

Corrigendum: Effects of Vertical Spatial Overlap on Phytoplankton Diversity under Experimentally Altered Lake Stratification Regimes.

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Since publishing the original article, additional work has led us to update the functional trait matrix used in the study. Specifically, we have revised the nutrition strategies of several of the nanophytoplankton taxa. Redoing the analyses using this updated trait matrix changed several of the original results and their interpretation. We hereby propose a corrected version of our results and of their interpretation to interested readers. Hereafter follow our corrections to the Abstract, Results, Discussion and Conclusion sections of the article, presented in their entirety here. Updated version of a corrected figure and table (Figure 4 and Table 2) are also presented. Sections of the text from the original article that have been corrected are underlined in the following sections. The Introduction and Methods sections of the paper were not altered by the correction process and can thus be found in the original article. The updated trait matrix is available as Supplementary Materials DataS1, as is an updated version of the supplementary Figure S4.

1 ***Abstract***

2 In phytoplankton communities, competitive exclusion might occur when functionally similar
3 species are impeded from regulating their positions along light and nutrient gradients to reduce
4 niche overlap. Greater spatial overlap (SO) between species due to water column mixing could
5 thus promote competitive exclusion, reducing community taxonomic diversity. However, greater
6 SO could also promote coexistence of functionally different taxa. Using data from a whole-lake
7 experiment, we investigated the effects of SO and other relevant environmental factors on
8 phytoplankton diversity across the water columns of lake basins with different thermocline
9 manipulations. We estimated the SO using an in situ fluorometer, and overall community
10 diversity microscopically. Using structured equation models, we estimated directional
11 relationships between phytoplankton diversity, SO, the lake physical structure and the
12 zooplankton community. No significant effect of SO on phytoplankton taxonomic or functional
13 diversity was observed. However, change in lake physical structure and in the zooplankton
14 community did affect diversity, with a negative response to increased top-down interactions.
15 Overall, the alteration of water column stratification structure and top-down interactions were
16 stronger drivers of phytoplankton diversity in our system than competitive interactions.

17 **Keywords:** diversity, composition, functional traits, competition, spatial ecology

18

19 ***Results***

20 *SEM for the taxonomic diversity*

21 The SE (structural equation) model for taxonomic diversity H' was not significant ($\chi^2 =$
22 $0.404, df = 1, p = 0.525$) and the goodness-of-fit index was high ($GFI = 0.996$), together
23 indicating a valid model adequately representing the observed data (Fig. 4a). The relationship of
24 H' with SO was not significant and neither was the direct relationship with $Thermo_{Depth}$.
25 However, taxonomic *Phytoplankton diversity* (H') was significantly positively affected by
26 $Meta_{width}$. H' was also significantly negatively affected by $Cladocera_{Biom}$ (*Zooplankton*
27 *community* compartment), which itself was negatively affected by $Thermo_{Depth}$ (but not by
28 $Meta_{width}$). A significant positive relationship was detected between $Thermo_{Depth}$ and SO , but not
29 between $Meta_{width}$ and SO . Although there was no direct significant relationship between

