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RESEARCH

Top-down vs. bottom-up effects on the planktonic microbial food web of a maritime Antarctic lake

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Abstract
This study evaluated the influence of nutrient (bottom-up) and biological (top-down) controls on the planktonic microbial food web of Lake Limnopolar, an oligotrophic lake in maritime Antarctica. Using a factorial experiment, we manipulated nutrient levels (nitrogen and phosphorus) and macrozooplankton abundance (absence/presence, and abundance increased by 30×) to simulate environmental variations. Nutrient additions led to moderate phytoplankton growth in the absence of zooplankton, indicating nutrient limitation. The presence of zooplankton enhanced algal growth, favoured chlorophytes over diatoms, and significantly increased bacterial abundance while reducing particularly ciliates. These results indicate a cascading effect typical of biological control and they highlight the role of biotic interactions, even in extreme environments where abiotic factors are thought to dominate. Understanding biological controls in such extreme environments is essential, as it underscores the complexity of ecosystem functioning in the polar regions, which are increasingly seen as early indicators of global change.

Keywords Trophic cascades · Biotic control · Microbial loop · Picoplankton · Byers peninsula · Antarctica · Protists · *Boeckella poppei*

Introduction

Most of the surface of Antarctica is permanently covered by ice because of the extremely low temperatures. However, the maritime Antarctic region presents a less extreme climatic regime compared to continental Antarctica, allowing ice melting during short periods and the occurrence of ice-free water bodies during these periods. Even in the maritime zone, however, physical control due to climatic restrictions has been considered the main factor controlling community structure and function in its freshwater bodies, with biotic interactions playing a minor role.

Antarctic lakes, including in maritime Antarctica, are microbially dominated ecosystems (Rochera et al. 2017; Picazo et al. 2019) with truncated food webs (Vincent et al. 2008). In these waterbodies, microorganisms, such as bacteria, heterotrophic protists, algae and cyanobacteria, but also sometimes zooplankton, are the main planktonic components (Laybourn-Parry et al. 2001; Toro et al. 2007). These microbe-dominated communities are confronted by continuous low temperatures as well as low energy supply and nutrient limitation (Dore and Priscu 2001; Laybourn-Parry 2001). Phytoplankton in continental Antarctic lakes can be adapted to low irradiance levels, low temperatures, and nutrient limitation (Dore and Priscu 2001; Henshaw and Laybourn-Parry 2002). These factors could also be important in maritime Antarctic lakes, which are generally oligotrophic. However, more productive lakes are located close to the sea where nutrient input is much increased by marine birds and mammals (Rochera et al. 2013a; Picazo et al. 2019), and nutrient enrichments can thus be a determinant of the trophic functioning of plankton communities in

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maritime Antarctic lakes and ponds. In a scenario of climate change, regional warming may promote strong variations in these communities, changing nutrient dynamics and productivity, thus influencing biotic interactions (Camacho et al. 2012, 2022).

The trophic structure of aquatic ecosystems can potentially be controlled not only by resource availability, but also by predation. Accordingly, trophic cascades (sensu Carpenter et al. 1985) have been observed within the microbial food web of oligotrophic waters (e.g., Samuelsson and Andersson 2003), where bacteria and flagellates may strongly increase their abundances under reduced predation. Lake food webs in continental Antarctica have been traditionally thought of as being structured by bottom-up forces (Roberts et al 2004), given the absence or low abundance of crustacean zooplankton (Vincent et al. 2008). However, the substantial presence of copepods and fairy shrimps in some lakes, such as those on the Byers Peninsula in maritime Antarctica (Rochera and Camacho 2019), challenges this notion.

In contrast with continental Antarctic lakes, the presence of metazoan plankton in lakes from the maritime Antarctica led us to speculate about the potential importance of top-down control at certain periods of the year (Rochera et al. 2017). From the point of view of classical food webs, maritime Antarctic lakes have been regarded as two-level systems (Laybourn-Parry and Pearce 2007), composed of primary producers and crustacean grazers. A foundational study by Hansson (1992) compared the response of phytoplankton to phosphorus increase in Swedish lakes, with functionally three trophic levels, with that of Antarctic lakes (functionally two trophic levels); there were differences in response showing that, at low or moderate productivity, food chain composition in addition to nutrients had decisive effects on the biomass development of planktonic algae. Although both nutrients and zooplankton grazing are important to phytoplankton and bacteria in lakes, the relative importance of grazing pressure for these two groups of organisms may vary. With attention to these two groups, Hansson et al (1993) observed that the phytoplankton in Antarctic lakes was strongly affected by grazing, whereas bacterial abundance was apparently not regulated by flagellate grazing. In addition to phytoplankton and bacteria, other microbial components, such as heterotrophic protists (nanoflagellates and ciliates), are also important in terms of abundance and biomass in Antarctic lakes, and deserve much more attention, since interactions among all these components of the planktonic community may play major roles in these ecosystems with simplified food webs (Petz et al. 2007).

The role of heterotrophic protists as possible controllers of bacterial abundance via grazing has long been known (Hahn and Höfle 2001). In temperate waters, it has been shown how indirect effect of zooplankton grazing on protists can favour bacteria by reducing grazing pressure (Zöllner et al. 2003). Cascading predation effects of macrozooplankton can potentially be transmitted by phagotrophic protists to the bacterial community (Hahn and Höfle 2001). The broad community impacts of trophic cascades are particularly evident in aquatic systems. For instance, an extensive analysis by Gasol et al (2002) on published data concluded that in most nutrient-poor aquatic environments bacterial abundance was regulated by predators. Additionally, analyses of bacterial growth and losses in continental Antarctic lakes have shown that bacteria, through grazing, may be an important source of carbon to higher trophic levels (Camacho 2006; Villaescusa et al. 2016; Rochera et al. 2017). Yet most studies to date on the relative importance of resources versus predation control have focused on planktonic communities in temperate aquatic systems or have been conducted in polar marine waters. In both of these environments, the relative complexity of food webs makes it difficult to distinguish the role of each factor when mimicking natural conditions, since many components of the original food web are usually excluded in manipulation experiments.

