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Limited functional responses of plankton food webs in northern lakes following diamond mining

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Abstract

There is a very limited understanding of the impact of diamond mining on the aquatic communities of lakes that receive mine activity residue. For aquatic communities, assessing the response to mining, which can affect multiple variables concurrently, is not trivial, especially when a species-by-species approach is used. Thus, we assessed diamond mine impacts using changes in plankton functional composition enabling an integrated evaluation of the ecological responses of aquatic ecosystems. Using a 19-year dataset, we evaluated the functional responses of lake phytoplankton and zooplankton to water quality changes associated with mining activities in five downstream lakes of the Ekati diamond mine (NWT, Canada). Our results demonstrate that diamond mine activity shifted phytoplankton functional composition toward edible diatoms, and more recently the rotifer communities toward parallelepiped types, while for crustacean zooplankton we observed a reinforcement of the original functional composition. Following functional, rather than taxonomic changes enabled a more mechanistic understanding of the processes behind the impacts and should facilitate the generalization of impacts to other sites, permitting comparison of community shifts with other mining types.

Keywords: phytoplankton; zooplankton; functional ecology; nitrate enrichment; mining; northern lakes; water quality; Northwest Territories, Canada; rotifer; crustacean

18 Introduction

19 Mining activities in northern Canada have steadily increased over recent decades, and are
20 expected to further grow by 91% between 2011 and 2020 (The Conference Board of Canada
21 2013). Diamond mining has played an important role in recent expansion, with Canada
22 becoming one of the main diamond producers in the world. Expansion is expected to continue as
23 several new mining projects (e.g. Renard Project in Quebec, Jay Pipe at Ekati in the NWT) start
24 operations within the next few years. As many different compounds are exported from a
25 diamond mine to neighboring lakes (ERM 2014), understanding the effects of such mining
26 activities on water quality and consequently on surrounding ecosystems is crucial to minimize
27 their impact, as well as to comply with federal, provincial and territorial laws on environmental
28 protection.

29 In lakes, plankton are trophically important organisms as they largely control the flux of
30 energy and matter in mostly unproductive northern ecosystems (Kling *et al.* 1992; Gu *et al.*
31 1994, 1996). Consequently, mining effects on plankton should ultimately also have repercussions
32 for the functioning of lake ecosystems, including higher trophic levels (e.g. fish). Moreover, as
33 they have short generation times, plankton communities respond quickly to disturbances and are
34 thus useful indicators to track environmental change, in addition to providing important insights
35 on the general response of aquatic ecosystems (Whitton *et al.* 1991; Carvalho *et al.* 2013;
36 Khalifa *et al.* 2015).

37 The Ekati Diamond Mine is Canada's first diamond mine and has been in operation since
38 1998. This study was conducted in the Koala Watershed, one of the main watersheds influenced
39 by the Ekati Diamond Mine. As a component of the Ekati Diamond Mine Aquatic Effects
40 Monitoring Program (AEMP), phytoplankton and zooplankton communities in Koala Watershed

lakes were sampled annually over 19 years, including two years before mining operations began, along with multiple water quality parameters and physical variables. We used these time-series to study how temporal changes in water quality impacted downstream plankton communities. We used a functional trait perspective for interpreting temporal community changes; specifically, we tracked changes in functional trait composition. Using a functional approach enabled us to establish a more general and mechanistic evaluation of the ecological response of lakes to mining activities with conclusions that could be more easily applied to other locations without reference to taxonomic detail. This approach is also likely to be more amenable to industry or other stakeholders, requiring less taxonomic expertise if easily attributed traits provide the same information as more taxonomically-based approaches; saving time and money but still permitting valid environmental impact assessments. However, the correspondence of taxonomic and functional conclusions remains to be verified and was thus one of the goals of this study.

The main route by which diamond mining activities should affect aquatic organisms is through changes in water quality, via the regulated release of several concentrated compounds from a mine to the environment. Through such releases, increased concentrations of some trace elements (e.g. copper, aluminium) could have toxic effects on living organisms (see AEMP appendix G, ERM 2014). However, it was previously determined that in the Koala Watershed, no water quality parameters exceeded the lowest known chronic effect values for the most sensitive plankton species (AEMP appendix G, ERM 2014). At the same time, the input of macronutrients (e.g. nitrogen, phosphorus) from watersheds is well known to influence the interaction of species within plankton food webs; affecting composition and diversity, with such effects being well established in the ecological literature, especially for phosphorus (Downing & McCauley 1992; Jeppesen *et al.* 2000a,b; Barnett & Beisner 2007; Orihel *et al.* 2012; Monchamp