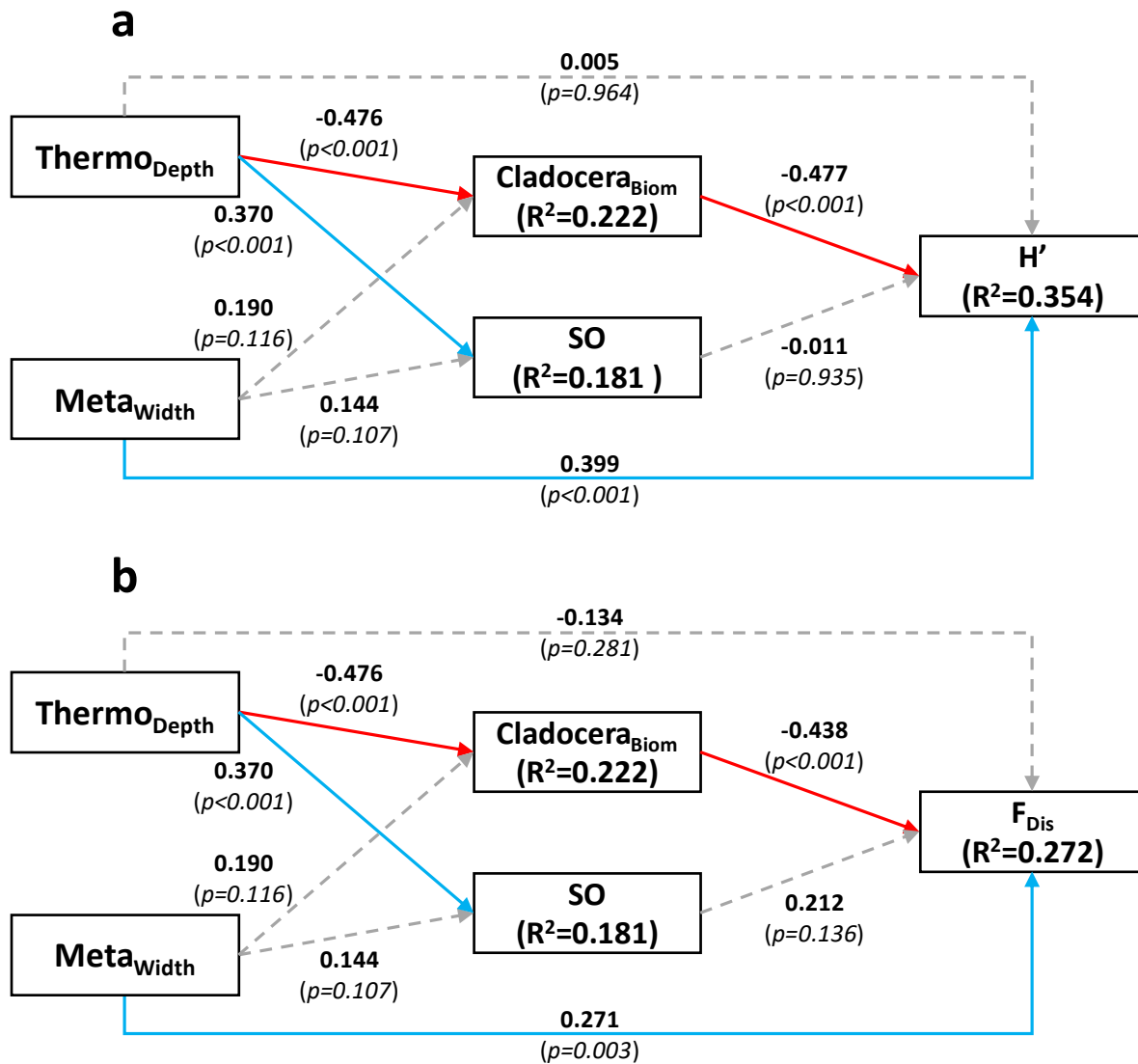
30 *Thermo*_{Depth} and *H'*, a larger epilimnion indirectly promoted the taxonomic *Phytoplankton*
31 *diversity* compartment because *Thermo*_{Depth} negatively affected *Cladocera*_{Biom} which itself
32 negatively affected *H'*. The model explained 18.1% of the variability in *SO*, 22.2% of the
33 variability in *Cladocera*_{Biom} and 35.4% of the variability in *H'*.

34 *SEM for the functional diversity*

35 The final SE model for *F*_{Dis} was very similar to the model for *H'* (Fig. 4b), as might be expected
36 given that the predictors for the compartments were the same. This SEM did not reveal a
37 significant effect of *SO* on *F*_{Dis}, indicating no influence of spatial overlap on functional
38 *Phytoplankton diversity*. The model Chi-square test was not significant ($\chi^2 = 0.405, df = 1, p =$
39 0.525) and the goodness-of-fit index index was *GFI* = 0.996, indicating that the model provided
40 an adequate fit to the data. This model explained 18.1%, 22.2% and 27.2 of the variability of *SO*,
41 *Cladocera*_{Biom} and *F*_{Dis} respectively.

