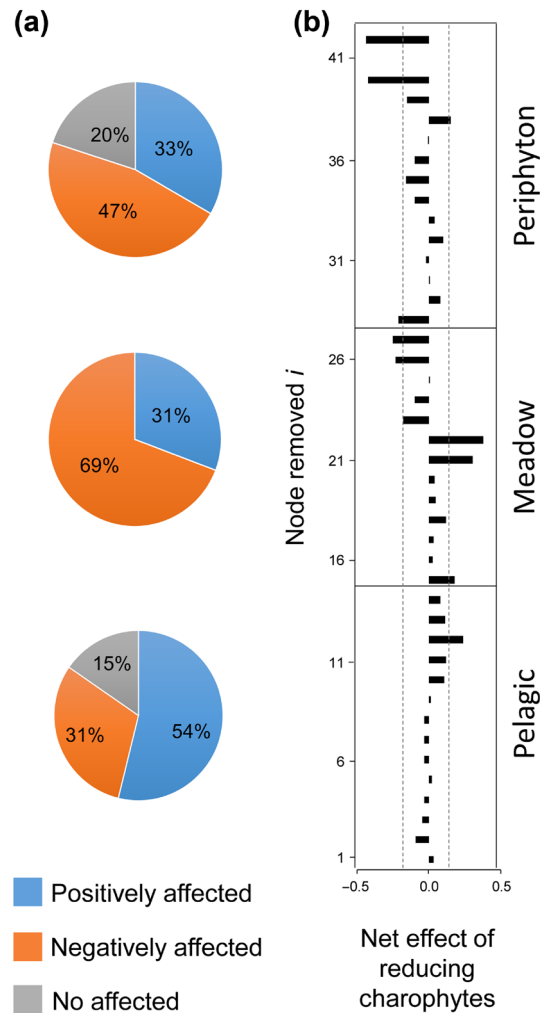


Figure 5. (a) Percentage of positively, negatively and unaffected nodes in each compartment of the network after the reducing the abundance of charophytes, and (b) detail of the net effects of reducing the equilibrium abundance of charophytes on equilibrium levels of each node of the network. Dashed lines represent  $\pm 95$ th percentile of the absolute value of deviations from the whole network. The correspondence between the number and the name of the nodes is shown in Table 4.

compartment is mainly due to the fact that these macroalgae are the life support for the elements of this community (Rojo et al. 2017). Among these elements, the gastropods are seriously damaged (Fig. 5b), since in addition to benefiting from their support they feed on the charophytes (Brönmark and Vermaat 1998, Semchenko et al. 2008). Copepods are also negatively affected, for reasons similar to the effect on their homologues in the meadow compartment (Fig. 5b).

## Conclusions

Through the study of the structure and sensitivity of the network of a complex aquatic community in a shallow environment dominated by macrophyte meadows recreated in

a mesocosm, we were able to identify which elements play critical roles for the integrity of the whole system. Our results highlight the importance of submerged macrophytes (such as charophytes) as a key highly-influential element on the rest of the elements in this system. These macrophytes are playing a foundation role, structuring the whole system. Furthermore, the determining function of the littoral habitats in these water bodies and, particularly, the key role played by large herbivores (such as cladocerans or copepodites) living within the submerged meadows, introduces the idea of a macrophyte–large herbivores tandem structurally crucial. The function of the lake with alternative states (macrophyte–plankton dominance) has been described for years, we now quantify both the relevance of their main agents and the shifts on their network due to the foreseeable global change. Our numerical characterization of the multi-interaction network in this system, contributes to better identification of species extremely relevant in conservation biology and open the gate to more complex views that encompass dynamics, environmental factors and relevant tandems between species with different roles in ecological networks.

## Speculations

Macrophyte-dominated shallow lakes exposed to changing climate will likely suffer from a negative impact on their constituent species, including charophytes. The loss of macrophytes would harm the efficiency of the macrophyte–herbivore tandem since much of the non-trophic relationships, along with the connections between the different habitats generated by these elements, would be lost. Consequently, the system will increase its modularity and, thus, become more vulnerable, favoring the shift towards a phytoplankton-dominated system. Therefore, the deterioration of ecosystem services provided by these ecosystems, such as the necessary maintenance of good water quality, as much as other cultural services associated with it, would occur.

In this context in which the network elements and the relationships they establish can be altered differentially by environmental changes, it is essential to accurate the measure of strengths of both trophic and non-trophic relationships. Moreover, the macrophyte-dominated multi-network includes elements of very different body size, from bacteria to plants, the latter being also, as we have described here, the foundation species. Thus, we expect to obtain substantial differences in link strength depending on whether they are measured: on a population basis or a per-individual or per-unit biomass basis. Establishing which of these metrics will be more sensitive to environmental perturbations suffered by the network and introducing tools such as the size spectrum of the community in its calculation seems to us exciting challenges.

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Supplementary material (available online as Appendix oik-06694 at <[www.oikosjournal.org/appendix/oik-06694](http://www.oikosjournal.org/appendix/oik-06694)>). Appendix 1.