Original Research:

The taxonomic and functional biogeographies of phytoplankton and zooplankton communities across boreal lakes

Nicolas F. St-Gelais*, Richard J. Vogt, Paul A. del Giorgio and Beatrix E. Beisner

Groupe de Recherche Interuniversitaire en Limnologie et en Environnement Aquatique (GRIL) and Department of Biological Science, University of Québec at Montréal, Montréal, QC, Canada.

Corresponding author: Nicolas F. St-Gelais (nicolas.fstgelais@gmail.com)

*Current address : Groupe de Recherche Interuniversitaire en Limnologie et en Environnement Aquatique (GRIL), Department of Biological Sciences, Université de Montréal, Montréal, Quebec, Canada

Orcid ID (NFS): 0000-0002-6721-5191

OrcidID (BEB): 0000-0001-6972-6887

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Abstract

Strong trophic interactions link primary producers (phytoplankton) and consumers (zooplankton) in lakes. However, the influence of such interactions on the biogeographical distribution of the taxa and functional traits of planktonic organisms in lakes has never been explicitly tested. To better understand the spatial distribution of these two major aquatic groups, we related the distributions of their taxa and functional traits across boreal lakes (104 for zooplankton and 48 for phytoplankton) to a common suite of environmental and spatial factors. We directly tested the degree of coupling in their taxonomic and functional distributions across the subset of common lakes. Phytoplankton functional composition responded mainly to properties related to water quality, while zooplankton composition responded more strongly to lake morphometry. Overall, the spatial distributions of phytoplankton and zooplankton were coupled at taxonomic and functional levels but after controlling for the effect of environmental drivers (water quality and morphometry) and dispersal limitation, no residual coupling could be attributed to trophic interactions. The lack of support for the role of trophic interactions as a driver coupling the distribution of plankton communities across boreal lakes indicates that taxon-specific and functional trait driven ecological interactions may not modulate large-scale spatial patterns of phytoplankton and zooplankton in a coordinated way. Our results point to community structuring forces beyond the phytoplankton-zooplankton trophic coupling itself, and which are specific to each trophic level: fish predation for zooplankton and resources for phytoplankton.

Introduction

The composition of ecological communities is the results of multiple assembly processes (Kraft and Ackerly 2014) occurring at different spatial scales (Declerck et al. 2011). At the local spatial scale, species interactions (e.g. competition, predation, mutualisms) are considered to be strong an assembly processes that structure ecological communities (Diamond 1975). However, the relative influence of such local species interactions on the larger-scale distributions of organisms within and across regions is not well understood (Gotelli et al. 2010, Wiens 2011).

In lakes, planktonic consumers (zooplankton) and primary producers (phytoplankton) interact strongly, mainly via trophic relationships (Porter 1977, Sterner 1989), and studies on trophic cascades have emphasized how changes at one trophic level can affect entire food webs (Carpenter et al. 1985). While the top-down and bottom-up effects have typically been studied for their influence on standing biomass, they also influence community composition (McQueen et al. 1989, Ghadouani et al. 2003). However, whether these strong local trophic interactions affect the larger-scale distribution of zooplankton and phytoplankton in lake metacommunities at regional scales is still unresolved.

At the regional scale, the effect of habitat on the taxonomic biogeography of zooplankton and phytoplankton is well studied (see Keller and Pitblado 1989, Pinel-Alloul et al. 1990, 1995, O'Brien et al. 2004, Soininen et al. 2011, Stomp et al. 2011). The taxonomic composition of both phytoplankton and zooplankton are known to respond strongly to lake nutrient concentrations (Keller and Pitblado 1989, Pinel-Alloul et al. 1990, Soininen et al. 2011), as well as to other environmental variables such as lake colour (dissolved organic carbon content, Wissel et al. 2003) and lake morphometry (e.g. lake depth, Pinel-Alloul et al. 1990). On the other hand, very few studies have examined the joint distributions of phytoplankton and zooplankton and

thus, the importance of the trophic link between, phytoplankton and zooplankton, as a potential constraining factor of their respective taxonomic distributions on a landscape, has never been directly tested. Soininen and colleagues (2007) compared the spatial concordance of distance-decay relationships of phytoplankton and zooplankton taxa across ponds, finding that they were significantly stronger for zooplankton than for phytoplankton, but only across drainage basins and not within basins. However, they did not directly assess the joint taxonomic distributions of the different plankton groups.

Although zooplankton exhibit prey selectivity (Vanderploeg 1981, Paffenhöfer 1984), individual taxa often feed on many different phytoplankton species (Knisely and Geller 1986). Zooplankton grazing thus reflects selection for certain functional traits (e.g. size, shape) that are common to a number of phytoplankton species, more than for specific taxonomic groups per se. Thus, if trophic interactions between phytoplankton and zooplankton are strongly constrained by functional traits of both plankton groups, then joint biogeographical concordances should be more evident for functional traits than for strict taxonomic classification. A temporal analogue of this idea is the repeatable seasonal succession observed in many temperate lakes and captured effectively by the PEG model, which characterizes the dynamic effects of such trophic interactions by referring to specific functional groups of phytoplankton and zooplankton (Sommer et al. 1986, 2012). If functional trophic interactions are equally spatially important, and repeatable, across a landscape, one would expect any biogeographical concordance between phytoplankton and zooplankton to be more observable using a functional trait lens than a taxonomic one. Functional traits currently available for lake plankton account mechanistically for trophic interaction potential related to food web responses, including resource availability

(phytoplankton), feeding behaviours (zooplankton), and predator evasion (phytoplankton and zooplankton, Barnett et al. 2007, Hébert et al. 2016).

In addition to trophic interactions, spatial coupling between phytoplankton and zooplankton, even at the functional trait level, could also arise from similar responses to environmental drivers (habitat selection). For example, the taxonomic distributions of both phytoplankton and zooplankton are known to respond independently to lake nutrient concentrations (Keller and Pitblado 1989, Pinel-Alloul et al. 1990, Soininen et al. 2011), which could result in coupled distributions at the landscape scale. Beyond lake nutrient status, however, expectations for common responses of both plankton groups to gradients of other environmental variables such as lake colour (dissolved organic carbon content), lake size, and stratification are not evident. Furthermore, when considering a suite of environmental variables, different variable types do not necessarily have the same spatial structure: water quality variables generally having stronger spatial structure than do those related to lake morphometry (Lapierre et al. 2015). Consequently, while there is good reason to expect that each plankton group will respond to environmental variation, such responses are unlikely to be coordinated across different environmental variable types. In fact, this lack of coordination could disrupt factors like trophic interactions or nutrients that are likely to promote joint distributions. Proximate (water quality) variables with their strong spatial structure across landscapes should therefore favour greater plankton biogeographical concordance, than will less structured habitat variables characterizing lake morphometry.