42 *Effect of SEM predictors on the diversity of individual traits*

43 We used *Thermo*_{Depth}, *Meta*_{Width}, *Cladocera*_{Biom} and *SO* as predictors in the permutation multiple
44 linear regression models on the individual trait variability indices (Table 2). *Thermo*_{Depth} had a
45 significant negative effect on the diversity of taxa cell sizes (*CW*_{var_{MLD}) and a significant positive}
46 effect on the diversity of pigments (*H*_{Pig}). *Meta*_{Width} significantly and positively affected the
47 diversity of the nitrogen fixation and pigment traits (*H*_{Fix} and *H*_{Pig} respectively). *Cladocera*_{Biom}
48 had a significant negative effect on diversity of the pigment trait (*H*_{Pig}) and the coloniality trait
49 (*H*_{Col}). *SO* did not significantly affect any individual trait diversity indices, although the p-values
50 for the diversity of silica fixation and mixotrophy traits were relatively close to the 0.05 threshold
51 (with a positive trend for *H*_{Si} and a negative trend for *H*_{Mix}).



52

53 **Figure 4.** Updated SE models for H' and F_{Dis} (a and b respectively). Dashed grey arrows
 54 represent non-significant relationships. Blue arrows represent significant positive relationship and
 55 red arrows represent significant negative relationship. Results shown are standardized
 56 coefficients and *p*-value (between parentheses), as well as R^2 scores for endogenous variables.

57

58

59 **Table 2.** Updated results of the permuted multiple linear regressions on the different trait
60 diversity indices. For each regression, the coefficients for each potential explanatory factor (from
61 left to right: *Thermocline depth*, *Metalimnion width*, *Zooplankton biomass* and *Spatial Overlap*)
62 are indicated along with associated *p*-values in parentheses. Significant coefficients and *p*-values
63 are indicated in bold. The only result that changed in significance from the original article is
64 underlined (the effect of *MetaWidth* on *H_{mix}*).

	<i>Thermo_{Depth}</i>	<i>Meta_{Width}</i>	<i>Cladocera_{Biom}</i>	<i>SO</i>
<i>CWvar_{MLD}</i>	157 (1.00E-04)	-15.3 (0.356)	3.05 (0.086)	69.7 (0.442)
<i>H_{Nfix}</i>	-9.05E-04 (0.401)	8.34E-03 (0.025)	2.40E-04 (0.117)	0.01 (0.390)
<i>H_{Si}</i>	3.07E-03 (0.354)	3.15E-04 (0.498)	-5.14E-04 (0.161)	0.16 (0.073)
<i>H_{Mix}</i>	-9.340E-03 (0.111)	<u>9.73E-03</u> <u>(0.127)</u>	-6.73E-04 (0.075)	-0.15 (0.069)
<i>H_{Col}</i>	-1.34E-03 (0.420)	0.0107 (0.092)	-1.46E-03 (0.001)	0.09 (0.154)
<i>H_{Pig}</i>	-0.033 (0.030)	0.0613 (0.001)	-2.55E-03 (0.008)	0.29 (0.100)

65

66 **Discussion**

67 We examined, in a whole-lake experimental context, whether altering the stratification structure
68 of the water column would reveal an influence of spatial overlap on community diversity. We
69 predicted that increased interspecific competition would reduce taxonomic diversity, but our
70 analyses revealed no such effect of *SO* on the Shannon diversity index (*H'*) of the community.
71 This absence of effect of community aggregation on *H'* could be explained if functional trait
72 differentiation is effective at precluding taxonomic diversity decline through niche partitioning.
73 However, while the slope coefficient of the linear relationship between of *SO* and *F_{Dis}* was
74 positive in our SEM, this trend was not significant. While we anticipated that higher *SO* would be
75 associated with a generally greater diversity of resource acquisition and morphology traits, the
76 expected positive effect of *SO* on functional diversity was not observed. Thus, a summary
77 measure of functional diversity like *F_{Dis}* might be somewhat inadequate to capture potentially
78 contrasting responses of individual traits to spatial overlap.

79 In our study, greater SO was associated with a deeper thermocline (Fig. 4a, 4b), and thus, by
80 definition, a wider mixed (epilimnetic) layer. Overall, this implies a larger portion of the water
81 column over which phytoplankton species cannot easily regulate their position and are thus
82 potentially susceptible to greater competition. Therefore, we expected to see a negative effect of
83 SO on H' . The absence of such signal indicates that the effect of spatial aggregation on diversity
84 might not be as straightforward as we initially assumed, and that species can coexist even when
85 spatial overlap is high – perhaps via coexistence of taxa utilizing different traits – although no
86 effect on F_{Dis} was observed either. The absence of effect of SO on H' and F_{Dis} could also simply
87 indicate that interspecific competition is not a strong driver of either taxonomic or functional
88 diversity in our system. In the context of our experiment, the physical structure of the
89 environment and top-down interactions appear to be more important drivers of diversity, as
90 $MetaWidth$ and $Cladocera_{Biom}$ significantly affected H' and F_{Dis} in our SE models (Fig. 4a).