et al. 2014). In terms of macronutrients, nitrogen (N), mainly in the form of nitrate (NO_3), is one of the main nutrient inputs to lakes of the Koala Watershed as a result of mining activities, and more specifically from blast residue. Nitrogen inputs thus typically occur without addition of phosphorus (P), leading to a change in the N:P ratio in lakes, which in itself should have important effects on plankton communities (Saros *et al.* 2012; Saros & Anderson 2015). For example, the global shift toward a greater propensity for cyanobacteria blooms in lakes has been attributed not only to increases in macronutrient concentrations, but to altered N:P ratios (Paerl & Otten 2013). Changes in phytoplankton composition in response to altered macronutrient stoichiometry should also trigger changes at the primary consumer (i.e. zooplankton) trophic level, and eventually potentially to higher trophic levels (e.g. fish), which respond to changes in size structure, quality or overall quantity of zooplankton.

We examined the impact of almost two decades of mining activity on the northern lake plankton communities downstream from Canada's first diamond mine using 19 years time series across five impacted and two reference lakes. We wanted to determine whether there has been a significant effect of mining activities, via water quality changes, on plankton functional composition, and to identify the drivers of community change.

Materials and methods

Study design and sampling

The Ekati Diamond Mine is located 100 km north of the tree line in the Canadian subarctic (64.71N, 110.62W, Northwest Territories) on a landscape of interconnected wetlands and lakes (Figure 1, Table1). Lakes in this watershed (Koala) are generally clear with low

nutrient concentration and low productivity (Rescan 2012). This study was conducted in five monitored lakes of the Koala Watershed, downstream of the mine's Long Lake Containment Facility (LLCF), which receives mining by-products such as processed kimberlite, as well as all water that has been used in mining activities. The LLCF is considered to be the main influence of the mine on the surrounding aquatic environment, as water meeting regulatory effluent criteria is periodically discharged downstream into Leslie Lake (Rescan 2012). Consequently, we expected to observe a decrease in detectable mine effects through the downstream watershed, as distance from the LLCF increased. To do so, we divided monitored lakes in two categories: lakes that were proximal to the LLCF (Leslie and Moose) and distal lakes (Nema, Slipper and S2). We compared the monitored Koala Watershed lakes to two reference lakes outside of the Koala Watershed to track natural temporal trends in subarctic lakes.

Plankton communities were sampled annually (from 1996 to 2014 in most lakes, including two pre-operational years), in early August, at the deepest point in each lake. Phytoplankton were sampled at 1m water depth with a Teflon-lined General Oceanics FLO bottle (GO-FLO; 5 L) and whole water samples were preserved in Lugol's iodine solution. Zooplankton (crustacean and rotifer) were sampled using vertical hauls of a conical 118 μ m mesh net (0.3m diameter) equipped with a General Oceanics flowmeter (Model 2030R). Zooplankton were preserved in 5% buffered formalin (final concentration). For phytoplankton and zooplankton, three samples were collected and analysed separately, with data then averaged for further analyses. Zooplankton counts were done on a series of 1 ml subsample aliquots that were counted sequentially until the target count of between 200 and 400 crustacean and rotifer zooplankton were identified. Organisms were identified to the most highly resolved taxonomic level possible. For phytoplankton, the volume of sample enumerated was dependent on overall

cell densities. The subsample was mixed and allowed to settle in an Utermohl-type settling chamber for approximately 24 hours and then examined and enumerated at 630× magnification using an inverted Leica microscope. Phytoplankton were identified to the lowest practical taxon using the transect method until at least 300 natural counting units were enumerated. For colonial algae, each colony was counted as one natural unit and cell numbers in each unit were recorded, or in the case of filaments, each filament was counted as one natural unit and the cell numbers of each filament were recorded (for details see ERM 2014).

Environmental variables were measured at the same time as the plankton sampling. Water quality samples were collected at 1m water depth using an acid-cleaned, Teflon-lined General Oceanics FLO bottle. All water quality samples were sent to ALS Global (Yellowknife, NWT) for analyses of water quality variables at the lowest available detection limit (for details see ERM 2014).

Response variables: Functional composition

The overarching objective of this study was to use a functional trait approach (functional composition) to understand the ecological implications of observed changes in the plankton communities of monitored lakes in the Koala Watershed, as well as the reference lakes. The selection of functional traits was thus critical for obtaining ecologically meaningful results. Selected traits for phytoplankton and zooplankton (rotifer and crustacean) were chosen to reflect how organisms interact with their environment (response or effect traits, see Hooper et al. 2005). Thus, we selected traits related to resource acquisition (e.g. pigment type, feeding strategy) and predation avoidance strategies (e.g. motility, relative swimming speed), as these functional traits are those that establish the trophic relationships in food webs, including responses to nutrient enrichment (Table 2). Phytoplankton trait values were obtained from the literature (see Longhi