In addition to environmental gradients, dispersal limitation is also known to structure phytoplankton and zooplankton communities (Heino et al. 2015). Previous work has indicated that phytoplankton generally respond to environmental factors while zooplankton are more

dispersal-limited (Beisner et al. 2006, De Bie et al. 2012, Padial et al. 2014). Such differential response patterns could also interfere with a strong biogeographical coupling of the groups. In fact, differential relative responses of phytoplankton and zooplankton to environmental factors and distances between lakes, make it necessary to control for their respective effects before assessing the importance of trophic interactions on the biogeographical coupling of the two plankton groups.

Here, we conducted the first comprehensive study that directly considers the relative importance of trophic interactions between planktonic organisms and relates these to the effects of environmental conditions and dispersal (landscape space) to affect the coupling of their biogeographical distributions. Understanding of the importance of trophic interactions at the landscape-scale has implications for the impact of broad-scale changes in zooplankton or phytoplankton composition (e.g. Jeziorski et al. 2015) on aquatic food webs. To investigate whether the signature of trophic interactions could be captured at a regional scale, we assessed the coupling between the taxonomic and functional distribution of lake zooplankton and phytoplankton communities.By using a stepwise framework, we first accounted for the effect of habitat selection and dispersal, and then assessed whether residual variation in the observed coupling between species and traits could be attribution to trophic interactions. This approach also allowed us to investigate the environmental and spatial factors influencing each group (phytoplankton or zooplankton) separately as well. Our study covers a large biogeographical range (longest distance between two lakes is 1 228 km) and examines more than 100 lakes clustered in three regions that together characterize geological and environmental variation in the boreal belt across one of Canada's largest provinces (Québec). Because trophic interactions are mediated by functional traits, we expected to observe a stronger residual coupling between the

distribution of zooplankton and phytoplankton traits compared to the taxonomical coupling. Furthermore, we expected the coupling to be even stronger when only traits specific to trophic interactions between zooplankton and phytoplankton were considered (e.g. pigment type, feeding strategy).

Materials and Methods

Study lakes and sampling

Crustacean zooplankton samples were collected from 104 lakes with a low level of anthropogenic disturbance, within three environmentally (Figure 1, Table 1) and geologically (Roy 2012) distinct regions of Quebec, Canada, during the years 2010 (Abitibi, May to October), 2011 (Chicoutimi, June to October) and 2012 (Schefferville, July to August). Integrated zooplankton samples were collected from the deepest point of each lake using a conical plankton net (110 µm mesh, 0.30 m mouth diameter), equipped with a flow meter (General Oceanics, USA), hauled vertically from 1 m above the sediments to the surface. Zooplankton samples were anaesthetized using carbonated water and were preserved in 75% (final concentration) ethanol. In a subset of 48 lakes, the phytoplankton community was also simultaneously sampled over the photic zone using a flexible PVC sampler tube and an integrated subsample (250 ml) was preserved in Lugol's solution.

Crustacean zooplankton were identified at the species level (but aggregated for analyses at the genus level to correspond to the phytoplankton data), using an inverted microscope (50-400X) and individuals were counted until a total of 200 individuals had been enumerated. For each taxon present in a lake, the length of 20 mature individuals was measured and biomass by

taxon was estimated using length-dry-mass regressions (McCauley 1984, Culver et al. 1985). Phytoplankton were enumerated at the genus level using the Ütermohl method on an inverted microscope at 400X magnification. Phytoplankton biomass was estimated from biovolume computed using cell and colony length measurements and corresponding geometric forms (Hillebrand et al. 1999). We also measured key limnological variables to characterize the lake and catchment environments. We used a multiparameter sonde (YSI, Yellow Springs Instruments, OH, USA) to measure pH (at 0.5m depth) and temperature (at 0.5m depth intervals, then averaged over the water column). Water samples were collected at 0.5m depth to measure the concentration of chlorophyll *a* (Chl-*a*), total phosphorus (TP), total nitrogen (TN), dissolved organic carbon (DOC) and coloured dissolved organic matter (CDOM). Chl-*a* was extracted with 90% hot ethanol and absorption was measured spectrophotometrically before and after acidification to account for phaeophytin (Lorenzen 1967, Nush 1980); TP was measured from water samples using the molybdenum-blue method following persulfate digestion (Cattaneo and Prairie 1995); TN was measured using nitrates after persulfate digestion; DOC concentration was measured on an O.I. Analytical (Texas, USA) TIC/TOC using 0.45 μm filtered water after sodium persulfate digestion; CDOM was measured using a UV/Vis UltroSpec 2100 spectrophotometer (Biochrom, Cambridge, UK) at 440 nm. Missing values (see Table 1) were imputed using an approach based on random forest (missForest R package, Stekhoven and Bühlmann 2012, NRMSE : 0.038). Lake depth was measured at sampling point using a Portable Water Depth Sounder Gauge (Cole-Parmer). Lake area was derived using ArcGIS V10 software (ESRI Inc., Redland, CA, USA) and catchment slope was estimated using a Digital Elevation Model (Canadian Digital Elevation Data). To visualize environmental differences between the three regions, we used a principal component analysis (PCA) using the *rda* function (vegan R

package, Oksanen et al. 2015). Finally, because our sampling was discontinuous on the landscape we used the Euclidean distance between lakes to characterize the effect of dispersal limitation on the distribution of taxa and functional traits.

Functional trait composition and diversity

Given that our objective was to test for a significant coupling between adjacent trophic groups, we selected functional traits (Table 2) that explicitly characterize the grazing interaction between phytoplankton and zooplankton, as well as their interaction with other trophic levels in the food web. For phytoplankton, we selected traits that define how they interface with resources (i.e. nutrients and light) and the zooplankton grazers (motility, edibility, colony formation). For zooplankton, we focused on traits that define how they consume phytoplankton (i.e. feeding type and trophic group). Phytoplankton trait values were obtained from a literature review (see (Longhi and Beisner 2010 for details) and included (i) capacity for N-fixation, (ii) silica demand, (iii) capacity for mixotrophy, (iv) pigment composition (v) cell motility and (vi) edibility to zooplankton (>35 μm linear dimension) and (vii) tendency to form colonies. Crustacean zooplankton traits values were also obtained from a literature review (see Barnett et al. 2007)) and included (i) feeding type and (ii) trophic group. For both zooplankton and phytoplankton, we also used the average individual biomass of each taxon as an integrative functional trait of body size (Brown et al. 2004, Litchman et al. 2013) related both to resource acquisition and grazer avoidance (Table 2).

Statistical analyses

To visualize how phytoplankton and zooplankton taxa and traits were distributed on the landscape, we used the percentage of lakes in which they occurred. We tested for differences in

taxonomic or functional composition between the three regions using the constrained ordination technique Canonical Analysis of Principal Coordinates (*CAP* BiodiversityR package; Anderson and Willis 2003). Using the CAP leave-one-out allocation success (% correct, Anderson and Willis 2003) we assessed the distinctiveness of regional composition using the proportion of correct allocation, which can be interpreted as the strength of the compositional differences between regions. Prior to the CAP ordination, taxonomic composition was Hellinger-transformed to reduce the effect of double zeros (Legendre and Gallagher 2001) and Bray-Curtis distance was used for the CAP ordination.

