

1 **Supplementary material**

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3 **Details on methods and supplementary results**

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5 **1. Molecular-phylogenetic tree reconstruction and phylogenetic signal in body size**

6 We created a molecular phylogenetic tree for 42 cladoceran species previously recorded in Belgium [1] following a recently developed protocol
7 [2]. To do this, we extracted information from Genbank on four molecular markers (COI, and 16S, 18S and 28S rDNA) for the 42 species using
8 the browser “Geneious V. R9.1” (available at <http://www.geneious.com/download>). *Sida crystallina* was included as an out-group because it is
9 hierarchically ancestor to all the other Cladoceran species represented in the phylogenetic tree [3]. We used the EMBL-EBI web-server
10 (<http://www.ebi.ac.uk/Tools/webservices/>) to align the sequences based on six different alignment tools (Clustal omega, Clustal W2, Kalign,
11 MAFFT, MUSCLE and PRANK). We then checked the quality of the aligned output files in Bioedit [4]. The best alignment per genetic marker
12 was chosen using MUMSA [5] (<http://msa.sbc.su.se/cgi-bin/msa.cgi>). Since all alignments presented an “average overlap score” above 0.5, we
13 used the highest “multiple overlap score” to select the best alignment model for each molecular marker. After selecting the best alignments, the
14 aligned sequences were trimmed using the automated 1 algorithm in the software Phylemon2 [6] (<http://phylemon2.bioinfo.cipf.es/>). Afterwards,
15 we concatenated all the aligned sequences in a supermatrix. Based on the literature (Table S1) a constraint tree was built [see also Helmus,

16 Keller [7] for a similar example of the application of a constraint tree for freshwater zooplankton phylogeny reconstruction]. This constraint tree
17 was used as the backbone of the phylogeny to constrain the deeper nodes of the tree. This allowed us to assess species evolutionary relationships
18 within uncontested groups of species and to estimate branch lengths based on molecular information contained in our supermatrix. The
19 constrained nodes are indicated in Fig. S1. We used the Maximum Likelihood (ML) approach for phylogenetic tree reconstruction and
20 bootstrapping was performed using RAxML (thorough ML searches and rapid bootstrapping algorithm) (<http://phylobench.vital-it.ch/raxml-bb/>)
21 [8]. Finally, we ultrametricized the tree using the Penalized Likelihood method [9] using the function *chronos* in the package **ape** in R (R Core
22 Team 2014). The phylogenetic tree is presented in figure S1 and a reduced phylogenetic tree (containing only the species used in this
23 experiment) can be found in Figure S3.

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25 **2. Phylogenetic signal in body size**

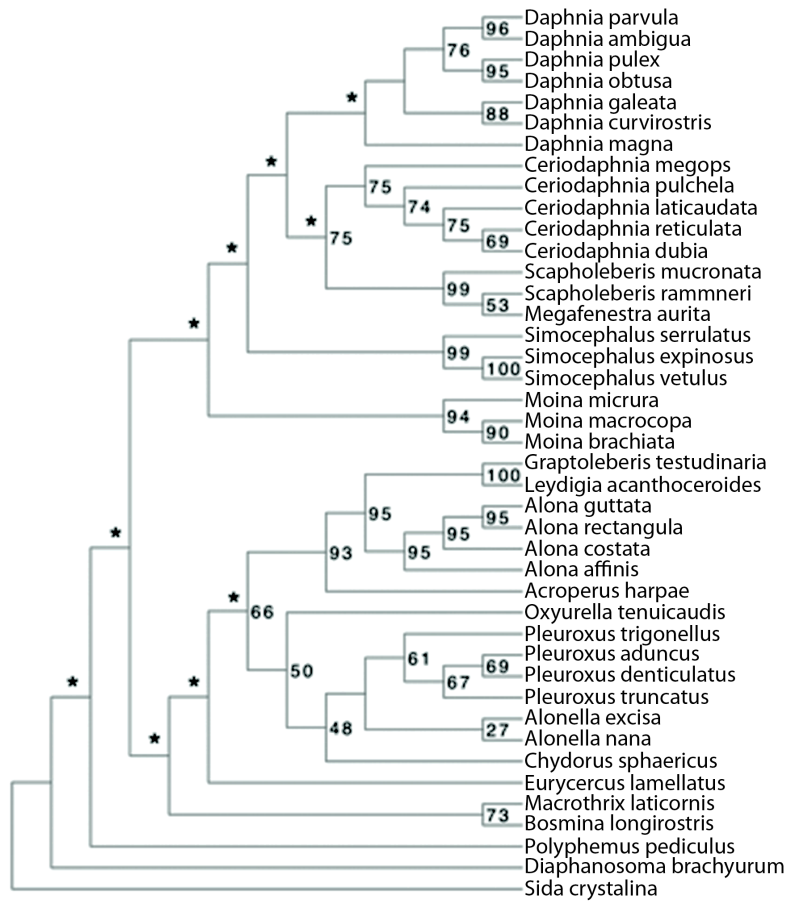
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27 Using the phylogenetic tree in figure S1 and body size values obtained from the literature, we tested for phylogenetic signal in body size of
28 Cladocera zooplankton (this test was part of another recent study; Gianuca et al. under review). To do this we used the K-statistic [10]. Obtained
29 K-values were compared to randomized K-values. Significant p-values indicate non-random correspondences between traits and phylogeny.