and Beisner 2010 for details) and included traits related primarily to resource acquisition: (i) capacity for N-fixation, (ii) silica demand, (iii) capacity for mixotrophy, (iv) pigment composition; and to predator avoidance: (v) cell motility and (vi) edibility to zooplankton ($< 35\mu\text{m}$ maximum linear dimension) and (vii) tendency to form colonies. Crustacean zooplankton traits values were also obtained from the literature (see Barnett, Finlay, & Beisner 2007) and included (i) feeding type, (ii) trophic group and (iii) relative swimming speed (RSS, categorical variable from 0 to 6 with 0 being relatively non-motile). Information on functional traits for rotifers is very limited relative to phytoplankton and crustacean zooplankton; the only available for comparison were morphological traits related to predator avoidance (see McCauley 1984): (i) shape, (ii) the presence of a spine and (iii) tendency to form colonies. With these traits, we estimated functional composition as our measure of functional change. Phytoplankton biomass was estimated from biovolumes using cell and colony dimensional length measurements and corresponding geometrical forms (Hillebrand *et al.* 1999). Zooplankton (rotifer and crustacean) biomass was similarly estimated using McCauley (1984) and Culver (1985).

To estimate functional composition (subsequently the “biotic matrix”), the relative presence (proportion) of each functional trait was calculated as the aggregated biomass of all organisms possessing a specific trait divided by the total community biomass, giving the relative biomass of each functional trait in a sample. For this study, organisms were identified at the species level when possible; however, since many functional traits are conserved within families or orders, it would have been possible to do the same functional analyses using a coarser taxonomical resolution.

Potential driving variables

Another objective of this study was to link spatial and temporal changes in plankton functional structure (biotic matrix) to environmental variation, to identify the likely drivers of the observed functional changes. Because plankton may respond to variation in different ecosystem properties, we divided potential drivers into four main categories: morphometric variables (mean depth and lake area), physical variables (mixing depth, secchi depth, water column stability and water column temperature), water quality variables and biotic variables (Table 3). Morphometric variables (Table 1) were not influenced by mining activities and thus represented a pure lake identity effect: testing whether lakes with different morphometries had intrinsically different functional composition. Mean depth was calculated using bathymetric surveys and lake area estimated from areal photos or topographic databases using GIS software. Second, lake physics as represented by thermal stratification structure and water column stability can impact plankton community functional composition given that plankton are drifting organisms. Variation in a lake's physical properties may be related to mining activities or to changes occurring at larger scales (e.g. climate warming). Mixing depth and water column stability were estimated from temperature profiles using the R package: Lake Analyzer (Read *et al.* 2011). Water column temperature was estimated as the mean temperature over the entire water column. The third group of variables encompassed water quality and were likely to be the main effects of mining activities on downstream lakes, via the influence of several chemical variables (see ERM 2014). To characterize water quality, we relied on traditional limnological water quality variables related to important macronutrients (i.e. nitrogen and phosphorus) and pH, turbidity, conductivity, alkalinity, dissolved solids and water hardness as well as potentially toxic compounds (Table S1). Water quality variables were averaged annually, and missing values were replaced by linearly interpolating the preceding and following years. For statistical analyses, we

reduced the number of water quality variables and established the main axes of variation by using the first two principal components (PC1 and PC2) of a Principal Components Analyses (PCA; Figure S1 and Table S1). The main axes of variation in water quality with respect to mining activities were thus represented by an increase in nitrogen (i.e. NO_3 , NO_2 and NH_4), water hardness, conductivity, and pH (PC1) with PC2 being related to phosphorus (TP and orthophosphate), metals and turbidity. Finally, because plankton communities do not respond solely to abiotic variables, we also included potential biotic driving variables to account for interaction between the trophic levels. As done for the environmental variables, we used PC1 and PC2 of a “biotic” PCA to capture the main axes of variation in functional trait composition in the other trophic level (phytoplankton for crustacean zooplankton and rotifer and vice-versa, Tables S2, S3, S4 and Figures S2, S3, S4).

Testing the effect of mining activities on plankton (PERMANOVA)