Global circulation models predict that the Arctic and Antarctic regions will experience accelerated climate change over the course of this century, and these effects are already being registered in the instrumental record (e.g., Bégin et al. 2021; González-Herrero et al. 2022). Thus, the knowledge of the structure and functioning of marine and freshwater ecosystems in polar regions will enhance their sentinel roles as early indicators of global change (Camacho et al. 2012, Castendyk et al. 2016). Relatively simple microbial communities, such as those studied here, can be used as indicators of microbial processes and responses to environmental change (Camacho 2006; Quesada et al. 2009). In a context of climate change in which alterations can greatly influence the distribution and availability of liquid water, which is essential for life and a vehicle for exchanging substances among ecosystems, variations in the temperature and rainfall patterns have a strong potential to alter ecological functioning and biological diversity in Antarctic lakes.

Our overall goal in the present study was to contribute towards understanding the indirect impacts of climate change on polar ecosystems, specifically the effects of a likely increase in nutrient supply and greater ecological interactions (Convey and Peck 2019). We aimed to simulate these effects for a maritime Antarctic lake, Lake Limnopolar, and to assess the relative importance of resource availability compared to predation in regulating the abundance of the different components of the lake's planktonic community. In this oligotrophic, cold-water environment, restrictions by abiotic variables such as nutrients, temperature and light availability might traditionally be expected to outweigh biotic interactions. We tested a set of these bottom-up versus

top-down effects by way of a factorial microcosm experiment, manipulating nutrient levels and macrozooplankton concentrations.

Material and methods

Study site

Lake Limnopolar is a small lake (22,172 m^2) located on Byers Peninsula, a 60.6 km^2 area that has landscapes that become free of ice and snow cover during the Antarctic summer. The region is a peninsula of Livingston Island, South Shetland Islands, Antarctica that extends from latitudes 62°34′35″ to 62°40′35″ S and from longitudes 60°54′14″ to 61°13′07″ W. Byers Peninsula is an Antarctic Site of Special Scientific Interest and has been designated as an Antarctic Special Protected Area (ASPA number 126, extending over a total area of 90.56 km^2), where all activities are highly restricted by international regulations to preserve its unique, pristine environments. This precludes conducting ecosystem-level manipulations, but allows for small-scale, non-impacting microcosm experiments, as performed here. The present study was conducted in the summer of 2002, with the experiment initiated on XX January. Entry to the ASPA was under the terms of a CPE permit to the project REN2000-0435 for participation in the Spanish Antarctic Campaign (Campaña Antártica Española) with reference CPE-EIA-2000-2.

Lake ice melting usually occurs on Byers Peninsula in late December or January, and the lake remains unfrozen through several weeks of the Antarctic summer. The lake is located c.a. 90 m a.s.l. in the central plateau of Byers Peninsula and has surface inflow and outflow at opposite ends of the lake. It is a cold monomictic waterbody, with a maximum depth of 5.5 m, and water temperatures from 2 to 6 °C in summer (Toro et al. 2007). The lake bottom is partially covered by the moss *Drepanocladus longifolius*; benthic moss communities have similarly been described in lakes elsewhere in Antarctica (Saługa et al. 2018). Part of the lake watershed is covered by microbial mats dominated by cyanobacteria, a common feature of the polar regions (Vincent et al. 2008), with high rates of primary production (Fernández-Valiente et al. 2007; Rochera et al. 2013b; Camacho et al. 2022).

Chemical analyses

Water samples were filtered through GF/F glass fibre filters and analyzed for inorganic nutrients (dissolved forms of nitrogen, phosphorus and silica) following standard analytical methods (APHA-AWWA-WPCF 2005). Ammonia was analysed by the phenol-hypochlorite method. Nitrate plus nitrite was determined after reduction of nitrate to nitrite and subsequent determination of nitrite with sulphanylamide and NNED. Soluble reactive phosphorus was measured by the phosphomolybdic acid-ascorbic method and soluble reactive silica by the vanodosilicomolybdic acid method. Dissolved organic carbon (DOC) concentrations in the filtered samples were determined by high temperature combustion using a Shimadzu TOC-5000 analyser. Potassium hydrogen phthalate ($C_8H_5KO_4$) was used as a standard. Further details on methods are given in Toro et al. (2007).

Biological and biochemical determinations

Heterotrophic picoplankton (HPP) abundance was determined after filtration through 0.2 µm black membrane filters and staining with DAPI, with the filters then examined under a Zeiss III fluorescence microscope at 1250×. The abundance of autotrophic picoplankton (APP) was determined by autofluorescence on the same type of filters and epifluorescence microscopy as described by MacIsaac and Stockner (1993), with separation of picocyanobacteria (PC) and picoeukaryotes (PE) based on their fluorescence characteristics. Nanoflagellates (NF) and ciliated protozoa were counted in a fluorescence microscope after filtration and staining with DAPI (Sherr et al. 1993). Phytoplankton were counted following the Utermöhl sedimentation method on formaldehyde fixed samples, using an inverted microscope at 400–1000×. Macrozooplankton were concentrated by filtration through a 150 µm size mesh and counted under an Olympus microscope at 200×.