30 Values of K higher than 1 indicate that traits are more conserved than expected by a Brownian Motion Evolutionary model (Blomberg *et al.*
31 2003). Values lower than 1 indicate that traits are less conserved along the phylogeny than expected by this model.

32 We found no evidence for phylogenetic signal in body size for the zooplankton species occurring in this Belgian metacommunity ($K = 0.04$, $p =$
33 0.243).

34



35

36 Figure S1: Best-scoring molecular-phylogenetic tree (Maximum Likelihood) showing the evolutionary relationships among 42 cladoceran
 37 species previously recorded in Belgium. Bootstrap values are given on the nodes (except for constrained nodes with supporting values lower
 38 than 50). Asterisks indicate which nodes were constrained based on previous expert knowledge (Table S1).

39

40 Table S1. References used to constrain deep nodes of the phylogeny and thus establish the main relationships among clades.

Family	References
Daphnidae	Adamowicz, Petrussek [11]
Moinidae	Braband, Richter [3]
Chydoridae	Sacherová and Hebert [12]
Euryceridae; Bosminidae;	Braband, Richter [3]; Helmus, Keller [7]
Polyphemus sp.; Sididae	

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43 **References**

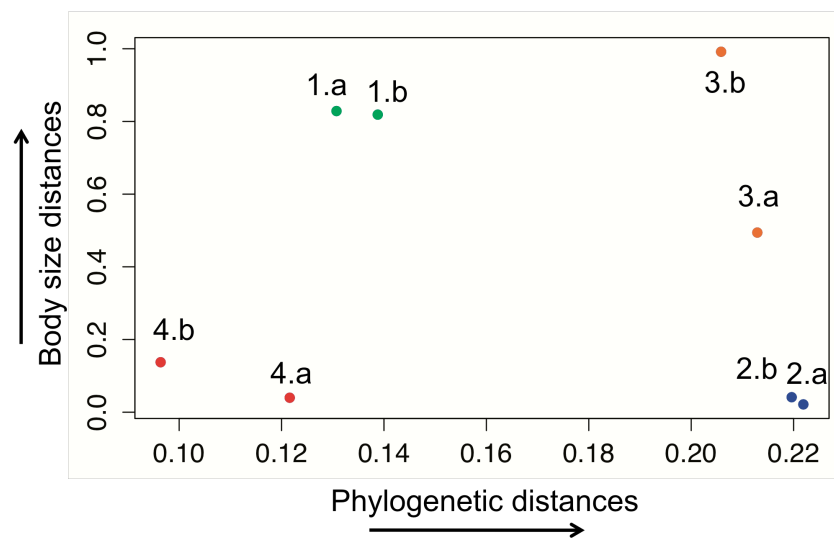
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45 1.
46 Adamowicz, S.J., Petrussek, A., Colbourne, J.K., Hebert, P.D.N. & Witt, J.D.S. (2009). The scale of divergence: A phylogenetic appraisal of
47 intercontinental allopatric speciation in a passively dispersed freshwater zooplankton genus. *Molecular phylogenetics and evolution*, 50,
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51 *Evolution; international journal of organic evolution*, 57, 717-745.
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80

81 **3. Experimental design and species characteristics**

82 We choose from the 42 species presented in Appendix S1 a set of eight species that are widespread in Europe (e.g., [13, 14]), relatively
83 abundant, easy to culture in the laboratory and that largely conform with our conceptual figure 2 presented in the main text. The functional trait
84 (body size) and phylogenetic distances among the species used in the grazing experiments are presented in Figure S2 below. More information
85 on species characteristics can be found in tables S2 and S3.