To test whether the effect of mining activities on plankton functional composition was significant, we used PERMANOVA, the permutational multivariate version of ANOVA, (Anderson 2001). We first tested the hypothesis that there was no significant interaction between lake *Type* (monitored vs reference) and *Year* (Time) on several response variables. If there was a significant *Type*Year* interaction, it would mean that functional differences between monitored and reference lakes changed through time, indicating an effect of mining activities. The response variable in the PERMANOVA model was the functional composition (biotic matrix). Because we expected the effect of mining activities to be greatest in lakes that were closer to the LLCF, following a significant monitored vs. reference difference, we then compared each group of lakes separately (proximal Leslie and Moose or distal Nema, Slipper and S2) to the reference lakes in a second set of PERMANOVAs to detect whether responses to mining activities were different or

delayed between these. P-values were obtained using 999 permutations under a reduced model constrained within lakes. Our unbalanced statistical design (i.e. five monitored and two reference lakes) could affect rejection rates in the PERMANOVA (Anderson & Walsh 2013), especially when all lakes are considered. Depending on which group of lakes had the largest variance, the PERMANOVA could either be too liberal or too conservative (Anderson & Walsh 2013). To compare the variance between reference (2) and monitored (5) lakes we used the *betadisper* function (*vegan R* library, Oksanen *et al.* 2015), and in all cases the variance between monitored lakes was more important than the variance between reference lakes. This indicates that the *p*-values of the PERMANOVA were conservative. In the PERMANOVAs comparing proximal (2) or distal (3) lakes to reference lakes (2), the design was almost balanced, leading to a less biased rejection rate.

Visualizing temporal trends in functional structure (Principal Response Curves)

When the interaction between *Type* and *Year* was significant (i.e., when an effect of mining activities was identified), multivariate techniques were used to visualize how the response was structured through time and to identify the drivers of the response in the biotic matrix. We expected the response to mining activities to be structured temporally, such that differences between lakes in the PERMANOVAs would increase with time. We used Principal Response Curves (PRCs) on the biotic matrix to (i) visualize the community functional response through time and (ii) to identify which functional traits responded the strongest. PRCs were conducted only when the effect of mining activities was statistically significant ($p < 0.05$) in the PERMANOVA. Visualizing temporal trends with multivariate data using standard ordination techniques (e.g. principal component analysis, redundancy analysis) is challenging because in ordination plots, sites will not often be organized in a chronological fashion (van den Brink *et al.*

2009). PRCs, an extension of redundancy analysis (RDA; using the equation: $Response \sim Treatment \times Time + condition(Time)$), is a statistical technique that enables the explicit representation of sites in a chronological order and graphically illustrates the temporal deviation of monitored from reference lakes by constraining the ordination using the interaction between treatment *Type* (monitored versus reference) and *Year*. In the context of the present study, PRC analysis was used to identify if and when the functional composition of monitored lakes began to deviate from those of reference lakes and to determine whether the timing of the response was related to the distance from the mine (by comparing the proximal vs. distal responses). In PRC diagrams, the effect (displayed on the y-axis) is the first principal component of the treatment effect over time (displayed on the x-axis), which represents the difference of the overall functional response of communities in monitored lakes compared to reference lakes (the zero line). Only the first PRC axis was retained in all cases. All PRCs were performed using the *prc* function (*vegan R* library, Oksanen *et al.* 2015).

PRCs also allowed for the identification of which traits in the biotic matrix were responsible for the differences between monitored and reference lakes and consequently those that responded to mining activities by using PRC “species” weights. As with such scores in other ordination techniques, the relative magnitude of the “species” weights represents how strongly a group (a trait in this case) loads onto the PRC axis, and thus represents the strength of the response by a specific trait (in this case). Also, the sign (+/-) associated to the weight represents whether the response was positive or negative relative to the control (reference) condition. Thus, traits with high absolute weights are the traits that mainly explain the functional difference between monitored and reference lakes. PRC analyses were also conducted on the taxonomic

data and compared to the PRC analyses for functional traits to determine whether similar patterns were observed by the two approaches.

Identifying drivers

Finally, to confirm that the observed responses were the result of mining activities, we investigated the drivers of the functional changes in the plankton biotic matrix using redundancy analysis (RDA) and variation partitioning. As potential explanatory variables we used water quality parameters (water quality PC1 and PC2) along with the other potential environmental drivers including the morphometric, physical, and biotic variables. We then partitioned the variance explained by the groups of variables (water quality, physical, morphometric and biotic) to better understand the potential sources of variation in plankton functional composition. However, because no variation was independently explained by physical variables in any variation partitioning, we only used water quality, morphometric and biotic variables (see Figure 3) to simplify interpretation.

Results

Effects of mining activities on lake plankton

The effect of mining activities (*Year*Type* in PERMANOVA) on phytoplankton functional composition was significant for proximal and distal lakes when compared to reference lakes (Table 4; main effects are in Table S5). For rotifers the effect of mining activities on functional composition was significant (Table 4). Finally, for crustacean zooplankton, the effect of mining activities on functional composition was significant both in proximal and distal lakes compared to reference lakes (Table 4).