To test whether the distributions of phytoplankton and zooplankton communities were coupled across the landscape, we used a hierarchical framework (Figure 2). First, we tested for significant coupling between the composition of the two groups at taxonomic and functional trait levels using a Procrustes analysis (Mardia et al. 1980) on the subset of lakes (48) for which both phytoplankton and zooplankton were sampled. Specifically, we tested the degree of concordance between the PCA ordinations of phytoplankton and zooplankton taxonomic and functional community compositions. For this analysis, species composition was transformed using the Hellinger transformation (Legendre and Legendre 1998, Legendre and Gallagher 2001), and functional trait composition was transformed using a logit transformation. We tested the significance of the Procrustes statistic using a permutation procedure (9999 simulation, *protest* function in vegan R package, Oksanen et al. 2015). To further investigate whether the coupling could be explained by a similar response of the two groups to environmental variation or by dispersal limitation (see Figure 2) we also tested for a significant coupling, using Procrustes analysis, after controlling for environmental factors (water quality characteristics and

morphometry) and space (using between-lake distance based on latitude and longitude coordinates).

To directly evaluate the relative importance of environmental and spatial variables as drivers of the taxonomic and functional composition of phytoplankton and zooplankton, we used distance-based redundancy analysis for taxonomic composition (dbRDA, Legendre and Legendre 1998) and multiple regression for each functional trait; both followed by variation partitioning (Borcard et al. 1992). We separated the environmental variables into two groups; water quality (WQ with Chl-*a*, TP, TN, DOC, water color, pH, temperature) and morphometry (M with lake area and depth). Prior to the RDAs and multiple regressions, we used a stepwise selection (based on AIC) of variables within each group of environmental variables. We identified shared variation between the two groups of environmental variables (WQ+M), and between environmental variables and space (S) as: WQ+S and M+S to determine whether environmental variables were spatially structured. Finally, to visualize the relationship between phytoplankton and zooplankton taxa and functional traits, we used an RDA with all taxa and all the functional traits combined.

Results

Biogeographical patterns

We observed important environmental differences between the three regions (Figure 1, Table 1). The first principal component (PC) mainly differentiated the Abitibi and Chicoutimi regions from the Schefferville region and represented differences that reflect latitude between the regions: lakes in Abitibi and Chicoutimi being warmer, darker (higher *CDOM*) with higher concentrations of chlorophyll *a*, dissolved organic carbon (*DOC*) and nutrients (*TN* and *TP*). The

second PC axis differentiated the Chicoutimi and Abitibi regions and was mainly related to morphometric differences: lakes in the Abitibi region were shallower, with flatter catchments and also with higher nutrient concentrations. Lakes in the Schefferville region were spread across the second PC axis indicating that lake morphometry and catchment characteristics were highly variable in this region.

The average percent occurrence (Figure 3a) was 24% for phytoplankton taxa (median 15%) and 21% for zooplankton taxa (Figure 3b, median 8%). Four phytoplankton taxa *Mallomonas* (90%), *Cryptomonas* (90%), *Dinobryon* (82%) and *Asterionella* (63%), were observed in more than 60% of lakes. For zooplankton *Leptodiaptomus* (89%), *Daphnia* (87%), *Bosmina* (84%) and *Holopedium* (64%) had occurrences greater than 60%. Of the 56 phytoplankton taxa, 34 (61%) were observed in all three regions, while across all 27 zooplankton taxa, 10 (37%) were observed in all regions. The differences in taxonomic composition between regions were significant for both groups, but were less pronounced for phytoplankton (% correct = 69%, p=0.01, Figure 4a) than for zooplankton (76%, p=0.01, Figure 4b).

For both phytoplankton and zooplankton, all functional traits were present in all three regions. All phytoplankton functional traits occurred in more than 90% of lakes, with the exception of two traits associated with cyanobacteria: presence of a vacuole for motility (63% of lakes) and the potential to fix nitrogen (58% of lakes). The occurrence of zooplankton functional traits ranged between 18% for carnivores and omnivore-herbivores, and 100% for herbivores, with the average occurrence of zooplankton traits being 64% (median 75%). Functional composition between regions did not differ for either phytoplankton, or zooplankton (% correct = 35%, p=0.69 and 49%, p=0.15 respectively, Figures 3c and 3d).

In the taxonomic Procrustes analyses we found a significant correlation between phytoplankton and zooplankton taxa (Table 3), indicating a taxonomic coupling between the distribution of the two groups. However, the correlation was not significant after controlling for water quality, morphometry and space together, suggesting that the observed taxonomic coupling cannot be attributed purely to trophic interactions but rather to both groups responding to the same environmental and spatial drivers. On the other hand, using functional traits, there was a significant coupling between the plankton groups only when traits related to trophic interactions between phytoplankton and zooplankton (i.e. without phytoplankton resource acquisition traits; including only motility, colonial and biovolume; Table 2) were used, but not when all traits were considered (Table 3). The significant trait correlation did not remain after controlling for water quality, morphometry or space (independently or together), indicating that the observed correlation was again likely induced by one of these drivers and not by trophic interactions directly.

Factors related to community composition

The RDA model explained 4% of the variation in phytoplankton taxonomic composition. For functional composition , up to 27% of variation in the biomass proportion of the different traits was explained in the multiple regressions (Figure 5), but no variation was associated with the distribution of mixotrophy, non-motility, nor biovolume. We observed no direct effect of spatial factors on phytoplankton functional trait variation, but a shared component between water quality and spatial factors indicating that the water quality variables driving the distribution of phytoplankton traits were spatially structured. Water quality variables were most consistently explanatory factors of the phytoplankton functional traits for which variation was explained (blue

bars; Figure 5). The exceptions were for the flagellated trait, which responded to lake morphometry, and the mixed pigment trait for which variation was shared between water quality and morphometry. After forward selection, the RDA of phytoplankton taxonomic composition was constrained by *TP*, *CDOM*, *pH* and lake area (Figure 6a). Based on the first axis, differences in taxonomic composition could be mainly explained by lake nutrient status, pH and coloured carbon content (*CDOM*). Similarly, functional composition also responded to nutrient status and carbon content as well as lake temperature and chlorophyll *a* concentration according to the first PC axis (Figure 6c). Functional traits related specifically to cyanobacteria (Pigment blue, N fixation and Motility-V) were positively associated with the first axis, while traits (Pigment and mixed brown, Si requiring, Motility-F) related to other key taxonomic groups (including chrysophytes, cryptophytes and diatoms) were negatively associated. In the taxonomic and functional RDA, the first two axes were significant.