86

87 Figure S2. Relationship between body size and phylogenetic distances among species pairs. The functional trait and phylogenetic axes were not
88 significantly correlated to each other ($_{adj}R^2 = -0.044$, $p = 0.873$). Codes are given in table S2. Treatment (i) is coloured in green and represent
89 closely related species that have diverged in body size; treatment (ii) in blue represent distantly related species that have converged in body size;

90 treatment (iii) in orange represent distantly related species that are dissimilar in body size; and treatment (iv) in red represent closely related
91 species that have similar body sizes. Note that species pair 3.a is comparatively less diverse in size than 3.b. This happened because we choose
92 species combinations based on body size data from the literature, which proved later on not to exactly match our own body size measurements.

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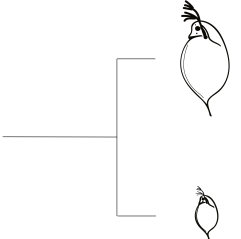
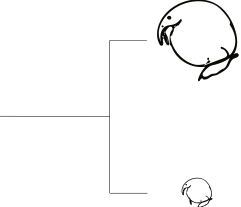
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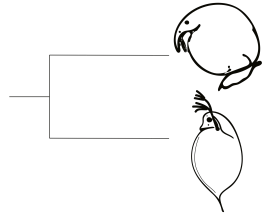
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103 Table S2: List of species combinations used in the experiments. Combinations were selected based on the following criteria: (1) HIGH FD -
 104 LOW PD, closely related species that differ strongly in body size; (2) HIGH FD - LOW PD; distantly related species that overlap in body size
 105 (cf. convergence); (3) HIGH FD - HIGH PD, distantly related species that differ strongly in body size; (4) LOW FD - LOW PD, closely related
 106 species that differ little in body size (cf. scenarios 3 and 4 represent body size conservatism along the phylogeny). For each treatment, there were
 107 two species combinations and for each species combination there were three replicates. For more details see methods and figure 2 in the main
 108 text.

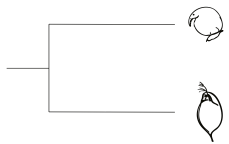
	Species combinations	Treatments	Description
	1.a (Daphniidae vs. Daphniidae) Small vs. Large <i>Daphnia ambigua</i> and <i>Daphnia magna</i>		
	1.b (Chydoridae vs. Chydoridae) Small vs. Large <i>Pleuroxus aduncus</i> and <i>Eurycercus lamellatus</i>	1	HIGH FD - LOW PD



2.a (Daphniidae vs. Chydoridae)

Large vs. Large

Daphnia curvirostris and *Eurycercus lamellatus*



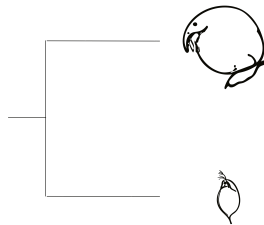
2.b (Daphniidae vs. Chydoridae)

Small vs. small

Daphnia ambigua and *Alona affinis*

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LOW FD - HIGH PD



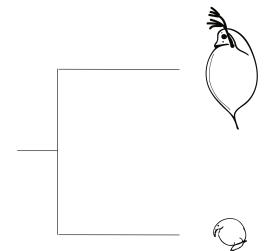
3.a (Daphniidae vs. Chydoridae)

Small vs. large

Daphnia ambigua and *Eurycercus lamellatus*

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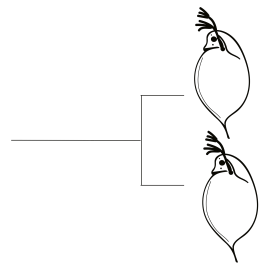
HIGH FD - HIGH PD



3.b (Daphniidae vs. Chydoridae)

Large vs. small

Daphnia magna and *Pleuroxus truncatus*



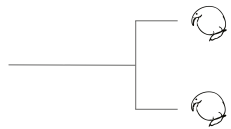
4.a (Daphniidae vs. Daphniidae)

Large vs. large

Daphnia pulex and *Daphnia curvirostris*

4

LOW FD - LOW PD



4.b (Chydoridae vs. Chydoridae)

Small vs. small

Pleuroxus aduncus and *Pleuroxus truncatus*

109

110 Table S3. List of species used in the experiments with their corresponding body size and biomass values. In the third column, “N” refers to the
 111 number of individuals used in each species combinations. When assessing individual grazing performances, we doubled that number to keep
 112 biomass constant among all treatments and replicates.