Responses of plankton communities to mining through time

The phytoplankton functional composition response curves (PRC) showed a clear temporal divergence of monitored lakes from reference lakes (horizontal vs. other lines in Figure 3a). Moreover, the timing of the functional composition response was proportional to the distance from the mine: the closest lake (Leslie) responded first, followed by Moose, and Nema. Slipper responded after 2005, while S2 only started to respond in 2010. Although differences between monitored and reference lakes were significant through the whole sampling period, the divergence in functional composition between these lakes clearly increased with time. Furthermore, following the initial responses in Leslie, Moose, and Nema (lakes proximal to the LLCF), the functional divergence of phytoplankton appears to have stabilized around the same level of difference from reference, despite variation in the downstream distance from the LLCF. Monitored lakes had greater biomass of edible and non-motile phytoplankton, and with high silica demand (Figure 3a), with decreases over time in mixotrophic, flagellated and colonial phytoplankton traits. The temporal pattern displayed by phytoplankton taxonomic composition (Figure S5) was also almost identical to functional composition, with *Cyclotella*, *Cryptomonas* and *Dinobryon* responding the most.

For rotifers, prior to 2009, the first PRC axis of functional composition showed mainly differences between distal lake S2 and the other lakes indicating lake-specific differences (Figure 3b). It was only after 2009 that a clear divergence of proximal lakes from reference and distal lakes occurred (Figure 3b). The functional composition divergence occurred later for rotifers than for phytoplankton, and does not have appear to have stabilized. The observed functional response in proximal lakes (Figure 3b) was a shift from conical species (predominantly of the genus *Kellicotia*) to parallelepiped species (predominantly of the genus *Keratella*, see Figure

S6). In the rotifer PRC, Nema and Slipper diverged from reference lakes in 2012, but in a different direction than Leslie and Moose (Figure 3b and Figure S6). This divergence was not the result of functional compositional shifts in Nema and Slipper but was rather the result of a change in reference lake rotifer composition (i.e. the zero line). In S2 there was important annual variation in the proportion of *Asplanchna*, a very large rotifer, while proximal lakes shifted from being dominated by *Kellicottia*, a species with long protective spines, to *Keratella* a species that has shorter spines.

For crustacean zooplankton, despite a significant effect of *Year*Type* detected in the PERMANOVA analyses, there was no directional temporal pattern in the differences between monitored and reference lakes, and no clear functional shift through time (Figure 3c). Together, these results suggest that there was a small effect of mining activities on crustacean zooplankton composition, but that the response was year-specific. In general, monitored lakes had greater abundances of herbivores, fast swimming (RSS6) stationary suspension feeders, characterized taxonomically by species within the *Calanoida* order, with lower relative dominance of slow swimming taxa and immature stages. Temporal patterns in crustacean zooplankton taxonomic composition were similar to the trends in functional composition, again, with no clear temporal pattern (Figure S7). Also, before mining activities started (in 1996 and 1997) there was a compositional shift in Moose and Nema (see figure 3c and figure S7). This shift was mainly explained by a decrease in the relative abundance of *Holopedium* in Nema and Moose.

Drivers of the functional response

Redundancy analyses were only performed on the functional composition data. For phytoplankton functional composition most of the variation, 47% of total and 89% of constrained variation in the RDA (Figure 4), was explained by the first axis. Functional traits loading

strongly on this axis were the same as those identified in the PRC (Figure 3a). Water quality PC1 (WQ PC1) loaded strongly on RDA axis 1, along with PC1 of crustacean zooplankton (Crust PC1), which also loaded positively on the first axis. On the other hand, lake morphometric variables (Mean Z and log.Lake Area) loaded negatively on the first axis, while physical variables (WC stability) loaded positively. Most of the explained variation in the phytoplankton functional RDA was independently explained by water quality (23%, $p < 0.0001$; Table 5). All the variation explained by morphometry was shared with water quality, biotic variables or both. A highly significant ($p = 0.001$) 6% fraction of the variation was also independently explained by biotic variables.

For rotifer functional composition, the first axis explained 33% and the second 13% of the variation in the RDA (Figure 5). Morphometry (Mean Z and log.Lake Area) and water quality PC1 (WQ PC1) had strong loadings on the first two axes, while crustacean PC2 (Crust PC2) loaded on the first axis and phytoplankton PC1 (Phyto PC1) on the second. The importance of lake area was consistent with the observation that S2, a large lake, was functionally different, dominated by ellipsoid species. Parallelepiped rotifers were associated to changes in water quality variables resulting from mining activities (WC PC1). Variation partitioning demonstrated that lake morphometry independently explained a large and significant (16%, $p < 0.0001$; Table 5) amount of the variation in the RDA. Water quality (17%, $p < 0.0001$) and biotic variables (6%, $p = 0.001$), also significantly independently explained variation in rotifer functional composition.