For zooplankton, the global RDA models explained 11% of the variation in taxonomic composition (Figure 5), and between 6% and 32% of the variation in the proportion of different functional traits (Figure 5). Similarly, zooplankton variation was shared between water quality and space, but spatial factors also independently explained a significant portion of the variation in zooplankton taxonomic composition, B-filtration, D-filtration and size. For taxonomic composition and most functional traits, a large portion of the variation was attributable to lake morphometry (yellow bars; Figure 5). However, for most traits, and taxonomic composition, some variation was either shared or explained independently by water quality.

In the subsequent RDA of zooplankton taxonomic composition, constrained by morphometric and water quality variables (Figure 6b), variables related to lake productivity (Chl*a*, *TP* and *TN*), carbon content (*DOC*) and temperature loaded on the first axis, while

variables relate to lake morphometry (lake depth and area) loaded more strongly on the second axis. For functional traits (Figure 6d), the first axis was mainly related to lake depth, with the second being related to lake area. The raptorial feeding trait was highly correlated with deep lakes and C-filtration with shallow lakes. B-filtration was positively correlated with lake area and size was negatively correlated with lake area while D-filtration was related to large and deep lakes and Stationary suspension to small and shallow lakes. In the taxonomic and functional RDA, the first two axes were significant.

Discussion

We observed a significant coupling between the landscape distributions of phytoplankton and zooplankton at both taxonomic and functional levels. However, after taking into account the coupling explained by environmental filtering and limitations to dispersal, we showed that in boreal lakes, no residual coupling between phytoplankton and zooplankton could be attributed to trophic interactions directly. Overall, the distribution of phytoplankton and zooplankton taxa and traits was strongly influenced by water quality and lake morphometry and to a lesser extent by dispersal limitation and these emerge as the main drivers coupling the distribution of phytoplankton and zooplankton across boreal lakes. Moreover, phytoplankton and zooplankton taxonomic composition displayed strong regional differences across the entire studied landscape, but not when considered through a functional trait lens. Thus, although we observed important taxonomic differences between regions, these differences did not translate into functional differences between regions, despite the large environmental gradients covered.

Regional differences in composition and environment

Significant differences in phytoplankton and zooplankton taxonomic composition between the three regions indicate a role for environmental factors or dispersal in influencing distributions at the regional scale. On the other hand, functional composition did not differ between regions, indicating that environmental variation or dispersal distances could not prevent any traits from being distributed across the entire landscape. However, the biogeographical overlap in traits does not mean that functional composition was similar across all lakes. Environmental factors related to water quality and lake morphometry explained a significant amount of variation in relative biomass of most functional traits across both plankton groups (Figure 5), indicating that the control of functional composition acts at sub-regional scales. We also observed that control of plankton taxonomic and functional composition by these lake characteristics was far more important than was the effect of dispersal limitation. Finally, comparing across plankton groups, dispersal limitation was more important for zooplankton than phytoplankton, supporting previous work (Beisner et al. 2006, De Bie et al. 2012), but here now also including functional traits.

Divergent responses of phytoplankton and zooplankton to their environment

Although both plankton groups responded strongly to environmental factors, the specific variable types accounting for the most variation in taxonomic and functional composition differed between phytoplankton and zooplankton. Phytoplankton taxonomic and functional trait compositions responded most consistently and strongly to water quality (i.e. their proximal environment). This is to be expected based on previously established strong relationships between composition and lake nutrient status for broad taxonomic groups, groups which were reflected in our functional (pigment) categorization (Watson et al. 1997). On the other hand, zooplankton taxonomic and functional composition both responded more strongly to lake

morphometry. However,some water quality effects were also evident for zooplankton, indicating overall an integrated response of zooplankton functional and taxonomic composition to their proximal environment (water quality) and habitat characteristics (lake morphometry).

Zooplankton response to water quality could either occur as a direct effect, or as an indirect bottom-up response to changes in phytoplankton community structure. For the more prevalent response of zooplankton to lake morphometry, this reflects variation in habitat characteristics, such as differences in lake physics (e.g. thermal stratification) or lake depth. We consider it most likely that this morphometry effect arose indirectly, operating via the trophic influence of fish predation, as previously observed (O'Brien et al. 2004). Specifically, lake depth influences fish community composition (Jackson and Harvey 1989),with larger volume lakes tending to have longer food chains (Post et al. 2000), thereby modulating the trophic cascade effect on zooplankton through planktivore fish feeding (Carpenter et al. 1985). The variation in zooplankton taxonomic and functional trait composition related to lake morphometry could thus result from local variation in fish composition (data which we did not have). Further evidence comes from the larger proportions of D-filtration in larger, deeper lakes: in larger lakes the presence of an extra trophic level (total of 4- levels) decreases fish zooplanktivory, resulting in reduced top-down pressure on large herbivorous cladocera, which are preferred prey for fish (Christoffersen et al. 1993). Repercussions throughout the community were observable in the ordination biplots (Figure 6d), with reductions in D-filtration being related to an increased proportion of stationary suspension herbivory, dominated by calanoid copepods. Also, the relative biomass of the C-filtration group was negatively related to lake depth, consistent with the fact that most species within this functional feeding type are littoral species and shallow lakes contain greater proportion of habitats that are littoral.

Uncoupled biogeographies of phytoplankton and zooplankton

Using the common currency of functional traits, the biogeography of phytoplankton and zooplankton were not coupled after controlling for environment and space. Even by only including phytoplankton traits reflecting trophic interactions (grazer avoidance traits) no coupling could be attributed to interactions between functional groups. Because no coupling between phytoplankton and zooplankton was observed after controlling for either water quality, morphometry or space, it suggests that one of these is the main driver of the observed coupling. Because water quality was an important driver for most phytoplankton, as well as zooplankton traits (see Figure 5), we can hypothesize that water quality was the main driver of the coupling between phytoplankton and zooplankton traits. The lack of support for a coupling induced by trophic interactions is surprising given the success of other functionally-based models to describe coupled plankton dynamics through time (e.g. PEG model). It suggests that trophic interactions are not consistent enough at the landscape level to result in a functional coupling of phytoplankton and zooplankton.

Taxonomically, we also observed a significant coupling between phytoplankton and zooplankton distribution and one that was stronger than observed functionally. The coupling between phytoplankton and zooplankton taxonomy only became non-significant after controlling for the combined effects of water quality, morphometry and space (Table 3), suggesting that the observed coupling resulted from a combined effect of these three groups of drivers across boreal lakes. While the greatest decrease in coupling was observed after controlling for space (Table 3), spatial factors could not explain any independent variation in the distribution of phytoplankton taxa (Figure 5). However, as most of the variation explained by water quality in the distribution of phytoplankton and zooplankton (taxonomic) was spatially structured (light blue bars in

Figure 5) the observed spatial impact is likely not a result of dispersal limitation, but rather the result of water quality variables that are spatially structured. Nevertheless, the coupling we observed at the taxonomic level is consistent with Bowman and colleagues (2008) who observed a spatial concordance between the community composition of phytoplankton and zooplankton in eight Canadian lakes.