Photosynthetic pigments were determined by high performance liquid chromatography (HPLC). Samples were filtered through glass fibre filters (Whatman GF/F) and extracted with 90% acetone for 24 h at – 20 ºC. The solvent was evaporated and then pigments were dissolved in a smaller volume of acetone to concentrate. A Waters 2690 liquid chromatograph equipped with a photo-diode array detector (Waters 996) was used to separate and identify individual compounds. Separation was performed on a Spherisorb S5 ODS2 chromatography column as described by Rochera et al. (2010). Peaks were identified according to their absorption spectra and concentrations were determined using standards prepared with commercially available purified pigments (DHI, Denmark). Chlorophyll-a (Chl-*a*) concentration was used to quantify the total biomass concentration of phytoplankton, and fucoxanthin and lutein were determined as taxa-specific carotenoids to quantify the relative abundances of diatoms and chlorophytes respectively.

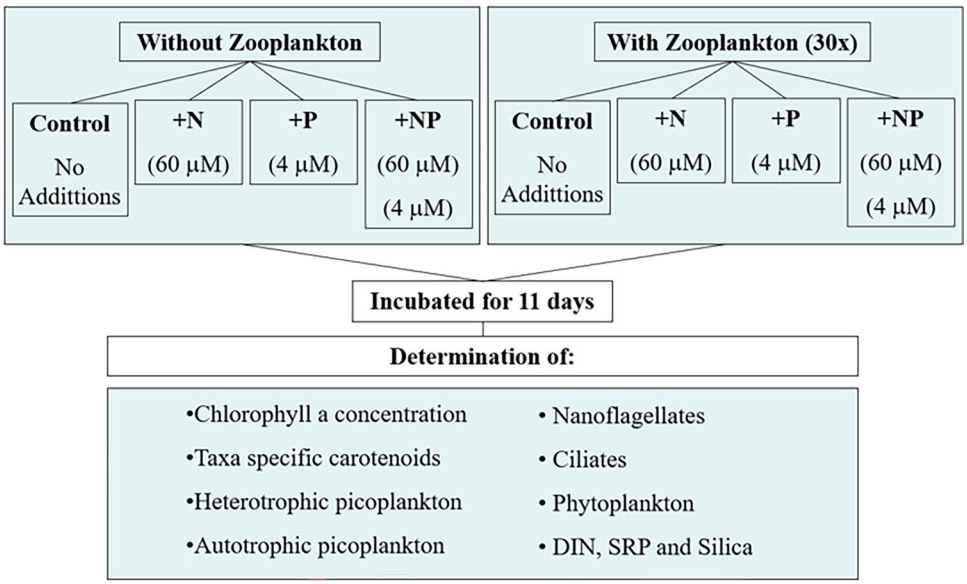

Fig. 1 Experimental design to evaluate the effect of nutrient additions in the absence and presence (30 × ambient levels) of zooplankton. Control treatments with no nutrient additions were included. Samples were incubated for 11 days, and then biological and chemical variables measured, including chlorophyll *a* concentration, taxa-specific carotenoids, heterotrophic and autotrophic picoplankton, nanoflagellates, ciliates, phytoplankton, and the concentration of dissolved nutrients

Experimental setting

Water for the incubation experiments was taken at 0.5 m depth in Lake Limnopolar in January, in the middle of the Antarctic summer. Five-liter clear plastic bottles were used for the incubations as microcosms. The experiment was based on a factorial design in which nutrient additions were combined with manipulations of zooplankton abundance (Fig. 1). Four nutrient conditions were assayed as follows: (C) control lake water without nutrient additions; (+N) lake water supplied with NH_4NO_3 at a final concentration of 60 µM N; (+P) lake water supplied with NaH_2PO_4 at a final concentration of 4 µM P; and (+NP) lake water supplied with NH_4NO_3 final concentration 60 µM N and Na_2HPO_4 final concentration 4 µM P. For each of these nutrient conditions two different manipulations of zooplankton abundance were performed: (F) lake water filtered through a 150 µm size mesh to remove all macrozooplankton (crustaceans); and (Z) macrozooplankton abundance increased 30 times to simulate the high zooplankton abundances detected in early summer, by concentrating the zooplankton with a 150 µm size mesh and subsequent resuspension. Consequently, 8 different treatments were assayed, with three replicates for each treatment. The microcosms were incubated in a small pond with flowing water for 10 days, to maintain natural conditions of temperature (approximate range from 2 to 8 °C) and irradiance. Silica enrichment was not considered since the concentrations of soluble reactive silica (SRSi) in the lake were much higher relative to both N and P (Toro et al. 2007).

At the end of the incubations, each microcosm was subsampled and filtered to determine the concentration of Chl-*a*, fucoxanthin, lutein, ammonia, nitrate + nitrite, orthophosphate, and silicate, as well as the abundance of heterotrophic bacteria, picocyanobacteria, picoeukaryotes, nanoflagellates and ciliates.

Statistical analyses were performed with the SPSS v11.0 statistical software. ANOVA tests were used to determine the significance of the differences among mean values of the different variables. Post-hoc analyses (Scheffe) were then performed to determine the differences among pairs of treatments. Significant differences were tested at 95% confidence levels ($p < 0.05$).

Size-preferences in ingestion of particles of different sizes by the copepod *Boeckella poppei* were measured by incubating copepods in lake water containing a solution of spherical latex beads (Bang-Labs Inc.) of 4 different diameters: 1, 5, 10 and 20 µm, which represented the main size classes of planktonic microorganisms in Lake Limnopolar. The incubation time (20 min) was determined based on preliminary time-course experiments.