The first axis of the crustacean zooplankton functional composition RDA explained 23% of the total variation and the second 9% of the constrained variation (Figure 6). On the first axis, water quality PC1 (WQ PC1), morphometric (mean Z), physical (Secchi Z and Thermocline Z), and biotic variables (Phyto PC1 and Phyto PC2) had strong loadings. As observed for

phytoplankton and rotifers, the functional traits that loaded strongly on the first axis were the same that responded in the PRC (i.e. Stat. susp., RSS 6, RSS 0 and Immature). Along the weaker second axis, rotifers and crustacean traits of carnivory and raptorial feeding loaded. One important difference in the variation partitioning (Table 5), was that water quality variables independently explained only 2% of the variation ($p=0.01$), while biotic and morphometric variables independently explained 8% and 10% of the variation ($p<0.0001$ for both). The variation independently explained by water quality was low, but could be more important because of the shared variation with morphometry and biotic variables.

Discussion

The aggregate effects of diamond mining activities on the functional composition of the plankton communities in the focal northern Canadian watershed were significant across community types. The response to mining activity of phytoplankton and rotifer communities had a strong temporal structure and was sequential: functional changes occurred first in proximal lakes, followed by distal lakes. The functional composition response in phytoplankton was strongly linked to water quality and appeared many years before that of the rotifers, suggesting a bottom-up progression of effects. The long-time-lag between the responses of the different trophic levels, occurring over several years was surprising considering that plankton organisms have very short generation times and can thus respond rapidly to a changing environment (McCormick & Cairns 1994; Stemberger & Lazorchak 1994); it is possible that longer development times combined with shorter growing seasons in more northerly environments would delay the response. A community response in the crustacean zooplankton was less obvious, and although there was a significant effect on functional composition in the

PERMANOVA, no directional temporal patterns in functional change in the crustacean zooplankton community were observed in the PRCs. The lack of a temporal directional trend suggests that the observed differences between monitored and reference lakes were year-specific rather than related to mining activities.

In the phytoplankton, although they responded strongly, traits related to edibility (edibility, motility, free-living) were probably not the only ones under selection, as the phytoplankton functional composition in monitored lakes shifted toward small centric diatoms, a functional group that is usually favored when unproductive lakes are enriched with nitrogen (see Saros and Anderson 2015 for a review). Although we cannot directly attribute the response of phytoplankton to N-enrichment, it is the most plausible explanation according to recent studies that have shown a strong response of small centric diatoms to this factor (Winder and Hunter 2008, Saros et al. 2012, 2014). On the other hand, the effect of lake thermal structure was not significant, although it has also been identified as a driver of small centric diatom abundance in northern lakes (see Rühland et al. 2015 for a review). The strong response we observed in traits related to phytoplankton edibility should favor a bottom-up trophic response of zooplankton.

Rotifer communities also shifted toward smaller species (mainly *Keratella*), and these shifts were related to nutrient (mainly N) enrichment. In the Great Lakes, a similar shift from *Kellicottia* dominance under oligotrophic conditions, to *Keratella* under eutrophic conditions has also been observed (Barbiero & Warren 2011). The response of rotifer functional composition to changes in phytoplankton and crustacean zooplankton functional composition also validates the presence of a trophic response. However, the restricted amount of functional knowledge on rotifer species in the literature limits the ecological interpretations we can draw for this group.

381 Given the shift toward more edible and accessible (non-colonial, non-motile)
382 phytoplankton biomass, we would expect highly competitive crustacean zooplankton (i.e. large,
383 filter-feeding cladocerans) to dominate (Lampert & Schober 1980). Furthermore, diatoms are of
384 high nutritional quality (Brett & Müller-Navarra 1997), especially for herbivorous filter-feeders
385 (Richman & Dodson 1983). However, we did not observe a concomitant shift toward
386 herbivorous filter-feeding cladocerans, but instead an increased dominance by the functional
387 traits reflecting the calanoid copepods; the group that had already dominated prior to mining
388 onset. One potential explanation for the absence of a shift toward filter-feeders could be that
389 while food quantity and quality increased, phytoplankton concentrations (average of 742
390 cells/ml, Figure 7) were almost always under or close to the lowest concentration limit for
391 cladocerans established at 1000 cells/ml (see Lampert and Schober 1980), except in Nema where
392 the average was 2732 cell/ml (Figure 7). Consequently, calanoids would have maintained a
393 competitive edge with their lower food concentration limit (see McNaught 1975). Because
394 cyclopoid copepods are one of the main predators of planktonic rotifers (Williamson 1983), we
395 also expected a response of crustacean zooplankton to functional changes in the rotifer
396 community, especially if a shift in potential edibility was observed. Although, both *Kellicottia*
397 and *Keratella* are common prey for many copepod species (Williamson 1983), and the fact that
398 we observe a negative relation between rotifers and raptorial and carnivorous zooplankton in the
399 crustacean zooplankton RDA (Figure 6 Axis 2), the functional shift in rotifers with mining, did
400 not affect overall community edibility in a way that influenced the primary crustacean
401 zooplankton response (Figure 6 Axis 1). In this RDA and in the variation partitioning, changes in
402 water quality resulting from mining (WQ PC1) had small but significant effects on crustacean
403 zooplankton functional composition, indicating only minor effects of mining activities on