It is also important to acknowledge that the coupling between plankton groups that we attribute to environmental factors could mask the effect of trophic interactions. For example, the concordant taxonomic response to phosphorus (*TP*, Figure 6 a and b), could indicate that phosphorus enrichment affecting phytoplankton triggers a response in the zooplankton, as would be expected with changes in edibility of phytoplankton along this nutrient gradient (e.g. Watson et al. 1997). Furthermore, for a large suite of environmental variables, spatial structure is likely to vary differently across variable types, with for example, water quality variables generally having stronger spatial structure than those related to lake morphometry (Lapierre et al. 2015). This difference in spatial structuring could disrupt factors like trophic interactions or nutrients that are likely to promote joint distributions. Proximate (water quality) variables with their strong spatial structure across landscapes should therefore favour greater plankton biogeographical concordance than would less structured habitat variables characterizing lake morphometry. Unfortunately, mechanistic links such as these are difficult to verify with observational studies like ours at these spatial scales, and would require smaller-scale experimental study to fully verify.

The goal of including functional traits was to assess whether biogeographical coupling would be more easily observed using a more mechanistic aggregation of organismal differentiation than is done by pure taxonomy. To this end, we selected functional traits that are

directly representative of interactions between phytoplankton and zooplankton and in resource acquisition. A lack of coupled biogeographies, after controlling for environmental variables was observed. Hence, for the suite of feeding related functional traits used in this study, we found no support for direct reciprocal influences of zooplankton and phytoplankton community structures across the landscape. Overall, our study points to other food web components such as fish predation and resource availability as independent drivers of each group's biogeography . Overall, although phytoplankton and zooplankton are known to share strong trophic connections in individual lakes (Porter 1977, Sterner 1989), which constitutes the basis of the main pathway for matter and energy transfer in aquatic environments, these patterns do not seem to constrain their joint biogeographical distributions. This result has implications for the predictability (or a lack thereof) regarding large-scale change in plankton taxonomic or functional compositions across lake landscapes with the spread of invasive species or the northward migration of species with climate warming.

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Table 1: Means and standard error (SE, in parenthesis) of environmental variables: water quality (WQ), and morphometry (M) with the number of missing values (#NA) and the transformation used.

Variable name (abbreviation)	***Category***	***# NA***	***Units***	***Transformation***	***Regional averages (Standard Error)***					
					Abitibi		***Chicoutimi***		***Schefferville***	
					Phyto.	***Zoo.***	***Phyto.***	***Zoo.***	***Phyto.***	***Zoo.***
Chlorophyll *a* (*Chl-a*)	WQ	0	ug/L	$\log_{10}$	3.1 (0.7)	3.7 (0.9)	1.9 (0.2)	2.0 (0.1)	0.9 (0.1)	0.8 (0.1)
Total Phosphorus (TP)	WQ	0	ug/L	$\log_{10}$	17.0 (3)	18.5 (3.5)	9.8 (0.9)	10.1 (0.6)	7.3 (0.7)	7.7 (0.6)
Total Nitrogen (TN)	WQ	0	mg/ L	$\log_{10}$	0.3 (0.05)	0.3 (0.04)	0.2 (0.02)	0.2 (0.0 1)	0.2 (0.02)	0.2 (0.01)
Dissolved organic carbon (DOC)	WQ	2	mg/ L	$\log_{10}$	12.4 (1.4)	12.4 (1.3)	7.5 (0.7)	7.7 (0.4)	3.8 (0.4)	4.0 (0.2)
Colored dissolved organic matter (CDOM)	WQ	1	1/m	$\log_{10}$	3.2 (0.7)	3.4 (0.8)	3.9 (0.5)	4.0 (0.4)	1.0 (0.2)	1.1 (0.1)
pH	WQ	1	-	-	7.4 (0.3)	7.5 (0.3)	6.9 (0.1)	7.0 (0.1)	6.8 (0.2)	7.1 (0.1)
Water temperature (0.5 m) (T_{water})	WQ	1	ºC	-	21.8 (1.1)	20.6 (1.0)	20.0 (0.5)	19.8 (0.3)	14.2 (0.2)	15.0 (0.2)
Maximum depth (Zmax)	M	0	m	$\log_{10}$	5.7 (1.4)	5.7 (1.2)	8.2 (1.5)	9.4 (1.1)	7.3 (1.5)	6.8 (0.9)
Lake Area (Area)	M	0	m^2	$\log_{10}$	5.89 (0.3)	5.7 (0.3)	6.1 (0.3)	6.2 (0.2)	6.0 (0.3)	6.1 (0.2)
Number of lakes					11	13	16	44	21	46

Table 2: Phytoplankton and zooplankton functional traits used in this study.

	Category	Traits	Values
Phytoplankton	Resource acquisition	Nitrogen fixation	yes/no
		Si requirement	yes/no
		Mixotroph	yes/no
		Pigments	Green Blue Brown Mixed
	Grazer avoidance	Motility	None F(Flagellated) V(Vacuole)
		Biovolume	numerical
		Colonial	yes/no
Zooplankton	Resource acquisition	Trophic Position	Carnivore Omnivore-Carnivore Omnivore Omnivore-Herbivores Herbivores Immature
		Feeding Type	B (Bosmina)-filtration C (Chydorus)-filtration D (Daphnia)-filtration S (Sidae)-filtration Stationary Suspension Raptorial

Table 3: Procrustes rotation analysis of species and the trait dataset correlation coefficients for phytoplankton (Phyto) and zooplankton (Zoo) after controlling (|) for water quality (WQ), morphometry (M) and space (S). Where * = 0.01<p<0.05, ** = 0.001<p<0.01,*** p<0.001, ns = non-significant.