Table 1 Soluble inorganic nutrients (N, P and Si), chlorophyll *a* concentration, bacterial abundance and dissolved organic carbon (DOC) concentrations during the Antarctic summer of 2001–2002 in Lake Limnopolar, and concentrations found at the beginning of the bioassay ($t = 0$). Mean and SD values are for 4 samples during summer

Variable	Mean ± SD	Concentration at $t = 0$
Ammonium (µM)	3.7 ± 2.7	1.2
Nitrate + nitrite (µM)	0.23 ± 0.11	0.33
Soluble reactive phosphorus (µM)	0.06 ± 0.03	0.04
Soluble reactive silica (µM)	49.6 ± 17.5	55.3
Chl-*a* (µg/l)	1.09 ± 0.76	0.25
Bacterioplankton (10^6 cells/ml)	1.76 ± 0.54	1.66
DOC (mg/l)	-	1.0

Results

Initial conditions

Table 1 shows the concentrations of inorganic (N, P, and Si) and organic (DOC) nutrients averaged for the studied Antarctic summer in Lake Limnopolar, as well as the measured values at the beginning of the bioassay experiment. Both N and P were found at low concentrations corresponding to ultra-oligotrophic to oligotrophic conditions: ammonium was the main form of inorganic nitrogen, soluble phosphorus was scarce, and Si was relatively abundant. Dissolved organic carbon concentrations were low and varied during this period around 1 mg C l^{-1}. Bacterial abundance was high, usually between 1.5 and 2.5×10^6 cells ml^{-1}.

During summer, Chl-*a* averaged 1.09 µg l^{-1} (SD 0.76). At the time of sampling Chl-*a* concentration in the samples of Lake Limnopolar used for the bioassay was lower (0.25 µg l^{-1}). The most abundant phototrophic cells were solitary picocyanobacteria (around 10^3 cells per ml), however the biomass dominants in the overall phytoplankton community were pennate diatoms and chlorophytes, and to a lesser extent, green nanoflagellates (mean diameter 6 µm). Among diatoms, those of the genera *Fragilaria* (20 µm average length), *Achnanthes* (7 µm), *Diploneis* (15 µm), *Gomphonema* (7 µm), *Nitzschia* (35 µm) and *Stauroneis* (12 µm) were the most abundant. The larger chlorophyte dominants were *Ankistrodesmus antarcticus* (15 µm average length) and desmids (*Cosmarium* spp., 80 µm average length).

The copepod *Boeckella poppei* was the only macrozooplankton species at the time of the bioassay (228 to 851 individuals m^{-3}), whose population was mainly composed of adults (89%), with juvenile stages less common (9% copepodites and 2% nauplii). The ciliate, *Balanion planctonicum*, of about 13 µm diameter (Petz 2007) was the only planktonic species of ciliated protist found in Lake Limnopolar in abundance. The anostracan *Branchinecta gainii* has also been found in this lake and others on the Byers Peninsula but was largely absent at the time of the bioassay.

Incubation responses

Both the nutrient enrichments and the manipulation of zooplankton abundance resulted in strong differences among the different treatments. Total depletion of soluble inorganic nutrients (N and P) occurred during incubation when nutrients were not added, both in the controls, where soluble N and P decreased to undetectable levels at the end of the bioassay, as well as in the treatments where a single nutrient was added and the other was completely exhausted during the incubation. The same pattern was found regardless of the presence or absence of zooplankton. This shows that the single addition of a nutrient in excess resulted in the induction of limitation by the other. However, in the N + P treatments, where both nutrients were added, final dissolved phosphorus concentrations were quite similar either with or without zooplankton, whereas the treatment with zooplankton had a 37% higher concentration of dissolved ammonia.

When zooplankton were absent, there was no effect of nutrients added individually, on total phytoplankton abundance (Chl-*a* concentration), although the combined addition of nitrogen and phosphorus (N + P) yielded significantly higher ($F = 19.70$; $p < 0.001$) Chl-*a* concentrations (Fig. 2a) compared to the other treatments. In contrast, when zooplankton was present at high abundances (30x) there was no significant difference in Chl-*a* concentration among the different conditions of nutrient addition; however, Chl-*a* was significantly higher ($F = 4.63$; $p = 0.016$) than in the treatments in which zooplankton was removed, except when both N and P were jointly added.

Concerning changes in the dominance of the main algal groups, the effect of zooplankton was stronger than that of the direct nutrient addition. As determined from ratios of the taxa-specific carotenoids, the presence of zooplankton at high abundance favoured the dominance of chlorophytes over diatoms as shown by the relatively higher lutein concentration in contrast to the lower fucoxanthin concentration (Fig. 2b and c). In the opposite treatment, zooplankton removal yielded higher relative abundances of diatoms and lower of chlorophytes as deduced from these pigment concentrations, but these differences were not significant for all nutrient treatments since the variability among replicates was high. Nevertheless, significant differences ($F = 27.33$; $p < 0.001$) emerged when the combined lutein to fucoxanthin ratio was considered (Fig. 2d), with a much increased dominance of chlorophytes (lutein) with respect to diatoms (fucoxanthin) associated with high zooplankton abundance, especially in the phosphorus amended treatments (both P and N + P). The abundance of picocyanobacteria was also favoured by the presence of zooplankton (Fig. 3a), showing statistically significant differences compared to the zooplankton lacking treatments ($F = 10.42$; $p = 0.005$), except for those microcosms jointly amended with both nutrients (N + P).