crustacean zooplankton. Given that during the implementation of mining activities there were non-directional changes in crustacean zooplankton functional composition, there is little evidence to indicate that crustacean zooplankton community composition in the studied lakes are related to the mine. Instead, the original community composition appears to have been reinforced in lakes impacted by mining activity. Thus, with respect to bottom-up effects propagating through the food web, mining effects on fish are likely to be minor as well. However, a full assessment of the fish communities would also need to be done to determine this more definitively, as there could be direct effects on fish that are beyond the influence of the food web.

By using a functional traits approach, we successfully synthesized shifts that took place at the taxonomic level: taxonomic composition principle response curves were highly similar to the response curves of functional composition for all zooplankton and phytoplankton. Overall, the functional approach offers promise to lake management in that it can reflect taxonomic shifts in plankton communities in response to environmental change.

Changes in water quality as a result of diamond mining activities have caused a functional shift in phytoplankton and rotifer communities in the lakes downstream of the mine. However, there are strong indications that after almost 20 years of activity, the impact of mining has been restricted to the base of the food web and that the functional structure of plankton communities is stabilizing over time. The weaker directional shift in crustacean zooplankton suggests minimal bottom-up effects are also likely for the fish communities in these lakes. For the mining industry, monitoring functional shifts in plankton traits composition at best, will enable an excellent and potentially broadened understanding of the impacts of mining activities on aquatic communities. Classifying individuals more broadly into coarse taxonomic groups (i.e. genus or family rather than species) is usually sufficient to allow the implementation of a

functional approach. Thus, this type of monitoring involves overall a smaller allocation of resources, expertise and time to classify taxa into functional groups. Our results show that it is likely to be a productive approach to assessing responses by aquatic communities to industrial perturbation and aid in environmental monitoring of lakes more generally.

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Table 1: Geographic coordinates of the study lakes, and their morphometric and physical characteristics. Physical characteristics were averaged over the 19-year sampling period and the standard error is reported in parentheses.

	Lake	Lat.	Long.	Area (m2)	Z _{mean} (m)	Z _{mixed} (m)	Z _{secchi} (m)	Temp. (oC)	Stability
Proximal	Leslie(Le)	64.68	-110.67	6.2E+05	5.8	9.4(0.7)	6.3(0.5)	13.1(0.5)	69(16)
	Moose(Mo)	64.68	-110.65	4.4E+02	1.5	6.9(0.4)	6(0.4)	12.7(0.4)	61(12)
Distal	Nema(Ne)	64.68	-110.72	7.8E+05	1.9	6.8 (0.5)	4(0.2)	14.1(0.5)	18(6.2)
	Slipper(Si)	64.61	-110.85	1.9E+06	3.2	10.5(0.5)	4.1(0.15)	12.5(0.4)	80(14)
	S2	64.61	-110.84	1.4E+08	11.5	4.5(0.4)	5.5(0.2)	12.2(0.4)	132(102)
Reference	Nanuq(Na)	64.92	-110.28	3.1E+07	28.5	15.9(1.1)	8.5(0.5)	10.7(0.25)	65(8)
	Counts(Co)	64.65	-110.29	1.2E+06	8.9	9.8(0.5)	5.4(0.2)	14(0.4)	23(8)

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Table2: Phytoplankton, rotifer and crustacean zooplankton functional traits and levels

Category	Traits	Values
Crustacean zooplankton	Trophic position	Carnivore
		Omnivore-Carnivore
	Resource acquisition	Omnivore
		Omnivore-Herbivore
Crustacean zooplankton	Feeding type	Herbivore
		Immature
	Feeding type	B(Bosmina)-filtration
		C(Chydorus)-filtration
Crustacean zooplankton	Feeding type	D(Daphnia)-filtration
		S(Sidae)-filtration
	Feeding type	Stationary Suspension
		Raptorial
Predation avoidance	Relative swimming speed (RSS)	0(slow)-6(fast)
Rotifer	Shape	Cone
		Cylinder
	Shape	Ellipsoid
		Parallelepiped
Rotifer	Spines	yes/no
	Colonial	yes/no
Phytoplankton	Nitrogen Fixation	yes/no
	Si requirement	yes/no
	Mixotroph	yes/no
	Resource acquisition	Green
Phytoplankton	Pigments	Blue
		Brown
	Pigments	Mixed
		Mixed
Phytoplankton	Motility	None
		Flagellated
	Predation avoidance	Vacuole
		Vacuole
Phytoplankton	Edible	yes/no
	Colonial	yes/no

Table 3: Variables used in the RDA as response and explanatory variables. “Other communities” refers to the principal components (PC1 and PC2) associated with PCAs done on all taxa excluding the focal community in each case (Tables S2, S3, S4 and Figure S2, S3 and S4). Z refers to depths of: mean lake (Mean Z), mixed layer depth (Z_{mixed}) and secchi depth (Z_{secchi}).