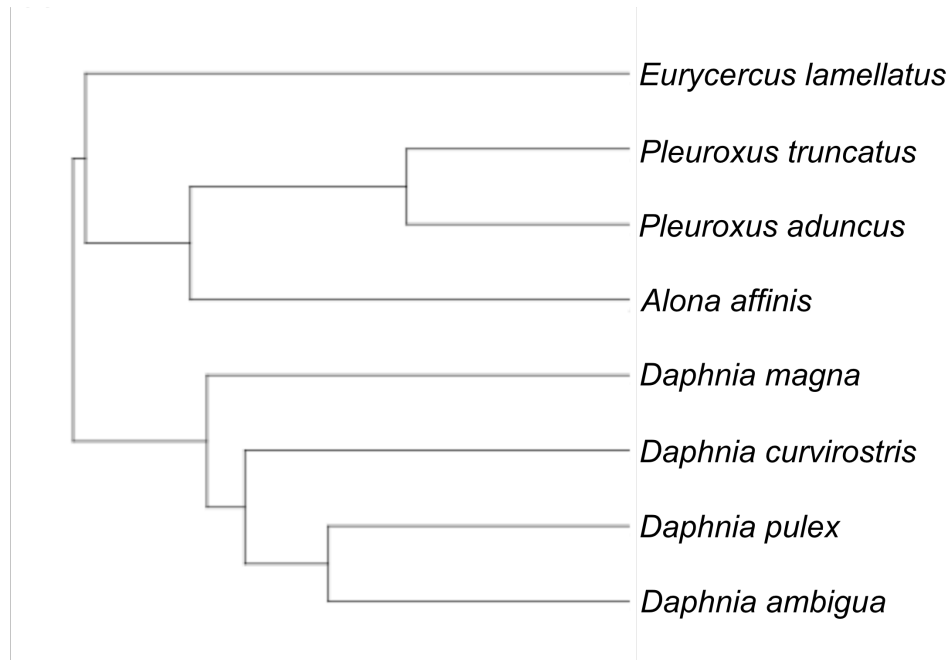
Species	Body size	Biomass	N
<i>Alona affinis</i>	0.88	3.36	25

<i>Daphnia ambigua</i>	0.94	3.9	22
<i>Daphnia curvirostris</i>	2.01	8.68	10
<i>Daphnia magna</i>	3.6	28.6	3
<i>Daphnia pulex</i>	1.9	8.1	10
<i>Eurycercus lamellatus</i>	1.95	7.96	10
<i>Pleuroxus aduncus</i>	0.52	2.06	35
<i>Pleuroxus truncatus</i>	0.63	2.4	35

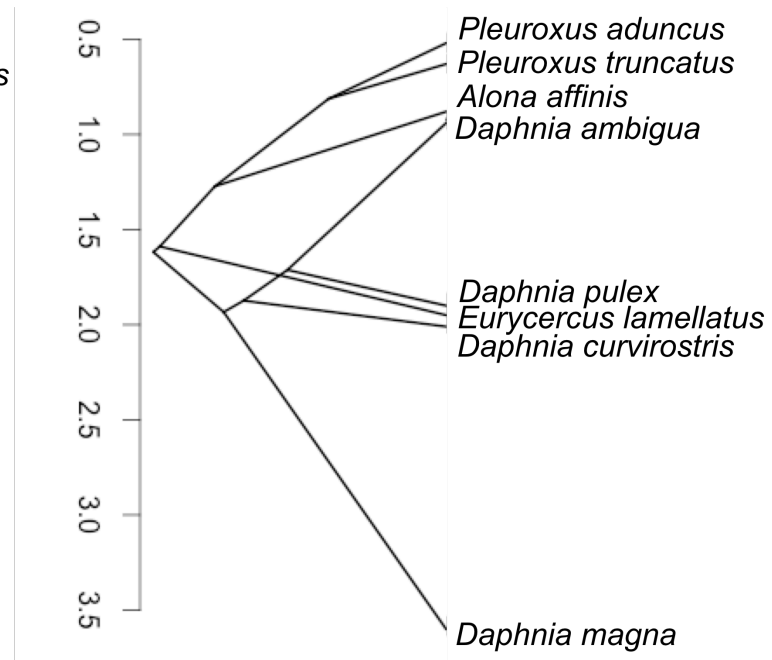
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(a)