91 The SEMs featured a direct positive effect of metalimnetic width on both diversity types, but not
92 a direct effect of thermocline depth itself. Focusing on functional diversity, further analyses
93 revealed an effect of metalimnetic width on the diversity of pigments and diazotrophy strategy
94 traits. A wider metalimnion implies a thicker stable layer covering a larger range of light
95 intensities and colors. Species with different light requirement, hence with different pigment
96 types, would be able to better coexist within a wider stratified layer by establishing at different
97 depth (Pérez *et al.*, 2007). The positive effect of $MetaWidth$ on H_{Nfix} appears to mostly be the result
98 of a taxonomic change in community contribution. Further investigation revealed that a larger
99 metalimnion, implying a larger stratified portion of the water column, favors buoyant
100 cyanobacteria that can use gas vacuoles to regulate their vertical positions (Fig. S4a) (Huisman *et*
101 *al.*, 2004; Walsby *et al.*, 1997). Because some cyanobacterial taxa are able to fix dinitrogen, a
102 larger metalimnion would then also contribute to a diversification (H_{Nfix}) of nitrogen fixation
103 strategy (Paerl, 1990).

104 While thermocline depth did not have a similar direct significant effect on the overall functional
105 diversity of the community, it did affect the diversity of several individual traits. In particular, a
106 deeper thermocline positively affected the diversity of community cell sizes and negatively
107 affected the diversity of pigments ($CWvar_{MLD}$ and H_{Pig} respectively). A larger mixed layer
108 induced by thermocline deepening might allow larger sinking diatoms to be more prevalent

109 where otherwise small non-sinking taxa would dominate. Indeed, Ptanick *et al.* (2003)
110 demonstrated in a mesocosm experiment that large fast sinking diatoms benefit from higher
111 mixing depths. Conversely, a deeper epilimnion could prevent some species from establishing at
112 the optimal light absorption depth for their accessory pigment composition, leading to a loss of
113 pigment diversity in the community; optimal adaptations being for varying light (more mixed
114 taxa) or for reduced light (those that are able to remain near or in the hypolimnion). These effects
115 of thermocline depth and metalimnetic width on phytoplankton diversity illustrate how the
116 physical environment shapes community composition.

117 Returning to the relationship between SO and F_{Dis} , we expected more extensive SO to be
118 associated with higher levels of functional differentiation. As our SEM results indicates, this was
119 not observed, at least not with a global index of trait diversity like F_{Dis} . A more detailed
120 investigation of the effect of SO on individual trait diversity could nuance this result. For
121 example, we expected functional differentiation of traits related to resource acquisition with more
122 SO because when spatial niche overlap occurs within the actively mixed layer, species need to
123 display different nutrient acquisition kinetics to avoid competitive exclusion (Sommer, 1984,
124 1985). However, we found no positive significant effect of SO on any individual trait diversities.
125 We noted a near-significant trend ($p = 0.073$) indicating that SO might promote a better balance
126 between silica-requiring taxa (i.e. diatoms and some chrysophytes) and non silica-requiring taxa
127 (greater H_{Si}). In contrast, we observed a negative trend between SO and the diversity of resource
128 acquisition strategies (H_{Mix} , $p = 0.069$). Further investigation revealed a trend to more dominance
129 by autotrophy with greater SO (Fig. S4b). Generalist phago-mixotrophs are likely to be more
130 competitive in a vertically structured community, as they can potentially grow at depths where
131 the growth rate of a specialized photo-autotroph is not positive. Observational studies have
132 shown that different nanoplankton nutrition strategies can display distinct vertical patterns
133 (Princiotta and Sanders, 2017; Romano, Symiakaki et al., 2021). Thus, reduced SO levels (i.e., a
134 more spatially structured nanophytoplankton community) could promote a better balance
135 between phago-mixotrophs and photo-autotrophs, although here this effect is rather weak.
136 Overall, our results indicate that SO does not act on the global functional diversity of the
137 community and that while vertical aggregation might have contrasting effects on traits related to
138 resource acquisition (promoting H_{Si} vs. lowering H_{Mix}), the effect on individual traits is weak.