Composition	Morphometry	Physical	Water Quality	Biotic
Response variables	Explanatory variables			
Community functional composition	Mean Z (m)	Z_{mixed} (m)	PC1	PC1 & PC2 Other communities
	Lake Area (m)	Z_{secchi} (m) Stability (RTR) Temperature(°C)	PC2	Total biomass of other communities

Table 4 Simplified PERMANOVA table, the interaction of *Year*Type* test for the significance of mining activities on functional composition. Based on 999 permutations. See Table S-1 for the full PERMANOVA table.

	Overall			Proximal			Distal		
	SS	F	p	SS	F	p	SS	F	p
Phytoplankton	1.1	4.2	0.005	2.2	5.4	0.007	1.5	3.8	0.04
Rotifer	1.2	3.4	0.045	2	16.8	0.001	1	3.8	0.07
Crustacean	2.8	3.5	0.002	3.9	10.7	0.003	2.6	3.8	0.009

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Table 5: Adjusted R^2 values from the variance partitioning on functional composition by plankton group shown as percentages for each fraction, as defined in Figure 2. Only the W, B and M fractions were statistically testable with significance (p-values) corresponding to * $p<.05$, ** $p<0.001$ and *** $p<0.0001$.

	W	B	M	W+M	W+B	B+M	W+B+M
Phytoplankton	0.23***	0.06**	0	0.03	0.5	0.03	0.08
Rotifer	0.17***	0.06**	0.16***	0	0.03	0.04	0
Crustacean zooplankton	0.02*	0.08***	0.1***	0.02	0.03	0.02	0.05

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Figure captions

Figure 1: Map of the lakes showing the LLCF proximal lakes: Leslie (Le) and Moose (Mo), and the distal lakes: Nema (Ne), Slipper (Sl) and station 2 of Lake de Gras (S2) in the Koala Watershed. Nanuq (Na) and Counts (Co) from a nearby watershed were reference lakes.

Figure 2: Venn diagram showing the structure of the variation partitioning analyses. Results for each fraction are shown in Table 5.

Figure 3: Principal Response Curves (PRC) for functional composition of: (a) phytoplankton, (b) rotifers, and (c) crustacean zooplankton in the monitored lakes (Leslie, Moose, Nema, Slipper and S2) vs. reference (Nanuq and Counts; the 0 line) lakes. The red lines indicate the year mining activities began (1998). Weights show the degree to which functional traits responded to mining activities. For clarity, only traits with an absolute weight > 0.5 are shown.

Figure 4: Ordination diagram of the phytoplankton functional composition RDA. Functional traits (response variables) are represented by crosses, and environmental variables (explanatory variables) by arrows. On each axis, constrained (const.) and total explained variation is reported. The unlabelled arrows correspond to the following variables starting in quadrant 1 (closest to 12:00 on a clock) and moving in a clockwise direction: WC temperature, Biomass crustacean, Rotifer PC2, Rotifer PC1, Thermocline Z, Biomass rotifer and Crustacean PC2.

Figure 5: Ordination diagram of the rotifer functional composition RDA. Functional traits (response variables) are represented by crosses and environmental variables (explanatory variables) by arrows. On each axis, constrained (const.) and total explained variation is

reported. The unlabelled arrows correspond to the following variables starting in quadrant 1 (closest to 12:00 on a clock) and moving in a clockwise direction: WQ stability, Secchi depth, Biomass phytoplankton, Crustacean PC1. Phytoplankton biomass, phytoplankton PC2, WC temperature and WQ PC2.

Figure 6: Ordination diagram of the crustacean zooplankton functional composition RDA.

Functional traits (response variables) are represented by crosses, and environmental variables (explanatory variables) are represented by arrows. On each axis, constrained (const.) and total explained variation is reported. The unlabelled arrows correspond to the following variables starting in quadrant 1 (closest to 12:00 on a clock) and moving in a clockwise direction: WQ stability and Biomass phytoplankton.

Figure 7: Boxplot of median total phytoplankton abundance in each lake. The grey horizontal line represents the *Daphnia* lower food concentration limit according to Lampert (1980).

Figure 1

Figure 2

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Figure 3

Figure 4

Figure 5

Figure 6

Figure 7