With respect to the effect of nutrient additions on chlorophytes and diatoms, some differences were apparent in the taxa-specific carotenoid concentrations (Fig. 2b, c), but these were not statistically significant. However, when the ratios between the taxa specific carotenoids were considered (lutein to fucoxanthin) those treatments including phosphorus additions (P and N + P) had significantly higher values ($F = 3.61$; $p = 0.037$) but only when zooplankton was present (Fig. 2d). Additionally, picocyanobacteria only showed statistically significant increases when both N and P were

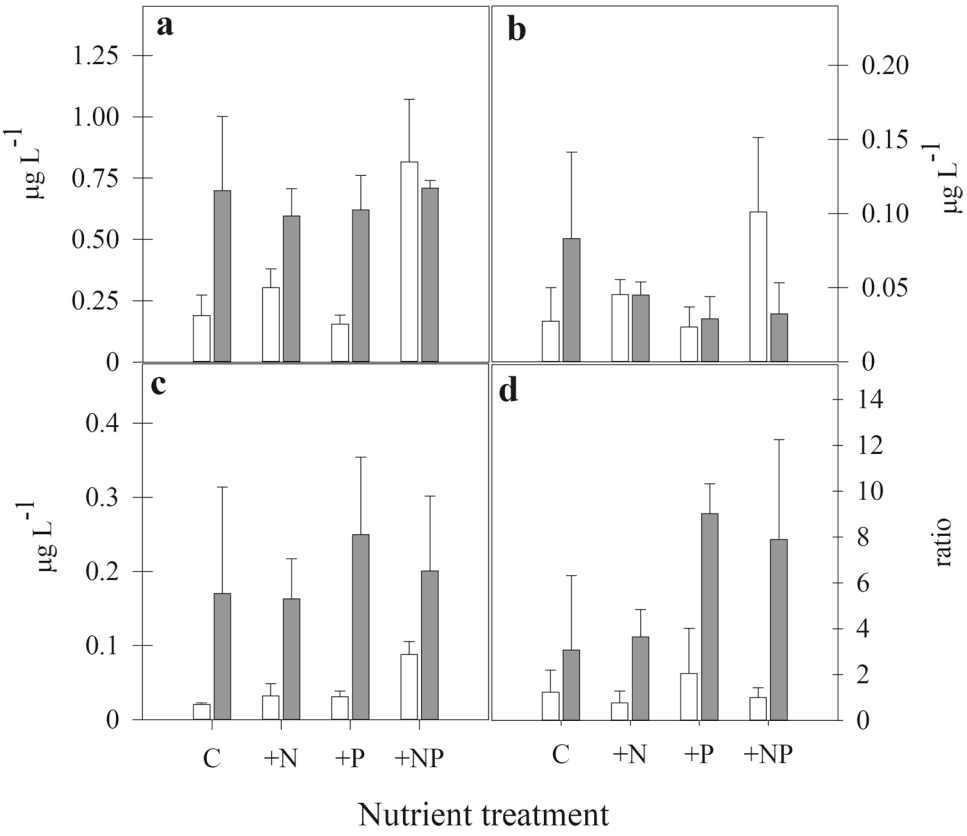

Fig. 2 Average pigment concentrations in the different nutrient treatments without (white) and with (grey) zooplankton. **a** Chlorophyll-*a*, **b** Fucoxanthin (taxon-specific for diatoms), **c** Lutein (taxon-specific for chlorophytes), **d** Phytoplankton community structure expressed as the ratio of lutein to fucoxanthin. Error bars indicate SD for triplicates

jointly added in the absence of zooplankton, but when zooplankton were present, any P additions, alone or combined with N, favoured picocyanobacterial growth with respect to the control and to the nitrogen amended treatments (Fig. 3a). Picoeukaryotes showed a similar pattern, with remarkable increases with zooplankton removal combined with any P enrichment (Fig. 3b).

Increased zooplankton abundance or removal also determined strong changes in the abundance of organisms directly involved in the microbial loop (bacterioplankton and heterotrophic protists). All treatments in which zooplankton was present showed significantly higher ($F = 29.95$; $p < 0.001$) bacterial abundance (Fig. 3c), except for + NP treatment. They also showed much lower abundance of ciliates (Fig. 3d), which almost disappeared in most microcosms with increased zooplankton abundance. Additionally, all treatments amended with phosphorus (P and N + P), either with or without zooplankton, showed significantly higher bacterial abundance ($F = 24.59$; $p < 0.001$) compared to those supplied only with N or to the controls without additions (Fig. 3c), whereas no significant differences were found in ciliate abundance in response to the nutrient additions (Fig. 3d).

In both the control and treatments supplemented with nitrogen (+ N and + NP), the densities of heterotrophic nanoflagellates were slightly higher (Fig. 3e), though not significantly, when the zooplankton were removed. In contrast, these increases were significantly higher ($F = 5.61$; $p < 0.008$) when only P was added. Conversely, despite the observed increases with full nutrient availability, no significant differences were observed in the abundances of phototrophic nanoflagellates (Fig. 3f), regardless of the presence or absence of zooplankton.

Experiments were also performed to determine the efficiency of the copepod *Boeckella poppei*, the dominant zooplankter in the lake, to ingest particles of different sizes. In these experiments four particle sizes were used simulating the main components of the microbial plankton. Among these, 10 µm diameter beads, corresponding to the size of ciliates and most diatoms, were by far the most ingested size (66%) by the adults, followed by the 20 µm diameter particles, whereas quite a few 5 µm diameter beads (the size of nanoflagellates) and almost none of 1 µm particles (representing picoplankton) were ingested by adult copepods. Copepodites ingested the 5 µm size particles more efficiently than the adults, although these juvenile stages were much scarcer in the zooplankton population at the time of the experiments.