	Control for	m12 squared	Correlation
Taxonomic	$Zoo_{taxo} \leftrightarrow Phyto_{taxo}$	0.85	*0.38****
	|wq	0.86	*0.37***
	|m	0.86	*0.37****
	|S	0.89	*0.34***
	|wq+m+S	0.92	ns
All traits	$Zoo_{funct} \leftrightarrow Phyto_{funct}$	0.92	ns
	|wq	0.92	ns
	|m	0.9	ns
	|S	0.92	ns
	|wq+m+S	0.93	ns
Without phytoplankton resource acquisition traits	$Zoo_{funct} \leftrightarrow Phyto_{funct}$	0.92	0.28*
	|wq	0.95	ns
	|m	0.94	ns
	|S	0.94	ns
	|wq+m+S	0.94	ns

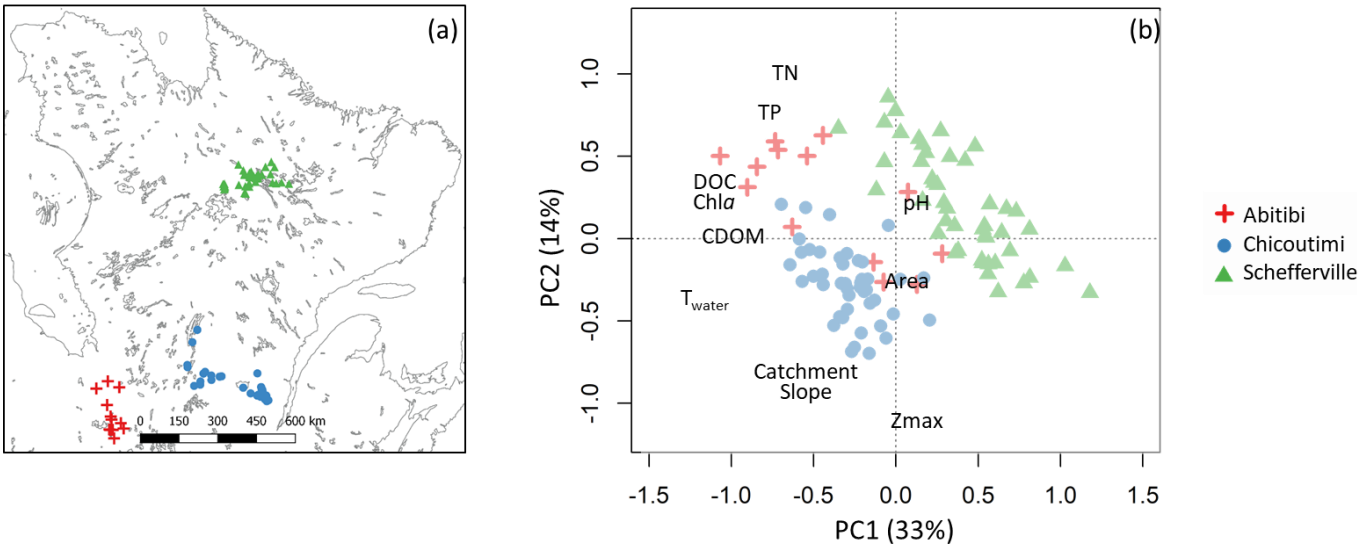

Figure 1: (a) Map of sampled lakes across three regions of boreal Quebec, Canada. (b) Lakes environmental principal component analysis (PCA) based on water quality and morphometric variables where the different symbols represent the three sampled regions. . Abbreviations are as follows total phosphorus (TP); total nitrogen (TN); dissolved organic carbon (DOC); chlorophyll a (*Chl-a*); coloured dissolved organic matter (CDOM); water temperature (Twater); lake maximum depth (Zmax); lake area (Area).

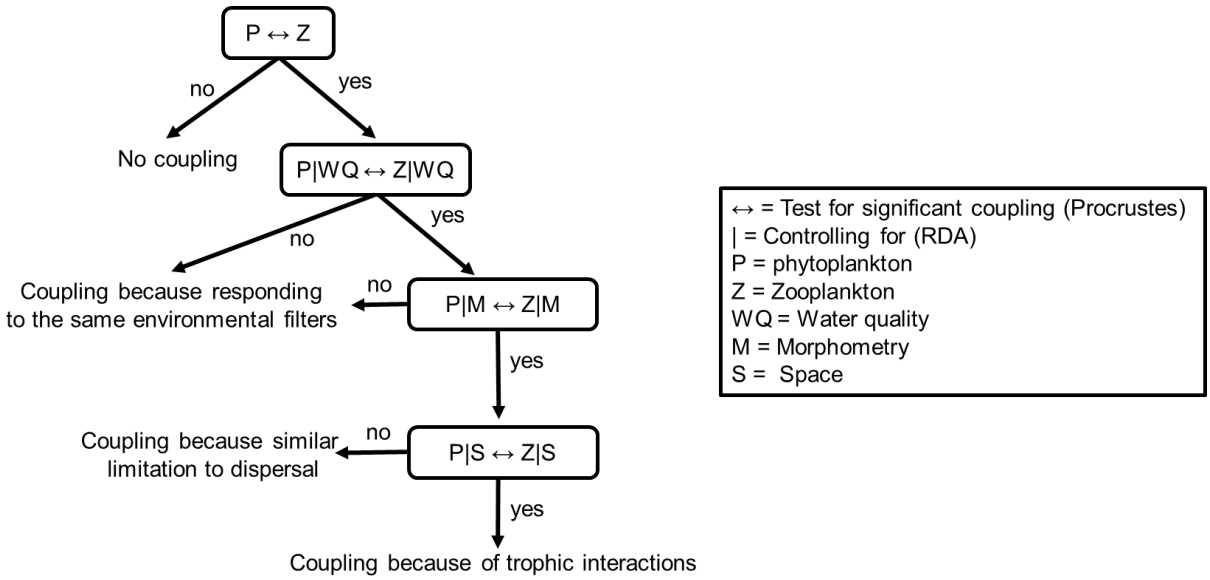

Figure 2: Conceptual framework used to test for a significant coupling between zooplankton and phytoplankton communities using taxonomy and functional traits, while controlling for environmental factors (water quality WQ, morphometry M) and dispersal limitation (space S).