(b)



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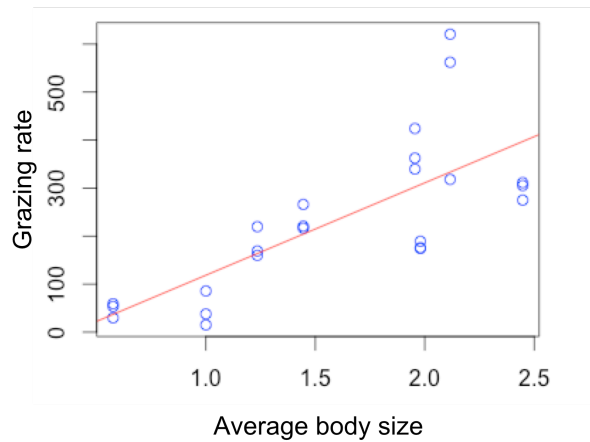
116 Figure S3: Phylogenetic and trait relationships among the cladoceran species used in this study. (a): the phylogenetic tree based on Maximum
117 Likelihood; (b) the evolutionary-traitgram, which posits the tips of the phylogeny according to a trait axis (here body size in mm) while keeping
118 the internal nodes according to evolutionary distance among species (genetic distance in this example). See Cadotte et al. (2013) for more details
119 on the evolutionary trait-gram.

120

121 **4. Average body sizes within communities and observed grazing rates**

122 To further test if increasing average body size within communities resulted in higher grazing rates, we use linear regression. In this analysis we
123 used average body size within communities as predictor and grazing rates as response variable. We found a strong positive correlation between
124 average body size within communities and observed grazing rates (Fig. S4) ($_{adj}R^2 = 0.56$; $p < 0.001$).

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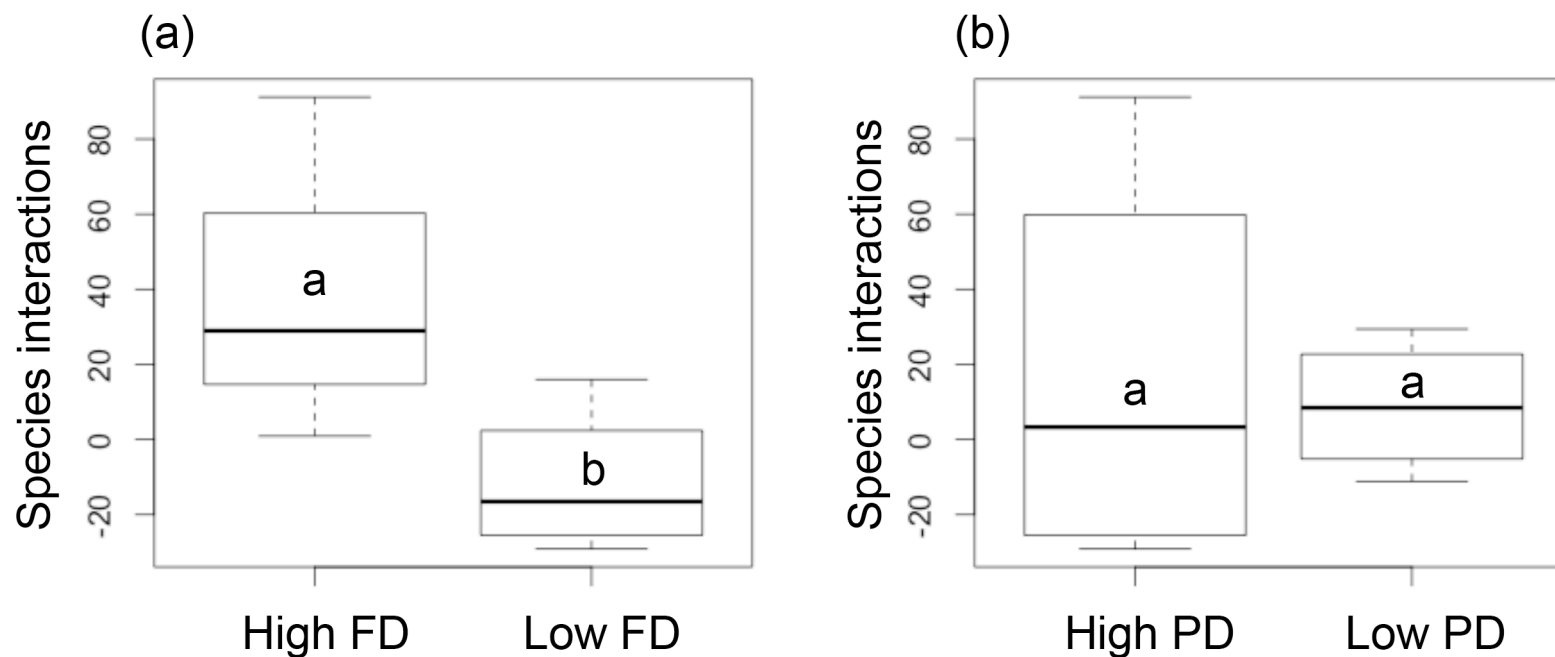
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127 Figure S4. Scatterplot depicting the positive correlation between increasing average community body size and total observed grazing rates.