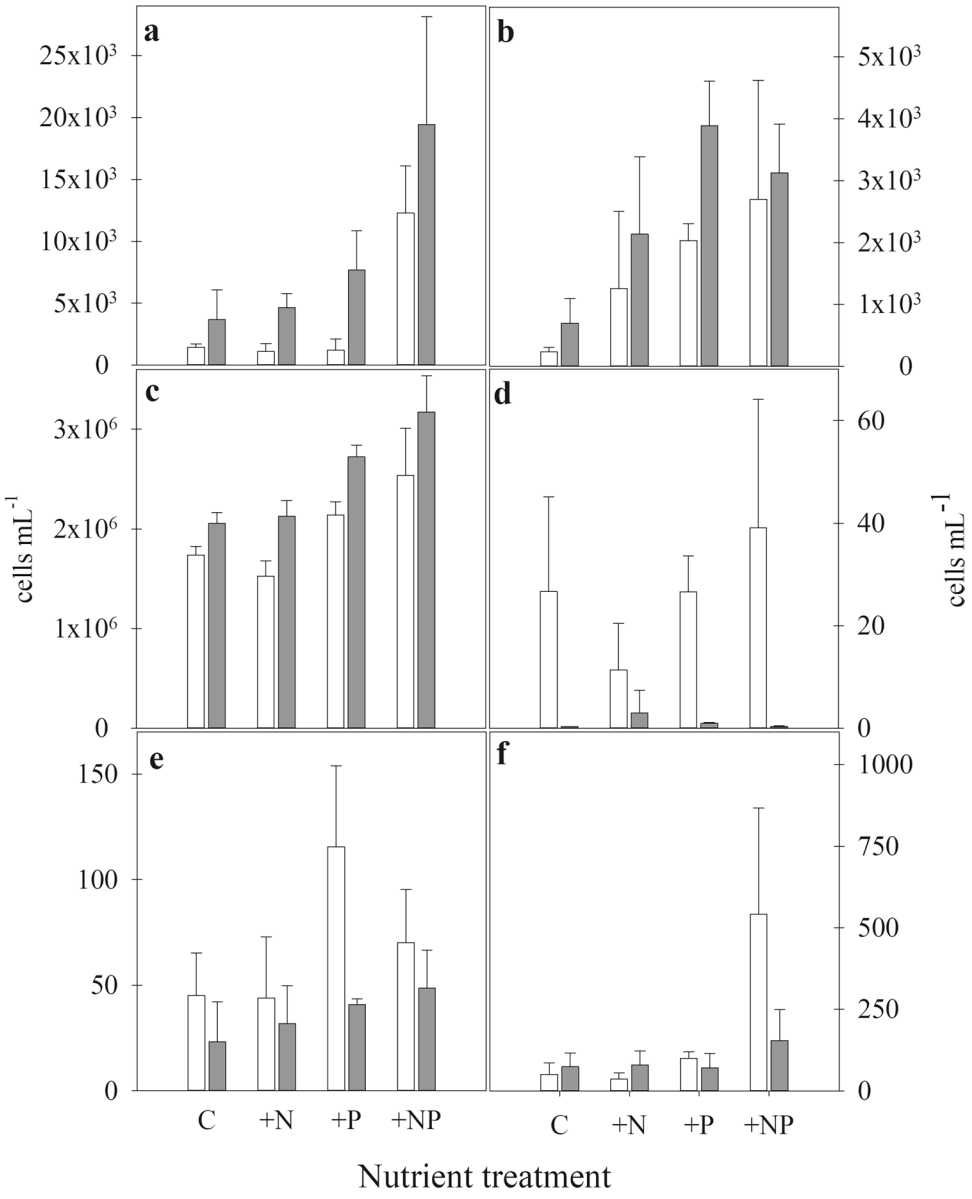

Fig. 3 Average abundances of microbial loop components measured in the incubation treatments without (white) and with (grey) zooplankton. **a** Picocyanobacteria, **b** Autotrophic picoeukaryotes, **c** Heterotrophic bacteria, **d** Ciliates, **e** Heterotrophic nanoflagellates, and **f** Phototrophic nanoflagellates. Error bars indicate SD for triplicates. Note the differences in scales for each variable

Discussion

The role of nitrogen and phosphorus as limiting nutrients for primary productivity in aquatic ecosystems has been widely discussed in the limnological literature. In many lakes of the northern hemisphere that have been subject to strong anthropogenic impacts and high nitrogen loads, the phytoplankton communities are phosphorus deficient during summer months (Vandergucht et al. 2013). Studies on nutrient deficiency in perennially ice-covered lakes from Antarctic Dry Valleys also found phosphorus limitation of phytoplankton growth, with the extent of P-limitation likely dependent on internal cycling (Dore and Priscu 2001). However, several studies performed in lakes with minimal human influence, especially those far from aerial nitrogen supply via atmospheric deposition, have found nitrogen to be a limiting nutrient for planktonic primary production. This includes lakes from certain locations of the northern hemisphere (Camacho et al. 2003), and N-limitation has been previously surmised for lakes on the Byers Peninsula (Picazo et al., 2019).