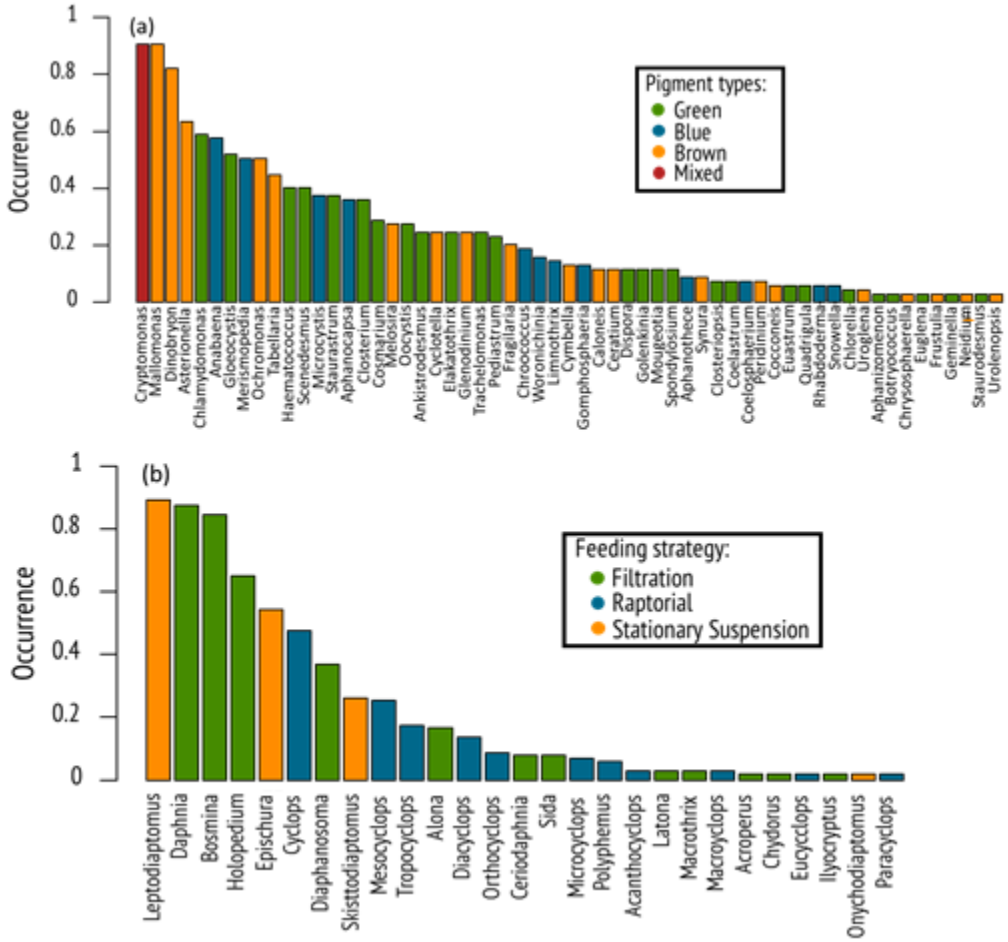

Figure 3: Ranked occurrence (percent of all lakes) of (a) phytoplankton and (b) zooplankton taxa.Bars and dots were coloured by Pigment trait type for phytoplankton and the Feeding strategy trait for zooplankton.

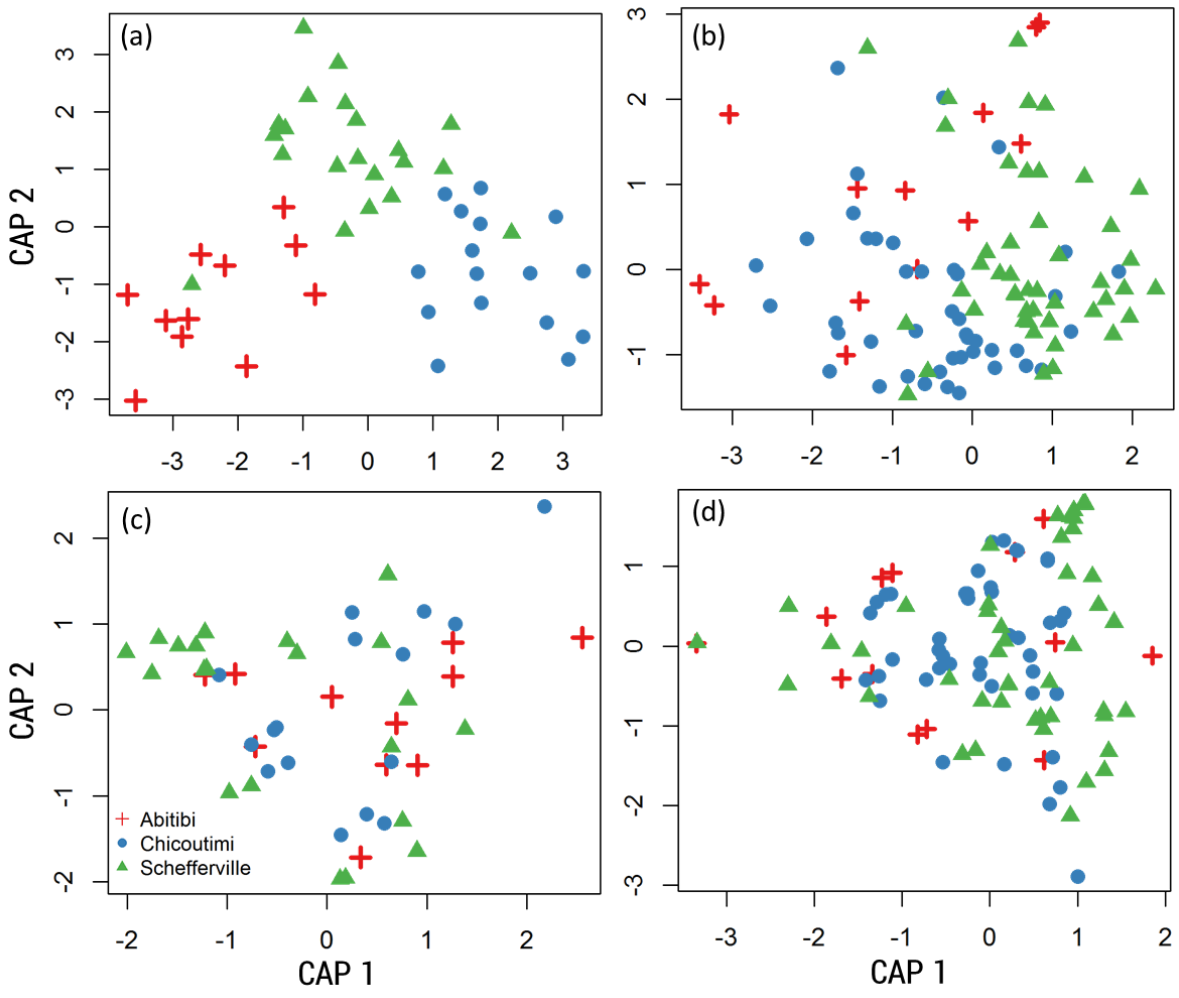

Figure 4: Two-dimensional scatter plot of canonical axes of the CAP ordinations for (a) phytoplankton and (b) zooplankton taxonomic composition and their respective functional trait compositions (c, d). Symbols represent the different regions. Strong regional differences occurred for taxonomic composition of phytoplankton (a, p=0.01) and zooplankton (b, p=0.01), but were not significant for functional trait composition (c and d).