139 Note that these results are conditioned by the selection of traits we could characterize and could
140 include in our analyses.

141 Grazing by zooplankton was also an important factor in regulating phytoplankton diversity in our
142 SEM analyses. In particular, cladoceran biomass was one of the main factors affecting,
143 negatively, phytoplankton both taxonomic and functional diversity. The *Cladocera_{Biom}* effect on
144 F_{Dis} was greater than SO in terms of the absolute values of the standardized relationship
145 coefficients, indicating that the zooplankton community was a more important driver of
146 *Functional diversity* than was SO in the context of our experiment. The negative grazing effect
147 runs counter to theory that states that zooplankton grazing pressure should promote
148 phytoplankton taxonomic diversity by reducing the amount of interspecific resource competition
149 (McCauley and Briand, 1979; Menge and Sutherland, 1976), even experimentally for evenness
150 (Sarnelle, 2005). However, detailed examination of phytoplankton communities under increasing
151 levels of cladoceran grazing has demonstrated concomitant shifts to dominance by larger or
152 colonial phytoplankton species (Sommer *et al.*, 2001), thereby reducing functional diversity, and
153 thus potentially taxonomic diversity where such species are rare, as is the case in our study lake
154 and as we observed. Indeed, individual trait diversity did demonstrate significantly reduced
155 diversity within traits associated with coloniality (H_{Col}) and pigments (H_{Pig}), indicating that
156 selective grazing by cladocerans can reduce the diversity of certain phytoplankton trait types as a
157 result. Accompanying declines in taxonomic diversity would be expected in a relatively closed
158 experimental system such as ours where selective feeding could remove entire taxa (based on
159 traits) without replacement by other more resistant species from adjacent lakes (none upstream of
160 our site) over the time scale of our experiment. Indeed, cladoceran feeding is known to be
161 selective, as observed in experiments demonstrating that cladoceran gut pigment composition is
162 significantly different from the pigment composition of the associated phytoplankton community
163 (Wong *et al.*, 2006).

164 It is important to note that our spectral measurements of phytoplankton vertical structure can only
165 approximate real values of SO , as they only inform on the pigment levels for four broad spectral
166 groups, but at fine spatial scales. For example, we cannot quantify spatial overlap between
167 chlorophyte taxa, as they all share the same green pigment detected spectrally. This leads to
168 difficulty in fully assessing SO at very fine taxonomic scales, similar to those at which diversity

169 was estimated. To utilize whole-lake experiments to their full potential, improved rapid tools to
170 assess both spatial overlap at fine spatial scales and taxonomic resolution are needed.

171 ***Conclusions***

172 Our study revealed that altering the thermal stratification structure of a lake, while controlling for
173 lake morphometry, chemistry and global community composition, can affect spatial overlap
174 between phytoplankton groups. Spatial overlap was not related to taxonomic diversity, indicating
175 that forced coexistence does not necessarily translate into competitive exclusion. While we could
176 have, in turn, expected vertical aggregation to favor functional diversity, as niche differentiation
177 along trait axes could have alleviated interspecific competition and precluded an effect of spatial
178 overlap on taxonomic diversity, we did not observe any effect of SO on global functional
179 dispersion (F_{Dis}). Although SO did not affect the global functional diversity of the nanoplankton
180 community, vertical aggregation appeared to have some contrasting effects on individual trait
181 diversity for resource acquisition. This highlights the importance of investigating the response of
182 individual traits to the environment, as global functional diversity measures, like functional
183 dispersion, fail to capture potentially contrasting effects of an environmental driver on multiple
184 traits. Globally our analyses revealed that the physical structure of the environment and cascading
185 top-down interactions are the stronger drivers of phytoplankton diversity (both taxonomic and
186 functional) in our system. To our knowledge, this study is the first to simultaneously assess the
187 relative effects of not only spatial overlap, but also grazing and the physical environment on
188 multiple dimensions of phytoplankton diversity.

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