Our results showed that increased nitrogen availability favoured algal growth in the Lake Limnopolar communities when zooplankton were absent. However, this growth response was moderate, and likely due to the low temperatures that maintained growth rates at low levels, as well as to the induction of a secondary nutrient limitation (P) once the nutrient in excess (N) was consumed. This latter effect often found in lakes, where colimitation by both N and P occurs and can change through time (Maberly et al. 2020). Our

results imply that nitrogen is more limiting than phosphorus for phytoplankton growth in Lake Limnopolar during the Antarctic summer, in accordance with the low atmospheric and direct sources of nitrogen in this region of the planet than in more anthropized areas (Shi et al. 2018). The relatively high bacterial abundance compared to other Antarctic lakes (e.g. Laybourn-Parry et al 2001), with concomitant production of phosphatases that may increase phosphorus recycling, may also help to avoid phosphorus limitation by allowing rapid resupply and turnover rates of the low orthophosphate concentrations found in lake water. As seen from the chemical analyses, silica is unlikely to be a limiting nutrient for diatom growth, and its relatively high concentrations in the lake water reflected its abundance in the main rock matrix component of the catchment (Lyons et al. 2013).

Nutrient cycling by top food web levels can substantially influence plankton community structure (Rogers et al. 2020), and supply of inorganic nutrients via excretion by zooplankters can maintain algal growth during short periods (Vanni 2002). In the conditions of our assays, algal growth appeared to be released from nitrogen limitation in the presence of abundant zooplankton; nitrogen excretion by these animals likely had a short-term fertilization effect, providing high enough N supply to support algal growth under the incubation conditions. This effect is also indicated by our observations of the higher ammonia concentrations when zooplankton were abundant, and by the depletion of ammonia in their absence.

The fertilization effect of the macrozooplankton appeared to compensate for the potential grazing effects on the phytoplankton, since all treatments including zooplankton yielded higher Chl-*a* concentrations compared to those in which zooplankton were removed. Zooplankton fertilization likely also included some phosphorus supply, since no differences in Chl-*a* concentrations were observed among the zooplankton-enriched treatments regardless of nutrient addition, whereas comparable growth in the zooplankton-lacking treatments was only achieved when both N and P were jointly added. The fertilization effect promoted by the high zooplankton abundance also favoured the growth of autotrophic picoplankton in the P-amended treatments (P and N + P). In the absence of zooplankton, increased picocyanobacteria abundance was only found when both nutrients were jointly supplemented, suggesting colimitation. For the heterotrophic bacteria, all treatments that included phosphorus amendments promoted increased cell abundance compared to those supplied only with N or to the controls without additions, indicating P-limitation of bacterial growth. Additionally, a possible supply of DOC excreted by zooplankton (Maas et al. 2020) could also benefit bacteria in the zooplankton amended microcosms. This combination of results shows how higher trophic levels may play an important role for the trophic functioning of the lake, not only as nutrient suppliers, but also as selective grazers favouring certain groups of microorganisms over others.

Biotic interactions, such as predation, have historically been considered of minor relevance in ecosystems subjected to extreme conditions (Krebs 2001). However, it has more recently been argued that biotic interactions may also contribute to the control of ecosystem biodiversity and function in the polar regions (Lee et al. 2019). Antarctic limnetic ecosystems experience severe temperature conditions during most of the year, but this severity is not so strong during the Antarctic summer, when air temperatures may increase over 0ºC and ice melting can occur at northern Antarctic latitudes (Rochera et al. 2010). Moreover, in the maritime Antarctica average temperatures have increased notably in the last decades (Sun and Hansen 2003; González-Herrero et al 2022). As a consequence, ice melting occurs every year in Byers Peninsula, one of the richer zones in limnetic ecosystems in the maritime Antarctica (Rochera et al. 2010; 2013a). Earlier and complete ice melting increases total water flux to the lakes, and consequently nutrient supply via runoff. Additionally, the growing period for planktonic and benthic organisms within the lake and its catchment is prolonged by the increase of length of the ice-free period, allowing increased light availability through the water column of ice-free lakes (Laybourn-Parry 2002; Rochera et al. 2017). This is likely to stimulate primary productivity (Dore and Priscu 2001; Henshaw and Laybourn-Parry 2002), as well as allow improved organic carbon supply and the potential for nitrogen fixation and phosphorus recycling by biotic activity. All these increases may act as feed-back mechanisms: the more nutrients supplied to the ecosystems, the greater the nutrient production and recycling rates are likely to be, especially when abiotic controls are less restrictive in summer.

The catchment area of Lake Limnopolar is greater than for many other lakes on the Byers Peninsula, and partially covered by cyanobacterial mats. Carbon and nitrogen fixation are active processes in these mats during summer (Fernandez-Valiente et al. 2007; Camacho et al. 2022), and are potential sources of DOC and nitrogen to the lake. Additionally, submerged mosses cover part of the lake bottom, and their primary production may also be an important source of organic carbon to the lake (Rochera et al. 2017). In Lake Limnopolar, it has been demonstrated that bacterial production patterns were strongly associated to DOC availability (Villaescusa et al 2013). Although DOC concentrations measured in Lake Limnopolar did not differ substantially from other oligotrophic polar lakes (e.g. Laybourn-Parry et al. 1995; 2001), the unusually high bacterial concentrations suggest that DOC turnover rates may be rapid, with continuous replenishment from phytoplankton photosynthesis, benthic production and allochthonous inputs from the catchment (Villaescusa et al. 2016). All of these DOC supply processes are likely to be favoured by increased

nutrient supply and water temperatures associated with climate warming. Nutrient enrichment effects may explain high biological production rates that lead to the high zooplankton densities sometimes observed in Lake Limnopolar (Camacho 2006), which as simulated in this experiment, may result in cascading food web effects on planktonic community structure and function.