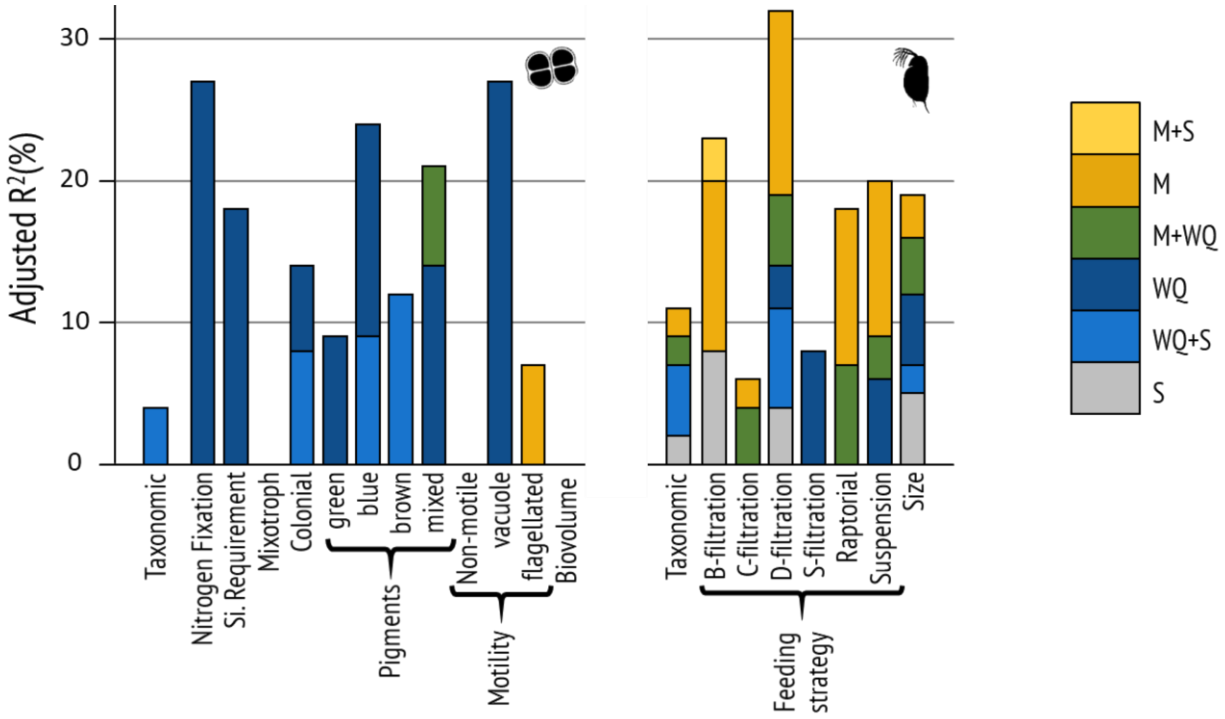

Figure 5: Variation explained (adjusted R^2) by environmental and spatial factors for phytoplankton (left panel) and zooplankton (right panel) taxonomic composition and each functional trait. Only regression and RDA models that were globally significant are displayed. The variation that was independently explained by a group of variables is represented if significant, and shared variation represents the sum of variation shared between any of the groups of variables, represented with a +. Abbreviations are as follows : morphometry (M) ; water quality (WQ) ; space (S).

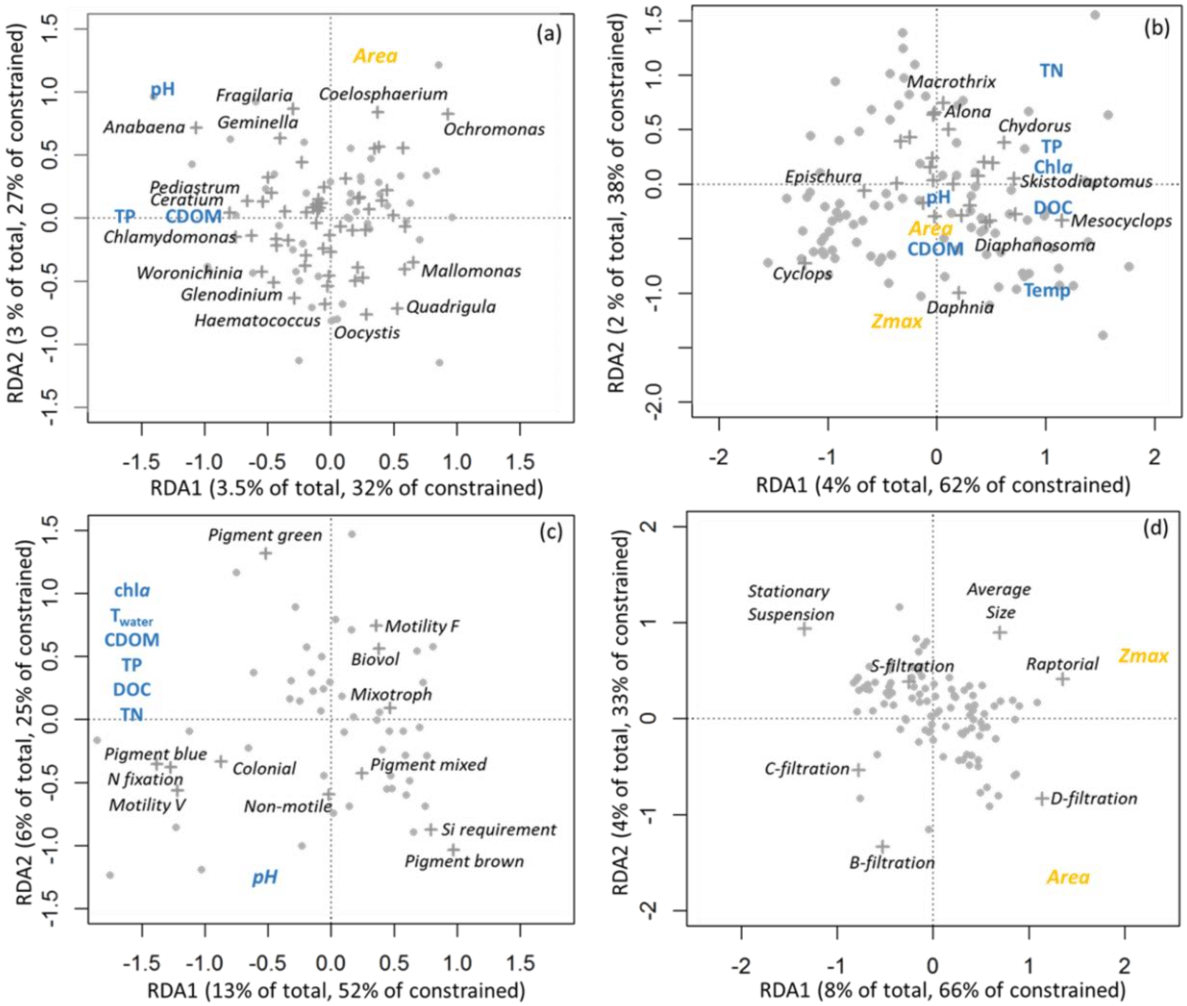

Figure 6: RDA ordination triplots of the (a, c) phytoplankton and (b,d) zooplankton composition classified by (a,b) taxonomy and (c,d) functional (c, d) traits. The RDA was constrained by environmental variables related to water quality (in blue) and by

variables related to lake morphometry (in yellow). Taxa and functional trait response variables are represented by crosses and lakes by dots.s. In the taxonomic RDAs (a, b), only taxa with loadings over 0.20 are displayed. Abbreviations are as follows total phosphorus (TP); total nitrogen (TN); dissolved organic carbon (DOC); chlorophyll a (*Chl-a*); coloured dissolved organic matter (CDOM); water temperature (T_{water}); lake maximum depth (Zmax); lake area (Area).