128

129

130 **5. Functional and phylogenetic diversity as predictors of species interactions and its impact on top-down control**



131

132 Figure S5. Relative increase or decrease in resource uptake efficiency for every species combination. Positive values refer to positive species
133 interactions; negative values refer to negative species interactions. Zero refers to a situation in which the two-species communities present a
134 grazing impact that is identical to the average of the two species in monoculture. Box plots depict the effect of functional diversity (FD) (a) and
135 phylogenetic diversity (PD) (b) on species interactions. HIGH FD refers to treatments (i) and (iii), whereas LOW FD refers to treatments (ii) and

136 (iv) in Figure 2. HIGH PD refers to treatments (ii) and (iii) in Figure 2, whereas LOW PD refers to treatments (i) and (iv). Distinct letters (a vs.
137 b) indicate significant differences ($p < 0.001$) based on two-way permutation univariate ANOVA.

138

139 **6. Variation partitioning analysis for species combinations that present phylogenetic signal in body size**

140

141 Here we constrained the variation partitioning analysis (see methods in the main text for details) to treatments where species combinations
142 presented size conservatism (i.e., treatments 3 and 4 only). This allowed us to assess the explanatory power provided by phylogeny on top-down
143 control when body size is conserved along the phylogeny.

144

145 **6.1 Results**

146 The results obtained when considering only experimental treatments designed to reflect trait conservatism indicated that phylogenetic and trait
147 diversity together explained a large proportion of grazing rate ($_{\text{adj}}R^2 = 0.287$). In this context, however, neither the pure effect of size diversity
148 nor the pure effect of phylogenetic diversity was significant ($_{\text{adj}}R^2 = 0.071$, $p = 0.194$; and $_{\text{adj}}R^2 = -0.061$, $p = 0.741$, respectively). This suggests

149 that the predictive power of phylogeny depends entirely on body size conservatism along the phylogeny and that phylogenetic distances do not
150 reflect any additional information on unmeasured effect traits relevant to this ecosystem function.

151

152 **7. Size-related grazing efficiency asymmetry or size-related niche differences as the main mechanism mediating top-down control?**

153

154 The idea of size-related niche differences as the main mechanism affecting top-down control is based on the assumption that smaller
155 zooplankton species are more efficient grazers on smaller phytoplankton while large zooplankton species are more efficient grazers on large
156 phytoplankton [15-17], thus resulting in feeding complementarity. In contrast, the hypothesis of size-related grazing efficiency asymmetry as the
157 main mechanism mediating top-down control is based on the assumption that larger and small zooplankton species overlap in their main food
158 particles, but the former are more efficient grazers over the entire spectrum of phytoplankton cell sizes [18-20].