Food chains in temperate limnetic ecosystems are typically composed of several trophic levels and energy transfer commonly follows the autotroph-herbivorous-carnivorous pathway (Krebs 2001). However, in low productivity aquatic ecosystems such as Antarctic lakes, any pathway allowing higher efficiency in energy transfer would permit marked increases in secondary production and a consequent accumulation of biomass in higher trophic levels, such as the copepod *Boeckella poppei* in Lake Limnopolar, that are not controlled by predators. In these cold, nutrient-poor waters, the microbial loop (Azam et al 1983; Li et al. 2014) may increase its relative importance as an energy transfer pathway with respect to the classical herbivore pathway, and any variation in the intensity and type of biotic interactions, such as those likely to result from climate change (Camacho et al. 2012), has the potential to greatly modify ecosystem functioning.

In the experimental manipulation of Lake Limnopolar samples undertaken here, the increase of macrozooplankton abundance or its withdrawal promoted deep changes in the abundance of the different components of the planktonic community. Among algae, additionally to the abovementioned fertilization effect, the presence of zooplankton selectively favoured chlorophytes over diatoms, as indicated by ratios of taxa specific carotenoids. Size and shape may play an important role, since the large size of the desmid (*Cosmarium* spp.) and the specialized shape of the other main chlorophyte (*Ankistrodesmus antarcticus*) likely makes them difficult for zooplankton to readily graze. Our experiments to determine the preferred particle-size ingested by copepods demonstrated that the size of most diatoms is within the range preferentially removed by *B. poppei*, and this selective grazing effect may explain why the presence or absence of copepods would favour one group of algae or the other. Large cell size and the development of structures or shapes that favour grazing avoidance are explanations that have been similarly evoked to explain patterns of algal dominance in other subantarctic lakes with *Boeckella* spp. as dominant zooplankters (Hansson and Tranvik 1996). In particular, these calanoid copepods are known to have much higher grazing impact on algae smaller than 25 µm of greatest axial lineal dimension (Edgar and Green 1994). Similarly, under laboratory controlled conditions, Steiner (2003) crossed nutrient manipulation and zooplankton presence/absence, and found that both predators and nutrients had an interactive effect, with the former favouring a shift to the dominance of large inedible taxa, although small edible algae displayed higher maximal growth rates allowing them to profit from increased nutrient availability.

Concerning the heterotrophic protist community, the size of the only important euplanktonic ciliate detected in the lake, *Balanion planctonicum*, coincides with the preferred particle size ingested by *Boeckella poppei*. In contrast nanoflagellates (both autotrophic and heterotrophic) are much smaller, and this particle size would not be efficiently grazed by the adult copepods that accounted for most of the zooplankters at the time of the bioassay. Although the particle size of nanoflagellates would be more efficiently captured by copepodites, the low relative abundance of these stages within the *Boeckella poppei* population at the time of the experiment explains why the population of planktonic ciliates was strongly affected by zooplankton grazing whereas the nanoflagellate populations largely escaped this effect. These results are consistent with those of Butler et al. (2005), who evaluated grazing by *B. poppei* in Sombre Lake (Signy Island) and found that the fastest clearance rates were on heterotrophic ciliates of around 18 µm in equivalent spherical diameter, similar in size to the dominant ciliate in our experiment.

These strong effects on ciliate abundance and changes in the protist clearance rates related to the developmental instar composition of copepod populations have also been reported in other lakes (Hansen 2000). A related but somewhat different effect of copepod grazing on protists was found in lakes of the subantarctic South Georgia Islands (Tranvik and Hansson 1997), where grazing by the copepod *Boeckella michaelsenii* on flagellates favoured bacterioplankton abundance, whereas the larger *Pseudoboeckella* (=*Boeckella) poppei* did not have an effect on microbial populations because of their preference for larger particle sizes. Cascading food web effects are also likely within the microbial loop in Lake Limnopolar, since the strong grazing pressure on ciliates would benefit bacteria (both autotrophic and heterotrophic), allowing them to increase their abundances in the absence of ciliate grazing. This reduced grazing pressure on picoplankton seems more likely to explain their increased populations than the possible effects of DOC release by copepods, since both autotrophic (picocyanobacteria) and heterotrophic (DAPI-stained bacteria) picoplankters increased in their abundance in the presence of zooplankton, although N and P enrichment by the zooplankton may have had an additional synergistic effect. Size-selective zooplankton predation on pelagic microorganisms has been suggested to have a potentially strong effect on the community structure and production of planktonic microorganisms in other Arctic (Bertilsson et al. 2003) and Antarctic (Swadling and Gibson 2000) freshwater environments and, specifically, on aquatic bacteria through an array of freshwater environments by a complex interplay of variables (Hahn

and Höfle 2001). Ciliates and calanoid copepods, the main groups involved in the trophic cascade in Lake Limnopolar, appear to have much higher ingestion rates than other grazers of comparable sizes (Hansen et al. 1997), and might therefore be expected to induce strong interactive changes across trophic levels.

The relative importance of the microbial loop for energy transfer in different lacustrine communities may determine the extent of cascading effects through the food web. In Lake Limnopolar, energy transfer via the microbial loop likely exerts a major influence on the ecosystem, with effects on heterotrophic microbes as well as on microbial phototrophs. Despite the expectation that abiotic factors control the biological structure and functioning of Antarctic lakes, our experimental results indicate that top-down biotic interactions can also play a key role in determining the food web configuration. Although our experiment simulated large scale increases in nutrients and zooplankton relative to background levels, the results imply that with ongoing climate change in the polar regions, cascading food web effects will likely modulate the ecosystem responses to shifts in environmental conditions.

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Author contributions Author contributions: AC conceived and designed the research, conducted field work, conducted laboratory analyses, wrote the manuscript, and provided funding. CR conducted laboratory analyses, analysed the data and prepared figures. ER, EF, WV, AQ conducted field work. AC and WV edited the manuscript. WV conducted DOC analyses. AQ organized field work and provided funding. All authors read and approved the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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