159

160 To test which of these two mechanisms better explain the relationship between body size compositional differences and top-down control, we
161 here complement the results from the ANOVA approach (see main text) using a linear regression approach. The main differences between such

162 approaches (ANOVA and linear regression) is that the former is based on an artificial cut of species groups based on body sizes (i.e., small vs.
163 large) whereas the latter is based on the continuous distributions of average body sizes among species.

164

165 Using grazing rate performances of each species in monoculture, we performed linear regressions to test the hypothesis that small zooplankton
166 species are more efficient grazers on small algae cells and that large zooplankton are more efficient grazers on large algae cells. For this analysis,
167 we used body size as a predictor and grazing rates as a response variable using a linear model in the R statistical package (R Core Team 2014).
168 Here we distinguished between (1) total grazing rates (i.e., the entire spectrum of phytoplankton cell sizes); (2) grazing rates on small algae cells;
169 and (3) grazing rates on large algae cells. Results from ANOVA and linear regression approaches were very similar.

170

171 **7.1 Results**

172 The linear regression analyses revealed that larger zooplankton species depleted both large and small phytoplankton more efficiently than small
173 zooplankton (large algae: $_{\text{adj}}R^2 = 0.70$, $p < 0.001$; small algae: $_{\text{adj}}R^2 = 0.26$, $p = 0.005$). The larger species were therefore superior grazers over the
174 entire spectrum of algae sizes ($_{\text{adj}}R^2 = 0.69$, $p < 0.001$) (Fig. S2c).

175

176 **8. Quantifying the pure and shared effects of community average size (CAS) and phylogenetic composition on community grazing rates**

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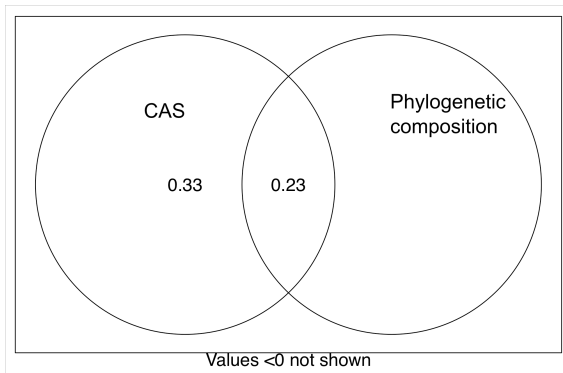
178 In a similar way that body size can be separated in two components (i.e., variance and mean), phylogenetic effects on grazing rates can also be
179 separated in a variance component (i.e., phylogenetic diversity within assemblages) and a compositional component (i.e., lineage composition).
180 For instance, two communities may present a pattern of low phylogenetic diversity (i.e., phylogenetic clustering) but differ a lot in terms of
181 lineage composition; e.g., communities composed only of Daphniidae species and communities composed only of Chydoriidae species. While
182 our study was carefully designed to separate the effects of body size and phylogenetic diversity, it was not possible to design it so as to
183 completely separate the effects of community average size (CAS) from that of phylogenetic composition. This happened due to the inexistence
184 of Chydoriidae species as large as *Daphnia magna* (i.e., our largest species) as well as Daphniidae species as small as *Pleuroxus aduncus* (i.e.,
185 our smallest species). In order to quantify the independent and shared contributions of community average size and phylogenetic composition on
186 grazing rates, we used variation partitioning (for more details on the method of variation partitioning, please see methods in the main text). In
187 this case, we used as a response variable the observed grazing rates and as predictors community average size and phylogenetic composition.
188 The variable phylogenetic composition was a categorical variable (1 = Daphniidae; 2 = Daphniidae and Cydoridae; 3 = Chydoridae).

189

190 **8.1. Results**

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192 Variation partitioning revealed that the explanatory variables CAS and phylogenetic composition and their intersections accounted for
193 approximately 56% of observed variation in grazing rates (Fig. S5). Community average size (CAS) accounted for 100 % of the total amount of
194 explained variation, either as a pure effect (33% of explained variation) or shared with phylogenetic composition (23% of explained variation).
195 The pure effect of CAS was highly significant ($p < 0.001$), whereas the pure effect of phylogenetic composition was not significant ($p = 0.452$).



196

197 Figure S6: Venn diagrams showing the pure and shared effects of community average size (CAS) and phylogenetic composition on community
198 grazing rates.

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201 **References**

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